

# Origin and diversification of hornbills (Bucerotidae)



Juan-Carlos Tecson Gonzalez

St. Anne's College

Thesis submitted for the degree of

Doctor of Philosophy

University of Oxford

Trinity term 2012

This page is intentionally left blank

# **Abstract**

## **Origin and diversification of hornbills (Bucerotidae)**

*DPhil thesis by Juan-Carlos T. Gonzalez, St Anne's College, submitted Trinity term 2012*

Hornbills (Aves: family Bucerotidae) are a charismatic group of Palaeotropical birds recognised for their distinctive morphology (casque) and behaviour (nest-sealing). Hornbill diet, habitat use, distribution and social system display pronounced interspecific variation, and their mutualistic interactions with tropical fruits provide vital ecosystem services. A wide range of species of hornbills across the Afrotropics, Indo-Malaya and Melanesia are of conservation concern. However, the evolutionary history of hornbills remains unclear and there are conflicting hypotheses about their origin, tempo of diversification and biogeography. Despite a comprehensive cladistic analysis of phenotypic data, there are unresolved taxonomic uncertainties within the family, and although a gradual accumulation of molecular data has revealed interesting phylogenetic relationships, methodological limitations, and incomplete sampling, has left gaps and produced incongruent results. Hence the evolutionary framework against which to interpret the diversity of this group is incomplete.

The aim of this thesis was to construct a well-resolved molecular phylogeny of hornbills, and to use it to address longstanding questions about the evolution and diversification of these remarkable birds. This thesis presents a broad phylogenetic and biogeographic analysis of the family Bucerotidae, based on a coalescence of molecular methods and comparative analyses, largely using historical samples and recently-developed bioinformatic approaches. I provide the first complete species-level molecular phylogeny of hornbills, derived from nuclear adenylate kinase 1 intron 5 and mitochondrial (mtDNA) cytochrome b genes, and also a comprehensive mtDNA phylogeny covering 98% of the taxa, with extensive sampling of Asian geo-isolates. Using these two phylogenetic trees, I sought to determine the tempo of divergence, trace the evolution of traits, identify ancestral areas and colonisation routes, and also calculate genetic divergence. In this part of the work, I stress the importance of (1) using historical samples, (2) calibrating time-trees

with fossil and molecular anchor-points, and (3) the use of a complete tree to test models of diversification and reconstruct ancestral states.

My findings confirm the monophyly of Bucerotidae, showing nearly distinct African and Asian lineages, with relatively congruent topologies across different phylogenetic methods and genes. In turn, these gene trees were comparable with previous cladistic analysis based on phenotypic data. As a result, I was able to resolve some taxonomic issues and propose generic changes. Comparative analyses of social behaviour revealed that cooperative breeding is an ancestral trait, and its evolution in hornbills (in contrast with some other taxa) is associated with stable environments in combination with frugivory, territoriality and reduced body size. Analysis of the evolution of diet with diversification showed that the shift to frugivory from faunivorous African ancestors influenced the rapid diversification of Afro-Asian forest hornbills, facilitated by their preference for humid forests and mutualistic interactions with hornbill-dispersed fruits (HDF). This dispersal of frugivorous lineages via the Palaeotropical Biotic Interchange promoted colonisation of Asia, with Sundaland being the centre of radiation for continental and insular Asian species. The gradual eastward colonisation from India to Melanesia matches palaeo-tectonic events that allowed dispersal across Huxley's, Wallace's and Lydekker's lines, and was congruent with historical biogeography of some HDFs. Finally, I used a combined analysis of genetic divergence and a standard scoring system for phenotypic data of Asian geo-isolates to provide evidence for quantitative delineation of species and propose changes in conservation status.

My findings reveal the evolutionary history of hornbills from their emergence in the Late Oligocene from African origins, with a switch to frugivory influencing successful colonisation of hornbills and HDFs in Asian forests, which combined to promote sociality. Overall, this thesis demonstrates that access to novel environments and innovations to ecological niche facilitate rapid diversification in an avian lineage, and that this process is further promoted by the interplay of these birds in complex mutualistic interactions with their food, as well as palaeo-climatic and palaeo-tectonic changes.



## Acknowledgements

This thesis is the culmination of a research project created and completed over three and half years, and this wouldn't be possible if not for the involvement and contributions of many people and organisations.

I would like to sincerely thank my advisory committee, Prof Ben C. Sheldon, Dr Nigel J. Collar and Dr. Joseph A. Tobias, for their diligent and brilliant supervision, and for having the patience and understanding to go through my results and improve the writing of my thesis. I will certainly try to promulgate the high standard of research and all important "evolutionary" life skills they have imbibed in me, and hopefully these would reflect in the final publications and other future work. I am extremely fortunate for having a cohesive supervisory group, as each of them imparts invaluable knowledge and expertise in the development and execution of my research. I am tremendously thankful for their critical review and constructive critique to improve my dissertation, and for their untiring assistance especially during the last months of writing.

I am extremely grateful for the substantial financial support of the following organisations for which my DPhil research would not have been completed: Ford Foundation, through the International Fellowship Program; Oxford University; the University of the Philippines; the North of England Zoological Society; the British Ornithologist's Union; and St Anne's College. I would also like to thank the following people and academic institutions for their invaluable assistance during the course of my DPhil in Oxford, Philippine Social Science Council; Institute for International Education, the British Council (UK and Manila), and the University of the Philippines Los Baños for allowing to go in study leave and pursue my doctorate in Zoology.

I extend my heartfelt thanks to the Department of Zoology for hosting my hornbill research and for the use of their facilities – EGI Molecular Ecology Lab and J.W. Jenkinson Lab for Evolution and Development and Zoology Sequencing Lab. I would like thank Prof Peter Holland for permitting me to conduct my extractions at his lab, Dr Tim Guilford to allow me to do field trials on hornbills, and to my college adviser Dr Martin Speight for all his sound advice. I am thankful to my PRS examiners Prof David MacDonald and Dr Andy Gosler for their insights to

earlier versions of my research. I am extremely thankful for the invaluable comments and suggestions of my thesis viva voce examiners, Dr Stephen Harris and Dr Alan Kemp. I also thank the Zoology Admin staff and Graduate Office for their assistance.

I am indebted to the support of the following museums, zoos and aviaries for providing invaluable tissue samples for my research: Natural History Museum Tring, Oxford University Museum of Natural History, UPLB Museum of Natural History, UP Diliman Institute of Biology, Mindanao State University Marawi, MSU Iligan Institute for Technology, American Museum of Natural History, Delaware Museum of Natural History, University Museum of Zoology Cambridge, National University of Singapore Raffles Museum, PAWB, Calintaan Wildlife Rescue Centre, Laguna Wildlife Park & Rescue Centre, Silliman University RB Gonzalez Natural History Museum, National Museum of the Philippines, Malagos Garden Resort, Opol Aviary, Sabin Hotel Aviary, West Visayas State University Mariit Wildlife Conservation Park, Negros Forests & Ecological Foundation Inc Biodiversity Conservation Centre, Pollilo Islands Biodiversity Conservation Foundation Inc, Chester Zoo, Zoological Society of London (London & Whipsnade Zoo), Ninoy Aquino Parks & Wildlife Rescue Centre and the Northern Sierra Madre Natural Park. Permits for collection and transport of samples were facilitated by DEFRA, US Fish & Wildlife, Protected Areas & Wildlife Bureau, Department of Environment & Natural Resources Regional and Provincial Offices and NSMNP Protected Areas Management Board. It has been a privilege to have extracted DNA from skins collected by E. Mayr, A.R. Wallace and D.S. Rabor.

To refrain from turning my acknowledgements section into one full chapter, I would like to thank everyone, both colleagues and friends, who have helped me go through this academic process directly or indirectly, either here in the UK, in the Philippines or elsewhere – and to be fair to everyone my apologies if I have not been able to mention your name directly. I have also acknowledged the assistance of particular individuals and institutions in each of the five data-chapters/papers. Nonetheless, I would like to thank the following: my Sierra Madre hornbill project field team, Wild Bird Club of the Philippines; Wildlife Conservation Society of the Philippines, Asian Hornbill Network, Oriental Bird Club, Zoological Society of London, Chester Zoo, BirdLife International, Association for Tropical Biology & Conservation, Philippine Biodiversity

Conservation Foundation, Ford-IFP Cohort 2007, U.P. Alumni Association U.K., OZOOMS, Alcasid & Dans clan, People of Palanan, Isabela, Institute of Biological Sciences-UPLB, Oxford Department of Zoology JRC, Avian NavGroup, St Anne's College MCR & SDG, flatmates at 15 Purcell, Oxford Philippines Society, Animal Biology Division-UPLB, and the Edward Grey Institute for Field Ornithology, most especially to my fellow DPhil students for their excellent camaraderie, all my D6 officemates, the Alexander Library and the Tropical Ecology and Evolution group.

To my family, both in the Philippines and the United States for their love and support.

I dedicate this thesis to four people who have helped me through my DPhil but are no longer with us to share this moment of completion, Prof Pedro Alviola III, Leonardo Co, Dean Corazon Lamug and Dr Josefino Hedreyda.

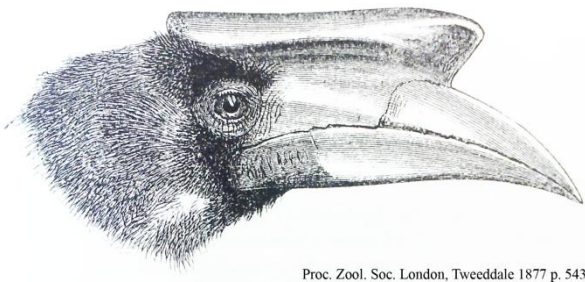
*Maraming salamat po*

# Contents

|                                                                                                                                                                                   | Page |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <b>Chapter 1</b> Introduction                                                                                                                                                     | 1    |
| <b>Chapter 2</b> A comprehensive molecular phylogeny for the hornbills<br>(Aves: Bucerotidae): nuclear and mitochondrial DNA<br>trees for a threatened avian radiation.           | 28   |
| <b>Chapter 3</b> Environmental stability and the evolution of cooperative<br>breeding in hornbills.                                                                               | 80   |
| <b>Chapter 4</b> Diet, dispersal and diversification: molecular phylogenies<br>of hornbills and their food-plants reveal the evolutionary<br>history of a classic mutualism.      | 118  |
| <b>Chapter 5</b> Dispersal across insular barriers in the Palaeotropics:<br>Phylogeny and historical biogeography of Afro-Asian hornbills.                                        | 185  |
| <b>Chapter 6</b> Combining genotypic and phenotypic data to address the<br>question of species limits: a case study of Asian hornbills.                                           | 243  |
| <b>Chapter 7</b> Discussion                                                                                                                                                       | 348  |
| <b>Appendices</b> Other papers on the Ecology and Ethno-ornithology<br>of Philippine Hornbills (Research papers completed<br>and presented as paper/poster while a DPhil student) | 378  |

## Chapter 1

### Introduction



*The geographical distribution of the hornbills seems therefore quite in accordance with the almost universal belief of modern naturalists, that the present position of every living thing upon the earth is the result of that long and complicated series of geological changes and organic modification which the globe has ever been and still is undergoing.*

Alfred Russel Wallace, 1863, The Bucerotidæ, or Hornbills, *The Intellectual Observer*

### **Introduction**

Patterns of diversification and biogeographic origins of biological diversity have been a focal area of research since Charles Darwin and Alfred Russel Wallace introduced their theory of evolution (Mayr, 1942; Newton, 2003). Understanding the processes underpinning speciation and radiation has sparked numerous investigations that interface evolutionary ecology with different traditional disciplines (e.g. genetics, geology, palaeontology), leading to the development of modern studies in molecular systematics, phylogeography, co-evolution and conservation biology (Newton, 2003; Price, 2008). I am interested in how these different inter-related fields can be used to understand the role of speciation and biogeography in promoting biological diversity in the tropics.

More than half of the world's species are found in the tropics (Mittermeier *et al.*, 2005; Lamoreux *et al.*, 2006), the warm belt around the earth between the Tropic of Cancer and Tropic of Capricorn. This is evident in the exceptionally rich assemblage of bird species occurring in the tropics of the New World (Neotropics) and Old World (Palaeotropics). Nearly all the global hotspots for avian species richness, endemism and threatened species occur in these two regions (BirdLife International, 2000; Orme *et al.*, 2005). Birds are the ideal taxonomic group to test the integrated roles of genetics, ecology and behaviour in understanding speciation (Price, 2008). In this thesis, I have sought to answer fundamental questions commonly raised in ornithology and evolutionary ecology: What drives an avian lineage to diversify? How are the species in the lineage related? Where did they originate from? When and how did they arrive at their present distribution?

### **Overview of approaches used to understand diversification**

To address these questions, I looked into the role of *molecular systematics* in assessing similarities between species within a distinct tropical avian group. Molecular methods helped unravel the information stored in genes to reconstruct phylogenetic relationships. Nucleotide

## CHAPTER 1

DNA (deoxyribonucleic acid) sequences serve as an invaluable detailed record of the phylogenetic relationships and times of divergence between organisms (Page & Holmes, 1998; Stearns & Hoekstra, 2005). Reconstruction of phylogenies is based on the principle of common ancestry and the branching process, whereby one species diverges to form more species along a lineage. The evolutionary history is supposedly illustrated in the constructed tree, and this has been instrumental in understanding avian lineages (Newton, 2003; Price, 2008).

I then built on this molecular approach, and investigated the role of *phylogeography* in shaping geographical distributions of genealogical lineages. It takes into account historical and spatial components (historical biogeography), and is thus able to identify the ancestral origins of taxonomic assemblages. It also determines the rationale for their current distribution based on dispersal and vicariance events (Avice, 2000). Although the study focused more on genetic traits, I recognise the importance of traditional biogeography and associated speciation processes inferred from morphological and distribution patterns (Mayr, 1942).

I also examined the role of biotic interactions in generating diversity, and how potentially two groups of organisms can co-evolve from reciprocal evolutionary relationships. These interactions may have been a major factor in promoting plant and animal diversification in the tropics (Ehrlich & Raven, 1964). Some theoretical models of speciation suggest a probable pairing between mutualistic plants and animals, although some of these forms of *co-evolution* (e.g. fruit-frugivore interactions) may be too diffused to promote diversity and therefore merit further study (Fleming *et al.*, 1993, Herrera & Pellmyr, 2002).

I also looked into the implications of species formation and diversification in addressing conservation issues in the tropics. Splitting into morphologically distinct populations has a key role in geographical (allopatric) speciation, which in turn translates into reproductive isolation, a key component in recognizing species limits (Newton, 2003; Price, 2008). Likewise, a good understanding of the speciation process requires a high level of resolution of taxonomic uncertainties and a clear, consistent approach to species delimitation. Therefore, sound inter-specific and intra-specific systematics based on a robust phylogeny leads to an informative classification and a precise taxonomy. This taxonomy is essential in *conservation biology*, as

phylogenetic and taxonomic distinctiveness can provide the measures by which priorities are set (Frankham *et al.*, 2002).

### **Molecular phylogenetics and avian lineages**

The use of molecular methods and *de novo* bio-informatics as tools for the analysis of patterns in evolution and ecology has produced a new wave of integrated studies in molecular phylogenetics, historical biogeography and analyses in trait evolution. Indeed, a phylogenetic tree constructed from DNA sequences can serve as a multifunctional device, facilitating studies that infer organismal phylogeny, genetic distances, comparative analyses of traits, reconstruction of ancestral states, and calculation of age and rates of diversification (Page & Holmes, 1998; Stearns & Hoekstra, 2005). The combination of molecular and traditional methods has proven useful in resolving key phylogenetic and biogeographic issues in birds. These methods have been instrumental in understanding the evolution of many avian lineages, most of which were done at the family level, from barbets, bee-eaters and cockatoos (Moyle, 2004; Marks *et al.*, 2007; White *et al.*, 2011) to bulbuls, cuckoo-shrikes and vangas (Moyle & Marks, 2006; Jønsson *et al.*, 2010; Reddy *et al.*, 2012).

Studies in molecular systematics have attempted to address issues regarding higher-level classification of birds through the use of different nucleotide loci (nuclear and mitochondrial), but have arrived at discordant results (Hackett *et al.*, 2008; Pacheco *et al.*, 2011). However, phylogenetic reconstructions among or within related avian lineages using robust datasets have generally provided novel and better resolved findings. A phylogenetic analysis of the starlings Sturnidae and thrashers Mimidae determined that the family of Philippine creepers Rhabdornithidae is a sister lineage to Indo-Pacific starlings and mynas, and that oxpeckers *Buphagus* should be excluded from Sturnidae and placed in their own family Buphagidae (Zuccon *et al.*, 2006; Lovette & Rubenstein, 2007). A more arresting example of these findings described how the rockfowl Picathartidae has been extended to include the rockjumpers *Chaetops* and the enigmatic Rail Babbler *Eupetes macrocerus* (Jønsson *et al.*, 2007). A phylogeny of the family



Timaliidae indicated that other oscine taxa (*Sylvia* and *Zosterops*) unexpectedly belonged to the babbler assemblage (Cibois, 2003). Indeed, the impact of molecular approaches on systematics and biogeography of birds in recent years has been profound. The stochastic processes and time-dependent evolution of biochemical characters circumvents problems associated with morphological characters (Newton, 2003).

Molecular phylogenies have also been used to calculate divergence time and diversification rates within avian groups, and to explain the radiation of bird species across key biogeographic areas. Historical biogeography in New World birds such as toucans and aracaris (Nahum *et al.*, 2003; Patel *et al.*, 2011), woodpeckers (Benz & Robbins, 2011) and parrots (Ribas *et al.*, 2005, Tavares *et al.*, 2006) have provided insights on how birds colonised and radiated across the Neotropics. Similar studies on birds in the Indo-Pacific provided the basis in understanding the dispersal and colonisation of Asia and Australasia. The phylogeography of trogons (Hosner *et al.*, 2010), racquet-tail parrots (Schweizer *et al.*, 2012) and ground-doves of the genus *Gallicolumba* (Jønsson *et al.*, 2011) have provided evidence on how birds diversified across Sundaland, Wallacea and Melanesia. Following these examples, I applied integrated techniques to investigate the evolution and radiation of another classic tropical avian family, one which is shrouded in taxonomic and biogeographic uncertainties – the hornbills.

### **The hornbills (Aves: Bucerotidae)**

Hornbills are a charismatic, distinctive and diverse group of Palaeotropical birds. They are focal species for studies in ecology and behaviour, owing to their conspicuous nature in the environment and their partiality to frugivory. Generally, they are easily recognised by their brilliant ornamentation, loud vocalisations and interesting behavioural traits (Kemp, 1995; Kinnaird & O'Brien, 2007). Wallace (1863) described hornbills as “large and clumsy birds, seldom adorned with bright-coloured plumage, but in many cases bearing a really prodigious bill”. The bill is indeed distinctive, being elongate, decurved, equally suited to handling animal prey or delicate fruits, and often supported by an elaborate ornamentation of the casque or facial skin (Figure 1.1).



**Figure 1.1:** Hornbills (Aves: family Bucerotidae) are a charismatic and diverse group of Palaeotropical birds with colourful facial skin and an elaborate horny casque on their long decurved bills. The diversity of forms is illustrated in the following species: (A) *Tockus deckeni*, (B) *Anthracoceros marchei*, (C) *Buceros bicornis*, (D) *Aceros walderi*, (E) *Penelopides panini*, and (F) *Rhyticeros undulatus*.

Hornbills are also renowned for their raucous resonating calls (deep resonant boom audible up to 5 km) and unique breeding behaviour (with the exception of the ground-hornbills), namely the strategy of walling up the entrance of their nest cavity (Kemp, 1995; 2001a). Hornbills range in size from 30 cm (dwarf hornbills) to 120 cm (ground-hornbills), with rounded wings and long tails (except for ground-hornbills). Bucerotidae are restricted to the tropical regions of Africa (south of the Sahara) and Asia (Oriental or Indo-Malayan), with one species reaching Australasia (particularly Melanesia) (Figure 1.2). They are often confused with toucans of the Neotropics as they possess convergent characters, but have entirely separate lineages (Wallace, 1863; Kemp, 1995).

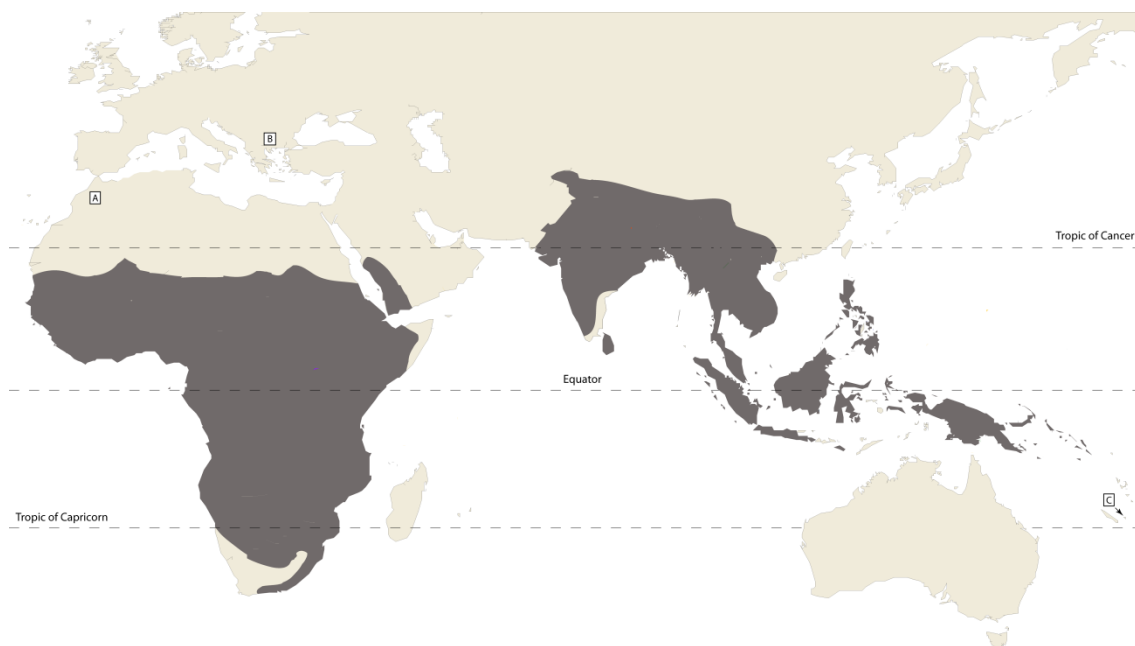


**Figure 1.2:** The present geographic range of extant hornbills (dark grey) and extralimital localities of three extinct species: (A) *Bucorvus brailloni* from Beni-Mellal, Morocco (Brunet, 1971), (B) *Euroceros bulgaricus* from Hadzhidimovo, Bulgaria (Boev & Kovachev, 2007), and (C) *Rhyticeros* sp. from Lifou, Loyalty Islands (Steadman, 2006).

## CHAPTER 1

Recent studies on hornbills have focused mainly on their ecology, behaviour and conservation, although there is growing interest in ethno-biology and genetics. Ecological studies vary extensively, and include aspects of movement patterns (Holbrook & Smith, 2000; Holbrook *et al.*, 2002), population density estimation (Marsden & Pilgrim, 2003; Shankar Raman & Mudappa, 2003; Sitompul *et al.*, 2004; Marsden, 2008), seed dispersal and frugivory (Luft *et al.*, 2002; Kitamura *et al.*, 2004; Kitamura, 2011), nest-hole characteristics (Klop *et al.*, 2000; Poonswad *et al.*, 2005), breeding strategies (Stanback *et al.*, 2002; Stauffer & Smith, 2004; Mills *et al.*, 2005), and feeding behaviour (Datta & Rawat, 2003; Hadiprakarsa & Kinnaird, 2004). Hornbills are well recognised in archaeology and anthropology due to their wide use among indigenous people, in folklore, artefacts and traditions (Bennett *et al.*, 1997; Oliver & Wilkinson, 2007; Gonzalez, 2011). Current use of molecular techniques has also facilitated research into phylogenetics (Kemp & Delpont, 2002; Hübner *et al.*, 2003; Viseshakul *et al.*, 2011) and population genetics (Delpont *et al.*, 2002; 2005; Sammler *et al.*, 2011).

Studies have also focused on the assessment of threats to hornbills and their importance to tropical conservation (Kemp, 2001b; Gonzalez, 2007; Trail, 2007). However, despite the increased attention placed on hornbill research in recent years, there are still several key gaps that need to be addressed in order to make sound conservation strategies. During the 2009 International Hornbill Conference, experts agreed on three major concerns, namely (1) the re-evaluation of species limits based on phylogenetic techniques, (2) filling in the gaps in ecological knowledge for poorly known West African and Philippine hornbills, and (3) outlining the ethno-biological importance of hornbills. Kemp & Kemp (2009) further emphasised the importance of understanding the origins of hornbills and the reasons for the rapid radiation of frugivorous hornbills in Asia and Africa (Davison, 2011). These recommendations highlight the need for a comprehensive phylogenetic study of Bucerotidae based on molecular data, as these can provide the basis to make salient analyses on diversification and genetic divergence.

## Taxonomy of the family Bucerotidae

Hornbill taxonomy is described as having a convoluted and unsettled history, given the inconsistency in taxonomic treatments across various monographs and global bird lists. Much earlier, Wallace (1863) recognised that the relations of the hornbills to other birds were rather obscure. Taxonomic treatments varied substantially between Elliot (1882), Peters (1945), Sanft (1960) and Kemp (1995; 2001a). Despite having a comprehensive cladistic analysis based on morphology, internal anatomy, behaviour and ontogeny (Kemp, 1995), some taxonomic enigmas remain unresolved (e.g. *Tropicranus*). Traits were shared by species across different genera, and were lacking a clear pattern to indicate unequivocal inter-generic relations and evolutionary history (Kemp, 2001a).

Generally, hornbill relationships at both the higher and species levels remain obscure. It is uncertain whether polytypic species in both African and Asian hornbills, such as those forming species-complexes, constitute a group of distinct species (Kemp, 2001b). Some proposed splits, such as the superspecies formed by *Penelopides panini* (Kemp, 1988) are contested in other taxonomic treatments (e.g. Dickinson *et al.*, 1991). Two key authorities, namely the *Handbook of the Birds of the World* (“HBW”: Kemp, 2001a) and IOC World Bird List (“IOC”: Gill & Donsker, 2012), have contrasting views, the former placing hornbills in the Order Coraciiformes and the latter in the Order Bucerotiformes. The number of inclusive taxa also varies, with 54 species for HBW versus 61 in IOC, and 24 and 27 subspecies, respectively. The overall number of hornbill species differs across other global bird lists, with 51 in Dickinson (2003), and 57 in Clements (2007) and BirdLife International ([www.birdlife.org/datazone/](http://www.birdlife.org/datazone/)).

Hornbills have traditionally been placed in the Order Coraciiformes together with kingfishers (Alcedinidae), bee-eaters (Meropidae), rollers (Coraciidae), hoopoes (Upupidae), todies (Todidae), motmots (Momotidae) and woodhoopoes (Phoeniculidae), based on a common pedal feature, syndactyly (Peters, 1945). They also share similar features in their altricial nestling stage, such as protrusion of the lower jaw and prolonged retention of pin-feathers or what is referred to as the “pin-cushion-like” stage (Kemp, 1995; 2001a). However, certain unique features of hornbills

## CHAPTER 1

(bilobular kidney, absence of secondary underwing coverts, fused atlas and axis, supra/basioccipital articulation), phylogenetic evidence (from DNA-DNA hybridisation, larger Z chromosome) and behavioural traits (walling up of nest entrance) have been speculated as sufficient to elevate hornbills to their own order (Kemp, 2001a). This was later supported by molecular studies covering a comprehensive nucleotide matrix (32,000 base pairs) of nuclear DNA sequences (Hackett *et al.*, 2008).

However, recent phylogenetic evidence based on a wide coverage of nearly 3,000 morphological characters (Livezey & Zusi, 2007) and a well-resolved molecular phylogeny of the complete avian mitochondrial genome (Pacheco *et al.*, 2011) supports the monophyly of member families of Coraciiformes, including hornbills. Given this agreement between morphological and molecular data across a wide scale of attributes (characters and gene regions), I have retained the traditional placement and treated Bucerotidae as a family embedded within the Coraciiformes. This taxonomic treatment is followed in other global checklists and handbooks (Dickinson, 2003; Clements, 2007; Perrins, 2009).

Kemp (2001a) recognised a single family Bucerotidae, with 14 genera in two subfamilies, Bucorvinae (ground-hornbills, 1 genus, 2 species) and Bucerotinae (nest-sealing hornbills, 13 genera, 52 species). However, in the 10 years since then, a number of changes have been made to the global hornbill list. The IOC World Bird List is a recognised and periodically updated on-line database. I accepted the changes made for Bucerotidae (and Bucorvidae) in the IOC list version 2.11 (Gill & Donsker, 2012), which has total of 61 species (Table 1.1), and an additional 27 subspecies (88 taxa).

# CHAPTER 1

**Table 1.1:** Relative distribution of 61 extant species of African and Asian hornbills and their current conservation status.

| Common name <sup>a</sup>         | Scientific name <sup>a</sup>     | Distribution               | Status <sup>b</sup> |
|----------------------------------|----------------------------------|----------------------------|---------------------|
| Northern Ground-Hornbill         | <i>Bucorvus abyssinicus</i>      | Mauritania to Ethiopia     | LC                  |
| Southern Ground-Hornbill         | <i>Bucorvus leadbeateri</i>      | Rwanda to South Africa     | VU                  |
| Crowned Hornbill                 | <i>Tockus alboterminatus</i>     | Ethiopia to South Africa   | LC                  |
| Bradfield's Hornbill             | <i>Tockus bradfieldi</i>         | Zimbabwe to Angola         | LC                  |
| Red-billed Dwarf Hornbill        | <i>Tockus camurus</i>            | Guinea to Angola           | LC                  |
| Damara Red-billed Hornbill       | <i>Tockus damarensis</i>         | Namibia                    | LC                  |
| Von der Decken's Hornbill        | <i>Tockus deckeni</i>            | Ethiopia to Tanzania       | LC                  |
| Northern Red-billed Hornbill     | <i>Tockus erythrorhynchus</i>    | Burkina Faso to Tanzania   | LC                  |
| African Pied Hornbill            | <i>Tockus fasciatus</i>          | Gambia to Angola           | LC                  |
| Eastern Yellow-billed Hornbill   | <i>Tockus flavirostris</i>       | Somalia to Tanzania        | LC                  |
| Black Dwarf Hornbill             | <i>Tockus hartlaubi</i>          | Sierra Leone to Uganda     | LC                  |
| Hemprich's Hornbill              | <i>Tockus hemprichii</i>         | Ethiopia to Kenya          | LC                  |
| Jackson's Hornbill               | <i>Tockus jacksoni</i>           | Uganda to Kenya            | LC                  |
| Western Red-billed Hornbill      | <i>Tockus kemp</i>               | Mauritania to Senegal      | LC                  |
| Southern Yellow-billed Hornbill  | <i>Tockus leucomelas</i>         | Angola to Mozambique       | LC                  |
| Monteiro's Hornbill              | <i>Tockus monteiri</i>           | Angola & Namibia           | LC                  |
| African Grey Hornbill            | <i>Tockus nasutus</i>            | Mauritania to South Africa | LC                  |
| Pale-billed Hornbill             | <i>Tockus pallidirostris</i>     | Angola to Mozambique       | LC                  |
| Tanzanian Red-billed Hornbill    | <i>Tockus ruahae</i>             | Tanzania                   | LC                  |
| Southern Red-billed Hornbill     | <i>Tockus rufirostris</i>        | Angola to South Africa     | LC                  |
| White-crested Hornbill           | <i>Tropicranus albocristatus</i> | Guinea to Uganda           | LC                  |
| Black-casqued Wattled Hornbill   | <i>Ceratogymna atrata</i>        | Guinea to Uganda           | LC                  |
| Yellow-casqued Wattled Hornbill  | <i>Ceratogymna elata</i>         | Senegal to Cameroon        | NT                  |
| White-thighed Hornbill           | <i>Bycanistes albotibialis</i>   | Benin, to Uganda           | LC                  |
| Silvery-cheeked Hornbill         | <i>Bycanistes brevis</i>         | Ethiopia to Mozambique     | LC                  |
| Trumpeter Hornbill               | <i>Bycanistes bucinator</i>      | Kenya to Angola            | LC                  |
| Brown-cheeked Hornbill           | <i>Bycanistes cylindricus</i>    | Sierra Leone to Ghana      | NT                  |
| Piping Hornbill                  | <i>Bycanistes fistulator</i>     | Senegal to Uganda          | LC                  |
| Black-and-white-casqued Hornbill | <i>Bycanistes subcylindricus</i> | Côte d' Ivoire to Kenya    | LC                  |
| Tickell's Brown Hornbill         | <i>Anorrhinus tickelli</i>       | Myanmar & Thailand         | NT                  |
| Austen's Brown Hornbill          | <i>Anorrhinus austeni</i>        | India to Vietnam           | NT                  |
| Bushy-crested Hornbill           | <i>Anorrhinus galeritus</i>      | Sunda Shelf excluding Java | LC                  |
| Malabar Grey Hornbill            | <i>Ocyceros griseus</i>          | Southwest India            | LC                  |
| Sri Lankan Grey Hornbill         | <i>Ocyceros gingalensis</i>      | Sri Lanka                  | LC                  |
| Indian Grey Hornbill             | <i>Ocyceros birostris</i>        | Pakistan to Bangladesh     | LC                  |
| Indian Pied Hornbill             | <i>Anthracoceros coronatus</i>   | India & Sri Lanka          | NT                  |
| Oriental Pied Hornbill           | <i>Anthracoceros albirostris</i> | India to Sunda Shelf       | LC                  |

## CHAPTER 1

|                        |                                 |                            |    |
|------------------------|---------------------------------|----------------------------|----|
| Palawan Hornbill       | <i>Anthracoceros marchei</i>    | Philippines: Palawan       | VU |
| Black Hornbill         | <i>Anthracoceros malayanus</i>  | Sunda Shelf excluding Java | VU |
| Sulu Hornbill          | <i>Anthracoceros montani</i>    | Philippines: Sulu group    | CR |
| Great Hornbill         | <i>Buceros bicornis</i>         | India to Sunda Shelf       | NT |
| Rhinoceros Hornbill    | <i>Buceros rhinoceros</i>       | Thailand to Sunda Shelf    | NT |
| Rufous Hornbill        | <i>Buceros hydrocorax</i>       | Eastern Philippines        | NT |
| Helmeted Hornbill      | <i>Rhinoplax vigil</i>          | Myanmar to Sunda Shelf     | NT |
| Sulawesi Hornbill      | <i>Penelopides exarhatus</i>    | Sulawesi, Indonesia        | LC |
| Tarictic Hornbill      | <i>Penelopides panini</i>       | Philippines: West Visayas  | EN |
| Luzon Hornbill         | <i>Penelopides manillae</i>     | Philippines: Luzon         | LC |
| Samar Hornbill         | <i>Penelopides samarensis</i>   | Philippines: East Visayas  | LC |
| Mindanao Hornbill      | <i>Penelopides affinis</i>      | Philippines: Mindanao      | LC |
| Mindoro Hornbill       | <i>Penelopides mindorensis</i>  | Philippines: Mindoro       | EN |
| White-crowned Hornbill | <i>Berenicornis comatus</i>     | Cambodia to Sunda Shelf    | NT |
| Rufous-necked Hornbill | <i>Aceros nipalensis</i>        | Bhutan to Vietnam          | VU |
| Wrinkled Hornbill      | <i>Aceros corrugatus</i>        | Cambodia to Sunda Shelf    | NT |
| Writhed Hornbill       | <i>Aceros leucocephalus</i>     | Philippines: Mindanao      | NT |
| Walden's Hornbill      | <i>Aceros waldeni</i>           | Philippines: West Visayas  | CR |
| Knobbed Hornbill       | <i>Aceros cassidix</i>          | Sulawesi, Indonesia        | LC |
| Narcondam Hornbill     | <i>Rhyticeros narcondami</i>    | Narcondam, India           | EN |
| Blyth's Hornbill       | <i>Rhyticeros plicatus</i>      | Moluccas to Solomons       | LC |
| Plain-pouched Hornbill | <i>Rhyticeros subruficollis</i> | Myanmar to Malaysia        | VU |
| Wreathed Hornbill      | <i>Rhyticeros undulatus</i>     | Bhutan to Sunda Shelf      | LC |
| Sumba Hornbill         | <i>Rhyticeros everetti</i>      | Sumba, Indonesia           | VU |

<sup>a</sup>Taxonomic treatment based on International Ornithological Congress World Bird List version 2.11 (Gill & Donsker 2012).

<sup>b</sup>Conservation status of hornbills according to the IUCN Red List assessments: LC, Least Concern; VU, Vulnerable; EN, Endangered; CR, Critically Endangered; NT, Near-threatened; assignment to categories follows BirdLife International (2000), apart from *Bucorvus leadbeateri* which was upgraded to VU from LC (BirdLife International, 2010; [www.birdlife.org/action/science/species/global\\_species\\_programme/whats\\_new.html](http://www.birdlife.org/action/science/species/global_species_programme/whats_new.html)).



One probable reason for the disparity in the global hornbill list has been the lack of cohesion in recognising proposed splits, which is sometimes attributed to insufficient evidence. Kemp (2001a) listed 54 hornbill species, but Gill & Donsker (2012) recognised seven additional splits from subspecies (also noted by Kemp, 2001a) that were later elevated to species status. Gill & Donsker (2012) indicated that the split of *Tockus erythrorhynchus* was based on recommendations by Kemp & Delport (2002), whereas reasons for splitting *Tockus jacksoni* and *Bycanistes cylindricus* were lacking. A robust tree topology arising from a detailed molecular phylogeny of hornbills can be used to analyse relationships and make inferences on their taxonomy. Earlier attempts to build this phylogeny have proved useful in addressing these issues by providing insights on taxonomic relationships, but these phylogenies were based on an incomplete sampling of taxa (Sibley & Ahlquist, 1990; Morin *et al.*, 1994; Srikwan & Woodruff, 1998; Delport *et al.*, 2002; Hübner *et al.*, 2003; Viseshakul *et al.*, 2011). A more comprehensive sequence matrix covering all species and subspecies is therefore essential. Molecular studies in combination with a detailed phenotypic analysis can provide powerful evidence to delimit species within the group (Helbig *et al.*, 2002; Tobias *et al.*, 2010).

### **Evolutionary history of hornbills**

Within the Coraciiformes, the Bucerotidae have close affinities with two other syndactylous families, the hoopoes Upupidae and woodhoopoes Phoeniculidae. They form the Suborder Bucerotes (Kinnaird & O'Brien, 2007, Mayr, 2007a), together with an allied extinct family Messelirrisoridae from the middle Eocene epoch (Mayr, 2000; 2006). All extant Bucerotes possess a decurved bill, oval eggs with pitted shells and tufted oil-glands. The Messelirrisoridae are hypothesised to form a stem lineage with the Upupidae and Phoeniculidae, which in turn form sister clades with the Bucerotidae within Bucerotes. Some morphological evidence also suggests an alternative sister group relationship between Suborder Bucerotes and Order Piciformes (woodpeckers and allies) (Olson, 1985; Mayr, 2006). Although this relationship with hoopoes and woodhoopoes is generally accepted (Olson, 1985; Kemp, 1995; Mayr, 2006; Kinnaird & O'Brien,

2007), the origins of the Bucerotidae remain unresolved. There are contrasting hypotheses about the ancestry of the hornbills, which is rendered all the more problematic by the apparent lack of any pre-Pliocene fossil arboreal Bucerotidae.

An earlier hypothesis suggested that hornbills arose in Asia (proto-*Tockus*) and emigrated to Africa via Arabia to form the diverse group of African *Tockus* (Kemp, 1976). However, a more complete phylogeny of hornbill genera suggested an African origin for *Tockus*, which then spread to Asia and became larger before re-colonising Africa as frugivorous forest hornbills and ground-hornbills (Kemp, 1979). Kinnaird & O'Brien (2007) painted a different scenario for hornbill evolution, again with African origins but with an ancestral ground-hornbill lineage referred to as proto-*Buceros*. Proto-*Buceros* was large-bodied, territorial and carnivorous. It gave rise to *Tockus* in Africa, but then spread to Asia to inhabit forests and shift to frugivory, which promoted radiation. Frugivorous forms then reinvaded Africa giving rise to African forest hornbills.

To date, only two pre-Quaternary fossil records exist for the Bucerotidae, both occurring in the Upper Miocene (5.3 to 11.6 Ma), and both involving ground-hornbills. A fossil of *Bucorvus brailloni* was taken from Beni-Mellal, Morocco (Brunet, 1971), and *Euroceros bulgaricus* was taken from Hadzhidimovo, Bulgaria (Boev & Kovachev, 2007), both at the margins of the Mediterranean Sea (Figure 1.2). All other fossils previously placed in the Bucerotidae, *Geiseloceros robustus*, *Cryptornis antiquus* and *Homalopus picoides*, are no longer valid (Olson, 1985; Mayr, 2007b). Several Quaternary (Late Paleolithic to Neolithic) fossils of arboreal hornbills have been found in Southeast Asia and Melanesia. These include *Rhinoplax vigil* from Niah Cave in Sarawak, Malaysia (Borneo); *Anthracoceros albirostris*, *R. vigil*, *Anorrhinus cf. tickelli*, *Buceros cf. bicornis*, and *Rhyticeros cf. undulatus* from Maxa I and Dieu Caves of Tonkin, Vietnam (Late Paleolithic deposits, dated 24,000–9,400 years B.P.: Boev & Kovachev, 2007); and *Rhyticeros* sp. from Lifou, Loyalty Islands (Steadman, 2006).

There is a growing consensus that a dated phylogeny based on molecular data may be able to help trace the evolutionary history of hornbills (Kemp, 2001a; Kemp & Kemp, 2009). Kinnaird & O'Brien (2007) reaffirmed this importance, stating that the application of advances in genetic research can provide a clearer picture of hornbill evolution. Reviews describing molecular

evidence and historical relations between hornbills were largely based on analysis using DNA-DNA hybridisation (Sibley & Ahlquist, 1990) and mitochondrial DNA sequences (Morin *et al.*, 1994; Srikwan & Woodruff, 1998; Hübner *et al.*, 2003). All four phylogenetic trees are incomplete and showed variable results. However, certain lineages appear to coincide between trees, particularly in placing ground-hornbills as the oldest lineage. This supports the theory of ancestral origins from Africa, which was based on a fossil record from North Africa (Brunet, 1971). Pinning down African origins became controversial with the discovery of fossil ground-hornbills from southern Europe (Boev & Kovachev, 2007). However, Kinnaird & O'Brien (2007) did explain that the Eocene tropical region extended from Europe to Africa, whereas Boev & Kovachev (2007) noted that tropical forest-steppes were predominant in southern Europe during the Miocene and Pliocene, and shared this biome with typical African fauna (e.g. ostriches, giraffes). A comprehensive phylogenetic analysis of hornbills taking into account detailed evidence on evolutionary divergence (molecular clock) is indeed crucial to understanding its origins.

This was achieved recently with an expanded mitochondrial DNA phylogeny covering 34 species, and all known hornbill genera (Viseshakul *et al.*, 2011). Divergence time was estimated from a linearised tree calibrated using the fossil *B. brailloni* (node age placed at 13 Ma). This tree allowed preliminary assumptions to be made about the radiation of basic hornbill genera and suggested that frugivorous clades originated in the mid-Eocene (~48 Ma), and split from the African crown-group around 53 Ma. Historical biogeography postulated that frugivorous Asian hornbills facilitated seed dispersal during the invasion of Southeast Asia by plant families from India (Viseshakul *et al.*, 2011). However, Viseshakul *et al.* (2011) demurred from earlier suggestions by Kinnaird & O'Brien (2007) that hornbills had African origins. Viseshakul *et al.* (2011) proposed an alternative Laurasian ancestry during the Early Eocene based on their dated phylogeny, which was in accordance with other fossil Coraciiformes (e.g. Messelirrisoridae: Mayr, 2006). Moreover, Viseshakul *et al.* (2011) noted that post-Palaeocene colonisation of Asia from Africa was less parsimonious due to oceanic barriers. It appears there are two confounding models for the origin of hornbills, the "Out-of-Africa" (Kinnaird & O'Brien, 2007) model and the "Out-of-Laurasia" model (Viseshakul *et al.*, 2011). Seemingly, an integrative analysis using complete

molecular data, with robust calibration of divergence time and reconstruction of ancestral ranges is needed to understand hornbill origins better.

Viseshakul *et al.* (2011) placed a caveat that their findings were unable to resolve basal branches within their phylogeny owing to low confidence values, and urged that further phylogenetic analyses be done on a well-resolved tree. To describe best the significance of attending to this requisite, I quote Kinnaird & O'Brien (2007) "Confirmation of this scenario will have to await the day when we finally have a reliable evolutionary tree for hornbills based on the DNA of all the species". The applications of a well-resolved tree covering all hornbill taxa are indeed considerable, as many novel techniques now allow complex analysis to understand processes, from speciation to trait development, and from reconstructing ancestral ranges to delimiting species.

### **Biogeography and conservation of Asian hornbills**

In the search for understanding where hornbills originated and how they diversified, further questions were raised regarding how continental and insular Asia was colonised, particularly Sundaland, Wallacea, the Philippines and Melanesia (Kinnaird & O'Brien, 2007). Asian hornbills have diverse forms and some have highly limited distributions, showing increased variation and endemism on small islands. About 32 out of the total 61 hornbill species occur only in Asia, ranging from Pakistan to the Solomon Islands (Table 1.1) (Kemp, 2001; Kinnaird & O'Brien, 2007).

Nearly all species of Asian hornbills are obligate frugivores, highly forest-dependent and often with limited ranges, whether continental or insular. Thus, they make ideal taxa for studies in evolutionary ecology and biogeography. All but one (*Ocyrceros birostris*) inhabit tropical evergreen forests and therefore are essential keystone mutualists to equally important forest trees (Sethi & Howe, 2009; Viseshakul *et al.*, 2011). They are invaluable contributors to reforestation and provide functional linkages that help maintain succession in tropical rainforests in Asia (Herrera & Pellmyr, 2002; Kitamura, 2011). They are described as candidate landscape-species for having a

significant role in the function of natural ecosystems (Kinnaird & O'Brien, 2007). However, dependence on forest has also been the root of their decline, given that Asia suffers one of the highest rates of deforestation in the world (Koh *et al.*, 2011; Miettinen *et al.*, 2011). At present, 10 species of hornbills are listed as threatened, of which nine are Asian (BirdLife International, 2010). Another 13 are near-threatened and gradually declining, of which 11 are from Asia.

The Sunda Shelf is regarded as a key hotspot for speciation given that a third of all hornbills are restricted to this region and the Philippines (Kinnaird & O'Brien, 2007). Ten species of endemic hornbills occur in the Philippines, of which four are polytypic (Dickinson, 2003). This represents nearly 30% of all Asian hornbills, and 17% of the world's total hornbill species (Gonzalez, 2007). Owing to their restricted ranges and the impacts from habitat loss and over-exploitation, five species are currently threatened. Continued threats from deforestation may further impact three other hornbills considered to be near-threatened, or lead to the extinction of two species classified as Critically Endangered (Collar *et al.*, 1999). Extensive deforestation in the Southeast Asia has left many islands prone to a high probability of avian extinction in the wild (Brooks *et al.*, 1997; Miettinen *et al.*, 2011). A better understanding of the distribution patterns and species limits in Asian hornbills would serve as a basis for setting conservation priorities within the group.

### **Thesis overview**

In this thesis, I use different approaches to examine the evolution of the family Bucerotidae based on well-resolved comprehensive molecular phylogenies derived largely from historical samples and covering nearly all species and subspecies. Overall, five data chapters address major concerns in hornbill research and answer long-standing questions about the origin and speciation of hornbills. These five data chapters also represent the different approaches used to understand hornbill evolution, namely (1) constructing a molecular phylogeny necessary for conducting comparative analyses; (2) tracing the evolution and causes of cooperative breeding; (3) historical divergence and causes of diversification; (4) reconstruction of ancestral origins and biogeographic analysis focused on Asian geo-isolates; and (5) resolving taxonomic issues through species delimitation as a basis for conserving Asian hornbills.

With these collective chapters, I provide a comprehensive picture of hornbill evolution and propose models describing the drivers of speciation, evolution of sociality and phylogeographic patterns. The ultimate goal of the thesis is to fill in the gaps in current research and resolve taxonomic issues, which are deemed invaluable in developing conservation strategies, particularly for poorly known insular species and subspecies. This thesis contains five stand-alone data chapters in addition to the general introduction (Chapter 1), and general discussion and conclusion (Chapter 7). All five data chapters (Chapters 2–6) are formatted for future submission, and each is accompanied by its own supplementary appendix, immediately following the chapter.

## CHAPTER 1

The aim of this thesis is to answer the following questions:

How are hornbills related in a resolved phylogeny, and how valid is their current taxonomy?

- In response to this question, I had constructed a comprehensive molecular phylogeny of hornbills to reveal taxonomic relations (Chapter 2)

What facilitated the development of sociality in hornbills? Was it climatic stability?

- To facilitate this, I had used the molecular phylogeny to trace the evolution and test the causes of cooperative breeding behaviour in hornbills (Chapter 3)

What caused hornbills to diversify and when did this occur?

- To answer this question, I used the molecular phylogeny to understand the evolutionary history of hornbills and drivers of diversification in Asia and Africa (Chapter 4)

Where did hornbills originate, and what patterns and paths of colonisation did they follow?

- In order to address this question, I used the molecular phylogeny to investigate routes for colonising Asia from Africa (Chapters 4 and 5)

How did hornbills colonise continental and insular Asia and Melanesia?

- To understand this, I used the molecular phylogeny to reconstruct ancestral ranges and explain phylogeographic relations amongst Asian hornbills (Chapter 5)

Do these geo-isolates represent valid species for conservation?

- In order to determine this, I used the molecular phylogeny of geo-isolates to determine species limits as the basis for setting conservation priorities in Asia (Chapter 6)

## **Authors' contributions**

**Juan-Carlos T. Gonzalez.** Collected three years of data from the field and laboratory; collected and processed samples and analysed data from museums/aviaries; conceived, analysed and wrote the first drafts of each manuscript, and corrected drafts.

**Ben C. Sheldon.** Overall guidance in research design and development of manuscripts; advised on statistical approaches; facilitated permits for field & laboratory work; and gave detailed comments on drafts.

**Joseph A. Tobias.** Overall guidance in research design; conceptualisation and development of manuscripts; advised on statistical approaches and analysis; commented on drafts and made revisions of manuscripts for submission to journals.

**Nigel J. Collar.** Overall guidance in writing and development of manuscripts; aided in data collection and analysis (Chapter 6); commented on and edited drafts.



## References

- Avice, J.C. (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.
- Bennett, E.L., Nyaoi, A.J., & Sompud, J. (1997) Hornbills *Buceros* spp. and culture in Northern Borneo: Can they continue to co-exist? *Biological Conservation*, **82**, 41–46.
- Benz, B. W., & Robbins, M. B. (2011) Molecular phylogenetics, vocalizations, and species limits in *Celeus* woodpeckers (Aves: Picidae). *Molecular Phylogenetics and Evolution*, **61**, 29–44.
- BirdLife International (2000) *Threatened Birds of the World*. Lynx Edicions and BirdLife International, Barcelona and Cambridge.
- BirdLife International (2010) Data zone search for Bucerotidae and Bucrovidae (Hornbills), <http://www.birdlife.org/datazone/index.html>.
- Brunet, J. (1971) Oiseaux miocènes de Beni Mellal (Maroc); un complément à leur étude. *Notes et Mémoires du Service géologique du Maroc*, **31**, 109–111.
- Boev, Z., & Kovachev, D. (2007) *Euroceros bulgaricus* gen. nov., sp. nov. from Hadzhidimovo (SW Bulgaria) (Late Miocene) – the first European record of Hornbills (Aves: Coraciiformes). *Geobios*, **40**, 39–49.
- Brooks, T.M., Pimm, S.L., & Collar, N.J. (1997) Deforestation predicts the number of threatened birds in insular Southeast Asia. *Conservation Biology*, **11**, 382–394.
- Cibois, A. (2003) Mitochondrial DNA phylogeny of babbler (Timaliidae). *The Auk*, **120**, 35–54.
- Clements, J.F. (2007) *The Clements Checklist of Birds of the World*, 6th ed. Cornell Univ. Press, New York.
- Collar, N.J., Mallari, N.A.D., & Tabaranza, B.R. Jr. (1999) *Threatened Birds of the Philippines*. Bookmark Inc., Makati City, Philippines.
- Davison, G.W.H. (2011) Foreword for The 5<sup>th</sup> International Hornbill Conference, Singapore 22<sup>nd</sup>-25<sup>th</sup> March 2009. *The Raffles Bulletin of Zoology*, **24**, 1–2.
- Datta, A., & Rawat, G.S. (2003) Foraging patterns of sympatric hornbills during the non-breeding season in Arunachal Pradesh, Northeast India. *Biotropica*, **35**, 208–218.
- Delport, W., Ferguson, J.W.H., & Bloomer, P. (2002) Characterization and evolution of the mitochondrial DNA control region in hornbills (Bucerotiformes). *Journal of Molecular Evolution*, **54**, 794–806.
- Delport, W., Kemp, A.C., & Ferguson, J.W.H. (2005) Structure of an African red-billed hornbill (*Tockus erythrorhynchus rufirostris* and *T. e. damarensis*) hybrid zone as revealed by morphology, behaviour, and breeding biology. *The Auk*, **121**, 565–586.

## CHAPTER 1

- Dickinson, E.C. ed. (2003) *The Howard and Moore Complete Checklist of the Birds of the World*, 3rd edn. Princeton University Press, Princeton, New Jersey.
- Ehrlich, P.R., & Raven, P.H. (1964) Butterflies and plants: a study in coevolution. *Evolution*, **18**, 586–608.
- Elliot, D.G. (1882) *A Monograph of the Bucerotidae, or Family of the Hornbills*. Taylor & Francis, London.
- Fleming, T.H., Venable, D.L., & Herrera M., L.G. (1993) Opportunism vs. specialization: the evolution of dispersal strategies in fleshy-fruited plants. *Vegetatio*, **107/108**, 107–120.
- Frankham, R., Ballou, J.D., & Briscoe, D.A. (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.
- Gill F., & Donsker, D. (2012) International Ornithological Congress World Bird List (v 2.11) Available from: <http://www.worldbirdnames.org/names/> (accessed April 2012).
- Gonzalez, J.C.T. (2007) Fragmented forests and isolated islands: the distribution and status of Philippine endemic hornbills. In: Kemp, A.C. & M.I. Kemp (eds.) *The Active Management of Hornbills and their Habitats for Conservation*, p. 51–74. CD-ROM Proceedings of the 4th International Hornbill Conference, Mabula Game Lodge, South Africa. Naturalists & Nomads, Pretoria.
- Gonzalez, J. C. T. (2011). Enumerating the ethno-ornithological importance of Philippine hornbills. *The Raffles Bulletin of Zoology*, **24**, 149–161.
- Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C.K., Braun, E.L., Braun, M.J., Chojnowski, J.L., Cox, W.A., Han, K.-L., Harshman, J., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Sheldon, F.H., Steadman, D.W., Witt, C.C., & Yuri, T. (2008) A phylogenomic study of birds reveals their evolutionary history. *Science* **27**, 1763–1768.
- Hadiprakarsa, Y.-Y., & Kinnaird, M.F. (2004) Foraging characteristics of an assemblage of four Sumatran hornbill species. *Bird Conservation International*, **13**: S53–S62.
- Helbig, A.J., Knox, A., Parkin, D.T., Sangster, G. & Collinson, M. (2002) Guidelines for assigning species rank. *Ibis*, **144**, 518–525.
- Herrera, C.M., & Pellmyr, O. (2002) *Plant-Animal Interactions: An Evolutionary Approach* (eds.). Blackwell Science, Oxford.
- Holbrook, K.M., & Smith, T.B. (2000) Seed dispersal and movement patterns in two species of *Ceratogymna* hornbills in a West African tropical lowland forest. *Oecologia*, **125**, 249–257.
- Holbrook, K.M., Smith, T.B., & Hardesty, B.D. (2002) Implications of long-distance movements of frugivorous rain forest hornbills. *Ecography*, **25**, 745–749.
- Hosner, P.A., Sheldon, F.H., Lim, H.C., & Moyle, R.G. (2010) Phylogeny and biogeography of the Asian trogons (Aves: Trogoniformes) inferred from nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics Evolution*, **57**, 1219–1225.

- Hübner, V.S.M., Prinzing, R., & Wink, M. (2003) Neue Erkenntnisse zur Taxonomie der Hornvögel (Aves: Bucerotiformes) und ihre Bedeutung für die Zucht in Menschenobhut. *Zoologische Garten N.F.*, **73**, 396–401.
- Jønsson, K.A., Fjeldså, J., Ericson, P.G.P., & Irestedt, M. (2007) Systematic placement of an enigmatic Southeast Asian taxon *Eupetes macrocerus* and implications for the biogeography of a main songbird radiation, the Passerida. *Biology Letters*, **3**, 323–326.
- Jønsson, K.A., Bowie, R.C.K., Nylander, J.A.A., Christidis, L., Norman, J.A., & Fjeldså, J. (2010) Biogeographical history of cuckoo-shrikes (Aves: Passeriformes): transoceanic colonisation of Africa from Australo-Papua. *Journal of Biogeography*, **37**, 1767–1781.
- Jønsson, K. A., Irestedt, M., Bowie, R. C. K., Christidis, L., & Fjeldså, J. (2011) Systematics and biogeography of Indo-Pacific ground-doves. *Molecular Phylogenetics and Evolution*, **59**, 538–43.
- Kemp, A.C. (1976) *A study of the ecology, behaviour and systematics of Tockus hornbills (Aves: Bucerotidae)*. Transvaal Museum memoir No. 20, Pretoria.
- Kemp, A.C. (1979) A review of the hornbills: biology and radiation. *Living bird*, **17**, 105–136.
- Kemp, A.C. (1988) The systematic and zoogeography of Oriental and Australasian hornbills (Aves: Bucerotidae). *Bonner Zoologische Beiträge*, **39**, 315–345.
- Kemp, A.C. (1995) *Bird families of the world. The Hornbills: Bucerotiformes*. Oxford University Press, Oxford.
- Kemp, A.C. (2001a) Family Bucerotidae (Hornbills). Pp. 436–523 in: de Hoyo, J., Elliott, A., & Sargatal, J., eds. (2001) *Handbook of the Birds of the World*. Vol. 6, Mousebirds to Hornbills. Lynx Edicions, Barcelona.
- Kemp, A.C. (2001b) The role of species limits and biology in the conservation of African hornbills. *Ostrich Supplement No.*, **15**, 200–204.
- Kemp, A.C., & Delport, W. (2002) Comments on the status of subspecies in the red-billed hornbill (*Tockus erythrorhynchus*) complex (Aves: Bucerotidae), with the description of a new taxon endemic to Tanzania. *Annals of the Transvaal Museum*, **39**, 1–8.
- Kemp, A.C., & M.I. Kemp (2009). The diversity and radiation of hornbills: How, when, where and why might these have occurred? *Abstracts of papers*, 5th International Hornbill Conference, Singapore Botanic Garden; National Parks Board, Singapore; Keynote paper 01.
- Kinnaird, M.F., & O'Brien, T.G. (2007) *The Ecology and Conservation of Asian Hornbills: Farmers of the Forest*. The University of Chicago Press, Chicago.
- Kitamura, S. (2011) Frugivory and seed dispersal by hornbills (Bucerotidae) in tropical forests. *Acta Oecologica*, **37**, 531–541.

- Kitamura, S., Suzuki, S., Yumoto, T., Poonswad, P., Chuailua, P., Plongmai, K., Noma, N., Maruhashi, T., & Suckasam, C. (2004) Dispersal of *Aglaia spectabilis*, a large-seeded tree species in a moist evergreen forest in Thailand. *Journal of Tropical Ecology*, **20**, 421–427.
- Klop, E., Curio, E., & Lastimoza, L.L. (2000) Breeding biology, nest site characteristics and nest spacing of the Visayan Tarictic Hornbill *Penelopides panini panini* on Panay, Philippines. *Bird Conservation International*, **10**, 17–27.
- Koh, L. P., Miettinen, J., Liew, S. C., & Ghazoul, J. (2011). Remotely sensed evidence of tropical peatland conversion to oil palm. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 5127–5132.
- Lamoreux, J. F., Morrison, J. C., Ricketts, T. H., Olson, D. M., Dinerstein, E., McKnight, M. W., & Shugart, H. H. (2006) Global tests of biodiversity concordance and the importance of endemism. *Nature*, **440**, 212–4.
- Livezey, B. C., & Zusi, R. L. (2007) Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. *Zoological Journal of the Linnean Society*, **149**, 1–95.
- Lovette, I.J., & Rubenstein, D.R. (2007) A comprehensive molecular phylogeny of the starlings (Aves: Sturnidae) and mockingbirds (Aves: Mimidae): Congruent mtDNA and nuclear trees for a cosmopolitan avian radiation. *Molecular Phylogenetics and Evolution*, **44**, 1031–1056.
- Luft, S., Tacud, B., Urbina, H., & Geronimo, F., 2002. Visual fruit preferences of Visayan Tarictic hornbills, *Penelopides panini panini* (Bucerotiformes: Bucerotidae), and Musky fruit Bats, *Ptenochirus jagori* (Megachiroptera: Pteropodidae), in cafeteria experiments. *Silliman Journal*, **43**, 42–58.
- Marks, B. D., Weckstein, J. D., & Moyle, R. G. (2007) Molecular phylogenetics of the bee-eaters (Aves: Meropidae) based on nuclear and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **45**, 23–32.
- Marsden, S.J. (2008) Estimation of parrot and hornbill densities using a point count distance sampling method. *Ibis*, **141**, 327–390.
- Marsden, S.J., & Pilgrim, J.D. (2003) Factors influencing the abundance of parrots and hornbills in pristine and disturbed forests on New Britain, PNG. *Ibis*, **145**, 45–53.
- Mayr, E. (1942) *Systematics and the origin of species from the viewpoint of a zoologist*. Columbia University Press, New York.
- Mayr, G. (2000) Tiny hoopoe-like birds from the Middle Eocene of Messel (Germany). *The Auk*, **117**, 964–970.
- Mayr, G. (2006) New specimens of the Eocene Messelirrisoridae (Aves: Bucerotes), with comments on the preservation of uropygial gland waxes in fossil birds from Messel and the phylogenetic affinities of Bucerotes. *Paläontologische Zeitschrift*, **80**, 390–405.

- Mayr, G. (2007a) Avian higher-level phylogeny: well-supported clades and what we can learn from a phylogenetic analysis of 2954 morphological characters. *Journal of Zoological Systematics and Evolutionary Research*, **46**, 63–72.
- Mayr, G. (2007b) Synonymy and actual affinities of the putative Middle Eocene “ New World vulture ” *Eocathartes* Lambrecht, 1935 and “ hornbill ” *Geiseloceros* Lambrecht, 1935 (Aves, Ameghinornithidae). *Paläontologische Zeitschrift*, **81**, 457–462.
- Miettinen, J., Shi, C., & Liew, S. C. (2011). Deforestation rates in insular Southeast Asia between 2000 and 2010. *Global Change Biology*, **17**, 2261–2270.
- Mills, M.S.L., Boix-Hinzen, C., & Du Plessis, M.A. (2005) Live or let live: life-history decisions of the breeding female Monteiro’s Hornbill *Tockus monteiri*. *Ibis*, **147**, 48–56.
- Mittermeier, R.A., Gil, P.R., Hoffman, M., Pilgrim, J., Brooks, T., Goettsch Mittermeier, C., Lamoreux, J., & Da Fonseca, G.A.B. (2005) *Hotspots revisited: earth’s biologically richest and most threatened terrestrial ecoregions*. CEMEX, Agrupación Sierra Madre, Mexico City.
- Morin, P.A., Messier, J., & Woodruff, D.S. (1994) DNA extraction, amplification, and direct sequencing from hornbill feathers. *Journal of the Scientific Society of Thailand*, **20**, 31–42.
- Moyle, R. G. (2004) Phylogenetics of barbets (Aves: Piciformes) based on nuclear and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **30**, 187–200.
- Moyle, R. G., & Marks, B. D. (2006) Phylogenetic relationships of the bulbuls (Aves: Pycnonotidae) based on mitochondrial and nuclear DNA sequence data. *Molecular Phylogenetics and Evolution*, **40**, 687–95.
- Nahum, L.A., Pereira S.L., de Campos Fernandes, F.M., Matioli, S.R. & Wajntal, A. (2003) Diversification of Ramphastinae (Aves, Ramphastidae) prior to the Cretaceous/Tertiary boundary as shown by molecular clock of mtDNA sequences. *Genetics and Molecular Biology*, **26**, 411–418.
- Newton, I. (2003) *The Speciation and Biogeography of Birds*. Academic Press, London.
- Oliver, W.L.R., & Wilkinson, R. (2007) Philippine Hornbills Conservation Programme. In: Kemp, A.C. & Kemp, M.I. (eds.) *The Active Management of Hornbills and their Habitats for Conservation*, p. 13–30. CD-ROM Proceedings of the 4th International Hornbill Conference, Mabula Game Lodge, Bela-bela, South Africa. Naturalists & Nomads, Pretoria.
- Olson S.L. (1985) The fossil record of birds. Pp.79–238, In: Farner, D.S., King, J.R., & Parkes, K.C., eds., *Avian Biology*, vol. 8, Academic Press, New York.
- Orme, C.D.L., Davies, R.G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V.A., Webster, A.J., Ding, T.-S., Rasmussen, P.C., Ridgely, R.S., Stattersfield, A.J., Bennett, P.M., Blackburn, T.M., Gaston, K.J., & Owens, I.P.F. (2005) Global hotspots of species richness are not congruent with endemism or threat. *Nature*, **436**, 1016–1019.

- Pacheco, M. A., Battistuzzi, F. U., Lentino, M., Aguilar, R. F., Kumar, S., & Escalante, A. A. (2011) Evolution of modern birds revealed by mitogenomics: timing the radiation and origin of major orders. *Molecular Biology and Evolution*, **28**, 1927–1942.
- Page, R.D.M., & Holmes, E.C. (1998) *Molecular Evolution: A Phylogenetic Approach*. Blackwell Science Ltd, Oxford.
- Patel, S., Weckstein, J. D., Patané, J. S. L., Bates, J. M., & Aleixo, A. (2011) Temporal and spatial diversification of *Pteroglossus* arazari (AVES: Ramphastidae) in the neotropics: constant rate of diversification does not support an increase in radiation during the Pleistocene. *Molecular Phylogenetics and Evolution*, **58**, 105–15.
- Perrins, C. (2009) *The Encyclopedia of Birds*. Oxford University Press, Oxford.
- Peters, J.L. (1945) *Check-List of Birds of the World*, Vol. V., Harvard University Press, Cambridge, MA.
- Poonswad, P., Sukkasem, C., Phataramata, S., Hayeemuida, S., Plongmai, K., Chuailua, P., Thiensongrusame, P., & Jirawatkavi, N. (2005) Comparison of cavity modification and community involvement as strategies for hornbill conservation in Thailand. *Biological Conservation*, **122**, 385–393.
- Price, T.D. (2008) *Speciation in Birds*. Roberts & Company Publishers, Colorado.
- Reddy, S., Driskell, A., Rabosky, D. L., Hackett, S. J., & Schulenberg, T. S. (2012) Diversification and the adaptive radiation of the vangas of Madagascar. *Proceedings of the Royal Society B*, published online 4 January 2012, doi: 10.1098/rspb.2011.2380.
- Ribas, C. C., Gaban-Lima, R., Miyaki, C. Y., & Cracraft, J. (2005) Historical biogeography and diversification within the Neotropical parrot genus *Pionopsitta* (Aves: Psittacidae). *Journal of Biogeography*, **32**, 1409–1427.
- Sammler, S., Bleidorn, C., & Tiedemann, R. (2011) Full mitochondrial genome sequences of two endemic Philippine hornbill species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. *BMC Genomics*, **12**, 35–45.
- Sanft, K. (1960) Bucerotidae (Aves/Upupae). *Das Tierreich*, **76**, 1–176.
- Shankar Raman, T.R., & D. Mudappa (2003) Correlates of hornbill distribution and abundance in rainforest fragments in the southern Western Ghats, India. *Bird Conservation International*, **13**, 199–212.
- Schweizer, M., Güntert, M., & Hertwig, S. T. (2012) Phylogeny and biogeography of the parrot genus *Prioniturus* (Aves: Psittaciformes). *Journal of Zoological Systematics and Evolutionary Research*, **50**, 145–156.
- Sethi, P., & Howe, H. F. (2009) Recruitment of Hornbill-Dispersed Trees in Hunted and Logged Forests of the Indian Eastern Himalaya. *Conservation biology*, **23**, 710–718.
- Sibley, C.G., & Ahlquist, J.E. (1990) *Phylogeny and Classification of Birds*. Yale University Press, New Haven, CT.

- Sitompul, A.F., Kinnaird, M.F., & O'Brien, T.G. (2004) Size matters: the effects of forest fragmentation and resource availability on the endemic Sumba Hornbill *Aceros everetti*. *Bird Conservation International*, **14**, S23–S37.
- Srikwan, S., & Woodruff, D.S. (1998) Molecular genetics and the conservation of hornbills in fragmented landscape. Pp. 257–263, In: Poonswad, P. (ed.) *The Asian Hornbills: Ecology and Conservation. Thai Studies in Biodiversity No.2*, Bangkok, Thailand.
- Stanback, M., Richardson, D.S., Boix-Hinzen, C., & Mendelsohn, J. (2002) Genetic monogamy in Monteiro's hornbill, *Tockus monteiri*. *Animal Behaviour*, **63**, 787–793.
- Stauffer, D.J., & Smith, T.B. (2004) Breeding and nest site characteristics of the Black-casqued Hornbill *Ceratogymna atrata* and White-thighed Hornbill *Ceratogymna cylindricus* in south-central Cameroon. *Ostrich*, **75**, 79–88.
- Steadman, D.W. (2006) *Extinction and biogeography of tropical Pacific birds*. University of Chicago Press, Chicago, IL.
- Stearns, S.C., & Hoekstra, R.F. (2005) *Evolution, an introduction*, 2nd edn. Oxford University Press, Oxford, UK.
- Tavares, E.S., Baker, A.J., Pereira, S.L., & Miyaki, C.Y. (2006) Phylogenetic Relationships and historical biogeography of neotropical parrots (Psittaciformes: Psittacidae: Arini) inferred from mitochondrial and nuclear DNA sequences. *Systematic Biology*, **55**, 454–470.
- Tobias, J.A., Seddon, N., Spottiswoode, C.N., Pilgrim, J.D., Fishpool, L.D.C., & Collar, N.J. (2010) Quantitative criteria for species delimitation. *Ibis*, **152** (4), 724–746.
- Trail, P.W. (2007) African hornbills: keystone species threatened by habitat loss, hunting and international trade. *Ostrich*, **78**, 609–613.
- Viseshakul, N., Charoennitikul, W., Kitamura, S., Kemp, A.C., Thong-aree, S., Surapunpitak, Y., Poonswad, P., & Ponglikitmongkol, M. (2011) A phylogeny of frugivorous hornbills linked to the evolution of Indian plants within Asian rainforests. *Journal of Evolutionary Biology*, **24**, 1533–1545.
- Wallace, A.R. (1863) The Bucerotidae, or Hornbills. *The Intellectual Observer* (June 1863), 309–316.
- White, N.E., Phillips, M.J., Gilbert, M.T.P., Alfaro-Núñez, A., Willerslev, E., Mawson, P.R., Spencer, P.B.S., & Bunce, M. (2011). The evolutionary history of cockatoos (Aves: Psittaciformes: Cacatuidae). *Molecular Phylogenetics and Evolution*, **59**, 615–22.
- Zuccon, D., Cibois, A., Pasquet, E. & Ericson, P. G. P. (2006). Nuclear and mitochondrial sequence data reveal the major lineages of starlings, mynas and related taxa. *Molecular Phylogenetics and Evolution*, **41**, 333–44.

## Chapter 2

### **A comprehensive molecular phylogeny for the hornbills (Aves: Bucerotidae): nuclear and mitochondrial DNA trees for a threatened avian radiation**



*Proc. Zool. Soc. London, Tweeddale 1877 p. 825*

*Any meaningful conclusions based on molecular studies will become available only when samples have been analysed for all species and subspecies of the Bucerotidae, and when the techniques have been applied to, and compared for, several different parts of the genetic code.*

Alan C. Kemp, 2001, Family Bucerotidae (Hornbills), *Handbook of the Birds of the World*



**A comprehensive molecular phylogeny for the hornbills (Aves: Bucerotidae): nuclear and mitochondrial DNA trees for a threatened avian radiation**

**Juan-Carlos T. Gonzalez<sup>1,2</sup>, Ben C. Sheldon<sup>1</sup>, Nigel J. Collar<sup>3</sup>, and Joseph A. Tobias<sup>1</sup>**

<sup>1</sup>*Department of Zoology, Edward Grey Institute for Field Ornithology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*

<sup>2</sup>*Institute of Biological Sciences, University of the Philippines Los Baños, College, Laguna 4031, Philippines*

<sup>3</sup>*BirdLife International, Wellbrook Court, Girton Road, Cambridge CB3 0NA, UK*

**Abstract**

The hornbills comprise a group of morphologically and behaviourally distinct Palaeotropical bird species that feature prominently in studies of ecology and conservation biology. Although the monophyly of hornbills is well established, previous phylogenetic hypotheses were based solely on mtDNA and limited sampling of species diversity. We used parsimony, maximum likelihood and Bayesian methods to reconstruct relationships among all 61 extant hornbill species, based on nuclear and mtDNA gene sequences extracted largely from historical samples. The resulting phylogenetic trees closely match vocal variation across the family but conflict with current taxonomic treatments. In particular, they highlight a new arrangement for the six major clades of hornbills and reveal that five groups traditionally treated as genera (*Tockus*, *Ocyrceros*, *Anthracoceros*, *Aceros*, *Penelopides*) are non-monophyletic. These findings resolve some longstanding problems in hornbill systematics, including the placement of ‘*Penelopides exharatus*’

## CHAPTER 2

(embedded in *Aceros*) and ‘*Tockus hartlaubi*’ (sister to *Tropicranus albocristatus*). We also confirm that an Asiatic lineage (*Berenicornis*) is sister to a trio of Afrotropical genera (*Tropicranus* [including ‘*Tockus hartlaubi*’], *Ceratogymna*, *Bycanistes*). We present a summary phylogeny as a robust basis for further studies of hornbill ecology, evolution and historical biogeography.

### **Keywords**

biogeography; Bucerotidae; classification; phylogeny; systematics

Running title: Molecular phylogeny of hornbills

Formatted for submission to Molecular Phylogenetics and Evolution

## 1. Introduction

The hornbills and ground-hornbills comprising the family Bucerotidae are charismatic land-birds that have long been the focus of research attention. Amongst evolutionary biologists, they are well known for their elaborate bill casques, cooperative breeding systems, and the unique strategy of self-incarceration, the females of many species sealing themselves into tree-holes for several weeks by plastering the entrances of their nest cavities (Moreau, 1934; Kemp, 2001). Amongst ecologists, hornbills are famed as keystone species (Trail, 2007), and implicated in the historical expansion of Palaeotropical forests (Viseshakul et al., 2011), because of their function as long-distance seed dispersers. They also play an important role in tribal cultures from South Africa to East Asia (Bennett et al., 1997). Unfortunately, as a corollary of their large size and need for extensive foraging areas, many hornbills are highly sensitive to hunting and habitat fragmentation, making them one of the most threatened components of tropical ecosystems (Kinnaird and O'Brien, 2007). Over a third of hornbill species are considered to be of conservation concern globally, including 62% (20/32) of Asian species (see Appendix A). Because of these attributes, hornbills are becoming increasingly prominent as study systems in ecology (e.g. Holbrook and Smith, 2000; Holbrook et al., 2002; Kitamura, 2011) and conservation biology (e.g. Sethi and Howe, 2009; Lenz et al., 2011).

The evolutionary history of the family has received less attention, yet the basic outline of hornbill systematics is now well established. Several anatomical features—including fused upper vertebrae (atlas and axis), long flattened upper eyelashes, and bilobular kidneys (Kemp 1995, 2001a)—are unique to hornbills, suggesting that they form a monophyletic clade. Relationships within the family have been estimated on the basis of a qualitative assessment of characters such as phenotype, vocalizations and breeding behaviour (Kemp and Crowe, 1985; Kemp, 1988), culminating in the publication of a consensus cladogram built using presence or absence of 26 such characters (Kemp, 1995). This tree has proved to be a useful framework for hornbill systematics,

particularly because its coverage (54 of 61 taxa) is reasonably comprehensive (Kinnaird and O'Brien, 2007).

Several quantitative assessments of hornbill relationships have been undertaken using molecular techniques, but all have been based on highly incomplete datasets. The first steps involved karyological studies focused on 7 species (Belterman and de Boer, 1984, 1990), and a 17-taxa tree constructed using DNA-DNA hybridization (Sibley and Ahlquist, 1990). These were followed by phylogenetic approaches generating partial cytochrome *b* (*cytb*) sequences (189 bp) for 11 taxa (Morin et al., 1994; Srikwan and Woodruff, 1998). The results of these preliminary analyses agreed on the placement of the genus *Bucorvus* (ground-hornbills) as a highly divergent sister clade to all other hornbills, perhaps warranting designation as a separate family (Kemp, 1995). They also showed that *Tockus* is an early branch of the Bucerotidae, and revealed the clustering of African and Asiatic taxa. Further sequencing led to expanded mitochondrial DNA (mtDNA) phylogenies for hornbills, first including 22 species (Hübner et al., 2003), then more recently all 34 species for which molecular data are currently available, i.e. 54% of species diversity in the family (Viseshakul et al., 2011).

The phylogeny published by Viseshakul et al. (2011) provided the most informative assessment of the historical relationships between major clades of hornbills, particularly as it contained at least one member from each genus. However, many nodes had low confidence in terms of bootstrap values, presumably because tree topology was based on variation in one mitochondrial gene across a limited set of species. Moreover, most species were only represented by partial *cytb* sequences (400–1043 bp), whereas complete genes (1143 bp) were only available for 15 species, i.e. 25% of species diversity in the family. Viseshakul et al. (2011) noted that a fuller understanding of phylogenetic relationships within the clade, as well as a better grasp of the timing of evolutionary events, could only be resolved by more comprehensive sampling of lineages and loci.

To address this issue we conducted the first complete species-level phylogenetic analysis for hornbills, based on both nuclear and mtDNA sequences. Given the rarity of well-preserved hornbill tissue in collections, and the difficulty of trapping or collecting arboreal species, we

mainly sequenced genetic material from captive individuals or historical samples, i.e. museum specimens. Our main goal was to provide the robust, comprehensive evolutionary tree needed to resolve questions relating to speciation, biogeography and evolution within the Bucerotidae (Kinnaird and O'Brien, 2007). In addition, we aimed to clarify the placement of certain taxa (e.g. *Berenicornis comatus*, *Tockus hartlaubi*, *T. camurus*, the genus *Ocyceros* and all Philippine taxa) which remained either unsampled or unresolved by Viseshakul et al. (2011).

## 2. Materials and methods

### 2.1 Taxon sampling, DNA extraction and sequence alignment

Our analyses were based on genetic material from all 61 currently recognized hornbill species (Table 2.1), including multiple representatives of most lineages. We sequenced a complete mtDNA *cytb* gene (1143 bp) for at least one individual from each of 59 hornbill species, and added partial (~400 bp) *cytb* genes for two additional taxa (*Tockus kemp*i and *T. damarensis*) by downloading sequences from GenBank. We also generated complete or partial sequences (500–703 bp) of a nuclear gene, cytosolic adenylate kinase 1 intron 5 (AK1 intron 5: Shapiro and Dumbacher, 2001), for 54 species. The combined length of nuclear and mitochondrial loci used in this study was 1,846 aligned nucleotides. The final dataset contained 162 genetic sequences for hornbills, with a mean of 2.66 sequences per species (Appendix B).

These data include the first *cytb* sequences for 23 species, including several African taxa (e.g. *Tockus hemprichii*, *T. bradfieldi*, *Bycanistes fistulator*, *B. cylindricus*, *Ceratogymna elata*) and rare Asian taxa (e.g. *Penelopides mindorensis*, *Anthracoceros montani*, *Rhyticeros everetti*, *R. narcondami*). A slightly larger total of 27 species were added to the previous mtDNA phylogeny because Viseshakul et al. (2011) did not include genes available on Genbank for *T. rufirostris*, *T. damarensis*, *T. monteiri*, and *Aceros waldeni*. We also added the first AK1 intron 5 sequences for 54 species.

## CHAPTER 2

For out-group samples we selected six species from related coraciiform families: *Phoeniculus purpureus* (Phoeniculidae), *Upupa epops* (Upupidae), *Coracias caudata*, *Eurystomus orientalis*, *E. glaucurus* (Coraciidae) and *Todiramphus sanctus* (Alcedinidae). We also included a more distantly related gruiform: *Rallus longirostris* (Rallidae). We downloaded *cytb* sequences from Genbank for all these species and AK1 intron 5 for *Rallus longirostris*, and in addition sequenced AK1 intron 5 for *Phoeniculus purpureus*. All sequences new to this study (in-groups and out-groups) have been deposited in GenBank; Appendix B gives accession numbers for both these and all previously reported sequences.

Genomic DNA was derived from several sources, including contemporary material (i.e. captive and wild-trapped individuals) for 18 species, and historical material (i.e. museum collections) for 57 species. For contemporary material, DNA was extracted from molted flight feathers and plucked pin-feathers (the latter preserved in 70–90% ethanol) following Morin et al. (1994). For historical material, we extracted DNA from toe-pads following Mundy et al. (1997). Processing of historical samples was done in a separate laboratory using stringent protocols to avoid cross-contamination with modern avian DNA, using extraction and polymerase chain reaction (PCR) controls (Vallianatos et al., 2002; Lerner and Mindell, 2005). In all cases, short fragments of genes (200–500 bp) were amplified using published and newly developed primers designed from existing GenBank sequences (Table S2.1, see Supplementary Material) using the online program PRIMER3 v.04.0 (Rozen and Skaletsky, 2000). We designed specific internal primers to improve amplification of fragments of degraded DNA and reduce risk of contamination. PCRs were performed using pre-optimized Qiagen HotStarTaq Master Mix in ABI 2720 thermal cyclers (Applied Biosystems, Foster City, CA) and purified using the Qiagen Mini-elute kit.

The PCR profile followed for AK1 intron 5 was as follows: touchdown of 15 min at 95°C, followed by 45 cycles of 95°C for 45 sec, 54°C for 60 sec, and 72°C for 60 sec, and a final extension phase at 72°C for 10 min. The equivalent profile for *cytb* was as follows: touchdown of 15 min at 95°C, followed by 45 cycles of 94°C for 30 sec, 52°C for 45 sec, and 72°C for 60 sec, and a final extension phase at 72°C for 10 min. Cycle sequencing reactions were run using the Big Dye Sequencing kit and analyzed in the ABI Prism Genetic Analyzer 377. Gene sequence contigs

were assembled and edited using SEQUENCHER v4.2 (Gene Codes Corp., Ann Arbor, MI) and BIOEDIT v7.0.5.3 (Hall 1999). Cytochrome *b* sequences were aligned using CLUSTALW v.2.0 (Larkin et al. 2007) and truncated following a prescribed start codon (ATG) and termination codon (TAA/TAG). MUSCLE (Edgar 2004) was used to align AK1 intron 5 and concatenate nuclear-mtDNA datasets manually in MEGA v.5.03 (Tamura et al. 2011). Final alignments are in FASTA and NEXUS format, and available upon request from the authors.

## 2.2 Phylogenetic analysis and tree construction

Preliminary analyses of both in-groups and out-groups showed that sequences from conspecific samples had very high similarity. As we are primarily concerned with interspecific relationships, we therefore selected a single representative of each species to include in phylogenetic trees or analyses to reduce computation times and to simplify tree topology. Sequences selected for analysis are highlighted in Appendix B.

Using a dataset of 54 in-group species for which both mitochondrial and nuclear genes were available, we conducted an analysis of sequence characters and a disparity index test for patterns of homogeneity following a Monte Carlo test of 500 replicates using MEGA5. Estimates of transition (Ts) and transversion (Tv) bias, and the substitution matrix, were calculated using Maximum Composite Likelihood (MCL) and Maximum Likelihood (ML) methods. We implemented a pairwise comparison to explore the congruence of phylogenetic signals and homogeneity of substitution patterns between nuclear, mtDNA and concatenated nuclear-mtDNA datasets. We then plotted genetic divergence estimates between pairs of taxa for the three datasets to compare *p*-distance values. To determine mean evolutionary site rates in five categories, we also conducted an ML estimate of evolutionary divergence in the concatenated dataset (with out-group taxa) following the Tamura-Nei model with  $\Gamma$  distribution obtained by bootstrapping 1000 pseudoreplicates (Tamura and Nei, 1993; Tamura et al., 2011). Analysis involved 54 nucleotide sequences and all positions containing gaps, with missing data eliminated.

## CHAPTER 2

We used Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian inference (BI) to conduct separate phylogenetic analyses for our three datasets (*cytb*, AK1 intron 5 and concatenated). Selection of best-fit models of evolution was implemented in MEGA5 and MRMODELTEST v2.3 (Nei and Kumar, 2000, Nylander, 2004), based on ML fits of 24 different nucleotide substitution models, using least scores of the Akaike Information Criterion (AIC) and ML values ( $\ln L$ ) (Table S2.2, see Supplementary Material). MP reconstruction was conducted in MEGA5 following the Closest-neighbor interchange option, and ML was drawn from PHYML v.3.0 (Guindon and Gascuel, 2003) using the approximate Likelihood-ratio test (LRT) based on a Shimodaira-Hasegawa-like procedure (Anisimova and Gascuel, 2006). We estimated robustness of clades using non-parametric bootstrapping with 1,000 pseudo-replicates (Felsenstein, 1985). BI was implemented in MRBAYES v.3.1 (Ronquist and Huelsenbeck, 2003) using default parameters and priors, with two independent Markov-chain Monte Carlo (MCMC) runs of 1 million generations sampled every 100 increments. We discarded a burn-in of 500 generations after runs reached stationary distribution (PRSF<0.1). Trees were visualized in FIGTREE v1.3.1 with clade robustness based on consistent posterior probabilities (PP). We considered 0.90–0.98 PP and 70–89 % bootstrap values (BS) as moderate support, and >0.98 PP and 90–100 % BS as strong support, following previous studies (e.g. Muellner et al., 2008; Siler et al., 2011).

To examine the robustness of our tree topology further we performed a Kishino-Hasegawa (KH) test of competing evolutionary hypotheses. This likelihood-based approach determines whether alternative trees are supported significantly less by the data than a hypothetical tree topology, and is best applied when a given topology can be specified *a priori* (Goldman et al., 2000). The ML majority-rule consensus tree did not support the analysis, but is highly congruent with the BI maximum clade credibility tree (see Results). Thus, we implemented the KH test using MP analysis in PAUP v4.0b10 (Swofford, 2002) to generate 80 alternative trees to compare against the single ‘best’ tree inferred from Bayesian analyses.



### 3. Results

#### 3.1 Sequence attributes and comparison of genes

In-group sequence characters exhibited a mean of 40% conserved sites and 60% variable sites across the three datasets, of which 40% were parsimony-informative (Table S2.3, see Supplementary Material). Compared with the out-group, sequence variations across the three datasets were relatively proportional to each other, with 1,219 variable and 837 parsimony-informative sites in the concatenated dataset. All pairwise comparisons for the pattern of homogeneity (disparity index test) exhibited significant ( $P$ -values  $< 0.05$ ) estimates, indicating that sequences have evolved different patterns of substitution across the three datasets (Table 2.2). Base composition was biased to adenine (A) and cytosine (C) in the in the *cytb* dataset, but biased to guanine (G) and cytosine in the nuclear dataset (Table 2.2). Base composition was more A–C rich in the concatenated dataset, which was consistent with the patterns found in other birds (e.g. Moyle and Marks, 2006; Marks et al., 2007). Relative substitution rates, empirical base frequencies and nucleotide composition bias varied slightly between the two methods (ML/MCL), but did not vary extensively between the three datasets. Patterns of transitions (Ts) and transversions (Tv) between nuclear and mtDNA were relatively similar, although overall Ts/Tv bias for both ML and MCL tests were higher in *cytb* than AK1 intron 5 (Table 2.2). Overall, comparative tests confirm that the use of relaxed substitution models and nucleotide composition bias shows no potentially deleterious effect on phylogenetic reconstructions (Tamura et al., 2011).

A pairwise comparison of divergence at mitochondrial and nuclear loci revealed a weak relationship within lineages (Fig. 2.1A), indicating heterogeneity in rates of molecular evolution. When we compared divergence in the concatenated dataset with divergence at nuclear (Fig. 1B) and mitochondrial (Fig. 2.1C) loci, we found strong congruence with mtDNA divergence—despite this representing only 62% of the combined sequence. Accordingly, the topology of the phylogenetic tree based on the concatenated dataset was strongly congruent with that based on mtDNA (Fig. 2.2), indicating that the final topology is primarily driven by the signal in the

mitochondrial data partition. Moderate congruence in genetic distance was observed between the nuclear gene and the two other datasets, but nonetheless the novel nuclear gene AK 1 intron 5 was comparable in phylogenetic signal to the mtDNA *cytb* gene, in line with previous studies (e.g. Shapiro and Dumbacher, 2001; Allen and Omland, 2003).

Overall mean distance for the concatenated AK1 intron 5 and *cytb* dataset was  $0.162 \pm 0.007$  (S.E.). We used the estimated value of the shape parameter for the discrete  $\Gamma$  distribution using ML (0.5019) to model evolutionary rates of base substitution  $\mu$  (per site, per million years) in 5 categories, giving an estimated average rate of 1.0. This was within the range for avian *cytb* of 0.7–2.0 (see Groombridge et al., 2002), and lower than the previous estimate of 1.59 for hornbills (Viseshakul et al., 2011). Several parsimony-informative indels (insertions/deletions) were recovered in AK1 intron 5, with a total of 13 insertions and 5 deletions across the different clades (Fig. 2.3; Table S2.4, see Supplementary Material). Five independent insertions differentiate *Bucorvus* from typical hornbills (Bucerotinae), which were in turn defined by two independent insertions. Long-tailed forest hornbills (*Berenicornis*, *Tropicranus*) were united by a 2 bp insertion and 1 bp deletion, and the large Afrotropical hornbills (*Ceratogymna*–*Bycanistes*) were united by a single insertion. Notably, all the Philippine *Penelopides* were unified with 1 bp insertion, as was the *Rhinoplax* clade. All *cytb* sequences for 61 hornbills had the same start codon (ATG), but varied in their terminal codons (TAA/TAG). The initiation codon for AK1 intron 5 (GTG/GCA) was similar to *Gallus gallus* (511 bp), but differed in the termination codon, which was CTG/CTC rather than AAG (Suminami et al., 1988).

### 3.2 Tree topology

Tree topology was approximately congruent across the three datasets (nuclear DNA, mtDNA, and concatenated), although somewhat divergent in the nuclear tree (Fig. 2.2A). In all cases, the genus *Bucorvus* (ground-hornbills) was recovered as a sister clade to a monophyletic Bucerotinae (nest-sealing hornbills). The latter group was subdivided into 6–7 prominent clades in the nuclear tree (Fig. 2.2A) but simplified in the mtDNA and concatenated trees into five clades,

with *Tockus* the most basal (Fig. 2.2B, 2C). We refer to these clades henceforth by their ancestral lineage: B = *Tockus* clade, C = *Berenicornis* clade, D = *Rhinoplax* clade, E = *Anorrhinus* clade, F = *Aceros* clade (see Fig. 2.3 and Appendix A for constituent species).

Focusing on results generated from the most deeply sampled, concatenated dataset, we found moderate to strong congruence in tree topology based on three separate analytical approaches (MP, BI and ML). Nodal values using MP were relatively low (Fig. S2.1, see Supplementary Material), but the overall topology was similar to the BI maximum clade credibility tree (Fig. 2.2C) and the ML majority-rule consensus tree (Fig. 2.3). Composition of major clades and placement of key taxa was consistent across all three topologies. For example, *Aceros cassidix* always grouped with other wreathed hornbills (*Rhyticeros*), *Penelopides exarhatus* with ‘wrinkled’ hornbills (*Aceros*), *Tockus hartlaubi* with *Tropicranus albocristatus*, and *Tockus camurus* with other ‘whistling’ *Tockus* (see Discussion).

The most strongly supported trees were constructed using BI (Fig. 2.2C) and ML (Fig. 2.3). The topology of the most strongly supported trees (BI and ML) only differed in the placement of 5 of 68 tips in the phylogeny. This included the re-arrangement of the whistling *Tockus* subclade, the separation of *Berenicornis comatus* from the *Tropicranus* clade (BS 70–90, PP >0.98), and the separation of *Anthracoceros malayanus* from the rainforest-dwelling *Ocyceros* clade (BS >90, PP >0.98). BI and ML trees were not only highly congruent, but also the most parsimonious as tree topology was moderately to strongly supported at >80% of nodes. In other words, only 20% of nodes had weaker node support (<70% BS and <90% PP).

Topological certainty of nodes were also based on moderate to strong support ( $\geq 70$  BS and  $\geq$  PP values; Lovette and Rubenstein 2007), and topology was consistent across three phylogenetic reconstructions (MP, BI and ML) for the monophyly of the *Tockus*, *Anorrhinus* and *Aceros* clades. Topology was also certain for the following monophyletic groups: clucking *Tockus*; *T. monteiri* with *T. erythrorhynchus* group; *T. erythrorhynchus* group; *T. flavirostris* group; *Ceratogymna*–*Bycanistes*; *T. hartlaubi* with *T. albocristatus*; *Rhyticeros*–*Penelopides*–writhed *Aceros* group; *Rhyticeros*; and Philippine *Penelopides*. The KH test implemented on 80 trees showed that all trees had topologies similar to the BI tree. Tree lengths deviated by 2.00–9.38, with

all trees having a  $t$ -value of 0.00 and  $P$ -value of 1.00. Thus, all 80 trees were not significantly different from the ‘best’ tree, indicating robust topology.

We selected the ML tree from the concatenated dataset to represent a complete phylogeny of hornbills (Fig. 2.3). Topology of this tree was highly congruent with the expanded BI tree (Fig. S2.2) constructed from a concatenated nuclear-mtDNA dataset of all 162 hornbill sequences (Appendix B).

## 4. Discussion

### 4.1 Phylogenetic framework for the Bucerotidae

The topology of the ML reconstruction of combined nuclear and mitochondrial datasets (Fig. 2.3) represents our best hypothesis of evolutionary relationships within the Bucerotidae. We recognize that some nodes remain weakly supported, and emphasize that deeper sampling of lineages and loci may be required to resolve these issues. However, we recommend the use of this topology as the most robust framework available for comparative analyses, or indeed for any study relying on knowledge of hornbill relationships.

Our results support prior analyses (Sibley and Ahlquist, 1990; Kemp, 1995; Hübner et al., 2003; Viseshakul et al., 2011) in identifying a basal split subdividing the Bucerotidae into two clades, a small one containing the ground-hornbills (*Bucorvus*) and a large one containing all other hornbills. This separation reflects notable phenotypic divergence, with ground-hornbills being the only wholly terrestrial members of the family, and differing from other hornbills in having one extra cervical vertebra (Kemp and Crowe, 1985). They also differ from most other Bucerotidae in lacking nest sanitation and in not using plaster to seal entrances to their nest cavities (Kemp, 1995, 2001). Our results support treatment of these clades as separate subfamilies, Bucorvinae and Bucerotinae, and suggest that divergence may be large enough to warrant treatment as separate families (Sibley and Ahlquist, 1990; Kemp, 1995).

Focusing within the Bucerotinae, the tree topology supported by our analyses largely agrees with that of the mtDNA phylogeny published by Viseshakul et al. (2011). For example, both trees placed *Tropicranus* basal to the *Ceratogymna*–*Bycanistes* clade, *Anorrhinus galeritus* basal to *A. austeni/tickelli*, and *Aceros nipalensis* basal to the *Aceros* clade. However, several novel insights are provided by our results. One example is *Berenicornis comatus*, which Viseshakul et al. (2011) left as enigmatic because different analyses disagreed whether it was basal to a clade containing Asiatic and Afrotropical genera (*Ocyceros*, *Tropicranus*, *Ceratogymna*, *Bycanistes*) or to the Asiatic *Rhinoplax*–*Buceros* lineage. Our findings strengthen support for the placement of *Berenicornis*—an Asiatic omnivorous species—as basal to a clade of Afrotropical insectivores (*Tropicranus*) and frugivores (*Ceratogymna*, *Bycanistes*). Another example is Asian *Ocyceros*, which Viseshakul et al. (2011) tentatively grouped with African *Tropicranus*, *Ceratogymna* and *Bycanistes*, but which our analyses revealed to be allied to *Anthracoceros* in an exclusively Asiatic clade. In addition, our results help to resolve the placement of *Tockus hartlaubi*, *T. camurus*, and several other less contentious species absent from previous analyses. These findings are summarized and placed in context in the following sections, which focus on each of the five major clades of the Bucerotinae identified by our analyses.

## 4.2 Phylogenetic relationships within major clades

### 4.2.1. *Tockus* clade

According to current taxonomic arrangements, *Tockus* currently represents the largest genus in the family with 18 species (see Table 2.1; Appendix A). Our tree provides some support for this diversity by confirming genetic divergence in all lineages proposed as species in the *T. erythrorhynchus* complex (Kemp and Delport, 2002). Another undescribed yellow-eyed red-billed hornbill form (nominate forms are brown-eyed) from northwest Kenya awaits further investigation (P. Stapelberg in litt., 2011 via A. Kemp). They also reveal that *T. monteiri* forms the basal lineage for this complex. Patterns of genetic divergence in our phylogeny are also consistent with the

taxonomic revisions in yellow-billed (split into *T. leucomelas* and *T. flavirostris*, Kemp and Crowe, 1988) and Von der Decken's hornbills (split into *T. deckeni* and *T. jacksoni*, Gill and Donsker, 2012). However, *T. deckeni* and *T. jacksoni* were never formally split but recognised as distinctive subspecies (Kemp, 1995, 2001).

All our trees show that the genus *Tockus* as currently defined is subdivided by a deep phylogenetic split into two major groups, which represent two different vocal types ('whistlers' and 'cluckers'). These findings support the suggestion of Hübner et al. (2003) that *Tockus* should be split into two genera demarcated by vocal phenotypes, both further subdivided by shallower divisions into subgenera. This arrangement is consistent with the evidence of DNA-DNA hybridization, morphology, and behaviour (e.g., nest-lining, hop/walk locomotion, etc.) (Kemp, 1976, 1994, 1995).

Our results also help to clarify the position of *Tockus camurus*, a contentious species previously placed in a subclade separate from the 'whistlers' and 'cluckers', and treated as *Incertae sedis* (Kemp, 1995). In the AK1 intron 5 tree (Fig. 2.2A), *T. camurus* forms a basal branch to all whistling and clucking *Tockus*, suggesting that they are derived from a smaller-bodied, finer-billed lineage. This is consistent with the idea that *Tockus* is derived directly from *Phoeniculus*-like ancestors (Kemp, 1979). It is also intuitive based on phenotype, as an examination of *T. camurus* suggests it to be a dwarf relative of *T. alboterminatus*, as noted by Elliot (1882), and an affinity previously proposed based on bill-color (Kemp, 1976).

More unexpectedly, the novel inclusion of *Tockus hartlaubi* in our analyses reveals that it only superficially resembles *Tockus*, and instead emerges in all analyses as sister to *Tropicranus albocristatus* in the *Berenicornis* clade. Thus, our findings reveal that *Tockus* is polyphyletic, even though the monophyly of the genus has never seriously been questioned. Kemp (1995) noted that *T. hartlaubi* had uncertain affinities, but he still placed the taxon in a subclade *incertae sedis* of *Tockus*. Again, this rearrangement makes sense on the basis of phenotype, as examination of museum specimens indicates that *T. hartlaubi* and *Tropicranus albocristatus* share several diagnostic characters (e.g., crest structure, graduated tail, etc.).

#### 4.2.2 *Berenicornis* clade

This clade contains three subclades with a heterogeneous mix of taxa, including Asiatic *Berenicornis* and Afrotropical *Tropicranus* (both primarily faunivorous) and Afrotropical *Ceratogymna* and *Bycanistes* (frugivorous). *Berenicornis* was previously subsumed within *Aceros* on the basis of genetic data (Hübner et al., 2003) and some external morphology or non-genetic cladogram (Kemp, 1995), but our analyses confirm that the relationship with *Aceros* is erroneous. *Berenicornis* was also included as a subgenus of *Aceros* based on the derived all-black adult female trait (Kemp, 1995). Instead, they provide stronger support for the tentative suggestion of Viseshakul et al. (2011) that *Berenicornis* should be reunited in a clade with Afrotropical *Tropicranus*, as first proposed by Peters (1945) on the basis of their shared crests and long, graduated tails. As reported above, we also show that *Tropicranus* is not a monotypic genus, as it merges with *Tockus hartlaubi* in all analyses.

These largely faunivorous lineages gave rise to two genera of Afrotropical frugivores, with strong support for a pair of sibling species (*Ceratogymna elata* and *C. atrata*) being basal to all *Bycanistes*, in line with the findings of Viseshakul et al. (2011). However, our analyses suggest a revised topology for *Bycanistes*, with *B. brevis* and *B. subcylindricus* basal and *B. fistulator* and *B. bucinator* representing the most recent split. The sister species of *B. fistulator* and *B. bucinator* belong to the subgenus *Bycanistes*, whereas the remaining taxa (*brevis*, *subcylindricus*, *cylindricus*, *albotibialis*) belong to the subgenus *Baryrhynchodes* Strand 1928 (Sanft, 1960; Kemp and Crowe, 1985; Kemp, 1995).

#### 4.2.3 *Rhinoplax* clade

This clade of four large forest frugivores in the genera *Rhinoplax* and *Buceros* diverged earlier than most other Asian clades and probably represents the oldest Asian lineage (Kemp, 1995; Viseshakul et al., 2011) pre-*Berenicornis*. *Rhinoplax* is basal to *Buceros* in nearly all topologies, and our novel data show that *Buceros hydrocorax* is basal to *B. bicornis* and *B. rhinoceros*. All four species collectively exhibit the distinctive strategy of cosmetic coloration using uropygial gland secretions (Delhey et al., 2007), a feature shared with wrinkled hornbills (*Aceros*).

#### 4.2.4 *Anorrhinus* clade

Our results confirm a close association between *Anorrhinus* and *Anthracoceros*, in contrast with the early phylogeny based on DNA-DNA hybridization (Sibley and Ahlquist, 1990), but in agreement with previous molecular phylogenies (Srikwan and Woodruff, 1998; Viseshakul et al., 2011). Unlike all previous studies, however, we show that *Anorrhinus* is basal, forming a sister clade with a combined *Ocyceros*–*Anthracoceros* clade. Our topology also reveals that there is only minor genetic divergence between *Anorrhinus galeritus* and *Anorrhinus austeni/tickelli* (previously treated as *Ptilolaemus*), thus supporting the merger of *Ptilolaemus* Ogilvie-Grant 1892 into *Anorrhinus* Reichenbach 1849 (Kemp 1995, 2001). However, we note that *Ptilolaemus* is distinctive in a number of features, including bill color, casque shape, and plumage, and could be considered a subgenus.

The recent mtDNA tree of Viseshakul et al. (2011) suggested that Asiatic grey (*Ocyceros*) and Asiatic pied (*Anthracoceros*) hornbills were distantly related, but all our trees identify an *Ocyceros*–*Anthracoceros* clade with strong support for the ancestral node. Close affinity of these genera had previously been suspected on the basis of plumage details (Kemp 1979, 1988, Kinnaird & O’Brien 2007), but our analyses provide the first molecular evidence. However, the structure of this clade is less easily resolved: topology is conflicting across different analyses and internal node support is typically moderate to low. Our nuclear DNA tree (Fig. 2.2A) suggests that *Ocyceros* and *Anthracoceros* are monophyletic, whereas our mtDNA (Fig. 2.2B) and concatenated (Fig. 2.2C, Fig. 2.3) trees recover two-way polyphyly of both genera. Given the deeper sampling and greater stability of our consensus trees, these findings suggest that (1) *Anthracoceros* is polyphyletic, with *A. malayanus* more closely allied to *Ocyceros*, and (2) *Ocyceros* is polyphyletic, with *O. birostris* perhaps warranting separation in its own genus. To our knowledge, this is the first time that compelling evidence for polyphyly of these genera has been presented (but see Srikwan and Woodruff, 1998). Further sampling of loci is needed to resolve phylogenetic relationships between *Ocyceros* and *Anthracoceros*.



If *Anthracoceros malayanus* is maintained in *Anthracoceros*, it is clearly basal to the clade. The remaining ‘pied hornbills’ (*A. coronatus*, *A. albirostris*, *A. marchei*) form a relatively simple monophyletic lineage, with *A. montani* as the earliest branch. This finding resolves the uncertainty surrounding the placement of this critically endangered hornbill: *A. montani* is a black-billed ‘pied hornbill’ (i.e. closely allied to the *Anthracoceros marchei*, *A. albirostris*, and *A. coronatus*) rather than a white-tailed ‘black hornbill’ (i.e. not related to the somewhat divergent *A. malayanus*).

#### 4.2.5 *Aceros* clade

*Aceros* Gray 1844 once applied to a diverse genus containing at least 10 species, yet our results reveal the complex evolutionary history, and three-fold polyphyly, of this earlier grouping. In effect, the name *Aceros* is only valid for the type species, *Aceros nipalensis*, which is basal to the clade. Focusing on the more derived lineages, the ‘wreath-billed’ hornbills can be split into the genus *Rhyticeros*, as proposed by Viseshakul et al. (2011). Our results clarify that this subclade contains two sister pairs (*undulatus*–*subruficollis* and *plicatus*–*narcondami*), with *R. everetti* forming a basal taxon. Although *Rhyticeros subruficollis* is now generally accepted as a full species, it was often considered a subspecies of *R. plicatus* (e.g. Deignan, 1963; Elbel, 1969) or juvenile of *R. undulatus* (Sanft, 1960), and its evolutionary relationships were uncertain (Rasmussen, 2000). Our analyses confirm that it is sister to the sympatric *R. undulatus*. They also consistently recovered *R. plicatus* and *R. narcondami* as sisters, confirming close affinities suggested by earlier treatments (Kemp, 2001; Dickinson, 2003).

Our results reveal that *A. corrugatus* forms a separate lineage, again supporting the preliminary results of Viseshakul et al. (2011). On the basis of novel sequences, we confirm that other ‘wrinkled’ hornbills—*A. waldeni* and *A. leucocephalus*—also belong in this subclade. A more unexpected discovery was that *Penelopides exarhatus* is a fourth member of the lineage, providing the first evidence that *Penelopides* Reichenbach, 1849 is polyphyletic. Unlike the other three ‘wrinkled’ hornbills, *P. exarhatus* does not have a knob-like casque and is a cooperative breeder (O’Brien and Kinnaird, 1994; Kemp 2001), producing a superficial similarity to *Penelopides*. All ‘wrinkled’ hornbills and *P. exarhatus* exhibits cosmetic colouration (Kemp 1995). With the

repositioning of *P. exarhatus*, all Philippine *Penelopides* form a recent monophyletic offshoot of the *Aceros* clade.

### 4.3 Congruence with vocal variation

Avian genetic data often correlate more closely with vocal variation than morphology (e.g. Rheindt et al., 2008; Patané et al. 2009; Wink et al., 2009), and thus it is worth noting that our consensus tree (Fig. 2.3) tends to group hornbill vocalizations into distinct types (see Appendix A), suggesting that we have accurately captured evolutionary history. Both ground-hornbills (*Bucorvus*) have distinct booming calls, while all members of *Tockus* divide cleanly into ‘whistlers’ and ‘cluckers’. African forest hornbills (*Ceratogymna*, *Bycanites*) have distinctive wailing calls. In Asia, all *Aceros* hornbills share barking and bleating calls, while the members of *Ocyeros*, *Anthracoceros* and *Anorrhinus* have cackling calls. All hornbills in the *Rhinoplax* clade use resonant honks, but the uniquely complex ‘song’ of *Rhinoplax* itself—one of the characteristic sounds of Sundaic forests—reinforces the evolutionary divergence of this basal lineage from the genus *Buceros*. Vocalizations also support more contentious taxonomic placements. In particular, *Berenicornis*, *Tropicranus* and *Tockus hartlaubi* exhibit surprisingly similar polysyllabic hooting or piping calls, providing further support for a relationship between these three taxa (which form a clade in the nuclear tree).

### 4.4 Taxonomic implications

Our results shed light on hornbill systematics, and suggest several changes in taxonomy, particularly the revision of genus boundaries. Within the *Tockus* clade, for example, the deep division into ‘cluckers’ and ‘whistlers’ strongly supports a generic revision, with *Tockus* Lesson 1830 retained for the former, and *Rhynchaceros* Gloger 1841 reinstated for the latter.

In the *Berenicornis* clade, our results reveal that *Berenicornis* itself is not allied to *Aceros* (*contra* Kemp, 1995) but to *Tropicranus*. However, we do not propose a return to the merger of *Berenicornis* into *Tropicranus* first adopted by Peters (1945), particularly as these lineages do not

form a monophyletic grouping in the concatenated tree. We thus retain *Berenicornis* and *Tropicranus* (includes *hartlaubi*) as monotypic genera on the basis of considerable genetic divergence and differences in size and ecology (Sanft, 1960; Kemp, 2001). We also retain the genera *Ceratogymna* Bonaparte 1854 and *Bycanistes* Cabanis and Heine 1860, given their genetic and phenotypic divergence (*contra* Kemp, 1995; Hübner et al., 2003).

Turning to the *Rhinoplax* clade, our results confirm that the genus *Rhinoplax* groups closely with, and is basal to, the genus *Buceros*, as suggested by Viseshakul et al. (2011). Treating *Rhinoplax* as *Buceros* is possible on phylogenetic grounds, but we prefer to retain *Rhinoplax* as a monotypic genus given its extreme vocal and morphological divergence. This treatment is also supported by highly divergent nuclear intron sequences (Fig. 2.2A).

Generic taxonomy in the *Anorrhinus* clade remains unclear, partly because of disparity between nuclear and mtDNA phylogenies. Judging from the AK1 intron 5 tree, both *Anthracoceros* Reichenbach 1849 and *Ocyceros* Hume 1873 are monophyletic. However, the mtDNA and concatenated trees suggest that taxonomic changes are required. Specifically, they imply that *Anthracoceros malayanus* should be grouped in the same genus as *Ocyceros griseus/gingalensis*, and *Ocyceros birostris* placed in a monotypic genus. As *birostris* is the type species of *Ocyceros* (assigned by Elliot, 1882), the *malayanus–griseus–gingalensis* trio could either be subsumed in *Anthracoceros* or placed in a separate genus. Given the degree of divergence between these subclades, we favour this latter course of action (which would require a new genus name to be introduced). For the moment, we prefer to maintain *Ocyceros* and *Anthracoceros* in their traditional format, but urge further sequencing of loci to resolve their evolutionary relationships.

In the *Aceros* clade, our results suggest that *A. corrugatus*, *A. waldeni* and *A. leucocephalus* do not form a monophyletic grouping with either *A. nipalensis* or *A. cassidix*. They should thus be removed from *Aceros* and placed in the genus *Cranobrontes* Riley 1921. We also show that *Cranobrontes* is actually a quartet of species, with the fourth being the Sulawesi endemic, *Penelopides exharatus*. This lineage is phenotypically divergent, and has been placed in its own genus (*Rhabdotorrhinus* Stresemann 1932). This treatment may be the best arrangement if

priority is given to phenotypic data, although it is not supported by our analyses, which recover a sister relationship with *corrugatus*. Finally, our findings clarify that another Sulawesi endemic, *A. cassidix*, could either be grouped with the genus *Rhyticeros*—to which it is basal—or separated into the monotypic genus *Cranorrhinus* Cabanis and Heine 1860. Although this latter treatment would help to emphasize the divergent phenotype of *cassidix*, we prefer to group it with *Rhyticeros* until further studies are undertaken, particularly as the range of *cassidix* is geographically nested between other forms of *Rhyticeros* to the west and east.

If the Bucerotinae is treated as a subfamily, the five distinctive subclades identified by our analyses (nodes B–F in Fig. 2.3) could be designated as tribes. Given that tribe designations are based on generic seniority, the appropriate names would be Tockini, Berenicornini, Bucerini, Acerini and Anorrhinini. A full summary of taxonomic recommendations is given in Appendix A.

#### 4.5 Implications for biogeography

It has long been proposed that hornbills are generally separated into African and Asiatic clades (Kemp and Crowe, 1985). One of the few instances of a biogeographical mismatch based on molecular evidence was the placement of Asiatic *Ocyrceros* within African genera by Viseshakul et al. (2011). Our deeper sampling revealed that *Ocyrceros* is best placed with other Asiatic species in the *Anorrhinus* clade, and thus fits with the traditional view of the historical biogeography of hornbills.

Our results also confirm an ancestral placement for African *Bucorvus* and *Tockus*, along with a more recent radiation of Asiatic clades, supporting the usual contention that hornbills originated in Africa, followed by an eastward colonization of South Asia, South-East Asia, and marginally Australasia (Kemp, 1995). In agreement with this ‘Out-of-Africa’ model, one of the most derived hornbill lineages, *Rhyticeros plicatus*, is the only member of the family to cross the key biogeographic barriers of Weber’s and Lydekker’s lines, ranging eastward to New Guinea and the islands of Northern Melanesia (Mayr and Diamond, 2001).

This hypothesis of a gradual progression eastward by hornbills has been challenged by a ‘Back-to-Africa’ model, wherein ancestral forest-dwelling hornbills recolonized Africa from Asia (Kinnaird and O’Brien, 2007). This proposal was based on early phylogenetic results showing close common ancestry of African and Asiatic forest hornbills—the former seemingly embedded in the latter (Sibley and Ahlquist, 1990; Hübner et al., 2003). A more recent phylogenetic study suggested that Sundaic *Berenicornis* and Afrotropical *Tropicranus* may represent surviving lineages of primitive radiations from the African *Tockus* clade (Viseshakul et al., 2011). This type of close relationship between Sundaic and African species occurs in other forest birds, such as pheasants Phasianidae (Kimball et al., 1997) and trogons Trogonidae (Hosner et al., 2010). However, the direction of colonization is often unclear.

Our results do not fully resolve this issue, but tend to weaken previous evidence for recolonization of Africa. In the nuclear tree (Fig. 2.2A), all African hornbills are ancestral to Asiatic hornbills, supporting the simplest version of the ‘Out-of-Africa’ hypothesis. Even in our consensus tree (Fig. 2.3), the branch leading to African forest hornbills (*Tropicranus*, *Ceratogymna*, *Bycanistes*) is ancestral to the diverse Asiatic clade. The only anomaly is the placement of *Berenicornis* as basal to a group of African genera (*Tropicranus*–*Ceratogymna*–*Bycanistes*). While this topology has potentially important biogeographical implications, we believe that it provides no watertight evidence for the ‘Back-to-Africa’ model. An alternative explanation is that a *Tockus*-like ancestor colonised Asia, with *Berenicornis* representing a surviving lineage of this invasion, and that the same ancestral lineage subsequently radiated in Africa. This series of events could produce the tree topology shown in our consensus tree (Fig. 2.3) without any return wave of colonization into Africa.

## Acknowledgments

We are grateful to the museums, zoos and aviaries listed in Appendix B for providing tissue samples, particularly American Museum of Natural History, Delaware Museum of Natural

History, Natural History Museum, Tring, Oxford University Museum of Natural History, and Cambridge University Museum of Zoology. We also acknowledge the generous assistance of M. Adams, R. Alves, R. Antolin, E. Batara, H. Bratcher, J. Cooper, A. Dans, J. de Leon, A. Davies, R. Domingo, N. Donato, C. Edwards, L. Estoya, J. Haile, P. Holland, P. Hosner, J.M. Justo, A. Kemp, L. Lastimoza, K. Lim, I. Lit Jr., M. Lowe, S. Nacua, M. Nowak-Kemp, P. Ong, A. Owen, R. Prys-Jones, R. Puentespiná, I. Sepil-Oz, P. Sweet, R. Urriza, A. Viojan, A. Walls, J. Woods and V. Yngente. Permits for collection and transport of samples were facilitated by Department of Environment, Food and Rural Affairs (DEFRA, UK), Fish and Wildlife Service (USA), Protected Areas and Wildlife Bureau and DENR Regional and Provincial Offices. This study was supported by the Ford Foundation International Fellowship Program, with further contributions from the British Ornithologists' Union, North of England Zoological Society and St Anne's College, Oxford.

## References

- Allen, E.S., Omland, K.E., 2003. Novel intron phylogeny supports plumage convergence in Orioles (*Icterus*). *Auk* 120, 961–969.
- Anisimova, M., Gascuel, O., 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Syst. Biol.* 55, 539–552.
- Belterman, R.H.R., de Boer, L.E.M., 1984. A karyological study of 55 species of birds, including karyotypes of 39 species new to cytology. *Genetica* 65, 39–82.
- Belterman, R.H.R., de Boer, L.E.M., 1990. A miscellaneous collection of bird karyotypes. *Genetica* 83, 17–29.
- Bennett, E.L., Nyaoi, A.J., Sompud, J., 1997. Hornbills *Buceros* spp. and culture in northern Borneo: can they continue to coexist? *Biol. Conserv.* 82, 41–46.
- BirdLife International, 2000. Threatened Birds of the World. Lynx Edicions and BirdLife International, Barcelona and Cambridge.
- Brown, J.W., Rest, J.S., Garcia-Moreno, J., Sorensen, M.D., Mindell, D.P., 2008. Strong mitochondrial DNA support for a Cretaceous origin of modern avian lineages. *BMC Biol.* 6, 6.

## CHAPTER 2

- Clements, J.F., 2007. The Clements Checklist of Birds of the World, sixth ed. Cornell University Press, New York, NY.
- Deignan, H. G., 1963. Checklist of the birds of Thailand. Bull. U.S. Nat. Mus. 226, 1–263.
- Delhey, K., Peters, A., Kempnaers, B., 2007. Cosmetic coloration in birds: occurrence, function, and evolution. American Naturalist 169, 145–158.
- Dickinson, E.C., 2003. The Howard and Moore Complete Checklist of the Birds of the World, third ed. Christopher Helm, London.
- Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
- Elbel, R. E., 1969. The taxonomic position of the hornbill *Rhyticeros plicatus subruficollis* (Blyth) as indicated by the Mallophaga. Condor 71, 434–435.
- Elliot, D.G., 1882. A Monograph of the Bucerotidae, or Family of the Hornbills. Taylor and Francis, London.
- Espinosa de los Monteros, A., 2000. Higher-level phylogeny of Trogoniformes. Mol. Phylogenet. Evol. 14, 20–34.
- Fain, M.G., Krajewski, C., Houde, P., 2007. Phylogeny of "core Gruiformes" (Aves: Grues) and resolution of the Limpkin-Sungrebe problem. Mol. Phylogenet. Evol. 43, 515–529.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
- Frith, C. B., Frith, D. W., 1983. A systematic review of the hornbill genus *Anthracoceros* (Aves, Bucerotidae). Zool. J. Linn. Soc. 78, 29–71.
- Fry, C.H., Keith, S., Urban, E.K., 1998. The Birds of Africa, Volume III: Parrots to Woodpeckers. Princeton University Press, Princeton, NJ.
- Gill, F., Donsker, D., 2012. International Ornithological Congress World Bird List (v 2.11). Accessed January 2012 from: <http://www.worldbirdnames.org/names/>
- Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–70.
- Groombridge, J.J., Jones, C.G., Bayes, M.K., van Zyl, A.J., Carillo, J., Nichols, R.A., Bruford, M.W., 2002. A molecular phylogeny of African kestrels with reference to divergence across the Indian Ocean. Mol. Phylogenet. Evol. 25, 267–277.
- Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41, 95–98.
- Helbig, A.J., Kocum, A., Seibold, I., Braun, M.J., 2005. A multi-gene phylogeny of aquiline eagles (Aves: Accipitriformes) reveals extensive paraphyly at the genus level. Mol. Phylogenet. Evol. 35, 147–164.

- Holbrook, K.M., Smith, T.B., 2000. Seed dispersal and movement patterns in two species of *Ceratogymna* hornbills in a West African tropical lowland forest. *Oecologia* 125, 249–257.
- Holbrook, K.M., Smith, T.B., Hardesty, B.D., 2002. Long-distance movements of frugivorous rainforest hornbills. *Ecography* 25, 745–749.
- Hosner, P.A., Sheldon, F.H., Lim, H.C., Moyle, R.G., 2010. Phylogeny and biogeography of the Asian trogons (Aves: Trogoniformes) inferred from nuclear and mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 57, 1219–1225.
- Hübner, V.S.M., Prinzing, R., Wink, M., 2003. Neue Erkenntnisse zur Taxonomie der Hornvögel (Aves: Bucerotiformes) und ihre Bedeutung für die Zucht in Menschenobhut. *Zool. Garten N.F.* 73, 396–401.
- Kemp, A.C., 1976. A study of the ecology, behaviour and systematics of *Tockus* hornbills (Aves: Bucerotidae), Trans. Mus. memoir No. 20, Transvaal Museum, Pretoria.
- Kemp, A.C., 1979. A review of the hornbills: biology and radiation. *Living Bird* 17, 105–136.
- Kemp, A.C., 1988. The systematics and zoogeography of Oriental and Australasian hornbills (Aves: Bucerotidae). *Bonn. Zool. Beitr.* 39, 315–345.
- Kemp, A.C., 1994. Congruence of phylogenies for *Tockus* hornbills (Bucerotiformes) based on behaviour, ecology, morphology, DNA hybridisation and ectoparasites. *J. Orn.* 135, 338.
- Kemp, A.C., 1995. *The Hornbills: Bucerotiformes*. Oxford University Press, Oxford.
- Kemp, A.C., 2001. Family Bucerotidae (Hornbills), In: del Hoyo, J., Elliott, A. Sargatal, J., (Eds.), *Handbook of the Birds of the World, Piciformes to Bucerotiformes*, vol. 6. Lynx Edicions, Barcelona, pp 436–523.
- Kemp, A. C., Crowe, T. M., 1985. The systematics and zoogeography of Afrotropical hornbills (Aves: Bucerotidae), In: Schuchmann, K.-L. (Ed.), *Proceedings of the International Symposium on African Vertebrates, Systematics, Phylogeny and Evolutionary Ecology*, Alexander Koenig Research Institute and Zoological Museum, Bonn, pp. 279–324.
- Kemp, A.C., Delpont, W., 2002. Comments on the status of subspecies in the red-billed hornbill (*Tockus erythrorhynchus*) complex (Aves: Bucerotidae), with the description of a new taxon endemic to Tanzania. *Annals. Trans. Mus.* 39, 1–8.
- Kennedy, R.S., Gonzales, P.C., Dickinson, E.C., Miranda, H.C. Jr., Fisher, T.H., 2000. *A guide to the birds of the Philippines*. Oxford University Press, Oxford.
- Kimball, R.T., Braun, E.L., Ligon, J.D., 1997. Resolution of the phylogenetic position of the Congo peafowl, *Afropavo congensis*: a biogeographic and evolutionary enigma. *Proc. R. Soc. Lond. B* 264, 1517–1523.
- Kinnaird, M.F., O'Brien, T.G., 2007. *The Ecology and Conservation of Asian Hornbills: Farmers of the Forest*. University of Chicago press, Chicago, IL.
- Kirchman, J.J., Hackett, S.J., Goodman, S.M., Bates, J.M., 2001. Phylogeny and systematics of ground rollers (Brachypteraciidae) of Madagascar. *Auk* 118, 849–863.



- Kitamura, S., 2011. Frugivory and seed dispersal by hornbills (Bucerotidae) in tropical forests. *Acta Oecologica* 37, 531–541.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chennam R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. ClustalW and ClustalX version 2. *Bioinformatics* 23, 2947–2948.
- Lenz, J., Fiedler, W., Caprano T., Friedrichs, W., Gaese, B. H., Wikelski, M., Böhning-Gaese, K., 2011. Seed-dispersal distributions by Trumpeter Hornbills in fragmented landscapes. *Proc. R. Soc. B Lond.* 278, 2257–2264.
- Lerner, H.R., Mindell, D.P., 2005. Phylogeny of eagles, Old World vultures, and other Accipitridae based on nuclear and mitochondrial DNA. *Mol. Phylogenet. Evol.* 37, 327–346.
- Lovette, I.J., Rubenstein, D.R., 2007. A comprehensive molecular phylogeny of the starlings (Aves: Sturnidae) and mockingbirds (Aves: Mimidae): Congruent mtDNA and nuclear trees for a cosmopolitan avian radiation. *Mol. Phylogenet. Evol.* 44, 1031–1056.
- Mayr, E., Diamond, J.M., 2001. *The Birds of Northern Melanesia: Speciation, Ecology & Biogeography*. Oxford University Press, Oxford.
- Marks, B.D., Weckstein, J.D., Moyle, R.G., 2007. Molecular phylogenetics of the bee-eaters (Aves: Meropidae) based on nuclear and mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.* 45, 23–32.
- Moreau, R.E. (1934) Breeding habits of hornbills. *Nature* 134, 899.
- Morin, P.A., Messier, J., Woodruff, D.S., 1994. DNA extraction, amplification, and direct sequencing from hornbill feathers. *J. Sci. Soc. Thailand* 20, 31–42.
- Moyle, R.G., Marks, B.D., 2006. Phylogenetic relationships of bulbuls (Aves: Pycnonotidae) based on mitochondrial and nuclear DNA sequence data. *Mol. Phylogenet. Evol.* 40, 687–695.
- Muellner, A.N., Pannell, C.M., Coleman, A., Chase, M.W., 2008. The origin and evolution of Indomalayan, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *J. Biogeogr.* 35, 1769–1789.
- Mundy, N., Unitt, P., Woodruff, D.S., 1997. Skin from feet of museum specimens as a non-destructive source of DNA for Avian Genotyping. *Auk* 114, 126–129.
- Nei, M., Kumar S., 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, Oxford.
- Nylander, J.A.A., 2004. MrModeltest version 2. Evolutionary Biology Centre, Uppsala University, Uppsala, (Program distributed by the author, [www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html](http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html)).
- O'Brien, T.G., Kinnaird, M.F., 1994. Notes on the density and distribution of the endemic Sulawesi Tarictic Hornbill (*Penelopides exarhatus*) in the Tangkoko Dua Saudara Nature Reserve, North Sulawesi. *Trop. Biodiv.* 2, 252–260.

- Pacheco, M.A., Battistuzzi, F.U., Lentino, M., Aguilar, R., Kumar, S., Escalante, A.A., 2011. Evolution of modern birds revealed by Mitogenomics: timing the radiation and origin of major orders. *Mol. Biol. Evol.* 28, 1927–1942.
- Patané, J.S.L., Weckstein, J.D., Aleixo, A., Bates, J.M., 2009. Evolutionary history of *Ramphastos* toucans: Molecular phylogenetics, temporal diversification, and biogeography. *Mol. Phylogenet. Evol.* 53, 923–934.
- Pratt, R.C., Gibb, G.C., Morgan-Richards, M., Phillips, M.J., Hendy, M.D., Penny, D., 2009. Toward resolving deep neoaves phylogeny: data, signal enhancement, and priors. *Mol. Biol. Evol.* 26, 313–326.
- Peters, J.L., 1945. Check-List of Birds of the World, Vol. V. Harvard University Press, Cambridge, MA.
- Rasmussen, P.C., 2000. A review of the taxonomy and status of the Plain-pouched Hornbill *Aceros subruficollis*. *Forktail* 16, 83–86.
- Rasmussen, P.C., Anderton, J.C., 2005. Birds of South Asia, The Ripley Guide, vol. 2. Lynx Edicions, Barcelona.
- Rheindt, F. E., Norman, J. A, Christidis, L., 2008. DNA evidence shows vocalizations to be a better indicator of taxonomic limits than plumage patterns in *Zimmerius* tyrant-flycatchers. *Mol. Phylogenet. Evol.* 48 (1), 150–156.
- Robson, C., 2009. A Field Guide to the Birds of South-East Asia. New Holland Publishers, London.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Rozen, S., Skaletsky, H.J., 2000. PRIMER 3.0. In: Krawetz, S., Misener, S. (Eds.) *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Humana Press, New Jersey.
- Sanft, K., 1960. Bucerotidae (Aves/Upupae). *Das Tierreich* 76, 1–176.
- Sethi, P., Howe, H.F., 2009. Recruitment of hornbill-dispersed trees in hunted and logged forests of the Indian Eastern Himalaya. *Conserv. Biol.* 23, 710–718.
- Shapiro, L. H., Dumbacher, J. P., 2001. Adenylate kinase intron 5: a new nuclear focus for avian systematics. *Auk* 118, 248–255.
- Sibley, C.G., Ahlquist, J.E., 1990. *Phylogeny and Classification of Birds*. Yale University Press, New Haven, CT.
- Sibley, C.G., Monroe Jr., B.L., 1990. *Distribution and Taxonomy of Birds of the World*. Yale University Press, New Haven, CT.
- Siler, C.D., Diesmos, A.C., Alcala, A.C., Brown, R.M., 2011. Phylogeny of Philippine slender skinks (Scincidae: *Brachymeles*) reveals underestimated species diversity, complex biogeographical relationships, and cryptic patterns of lineage diversification. *Mol. Phylogenet. Evol.* 59, 53–65.

- Srikwan, S., Woodruff, D.S., 1998. DNA sequence variation and hornbill conservation. In: Poonswad, P. (ed.) *The Asian Hornbills: Ecology and Conservation*. Thai Studies in Biodiversity No.2, BIOTEC-NSTDA, Bangkok, pp. 69–82.
- Suminami, Y., Kishi, F., Torigoe, T., Nakazawa, A., 1988. Structure and complete nucleotide sequence of the gene encoding chicken Cytosolic Adenylate Kinase. *J. Biochem.* 103, 611–617.
- Swofford, D.L., 2002. *PAUP\*. Phylogenetic Analysis Using Parsimony*. Version 4. Sinauer, Sunderland, MA.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.* 28, 2731–2739.
- Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10, 512–526.
- Trail, P. W., 2007. African hornbills: keystone species threatened by habitat loss, hunting and international trade. *Ostrich*, 78(3), 609–613.
- Tréca, B., Énard, C., 2000. A new subspecies of the red-billed hornbill *Tockus erythrorhynchus* from West Africa. *Ostrich* 71 (3&4), 363–366.
- Vallianatos, M., Loughheed, S.C., Boag, P.T., 2002. Conservation genetics of the loggerhead shrike (*Lanius ludovicianus*) in central and eastern North America. *Conserv. Gen.* 3, 1–13.
- Viseshakul, N., Charoenitikul, W., Kitamura, S., Kemp, A.C., Thong-aree, S., Surapunpitak, Y., Poonswad, P., Ponglikitmongkol, M., 2011. A phylogeny of frugivorous hornbills linked to the evolution of Indian plants within Asian rainforests. *J. Evol. Biol.* 24, 1533–1545.
- Wink, M., El-Sayed, A.-A., Sauer-Gürth, H., Gonzalez, J., 2009. Molecular phylogeny of owls (Strigiformes) inferred from DNA sequences of the mitochondrial cytochrome b and the nuclear RAG-1 gene, *Ardea* 97(4), 581–591.

## CHAPTER 2

**Table 2.1:** Taxonomy and nomenclature of all hornbill species included in this study, and comparison with six taxonomic treatments of this group. Classification follows Gill and Donsker (2012), which contained updates for Bucerotidae based on Kemp and Delport (2002) and Viseshakul et al. (2011).

| Genus                        | Species                         | Peters (1945)             | Sanft (1960)              | Sibley and Monroe<br>(1990) | Kemp (1995)               | Kemp (2001)               | Dickinson<br>(2003)       | Clements (2007)           |
|------------------------------|---------------------------------|---------------------------|---------------------------|-----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| <i>Bucorvus</i> <sup>a</sup> | <i>abyssinicus</i>              | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Bucorvus</i> <sup>a</sup> | <i>leadbeateri</i>              | —                         | <i>cafer</i>              | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>ruahae</i> <sup>b</sup>      | NC                        | NC                        | NC                          | NC                        | NC                        | NC                        | NC                        |
| <i>Tockus</i>                | <i>kempi</i> <sup>b</sup>       | NC                        | NC                        | NC                          | NC                        | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> |
| <i>Tockus</i>                | <i>damarensis</i> <sup>b</sup>  | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> | NC                          | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> |
| <i>Tockus</i>                | <i>rufirostris</i> <sup>b</sup> | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> | NC                          | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> | ssp <i>erythrorynchus</i> |
| <i>Tockus</i>                | <i>erythrorynchus</i>           | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>monteiri</i>                 | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>deckeni</i>                  | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>jacksoni</i>                 | —                         | syn <i>deckeni</i>        | —                           | ssp <i>deckeni</i>        | ssp <i>deckeni</i>        | —                         | —                         |
| <i>Tockus</i>                | <i>leucomelas</i>               | ssp <i>flavirostris</i>   | ssp <i>flavirostris</i>   | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>flavirostris</i>             | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>fasciatus</i>                | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>hemprichii</i>               | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>nasutus</i>                  | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>pallidirostris</i>           | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>bradfieldi</i>               | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>alboterminatus</i>           | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>camurus</i>                  | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tockus</i>                | <i>hartlaubi</i>                | —                         | —                         | —                           | —                         | —                         | —                         | —                         |
| <i>Tropicranus</i>           | <i>albocristatus</i>            | <i>Berenicornis</i>       | —                         | —                           | —                         | —                         | —                         | —                         |

## CHAPTER 2

|                      |                                 |                          |                          |                     |                        |                        |                        |                    |
|----------------------|---------------------------------|--------------------------|--------------------------|---------------------|------------------------|------------------------|------------------------|--------------------|
| <i>Berenicornis</i>  | <i>comatus</i>                  | —                        | —                        | <i>Aceros</i>       | <i>Aceros</i>          | —                      | —                      | <i>Aceros</i>      |
| <i>Ceratogymna</i>   | <i>atrata</i>                   | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Ceratogymna</i>   | <i>elata</i>                    | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Bycanistes</i>    | <i>fistulator</i>               | <i>ssp bucinator</i>     | —                        | <i>Ceratogymna</i>  | <i>Ceratogymna</i>     | —                      | —                      | <i>Ceratogymna</i> |
| <i>Bycanistes</i>    | <i>bucinator</i>                | —                        | —                        | <i>Ceratogymna</i>  | <i>Ceratogymna</i>     | —                      | —                      | <i>Ceratogymna</i> |
| <i>Bycanistes</i>    | <i>cylindricus</i>              | —                        | —                        | <i>Ceratogymna</i>  | <i>Ceratogymna</i>     | —                      | —                      | <i>Ceratogymna</i> |
| <i>Bycanistes</i>    | <i>albotibialis</i>             | <i>ssp cylindricus</i>   | <i>ssp cylindricus</i>   | <i>Ceratogymna</i>  | <i>ssp cylindricus</i> | <i>ssp cylindricus</i> | <i>ssp cylindricus</i> | <i>Ceratogymna</i> |
| <i>Bycanistes</i>    | <i>subcylindricus</i>           | —                        | —                        | <i>Ceratogymna</i>  | <i>Ceratogymna</i>     | —                      | —                      | <i>Ceratogymna</i> |
| <i>Bycanistes</i>    | <i>brevis</i>                   | —                        | —                        | <i>Ceratogymna</i>  | <i>Ceratogymna</i>     | —                      | —                      | <i>Ceratogymna</i> |
| <i>Buceros</i>       | <i>rhinoceros</i>               | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Buceros</i>       | <i>bicornis</i>                 | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Buceros</i>       | <i>hydrocorax</i>               | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Rhinoplax</i>     | <i>vigil</i>                    | —                        | —                        | <i>Buceros</i>      | <i>Buceros</i>         | —                      | —                      | <i>Buceros</i>     |
| <i>Anorrhinus</i>    | <i>tickelli</i>                 | <i>Ptilolaemus</i>       | <i>Ptilolaemus</i>       | —                   | —                      | —                      | —                      | —                  |
| <i>Anorrhinus</i>    | <i>austeni</i> <sup>c</sup>     | <i>ssp tickelli</i>      | <i>ssp tickelli</i>      | <i>ssp tickelli</i> | —                      | —                      | <i>ssp tickelli</i>    | —                  |
| <i>Anorrhinus</i>    | <i>galeritus</i>                | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Anthracoceros</i> | <i>marchei</i>                  | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Anthracoceros</i> | <i>albirostris</i> <sup>d</sup> | ‘ <i>malabaricus</i> ’   | <i>ssp coronatus</i>     | —                   | —                      | —                      | —                      | —                  |
| <i>Anthracoceros</i> | <i>coronatus</i>                | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Anthracoceros</i> | <i>montani</i>                  | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Anthracoceros</i> | <i>malayanus</i>                | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Ocyceros</i>      | <i>griseus</i>                  | <i>Tockus</i>            | <i>Tockus</i>            | —                   | —                      | —                      | —                      | —                  |
| <i>Ocyceros</i>      | <i>gingalensis</i>              | <i>ssp griseus</i>       | <i>ssp griseus</i>       | —                   | —                      | —                      | —                      | —                  |
| <i>Ocyceros</i>      | <i>birostris</i>                | <i>Tockus</i>            | <i>Tockus</i>            | —                   | —                      | —                      | —                      | —                  |
| <i>Aceros</i>        | <i>nipalensis</i>               | —                        | —                        | —                   | —                      | —                      | —                      | —                  |
| <i>Aceros</i>        | <i>waldeni</i>                  | <i>ssp leucocephalus</i> | <i>ssp leucocephalus</i> | —                   | —                      | —                      | —                      | —                  |

## CHAPTER 2

|                    |                                 |                     |                      |               |                    |                    |                   |               |
|--------------------|---------------------------------|---------------------|----------------------|---------------|--------------------|--------------------|-------------------|---------------|
| <i>Aceros</i>      | <i>leucocephalus</i>            | –                   | <i>Rhyticeros</i>    | –             | –                  | –                  | –                 | –             |
| <i>Aceros</i>      | <i>corrugatus</i>               | –                   | <i>Rhyticeros</i>    | –             | –                  | –                  | –                 | –             |
| <i>Aceros</i>      | <i>cassidix</i>                 | –                   | <i>Rhyticeros</i>    | –             | –                  | –                  | –                 | –             |
| <i>Rhyticeros</i>  | <i>plicatus</i>                 | <i>Aceros</i>       | –                    | <i>Aceros</i> | <i>Aceros</i>      | –                  | –                 | <i>Aceros</i> |
| <i>Rhyticeros</i>  | <i>narcondami</i>               | <i>Aceros</i>       | –                    | <i>Aceros</i> | <i>Aceros</i>      | –                  | –                 | <i>Aceros</i> |
| <i>Rhyticeros</i>  | <i>undulatus</i>                | <i>Aceros</i>       | –                    | <i>Aceros</i> | <i>Aceros</i>      | –                  | –                 | <i>Aceros</i> |
| <i>Rhyticeros</i>  | <i>everetti</i>                 | <i>Aceros</i>       | –                    | <i>Aceros</i> | <i>Aceros</i>      | –                  | –                 | <i>Aceros</i> |
| <i>Rhyticeros</i>  | <i>subruficollis</i>            | <i>ssp plicatus</i> | <i>syn undulatus</i> | <i>Aceros</i> | <i>Aceros</i>      | –                  | –                 | <i>Aceros</i> |
| <i>Penelopides</i> | <i>manillae</i> <sup>e</sup>    | <i>ssp panini</i>   | <i>ssp panini</i>    | –             | –                  | –                  | <i>ssp panini</i> | –             |
| <i>Penelopides</i> | <i>mindorensis</i> <sup>e</sup> | <i>ssp panini</i>   | <i>ssp panini</i>    | –             | –                  | –                  | <i>ssp panini</i> | –             |
| <i>Penelopides</i> | <i>affinis</i> <sup>e</sup>     | <i>ssp panini</i>   | <i>ssp panini</i>    | –             | –                  | –                  | <i>ssp panini</i> | –             |
| <i>Penelopides</i> | <i>samarensis</i> <sup>e</sup>  | <i>ssp panini</i>   | <i>ssp panini</i>    | –             | <i>ssp affinis</i> | <i>ssp affinis</i> | <i>ssp panini</i> | –             |
| <i>Penelopides</i> | <i>exarhatus</i>                | –                   | –                    | –             | –                  | –                  | –                 | –             |
| <i>Penelopides</i> | <i>panini</i>                   | –                   | –                    | –             | –                  | –                  | –                 | –             |

– indicates congruence with IOC World Bird List ver 2.11 (Gill and Donsker, 2012); ssp: treated as a subspecies of the named species (i.e. *ssp affinis* means subspecies of *affinis*); syn: synonym of the named species (i.e. *syn deckeni* means synonym of *deckeni*); NC: not considered

<sup>a</sup>Sometimes treated as separate family, Bucorvidae

<sup>b</sup>Recently described taxa (Tréca and Énard, 2000; Kemp and Delport, 2002)

<sup>c</sup>Split from *A. tickelli* (Kemp, 1988; Kemp, 1995; Rasmussen and Anderton, 2005)

<sup>d</sup>Previously treated by Peters (1945) as *A. malabaricus* and *A. coronatus convexus* (see Frith and Frith, 1983)

<sup>e</sup>Split from *P. panini* (Kemp, 1995; Kemp, 2001)

**Table 2.2:** Estimated model parameters for relative substitution rates, nucleotide frequencies and overall mean pairwise genetic distances calculated for two loci using maximum likelihood (ML) and maximum composite likelihood (MCL). Although mtDNA cytochrome *b* (*cytb*) sequences were available for 61 species, analyses were based on 54 species as this was the total number sequenced for Nuclear adenylate kinase 1 intron 5 (AK1 intron 5). Only one mtDNA and one nuclear DNA sequence per species was included in analyses.

|                                               | Nuclear intron<br>locus<br>(AK1 intron 5) | mtDNA coding<br>gene<br>( <i>cytb</i> ) | Concatenated loci<br>(AK1 intron 5 +<br><i>cytb</i> ) |
|-----------------------------------------------|-------------------------------------------|-----------------------------------------|-------------------------------------------------------|
| Relative substitution rates <sup>a</sup>      |                                           |                                         |                                                       |
| Total positions (gaps eliminated)             | 608 (187)                                 | 1143 (852)                              | 1751 (1039)                                           |
| Overall mean distance (d) + S.E.              | 0.13 + 0.07                               | 0.17 + 0.01                             | 0.16 + 0.01                                           |
| Nucleotide frequencies (ML/MCL)               |                                           |                                         |                                                       |
| Adenine (A)                                   | 0.19 / 0.18                               | 0.28 / 0.27                             | 0.25 / 0.25                                           |
| Cytosine (C)                                  | 0.31 / 0.34                               | 0.36 / 0.14                             | 0.34 / 0.18                                           |
| Guanine (G)                                   | 0.31 / 0.26                               | 0.14 / 0.35                             | 0.19 / 0.33                                           |
| Thymine (T)                                   | 0.19 / 0.22                               | 0.23 / 0.24                             | 0.22 / 0.24                                           |
| Overall transition/transversion bias (ML/MCL) | 0.93 / 1.14                               | 2.45 / 2.20                             | 2.12 / 1.97                                           |
| Maximum log likelihood (ML)                   | - 5171.67                                 | - 16471.47                              | - 22086.37                                            |
| Nucleotide substitution pattern (ML/MCL)      |                                           |                                         |                                                       |
| A-C (Tv)                                      | 0.07 / 3.93                               | 0.05 / 4.13                             | 0.06 / 4.24                                           |
| A-G (Tr)                                      | 0.16 / 11.37                              | 0.12 / 22.92                            | 0.15 / 19.14                                          |
| A-T (Tv)                                      | 0.05 / 3.93                               | 0.04 / 4.13                             | 0.04 / 4.24                                           |
| C-G (Tv)                                      | 0.07 / 5.95                               | 0.02 / 5.31                             | 0.03 / 5.57                                           |
| C-T (Tv)                                      | 0.10 / 9.58                               | 0.13 / 14.05                            | 0.13 / 14.04                                          |
| MCL Transition/transversion rate ratios       |                                           |                                         |                                                       |
| K <sub>1</sub> (purines)                      | 2.89                                      | 5.55                                    | 4.52                                                  |
| K <sub>2</sub> (pyrimidines)                  | 1.95                                      | 3.85                                    | 3.57                                                  |
| Γ statistic                                   | -1.151                                    | -3.899                                  | -1.857                                                |
| Treeness                                      | 0.422                                     | 0.281                                   | 0.339                                                 |

<sup>a</sup>relative substitution rates and statistics: Tr = transitional substitution rate; Tv = transversional substitution rate; Γ = Pybus-Harvey gamma statistic; S.E. = standard error.

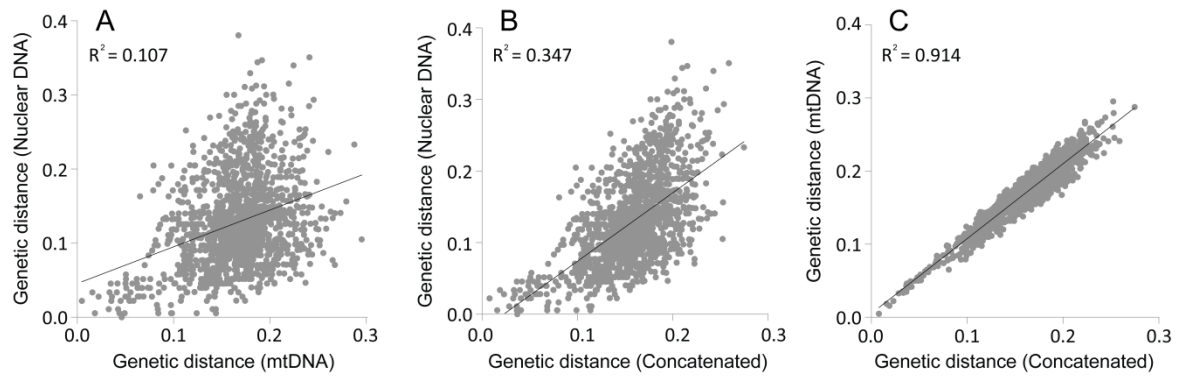


**Figure 2.1:** Relative divergence compared between different loci based on pairwise distances among 54 hornbill species. Comparisons were made between (A) divergence in the nuclear locus AK1 intron 5 and the protein-coding mtDNA *cytb* gene, and then between (B) AK1 intron 5 and (C) *cytb* against the concatenated nuclear-mtDNA dataset. All comparisons were based on Maximum Likelihood distances calculated using locus-specific substitution models.



## CHAPTER 2

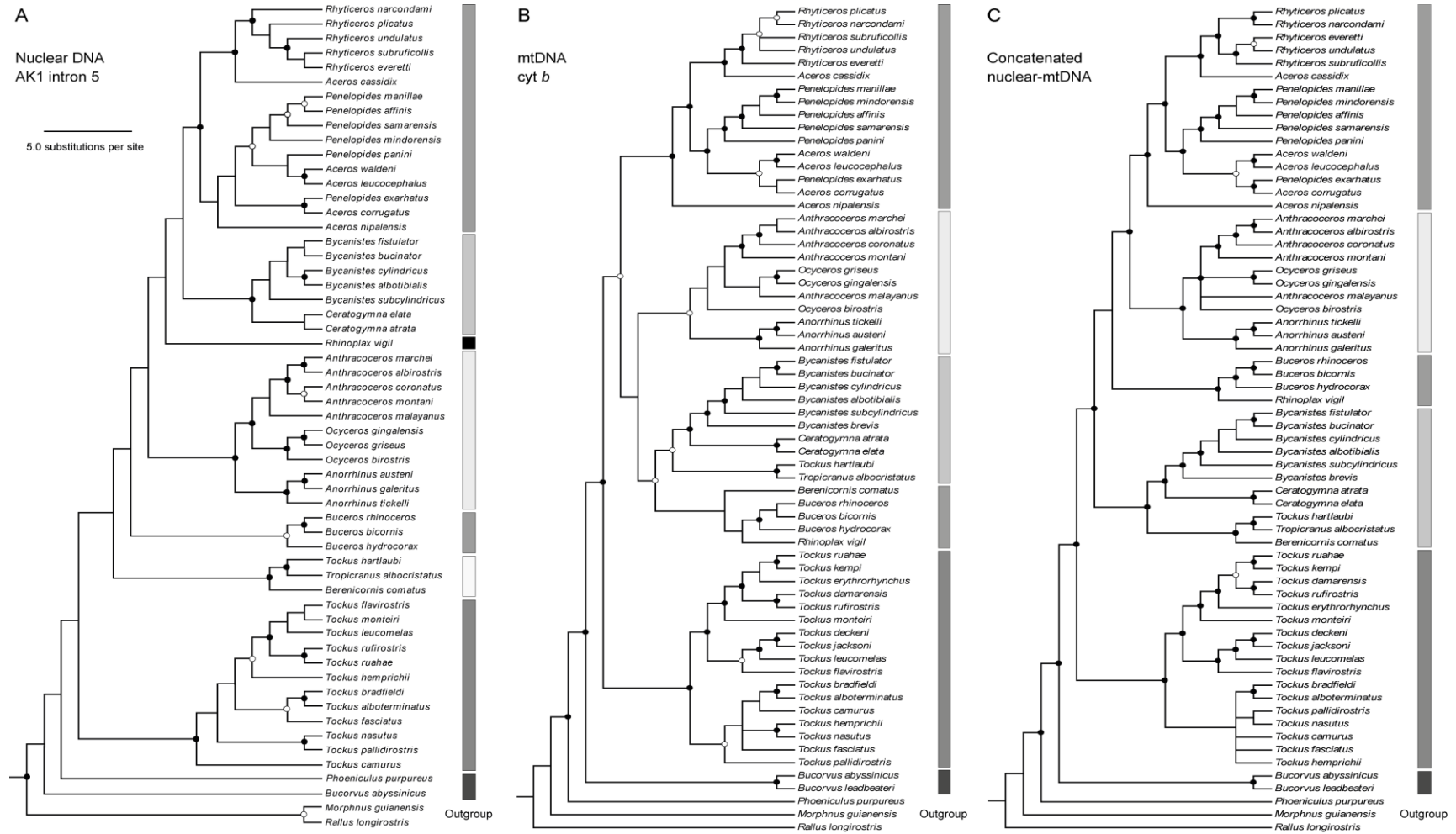


**Figure 2.2:** Bayesian consensus trees of hornbills (100% species coverage) derived from aligned sequences of (A) nuclear loci AK1 intron 5, (B) mtDNA cytb and (C) combined nuclear–mtDNA dataset. Black circles denote nodes with highly supported (>98%) and white circles denote nodes with moderately supported (90-97%) posterior probabilities. Shaded bars represent major clades (to highlight areas of incongruence between analyses, and with the maximum likelihood tree [Fig. 2.3]).

## CHAPTER 2

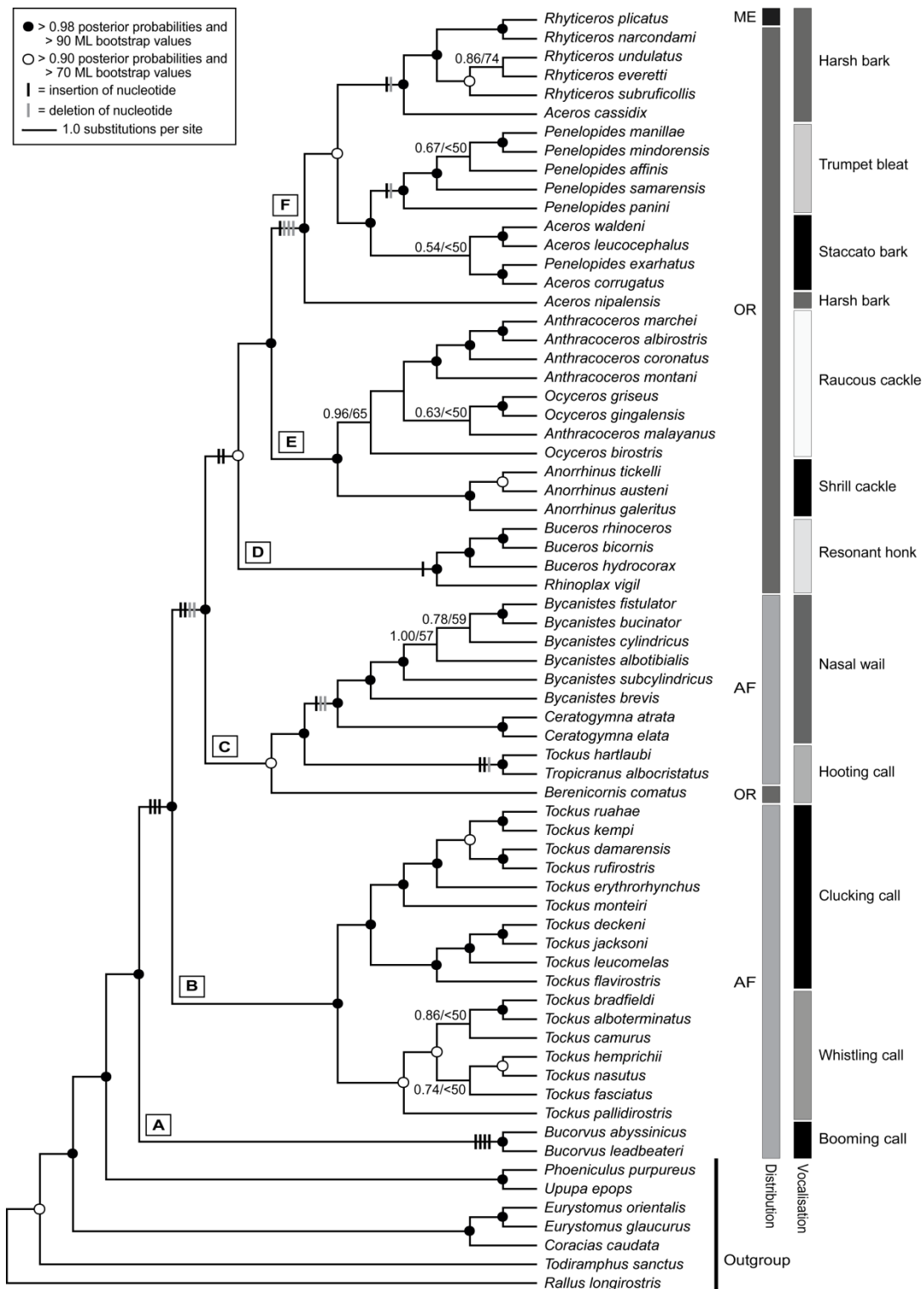


**Figure 2.3:** Maximum likelihood (ML) phylogeny of hornbills (100% species coverage) estimated from concatenated nuclear and mtDNA sequences. Squares indicate major clades: A = *Bucorvus* clade; B = *Tockus* clade; C = *Berenicornis* clade; D = *Rhinoplax* clade; E = *Anorrhinus* clade; F = *Aceros* clade. Vertical slash indicates insertions and deletions for nuclear locus AK1 intron 5. Circles and numerical values at nodes correspond to support values (also included for the Bayesian tree [Fig. 2.2C], as this was highly congruent). Shaded bars on right refer to representative biogeographical regions (ME: Melanesian; OR: Oriental; AF: Afrotropical) and vocalizations (see Appendix A for source of vocal data).

## Appendix A

Taxonomic recommendations, casque design, vocal type, and conservation status of hornbills.

| Clade                   | Taxonomic recommendation <sup>a</sup> | Casque design <sup>b</sup> | Vocalizations  | Status <sup>c</sup> | Sources <sup>d</sup> |
|-------------------------|---------------------------------------|----------------------------|----------------|---------------------|----------------------|
| A - <i>Bucorvus</i>     | <i>Bucorvus abyssinicus</i>           | High cowl-like curve       | Booming call   | LC                  | 1,2,3                |
|                         | <i>Bucorvus leadbeateri</i>           | Low ridge at base          | Booming call   | VU                  | 1,2,3                |
| B - <i>Tockus</i>       | <i>Tockus ruahae</i>                  | Slight ridge               | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus kemp</i>                    | Slight ridge               | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus damarensis</i>              | Slight ridge               | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus rufirostris</i>             | Slight ridge               | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus erythrorhynchus</i>         | Slight ridge               | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus monteiri</i>                | Low grooved ridge          | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus deckeni</i>                 | Low ridge (complete)       | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus jacksoni</i>                | Low ridge (complete)       | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus leucomelas</i>              | Low ridge (complete)       | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Tockus flavirostris</i>            | Low ridge (complete)       | Clucking call  | LC                  | 1,2,3                |
|                         | <i>Rhynchaceros bradfieldi</i>        | Low ridge (attenuated)     | Whistling call | LC                  | 1,2,3                |
|                         | <i>Rhynchaceros alboterminatus</i>    | High ridge (attenuated)    | Whistling call | LC                  | 1,2,3                |
|                         | <i>Rhynchaceros fasciatus</i>         | High ridge (attenuated)    | Whistling call | LC                  | 1,2,3                |
|                         | <i>Rhynchaceros hemprichii</i>        | Low ridge (attenuated)     | Whistling call | LC                  | 1,2,3                |
|                         | <i>Rhynchaceros nasutus</i>           | Low ridge (attenuated)     | Whistling call | LC                  | 1,2,3                |
|                         | <i>Rhynchaceros camurus</i>           | Low ridge (attenuated)     | Whistling call | LC                  | 1,2,3                |
|                         | <i>Rhynchaceros pallidirostris</i>    | Low ridge (attenuated)     | Whistling call | LC                  | 1,2,3                |
| C - <i>Berenicornis</i> | <i>Bycanistes fistulator</i>          | Projecting low cylinder    | Nasal wail     | LC                  | 1,2,3                |
|                         | <i>Bycanistes bucinator</i>           | Projecting low cylinder    | Nasal wail     | LC                  | 1,2,3                |
|                         | <i>Bycanistes cylindricus</i>         | Projecting high cylinder   | Nasal wail     | NT                  | 1,2,3                |
|                         | <i>Bycanistes albotibialis</i>        | Projecting high cylinder   | Nasal wail     | LC                  | 1,2,3                |
|                         | <i>Bycanistes subcylindricus</i>      | Projecting high cylinder   | Nasal wail     | LC                  | 1,2,3                |
|                         | <i>Bycanistes brevis</i>              | Projecting high cylinder   | Nasal wail     | LC                  | 1,2,3                |
|                         | <i>Ceratogymna atrata</i>             | Projecting high cylinder   | Nasal wail     | LC                  | 1,2,3                |
|                         | <i>Ceratogymna elata</i>              | Curved high cylinder       | Nasal wail     | NT                  | 1,2,3                |
|                         | <i>Tropicranus hartlaubi</i>          | Low ridge (attenuated)     | Hooting call   | LC                  | 1,2,3                |
|                         | <i>Tropicranus albocristatus</i>      | High ridge (attenuated)    | Hooting call   | LC                  | 1,2,3                |
|                         | <i>Berenicornis comatus</i>           | High ridge (attenuated)    | Hooting call   | NT                  | 2,3,6                |
| D - <i>Buceros</i>      | <i>Buceros rhinoceros</i>             | Cylindrical block          | Resonant honk  | NT                  | 2,3,6                |
|                         | <i>Buceros bicornis</i>               | Bifurcated block           | Resonant honk  | NT                  | 2,3,5,6              |
|                         | <i>Buceros hydrocorax</i>             | Pointed block              | Resonant honk  | NT                  | 2,3,4                |
|                         | <i>Rhinoplax vigil</i>                | Broad ridged block         | Resonant honk  | NT                  | 2,3,6                |
| E - <i>Anorrhinus</i>   | <i>Anthracoceros marchei</i>          | Cylinder with blade        | Raucous cackle | VU                  | 2,3,4                |
|                         | <i>Anthracoceros albirostris</i>      | Cylinder with blade        | Raucous cackle | LC                  | 2,3,5,6              |
|                         | <i>Anthracoceros coronatus</i>        | Cylinder with blade        | Raucous cackle | NT                  | 2,3,5                |
|                         | <i>Anthracoceros montani</i>          | Cylinder with blade        | Raucous cackle | CR                  | 2,3,4                |
|                         | <i>Anthracoceros malayanus</i>        | Cylinder with blade        | Raucous cackle | NT                  | 2,3,6                |
|                         | <i>Ocyrceros griseus</i>              | Low ridge                  | Raucous cackle | LC                  | 2,3,5                |

## CHAPTER 2

|                   |                                   |                     |                |    |         |
|-------------------|-----------------------------------|---------------------|----------------|----|---------|
| F - <i>Aceros</i> | <i>Ocyceros gingalensis</i>       | Low ridge           | Raucous cackle | LC | 2,3,5   |
|                   | <i>Ocyceros birostris</i>         | Pointed blade       | Raucous cackle | LC | 2,3,5   |
|                   | <i>Anorrhinus tickelli</i>        | Low ridge           | Shrill cackle  | NT | 2,3,6   |
|                   | <i>Anorrhinus austeni</i>         | Low ridge           | Shrill cackle  | NT | 2,3,5,6 |
|                   | <i>Anorrhinus galeritus</i>       | Low ridge           | Shrill cackle  | LC | 2,3,6   |
|                   | <i>Aceros nipalensis</i>          | Low ridge           | Harsh bark     | VU | 2,3,5,6 |
|                   | <i>Rhyticeros plicatus</i>        | Low wreath          | Harsh bark     | LC | 2,3,6   |
|                   | <i>Rhyticeros narcondami</i>      | Low wreath          | Harsh bark     | EN | 2,3,5   |
|                   | <i>Rhyticeros undulatus</i>       | Low wreath          | Harsh bark     | LC | 2,3,5,6 |
|                   | <i>Rhyticeros everetti</i>        | Low wreath          | Harsh bark     | VU | 2,3,6   |
|                   | <i>Rhyticeros subruficollis</i>   | Low wreath          | Harsh bark     | VU | 2,3,6   |
|                   | <i>Rhyticeros cassidix</i>        | High wrinkled knob  | Harsh bark     | LC | 2,3,6   |
|                   | <i>Cranobrontes waldeni</i>       | High wrinkled ridge | Staccato bark  | CR | 2,3,4   |
|                   | <i>Cranobrontes leucocephalus</i> | High wrinkled ridge | Staccato bark  | NT | 2,3,4   |
|                   | <i>Cranobrontes exarhatus</i>     | Low grooved ridge   | Staccato bark  | LC | 2,3,6   |
|                   | <i>Cranobrontes corrugatus</i>    | High wrinkled ridge | Staccato bark  | NT | 2,3,6   |
|                   | <i>Penelopides manillae</i>       | Low half ridge      | Trumpet bleat  | LC | 2,3,4   |
|                   | <i>Penelopides mindorensis</i>    | Low half ridge      | Trumpet bleat  | EN | 2,3,4   |
|                   | <i>Penelopides affinis</i>        | Low half ridge      | Trumpet bleat  | LC | 2,3,4   |
|                   | <i>Penelopides samarensis</i>     | Low half ridge      | Trumpet bleat  | LC | 2,3,4   |
|                   | <i>Penelopides panini</i>         | Low half ridge      | Trumpet bleat  | EN | 2,3,4   |

<sup>a</sup>Taxonomic treatment recommended on the basis of new data present in this paper, to compare with traditional hypotheses (Table 2.1).

<sup>b</sup>Descriptions of the distinctive casque situated on top of hornbill beaks described on basis of key literature.

<sup>c</sup>Conservation status of hornbills according to the IUCN Red List assessments: LC, Least Concern; VU, Vulnerable; EN, Endangered; CR, Critically Endangered; assignment to categories follows BirdLife International (2000), apart from *Bucorvus leadbeateri* which was recently upgraded from LC to VU.

<sup>d</sup>Sources: 1, Fry et al., 1998; 2, Kemp, 1995; 3, Kemp, 2001; 4, Kennedy et al., 2000; 5, Rasmussen and Anderton, 2005; 6, Robson, 2009.

## Appendix B

List of 171 samples used in this study, representing 61 hornbill (in-group) species and 7 out-group species, with details of museum registry or source material, geographic origin, gene regions, and GenBank accession numbers.

| Taxon                       | Institutional source <sup>a</sup> and sample number | Type <sup>b</sup> | Locality                     | Mitochondrial coding genes: cytochrome <i>b</i> | Nuclear intron loci: adenylate kinase 1 intron 5 |
|-----------------------------|-----------------------------------------------------|-------------------|------------------------------|-------------------------------------------------|--------------------------------------------------|
| In-group                    |                                                     |                   |                              |                                                 |                                                  |
| <i>Aceros cassidix</i>      | BMNH 1969.32.18                                     | S                 | Indonesia, Sulawesi          | XXX <sup>d</sup>                                | XXX <sup>d</sup>                                 |
|                             | BMNH 88.10.30.140                                   | S                 | Indonesia, Sulawesi          | XXX                                             | -                                                |
|                             | UMZC 25/Buc/1/a/1                                   | S                 | Indonesia, Sulawesi          | XXX                                             | -                                                |
| <i>Aceros corrugatus</i>    | OUMNH B05362                                        | S                 | Malaysia, Borneo, Sarawak    | XXX <sup>d</sup>                                | XXX <sup>d</sup>                                 |
|                             | NUS 3.11111                                         | S                 | Indonesia, Sumatra           | XXX                                             | -                                                |
|                             | NEZS ACA1                                           | F                 | United Kingdom, Captive bird | XXX                                             | -                                                |
| <i>Aceros leucocephalus</i> | LWPRC P45                                           | F                 | Philippines, Captive bird    | XXX <sup>d</sup>                                | -                                                |
|                             | OPAV P08                                            | F                 | Philippines, Captive bird    | -                                               | XXX <sup>d</sup>                                 |
|                             | MSUIL 56487                                         | S                 | Philippines, Dinagat         | XXX                                             | -                                                |
|                             | NFEFI P05                                           | F                 | Philippines, Captive bird    | XXX                                             | -                                                |
| <i>Aceros nipalensis</i>    | BMNH 1941.12.1.827                                  | S                 | Myanmar, Hmu-Chanka          | XXX <sup>d</sup>                                | XXX <sup>d</sup>                                 |
|                             | UMZC 25/Buc/1/f/1                                   | S                 | India, Darjeeling            | XXX                                             | -                                                |
|                             | BMNH 87.9.1.202                                     | S                 | India, Sikkim                | XXX                                             | -                                                |
| <i>Aceros waldeni</i>       | UPLB 2103                                           | S                 | Philippines, Negros          | XXX <sup>d</sup>                                | -                                                |
|                             | WVSU P19                                            | F                 | Philippines, Panay           | XXX                                             | XXX <sup>d</sup>                                 |
|                             | BMNH 96.4.15.98                                     | S                 | Philippines, Guimaras        | XXX                                             | -                                                |
| <i>Anorrhinus austeni</i>   | BMNH 1904.7.24.1                                    | S                 | India, Assam                 | XXX <sup>d</sup>                                | -                                                |

## CHAPTER 2

|                                  |                      |   |                              |                  |                  |
|----------------------------------|----------------------|---|------------------------------|------------------|------------------|
|                                  | BMNH 1932.5.14.31    | S | Laos, Phou-Kong-Ntoul        | XXX              | XXX <sup>d</sup> |
| <i>Anorrhinus galeritus</i>      | OUMNH B05356         | S | Malaysia, Borneo, Sabah      | XXX <sup>d</sup> | -                |
|                                  | UMZC 25/Buc/2/a/6    | S | Malaysia, Borneo, Sarawak    | XXX              | XXX <sup>d</sup> |
|                                  | OUMNH B05359         | S | Malaysia, Borneo, Sarawak    | XXX              | -                |
| <i>Anorrhinus tickelli</i>       | BMNH 1924.12.22.202  | S | Thailand, Sawan              | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                  | BMNH 83.4.54         | S | Myanmar, Tenasserim          | XXX              | -                |
|                                  | GenBank <sup>c</sup> |   | Thailand                     | GU257907         | -                |
| <i>Anthracoceros albirostris</i> | BMNH 1949.25.878     | S | India, Doon Valley           | XXX <sup>d</sup> | -                |
|                                  | OUMNH B05374         | S | Malaysia, Pahang             | XXX              | XXX <sup>d</sup> |
|                                  | OUMNH B05375         | S | Malaysia, Borneo, Sabah      | XXX              | -                |
| <i>Anthracoceros coronatus</i>   | BMNH 1948.57.16      | S | Sri Lanka, Uva Province      | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                  | BMNH 1926.12.23.1494 | S | India, Karnataka             | XXX              | -                |
|                                  | OUMNH B05371         | S | India, Hindostan             | XXX              | -                |
| <i>Anthracoceros malayanus</i>   | BMNH 1921.10.24.1    | S | Indonesia, Sumatra           | XXX <sup>d</sup> | -                |
|                                  | ZSL AMA1             | F | United Kingdom, Captive bird | XXX              | XXX <sup>d</sup> |
|                                  | OUMNH B05370         | S | Malaysia, Borneo, Sarawak    | XXX              | -                |
| <i>Anthracoceros montani</i>     | AMNH 802255          | S | Philippines, Tawi-Tawi       | XXX <sup>d</sup> | -                |
|                                  | DMNH 27721           | S | Philippines, Batu-batu       | XXX              | XXX <sup>d</sup> |
|                                  | MSUM Sulu            | S | Philippines, Sanga-sanga     | XXX              | -                |
| <i>Anthracoceros marchei</i>     | MGR PHB1             | F | Philippines, Captive bird    | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                  | NMP 014884           | S | Philippines, Calamianes      | XXX              | -                |
|                                  | DMNH 37064           | S | Philippines, Balabac         | XXX              | -                |
| <i>Berenicornis comatus</i>      | BMNH 1935.10.22.163  | S | Malaysia, Borneo, Sarawak    | XXX <sup>d</sup> | -                |
|                                  | BMNH 1882.7.24.12    | S | Indonesia, Sumatra           | XXX              | XXX <sup>d</sup> |
|                                  | AMNH 644968          | S | Indonesia, Sumatra           | XXX              | -                |

## CHAPTER 2

|                                  |                      |   |                              |                  |                  |
|----------------------------------|----------------------|---|------------------------------|------------------|------------------|
| <i>Buceros bicornis</i>          | BMNH 1925.12.23.1493 | S | India, Karnataka             | XXX <sup>d</sup> | -                |
|                                  | NEZS BBA1            | F | United Kingdom, Captive bird | XXX              | XXX <sup>d</sup> |
|                                  | NUS 3.11132          | S | Indonesia, Sumatra           | XXX              | -                |
| <i>Buceros hydrocorax</i>        | PAWB P47             | F | Philippines, Captive bird    | XXX <sup>d</sup> | -                |
|                                  | SMNP BHSA1           | F | Philippines, Luzon           | XXX              | XXX <sup>d</sup> |
|                                  | UPD 043              | S | Philippines, Luzon           | XXX              | -                |
| <i>Buceros rhinoceros</i>        | NEZS BRA1            | F | United Kingdom, Captive bird | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                  | AMNH 122436          | S | Indonesia, Java              | XXX              | -                |
|                                  | BMNH 88.10.30.207    | S | Indonesia, Sumatra           | XXX              | -                |
| <i>Bucorvus abyssinicus</i>      | ZSL BAB1             | F | United Kingdom, Captive bird | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                  | ZSL BAA2             | F | United Kingdom, Captive bird | XXX              | -                |
| <i>Bucorvus leadbeateri</i>      | BMNH 1932.5.5.356    | S | Botswana, Mochaba river      | XXX <sup>d</sup> | -                |
|                                  | GenBank <sup>c</sup> | - | -                            | NC015199         | -                |
| <i>Bycanistes albotibialis</i>   | BMNH 1951.34.661     | S | Nigeria, Kumba River         | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                  | OUMNH B016444        | S | Cameroon                     | XXX              | -                |
| <i>Bycanistes brevis</i>         | BMNH 1942.12.1.3     | S | Ethiopia, Dilla              | XXX <sup>d</sup> | -                |
|                                  | GenBank <sup>c</sup> |   | Germany, Captive bird        | AF346915         | -                |
|                                  | GenBank <sup>c</sup> | - | -                            | NC015201         | -                |
| <i>Bycanistes bucinator</i>      | UMZC 25/Buc/7/b/1    | S | Zambia, Serenje              | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                  | GenBank <sup>c</sup> |   | Germany, Captive bird        | AF346920         | -                |
| <i>Bycanistes cylindricus</i>    | BMNH 1977.20.473     | S | Liberia, Mt Nimba            | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                  | BMNH 1934.3.16.68    | S | Ghana, Ashanti               | XXX              | -                |
| <i>Bycanistes fistulator</i>     | BMNH 1948.22.10      | S | Nigeria, Yankari             | XXX <sup>d</sup> | -                |
|                                  | BMNH 1926.8.8.163    | S | Cameroon, North Yaounde,     | XXX              | XXX <sup>d</sup> |
| <i>Bycanistes subcylindricus</i> | BMNH 1955.59.1195    | S | Nigeria, Ahoada              | XXX <sup>d</sup> | XXX <sup>d</sup> |

## CHAPTER 2

|                                |                       |   |                              |                  |                  |
|--------------------------------|-----------------------|---|------------------------------|------------------|------------------|
|                                | BMNH 1939.10.2.32     | S | Sudan, Iloma plateau         | XXX              | -                |
|                                | GenBank <sup>c</sup>  |   | Germany, Captive bird        | AF346924         | -                |
| <i>Ceratogymna atrata</i>      | OUMNH B16445          | S | Cameroon                     | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                | GenBank <sup>c</sup>  |   | Germany, Captive bird        | AF346912         | -                |
| <i>Ceratogymna elata</i>       | BMNH 1934.3.16.66     | S | Ghana, Ashanti               | XXX <sup>d</sup> | -                |
|                                | BMNH 1951.34.663      | S | Nigeria, Kumba River         | XXX              | XXX <sup>d</sup> |
| <i>Ocyrceros birostris</i>     | BMNH S/2002.22.1      | T | United Kingdom, Captive bird | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                | AMNH 257649           | S | India, Bombay                | XXX              | -                |
|                                | OUMNH B05558          | S | India, Saharimpur            | XXX              | -                |
| <i>Ocyrceros gingalensis</i>   | BMNH 1940.12.3.159    | S | Sri Lanka, Uva Province      | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                | BMNH 1940.12.3.162    | S | Sri Lanka, Uva Province      | XXX              | -                |
| <i>Ocyrceros griseus</i>       | BMNH 1949.WhI.1.16521 | S | India, Karnataka             | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                | AMNH 344282           | S | India, Mysore                | XXX              | -                |
|                                | UMZC 25 Buc/12/i/1    | S | India, Madras                | XXX              | -                |
| <i>Penelopides affinis</i>     | UPLB 2081             | S | Philippines, Mindanao        | XXX <sup>d</sup> | -                |
|                                | PAWB WA0010           | F | Philippines, Captive bird    | XXX              | XXX <sup>d</sup> |
|                                | MSUIL 53258           | S | Philippines, Mindanao        | XXX              | -                |
| <i>Penelopides exarhatus</i>   | BMNH 1888.10.30.146   | S | Indonesia, Sulawesi          | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                | BMNH Y3.5.12.2423     | S | Indonesia, Sulawesi          | XXX              | -                |
|                                | UMZC 25 Buc/9/a/1     | S | Indonesia, Sulawesi          | XXX              | -                |
| <i>Penelopides manillae</i>    | SMNP PMSA1            | F | Philippines, Luzon           | XXX <sup>d</sup> | -                |
|                                | SMNP PMC01FT          | F | Philippines, Luzon           | XXX              | XXX <sup>d</sup> |
|                                | DMNH 70334            | S | Philippines, Palau           | XXX              | -                |
| <i>Penelopides mindorensis</i> | CWRC P12              | F | Philippines, Mindoro         | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                | NMP 0-9600            | S | Philippines, Mindoro         | XXX              | -                |



## CHAPTER 2

|                                 |                      |   |                           |                  |                  |
|---------------------------------|----------------------|---|---------------------------|------------------|------------------|
|                                 | UPD PSO-402          | S | Philippines, Mindoro      | XXX              | -                |
| <i>Penelopides panini</i>       | BMNH 1896.4.15.70    | S | Philippines, Masbate      | XXX <sup>d</sup> | -                |
|                                 | WVSU P25             | F | Philippines, Panay        | -                | XXX <sup>d</sup> |
|                                 | NMP 0-06363          | S | Philippines, Negros       | XXX              | -                |
|                                 | UMZC 25 Buc/9/b/1    | S | Philippines, Guimaras     | XXX              | -                |
| <i>Penelopides samarensis</i>   | BMNH 673.97.5.13.492 | S | Philippines, Samar        | XXX <sup>d</sup> | -                |
|                                 | SBAV P01             | F | Philippines, Leyte        | -                | XXX <sup>d</sup> |
|                                 | NMP 0-12255          | S | Philippines, Samar        | XXX              | -                |
|                                 | UPLB 2073            | S | Philippines, Leyte        | XXX              | -                |
| <i>Rhinoplax vigil</i>          | OUMNH B05388         | S | Malaysia, Borneo, Sarawak | XXX <sup>d</sup> | -                |
|                                 | OUMNH B05387         | S | Malaysia, Borneo, Sarawak | XXX              | XXX <sup>d</sup> |
|                                 | NUS 3.11140          | S | Malaysia, Pahang          | XXX              | -                |
| <i>Rhyticeros everetti</i>      | BMNH 1898.12-8.71    | S | Indonesia, Sumba          | XXX <sup>d</sup> | XXX <sup>d</sup> |
|                                 | AMNH 346740          | S | Indonesia, Sumba          | XXX              | -                |
|                                 | AMNH 346744          | S | Indonesia, Sumba          | XXX              | -                |
| <i>Rhyticeros narcondami</i>    | UMZC 25/Buc/1/e/1    | S | India, Narcondam          | XXX <sup>d</sup> | -                |
|                                 | BMNH 1920.10.29.1    | S | India, Narcondam          | XXX              | XXX <sup>d</sup> |
|                                 | BMNH 1920.10.29.2    | S | India, Narcondam          | XXX              | -                |
| <i>Rhyticeros plicatus</i>      | BMNH 1911.12.20.891  | S | Indonesia, West Papua     | XXX <sup>d</sup> | -                |
|                                 | OPAV P13             | F | Philippines, Captive bird | XXX              | XXX <sup>d</sup> |
|                                 | AMNH 300745          | S | Indonesia, Waigen         | XXX              | -                |
| <i>Rhyticeros subruficollis</i> | BMNH 1888.10.30.119  | S | Myanmar, Tonghoo          | XXX <sup>d</sup> | -                |
|                                 | OUMNH B05366         | S | Malaysia, Borneo, Sarawak | -                | XXX <sup>d</sup> |
|                                 | BMNH 1887.9.1.243    | S | Myanmar, Mandalay         | XXX              | -                |
|                                 | GenBank <sup>c</sup> |   | Thailand                  | GU257914         | -                |

## CHAPTER 2

|                               |                      |   |                              |                       |                  |
|-------------------------------|----------------------|---|------------------------------|-----------------------|------------------|
| <i>Rhyticeros undulatus</i>   | BMNH 1887.9.1.224    | S | India, Manipur               | XXX <sup>d</sup>      | -                |
|                               | BMNH 1857.6.10.7     | S | Indonesia, Java              | XXX                   | XXX <sup>d</sup> |
|                               | OUMNH B05366         | S | Malaysia, Borneo, Sarawak    | XXX                   | -                |
| <i>Tockus alboterminatus</i>  | OUMNH B16328         | S | Kenya                        | XXX                   | -                |
|                               | UMZC 25/Buc/12/a/3   | S | Zimbabwe, Umtali             | XXX                   | XXX <sup>d</sup> |
|                               | OUMNH B02061         | S | South Africa                 | XXX <sup>d</sup>      | -                |
| <i>Tockus bradfieldi</i>      | BMNH 1950.50190      | S | Botswana, Ngamiland          | XXX <sup>d</sup>      | -                |
|                               | BMNH 1944.10.26.7    | S | Botswana, Ngamiland          | XXX                   | XXX <sup>d</sup> |
| <i>Tockus camurus</i>         | BMNH 1977.20.3088    | S | Liberia, Mt Nimba            | XXX <sup>d</sup>      | XXX <sup>d</sup> |
|                               | BMNH 1951.34.260     | S | Cameroon, Kumba              | XXX                   | -                |
| <i>Tockus damarensis</i>      | GenBank <sup>c</sup> |   | Namibia, Damaraland          | AY054443 <sup>d</sup> | -                |
|                               | GenBank <sup>c</sup> |   | Namibia, Damaraland          | AY054443              | -                |
| <i>Tockus deckeni</i>         | BMNH 1940.1.12       | S | Tanzania, Shinyanga          | XXX <sup>d</sup>      | -                |
|                               | GenBank <sup>c</sup> |   | Germany, Captive bird        | AF345931              | -                |
| <i>Tockus erythrorhynchus</i> | ZSL TE1              | F | United Kingdom, Captive bird | XXX <sup>d</sup>      | -                |
|                               | ZSL TE2              | F | United Kingdom, Captive bird | XXX                   | -                |
| <i>Tockus fasciatus</i>       | OUMNH B16442         | S | Cameroon                     | XXX <sup>d</sup>      | XXX <sup>d</sup> |
|                               | OUMNH B16441         | S | Cameroon                     | XXX                   | -                |
|                               | BMNH 1977.20.451     | S | Liberia, Mt Nimba            | XXX                   | -                |
| <i>Tockus flavirostris</i>    | BMNH 1923.8.7.5793   | S | Somalia, Waisangli           | XXX <sup>d</sup>      | -                |
|                               | BMNH 1945.40.56      | S | Sudan, East district         | XXX                   | XXX <sup>d</sup> |
| <i>Tockus hartlaubi</i>       | BMNH 1977.20.460     | S | Liberia, Mt Nimba            | XXX <sup>d</sup>      | XXX <sup>d</sup> |
|                               | BMNH 1977.20.456     | S | Liberia, Mt Nimba            | XXX                   | -                |
|                               | BMNH 1975.6.2        | S | Uganda, Ituri                | XXX                   | -                |
| <i>Tockus hemprichii</i>      | BMNH 1946.5.1288     | S | Ethiopia, Yavello            | XXX <sup>d</sup>      | -                |

## CHAPTER 2

|                                  |                      |   |                               |                       |                  |
|----------------------------------|----------------------|---|-------------------------------|-----------------------|------------------|
|                                  | BMNH 1939.10.1.453   | S | Sudan, Didinga hills          | XXX                   | XXX <sup>d</sup> |
| <i>Tockus jacksoni</i>           | BMNH 1971.16.16      | S | Kenya, Kangetet               | XXX <sup>d</sup>      | -                |
|                                  | GenBank <sup>c</sup> |   | Germany, Captive bird         | XXX                   | -                |
| <i>Tockus kemp</i>               | GenBank <sup>c</sup> |   | Gambia                        | AY423372 <sup>d</sup> | -                |
| <i>Tockus leucomelas</i>         | OUMNH B18556         | S | Zimbabwe                      | XXX <sup>d</sup>      | XXX <sup>d</sup> |
|                                  | BMNH 1911.12.18.176  | S | Angola, Marro da Cruz         | XXX                   | -                |
|                                  | UMZC 25 Buc/12/h/1   | S | Zimbabwe, Sabi Valley         | XXX                   | -                |
| <i>Tockus monteiri</i>           | BMNH 1957.35.43      | S | Angola, Sa de Bandeira        | XXX <sup>d</sup>      | -                |
|                                  | BMNH 1950.50.188     | S | Namibia, Damaraland           | XXX                   | XXX <sup>d</sup> |
| <i>Tockus nasutus</i>            | OUMNH B18098         | S | Tanzania                      | XXX <sup>d</sup>      | -                |
|                                  | UMZC 25/Buc/12/n/2   | S | Zimbabwe, Nuanetsi            | XXX                   | XXX <sup>d</sup> |
|                                  | UMZC 25/Buc/12/n/1   | S | Namibia, Damaraland           | XXX                   | -                |
| <i>Tockus pallidirostris</i>     | BMNH 1957.35.42      | S | Angola, Teixeira de Sousa     | XXX <sup>d</sup>      | -                |
|                                  | UMZC 25/Buc/12/o/1   | S | Zambia, Kalulushi             | XXX                   | XXX <sup>d</sup> |
| <i>Tockus ruahae</i>             | OUMNH B17998         | S | Tanzania, Serengeti           | XXX <sup>d</sup>      | XXX <sup>d</sup> |
|                                  | OUMNH B17997         | S | Tanzania, Serengeti           | XXX                   | -                |
|                                  | GenBank <sup>c</sup> |   | Tanzania, Ruaha National Park | AY423370              | -                |
| <i>Tockus rufirostris</i>        | UMZC 25/Buc/12/f/3   | S | Malawi, Hewe River            | XXX <sup>d</sup>      | XXX <sup>d</sup> |
|                                  | GenBank <sup>c</sup> |   | Zimbabwe                      | AF082071              | -                |
| <i>Tropicranus albocristatus</i> | BMNH 1977.20.3087    | S | Liberia, Mt Nimba             | XXX <sup>d</sup>      | XXX <sup>d</sup> |
|                                  | OUMNH B16443         | S | Cameroon                      | XXX                   | -                |
|                                  | BMNH 1935.7.17.23    | S | Ghana, Ashanti                | XXX                   | -                |
| <i>Out-group</i>                 |                      |   |                               |                       |                  |
| <i>Phoeniculus purpureus</i>     | NEZS PP1             | F | United Kingdom, Captive bird  | XXX <sup>d</sup>      | XXX <sup>d</sup> |
|                                  | GenBank <sup>c</sup> | - | -                             | EU167012              | -                |

## CHAPTER 2

|                              |                      |                             |                       |                       |
|------------------------------|----------------------|-----------------------------|-----------------------|-----------------------|
| <i>Upupa epops</i>           | GenBank <sup>c</sup> | -                           | EU167030 <sup>d</sup> | -                     |
|                              | GenBank <sup>c</sup> | United States, Captive bird | U89189                | -                     |
| <i>Eurystomus orientalis</i> | GenBank <sup>c</sup> | -                           | EU344978 <sup>d</sup> | -                     |
| <i>Eurystomus glaucurus</i>  | GenBank <sup>c</sup> | -                           | AF407452 <sup>d</sup> | -                     |
| <i>Coracias caudatus</i>     | GenBank <sup>c</sup> | United States, Captive bird | CCU89184 <sup>d</sup> | -                     |
| <i>Todiramphus sanctus</i>   | GenBank <sup>c</sup> | New Zealand                 | EU410189 <sup>d</sup> | -                     |
| <i>Morphnus guianensis</i>   | GenBank <sup>c</sup> | Peru, Venezuela             | AY987269 <sup>d</sup> | AJ601481 <sup>d</sup> |
| <i>Rallus longirostris</i>   | GenBank <sup>c</sup> | North America               | DQ485908 <sup>d</sup> | AF307901 <sup>d</sup> |

<sup>a</sup>Institutional sources of samples: AMNH: American Museum of Natural History, New York, USA; BMNH: Natural History Museum, Tring, UK; CWRC: Calintaan Wildlife Rescue Centre, Occidental Mindoro, Philippines; DMNH: Delaware Museum of Natural History, Delaware, USA; LWPRC: Laguna Wildlife Park and Rescue Centre, Laguna, Philippines; MGR: Malagos Garden Resort, Davao City, Philippines; MSUIL: Mindanao State University Natural Science Museum, Iligan City, Philippines; MSUM: Mindanao State University Aga Khan Museum, Marawi City, Philippines; NFEFI: Negros Forests and Ecological Foundation Inc. Biodiversity Conservation Centre, Bacolod City, Philippines; NEZS: North of England Zoological Society, Cheshire, UK; NMP: National Museum of the Philippines, Manila, Philippines; SMNP: Northern Sierra Madre Natural Park, Isabela, Philippines; NUS: National University of Singapore, Raffles Museum Zoological Research Collection, Singapore; OPAV: Opol Aviary, Misamis Oriental, Philippines; OUMNH: Oxford University Museum of Natural History, Oxford, UK; PAWB: Protected Areas and Wildlife Bureau Wildlife Rescue Centre, Manila, Philippines; SHAV: Sabin Hotel Aviary, Ormoc, Leyte, Philippines; UMZC: Cambridge University Museum of Zoology, Cambridge, UK; UPD: University of the Philippines Diliman Institute of Biology Collection, Quezon City, Philippines; UPLB: University of the Philippines Los Baños Museum of Natural History, Laguna, Philippines; WVSU: West Visayas State University Mariit Wildlife Conservation Park, Iloilo, Philippines; ZSL: Zoological Society of London, London, UK.

<sup>b</sup>Sample types for sequences generated in this study: F = feather sample; T = preserved muscle tissue; S = skin shavings or toe-pad sample from museum skin.

<sup>c</sup>Genbank sequences derived from published phylogenetic analyses: Brown et al., 2008; Espinosa de los Monteros, 2000; Fain et al., 2007; Helbig et al., 2005; Hübner et al., 2003; Kemp and Delport, 2002; Kirchman et al., 2001; Lerner and Mindell 2005; Pacheco et al., 2011; Pratt et al., 2009; Shapiro and Dumbacher, 2001; Viseshakul et al., 2011.

<sup>d</sup>Sequences indicated were selected to form a reduced sequence matrix to simplify analyses and to construct the final phylogenetic trees.

## Supplementary information

**Table S2.1:** List of primers used for PCR amplification and sequencing.

| Gene region <sup>a</sup> | Primer name <sup>b</sup>       | Sequence                       | Location /Tm <sup>c</sup> |
|--------------------------|--------------------------------|--------------------------------|---------------------------|
| Nuclear DNA              | AvianAK5b+F                    | 5'-ATTGACGGCTACCCTCGCGAGGTG-3' | External / 67.8           |
| AK1 intron 5             | AvianAK5c-R                    | 5'-CACCCGCCCCGCTGGTCTCTCC-3'   | External / 69.6           |
|                          | <i>Buceros</i> AK5b-for        | 5'-TCTCTCCAGGATGTCTGTGG-3'     | Internal / 59.4           |
|                          | <i>Buceros</i> AK5a-rev        | 5'-ATCACCAGTGCGCCAGTACT-3'     | Internal / 59.4           |
|                          | <i>Buceros</i> AK5b-R          | 5'-TTTCACCATCGTCTCCTTCC-3'     | Internal / 57.3           |
| mtDNA <i>cytb</i>        | <i>Tockus</i> CytbLT-F         | 5'-GCCCTGTCCATCCTAATCAC-3'     | External / 59.4           |
|                          | <i>Tockus</i> CytbLT-R         | 5'-CGACGGATGAGAAAGCTAGG-3'     | Internal / 59.4           |
|                          | <i>Aceros</i> CytbI-F          | 5'-ACATCTCCGCCTGATGAAAC-3'     | Internal / 57.3           |
|                          | <i>Aceros</i> CytbI-R          | 5'-GTGGGTTGTTTGAGCCTGAT-3'     | Internal / 57.3           |
|                          | <i>Anthracoceros</i> CytbI-R   | 5'-GGTAGGACATAGCCCACGAA-3'     | Internal / 59.4           |
|                          | <i>Anthracoceros</i> CytbII-F  | 5'-GGGCATCATCCTCCTACTCA-3'     | Internal / 59.4           |
|                          | <i>Anthracoceros</i> CytbII-R  | 5'-GTGGGTTGTTTGAGCCTGAT-3'     | Internal / 59.4           |
|                          | <i>Aceros</i> CytbII-F         | 5'-ACACTTCTCCTCCCGTTCA-3'      | Internal / 57.3           |
|                          | <i>Aceros</i> CytbII-R         | 5'-TCATTATCGGCCAACTAGCC-3'     | Internal / 57.3           |
|                          | <i>Anthracoceros</i> CytbIII-F | 5'-GCCCTACACTTTCTCCTCCC-3'     | Internal / 61.4           |
|                          | <i>Anthracoceros</i> CytbIII-R | 5'-TATTGGGGATTGAGCGTAGG-3'     | Internal / 57.3           |
|                          | <i>Anthracoceros</i> CytbIV-F  | 5'-AACTTCACACCAGCAAACCC-3'     | Internal / 57.3           |
|                          | <i>Tockus</i> CytbRT-F         | 5'-GCCCCCTCTCTCAAATCCTA-3'     | Internal / 59.4           |
|                          | <i>Tockus</i> CytbRT-R         | 5'-GGTTGAGGTCTTCAGTCTTTGG-3'   | External / 60.3           |

<sup>a</sup>AK1 intron 5 (AK5 in primer names): cytosolic adenylate kinase 1 intron 5 gene (500-700bp); *cytb* (Cytb in primer names): cytochrome *b* gene (1143bp),

<sup>b</sup>Primers were designed from the following GenBank sequences: *Buceros*AK5 (XXX, this study); *Tockus*Cytb (AF082071, Mindell et al., 1998), *Aceros*Cytb (AF346946, Hübner et al., 2003), and *Anthracoceros*Cytb (U89190, van Tuinen et al., 2000); New primers developed using the online program PRIMER3 v.04.0 (Rozen & Skaletsky 1998); AvianAK5 from Shapiro and Dumbacher (2001).

<sup>c</sup>Tm: melting temperature of primer sequence.

## CHAPTER 2

**Table S2.2:** Sequence characters of nuclear, mtDNA and concatenated genes for 61 in-group Bucerotidae and five out-group taxa.

|                | Conserved |       | Variable |       | Parsimony   |       |             |
|----------------|-----------|-------|----------|-------|-------------|-------|-------------|
| In-group only  | sites     | C%    | sites    | V%    | informative | Pi%   | Total sites |
| AK1 intron 5   | 288       | 47.37 | 319      | 52.47 | 188         | 30.92 | 608         |
| <i>cytb</i>    | 395       | 34.56 | 748      | 65.44 | 551         | 48.21 | 1143        |
| Concatenated   | 663       | 37.89 | 1087     | 62.11 | 745         | 42.57 | 1750        |
| Average        |           | 39.94 |          | 60.01 |             | 40.57 |             |
| with Out-group |           |       |          |       |             |       |             |
| AK1 intron 5   | 191       | 27.17 | 433      | 61.59 | 231         | 32.86 | 703         |
| <i>cytb</i>    | 357       | 31.23 | 786      | 68.77 | 606         | 53.02 | 1143        |
| Concatenated   | 545       | 29.52 | 1219     | 66.04 | 837         | 45.34 | 1846        |
| Average        |           | 29.31 |          | 65.47 |             | 43.74 |             |

Gene regions used, AK1 intron 5: nuclear loci cytosolic adenylate kinase 1 intron 5 gene; *cytb*: mtDNA cytochrome *b* gene;

**Table S2.3:** Summary of alignment features and models of sequence evolution for three datasets (Nuclear DNA AK1 intron 5; mtDNA *cytb*; Concatenated AK1 intron 5-*cytb*).

| Gene region <sup>a</sup>                  | Size    | Model<br>Likelihood | AICc <sup>c</sup> | BIC <sup>c</sup> | <i>lnL</i> <sup>c</sup> | Method <sup>d</sup> |
|-------------------------------------------|---------|---------------------|-------------------|------------------|-------------------------|---------------------|
| Nuclear DNA AK1<br>intron 5               | 703 bp  | T92 + $\Gamma$      | 13064.24          | 14009.20         | -6417.68                | MEGA5               |
|                                           |         | GTR+ $\Gamma$ +I    | 13038.81          | 13512.63         | 6377.39                 | mrModeltest         |
| mtDNA <i>cytb</i>                         | 1143 bp | TN93 + $\Gamma$ +I  | 44435.60          | 45757.53         | -22075.54               | MEGA5               |
|                                           |         | GTR+ $\Gamma$ +I    | 44213.16          | 44901.91         | -21939.03               | mrModeltest         |
| Concatenated AK1<br>intron 5- <i>cytb</i> | 1846 bp | TN93 + $\Gamma$     | 58403.40          | 59755.87         | -29060.52               | MEGA5               |
|                                           |         | GTR + $\Gamma$      | 44213.16          | 44901.91         | -21939.03               | mrModeltest         |

<sup>a</sup>gene region used, AK1 intron 5: cytosolic adenylate kinase 1 intron 5 gene; *cytb*: cytochrome *b* gene.

<sup>b</sup>nucleotide substitution models, T92: Tamura 3-parameter model; TN93: Tamura-Nei model; GTR: General Time Reversal model; +  $\Gamma$ : discrete gamma distribution with 5 rate categories; +I: with invariable sites.

<sup>c</sup>statistical inference used for model selection, AICc: corrected Akaike information criterion; BIC: Bayesian information criterion; *lnL*: Maximum likelihood estimate.

<sup>d</sup>method of model selection used, MEGA5: Molecular Evolutionary Genetics Analysis version 5 (Tamura et al., 2011) for selecting best substitution model for use in maximum likelihood analyses; mrModeltest version 2 (Nylander 2004) for selecting best substitution model for use in Bayesian analyses.

**Table S2.4:** Summary of base insertions and deletions for nuclear DNA cytosolic adenylate kinase 1 intron 5 based on a comparison of 61 in-group species of Bucerotidae.

| Insertions <sup>a</sup> | Taxa included                                                                                                                                                                                                                        |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 bp (A)                | <i>Rhinoplax</i> clade, <i>Anorrhinus</i> , <i>Ocyceros</i> , <i>Anthracoceros</i> and <i>Aceros</i> clade, except <i>Aceros</i> and <i>Penelopides</i> (1 bp deletion)                                                              |
| 2 bp (AG/GG)            | <i>Berenicornis</i> , <i>Tropicranus</i> , <i>Tockus hartlaubi</i>                                                                                                                                                                   |
| 1 bp (C/T)              | <i>Bucorvus</i> , <i>Buceros</i> , <i>Anorrhinus</i> , <i>Ocyceros</i> , <i>Anthracoceros</i>                                                                                                                                        |
| 2 bp (AG)               | All except <i>Ceratogymna</i> , <i>Bycanistes</i> , <i>Rhinoplax</i> clade, <i>Anorrhinus</i> - <i>Anthracoceros</i> clade and <i>Aceros</i> clade (1 bp deletion)                                                                   |
| 1 bp (T)                | <i>Bucorvus</i> and <i>Penelopides</i> - <i>Aceros</i> clade                                                                                                                                                                         |
| 1 bp (T)                | All <i>Aceros</i> except <i>A. cassidix</i> (1 bp deletion)                                                                                                                                                                          |
| 1 bp (T)                | All Philippine <i>Penelopides</i>                                                                                                                                                                                                    |
| 1 bp (A/G/T)            | <i>Bucorvus</i> and <i>Rhinoplax</i> - <i>Buceros</i>                                                                                                                                                                                |
| 1 bp (G)                | <i>Bycanistes</i> - <i>Ceratogymna</i> ,                                                                                                                                                                                             |
| 1 bp (T)                | <i>Buceros</i> , <i>Anorrhinus</i> , <i>Ocyceros</i> , <i>Anthracoceros</i> , <i>Bycanistes</i> - <i>Ceratogymna</i> , <i>Rhyticeros</i> - <i>Aceros</i> clade except <i>Berenicornis</i> - <i>Tropicranus</i> clade (1 bp deletion) |
| 1 bp (A)                | All except <i>Penelopides</i> - <i>Aceros</i> clade and <i>Aceros nipalensis</i> (1 bp deletion)                                                                                                                                     |

<sup>a</sup>Nucleotide bases, A: adenine, C: cytosine, G: guanine, T: thymine; bp: base pairs.



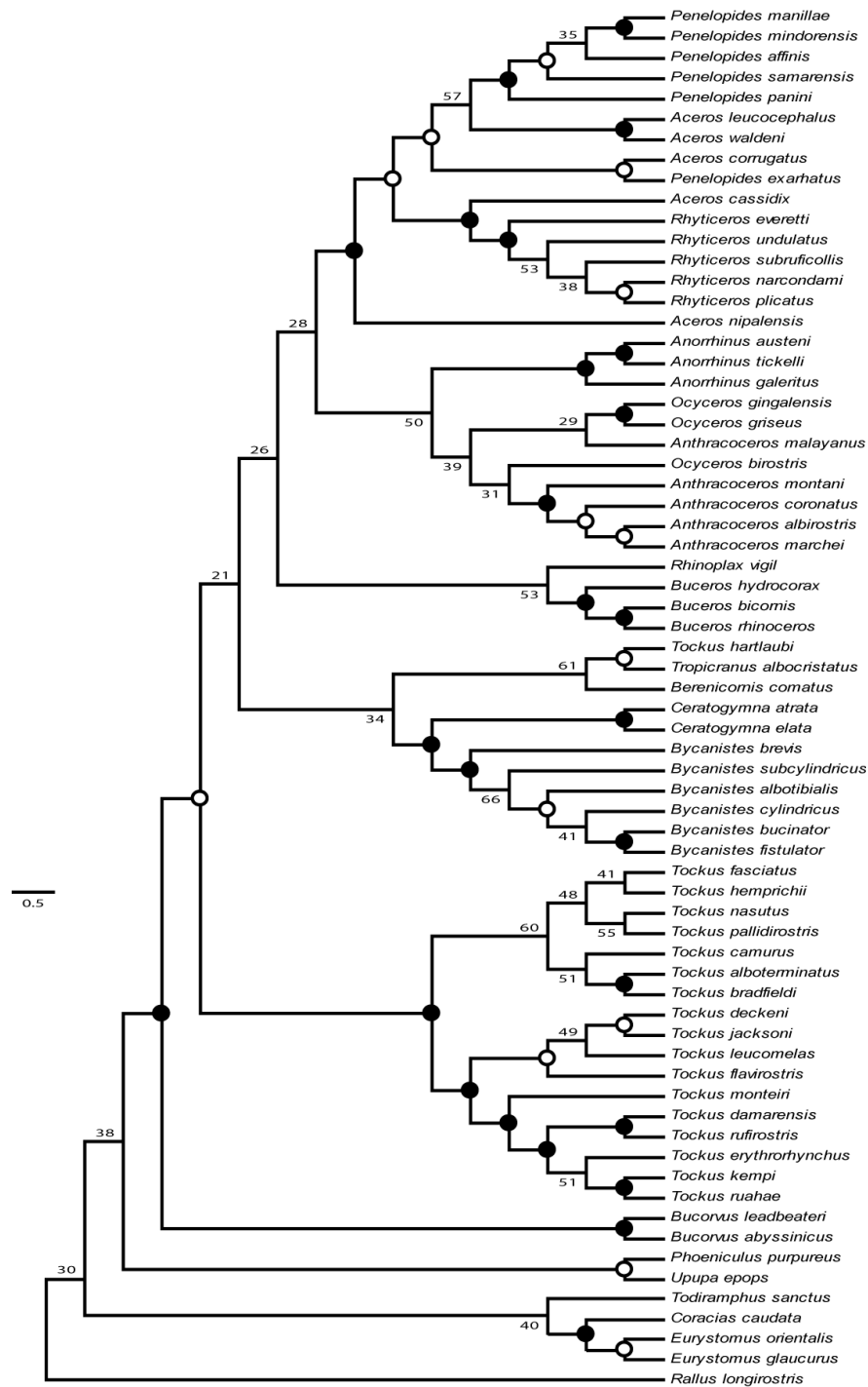


**Figure S1.1:** Maximum Parsimony phylogeny of hornbills (Bucerotidae) based on concatenated nuclear DNA-mtDNA sequences representing 61 in-group species and five out-group species. Numbers and circles on nodes indicate bootstrap values, with black circles indicating strong support at  $\geq 90\%$ , and white circles indicating moderate support at 70-90%. Support values  $< 70\%$  are labelled on nodes.



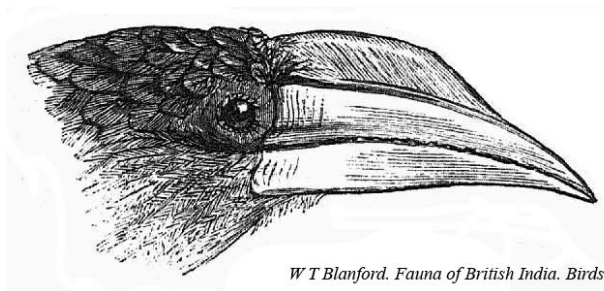
**Figure S2.2:** Bayesian phylogeny of Bucerotidae based on concatenated nuclear DNA-mtDNA sequences covering 169 samples, and representing 61 in-group species and eight out-group species. Numbers on nodes indicate posterior probabilities, with  $\geq 97\%$  as showing strong support.

## References

- Mindell, D. P., Sorenson, M. D., Dimcheff, D. E., 1998. Multiple independent origins of mitochondrial gene order in birds. *Proc. Nat. Acad. Sci. USA* 95(18), 10693–10697.
- van Tuinen, M., Sibley, C. G., Hedges, S. B., 2000. The early history of modern birds inferred from DNA sequences of nuclear and mitochondrial ribosomal genes. *Mol. Biol. Evol.*, 17(3), 451–457.

## Chapter 3

### Environmental stability and the evolution of cooperative breeding in hornbills



*The Brown Hornbill breeds cooperatively with one to five helpers, but two nest helpers are sufficient to reduce the feeding load of the breeding male by as much as 40%. Nests of this species showed a low chick survival rate (43%) if there were no helpers. Besides helping the breeding male in feeding, nest helpers contribute to protecting the brood from invaders.*

Pilai Poonswad, 1993, *Comparative ecology of sympatric hornbills (Bucerotidae) in Thailand*

## **Environmental stability and the evolution of cooperative breeding in hornbills**

**Juan-Carlos T. Gonzalez<sup>1,2</sup>, Ben C. Sheldon<sup>1</sup>, Nigel J. Collar<sup>3</sup> and Joseph A. Tobias<sup>1</sup>**

<sup>1</sup>*Department of Zoology, Edward Grey Institute for Field Ornithology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*

<sup>2</sup>*Institute of Biological Sciences, University of the Philippines Los Baños, College, Laguna 4031, Philippines*

<sup>3</sup>*BirdLife International, Wellbrook Court, Girton Road, Cambridge CB3 0NA, UK*

### **SUMMARY**

The origin of cooperation in social animals has long been the focus of debate in evolutionary biology, yet the role of environmental factors remains unclear. Some studies conclude that cooperation over reproduction is associated with environmental stability, whereas in contrast a recent global analysis in birds provides evidence for the opposite pattern, implying that cooperative breeding may be a ‘bet-hedging’ strategy associated with climatic uncertainty. One explanation for this disparity is that global patterns may be driven by processes relevant to insectivores, which make up the bulk of cooperatively breeding birds. Here we use comparative phylogenetic methods to assess the relationship between climate and reproduction in hornbills (Bucerotidae), an avian family characterised by frugivory and faunivory. We show that cooperation is positively associated with both inter- and intra-annual climatic stability, as well as with smaller body size, frugivory and territoriality. These findings suggest that the relationship between cooperation and climatic uncertainty is reversed in frugivores, perhaps because they are less sensitive than insectivores to fluctuations in temperature and precipitation. In such cases, climatic stability may promote

## CHAPTER 3

cooperation by increasing survival and population density, and thus reducing the availability of territories for young birds, whose best strategy may be to cooperate with dominant breeders. We conclude that the mixed results of previous studies are likely to reflect taxon-dependent interactions between diet, climate and cooperation.

### **Keywords**

hornbills; comparative analysis; cooperative breeding; phylogeny

**Running title:** Evolution of cooperative breeding in hornbills

Formatted for submission to Proceedings of the Royal Society, London *B*

## 1. INTRODUCTION

The origin and evolution of cooperative breeding behaviour have had a long and continuing debate, and causes for its development are much studied (Brown 1987; Stacey & Koenig 1990; Hatchwell 2007). This remarkable behaviour involves non-reproductive members of a social group in providing aid to improve survival of offspring of other individuals (Ridley et al. 2005). These groups are usually formed by retention of young on the natal territory, wherein juveniles delayed their dispersal to aid their parents. As a result, helpers gain indirect fitness benefits from helping raise kin, or gain direct benefits from group-living (e.g., resource defence, increased predator detection) (Hatchwell & Komdeur 2000; Ridley et al. 2005). Cooperative breeding (CB) in birds has been attributed to a wide range of different drivers, including altriciality, longevity, kinship, promiscuity and climatic variation (Ligon & Burt 2004; Hatchwell 2009; Cornwallis *et al.* 2010). This is often associated with biotic factors (e.g. body mass, primary diet) that exhibit a strong phylogenetic signal, suggesting that life-history traits may predispose lineages to exhibit CB behaviour (Arnold & Owens 1999). However, CB is generally rare in birds, being recorded in only 831 (8.4%) out of the 9,845 species (Jetz & Rubenstein 2011). It is not randomly distributed across avian families and rather recurrent in certain taxonomic groups, such as Corvidae and Timaliidae, and certain ecological guilds, such as ground-foraging insectivores in savannas and woodlands (Stacey & Koenig 1990).

The general conception is that CB predominates in birds found in stable-temperature non-forest habitats (Ford *et al.* 1988; du Plessis *et al.* 1995; Hatchwell & Komdeur 2000). But, there are contrasting ideas about the role of environmental stability in the evolution of CB. Some evidence suggests that stability promotes CB by allowing long-lived populations to persist at carrying capacity and with year-round territoriality, thus providing offspring little opportunity to establish territories (Arnold & Owens 1999; Covas & Griesser 2007; Koenig et al. 2011). However, recent work on birds (Rubenstein & Lovette 2007; Jetz & Rubenstein 2011) provides an opposing view, which proposes a key role for environmental variability in promoting evolution of CB, both at the

continental and global scale (Strassman et al. 2011). This robust global dataset suggests that environmental uncertainty and biotic factors are important predictors of CB, with positive associations existing in regions with high variation in inter-annual rainfall, and more evident in non-forest habitats in Africa and Australia (Jetz & Rubenstein 2011). This contrasting view holds true in African starlings, wherein climatic uncertainty promotes CB as a bet-hedging strategy (Rubenstein 2011).

Koenig et al. (2011) summarizes these two contrasting scenarios based on two hypotheses. One is driven by extrinsic constraints (habitat saturation hypothesis), where environmental stability and resource predictability persuades young birds to remain as helpers, and the other driven by intrinsic constraints (hard life hypothesis), where high environmental variability and resource unpredictability encourages helping. However, there are problems with applying a single hypothesis at a global scale, since independent processes affecting a focal trait may be obscured by global correlations. Jetz & Rubenstein(2011) noted increasing rarity of CB with distance from the equator, and a slight prevalence of CB in songbirds over non-passerines. At a smaller scale, Rubenstein & Lovette (2007) found CB as an adaptive response in African starlings to maintain reproductive success in temporally variable environments. In contrast, Ligon & Burt (2004) suggested that environmental unpredictability and strong seasonality of food supply facilitated the loss of CB in Scrub Jays. Whilst in other clades such as bee-eaters and woodhooops, variability in ecologies had minimal effect on the maintenance of CB (Ligon & Burt 2004). Hence, different taxa may have different responses to environmental instability and further analyses within other CB clades may shed light on the influence of variability (Cockburn & Russell 2011).

One possible reason for this disparity in global patterns is that CB development may be driven by processes relevant to diet. Based on the global dataset (Jetz & Rubenstein 2011), the largest proportion of CB bird species is insectivorous (prey mainly on invertebrate arthropods, 59.5%). Other CB birds are omnivores (generalists with broad dietary intake, 18.7%), herbivores (mainly eat fruit and seeds, 18.4%) and carnivores (prey mainly on vertebrates, 3.5%). This supports the claim that environmental uncertainty is associated with insectivory, since CB predominates in insectivorous birds from non-forest habitats with aseasonal insect populations



(Ford *et al.* 1988; du Plessis *et al.* 1995; Hatchwell & Komdeur 2000). We presume that non-insectivores are not associated with climatic variability in the same way as insectivores, instead other factors may drive the evolution of CB in frugivorous birds.

In this paper, we aim to test the “environmental uncertainty hypothesis” (Jetz & Rubenstein 2011) in a large clade of birds where CB is common and that are mainly frugivorous - a feeding specialisation differing from other clades that have been tested. CB is generally uncommon in frugivorous birds and evident only in aracaris, African barbets, trumpeters, hornbills, magpies and jays (Sherman 1995, Ekman & Ericson 2006). We focus our comparative evolutionary analysis on hornbills (Bucerotidae), an avian family characterised by frugivory (feeds on fruit) or faunivorey (eats both invertebrates and vertebrates). It displays an exceptionally high level of CB, exhibiting three times more documented CB species than would be expected by chance alone (Kinnaird & O’Brien 2007). However, the origins or function of CB in the Bucerotidae remain unstudied, and explanations for the evolution of such behaviour have not been fully tested (Kemp 2001). To address this, we examined the relationship between climatic conditions (variation in temperature and rainfall) and the preponderance of CB in hornbills, and the potential influence of key biotic factors as alternative predictors or in combination with climate (e.g. habitat choice, primary diet, body mass, nest-hole size and territoriality). To understand these relationships, we conducted three sets of comparative analyses: (1) a test of the association between CB and biotic factors, using a well-resolved hornbill phylogeny to determine origin and evolution of CB in hornbills; (2) tests of the association of CB with environmental data and biotic factors (without phylogenetic control); and (3) tests of the association of CB with environmental data and biotic factors, but with control for phylogenetic effects.

## 2. MATERIALS AND METHODS

### 2.1 The Hornbills

The family Bucerotidae comprises 61 species of territorial cavity-nesters occurring in tropical forests and savannas of Asia and Africa (Kemp 1995, 2001). They are an ideal study system for testing hypotheses on sociality, since nearly 41% of species are cooperatively breeding, and they also belong to an ancestral lineage predisposed to CB (Ligon & Burt 2004). Arnold & Owens (1999) established this lineage in proving that CB is strongly evolved in hoopoes, woodhoopoes and hornbills (suborder Bucerotes). There are generally two types of CB systems, one involves mature non-breeders ("helpers-at-the-nest") that have no shared paternity, and the other that involves some degree of shared paternity (Ehrlich *et al.* 1988). Of these, the helper-at-the-nest system is the type usually exhibited by hornbills (Kinnaird & O'Brien 2007). This system is confirmed in at least ten species, but suspected in 15 (see Table S3.1, Supplementary information).

For data on social system, we categorized species as either cooperative or non-cooperative breeders, disregarding whether a species was classified as obligate, facultative or suspected. This primarily follows Jetz & Rubenstein (2011), which cited mainly Cockburn (2006), and recognized 22 cooperative hornbills from 53 species. In contrast, Kinnaird & O'Brien (2007) recognised only 13 cooperative species because studies failed to verify CB or because data are insufficient for some suspected species. Kinnaird & O'Brien (2007) accepts group-living patterns as an indication of CB but rejects the hypothesis that dimorphic juvenile plumage is indicative of natal philopatry, and consequently CB. Our dataset on social system also varies from combined notes in Kemp (1995, 2001). Our expanded dataset accepts group territoriality in hornbills as a form of cooperative breeding (Theuerkauf *et al.* 2009) but retains all suspected species, given that long-term field studies necessary to disprove or confirm CB are lacking. Like Jetz & Rubenstein (2011), we included *Anorrhinus austeni*, and excluded *Tockus alboterminatus*. We added *Bycanistes subcylindricus* as cooperative from Ligon & Burt (2004), and adopted the split of *Penelopides*

*samarensis* from *P. affinis*, then retaining both as cooperative. The proportion of CB and non-CB species was quantified relative to different variables tested, but done without phylogenetic control. Alternatively, we used comparative analyses of independent contrasts to control for phylogenetic effects using a well-resolved hornbill phylogeny.

## 2.2 Phylogeny Construction

A highly resolved multi-locus phylogeny of all 61 hornbill species was constructed from a concatenated dataset of nuclear DNA (adenylate kinase 1 intron 5, 703 bp) and mtDNA (cytochrome *b*, 1,143 bp) amplified by Polymerase Chain Reaction from historical samples collected largely from museum specimens, as described in Gonzalez et al. (in prep., see Chapter 2). From this concatenated dataset, a single maximum credibility clade (MCC) tree was annotated from 4,500 trees constructed from Bayesian Markov Chain Monte Carlo (MCMC) simulations. Trees were calibrated using both fossil and molecular anchorpoints implemented in the program BEAST v.1.6.1 (Drummond & Rambaut 2007). Details of the constructed molecular chronogram of Bucerotidae are described in Gonzalez & Tobias (in prep., see Chapter 4). The MCC tree was trimmed to remove out-groups, and was tested to be ultrametric (test performed in R package *ape*, Paradis et al. 2004). Comparative analyses of independent contrasts (CAIC) was instigated against the phylogeny to test for evolutionary relationships between incidence of sociality and various environmental and biotic variables. A summary of selected biotic variables against the well-resolved ultrametric tree used in this study is shown in Fig. 3.1.

## 2.3 Comparative Data Sources

Tests conducted against variation in climatic conditions (temperature and rainfall) were based primarily on environmental data derived from Jetz & Rubenstein (2011). These climatic conditions represent the prevailing environmental data within the distributional range of a species and therefore indicate climatic niches for each species. We extracted data for both CB and non-CB hornbills from the global dataset focusing on six variables, which included mean annual values,

inter-annual (standard deviation from the mean of data taken over a span of 3 years) and intra-annual variance (standard deviation from the mean of data taken across 3 months) (see Table S3.1, Supplementary information). Inter-annual variance in temperature and precipitation describes environmental variability between years (long term), and intra-annual variance describes environmental variability within a year (short term).

Tests for associations of CB with key biotic factors were based on six variables, namely habitat choice, primary diet, body mass, nest-hole size and territoriality (see Table S3.1, Supplementary information). Data for primary diet and preferred habitat were derived mainly from Kemp (2001), with notes from Carrière *et al.* (2002) and Kitamura (2011). We categorized diet as either predominantly frugivorous or non-frugivorous (faunivorous), and classified habitat as either humid forest or drylands (referring to savanna grasslands, desert thornveld and dry woodland). Data for hornbill body mass were extracted from Dunning (2008), with added notes from Kemp (1995, 2001) and Kemp & Delport (2002). Data for territoriality was derived primarily from Kemp (2001), and nest-hole size was adopted from Galama *et al.* (2002), who classified hornbill nest-holes into four class-sizes.

## 2.4 Statistical Analysis

### 2.4.1 Trait evolution and ancestral state reconstruction

Two methods were implemented to imply ancestral origins and trace the evolution of CB in hornbills using the well-resolved hornbill phylogeny. We used the reversible-jump (RJ) MCMC method in BAYESTRAITS to test for correlation between discrete binary traits and phylogenetic trees, as well as to determine root combinations that imply ancestral origins (Pagel & Meade 2006). Correlated evolution was tested through model comparison wherein two traits were allowed to evolve independently and fitted to the data and phylogeny using log-likelihood ( $Lh$ ). To test for correlated trait evolution, we ran RJMCMC for both Dependent (Dep) and Independent (Ind) models each at 20 million iterations with a burnin of 2 million. We followed a uniform rate deviation parameter of 10 to maintain an acceptance value of approximately 20-40% and denote

adequate mixing. The likelihood ratio test (LRT) was used to compare the two models, based on the differences in maximum  $Lh$  values between models ( $2 * Lh$  Ind model –  $Lh$  Dep model). Significance of model  $Lh$  was based on p-values calculated from chi-squared distributions of the LRT with degrees of freedom equal to the difference in number of parameters estimated (Ekman & Ericson 2006). Ancestral state reconstruction of hornbill sociality was implemented in the program MESQUITE, version 2.74 (Maddison & Maddison 2010). Stochastic character mapping for categorical variables was used to trace the history of CB within the well-resolved Bayesian MCC tree (see Methods 2.2). Marginal probability reconstruction was done using current probability models, such as estimates for Mk.

#### ***2.4.2 Comparison of environmental conditions and biotic factors with CB***

The proportion of CB and non-CB species was quantified relative to different variables tested, both for environmental conditions and biotic factors. These analyses involved simple comparative proportions of hornbills conducted without the effect of phylogenetic control. We tested the prevalence of CB hornbills in variable environments using scatter plots of mean niche positions filled by both CB and non-CB hornbills, and these niches were based on the inter-annual and intra-annual variation in temperature and precipitation. Scatter plots were constructed separately for temperature and precipitation in the statistical program R version 2.13.1 (R Development Core Team 2011) with climatic niche axes represented by inter-annual (among years, y-axis) and intra-annual variation (within year, x-axis). We then compared the proportion of CB and non-CB hornbills based on three different biotic variables: preferred habitats, primary diet and territoriality. All characters are discrete and treated as binary, and plotted on a simple bar graph using Microsoft Excel, with proportion of species on the y-axis. For comparisons of CB with environmental and biotic variables, results were tested for independence using the Chi-squared test in the R package MASS (Venables & Ripley 2002).

#### ***2.4.3 Comparative analyses of independent contrasts with CB***

## CHAPTER 3

Comparative analyses of independent contrasts (CAIC) was implemented to test for phylogenetically-dependent trait evolution. This was instigated against the well-resolved hornbill phylogeny (see Methods 2.2) to test for evolutionary relationships between incidence of sociality and various environmental and biotic variables, and to control for phylogenetic effects. Multivariate analyses were carried out in four different methods in R, version 2.13.1 (R Development Core Team 2011), included in the following R packages: comparative analysis of phylogenetics and evolution in R (Caper) package (Orme et al. 2012), analyses of phylogenetics and evolution (Ape) package (Paradis *et al.* 2004), and the MCMC generalized linear mixed models (MCMCglmm) package (Hadfield 2010).

The package Caper fits phylogenetically generalised least squares (PglS) models and implements two CAIC algorithms, particularly the crunch function for continuous variables and brunch function for categorical variables (using binary discrete characters). This performs a regression on the contrasts and provides both the F statistic and p-value at appropriate degrees of freedom. In both CAIC functions, all binary traits were factored and all numerical data were log transformed. We then performed a comparison of different models involving relationships and interactions of two different traits with social system. This was done primarily in Caper, using the crunch function, and the values derived from the Akaike information criterion (AIC) and analysis of variance (ANOVA) were used to support model inference. We found that body mass was significantly correlated with nest-hole size ( $R^2=0.62$ ,  $F_{1,59}=96.74$ ,  $p < 0.0001$ ) and given that nest-hole data were stratified into four sizes and performed less as continuous data, we decided to omit nest-hole size in the final CAIC analyses.

The package MCMCglmm was used to test correlated random or fixed effects arising from our hornbill phylogeny by calculating the fit of generalized linear mix models (glmm) using the Markov chain Monte Carlo (MCMC). MCMCglmm ran for 20,000,000 iterations, with a burn-in of 10%, and was thinned for every 100 intervals. The categorical function was used to accommodate comparison between continuous and binary data. Flat non-informative priors were adopted for both R and G structures, with fixed effects at  $V=1$  (covariance matrix) and uniform low degree of belief parameters of  $\nu = 0.002$  across all parameters. Estimates of the posterior mean with 95% lower

and upper confidence intervals and p-values for correlations between traits and social system were reported. The calculated deviance information criterion (DIC) and effective sample size (ESS) were used to evaluate convergence of simulations, along with diagnosis of plotted samples showing asymptotically standard normal distribution.

### 3. RESULTS

#### 3.1 Cooperative breeding in hornbills

An overview of cooperatively breeding hornbill species (25/61 or 41%) indicates that CB behaviour occurs widely within the family, from the smallest (*Tockus camurus*, 30 cm) to the largest species (*Bucorvus leadbeateri*, 120 cm), and the largest obligate CB species in the world. Four genera (4/14 or 28.6%, *Bucorvus*, *Ceratogymna*, *Anorrhinus* and *Penelopides*) contain exclusively CB species. However, it is poorly represented in the genus *Tockus* (1/16 or 6.3%, *T. camurus*) and none exists in the genus *Rhyticeros*. Prevalence of CB behaviour is more evident in Indo-Malayan (17/25 or 68%) than in Afrotropical hornbills (8/25 or 32%), which suggest that hornbills do not necessarily follow the global trend (CB prevalent in African and Australia, Jetz & Rubenstein 2011). Thus, Asian hornbills comprised 17% of the known CB birds in the Indo-Malayan region (n=98, Jetz & Rubenstein 2011). From the 28 species of Indo-Malayan hornbills (count excludes 4 species from Wallacea and Melanesia), 15 are CB, which is considerably more than the 12 which are non-CB ( $\chi^2 = 44.29$ , df=1,  $p < 0.0001$ ). Based on the molecular phylogeny used (Fig. 3.1, original chronogram in Gonzalez & Tobias, in prep., see Chapter 4), both subfamilies of Bucerotidae have CB species, and all five major clades of hornbills within the subfamily Bucerotinae harbour CB species. It is most represented in the *Anorrhinus* clade (8/11 or 72.7%), followed by the *Berenicornis* clade (6/11 or 54.5%), *Rhinoplax* clade (2/4 or 50%) and *Aceros* clade (6/16 or 37.5%).

#### 3.2 Ancestral reconstruction in hornbills

Results of BAYESTRAITS analysis indicated no correlated evolution between social system with primary diet, preferred habitat, territoriality and body mass (Table 3.1). These characters evolved independently of each other. However, posterior probabilities (PP) of root characters suggests that ancestral origins of cooperative breeding (CB) coincide with large body mass ( $>0.5$  kg, PP=1.00), and to some extent occupancy of humid forests and territorial behaviour (PP=0.50), but both are poorly resolved due to low support values. Ancestral state reconstructions corroborate that CB behaviour is ancestral in hornbills (Table 3.1 and Fig. 3.2). Cooperative living as a root trait in hornbills coincides independently with body mass greater than 0.5 kg, occupancy of humid forests and exhibiting territorial behaviour, suggesting that cooperative hornbills originated from either large, territorial or rainforest-dwelling species. The distribution of CB across hornbill lineages implies that this ancestral strategy persisted in at least four major clades (*Bucorvus*, *Berenicornis*, *Rhinoplax* and *Anorrhinus* clades), and re-occurs partially in two others (*Tockus* and *Aceros* clades). Only ground-hornbills (*Bucorvus*) and Philippine Tarictic hornbills (*Penelopides*) represent subclades that exhibit exclusively CB behaviour, although it is exclusive in two other genera (*Anorrhinus*, *Berenicornis*). Prevalence in certain genera and major clades suggest that these lineages exhibit some degree of predisposition for CB development.

### 3.3 Environmental variability and CB in hornbills

Jetz & Rubenstein (2011) presented the relationship between environmental uncertainty and CB in birds at a global scale, showing a positive association with variation in rainfall. At the family level, our data strongly suggest that CB in hornbills is not driven by environmental variability (Fig. 3.3). In contrast, cooperatively breeding species of hornbills tend to be found in habitats that experience lower degrees of variability in temperature (Fig. 3.3A) and lower degrees of variability in precipitation (Fig. 3.3B), both inter-annually (across 3 years) and intra-annually (within a year and across 3 months). This supports the hypothesis that sociality in hornbills probably developed in stable rather than variable climatic conditions. Although four prominent outliers can be observed, most (21/25 or 84%) CB hornbills occupy stable humid forests ( $\chi^2=11.56$ ,



df=1,  $p < 0.01$ ). These outliers represent species inhabiting variable dry woodlands and savannah grasslands, particularly in Africa and India, and these species appear to follow the global trend.

Quantitative comparison was used to test the association of biotic factors with CB in hornbills, particularly addressing the role of diet (frugivory/faunivory), habitat choice (humid/drylands) and territoriality. Comparative results revealed that dietary type influences the incidence of CB in hornbills, and showed more association with CB than habitat choice, territoriality and body mass (Figure 3.4, body mass not shown, see Supplementary information, Table S3.3). The proportion of frugivorous CB hornbills is significantly higher ( $\chi^2=4.90$ , df=1,  $p=0.03$ ) than non-CB frugivorous hornbills. This indicates that CB is more pronounced in frugivorous hornbills than faunivorous hornbills. This supports the hypothesis that frugivory has a more pronounced influence on the development of CB in hornbills than faunivory or more specifically insectivory, as shown in the global trend (Jetz & Rubenstein 2011).

However, the proportion of CB hornbills inhabiting humid forests ( $\chi^2=3.41$ , df=1,  $p=0.07$ ) was not considerably higher than non-CB hornbills. This suggests that forest-living is not crucial for CB development amongst hornbills based on this analysis. Moreover, the proportion of territorial CB hornbills is also not significantly higher than territorial non-CB hornbills ( $\chi^2=0.37$ , df=1,  $p=0.54$ ). Thus, territoriality also may have a rather benign impact on the development of CB in hornbills. Likewise, the number of CB hornbills weighing more than 0.5 kg is not proportionately higher than the number of non-CB hornbills of similar mass ( $\chi^2=1.07$ , df=1,  $p=0.30$ ). This indicates that increase in body mass does not exhibit a strong influence on CB development in hornbills. Given these results, a direct quantification of interactions between CB and biotic factors did not reveal significant associations other than diet, thus a more resolved analyses controlling for phylogenetic effects might provide a better picture of these relationships.

### 3.4 Comparative analyses of independent contrasts with CB

Results reversed dramatically when tests for predictors were controlled for phylogeny, compared with the initial non-phylogenetically controlled quantitative interactions. Analysis based

on CAIC proved important for territorial behaviour (particularly during the breeding season) but not for frugivory. Results were consistent across three comparative analyses, namely MCMCglmm, pglms and CAIC (based on brunch and crunch algorithms) (Table 3.2). However, analysis for correlated trait evolution yielded no significant correlation of social system with preferred habitat, dietary type, territoriality and/or body mass (for details of datasets, see Supplementary information, Table S3.1). Among the four biotic factors tested, only territoriality was positively correlated with sociality. No significant relationship with CB was observed for preferred habitat, dietary type or body mass. This suggests that maintenance of a territory (referring to nest and nest-surroundings) is important in the development of CB in hornbills. For environmental variables, social system did not show any relationship with mean annual temperature and precipitation, or with inter-annual variation in temperature. However, significant negative relationships were apparent with intra-annual variation in temperature and precipitation, and inter-annual variation of rainfall. This negative relationship with environmental variability supports our hypothesis that CB development in hornbills is predicted by stable conditions rather than variable fluctuations.

Further comparisons of social system with combined pairs of variables yielded similar models indicating key predictors for CB in hornbills (Table 3.2). However, no trait interactions were observed in any of the models selected. Based on AIC, sociality in hornbills fitted more often with territoriality (AIC=2.71). We therefore consider territorial behaviour a strong predictor of CB in hornbills, whereas, the relationship of social system with body mass and variation in precipitation were equally significant (ANOVA, both with  $p$ -value=0.03), both within (intra-annual variation, AIC=-88.45) and among years (inter-annual variation, AIC=-87.74). Subsequently, these two models (selection based on AIC) indicate that body mass and rainfall variability are important predictors of CB in hornbills. Therefore, these models (estimates shown in Table 3.2, model selection in Table 3.3) highlight the negative relationship between intra-annual (-0.18) and inter-annual (-0.29) variability in precipitation to CB, thus providing further support to our environmental stability hypothesis. There are exceptions, since three of the 25 CB species of hornbills occur in environmentally variable savannah-woodland habitats, in particular faunivorous ground-hornbills (*Bucorvus*) from Africa and *O. birostris* from India. Model selection also stressed

the negative relationship between CB and body size, suggesting that a reduction in body size would likely predict the incidence of CB in hornbills. This is evident in some small-bodied CB species, including *T. camurus*, *B. fistulator*, *O. birostris*, *O. gingalensis* and all *Penelopides* hornbills. However, this relationship is not particularly water-tight, as some of the largest hornbills are also CB species, particularly ground-hornbills (*Bucorvus*), some Asia forest hornbills (*Buceros*) and wattled hornbills (*Ceratogymna*).

## 4. DISCUSSION

### 4.1 Cooperation is ancestral in hornbills

We found CB to be ancestral in hornbills, as it is an ancestral trait within other Bucerotes (hoopoes and woodhoopoes) and closely-related Coraciiformes (bee-eaters and kingfishers) (Ligon & Burt 2004; Cockburn 2006). It is ancestral in Bucorvinae, and therefore to Bucerotinae, since ground-hornbills represent an early separation from that group. Both ground-hornbills is cooperative, but only 40% of typical hornbills are cooperative. The pattern found in hornbills, is that CB persists in all clades and but more predominant in several lineages, such as *Bucorvus*, *Ceratogymna*, *Anorrhinus*, *Ocyceros* and *Penelopides* (Kinnaird & O'Brien 2007). This high incidence of CB in Bucerotidae, Upupidae and Phoeniculidae suggests helping was conserved throughout the Bucerotes lineage. Overall, CB appears to be ancestral in many avian groups, including the passerine parvorders Corvida and Passerida (Pruett-Jones 2004). In corvids, it exhibits high plasticity, given that multiple transitions of family cohesion and alloparental care occurred across the entire corvid tree (Ekman & Ericson 2006). It is also prevalent in primarily arboreal ancient orders such as Coliiformes, Coraciiformes and Piciformes (Ligon & Burt 2004). CB is also an ancestral trait in Piciformes, particularly for African barbets, toucan-barbet and aracaris, which had evolved independently across the barbet-toucan lineage (Restrepo & Mondragon 1998; Short & Horne 2002).

## 4.2 Environmental stability promotes CB in hornbills

Recent global analyses have suggested that environmental uncertainty is a key component in predicting the occurrence of CB in birds (Jetz & Rubenstein 2011), with CB more likely to occur in variable environments. The interpretation of these findings is that CB is an adaptive response to permit continuous breeding and improve reproductive success in such variable and unpredictable habitats, later described as a bet-hedging strategy (Rubenstein 2011). In contrast, we show that in hornbills, CB is associated with more stable environments. Cooperatively breeding species of hornbills were more likely to be found in habitats with lower degrees of variation in rainfall. These findings were robust and statistically supported by comparative analyses with (CAIC and AIC/ANOVA tests) and without (scatter plot and  $\chi^2$  tests) phylogenetic control. Prevalence of CB hornbills in tropical humid forests supports the environmental stability hypothesis, since stable rainforests exhibit low variation in temperature and rainfall. Only a fraction (16%) of CB hornbills inhabits variable seasonal habitats (*B. abyssinicus*, *B. leadbeateri*, *B. bucinator*, *O. birostris*). Environmental stability is probably the mechanism influencing CB behaviour in hornbills, since climatic stability is more pronounced in the tropics, leading to minor fluctuations in seasonal resource availability. This is a probable reason why CB birds abound in the tropics (Gaston 1978) and nearer the equator (Cornwallis *et al.* 2010, Jetz & Rubenstein 2011). This same stability drives sociality in Australian birds (Ford *et al.* 1988) and possibly in other tropical birds (Restrepo & Mondragon 1998, Sherman 1995). Apparently, living in a relatively stable climate encourages year-round occupancy of a territory, causing low turnover of occupants and minimizing dispersal (Hatchwell & Komdeur 2000).

## 4.3 Stability and frugivory facilitates cooperation in hornbills

There is a general predominance of cooperative insectivorous species inhabiting unpredictable environments in the global dataset (Jetz & Rubenstein 2011), and it is likely that this large number of CB insectivores is driving the overall trends. However, this trend may not always be true for non-insectivores. Alternatively, we found CB to be dominant in the largely frugivorous

and faunivorous Bucerotidae, and all except three (both *Bucorvus*, *T. camurus*) are frugivores. Hornbills do not follow the global trend and are not driven by environmental uncertainty due to their prevalence in stable environments, since variability presumably affects aseasonal insect abundance (Ford *et al.* 1988; du Plessis *et al.* 1995). Being less dependent on insects, large frugivorous hornbills rely on the seasonal abundance of certain energy-rich “hornbill-dispersed” fruits and asynchronous figs. Although *B. bucinator* and *O. birostris* inhabits forests interspersed with savannas and dry woodland (variable habitats), their affinity to fruits may have influenced persistence of CB behaviour. Hornbills are also reliant on vertebrate prey during the breeding period, and it is likely that CB is motivated by difficulty in acquiring sufficient vertebrate prey to improve breeding output. Further study is needed to understand whether partiality to vertebrate faunivory in the nestling diet influenced the development to CB.

Presumably, group territorial defence is rare in frugivores (Brown 1987), since frugivory may not encourage maintenance of year-round territories due to unpredictability of widely spaced fruiting syndromes, so forcing frugivores to be nomadic (Arnold & Owens 1998). However, some hornbills, known to feed almost exclusively on aseasonal figs, counter this unpredictability through group living and expanded territories (Kinnaird & O’Brien 2007, Gautier-Hion & Michaloud 1989). Incidentally, frugivory does not impede the evolution of CB, but may not necessarily constrain it due to plasticity in diet. Competition for nesting cavities rather than frugivory promulgates territorial behaviour and helping in toucan barbets (Restrepo & Mondragon 1998). Nonetheless, trends driving sociality in hornbills may also be apparent in other frugivorous CB birds. Of the 76 species of rainforest birds regarded as cooperative, territorial and frugivorous, approximately 22.4% of these are hornbills. Also included were aracaris, toucanets, Lybiid barbets, corvids, tanagers and trumpeters (Cockburn 2006, Jetz & Rubenstein 2011).

#### **4.4 Does body size influence sociality in hornbills?**

Hornbills are probably one of the largest CB birds, and ground-hornbills are enormous apart from the Rhea, Crowned Eagle and Bearded Vulture (Jetz & Rubenstein 2011). However, CB

behaviour in Bucerotidae ranges widely in size and habits, from the diminutive territorial insectivorous *T. camurus* of tropical humid forests to large faunivorous ground-hornbills that inhabit savannahs and dry woodlands (Kemp 2001, Kinnaird & O'Brien 2007). Alongside diet, body mass was indicated as a strong overall predictor of CB and exhibits a strong phylogenetic signal in the global dataset (Jetz & Rubenstein 2011). Model combinations (based on CAIC) selected decreasing body mass to be a significant predictor of CB in hornbills. This result is congruent with African starlings, wherein reduction in body size is associated with CB (Rubenstein & Lovette 2009). Similarly, higher incidence of CB was noted in other smaller African birds, suggesting an association between decrease in body mass and energetic factors (du Plessis *et al.* 1995). This result is rather confounding since body size is usually correlated with longevity, as most large birds are long-lived, thus linking increased size with survival rate (Blumstein & Møller 2008). By comparison, the smallest CB hornbill (*T. camurus*) is still relatively large by passerine standards. It is rather unclear how smaller body size in hornbills is linked to CB behaviour, and further analysis is needed.

One possible explanation is that body size relates to nest-hole size, which is a proxy for nest availability. Presumably, competition for nest-cavities is greater among smaller hornbills, as many cavity-nesting birds are small and there maybe more small holes than large ones. Even among small cavity-nesting species, size and abundance of suitable cavities were a limiting resource (Dobkin *et al.* 1993). Hornbills are a classic example of a specialized cavity-nester, as they practise a unique behaviour of nest-sealing (Kemp 1995). The availability of ample-sized safe nest sites restrains successful reproduction in hornbills (Poonswad *et al.* 2005). Likewise, the shortage of suitable nest cavities may be a limiting factor within guarded territories and thus reduces opportunities for breeding (du Plessis *et al.* 1995). African barbets, todies, aracaris, jacamars, bee-eaters, hoopoes, woodhoopoes, parrots and woodpeckers are cavity-nesting birds that have equally high incidences of CB species (Cockburn 2006).

#### **4.5 Territoriality**

Our findings based on phylogenetically controlled CAIC substantiates the significant positive relationship between social system and territoriality. This relationship reaffirms the importance of territorial behaviour as a precursor to group defence and cooperation. Earlier studies suggested that territorial behaviour is a key predictor in the development of CB in many bird species, since territoriality defines the limits of a territory (Ridley *et al.* 2005). Territory inheritance drives CB in long-lived species like hornbills, and consequences leading to philopatry explains why CB is often found in stable environments (Ridley *et al.* 2005). Group territoriality is a recognized form of CB, since cooperative territory-defence improves breeding success (Theuerkauf *et al.* 2009). Helpers improve reproductive success by feeding and guarding nestlings, and by defending territories from cavity competitors given the limited cavities for nesting (Restrepo & Mondragon 1998).

Cavities are regarded as a critical limiting resource that influenced extreme territoriality and development of CB in Green Woodhoopoes, but also obligatory for roosting (Ligon & Ligon 1990). Given that many CB hornbills have small body masses, appropriating the right-sized cavity that is suitable for plastering is indeed difficult. The habitat saturation hypothesis maintains that finding this rare commodity essential for breeding restricts the need to disperse. With this, the benefits of philopatry appear obvious, particularly if young birds are constrained from dispersing due to shortage of breeding territories. It seems that habitat saturation and territory quality are involved in the development of sociality (Komdeur 1992), and are more evident for specialized cavity-nesters like hornbills. A critical shortage of suitable breeding territories, along with risks associated with breeding failure or dispersal due to inexperience, may force young birds to remain in natal groups,.

#### **4.6 Integrating the environmental stability hypothesis with CB**

A combination of factors probably influenced the evolution of CB in Bucerotidae. This involves the integration of ancestry, stable warm humid habitats, frugivory, reduced body size and territoriality, all interacting in some degree to promote delayed dispersal and helping in 25

hornbills species. Our findings highlight the role of environmental stability, frugivory and territoriality as strong predictors of CB in hornbills. In long-lived frugivores like hornbills, stable inter-annual and intra-annual climatic stability promote cooperation for the following reasons: (1) extended survival of territorial pairs and longer temporal occupancy of territories; (2) increased population density has limiting effects on inherently seasonal fruit crops instigating philopatry via the habitat saturation hypothesis; (3) facilitated stable habitats that encourage group territoriality and monopoly of limited resources (e.g. fruit, nest sites) or afford eventual territory inheritance; (4) and impacted less on food variability since frugivores are less sensitive to fluctuations in climate than insectivores. Kinnaird & O'Brien (2007) agree that the relatively high incidence of CB in hornbills can be attributed to the combined effects of saturation, philopatry, ecological limits and ancestry.

Overall, we can find similarities and differences between CB hornbills with other cooperative frugivores (e.g. aracaris, toucan barbet, trumpeters). They all inhabit stable tropical environments that promote longevity in monogamous pairs (Cornwallis *et al.* 2010) and are conducive to philopatry (Brown 1987, Hatchwell 2009), thus facilitating delayed dispersal, cooperation and group territoriality (Gaston 1978). Although some frugivorous CB birds have predisposed lineages for cooperation, this does not necessarily guarantee its emergence (Kinnaird & O'Brien 2007), thus reiterating the importance of sedentariness and habitation of relatively invariable warm climates, which leads to the presumption that CB is probably driven by both life-history predisposition and ecological facilitation. Essentially, longevity and territoriality constrains hornbills and other CB frugivores to be sociable, since low adult mortality is associated with being sedentary in the tropics and results in reduced population turnover and reduced dispersal (Hatchwell & Komdeur 2000). Delayed dispersal as a result of philopatry (Cockburn & Russell 2011) is a consequence of territory inheritance (Ridley *et al.* 2005).

Environmental stability encourages aseasonal resource availability and maintenance of year-round territories leading to habitat saturation, and eventually ecological limitation (Ridley *et al.* 2005). In the same way, the life history hypothesis also explains habitat saturation as prevalent in certain CB species, since a slow life history within an invariable climate predisposes certain



avian families to CB due to occupancy of year-round territories (Arnold & Owens 1998). Thus, guarding limited resources, particularly inadequate suitable nesting cavities and aseasonal fruit sources influence the development of group territoriality in cavity-nesting frugivores such as hornbills, barbets and aracaris. To some extent, our findings agree with Kemp (2001) and Kinnaird & O'Brien (2007) that both the habitat saturation hypothesis and the expanded ecological constraints hypothesis best explain how CB behaviour came about in hornbills. In fact, we find more CB hornbills in Asia (17 species) than in Africa (8 species), where they are predominantly frugivorous, territorial and generally occur in warm humid forests with stable temperature and precipitation. But as a point of contention, ground-hornbills (and even *T. camurus*) do not necessarily fall within the general dogma for frugivorous hornbills, and therefore other factors possibly influence their expression of CB behaviour.

## 5 CONCLUSION

Cockburn & Russell (2011) criticized the benchmark findings of Jetz & Rubenstein (2011), suggesting that the key role of environmental uncertainty in influencing CB behaviour is generally relevant for the broad comparison of the world's birds. We concur with Cockburn & Russell (2011) that CB is equally associated with seasonal and predictable environments. However, both hypotheses do not necessarily apply to the entire assemblage of CB birds, as there are always exceptions. We believe environmental variability is more broadly associated with insectivory in the global dataset and a different scenario impacts on sociality in non-insectivores. Here, we conclude that environmental stability plays a key role in the evolution of CB in predominantly frugivorous hornbills. Climatic stability promotes cooperation as a result of philopatry in these long-lived birds and delay dispersal into limited breeding territories. Overall, the evolution of sociality in hornbills may be an integration of diet, stable tropical humid forest habitats, territoriality and a lineage predisposed to CB behaviour. Hence, global trends may or may not apply to particular species or groups that occupy certain habitats, distinct distributions or even trophic groups. Confounding

results between globally driven insectivores and frugivorous hornbills therefore reflect possible taxon-dependent interactions. Indeed, environmental and biotic factors underpinning cooperation (e.g. climate, diet) vary across avian groups. More studies on other CB clades are needed, particularly on other frugivorous cooperative species to help resolve such issues as the influence of size and aseasonality of some fruiting syndromes. Effect of seasonal faunivory to support hornbill nestling diet also merits further study. Detail studies on other hornbills are also needed to determine the extent of CB behaviour within Bucerotidae, as breeding behaviour is still poorly known in some species. Similarly, hornbills suspected of CB warrant further confirmation. In retrospect, our findings support the premise that an interplay between intrinsic biology and extrinsic environmental factors influence the evolution of sociality in animals.

### Acknowledgements

We are grateful to C. Cooney for his assistance on the use of comparative analyses in R and for invaluable comments from C. Cornwallis, D. Orme and A. Pigot. We acknowledge D. Rubenstein and W. Jetz for allowing us to use their global dataset. This research was in part supported by the Ford Foundation International Fellowship Program, British Ornithologists' Union and North of England Zoological Society.

### REFERENCES

- Arnold, K.E. & Owens, I.P.F. 1998 Cooperative breeding in birds: a comparative test of the life history hypothesis. *Proceedings of the Royal Society B*, **265**, 739–745.
- Arnold, K.E. & Owens, I.P.F. 1999 Cooperative breeding in birds: the role of ecology. *Behavioural Ecology*, **10**, 465–471.
- Blumstein, D.T. & Møller, A.P. Is sociality associated with high longevity in North American birds? *Biology Letters*, **4** (2), 146–148.
- Brown, J.L. 1987 *Helping and communal breeding in birds: ecology and evolution*. Princeton, New Jersey: Princeton University Press.

- Carrière, S.M., André, M., Letourmy, P., Olivier, I. & McKey, D.B. 2002. Seed rain beneath remnant trees in a slash-and-burn agricultural system in southern Cameroon. *Journal of Tropical Ecology*, **18**, 353–374.
- Cockburn, A. 2006 Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society B*, **273**, 1375–1383.
- Cockburn, A. & Russell, A.F. 2011 Cooperative breeding: a question of climate? *Current Biology*, **21**, R195–197.
- Cornwallis, C.K., West, S.A., Davis, K.E. & Griffin, A.S. 2010 Promiscuity and the evolutionary transition to complex societies. *Nature*, **466**, 969–974.
- Covas, R. & Griesser, M. 2007. Life history and the evolution of family living in birds. *Proceedings of the Royal Society B*, **274** (1616), 1349–57.
- Dobkin, D.S., Rich, A.C., Pretare, J.A. & Pyle, W.H. 1993. Nest-site relationships among cavity-nesting birds of riparian and snowpocket Aspen woodlands in the northwestern Great Basin. *The Condor*, **97**, 694–707.
- Drummond, A. J. & Rambaut, A. 2007 BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Dunning, J.B. 2008 *CRC handbook of avian body masses*, 2nd ed., Boca Raton, Florida: CRC Press, Taylor & Francis Group.
- Du Plessis, M.A., Siegfried, W.R. & Armstrong, A.J. 1995 Ecological and life-history correlates of cooperative breeding in South African birds. *Oecologia*, **102**, 180–188.
- Ekman, J. & Ericson, P.G.P. 2006 Out of Gondwanaland; the evolutionary history of cooperative breeding and social behaviour among crows, magpies, jays and allies. *Proceedings of the Royal Society B*, **273**, 1117–1125.
- Ford, H.A., Bell, H., Nias, R. & Noske, R. 1988 The relationship between ecology and the incidence of cooperative breeding in Australian birds. *Behavioral Ecology and Sociobiology*, **22**, 239–249.
- Galama, W., King, C. & Brouwer, K. 2002. EAZA Hornbill Management and Husbandry Guidelines, 1<sup>st</sup> edition. EAZA Hornbill TAG, Amsterdam: National Foundation for Research in Zoological Gardens.
- Gaston, A.J. 1978 The evolution of group territorial behaviour and cooperative breeding. *The American Naturalist*, **112**, 1091–1100.
- Gautier-Hion, A. & Michaloud, G. 1989 Are figs always keystone resources for tropical frugivorous vertebrates? A test in Gabon. *Ecology*, **70**, 1826–1833.
- Hadfield, J.D. 2010 MCMC methods for Multi-response Generalised Linear Mixed Models: The MCMCglmm R Package. *Journal of Statistical Software*, **33**, 1–22.
- Hatchwell, B.J. 2007 Avian reproduction: role of ecology in the evolution of cooperative breeding. *Current Biology*, **17**, R845–R847.

- Hatchwell, B.J. 2009 The evolution of cooperative breeding in birds: kinship, dispersal and life history. *Philosophical Transactions of the Royal Society B*, **364**, 3217–3227.
- Hatchwell, B.J. & Komdeur, J. 2000 Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour*, **59**, 1079–1086.
- Jetz, W. & Rubenstein, D.R. 2011 Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, **21**, 1–7.
- Johns, A.D. 1987. The use of primary and selectively logged rainforest by Malaysian hornbills (Bucerotidae) and implications for their conservation. *Biological Conservation*, **40**, 179–190.
- Kemp, A.C. 1995 *Bird families of the world. The Hornbills: Bucerotiformes*. Oxford, U.K.: Oxford University Press.
- Kemp, A.C. 2001 Family Bucerotidae (Hornbills). In *Handbook of the Birds of the World. Vol. 6. Mousebirds to Hornbills* (ed. J. de Hoyo, A. Elliott & J. Sargatal), pp. 436–523. Barcelona, Spain: Lynx Edicions.
- Kemp, A.C. & Delport, W. 2002. Comments on the status of subspecies in the red-billed hornbill (*Tockus erythrorhynchus*) complex (Aves: Bucerotidae), with the description of a new taxon endemic to Tanzania. *Annals of the Transvaal Museum*, **39**, 1–8.
- Kinnaird, M.F. & O'Brien, T.G. 2007 *The ecology and conservation of Asian hornbills: farmers of the forest*. Chicago, Illinois: The University of Chicago Press.
- Kitamura, S. 2011. Frugivory and seed dispersal by hornbills (Bucerotidae) in tropical forests. *Acta Oecologica*, **37**, 531–541.
- Komdeur, J. 1992 Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. *Nature*, **358**, 493–495.
- Koenig, W. D., Walters, E. L. & Haydock, J. 2011. Fitness consequences of within-brood dominance in the cooperatively breeding acorn woodpecker. *Behavioral Ecology and Sociobiology*, **65** (12), 2229–2238. doi:10.1007/s00265-011-1231-1
- Ligon, J.D. & Burt, D.B. 2004 Evolutionary origins. In *Ecology and Evolution of Cooperative Breeding in Birds* (ed. W.D. Koenig & J.L. Dickinson), pp. 5–34. Cambridge, U.K.: Cambridge University Press.
- Ligon, J.D. & Ligon, S.H. 1990 Green Woodhoopoes: life history traits and sociality. In *Cooperative breeding in birds: long-term studies of ecology and behaviour* (ed. P.B. Stacey & W.D. Koenig), pp. 31–68. Cambridge, U.K.: Cambridge University Press.
- Maddison, W. & Maddison, D. 2010 Mesquite: a modular system for evolutionary analysis, v. 2.74. (see <http://mesquiteproject.org>.)
- Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N. & Pearse, W. 2012. Caper: Comparative Analyses of Phylogenetics and Evolution in R, version 5.

- Pagel, M. & Meade, A. 2006 Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *The American Naturalist*, **167**, 808–825.
- Paradis E., Claude, J. & Strimmer, K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.
- Poonswad, P., Sukkasem, C., Phataramata, S., Hayeemuida, S., Plongmai, K., Chuailua, P., Thiensongrusame, P. & Jirawatkavi, N. 2005 Comparison of cavity modification and community involvement as strategies for hornbill conservation in Thailand. *Biological Conservation*, **122**, 385–393.
- Pruett-Jones, S. 2000 Summary. In *Ecology and Evolution of Cooperative Breeding in Birds*. (ed. W.D. Koenig & J.L. Dickinson), pp. 228–238. Cambridge, U.K.: Cambridge University Press.
- R Development Core Team. 2011 R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna)(<http://www.r-project.org>).
- Restrepo, C. & Mondragon, M.L. 1998 Cooperative breeding in the frugivorous toucan barbet (*Semnornis ramphastinus*). *The Auk*, **115**, 1–4.
- Ridley, J., Yu, D.W. & Sutherland, W.J. 2005 Why long-lived species are more likely to be social: the role of local dominance. *Behavioural Ecology*, **16**, 358–363.
- Rubenstein, D. R. 2011. Spatiotemporal environmental variation, risk aversion , and the evolution of cooperative breeding as a bet-hedging strategy. *Proceedings of the National Academy of Science*, **108** (2), 10816–10822. doi:10.1073/pnas.1100303108
- Rubenstein, D.R. & Lovette, I.J. 2007 Temporal environmental variability drives the evolution of cooperative breeding in birds. *Current Biology*, **17**, 1414–1419.
- Rubenstein, D.R. & Lovette, I.J. 2009 Reproductive skew and selection on female ornamentation in social species. *Nature*, **462** (7274), 786–9.
- Sherman, P.T. 1995 Social organization of cooperatively polyandrous White-winged Trumpeters (*Psophia leucoptera*). *The Auk*, **112**, 296–309.
- Short, L.L. & Horne, J.F.M. 2002. Family Capitonidae (barbets) and Family Ramphastidae (toucans). In *Handbook of the Birds of the World. Vol. 7. Jacamars to Woodpeckers* (ed. J. de Hoyo, A. Elliott & D.A. Christie), pp. 140–272. Barcelona, Spain: Lynx Edicions.
- Stacey, P.B. & Koenig, W.D. 1990 *Cooperative breeding in birds: long-term studies of ecology and behaviour*. Cambridge, U.K.: Cambridge University Press.
- Strassmann, J.E., Queller, D.C., Avise, J.C. & Ayala, F.J. (eds.) 2011. In the Light of Evolution, Volume V: Cooperation and Conflict. Washington DC: National Academies Press.
- Theuerkauf, J., Rouys, S., Mériot, J.M. & Gula, R. 2009 Group territoriality as a form of cooperative breeding in the flightless Kagu (*Rhynochetos jubatus*) of New Caledonia. *The Auk*, **126**, 371–375.

## CHAPTER 3

Venables, W. N. & Ripley, B. D. 2002 Modern Applied Statistics with S. Fourth Edition. New York: Springer.

**Table 3.1:** Evolutionary pathway of cooperative breeding in hornbills based on likelihood ratio of discrete models using RJMCMC in BAYESTRAITS (Pagel & Meade 2006).

| Character      | In Lh  | De Lh  | LRT    | p-value | $\alpha$ | $\beta$ | Root posterior probability |
|----------------|--------|--------|--------|---------|----------|---------|----------------------------|
| Habitat        | -79.15 | -80.41 | -2.51  | 1.00    | 0.07     | 0.07    | 0.50 (Coop, Humid forest)  |
| Primary diet   | -74.03 | -71.37 | -5.32  | 1.00    | 0.06     | 0.06    | 0.50 (Noncoop, Nonfruit)   |
| Territoriality | -81.57 | -74.69 | -13.76 | 1.00    | 0.09     | 0.09    | 0.50 (Coop, Territorial)   |
| Body mass      | -72.92 | -70.69 | -4.46  | 1.00    | 13.10    | 13.10   | 1.00 (Coop, Large)         |

Abbreviations used, In: Independent; De: Dependent; Lh: Log likelihood; LRT: Likelihood ratio test;  $\alpha$ : forward transition;  $\beta$ : reverse transition; Coop: Cooperative breeding; Noncoop: Non-cooperative breeding.

**Table 3.2:** Comparative analysis of independent contrasts between social system in hornbills against selected biotic and environmental variables, based on four different regression methods taking into account phylogenetic non-independence. Estimates were made using the R package caper (Orme et al. 2012), which calculated fits of linear models using pgl, crunch and brunch algorithms, and MCMCglmm (Hadfield 2010), which calculated fit of generalized linear mix models (glmm) using the Markov chain Monte Carlo (MCMC) method. Abbreviations used were based from R packages caper and MCMCglmm.

| Variables <sup>a</sup> | pgls    | MCMCglmm  |                  | brunch/crunch |                          |
|------------------------|---------|-----------|------------------|---------------|--------------------------|
|                        | t-value | Posterior | DIC <sup>b</sup> | Estimate      | F-statistic <sup>c</sup> |
| Preferred habitat      | 0.07    | 59.56     | 1.68             | 0.01          | 0.00                     |
| Primary diet           | 1.50    | 119.5     | 1.45             | 0.07          | 0.07                     |
| Territoriality         | 2.46*   | 247.68    | 1.45*            | 0.52          | 6.07                     |
| Body mass              | -0.50   | -55.54    | 1.52             | -0.18         | 3.25                     |
| Mean annual Temp       | 0.73    | 876.9     | 1.44             | 0.50          | 0.53                     |
| Mean annual Precip     | 0.31    | 153.6     | 1.43             | 0.04          | 0.09                     |
| Temp Var within year   | -2.37*  | -126.87   | 1.24*            | -0.18         | 5.63*                    |
| Temp Var among years   | -1.63   | -298.8    | 1.61             | -0.52         | 2.65                     |
| Precip Var within year | -2.29*  | -124.82   | 1.34*            | -0.18         | 5.24*                    |
| Precip Var among years | -2.28*  | -202.31   | 1.34*            | -0.29         | 5.19*                    |

<sup>a</sup>Temp: temperature; Precip: precipitation; Var: variation

<sup>b</sup>MCMCglmm based on DIC: deviance information criterion

<sup>c</sup>F-statistic calculated each with 1 and 59 degrees of freedom

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .



**Table 3.3:** Fitted models testing relationship and interaction of social system with trait evolution in hornbills using comparative phylogenetic analysis of independent contrasts. Models were selected based on calculated Akaike information criterion (AIC) and analysis of variance(ANOVA) of different brunch and crunch model combinations using the R package caper (Orme et al. 2012).

| Brunch (3 model combinations) <sup>a</sup> | ANOVA <sup>b</sup> | AIC    | Best-fit model <sup>a</sup> |
|--------------------------------------------|--------------------|--------|-----------------------------|
| Diet & Territory                           | n.s.               | 2.71   | Territory                   |
| Habitat & Territory                        | n.s.               | 2.71   | Territory                   |
| Territory & Nest hole                      | n.s.               | 2.71   | Territory                   |
| Diet & Nest hole                           | n.s.               | 3.99   | Nest hole                   |
| Habitat & Nest hole                        | n.s.               | 3.99   | Nest hole                   |
| Diet & Habitat                             | n.s.               | 4.09   | Diet                        |
| Crunch (4 model combinations)              | ANOVA              | AIC    | Best-fit model              |
| Body mass & VarPrecip3mo                   | 0.03               | -88.45 | Body mass & VarPrecip3mo    |
| Body mass & VarPrecip3yr                   | 0.03               | -87.74 | Body mass & VarPrecip3yr    |
| Body mass & VarTemp3mo                     | n.s.               | -85.27 | Body mass & VarTemp3mo      |
| AveTemp & VarTemp3mo                       | n.s.               | -84.01 | VarTemp3mo                  |
| AvePrecip & VarTemp3mo                     | n.s.               | -84.01 | VarTemp3mo                  |
| VarTemp3mo & VarTemp3yr                    | n.s.               | -84.01 | VarTemp3mo                  |
| VarTemp3mo & VarPrecip3mo                  | n.s.               | -84.01 | VarTemp3mo                  |
| VarTemp3mo & VarPrecip3yr                  | n.s.               | -84.01 | VarTemp3mo                  |
| AveTemp & VarPrecip3mo                     | n.s.               | -83.65 | VarPrecip3mo                |
| AvePrecip & VarPrecip3mo                   | n.s.               | -83.65 | VarPrecip3mo                |
| VarTemp3yr & VarPrecip3mo                  | n.s.               | -83.65 | VarPrecip3mo                |
| VarPrecip3mo & VarPrecip3yr                | n.s.               | -83.65 | VarPrecip3mo                |
| AveTemp & VarPrecip3yr                     | n.s.               | -83.61 | VarPrecip3yr                |
| AvePrecip & VarPrecip3yr                   | n.s.               | -83.61 | VarPrecip3yr                |
| VarTemp3yr & VarPrecip3yr                  | n.s.               | -83.61 | VarPrecip3yr                |
| Body mass & VarTemp3yr                     | n.s.               | -82.71 | Body mass & VarTemp3yr      |
| Body mass & AveTemp                        | n.s.               | -81.77 | Body mass                   |
| Body mass & AvePrecip                      | n.s.               | -81.77 | Body mass                   |
| AveTemp & VarTemp3yr                       | n.s.               | -81.18 | VarTemp3yr                  |
| AvePrecip & VarTemp3yr                     | n.s.               | -81.18 | VarTemp3yr                  |
| AveTemp & AvePrecip                        | n.s.               | -79.08 | AveTemp                     |

<sup>a</sup>Abbreviations used in models, Ave: average; Temp: temperature; Precip: Precipitation; mo: months; yr: year;

<sup>b</sup>results of ANOVA, n.s.: not significant.

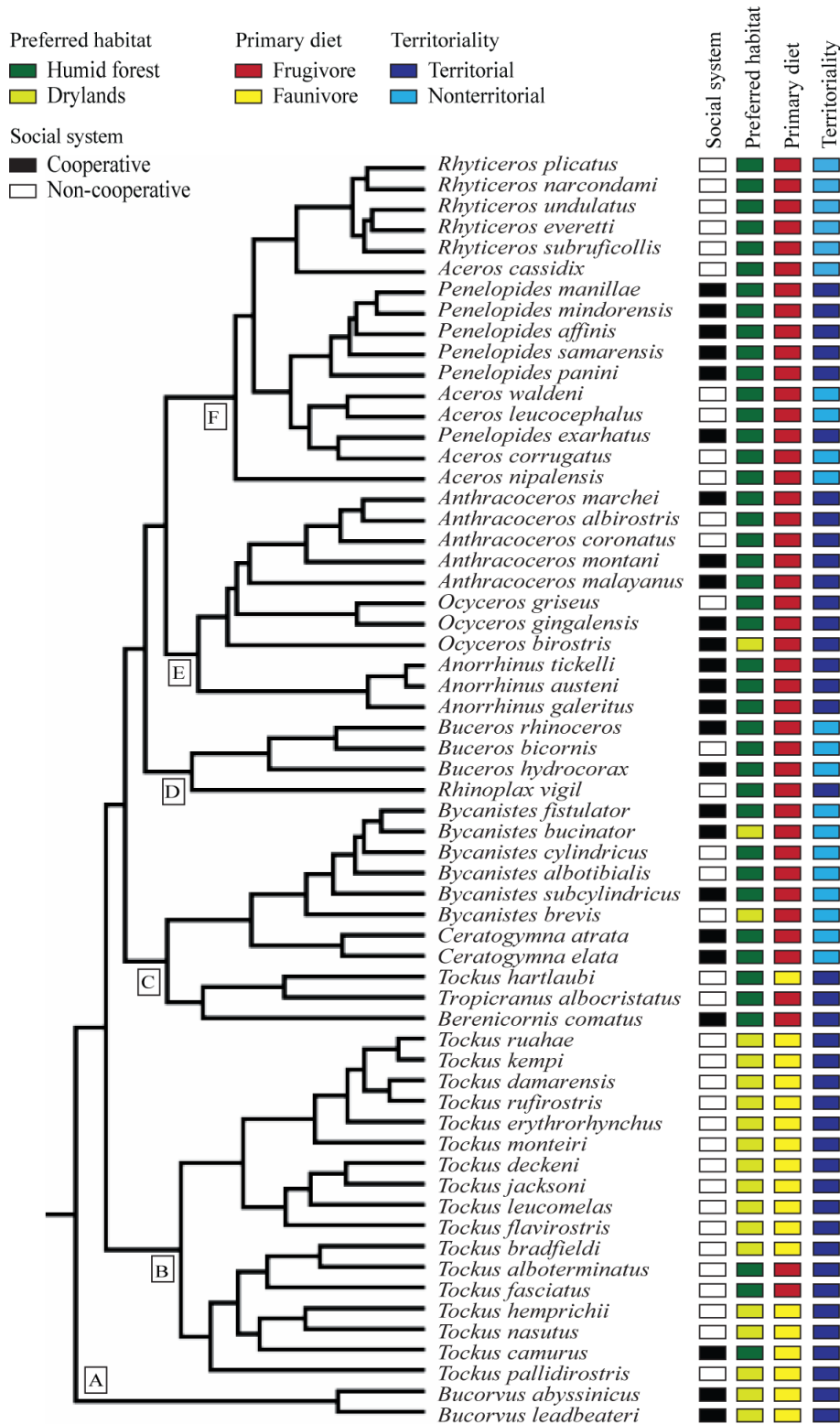


**Figure 3.1:** Molecular phylogeny of hornbills and their social system in relation to preferred habitat, primary diet and territoriality. This ultrametric Bayesian topology is based on concatenated nuclear and mitochondrial genes. All characters are discrete and treated as binary, with a key given above the tree. Boxes with letters at key nodes indicate major clades within Bucerotidae, this includes A: *Bucorvus* clade; B: *Tockus* clade; C: *Berenicornis* clade; D: *Rhinoplax* clade; E: *Anorrhinus* clade; and F: *Aceros* clade.

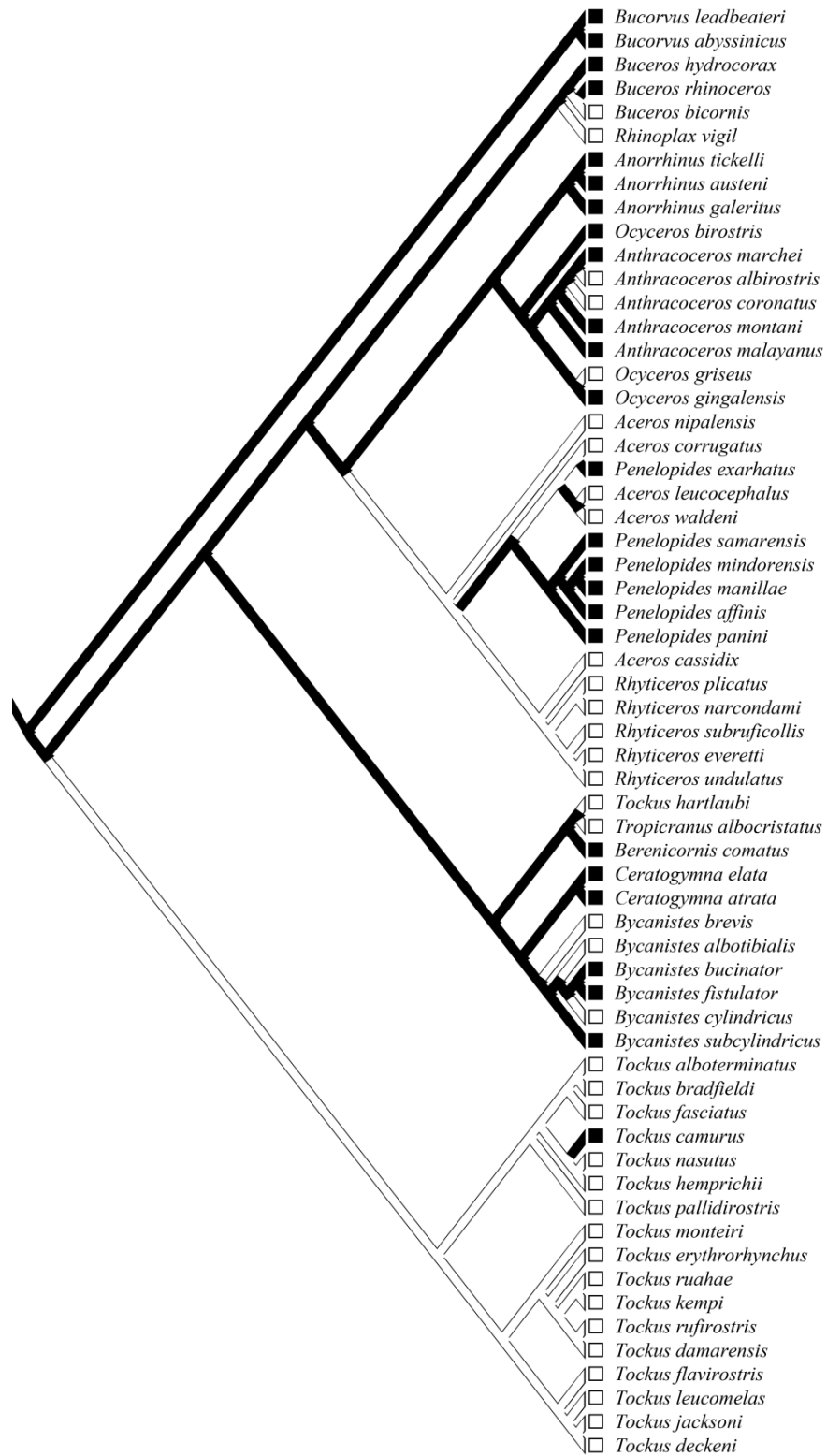


**Figure 3.2:** Ancestral state reconstruction of social system in hornbills using stochastic character mapping constructed in Mesquite v2.74 (Maddison & Maddison 2010). Black squares and shading indicate cooperative breeding species and lineages, while white squares and shading indicate non-cooperative breeding species and lineages.

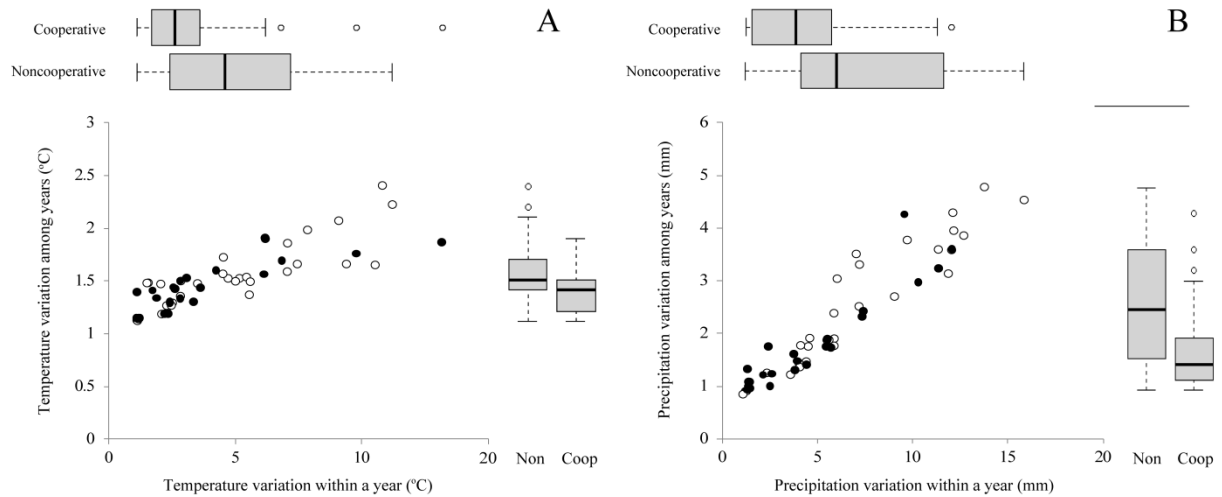


**Figure 3.3:** Prevalence of cooperative breeding hornbills in stable environments with low variability in temperature and precipitation. Individual circles in the scatterplots represent climatic niche position of a single species, black circles refer to cooperative (“Coop”) breeders and white circles refer to non-cooperative (“Non”) breeding hornbills; box plots summarize these positions for a single axis, with ends of boxes indicating position of upper and lower quartile of data, and small open circles refer to outliers.

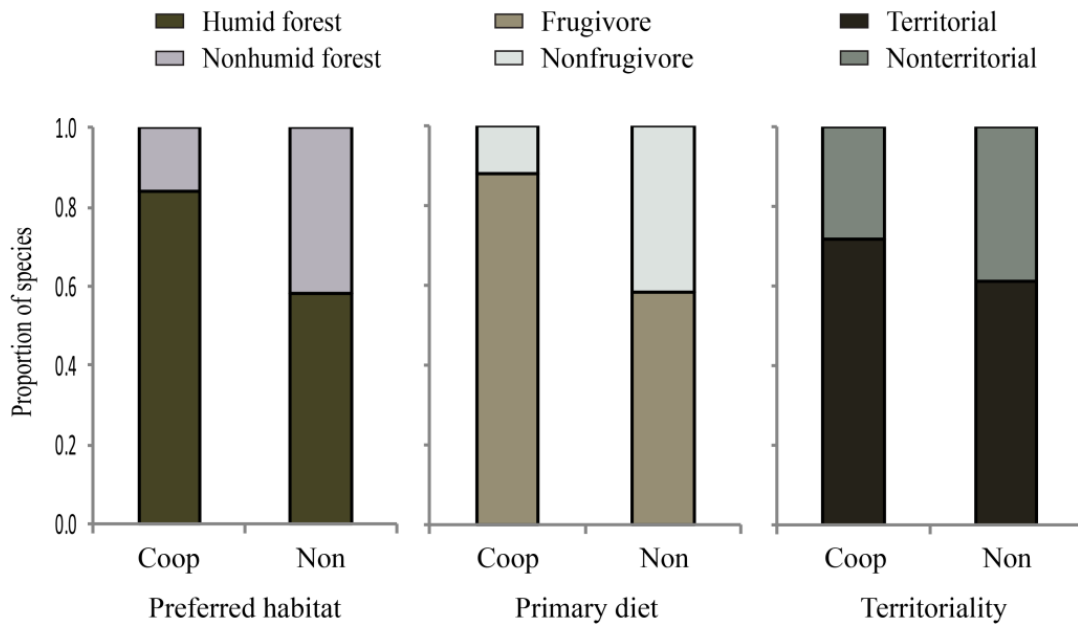


**Figure 3.4:** Proportion of cooperative and non-cooperative breeding hornbills based on different preferred habitats, primary diet and territoriality. All characters are discrete and treated as binary, with a key given above each bar graph. Columns separated into cooperative (“Coop”) breeders and non-cooperative (“Non”) breeding hornbills.

## Supplementary information

**Table S3.1:** Dataset of categorical and continuous biotic variables used for comparative analyses in hornbills.

| Taxon                            | Social <sup>a</sup><br>System | Preferred <sup>b</sup><br>habitat | Primary <sup>c</sup><br>diet | Territoriality <sup>d</sup> | Nest <sup>e</sup><br>hole | Body <sup>f</sup><br>mass |
|----------------------------------|-------------------------------|-----------------------------------|------------------------------|-----------------------------|---------------------------|---------------------------|
| <i>Aceros cassidix</i>           | NonC                          | Humid                             | Frug                         | NonT                        | 350                       | 2360                      |
| <i>Aceros corrugatus</i>         | NonC                          | Humid                             | Frug                         | NonT                        | 350                       | 1590                      |
| <i>Aceros leucocephalus</i>      | NonC                          | Humid                             | Frug                         | NonT                        | 350                       | 1086                      |
| <i>Aceros nipalensis</i>         | NonC                          | Humid                             | Frug                         | NonT                        | 350                       | 2385                      |
| <i>Aceros waldeni</i>            | NonC                          | Humid                             | Frug                         | NonT                        | 350                       | 1748.8                    |
| <i>Anorrhinus austeni</i>        | Coop <sup>g</sup>             | Humid                             | Frug                         | Terri                       | 350                       | 1079.5                    |
| <i>Anorrhinus galeritus</i>      | Coop <sup>g</sup>             | Humid                             | Frug                         | Terri                       | 350                       | 1172                      |
| <i>Anorrhinus tickelli</i>       | Coop <sup>g</sup>             | Humid                             | Frug                         | Terri                       | 350                       | 792.6                     |
| <i>Anthracoceros albirostris</i> | NonC                          | Humid                             | Frug                         | Terri                       | 350                       | 892.9                     |
| <i>Anthracoceros coronatus</i>   | NonC                          | Humid                             | Frug                         | Terri                       | 350                       | 806.7                     |
| <i>Anthracoceros malayanus</i>   | Coop <sup>h</sup>             | Humid                             | Frug                         | Terri                       | 350                       | 1050                      |
| <i>Anthracoceros marchei</i>     | Coop <sup>h</sup>             | Humid                             | Frug                         | Terri                       | 350                       | 750                       |
| <i>Anthracoceros montani</i>     | Coop <sup>h</sup>             | Humid                             | Frug                         | Terri                       | 200                       | 714.6                     |
| <i>Berenicornis comatus</i>      | Coop <sup>g</sup>             | Humid                             | Frug                         | Terri                       | 350                       | 1470                      |
| <i>Buceros bicornis</i>          | NonC                          | Humid                             | Frug                         | NonT                        | 600                       | 2790.8                    |
| <i>Buceros hydrocorax</i>        | Coop <sup>g</sup>             | Humid                             | Frug                         | NonT                        | 350                       | 1409.6                    |
| <i>Buceros rhinoceros</i>        | Coop <sup>h</sup>             | Humid                             | Frug                         | NonT                        | 600                       | 2371.6                    |
| <i>Bucorvus abyssinicus</i>      | Coop <sup>i</sup>             | NonH                              | NonF                         | Terri                       | 600                       | 4000                      |
| <i>Bucorvus leadbeateri</i>      | Coop <sup>g</sup>             | NonH                              | NonF                         | Terri                       | 600                       | 3743.6                    |
| <i>Bycanistes albotibialis</i>   | NonC                          | Humid                             | Frug                         | NonT                        | 350                       | 1137.8                    |
| <i>Bycanistes brevis</i>         | NonC                          | NonH                              | Frug                         | NonT                        | 350                       | 1232.8                    |
| <i>Bycanistes bucinator</i>      | Coop <sup>h</sup>             | NonH                              | Frug                         | NonT                        | 200                       | 639.4                     |
| <i>Bycanistes cylindricus</i>    | NonC                          | Humid                             | Frug                         | NonT                        | 350                       | 921                       |
| <i>Bycanistes fistulator</i>     | Coop <sup>i</sup>             | Humid                             | Frug                         | NonT                        | 115                       | 508.1                     |
| <i>Bycanistes subcylindricus</i> | Coop <sup>h</sup>             | Humid                             | Frug                         | NonT                        | 350                       | 1195.4                    |
| <i>Ceratogymna atrata</i>        | Coop <sup>i</sup>             | Humid                             | Frug                         | NonT                        | 350                       | 1194.8                    |
| <i>Ceratogymna elata</i>         | Coop <sup>i</sup>             | Humid                             | Frug                         | NonT                        | 350                       | 1917                      |
| <i>Ocyrceros birostris</i>       | Coop <sup>h</sup>             | NonH                              | Frug                         | Terri                       | 115                       | 375                       |
| <i>Ocyrceros gingalensis</i>     | Coop <sup>h</sup>             | Humid                             | Frug                         | Terri                       | 115                       | 238                       |
| <i>Ocyrceros griseus</i>         | NonC                          | Humid                             | Frug                         | Terri                       | 115                       | 292                       |
| <i>Penelopides affinis</i>       | Coop <sup>g</sup>             | Humid                             | Frug                         | Terri                       | 200                       | 456                       |
| <i>Penelopides exarhatus</i>     | Coop <sup>g</sup>             | Humid                             | Frug                         | Terri                       | 200                       | 370                       |

### CHAPTER 3

|                                  |                   |       |      |       |     |        |
|----------------------------------|-------------------|-------|------|-------|-----|--------|
| <i>Penelopides manillae</i>      | Coop <sup>g</sup> | Humid | Frug | Terri | 200 | 460.9  |
| <i>Penelopides mindorensis</i>   | Coop <sup>h</sup> | Humid | Frug | Terri | 200 | 462    |
| <i>Penelopides panini</i>        | Coop <sup>g</sup> | Humid | Frug | Terri | 200 | 470.5  |
| <i>Penelopides samarensis</i>    | Coop <sup>g</sup> | Humid | Frug | Terri | 200 | 478.4  |
| <i>Rhinoplax vigil</i>           | NonC              | Humid | Frug | Terri | 600 | 2887.6 |
| <i>Rhyticeros everetti</i>       | NonC              | Humid | Frug | NonT  | 200 | 1748.8 |
| <i>Rhyticeros narcondami</i>     | NonC              | Humid | Frug | NonT  | 200 | 697.2  |
| <i>Rhyticeros plicatus</i>       | NonC              | Humid | Frug | NonT  | 350 | 1703.8 |
| <i>Rhyticeros subruficollis</i>  | NonC              | Humid | Frug | NonT  | 350 | 2042   |
| <i>Rhyticeros undulatus</i>      | NonC              | Humid | Frug | NonT  | 350 | 2214.6 |
| <i>Tockus albeterminatus</i>     | NonC              | Humid | Frug | Terri | 115 | 223.7  |
| <i>Tockus bradfieldi</i>         | NonC              | NonH  | NonF | Terri | 115 | 196.5  |
| <i>Tockus camurus</i>            | Coop <sup>g</sup> | Humid | NonF | Terri | 115 | 103.8  |
| <i>Tockus damarensis</i>         | NonC              | NonH  | NonF | Terri | 115 | 192.5  |
| <i>Tockus deckeni</i>            | NonC              | NonH  | NonF | Terri | 115 | 169.5  |
| <i>Tockus erythrorhynchus</i>    | NonC              | NonH  | NonF | Terri | 115 | 154    |
| <i>Tockus fasciatus</i>          | NonC              | Humid | Frug | Terri | 115 | 258.8  |
| <i>Tockus flavirostris</i>       | NonC              | NonH  | NonF | Terri | 115 | 216.7  |
| <i>Tockus hartlaubi</i>          | NonC              | Humid | NonF | Terri | 115 | 98.5   |
| <i>Tockus hemprichii</i>         | NonC              | NonH  | NonF | Terri | 200 | 297    |
| <i>Tockus jacksoni</i>           | NonC              | NonH  | NonF | Terri | 115 | 167.7  |
| <i>Tockus kemp</i>               | NonC              | NonH  | NonF | Terri | 115 | 138.6  |
| <i>Tockus leucomelas</i>         | NonC              | NonH  | NonF | Terri | 115 | 188.3  |
| <i>Tockus monteiri</i>           | NonC              | NonH  | NonF | Terri | 115 | 289.8  |
| <i>Tockus nasutus</i>            | NonC              | NonH  | NonF | Terri | 200 | 179.6  |
| <i>Tockus pallidirostris</i>     | NonC              | NonH  | NonF | Terri | 115 | 233    |
| <i>Tockus ruahae</i>             | NonC              | NonH  | NonF | Terri | 115 | 126.5  |
| <i>Tockus rufirostris</i>        | NonC              | NonH  | NonF | Terri | 115 | 134.3  |
| <i>Tropicranus albocristatus</i> | NonC              | Humid | Frug | Terri | 200 | 289.4  |

Abbreviations used, Coop: Cooperative breeding; NonC: Noncooperative breeding; Humid: Humid forests; NonH: Nonhumid forests; Frug: Frugivorous; NonF: Nonfrugivorous; Terri: Territorial; NonT: Non-territorial.

<sup>a</sup>Social system compiled from Cockburn 2006, with modifications from Brown 1987, Kemp 1995, Kemp 2001 and Jetz & Rubenstein 2011.

<sup>b</sup>Primary habitat derived from Kemp 2001 and Kinnaird & O'Brien 2007.

<sup>c</sup>Predominant diet based on Kemp 2001, with changes from Carrière et al. 2002 and Kitamura 2011.

<sup>d</sup>Territoriality compiled from Johns 1987, Kemp 1995, Kemp 2001 and Kinnaird & O'Brien 2007.

<sup>e</sup>Nest hole dimensions based entirely on categories by Galama et al. 2002.

<sup>f</sup>Body mass derived from Dunning 2008, with additions from Kemp 1995 and Kemp & Delport 2002.

## CHAPTER 3

<sup>g</sup>confirmed obligate Coop (Kemp 1995, Kemp 2001)

<sup>h</sup>confirmed facultative Coop (Kemp 1995, Kemp 2001)

<sup>i</sup>questionable/suspected and unconfirmed facultative Coop (Kemp 1995, Kemp 2001)



# CHAPTER 3

**Table S3.2:** Dataset of environmental variables used for comparative analyses in hornbills, derived from Jetz & Rubenstein (2011). Temperature is given in degrees Celsius and precipitation is given in millilitres.

|                                  | Mean <sup>a</sup> | AveVar <sup>b</sup> | AveVar <sup>c</sup> | Mean <sup>d</sup> | AveVar <sup>e</sup> | AveVar <sup>f</sup> |
|----------------------------------|-------------------|---------------------|---------------------|-------------------|---------------------|---------------------|
|                                  | annual            | Temp                | Temp                | annual            | Precip              | Precip              |
| Taxon                            | Temp              | 3months             | 3years              | Precip            | 3months             | 3years              |
| <i>Aceros cassidix</i>           | 25.3              | 1.1                 | 1.1                 | 2089.5            | 1.5                 | 1.1                 |
| <i>Aceros corrugatus</i>         | 25.9              | 1.1                 | 1.1                 | 2776.3            | 1.2                 | 0.9                 |
| <i>Aceros leucocephalus</i>      | 25.7              | 1.5                 | 1.5                 | 2697.7            | 1.4                 | 1                   |
| <i>Aceros nipalensis</i>         | 21.6              | 10.5                | 1.7                 | 1889.7            | 5.9                 | 1.9                 |
| <i>Aceros waldeni</i>            | 26.8              | 2.1                 | 1.2                 | 2419.5            | 2.4                 | 1.3                 |
| <i>Anorrhinus austeni</i>        | 23.9              | 9.8                 | 1.8                 | 1751.8            | 5.5                 | 1.7                 |
| <i>Anorrhinus galeritus</i>      | 26                | 1.2                 | 1.1                 | 2734.6            | 1.3                 | 0.9                 |
| <i>Anorrhinus tickelli</i>       | 23.1              | 4.3                 | 1.6                 | 1998.4            | 7.4                 | 2.4                 |
| <i>Anthracoceros albirostris</i> | 24.8              | 7                   | 1.6                 | 2169.8            | 4.5                 | 1.8                 |
| <i>Anthracoceros coronatus</i>   | 26                | 9.4                 | 1.7                 | 1461.7            | 9.7                 | 3.8                 |
| <i>Anthracoceros malayanus</i>   | 26                | 1.1                 | 1.1                 | 2761.1            | 1.2                 | 0.9                 |
| <i>Anthracoceros marchei</i>     | 26.9              | 1.9                 | 1.3                 | 2135.1            | 2.5                 | 1                   |
| <i>Anthracoceros montani</i>     | 26.8              | 1.1                 | 1.4                 | 1872.5            | 1.3                 | 1.3                 |
| <i>Berenicornis comatus</i>      | 24.3              | 1.2                 | 1.2                 | 1584.1            | 1.5                 | 1                   |
| <i>Buceros bicornis</i>          | 24.6              | 7.4                 | 1.7                 | 2067.7            | 5.6                 | 1.9                 |
| <i>Buceros hydrocorax</i>        | 25.9              | 2.6                 | 1.4                 | 2655.1            | 2.6                 | 1.2                 |
| <i>Buceros rhinoceros</i>        | 25.9              | 1.1                 | 1.1                 | 2743.8            | 1.4                 | 1                   |
| <i>Bucorvus abyssinicus</i>      | 25.3              | 6.2                 | 1.9                 | 1013.5            | 12.1                | 3.6                 |
| <i>Bucorvus leadbeateri</i>      | 21.8              | 6.8                 | 1.7                 | 961.8             | 11.3                | 3.2                 |
| <i>Bycanistes albotibialis</i>   | 24.6              | 2.8                 | 1.5                 | 1770.7            | 5.5                 | 1.9                 |
| <i>Bycanistes brevis</i>         | 21.7              | 5.1                 | 1.5                 | 982.5             | 5.9                 | 2.4                 |
| <i>Bycanistes bucinator</i>      | 22.1              | 6.1                 | 1.6                 | 1015.6            | 10.3                | 3                   |
| <i>Bycanistes cylindricus</i>    | 25.8              | 2.5                 | 1.3                 | 2069.3            | 4.4                 | 1.5                 |
| <i>Bycanistes fistulator</i>     | 24.8              | 2.8                 | 1.3                 | 1736              | 5.7                 | 1.7                 |
| <i>Bycanistes subcylindricus</i> | 23.8              | 2.8                 | 1.5                 | 1491.9            | 5.5                 | 1.9                 |
| <i>Ceratogymna atrata</i>        | 24.6              | 2.4                 | 1.3                 | 1771              | 4.4                 | 1.4                 |
| <i>Ceratogymna elata</i>         | 26.2              | 3.4                 | 1.3                 | 2033.9            | 7.3                 | 2.3                 |
| <i>Ocyrceros birostris</i>       | 25.6              | 13.2                | 1.9                 | 1127.5            | 9.6                 | 4.3                 |
| <i>Ocyrceros gingalensis</i>     | 27.1              | 3.1                 | 1.5                 | 1755.5            | 2.4                 | 1.8                 |
| <i>Ocyrceros griseus</i>         | 25.8              | 5.5                 | 1.4                 | 1835.8            | 12.7                | 3.9                 |
| <i>Penelopides affinis</i>       | 25.8              | 1.7                 | 1.4                 | 2650.6            | 1.4                 | 1                   |
| <i>Penelopides exarhatus</i>     | 24.8              | 1.1                 | 1.1                 | 2127              | 1.5                 | 1.1                 |
| <i>Penelopides manillae</i>      | 25.9              | 3.6                 | 1.4                 | 2739.5            | 4                   | 1.5                 |

# CHAPTER 3

|                                  |      |      |     |        |      |     |
|----------------------------------|------|------|-----|--------|------|-----|
| <i>Penelopides mindorensis</i>   | 26.4 | 2.6  | 1.4 | 2421.2 | 3.8  | 1.6 |
| <i>Penelopides panini</i>        | 26.9 | 2.2  | 1.2 | 2448.6 | 2.1  | 1.2 |
| <i>Penelopides samarensis</i>    | 26.5 | 2.3  | 1.2 | 2644.9 | 1.4  | 1.1 |
| <i>Rhinoplax vigil</i>           | 26   | 1.2  | 1.1 | 2754.5 | 1.3  | 0.9 |
| <i>Rhyticeros everetti</i>       | 25.9 | 2.3  | 1.3 | 2776.3 | 4.1  | 1.8 |
| <i>Rhyticeros narcondami</i>     | 27   | 2    | 1.5 | 2980   | 4.6  | 1.9 |
| <i>Rhyticeros plicatus</i>       | 25.3 | 1.5  | 1.5 | 2856.5 | 1.1  | 0.9 |
| <i>Rhyticeros subruficollis</i>  | 24.7 | 4.7  | 1.5 | 2363.8 | 7.2  | 2.5 |
| <i>Rhyticeros undulatus</i>      | 25.2 | 5    | 1.5 | 2342.6 | 4    | 1.5 |
| <i>Tockus alboterminatus</i>     | 22.2 | 5.4  | 1.5 | 1033.8 | 9.1  | 2.7 |
| <i>Tockus bradfieldi</i>         | 21.6 | 10.8 | 2.4 | 571.2  | 15.9 | 4.5 |
| <i>Tockus camurus</i>            | 24.8 | 2.4  | 1.3 | 1836.4 | 3.8  | 1.3 |
| <i>Tockus damarensis</i>         | 20.9 | 9.1  | 2.1 | 527.1  | 13.8 | 4.8 |
| <i>Tockus deckeni</i>            | 23.9 | 3.5  | 1.5 | 613.5  | 7.2  | 3.3 |
| <i>Tockus erythrorhynchus</i>    | 24.1 | 7.8  | 2   | 686.5  | 12.2 | 4   |
| <i>Tockus fasciatus</i>          | 25   | 2.8  | 1.4 | 1719.3 | 5.9  | 1.8 |
| <i>Tockus flavirostris</i>       | 24.7 | 4.5  | 1.6 | 480.3  | 7.1  | 3.5 |
| <i>Tockus hartlaubi</i>          | 24.9 | 2.3  | 1.3 | 1795   | 3.6  | 1.2 |
| <i>Tockus hemprichii</i>         | 22.4 | 4.5  | 1.7 | 725.8  | 6    | 3.1 |
| <i>Tockus jacksoni</i>           | 25.5 | 4.5  | 1.6 | 595.3  | 7.1  | 3.5 |
| <i>Tockus kemp</i>               | 26.6 | 6.2  | 1.9 | 266.2  | 12.1 | 3.6 |
| <i>Tockus leucomelas</i>         | 20.9 | 11.2 | 2.2 | 539.6  | 12.1 | 4.3 |
| <i>Tockus monteiri</i>           | 20.4 | 9.1  | 2.1 | 360.3  | 13.8 | 4.8 |
| <i>Tockus nasutus</i>            | 24.2 | 7    | 1.9 | 830    | 11.4 | 3.6 |
| <i>Tockus pallidirostris</i>     | 22.2 | 5.6  | 1.5 | 1182.9 | 11.9 | 3.2 |
| <i>Tockus ruahae</i>             | 21.2 | 4.5  | 1.6 | 1002   | 7.1  | 3.5 |
| <i>Tockus rufirostris</i>        | 21.3 | 11.2 | 2.2 | 754.7  | 12.1 | 4.3 |
| <i>Tropicranus albocristatus</i> | 25   | 2.5  | 1.3 | 1860.5 | 4.1  | 1.4 |

Abbreviations used, AveVar: Average variation (Standard deviation from average); Temp: temperature;

Precip: precipitation

<sup>a</sup>Mean annual temperature.

<sup>b</sup>Standard deviation of average temperature over 3 months.

<sup>c</sup>Standard deviation of average temperature over 3 years.

<sup>d</sup>Mean annual precipitation.

<sup>e</sup>Standard deviation of monthly average precipitation.

<sup>f</sup>Standard deviation of yearly average precipitation.

## CHAPTER 3

**Table S3.3:** Proportion of cooperative and non-cooperative species of the world's birds and hornbills based on differences in diet, habitat preference, territoriality and body size derived from Jetz & Rubenstein (2011).

|                         | Global birds |                 | Hornbills   |                 |
|-------------------------|--------------|-----------------|-------------|-----------------|
|                         | Cooperative  | Non-cooperative | Cooperative | Non-cooperative |
| Humid forest habitat    | 365 (4.0%)   | 2877 (31.5%)    | 21 (34.4%)  | 21 (34.4%)      |
| Nonhumid forest habitat | 466 (5.1%)   | 5413 (59.4%)    | 4 (6.6%)    | 15 (24.6%)      |
| Frugivorous diet        | 198 (2.2%)   | 2391 (26.2%)    | 22 (36.1%)  | 21 (34.4%)      |
| Nonfrugivorous diet     | 649 (7.1%)   | 6948 (76.2%)    | 3 (4.9%)    | 15 (24.5%)      |
| Territorial species     | 739 (8.1%)   | 6758 (74.1%)    | 18 (29.5%)  | 22 (36.1%)      |
| Nonterritorial species  | 108 (1.2%)   | 2581 (28.3%)    | 7 (11.5%)   | 14 (23.0%)      |
| Large body mass         | 55 (0.6%)    | 792 (8.7%)      | 16 (26.2%)  | 17 (27.9%)      |
| Small body mass         | 787 (8.6%)   | 7584 (83.2%)    | 9 (14.8%)   | 19 (31.5%)      |

## Chapter 4

### **Diet, dispersal and diversification: molecular phylogenies of hornbills and their food-plants reveal the evolutionary history of a classic mutualism**



*Proc. Zool. Soc. London, Tweeddale 1877 p. 825*

*As a consequence, geneticists described evolution simply as a change in gene frequencies in populations, totally ignoring the fact that evolution consists of the two simultaneous but quite separate phenomena of adaptation and diversification.*

Ernst W. Mayr, 1992, *Speciational Evolution or Punctuated Equilibria*

## **Diet, dispersal and diversification: molecular phylogenies of hornbills and their food-plants reveal the evolutionary history of a classic mutualism**

**Juan-Carlos T. Gonzalez<sup>1,2</sup> and Joseph A. Tobias<sup>1</sup>**

<sup>1</sup>*Edward Grey Institute, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*

<sup>2</sup>*Institute of Biological Sciences, University of the Philippines Los Baños, Laguna 4031, Philippines*

### **Abstract**

Mutualistic plant-vertebrate interactions are often proposed as drivers of diversification, for example when coevolutionary adaptation results in reproductively isolated lineages of both flowering plants and their pollinators. An alternative hypothesis of co-diversification involves mutual facilitation of access to novel environments, such as may occur with seed-producing plants and seed dispersing animals. However, this mechanism is difficult to assess directly, and its role in shaping broad-scale patterns of diversity remains unclear. Here, we use fossil-dated molecular phylogenies to examine the evolutionary history of trophic interactions, the timing of colonization events, and the tempo of diversification in a classic mutualism between hornbills and their food-plants. We show that the switch to frugivory occurred ~25 Ma, and was followed by accelerated diversification in frugivorous versus non-frugivorous hornbill lineages, coinciding with the rapid colonization of humid forests across Africa and into Asia. Phylogenetic data also reveal that major bursts of geographic expansion and diversification in hornbill food plants followed the emergence of hornbills, presumably because hornbills

increased the transfer of seeds across unsuitable habitats, including sea-crossings. These results indicate that frugivory is an ecological innovation that can promote biotic diversification, both directly in animals, and indirectly in their food plants, via the mechanism of mutualistic biotic interaction.

### **Keywords**

biotic interchange, Bucerotidae, colonization, dietary shift, diversification, ecological opportunity, frugivory, hornbills, molecular phylogenetics, Palaeotropics

**Running title:** Diet, dispersal and diversification

Formatted for submission to Molecular Ecology

## Introduction

The role of abiotic factors and biotic interactions in understanding distribution patterns and speciation events is an established paradigm in evolutionary ecology (Herrera & Pellymyr 2002; Winkler *et al.* 2009). Both biotic interactions and abiotic factors serve as an impetus for diversification of tropical taxa and contribute extensively to the generation of global biological diversity (Bolmgren & Eriksson 2005; Couvreur *et al.* 2011). However, there are contentions aimed at distinguishing whether biotic or abiotic interactions have a key role in constraining species diversification, thus sparking a number of recent approaches (Ezard *et al.* 2011; Condamine *et al.* 2012). To address this, there has been increased focus on investigating the importance of biotic interactions in promoting diversification (Winkler *et al.* 2009; Rojas *et al.* 2011). Biotic interactions are generally described as relationships occurring among living organisms, and may have positive, negative or neutral effects (Lidicker 1979). Both mutualistic (e.g. frugivory) and antagonistic (e.g. herbivory) associations presumably create and maintain biodiversity (Fleming *et al.* 2009). In fact, these mutual interactions between plants and their pollinators/dispersers are vital to the expansion of terrestrial plant diversity (Ehrlich & Raven 1964, Herrera & Pellymyr 2002).

Mutualistic plant-vertebrate interactions such as frugivory and pollination are often postulated to be important drivers of diversification (Bolmgren & Eriksson 2005; Fleming *et al.* 2009). Some of these interactions are well-defined co-evolutionary adaptations, such as between lineages of both flowering plants and their pollinators (Kiestner *et al.* 1984), and some interactions may show highly congruent phylogenies (Herre *et al.* 1996; Pellmyr 2003). Alternatively, fruits and their dispersal agents follow a different hypothesis of co-diversification, which usually involves ecological innovations or mutual access to novel environments (Lambert & Garber 1998; Rojas *et al.* 2011). In a recent review of fruit and frugivore evolutionary history, Fleming & Kress (2011) proposed that frugivorous birds and mammals radiated extensively after the appearance of major food plants, most of which

emerged during the Oligocene (~30 Ma). However, they pointed out that some temporal congruence occurred in first appearance of several plant–animal combinations (e.g. Cactus & Leaf-nosed bats, *Loranthus* & Honey-eaters), but were uncertain if these matches represented causal co-radiation or simply temporal correlation.

Co-radiation is relatively unknown in frugivores, since relationships between fruit and frugivore phylogenies are still poorly understood (Burns & Lake 2009). Only recently, molecular phylogenetics has allowed dating of lineages along evolutionary time, and this provided an opportunity to compare colonisation histories between these mutualistic interactions (Quental & Marshall 2011). Some phylogenetic comparisons found strong support for parallel diversification between fruits and frugivores (Rojas *et al.* 2011), while others matched the tempo of frugivore divergence with general floristic colonisation (Viseshakul *et al.* 2011). Overall, assessing the mechanism involved in co-diversification has been challenging, and more detailed comparisons of mutualistic interactions are necessary to understand their role in shaping broad-scale patterns of diversity.

To investigate the processes underpinning parallel diversification in fruit-bearing trees and their dispersal agents, we test the hypothesis of mutual facilitation to novel environments and novel innovations on a classic mutualism between hornbills and their food plants. Hornbills are an ideal study system and evolutionary congruence with mutualistic fruit trees have been highlighted previously, but not investigated in detail (Fleming & Kress 2011). Frugivorous hornbills (family Bucerotidae) belong to a clade of ancestrally carnivorous birds with lineages developing into obligate frugivores in tropical Asia and Africa (Kemp 2001). Kemp (1995) had emphasised the importance of comparing evolutionary pathways between hornbills and hornbill-dispersed fruits (HDFs) to further understand the basis for their diversity in the Palaeotropics. However, previous molecular phylogenies were incomplete and showed incongruent estimates of divergence, particularly with the split from basal carnivorous ground-hornbills (33 Ma, Hübner *et al.* 2003 and 52 Ma, Viseshakul *et al.* 2011). Thus, a complete



evolutionary tree based on DNA of all hornbill species is crucial in explaining their dramatic radiation as frugivores in Asia and Africa (Kinnaird & O'Brien 2007).

Both hornbills and large seeded hornbill-dispersed fruits (HDFs, e.g. Annonaceae, Burseraceae, Meliaceae, Myristicaceae) have disjunct distributions in tropical Africa and Asia, and both have proposed ancestral origins in Africa (Weeks *et al.* 2005; Kinnaird & O'Brien 2007; Muellner *et al.* 2008; Zhou *et al.* 2011). It is likely that hornbills facilitated dispersal of HDFs from Africa to Asia, crossing through the Palaeotropical Biotic Interchange (PBI). The PBI is a biogeographic event that facilitated exchange of terrestrial flora and fauna between tropical Africa and Asia during the late Oligocene. It represents an Old World inter-continental link that complements the well-studied Great American Biotic Interchange in the New World (Stehli & Webb, 1985; DaCosta & Klicka, 2008). The closure of the Tethyan Seaway around 25–23 Ma (Harzhauser *et al.* 2007) abated dispersal of biotic elements from East Africa through the Arabian Peninsula towards South Asia (Antoine *et al.* 2003). This connection pre-dates the *Gomphotherium* land-bridge formed in the mid-Miocene (18–17 Ma), which allowed direct exchange of land mammals between Africa and Eurasia (Koufos *et al.* 2005). Growing evidence supports an earlier trans-Tethyan dispersal occurring between late Oligocene and early Miocene, which encouraged Afro-Arabian and Eurasian mammalian turnover (Antoine *et al.* 2003; Kappelman *et al.* 2003; Zalmout *et al.* 2010; Leakey *et al.* 2011).

To understand the evolutionary history and biogeographic implications of trophic interactions between hornbills and HDFs, we constructed fossil-dated molecular phylogenies of both hornbills and key HDFs (Meliaceae, Myristicaceae) to provide a more resolved timing of colonisation events and tempo of diversification.

## Materials and methods

### Ecology and biogeography of hornbills and their food plants

We compiled data on geographical distribution and ecology for all 61 species of hornbills, following Kemp (2001). Distribution was simplified to two major biogeographic regions –African (Africa and Arabian Peninsula) and Asian (India to Southeast Asia, including Melanesia). Ecology of hornbills was classified based on their preferred habitat (humid forests versus drylands) and diet (frugivory versus faunivory). Humid forests were largely represented by lowland evergreen rainforests and hill forests, whereas drylands included savanna grasslands, dry deciduous woodland and desert thornveld. We derived predominant diet mainly from Kemp (2001), Carrière *et al.* (2002) and Kitamura (2011). Hornbills are generally omnivorous but have biases to either fruit or animal food. Species with a fruit-dominated diet (feed regularly on fruits) were categorized as frugivorous, and those noted to feed largely on insects, large arthropods and vertebrates were labelled faunivorous (Gray *et al.* 2007).

### Phylogenetic reconstruction and estimation of divergence times in hornbills

A total of 61 species were used to generate a complete species-level molecular phylogeny of the Family Bucerotidae. Five species were selected as the outgroup (see Table S4.1, see Supplementary Information). The dataset was compiled from complete and partial sequences of the mitochondrial DNA, cytochrome *b* gene (mtDNA *cytb*) and the nuclear gene, cytosolic adenylate kinase 1 intron 5 (AK1 intron 5, Shapiro & Dumbacher 2001). Sequences were derived from GenBank, based on the phylogenetic analysis described in Gonzalez *et al.* (in prep., see Chapter 2) with Accession Nos. XXX-XXX for *cytb* and XXX-XXX for AK1 intron 5 (Table S4.1; see Supporting Information).

Sequences were aligned and concatenated in MEGA v.5.03 (Tamura *et al.* 2011). Final alignments are in FASTA and NEXUS format, and available upon request from the authors. Phylogenetic analysis of the concatenated *cytb*-AK1 intron 5 dataset was conducted using

Bayesian inference following the General Time Reversal model in MRMODELTEST v2.3 (Nylander 2004) based on least scores of the Akaike Information Criterion (AIC) (Table S4.2/ see Supporting Information). A molecular clock test was performed in MEGA5 to determine significant deviation from the null hypothesis of equal evolutionary rate. The test rejected a strict molecular clock ( $\ln L = -27076.3$ ) in favour of the relaxed clock ( $\ln L = -26868.3$ ) at  $P < 0.05$  (Tamura *et al.* 2011).

Tree construction and divergence time estimation were performed in BEAST v.1.6.1 using templates from BEAUTi v.1.6.1 (Drummond & Rambaut 2007). We implemented three independent Markov-chain Monte Carlo (MCMC) runs of 20 million generations, sampled every 10,000 with burn-ins of 2 million. Substitution rates were estimated under an uncorrelated lognormal relaxed clock model, constrained at 0.005 based from estimates for Coraciiformes *cytb* by Pacheco *et al.* (2011). Taxon sets were selected to include the stem to calibrate for “time to most recent common ancestor” ( $t_{\text{MRCA}}$ ), using both molecular divergence data and dated fossil records (Van Tuinen & Dyke 2004). We used molecular estimates from Pacheco *et al.* (2011) to root the tree to 78.56 Ma (A), the molecular age between rollers and hornbills. Nodes representing  $t_{\text{MRCA}}$  (B) between *Bucorvus* and Bucerotinae were calibrated at 47.06 Ma, and between *Aceros* and *Bycanistes* (C) at 31.56 Ma (Pacheco *et al.* 2011). Three fossils were used as a basis for Bucerotidae divergence, namely *Primobucco mcgrewi* dated at 51.66 Ma (Ksepka & Clarke 2010) for  $t_{\text{MRCA}}$  of Coraciidae (D); *Messelirrisor* at 47 Ma (Mayr 2006) for the  $t_{\text{MRCA}}$  of Upupidae and Phoeniculidae (E); and *Bucorvus brailloni* which occurred between 7 and 11.5 Ma (Brunet 1971) for Bucorvinae  $t_{\text{MRCA}}$  (F). We selected the normally distributed prior, with a fixed standard deviation of  $\pm 3.0$  for fossil dates and  $\pm 10.0$  for molecular dates.

Results were diagnosed using TRACER v1.5 (Rambaut & Drummond 2007) and combined trace files confirmed convergence at >200 Effective Sample Sizes. Independent runs were merged in LOGCOMBINER v.1.6.1 (Drummond & Rambaut 2007), each with a burn-in of 500. A single 50% maximum clade credibility (MCC) tree was generated from 4,504 trees using TREEANNOTATOR v.1.6.1. The target MCC tree was visualized in FIGTREE v1.3.1 (Rambaut

2006) with clade robustness based on consistent posterior probabilities (PP). We considered 0.90–0.98 PP as moderate support and >0.98 PP as strong support (Muellner *et al.* 2008). Error bars indicate the 95% highest posterior densities (HPD), which correspond to credible interval values of estimated node ages.

### **Ancestral states reconstruction**

RASP v1.107 (Yu *et al.* 2011) was used to infer the ancestral state of diet preference and biogeographic histories to obtain a more detailed picture of the evolution of frugivory in hornbills. Bayesian binary MCMC analysis in RASP calculates ancestral states based on optimal distributions at each node, providing a statistical support value of the ancestor for enclosed terminals visualized as pie charts. A single tree was condensed from 4,504 BEAST trees handling both soft and hard polytomies. For analysis of ancestral ranges, we assigned distribution of taxa based on affinities for Africa or Asia (Table S4.1; see Supporting Information). The frequency of ancestral states was generated using MCMC with 50,000 iterations, sampled every 100 replicates with 10 heated chains using the F18 model with  $\Gamma$  distribution, combined from two independent runs.

We used BAYESTRAITS v1.0 (Pagel *et al.* 2004) to test the validity of faunivory as the ancestral character for hornbills. Trait evolution was estimated from instantaneous forward and reversed rate coefficients between specific character states and computed probabilities of state change in a given branch of the phylogeny. We assume that faunivory and occupancy of drylands are ancestral states, while frugivory and occupancy of humid forests are derived states. We tested our hypothesis with three models of evolution, using Multistate to determine the directional pathway from a root state and used both Discrete-dependent (DDe) and Discrete-independent (DIn) to determine correlations between binary traits. We reconstructed ancestral states of diet and habitat using the reversible-jump MCMC method set with an exponential hyperprior and ratedev parameter of 10 to achieve 20–40% acceptance. Character states for each clade were constrained by applying the “AddMRCA” prior. Simulations ran for 5,050,000

iterations and had a burn-in of 50,000. The likelihood ratio statistic ( $2 \times \log\text{-likelihood DDe} - \log\text{-likelihood DIn}$ ) was calculated to ascertain correlation between traits, with significance assessed against a chi-squared distribution with four degrees of freedom. The log-BayesFactor test ( $2 \times \text{harmonic mean DDe} - \text{harmonic mean DIn}$ ) was also used to show indicate correlation, where a value  $>10$  indicates very strong support to correlated trait evolution (Pagel & Meade 2006). Transitions among the four combinations of states arising from binary traits are identified by rate coefficients, and the root assigned by probabilities.

### Testing congruence of diversification patterns

Hornbill and HDF diversification were compared using multi-locus chronograms of plant families. Meliaceae and Myristicaceae contain largely animal-dispersed taxa, whereas wind-dispersed Dipterocarpaceae was the control group (Doyle *et al.* 2004, Gamage *et al.* 2006, Muellner *et al.* 2008). The Aglaieae tribe was used as a proxy for HDFs within Meliaceae, and Afro-Asian nutmegs for HDFs within Myristicaceae. We fitted speciation models to determine if phylogenies follow expected patterns under different hypotheses. Density-dependent speciation is described either by rapid radiation that gradually declines or by a slow initial increase, which gradually speeds-up (Rabosky & Lovette 2008). Both are linked as a response to gradual climatic, tectonic or biotic changes (McKenna & Farrell 2006, Couvreur *et al.* 2011). Diversification pulses are driven by the same changes but with more abrupt responses, such as volcanic uplifts or extreme variations in thermal maxima (Esselstyn *et al.* 2009), as well as by sudden biotic shifts in reproductive strategy (Chakrabarty *et al.* 2011), habitat preference and diet (Fordyce 2010, Rojas *et al.* 2011). The null hypothesis follows a constant rate of diversification associated with more stable tropical ecosystems. Matching models of diversification support the assumption that direct trophic interactions facilitated co-radiation between hornbills and HDFs.

We reconstructed phylogenies of three plant families based on concatenated sequences downloaded from GenBank (Table S4.3/ see Supporting Information). A total of 66 taxa for

Myristicaceae, 78 taxa for Meliaceae and 102 taxa for Dipterocarpaceae including outgroups were examined. Divergence times were estimated using BEAST with the same parameters and priors as hornbills. For details of calibration and gene regions used, see supporting information. Trees were pruned to represent key clades for hornbills (e.g. Afro-Asian frugivores, African faunivores) and HDFs. We inferred rate variation for each Ultrametric tree based on the vector of branching times, with all analyses implemented in R (R Development Core Team 2008), utilizing functions from the package APE v2.7-3 (Paradis *et al.* 2004) and LASER v2.3 (Rabosky 2006).

To visualize the accumulated lineages, we plotted histograms of branching times and used semilogarithmic lineage-through-time (LTT) plots to examine rates of diversification. The unpruned hornbill phylogeny and 4,504 sub-sampled trees were used to construct an empirical LTT curve with 95% confidence intervals. Estimated crown ages of HDFs and non-HDFs were based on reconstructed chronograms and published accounts of divergence times juxtaposed to the hornbill LTT, alongside known palaeoclimatic events. The Pybus-Harvey  $\gamma$  statistic was calculated to estimate divergence tempo and measure change in diversification. Corresponding *P*-values indicate deviation from the null hypothesis that clades diversified with constant rates following a pure-birth process (Pybus & Harvey 2000). Positive values of  $\gamma$  indicate increasing rates following a slow initial period, whereas negative values imply recent decelerating diversification after early rapid accumulation (Rabosky & Lovette 2008).

The Monte Carlo constant rates (MCCR) test was used to address type I error and accommodate incomplete sampling (Pybus & Harvey 2000). This test calculates the critical value of  $\gamma$  at  $\alpha=0.05$  for a given dataset from 5,000 simulated pure-birth trees (Fordyce 2010). We evaluated the fit of six models of diversification using maximum likelihood with the function `fitdAICRC` in LASER. This model-fitting approach implements the  $\Delta\text{AIC}_{\text{RC}}$  test statistic to determine temporal variation in diversification rates, using a variety of rate-constant and rate-variable models. The available options in the model set include pure-birth and birth-death for rate-constant models, exponential and logistic density-dependent rate-variable models, and two

versions of the Yule- $n$ -rate (diversification pulse) model. The calculated  $\Delta\text{AIC}_{\text{RC}}$  test statistic is the difference in AIC scores between the best rate-constant ( $\text{AIC}_{\text{RC}}$ ) and rate-variable models ( $\text{AIC}_{\text{RV}}$ ), selected from least AIC scores. A positive  $\Delta\text{AIC}_{\text{RC}}$  value implies that the rate-variable model provides the best fit (Esselstyn *et al.* 2009). We addressed type I error by calculating a critical value of the mean  $\Delta\text{AIC}_{\text{RC}}$  for rejecting the rate-constant model by evaluating the distribution of  $\Delta\text{AIC}_{\text{RC}}$  across 5,000 simulated pure-birth trees.

## Results

### Phylogenetic analyses and divergence times

A total of 1,846 characters were used in the combined *cytb*-AK1 intron 5 dataset for all 61 hornbill species. Ingroup sequence characters exhibited 38% conserved sites and 62% variable sites, of which 43% were parsimony-informative. The Bayesian MCC tree provides a robust multi-locus phylogeny, with >80% of nodes having >90% PP nodal support. Moderate to strong support for consistency of topology included: (i) placement of *Berenicornis comatus* in the Afrotropical frugivore clade; (ii) monophyly of Asian frugivores, and (iii) placement of *Tockus hartlaubi* within the African frugivore clade. Hornbills were separated into six distinct clades, including ground-hornbills (Bucorvinae), small African faunivores (*Tockus* clade) and four subclades of Afro-Asian frugivores (Fig. 1b). The crown age for Bucerotidae was estimated at 29.54 Ma, appearing at the lower Oligocene. A 40 million year gap separates this from the split of Bucerotes estimated at 77.8 Ma. Fig. 1a shows the predicted distribution of megathermal humid forests during the early Eocene and late Oligocene based on reconstructed plate-tectonic maps and approximated coverage of tropical rainforests (see Supporting Information for details).

In our Bayesian chronogram (Fig. 1b) we estimated the crown age for Bucorvinae at 7.35 Ma and 27.0 Ma for Bucerotinae. The crown age for African small faunivorous hornbills (20.64 Ma) was 5 million years younger than the crown age for frugivorous hornbills (25.37

Ma) during the late Oligocene. A further split of frugivorous *Tockus* occurred at 13.32 Ma at the middle Miocene. Crown age for the *Berenicornis* clade was 18.78 Ma, nested within the Afrotropical frugivore clade that coalesced in the early Miocene (21.81 Ma), and matches the crown age for Asian frugivores (23.64 Ma). The relatively large monophyletic *Aceros* clade diverged at around 16.03 Ma. Mean node ages and credible intervals of these hornbill clades are summarized in Table S4.7. All recognized genera of Bucerotidae had already appeared during the middle Miocene (15–17 Ma). Semi-logarithmic LTT plot of hornbills and out-groups (Fig. 1c) follows the fitted Yule-3-rate model. Initially, it shows a slow rate from 80 to 30 Ma, with an abrupt increase 30 Ma and then a slow-down after 5 Ma. Initial and final net diversification rates were equal.

### Reconstruction of ancestral states in hornbills

Faunivory clearly represents the ancestral diet in Bucerotidae, similar to their allied out-groups, Upupidae, Phoeniculidae and Coraciidae. Ancestral character reconstruction indicated an abrupt change from faunivory to frugivory within the hornbill phylogeny (Fig. 2a), with moderate (PP=0.80–0.99) to strong support (PP=1.00) at nodes of key clades (Table S4.7, see Supporting Information). Only the node covering *T. alboterminatus* and *T. fasciatus* exhibited poor support (PP<0.50). The shift in character state occurred between Crown Bucerotinae (node 1) and Afro-Asian frugivores (node 4) prompting a change in diet from faunivory to frugivory. Additional diet shifts were observed in two other nodes, a secondary shift to frugivory within the predominantly faunivorous *Tockus* clade (node 2) and a reversion to faunivory (*Tockus hartlaubi*) within the *Berenicornis* clade (node 3).

BAYESTRAITS analysis confirms faunivory as the root diet for hornbills, with shifts in predominant diet traceable along examined nodes based on the MULTISTATE model (Table S4.5, see Supporting Information). As in RASP, out-group taxa, Bucorvinae and the *Tockus* clade presented faunivory as their root diet, with drylands as root habitat, showing moderate to strong support (PP=>0.85). Unlike RASP, the sudden shift to derived states is evident in the



Bucerotidae node. Subsequent clades show generally derived states with strong support (PP=1.00), except for Asian frugivores and the *Aceros* clade.

Results indicated that trait evolution between diet and habitat is significantly correlated ( $P=0.001$ ), following the discrete-dependent model. Log-BayesFactor (BF) values express a more refined acceptance of correlated trait evolution, with very strong support (BF>10) given to Upupidae and Phoeniculidae, *Berenicornis* clade and Asian frugivores. Strong support (BF at 5–10) was given to all other clades except African frugivores (BF<1.0). Strong and weak rate coefficients between binary traits illustrate the transition between ancestral (0,0) and derived (1,1) states, and the combination of transitions appears constant across all nodes (Fig. 3b). Directional pathways indicate that ancestral African faunivores changed occupancy from drylands to humid forest, a likely scenario but weakly supported. The transition of faunivorous hornbills inhabiting humid forest to a frugivorous diet is strongly supported.

Alternatively, strong support is shown for the transition of frugivorous hornbills occupying drylands to humid forests. A reversion to drylands is also likely but with weaker support, as exemplified by some East African hornbills. Reconstruction of ancestral ranges confirms an African origin for Bucerotidae (Fig. S3, see Supporting Information) with nearly all nodes strongly supported (PP >0.90). Our results clearly show the separation of African and Asian clades between nodes 4 and 5, with the transition occurring from 25.4 Ma ( $\pm 5.29$ ) to 23.6 Ma ( $\pm 5.16$ ) across the Oligocene–Miocene boundary (Fig. 2a). Dispersal into Asia may have occurred not once but twice. It is possible that a second dispersal into Asia occurred before node 6, which marks the split between *Berenicornis* and *Tropicranus* during the lower Miocene (18.8 Ma).

### **Divergence times for hornbill-dispersed fruits**

Phylogenetic reconstruction of three plant families were congruent with topologies described in previous analyses (Muellner *et al.* 2008, Sauquet *et al.* 2003, Gamage *et al.* 2006), but node ages differed for key clades, possibly owing to the variable resolution given by

concatenated datasets, added taxa and multiple calibration points (Fig. S1, see Supporting Information). A strict molecular clock was rejected for all reconstructions, thus accepting the relaxed clock. Results significantly differed ( $P < 0.05$ ) from the null hypothesis for Myristicaceae (lnL-8347.8), Meliaceae (lnL-14390.4) and Dipterocarpaceae (lnL-11452.8). Prior patterns of diversification were lacking, and divergence estimates were only available previously for Meliaceae and Myristicaceae (Table S4.7, see Supporting Information). Crown age for the Aglaieae tribe was younger at 24 Ma compared to estimates by Muellner *et al.* (2008), while crown age of Myristicaceae was older at 30 Ma compared to Doyle *et al.* (2004). The crown age for wind-dispersed Dipterocarpaceae was estimated at 47 Ma.

### **Diversification patterns in hornbills and hornbill-dispersed fruits**

Patterns of diversification between hornbills and plant families were examined from values of the  $\gamma$  statistic, fitted models of diversification and LTT plots, calculated from chronograms subsequently pruned to analyse key clades (e.g. Afro-Asian frugivores, *Tockus* clade). Results of the MCCR test and likelihood-based model fitting approach are summarized in Table 4.1. Seven of the nine clades exhibited negative  $\gamma$  values, suggesting that branching events occurred earlier in the tree as opposed to closer to the tips. This indicates that cladogenetic events are distributed disproportionately towards the root of the tree, and thus plausible rapid initial diversification. Conversely, Myristicaceae and Dipterocarpaceae showed a high positive  $\gamma$ . Negative values also indicated incomplete taxon sampling, but given that our dataset covers all species of hornbills, this bias is reduced (Pybus & Harvey 2000). However, this condition is evident for plant families, which was clearly under-represented.

The MCCR test was significant for all hornbill clades but not for all plant clades. The critical value of the mean  $\Delta AIC_{rc}$  was calculated for all ten clades (Table S4.6/see Supporting Information). Results for African frugivores, Afro-Asian nutmegs, Meliaceae, Myristicaceae and Dipterocarpaceae were not significant ( $P > 0.05$ ), indicating that the null distribution follows the rate-constant model (CRD) in half the clades tested. The remaining clades generally follow

the yulenrate model, except for the *Tockus* clade, which follows a logistic density-dependent model (DDL). Notably, African frugivores follow the same rate-constant diversification model as Afro-Asian nutmegs. Asian frugivores exhibit the same rate-variable model as the Asian Aglaieae tribe. Shifts in net diversification rates are evident among frugivorous hornbills and frugivore-dispersed plant clades, except for African frugivores.

Semi-logarithmic LTT plots visualize the fitted models (Fig. S2, see Supporting Information) and show which points in evolutionary time rate changes occurred. Yulenrate models show a rapid diversification across a narrow band of time, confirming that instantaneous shifts occurred amongst these clades, particularly frugivorous hornbills and Aglaieae tribe. LTT plots of African frugivores, Dipterocarpaceae and Meliaceae exhibit linear curves typical of CRD, whereas faunivorous hornbills show a curve typical for DDL. Net diversification rates were significantly faster ( $sd \pm 0.022$ ,  $z = 1.998$ ,  $P = 0.046$ ) for Afro-Asian frugivores ( $r = 0.332$ ) than faunivorous hornbills ( $r = 0.271$ ), supporting the assumption that a shift to frugivory influenced an increase in net diversification rates. Although similar models were found between frugivorous hornbills and Myristicaceae, and between Asian frugivores and Aglaieae tribe, their trends and shift-times do not coincide. Frugivorous hornbills exhibited a slow-down after 21.8 Ma and 3.7 Ma, while Myristicaceae accelerated at 30.2 Ma then slowed down at 0.69 Ma. Asian frugivores showed a slow-down at 4.47 Ma, while Aglaieae had a slow-down at 2.82 Ma (Table 4.1).

Histograms of branching times of each clade show accumulation of lineages at certain node ages, thus identifying the time where diversification occurred most and where lineages had first emerged (Fig. 3). Proportion of lineages was high for frugivorous hornbills at the 5-10 Ma range, and congruent to the distribution of lineages in Aglaieae and Afro-Asian Myristicaceae. No lineages appeared prior to 30 Ma for these three datasets.

### Comparison between hornbills and hornbill-dispersed fruits

We compared estimates of divergence times from chronograms of hornbills and plant families, alongside published dates for other key families with known HDFs (Table S4.7, see Supporting Information). Many plant groups associated with hornbills were already present prior to the emergence of frugivorous hornbills, including Meliaceae, Burseraceae, *Ficus* and *Artocarpus* (Moraceae), *Cinnamomum* (Lauraceae), short-branch clade Annonaceae, and various palms (Arecoideae and Coryphoideae). Families of minor HDFs (<15 taxa as HDF like Arecaceae) and non-HDFs (e.g. wind-dispersed Dipterocarpaceae and Bignoniaceae, water-dispersed Sonneratiaceae) were established long before crown hornbills appeared (Fig. 1c). Mean ages of plant clades harbouring known HDFs (e.g. crown Myristicaceae, *Aphanamixis*–*Aglaiae*) converge with the emergence of frugivorous hornbills between 20 and 30 Ma (Fig. 1c). Crown ages of either Afrotropical or Asian frugivores coincide with key HDFs, namely *Canarium*, *Amoora*–*Neoaglaia*, *Guarea*–*Ruagea* and *Coelocaryon*–*Paramyristica*.

## Discussion

### Phylogeny and divergence times in frugivorous hornbills

Our complete species-level molecular phylogeny is a well-resolved representation of the evolutionary history of Bucerotidae. It is concurrent with other molecular analyses but differs in several aspects of the topology (Hübner *et al.* 2003, Viseshakul *et al.* 2011). The stem lineage of hornbills agrees with proposed ages between Upupidae, Phoeniculidae and Bucerotidae (Bucerotes), but differs largely in bucerotid crown age in previous analyses (Hackett *et al.* 2008, Pacheco *et al.* 2011). Viseshakul *et al.* (2011) marked the emergence of frugivorous hornbills at 47–48 Ma, which occurred after the collision of India with mainland Asia (50–55 Ma, Najman *et al.* 2010) suggesting that hornbills assisted the rapid colonisation of Indian flora during the early Eocene climatic optimum. This pre-dates the emergence of key HDFs (e.g. crown Myristicaceae), crown Dipterocarpaceae and even the *Ficus* clade (Moraceae). Viseshakul *et al.*

(2011) also noted divergence of African ground-hornbills from the crown-group at 52–53 Ma. Although this scenario is plausible, there appears to be some palaeobiogeographic uncertainty.

How did the crown-group colonise both Africa and Asia given the separation by the Tethys Sea? At the early Eocene, India merged with Asia facilitating early floral invasions, but Afro-Arabia was still detached from Eurasia (Allen & Armstrong 2008). Our findings agree with the proposition of Kinnaird & O'Brien (2007) that hornbills arose in the Oligocene (30–35 Ma) and radiated rapidly during the late Miocene and Pliocene periods. This also coincides with the crown age for Bucerotinae (33 Ma) estimated by Hübner *et al.* (2003) using a molecular clock. This suggests that the crown-group of frugivorous hornbills evolved after Afro-Arabia and India had merged with Asia forming the *Gomphotherium* land-bridge which facilitated biotic interchange (Pook *et al.* 2009, Zhou *et al.* 2011). This also agrees with more robust avian colonisation scenarios between Asia and Africa during the Oligocene (Jönsson *et al.* 2010).

Emergence of frugivorous clades concurs with the distribution of closed-canopy megathermal rainforests and monsoonal forests in Equatorial Africa, India, Indochina and Sundaland during warming events in the Oligocene and Miocene, following the terminal Eocene cooling (Morley 2000). The split between Asian and Afrotropical frugivores coincides with the late Oligocene warming event (LOWE) between 23.5 and 26.7 Ma (Winkler *et al.* 2009, Rojas *et al.* 2011), and the Miocene climatic optima (MCO at 18–20 Ma, Böhme 2003). Expansion of humid forests allowed hornbills to disperse along the Horn of Africa into Arabia then to India and Sundaland. Evidence of rainforests in Ethiopia and Arabian Peninsula during the Oligocene–Miocene provides a more plausible pathway for hornbill routes between Africa and Asia (Jacobs 2004).

Estimated age of Bucorvinae was concurrent with estimates for fossil ground-hornbills, *Bucorvus brailloni* (Brunet 1971) and *Euroceros bulgaricus* (Boev & Kovachev 2007). Savanna grasslands prevailed in Africa and Eurasia during the late Miocene. Our dates for frugivorous hornbills were half the age estimated by Viseshakul *et al.* (2011), and several factors may have led to these differences. A single erroneous fossil calibration (13 Ma) was used, since Brunet

(1971) mentioned *B. brailloni* was from the Miocène supérieur or Upper Miocene (7–11.5 Ma). The use of accurate fossil dates is imperative for constructing time-trees, as fossils should be stratigraphically well-identified (Ho & Phillips 2009).

The lack of error bars restricted understanding of estimated age range and the node representing crown frugivores was poorly supported. Error and confidence intervals are inherent in molecular dating (Donoghue & Benton 2007). Calibrations with both fossil and molecular dates on a relaxed clock of multiple genes offer higher resolution over a single-point calibration of one gene following an untested strict clock (Van Tuinen & Dyke 2004, Donoghue & Benton 2007). We reiterate the importance of using a robust well-resolved tree calibrated with multiple anchor-points. Therefore, our proposed late Oligocene emergence represents a more likely scenario of divergence in frugivorous hornbills.

### **Ancestral state reconstructions and shift to frugivory**

We confirm faunivory as the ancestral diet in hornbills, with Bucorvinae and small African *Tockus* as basal clades (Kemp 2001). Although hornbills are generally omnivorous, they clearly separate into clades which are predominantly faunivorous and frugivorous, and clades which inhabit drylands and humid forests. Kinnaird & O'Brien (2007) regarded frugivory as a fundamental specialization which allowed Asian hornbills to inhabit forests and radiate. Frugivory led to the development of a gular pouch and large home ranges to access patchy distribution of fruits. Our data suggested that a switch to frugivory was strongly correlated with occupancy of humid forests during the Oligocene. A shift to frugivory was also observed in phyllostomid bats and anthropoid primates during this period (Rojas *et al.* 2011, Ramdarshan *et al.* 2010). Many modern frugivores evolved during the Oligocene and Miocene regardless of stem-group age (Fleming & Kress 2011). Our well-resolved trees with moderate to high topological support confirm the placement of *Berenicornis* in the Afrotropical frugivore clade, an ancestral lineage leading to the diet shift and instrumental to the colonisation of Asia. Indeed, *Berenicornis comatus* best represents the plausible ancestral labile hornbill, as it is equally

faunivorous and frugivorous like *Tropicranus* (although mainly faunivorous). Clustering with *Tropicranus albocristatus* and *Tockus hartlaubi* implies close affinities with more ancestral African faunivores as previously realized. Unlike most Asian frugivores, *Berenicornis* does not have an extensive bare facial skin and a non-inflatable gular pouch (Kemp 1995).

This innovation in diet greatly influenced the successful radiation of hornbills into Asia and Africa. Pathways indicate that occupancy of humid forest encouraged the shift from faunivory to frugivory, due to ample nesting and fruit resources. In most cases, fruits and flowers evolved first before their pollinators and dispersal vectors (Fleming & Kress 2011). Rapid diversification of various plant families following the first and second wave of floral invasions of Asia from Africa (Morley 2000, Couvreur *et al.* 2011) provided an opportunity for hornbills to occupy a novel niche. Their elongated bills and extendible gular pouch are adaptable tools for frugivory, particularly for the handling and procurement of large-seeded fruit, with complex dehiscent capsules (Kitamura 2011). Non-HDFs (water or wind-dispersed) and minor HDFs evolved much earlier than key HDFs as they were not reliant on bucerotichory (dispersal of fruits by hornbills). Key HDF clades were radiating about the same time as frugivorous hornbills, with similar trends and models of diversification. However, our results do not show a strong congruence in net diversification rates, with no match in topology as often observed in more closely associated mutualisms (McKenna *et al.* 2009, McLeish *et al.* 2010, Fordyce 2010). This robust temporal concordance approximates complementary radiation between herbivores and their host plants, which demonstrates evolutionary responses often associated with biotic interactions, as well as tectonic and climatic events (McKenna & Farrell 2006, Fordyce 2010). Most of these families (e.g. Annonaceae, Meliaceae) evolved much earlier than hornbills but equally diversified within the Oligocene–Miocene climatic optima. Beyond this initial diet shift, a secondary transition to frugivory occurred within the largely faunivorous *Tockus* clade, similarly driven by occupancy of humid forest by *T. fasciatus* and *T. alboterminatus* (Kitamura 2011). Conversely, the reversion to faunivory (also lack of gular

pouch and single item delivery to nest) by *T. hartlaubi* (and possibly *Tropicranus*) within the frugivore clade requires further investigation.

### **Historical biogeography of frugivorous hornbills**

We confirm Africa as the ancestral area for Bucerotidae and, based on divergence time, we propose that dispersal into Asia was facilitated by land-bridges formed after the collision of Afro-Arabia and India. This agrees with the Out-of-Africa hypothesis proposed by Kinnaird & O'Brien (2007) but deviates from Eurasian origins postulated by Viseshakul *et al.* (2011). Reconstructions also ascribe affinity of frugivorous hornbills to humid forests, since frugivory was probably constrained by availability of diverse fruit resources and nesting sites. Divergence dates concur with two significant warming events promoting expansion of humid forest and colonisation routes for Asian hornbills (Böhme 2003, Rojas *et al.* 2011). The width of barriers such as Bab-el-Mandeb and Strait of Hormuz were sufficiently narrow to allow biotic exchange during temporal drops in sea level (Otero & Gayet 2001). Afro-Arabian connection with Eurasia was completed at 25 Ma coinciding with LOWE (Allen & Armstrong 2008). This also supports dispersal of frugivorous anthropoid primates from Africa to Asia during the Oligocene (Ramdarshan *et al.* 2010).

The aridification and contraction of humid forests between the Arabian Peninsula and Pakistan during the Miocene–Pliocene left a gap between hornbill distributions. About 3,000 km currently separate the Afro-Arabian faunivore *T. nasutus* and Indo-Pakistani frugivore *Ocyrceros birostris*. The *Berenicornis*–*Tropicranus* split, leading to the secondary colonisation of Sundaland, coincides with the MCO. Ectothermic vertebrates followed similar migration events at 18–20 Ma, confirmed by the coexistence of thermophilic taxa during the Ottnangian thermal peak (Böhme 2003). The warm and humid mid-Miocene optimum allowed expansion of both humid forests and tropical grasslands in Africa and Asia, facilitating biotic exchange (Otero & Gayet 2001, Zhou *et al.* 2011). Similarities between Sundaic *Berenicornis* and Afrotropical forest hornbills (*Tropicranus*, *Ceratogymna*) confirm their basal African lineage.



This close relationship between African and Indo-Malayan taxa is also found in bird groups such as *Xenoperdix*, *Afropavo* and *Apaloderma*. These Afro-Oriental assemblages were assumed to be western relicts that inhabited Miocene forests across Africa and southern Asia (Dinesen *et al.* 1994).

### **Hornbill-dispersed fruits drive hornbill diversification**

The greater diversity of typical or nest-sealing hornbills than in ground-hornbills Bucorvinae has been attributed to the adoption of a novel nesting strategy, since female incarceration is unique to Bucerotidae (Kemp 2001). However, the innovation in diet represents a more likely impetus for hornbill diversification given the successful radiation of frugivorous clades in Asia and Africa. Speciation rates in hornbills are not constant and exhibit abrupt changes and slow-downs even for inclusive subclades within the phylogeny. Diversification pulses and density-dependent models were evident for hornbills and some key HDFs. The gradual deceleration in diversification during the Pliocene after an explosive early accumulation suggests that potential niches were gradually being filled, as an aftermath of coupling between lineage and ecological diversification mediated by ecological interactions (Rabosky & Lovette 2008). Non-HDFs and minor HDFs tend to follow a constant rate of diversification, indicating that these clades continued to diversify throughout their evolutionary history without any evidence of deceleration (Couvreur *et al.* 2010). African frugivores and Afro-Asian nutmegs follow rate-constant models, suggesting that both are still in the process of speciation. Although there is agreement in terms of divergence times and models of diversification, detectable rates and shift-times were not matched. Other factors probably influence overall diversification of tropical plant families. Ages of non-HDFs, minor HDFs and basal lineages of HDF-inclusive families pre-date the emergence of hornbills. Further studies are needed to determine if co-evolution characterizes linkages between hornbills and HDFs.

Unlike previous broad comparisons (Viseshakul *et al.* 2011, Fleming & Kress 2011), we focused our analysis on frugivorous hornbills and key clades of HDFs. Given the

concordance, we can assume that the shift to frugivory was facilitated by the emergence of HDFs. Likewise, radiation of HDFs in Africa and Asia were influenced by bucerotichory. Hornbills serve as efficient mobile links to large-seeded fruits with fleshy drupes attributed to Annonaceae (*Xylopia*), Lauraceae (*Cinnamomum*) and Burseraceae (*Dacryodes*) or arillate capsules of Meliaceae (*Aglaia*) and Myristicaceae (*Knema*) (Kitamura 2011). The massive seeds of Burseraceae are well adapted for endozoochory, dispersed widely by hornbills and primates (Weeks *et al.* 2005). Many species of Annonaceae have comparatively large fleshy pericarps like *Uvaria* and *Pseuduvaria*, so hornbills serve as important volant seed-dispersers (Zhou *et al.* 2011). Large-seeds of dehiscent fruits of section *Amoora* in Aglaieae can only be dispersed by large birds such as hornbills (*Buceros*, *Anthracoseros*) and pigeons (*Ducula*) over wide distances (Kitamura *et al.* 2004, Muellner *et al.* 2008). Asian hornbills also disperse seeds of closely related *Dysoxylum* and *Chisocheton* (Kitamura 2011).

Myristicaceae is a cohesive pantropical family with capsular fruits dispersed by long-billed hornbills and toucans Ramphastidae (Sauquet *et al.* 2003). Generally, hornbills and HDFs have West Gondwanan origins following the same Out-of-Africa dispersal, radiating eastward to Asia and Australasia (Doyle *et al.* 2004, Muellner *et al.* 2006). Dispersion was achieved either through earlier Eocene–Oligocene boreotropical land-bridges or the later Miocene *Gomphotherium* land-bridge (Weeks *et al.* 2005, Zhou *et al.* 2011). Complex megathermal flora included most of these key rainforest families dispersed from Africa *en route* to Asia via India and biotic connections persisted in the Miocene due to contiguous humid forests (Morley 2000).

## Conclusions

Our molecular phylogeny of 61 hornbill species facilitated reconstruction of ancestral states, inferred divergence times and mapped dispersal routes. We reiterate the importance of calibrating with multiple fossil and molecular anchor-points to achieve a robust chronogram. Our findings indicate that hornbills emerged much later than previously suggested, and our

estimates agree with floristic, palaeo-climatic and biogeographic events, which support hornbill dispersal through Palaeotropical Biotic Interchange from Africa to Asia. Further studies are needed to determine the colonisation routes taken by hornbills to reach Southeast Asia and Melanesia, and should cover all taxa and representative geographic populations. Our tree reaffirms the evolution of Asian hornbills from ancestral African ancestors initiated by a novel shift to frugivory and correlated with historical expansion of humid forests. Warming events promoted dispersal of both frugivorous hornbills and hornbill-dispersed fruits. This unequivocal temporal agreement supports our proposition that HDFs facilitated radiation of Afro-Asian frugivorous hornbills, but also limiting their occupancy to rainforests. We speculate that coevolution with hornbills predispose HDF to colonise Asiatic forests early, giving them a precursor advantage when niches were relatively open.

Concurrence of divergence times open up new avenues for research in understanding the links between large-seeded fruits and their dispersal agents to determine drivers for co-evolution and parallel cladogenesis. It is likely that permutation between land-bridge formations, warming events, humid forest expansion, diet shift and emergence of HDFs served as the impetus for the successful radiation of frugivorous hornbills into Africa and Asia. This combination of biotic interactions and abiotic changes influenced rapid diversification in hornbills. Biotic interactions not only have important consequences for ecosystem function and stability, but also have a key role in promoting and expanding biological diversity in tropical systems.

## **Acknowledgements**

We are grateful to numerous institutions for research permission and access to museum material (see Supporting Information for full list) and to N.J. Collar, C. Pannell and B.C. Sheldon, for support. We also thank various people who offered information or assistance, including D. Allen, A. Dans, C. Edwards, A. Escalante, J. Haile, P. Holland, G. Mayr and A.

Pigot. This research was supported by a Ford Foundation International Fellowship, with additional funding from the North of England Zoological Society, British Ornithologists' Union, and St. Anne's College, Oxford.

## References

- Allen MB, Armstrong HA (2008) Arabia-Eurasia collision and the forcing of mid-Cenozoic global cooling. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **265**, 52-58.
- Antoine P-O, Welcomme J-L, Marivaux L, Baloch I, Benammi M, Tass P (2003) First record of Paleogene Elephantoidea (Mammalia, Proboscidea) from the Bugti Hills of Pakistan. *Journal of Vertebrate Paleontology*, **23**, 977-980.
- Boev Z, Kovachev D (2007) *Euroceros bulgaricus* gen. nov., sp. nov. from Hadzhidimovo (SW Bulgaria) (Late Miocene) – the first European record of hornbills (Aves: Coraciiformes). *Geobios*, **40**, 39-49
- Böhme M (2003) The Miocene Climatic Optimum: evidence from ectothermic vertebrates of Central Europe. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **185**, 389-401.
- Bolmgren K, Eriksson O (2005) Fleshy fruits – origins, niche shifts, and diversification. *Oikos*, **109**, 255-272.
- Brunet J (1971) Oiseaux miocènes de Beni Mellal (Maroc); un complément à leur étude. *Notes et Mémoires du Service géologique du Maroc*, **31**, 109-111.
- Burns KC, Lake B (2009) Fruit-frugivore interactions in two southern hemisphere forests: allometry, phylogeny and body size. *Oikos*, **118**, 1901-1907.
- Carrière SM, André M, Letourmy P, Olivier I, McKey DB (2002) Seed rain beneath remnant trees in a slash-and-burn agricultural system in southern Cameroon. *Journal of Tropical Ecology*, **18**, 353-374.
- Chakrabarty P, Davis MP, Smith WL, Baldwin ZH, Sparks JS (2011) Is sexual selection driving diversification of the bioluminescent ponyfishes (Teleostei: Leiognathidae)? *Molecular Ecology*, **20**, 2818-2834.
- Condamine FL, Sperling FAH, Wahlberg N, Rasplus J-Y, Kergoat GJ (2012) What causes latitudinal gradients in species diversity? Evolutionary processes and ecological constraints on swallowtail biodiversity. *Ecology Letters*, **15**, 267-77.
- Couvreux LP, Forest F, Baker WJ (2011) Origin and global diversification patterns of tropical rain forests: inferences from a complete genus-level phylogeny of palms. *BMC Biology*, **9**, 44-56.

- DaCosta JM, Klicka J (2008) Blackwell Publishing Ltd The Great American Interchange in birds: a phylogenetic perspective with the genus *Trogon*. *Molecular Ecology*, **17**, 1328–1343.
- Dinesen L, Lehmborg T, Svendsen JO, Hansen LA, Fjeldså J (1994) A new genus and species of perdicine bird (Phasianidae, Perdicini) from Tanzania; a relict form with Indo-Malayan affinities. *Ibis*, **136**, 2-11.
- Donoghue PCJ, Benton MJ (2007) Rocks and clocks: calibrating the Tree of Life using fossils and molecules. *Trends in Ecology and Evolution*, **22**, 424-431.
- Doyle JA, Sauquet H, Scharaschkin T, Le Thomas A (2004) Phylogeny, molecular and fossil dating, and biogeographic history of Annonaceae and Myristicaceae (Magnoliales). *International Journal of Plant Sciences*, **165**, 55-67.
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. *Evolution*, **18**, 586-608.
- Ehrlich PR, Dobkin DS, Wheye D (1988) The Birder's Handbook: A field guide to the natural history of North American birds. Simon and Schuster Inc., New York.
- Esselstyn JA, Timm RM, Brown RM (2009) Do geological or climatic processes drive speciation in dynamic archipelagos? The evolution of shrew diversity on SE Asian islands. *Evolution*, **63**, 2595-2610.
- Ezard THG, Aze T, Pearson PN, Purvis A (2011) Interplay Between Changing Climate and Species' Ecology Drives Macroevolutionary Dynamics. *Science*, **332**, 349-351.
- Fleming TH, Geiselman C, Kress WJ (2009) The evolution of bat pollination: a phylogenetic perspective. *Annals of Botany*, **104**, 1017–1043.
- Fleming TH, Kress WJ (2011) A brief history of fruits and frugivores. *Acta Oecologica*, DOI: 10.1016/j.actao.2011.01.016.
- Fordyce JA (2010) Host shifts and evolutionary radiations of butterflies. *Proceedings of the Royal Society of London B*, **277**, 3735-3743.
- Gamage DT, de Silva MP, Inomata N, Yamazaki T, Szmidt AE (2006) Comprehensive molecular phylogeny of the sub-family Dipterocarpoideae (Dipterocarpaceae) based on chloroplast DNA sequences. *Genes and Genetic Systems*, **81**, 1-12.
- Gray MA, Baldauf SL, Mayhew PJ, Hill JK (2007) The response of avian feeding guilds to tropical forest disturbance. *Conservation Biology*, **21**, 133-141.
- Hackett SJ, Kimball RT, Reddy S, Bowie RCK, Braun EL, Braun MJ, Chojnowski JL, Cox WA, Han K-L, Harshman J, Huddleston CJ, Marks BD, Miglia KJ, Moore WS, Sheldon

- FH, Steadman DW, Witt CC, Yuri T (2008) A phylogenomic study of birds reveals their evolutionary history. *Science*, **27**, 1763-1768.
- Harzhauser M, Kroh A, Mandic O, Piller WE, Göhlich U, Reuter M, Berning B (2007) Biogeographic responses to geodynamics: A key study all around the Oligo–Miocene Tethyan Seaway. *Zoologischer Anzeiger*, **246**, 241–256.
- Hay WW, DeConto R, Wold CN, Wilson KM, Voigt S, Schulz M, Wold-Rosby A, Dullo W-C, Ronov AB, Balukhovskiy AN, Soeding E (1999) Alternative Global Cretaceous Paleogeography, in Barrera E, Johnson C (eds.), *The Evolution of Cretaceous Ocean/Climate Systems, Geological Society of America Special Paper*, **332**, pp. 1-47.
- Herre EA, Machado CA, Bermingham E, Nason JD, Windsor DM, McCafferty SS, Van Houten W, Bachmann K (1996) Molecular phylogenies of figs and their pollinator wasp. *Journal of Biogeography*, **23**, 521–530.
- Herrera CM, Pellmyr O (2002) *Plant-Animal Interactions: An Evolutionary Approach* (eds.). Blackwell Science, Oxford.
- Ho SYW, Phillips MJ (2009) Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Systematic Biology*, **38**, 367-380.
- Hübner VSM, Prinzing R, Wink M (2003) Neue Erkenntnisse zur Taxonomie der Hornvögel (Aves: Bucerotiformes) und ihre Bedeutung für die Zucht in Menschenobhut. *Zoologische Garten N.F.*, **73**, 396-401.
- Jacobs BF (2004) Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society of London B*, **359**, 1573-1583.
- Jönsson KA, Bowie RCK, Nylander JAA, Christidis L, Norman JA, Fjeldså J (2010) Biogeographical history of cuckoo-shrikes (Aves: Passeriformes): transoceanic colonisation of Africa from Australo-Papua. *Journal of Biogeography*, **37**, 1767-1781.
- Kappelman J, Rasmussen TD, Sanders WJ, Feseha M, Bown T, Copeland P, Crabaugh J, Fleagle J, Glantz M, Gordon A, Jacobs B, Maga M, Muldoon K, Pan A, Pyne L, Richmond B, Ryan T, Seiffert ER, Sen S, Todd L, Wiemann MC, Winkler A (2003) Oligocene mammals from Ethiopia and faunal exchange between Afro-Arabia and Eurasia. *Nature*, **426**, 549–552.
- Kemp AC (1995) *The Hornbills: Bucerotiformes*. Oxford University Press, Oxford.
- Kemp AC (2001) Family Bucerotidae (Hornbills). Pp. 436-523 in: de Hoyo J, Elliott A, Sargatal J, eds. (2001) *Handbook of the Birds of the World*. Vol. 6. Mousebirds to Hornbills. Lynx Edicions, Barcelona.
- Kiester AR, Lande R, Schemske DW (1984) Models of coevolution and speciation in plants and their pollinators. *The American Naturalist*, **124**, 220–243

- Kinnaird MF, O'Brien TG (2007) *The ecology and conservation of Asian hornbills: farmers of the forest*. The University of Chicago press, Chicago.
- Kitamura S, Suzuki S, Yumoto T, Poonswad P, Chuailua P, Plongmai K, Noma N, Maruhashi T, Suckasam C (2004) Dispersal of *Aglaia spectabilis*, a large-seeded tree species in a moist evergreen forest in Thailand. *Journal of Tropical Ecology*, **20**, 421-427.
- Kitamura S (2011) Frugivory and seed dispersal by hornbills (Bucerotidae) in tropical forests. *Acta Oecologica*, **37**, 531-541.
- Koufos GD, Kostopoulos DS, Vlachou TD (2005) Neogene/Quaternary mammalian migrations in Eastern Mediterranean. *Belgian Journal of Zoology*, **135**, 181-190.
- Ksepka DT, Clarke JA (2010) *Primobucco mcgrewi* (Aves: Coracii) from the Eocene Green river formation: new anatomical data from the earliest constrained record of stem rollers. *Journal of Vertebrate Paleontology*, **30**, 215-225.
- Lambert JE, Garber PA (1998) Evolutionary and ecological implications of primate seed dispersal. *American Journal of Primatology*, **45**, 9-28.
- Leakey M, Grossman A, Gutiérrez M, Fleagle JG (2011) Faunal change in the Turkana basin during the Late Oligocene and Miocene. *Evolutionary Anthropology*, **20**, 238-253.
- Lidicker WZ Jr (1979) A clarification of interactions in ecological systems. *BioScience*, **29**, 475-477.
- Mayr G (2006) New specimens of the Eocene Messelirrisoridae (Aves: Bucerotes), with comments on the preservation of uropygial gland waxes in fossil birds from Messel and the phylogenetic affinities of Bucerotes. *Paläontologische Zeitschrift*, **80/4**, 390-405.
- McKenna DD, Farrell BD (2006) Tropical forests are both evolutionary cradles and museums of leaf beetle diversity. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 10947-10951.
- McKenna DD, Sequeira AS, Marvaldi AE, Farrell BD (2009) Temporal lags and overlap in the diversification of weevils and flowering plants. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 7083-7088.
- McLeish MJ, van Noort S, Tolley KA (2010) African parasitoid fig wasp diversification is a function of *Ficus* species ranges. *Molecular Phylogenetics and Evolution*, **57**, 122-134.
- Morley RJ (2000) *Origin and Evolution of Tropical Rain Forests*. Wiley, New York.
- Muellner AN, Savolainen V, Samuel R, Chase MW (2006) The mahogany family "out-of-Africa": Divergence time estimation, global biogeographic patterns inferred from plastid rbcL DNA sequences, extant, and fossil distribution of diversity. *Molecular Phylogenetics and Evolution*, **40**, 236-250.

- Muellner AN, Pannell CM, Coleman A, Chase MW (2008) The origin and evolution of Indomalayan, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *Journal of Biogeography*, **35**, 1769-1789.
- Najman Y, Appel E, Boudagher-Fadel M, Bown P, Carter A, Garzanti E, Godin L, Han J, Liebke U, Oliver G, Parrish R, Vezzoli G (2010) Timing of India-Asia collision: Geological, biostratigraphic and palaeomagnetic constraints. *Journal of Geophysical Research*, **115**, 1-18.
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Otero O, Gayet M (2001) Palaeoichthyofaunas from the Lower Oligocene and Miocene of the Arabian Plate: palaeoecological and palaeobiogeographical implications. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **165**, 141-169.
- Pacheco MA, Battistuzzi FU, Lentino M, Aguilar R, Kumar S, Escalante AA (2011) Evolution of modern birds revealed by Mitogenomics: timing the radiation and origin of major orders. *Molecular Biology and Evolution*, **28**, 1927-1942.
- Pagel M, Meade A (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *American Naturalist*, **167**, 808-825.
- Pagel M, Meade A, Baker D (2004) Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology*, **53**, 673-684.
- Paradis E, Claude J, Strimmer K (2004) APE: analysis of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289-290.
- Pellym O (2003) Yuccas, Yucca moths, and coevolution: a review. *Annals of the Missouri Botanical Garden*. **90**, 35-55.
- Pook CE, Joger U, Stümpel N, Wüster W (2009) When continents collide: Phylogeny, historical biogeography and systematics of the medically important viper genus *Echis* (Squamata: Serpentes: Viperidae). *Molecular Phylogenetics and Evolution*, **53**, 792-807.
- Pybus OG, Harvey PH (2000) Testing macro-evolutionary models using incomplete molecular phylogenies. *Proceedings of the Royal Society of London B*, **267**, 2267-2272.
- Quental TB, Marshall CR (2011) The Molecular Phylogenetic Signature of Clades in Decline. *PLoS ONE* **6**(10): e25780. doi:10.1371/journal.pone.0025780
- Rabosky DL (2006) Likelihood methods for inferring temporal shifts in diversification rates. *Evolution*, **60**, 1152-1164.
- Rabosky DL, Lovette IJ (2008) Density-dependent diversification in North American wood warblers. *Proceedings of the Royal Society of London B*, **275**, 2363-2371.
- Rambaut A (2006) FigTree: Tree Figure Drawing Tool. Edinburgh: University of Edinburgh.



- Rambaut A, Drummond AJ (2007) Tracer (v.1.5) Available from <http://beast.bio.ed.ac.uk/Tracer> (accessed April 2011).
- Ramdarshan A, Merceron G, Tafforeau P, Marivaux L (2010) Dietary reconstruction of the Amphipithecidae (Primates, Anthropoidea) from the Paleogene of South Asia and paleoecological implications. *Journal of Human Evolution*, **59**, 96-108.
- R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Rojas D, vale A, Ferrero V, Navarro L (2011) When did plants become important to leaf-nosed bats? Diversification of feeding habits in the family Phyllostomidae. *Molecular Ecology*, **20**, 2217-2228.
- Sauquet H, Doyle JA, Scharaschkin T, Borsch T, Hilu KW, Chatrou LW, Le Thomas A (2003) Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implication for character evolution. *Botanical Journal of the Linnean Society*, **142**, 125-186.
- Shapiro LH, Dumbacher JP (2001) *Adenylate kinase* intron 5: a new nuclear focus for avian systematics. *The Auk*, **118**, 248-255.
- Stehli FG, Webb SD (eds) (1985) *The great American biotic interchange. Topics in geobiology*. Plenum Press, New York.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731-2739.
- Van Tuinen M, Dyke GJ (2004) Calibration of galliform molecular clocks using multiple fossils and genetic partitions. *Molecular Phylogenetics Evolution*, **30**, 74-86.
- Venditti C, Meade A, Pagel M (2010) Phylogenies reveal new interpretation of speciation and the Red Queen. *Nature*, **463**, 349-352.
- Viseshakul N, Charoennitikul W, Kitamura S, Kemp AC, Thong-aree S, Surapunpitak Y, Poonswad P, Ponglikitmongkol M (2011) A phylogeny of frugivorous hornbills linked to the evolution of Indian plants within Asian rainforests. *Journal of Evolutionary Biology*, **24**, 1533-1545.
- Weeks A, Daly DC, Simpson BB (2005) The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution*, **35**, 85-101.
- Winkler I, Mitter C, Scheffer SJ (2009) Repeated climate-linked host shifts have promoted diversification in a temperate clade of leaf-mining flies. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 18103-18108.

- Yu Y, Harris AJ, He XJ (2011) RASP (Reconstruct Ancestral State in Phylogenies). Available at <http://mnh.scu.edu.cn/soft/blog/RASP>.
- Zalmout IS, Sanders WJ, MacLachy LM, Gunnell1 GF, Al-Mufarreah YA, Ali MA, Nasser A-AH, Al-Masari AM, Al-Sobhi SA, Nadhra AO, Matari AH, Wilson JA, Gingerich PD (2010) New Oligocene primate from Saudi Arabia and the divergence of apes and Old World monkeys. *Nature*, **466**, 360–365.
- Zhou L, Su YCF, Thomas DC, Saunders RMK (2011) ‘Out-of-Africa’ dispersal of tropical floras during the Miocene climatic optimum: evidence from *Uvaria* (Annonaceae). *Journal of Biogeography*. **39**, 322–335.

**Table 4.1:** Diversification trends in hornbills and associated tropical plant families based from the Pybus-Harvey  $\gamma$  statistic and best-fit model selection. Model names derived from R package LASER (Rabosky 2006), selected from AIC scores and  $P$ -values.

| Taxa group              | $\gamma$ statistic | $P$ -value <sup>1</sup> | $\Delta\text{AIC}_{\text{RC}}$ | $P$ -value <sup>2</sup> | best model | r     |
|-------------------------|--------------------|-------------------------|--------------------------------|-------------------------|------------|-------|
| Hornbills               | -4.217             | 0.000                   | 22.776                         | 0.000                   | yule2rate  | 0.126 |
| Frugivorous hornbills   | -3.488             | 0.000                   | 18.258                         | 0.000                   | yule3rate  | 0.332 |
| Faunivorous hornbills   | -2.449             | 0.002                   | 7.013                          | 0.013                   | DDL        | 0.271 |
| Asian frugivore clade   | -2.942             | 0.000                   | 13.306                         | 0.001                   | yule2rate  | 0.137 |
| African frugivore clade | -1.324             | 0.018                   | 1.859                          | 0.130                   | purebirth  | 0.070 |
| <i>Aglaiaeae</i> tribe  | -1.330             | 0.798                   | 13.873                         | 0.001                   | yule2rate  | 0.110 |
| Afro-Asian nutmegs      | 0.598              | 0.725                   | 0.528                          | 0.907                   | purebirth  | 0.090 |
| Myristicaceae           | -1.330             | 1.000                   | -6.059                         | 1.000                   | birthdeath | 0.091 |
| Meliaceae               | -0.622             | 1.000                   | 2.096                          | 0.177                   | purebirth  | 0.059 |
| Dipterocarpaceae        | 4.178              | 1.000                   | -2.001                         | 1.000                   | purebirth  | 0.082 |

$P$ -values derived from critical values of the ( $P$ -value<sup>1</sup>) MCCR test of  $\gamma$  statistic and ( $P$ -value<sup>2</sup>) mean  $\Delta\text{AIC}$  compared to 5000 simulated trees of the null distribution, r = net diversification rates.

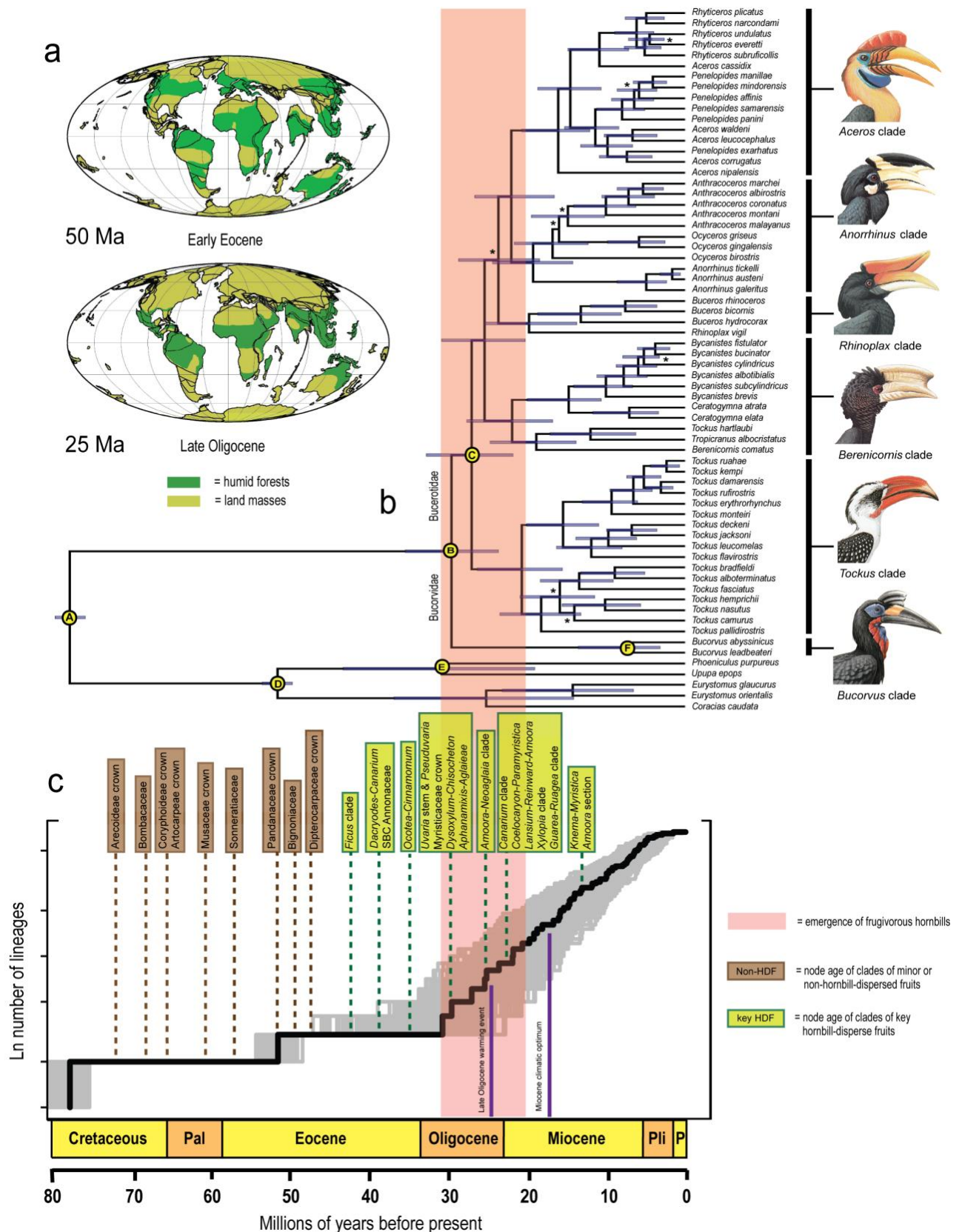


**Fig. 4.1: Area, tempo and mode of hornbill diversification.** (a) Predicted distribution of humid forests during the early Eocene and late Oligocene based on Morley (2000) with modifications (see Supplementary Information, reconstructed maps from Hay *et al.* 1999).

## CHAPTER 4

Emergence of frugivorous hornbills at 25.4 Ma ( $\pm$  5.3 Ma) coincide with formation of the Palaeotropical Biotic Interchange and expansion of humid forests; **(b)** Bayesian chronogram of Bucerotidae with calibration points in yellow circles, with A-C representing molecular divergence and D-F representing fossil dates (see methods for details). Light blue error bars denote the 95% highest posterior densities of each node and asterisks indicate nodes with low to moderate support ( $<0.90$  PP); and **(c)** Semilogarithmic LTT plot based on a maximum clade credibility tree with confidence intervals shown in grey and dashed lines with coloured boxes indicating node ages of key clades of HDFs ( $>15$  hornbill-dispersed taxa) and minor or non-HDFs ( $<15$  hornbill-dispersed taxa). Illustrations are reproduced from Handbook of the Birds of the World with the permission of Lynx Edicions.

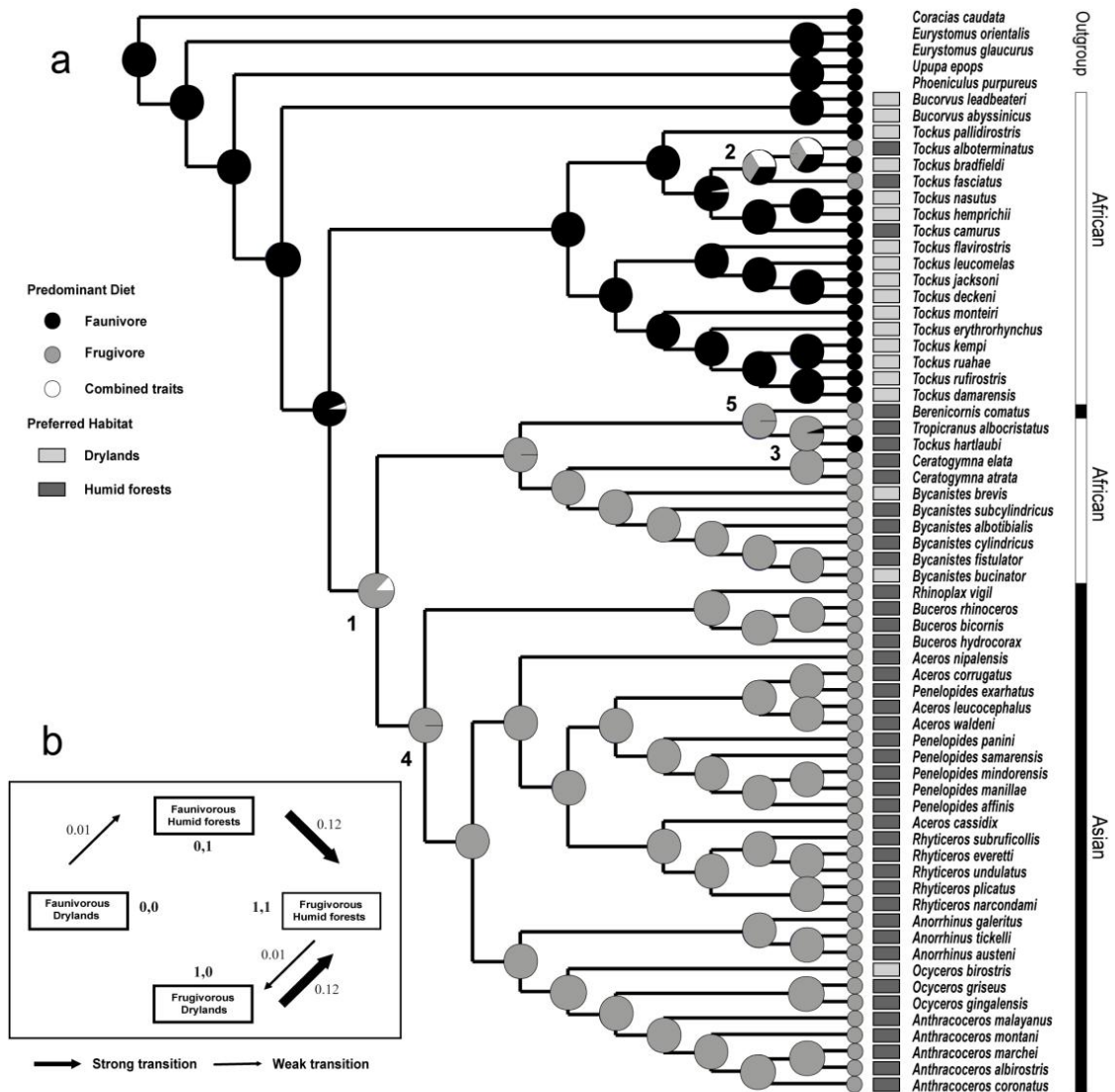


**Fig. 4.2: Evolution of frugivory in hornbills in relation to habitat and biogeography. (a)** Bayesian ancestral character reconstruction of primary diet with strong support at all nodes (PP>0.99). Pie charts represent proportional support for reconstructed states. Numbered nodes indicate changes in diet and distribution along the phylogeny. Nodes 1 & 2 indicated a shift to frugivory, node 3 was a reversion to faunivory, and nodes 4 & 5 marked two colonisations of Asia from Africa. **(b)** Correlated trait evolution pathways from ancestral (0,0) to derived (1,1) diet and preferred habitat under the DISCRETE dependent model using BAYESTRAITS. Arrows indicate strengths of transitions between ancestral and derived states based on rate coefficients from RJMCMC analysis (details in methods).

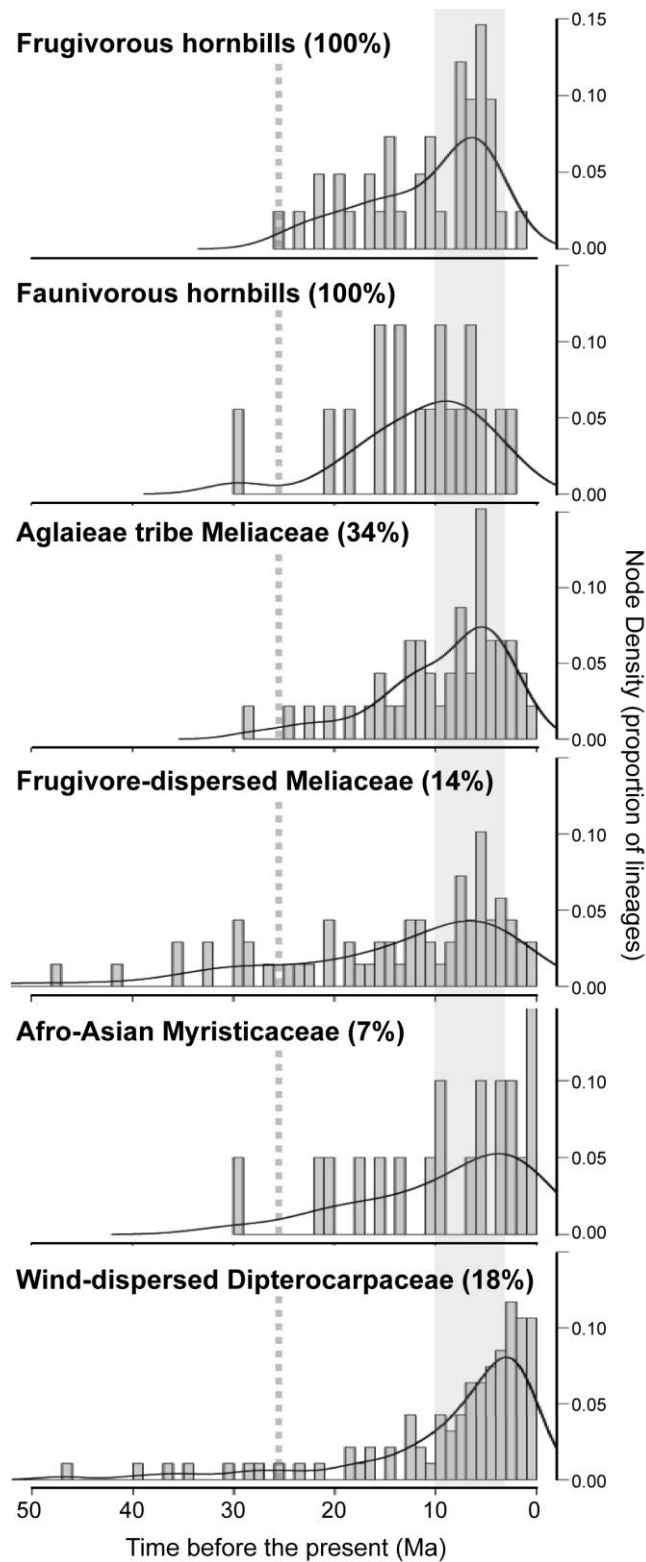


**Fig. 4.3: Patterns of lineage accumulation in hornbills and associated plant families.**

Distribution of lineages across time of hornbills, frugivore-dispersed and wind-dispersed plant families. Grey bar indicates peak accumulation of lineages and vertical dashed line denotes time of emergence for frugivorous hornbills. Percentage values refer to the proportion of taxon sampled in each clade.

## Supplementary information

### **Methods S4.1:** Divergence estimation in associated tropical plant families.

Reconstruction of a well-resolved chronogram of three associated tropical plant families were implemented using multiple-genes and calibrated fossil anchor-points. Representative plant taxa were selected for available sequences of the large subunit of the ribulose-biphosphate carboxylase chloroplast gene (*rbcL*), chloroplast maturase K gene (*matK*), mtDNA nicotinamide adenine dinucleotide dehydrogenase gene (*NADH*), ribosomal protein S16 (*rps16*), ribosomal DNA intergenic spacer (IGS) region and internal transcribed spacer (ITS) region of the 18S-5.8S-26S nuclear ribosomal cistron (Alvarez & Wendel 2003, Arjen de Groot *et al.* 2011). Sequences were derived entirely from GenBank (see Table S5) based on a combination of studies, including Scora & Bergh (1990), Qui *et al.* (1993), Kajita *et al.* (1998), Muellner *et al.* (2003), Sauquet *et al.* (2003), Doyle *et al.* (2004), Richardson *et al.* (2004), Muellner *et al.* (2006), Gamage *et al.* (2006), Newmaster *et al.* (2007), Muellner *et al.* (2008) and Guzmán & Vargas (2009). Datasets include out-groups of closely-related families necessary for calibration: 15 related Magnoliales (e.g. Magnoliaceae, Degeneriaceae and Annonaceae) for Myristicaceae, 30 related Meliaceae for the tribe Aglaia and 10 related Malvales (e.g. Cistaceae, Muntingiaceae and Sarcolaenaceae).

Divergence times were estimated using BEAST with the same clock model, iterations, burn-ins and priors used for hornbills. Myristicaceae was calibrated with two fossil anchor-points, Miocene *Magnolia latahensis* (dated 17-20 Ma) used to constrain the *Magnolia* clade and the early Eocene *Myristicacarpum* (dated 56-49 Ma) representing the stem relative to the nutmeg crown group (Golenberg *et al.* 1990, Kim *et al.* 2004). Tribe Aglaieae and Meliaceae were calibrated with two fossils, *Toona sulcata* was used as  $t_{\text{MRCA}}$  for Cedreleae at 48.6 Ma and a fossil *Guarea* (dated 23.03 Ma) was used to calibrate the node between *Guarea* and *Ruagea* following Muellner *et al.* (2008). Dipterocarpaceae was calibrated using two fossil anchor-points, at 53 Ma with palynofossil Dipterocarps (e.g. *Dipterocarpuspollenites*, *Albertipollenites*)



from India (Ducousso *et al.* 2004, Dutta *et al.* 2011) and at 67 Ma based on a Late Maastrichtian fossil Malvales used to constrain the node between Rutaceae and Aceraceae (Wikström *et al.* 2001).

**Methods S4.2:** Plate-tectonic map reconstruction with predicated distribution of humid forest.

We reconstructed plate-tectonic maps for Early Eocene (50 Ma) and Late Oligocene (25 Ma) periods using an online interactive mapping system ([www.odsn.de/odsn/services/paleomap/paleomap.html](http://www.odsn.de/odsn/services/paleomap/paleomap.html)) developed by the Ocean Drilling Stratigraphic Network (ODSN). The ODSN Plate Tectonic Reconstruction Service was established by GEOMAR, the Research Centre for Marine Geosciences / Kiel and the Geological Institute of the University Bremen. The map was generated using default parameters for boundaries, annotation and gridline intervals, and we selected the Mollweide projection to illustrate our reconstructions, and tectonic plates were moved relative to Africa (Hay *et al.* 1999).

Distribution of humid forest during the Early Eocene and Late Oligocene were based on the predicted coverage of tropical rainforest (TRF) adopted mostly from Morley (2000) and inclusion of seasonal monsoon forests from Bush & Flenley (2006). Amendments to this distribution were derived from the extension of TRF in South America based on Hoorn *et al.* (2010), and the extension of TRF during the Oligocene derived from occurrence of thermophilic organisms (e.g. palms, arboreal primates, tropical freshwater fish) in Ethiopia (Kappelman *et al.* 2003, Jacobs 2006, Couvreur *et al.* 2011), Saudi Arabia (Privé-Gill *et al.* 1999, Otero & Gayet 2011, Zalmout *et al.* 2010), Oman (Kappelman *et al.* 2003, Seiffert 2007, Zalmout *et al.* 2010), Yemen (Kappelman *et al.* 2003), Iran (Koufos & Konidaris 2011) and Pakistan (Marivaux *et al.* 2001).

# CHAPTER 4

**Table S4.1:** List of 61 in-group (in alphabetical order) and eight out-group samples used in this study, with details of distribution, predominant diet, habitat preferences and GenBank accession numbers.

| Taxon                            | Distribution <sup>a</sup> | Predominant diet <sup>a</sup> | Preferred Habitat <sup>a</sup> | Nuclear AK1 intron 5 <sup>b</sup> | mtDNA cytb <sup>b</sup> |
|----------------------------------|---------------------------|-------------------------------|--------------------------------|-----------------------------------|-------------------------|
| In-group                         |                           |                               |                                |                                   |                         |
| <i>Aceros cassidix</i>           | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Aceros corrugatus</i>         | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Aceros leucocephalus</i>      | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Aceros nipalensis</i>         | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Aceros waldeni</i>            | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Anorrhinus austeni</i>        | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Anorrhinus galeritus</i>      | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Anorrhinus tickelli</i>       | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Anthracoceros albirostris</i> | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Anthracoceros coronatus</i>   | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Anthracoceros malayanus</i>   | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Anthracoceros montani</i>     | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Anthracoceros marchei</i>     | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Berenicornis comatus</i>      | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Buceros bicornis</i>          | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Buceros hydrocorax</i>        | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Buceros rhinoceros</i>        | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Bucorvus abyssinicus</i>      | Africa                    | Faunivory                     | Drylands                       | XXX                               | XXX                     |
| <i>Bucorvus leadbeateri</i>      | Africa                    | Faunivory                     | Drylands                       | -                                 | XXX                     |
| <i>Bycanistes albotibialis</i>   | Africa                    | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Bycanistes brevis</i>         | Africa                    | Frugivory                     | Drylands                       | -                                 | XXX                     |
| <i>Bycanistes bucinator</i>      | Africa                    | Frugivory                     | Drylands                       | XXX                               | XXX                     |
| <i>Bycanistes cylindricus</i>    | Africa                    | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Bycanistes fistulator</i>     | Africa                    | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Bycanistes subcylindricus</i> | Africa                    | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Ceratogymna atrata</i>        | Africa                    | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Ceratogymna elata</i>         | Africa                    | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Ocyceros birostris</i>        | Asia                      | Frugivory                     | Drylands                       | XXX                               | XXX                     |
| <i>Ocyceros gingalensis</i>      | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Ocyceros griseus</i>          | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Penelopides affinis</i>       | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Penelopides exarhatus</i>     | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Penelopides manillae</i>      | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Penelopides mindorensis</i>   | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Penelopides panini</i>        | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Penelopides samarensis</i>    | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |
| <i>Rhinoplax vigil</i>           | Asia                      | Frugivory                     | Humid forests                  | XXX                               | XXX                     |

## CHAPTER 4

|                                  |        |           |               |     |                       |
|----------------------------------|--------|-----------|---------------|-----|-----------------------|
| <i>Rhyticeros everetti</i>       | Asia   | Frugivory | Humid forests | XXX | XXX                   |
| <i>Rhyticeros narcondami</i>     | Asia   | Frugivory | Humid forests | XXX | XXX                   |
| <i>Rhyticeros plicatus</i>       | Asia   | Frugivory | Humid forests | XXX | XXX                   |
| <i>Rhyticeros subruficollis</i>  | Asia   | Frugivory | Humid forests | XXX | XXX                   |
| <i>Rhyticeros undulatus</i>      | Asia   | Frugivory | Humid forests | XXX | XXX                   |
| <i>Tockus alboterminatus</i>     | Africa | Frugivory | Humid forests | XXX | XXX                   |
| <i>Tockus bradfieldi</i>         | Africa | Faunivory | Drylands      | XXX | XXX                   |
| <i>Tockus camurus</i>            | Africa | Faunivory | Humid forests | XXX | XXX                   |
| <i>Tockus damarensis</i>         | Africa | Faunivory | Drylands      | -   | AY054443 <sup>c</sup> |
| <i>Tockus deckeni</i>            | Africa | Faunivory | Drylands      | -   | XXX                   |
| <i>Tockus erythrorhynchus</i>    | Africa | Faunivory | Drylands      | -   | XXX                   |
| <i>Tockus fasciatus</i>          | Africa | Frugivory | Humid forests | XXX | XXX                   |
| <i>Tockus flavirostris</i>       | Africa | Faunivory | Drylands      | XXX | XXX                   |
| <i>Tockus hartlaubi</i>          | Africa | Faunivory | Humid forests | XXX | XXX                   |
| <i>Tockus hemprichii</i>         | Africa | Faunivory | Drylands      | XXX | XXX                   |
| <i>Tockus jacksoni</i>           | Africa | Faunivory | Drylands      | -   | XXX                   |
| <i>Tockus kempii</i>             | Africa | Faunivory | Drylands      | -   | AY423372 <sup>c</sup> |
| <i>Tockus leucomelas</i>         | Africa | Faunivory | Drylands      | XXX | XXX                   |
| <i>Tockus monteiroi</i>          | Africa | Faunivory | Drylands      | XXX | XXX                   |
| <i>Tockus nasutus</i>            | Africa | Faunivory | Drylands      | XXX | XXX                   |
| <i>Tockus pallidirostris</i>     | Africa | Faunivory | Drylands      | XXX | XXX                   |
| <i>Tockus ruahae</i>             | Africa | Faunivory | Drylands      | -   | XXX                   |
| <i>Tockus rufirostris</i>        | Africa | Faunivory | Drylands      | -   | XXX                   |
| <i>Tropicranus albocristatus</i> | Africa | Frugivory | Humid forests | XXX | XXX                   |
| <i>Out-group</i>                 |        |           |               |     |                       |
| <i>Phoeniculus purpureus</i>     | Africa | Faunivory | Drylands      | XXX | XXX                   |
| <i>Upupa epops</i>               | Africa | Faunivory | Drylands      | -   | EU167030 <sup>c</sup> |
| <i>Eurystomus orientalis</i>     | Asia   | Faunivory | Humid forests | -   | EU344978 <sup>c</sup> |
| <i>Eurystomus glaucurus</i>      | Africa | Faunivory | Drylands      | -   | AF407452 <sup>c</sup> |
| <i>Coracias caudatus</i>         | Africa | Faunivory | Drylands      | -   | CCU89184 <sup>c</sup> |

<sup>a</sup>Dataset on distribution, preferred diet and habitat based on Kemp (1976), Kemp (1995), Kemp (2001), Carriene *et al.* (2002), Gray *et al.* (2007) and Kitamura (2011).

<sup>b</sup>Abbreviations used for gene regions, AK1 intron 5: adenylate kinase 1 intron 5 gene; *cytb*: cytochrome *b* gene.

<sup>c</sup>Genbank sequences derived from published phylogenetic analyses: Brown *et al.*, 2008; Espinosa de los Monteros, 2000; Fain *et al.*, 2007 Kemp and Delport, 2002; Kirchman *et al.*, 2001; Pratt *et al.*, 2009.

## CHAPTER 4

**Table S4.2:** Summary of alignment features and models of sequence evolution for four datasets.

| Taxon            | Concatenated Gene region <sup>a</sup> | Size    | Model Likelihood <sup>b</sup> | AIC/AICc <sup>b</sup> | <i>lnL</i> <sup>b</sup> |
|------------------|---------------------------------------|---------|-------------------------------|-----------------------|-------------------------|
| Hornbills        | AK1 intron 5- <i>cytb</i>             | 1846 bp | GTR+ $\Gamma$ +I              | 53831.97              | -26905.97               |
| Myristicaceae    | matK- <i>rbcL</i> -IGS-NADH           | 2740 bp | GTR+ $\Gamma$ +I              | 16820.10              | - 8400.05               |
| Meliaceae        | ITS-rps16                             | 1638 bp | GTR+ $\Gamma$ +I              | 30596.85              | -14366.39               |
| Dipterocarpaceae | matK- <i>rbcL</i>                     | 3163 bp | GTR+ $\Gamma$                 | 25356.43              | -11458.59               |

<sup>a</sup>Gene regions used, AK1 intron 5: adenylate kinase 1 intron 5 gene; *cytb*: cytochrome *b* gene. *matK*: maturase K gene; *rbcL*: ribulose-biphosphate carboxylase gene; IGS: intergenic spacer region; NADH: nicotinamide adenine dinucleotide dehydrogenase gene; ITS: internal transcribed spacer region; rps16: ribosomal protein S16.

<sup>b</sup>Abbreviations used, GTR: General Time Reversal model; +  $\Gamma$ : discrete gamma distribution with 5 rate categories; +I: with invariable sites; AICc: corrected Akaike Information Criterion; *lnL*: Maximum Likelihood value;

# CHAPTER 4

**Table S4.3:** List of in-group and out-group sequences of plants used to reconstruct phylogenies of family Meliaceae, Myristicaceae and Dipterocarpaceae in this study, with details of gene regions and GenBank accession numbers.

| Taxon                              | GenBank Accession Numbers of represented gene regions <sup>a</sup> |             |            |             |
|------------------------------------|--------------------------------------------------------------------|-------------|------------|-------------|
| <b>In-group (Myristicaceae)</b>    | <b>maturase K</b>                                                  | <b>rbcL</b> | <b>IGS</b> | <b>NADH</b> |
| <i>Brochoneura acuminata</i>       | -                                                                  | -           | AY220364   | -           |
| <i>Cephalosphaera usambarensis</i> | -                                                                  | -           | AY220367   | -           |
| <i>Coelocaryon oxycarpum</i>       | AY220444                                                           | -           | AY220368   | AY218181    |
| <i>Coelocaryon preussii</i>        | AY743475                                                           | AY743437    | -          | -           |
| <i>Compsonaura atopa</i>           | EU090469                                                           | EU090508    | -          | -           |
| <i>Compsonaura capitellata-A</i>   | EU090471                                                           | EU090512    | -          | -           |
| <i>Compsonaura capitellata-B</i>   | EU090474                                                           | EU090513    | -          | -           |
| <i>Compsonaura debilis-A</i>       | EU090476                                                           | EU090514    | -          | -           |
| <i>Compsonaura debilis-B</i>       | EU090477                                                           | EU090515    | -          | -           |
| <i>Compsonaura excelsa-A</i>       | EU090480                                                           | EU090519    | -          | -           |
| <i>Compsonaura excelsa-B</i>       | EU090481                                                           | EU090520    | -          | -           |
| <i>Compsonaura mexicana-A</i>      | EU090490                                                           | EU090528    | -          | -           |
| <i>Compsonaura mexicana-B</i>      | EU090491                                                           | EU090529    | -          | -           |
| <i>Compsonaura mutisii-A</i>       | EU090495                                                           | EU090533    | -          | -           |
| <i>Compsonaura mutisii-B</i>       | EU090496                                                           | EU090534    | -          | -           |
| <i>Compsonaura sprucei-A</i>       | EU090501                                                           | EU090538    | -          | -           |
| <i>Compsonaura sprucei-B</i>       | AY220445                                                           | EU090539    | -          | -           |
| <i>Compsonaura sprucei-C</i>       | EU090502                                                           | EU090540    | AY220369   | -           |
| <i>Compsonaura ulei-A</i>          | EU090504                                                           | EU090541    | -          | -           |
| <i>Compsonaura ulei-B</i>          | EU090505                                                           | EU090542    | -          | -           |
| <i>Gymnacranthera farquhariana</i> | -                                                                  | -           | AY220370   | -           |
| <i>Haematodendron glabrum</i>      | -                                                                  | -           | AY220371   | -           |
| <i>Horsfieldia basifissa-A</i>     | GQ248135                                                           | FJ976140    | -          | -           |
| <i>Horsfieldia basifissa-B</i>     | -                                                                  | GQ248618    | -          | -           |
| <i>Horsfieldia hellwigii</i>       | -                                                                  | GQ248619    | -          | -           |
| <i>Horsfieldia punctatifolia</i>   | AY220448                                                           | -           | AY220372   | AY218184    |
| <i>Iryanthera hostmanni</i>        | -                                                                  | -           | AY220373   | -           |
| <i>Iryanthera lancifolia</i>       | EU090506                                                           | -           | -          | -           |
| <i>Iryanthera sagotiana-A</i>      | FJ514726                                                           | -           | -          | -           |
| <i>Iryanthera sagotiana-B</i>      | -                                                                  | FJ038128    | -          | -           |
| <i>Knema latericia</i>             | AF197767                                                           | L12653      | AF040694   | AY394740    |
| <i>Knema laurina</i>               | AY220450                                                           | -           | -          | AY218186    |
| <i>Knema sp. SH-2010-A</i>         | -                                                                  | AB586431    | -          | -           |
| <i>Knema sp. SH-2010-B</i>         | -                                                                  | AB586432    | -          | -           |
| <i>Mauloutchia chapelieri</i>      | AY220451                                                           | AF197594    | AY220374   | AY218187    |
| <i>Myristica fatua</i>             | GQ248165                                                           | GQ248653    | -          | -           |
| <i>Myristica fragrans-A</i>        | EU669472                                                           | AF206798    | -          | -           |
| <i>Myristica fragrans-B</i>        | -                                                                  | AY298839    | -          | -           |

# CHAPTER 4

|                                |                   |              |            |             |
|--------------------------------|-------------------|--------------|------------|-------------|
| <i>Myristica globosa</i>       | GQ248166          | GQ248654     | -          | -           |
| <i>Myristica maingayi</i> -A   | AY220452          | -            | -          | -           |
| <i>Myristica maingayi</i> -B   | DQ401374          | -            | -          | -           |
| <i>Myristica</i> sp. 1 GW2420  | -                 | FJ976153     | -          | -           |
| <i>Paramyristica sepicana</i>  | -                 | FJ976152     | -          | -           |
| <i>Pycnanthus angolensis</i>   | AY220453          | -            | AY220378   | AY218189    |
| <i>Virola koschnyi</i>         | EU669473          | -            | -          | -           |
| <i>Virola kwatae</i>           | FJ514688          | FJ038129     | -          | -           |
| <i>Virola michelii</i> -A      | FJ514689          | FJ038131     | -          | -           |
| <i>Virola michelii</i> -B      | AY220454          | FJ038132     | -          | -           |
| <i>Virola multiflora</i>       | GQ982125          | GQ981913     | -          | -           |
| <i>Virola nobilis</i>          | GQ982126          | GQ981914     | -          | -           |
| <i>Virola sebifera</i>         | EU090507          | GQ981915     | -          | -           |
| <b>Out-group (Magnoliales)</b> | <b>maturase K</b> | <b>rbcl</b>  | <b>IGS</b> | <b>NADH</b> |
| <i>Ambavia gerrardii</i>       | AY220435          | HM173806     | AY220358   | AY218168    |
| <i>Anaxagorea acuminata</i>    | AY220436          | -            | -          | -           |
| <i>Anaxagorea luzonensis</i>   | AY518883          | -            | -          | -           |
| <i>Asimina triloba</i>         | AY743479          | AY743441     | AY220359   | -           |
| <i>Cananga odorata</i>         | HM173746          | -            | AY220360   | -           |
| <i>Degeneria roseiflora</i>    | AB055548          | -            | AY220361   | AY218174    |
| <i>Galbulimima belgraveana</i> | AY220441          | -            | AY220362   | AY218176    |
| <i>Liriodendron chinense</i>   | AF123481          | AY841593     | AF123481   | AF107996    |
| <i>Liriodendron tulipifera</i> | HQ619818          | HQ619757     | AF123480   | AF107997    |
| <i>Magnolia grandiflora</i>    | AF548640          | AY298837     | -          | AF107940    |
| <i>Magnolia latahensis</i>     | -                 | X54344       | -          | AY339984    |
| <i>Magnolia salicifolia</i>    | -                 | L12656       | -          | AF107955    |
| <i>Malmea dielsiana</i>        | AY238964          | AY238955     | AY238948   | AY841410    |
| <i>Monodora Myristica</i>      | EU169700          | AY743447     | AY743466   | EF179305    |
| <i>Polyalthia suberosa</i>     | AY518833          | AY319038     | AY238949   | AY841417    |
| <b>In-group (Aglaia clade)</b> | <b>ITS</b>        | <b>rps16</b> |            |             |
| <i>Aglaia archboldiana</i>     | AY695524          | AY695596     |            |             |
| <i>Aglaia vitiensis</i>        | AY695570          | AY695641     |            |             |
| <i>Aglaia basiphylla</i>       | AY695527          | AY69559      |            |             |
| <i>Aglaia samoensis</i>        | AY695557          | AY695629     |            |             |
| <i>Aglaia sapindina</i>        | AY695558          | AY695630     |            |             |
| <i>Aglaia crassinervia</i>     | AY695530          | AY695602     |            |             |
| <i>Aglaia sexipetala</i>       | AY695531          | AY695631     |            |             |
| <i>Aglaia tomentosa</i>        | AY695565          | AY695639     |            |             |
| <i>Aglaia exstipulata</i>      | AY695543          | AY695617     |            |             |
| <i>Aglaia tenuicaulis</i>      | AY695564          | AY695636     |            |             |
| <i>Aglaia glabrata</i>         | AY695547          | AY695619     |            |             |
| <i>Aglaia coriacea</i>         | AY695529          | AY695601     |            |             |
| <i>Aglaia odorata</i>          | AY695551          | AY695623     |            |             |
| <i>Aglaia oligophylla</i>      | AY695554          | AY695626     |            |             |

## CHAPTER 4

|                                         |            |              |
|-----------------------------------------|------------|--------------|
| <i>Aglaia simplicifolia</i>             | AY695560   | AY695632     |
| <i>Aglaia elaeagnoidea</i>              | AY695536   | AY695608     |
| <i>Aglaia chittagonga</i>               | AY695528   | AY695600     |
| <i>Aglaia elliptica</i>                 | AY695540   | AY695612     |
| <i>Aglaia korthalsii</i>                | AY695549   | AY695621     |
| <i>Aglaia edulis</i>                    | AY695533   | AY695607     |
| <i>Aglaia leucophylla</i>               | AY695550   | AY695623     |
| <i>Aglaia argentea</i>                  | AY695526   | AY695598     |
| <i>Aglaia eximia</i>                    | AY695541   | AY695613     |
| <i>Aglaia grandis</i>                   | AY695548   | AY695620     |
| <i>Aglaia pachyphylla</i>               | AY695555   | AY695627     |
| <i>Aglaia forbesii</i>                  | AY695546   | AY695618     |
| <i>Aglaia periviridis</i>               | AY695556   | AY695628     |
| <i>Aglaia silvestris</i>                | AY695561   | AY695633     |
| <i>Aglaia lawii</i>                     | AY695574   | AY695646     |
| <i>Aglaia teysmanniana-A</i>            | AY695537   | AY695653     |
| <i>Aglaia teysmanniana-B</i>            | AY695538   | AY695652     |
| <i>Aglaia australiensis</i>             | AY695571   | AY695642     |
| <i>Aglaia meridionalis</i>              | AY695577   | AY695648     |
| <i>Aglaia cucullata</i>                 | AY695572   | AY695643     |
| <i>Aglaia macrocarpa</i>                | AY695576   | AY695647     |
| <i>Aglaia rugulosa</i>                  | AY695578   | AY695649     |
| <i>Aglaia spectabilis</i>               | AY695579   | AY695651     |
| <i>Aglaia odoratissima</i>              | AY695553   | AY695625     |
| <i>Lansium domesticum</i>               | AY695586   | AY695658     |
| <i>Lansium membranaceum</i>             | DQ861611   |              |
| <i>Reinwardtiodendron cinereum</i>      | AY695588   | AY695659     |
| <i>Reinwardtiodendron humile</i>        | DQ861612   | -            |
| <i>Reinwardtiodendron kinabaluense</i>  | AY695589   | AY695660     |
| <i>Reinwardtiodendron kostermanisii</i> | DQ861634   | -            |
| <i>Aphanamixis borneensis</i>           | AY695583   | AY695654     |
| <i>Aphanamixis polystachya</i>          | AY695584   | AY695655     |
| <i>Aphanamixis sumatrana</i>            | AY695585   | AY695656     |
| <i>Sphaerosacme decandra</i>            | AY695590   | -            |
| <b>Out-group (Meliaceae)</b>            | <b>ITS</b> | <b>rps16</b> |
| <i>Cabrlea canjerana</i>                | DQ861617   | -            |
| <i>Turreanthus sp.</i>                  | DQ861614   | -            |
| <i>Guarea glabra</i>                    | AY695591   | AY695661     |
| <i>Guarea macrophylla</i>               | -          | EU853744     |
| <i>Ruarea pubescens</i>                 | AY695593   | AY695663     |
| <i>Heckeldora staudtii</i>              | AY695592   | AY695662     |
| <i>Dysoxylum arborescens</i>            | DQ499101   | -            |
| <i>Dysoxylum guadichaudianum</i>        | DQ861619.  | -            |
| <i>Dysoxylum spectabile</i>             | DQ499100   | -            |

# CHAPTER 4

|                                     |                   |             |
|-------------------------------------|-------------------|-------------|
| <i>Chisocheton macrophyllus</i>     | DQ861613.         | -           |
| <i>Synoum glandulosum</i>           | DQ861618          | -           |
| <i>Anthocarapa nitidula</i>         | DQ861616          | -           |
| <i>Trichilia cipo</i>               | FJ037837          | -           |
| <i>Trichilia surinamensis</i>       | FJ037839          | -           |
| <i>Pterorhachis zenkeri</i>         | DQ861628          | -           |
| <i>Ekebergia capensis</i>           | DQ861623          | -           |
| <i>Cipadessa baccifera</i>          | DQ861627          | -           |
| <i>Pseudoclausena chrysogyne</i>    | DQ861602          | -           |
| <i>Sandoricum koetjape</i>          | DQ861600          | -           |
| <i>Sandoricum vidalii</i>           | DQ861601          | -           |
| <i>Azadirachta indica</i>           | AY695594          | AY695664    |
| <i>Melia azedarach</i>              | AY695595          | AY695665    |
| <i>Carapa guianensis</i>            | FJ518873          | EU853727    |
| <i>Carapa surinamensis</i>          | FJ518891          | -           |
| <i>Cedrela fissilis</i>             | FJ462475          | EU853729    |
| <i>Cedrela odorata</i>              | GU338247          | -           |
| <i>Toona australis</i>              | FJ462489          | -           |
| <i>Toona ciliata</i>                | FJ462488          | EF126632    |
| <i>Toona sinensis</i>               | FJ462490          | -           |
| <i>Sweitenia macrophylla</i>        | FJ518905          | -           |
| <b>In-group (Dipterocarpaceae)</b>  | <b>maturase K</b> | <b>rbcL</b> |
| <i>Anisoptera laevis</i>            | AB006370          | -           |
| <i>Anisoptera marginata</i>         | AJ581409          | Y15144      |
| <i>Anisoptera oblonga</i>           | AB006371          | -           |
| <i>Cotylelobium malayanum</i>       | AB246414          | -           |
| <i>Cotylelobium scabriusculum</i>   | AB246415          | -           |
| <i>Dipterocarpus alatus</i>         | AB246473          | -           |
| <i>Dipterocarpus baudii</i>         | AB006376          | -           |
| <i>Dipterocarpus cornutus</i>       | AB246472          | -           |
| <i>Dipterocarpus glandulosus</i>    | AB246477          | -           |
| <i>Dipterocarpus hispidus</i>       | AB246476          | -           |
| <i>Dipterocarpus insignis</i>       | AB246475          | -           |
| <i>Dipterocarpus kerrii</i>         | AB006375          | -           |
| <i>Dipterocarpus palembanicus</i>   | AB295903          | -           |
| <i>Dipterocarpus zeylanicus</i>     | AB246474          | -           |
| <i>Dryobalanops aromatica</i>       | AB006377          | -           |
| <i>Dryobalanops oblongifolia</i>    | AB006378          | -           |
| <i>Hopea discolor</i>               | AB246458          | -           |
| <i>Hopea hainanensis</i>            | -                 | AY328198    |
| <i>Hopea helferi</i>                | AB24645           | -           |
| <i>Hopea jucunda</i>                | AB246459          | -           |
| <i>Hopea jucunda subsp. modesta</i> | AB246460          | -           |
| <i>Hopea latifolia</i>              | AB246456          | -           |



# CHAPTER 4

|                                      |          |          |
|--------------------------------------|----------|----------|
| <i>Hopea nervosa</i>                 | AB006384 | -        |
| <i>Hopea odorata</i>                 | AB006385 | -        |
| <i>Hopea subalata</i>                | AB246455 | -        |
| <i>Hopea wightiana</i>               | AB246461 | -        |
| <i>Monotes madagascariensis</i>      | AB246478 | -        |
| <i>Neobalanocarpus heimii</i>        | AB006383 | -        |
| <i>Pakaraimaea dipterocarpacea-A</i> | -        | DQ406587 |
| <i>Pakaraimaea dipterocarpacea-B</i> | -        | DQ406586 |
| <i>Parashorea chinensis</i>          | AY305717 | -        |
| <i>Parashorea kwangsiensis</i>       | AY305718 | -        |
| <i>Parashorea lucida</i>             | AB006382 | -        |
| <i>Pseudomonotes tropenbosii</i>     | -        | AF030238 |
| <i>Shorea acuminata</i>              | AB246440 | -        |
| <i>Shorea affinis</i>                | AB246471 | -        |
| <i>Shorea assamica</i>               | AB246453 | -        |
| <i>Shorea bullata</i>                | AB246435 | -        |
| <i>Shorea congestiflora</i>          | AB246463 | -        |
| <i>Shorea cordifolia</i>             | AB246462 | -        |
| <i>Shorea curtisii</i>               | AB246433 | -        |
| <i>Shorea disticha</i>               | AB246465 | -        |
| <i>Shorea dyeri</i>                  | AB246446 | -        |
| <i>Shorea elliptica</i>              | AB246444 | -        |
| <i>Shorea fallax</i>                 | AB246434 | -        |
| <i>Shorea gardneri</i>               | AB246468 | -        |
| <i>Shorea kunstleri</i>              | AB295896 | -        |
| <i>Shorea laevis</i>                 | AB246449 | -        |
| <i>Shorea leprosula</i>              | AB246439 | -        |
| <i>Shorea lissophylla</i>            | AB246447 | -        |
| <i>Shorea macrophylla</i>            | AB246441 | -        |
| <i>Shorea macroptera</i>             | AB246438 | -        |
| <i>Shorea megistophylla</i>          | AB246464 | -        |
| <i>Shorea multiflora</i>             | AB246451 | -        |
| <i>Shorea ovalifolia</i>             | AB246467 | -        |
| <i>Shorea ovalis</i>                 | AB006380 | -        |
| <i>Shorea pallescens</i>             | AB246448 | -        |
| <i>Shorea parvifolia</i>             | AB246437 | -        |
| <i>Shorea pinanga</i>                | AB246445 | -        |
| <i>Shorea quadrinervis</i>           | AB246436 | -        |
| <i>Shorea richetia</i>               | AB246442 | -        |
| <i>Shorea seminis</i>                | AB246450 | -        |
| <i>Shorea smithiana</i>              | AB295902 | -        |
| <i>Shorea splendens</i>              | AB246443 | -        |
| <i>Shorea stipularis</i>             | AB246454 | -        |
| <i>Shorea talura</i>                 | -        | AJ247623 |

## CHAPTER 4

|                                   |                   |             |
|-----------------------------------|-------------------|-------------|
| <i>Shorea trapezifolia</i>        | AB246466          | -           |
| <i>Shorea worthingtonii</i>       | AB246469          | -           |
| <i>Shorea xanthophylla</i>        | AB246452          | -           |
| <i>Shorea zeylanica</i>           | AB246470          | -           |
| <i>Stemonoporus acuminatus</i>    | AB246422          | -           |
| <i>Stemonoporus bullatus</i>      | AB246426          | -           |
| <i>Stemonoporus canaliculatus</i> | AB246425          | -           |
| <i>Stemonoporus gilimalensis</i>  | AB246423          | -           |
| <i>Stemonoporus kanneliyensis</i> | AB246429          | -           |
| <i>Stemonoporus lancifolius</i>   | AB246430          | -           |
| <i>Stemonoporus reticulatus</i>   | AB246427          | -           |
| <i>Stemonoporus scalarinervis</i> | AB246424          | -           |
| <i>Stemonoporus wightii</i>       | AB24642           | -           |
| <i>Upuna borneensis</i>           | AB006374          | -           |
| <i>Vateria copallifera</i>        | AB246431          | -           |
| <i>Vateriopsis seychellarum</i>   | AB246432          | -           |
| <i>Vatica affinis</i>             | AB246421          | -           |
| <i>Vatica affinis</i>             | AB246421          | -           |
| <i>Vatica bella</i>               | AB246416          | -           |
| <i>Vatica chinensis</i>           | AB246420          | -           |
| <i>Vatica coriacea</i>            | AB246418          | -           |
| <i>Vatica mangachapoi</i>         | -                 | AY328199    |
| <i>Vatica micrantha</i>           | AB246419          | -           |
| <i>Vatica odorata</i>             | AB006373          | -           |
| <i>Vatica pauciflora</i>          | AB246417          | -           |
| <b>Out-group (Malvales)</b>       | <b>maturase K</b> | <b>rbcL</b> |
| <i>Acer saccharum</i>             | AY724265          | -           |
| <i>Cistus ladanifer</i>           | FM179939          | FJ225872    |
| <i>Cistus symphytifolius</i>      | GQ927023          | FJ225864    |
| <i>Citrus trifoliata</i>          | HM163960          | GQ436737    |
| <i>Leptolaena multiflora</i>      | -                 | AY157716    |
| <i>Leptolaena sp. E09</i>         | -                 | AY157719    |
| <i>Muntingia calabura</i>         | FM179930          | AY328197    |
| <i>Sarcolaena oblongifolia</i>    | -                 | U26337      |
| <i>Sarcolaena sp.</i>             | -                 | Y15147      |
| <i>Tilia kiusiana</i>             | AB006386          | -           |

<sup>a</sup>Gene regions used, *matK*: maturase K gene; *rbcL*: ribulose-biphosphate carboxylase gene; IGS: intergenic spacer region; NADH: nicotinamide adenine dinucleotide dehydrogenase gene; ITS: internal transcribed spacer region; rps16: ribosomal protein S16.

## CHAPTER 4

**Table S4.4:** Ancestral state reconstructions of hornbill diet based on Bayesian MCMC analysis in RASP (Yu *et al.* 2011) indicating ancestral character states and shift in clades.

| Taxa group                                     | Faunivory (A) | Frugivory (B) | Combined (AB) | Probability | RASP route               |
|------------------------------------------------|---------------|---------------|---------------|-------------|--------------------------|
| Upupidae-Phoeniculidae                         | 99.95         | 0.00          | 0.05          | 1.0         | A→A <sup>^</sup> A→A A   |
| Bucerotidae                                    | 99.60         | 0.00          | 0.40          | 1.0         | A→A <sup>^</sup> A→A A   |
| <i>Bucorvus</i> clade                          | 99.97         | 0.00          | 0.03          | 0.95        | A→A <sup>^</sup> A→A A   |
| Faunivorous hornbills                          | 99.80         | 0.00          | 0.20          | 0.81        | A→AB→A B                 |
| <i>T. fasciatus</i> - <i>T. alboterminatus</i> | 33.77         | 33.19         | 33.04         | 0.38        | A→AB <sup>^</sup> A→AB A |
| Bucerotinae                                    | 94.90         | 0.40          | 4.86          | 1.0         | A→A <sup>^</sup> A→A A   |
| Frugivorous hornbills                          | 0.78          | 87.32         | 11.90         | 0.87        | B→B <sup>^</sup> B→B B   |
| <i>Berenicornis</i> clade                      | 0.00          | 99.48         | 0.52          | 0.99        | B→B <sup>^</sup> B→B B   |
| African frugivore clade                        | 0.00          | 99.54         | 0.45          | 0.85        | B→B <sup>^</sup> B→B B   |
| Asian frugivore clade                          | 0.00          | 99.58         | 0.42          | 1.0         | B→B <sup>^</sup> B→B B   |
| <i>Aceros</i> clade                            | 0.00          | 99.90         | 0.05          | 1.0         | B→B <sup>^</sup> B→B B   |

# CHAPTER 4

**Table S4.5:** Correlated trait evolution of hornbill diet and preferred habitat based on Multistate Likelihood and Discrete Reversible-jump MCMC analysis in BAYESTRAITS (Pagel & Meade 2006), indicating root states and transitions between trait combinations.

| Taxa group                         | Multistate<br>root state | Root<br>P | Dependent<br>-logLH/-HM | Independent<br>-logLH/-HM | LRT $\chi^2$<br>(P-value) | Bayes<br>Factor | Transition<br>strong | Transition<br>weak |
|------------------------------------|--------------------------|-----------|-------------------------|---------------------------|---------------------------|-----------------|----------------------|--------------------|
| Upupidae & Phoeniculidae           | 0,0                      | 0.85      | 35.60/42.22             | 44.73/55.11               | 18.3(0.001)               | 26.08           | q24,q34              | q12, q43           |
| Bucerotidae                        | 0,0                      | 0.68      | 35.39/42.50             | 44.73/46.9                | 18.7(0.001)               | 8.81            | q34                  | q21,q43            |
| <i>Bucorvus</i> clade              | 0,0/1,0                  | 1.00      | 35.61/42.38             | 44.73/46.65               | 18.3(0.001)               | 8.54            | q24,q34              | q12,q43            |
| Faunivorous hornbills              | 0,0                      | 1.00      | 35.60/43.32             | 44.73/47.21               | 18.3(0.001)               | 7.78            | q24,q34              | q12,q43            |
| <i>T. fasciatus-alboterminatus</i> | 0,1/1,1                  | 0.87      | 35.61/42.26             | 44.73/46.73               | 18.2(0.001)               | 8.92            | q24,q34              | q12,q43            |
| Bucerotinae                        | 1,1                      | 0.84      | 35.62/42.67             | 44.73/47.42               | 18.2(0.001)               | 9.51            | q24,q34              | q12,q43            |
| Frugivorous hornbills              | 1,1                      | 1.00      | 35.60/42.22             | 44.73/46.9                | 18.3(0.001)               | 9.34            | q24,q34              | q12,q43            |
| <i>Berenicornis</i> clade          | 1,1                      | 1.00      | 35.61/44.97             | 44.73/55.08               | 18.3(0.001)               | 20.23           | q24,q34              | q12,q43            |
| African frugivore clade            | 1,1                      | 1.00      | 35.61/46.36             | 44.73/46.63               | 18.3(0.001)               | 0.56            | q24,q34              | q12,q43            |
| Asian frugivore clade              | 0,1                      | 1.00      | 35.62/41.43             | 44.73/56.09               | 18.2(0.001)               | 29.32           | q24,q34              | q12,q43            |
| <i>Aceros</i> clade                | 0,1/1,1                  | 1.00      | 35.61/42.46             | 44.73/46.62               | 18.3(0.001)               | 8.32            | q24,q34              | q12,q43            |

Binary traits refer to 0,0 for ancestral traits (faunivore-non humid forest) and 1,1 for derived traits (frugivore-humid forest), LH =log-likelihood, HM=harmonic mean, LRT=likelihood ratio test, Root P=posterior probability of root/node.

# CHAPTER 4

**Table S4.6:** Rate-constant and rate-variable models of diversification fitted on the phylogeny of hornbills and associated tropical plant families. Model names derived from R package LASER (Rabosky 2006).

| Clade group             | Model      | Type | LH      | AIC           | r1    | r2    | r3    | st1    | st2   |
|-------------------------|------------|------|---------|---------------|-------|-------|-------|--------|-------|
| Hornbills               | birthdeath | RC   | -16.662 | 37.323        | 0.084 | 0.000 | 0.000 | 0.000  | 0.000 |
| (61/61)                 | pureBirth  | RC   | -16.662 | <b>35.323</b> | 0.084 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDX        | RV   | -11.123 | 26.247        | 0.468 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDL        | RV   | -6.433  | 16.865        | 0.209 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | yule2rate  | RV   | -3.274  | <b>12.547</b> | 0.126 | 0.021 | 0.000 | 4.811  | 0.000 |
|                         | yule3rate  | RV   | -1.943  | 13.887        | 0.126 | 0.034 | 0.007 | 4.811  | 2.262 |
| Frugivorous hornbills   | birthdeath | RC   | -24.139 | 52.278        | 0.086 | 0.000 | 0.000 | 0.000  | 0.000 |
| (42/61)                 | pureBirth  | RC   | -24.139 | <b>50.278</b> | 0.086 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDX        | RV   | -19.606 | 43.212        | 0.561 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDL        | RV   | -16.786 | 37.571        | 0.222 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | yule2rate  | RV   | -13.027 | 32.054        | 0.128 | 0.012 | 0.000 | 4.068  | 0.000 |
|                         | yule3rate  | RV   | -11.010 | <b>32.021</b> | 0.332 | 0.119 | 0.007 | 21.812 | 3.702 |
| Faunivorous hornbills   | birthdeath | RC   | -22.507 | 49.014        | 0.078 | 0.000 | 0.000 | 0.000  | 0.000 |
| (17/61)                 | pureBirth  | RC   | -22.507 | <b>47.014</b> | 0.078 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDX        | RV   | -19.765 | 43.530        | 0.622 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDL        | RV   | -18.001 | <b>40.002</b> | 0.271 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | yule2rate  | RV   | -18.757 | 43.514        | 0.163 | 0.039 | 0.000 | 8.852  | 0.000 |
|                         | yule3rate  | RV   | -18.204 | 46.408        | 0.163 | 0.063 | 0.024 | 8.852  | 5.121 |
| Asian frugivore clade   | birthdeath | RC   | -25.024 | 54.048        | 0.087 | 0.000 | 0.000 | 0.000  | 0.000 |
| (31/61)                 | pureBirth  | RC   | -25.024 | <b>52.048</b> | 0.087 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDX        | RV   | -21.805 | 47.609        | 0.505 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDL        | RV   | -19.475 | 42.950        | 0.232 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | yule2rate  | RV   | -16.371 | <b>38.741</b> | 0.137 | 0.015 | 0.000 | 4.468  | 0.000 |
|                         | yule3rate  | RV   | -14.717 | 39.435        | 0.194 | 0.120 | 0.008 | 14.476 | 4.068 |
| African frugivore clade | birthdeath | RC   | -17.819 | 39.638        | 0.070 | 0.000 | 0.000 | 0.000  | 0.000 |
| (11/61)                 | pureBirth  | RC   | -17.819 | <b>37.638</b> | 0.070 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDX        | RV   | -16.907 | 37.815        | 0.297 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDL        | RV   | -15.889 | <b>35.778</b> | 0.218 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | yule2rate  | RV   | -16.514 | 39.028        | 0.105 | 0.032 | 0.000 | 5.905  | 0.000 |
|                         | yule3rate  | RV   | -15.446 | 40.892        | 0.095 | 0.146 | 0.018 | 7.735  | 5.160 |
| <i>Aglaia</i> clade     | birthdeath | RC   | -24.397 | 52.794        | 0.080 | 0.000 | 0.000 | 0.000  | 0.000 |
| (50/146)                | pureBirth  | RC   | -24.397 | <b>50.794</b> | 0.080 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDX        | RV   | -24.335 | 52.669        | 0.095 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | DDL        | RV   | -23.173 | 50.346        | 0.113 | 0.000 | 0.000 | 0.000  | 0.000 |
|                         | yule2rate  | RV   | -15.460 | <b>36.921</b> | 0.110 | 0.023 | 0.000 | 2.817  | 0.000 |
|                         | yule3rate  | RV   | -14.739 | 39.479        | 0.101 | 0.796 | 0.012 | 1.829  | 1.802 |
| Afro-Asian nutmegs      | birthdeath | RC   | -22.198 | 48.396        | 0.064 | 0.000 | 0.000 | 0.000  | 0.000 |
| (23/300)                | pureBirth  | RC   | -22.401 | <b>46.801</b> | 0.090 | 0.000 | 0.000 | 0.000  | 0.000 |

# CHAPTER 4

|                              |            |    |         |               |       |       |       |        |       |
|------------------------------|------------|----|---------|---------------|-------|-------|-------|--------|-------|
| Myristicaceae<br>(51/500)    | DDX        | RV | -22.401 | 48.772        | 0.079 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | DDL        | RV | -22.386 | 48.802        | 0.090 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | yule2rate  | RV | -22.040 | 50.081        | 0.079 | 0.119 | 0.000 | 3.334  | 0.000 |
|                              | yule3rate  | RV | -18.665 | <b>47.329</b> | 0.084 | 9.892 | 0.086 | 9.451  | 9.440 |
|                              | birthdeath | RC | -4.958  | <b>5.916</b>  | 0.091 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | pureBirth  | RC | -3.814  | 5.629         | 0.142 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | DDX        | RV | -4.615  | 5.229         | 0.072 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | DDL        | RV | -3.814  | 3.628         | 0.142 | 0.000 | 0.000 | 0.000  | 0.000 |
| Meliaceae<br>(78/550)        | yule2rate  | RV | -6.034  | 6.068         | 0.108 | 0.197 | 0.000 | 3.410  | 0.000 |
|                              | yule3rate  | RV | -13.148 | <b>16.295</b> | 0.143 | 2.037 | 0.028 | 0.734  | 0.693 |
|                              | birthdeath | RC | -30.082 | 64.165        | 0.059 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | pureBirth  | RC | -30.082 | <b>62.165</b> | 0.059 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | DDX        | RV | -30.009 | 64.018        | 0.071 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | DDL        | RV | -29.746 | 63.491        | 0.070 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | yule2rate  | RV | -27.034 | <b>60.069</b> | 0.066 | 0.025 | 0.000 | 2.713  | 0.000 |
|                              | yule3rate  | RV | -25.827 | 61.653        | 0.066 | 1.147 | 0.033 | 3.728  | 3.715 |
| Dipterocarpaceae<br>(91/500) | birthdeath | RC | 27.340  | 50.679        | 0.031 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | pureBirth  | RC | 18.183  | <b>34.365</b> | 0.082 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | DDX        | RV | 27.182  | 50.365        | 0.017 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | DDL        | RV | 18.182  | <b>32.364</b> | 0.082 | 0.000 | 0.000 | 0.000  | 0.000 |
|                              | yule2rate  | RV | 28.209  | 50.418        | 0.042 | 0.114 | 0.000 | 12.861 | 0.000 |
|                              | yule3rate  | RV | 31.530  | 53.059        | 0.042 | 0.123 | 0.029 | 12.861 | 0.677 |

---

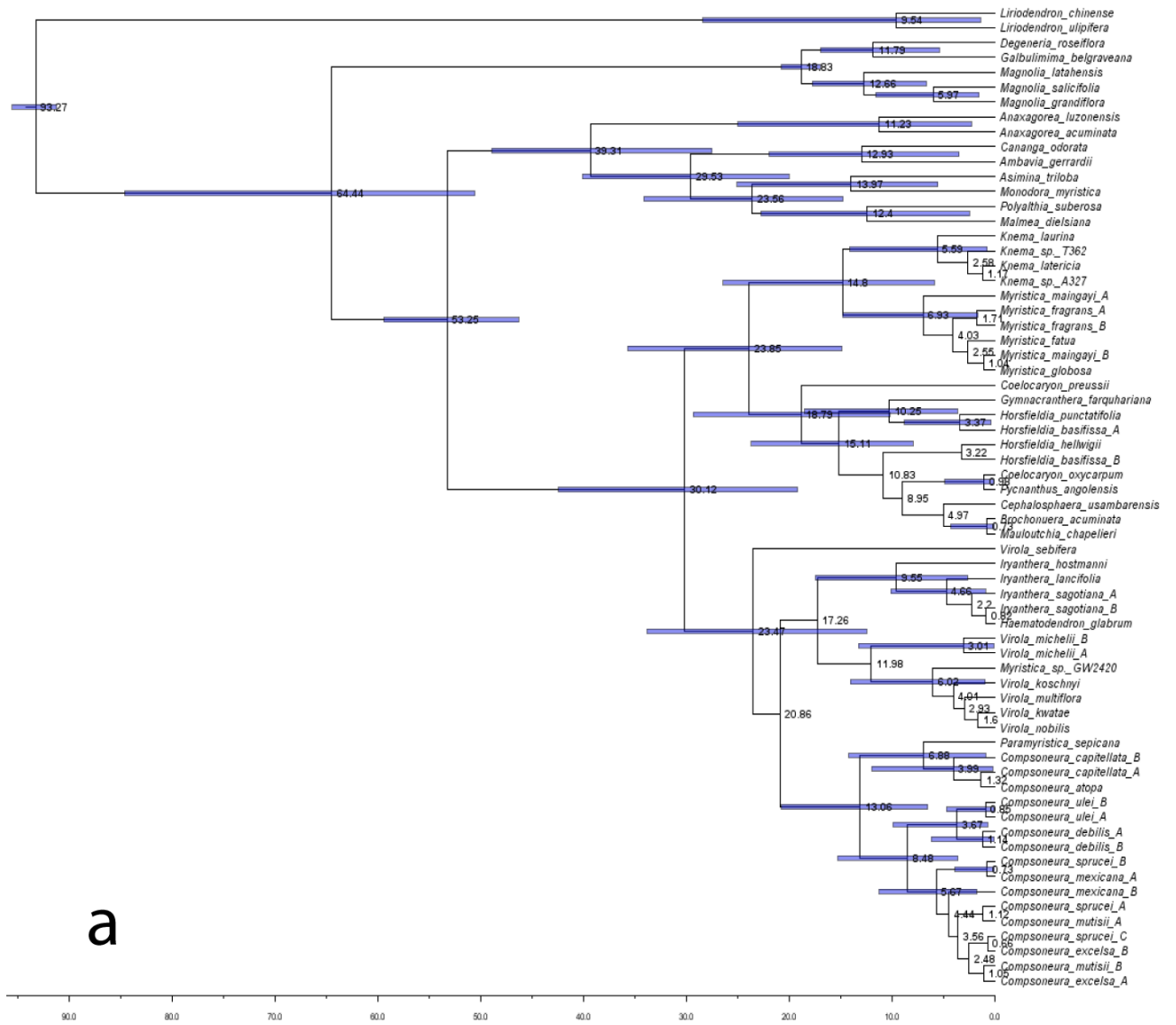
Rate-constant (RC) and rate-variable (RV) models, weighted scores for Likelihood (LH) and Akaike Information Criterion (AIC), net diversification rates (r), shift-times (st).

# CHAPTER 4

**Table S4.7:** Mean node ages of hornbills and associated tropical plant families, indicating confidence intervals based on 95% highest posterior density and node support.

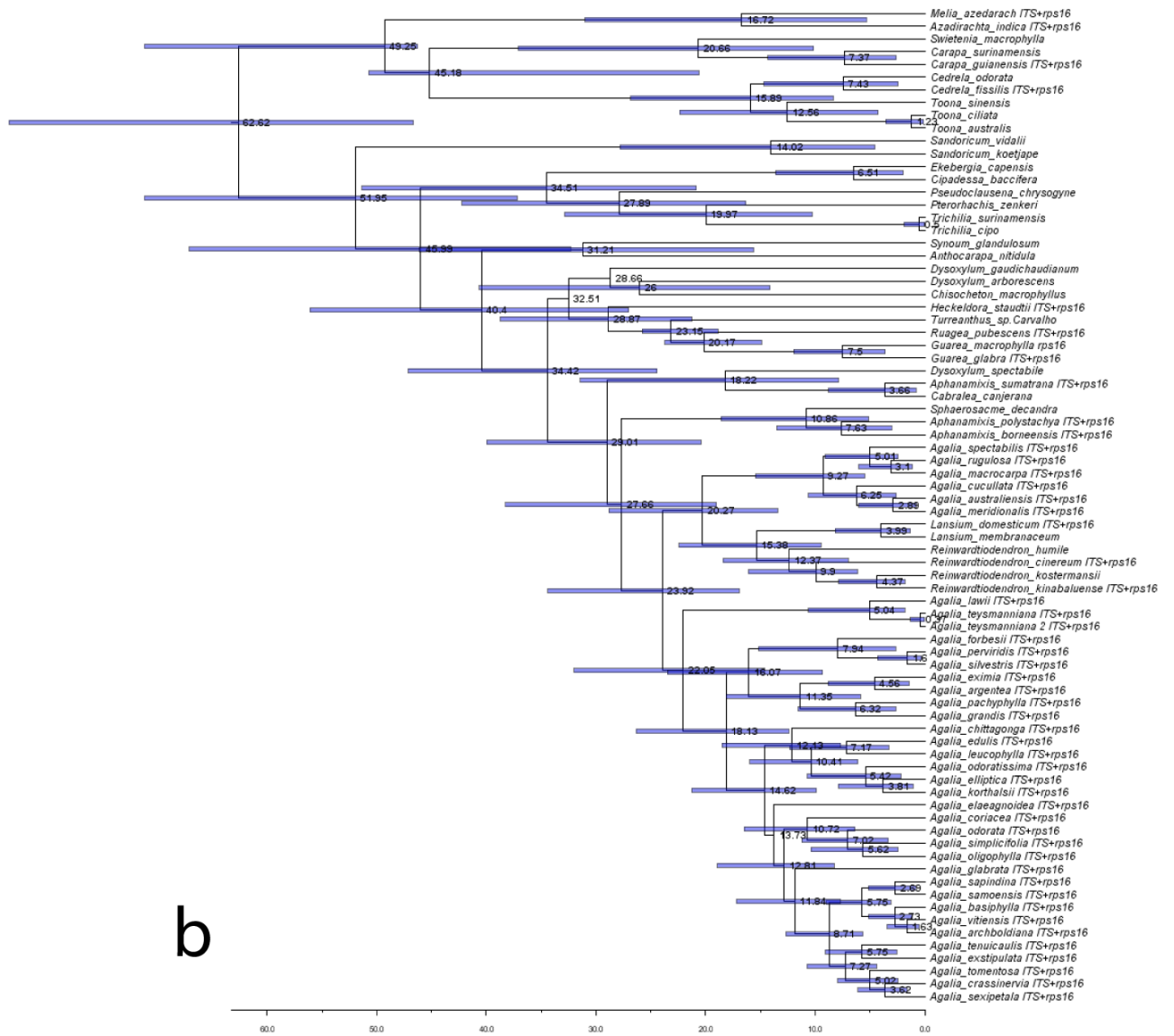
| Taxa group              | Clade                                         | Mean node age (Ma) | 95% confidence intervals (Ma) | Node support (PP or BS) |
|-------------------------|-----------------------------------------------|--------------------|-------------------------------|-------------------------|
| Family Bucerotidae      | Bucerotidae                                   | 29.54              | 23.66-35.4                    | 1.00                    |
|                         | Bucorvus clade                                | 7.35               | 3.15-13.5                     | 1.00                    |
|                         | Faunivorous hornbills                         | 20.64              | 15.51-26.21                   | 1.00                    |
|                         | <i>T. fasciatus-alboterminatus</i>            | 13.32              | 5.04-13.2                     | 0.93                    |
|                         | Bucerotinae                                   | 27.0               | 21.78-32.73                   | 1.00                    |
|                         | Frugivorous hornbills                         | 25.37              | 20.17-30.75                   | 1.00                    |
|                         | <i>Berenicornis</i> clade                     | 18.78              | 13.75-24.60                   | 0.96                    |
|                         | African frugivore clade                       | 21.81              | 16.74-27.54                   | 1.00                    |
|                         | Asian frugivore clade                         | 23.64              | 18.35-28.66                   | 0.71                    |
|                         | <i>Aceros</i> clade                           | 16.03              | 12.01-20.59                   | 1.00                    |
| Family Meliaceae        | <i>Amoora-Lansium</i> <sup>1</sup>            | 30.0               | 22.0-38.0                     | 0.61                    |
|                         | <i>Amoora-Lansium</i>                         | 24.01              | 13.59-47.11                   | 0.99                    |
|                         | <i>Amoora</i> sect. only                      | 9.27               | 5.48-15.46                    | 1.00                    |
|                         | <i>Amoora-Neoaglaia</i>                       | 27.4               | 15.59-49.65                   | 1.00                    |
|                         | <i>Dysoxylum-Chisocheton</i>                  | 28.66              | 24.42-47.11                   | 0.30                    |
|                         | <i>Aphanamixis-Aglaia</i>                     | 29.01              | 20.41-39.96                   | 0.99                    |
| Family Myristicaceae    | Nutmeg crown group <sup>2</sup>               | 19.0               | 17.0-21.0                     | NA                      |
|                         | Nutmeg crown group                            | 30.12              | 19.14-42.43                   | 1.00                    |
|                         | <i>Knema-Coelocaryon</i>                      | 23.85              | 14.86-35.66                   | 0.75                    |
|                         | <i>Knema-Myristica</i>                        | 14.8               | 5.83-26.39                    | 0.98                    |
|                         | <i>Virola-Paramyristica</i>                   | 23.17              | 12.4-33.79                    | 0.88                    |
| Family Dipterocarpaceae | Dipterocarp clade                             | 37.22              | 31.98-53.22                   | 0.94                    |
|                         | <i>Hopea-Shorea</i>                           | 30.3               | 21.55-37.57                   | 1.00                    |
| Family Annonaceae       | Short-branch Clade <sup>3</sup>               | 39.84              | 26.86-55.17                   | 1.00                    |
|                         | <i>Xylopia-Asimina</i> <sup>3</sup>           | 46.62              | 35.68-57.1                    | 1.00                    |
|                         | <i>Onychopetalum-Pseuduvaria</i> <sup>3</sup> | 29.1               | 20.18-39.19                   | 1.00                    |
| Family Burseraceae      | <i>Dacryodes-Canarium</i> <sup>4</sup>        | 38.0               | 32.1-43.9                     | 1.00                    |
|                         | <i>Dacryodes</i> clade <sup>4</sup>           | 27.0               | 18.1-35.1                     | 1.00                    |
|                         | <i>Canarium</i> clade <sup>4</sup>            | 26.0               | 23.3-29.3                     | 1.00                    |
| Family Arecaceae        | Arecoideae crown <sup>5</sup>                 | 73.6               | 66.1-81.3                     | NA                      |
|                         | Coryphoideae crown <sup>5</sup>               | 66.0               | 51.4-80.0                     | NA                      |
| Family Moraceae         | <i>Ficus</i> clade <sup>6</sup>               | 43.3               | 40.1-51.0                     | 100                     |
|                         | <i>Artocarpus</i> clade <sup>6</sup>          | 65.1               | 52.2-80.6                     | 100                     |
| Family Lauraceae        | <i>Ocotea-Cinnamomum</i> <sup>7</sup>         | 35.0               | 29.0-41.0                     | 86                      |

Additional plant node ages from (1) Muellner *et al.* 2008, (2) Doyle *et al.* 2004, (3) Su & Saunders 2009, (4) Weeks *et al.* 2005, (5) Couvreur *et al.* 2011, (6) Zerega *et al.* 2005, (7) Chanderbali *et al.* (2001) and Liao *et al.* (2010). Node support given as Bayesian posterior probability (PP) or Bootstrap values (BS).





# CHAPTER 4



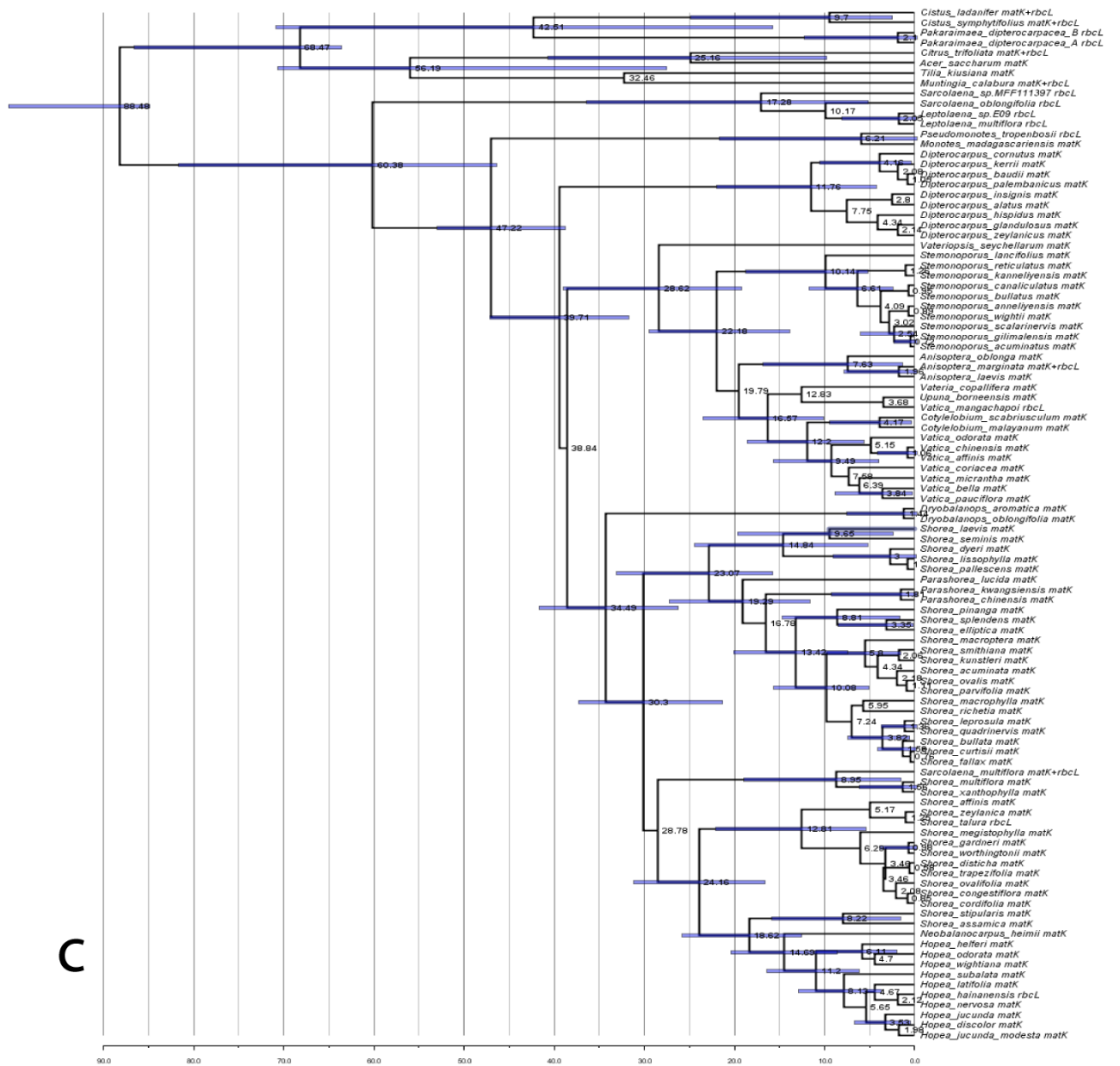


**Figure S4.1:** Chronograms of associated plant families (a) Myristicaceae, (b) Meliaceae and (c) Dipterocarpaceae constructed using BEAST and calibrated based on fossil anchor-points discussed in methods S1. Numbers on each node indicated mean divergence times and light blue bars represent 95% highest posterior densities.

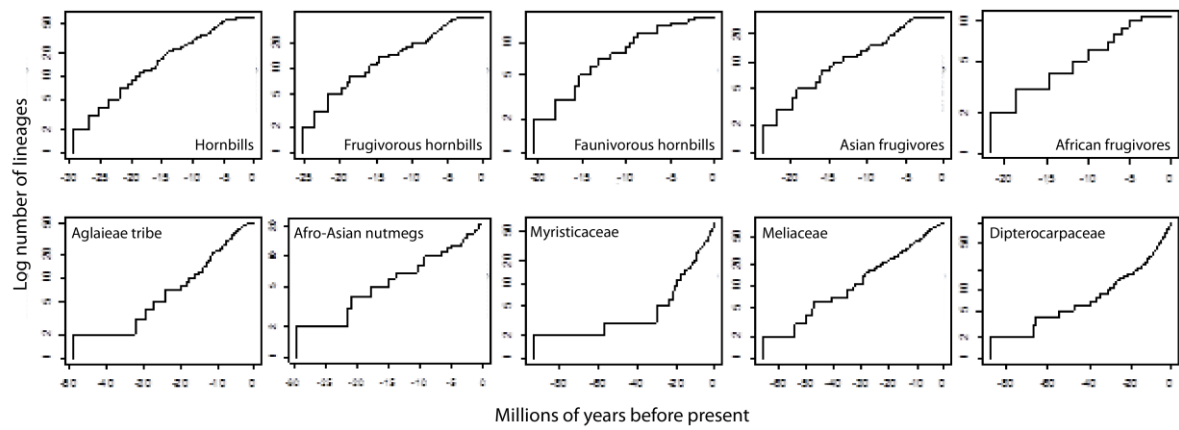


**Figure S4.2:** Lineage-through-time plots (Log number of lineages) of hornbills and associated plant families. Plots are illustrated based on key clades of hornbills and plant families considered to be frugivore-dispersed (Meliaceae and Myristicaceae), including key clades of HDFs (Aglaieae tribe and Afro-Asian nutmegs) and wind-dispersed Dipterocarpaceae.

result of combined:

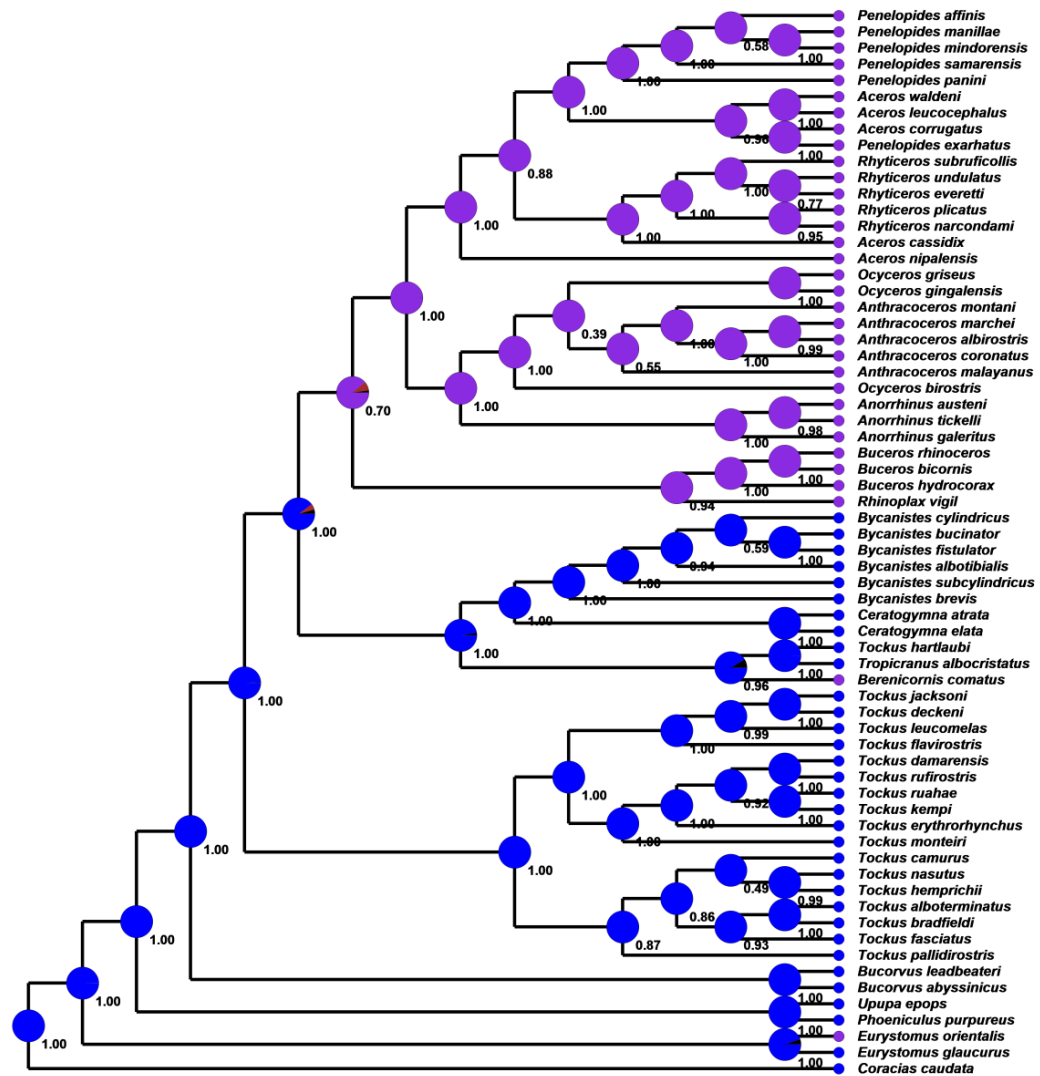


**Figure S4.3:** Ancestral area reconstruction of hornbills using RASP based on binary distribution in Asia (purple) and Africa (blue). Numbers on nodes indicate nodal support based on posterior probabilities and pie charts denote optimal distribution at each node.

## References

- Arjen de Groot G, During HJ, Mass JW, Schneider H, Vogel JC, Erkens RHJ (2011) Use of *rbcL* and *trnL-F* as a two-locus DNA barcode for identification of NW-European ferns: an ecological perspective. *PLOS One*, **6** (e16371), 1-10.
- Alvarez I, Wendel JF (2003) Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution*, **29**, 417–434.
- Brown JW, Rest JS, Garcia-Moreno J, Sorensen MD, Mindell DP (2008) Strong mitochondrial DNA support for a Cretaceous origin of modern avian lineages. *BMC Biology*, **6**, 6.
- Bush MB, Flenley JR (2007) *Tropical Rainforest Responses to Climatic Change*. Springer-Praxis, UK.
- Carrière SM, André M, Letourmy P, Olivier I, McKey DB (2002) Seed rain beneath remnant trees in a slash-and-burn agricultural system in southern Cameroon. *Journal of Tropical Ecology*, **18**, 353-374.
- Chanderbali ASH, Van der Werff H, Renner SS (2001) The relationships and historical biogeography of Lauraceae: Evidence from the chloroplast and nuclear genomes. *Annals of the Missouri Botanical Garden*, **88**, 104-134.
- Couvreur LP, Forest F, Baker WJ (2011) Origin and global diversification patterns of tropical rain forests: inferences from a complete genus-level phylogeny of palms. *BMC Biology*, **9**, 44-56.
- Doyle JA, Sauquet H, Scharaschkin T, Le Thomas A (2004) Phylogeny, molecular and fossil dating, and biogeographic history of Annonaceae and Myristicaceae (Magnoliales). *International Journal of Plant Sciences*, **165**, 55-67.
- Ducousso M, Béna G, Bourgeois C, Buyck B, Eyssartier G, Vincelette M, Rabevohitra R, Randrihasipara L, Dreyfus B, Prin Y (2003) The last common ancestor of Sarcolaenaceae and Asian dipterocarp trees was ectomycorrhizal before the India–Madagascar separation, about 88 million years ago. *Molecular Ecology*, **13**, 231-236.
- Dutta S, Tripathi SM, Mallick M, Mathews RP, Greenwood PF, Rao MR, Summons RE (2011) Eocene out-of-India dispersal of Asian Dipterocarps. *Review of Palaeobotany and Palynology*, **166**, 63-68.
- Espinosa de los Monteros A (2000) Higher-level phylogeny of Trogoniformes. *Molecular Phylogenetics and Evolution*, **14**, 20–34.
- Fain, M.G., Krajewski, C., Houde, P., 2007. Phylogeny of "core Gruiformes" (Aves: Grues) and resolution of the Limpkin-Sungrebe problem. *Molecular Phylogenetics and Evolution*, **43**, 515–529.

- Gamage DT, de Silva MP, Inomata N, Yamazaki T, Szmidt AE (2006) Comprehensive molecular phylogeny of the sub-family Dipterocarpoideae (Dipterocarpaceae) based on chloroplast DNA sequences. *Genes and Genetic Systems*, **81**, 1-12.
- Golenberg EM, Giannasi DE, Clegg MT, Smiley CJ, Durbin M, Henderson D, Zurawski G (1990) Chloroplast DNA sequence from a Miocene *Magnolia* species. *Nature*, **344**, 656-658.
- Gray MA, Baldauf SL, Mayhew PJ, Hill JK (2007) The response of avian feeding guilds to tropical forest disturbance. *Conservation Biology*, **21**, 133-141.
- Guzmán B, Vargas P (2009) Historical biogeography and character evolution of Cistaceae (*Malvales*) based on analysis of plastid *rbcL* and *trnL-trnF* sequences. *Organisms Diversity and Evolution*, **9**, 83-99.
- Hay WW, DeConto R, Wold CN, Wilson KM, Voigt S, Schulz M, Wold-Rossby A, Dullo W-C, Ronov AB, Balukhovsky AN, Soeding E (1999) Alternative Global Cretaceous Paleogeography, in Barrera E, Johnson C (eds.), *The Evolution of Cretaceous Ocean/Climate Systems, Geological Society of America Special Paper*, **332**, pp. 1-47.
- Hoorn C, Wesselingh FP, ter Steege H, Bermudez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T, Antonelli A (2010) Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science*, **330**, 927-931.
- Jacobs BF (2004) Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society of London B*, **359**, 1573-1583.
- Kajita T, Kamiya K, Nakamura K, Tachida H, Wickneswari R, Tsumura Y, Yoshimaru H, Yamazaki T (1998) Molecular phylogeny of Dipterocarpaceae in Southeast Asia based on nucleotide sequences of *matK*, *trnL* intron, and *trnL-trnF* intergenic spacer region in Chloroplast DNA. *Molecular Phylogenetics Evolution*, **10**, 202-209.
- Kappelman J, Rasmussen DT, Sanders WJ, Feseha M, Bown T, Copeland P, Crabaugh J, Fleagle J, Glantz M, Gordon A, Jacobs B, Maga M, Muldoon K, Pan A, Pyne L, Richmond B, Ryan T, Seiffert ER, Sen S, Todd L, Weimann MC, Winkler A (2003) Oligocene mammals from Ethiopia and faunal exchange between Afro-Arabia and Eurasia. *Nature*, **426**, 549-552.
- Kemp AC (1976) *A study of the ecology, behaviour and systematics of Tockus hornbills (Aves: Bucerotidae)*. Transvaal Museum memoir No. 20, Pretoria, 137 p.
- Kemp AC (1995) *Bird families of the world. The Hornbills: Bucerotiformes*. Oxford University Press, Oxford, 318 p.

- Kemp AC (2001) Family Bucerotidae (Hornbills). Pp. 436-523 in: de Hoyo J, Elliott A, Sargatal J, eds. (2001) *Handbook of the Birds of the World*. Vol. 6. Mousebirds to Hornbills. Lynx Edicions, Barcelona.
- Kemp AC, Delpont W (2002) Comments on the status of subspecies in the red-billed hornbill (*Tockus erythrorhynchus*) complex (Aves: Bucerotidae), with the description of a new taxon endemic to Tanzania. *Annals of the Transvaal Museum*, **39**, 1–8.
- Kim S, Soltis DE, Soltis PS, Suh Y (2004) DNA sequences from Miocene fossils: an NDHF sequence of *Magnolia latahensis* (Magnoliaceae) and an RBCL sequence of *Persea pseudocarolinensis* (Lauraceae). *American Journal of Botany*, **91**, 615-620.
- Kirchman JJ, Hackett SJ, Goodman SM, Bates JM (2001) Phylogeny and systematics of ground rollers (Brachypteraciidae) of Madagascar. *The Auk*, **118**, 849–863.
- Kitamura S (2011) Frugivory and seed dispersal by hornbills (Bucerotidae) in tropical forests. *Acta Oecologica* (article in press) doi:10.1016/j.actao.2011.01.015.
- Koufous GD, Konidaris GE (2011) Late Miocene carnivores of the Greco-Iranian Province: Composition, guild structure and palaeoecology. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **305**, 215-226.
- Liao P-C, Kuo D-C, Lin C-C, Ho K-C, Lin T-P, Hwang S-Y (2010) Historical spatial range expansion and a very recent bottleneck of *Cinnamomum kanehirae* Hay. (Lauraceae) in Taiwan inferred from nuclear genes. *BMC Evolutionary Biology*, **10**, 124-141.
- Marivaux L, Welcomme J-L, Antoine P-O, Métails G, Baloch IM, Benammi M, Chaimanee Y, Ducrocq S, Jaeger J-J (2005) A fossil lemur from the Oligocene of Pakistan. *Science*, **294**, 587-592.
- Morley RJ (2000) *Origin and Evolution of Tropical Rain Forests* (Wiley, New York).
- Muellner AN, Samuel R, Johnson SA, Cheek M, Pennington TD, Chase MW (2003) Molecular phylogenetics of Meliaceae (Sapindales) based on nuclear and plastid DNA sequences. *American Journal of Botany*, **90**, 471-480.
- Muellner AN, Savolainen V, Samuel R, Chase MW (2006) The mahogany family “out-of-Africa”: Divergence time estimation, global biogeographic patterns inferred from plastid rbcL DNA sequences, extant, and fossil distribution of diversity. *Molecular Phylogenetics and Evolution*, **40**, 236-250.
- Muellner AN, Pannell CM, Coleman A, Chase MW (2008) The origin and evolution of Indomalayan, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *Journal of Biogeography*, **35**, 1769-1789.
- Newmaster SG, Fazekas AJ, Steeves RAD, Janovec J (2007) Testing candidate plant barcode regions in the Myristicaceae. *Molecular Ecology Notes*, doi: 10.1111/j.1471-8286.2007.02002.x.

- Otero O, Gayet M (2001) Palaeoichthyofaunas from the Lower Oligocene and Miocene of the Arabian Plate: palaeoecological and palaeobiogeographical implications. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **165**, 141-169.
- Pratt RC, Gibb GC, Morgan-Richards M, Phillips MJ, Hendy MD, Penny D (2009) Toward resolving deep neoaves phylogeny: data, signal enhancement, and priors. *Molecular Biology and Evolution* **26**, 313–326.
- Pagel M, Meade A (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *American Naturalist*, **167**, 808–825.
- Privé-Gill C, Thomas H, Lebret P (1999) Fossil wood of *Sindora* (Leguminosae, Caesalpiniaceae) from the Oligocene-Miocene of Saudi Arabia: palaeobiogeographical considerations. *Review of Palaeobotany and Palynology*, **107**, 191-199.
- Qiu YL, Chase MW, Les DH, Parks CR (1993) Molecular phylogenetics of the Magnoliidae: Cladistic analyses of nucleotide sequences of the plastid gene *rbcL*. *Annals of the Missouri Botanical Gardens*, **80**, 587-606.
- Rabosky DL (2006) Likelihood methods for inferring temporal shifts in diversification rates. *Evolution*, **60**, 1152-1164.
- Richardson JE, Chatrou LW, Mols JB, Erkens RHJ, Pirie MD (2004) Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. *Philosophical Transactions of the Royal Society of London B*, **359**, 1495-1508.
- Sauquet H, Doyle JA, Scharaschkin T, Borsch T, Hilu KW, Chatrou LW, Le Thomas A (2003) Phylogenetic analysis of Magnoliales and Myristicaceae based on multiple data sets: implication for character evolution. *Botanical Journal of the Linnean Society*, **142**, 125-186.
- Scora RW, Bergh B (1990) The origins and taxonomy of Avocado (*Persea americana*) Mill. Lauraceae. *Acta Horticultura*, **275**, 387-394.
- Seiffert ER (2007) Evolution and extinction of Afro-Arabian primates near the Eocene-Oligocene boundary. *Folia Primatologica* (Basel), **78**, 314-327.
- Su YCF, Saunders RMK (2009) Evolutionary divergence times in the Annonaceae: evidence of a late Miocene origin of *Pseudeuvaria* in Sundaland with subsequent diversification in New Guinea. *BMC Evolutionary Biology*, **9**, 153-172.
- Weeks A, Daly DC, Simpson BB (2005) The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution*, **35**, 85-101.
- Wikström N, Savolainen V, Chase MW (2001) Evolution of the Angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London B*, **268**, 2211-2220.



## CHAPTER 4

- Yu Y, Harris AJ, He XJ (2010) S-DIVA (statistical dispersal-vicariance analysis): a tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution*, **56**, 848-850.
- Zalmout IS, Sanders WJ, MacLatchy LM, Gunnell GF, Al-Mufarreh YA, Ali MA, Nasser A-A, Al-Masari AM, Al-Sobhi SA, Nadhra AO, Matari AH, Wilson JA, Gingerich PD (2010) New Oligocene primate from Saudi Arabia and the divergence of apes and Old World monkeys. *Nature*, **466**, 360-365 (doi:10.1038/nature09094).
- Zerega NJC, Clement WL, Datwyler SL, Weiblen GD (2005) Biogeography and divergence times in the mulberry family (Moraceae). *Molecular Phylogenetics and Evolution*, **37**, 402-416.

## Chapter 5

### Dispersal across insular barriers in the Palaeotropics: Phylogeny and historical biogeography of Afro-Asian hornbills



*W T Blanford. Fauna of British India. Birds*

*God is great! That surely is the bird! So much for the phoenix.* A collection of responses by Richard Owen after being presented with a head and beak of the Helmeted Hornbill or *Buceros galeatus*, a native of Ceylon (note: there are no *Buceros bicornis* in Sri Lanka).

Richard S. Owen, 1894, The Phoenix p. 5 (1855–56), *The Life of Richard Owen*.

## **Dispersal across continental and insular barriers in the Palaeotropics: Phylogeny and historical biogeography of Afro-Asian hornbills**

**Juan-Carlos T. Gonzalez<sup>1,2</sup>, Ben C. Sheldon<sup>1</sup>, Nigel J. Collar<sup>3</sup> and Joseph A. Tobias<sup>1</sup>**

<sup>1</sup>*Department of Zoology, Edward Grey Institute for Field Ornithology, University of Oxford,  
South Parks Road, Oxford OX1 3PS, UK*

<sup>2</sup>*Institute of Biological Sciences, University of the Philippines Los Baños, Laguna 4031,  
Philippines*

<sup>3</sup>*BirdLife International, Wellbrook Court, Girton Road, Cambridge CB3 0NA, UK*

### **Abstract**

**Aim** Recent phylogeographic studies have postulated different dispersal-vicariance regimes for organisms distributed across the Afrotropical, Indo-Malayan and Melanesian regions. Some have attempted to determine how certain taxonomic groups traversed major biogeographical barriers such as Wallace's and Lydekker's lines to inhabit fringing archipelagos. However, incomplete sampling across these regions has limited understanding of historical distributions crucial to identifying ancestral colonization routes. Hornbills are an ideal model system for testing hypotheses on when, where and how the continental and insular Palaeotropics were colonized.

**Location** sub-Saharan Africa, South Asia, Southeast Asia, Melanesia

**Methods** Complete sampling of geo-isolates can be used to reconstruct historical biogeography and trace routes taken across biogeographical barriers. Combined likelihood and Bayesian approaches were used to construct a comprehensive phylogeny of African and Asian hornbills

based on mtDNA Cytochrome *b* sequences derived from historical and contemporary samples representing 78 taxa in 160 geo-isolates. A dated phylogeny was obtained using BEAST calibrated with both molecular and fossil anchor-points. Biogeographical inferences were derived from reconstructed ancestral areas using Bayesian MCMC analysis implemented in RASP.

**Results** The comprehensive *cytb* phylogeny of hornbill geo-isolates provides a more resolved view of intra-specific relations, congruent with a species-level chronogram based on concatenated nuclear-mtDNA sequences. Both chronograms place crown age for Bucerotidae in the late Oligocene. Divergence of continental and insular clades occurred during the Miocene, coinciding with key tectonic and palaeo-ecological events. Hornbills traversed Huxley's and Wallace's line in the early to mid-Miocene, while Lydekker's line was traversed during the late Miocene. Bayesian ancestral area analyses confirmed dispersal from Africa to Southeast Asia, with dispersal-vicariance events in African hornbills coinciding with historical changes in habitat and aridity. Reconstruction indicates India-Indochina and Sundaland as important ancestral areas for continental and insular Asian hornbills; Sundaland served as the jump-off point for radiation into Philippines and Wallacea via different Miocene routes.

**Main conclusions** Bucerotidae follows an "Out-of-Africa" dispersal into tropical Asia. Historical distribution of African and continental Asian hornbills was influenced by changes in palaeo-environment, while tectonic movements facilitated insular dispersal in Asian hornbills. Central Africa, India, Sundaland, Sulawesi and Philippines served as core ancestral areas to hornbill speciation. The unique assemblage and distinct colonization routes taken by Philippine hornbills supports the separation of this region as a biogeographic zone distinct from Wallacea.

### **Keywords**

Hornbills, historical biogeography, ancestral area reconstruction, dated phylogeny, Wallace's line, Huxley's line, Philippines, Wallacea, Sundaland

**Running title:** Historical biogeography of Afro-Asian hornbills

Formatted for Journal of Biogeography

## INTRODUCTION

Permeability of biogeographic boundaries has been an important focus for research in evolutionary biogeography (Morrone, 2009; Albert & Crampton, 2010; Lohman *et al.*, 2011). Depending on the interaction between the habitat and the organism, continuous landscapes can act both as a bridge to facilitate dispersal and as a barrier to impede or filter movement and gene flow. Allopatric speciation eventually results from the accumulated genetic differentiation resulting between spatially disjunct populations caused by dispersal or vicariance (Avice, 2000; Newton, 2003; Beldade *et al.*, 2009). Various models have been presented to understand how these landscapes can either facilitate or impede speciation since biogeography was introduced into the modern evolutionary synthesis (Mayr, 1942; Simpson 1940; 1977). Simpson's concept of dispersal probability and centre-of-origin emphasizes the constraints on dispersal and paths of faunal interchange (Heads, 2009; Morrone, 2009). Simpson (1940) defined corridors as routes that do not impede movements, while filter bridges restrict movements, and sweepstakes routes allow chance events to lead to the crossing of formidable barriers. These constraints coincide with dispersalist idea of the centre-of-origin model, which postulates the biotic expansion and contraction of a taxon, in response to insuperable barriers (Simpson, 1940). A central origin approach characterizes the "Out-of-Africa model", originally proposed for human evolution, but now widely used for explaining the biogeography of various taxa with African origins dispersing out into the Pantropics (Weeks *et al.*, 2005; Zhou *et al.*, 2011). Extensive use of molecular techniques in phylogeography now allows us to test these concepts and trace evolutionary pathways of organisms across biogeographic boundaries (Avice, 2000).

Recent phylogeographic studies have described a number of different dispersal-vicariance regimes for organisms distributed across the Palaeotropics (Weeks *et al.*, 2005; Jönsson *et al.*, 2010; Bacon *et al.*, 2012). These studies determine mechanisms by which certain taxonomic groups traversed major biogeographical barriers, such as Wallace's and Lydekker's lines to inhabit fringing archipelagos of the Indo-Pacific region, from India to Southeast Asia

and Melanesia (Jansa *et al.*, 2006; Müller & Beheregaray, 2010; Schweizer *et al.*, 2012). These barriers determine the division between the Indo-Malayan and Australasian realms, with Wallace's line following the edge of the Sunda Shelf and Lydekker's line following the edge of the Sahul Shelf (Newton, 2003). Alternatively, Huxley's line (also known as the Merrill-Dickerson line) changes the position of the Philippines to lie to the east of Wallace's original division (Diamond & Gilpin, 1983; Van Welzen *et al.*, 2011). The islands between Wallace's and Lydekker's lines constitute the transitional zone called Wallacea, which sometimes includes or excludes the Philippines (Vallejo, 2011). Evidence for the "downstream" dispersal from Africa to Asia and eastward into Australasia is available for some Palaeotropical flora. Historical biogeography of *Aglaia* and *Uvaria* suggested that ancestral forms crossed Wallace's and Lydekker's line during the Miocene (Muellner *et al.*, 2008; Zhou *et al.*, 2011). However, it is unclear whether different faunal elements follow the same tempo of dispersal from the Sunda Shelf to the Sahul Shelf.

Our study focuses on identifying processes that facilitated crossing of biogeographic borders from Africa to Australasia. We applied molecular methods to determine the tempo of phylogenetic evolution and understand the influence of palaeo-environments and tectonic movements on the diversification and biogeography of a classic Palaeotropical group. Hornbills (Family Bucerotidae) are an ideal model system for tracing these evolutionary pathways and testing hypotheses about when, where and how continental and insular Palaeotropics were colonized. Hornbills are exclusively distributed in the Palaeotropics, from the coastal tropics of West Africa to the Solomon Islands (Fig. 1). Some 61 species inhabit a wide variety of habitats, from the dry desert thornvelds and savannah grasslands of Africa to humid tropical evergreen rainforests of Southeast Asia (Kemp, 1995). Half of the species inhabit tropical Asia, distributed along continental India, Indo-China and Sundaland, and insular regions of the Philippines, Wallacea and Melanesia.

The evolutionary history of Bucerotidae has for many years been uncertain due to depauperate fossil evidence and absence of a resolved dated phylogeny. However, some

evidence suggested that hornbill radiation probably intensified during the late Miocene and Pliocene (Kemp, 2001; Kinnaird & O'Brien, 2007). Previous molecular phylogenies have provided valuable insights into the tempo of hornbill speciation (Hübner *et al.*, 2003; Viseshakul *et al.*, 2011), but present a different timing scenario (i.e., Eocene emergence) from a more recent species-level multi-locus phylogenetic tree (Gonzalez & Tobias, in prep., see Chapter 4). Gonzalez & Tobias (in prep) discussed the importance of the Palaeotropical biotic interchange during two key warming events on the dispersal and diversification of hornbills from Africa to Asia. Their dated phylogeny of concatenated nuclear and mitochondrial DNA (mtDNA) sequences suggests that the crown-group of hornbills evolved during the late Oligocene, hence long after splitting from the ancestral Bucerotes in the Cretaceous (Pacheco *et al.*, 2011) and after the Eocene collision of India to Asia (Najman *et al.*, 2010; Viseshakul *et al.*, 2011). Ancestral area reconstruction reaffirms Africa as the centre-of-origin for Bucerotidae, and these findings (Gonzalez & Tobias, in prep) support the claim by Kinnaird & O'Brien (2007) regarding the colonization of Afrotropical hornbills into Asia.

To understand further the potential dispersal routes taken to colonize Asia, we compared reconstructions between the concatenated nuclear-mtDNA chronogram from Gonzalez & Tobias (in prep) and a comprehensive phylogeny of hornbill “geo-isolates”. This representation of molecular “geo-isolates” or sequences of species, subspecies, regional and island subpopulations can provide a robust time-tree to reconstruct the historical biogeography of Bucerotidae. Our phylogeographic analysis aims to trace evolutionary pathways taken by hornbills and answer the following questions: (1) When did hornbills traverse key biogeographic barriers, such as Wallace’s, Huxley’s and Lydekker’s lines? (2) Which palaeo-geologic and palaeo-climatic events influenced radiation of hornbills across the continental and insular Palaeotropics?



## MATERIALS AND METHODS

### Taxon sampling and DNA sequencing

Over 500 historical and contemporary samples were collected from various museums and aviaries worldwide to form a comprehensive dataset for Family Bucerotidae (Table S5.1/ see supporting information). This represents 160 geo-isolates of African and Asian hornbills covering nearly all known species and subspecies, with emphasis on regional and insular populations of Asian hornbills. We used only a single locus for our analysis, targeting the mitochondrial DNA cytochrome *b* gene (*cytb*). Classification follows the current International Ornithological Congress World Bird List version 2.11 (Gill & Donsker, 2012). Genomic DNA was extracted from historical (toe-pads) and contemporary sources (molted flight feathers and pin-feathers preserved in 70–90% ethanol) using modified protocols for the DNeasy blood & tissue kit and QiaQuick PCR purification kit (Qiagen, West Sussex, UK), following Morin *et al.* (1994) and Mundy *et al.* (1997). Historical samples were processed in a separate non-avian laboratory (J.W. Jenkinson Laboratory for Evolution & Development, Oxford) and used both extraction and polymerase chain reaction (PCR) controls to minimize cross-contamination (Vallianatos *et al.*, 2002; Lerner & Mindell, 2005).

Short fragments (200-500 bp) of the *cytb* gene were amplified using specific primers designed from GenBank sequences (Table S5.2/ see supporting information) using the online program PRIMER3 version 4.0 (Rozen & Skaletsky, 1998) to improve amplification and reduce plausibility of contaminants. PCR reactions were completed using Qiagen HotStarTaq Master Mix in ABI 2720 thermal cyclers (Applied Biosystems, Foster City, CA) with the following PCR profile: touchdown of 15 min at 95 °C, followed by 45 cycles of 94 °C for 30 seconds, 52 °C for 45 seconds, and 72 °C for 60 seconds, and a final extension phase at 72 °C for 10 minutes. Amplicons were visualized in 2% Agarose gel (with 2.5 µl Ethidium Bromide) and purified through the Qiagen Mini-elute kit. Cycle sequencing was performed using the Big Dye Sequencing kit, purified through standard 2-propanol precipitation and analyzed through the

ABI Prism Genetic Analyzer 377. Sequence fragments were edited through visual review of ambiguous bases from trace files of digital chromatograms and contigs concatenated using BIOEDIT version 7.0.5.3 (Hall, 1999) and SEQUENCHER version 4.2 (Gene Codes Corp., Ann Arbor, MI). Novel *cytb* sequences were uploaded into GenBank with Accession Nos. XXX-XXX (Table S5.1/ see supporting information).

### **Sequence alignments and phylogenetic analyses**

Cytochrome *b* sequences were aligned in CLUSTALW version 2.0 (Larkin *et al.*, 2007) and truncated to 1,143bp using a prescribed start codon (ATG) and termination codon (TAA/TAG) following, Liu *et al.* (2012). Phylogenetic and molecular evolutionary analyses were done using Maximum Likelihood (ML) and Bayesian inference (BI). ML fits of 24 different nucleotide substitution models indicated GTR (general time reversal model) with discrete Gamma distribution and assumed invariable sites as the best model based on least scores of the Bayesian Information Criterion (BIC, 80327.8), corrected Akaike Information Criterion (AIC, 72716.8) and ML values (lnL, -35647.9) conducted in MEGA version 5.03 (Tamura *et al.*, 2011). The same model was selected by AIC (70874.9) and lnL (-35427.5) using MRMODELTEST version 2.3 (Nylander, 2004). ML was conducted using MEGA5 and robustness of clades was estimated using non-parametric bootstrapping with 1,000 pseudo-replicates (Felsenstein, 1985) and followed the Closest-neighbor interchange heuristic method using the selected best-fit model (GTR+I+ $\Gamma$ ). An added ML analysis was drawn from PHYML version 3.0 in SEAVIEW version 4.0 using the approximate Likelihood-ratio test (LRT) based on a Shimodaira-Hasegawa-like procedure (Guindon & Gascuel, 2003).

For BI, we performed two independent runs of the Metropolis-coupled Markov-chain Monte Carlo (MCMC) analyses in MRBAYES version 3.1 (Ronquist & Huelsenbeck, 2003). Each run consisted of three heated chains and one cold chain, set with default parameters and priors, for 20 million iterations, sampled every 1,000 generations and logged for every 10,000 increments. We discarded a burn-in of 1,000 after runs reached stationary distribution

(PRSF<0.1). A consensus tree derived from 10,000 trees was visualized in FIGTREE version 1.3.1 (Rambaut, 2006) with clade robustness based on consistent posterior probabilities (PP) and consolidated with ML results using MEGA5. We considered 70–89 % bootstrap values (BV) and 0.90–0.95 PP as moderate support, and 90–100 % BV and >0.95 PP as strong support (Muellner *et al.*, 2008; Siler *et al.*, 2010).

### Timing of divergence

The molecular clock test was performed in MEGA5 comparing ML values for the given topology with and without the molecular clock constraints under the GTR+ $\Gamma$ +I model to determine significant deviation from the null hypothesis of equal evolutionary rate. The LRT rejected our strict molecular clock ( $\ln L$  -36277.04) in favour of the relaxed clock ( $\ln L$  -35374.14) at  $P<0.05$  (Tamura *et al.*, 2007). Divergence time estimation was performed in BEAST version 1.6.1 using templates from BEAUti version 1.6.1 (Drummond & Rambaut, 2007). Two independent MCMC runs of 10 million iterations each were implemented generally following default tree parameters and priors, but topology was constrained with multiple tree priors each set for monophyly. Trees were sampled every 1,000 generations and logged at every 1,000, with burn-ins of 10%. Substitution rates were estimated under an uncorrelated lognormal relaxed clock model following the Yule speciation process.

Selected taxon sets were calibrated at the stem for “time to most recent common ancestor” ( $t_{\text{MRCA}}$ ), using both dated fossil records and recent molecular divergence data (Ho & Phillips, 2009). Divergence estimates were derived from Pacheco *et al.* (2011), one of which constrains the tree root height at 78.56 Ma (s.d. 1.0 Ma), the molecular age between hornbills and rollers. The node representing  $t_{\text{MRCA}}$  for Bucerotidae was calibrated at 47.06 Ma (s.d. 10.0 Ma), and  $t_{\text{MRCA}}$  for forest hornbills (excluding the *Tockus* clade) was calibrated at 31.56 Ma (s.d. 10.0 Ma) (Pacheco *et al.*, 2011). Three fossils were used as a basis for Bucerotidae divergence, including *Bucorvus brailloni* as  $t_{\text{MRCA}}$  for Bucorvinae calibrated at 7.0 Ma (s.d. 5.0 Ma). *B. brailloni* occurred between 7.0 and 11.5 Ma in northwest Africa (Brunet, 1971) around the same

period as *Euroceros bulgaricus* in southern Europe (Boev & Kovachev, 2007). Phylogeny was calibrated at the stem of two out-group clades, using *Primobucco mcgrewi* as  $t_{MRCA}$  for Coraciiformes dated at 51.66 Ma (s.d. 1.0 Ma, Ksepka & Clarke, 2010) and *Messelirrisor* as  $t_{MRCA}$  for Upupiformes dated at 47 Ma (s.d. 5.0 Ma, Mayr, 2006). Convergence was diagnosed using TRACER version 1.5 (Rambaut & Drummond, 2007) with nearly all values showing Effective Sample Sizes (ESS) above 100 suggesting valid estimates of the posterior distribution. Independent runs were merged in LOGCOMBINER version 1.6.1, each with a burn-in of 1,000. A single 50% maximum clade credibility (MCC) tree was generated using TREEANNOTATOR version 1.6.1 (Drummond & Rambaut, 2007). Resulting MCC tree was visualized in FIGTREE with node bars indicating 95% highest posterior densities (HPD) corresponding to credible interval values of estimated node ages.

### **Ancestral range reconstruction**

Non-parametric empirical Bayesian binary MCMC (BBM) analysis was used to infer biogeographic histories in hornbills using the program RASP version 2.0 (Reconstruct Ancestral State in Phylogenies, Yu *et al.*, 2011). RASP calculates ancestral states based on optimal distributions at each node providing support value (posterior probability) of the ancestor for enclosed terminals. BMM analysis of the single MCC tree derived from BEAST provides a measure of statistical support for each node. Frequency of ancestral states was plotted as marginal distributions generated from two independent runs of BMM each with 500,000 cycles, sampled for every 100 and with 10 heated chains (temperature at 0.1) following the Felsenstein 1981 model (F81) with  $\Gamma$  distribution. For the concatenated nuclear DNA-mtDNA chronogram, four biogeographic regions were used for ancestral area reconstructions. These comprised (i) Africa and Arabian Peninsula (Ethiopian realm), (ii) Indo-Malayan realm, (iii) the Philippines, and (iv) Wallacea-Melanesia. For the complete *cytb* chronogram, we assigned distribution of taxa based on more specific biogeographic areas, including (A) South & East Africa, (B) North & West Central Africa, (C) India and Sri Lanka, (D) Indo-China (including Andamans group),

(E) Sundaland, (F) Philippines (excluding Palawan group), (G) Wallacea and Melanesia. Biogeographic areas and regional limits were based on Kreft & Jetz (2010). To illustrate the palaeo-environment in specific time periods, plate-tectonic maps were reconstructed in an on-line program developed by ODSN-GEOMAR (Hay *et al.*, 1999). Additional comparisons were made with reconstructed plate-tectonic maps of Southeast Asia and Southwest Pacific by Hall (1998) and Hall *et al.* (2011). Palaeo-vegetation maps of Africa for these four time periods (20 Ma, 10 Ma, 5 Ma and present day) were re-constructed from Dowsett *et al.* (1999), Mistry (2000), Bush & Flenley (2007) and Edwards *et al.* (2010).

## RESULTS

### *Sequence variation and phylogenetic analysis*

Extractions were successful for 388 historical and 90 contemporary samples, but only 300 amplified sufficient lengths of mtDNA *cytb* (~900bp). An additional 42 *cytb* sequences were downloaded from NCBI GenBank to complete the dataset. Partial coding sequences (cds) were available for 78 out of 79 recognized species and subspecies (Kemp 2001), with a complete *cytb* cds for 57 out of 61 hornbill species. Only *Bycanistes fistulator fistulator* was absent from the collated dataset. The large sequence matrix contains 351 sequences, ranging in size from 354 bp to 1,143 bp, and includes nine out-group taxa and 342 in-group taxa (Table S5.1/ see supporting information). The sequence matrix including the out-group (rollers, hoopoes and woodhoopes) consisted of 1,143 characters, of which 373 sites are conserved, 770 are variable sites, and 689 are parsimony informative sites. By comparison, the in-group dataset of hornbills had 402 conserved sites, 741 variable sites, and 661 parsimony informative sites. A comprehensive mtDNA *cytb* phylogeny of hornbills (Bucerotidae) was constructed using Bayesian ( $\ln L -35060.4$ ) and ML analysis ( $\ln L -35190.5$ ) which hypothesized relationships between 160 geo-isolates in 78 taxa (see Fig. 5.2). Support for nodes of clades formed were based on Bayesian posterior probabilities (PP) and ML bootstrap value (BS). The majority of

the clades (c. 75%) had PP  $\geq$  90, and BS  $\geq$  70 showing moderate to strong support for the tree topology.

The inferred topologies were congruent between the species-level multi-locus tree (Fig. 5.1, based on Gonzalez *et al.*, in prep., see Chapter 2) and the expanded single-locus (*cytb*) geo-isolate tree (Fig. 5.2), although some differences are apparent. Ten discrepancies occur in areas receiving low nodal support, including placements of *Tockus camurus*, *T. pallidirostris*, *Rhyticeros everetti* and *R. undulatus*. Some nodes such as *Anthracoceros malayanus* were poorly supported in both trees and therefore remain unresolved. Inter-specific comparison indicated that the concatenated nuclear-mtDNA phylogeny was slightly more resolved than the partitioned Bayesian *cytb* tree. However, certain positions in the *cytb* topology provided better resolution for some inter-specific relationships, such as the placement of *T. camurus* within the orange-billed whistling clade, the subtree formed between *T. pallidirostris* and *T. nasutus*, and the placement of *R. everetti* as basal to wreathed hornbills. Most clades appear robust between trees, including clustering of *T. hartlaubi* with *Tropicranus albocristatus*, placement of *Berenicornis comatus* in the Afro-forest clade, polyphyly of *Ocyceros* and *Tockus*, and sister clades of *Buceros bicornis* and *B. rhinoceros*. Our analyses repeatedly recovered basal positions for *T. monteiri* with the *T. erythrorhynchus* complex, *Rhinoplax vigil* with the *Buceros* clade, *Ceratogymna* with the *Bycanistes* clade, *Aceros nipalensis* with the larger *Aceros* clade, and *A. cassidix* with the *Rhyticeros* clade. They also recovered monophyly of Philippine *Penelopides*, reaffirming the placement of *P. exarhatus* in the wrinkled *Aceros* clade. *Penelopides panini* from Central Philippines remains basal to northern and southern species of *Penelopides*.

### **Intra-specific relationships within the *cytb* phylogeny**

The *cytb* phylogeny allowed well-resolved inferences about intra-specific relationships among subspecies and subpopulations not included in the concatenated tree (Fig. 5.2), in particular, the well sampled geo-isolates of Southeast Asian hornbills, such as *Rhyticeros plicatus* and Philippine populations. Polyphyly within the *R. plicatus* clade suggested plausible

segregation of clinal variants, previously unresolved by morphological analysis. Six subspecies of *R. plicatus* originally described (Kemp, 1995) were later subsumed into a monotypic species by Kemp (2001), but then subsequently accepted as valid by Gill & Donsker (2012). Our analysis suggested four clades are clustered within this group, which includes the disjunct *R. narcondami* nested together with clade A from Seram and New Guinea, clade B from Bismarck and Solomon islands, and clade C from Waigen and North Moluccas islands. *R. undulatus* is sister to this predominantly Wallacean-Melanesian clade, but in itself formed Indochinese and Sundaland clusters. The subspecies *B. rhinoceros borneoensis* from Borneo was basal to sister clades of the nominate form (from the Malay Peninsula and Sumatra) and Javan *silvestris*. Strong support was observed for sister clades of *B. bicornis* from India and Southeast Asia, and between the nominate *Anthracoceros albirostris* from India-Indochina and *convexus* from Sundaland.

In the Philippines, 10 species of hornbills are grouped into four clades, three of which are syntopic (*Buceros*, *Aceros* and *Penelopides*), and one allopatric (*Anthracoceros*). *B. hydrocorax* retains its positions as basal to other *Buceros* species, but the East-Visayan subspecies *semigaleatus* was basal to sister clades *hydrocorax* from Luzon and *mindanensis* from Mindanao. *Aceros waldeni* from West Visayas is sister to *A. leucocephalus*, which in turn formed three allopatric clades from Western Mindanao, Dinagat and Siargao islands, and Eastern Mindanao. *Penelopides manillae subniger* from Polillo is a sister clade to *P. manillae manillae*, and the extinct *P. panini ticaensis* is a sister with *P. panini panini* from Masbate. The enigmatic *P. affinis basilanicus* has clustered with *P. affinis affinis* from Western Mindanao, a relationship also observed in other Philippine birds (Dickinson *et al.*, 1991). *Anthracoceros montani* and *A. marcheii* are sister clades, with the latter forming three allopatric clades from Balabac, Calamianes and mainland Palawan.

### Divergence time estimation

Estimation of divergence time was congruent between the concatenated (Fig. 5.1) and mtDNA *cytb* chronograms (Figs. 3-6). Bayesian posterior probabilities (PP) showed moderate to strong support to 85% of the nodes in the time-tree. Divergence time estimates between key clades within the *cytb* phylogeny are summarised in Table 5.1 (stem ages in Table S5.3, see supporting information). We based divergence time of taxa on crown ages of key clades forming monophyly, with emphasis given to taxa basal to nodes representing shifts in ancestral range. Nearly all the key clades investigated showed strong node support ( $\geq 95\%$ ), and the span of confidence intervals was usually between 5–10 Ma.

Bucorvinae began to diverge from Bucerotinae in the early Oligocene (32–28 Ma), but the crown-group of ground hornbills did not appear until late Miocene (8–6 Ma). This approximates the age estimated for two fossil ground hornbills (Brunet, 1971; Boev & Kovachev, 2007). Tempo of colonization of Asian hornbills from Africa was consistent between the two chronograms, since both evolved during the Oligocene (28–25 Ma). The timing between the two time-trees is similar but not exact, with estimates for the *cytb* tree being 2–5 million years older than the concatenated tree. However, these crown ages are within the bounds of confidence intervals (HPD). Given this degree of congruence, we used the *cytb* tree as the basis for addressing colonization histories of 78 hornbill taxa and 160 geo-isolates. This expanded tree is presented separately for three broad geographical groupings, specifically African hornbills (Bucorvids, *Tockus* and Afro-forest hornbills, Fig. 5.3–5.4), continental Asian (*Buceros*, *Ocyceros*, *Anorrhinus*, *Anthracoceros*, Fig. 5.5) and insular Asian hornbills (*Aceros*, *Penelopides*, *Rhyticeros*, Fig. 5.6).

The earliest Bucerotinae lineages were formed during the mid-Oligocene (29.5 Ma), which diverged to form ancestral *Tockus* and frugivorous forest hornbills (Fig. 5.3, node 3). By the late Oligocene (24.8 Ma), *Tockus* had split into two main clades (Fig. 5.3, nodes 5 & 6). Predominantly found in more arid habitats, forest-dwelling *Tockus* began to diverge around 8.7 (*T. camurus*, Fig. 5.3, node 11) and 7.8 Ma (*T. fasciatus*, Fig. 3, node 13). Frugivorous hornbills



had split into two clades, Afro-tropical and Asian (25–28 Ma), which coincides with the late Oligocene warming event that allowed ancestral frugivorous forms to colonize Asia (Gonzalez & Tobias, in prep., see Chapter 4). This earlier arrival in Asia allowed the Asian lineage to radiate extensively, expanding eastward into oceanic islands. However, one later arrival (*Berenicornis*) that split off from Afro-tropical hornbills did not diversify further (Fig. 5.3, node 4). The African forest clade diverged further into three groups of African forest hornbills, *Tropicranus* at 16.3 Ma (Fig. 5.3, node 8), *Bycanistes* at 12 Ma (Fig. 5.3, node 10) and *Ceratogymna* at 7.9 Ma (Fig. 5.3, node 9). Predominantly found in humid forests, some split off to inhabit drier forests more recently around 4.0–4.2 Ma (*B. brevis* and *B. bucinator*) (Fig. 5.3, nodes 15 & 16).

Lineages from both the continental clade (*Buceros*, *Anthracoceros*, *Anorrhinus*) and insular clade (*Aceros*, *Rhyticeros*, *Penelopides*) represent multiple colonizations of insular Southeast Asia (Sunda shelf islands, Philippines, Wallacea) and Melanesia, which were traversed between early to late Miocene (c. 20–10 Ma). The Sunda shelf, with five lineages, was colonized throughout the Miocene, from 22.2 Ma to 5.14 Ma, earliest by the *Anorrhinus* clade (Fig. 5.5, node 18) and latest by *Berenicornis* (Fig. 5.3, node 7). The Philippines were colonized by four hornbill lineages during the middle to late Miocene, earliest by *Buceros* at 13.8 Ma (Fig. 5.5, node 22, representing dispersal route IIa), then *Penelopides* at 11.95 Ma (Fig. 5.6, node 36), *Aceros* at 9.75 Ma (Fig. 5.6, node 37) and *Anthracoceros* at 8.5 Ma (Fig. 5.5, node 29 representing dispersal route IIb). Wallacea, with only two lineages, was colonized between 16.9 Ma (*Rhyticeros* clade, Fig. 5.6, node 33) and 14.84 Ma (*Aceros-Penelopides* clade, Fig. 5.6, node 35). Finally, a single lineage spread through Melanesia, between 5.9 Ma (*R. plicatus* B, Solomon islands & Bismarck archipelago, Fig. 5.6, node 43) and 3.5 Ma (*R. plicatus* A, Seram & New Guinea, Fig. 5.6, node 42).

### Ancestral area analyses

Bayesian MCMC ancestral area analysis implemented on the concatenated nuclear-mtDNA chronogram recovers Africa as the ancestral area (c. 24–18 Ma) as postulated by Gonzalez & Tobias (in prep., see Chapter 4) (see Fig. 5.1, route I). However, this expanded distribution set covering four biogeographic regions establishes the colonization of insular Southeast Asia as having occurred from 14–10 Ma (II). Four regions comprise core radiations of hornbills in the Palaeotropics, namely Africa and Arabian Peninsula, which contains 29 species, Indo-Malayan region with 18 species, Philippines with 10 species, and then Wallacea and Melanesia with 4 species. Under this perspective, we find the same two colonization events leading to Asia during the late Oligocene and early Miocene, but now placing the Indo-Malayan region as the core ancestral area for Asian hornbills. Insular forms radiated during the middle Miocene via Sundaland to colonize Wallacea and Melanesia (IIa) and the Philippines (IIb).

Although the current tree covers all 61 recognized species (Gill & Donsker, 2012), the absence of representative subspecies and subpopulations provides limited resolution as to how these continental and insular Asian hornbills evolved, and what routes were taken to reach the islands. Incomplete sampling of populations across the geographic range of a taxonomic group signifies gaps, compromising the analysis of historical distributions crucial to mapping ancestral colonization routes (Noonan & Wray, 2006). To fill in these gaps, our comprehensive ancestral area reconstruction using larger mtDNA *cytb* chronogram of hornbill geo-isolates provides a more robust analysis of the historical distribution of hornbills across Africa, continental Asia and insular Southeast Asia (Figs. 3–6). The ancestral area for Bucerotes, the node between hornbills and out-groups (Phoeniculidae, Upupidae), was estimated to be South-East Africa, with a probability of 0.58 (node not shown in figures).

### **Ancestral areas for African hornbills**

Optimal distribution of key hornbill clades are summarized in Table 5.1, with posterior probabilities showing strong support for nearly all nodes examined (detailed probabilities of distributions given in Table S5.3, see supporting information). Ancestral area reconstruction for

African hornbills suggests that the *Bucorvus* clade (node 1) has no specific regional origins within Africa, but may possibly have south-east African ancestors. The *Tockus* clade shows greater probability for an ancestral south-east African lineage, whereas *Berenicornis* clade (node 4) is most likely to have an ancestral lineage in the north-west. Four species within the *Tockus* clade (node 3) dispersed into the north-west (see Fig. 5.3, I) and conversely, two species within the *Berenicornis* clade dispersed into the south-east (Fig 3, II). These dispersal events are not directly associated with plate-tectonic events, since the formation of the complex East African Rift System (c. 10 Ma, Couvreur *et al.*, 2008) has little relation to the distribution of major clades of African hornbills. However, the Great Rift Valley may have a bearing on the distribution of some *Tockus* species that are restricted to the Somalian plate, such as *T. deckeni* and *T. flavirostris*. The distribution of species within these African clades appears to follow range limits of vegetation types (humid forest, dry forest and grassland/desert scrubland). To test this, we plotted the historical changes in vegetation across Africa from the time of inception of these hornbill clades (20 Ma to present; see Fig 4).

### Historical habitats constrained African hornbills

African clades were established based on their habitat preference, with the predominantly arboreal frugivorous *Berenicornis* clade (Fig. 5.4, node 4) being nearly exclusive to humid forest and the *Tockus* clade (Fig. 5.4, node 3) occurring in variety of habitats, but mostly drylands. The extent of humid forest was larger (Fig. 5.4, palaeo-habitat map I) at 20 Ma, and surrounded mainly by dry woodland, but seemingly little has changed with the Guinea-Congolian rainforest block of central Africa (Bush & Flenley, 2007). *Bycanistes bucinator* (Fig. 5.4, node 15) dispersed out of this forest block (Fig. 5.4, palaeo-habitat map II) to inhabit dry forests, while *B. brevis* (Fig. 5.4, node 16) remained in relict fragments of retreating coastal lowland and Afro-montane forest in the eastern part (Fig. 5.4, palaeo-habitat map III). During the mid-Miocene, the *Tockus* clade splits into two key clades, the ‘whistling’ clade (Fig. 5.4, node 5), which is predominantly arboreal, and the ‘clucking’ clade (Fig. 5.4, node 6) which are

predominantly terrestrial (Kemp, 1995). This difference in habitat preference may have been influenced by the expansion and contraction of dry forest (e.g. *Brachystegia* woodland), increasing aridity and expanding grasslands ( $C_4$  grasses, Jacobs 2004). The ‘clucking’ clade remained exclusive to savannah grasslands and desert scrublands, while the ‘whistling’ clade shifted to varied woody habitats, including humid forests (*T. camurus*, *T. alboterminatus*, *T. fasciatus*), dry woodland (*T. bradfieldi*, *T. nasutus*, *T. pallidirostris*) and dry montane woodland (*T. hemprichii*). Expanding grasslands probably influenced isolation and divergence between *T. hemprichii* and *T. fasciatus* (Fig. 5.4, node 13 and palaeo-habitat map II).

### **Ancestral areas for continental Asian hornbills**

Asian hornbills collectively have Indo-Sundaic origins, with a greater probability of Sundaland as the ancestral area (Fig. 5.3, branch opposite node 4). However, this frugivorous clade can be separated into two equally diverse subclades, based on their distributional limits. Continental Asian hornbills are predominantly restricted to mainland tropical Asia, with several species inhabiting Sundaland and the Philippines (Fig. 5.5). They show Sundaic origins (Fig. 5.5, dispersal route I) and ancestral forms probably arrived in the late Oligocene (25 Ma) when the Sunda Shelf was a massive peninsula. Sundaland was the ancestral area for both the *Rhinoplax* clade (Fig. 5.5, node 17) and *Anorrhinus* clade (Fig. 5.5, node 18). One *Buceros* lineage (Fig. 5.5, node 22, dispersal route IIa) was able to disperse into the Philippines in the mid-Miocene (15 Ma) and one *Ocyceros* lineage colonized Sri Lanka (Fig. 5.5, node 24, dispersal route IIb) at about the same time. Two forms of *A. albirostris* diverged in the Pliocene (Fig. 5.5, node 28, dispersal route IIIa), with the nominate form restricted to India and Indo-China, and *convexus* restricted to the Sunda Shelf. Colonisation of *Anthracoceros* (Fig. 5.5, node 29, dispersal route IIIb) in the Philippines was restricted to the outer edges of Sundaland, particularly Palawan by *A. marchei* (Fig. 5.5, node 30) and the Sulu archipelago by *A. montani* (Fig. 5.5, node 31).

### Ancestral areas for insular Asian hornbills

Insular Asian hornbills predominantly inhabit the fringing archipelagos of the Indo-Pacific, including the Sundaic islands, Philippines, Wallacea and Melanesia (Fig. 5.6). They trace their origins from India and Indo-China (Fig. 5.6, node 32), but sometime in the mid-Miocene (20–15 Ma) diverged into two main lineages. The *Rhyticeros* clade (Fig. 5.6, node 33, dispersal route Ia) colonized Wallacea and expanded into New Guinea and the Solomon islands, while the *Aceros-Penelopides* clade (Fig. 5.6, node 34, dispersal route Ib) radiated in the Philippines, with one subclade inhabiting Sundaland and Wallacea. The *Rhyticeros plicatus* complex (Fig. 5.6, node 40) diverged during late Miocene (5–8 Ma) into two subclades, formed by sister clades of *R. narcondami* (Fig. 5.6, node 41) and *R. plicatus* A from New Guinea (Fig. 5.6, node 42, dispersal route IIa) and sister clades of *R. plicatus* B (Fig. 5.6, node 43) from the Solomon islands and C (Fig. 5.6, node 44) from the Moluccan islands. *Rhyticeros undulatus* (Fig. 5.6, node 39) is possibly part of this species complex, and the probability index indicates Indo-China as their ancestral area. Plate-tectonic maps showed island formations during late Miocene, which may have provided dispersal routes for *Rhyticeros* to radiate across Sundaland, Wallacea and Melanesia. *Penelopides exarhatus* (Fig. 5.6, node 45, dispersal route IIb) diverged from *A. corrugatus* about the same time, indicating that routes were open between Sundaland and Sulawesi. West Sulawesi was formed from the Sunda Shelf during the Miocene (Hall, 1998). The monophyletic Philippine clade *Penelopides* (Fig. 5.6, node 36) separated into southern and northern clades (Fig. 5.6, dispersal route IIc) during late Miocene, suggesting that land-bridge connections allowed dispersal between major Pleistocene Aggregate Island Complexes (PAIC, Siler *et al.*, 2011). Branching patterns amongst geo-isolates within each of the *Penelopides* species suggest possible dispersal routes within the clade. *Penelopides manillae* diverges from *P. mindorensis* within the northern clade. In turn, *P. manillae subniger* from Polillo diverges from the nominate mainland form, which splits off further into southern and northern populations. *Penelopides manillae* from Palaui Island represents the northernmost population, which branches out from the northern Luzon subclade. Detailed branching patterns

can also be observed on well-sampled species with multiple geo-isolates, including *R. plicatus*, *R. undulatus*, *P. panini* and *A. leucocephalus*.

## DISCUSSION

### Historical biogeography of hornbills

Our results support the “Out-of-Africa” model (Kinnaird & O’Brien, 2007, Gonzalez & Tobias, in prep., see Chapter 4), placing Africa as the ancestral area of Bucerotidae, which radiated in both tropical Africa and Asia. Posterior estimates of Bucerotidae crown age (29.54 Ma concatenated data; 31.85 Ma *cytb* only) coincide with proposed late Oligocene origin (Kinnaird & O’Brien, 2007; Gonzalez & Tobias, in prep., see Chapter 4). All putative hornbill fossils (*Geiseloceros*, *Cryptornis*, *Homalopus*) previously described from Europe have subsequently been re-assigned to be non-hornbills (Olson, 1985; Mayr, 2005), thus maintaining African affinities. The remaining valid fossil Bucerotidae are apparently too young to be regarded as ancestral (Boev & Kovachev, 2007). Here, we discuss the historical biogeography of hornbills based on three major clades within the dated mtDNA phylogeny – Afrotropical, and Continental and Insular Asian hornbills. These three clades show a predominant distribution more or less bounded by biogeographic barriers. Afrotropical hornbills generally did not traverse any biogeographic barrier and remained exclusive to Africa and Arabia, with the exception of lineages leading to *Berenicornis* and the fossil *Euroceros* (Boev & Kovachev, 2007). Continental Asian hornbills generally occur within the Indo-Malayan region bounded by Engler’s line on the west (Burkill, 1943) and Wallace’s line on the east. Insular Asian hornbills predominantly occur in Southeast Asia, mostly beyond Wallace’s line (and Huxley’s line) and marginally crossed Lydekker’s line (Simpson, 1977).

### Continental African hornbills and environmental instability

African hornbills occupy varied habitat types from humid forests to arid savannah grasslands (Kemp, 1995; Muñoz *et al.*, 2003). Clades follow three distinct ecological groups: *Bucorvus* are predominantly terrestrial, faunivorous, and inhabit open savannah grasslands; the members of the *Berenicornis* clade are predominantly frugivorous, arboreal and inhabit humid forests. Finally, the *Tockus* clade is a combination of the two, with subclades being more arboreal or terrestrial. Ancestral area analysis indicated the *Tockus* clade to have a south-eastern origin within Africa, and the *Berenicornis* clade to have north-west origins, but each has several species dispersing out of the ancestral area. Tectonic events were unable to explain the branching patterns within the phylogeny and these clades do not correspond to the distribution of chorotypes (defined as groups of species with similar distribution) proposed by Muñoz *et al.* (2003). However, each key African clade is well represented in the three chorotypes: west-central forest, eastern open savanna, and southern montane and coastal woodland (Muñoz *et al.*, 2003). Apart from the East African Rift System, the African continent is geologically stable (Kemp, 1995). The biogeography of African clades appears to be influenced by changes in palaeo-climate that shaped the landscape during the Oligocene-Miocene. Habitat changes rather than geologic vicariance facilitated speciation on the continent, and this can be attributed to increasing aridity and expanding grasslands (Kemp & Crowe, 1985).

Proliferation of savannah grasslands (dominance of C<sub>4</sub> type grasses) in the middle Miocene (Jacobs, 2004; Edwards *et al.*, 2010) is thought to have encouraged diversification of terrestrial *Tockus* and *Bucorvus* (Kemp, 1995). Historically, ground hornbills expanded their range north of the Sahara, with fossils from Morocco and southwest Bulgaria both from the Miocène supérieur (Boev & Kovachev, 2007). Drier climates in the late Miocene allowed expansion of savannahs and African fauna into the Mediterranean region of Europe and northern Africa (Jacobs, 2004; Barry Cox & Moore, 2005). Varying degrees of aridity facilitated adaptation of *Tockus* to different wooded habitats, from moist coastal forests to arid desert thornvelds. Even within clades, taxa appear to be allopatric based on habitat preference, and become somewhat ecologically isolated. Frugivorous *Tockus fasciatus* and insectivorous *T.*

*camurus* inhabit humid forests of West-Central Africa, *T. alboterminatus* inhabits montane, riparian and coastal forests of South-east Africa, *T. hemprichii* is a specialist of montane *Euphorbia-Ficus* woodland, and *T. bradfieldi* has a relict distribution in mopane-teak woodland. *Tockus nasutus* is a non-specialist to drier *Acacia* woodlands, savannahs and arid steppe, but *T. pallidirostris* is restricted to moist miombo (*Brachystegia*) woodland (Kemp, 1976). Conversely, the formation of the East African Rift System probably restricted some *Tockus* to the drier habitats on the Somalian plate, including *T. deckeni*, *T. jacksoni* and *T. flavirostris*. Couvreur *et al.* (2008) indicated that the uplift of the rift system played a significant role in aridification of eastern Africa, and its timing (c. 10 Ma) coincides with extension of savannas (Jacobs, 2004).

Vicariance between sister-species can be attributed to formation of habitat barriers such as expansive grasslands or forests. Dense miombo woodland across central Africa separated the two ground hornbills. The expansion of Miombo woodland also splits the ranges of *Tockus erythrorhynchus* complex, and both species-subspecies pairs of *T. nasutus* and *T. flavirostris* (Kemp, 1995). *Tockus hemprichii* from the Ethiopian highlands was isolated by expanding arid savannahs (Jacobs, 2004), and two subspecies of *T. pallidirostris* were separated by the arid Luangwa valley (Kemp, 1995). We reiterate the importance of the historically persistent Guinea-Congolian rainforests in the development of African forest hornbills, which probably gave rise to ancestral Asian hornbills. Contraction of rainforest refugia during climatic perturbations isolated populations within Central Africa and induced speciation and subspeciation. Global cooling from the mid-Miocene to mid-Pliocene resulted in retraction of moist megathermal forests (Bush & Flenley, 2007). The separation of Upper and Lower Guinea forests by Dahomey Gap facilitated splits of *B. albotibialis* and *B. cylindricus*, and *B. s. subcylindricus* and *B. s. subquadratus* (Kemp & Crowe, 1985; Kemp, 1995). Adult plumage dimorphism is reversed in the *Ceratogymna/Bycanistes* clade with all-black males compared to the *Aceros* clade (including *Berenicornis*) with all-black females (Kemp, 1995).



### Continental Asian hornbills and core Sundaland refugia

Major clades of continental and insular Asian hornbills diverged about the same time from the ancestral African stock that first crossed the narrowing Tethyan Seaway (Harzhauser *et al.*, 2007) from Africa to Asia during the Oligocene-Miocene boundary. Formation of the “Palaeotropical biotic interchange” linking East Africa, the Arabian Peninsula and India, and the expansion of humid forests during warming events was crucial to the dispersal of hornbills to Asia (Gonzalez & Tobias, in prep., see Chapter 4). This narrow humid forest corridor facilitated biotic exchange between tropical Africa and Asia, as it did for Palaeotropical flora and primates (Zhou *et al.*, 2011). However, it did not facilitate dispersal of *Tockus* and *Bucorvus* clades into Asia, despite *Tockus nasutus* occurring on the Arabian Peninsula. The opposite scenario was postulated by Kemp (1976), with proto-*Tockus* originating from India, giving rise to *Ocyceros*, but also dispersing to Africa via Arabia. This was later corrected by Kemp (1995) placing *Tockus* as the earliest offshoot of Bucerotinae, which was congruent with evolution of African proto-*Buceros* (Kinnaird & O’Brien, 2007). The evidence thus supports our earlier hypothesis (Gonzalez & Tobias, in prep., see Chapter 4) that a generally frugivorous proto-*Berenicornis* evolved from ancestral Bucerotinae after splitting from the generally faunivorous *Tockus* clade.

One lineage gave rise to the predominantly African *Berenicornis* clade and the other crossed Engler’s line through the Palaeotropical biotic interchange. The Guinea-Congolian megathermal rainforest had persisted since the Oligocene (Bush & Flenley, 2007) and is likely to have been the ancestral area for proto-*Berenicornis*. Subsequently, a secondary dispersal through the Palaeotropical biotic interchange led to *Berenicornis* arriving in Sundaland, as the *Gomphotherium* land-bridge had closed the Tethyan Seaway (early Miocene, c. 18–17 Ma, Koufos *et al.*, 2005). Kinnaird & O’Brien (2007) emphasized the importance of Sundaland as a hotspot for hornbill radiation, harbouring one-third of the current Asian taxa. Both Sundaland and Guinea-Congolian forests harbour diverse assemblages of frugivorous hornbills and were crucial rainforest refugia (Bush & Flenley, 2007). Thus provides further support to the complex biogeographic connection between African and Southeast Asian lineages (Hosner *et al.*, 2010).

Sundaland is the ancestral area for continental Asian hornbills (*Buceros* and *Anorrhinus* clades), which allowed subsequent clades to radiate north to India and east to the Philippines. The continental core of Sundaland has been defined to comprise the Sunda Shelf islands (including Palawan), Thai–Malay Peninsula and Indo-China (Hall & Morley, 2004); this core formed a massive peninsula up until the Pleistocene (including part of West Sulawesi, Hall 1998). The Sunda Shelf subregion was divided at the Isthmus of Kra, separating this unique biogeographic zone we now recognise as Sundaland (Müller & Beheregaray, 2010). A subsequent drop in sea level isolated the Sunda Shelf islands, which separated subspecies of Sundaic hornbills such as *B. rhinoceros*. Divergence time estimates suggests that divergence of current subspecies occurred earlier than the sea level drops. Cyclic expansion and contraction of forest refugia may have facilitated this early divergence (Kinnaird & O’Brien, 2007). The same sea level changes isolated *A. marchei* on Palawan and *A. montani* of the Sulu archipelago. Both *Anthracoceros* marginally colonized the Philippines, inhabiting the outer edge of the Sunda Shelf. The only unique clade crossing beyond Sundaland is *Buceros hydrocorax*, which colonized the Oceanic Philippines via the Sulu Arc during the mid-Miocene.

Vicariance brought by expansion of the Chao Phraya River Basin and Myanmar Central Basin separated Tenasserim from Indo-China, facilitating divergence between *A. austeni* and *A. tickelli* (Hall & Morley, 2004). The uplift of the Himalayas and Indo-Burmese ranges was not a formidable barrier to dispersal for hornbills, given the interchange of many taxa between India and Indo-China. The Pied Hornbill complex (*Anthracoceros*) spans from India to the Philippines, and has close affinities with *Ocyceros* and *A. malayanus* (Kemp, 1995). India is the probable ancestral area for *Anthracoceros* and *Ocyceros*, following divergence from a Sundaic ancestor shared with *Anorrhinus*. *Anthracoceros* marginally colonized the Philippines, with endemic species inhabiting the eastern outer-edge of the Sunda Shelf. Alternatively, *Ocyceros* (and to some extent *Anthracoceros*) may have originated as an Indian relic from the Africa-to-Asia crossing rather than as a re-coloniser from Sundaland. *Ocyceros griseus* from the Western Ghats of India and *O. gingalensis* from Sri Lanka diverged much earlier, and preference for

humid forest limited their range, unlike *O. birostris*, which prefers dry woodland. *Anthracoceros coronatus* appears to be a recent arrival to Sri Lanka. Adaptation to drier woodland habitats due to limited rainforest refugia in India (Western Ghats) encouraged divergence among *Oryceros*. Speciation among continental Asian hornbills appears to be a combination of tectonic movements and vicariance events (Kemp, 1988). Vicariance brought by expanding non-humid habitats, sea level changes and distribution of rainforest refugia brought about by cyclic oscillations in the palaeo-climate (Kinnaird & O'Brien, 2007).

### **Insular Asian hornbills and Plate-tectonic dynamics**

Insular Asian hornbills comprise a single lineage (*Aceros* clade) that successfully radiated across the core of Sundaland and much of the Malesian archipelago. They are largely frugivorous and dependent on humid forest, but some are known to be strong fliers, flock-forming species and probably island-hopped over short distances to reach Melanesia (Kemp, 1995; Kinnaird & O'Brien, 2007). *Rhyticeros* probably developed in Wallacea, and spread out to colonize Melanesia, and re-colonise Sundaland and Indo-China. *Aceros cassidix* is basal to the *Rhyticeros* clade (Hübner *et al.*, 2003; Viseshakul *et al.*, 2011) and is unique to Sulawesi. It has close affinities with *A. corrugatus* from Sundaland, which is the likely ancestral area of both insular clades, as it intersperses Indo-China (and basal *A. nipalensis*) with the Philippines and Wallacea. *Aceros cassidix* fills the gap in distribution of *Rhyticeros* in Malesia, and has close phylogenetic affinities with *R. everetti* of Sumba, diverging about 13 Ma. Western Sulawesi and Sumba were part of the Banda Arc (Southeast Sundaland) during the mid-Miocene (15 Ma) and slowly drifted with the spread of the North Banda Sea (Hall *et al.*, 2011). This contradicts the vicariant dispersal hypothesis noted for *R. everetti* (Kinnaird & O'Brien, 2007).

Given their probable Wallacean origins, the spread of the *Rhyticeros* clade occurred west and east of Wallace's line (the Makassar straits). Sulawesi was formed by collision of West Sulawesi from the Sunda Shelf and East Sulawesi from the Sahul Shelf, gradually merging from early to late Miocene (c. 20–10 Ma, Hall, 1998). The clade formed by the *R. undulatus*

and *R. plicatus* complex diverged from *R. subruficollis* around 10 Ma, which coincides with close associations between West Sulawesi, the Tukang Besi Platform (East Sulawesi), Seram and Bird's Head (west New Guinea). Formation of the Central Range orogeny represents the closest proximity between the Sunda and Sahul Shelves (Su & Saunders, 2009). The Halmahera Arc showed close associations with New Guinea Terranes (northwest New Guinea) and Solomon Arc around 8 Ma (Hall *et al.*, 2011). Shallow seas connected the islands between the Sunda Shelf and Sahul Shelf, reaching east to the Solomon Islands during the Miocene-Pliocene boundary (c.5 Ma, Hall, 1998). This probably facilitated dispersal of ancestral *R. plicatus* throughout the Malesian archipelago, which stretches from the Andaman Islands to Solomon Islands (Beck *et al.*, 2006). Divergence of New Guinea clades of *R. plicatus* from Seram occurred in the Pliocene (3.5 Ma), and by the time it colonized eastern New Guinea (2.5 Ma), it was still connected to Australia. A land bridge at the Torres Strait was intermittent in between Pleistocene glacial oscillations (Taylor *et al.*, 1983). It is uncertain if lack of suitable habitat along this 150 km expanse prevented hornbills from colonizing Australia. The disjunct relict distribution of *R. narcondami* can be attributed to the connection of the Andaman Islands to Sumatra and Java, linking this eastward to West Sulawesi and the *R. plicatus* complex (Hall & Morley, 2004).

*Rhyticeros* probably dispersed through the southern part of West Sulawesi to reach Seram and the eastern passage to Melanesia. Narrow passages separated island clusters of Wallacea and Melanesia during the middle to late Miocene (c.15–10 Ma, Hall, 1998) from Seram and Bird's Head (still detached from New Guinea) to Northern Moluccas, then the New Guinea Terranes (detached northern part of New Guinea) east to the Bismarck archipelago and Solomon Islands. Shallow seas form a northern route that extends from northeast Borneo through the Sulu Arc (15–10 Ma), and Western Mindanao Arc towards the Central Visayan Islands of Negros and Panay (Hall & Morley, 2004; Hall *et al.*, 2011). This route was taken by an ancestral *Aceros* clade to colonize the Philippines, and evolved into a monophyletic group of small hornbills, *Penelopides*. This does not concur with any of three scenarios given by

Kinnaird & O'Brien (2007) about the origins of *Penelopides*, although it was suggested that molecular analysis was necessary to resolve this.

A key part missing from these scenarios was the polyphyly of *Penelopides*, and that Philippine *Penelopides* evolved independently of *P. exarhatus*. The basal position of *Penelopides panini* within the clade suggests the Central Visayas as the ancestral area, although further analysis is needed to confirm this. This is congruent with the phylogeographic arrangements in other taxa (Jones & Kennedy, 2008; Esselstyn *et al.*, 2009). *Penelopides* spread through the archipelago and developed into its representative species in each PAIC. Land bridges formed by tectonic movements allowed dispersal, but vicariance events isolated islands facilitating speciation. To understand the routes of colonisation taken between PAIC, a more detail ancestral area analysis of *Penelopides* is needed. Node ages between intra-specific clades provide a basis for delineating dispersal histories within polytypic taxa, and possibly indicate timing of diffusion across PAIC barriers (Siler *et al.*, 2010). Another lineage of *Aceros* allied to *A. corrugatus* colonized the Philippines about the same time as ancestral *Penelopides*, and probably by the same route. Occurring in sympatry at Central Visayas encouraged differentiation. Western Sulawesi was still part of Sundaland when *P. exarhatus* diverged from *A. corrugatus* (Hall *et al.*, 2011). Similarly, sympatry with *A. cassidix* in Sulawesi encouraged differentiation. Therefore, the West Sulawesi and Sulu Arc are important routes taken by insular *Aceros* from Sundaland to colonize the Philippines, Wallacea and Melanesia. Complex plate-tectonics and dispersal abilities facilitated speciation amongst insular Asian hornbills (Kemp, 1988).

### **Implications for biogeography and taxonomy**

Asian hornbill lineages followed a number of different models of speciation and faunal interchange to spread across the Indo-Pacific. Their diversification follows a pattern similar to the Darwinian centre-of-origin (Simpson, 1940), placing Africa as the centre for all hornbills. The Guinea-Congolian forest block possibly served as the centre for Afrotropical forest

hornbills (*Berenicornis* clade, giving rise to Asian clade) and South-East African woodlands as the centre for the *Tockus* clade. Tropical rainforests persisted in Cameroon from Late Eocene to Late Oligocene, when woodlands were first recorded in Tanzania (Jacobs, 2004). The Palaeotropical biotic interchange allowed ancestral frugivorous hornbills to colonize Asia and diversify, facilitated by warming events that also provided corridors of humid forests from the Horn of Africa to India and Sundaland (Gonzalez & Tobias, in prep., see Chapter 4). India served as the centre for *Oryx*-*Antilocapra* and *Aceros* radiation, while Sundaland served as centre for the *Buceros* and *Anorhynchus* radiation. Both biogeographic areas represent the cradle of Asian hornbill speciation (Kinnaird & O'Brien, 2007). Tectonic movements provided opportunistic filter bridges that allowed limited colonisation into the Philippines (via the Sulu Arc and Palawan chain) and Wallacea (via Western Sulawesi). Both served as centres for the radiation of *Penelopides* and *Rhyticeros* clades. This can be attributed to regional mechanisms driving diversification such as the formation of the PAIC in the Philippines (Esselstyn *et al.*, 2009; Siler *et al.*, 2010). Formation of the exclusive *Penelopides* clade and development of a unique assemblage of hornbills different from Wallacea suggests separation of the Philippines as a unique biogeographic zone.

Diversity among Asian hornbills decreased after crossing different biogeographic barriers following their eastward colonisation towards Melanesia. The Sunda Shelf was a massive peninsula harbouring forest refugia deemed crucial during climatic cycles, which facilitated both dispersal and speciation by vicariance (Hall & Morley, 2004; Kinnaird & O'Brien, 2007). The Isthmus of Kra is the remnant of a permeable barrier that defines the distribution of Sundaland endemics on the northwest. The outer edge of the shelf is outlined by Wallace's and Huxley's lines on the east (Van Welzen *et al.*, 2011). Given the substantial number of hornbills shared between Indo-China and Sundaland, the Isthmus of Kra did not prove an effective barrier to dispersal. In comparison, three lineages crossed Huxley's line to colonize the Philippines via an opportunistic filter bridge formed by the Sulu Arc (Hall, 1998). *Buceros* probably used the same route to reach the Oceanic Philippines as *Aceros* and ancestral

*Penelopides* during the mid-Miocene. Given this, a sweepstake route is therefore a less likely event for the Philippines. This also supports the strong biogeographic connections between Borneo and the Philippines (Diamond & Gilpin, 1983; Hosner *et al.*, 2010). *Anthracosceros* arrived much later via land bridges from Sundaland but was unable to cross beyond to the Oceanic Philippines. Deep water separating Palawan and the Sulu Arc from eastern islands of the Philippines were an effective barrier to the eastward dispersal of hornbills during the Pliocene when most of the PAIC had been formed (Esselstyn *et al.*, 2009; Siler *et al.*, 2010).

Wallace's line seems to have acted as a more effective barrier and a weak filter bridge, allowing only two lineages to colonize and facilitating the development of *Rhyticeros*. The *Rhyticeros* clade reinvaded Sundaland and Indo-China, and also managed to cross over to the Sahul Shelf. Given their greater ability to disperse, they were likely candidates for permeating Wallace's and Lydekker's lines, either through opportunistic sweepstakes routes or filter bridges. The extreme distance between sister-clades of *R. plicatus* and *R. narcondami* suggests that a sweepstakes route was the probable explanation for reaching the isolated volcanic island. It had followed the Sumatran-Javan Arc towards the Andaman Islands from Wallacea (Hall, 1998), thus supporting the vicariant dispersal hypothesis noted by Kinnaird & O'Brien (2007). The unique environment of Wallacea served as a filter to hornbill dispersal, allowing only *Rhyticeros* to permeate Lydekker's line. It was a strong biogeographic barrier causing major faunal discontinuity across the Malesian archipelago (Beck *et al.*, 2006; Van Welzen *et al.*, 2011). Permeability of barriers provided the constraints to the dispersal of hornbill lineages eastward to Melanesia. Similar scenarios were proposed for migration routes of rainforest trees, *Pseuduvaria* and *Aglaia* across Southeast Asia (Muellner *et al.*, 2008; Su & Saunders, 2009). Dispersal by hornbills facilitated the colonisation of Asia by hornbill-dispersed fruits (*Uvaria*, *Canarium*, *Aglaia* sect *Amoora*), with the same eastward downstream expansion to Papuo-Australia from Africa (Weeks *et al.*, 2005; Muellner *et al.*, 2008; Zhou *et al.*, 2011).

Phylogeographic analysis of geo-isolates revealed interesting inter-specific and intra-specific relationships, several of which warrant further taxonomic work. Our mtDNA *cytb*

phylogeny reaffirms earlier recommendations addressing taxonomic enigmas associated with a multilocus tree (Gonzalez *et al.*, in prep., see Chapter 2). We provided resolution regarding the placement of *Berenicornis* with *Tropicranus* and *T. hartlaubi*, separation of *Tockus* clade into “whistlers” and “cluckers”, and nesting of *P. exarhatus* within the wrinkled *Aceros* clade (Hübner *et al.*, 2003; Viseshakul *et al.*, 2011). We also provided resolution to understanding the branching patterns for the *T. erythrorhynchus* complex and *Bycanistes* clade (Kemp & Delport, 2002; Viseshakul *et al.*, 2011). Novel findings different from the concatenated tree include placement of *T. camurus* with *T. alboterminatus* and *T. bradfieldi*, sister-species formation of *T. pallidirostris* and *T. nasutus*, and in-depth sampling of the *R. plicatus* complex. These issues were previously raised as taxonomic enigmas (Kemp, 1995; Kinnaird & O’Brien, 2007). Several issues are raised that deserve further investigation. The lack of congruence between early divergence ages of *B. rhinoceros* subspecies versus relatively recent divergence age between *A. austeni* and *A. tickelli* requires further study. Additional review is needed on the unclear clustering of geo-isolates from the Philippines, including *P. affinis basilanicus* with *P. affinis affinis* from Western Mindanao, and *P. panini ticaensis* with *P. panini panini* from Masbate. Likewise, further study is needed on allopatric clades formed within monotypic species such as *A. leucocephalus* and *A. marchei*.

## CONCLUSION

The historical biogeography of hornbills illustrates different scenarios for radiation of Afrotropical, continental Asian and insular Asian-Melanesian hornbills based on diet, habitat and tectonic distribution. Faunivorous hornbills diversified in geologically stable, but environmentally unstable, continental Africa, whereas frugivorous hornbills radiated across stable forest habitats but geologically dynamic Southeast Asia. Ancestral reconstruction reiterates the importance of Central Africa, India, Sundaland, the Philippines and Wallacea as centres for hornbill speciation. Subsequently, central African humid forests served as the origin



for frugivorous hornbills, whereas Sundaland was a jump off point for hornbills to colonize insular Southeast Asia. Although timing coincides well with other phylogeographic studies, pre-Quaternary fossil evidence of arboreal bucerotids will be crucial to reaffirming our hypothesis. Moreover, colonisation of Asia by frugivorous hornbills mirrors the biogeography of some hornbill-dispersed fruits, and warrants further study to determine the degree of co-evolution involved.

Tectonic movements allowed hornbills to disperse beyond the Sunda Shelf and traverse Wallace's line (early to mid Miocene) and Lydekker's line (late Miocene) towards the Sahul Shelf. Phylogenetic reconstructions coincide with plate-tectonic reconstructions of Southeast Asia and the Southwest Pacific, confirming the importance of these geological events in shaping the distribution of species in the region and defining biogeographical limits. Crossing permeable boundaries from India to Melanesia allowed hornbills to diversify, despite the limitations of frugivory and dependence on humid forests. However, Lydekker's line and Wallace's line were more effective filters than Huxley's line. Asian taxa followed different pathways of faunal interchange to colonize the Indo-Pacific islands, and lineages used different routes (Sulu Arc, West Sulawesi) at different times during the Miocene. Given the difference in colonisation routes and unique assemblage formed (*Penelopides*), our findings support separating the Philippines as a distinct biogeographic area from Wallacea.

There is a need to resolve taxonomic issues presented here, including allopatric clades of geo-isolates from the Philippines and the enigmatic *Rhyticeros plicatus* complex. Intra-specific and intra-subspecific clustering of geo-isolates suggests further analysis of the relationships based on established standards for species delimitation. Resulting genes from these geo-isolates provide a framework for comparison of genetic divergence with quantitative approaches for measuring phenotypic divergence. This comprehensive sequence matrix of geo-isolates can be used to run more detailed analyses (island biogeography, refugia hypotheses) and test models of colonisation routes for Philippine and Wallacean-Melanesian hornbills. Together with our biogeographic analysis, subsequent analysis of species limits can support

## CHAPTER 5

identification of key geo-isolates, which in turn provide the basis for re-setting conservation priorities within the region.

## ACKNOWLEDGEMENTS

We are grateful to the museums, zoos and aviaries listed in Table S5.1 (see supporting information) for providing tissue samples, particularly American Museum of Natural History, Delaware Museum of Natural History, Natural History Museum, Tring, Oxford University Museum of Natural History, and Cambridge University Museum of Zoology. We also acknowledge the generous assistance of M. Adams, E. Alcala, R. Alves, R. Antolin, E. Batara, H. Bratcher, J. Cooper, E. Cosico, A. Dans, J. de Leon, A. Davies, R. Domingo, N. Donato, C. Edwards, L. Estoya, J. Haile, P. Holland, P. Hosner, J.M. Justo, A. Kemp, L. Lastimoza, K. Lim, M.T. Lim, I. Lit Jr., M. Lowe, S. Nacua, M. Nowak-Kemp, P. Ong, A. Owen, R. Prys-Jones, R. Puentespiña, I. Sepil-Oz, P. Sweet, R. Urriza, A. Viojan, A. Walls, R. Wilkinson, J. Woods and V. Yngente. Permits for collection and transport of samples were facilitated by Department of Environment, Food and Rural Affairs (DEFRA, UK), Fish and Wildlife Service (USA), Protected Areas and Wildlife Bureau and DENR Regional and Provincial Offices. Historical samples were processed at the J.W. Jenkinson Laboratory for Evolution and Development, University of Oxford. This study was supported by the Ford Foundation International Fellowship Program, with further contributions from the British Ornithologists' Union, North of England Zoological Society and St Anne's College, Oxford.

## REFERENCES

- Albert JS, Crampton WGR (2010) The Geography and Ecology of Diversification in Neotropical Freshwaters. *Nature Education Knowledge*, **1**(10), 13
- Avice JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.
- Bacon CD, Baker WJ, Simmons MP (2012) Miocene Dispersal Drives Island Radiations in the Palm Tribe Trachycarpeae (Arecaceae). *Systematic Biology*, published online, doi: 10.1093/sysbio/syr123.
- Barry Cox C, Moore PD (2005) *Biogeography: an ecological and evolutionary approach*, 7<sup>th</sup> ed. Blackwell Publishing, Oxford.
- Beldade R, Heiser JB, Robertson DR, Gasparini JL, Floeter SR, Bernardi G (2009) Historical biogeography and speciation in the Creole wrasses (Labridae, *Clepticus*). *Marine Biology*, **156**, 679–687.
- Beck J, Kitching IJ, Linsenmair KE (2006) Wallace's line revisited: has vicariance or dispersal shaped the distribution of Malesian hawkmoths (Lepidoptera: Sphingidae)? *Biological Journal of the Linnean Society*, **89**, 455–468
- Boev Z, Kovachev D (2007) *Euroceros bulgaricus* gen. nov., sp. nov. from Hadzhidimovo (SW Bulgaria) (Late Miocene) – the first European record of hornbills (Aves: Coraciiformes). *Geobios*, **40**, 39–49
- Brunet J (1971) Oiseaux miocènes de Beni Mellal (Maroc); un complément à leur étude. *Notes et Mémoires du Service géologique du Maroc*, **31**, 109–111.
- Burkill IH (1943) The biogeographic division of the Indo-Australian archipelago. *Proceedings of the Linnean Society*, **154**, 127–138.
- Bush MB, Flenley JR (2007) *Tropical Rainforest Responses to Climatic Change*. Springer-Praxis, UK.
- Couvreux TLP, Chatrou LW, Sosef MSM, Richardson JE (2008) Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMC Biology*, **6**, 54.
- Diamond JM, Gilpin ME (2009) Biogeographic Umbilici and the Origin of the Philippine Avifauna. *Oikos*, **41**, 307–321.
- Dickinson EC, Kennedy RS, Parkes KC (1991) *Birds of the Philippines, An annotated Checklist*, BOU Checklist no. 12. British Ornithologist's Union, Tring.

- Dowsett HJ, Barron JA, Poore RZ, Thompson RS, Cronin TM, Ishman SE, Willard DA (1999) Middle Pliocene Paleoenvironmental Reconstruction: PRISM2. U.S. Geological Survey Open File Report 99-535 ([pubs.usgs.gov/of/1999/of99-535/](http://pubs.usgs.gov/of/1999/of99-535/)).
- Drummond A J, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Edwards EJ, Osborne CP, Strömberg CAE, Smith SA, C<sub>4</sub> Grasses Consortium (2010) The Origins of C<sub>4</sub> Grasslands: Integrating Evolutionary and Ecosystem Science. *Science*, **328**, 587–591.
- Esselstyn JA, Oliveros CH, Moyle RG, Peterson AT, McGuire JA, Brown RM (2010) Integrating phylogenetic and taxonomic evidence illuminates complex biogeographic patterns along Huxley's modification of Wallace's Line. *Journal of Biogeography*, **37**, 2054–2066.
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.
- Gill F, Donsker D (2012) IOC World Bird List (v 2.11) Available from: <http://www.worldbirdnames.org/names/> (accessed April 2011).
- Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**, 696–704.
- Hall R (1998) The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. *Biogeography and Geological Evolution of SE Asia* (ed. by R. Hall & J.D. Holloway), pp. 99–131. Backhuys Publishers, Leiden.
- Hall R, Cottam MA, Wilson MEJ (2011) *The SE Asian Gateway: History and Tectonics of the Australia-Asia Collision, Special publication 355*. The Geological Society, Bath.
- Hall R, Morley CK (2004) Sundaland Basins. *Continent-Ocean Interactions within the East Asian Marginal Seas Vol. 149* (ed. by P. Clift, P. Wang, W. Kuhnt & D. Hayes), pp. 55–85. American Geophysical Union, Washington D.C.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.
- Harzhauser M, Kroh A, Mandic O, Piller WE, Göhlich U, Reuter M, Berning B (2007) Biogeographic responses to geodynamics: A key study all around the Oligo–Miocene Tethyan Seaway. *Zoologischer Anzeiger*, **246**, 241–256
- Hay WW, DeConto R, Wold CN, Wilson KM, Voigt S, Schulz M, Wold-Rosby A, Dullo W-C, Ronov AB, Balukhovskiy AN, Soeding E (1999) Alternative Global Cretaceous Paleogeography. *The Evolution of Cretaceous Ocean/Climate Systems, GSA Special Paper 332* (ed. by E. Barrera & C. Johnson), pp. 1–47. The Geological Society of America, Boulder, CO.

- Heads M (2009) Darwin's changing views on evolution: from centres of origin and teleology to vicariance and incomplete lineage sorting. *Journal of Biogeography*, **36**, 1018–1026.
- Ho SYW, Phillips MJ (2009) Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Systematic Biology*, **38**, 367–380.
- Hosner PA, Sheldon FH, Lim HC, Moyle RG (2010) Phylogeny and biogeography of the Asian trogons (Aves: Trogoniformes) inferred from nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics Evolution*, **57**, 1219–1225.
- Hübner VSM, Prinzing R, Wink M (2003) Neue Erkenntnisse zur Taxonomie der Hornvögel (Aves: Bucerotiformes) und ihre Bedeutung für die Zucht in Menschenobhut. *Zoologische Garten N.F.*, **73**, 396–401.
- Jacobs BF (2004) Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society of London B*, **359**, 1573–1583.
- Jansa SA, Barker FK, Heaney LR (2006) The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondrial and nuclear gene sequences. *Systematic Biology*, **55**, 73–88.
- Jones AW, Kennedy RS (2008) Evolution in a tropical archipelago: comparative phylogeography of Philippine fauna and flora reveals complex patterns of colonization and diversification. *Biological Journal of the Linnean Society*, **95**, 620–639.
- Jönsson KA, Bowie RCK, Moyle RG, Irestedt M, Christidis L, Norman JA, Fjeldså J (2010) Phylogeny and biogeography of Oriolidae (Aves: Passeriformes). *Ecography*, **33**, 232–241.
- Kemp AC (1976) *A study of the ecology, behavior and systematics of Tockus hornbills (Aves: Bucerotidae)*, The Transvaal Museum Memoir No. 20. The Transvaal Museum, Pretoria.
- Kemp AC (1995) *Bird families of the world, The Hornbills: Bucerotiformes*. Oxford University Press, Oxford.
- Kemp AC (2001) Family Bucerotidae (Hornbills). *Handbook of the Birds of the World. Vol. 6. Mousebirds to Hornbills* (ed. by J. de Hoyo, A. Elliott & J. Sargatal), pp. 436–523.. Lynx Edicions, Barcelona.
- Kemp AC, Crowe TM (1985) The systematics and zoogeography of Afrotropical hornbills (Aves: Bucerotidae). *Proceedings of the International Symposium African Vertebrates: Systematics, Phylogeny and Evolutionary Ecology* (ed. by K.-L. Schuchmann), pp. 279–324. Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonner.
- Kemp AC (1988) The systematics and zoogeography of Oriental and Australasian hornbills (Aves: Bucerotidae). *Bonner Zoologische Beiträge*, **39**, 315–345.

- Kinnaird MF, O'Brien TG (2007) *The ecology and conservation of Asian hornbills: farmers of the forest*. The University of Chicago press, Chicago.
- Körsgen S, Kantz R, Weinelt M (2011) planiglobe<sup>®</sup> online map creation. kk+w digitale-digitale kartografie GmbH. Kiel, Germany. [http://www.planiglobe.com/omc\\_set.html](http://www.planiglobe.com/omc_set.html). Accessed December 2011
- Koufos GD, Kostopoulos DS, Vlachou TD (2005) Neogene/Quaternary mammalian migrations in Eastern Mediterranean. *Belgian Journal of Zoology*, **135**, 181–190.
- Kreft H, Jetz W (2010) A framework for delineating biogeographic regions based on global species distributions. *Journal of Biogeography*, **37**, 2029–2053
- Ksepka DT, Clarke JA (2010) *Primobucco mcgrewi* (Aves: Coracii) from the Eocene Green river formation: new anatomical data from the earliest constrained record of stem rollers. *Journal of Vertebrate Paleontology*, **30**, 215–225.
- Larkin MA, Blackshields G, Brown NP, Chennam R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) ClustalW and ClustalX version 2. *Bioinformatics*, **23**, 2947–2948.
- Lerner HRL, Klaver MC, Mindell DP (2008) Molecular phylogenetics of the Buteonine birds of prey (Accipitridae). *The Auk*, **304**, 304–315.
- Liu G, Zhou L, Gu C (2012) Complete sequence and gene organization of the mitochondrial genome of scaly-sided merganser (*Mergus squamatus*) and phylogeny of some Anatidae species. *Molecular Biology Reports*, **39**, 2139–2145.
- Lohman DJ, de Bruyn M, Page T, von Rintelen K, Hall R, Ng PKL, Shih T-H, Carvalho GR, von Rintelen T (2011) Biogeography of the Indo-Australian Archipelago. *The Annual Review of Ecology, Evolution, and Systematics*, **42**, 205–26.
- Mayr E (1942) *Systematics and the origin of species from the viewpoint of a zoologist*. Columbia University Press, New York.
- Mayr G (2005) The Paleogene fossil record of birds in Europe. *Biological Review*, **80**, 515–542.
- Mayr G (2006) New specimens of the Eocene Messelirrisoridae (Aves: Bucerotes), with comments on the preservation of uropygial gland waxes in fossil birds from Messel and the phylogenetic affinities of Bucerotes. *Paläontologische Zeitschrift*, **80/4**, 390–405.
- Mistry J (2000) *World Savannas, Ecology and Human Use*. Pearson Education Ltd., U.K..
- Morin PA, Messier J, Woodruff DS (1994) DNA extraction, amplification, and direct sequencing from hornbill feathers. *Journal of the Scientific Society of Thailand*, **20**, 31–42.

- Muellner AN, Pannell CM, Coleman A, Chase MW (2008) The origin and evolution of Indomalayan, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *Journal of Biogeography*, **35**, 1769–1789.
- Müller CJ, Beheregaray LB (2010) Palaeo island-affinities revisited-biogeography and systematics of the Indo-Pacific genus *Cethosia* Fabricius (Lepidoptera: Nymphalidae). *Molecular Phylogenetics and Evolution*, **57**, 314–26.
- Morrone JJ (2009) *Evolutionary Biogeography: An Integrative Approach with Case Studies*. Columbia University Press, New York.
- Mundy N, Unitt P, Woodruff DS (1997) Skin from feet of museum specimens as a non-destructive source of DNA for Avian Genotyping. *The Auk*, **114**, 126–129.
- Muñoz A, Román R, Real R, Olivero J, Márquez AL, Guerrero JC, Bárcena SB, and Vargas JM (2003) Biogeographical zonation of African hornbills and their biotic and geographic characterizations, *Ostrich*, **74**, 39–47.
- Najman Y, Appel E, Boudagher-Fadel M, Bown P, Carter A, Garzanti E, Godin L, Han J, Liebke U, Oliver G, Parrish R, Vezzoli G (2010) Timing of India-Asia collision: Geological, biostratigraphic and palaeomagnetic constraints. *Journal of Geophysical Research*, **115**, 1-18.
- Newton I (2003) *The Speciation and Biogeography of Birds*. Academic Press, Amsterdam.
- Noonan BP, Wray KP (2006) Neotropical diversification: the effects of a complex history on diversity within the poison frog genus *Dendrobates*. *Journal of Biogeography*, **33**(6), 1007–1020.
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Olson SL (1985) The fossil record of birds. *Avian Biology* Vol. VIII. (ed. by D.S. Farner, J.R. King & K.C. Parkes), pp. 79–252. Academic Press, London.
- Pacheco MA, Battistuzzi FU, Lentino M, Aguilar R, Kumar S, Escalante AA (2011) Evolution of modern birds revealed by Mitogenomics: timing the radiation and origin of major orders. *Molecular Biology and Evolution*, **28**, 1927–1942.
- Rambaut A (2006) FigTree: Tree Figure Drawing Tool. Edinburgh: University of Edinburgh.
- Rambaut A, Drummond AJ (2007) Tracer (v.1.5) Available from <http://beast.bio.ed.ac.uk/Tracer> (accessed April 2011).
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.
- Rozen S, Skaletsky HJ (2000) PRIMER 3.0. In: *Bioinformatics methods and protocols: methods in molecular biology* (ed. By S. Krawetz & S. Misener), pp.365–386. Humana Press, New Jersey.



- Schweizer M, Güntert M, Hertwig ST (2012) Phylogeny and biogeography of the parrot genus *Prioniturus* (Aves: Psittaciformes). *Journal of Zoological Systematics and Evolutionary Research*, **50**: 145–156.
- Siler CD, Diesmos AC, Alcala AC, Brown RM (2011) Phylogeny of Philippine slender skinks (Scincidae: *Brachymeles*) reveals underestimated species diversity, complex biogeographical relationships, and cryptic patterns of lineage diversification. *Molecular Phylogenetics Evolution*, **59**, 53–65.
- Simpson GG (1940) Mammals and Land Bridges. *Journal of the Washington Academy of Sciences*, **30**, 137–163.
- Simpson GG (1977) Too many lines; the limits of the Oriental and Australian Zoogeographic regions. *Proceedings of the American Philosophical Society*, **121**, 107–120.
- Su YCF, Saunders RMK (2009) Evolutionary divergence times in the Annonaceae: evidence of a late Miocene origin of *Pseuduvaria* in Sundaland with subsequent diversification in New Guinea. *BMC Evolutionary Biology*, **9**, 153.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* (published online, doi: 10.1093/molbev/msr121).
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596–1599.
- Taylor JM, Calaby JH, Smith SC (1983) Native *Rattus*, Land Bridges, and the Australian Region. *Journal of Mammalogy*, **64**, 463–475.
- Weeks A, Daly DC, Simpson B B (2005) The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution*, **35**, 85–101.
- Vallejo VB (1998) The Philippines in Wallacea, pp. 27–42, In: Telnov D, *Biodiversity, biogeography and nature conservation in Wallacea and New Guinea*, Vol 1, The Entomological Society of Latvia.
- Vallianatos M, Loughheed SC, Boag PT (2002) Conservation genetics of the loggerhead shrike (*Lanius ludovicianus*) in central and eastern North America. *Conservation Genetics*, **3**, 1–13.
- Van Welzen PC, Parnell JAN, Slik JWF (2011) Wallace 's Line and plant distributions : two or three phytogeographical areas and where to group Java?, *Biological Journal of the Linnean Society*, **103**, 531–545.

- Viseshakul N, Charoennitikul W, Kitamura S, Kemp AC, Thong-aree S, Surapunpitak Y, Poonswad P, Ponglikitmongkol M (2011) A phylogeny of frugivorous hornbills linked to the evolution of Indian plants within Asian rainforests. *Journal of Evolutionary Biology*, **24**, 1533–1545.
- Yu Y, Harris AJ, He XJ (2011) RASP (Reconstruct Ancestral State in Phylogenies) [VERSION]. Available at <http://mnh.scu.edu.cn/soft/blog/RASPP>
- Zhou L, Su YCF, Thomas DC, Saunders RMK (2011) ‘Out-of-Africa’ dispersal of tropical floras during the Miocene climatic optimum: evidence from *Uvaria* (Annonaceae). *Journal of Biogeography*, **39**, 322–335.

**Table 5.1:** Posterior estimates of divergence time in Afro-Asian hornbills based on mtDNA *cytb* sequences using BEAST analysis, with results of ancestral area reconstructions using Bayesian MCMC analysis. Nodes selected represent key clades or nodes showing shifts in ancestral range.

| Node | Clade taxa                      | Divergence estimates (Ma) |             | Ancestral |    | Bayesian |
|------|---------------------------------|---------------------------|-------------|-----------|----|----------|
| No.  |                                 | Crown age                 | 95% HPD     | Area (%)  |    | PP       |
| 1    | <i>Bucorvus</i> clade           | 8.7                       | 3.84–16.24  | 0.52      | AB | 100      |
| 2    | Bucerotidae                     | 29.48                     | 24.45–35.25 | 0.86      | AB | 100      |
| 3    | <i>Tockus</i> clade             | 24.8                      | 19.69–30.30 | 0.73      | A  | 100      |
| 4    | <i>Berenicornis</i> clade       | 25.68                     | 19.63–31.53 | 0.5       | B  | 100      |
| 5    | Whistling <i>Tockus</i> clade   | 20.55                     | 15.80–25.63 | 0.63      | A  | 100      |
| 6    | Clucking <i>Tockus</i> clade    | 20.5                      | 15.04–26.38 | 0.96      | A  | 100      |
| 7    | <i>Berenicornis</i>             | 5.14                      | 2.24–9.37   | 0.98      | E  | 100      |
| 8    | <i>Tropicranus</i>              | 16.26                     | 10.98–22.62 | 0.99      | B  | 100      |
| 9    | <i>Ceratogymna</i>              | 7.89                      | 4.03–13.44  | 0.95      | B  | 100      |
| 10   | <i>Bycanistes</i>               | 11.95                     | 8.19–16.00  | 0.97      | B  | 100      |
| 11   | <i>T. camurus</i>               | 8.72                      | 2.81–15.20  | 0.87      | B  | 100      |
| 12   | <i>T. nasutus</i>               | 8.49                      | 4.26–13.21  | 0.87      | AB | 100      |
| 13   | <i>T. fasciatus</i>             | 7.75                      | 3.40–13.56  | 0.89      | B  | 100      |
| 14   | <i>T. erythrorhynchus</i>       | 1.52                      | 0.38–3.38   | 0.95      | A  | 100      |
| 15   | <i>B. bucinator</i>             | 3.96                      | 1.19–7.20   | 0.92      | A  | 100      |
| 16   | <i>B. brevis</i>                | 4.23                      | 2.25–7.04   | 0.93      | A  | 100      |
| 17   | <i>Rhinoplax</i> clade          | 21.8                      | 17.28–27.32 | 0.95      | E  | 100      |
| 18   | <i>Anorhinus</i> clade          | 22.26                     | 17.11–27.40 | 0.61      | E  | 100      |
| 19   | <i>Rhinoplax</i>                | 9.83                      | 5.47–15.57  | 0.99      | E  | 100      |
| 20   | <i>Buceros</i>                  | 13.82                     | 9.13–18.70  | 0.86      | E  | 100      |
| 21   | <i>Anorhinus</i>                | 13.83                     | 8.05–19.67  | 0.52      | E  | 100      |
| 22   | <i>B. hydrocorax</i>            | 13.82                     | 9.13–18.70  | 0.88      | F  | 100      |
| 23   | <i>B. rhinoceros</i>            | 9.04                      | 5.20–13.41  | 0.96      | E  | 100      |
| 24   | <i>O. griseus</i> clade         | 11.71                     | 6.88–16.95  | 0.98      | C  | 100      |
| 25   | <i>A. malayanus</i>             | 9.52                      | 5.14–14.86  | 0.96      | E  | 100      |
| 26   | <i>O. birostris</i>             | 6.78                      | 3.62–10.29  | 0.99      | C  | 100      |
| 27   | Pied <i>Anthracoceros</i>       | 11.79                     | 8.02–16.51  | 0.79      | C  | 100      |
| 28   | <i>A. albirostris</i>           | 3.99                      | 1.85–7.00   | 0.67      | C  | 100      |
| 29   | Philippine <i>Anthracoceros</i> | 8.52                      | 5.15–12.65  | 0.49      | E  | 93       |
| 30   | <i>A. montani</i>               | 4.02                      | 1.91–6.95   | 0.97      | F  | 100      |
| 31   | <i>A. marchei</i>               | 4.47                      | 2.17–7.62   | 0.99      | E  | 100      |
| 32   | <i>Aceros</i> clade             | 22.81                     | 18.11–27.78 | 0.5       | C  | 100      |
| 33   | <i>Rhyticeros</i> clade         | 16.86                     | 12.56–21.98 | 0.91      | G  | 100      |

## CHAPTER 5

|    |                               |       |             |      |     |     |
|----|-------------------------------|-------|-------------|------|-----|-----|
| 34 | <i>Aceros-Penelopides</i>     | 16.36 | 12.57–20.98 | 0.94 | F   | 95  |
| 35 | <i>Aceros-P. exarhatus</i>    | 14.84 | 10.92–19.20 | 0.92 | F   | 100 |
| 36 | Philippine <i>Penelopides</i> | 11.95 | 8.59–16.48  | 0.99 | F   | 100 |
| 37 | Philippine <i>Aceros</i>      | 9.75  | 1.39–8.90   | 0.99 | F   | 100 |
| 38 | <i>R. everetti</i>            | 5.68  | 2.25–11.21  | 0.99 | G   | 100 |
| 39 | <i>R. undulatus</i>           | 6.84  | 4.17–9.92   | 0.91 | CDE | 100 |
| 40 | <i>R. plicatus</i> clade      | 7.99  | 5.36–11.19  | 0.68 | D   | 100 |
| 41 | <i>R. narcondami</i>          | 2.58  | 0.82–5.56   | 0.99 | D   | 100 |
| 42 | <i>R. plicatus</i> A          | 3.52  | 1.53–5.92   | 0.89 | G   | 100 |
| 43 | <i>R. plicatus</i> B          | 5.94  | 3.48–9.00   | 0.96 | H   | 86  |
| 44 | <i>R. plicatus</i> C          | 3.97  | 2.04–6.14   | 0.99 | G   | 100 |
| 45 | <i>P. exarhatus</i>           | 7.04  | 3.73–11.12  | 0.96 | G   | 100 |

For node numbers refer to Figures 3, 5 & 6. Crown age and 95% highest posterior density (HPD) intervals are in millions of years before the present (Ma). Ancestral area indicated by highest probability among the optimal distribution, represented by biogeographic areas indicated by capital letters. Biogeographic areas were based on Kreft & Jetz (2010) and are as follows: A: South & East Africa; B: North & West Africa; C: India & Sri Lanka; D: Indo-China; E: Sundaland; F: Philippines; G: Wallacea; H: Melanesia. Node support for these reconstructions is indicated by posterior probabilities (PP), where  $\geq 0.95$  is strongly supported.

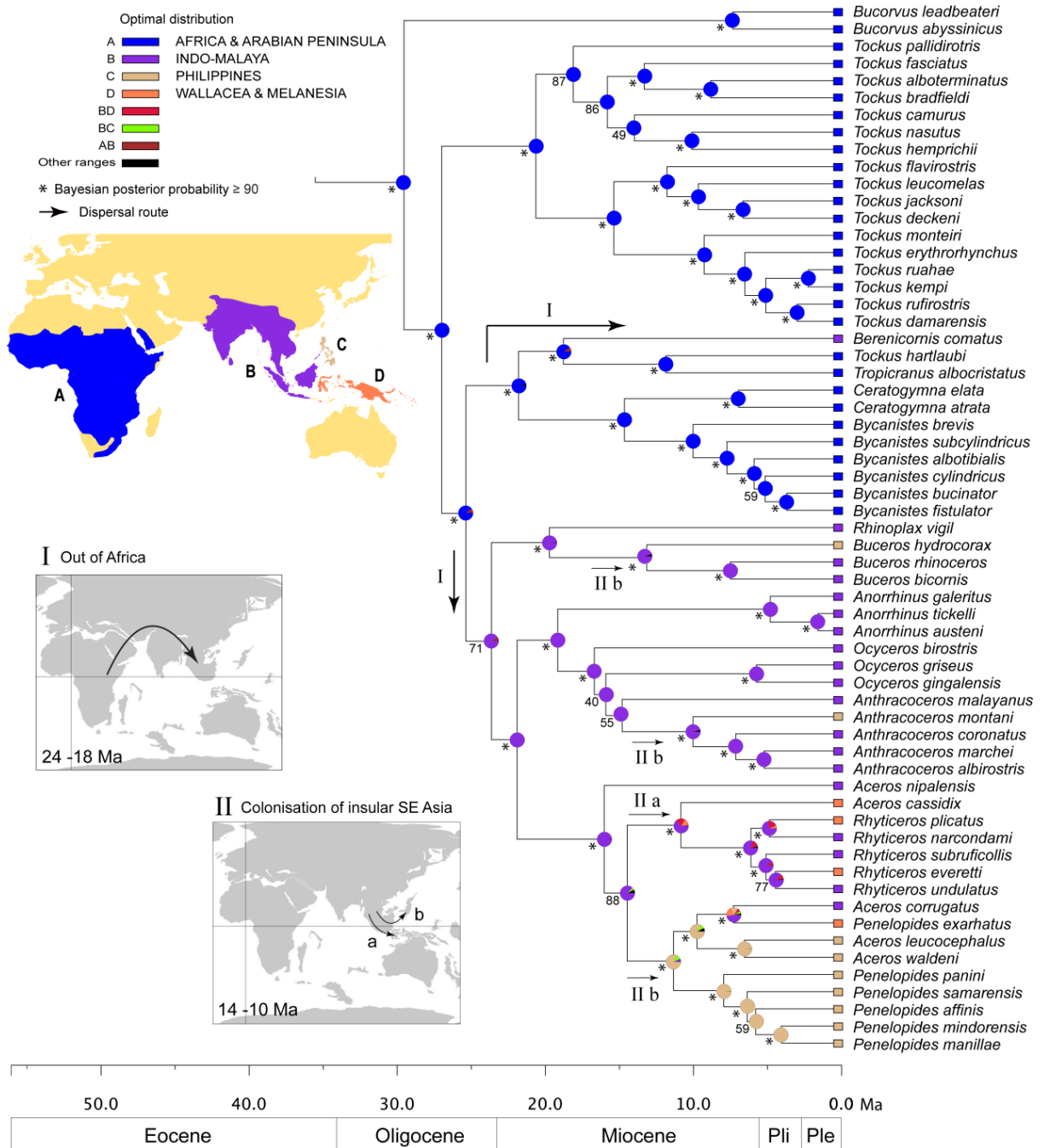


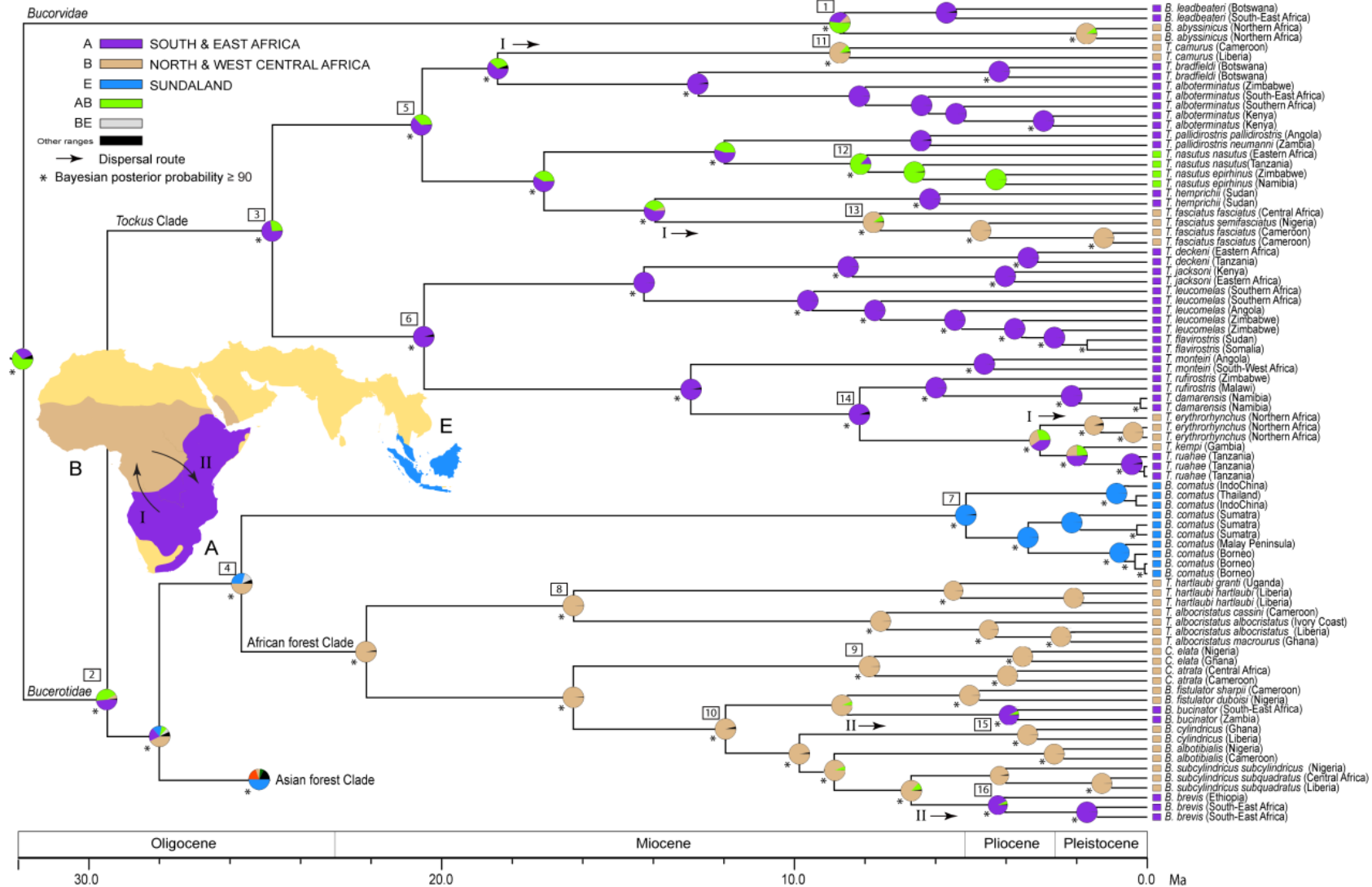
**Figure 5.1:** Historical biogeography of Afro-Asian hornbills (Bucerotidae) using Bayesian MCMC analysis in RASP (Yu et al. 2011) derived from a dated tree of concatenated nuclear-mtDNA sequences. Plate-tectonic maps reconstructed using the ODSN online service. Numbers at nodes indicate Bayesian posterior probabilities  $< 90$ . Tree rooted using outgroups (not shown) which include rollers, a hoopoe and a woodhoopoe. Biogeographic areas used are as follows: A: Africa and Arabian Peninsula; B: Indo-Malaya; C: Philippines; and D: Wallacea and Melanesia.



**Figure 5.2:** Hypothesized relationships of hornbills (Bucerotidae), illustrated by Bayesian phylogeny of 78 taxa based on mtDNA *cytb* sequences. Nodes with  $\geq 70\%$  ML bootstrap values and  $\geq 90\%$  Bayesian posterior probabilities were considered moderate to strongly supported and are indicated by black circles. Illustrations of hornbills are reproduced from Handbook of the Birds of the World with the permission of Lynx Edicions.

# CHAPTER 5

## African Hornbills



## CHAPTER 5

**Figure 5.3;** Ancestral area reconstruction of major clades of African hornbills (Bucorvidae, *Tockus* and *Tropicranus-Ceratogymna*), using Bayesian MCMC analysis derived from a dated tree of mtDNA *cytb* sequences. Tree rooted using outgroups (not shown) which include rollers, hoopoe and woodhoopoe. Arrows refer to possible dispersal routes taken by certain clades based on most likely ancestral ranges. Transition I indicates dispersion of ancestrally southeastern clade to northwest Africa, and transition II refers to a north-to-south dispersal. Numbers in boxes on nodes indicate key clades described in Table 5.1. Maps based from Körsen *et al.* (2011). Biogeographic areas were based on Kreft & Jetz (2010) and are as follows: A: South & East Africa; B: North & West Africa; and E: Sundaland.



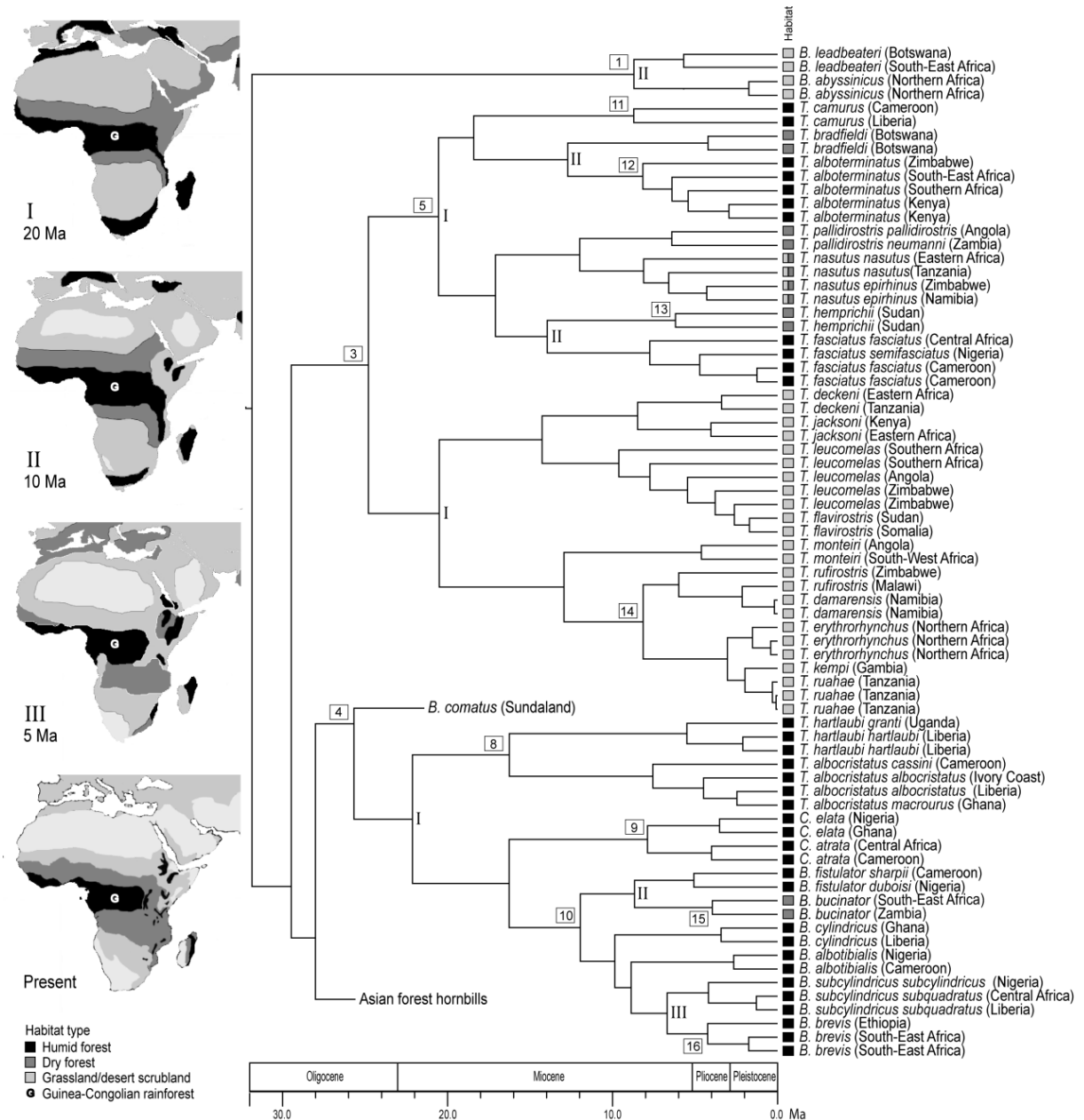


**Figure 5.4:** Historical habitats of African hornbills (Bucorvidae, *Tockus* and *Tropicranus*-*Ceratogymna* clades) based from a dated tree of mtDNA *cytb* sequences. Tree rooted using out-groups (not shown) which include rollers, a hoopoe and a woodhoopoe. Plate-tectonic maps reconstructed using the ODSN online service, with historical vegetation drawn to indicate possible ancestral habitats. Numbered nodes indicate possible ancestral habitats based on palaeo-vegetation maps derived from Dowsett *et al.* (1999), Bush & Flenley (2007) and Edwards *et al.* (2010). Numbers in boxes on nodes indicate key clades described in Table 5.1.

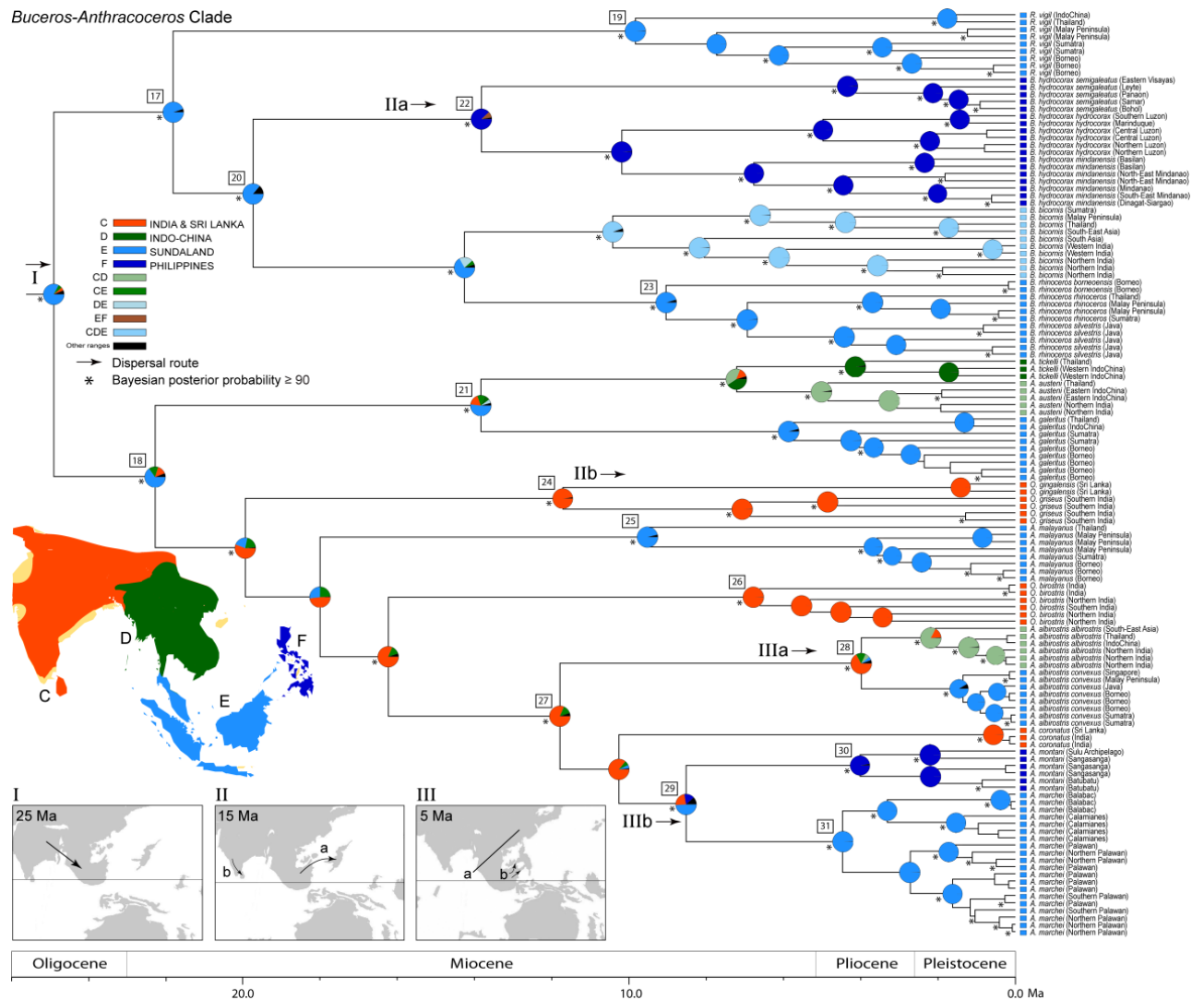


**Figure 5.5:** Ancestral area reconstruction of the continental Asian hornbills (*Buceros-Anthracoceros* clade), using Bayesian MCMC analysis derived from a dated tree of mtDNA *cytb* sequences. Tree rooted using out-groups (not shown) which include rollers, a hoopoe and a woodhoopoe. Maps based from Körsgen et al. (2011). Biogeographic regions based on Kreft & Jetz (2010) and plate-tectonic maps reconstructed using the ODSN online service. Numbered nodes and arrows refer to possible dispersal times and ancestral routes taken by hornbills, and drawn on plate-tectonic maps. Numbers in boxes on nodes indicate key clades described in Table 5.1. Biogeographic areas were based on Kreft & Jetz (2010) and are as follows: C: India & Sri Lanka; D: Indo-China; E: Sundaland; and F: Philippines.

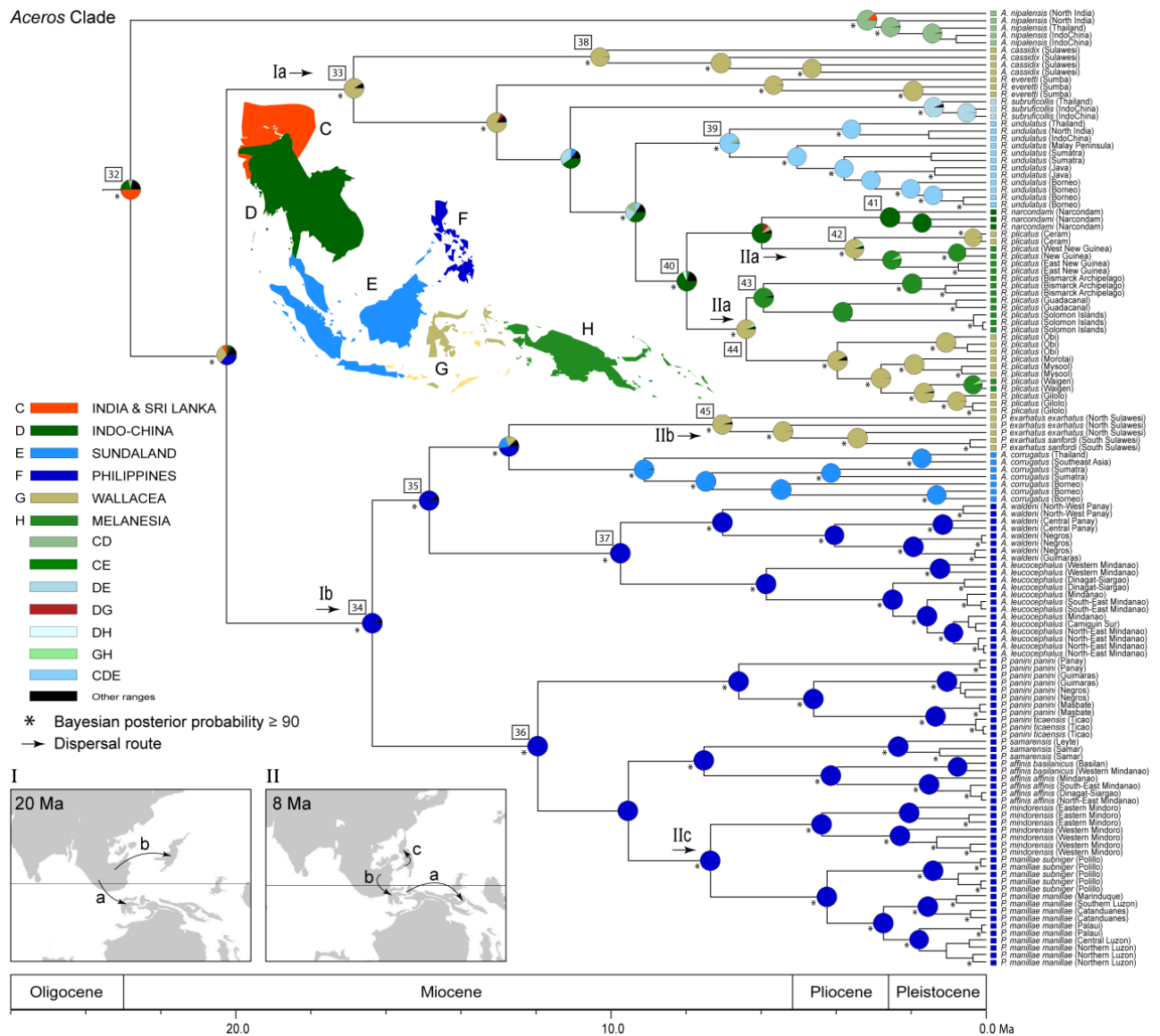


**Figure 5.6:** Ancestral area reconstruction of the insular Asian hornbills (*Aceros* clade), using Bayesian MCMC analysis derived from a dated tree of mtDNA *cytb* sequences. Tree rooted using out-groups (not shown) which include rollers, a hoopoe and a woodhoopoe. Maps based from Körsgen et al. (2011). Biogeographic regions based on Kreft & Jetz (2010) and plate-tectonic maps reconstructed using the ODSN online service. Numbered nodes and arrows refer to possible dispersal times and ancestral routes taken by hornbills, and drawn on plate-tectonic maps. Numbers in boxes on nodes indicate key clades described in Table 5.1. Biogeographic areas were based on Kreft & Jetz (2010) and are as follows: C: India & Sri Lanka; D: Indo-China; E: Sundaland; F: Philippines; G: Wallacea; and H: Melanesia.

## Supplementary information

**Table S5.1:** List of 342 in-group and nine out-group samples used in this study, with details of GenBank accession number of mtDNA Cytochrome *b* sequences, equivalent museum registry or source material and their geographic origin.

| Taxa                                         | GenBank No.           | Museum number <sup>a</sup> | Geo-isolate        |
|----------------------------------------------|-----------------------|----------------------------|--------------------|
| <i>Aceros nipalensis</i>                     | XXX                   | NHM1f1                     | North India        |
| <i>Aceros nipalensis</i>                     | XXX                   | NHM91202                   | North India        |
| <i>Aceros nipalensis</i>                     | GU257913 <sup>b</sup> | -                          | Thailand           |
| <i>Aceros nipalensis</i>                     | XXX                   | NHM121827                  | IndoChina          |
| <i>Aceros nipalensis</i>                     | XXX                   | NHM222200                  | IndoChina          |
| <i>Anorrhinus austeni</i>                    | XXX                   | NHM7241                    | North India        |
| <i>Anorrhinus austeni</i>                    | XXX                   | NHM7242                    | North India        |
| <i>Anorrhinus austeni</i>                    | GU257908 <sup>b</sup> | -                          | Thailand           |
| <i>Anorrhinus austeni</i>                    | XXX                   | NHM51431                   | East IndoChina     |
| <i>Anorrhinus austeni</i>                    | XXX                   | NHM(unknown)               | East IndoChina     |
| <i>Anorrhinus galeritus</i>                  | XXX                   | NHM91170                   | IndoChina          |
| <i>Anorrhinus galeritus</i>                  | GU257909 <sup>b</sup> | -                          | Thailand           |
| <i>Anorrhinus galeritus</i>                  | XXX                   | OUM5356                    | Borneo             |
| <i>Anorrhinus galeritus</i>                  | XXX                   | OUM5357                    | Borneo             |
| <i>Anorrhinus galeritus</i>                  | XXX                   | OUM5358                    | Borneo             |
| <i>Anorrhinus galeritus</i>                  | XXX                   | OUM5359                    | Borneo             |
| <i>Anorrhinus galeritus</i>                  | XXX                   | UMZC2a6                    | Borneo             |
| <i>Anorrhinus galeritus</i>                  | XXX                   | NUS3.11003                 | Sumatra            |
| <i>Anorrhinus galeritus</i>                  | XXX                   | AMNH645268                 | Sumatra            |
| <i>Anorrhinus tickelli</i>                   | GU257907 <sup>b</sup> | -                          | Thailand           |
| <i>Anorrhinus tickelli</i>                   | XXX                   | NHM22202                   | West IndoChina     |
| <i>Anorrhinus tickelli</i>                   | XXX                   | NHM83454                   | West IndoChina     |
| <i>Anthracoceros albirostris albirostris</i> | XXX                   | OUM5577                    | North India        |
| <i>Anthracoceros albirostris albirostris</i> | XXX                   | NHM25878                   | North India        |
| <i>Anthracoceros albirostris albirostris</i> | XXX                   | OUM5568                    | North India        |
| <i>Anthracoceros albirostris albirostris</i> | GU257911 <sup>b</sup> | -                          | Thailand           |
| <i>Anthracoceros albirostris albirostris</i> | XXX                   | NHM26260                   | IndoChina          |
| <i>Anthracoceros albirostris albirostris</i> | AAU89190 <sup>b</sup> | -                          | Captive, Singapore |
| <i>Anthracoceros albirostris convexus</i>    | XXX                   | OUM5374                    | Malay Peninsula    |
| <i>Anthracoceros albirostris convexus</i>    | GU257912 <sup>b</sup> | -                          | Singapore          |

# CHAPTER 5

|                                           |                       |            |                  |
|-------------------------------------------|-----------------------|------------|------------------|
| <i>Anthracoceros albirostris convexus</i> | XXX                   | OUM5375    | Borneo           |
| <i>Anthracoceros albirostris convexus</i> | XXX                   | OUM5376    | Borneo           |
| <i>Anthracoceros albirostris convexus</i> | XXX                   | OUM5378    | Borneo           |
| <i>Anthracoceros albirostris convexus</i> | XXX                   | OUM5373    | Sumatra          |
| <i>Anthracoceros albirostris convexus</i> | XXX                   | OUM5377    | Sumatra          |
| <i>Anthracoceros albirostris convexus</i> | XXX                   | NHM3026    | Java             |
| <i>Anthracoceros coronatus</i>            | XXX                   | NHM31494   | India            |
| <i>Anthracoceros coronatus</i>            | XXX                   | OUM5371    | India            |
| <i>Anthracoceros coronatus</i>            | XXX                   | NHM85716   | Sri Lanka        |
| <i>Anthracoceros malayanus</i>            | GU257910 <sup>b</sup> | -          | Thailand         |
| <i>Anthracoceros malayanus</i>            | XXX                   | NHM23355   | Malay Peninsula  |
| <i>Anthracoceros malayanus</i>            | XXX                   | OUM5369    | Malay Peninsula  |
| <i>Anthracoceros malayanus</i>            | XXX                   | NHM60148   | Borneo           |
| <i>Anthracoceros malayanus</i>            | XXX                   | OUM5370    | Borneo           |
| <i>Anthracoceros malayanus</i>            | XXX                   | UMZC3c3    | Borneo           |
| <i>Anthracoceros malayanus</i>            | XXX                   | NHM10241   | Sumatra          |
| <i>Anthracoceros malayanus</i>            | XXX                   | ZSLAMA1    | Captive, UK      |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP010100  | Calamianes       |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP014884  | Calamianes       |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP015297  | Calamianes       |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP074950  | Calamianes       |
| <i>Anthracoceros marchei</i>              | XXX                   | UPLB2128   | North Palawan    |
| <i>Anthracoceros marchei</i>              | XXX                   | PAWBWRCP42 | Captive, Palawan |
| <i>Anthracoceros marchei</i>              | XXX                   | NHM15102   | North Palawan    |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP14646   | North Palawan    |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP18033   | North Palawan    |
| <i>Anthracoceros marchei</i>              | XXX                   | UPLB2129   | North Palawan    |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP016137  | North Palawan    |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP04993   | South Palawan    |
| <i>Anthracoceros marchei</i>              | XXX                   | MGRHB1     | Captive, Palawan |
| <i>Anthracoceros marchei</i>              | XXX                   | MGRP43     | Captive, Palawan |
| <i>Anthracoceros marchei</i>              | XXX                   | OpolAviP15 | Captive, Palawan |
| <i>Anthracoceros marchei</i>              | XXX                   | OpolAviP41 | Captive, Palawan |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP06976   | South Palawan    |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP068200  | Balabac          |
| <i>Anthracoceros marchei</i>              | XXX                   | NMP068210  | Balabac          |
| <i>Anthracoceros marchei</i>              | XXX                   | DMNH37064  | Balabac          |
| <i>Anthracoceros montani</i>              | XXX                   | AMNH802255 | Sulu archipelago |
| <i>Anthracoceros montani</i>              | XXX                   | MSUSulu    | Sangasanga       |

# CHAPTER 5

|                                        |                       |             |                       |
|----------------------------------------|-----------------------|-------------|-----------------------|
| <i>Anthracoceros montani</i>           | XXX                   | DMNH27718   | Sangasanga            |
| <i>Anthracoceros montani</i>           | XXX                   | DMNH27720   | Sangasanga            |
| <i>Anthracoceros montani</i>           | XXX                   | DMNH27721   | Batubatu              |
| <i>Anthracoceros montani</i>           | XXX                   | NHM1638     | Batubatu              |
| <i>Berenicornis comatus</i>            | GU257917 <sup>b</sup> | -           | Thailand              |
| <i>Berenicornis comatus</i>            | XXX                   | NHM12079    | IndoChina             |
| <i>Berenicornis comatus</i>            | XXX                   | NHM91161    | IndoChina             |
| <i>Berenicornis comatus</i>            | XXX                   | NUS3.11082  | Malay Peninsula       |
| <i>Berenicornis comatus</i>            | XXX                   | NHM22163    | Borneo                |
| <i>Berenicornis comatus</i>            | XXX                   | NHM60617    | Borneo                |
| <i>Berenicornis comatus</i>            | XXX                   | NUS3.11084  | Borneo                |
| <i>Berenicornis comatus</i>            | XXX                   | NHM72412    | Sumatra               |
| <i>Berenicornis comatus</i>            | XXX                   | AMNH644968  | Sumatra               |
| <i>Berenicornis comatus</i>            | XXX                   | NUS3.11085  | Sumatra               |
| <i>Buceros bicornis</i>                | XXX                   | OUM14076    | North India           |
| <i>Buceros bicornis</i>                | XXX                   | OUM14077    | North India           |
| <i>Buceros bicornis</i>                | XXX                   | UMZC5a5     | North India           |
| <i>Buceros bicornis</i>                | XXX                   | NHM79167    | West India            |
| <i>Buceros bicornis</i>                | XXX                   | NHM231493   | West India            |
| <i>Buceros bicornis</i>                | AF346955 <sup>b</sup> | -           | Captive, Germany      |
| <i>Buceros bicornis</i>                | GU257920 <sup>b</sup> | -           | Thailand              |
| <i>Buceros bicornis</i>                | XXX                   | UMZC5a1     | Malay Peninsula       |
| <i>Buceros bicornis</i>                | XXX                   | NUS3.11132  | Sumatra               |
| <i>Buceros bicornis</i>                | XXX                   | NEZSBBB1    | Captive, SE Asia      |
| <i>Buceros hydrocorax hydrocorax</i>   | XXX                   | NSMNPBJA    | North Luzon           |
| <i>Buceros hydrocorax hydrocorax</i>   | XXX                   | UPLB2132    | North Luzon           |
| <i>Buceros hydrocorax hydrocorax</i>   | XXX                   | PAWBWRCP47  | Captive, Luzon        |
| <i>Buceros hydrocorax hydrocorax</i>   | XXX                   | UPDIB43     | Central Luzon         |
| <i>Buceros hydrocorax hydrocorax</i>   | XXX                   | UPLB2133    | South Luzon           |
| <i>Buceros hydrocorax hydrocorax</i>   | XXX                   | UPDIB344    | Marinduque            |
| <i>Buceros hydrocorax mindanensis</i>  | XXX                   | OpolAviP10  | Captive, Mindanao     |
| <i>Buceros hydrocorax mindanensis</i>  | XXX                   | UPLB2070    | Northeast Mindanao    |
| <i>Buceros hydrocorax mindanensis</i>  | XXX                   | MSUNSM56503 | Dinagat Siargao       |
| <i>Buceros hydrocorax mindanensis</i>  | XXX                   | NFEFIP32    | Captive, Mindanao     |
| <i>Buceros hydrocorax mindanensis</i>  | XXX                   | UPLB2171    | Southeast Mindanao    |
| <i>Buceros hydrocorax mindanensis</i>  | XXX                   | NHM10309    | Basilan               |
| <i>Buceros hydrocorax mindanensis</i>  | XXX                   | NMP45111    | Basilan               |
| <i>Buceros hydrocorax semigaleatus</i> | XXX                   | PAWBWRCP17  | Captive, East Visayas |
| <i>Buceros hydrocorax semigaleatus</i> | XXX                   | UPLB2140    | Leyte                 |

# CHAPTER 5

|                                                 |                       |            |                  |
|-------------------------------------------------|-----------------------|------------|------------------|
| <i>Buceros hydrocorax semigaleatus</i>          | XXX                   | NHM103019  | Panaon           |
| <i>Buceros hydrocorax semigaleatus</i>          | XXX                   | UPLB2147   | Samar            |
| <i>Buceros hydrocorax semigaleatus</i>          | XXX                   | UPLB2136   | Bohol            |
| <i>Buceros rhinoceros borneoensis</i>           | XXX                   | OUM5384    | Borneo           |
| <i>Buceros rhinoceros borneoensis</i>           | XXX                   | UMZC5c6    | Borneo           |
| <i>Buceros rhinoceros rhinoceros</i>            | GU257919 <sup>b</sup> | -          | Thailand         |
| <i>Buceros rhinoceros rhinoceros</i>            | XXX                   | AMNH645025 | Malay Peninsula  |
| <i>Buceros rhinoceros rhinoceros</i>            | XXX                   | NHMMCS21   | Malay Peninsula  |
| <i>Buceros rhinoceros rhinoceros</i>            | XXX                   | NHM1030207 | Sumatra          |
| <i>Buceros rhinoceros silvestris</i>            | XXX                   | AMNH122436 | Java             |
| <i>Buceros rhinoceros silvestris</i>            | AF346956 <sup>b</sup> | -          | Captive, Java    |
| <i>Buceros rhinoceros silvestris</i>            | AF346957 <sup>b</sup> | -          | Captive, Java    |
| <i>Buceros rhinoceros silvestris</i>            | XXX                   | NEZSBRA1   | Captive, Java    |
| <i>Buceros rhinoceros silvestris</i>            | XXX                   | NEZSBRA2   | Captive, Java    |
| <i>Bucorvus abyssinicus</i>                     | XXX                   | ZSLBAA2    | Captive, UK      |
| <i>Bucorvus abyssinicus</i>                     | XXX                   | ZSLBAB1    | Captive, UK      |
| <i>Bucorvus leadbeateri</i>                     | XXX                   | NHM55356   | Botswana         |
| <i>Bucorvus leadbeateri</i>                     | NC015199 <sup>b</sup> | -          | Captive?         |
| <i>Bycanistes brevis</i>                        | AF346915 <sup>b</sup> | -          | Captive, Germany |
| <i>Bycanistes brevis</i>                        | NC015201 <sup>b</sup> | -          | Captive?         |
| <i>Bycanistes brevis</i>                        | XXX                   | NHM421213  | Ethiopia         |
| <i>Bycanistes subcylindricus subcylindricus</i> | XXX                   | NHM591195  | Nigeria          |
| <i>Bycanistes subcylindricus subquadratus</i>   | XXX                   | NHM10232   | Liberia          |
| <i>Bycanistes subcylindricus subquadratus</i>   | AF346924 <sup>b</sup> | -          | Captive, Germany |
| <i>Bycanistes bucinator</i>                     | AF346920 <sup>b</sup> | -          | Captive, Germany |
| <i>Bycanistes bucinator</i>                     | XXX                   | UMZC7b1    | Zambia           |
| <i>Bycanistes albotibialis</i>                  | XXX                   | OUM16444   | Cameroon         |
| <i>Bycanistes albotibialis</i>                  | XXX                   | NHM34661   | Nigeria          |
| <i>Bycanistes cylindricus</i>                   | XXX                   | NHM20473   | Liberia          |
| <i>Bycanistes cylindricus</i>                   | XXX                   | NHM31668   | Ghana            |
| <i>Bycanistes fistulator duboisi</i>            | XXX                   | NHM88163   | Cameroon         |
| <i>Bycanistes fistulator sharpie</i>            | XXX                   | NHM82210   | Nigeria          |
| <i>Ceratogymna atrata</i>                       | XXX                   | OUM16445   | Cameroon         |
| <i>Ceratogymna atrata</i>                       | AF346912 <sup>b</sup> | -          | Captive, Germany |
| <i>Ceratogymna elata</i>                        | XXX                   | NHM31666   | Ghana            |
| <i>Ceratogymna elata</i>                        | XXX                   | NHM34663   | Nigeria          |
| <i>Aceros corrugatus</i>                        | GU257916 <sup>b</sup> | -          | Thailand         |
| <i>Aceros corrugatus</i>                        | HM755883 <sup>b</sup> | -          | Captive?         |
| <i>Aceros corrugatus</i>                        | XXX                   | NUS3.11132 | Sumatra          |

# CHAPTER 5

|                                    |                       |              |                     |
|------------------------------------|-----------------------|--------------|---------------------|
| <i>Aceros corrugatus</i>           | XXX                   | NUS3.11111   | Sumatra             |
| <i>Aceros corrugatus</i>           | XXX                   | OUM5360      | Borneo              |
| <i>Aceros corrugatus</i>           | XXX                   | OUM5362      | Borneo              |
| <i>Aceros corrugatus</i>           | XXX                   | NEZSACA1     | Captive, Borneo     |
| <i>Aceros leucocephalus</i>        | XXX                   | LWPRCP45     | Captive, Mindanao   |
| <i>Aceros leucocephalus</i>        | XXX                   | OpolAviFP07  | Captive, Mindanao   |
| <i>Aceros leucocephalus</i>        | XXX                   | OpolAviMP07  | Captive, Mindanao   |
| <i>Aceros leucocephalus</i>        | XXX                   | NMP0205      | Northeast Mindanao  |
| <i>Aceros leucocephalus</i>        | XXX                   | UPLB2113     | Northeast Mindanao  |
| <i>Aceros leucocephalus</i>        | XXX                   | NHM15130     | Camiguin            |
| <i>Aceros leucocephalus</i>        | XXX                   | MSUNSM56487  | Dinagat Siargao     |
| <i>Aceros leucocephalus</i>        | XXX                   | MSUNSM56488  | Dinagat Siargao     |
| <i>Aceros leucocephalus</i>        | XXX                   | NFEFIP05     | Captive, Mindanao   |
| <i>Aceros leucocephalus</i>        | XXX                   | UPLB2112     | Southeast Mindanao  |
| <i>Aceros leucocephalus</i>        | XXX                   | UPLB2121     | Southeast Mindanao  |
| <i>Aceros leucocephalus</i>        | XXX                   | UPLB2109     | West Mindanao       |
| <i>Aceros leucocephalus</i>        | XXX                   | UPLB2126     | West Mindanao       |
| <i>Aceros waldeni</i>              | HQ834454 <sup>b</sup> | -            | West Panay          |
| <i>Aceros waldeni</i>              | HQ834455 <sup>b</sup> | -            | West Panay          |
| <i>Aceros waldeni</i>              | XXX                   | MariitWCPP19 | Captive, East Panay |
| <i>Aceros waldeni</i>              | XXX                   | MtBaloyP21   | Captive, East Panay |
| <i>Aceros waldeni</i>              | XXX                   | NHM41598     | Guimaras            |
| <i>Aceros waldeni</i>              | XXX                   | NMP03939     | Negros              |
| <i>Aceros waldeni</i>              | XXX                   | UPLB2103     | Negros              |
| <i>Aceros waldeni</i>              | XXX                   | UPLB2104     | Negros              |
| <i>Oryceros birostris</i>          | XXX                   | AMNH257649   | North India         |
| <i>Oryceros birostris</i>          | XXX                   | NHM15902     | North India         |
| <i>Oryceros birostris</i>          | XXX                   | OUM5558      | North India         |
| <i>Oryceros birostris</i>          | XXX                   | NHM2002.22.1 | India               |
| <i>Oryceros birostris</i>          | XXX                   | ZSLOBA1      | Captive, UK         |
| <i>Oryceros birostris</i>          | XXX                   | NHM16530     | S India             |
| <i>Oryceros gingalensis</i>        | XXX                   | NHM123162    | Sri Lanka           |
| <i>Oryceros gingalensis</i>        | XXX                   | NHM3159      | Sri Lanka           |
| <i>Oryceros griseus</i>            | XXX                   | AMNH344282   | South India         |
| <i>Oryceros griseus</i>            | XXX                   | NHM16521     | South India         |
| <i>Oryceros griseus</i>            | XXX                   | UMZC12i1     | South India         |
| <i>Oryceros griseus</i>            | XXX                   | UMZC12i2     | South India         |
| <i>Penelopides affinis affinis</i> | XXX                   | UPLB2089     | Northeast Mindanao  |
| <i>Penelopides affinis affinis</i> | XXX                   | UPLB2090     | Dinagat Siargao     |



# CHAPTER 5

|                                        |                       |              |                       |
|----------------------------------------|-----------------------|--------------|-----------------------|
| <i>Penelopides affinis affinis</i>     | XXX                   | PAWBWRCP22   | Captive, Mindanao     |
| <i>Penelopides affinis affinis</i>     | XXX                   | UPLB2081     | Southeast Mindanao    |
| <i>Penelopides affinis affinis</i>     | XXX                   | MSUNSM53258  | West Mindanao         |
| <i>Penelopides affinis basilanicus</i> | XXX                   | NHM52213     | Basilan               |
| <i>Penelopides exarhatus exarhatus</i> | XXX                   | NHM30146     | North Sulawesi        |
| <i>Penelopides exarhatus exarhatus</i> | XXX                   | NHM5122423   | North Sulawesi        |
| <i>Penelopides exarhatus exarhatus</i> | XXX                   | UMZC9a1      | North Sulawesi        |
| <i>Penelopides exarhatus sanfordi</i>  | XXX                   | NHM12287     | South Sulawesi        |
| <i>Penelopides exarhatus sanfordi</i>  | XXX                   | ZSLPE1       | Captive, UK           |
| <i>Penelopides manillae manillae</i>   | XXX                   | DMNH70334    | Palau                 |
| <i>Penelopides manillae manillae</i>   | XXX                   | DMNH70335    | Palau                 |
| <i>Penelopides manillae manillae</i>   | XXX                   | NSMNPPMC01   | North Luzon           |
| <i>Penelopides manillae manillae</i>   | XXX                   | NSMNPPMSA1   | North Luzon           |
| <i>Penelopides manillae manillae</i>   | XXX                   | UPLB2056     | North Luzon           |
| <i>Penelopides manillae manillae</i>   | XXX                   | UPLB2061     | Central Luzon         |
| <i>Penelopides manillae manillae</i>   | XXX                   | NMP16625     | Catanduanes           |
| <i>Penelopides manillae manillae</i>   | XXX                   | NMP16582     | Catanduanes           |
| <i>Penelopides manillae manillae</i>   | XXX                   | UPLB2057     | South Luzon           |
| <i>Penelopides manillae manillae</i>   | XXX                   | UPDIB341     | Marinduque            |
| <i>Penelopides manillae subniger</i>   | XXX                   | PESPPMSE1    | Polillo               |
| <i>Penelopides manillae subniger</i>   | XXX                   | PESPPMSE2    | Polillo               |
| <i>Penelopides manillae subniger</i>   | XXX                   | UPDIBPSO0398 | Polillo               |
| <i>Penelopides manillae subniger</i>   | XXX                   | UPDIBPSO0400 | Polillo               |
| <i>Penelopides manillae subniger</i>   | XXX                   | UPDIBPSO0403 | Polillo               |
| <i>Penelopides mindorensis</i>         | XXX                   | CWRCP12      | Captive, West Mindoro |
| <i>Penelopides mindorensis</i>         | XXX                   | NMP9600      | West Mindoro          |
| <i>Penelopides mindorensis</i>         | XXX                   | NMP9601      | West Mindoro          |
| <i>Penelopides mindorensis</i>         | XXX                   | NMP09599     | West Mindoro          |
| <i>Penelopides mindorensis</i>         | XXX                   | UPDIBPSO0402 | East Mindoro          |
| <i>Penelopides mindorensis</i>         | XXX                   | NMP01261     | East Mindoro          |
| <i>Penelopides mindorensis</i>         | XXX                   | UPLB2143     | East Mindoro          |
| <i>Penelopides panini panini</i>       | HQ834451 <sup>b</sup> | -            | Panay                 |
| <i>Penelopides panini panini</i>       | HQ834459 <sup>b</sup> | -            | Panay                 |
| <i>Penelopides panini panini</i>       | XXX                   | NMP06363     | Negros                |
| <i>Penelopides panini panini</i>       | XXX                   | UPLB2062     | Negros                |
| <i>Penelopides panini panini</i>       | XXX                   | NHM30100     | Guimaras              |
| <i>Penelopides panini panini</i>       | XXX                   | UMZC9b1      | Guimaras              |
| <i>Penelopides panini panini</i>       | XXX                   | NMP012802    | Masbate               |
| <i>Penelopides panini panini</i>       | XXX                   | NHM41570     | Masbate               |

# CHAPTER 5

|                                     |                       |            |                      |
|-------------------------------------|-----------------------|------------|----------------------|
| <i>Penelopides panini ticaensis</i> | XXX                   | DMNH11381  | Ticao                |
| <i>Penelopides panini ticaensis</i> | XXX                   | DMNH11382  | Ticao                |
| <i>Penelopides panini ticaensis</i> | XXX                   | MNH11384   | Ticao                |
| <i>Penelopides samarensis</i>       | XXX                   | NHM13492   | Samar                |
| <i>Penelopides samarensis</i>       | XXX                   | NMP12255   | Samar                |
| <i>Penelopides samarensis</i>       | XXX                   | UPLB2073   | Leyte                |
| <i>Rhinoplax vigil</i>              | GU257918 <sup>b</sup> | -          | Thailand             |
| <i>Rhinoplax vigil</i>              | XXX                   | NHM201423  | Indo-China           |
| <i>Rhinoplax vigil</i>              | XXX                   | NHM412864  | Malay Peninsula      |
| <i>Rhinoplax vigil</i>              | XXX                   | NUS3.11140 | Malay Peninsula      |
| <i>Rhinoplax vigil</i>              | XXX                   | AMNH644995 | Sumatra              |
| <i>Rhinoplax vigil</i>              | XXX                   | NUS3.11143 | Sumatra              |
| <i>Rhinoplax vigil</i>              | XXX                   | OUM5388    | Borneo               |
| <i>Rhinoplax vigil</i>              | XXX                   | OUMN5387   | Borneo               |
| <i>Rhinoplax vigil</i>              | XXX                   | OUMNH5389  | Borneo               |
| <i>Rhyticeros cassidix</i>          | AF346938 <sup>b</sup> | -          | Captive, Sulawesi    |
| <i>Rhyticeros cassidix</i>          | XXX                   | NHM30140   | Sulawesi             |
| <i>Rhyticeros cassidix</i>          | XXX                   | NHM3218    | Sulawesi             |
| <i>Rhyticeros cassidix</i>          | XXX                   | UMZC1a1    | Sulawesi             |
| <i>Rhyticeros everetti</i>          | XXX                   | AMNH346740 | Sumba                |
| <i>Rhyticeros everetti</i>          | XXX                   | AMNH346744 | Sumba                |
| <i>Rhyticeros everetti</i>          | XXX                   | NHM12871   | Sumba                |
| <i>Rhyticeros narcondami</i>        | XXX                   | NHM10291   | Narcondam            |
| <i>Rhyticeros narcondami</i>        | XXX                   | NHM10292   | Narcondam            |
| <i>Rhyticeros narcondami</i>        | XXX                   | UMZC1e1    | Narcondam            |
| <i>Rhyticeros plicatus</i>          | XXX                   | AMNH645221 | Bismarck archipelago |
| <i>Rhyticeros plicatus</i>          | XXX                   | AMNH645225 | Bismarck archipelago |
| <i>Rhyticeros plicatus</i>          | XXX                   | NHM32993   | Bismarck archipelago |
| <i>Rhyticeros plicatus</i>          | XXX                   | AMNH645227 | Solomon Islands      |
| <i>Rhyticeros plicatus</i>          | XXX                   | AMNH645226 | Solomon Islands      |
| <i>Rhyticeros plicatus</i>          | XXX                   | NHM11717   | Solomon Islands      |
| <i>Rhyticeros plicatus</i>          | XXX                   | OUM3983    | Guadacanal           |
| <i>Rhyticeros plicatus</i>          | XXX                   | OUM3984    | Guadacanal           |
| <i>Rhyticeros plicatus</i>          | XXX                   | NHM28121   | Ceram                |
| <i>Rhyticeros plicatus</i>          | XXX                   | NHM28122   | Ceram                |
| <i>Rhyticeros plicatus</i>          | XXX                   | NHM23023   | East New Guinea      |
| <i>Rhyticeros plicatus</i>          | XXX                   | OUM5368    | East New Guinea      |
| <i>Rhyticeros plicatus</i>          | XXX                   | NHM20891   | West New Guinea      |
| <i>Rhyticeros plicatus</i>          | XXX                   | OpolAviP13 | Captive, New Guinea  |

# CHAPTER 5

|                                       |                       |            |                  |
|---------------------------------------|-----------------------|------------|------------------|
| <i>Rhyticeros plicatus</i>            | XXX                   | AMNH300745 | Waigen           |
| <i>Rhyticeros plicatus</i>            | XXX                   | AMNH645190 | Waigen           |
| <i>Rhyticeros plicatus</i>            | XXX                   | AMNH467670 | Gilolo           |
| <i>Rhyticeros plicatus</i>            | XXX                   | NHM22439   | Gilolo           |
| <i>Rhyticeros plicatus</i>            | XXX                   | NHM22440   | Gilolo           |
| <i>Rhyticeros plicatus</i>            | XXX                   | NHM2814    | Morotai          |
| <i>Rhyticeros plicatus</i>            | XXX                   | AMNH645189 | Mysool           |
| <i>Rhyticeros plicatus</i>            | XXX                   | NHM30128   | Mysool           |
| <i>Rhyticeros plicatus</i>            | XXX                   | AMNH645182 | Obi              |
| <i>Rhyticeros plicatus</i>            | XXX                   | NHM27191   | Obi              |
| <i>Rhyticeros plicatus</i>            | XXX                   | NHM3123    | Obi              |
| <i>Rhyticeros subruficollis</i>       | GU257914 <sup>b</sup> | -          | Thailand         |
| <i>Rhyticeros subruficollis</i>       | XXX                   | NHM30119   | Indo-China       |
| <i>Rhyticeros subruficollis</i>       | XXX                   | NHM91243   | Indo-China       |
| <i>Rhyticeros undulatus</i>           | XXX                   | NHM91224   | North India      |
| <i>Rhyticeros undulatus</i>           | XXX                   | NUS3.11153 | Indo-China       |
| <i>Rhyticeros undulatus</i>           | GU257915 <sup>b</sup> | -          | Thailand         |
| <i>Rhyticeros undulatus</i>           | XXX                   | NHMFMSM228 | Malay Peninsula  |
| <i>Rhyticeros undulatus</i>           | XXX                   | OUM5364    | Sumatra          |
| <i>Rhyticeros undulatus</i>           | XXX                   | NUS3.11166 | Sumatra          |
| <i>Rhyticeros undulatus</i>           | XXX                   | NHM43216   | Borneo           |
| <i>Rhyticeros undulatus</i>           | XXX                   | OUM5365    | Borneo           |
| <i>Rhyticeros undulatus</i>           | XXX                   | OUM5366    | Borneo           |
| <i>Rhyticeros undulatus</i>           | XXX                   | OUM5367    | Borneo           |
| <i>Rhyticeros undulatus</i>           | XXX                   | AMNH576107 | Java             |
| <i>Rhyticeros undulatus</i>           | XXX                   | NUS3.11167 | Java             |
| <i>Tockus alboterminatus</i>          | XXX                   | UMZC12a3   | Zimbabwe         |
| <i>Tockus alboterminatus</i>          | XXX                   | NHM02061   | South Africa     |
| <i>Tockus alboterminatus</i>          | XXX                   | OUM16327   | Kenya            |
| <i>Tockus alboterminatus</i>          | XXX                   | OUM16328   | Kenya            |
| <i>Tockus alboterminatus</i>          | AF346925 <sup>b</sup> | -          | Captive, Germany |
| <i>Tockus bradfieldi</i>              | XXX                   | NHM10267   | Botswana         |
| <i>Tockus bradfieldi</i>              | XXX                   | NHM50190   | Botswana         |
| <i>Tockus camurus</i>                 | XXX                   | NHM203088  | Liberia          |
| <i>Tockus camurus</i>                 | XXX                   | NHM34260   | Cameroon         |
| <i>Tockus fasciatus fasciatus</i>     | XXX                   | OUM16441   | Cameroon         |
| <i>Tockus fasciatus fasciatus</i>     | XXX                   | OUM16442   | Cameroon         |
| <i>Tockus fasciatus fasciatus</i>     | AF346928 <sup>b</sup> | -          | Captive, Germany |
| <i>Tockus fasciatus semifasciatus</i> | XXX                   | NHM20451   | Nigeria          |

# CHAPTER 5

|                                                |                       |                 |                  |
|------------------------------------------------|-----------------------|-----------------|------------------|
| <i>Tockus hemprichii</i>                       | XXX                   | NHM101453       | Sudan            |
| <i>Tockus hemprichii</i>                       | XXX                   | NHM51288        | Sudan            |
| <i>Tockus nasutus epirhinus</i>                | XXX                   | UMZC12n1        | Namibia          |
| <i>Tockus nasutus epirhinus</i>                | XXX                   | UMZC12n2        | Zimbabwe         |
| <i>Tockus nasutus nasutus</i>                  | XXX                   | OUM18098        | Tanzania         |
| <i>Tockus nasutus nasutus</i>                  | AF346934 <sup>b</sup> | -               | Captive, Germany |
| <i>Tockus pallidirostris neumanni</i>          | XXX                   | UMZC12o1        | Zambia           |
| <i>Tockus pallidirostris pallidirostris</i>    | XXX                   | NHM73542        | Angola           |
| <i>Tockus deckeni</i>                          | XXX                   | NHM40112        | Tanzania         |
| <i>Tockus deckeni</i>                          | AF345931 <sup>b</sup> | -               | Captive, Germany |
| <i>Tockus jacksoni</i>                         | XXX                   | NHM11616        | Kenya            |
| <i>Tockus jacksoni</i>                         | AF346930 <sup>b</sup> | -               | Captive, Germany |
| <i>Tockus flavirostris</i>                     | XXX                   | NHM4056         | Sudan            |
| <i>Tockus flavirostris</i>                     | XXX                   | NHM875703       | Somalia          |
| <i>Tockus leucomelas</i>                       | XXX                   | NHM121817       | Angola           |
| <i>Tockus leucomelas</i>                       | XXX                   | OUM18556        | Zimbabwe         |
| <i>Tockus leucomelas</i>                       | XXX                   | UMZC12h1        | Zimbabwe         |
| <i>Tockus leucomelas</i>                       | AF346933 <sup>b</sup> | -               | Captive, Germany |
| <i>Tockus leucomelas</i>                       | AF346932 <sup>b</sup> | -               | Captive, Germany |
| <i>Tockus monteiri</i>                         | XXX                   | NHM50188        | Southwest Africa |
| <i>Tockus monteiri</i>                         | XXX                   | NHM73543        | Angola           |
| <i>Tockus erythrorhynchus</i>                  | XXX                   | ZSLTE1          | Captive, UK      |
| <i>Tockus erythrorhynchus</i>                  | XXX                   | ZSLTE2          | Captive, UK      |
| <i>Tockus erythrorhynchus</i>                  | AF346927 <sup>b</sup> | -               | Captive?         |
| <i>Tockus damarensis</i>                       | AY054443 <sup>b</sup> | -               | Namibia          |
| <i>Tockus damarensis</i>                       | AY054447 <sup>b</sup> | -               | Namibia          |
| <i>Tockus rufirostris</i>                      | AF082071 <sup>b</sup> | UMMZA767        | Zimbabwe         |
| <i>Tockus rufirostris</i>                      | XXX                   | UMZC12f3        | Malawi           |
| <i>Tockus kemp</i>                             | AY423372 <sup>b</sup> | Live, C. Barlow | Gambia           |
| <i>Tockus ruahae</i>                           | AY423371 <sup>b</sup> | TM78009         | Tanzania         |
| <i>Tockus ruahae</i>                           | XXX                   | NHM17997        | Tanzania         |
| <i>Tockus ruahae</i>                           | XXX                   | NHM17998        | Tanzania         |
| <i>Tockus hartlaubi granti</i>                 | XXX                   | NHM197562       | Uganda           |
| <i>Tockus hartlaubi hartlaubi</i>              | XXX                   | NHM20460        | Liberia          |
| <i>Tockus hartlaubi hartlaubi</i>              | XXX                   | NHM20456        | Liberia          |
| <i>Tropicranus albocristatus albocristatus</i> | GU257921 <sup>b</sup> | BioBankSAfrica  | Ivory Coast      |
| <i>Tropicranus albocristatus albocristatus</i> | XXX                   | NHM203087       | Liberia          |
| <i>Tropicranus albocristatus cassini</i>       | XXX                   | OUM16443        | Cameroon         |
| <i>Tropicranus albocristatus macrourus</i>     | XXX                   | NHM71723        | Ghana            |

## CHAPTER 5

|                              |                       |            |              |
|------------------------------|-----------------------|------------|--------------|
| <i>Phoeniculus purpureus</i> | XXX                   | NEZSPP1    | Captive, UK  |
| <i>Phoeniculus purpureus</i> | EU167012 <sup>b</sup> | -          | Captive, USA |
| <i>Upupa epops</i>           | EU167030 <sup>b</sup> | -          | Captive, USA |
| <i>Upupa epops</i>           | U89189 <sup>b</sup>   | -          | Captive, USA |
| <i>Eurystomus glaucurus</i>  | AF407452 <sup>b</sup> | FMNH384689 | Madagascar   |
| <i>Eurystomus orientalis</i> | AF407451 <sup>b</sup> | FMNH350940 | Philippines  |
| <i>Eurystomus orientalis</i> | EU344978 <sup>b</sup> | -          | Australasia  |
| <i>Coracias caudata</i>      | U89184 <sup>b</sup>   | NMWN750    | Namibia      |
| <i>Coracias garrulus</i>     | AF407448 <sup>b</sup> | FMNH347816 | Pakistan     |

<sup>a</sup>AMNH: American Museum of Natural History, New York, USA; BMNH: Natural History Museum, Tring, UK; DMNH: Delaware Museum of Natural History, Delaware, USA; OUMNH: Oxford University Museum of Natural History, Oxford, UK; UMZC: Cambridge University Museum of Zoology, Cambridge, UK; UPLBMNH: University of the Philippines Los Baños Museum of Natural History, Laguna, Philippines; ZSL: Zoological Society of London, London, UK; NEZS: North of England Zoological Society, Cheshire, UK; PAWB WRC: Protected Areas and Wildlife Bureau Wildlife Rescue Centre, Manila, Philippines; NSMNP: Northern Sierra Madre Natural Park, Isabela, Philippines; LWPRC: Laguna Wildlife Park and Rescue Centre, Laguna, Philippines; WVSU-MWCP: West Visayas State University Mariit Wildlife Conservation Park, Iloilo, Philippines; MGR: Malagos Garden Resort, Davao City, Philippines; CWRC: Calintaan Wildlife Rescue Centre, Occidental Mindoro, Philippines; MSUNSM: Mindanao State University Natural Science Museum, Iligan City, Philippines; MSU Marawi: Mindanao State University Aga Khan Museum, Marawi City, Philippines; NMP: National Museum of the Philippines, Manila, Philippines; UPDIB: University of the Philippines Diliman Institute of Biology Collection, Quezon City, Philippines; Silliman University R.B. Gonzales Natural Science Museum, Dumaguete City, Philippines; NFEFI: Negros Forests and Ecological Foundation Inc. Biodiversity Conservation Centre, Bacolod City, Philippines; OpolAvi: Opol Aviary, Misamis Oriental, Philippines; PESP: Polillo Ecology Stewardship Project; FMNH: Field Museum of Natural History, Chicago, USA.

<sup>b</sup>Genbank sequences derived from published phylogenetic analyses: Brown *et al.*, 2008; Espinosa de los Monteros, 2000; Fain *et al.*, 2007 Hübner *et al.*, 2003; Kemp and Delpont, 2002; Kirchman *et al.*, 2001; Pacheco *et al.*, 2011; Pratt *et al.*, 2009; Sammler *et al.*, 2011; Srikwan & Woodruff, 1998; Viseshakul *et al.*, 2011.

**Table S5.2:** Primers used for PCR amplification and sequencing of mtDNA *cytb*<sup>a</sup>.

| Primer name <sup>b</sup> | Sequence (5' - 3')     | Location /Tm <sup>c</sup> |
|--------------------------|------------------------|---------------------------|
| TockusCytbLT-F           | GCCCTGTCCATCCTAATCAC   | External / 59.4           |
| TockusCytbLT-R           | CGACGGATGAGAAAGCTAGG   | Internal / 59.4           |
| AcerosCytbI-F            | ACATCTCCGCCTGATGAAAC   | Internal / 57.3           |
| AcerosCytbI-R            | GTGGGTTGTTTGAGCCTGAT   | Internal / 57.3           |
| AnthracoserosCytbI-R     | GGTAGGACATAGCCCACGAA   | Internal / 59.4           |
| AnthracoserosCytbII-F    | GGGCATCATCCTCCTACTCA   | Internal / 59.4           |
| AnthracoserosCytbII-R    | GTGGGTTGTTTGAGCCTGAT   | Internal / 59.4           |
| AcerosCytbII-F           | ACACTTTCTCCTCCCGTTCA   | Internal / 57.3           |
| AcerosCytbII-R           | TCATTATCGGCCAACTAGCC   | Internal / 57.3           |
| AnthracoserosCytbIII-F   | GCCCTACACTTTCTCCTCCC   | Internal / 61.4           |
| AnthracoserosCytbIII-R   | TATTGGGGATTGAGCGTAGG   | Internal / 57.3           |
| AnthracoserosCytbIV-F    | AACTTCACACCAGCAAACCC   | Internal / 57.3           |
| TockusCytbRT-F           | GCCCCCTCTCTCAAATCCTA   | Internal / 59.4           |
| TockusCytbRT-R           | GGTTGAGGTCTTCAGTCTTTGG | External / 60.3           |

<sup>a</sup>*cytb*: cytochrome b gene (1,143bp);

<sup>b</sup>Primers were designed from the following GenBank sequences: *TockusCytb* (AF082071, Mindell et al., 1998), *AcerosCytb* (AF346946, Hübner et al., 2003), and *AnthracoserosCytb* (U89190, van Tuinen et al., 2000); New primers developed using the online program PRIMER3 v.04.0 (Rozen & Skaletsky 1998).

<sup>c</sup>Tm: melting temperature of primer sequence.

**Table S5.3:** Posterior estimates<sup>a</sup> of stem age (Ma) of key clades of Afro-Asian hornbills (Bucerotidae) based on mtDNA *cytb* sequences using BEAST analysis, with results of optimal distributions based on ancestral area reconstructions using RASP analysis (see abbreviations below).

| Node <sup>b</sup> | Stem <sup>c</sup> | Optimal distributions <sup>d</sup> |      |   |   |      |   |   |   |  |      |      |    |     |    |    |    |    |    |
|-------------------|-------------------|------------------------------------|------|---|---|------|---|---|---|--|------|------|----|-----|----|----|----|----|----|
| number            | Age               | A                                  | B    | C | D | E    | F | G | H |  | AB   | BE   | CD | CDE | CE | DE | DH | EF | GH |
| 1                 | 31.9              | 0.36                               | 0.11 | - | - | -    | - | - | - |  | 0.52 | -    | -  | -   | -  | -  | -  | -  | -  |
| 2                 | 31.9              | 0.12                               | -    | - | - | -    | - | - | - |  | 0.86 | -    | -  | -   | -  | -  | -  | -  | -  |
| 3                 | 29.5              | 0.73                               | -    | - | - | -    | - | - | - |  | 0.27 | -    | -  | -   | -  | -  | -  | -  | -  |
| 4                 | 28                | -                                  | 0.5  | - | - | 0.29 | - | - | - |  | -    | 0.14 | -  | -   | -  | -  | -  | -  | -  |
| 5                 | 24.8              | 0.63                               | -    | - | - | -    | - | - | - |  | 0.36 | -    | -  | -   | -  | -  | -  | -  | -  |
| 6                 | 24.8              | 0.96                               | -    | - | - | -    | - | - | - |  | -    | -    | -  | -   | -  | -  | -  | -  | -  |
| 7                 | 25.7              | -                                  | -    | - | - | 0.98 | - | - | - |  | -    | -    | -  | -   | -  | -  | -  | -  | -  |
| 8                 | 22.1              | -                                  | 0.99 | - | - | -    | - | - | - |  | -    | -    | -  | -   | -  | -  | -  | -  | -  |
| 9                 | 16.3              | -                                  | 0.95 | - | - | -    | - | - | - |  | -    | -    | -  | -   | -  | -  | -  | -  | -  |
| 10                | 16.3              | -                                  | 0.97 | - | - | -    | - | - | - |  | -    | -    | -  | -   | -  | -  | -  | -  | -  |
| 11                | 18.4              | -                                  | 0.87 | - | - | -    | - | - | - |  | 0.11 | -    | -  | -   | -  | -  | -  | -  | -  |
| 12                | 14.3              | 0.13                               | -    | - | - | -    | - | - | - |  | 0.87 | -    | -  | -   | -  | -  | -  | -  | -  |
| 13                | 14                | -                                  | 0.89 | - | - | -    | - | - | - |  | 0.09 | -    | -  | -   | -  | -  | -  | -  | -  |
| 14                | 3.04              | 0.95                               | -    | - | - | -    | - | - | - |  | -    | -    | -  | -   | -  | -  | -  | -  | -  |
| 15                | 8.67              | 0.92                               | -    | - | - | -    | - | - | - |  | 0.06 | -    | -  | -   | -  | -  | -  | -  | -  |
| 16                | 6.69              | 0.93                               | -    | - | - | -    | - | - | - |  | 0.06 | -    | -  | -   | -  | -  | -  | -  | -  |
| 17                | 24.9              | -                                  | -    | - | - | 0.95 | - | - | - |  | -    | -    | -  | -   | -  | -  | -  | -  | -  |

# CHAPTER 5

|    |       |   |   |      |      |      |      |      |   |   |   |      |      |      |      |   |      |   |
|----|-------|---|---|------|------|------|------|------|---|---|---|------|------|------|------|---|------|---|
| 18 | 24.9  | - | - | 0.14 | -    | 0.61 | -    | -    | - | - | - | -    | -    | 0.15 | -    | - | -    | - |
| 19 | 21.8  | - | - | -    | -    | 0.99 | -    | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 20 | 21.8  | - | - | -    | -    | 0.86 | -    | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 21 | 22.3  | - | - | 0.18 | 0.09 | 0.52 | -    | -    | - | - | - | -    | -    | 0.12 | 0.06 | - | -    | - |
| 22 | 19.7  | - | - | -    | -    | -    | 0.88 | -    | - | - | - | -    | -    | -    | -    | - | 0.09 | - |
| 23 | 14.3  | - | - | -    | -    | 0.96 | -    | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 24 | 19.9  | - | - | 0.98 | -    | -    | -    | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 25 | 18    | - | - | -    | -    | 0.96 | -    | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 26 | 16.2  | - | - | 0.99 | -    | -    | -    | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 27 | 16.2  | - | - | 0.79 | -    | -    | -    | -    | - | - | - | -    | -    | 0.14 | -    | - | -    | - |
| 28 | 11.8  | - | - | 0.67 | -    | 0.06 | -    | -    | - | - | - | 0.07 | -    | 0.15 | -    | - | -    | - |
| 29 | 10.3  | - | - | 0.23 | -    | 0.49 | 0.16 | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 30 | 8.52  | - | - | -    | -    | -    | 0.97 | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 31 | 8.52  | - | - | -    | -    | 0.99 | -    | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 32 | 26.9  | - | - | 0.5  | 0.21 | -    | -    | -    | - | - | - | 0.07 | -    | -    | -    | - | -    | - |
| 33 | 20.3  | - | - | -    | -    | -    | -    | 0.91 | - | - | - | -    | -    | -    | -    | - | -    | - |
| 34 | 20.25 | - | - | -    | -    | -    | 0.94 | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 35 | 16.4  | - | - | -    | -    | -    | 0.92 | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 36 | 16.4  | - | - | -    | -    | -    | 0.99 | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 37 | 14.8  | - | - | -    | -    | -    | 0.99 | -    | - | - | - | -    | -    | -    | -    | - | -    | - |
| 38 | 13.1  | - | - | -    | -    | -    | -    | 0.99 | - | - | - | -    | -    | -    | -    | - | -    | - |
| 39 | 9.34  | - | - | -    | -    | -    | -    | -    | - | - | - | 0.07 | 0.91 | -    | -    | - | -    | - |



## CHAPTER 5

|    |      |   |   |   |      |   |   |      |      |   |   |   |   |   |   |      |   |      |
|----|------|---|---|---|------|---|---|------|------|---|---|---|---|---|---|------|---|------|
| 40 | 9.34 | - | - | - | 0.68 | - | - | -    | 0.06 | - | - | - | - | - | - | 0.06 | - | -    |
| 41 | 5.98 | - | - | - | 0.99 | - | - | -    | -    | - | - | - | - | - | - | -    | - | -    |
| 42 | 5.98 | - | - | - | -    | - | - | 0.89 | -    | - | - | - | - | - | - | -    | - | 0.06 |
| 43 | 6.4  | - | - | - | -    | - | - | -    | 0.96 | - | - | - | - | - | - | -    | - | -    |
| 44 | 6.4  | - | - | - | -    | - | - | 0.99 | -    | - | - | - | - | - | - | -    | - | -    |
| 45 | 12.7 | - | - | - | -    | - | - | 0.96 | -    | - | - | - | - | - | - | -    | - | -    |

---

<sup>a</sup>Node support for these reconstructions is indicated by posterior probabilities (PP), where  $\geq 0.95$  is strongly supported.

<sup>b</sup>For node numbers refer to Table 1 and Figures 3-6.

<sup>c</sup>Stem ages are in millions of years before the present (Ma)

<sup>d</sup>Ancestral areas are represented by biogeographic areas indicated by capital letters. Biogeographic areas were based on Kreft & Jetz (2010) and are as follows: A: South & East Africa; B: North & West Africa; C: India & Sri Lanka; D: Indo-China; E: Sundaland; F: Philippines; G: Wallacea; H: Melanesia.



**Figure S5.1:** Bayesian cladogram for 78 taxa of hornbills (Bucerotidae) based on mtDNA Cytb sequences. Nodes with  $\geq 70\%$  ML bootstrap values (BS) and  $\geq 90\%$  Bayesian posterior probabilities (PP) were considered moderate to strongly supported, and are indicated by black circles. Numbers on nodes indicate BS values  $< 70\%$  and PP  $< 90\%$ .

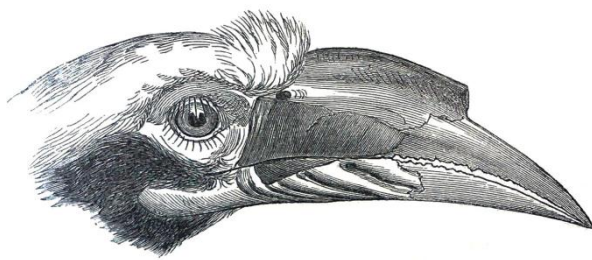
## References

- Brown, J.W., Rest, J.S., Garcia-Moreno, J., Sorensen, M.D. & Mindell, D.P. (2008) Strong mitochondrial DNA support for a Cretaceous origin of modern avian lineages. *BMC Biology*, **6**, 6.
- Espinosa de los Monteros, A. (2000) Higher-level phylogeny of Trogoniformes. *Molecular Phylogenetics and Evolution*, **14**, 20–34.
- Fain, M.G., Krajewski, C. & Houde, P. (2007) Phylogeny of "core Gruiformes" (Aves: Grues) and resolution of the Limpkin-Sungrebe problem. *Molecular Phylogenetics and Evolution*, **43**, 515–529.
- Hübner, V.S.M., Prinzinger, R. & Wink, M. (2003) Neue Erkenntnisse zur Taxonomie der Hornvögel (Aves: Bucerotiformes) und ihre Bedeutung für die Zucht in Menschenobhut. *Zoologische Garten N.F.*, **73**, 396–401.
- Kemp, A.C. & Delport, W. (2002) Comments on the status of subspecies in the red-billed hornbill (*Tockus erythrorhynchus*) complex (Aves: Bucerotidae), with the description of a new taxon endemic to Tanzania. *Annals of the Transvaal Museum*, **39**, 1–8.
- Kirchman, J.J., Hackett, S.J., Goodman, S.M. & Bates, J.M. (2001) Phylogeny and systematics of ground rollers (Brachypteraciidae) of Madagascar. *The Auk*, **118**, 849–863.
- Kreft, H. & Jetz, W. (2010) A framework for delineating biogeographic regions based on global species distributions. *Journal of Biogeography*, **37**, 2029–2053.
- Mindell, D. P., Sorenson, M. D. & Dimcheff, D. E. (1998) Multiple independent origins of mitochondrial gene order in birds. *Proceedings of National Academy of Sciences USA*, **95**, 10693–10697.
- Pacheco, M.A., Battistuzzi, F.U., Lentino, M., Aguilar, R., Kumar, S. & Escalante, A.A. (2011) Evolution of modern birds revealed by Mitogenomics: timing the radiation and origin of major orders. *Molecular Biology and Evolution*, **28**, 1927–1942.
- Pratt, R.C., Gibb, G.C., Morgan-Richards, M., Phillips, M.J., Hendy, M.D. & Penny, D. (2009) Toward resolving deep neoaves phylogeny: data, signal enhancement, and priors. *Molecular Biology and Evolution*, **26**, 313–326.
- Rozen S, Skaletsky HJ (2000) PRIMER 3.0. In: *Bioinformatics methods and protocols: methods in molecular biology* (ed. By S. Krawetz & S. Misener), pp.365–386. Humana Press, New Jersey.
- Sammler, S., Bleidorn, C. & Tiedemann, R. (2011) Full mitochondrial genome sequences of two endemic Philippine hornbill species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. *BMC Genomics*, **12**, 35–45.

- Srikwan, S. & Woodruff, D.S. (1998) DNA sequence variation and hornbill conservation. *The Asian Hornbills: Ecology and Conservation. Thai Studies in Biodiversity No.2* (ed. by P. Poonswad), pp. 69–82. BIOTEC-NSTDA, Bangkok.
- Viseshakul, N., Charoennitikul, W., Kitamura, S., Kemp, A.C., Thong-aree, S., Surapunpitak, Y., Poonswad, P. & Ponglikitmongkol, M. (2011) A phylogeny of frugivorous hornbills linked to the evolution of Indian plants within Asian rainforests. *Journal of Evolutionary Biology*, **24**, 1533–1545.
- van Tuinen, M., Sibley, C. G. & Hedges, S. B. (2000) The early history of modern birds inferred from DNA sequences of nuclear and mitochondrial ribosomal genes. *Molecular Biology and Evolution*, **17**, 451–457.

## Chapter 6

### Combining genotypic and phenotypic data to address the question of species limits: a case study of Asian hornbills



*Proc. Zool. Soc. London, Tweeddale 1877 p. 825*

*Scattered as the species are over many countries, it has not fallen to the lot of any one ornithologist to observe all of them in their native haunts; but beside what could be gathered from published accounts, to be found in various journals written in many languages, I have been most kindly aided by those who have enjoyed opportunities of observing certain species in the localities where the birds dwell.*

Daniel Giraud Elliot, 1882, *Monograph of the Bucerotidae or family of the hornbills*.

## **Combining genotypic and phenotypic data to address the question of species limits: a case study of Asian hornbills**

**Juan-Carlos T. Gonzalez<sup>1,2</sup>, Ben C. Sheldon<sup>1</sup>, Nigel J. Collar<sup>3</sup> and Joseph A. Tobias<sup>1</sup>**

<sup>1</sup>*Department of Zoology, Edward Grey Institute for Field Ornithology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*

<sup>2</sup>*Institute of Biological Sciences, University of the Philippines Los Baños, Laguna 4031, Philippines*

<sup>3</sup>*BirdLife International, Wellbrook Court, Girton Road, Cambridge CB3 0NA, UK*

### **SUMMARY**

Clarity and confidence over species limits are crucial for setting conservation priorities. However, taxonomy of birds in tropical regions, where most threatened species occur, is relatively poorly studied or not well-resolved. An integrated approach that makes use of both genotypic and phenotypic data offers an important and perhaps the best option for making both rapid and reliable progress in taxonomic reappraisals of such taxa-rich regions as the Indo-Malayan and Papuo-Australian regions. Plate tectonics and glaciation events have separated most species in these regions into geographically isolated populations, some of which have been split recently, but the taxonomic status of these ‘geo-isolates’ remains uncertain and unstable. We focus here on the diverse but taxonomically enigmatic group of forest hornbill species (Bucerotidae) from Asia and Melanesia, nine of which are currently threatened. Molecular divergence based on the corrected distance method and quantitative criteria for evaluating phenotypic data were used to compare differences for 54 pairs of species, subspecies and geo-isolates, and to help re-assess species limits. Molecular data were based on partial sequences of the mtDNA cytochrome *b* gene derived largely from historical material. Phenotypic data were calculated from a standard scoring system (Tobias

*et al.* 2010 *Ibis* 152: 724-746) that grades morphological and vocal differences. Results confirmed 11 recent splits of hornbills as valid species, including those in the Philippine genera *Penelopides* and *Aceros*. Calculated phenotypic scores suggest elevating Mindanao Rufous Hornbill *Buceros (hydrocorax) mindanensis*, Basilan Tarictic Hornbill *Penelopides (affinis) basilanicus* and Solomon Islands Hornbill *Rhyticeros (plicatus) mendanae* to species status. All but two currently recognized subspecies are retained, and some previously subsumed subspecies are proposed for resurrection. Intra-specific and intra-subspecific evaluation of geo-isolates reveals potentially cryptic taxa that merit further investigation. Our results suggest that phenotypic differentiation in Asian hornbills is correlated with molecular divergence, which disagrees with more discordant results from temperate birds. This supports the view that genetic and phenotypic data perform best to delineate species when used in combination. The resulting novel splits indicate reduced extent of occurrence and warrants re-evaluation of conservation status.

### **Keywords**

Bucerotidae, hornbills, species limits, Asia, genetic distance, phenotypic divergence

**Running title:** Species limits in Asian hornbills

Formatted for *Ibis*

## INTRODUCTION

Delimiting species is crucial to understanding biogeography, ecology and conservation biology, as a species is the fundamental unit whereby biological diversity is typically defined (Sites & Marshall 2004, Price 2008, Tobias *et al.* 2010). The importance of species limits to conservation is particularly critical, as actions and efforts depend on a clear, consistent and stable taxonomic status for their target taxa (Collar 1997). Resolving taxonomic issues to gear conservation priorities to taxa most at risk has been a major area of concern, particularly in the biologically diverse tropics (Solórzano *et al.* 2004, Gamauf *et al.* 2005). The use of molecular methods as a basis for delimiting taxa has been instrumental in resolving taxonomic issues and conserving critically threatened species previously regarded as conspecific (Garrido *et al.* 2002, Martínez-Cruz & Godoy 2007, Mittermeier *et al.* 2009), or shifting priorities to the importance of evolutionary significant units (ESU) in conservation (Hey *et al.* 2003, Solórzano *et al.* 2004, Peterson 2006). An ESU has multiple definitions, but it is broadly defined as a population that merits conservation priority or management due to its high genetic and/or ecological distinctiveness, irrespective of its formal taxonomic status. Three frameworks relating to reproductive isolation and adaptation, reciprocal monophyly, and level of gene exchange among populations are used to determine ESUs (Allendorf & Luikart 2006).

Genetic divergence has frequently been used in assessments of species status (Hickerson *et al.* 2006, Fregin *et al.* 2012), but the application of measures of such divergence in resolving taxonomic uncertainty has been criticized over the years, owing to the inconsistency of estimates that result from the different techniques and genes used (Price 2008, Winker 2009, Tobias *et al.* 2010, Fregin *et al.* 2012). Some of the overall difficulty in defining species limits also lies in the species concept used, rather than the choice of methods (Helbig *et al.* 2002). Conservationists face a considerable dilemma in this regard, as one species concept may lead (or be thought to lead) to under-estimating levels of divergence and thus not prompting essential conservation strategies (Gamauf *et al.* 2005), while another may lead to such ‘taxonomic inflation’ that too many ‘species’



present themselves for attention (Isaac *et al.* 2004). However, since speciation involves both the phenotype and genotype, the potential for an integrative approach in taxonomy is enormous, and a combined phenotypic and genetic analysis ought in theory to provide the most robust evaluation for defining species limits with confidence. Attempts at combining various analyses have proved useful in resolving taxonomic issues for many bird groups (Ribas *et al.* 2005, , Benz *et al.* 2006, Sangster *et al.* 2011).

Winker (2009) has described a model for the speciation process based on three routes of divergence, (1) when differentiation is driven largely by adaptive divergence, (2) when it is driven largely by neutral divergence, and (3) when phenotypic and genetic divergence rates are coupled. However, evidence for this correlation is limited in birds (Winker 2009), but although a linear relationship is questioned, both phenotypic and genetic data can be used comparatively as an objective measure of overall divergence (Helbig *et al.* 2002). There is evidence for congruence between taxonomic subspecies and molecular phylogenetic data (Phillimore & Owens 2006). However, the levels of differentiation may vary across biogeographical regions, and between continental and insular populations (Phillimore & Owens 2006). Putting these regional differences into consideration, we focus our analysis on the differentiation of tropical island populations, which harbour a high proportion of the world's 'endemic' and threatened species (Stattersfield *et al.* 1998). We reiterate the importance of using standard methods to make a quantifiable test and support an objective evaluation of taxonomic status. Two recent papers, one on the use of corrected genetic distances (Fregin *et al.* 2012), and the other on quantitative criteria in species delimitation (Tobias *et al.* 2010), provide the refined protocol needed for achieving such an aim. By combining genotypic and phenotypic data, we aim to address the question of species limits on a diverse group of tropical birds with many allopatric insular populations, unresolved taxonomic relationships and relevant conservation issues. It is this concordance across multiple data types (genetic, morphological, behavioural, life history and geographical), coupled with reproductive isolation, that binds criteria for delineating taxa (Allendorf & Luikart 2006).

Asian hornbills (Bucerotidae) are an ideal model system for testing a combined set of standardized criteria for species delimitation. They are a diverse and charismatic group of generally

frugivorous birds restricted to the tropical rainforests of the Indo-Malayan and Papuo-Australian regions (Kinnaird & O'Brien 2007). They include nine globally threatened species and 11 near-threatened species (BirdLife International 2004). Their history is riddled with taxonomic uncertainty, and taxonomic treatments vary across different monographs and global bird lists (Gonzalez *et al.* in prep., see Chapters 2 and 5). Recent revisions of species limits resulting in new species ('splits') have been contested and numerous insular populations contain clinal variants that require further scrutiny (Table 6.1). Philippine Tarictic Hornbills (*Penelopides*), which Collar *et al.* (1999) described as having a convoluted taxonomic history, are an ideal example. *Penelopides panini* was split by Kemp (1988, 1995) into four species, but these were not recognised by other authors (Dickinson *et al.* 1991, Kennedy *et al.* 2000). Clinal variations among insular forms of hornbill also led to various subspecies being subsumed into monotypic taxa (e.g. *Rhyticeros plicatus* in Kemp 2001a). But recent work on other Philippine birds has shown that island populations have the potential of harbouring taxa with weak phenotypic characterization but high levels of genetic divergence (Lohman *et al.* 2010). Molecular analysis of hornbills has already indicated the existence of genetically distinctive populations within a recognized monotypic species, as was the case for *Bucorvus leadbeateri* (Kemp 2001b, Hübner *et al.* 2003), but the question remains as to what taxonomic status should such populations possess? In this study, we apply the combined approach to re-examine species limits in Asian hornbills, focusing on pairwise comparisons of accepted species, subspecies and geographically isolated populations or 'geo-isolates'.

## METHODS

### Selection of taxonomic pairs of hornbills

Our combined analysis was designed to test the validity of contested species (Table 6.1), determine which subspecies are eligible to be elevated to species status, re-evaluate the status of subsumed subspecies, and identify cryptic taxa within a pool of undifferentiated geo-isolates. We

selected a total of 54 pairs of Asian hornbills in this study and these were categorized into four taxonomically stratified groups:

- 1) Species: hornbill pairs that represent either recent splits, or have been contested in the past and therefore require further analysis to confirm their status.
- 2) Subspecies: hornbill pairs of subspecies currently recognized in the IOC Word Bird List version 2.10 (Gill & Donsker 2012).
- 3) Subsumed taxa: pairs of hornbill geo-isolates previously described as subspecies but no longer recognized as valid due to lack of supporting evidence or because they are now regarded as clinal variants. Subspecies not listed in the current IOC Word Bird List.
- 4) Cryptic taxa: hornbill pairs representing undifferentiated geo-isolates not recognized previously as either species or subspecies but shown to possess separate genetic identities through the research undertaken in this study. These include intra-specific or intra-subspecific populations that have clustered into subclades within particular taxa in the completed molecular phylogeny (Gonzalez *et al.* in prep., Chapter 5, see Fig S5.1, supplementary data).

### **Phenotypic differentiation using quantitative criteria**

Phenotypic scores were derived from a simple quantitative scoring system used for delineating species based on objective criteria for assessing the degree of phenotypic difference between taxa (Tobias *et al.* 2010). This approach was developed for measuring phenotypic divergence using undisputed species as a basis for establishing ‘thresholds’ for a taxonomic scoring system. Total score was based on the sum of five key characters, including morphological (e.g. size and shape based on biometrics), visual (e.g. colour and pattern of plumage and soft parts), acoustic (e.g. descriptive measurement of pitch and pace of vocalizations), behavioural or ecological (e.g. unique social system, restricted habitat preference), and geographical relationships (e.g. parapatry in narrow or broad hybrid zones). A simple point-based system was used to determine four degrees of magnitude of phenotypic differentiation between taxa, namely minor

(score of 1), medium (score of 2), major (score of 3), and exceptional differences (score of 4). Pairs with a cumulative score of 7 are treated as species.

The strength of morphological characters was scored according to calculated effect sizes ( $\theta$ ) based on means and standard deviations of 10 measurements, presented as values of Cohen's  $d$  (Cohen 1969). We calculated the value of Cohen's  $d$  based on the difference between two means ( $\mu$ ) of each hornbill pair, divided by the pooled variance ( $\sigma_{\text{pooled}}$ ). Pooled variance takes into account the standard deviation ( $\sigma$ ) and sample size ( $n$ ) of each of the pairs. A standardized formula was derived from Hartung *et al.* (2008), which was modified from Cohen (1969). A version of the formula applicable to the Microsoft Excel program was integrated from an on-line matrix (See Kin Hai, unpublished data). Character differences were scored based on calculated  $d$  values of effect sizes, with 0.2–2 as minor, 2–5 as medium, 5–10 as major, and  $> 10$  as exceptional.

$$\text{Cohen's } d = \mu_1 - \mu_2 / \sigma_{\text{pooled}} ; \text{ where } \sigma_{\text{pooled}} = \sqrt{[(n_1-1) \sigma_1^2 + (n_2-1) \sigma_2^2 / (n_1+n_2)]}$$

Ten morphological measurements were included in the study derived from a list of 15 body and bill-casque morphometrics used for hornbills by Lua & Nakkuntod (1998) (see Table S6.1, supplementary data). These included tarsus length, wing length, tail length, length of terminal band of central tail feather (CTF), bill length, bill width, bill height, casque length, casque width and casque height. Nearly all measurements were taken from museum skins of adult male birds deposited in various museum collections in the United Kingdom, United States, Singapore and Philippines (see Table S6.2, supplementary data). Biometrics were taken largely by one researcher (JCG), although measurements of selected taxa were also taken by three other researchers (NJC, J. Woods, J. Dean) following the same morphometric regime. Additional measurements were taken from wild-caught birds, and from published literature using the same measurement regime (e.g. Mayr 1934, Lua & Nakkuntod 1998). Alternatively, measurements of adult female birds were used in support of analysis for poorly sampled males (these are indicated in the dataset). Scores generated for biometrics were derived from the largest increase (positive value) and the largest decrease (negative value) in effect size.

Scores for plumage and soft parts were derived largely from the literature (Kemp 1995, Kemp 2001a), including original descriptions (e.g. Hartert 1924, Mayr 1934) and direct examination of museum skins and their labels. Soft parts were also scored from on-line image databases and photographs of live birds. Plumage scores were based on a consensus of three independent observers, following the categories set in Tobias *et al.* (2010). A minor difference (score of 1) involves a weak diffusion in plumage or soft part (slightly different wash), medium (score of 2) refers to a distinctly different tone over a significant part of plumage, major (score of 3) refers to contrasting hues or patterns, and exceptional (score of 4) refers to radical changes in plumage pattern or colour. Overall score for visual character was based on a summation of the three highest scores for plumage and bare parts, deemed independent to minimize the degree of covariance.

Acoustic scores were basically derived from descriptions of vocalizations taken mostly from the literature, but some taxa were evaluated using available recordings of hornbill calls in the on-line database Xeno-Canto Asia (<http://www.xeno-canto.org/asia/>). A notable change in pitch and pace was given a minimum score of 1. Due to the limitations of acquiring a complete set of suitable sound recordings for all hornbill pairs, a quantitative measurement of acoustic characters using effect sizes of standard temporal and spectral measures was not conducted. Given these limitations, only one vocal character was included in the scoring system. One behavioural or ecological character—allowed a single score of 2 by Tobias *et al.* (2010)—was included in the overall score, together with a score for geographic relationship. The geographical relationship between hornbill pairs was based on definitions for parapatry and allopatry, wherein pairs known to be allopatric achieve no score. Only pairs known to occur in parapatry or in contact zones were scored, with birds occurring in true parapatry earning a score of 3 (major magnitude), pairs in a narrow hybrid zone a score of 2 (medium) and pairs in a broad hybrid zone a score of 1 (minor) (Tobias *et al.* 2010).

### **Molecular divergence using corrected distance estimation**

Molecular divergence was based on pairwise comparisons of partial mitochondrial cytochrome *b* (mtDNA Cytb) sequences derived from a comprehensive sequence matrix of Afro-Asian hornbills from Gonzalez *et al.* in prep. (see Chapter 5). A total of 339 mtDNA Cytb sequences were used in the aligned sequence matrix, based largely on historical material (see Table S6.3, supplementary data). We followed the recommendations of Fregin *et al.* (2012) on the calculation of corrected genetic distances using mtDNA Cytb sequences. Corrected distances were calculated using the optimal substitution model for sequence divergence. Fregin *et al.* (2012) suggested comparison of (1) only a single locus; (2) use of perfectly homologous regions with the same length and locus position; (3) and selection of the option for ‘complete deletion’ to avoid use of uncertain nucleotides in the sequence matrix. To fulfil these requirements, we truncated the aligned sequence matrix to 909 base pairs (bp), and all samples fewer than 909 were excluded. About 266 of the total 339 sequences were exclusively Asian, and this sequence data included 411 conserved sites, 498 variable sites, and 465 parsimony-informative sites. There was an average nucleotide proportion of 23.6% Thymine, 35.3% Cytosine, 26.9% Adenine, and 14.3% Guanine across three positions within the truncated sequence.

Best-fit model selection was conducted using MEGA version 5.0 (Tamura *et al.* 2011) based on Maximum Likelihood (*lnL*) fits of 24 different nucleotide substitution models. Models with the lowest Bayesian Information Criterion scores (BIC) described the best substitution pattern. The Tamura-Nei model (TN93) was selected based on 685 parameters, with a BIC score of 68,524.58, corrected Akaike Information Criterion (AICc) value of 61,240.38 and *lnL* value of –29,933.66. Non-uniformity of evolutionary rates was modelled using a discrete Gamma distribution (+ $\Gamma$ ) with five rate categories (Nei & Kumar 2000, Tamura *et al.* 2011). Mean genetic distances (and standard deviation) were calculated from resulting ‘genetic differentiation statistic’ compiled for each hornbill pair, derived from the extensive genetic distance matrix based on pairwise comparisons of 339 samples. A genetic distance of 4.0% was used as a simple ‘threshold’, which was considered a general consensus estimate for molecular divergence. Genomic

incompatibilities between allopatric populations build up after two million years of isolation, and these genetic incompatibilities cause infertility and inviability after completion of the speciation process. The *cytb* clock indicates a rate of 2% divergence per million years, which was used as the standard for protein-coding regions of mtDNA (Price & Bouvier 2002). Genetic distance (4%) is therefore a product of divergence rate (2% per million years) multiplied by two million years (time for completion of speciation process) (Price 2008). However, we emphasize that we only use this percentage to compare our estimates of genetic distance with resulting phenotypic scores, and are not confirming any cut-offs (Tobias *et al.* 2010). Recent evidence is still unable to resolve the complexity between genetic divergence and reproductive isolation, and estimates for genetic divergence vary extensively across different taxonomic groups, gene regions and methods (Fregin *et al.* 2012).

## RESULTS

We completed a comprehensive comparison of 54 pairs of hornbills, based on combined phenotypic scores (biometrics, plumage/bare parts, voice, behaviour/ecology, geographical relationship) and genetic distance values (mtDNA *Cytb*). These are represented in four groups (Table 6.2, Table S6.4, supplementary data): 11 species pairs (pairs 1–11, 20.4%), 17 subspecies pairs (pairs 12–28, 31.5%), 12 geo-isolate pairs based on subsumed subspecies (pairs 29–40, 22.2%), and 14 undifferentiated geo-isolates potentially representing cryptic taxa (pairs 40–54, 25.9%). About one-third ( $n=16$ ) of the hornbill pairs tested had phenotypic scores  $\geq 7.0$  (Fig. 6.1A) and were recognized as distinct species. This includes all 11 species pairs, of which seven had scores above 9.0 and were regarded to have achieved substantial phenotypic differentiation. Added to this were three subspecies pairs and two pairs of geo-isolates from subsumed taxa.

A quarter of the remaining pairs occurring below the ‘threshold’ had scores  $< 3.0$ , and were regarded as pairs with low phenotypic differentiation. These included pairs representing undifferentiated geo-isolates, several subsumed subspecies and two subspecies pairs (*Aceros c.*

*corrugatus*–*A. c. rugosus* and *Rhyticeros u. undulatus*–*R. u. aequabilis*). Although no ‘threshold’ for delineating subspecies was currently available for this scoring system, we considered a score of  $< 3.0$  to be low for subspecific recognition and therefore grouped them separately from other subspecies (referred to as “subspecies?” in Table 6.2). We based this assumption on resulting phenotypic scores of hornbill pairs for subspecies and subsumed taxa, wherein 15 of the 17 (88.2%) currently recognized subspecies had scores  $\geq 3.0$ , and 5 of the 12 (41.7%) subsumed taxa had scores  $\geq 3.0$ . All the pairs with scores  $< 3.0$  had no visible plumage differences (based on skins examined at Natural History Museum, Tring) and therefore no plumage scores were given. This artificial sub-grouping of geo-isolates into two sets of subspecies is used only as a basis for comparison in this study and we recognise that more stringent testing is required to produce a more acceptable value. We also do not discount the possibility that further analysis of plumage using other methods (e.g. spectrophotometry) may reveal differences unforeseen in our examination of skins. Moreover, a population may garner a score of 2 based on one medium character, rather than a sum of two minor characters. Hence, this medium character deserves recognition and merits further study.

In the molecular data, we calculated the mean inter-specific genetic distance of 32 species pairs in our mtDNA *cytb* dataset at 7.9% (range 3.4–19.4%). Mean inter-generic genetic distance was estimated at 12.1% (range 6.4–20%,  $n=32$ ). Thus we found wide variation in genetic distance amongst hornbill pairs at the generic and species level, with overlapping ranges. Only 17 pairs of hornbills had genetic distances above the 4% consensus ‘threshold’ for mtDNA *cytb* coding region (Fig. 6.1B), suggesting these pairs exhibited substantial genetic divergence for species recognition (based on our artificial threshold, adopted from Price 2008). Of these, only four had a distance score  $> 8.0$  (*Ocyrceros* and *Buceros*) (Table 6.2). Only eight (15%) of the 54 hornbill pairs had phenotypic scores  $\geq 7.0$  and genetic distances  $\geq 4\%$ , and six of these were species pairs (Table 6.2). Apparently, only 55% of the species pairs were both phenotypically differentiated and genetically divergent. Two other pairs represent subspecies pairs that had crossed both ‘thresholds’, and were therefore eligible for elevated species status. By contrast, nine pairs had phenotypic scores below the ‘threshold’, but had genetic distances  $> 4\%$ , and seven of these were subspecies pairs, but only



one had a phenotypic score approaching 7 (*Penelopides e. exarhatus*–*P. e. sanfordi*). Three pairs of geo-isolates had phenotypic scores < 3.0, suggesting that despite their weak morphological divergence they have achieved considerable genetic differentiation and may represent cryptic taxa: further study is needed to determine the degree of phenotypic divergence between these geo-isolates.

### Comparing genotypic and phenotypic data

We compared the highest biometric differences with genetic distance in the 54 hornbill pairs using a linear model to determine the relationship between phenotype and genotype (Fig. 6.2). These biometric values represent the largest difference between means of hornbill pairs among the ten measurements taken. A low but positive linear relationship was observed between morphological differentiation and molecular divergence. However, no pattern exists between the taxon groups. One key concern raised about the biometric dataset was the presence of small sample sizes in some of the hornbill pairs. About 13 pairs in the dataset had sample sizes below six individuals, and these represented taxa or geo-isolates that were rare in museum collections. These include the extinct *Penelopides panini ticaensis* and poorly known *Penelopides affinis basilanicus*. To determine the impact of these small sample sizes on our analysis, we also plotted a linear model for 41 pairs with sample sizes of under six individuals. Regression line for 54 pairs ( $r=0.18$ ;  $p=0.20$ ) was not significantly different from the regression for 41 pairs ( $r=0.18$ ;  $p=0.25$ ). We found very little difference in the slopes between the two models (8.71 and 8.97), indicating that our limited sampling effort had minimal impact on the overall analysis (Fig S6.2, see supplementary data). However, we do consider the importance of an ideal sampling regime, wherein all sample sizes are based on measurements of more than 10 adult male birds across ten biometric characters.

Conversely, a moderately positive relationship was observed between phenotypic score and genetic distance (Fig. 6.3,  $r=0.41$ ;  $p=0.002$ ; slope  $a=1.37$ ). A regression line based on 41 hornbill pairs (excluding pairs with sample size < 6) showed similar results ( $r=0.37$ ;  $p=0.017$ ; slope

a=1.31) and suggests that smaller sample sizes in the dataset did not cause deleterious impacts on the analysis. These poorly sampled pairs have limited effects on fitting the regression line, as only a few of the far outliers are poorly sampled. Although their removal improves the regression fit, there is a lot of scatter of points on either side of the regression line. Despite this, we can confirm that phenotypic divergence is correlated with genetic divergence in Asian hornbills.

Compared with the results for highest biometric difference, we observed a moderately positive linear relationship between phenotypic score and genetic distance (Fig. 6.3). Eight hornbill pairs were plotted in a section above the ‘thresholds’ for phenotypic differentiation and molecular divergence. Placement in this region suggests stronger support for the validity of their species status. The majority of the pairs occur in a section below the two ‘thresholds’, and therefore represent taxa ineligible for species status but which may be categorized as subspecies. Given the lack of a subspecific ‘threshold’, we consider scores below 3.0 to be less phenotypically differentiated. To illustrate the level of differentiation shown in Fig 6.3, we added ellipses to designate possible taxonomic groups within the model, with group A representing species and B representing subspecies. Group (C), represents a cluster of less differentiated geo-isolates that have low phenotypic scores, and their subspecies status requires further investigation.

However, we must not discount the importance of two other sections in the model. In Fig. 6.3, pairs above the dashed line but left of the dotted line represent species perceived to have achieved substantial phenotypic differentiation but which exhibit relatively low molecular divergence. By contrast, pairs plotted below the dashed line and right of the dotted line represent geo-isolates that exhibit high molecular divergence but show insufficient quantifiable phenotypic differentiation. Geo-isolates from group C occurring in this section represent probable cryptic taxa, and therefore require further scrutiny to identify their taxonomic status, since some may possess an unidentified but stable phenotypic character. Inclusive species pairs in group A and subspecies in group B exhibit different levels of phenotypic and genetic differentiation. We can safely designate group A and B as definite ESUs. Nonetheless, some pairs in group C may have achieved sufficient difference to be considered as ESUs.

### Delineating species in Asian hornbills

We provide evidence for validating the taxonomic status of eleven species pairs based on calculated phenotypic scores and genetic distances (Table 6.2). Details of scoring results for evaluating phenotypic differentiation are presented in Table S6.4 (see supplementary information) and estimates of molecular divergence in Table S6.5 (see supplementary information). From the 11 species pairs, we confirm six pairs, based on their phenotypic scores and genetic distances (score  $\geq 7$ , distance  $>4\%$ ). These recent but sometimes resisted splits include *Aceros leucocephalus* and *A. waldeni* (10, 5.36%); *Anthracoceros albirostris* and *A. coronatus* (7, 5.68%); *Ocyceros griseus* and *O. gingalensis* (13, 9.26%); *Penelopides panini* and *P. manillae* (11, 4.53%); *P. panini* and *P. mindorensis* (12, 4.75%); and *Rhyticeros subruficollis* and *R. undulatus* (10, 4.15%). Also from the 11 species pairs, we validate the status for another five pairs based on their cumulative phenotypic scores  $\geq 7$ . However, their calculated genetic distance was below the consensus threshold of 4%. These are *Penelopides affinis* and *P. manillae* (9, 3.52%); *Anorrhinus austeni* and *A. tickelli* (10, 2.86%); *P. panini* and *P. affinis* (11, 3.4%); *P. samarensis* and *P. affinis* (7, 2.06%); and *Rhyticeros narcondami* and *R. plicatus* (7, 3.4%).

In addition to the eleven species pairs validated by the standard scoring system, five other hornbill pairs qualify for species status, based on calculated scores  $\geq 7$ . Two of these pairs exhibit both high phenotypic differentiation and molecular divergence. These are *Buceros hydrocorax hydrocorax* and *B. h. mindanensis* (9, 8.85%) and *B. h. hydrocorax* and *B.h. semigaleatus* (9, 11.56%). A comparison between *B. h. mindanensis* and *B. h. semigaleatus* indicated high genetic distance, but a low phenotypic score (4, 8.22%), and therefore these two taxa do not differ sufficiently to be regarded as distinct species. Three other pairs have achieved substantial phenotypic differentiation but possess low levels of molecular divergence. These include *Penelopides affinis basilanicus* and *P. affinis affinis* (7, 1.27%); *R. plicatus* from the Solomon Islands and *R. plicatus ruficollis* (8, 3.32%); and *R. plicatus* from the Solomon Islands and *R. plicatus* from Seram–New Guinea (8, 3.88%). Parallel comparisons of *R. p. ruficollis* with other geo-isolates indicated phenotypic scores were insufficient to split them into distinct species (*R. p.*

*jungei*: 4, 2.57%; *R. p. plicatus*: 5, 1.49%). From this analysis, the following taxa are proposed to be elevated to species: *Buceros hydrocorax* (monotypic), *B. mindanensis* (*mindanensis* & *semigaleatus*), *Penelopides basilanicus* (monotypic) and *Rhyticeros mendanae* (*mendanae* & *harterti*). The reasons for proposed taxonomic changes are detailed in Appendix A (see supplementary data).

## DISCUSSION

### Coupling of phenotypic and genotypic data

Our results from Asian hornbills provide some indication that measures for phenotypic differentiation and molecular divergence are correlated, given that comparable measures of divergence are used. This slightly deviates from the assumption that molecular divergence is not linearly correlated with phenotypic divergence or may exhibit discordance (Helbig *et al.* 2002, Humphries & Winker 2011). However, earlier studies testing this relationship were focused mostly on temperate species, particularly from the Nearctic and Palearctic regions (Phillimore & Owens 2006, Humphries & Winker 2011). Our findings suggest that regional differences may be at work here, and that tropical regions exhibit a more linear relationship than temperate areas. This regional disparity may be fuelling the confusion regarding assumptions on the relationship of genetic and phenotypic divergence. Further analysis is needed to help resolve these confounding results in order to understand the mechanisms within the speciation process.

We attribute this correlation and coupling of phenotypic and genotypic axes to the stratified differentiation formed by three groups of hornbill pairs, which represent hierarchical levels of divergence along the regression line. Pairs belonging to group A and occurring above the 4% consensus divergence represent the ideal taxa for delineation, being highly differentiated both phenotypically and genetically. They represent the conceptual form of a species following the route based on the coupling of phenotypic and genotypic divergence rates (Winker 2009). Despite

looking superficially similar in morphology, we now find that *Buceros hydrocorax* and *B. mindanensis* also followed this coupled route.

Further down the regression line, hornbill pairs belonging to group B and occurring above the 4% consensus divergence represent taxa that we cannot accept as valid species. These taxa have not achieved sufficient phenotypic differentiation, but rather represent enroute populations driven largely by neutral divergence (Winker 2009). In this section of the model, we retain these less phenotypically differentiated but genetically divergent geo-isolates as subspecies, and regard them as ‘incipient species’. These geo-isolates are currently undergoing speciation and are perhaps reproductively isolated, but further work is required to ascertain if this route may lead to elevating their status to species. At the extreme end of the regression line nearest zero, hornbill pairs belonging to group C and occurring above the 4% consensus divergence rate also follow the same route as group B. However, these geo-isolates are phenotypically undifferentiated but sufficiently divergent at the molecular level and therefore represent distinct populations that may be potential new subspecies. On the proximal end of the line, in a section of the model defined as achieving panmixia (Winker 2009), there is high sequence similarity between geo-isolates.

### **Taxonomic recommendations for Asian hornbills**

Table 6.3 summarizes the proposed taxonomic treatments derived from earlier phylogenetic analysis and the results of species-limit analysis in this study. Congruent phenotypic and genetic data confirm species status for 16 hornbill pairs, 11 of which have in recent years been recognized as valid species by some authorities, with another four entirely new elevations to species being proposed. In the case of hornbill pairs straddling the edge of the ‘threshold’, with a cumulative phenotypic score of 6, it may be that additional biometric sampling, further analysis of vocalizations, and detailed investigation of plumage differences will add to their scores and thus establish them as full species. *Penelopides manillae subniger* is an interesting candidate in this regard, as its plumage scores achieve borderline status while its biometrics add nothing, despite being explicitly described as ‘larger’ than nominate *manillae* (McGregor 1910). Conflicting results

concerning the biometrics of *P. manillae subniger* suggests further analysis is in needed to resolve this.

### **Limitations, gaps and directions for species delineation**

It is not acceptable to elevate taxa to species status purely for conservation reasons (Gamauf *et al.* 2005; Groves *et al.*, 2010). Instead, by contrast, conservation strategies and planning need to be constantly alert to new taxonomic evaluations, and as far as possible should be open to other considerations such as ESUs (Gamauf *et al.* 2005). Taxonomic treatments of allopatric forms may be said to represent hypotheses that are open to new evidence and are dependent on the quality of the evaluation (Collar 2011). In this regard, limited sampling for the analysis of key phenotypic characters is particularly problematic. Ideally, >10 individuals are needed for a robust evaluation, but museum studies are often faced with fewer numbers of specimens. Some specimens are rare because the species they represent are rare, and probably even the total number of skins worldwide is limited for taxa such as *P. p. ticaensis* and *P. a. basilanicus*, which may not be represented by ten adult male skins in all the world's collections. The lack of sufficient male skins for biometric evaluation was the reason why we excluded *R. plicatus harterti* from our analysis.

Tobias *et al.* (2010) recommended the use of spectrophotometry to improve the scoring of plumage characters. The application of spectrophotometric techniques to determine the degree of difference in hues and gloss can help quantify these characters, and apply effect size calculations. However, not all spectral measurements are applicable to Asian hornbills. An examination of 28 species of hornbills based on museum skins indicated that UV phenomena were absent in the group. It is probable that UV plays a minimal role in intra-specific communication amongst hornbills (Mullen & Pohland 2008). Nonetheless, there are other parts of the plumage colour spectrum (four retinal cone types) that can be measured, including other variables like colour contrast, hue contrast and diversity, and chroma (Stoddard and Prum 2008). There is also a need to identify which body parts are actually based on cosmetic colouration (use of preen oil). Coloured

urophygial gland secretion is an ideal example of cosmetic colouration in birds, and about eight species of hornbill elaborately apply these secretions to their bill, casque and plumage. Secretions range from yellow to red, and oxidize quickly, thus requiring frequent reapplication. This results in substantial individual variation in the extent of colouration of plumage and soft parts (Delhey *et al.* 2007).

A more in-depth vocal analysis of hornbill calls is also needed. Calls of hornbills can be analysed to quantify the same seven standard temporal and spectral measures used for song structure (Tobias *et al.* 2010). All seven variables (e.g. pace, bandwidth, peak frequency) were quantifiable from the results of a multivariate analysis of vocalizations of *A. waldeni* and *P. panini* (Policht *et al.* 2009). Likewise, Policht *et al.* (2009) suggested that further vocal comparisons within the genus *Penelopides* may provide data that can assist in the re-assessment of this group. However, the major limitations in using this technique were the availability of suitable sound and voice material from all hornbill species and their geo-isolates. Consistency in the quality, length and coverage of these calls is equally limited. Development of specific primers (Gonzalez *et al.* in prep., see Chapter 2) and molecular markers for hornbills (Chamutpong *et al.* 2009, Sammler *et al.* 2012) can push phylogeographic studies further, to provide an improved understanding of divergence rates and to help disentangle the genetic relationships among geo-isolates. We also need to address questions regarding designation of a standard ‘threshold’ for subspecies delimitation. For the purpose of comparison, we had used a simple ‘threshold’ of 3.0 in this study, but no significant tests were done to define selection of this arbitrary value. Therefore, a similar test used to designate the species ‘threshold’ (Tobias *et al.* 2010) could be done to establish a more standard quantification of phenotypic differentiation for subspecific status. This would be a valuable contribution, as subspecies provide a useful tool in the study of evolutionary and conservation biology (Phillimore & Owens 2006).

### **Species limits suggest re-setting of priorities**

A summary of taxonomic recommendations and associated conservation status of hornbills is presented in Table 6.3. We include our proposal to elevate three subspecies of Asian hornbills to species, which separates the populations into smaller areas of occupancy and thus increases the likelihood that their conservation status will change to threatened.

Kemp (1995, 2001a) already raised the possibility of a split between *Buceros (hydrocorax) hydrocorax* and *B. (h.) mindanensis*, and suggested that a reassessment of their conservation status was required. Given their smaller effective populations, both species can be considered potentially threatened based on IUCN Red List Categories and Criteria (IUCN Standards & Petitions Subcommittee 2010). *Buceros (hydrocorax) hydrocorax* has a potential extent of occurrence of 212,460 km<sup>2</sup> on Luzon and Marinduque islands, but only 25,297 km<sup>2</sup> of this area is forested based on estimates in 1999 (ESSC 1999), whereas *B. (h.) mindanensis* has a total extent of occurrence of 97,642 km<sup>2</sup> on Mindanao and adjacent islands, but only 27,878 km<sup>2</sup> remained forested in 1999 (ESSC 1999). Moreover, *B. (h.) semigaleatus* occur in only 24,134 km<sup>2</sup> on the East Visayan Islands where only 5,381 km<sup>2</sup> of forests remained in 1999 (Gonzalez 2007). All three hornbill populations occur on multiple islands, and each is severely fragmented. There has been a notable decline in all three populations, owing to continued habitat degradation and direct exploitation (Collar *et al.* 1999). In particular, *B. (h.) hydrocorax* is still hunted for use in ceremonial head-dresses in northern Luzon (Gonzalez 2011).

*Penelopides (affinis) basilanicus* has an extremely small range, being unique to Basilan Island off the southwest tip of Mindanao. Basilan inherently has a small land area (1,240 km<sup>2</sup>) and is seriously deforested with only 9.6% forest cover remaining, as much of the island has been logged and converted to coconut plantations (ESSC, 1999). Any remaining fragments of lowland evergreen rainforest may occur in the central mountain range (ESSC, 1999; Gonzalez, 2007), which is heavily disturbed by on-going civil war. Even without direct human disturbance, the remaining forest is only 119 km<sup>2</sup>, and this small patch is likely to be fragmented. Given the limited forest area and lack of recent reports, there is the likelihood that both *B. (hydrocorax) mindanensis* (previously *basilanicus*) and *(affinis) basilanicus* are extinct on Basilan. High risks involved in



conducting studies on the island restrict the information available for both species of hornbills on Basilan.

*Rhyticeros (plicatus) mendanae* has a total extent of occurrence of 23,019 km<sup>2</sup> on the eastern Solomon Islands, whereas *R. (plicatus) harterti* has a total extent of occurrence of 9,300 km<sup>2</sup> on the western Solomon Islands, and *R. (plicatus) dampieri* has a total extent of occurrence of 49,700 km<sup>2</sup> on the Bismarck Archipelago (Mayr 1934). Although many of the islands in the Solomon Islands and Bismarck Archipelago are intact, the population on the islands may be low. Mayr & Diamond (2001) described the population of Blyth's Hornbill in the Solomon Islands as inherently small. Although the collective area of occurrence is above 20,000 km<sup>2</sup>, it actually comprises many small islands and the degree of migration of this rather vagile species between islands is not known (Mayr 1934, Mayr & Diamond 2001). Dutson (2011) described this *R. plicatus* complex to be fairly common in Northern Melanesia but rare where hunted.

### **Applications to Afrotropical hornbills**

Future investigations using the same methods applied to Asian hornbills may help resolve issues of species limits between similar contested taxa and recent splits in African hornbills. These issues have been thoroughly discussed in Kemp (2001b), which raised the importance of species limits in understanding the biology of African hornbills, and its role in conservation management. The importance of genetic studies in delineating the status of a possible seven species and nine subspecies would have significant implications for conservation. Among interesting couplings of hornbills that may merit re-evaluation are *Tockus jacksoni* and *T. deckeni*; *Bycanistes albotibialis* and *B. cylindricus*; and the *T. erythrorhynchus* species complex. Similar tests for species delimitation can be used on recognized subspecies of African hornbills, including *T. fasciatus semifasciatus*, *Tropicranus albocristatus cassini*, *T. nasutus epirhinus*, *B. subcylindricus subquadratus*, *B. fistulator sharpei* and *T. pallidirostris neumanni*, as well as on poorly known subsumed subspecies (*T. alboterminatus suahelicus* and *T. leucomelas elegans*). Kemp (1995) noted that clear morphological and vocal differences between *subcylindricus* and *subquadratus*

may warrant their taxonomic revision. Possible cryptic populations exist among monotypic hornbills such as in *Bucorvus leadbeateri*, *Bycanistes bucinator* and *B. brevis* (Hübner *et al.* 2003). An undescribed yellow-eyed form within the *Tockus erythrorhynchus* complex (nominate forms are brown-eyed) from NW Kenya awaits further investigation (P. Stapelberg in litt., 2011 via A. Kemp).

## CONCLUSION

Models depicting ideal routes to speciation rely on the coupling of phenotypic and genotypic divergence rates (Winker 2009), and can only be understood when there is some degree of molecular and morphological correlation. Quantitative measures of phenotypic differentiation in Asian hornbills have been shown to be correlated with estimated rates of molecular divergence. Although discordance and non-linearity between genotypic and phenotypic data appears to be the norm for temperate regions (e.g. Nearctic, Palearctic), our findings from tropical Asia support a linear relationship. However, more studies are needed to determine the degree of regional variation between these relationships, and their implications for understanding the speciation process. Our results support the view that genetic and phenotypic data perform best when used in combination to evaluate species limits, as each provides a comparable measure of divergence. The standard scoring system (Tobias *et al.*, 2010) allowed us to validate the species status of contested hornbill species and elevate three from subspecies, thus increasing the number of hornbill species to 64. However, we must not disregard the importance of subspecies and geo-isolates, and their role as ‘incipient’ species. Therefore, additional sampling and analysis is needed to evaluate populations that we have assessed as having borderline status. The use of quantitative approaches to scoring vocalizations, displays and plumage would greatly improve the means by which species delimitation is evaluated. Further work can be directed to addressing issues on cryptic species, or taxa exhibiting high genetic divergence but limited phenotypic differentiation. There is also a need to determine a ‘threshold’ for subspecies delimitation within the existing scoring system. A

## CHAPTER 6

standard system for delineating subspecies based on a combined approach may help distinguish differentiation among geo-isolates, and provide support for recognising populations that necessitate conservation priority.

## ACKNOWLEDGEMENTS

We are grateful for the assistance of the following for providing the source materials used in this study: R. Prys-Jones, M. Adams, J. Cooper, Natural History Museum Tring; M. Nowak-Kemp, Oxford University Museum of Natural History; P. Sweet, American Museum of Natural History; J. Woods, Delaware Museum of Natural History; UMZC: Cambridge University Museum of Zoology; I. Lit Jr, E. Cosico, University of the Philippines Los Baños Museum of Natural History; J. Graham, A. Walls, J. Ellis, Zoological Society of London; Roger Wilkinson, A. Owen, North of England Zoological Society; M.T. Lim, J. de Leon, T Protected Areas and Wildlife Bureau Wildlife Rescue Centre; R. Antolin, Northern Sierra Madre Natural Park; N. Donato, Laguna Wildlife Park and Rescue Centre; L. Lastimoza, West Visayas State University Mariit Wildlife Conservation Park; R. Puentespiña, Malagos Garden Resort; L. Estoya, Calintaan Wildlife Rescue Centre; E. Batara, Mindanao State University Iligan Institute for Technology Natural Science Museum; S. Nacua, Boni, Mindanao State University Marawi Aga Khan Museum; R. Uriza, National Museum of the Philippines; Perry Ong, I. Fontanilla, University of the Philippines Diliman Institute of Biology Collection; R. Domingo, R. Dahonog Opol Aviary; B. Malayang, E. Alcala, Silliman University R.B. Gonzales Natural Science Museum, Dumaguete City, Philippines; M.J. Justo, M. Ledesma, NFEFI: Negros Forests and Ecological Foundation Inc. Biodiversity Conservation Centre, Bacolod City, Philippines; A. Viojan, M. Larrazzabal, Sabin Hotel Aviary, Ormoc City, Leyte, Philippines; V. Yngente, PESP: Polillo Ecology Stewardship Project, Quezon, Philippines; We deeply thankful for the additional measurements taken by Jean Woods and James Dean. DNA analysis was conducted at the Department of Zoology, particularly at the J.W. Jenkinson Laboratory, EGI Molecular Ecology Lab and Sequencing Lab. This research was supported through Ford Foundation International Fellowship Program, University of the Philippines, North of England Zoological Society and British Ornithologist's Union.

## REFERENCES

- Allendorf, F. W. & Luikart, G. 2006. *Conservation and the Genetics of Populations*. Wiley-Blackwell, 664 p.
- Benz, B. W., Robbins, M. B. & Peterson, A. T. 2006. Evolutionary history of woodpeckers and allies (Aves: Picidae): placing key taxa on the phylogenetic tree. *Molecular phylogenetics and evolution*, **40**(2): 389-99.
- BirdLife International. 2004. *Threatened Birds of the World 2004*. Cambridge: BirdLife International. CD-ROM.
- Coates, B. J. & Bishop, K. D. 1997. *A guide to the birds of Wallacea: Sulawesi, the Moluccas, and Lesser Sunda Islands, Indonesia*. Dove Publications, Alderley, Queensland, Australia.
- Chamutpong, S., Saito, D. S., Viseshakul, N., Nishiumi, I., Poonswad, P., & Ponglikitmongkol, M. 2009. Isolation and characterization of microsatellite markers from the great hornbill, *Buceros bicornis*. *Molecular ecology resources*, **9**(2), 591-3.
- Cohen, J. 1969. *Statistical Power Analysis for the Behavioral Sciences*. New York: Academic Press.
- Collar, N. J. 1997. Taxonomy and conservation: chicken and egg. *Bulletin of the British Ornithologist's Club*, **117**: 122-136.
- Collar, N.J. 2011. Species limits in some Philippine birds including the Greater Flameback *Chrysocolaptes lucidus*. *Forktail* **27**: 29-38.
- Collar N.J., Mallari, N.A.D. & Tabaranza, B.R. 1999. *Threatened Birds of the Philippines: The Haribon Foundation/Birdlife International Red Data Book*. Makati City, Philippines: Bookmark Inc., 559 p.
- Delhey, K., Peters, A. & Kempnaers, B. 2007. Cosmetic Coloration in Birds: Occurrence, Function, and Evolution. *The American Naturalist* **169**: S145-S158.
- Dickinson, E.C., Kennedy, R.S. & Parkes, K.C. 1991. *The birds of the Philippines: an annotated checklist*. Tring, U.K.: British Ornithologists' Union (Checklist 12).
- Dickinson, E.C. (ed) 2003. *The Howard and Moore Complete Checklist of the Birds of the World*. (3rd edn). Princeton University Press, Princeton, New Jersey. Environmental Science for Social Change, Inc. (ESSC) 1999. *Decline of the Philippine Forest*. The Bookmark, Inc., Makati City.
- Dutson, G. 2011. *Birds of Melanesia*. London: Christopher Helm.
- Environmental Sciences for Social Change, Inc. (ESSC) 1999. *Decline of the Philippine Forest*. The Bookmark, Inc., Makati City.

- Fregin, S., Haase, M., Olsson, U., & Alström, P. 2012. Pitfalls in comparisons of genetic distances: a case study of the avian family Acrocephalidae. *Molecular Phylogenetics and Evolution* **62**(1): 319-28. Elsevier Inc.
- Frith, C.B. & Frith D.W. 1983. A systematic review of the hornbill genus *Anthracoceros* (Aves, Bucerotidae). *Zoological Journal of the Linnean Society* **78**: 29-71.
- Fry, C.H., Keith, S. & Urban, E.K. 1988. *The Birds of Africa, Vol. III*. Academic Press, London
- Gamauf, A., Gjershaug, J.-O., Røv, N., Kvaløy, K. & Haring, E. 2005. Species or subspecies? The dilemma of taxonomic ranking of some South-East Asian hawk-eagles (genus *Spizaetus*). *Bird Conservation International*, **15**(01): 99-117.
- Garrido, O. H., Kirwan, G. M. & Capper, D. R. 2002. Species limits within Grey-headed Quail-dove *Geotrygon caniceps* and implications for the conservation of a globally threatened species. *Bird Conservation International*, **12**(02): 169-187.
- Gill, F. & Donsker, D. 2012. *International Ornithological Congress World Bird List* (version 2.11). Available from: <http://www.worldbirdnames.org/names/> (accessed April 2011).
- Gonzalez, J.C.T. 2007. Fragmented forests and isolated islands: the distribution and status of Philippine endemic hornbills. In: Kemp, A. C. & M. I. Kemp (eds.), *Proceedings the Fourth International Hornbill Workshop. The Active Management of Hornbills and Their Habitats for Conservation*. Naturalists & Nomads. Pp. 51–74.
- Gonzalez, J.C.T. 2011. Enumerating the ethno-ornithological importance of Philippine hornbills. *The Raffles Bulletin of Zoology Supplement No. 24*: 149–161.
- Grimmett, R., Inskipp, C. & Inskipp, T. 1998. *Birds of the Indian subcontinent*. Christopher Helm, London.
- Groves, C.P., Fernando, P., & Robovsky, J. 2010. The sixth rhino: a taxonomic re-assessment of the critically endangered Northern White Rhinoceros. *PLoS ONE* **5**: e9703. doi:10.1371/journal.pone.0009703.
- Hartert, E. 1924. *Rhyticeros plicatus mendanae*, subsp. nov. *Bulletin of the British Ornithologist's Club*, **45**: 46.
- Hartung, J., Knapp, G. & Sinha, B.K. 2008. *Statistical Meta-Analysis with Applications*. John Wiley & Sons, Inc., 244 p.
- Helbig, A. J., Knox, A. G., Parkin, D. T., Sangster, G. & Collinson, M. 2002. Guidelines for assigning species rank. *Ibis*, **144**, 518-525.
- Hey, J., Waples, R. S., Arnold, M. L., Butlin, R. K. & Harrison, R. G. 2003. Understanding and confronting species uncertainty in biology and conservation. *Trends in Ecology & Evolution*, **18**(11): 597-603.
- Hickerson, M. J., Meyer, C. P. & Moritz, C. 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. *Systematic biology*, **55**(5): 729-39.

- Hübner, V.S.M., Prinzinger, R. & Wink, M. 2003. Neue Erkenntnisse zur Taxonomie der Hornvögel (Aves: Bucerotiformes) und ihre Bedeutung für die Zucht in Menschenobhut. *Zoologische Garten N.F.*, **73**: 396-401.
- Humphries, E.M. & Winker, K. 2011. Discord reigns among nuclear, mitochondrial and phenotypic estimates of divergence in nine lineages of trans-Beringian birds. *Molecular Ecology*, **20**:573-583.
- Isaac, N. J. B., Mallet, J. & Mace, G. M. 2004. Taxonomic inflation: its influence on macroecology and conservation. *Trends in Ecology & Evolution*, **19**(9): 464-9.
- IUCN Standards & Petitions Subcommittee. 2010. *Guidelines for Using the IUCN Red List Categories and Criteria* (version 8.1). Prepared by the Standards and Petitions Subcommittee in March 2010. Downloadable from <http://intranet.iucn.org/webfiles/doc/SSC/RedList/RedListGuidelines.pdf>.
- Kemp, A. C. 1988. The systematics and zoogeography of Oriental and Australasian hornbills (Aves: Bucerotidae). *Bonner Zoologische Beiträge* **39**: 315-345.
- Kemp, A.C. 1995. *Bird families of the world, The Hornbills: Bucerotiformes*. Oxford: Oxford University Press, 318 p.
- Kemp, A.C. 2001a. Family Bucerotidae (Hornbills). In de Hoyo, J., Elliott, A. & Sargatal, J. (eds) *Handbook of the Birds of the World Vol. 6, Mousebirds to Hornbills*: 436-523. Barcelona; Lynx Edicions.
- Kemp, A.C. 2001b. The role of species limits and biology in the conservation of African hornbills. *Ostrich Supplement No. 15*: 200-204.
- Kennedy, R. S., Gonzales, P. C., Dickinson, E. C., Miranda H. C. & Fisher, T. H. 2000. *A guide to the birds of the Philippines*. Oxford, U.K.: Oxford University Press.
- Kinnaird, M.F. & O'Brien, T.G. 2007. *The ecology and conservation of Asian hornbills: farmers of the forest*. The University of Chicago press, Chicago, 315 p.
- Lohman, D. J., Ingram, K. K., Prawiradilaga, D. M., Winker, K., Sheldon, F. H., Moyle, R. G., Ng, P. K. L., Ong, P.S., Wang, L.K., Braile, T.M., Astuti, D. & Meier, R. 2010. Cryptic genetic diversity in 'widespread' Southeast Asian bird species suggests that Philippine avian endemism is gravely underestimated. *Biological Conservation*, **143**(8): 1885-1890.
- Lua, H.K. & Nakkuntod, S. 1998. Hornbills in the Singapore Zoological Reference Collection. In Poonswad, P. (ed) *The Asian Hornbills: Ecology and Conservation. Thai Studies in Biodiversity No. 2*: 49-69.
- Martínez-Cruz, B. & Godoy, J. A. 2007. Genetic evidence for a recent divergence and subsequent gene flow between Spanish and Eastern imperial eagles. *BMC evolutionary biology*, **7**: 170.
- Mayr, E. 1934. Birds collected during the Whitney South Sea Expedition. XXVIII, notes on some birds from New Britain, Bismarck Archipelago. *American Museum Novitates* **709**: 7-22.

- Mayr, E. & Diamond, J. 2001. *The Birds of Northern Melanesia: Speciation, Ecology, and Biogeography*. Oxford: Oxford University Press.
- McGregor, R.C. 1910. Birds collected in the island of Polillo, Philippines. *Philippine Journal of Science*, **5**: 103-114.
- Mittermeier, R. A., Wallis, J., Rylands, A. B., Ganzhorn, J. U., Oates, J. F., Williamson, E. A., Palacios, E., Heymann, E.W., Kieruff, M.C.M., Yongcheng, L., Supriatna, J., Roos, C., Walker, S., Cortés-Ortiz, L. & Schwitzer, C. (eds.) 2009. *Primates in Peril: the world's 25 most endangered primates 2008-2010*. IUCN/SSC Primate Specialist Group, International Primatological Society and Conservation International (<http://www.primate-sg.org>).
- Mullen, P. & Pohland, G. 2008. Studies on UV reflection in feathers of some 1000 bird species: are UV peaks in feathers correlated with violet-sensitive and ultraviolet-sensitive cones? *Ibis* **150**: 59-68.
- Nei, M. & Kumar, S. 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
- Peterson, A. T. 2006. Taxonomy is important in conservation: a preliminary reassessment of Philippine species-level bird taxonomy. *Bird Conservation International*, **16**(02): 155.
- Phillimore, A.B. & Owens, I.P.F., 2006. Are subspecies useful in evolutionary and conservation biology? *Proceedings of the Royal Society of London B* **273**: 1049–1053.
- Policht, R., Petrú, M., Lastimoza, L. & Suarez, L. 2009. Potential for the use of vocal individuality as a conservation research tool in two threatened Philippine hornbill species, the Visayan Hornbill and the Rufous-headed Hornbill. *Bird Conservation International*, **19**:83–97.
- Price, T.D. & Bouvier, M.M. 2002. The evolution of F<sub>1</sub> postzygotic incompatibilities in birds. *Evolution*, **56**(10): 2083-2089.
- Price, T.D. 2008. *Speciation in Birds*. Colorado: Roberts & Company Publishers.
- Rasmussen, P.C. 2000. A review of the taxonomy and status of the Plain-pouched Hornbill *Aceros subruficollis*. *Forktail* **16**: 83-86.
- Rasmussen, P. & Anderton, J. 2005. *Birds of south Asia: the Ripley guide*. Vols. 1 and 2. Smithsonian Institution and Lynx Edicions, Washington D.C. and Barcelona.
- Ribas, C. C., Gaban-Lima, R., Miyaki, C. Y. & Cracraft, J. 2005. Historical biogeography and diversification within the Neotropical parrot genus *Pionopsitta* (Aves: Psittacidae). *Journal of Biogeography*, **32**(8): 1409-1427.
- Robson, C. 2009. *A field guide to the birds of South-East Asia*. New Holland, London.
- Sammler, S., Havenstein, K., & Tiedemann, R. 2012. Fourteen new microsatellite markers for the Visayan tarictic hornbill (*Penelopides panini*) and their cross-species applicability among other endangered Philippine hornbills. *Conservation Genetics Resources*, **4**, 435–438.
- Sanft, K. 1960. Aves/Upupae Bucerotidae. *Das Tierreich* **76**: 1-174.



- Sangster, G., Collinson, J. M., Crochet, P.-A., Knox, A. G., Parkin, D. T., Svensson, L., & Votier, S. C. 2011. Taxonomic recommendations for British birds: seventh report. *Ibis*, **153**(4): 883-892.
- Sites, J. W. & Marshall, J. C. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution, and Systematics*, **35**(1): 199-227.
- Solórzano, S., Baker, A.J. & Oyama, K. 2004. Conservation priorities for resplendent quetzals based on analysis of mitochondrial DNA control region sequences. *Condor* **106**: 449–456..
- Stattersfield, A. J., Crosby, M. J., Long, M. J. & Wege, D. C. 1998. *Endemic bird areas of the world: priorities for biodiversity conservation*. Cambridge, U.K.: BirdLife International (Conservation Series 7).
- Stoddard, M.C. & Prum, R.O. 2008. Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of New World buntings. *The American Naturalist*, **171** 96): 755-776.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* **28**:2731-2739.
- Tobias, J. A., Seddon, N., Spottiswoode, C. N., Pilgrim, J. D., Fishpool, L. D.C. & Collar, N. J. 2010. Quantitative criteria for species delimitation. *Ibis* **152**(4): 724-746.
- Wells, D.R. 1999. *The Birds of the Thai-Malay Peninsula, 1. Non-passerines*. London, Academic Press.
- Winker, K. 2009. Reuniting phenotype and genotype in biodiversity research. *BioScience*, **59**(8): 657-665.

## CHAPTER 6

**Table 6.1:** Recent changes to the taxonomy of Asian hornbills (Bucerotidae) and issues previously contested regarding their taxonomic status. Current taxonomy is based on the IOC World Bird List version 2.11 (Gill & Donsker 2012).

| Taxa                                       | Distribution                 | Taxonomic Status <sup>a</sup> | Issue/Remarks                            | Source <sup>b</sup> |
|--------------------------------------------|------------------------------|-------------------------------|------------------------------------------|---------------------|
| <i>Aceros waldeni</i>                      | West Visayas, Philippines    | Monotypic species             | Split from <i>A. leucocephalus</i>       | 3,4,6               |
| <i>Anorrhinus austeni</i>                  | Tenasserim, West Indo-China  | Monotypic species             | Split from <i>A. tickelli</i>            | 3,4,7               |
| <i>Anthracosceros coronatus</i>            | India and Sri Lanka          | Monotypic species             | Conspecific with <i>A. albirostris</i>   | 2,4,6               |
| <i>Ocyrceros griseus</i>                   | Western Ghats, India         | Monotypic species             | Split from <i>O. gingalensis</i>         | 3,4,6               |
| <i>Penelopides affinis</i>                 | Mindanao, Philippines        | Polytypic species             | Split from <i>P. panini</i>              | 3,4,6               |
| <i>Penelopides manillae</i>                | Luzon, Philippines           | Polytypic species             | Split from <i>P. panini</i>              | 3,4,6               |
| <i>Penelopides mindorensis</i>             | Mindoro, Philippines         | Monotypic species             | Split from <i>P. panini</i>              | 3,4,6               |
| <i>Penelopides samarensis</i>              | East Visayas, Philippines    | Monotypic species             | Split from <i>P. panini</i>              | 6?                  |
| <i>Rhyticeros narcondami</i>               | Narcondam, Andamans          | Monotypic species             | Conspecific with <i>R. plicatus</i>      | 3,4,6               |
| <i>Rhyticeros subruficollis</i>            | Tenasserim, West Indo-China  | Monotypic species             | Conspecific with <i>R. plicatus</i>      | 3,4,6               |
| <i>Rhyticeros undulatus</i>                | India, Indo-China, Sundaland | Polytypic species             | Conspecific with <i>R. subruficollis</i> | 3,4,6               |
| <i>Aceros corrugatus rugosus</i>           | Sumatra and Malay Peninsula  | Valid subspecies              | Possible clinal variant                  | 4                   |
| <i>Anthracosceros albirostris convexus</i> | Sundaland                    | Valid subspecies              | Subspecies recognized                    | 2                   |
| <i>Anthracosceros malayanus deminutus</i>  | Borneo                       | Subsumed subspecies           | Represent clinal variant                 | 2                   |
| <i>Anorrhinus galeritus carinatus</i>      | Malay Peninsula              | Subsumed subspecies           | Represent clinal variant                 | 4                   |
| <i>Anorrhinus galeritus minor</i>          | Borneo                       | Subsumed subspecies           | Represent clinal variant                 | 4                   |
| <i>Buceros bicornis homrai</i>             | India, Indo-China, Sundaland | Subsumed subspecies           | Represent clinal variant                 | 4                   |
| <i>Buceros hydrocorax basilanicus</i>      | Basilan, Philippines         | Subsumed subspecies           | Represent clinal variant                 | 1                   |
| <i>Buceros hydrocorax mindanensis</i>      | Mindanao, Philippines        | Valid subspecies              | Suggested elevation unresolved           | 4                   |
| <i>Buceros hydrocorax semigaleatus</i>     | East Visayas, Philippines    | Valid subspecies              | Suggested elevation unresolved           | 4                   |
| <i>Buceros rhinoceros borneoensis</i>      | Borneo                       | Valid subspecies              | Recognition possibly superficial         | 4                   |
| <i>Buceros rhinoceros silvestris</i>       | Java                         | Valid subspecies              | Recognition possibly superficial         | 4                   |
| <i>Penelopides affinis basilanicus</i>     | Basilan, Philippines         | Valid subspecies              | Subspecies recognized                    | 4                   |
| <i>Penelopides exarhatus sanfordi</i>      | Sulawesi, Wallacea           | Valid subspecies              | Subspecies recognized                    | 4                   |
| <i>Penelopides manillae subniger</i>       | Polillo, Philippines         | Valid subspecies              | Subspecies recognized                    | 4                   |
| <i>Penelopides panini ticaensis</i>        | Ticao, Philippines           | Valid subspecies              | Subspecies recognized                    | 4                   |
| <i>Rhyticeros plicatus dampieri</i>        | Bismarck Archipelago         | Valid subspecies              | Represent clinal variant                 | 4                   |
| <i>Rhyticeros plicatus jungei</i>          | East New Guinea              | Valid subspecies              | Represent clinal variant                 | 4                   |
| <i>Rhyticeros plicatus harterti</i>        | West Solomon Islands         | Valid subspecies              | Represent clinal variant                 | 4                   |
| <i>Rhyticeros plicatus mendanae</i>        | East Solomon Islands         | Valid subspecies              | Represent clinal variant                 | 4                   |
| <i>Rhyticeros plicatus ruficollis</i>      | North Moluccas & West Papua  | Valid subspecies              | Represent clinal variant                 | 4                   |
| <i>Rhyticeros undulatus aequabilis</i>     | Borneo                       | Valid subspecies              | Possible clinal variant                  | 4,5                 |
| <i>Rhyticeros undulatus ticehursti</i>     | North India                  | Subsumed subspecies           | Represent clinal variant                 | 4,5                 |

<sup>a</sup>Not considered valid by Kemp (2001a).

<sup>b</sup>Source: 1: Sanft 1960; 2: Frith & Frith 1983; 3: Kemp 1988; 4: Kemp 1995; 5: Rasmussen 2000; 6: Dickinson 2003; 7: Rasmussen & Anderton 2005.

## CHAPTER 6

**Table 6.2:** Phenotypic scores and molecular divergence of 54 pairs of hornbills examined for species delimitation. Total score was derived from biometrics, plumage and bare parts, vocalization, behaviour and ecology, and geographic relationship using a standard scoring system (Tobias *et al.* 2010). Molecular divergence was estimated based on best-fit model method for corrected genetic distance (Fregin *et al.* 2012) using mtDNA Cytb sequences.

| Pair No. | Genus Species                    | Subspecies        | Geo-isolate        | Biometrics <sup>a</sup> | Plumage & bare parts <sup>b,c</sup> | Vocalization <sup>d</sup> | Behaviour & Ecology | Geographical relationship | Phenotypic Score | Taxonomic status | Molecular divergence <sup>e</sup> | Conservation Status <sup>f</sup> |
|----------|----------------------------------|-------------------|--------------------|-------------------------|-------------------------------------|---------------------------|---------------------|---------------------------|------------------|------------------|-----------------------------------|----------------------------------|
| 1        | <i>Aceros leucocephalus</i>      |                   | Mindanao           | 3                       | 6                                   | 1                         | 0                   | 0                         | 10               | species          | 5.36                              | NT                               |
|          | <i>Aceros waldeni</i>            |                   | Negros-Panay       |                         |                                     |                           |                     |                           |                  |                  |                                   | CR                               |
| 2        | <i>Anorrhinus austeni</i>        |                   | East Indo-China    | 3                       | 7                                   | 0                         | 0                   | 0                         | 10               | species          | 2.86                              | NT                               |
|          | <i>Anorrhinus tickelli</i>       |                   | West Indo-China    |                         |                                     |                           |                     |                           |                  |                  |                                   | NT                               |
| 3        | <i>Anthracoceros albirostris</i> |                   | India-Sundaland    | 2                       | 4                                   | 1                         | 0                   | 0                         | 7                | species          | 5.68                              | LC                               |
|          | <i>Anthracoceros coronatus</i>   |                   | India-Sri Lanka    |                         |                                     |                           |                     |                           |                  |                  |                                   | NT                               |
| 4        | <i>Ocyrceros griseus</i>         |                   | India              | 5                       | 6                                   | 1                         | 1                   | 0                         | 13               | species          | 9.26                              | LC                               |
|          | <i>Ocyrceros gingalensis</i>     |                   | Sri Lanka          |                         |                                     |                           |                     |                           |                  |                  |                                   | LC                               |
| 5        | <i>Penelopides affinis</i>       |                   | Mindanao           | 2                       | 6                                   | 1                         | 0                   | 0                         | 9                | species          | 3.52                              | LC                               |
|          | <i>Penelopides manillae</i>      |                   | Greater Luzon      |                         |                                     |                           |                     |                           |                  |                  |                                   | LC                               |
| 6        | <i>Penelopides panini</i>        |                   | Negros-Panay       | 3                       | 7                                   | 1                         | 0                   | 0                         | 11               | species          | 4.53                              | EN                               |
|          | <i>Penelopides manillae</i>      |                   | Greater Luzon      |                         |                                     |                           |                     |                           |                  |                  |                                   | LC                               |
| 7        | <i>Penelopides panini</i>        |                   | Negros-Panay       | 3                       | 8                                   | 1                         | 0                   | 0                         | 12               | species          | 4.75                              | EN                               |
|          | <i>Penelopides mindorensis</i>   |                   | Mindoro            |                         |                                     |                           |                     |                           |                  |                  |                                   | EN                               |
| 8        | <i>Penelopides panini</i>        |                   | Negros-Panay       | 3                       | 7                                   | 1                         | 0                   | 0                         | 11               | species          | 3.40                              | EN                               |
|          | <i>Penelopides affinis</i>       |                   | Mindanao           |                         |                                     |                           |                     |                           |                  |                  |                                   | LC                               |
| 9        | <i>Penelopides samarensis</i>    |                   | East Visayas       | 2                       | 5                                   | 0                         | 0                   | 0                         | 7                | species          | 2.06                              | LC                               |
|          | <i>Penelopides affinis</i>       |                   | Mindanao           |                         |                                     |                           |                     |                           |                  |                  |                                   | LC                               |
| 10       | <i>Rhyticeros narcondami</i>     |                   | Narcondam          | 3                       | 3                                   | 1                         | 0                   | 0                         | 7                | species          | 3.40                              | EN                               |
|          | <i>Rhyticeros plicatus</i>       |                   | Wallacea-Melanesia |                         |                                     |                           |                     |                           |                  |                  |                                   | LC                               |
| 11       | <i>Rhyticeros subruficollis</i>  |                   | Indo-China         | 3                       | 6                                   | 1                         | 0                   | 0                         | 10               | species          | 4.15                              | VU                               |
|          | <i>Rhyticeros undulatus</i>      |                   | India-Sundaland    |                         |                                     |                           |                     |                           |                  |                  |                                   | LC                               |
| 12       | <i>Aceros corrugatus</i>         | <i>corrugatus</i> | Borneo             | 2                       | 0                                   | 0                         | 0                   | 0                         | 2                | subspecies?      | 7.09                              | NT                               |

# CHAPTER 6

|    |                                   |                     |                         |   |   |   |   |   |   |            |       |    |
|----|-----------------------------------|---------------------|-------------------------|---|---|---|---|---|---|------------|-------|----|
|    | <i>Aceros corrugatus</i>          | <i>rugosus</i>      | Malay Peninsula-Sumatra |   |   |   |   |   |   |            |       | NT |
| 13 | <i>Anthracosceros albirostris</i> | <i>albirostris</i>  | Indo-China              | 2 | 4 | 0 | 0 | 0 | 6 | subspecies | 1.88  | LC |
|    | <i>Anthracosceros albirostris</i> | <i>convexus</i>     | Sundaland               |   |   |   |   |   |   |            |       | LC |
| 14 | <i>Buceros bicornis</i>           | <i>homrai</i>       | India-IndoChina         | 4 | 0 | 0 | 0 | 0 | 4 | subspecies | 4.65  | NT |
|    | <i>Buceros bicornis</i>           | <i>bicornis</i>     | Sundaland               |   |   |   |   |   |   |            |       | NT |
| 15 | <i>Buceros hydrocorax</i>         | <i>hydrocorax</i>   | Greater Luzon           | 2 | 7 | 0 | 0 | 0 | 9 | species    | 8.85  | NT |
|    | <i>Buceros hydrocorax</i>         | <i>mindanensis</i>  | Mindanao                |   |   |   |   |   |   |            |       | NT |
| 16 | <i>Buceros hydrocorax</i>         | <i>hydrocorax</i>   | Greater Luzon           | 2 | 7 | 0 | 0 | 0 | 9 | species    | 11.56 | NT |
|    | <i>Buceros hydrocorax</i>         | <i>semigaleatus</i> | East Visayas            |   |   |   |   |   |   |            |       | NT |
| 17 | <i>Buceros hydrocorax</i>         | <i>mindanensis</i>  | Mindanao                | 2 | 2 | 0 | 0 | 0 | 4 | subspecies | 8.22  | NT |
|    | <i>Buceros hydrocorax</i>         | <i>semigaleatus</i> | East Visayas            |   |   |   |   |   |   |            |       | NT |
| 18 | <i>Buceros rhinoceros</i>         | <i>rhinoceros</i>   | Malay Peninsula-Sumatra | 2 | 2 | 0 | 0 | 0 | 4 | subspecies | 5.56  | NT |
|    | <i>Buceros rhinoceros</i>         | <i>borneoensis</i>  | Borneo                  |   |   |   |   |   |   |            |       | NT |
| 19 | <i>Buceros rhinoceros</i>         | <i>rhinoceros</i>   | Malay Peninsula-Sumatra | 2 | 2 | 0 | 0 | 0 | 4 | subspecies | 4.35  | NT |
|    | <i>Buceros rhinoceros</i>         | <i>silvestris</i>   | Java                    |   |   |   |   |   |   |            |       | NT |
| 20 | <i>Buceros rhinoceros</i>         | <i>borneoensis</i>  | Borneo                  | 2 | 1 | 0 | 0 | 0 | 3 | subspecies | 4.48  | NT |
|    | <i>Buceros rhinoceros</i>         | <i>silvestris</i>   | Java                    |   |   |   |   |   |   |            |       | NT |
| 21 | <i>Penelopides affinis</i>        | <i>basilanicus</i>  | Basilan                 | 3 | 4 | 0 | 0 | 0 | 7 | species    | 1.27  | LC |
|    | <i>Penelopides affinis</i>        | <i>affinis</i>      | Mindanao                |   |   |   |   |   |   |            |       | LC |
| 22 | <i>Penelopides exarhatus</i>      | <i>exarhatus</i>    | North Sulawesi          | 2 | 3 | 0 | 0 | 0 | 5 | subspecies | 4.93  | LC |
|    | <i>Penelopides exarhatus</i>      | <i>sandfordi</i>    | South Sulawesi          |   |   |   |   |   |   |            |       | LC |
| 23 | <i>Penelopides manillae</i>       | <i>manillae</i>     | mainland Luzon          | 2 | 4 | 0 | 0 | 0 | 6 | subspecies | 1.28  | LC |
|    | <i>Penelopides manillae</i>       | <i>subniger</i>     | Polillo                 |   |   |   |   |   |   |            |       | LC |
| 24 | <i>Penelopides panini</i>         | <i>panini</i>       | Negros-Panay            | 4 | 2 | 0 | 0 | 0 | 6 | subspecies | 2.10  | EN |
|    | <i>Penelopides panini</i>         | <i>ticaensis</i>    | Ticao                   |   |   |   |   |   |   |            |       | EX |
| 25 | <i>Rhyticeros plicatus</i>        | <i>ruficollis</i>   | West NG-N Moluccas      | 2 | 1 | 0 | 0 | 1 | 4 | subspecies | 2.57  | LC |
|    | <i>Rhyticeros plicatus</i>        | <i>jungei</i>       | East New Guinea         |   |   |   |   |   |   |            |       | LC |
| 26 | <i>Rhyticeros plicatus</i>        | <i>harterti</i>     | West Solomon Islands    | 2 | 2 | 0 | 0 | 0 | 4 | subspecies | 2.99  | LC |
|    | <i>Rhyticeros plicatus</i>        | <i>mendanae</i>     | East Solomon Islands    |   |   |   |   |   |   |            |       | LC |
| 27 | <i>Rhyticeros plicatus</i>        | <i>jungei</i>       | East New Guinea         | 2 | 1 | 0 | 0 | 1 | 4 | subspecies | 1.52  | LC |

# CHAPTER 6

|    |                                |                        |                         |   |   |   |   |   |   |             |      |    |
|----|--------------------------------|------------------------|-------------------------|---|---|---|---|---|---|-------------|------|----|
|    | <i>Rhyticeros plicatus</i>     | <i>ruficollis</i>      | West New Guinea         |   |   |   |   |   |   |             |      | LC |
| 28 | <i>Rhyticeros undulatus</i>    | <i>undulatus</i>       | Sundaland               | 2 | 0 | 0 | 0 | 0 | 2 | subspecies? | 2.46 | LC |
|    | <i>Rhyticeros undulatus</i>    | <i>aequabilis</i>      | Borneo                  |   |   |   |   |   |   |             |      | LC |
| 29 | <i>Anorhinus galeritus</i>     | <i>carinatus</i>       | Malay Peninsula         | 1 | 1 | 0 | 0 | 0 | 2 | subspecies  | 1.56 | LC |
|    | <i>Anorhinus galeritus</i>     | <i>minor</i>           | Borneo                  |   |   |   |   |   |   |             |      | LC |
| 30 | <i>Anorhinus galeritus</i>     | <i>galeritus</i>       | Sumatra                 | 1 | 0 | 0 | 0 | 0 | 1 | subspecies? | 1.64 | LC |
|    | <i>Anorhinus galeritus</i>     | <i>carinatus</i>       | Malay Peninsula         |   |   |   |   |   |   |             |      | LC |
| 31 | <i>Anorhinus galeritus</i>     | <i>carinatus</i>       | Malay Peninsula         | 1 | 1 | 0 | 0 | 0 | 2 | subspecies  | 1.58 | LC |
|    | <i>Anorhinus galeritus</i>     | <i>ssp.</i>            | Borneo-Sumatra          |   |   |   |   |   |   |             |      | LC |
| 32 | <i>Anthracoceros malayanus</i> | <i>deminutus</i>       | Borneo                  | 2 | 0 | 0 | 0 | 0 | 2 | subspecies? | 2.72 | NT |
|    | <i>Anthracoceros malayanus</i> | <i>malayanus</i>       | Malay Peninsula-Sumatra |   |   |   |   |   |   |             |      | NT |
| 33 | <i>Buceros bicornis</i>        | <i>homrai</i>          | India                   | 2 | 0 | 0 | 0 | 0 | 2 | subspecies? | 5.49 | NT |
|    | <i>Buceros bicornis</i>        | <i>ssp.</i>            | IndoChina-Sundaland     |   |   |   |   |   |   |             |      | NT |
| 34 | <i>Buceros hydrocorax</i>      | <i>mindanensis</i>     | Mindanao                | 2 | 0 | 0 | 0 | 0 | 2 | subspecies? | 3.98 | NT |
|    | <i>Buceros hydrocorax</i>      | <i>basilanicus</i>     | Basilan                 |   |   |   |   |   |   |             |      | NT |
| 35 | <i>Buceros hydrocorax</i>      | <i>basilanicus (F)</i> | Basilan                 | 2 | 0 | 0 | 0 | 0 | 2 | subspecies? | 3.98 | NT |
|    | <i>Buceros hydrocorax</i>      | <i>mindanensis (F)</i> | Mindanao                |   |   |   |   |   |   |             |      | NT |
| 36 | <i>Rhyticeros plicatus</i>     | <i>ssp.</i>            | Solomon Islands         | 3 | 4 | 1 | 0 | 0 | 8 | species     | 3.32 | LC |
|    | <i>Rhyticeros plicatus</i>     | <i>ruficollis</i>      | North Moluccas          |   |   |   |   |   |   |             |      | LC |
| 37 | <i>Rhyticeros plicatus</i>     | <i>ssp.</i>            | Seram-New Guinea        | 2 | 5 | 1 | 0 | 0 | 8 | species     | 3.88 | LC |
|    | <i>Rhyticeros plicatus</i>     | <i>ssp.</i>            | Solomon Islands         |   |   |   |   |   |   |             |      | LC |
| 38 | <i>Rhyticeros plicatus</i>     | <i>plicatus</i>        | Ceram                   | 3 | 2 | 0 | 0 | 0 | 5 | subspecies  | 1.49 | LC |
|    | <i>Rhyticeros plicatus</i>     | <i>ssp.</i>            | New Guinea              |   |   |   |   |   |   |             |      | LC |
| 39 | <i>Rhyticeros undulatus</i>    | <i>ticehursti</i>      | India-IndoChina         | 1 | 2 | 0 | 0 | 0 | 3 | subspecies  | 2.67 | LC |
|    | <i>Rhyticeros undulatus</i>    | <i>ssp.</i>            | Sundaland               |   |   |   |   |   |   |             |      | LC |
| 40 | <i>Rhyticeros undulatus</i>    | <i>ticehursti</i>      | India-IndoChina         | 2 | 2 | 0 | 0 | 0 | 4 | subspecies  | 2.78 | LC |
|    | <i>Rhyticeros undulatus</i>    | <i>aequabilis</i>      | Borneo                  |   |   |   |   |   |   |             |      | LC |
| 41 | <i>Aceros leucocephalus</i>    |                        | West Mindanao           | 2 | 0 | 0 | 0 | 1 | 3 | subspecies  | 3.90 | NT |
|    | <i>Aceros leucocephalus</i>    |                        | East Mindanao           |   |   |   |   |   |   |             |      | NT |
| 42 | <i>Anthracoceros coronatus</i> |                        | India                   | 2 | 0 | 0 | 0 | 0 | 2 | subspecies? | 0.11 | NT |

## CHAPTER 6

|    |                                |                    |                 |   |   |   |   |   |   |             |      |    |
|----|--------------------------------|--------------------|-----------------|---|---|---|---|---|---|-------------|------|----|
|    | <i>Anthracoceros coronatus</i> |                    | Sri Lanka       |   |   |   |   |   |   |             |      | NT |
| 43 | <i>Anthracoceros marchei</i>   |                    | Balabac         | 2 | 0 | 0 | 0 | 0 | 2 | subspecies  | 1.28 | VU |
|    | <i>Anthracoceros marchei</i>   |                    | Palawan         |   |   |   |   |   |   |             |      | VU |
| 44 | <i>Anthracoceros marchei</i>   |                    | Calamianes      | 2 | 0 | 0 | 0 | 0 | 2 | subspecies? | 1.20 | VU |
|    | <i>Anthracoceros marchei</i>   |                    | Palawan         |   |   |   |   |   |   |             |      | VU |
| 45 | <i>Berenicornis comatus</i>    |                    | Malay Peninsula | 1 | 0 | 0 | 0 | 0 | 1 | subspecies? | 1.57 | NT |
|    | <i>Berenicornis comatus</i>    |                    | Borneo-Sumatra  |   |   |   |   |   |   |             |      | NT |
| 46 | <i>Penelopides affinis</i>     | <i>affinis</i>     | West Mindanao   | 2 | 0 | 0 | 0 | 1 | 3 | subspecies  | 1.62 | LC |
|    | <i>Penelopides affinis</i>     | <i>affinis</i>     | East Mindanao   |   |   |   |   |   |   |             |      | LC |
| 47 | <i>Penelopides manillae</i>    | <i>manillae</i>    | South Luzon     | 2 | 2 | 0 | 0 | 1 | 5 | subspecies  | 1.04 | LC |
|    | <i>Penelopides manillae</i>    | <i>manillae</i>    | North Luzon     |   |   |   |   |   |   |             |      | LC |
| 48 | <i>Penelopides mindorensis</i> |                    | West Mindoro    | 2 | 2 | 0 | 0 | 1 | 5 | subspecies  | 2.10 | EN |
|    | <i>Penelopides mindorensis</i> |                    | East Mindoro    |   |   |   |   |   |   |             |      | EN |
| 49 | <i>Rhinoplax vigil</i>         |                    | Malay Peninsula | 2 | 0 | 0 | 0 | 0 | 2 | subspecies? | 4.40 | NT |
|    | <i>Rhinoplax vigil</i>         |                    | Borneo-Sumatra  |   |   |   |   |   |   |             |      | NT |
| 50 | <i>Penelopides affinis</i>     | <i>affinis</i>     | Dinagat/Siargao | 2 | 2 | 0 | 0 | 0 | 4 | subspecies  | 1.85 | LC |
|    | <i>Penelopides affinis</i>     | <i>affinis</i>     | East Mindanao   |   |   |   |   |   |   |             |      | LC |
| 51 | <i>Penelopides affinis</i>     | <i>affinis</i>     | Dinagat/Siargao | 3 | 3 | 0 | 0 | 0 | 6 | subspecies  | 2.13 | LC |
|    | <i>Penelopides samarensis</i>  |                    | East Visayas    |   |   |   |   |   |   |             |      | LC |
| 52 | <i>Penelopides affinis</i>     | <i>affinis (F)</i> | East Mindanao   | 2 | 0 | 0 | 0 | 1 | 3 | subspecies  | 1.62 | LC |
|    | <i>Penelopides affinis</i>     | <i>affinis (F)</i> | West Mindanao   |   |   |   |   |   |   |             |      | LC |
| 53 | <i>Penelopides panini</i>      | <i>panini</i>      | Masbate         | 2 | 1 | 0 | 0 | 0 | 3 | subspecies  | 2.02 | EN |
|    | <i>Penelopides panini</i>      | <i>panini</i>      | Negros-Panay    |   |   |   |   |   |   |             |      | EN |
| 54 | <i>Penelopides panini</i>      | <i>panini</i>      | Masbate         | 4 | 1 | 0 | 0 | 0 | 5 | subspecies  | 2.85 | EN |
|    | <i>Penelopides panini</i>      | <i>ticaensis</i>   | Ticao           |   |   |   |   |   |   |             |      | EN |

All skins measured refer to adult males, except for those indicated with (F), which refer to female skins only, and measurements were taken in millimetres; Plumage scores noted from skins examined and from literature; Vocalizations, behaviour and geographic relationships were based largely from literature.

<sup>a</sup> Total score represents the two highest or lowest *d* values among the ten measurements; Scoring system for Cohen's *d* value: 1=minor (0.2–2), 2=medium (2–5), 3=major (5–10), 4=exceptional(>10).

## CHAPTER 6

<sup>b</sup> Total score is the sum of scores from three (3) plumage characters including bare parts.

<sup>c</sup> Additional measurements and plumage descriptions were taken from Mayr (1934), Kemp (1995), Lua & Nakkuntod (1998), and Kemp (2001a).

<sup>d</sup> Vocalizations, behaviour and geographic relationships were largely based on the following sources: Fry et al. 1988; Kemp 1995; Kemp 2001a; Kennedy et al. 2000; Rasmussen & Anderton 2005; Robson 2009; Grimmett *et al.* 1998; Wells 1999; Coates & Bishop 1997.

<sup>e</sup> Nucleotide divergence based on pairwise comparisons of mtDNA Cytochrome *b* truncated to 909 bp, calculated using lnL best-fit model TN93+G based on Fregin *et al.* (2012).

<sup>f</sup> Conservation status of hornbills according to the IUCN Red List assessments: LC, Least Concern; VU, Vulnerable; EN, Endangered; CR, Critically Endangered; NT, Near-threatened; assignment to categories follows BirdLife International (2004).

# CHAPTER 6

**Table 6.3:** Taxonomic recommendations and conservation status of hornbills (Bucerotidae) based on proposed changes from Gonzalez *et al.* (in prep., see Chapter 2) and this study.

| Clade                   | Current taxonomy (IOC 2.10)      | Taxonomic recommendation <sup>a</sup> | Status <sup>b</sup> |
|-------------------------|----------------------------------|---------------------------------------|---------------------|
| A - <i>Bucorvus</i>     | <i>Bucorvus abyssinicus</i>      | <i>Bucorvus abyssinicus</i>           | LC                  |
|                         | <i>Bucorvus leadbeateri</i>      | <i>Bucorvus leadbeateri</i>           | VU                  |
| B - <i>Tockus</i>       | <i>Tockus ruahae</i>             | <i>Tockus ruahae</i>                  | LC                  |
|                         | <i>Tockus kemp</i>               | <i>Tockus kemp</i>                    | LC                  |
|                         | <i>Tockus damarensis</i>         | <i>Tockus damarensis</i>              | LC                  |
|                         | <i>Tockus rufirostris</i>        | <i>Tockus rufirostris</i>             | LC                  |
|                         | <i>Tockus erythrorhynchus</i>    | <i>Tockus erythrorhynchus</i>         | LC                  |
|                         | <i>Tockus monteiri</i>           | <i>Tockus monteiri</i>                | LC                  |
|                         | <i>Tockus deckeni</i>            | <i>Tockus deckeni</i>                 | LC                  |
|                         | <i>Tockus jacksoni</i>           | <i>Tockus jacksoni</i>                | LC                  |
|                         | <i>Tockus leucomelas</i>         | <i>Tockus leucomelas</i>              | LC                  |
|                         | <i>Tockus flavirostris</i>       | <i>Tockus flavirostris</i>            | LC                  |
|                         | <i>Tockus bradfieldi</i>         | <i>Rhynchaceros bradfieldi</i>        | LC                  |
|                         | <i>Tockus alboterminatus</i>     | <i>Rhynchaceros alboterminatus</i>    | LC                  |
|                         | <i>Tockus fasciatus</i>          | <i>Rhynchaceros fasciatus</i>         | LC                  |
|                         | <i>Tockus hemprichii</i>         | <i>Rhynchaceros hemprichii</i>        | LC                  |
|                         | <i>Tockus nasutus</i>            | <i>Rhynchaceros nasutus</i>           | LC                  |
|                         | <i>Tockus camurus</i>            | <i>Rhynchaceros camurus</i>           | LC                  |
|                         | <i>Tockus pallidirostris</i>     | <i>Rhynchaceros pallidirostris</i>    | LC                  |
| C - <i>Berenicornis</i> | <i>Bycanistes fistulator</i>     | <i>Bycanistes fistulator</i>          | LC                  |
|                         | <i>Bycanistes bucinator</i>      | <i>Bycanistes bucinator</i>           | LC                  |
|                         | <i>Bycanistes cylindricus</i>    | <i>Bycanistes cylindricus</i>         | NT                  |
|                         | <i>Bycanistes albotibialis</i>   | <i>Bycanistes albotibialis</i>        | LC                  |
|                         | <i>Bycanistes subcylindricus</i> | <i>Bycanistes subcylindricus</i>      | LC                  |
|                         | <i>Bycanistes brevis</i>         | <i>Bycanistes brevis</i>              | LC                  |
|                         | <i>Ceratogymna atrata</i>        | <i>Ceratogymna atrata</i>             | LC                  |
|                         | <i>Ceratogymna elata</i>         | <i>Ceratogymna elata</i>              | NT                  |
|                         | <i>Tropicranus hartlaubi</i>     | <i>Tropicranus hartlaubi</i>          | LC                  |
|                         | <i>Tropicranus albocristatus</i> | <i>Tropicranus albocristatus</i>      | LC                  |
|                         | <i>Berenicornis comatus</i>      | <i>Berenicornis comatus</i>           | NT                  |
| D - <i>Buceros</i>      | <i>Buceros rhinoceros</i>        | <i>Buceros rhinoceros</i>             | NT                  |
|                         | <i>Buceros bicornis</i>          | <i>Buceros bicornis</i>               | NT                  |
|                         | <i>Buceros hydrocorax</i>        | <i>Buceros hydrocorax</i>             | VU <sup>c</sup>     |
|                         |                                  | <i>Buceros mindanensis</i>            | VU <sup>c</sup>     |
|                         | <i>Rhinoplax vigil</i>           | <i>Rhinoplax vigil</i>                | NT                  |
| E - <i>Anorrhinus</i>   | <i>Anthracoceros marchei</i>     | <i>Anthracoceros marchei</i>          | VU                  |
|                         | <i>Anthracoceros albirostris</i> | <i>Anthracoceros albirostris</i>      | LC                  |



## CHAPTER 6

|                   |                                 |                                   |                 |
|-------------------|---------------------------------|-----------------------------------|-----------------|
|                   | <i>Anthracoceros coronatus</i>  | <i>Anthracoceros coronatus</i>    | NT              |
|                   | <i>Anthracoceros montani</i>    | <i>Anthracoceros montani</i>      | CR              |
|                   | <i>Anthracoceros malayanus</i>  | <i>Anthracoceros malayanus</i>    | NT              |
|                   | <i>Oryx capensis</i>            | <i>Oryx capensis</i>              | LC              |
|                   | <i>Oryx capensis</i>            | <i>Oryx capensis</i>              | LC              |
|                   | <i>Oryx capensis</i>            | <i>Oryx capensis</i>              | LC              |
|                   | <i>Anorhynchus tickelli</i>     | <i>Anorhynchus tickelli</i>       | NT              |
|                   | <i>Anorhynchus austeni</i>      | <i>Anorhynchus austeni</i>        | NT              |
|                   | <i>Anorhynchus galeritus</i>    | <i>Anorhynchus galeritus</i>      | LC              |
| F - <i>Aceros</i> | <i>Aceros nipalensis</i>        | <i>Aceros nipalensis</i>          | VU              |
|                   | <i>Rhyticeros plicatus</i>      | <i>Rhyticeros plicatus</i>        | LC              |
|                   |                                 | <i>Rhyticeros mendanae</i>        | VU <sup>c</sup> |
|                   | <i>Rhyticeros narcondami</i>    | <i>Rhyticeros narcondami</i>      | EN              |
|                   | <i>Rhyticeros undulatus</i>     | <i>Rhyticeros undulatus</i>       | LC              |
|                   | <i>Rhyticeros everetti</i>      | <i>Rhyticeros everetti</i>        | VU              |
|                   | <i>Rhyticeros subruficollis</i> | <i>Rhyticeros subruficollis</i>   | VU              |
|                   | <i>Aceros cassidix</i>          | <i>Rhyticeros cassidix</i>        | LC              |
|                   | <i>Aceros waldeni</i>           | <i>Cranobrontes waldeni</i>       | CR              |
|                   | <i>Aceros leucocephalus</i>     | <i>Cranobrontes leucocephalus</i> | NT              |
|                   | <i>Penelopides exarhatus</i>    | <i>Cranobrontes exarhatus</i>     | LC              |
|                   | <i>Aceros corrugatus</i>        | <i>Cranobrontes corrugatus</i>    | NT              |
|                   | <i>Penelopides manillae</i>     | <i>Penelopides manillae</i>       | LC              |
|                   | <i>Penelopides mindorensis</i>  | <i>Penelopides mindorensis</i>    | EN              |
|                   | <i>Penelopides affinis</i>      | <i>Penelopides affinis</i>        | LC              |
|                   |                                 | <i>Penelopides basilanicus</i>    | VU <sup>c</sup> |
|                   | <i>Penelopides samarensis</i>   | <i>Penelopides samarensis</i>     | LC              |
|                   | <i>Penelopides panini</i>       | <i>Penelopides panini</i>         | EN              |

<sup>a</sup> Taxonomic treatment recommended on the basis of new data present in this paper, to compare with recent International Ornithological Congress World Bird List version 2.11 (Gill & Donsker 2012).

<sup>b</sup> Conservation status of hornbills according to the IUCN Red List assessments: LC, Least Concern; VU, Vulnerable; EN, Endangered; CR, Critically Endangered; NT, Near-threatened; assignment to categories follows BirdLife International (2004), apart from *Bucorvus leadbeateri* which was upgraded to VU from LC (BirdLife International 2010; [www.birdlife.org/action/science/species/global\\_species\\_programme/whats\\_new.html](http://www.birdlife.org/action/science/species/global_species_programme/whats_new.html)).

<sup>c</sup> Proposed conservation status based on small effective population following elevated species status, based on IUCN Standards and Petitions Subcommittee (2010).

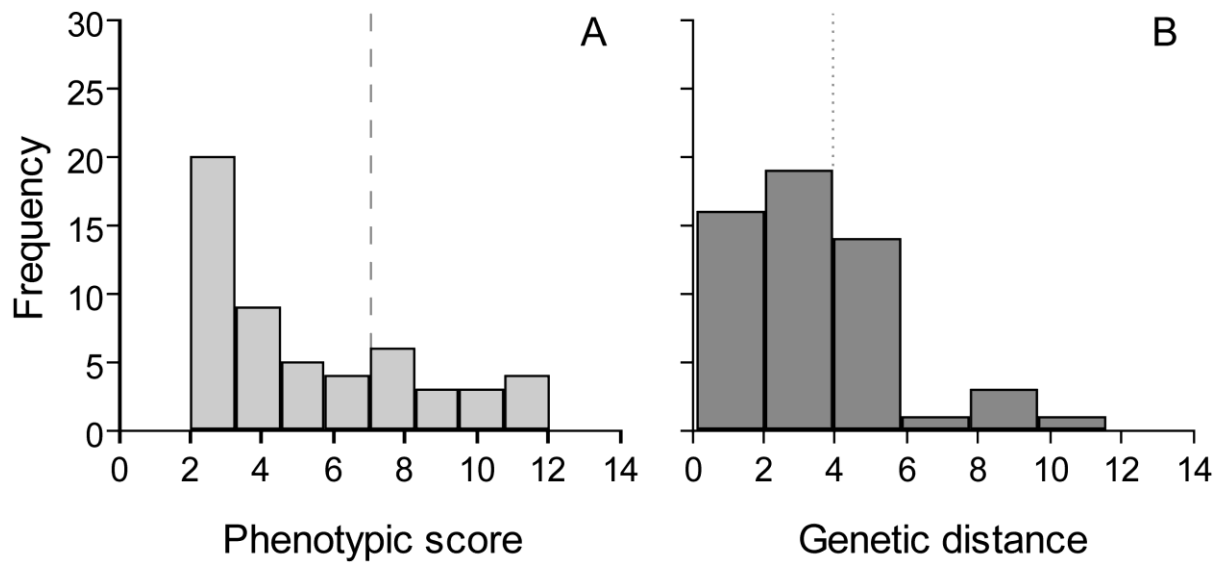


**Figure 6.1:** Frequency of phenotypic scores (A) and genetic distance (B) to show levels of differentiation between 54 hornbill pairs based on a standard scoring system (Tobias *et al.* 2010) and corrected pairwise distance analysis of mtDNA Cytb sequences. Dashed line in A indicates a fixed threshold for species level phenotypic divergence using the scoring system, and dotted line in B indicates a simple ‘threshold’ for species level genetic divergence (mtDNA coding regions: Price 2008).

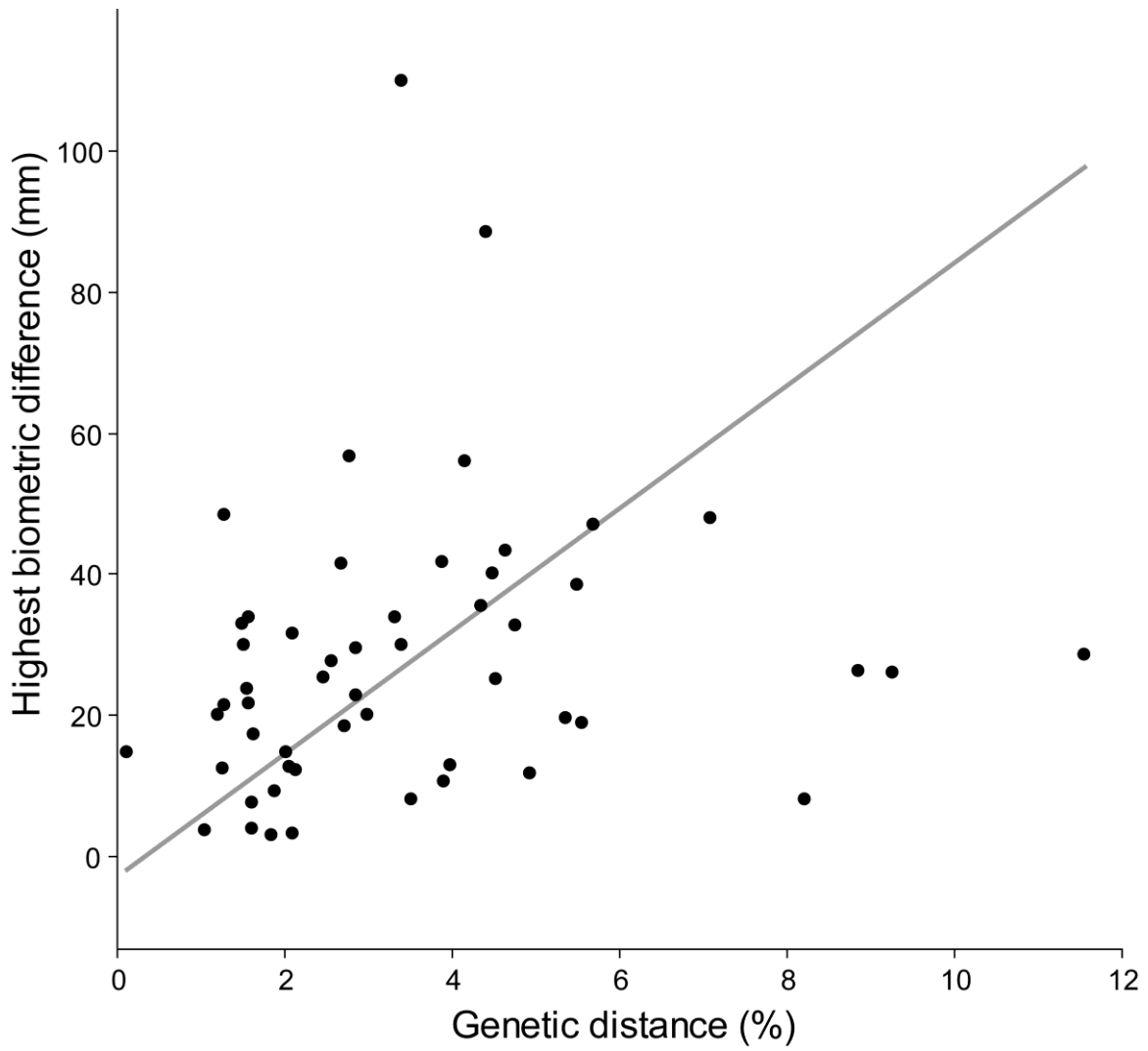


**Figure 6.2:** Linear model of highest biometric differences and genetic distance of 54 hornbill pairs to show relationship between levels of differentiation, based on the greatest difference between means of 10 morphological measurements, and corrected pairwise distances using mtDNA Cytb sequences.

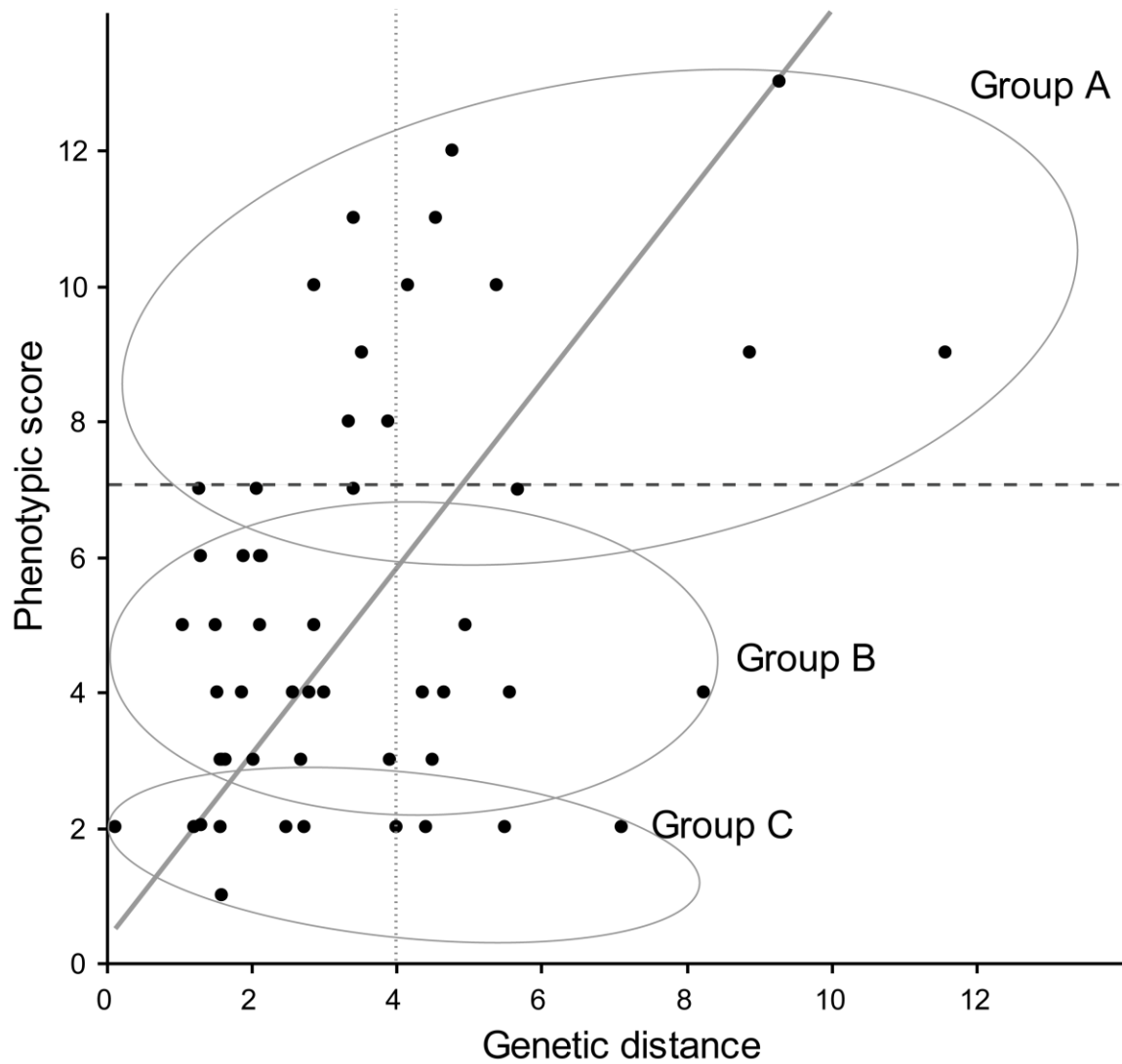


**Figure 6.3:** Linear model of phenotypic scores and genetic distance of 54 hornbill pairs to show relationship between levels of differentiation based on a standard scoring system (Tobias *et al.* 2010) and corrected pairwise distance analysis of mtDNA Cytb sequences. Dashed line indicates a fixed ‘threshold’ for species level phenotypic divergence using the scoring system, and dotted line indicates a simple ‘threshold’ for species level genetic divergence (mtDNA coding regions: Price 2008). Ellipses indicate observed taxonomic groups based on phenotypic scores, wherein group A represents species, B represents subspecies, and C represents conspecific populations within a taxon (species or subspecies).

## Supplementary Information

**Appendix A.** Suggested and supported changes for four elevated taxa of hornbills based on our analysis of phenotypic differentiation and molecular divergence using standard quantitative methods.

### ***Buceros (hydrocorax) hydrocorax* and *B. (h.) mindanensis***

Elliott (1882) and McGregor (1909-10) noted three species of *Hydrocorax* (= *Buceros*) from the Philippines, *hydrocorax* (Luzon Calao), *mindanensis* (Mindanao Calao), and *semigaleatus* (Intermediate Calao). Plumage colour did not differ, but taxa were readily distinguished by shape and colour of bill and casque. These were lumped into a single polytypic species *Buceros hydrocorax* (with 4 subspecies) by Peters (1945), which include the subspecies *basilanicus* described by Hachisuka (1934). This was adopted by Delacour & Mayr (1946), who noted *basilanicus* as doubtfully distinct. Sanft (1960) recognized this and subsumed *basilanicus* into *mindanensis*, which was followed by duPont (1971), Dickinson *et al.* (1991) and Kennedy *et al.* (2000). Kemp (2001) considered *basilanicus* as inseparable from *mindanensis*.

Separation of the form *mindanensis* and *semigaleatus* from the distinct monotypic nominate form has been suggested before (Kemp 1995, 2001), on the basis of differences in bare parts, presumed absence of bare gular skin and unique juvenile plumage. The nominate form of Rufous Hornbill was also noted to be larger than the other races, and soft part colours varied markedly between subspecies (Kennedy *et al.* 2000). Alternatively, Kemp (1995) referred to this group as Great Philippine Hornbill, but with specific regional names for each form: Great Luzon Hornbill (*B. h. hydrocorax*), Great Mindanao Hornbill (*B. h. mindanensis*), and Great Samar Hornbill (*B. h. semigaleatus*).

The form *mindanensis* differs from *hydrocorax* in having the outer/distal half of the bill whitish to pale yellow (not entirely crimson) (2, score based on Tobias *et al.*, 2010, see methods); circumorbital skin sooty black with yellow patch under eye (not completely yellow) (1); feet and legs more coral red (red-brown in nominate form) (u[1]); brownish grey claws (as opposed to

black)(u[1]); presence of black band across central tail in juvenile plumage (absent in juvenile *hydrocorax*) (3); an overall smaller bill (*hydrocorax* mean bill height  $59.2 \pm 3.01$ ,  $n=13$ ; *mindanensis*  $51.9 \pm 3.50$ ,  $n=35$ ;  $\sigma=2.20$ ) (2) and total score 9.

Molecular divergence was high between *hydrocorax* and *mindanensis* at 8.9 % mtDNA Cytb genetic distance, which is comparably high over most distance estimates. The same differences in colour of soft parts were observed between *hydrocorax* and *semigaleatus*, as above. However, the reduced casque in *semigaleatus* was more prominent (*semigaleatus* mean casque length  $133.0 \pm 11.63$ ,  $n=17$ ;  $\sigma=2.45$ ) (2), total score also 9. Molecular divergence shows a very high genetic distance between *hydrocorax* and *semigaleatus* at 11.6 %, well above the 4 % threshold for mtDNA Cytb. Our results concur with Kemp (1995, 2001), who proposes *B. hydrocorax* as a distinct monotypic species, separate from *mindanensis* and *semigaleatus* ('Southern Rufous Hornbill') given sufficient differences in colour of soft parts and distinct juvenile plumage.

A comparison of the two similar forms, *mindanensis* and *semigaleatus*, indicated no distinct difference in colour of plumage and soft parts. They also share similar juvenile plumages that were regarded distinctive from juvenile *hydrocorax* (Kemp 1995). However, they may differ to some extent in the amount of yellow and ivory in the distal part of the bill, and in the degree of yellow of the circumorbital skin below the eye, described as greenish yellow in *semigaleatus* (1) (Kennedy *et al.* 2000), which might be attributed to individual variation or amount of cosmetic colouration. Application of gamboge-yellow preen oil may cause variation in amount of yellow on pale bill and stain of yellowish wash on the white tail (McGregor 1909-1910, Delacour & Mayr 1946). They differ slightly in the bill and casque, where *mindanensis* has a longer bill (*mindanensis* bill length  $158.2 \pm 22.90$ ,  $n=35$ ; *semigaleatus*  $150.4 \pm 10.45$ ,  $n=17$ ;  $\sigma=0.40$ ) (1), but narrower casque (*mindanensis* casque width  $37.1 \pm 3.04$ ,  $n=35$ ; *semigaleatus*  $38.8 \pm 1.92$ ,  $n=17$ ;  $\sigma=-0.62$ )(1), total score 3. The only readily distinguishable difference between these two forms is the shape of the front part of the casque, which is a shallow wedge in *semigaleatus* that joins with the upper mandible in a continuous line, and not a projecting blade as in *mindanensis* (Kemp 1995, Kennedy *et al.* 2000).

The score is below the threshold for species and thus relationship is retained as subspecies, with *B. (hydrocorax) mindanensis* Tweeddale 1877 given priority over *B. (h.) semigaleatus* Tweeddale 1878. However, genetic divergence was surprisingly high between the two taxa at 8.2 %, and higher than most estimates for other recognized species. Given this high genetic divergence, we should treat *semigaleatus* as a distinct conservation unit until further evidence (e.g. vocalization, behaviour, phylogeography) can be used to assess further its taxonomic status with respect to *mindanensis*.

### ***Penelopides (affinis) basilanicus***

The Mindanao or Allied Tarictic Hornbill (*Penelopides affinis*) was first described by Tweeddale in 1877, and two closely related taxa were subsequently described by Steere in 1890, the Basilan Tarictic (*P. basilanica*) and Samar Tarictic (*P. samarensis*). Differences between *basilanica* (= *basilanicus*) and *affinis* were deemed slight, but were readily distinguishable in the specimens secured by the Bureau of Science, and the taxa thus were accepted as valid by McGregor (1909-10). On the other hand, *samarensis* was regarded as ‘a well-marked species’, though very similar to *affinis*. Steere (1890) originally described *basilanicus* as ‘much like *P. affinis* of Mindanao’, but noted the diagnostic differences to be the greater extent of rufous in the tail and the flesh-coloured base to the mandibles (McGregor 1909-10).

As in the case of *Buceros hydrocorax*, all the separate island taxa of *Penelopides* hornbills in the Philippines were lumped into a single polytypic species (*P. panini* with eight subspecies) by Peters (1945), and this arrangement was adopted by Delacour & Mayr (1946), Sanft (1960), duPont (1971), Dickinson *et al.* (1991) and Kennedy *et al.* (2000). It was Kemp (1988) who recognized separating *Penelopides* into four more closely related species, and placed *basilanicus* and *samarensis* under a polytypic species, *P. affinis*. This separation into four species was carried on by Kemp (1995, 2001) and was accepted by BirdLife International (2000, 2004), Peterson (2006) and the IOC (Gill & Donsker 2012). However, Sanft (1960) suggested that differences in *basilanicus* were of equal magnitude with the other seven subspecies, putting emphasis on the distinctive colour of the bill-casque and tail patterns.

The form *basilanicus* differs from *affinis* in having the rufous area on the tail more extensive and the black base being very minimal, thus the rufous on its tail appears less as a central band (vs rufous less extensive and black base moderate) (2); black patch at the base of upper mandible is lacking, thus exhibits a flesh patch which is heavily grooved in some specimens (black and ungrooved in *affinis*) (2); the casque and bill tip is generally flesh-coloured (not horn-coloured in *affinis*), but further comparison is needed to confirm this (u[1]); the pattern of grooves are more pronounced and ordered (less ordered in *affinis*), but further comparisons are also needed (u[1]); and though described as similar in size to *affinis*, it has a longer bill (*affinis* bill length  $78.0 \pm 5.84$ ,  $n=38$ ; *basilanicus*  $90.4 \pm 11.14$ ,  $n=3$ ;  $\sigma=2.39$ ) (2); and a narrower gape (*affinis* bill length  $30.7 \pm 2.00$ ,  $n=38$ ; *basilanicus*  $27.6 \pm 5.01$ ,  $n=3$ ;  $\sigma=-1.40$ ) (1), total score of 7. Molecular divergence was relatively low between *affinis* and *basilanicus* at 1.3%, but comparable with distances found between *affinis* and *samarensis* (2.1%).

The identity of *Penelopides samarensis* as a distinct species from *P. affinis* was validated in our results and therefore resolves the issue regarding its contested species status. Similarly, we propose elevating *basilanicus* to species status, given the reasons stated above. These three taxa forms a unique subclade within monophyletic Philippine *Penelopides*, and representing the islands of Greater Mindanao (which includes Basilan and the East Visayas), which we recognize as the *Penelopides affinis* species complex (Fig. S3). However, there are two issues that still require resolution, which is the status of *P. affinis* geo-isolates from Dinagat and Siargao islands, and those from the Zamboanga Peninsula in Western Mindanao. McGregor (1909-10) noted that specimens secured from Dinagat probably belonged to *P. samarensis*, yet our phenotypic scores and genetic analysis suggested otherwise. It appears that birds from Dinagat and Siargao islands represent an intermediate form between *samarensis* and *affinis*, which is indicated by the sparse beige feathers on the rump coupled with its geographic location. Further study is needed to determine their status. Given their phenotypic scores with *affinis* (4) and *samarensis* (6) are moderate to high, it is likely that this geo-isolate represents a distinct subspecies. By contrast, molecular divergence is low at 1.85 % and 2.13 %, respectively.



Based on phenotypic scores of both adult male and female populations of *P. affinis* from Western Mindanao and Eastern Mindanao, little to moderate differentiation has occurred between them (both scores 3). Although a threshold for separating subspecies has not been fully established (or tested), we consider a score of 3 to be a sufficient value to merit further studies on the taxonomic status of Western and Eastern Mindanao Tarictic Hornbills. Molecular divergence was substantially low to moderate at 1.62 %, but a similar distance to other taxa assigned as valid subspecies. However, this is not the only issue raised for the Western Mindanao geo-isolate of *P. affinis*, since we observed the clustering of Western Mindanao *P. affinis* with *P. (affinis) basilanicus*, thus forming a subclade. A similar case was observed for *P. panini panini* Masbate and *P. panini ticaensis*, as well as *R. narcondami* and *R. plicatus ruficollis*. Reciprocal monophyly was not achieved within the mtDNA phylogeny and often one taxon is nested within the other, which is paraphyletic (Allendorf & Luikart 2006).

A comparison between *Penelopides samarensis* and *P. basilanicus* (not shown in the tables), indicated that phenotypic scores were sufficiently divergent to separate the two as species. The total score for biometrics and plumage/soft parts was 8.0, and these didn't include a score for vocalisation, since calls were unavailable for *P. basilanicus*. The phenotypic score was based on biometrics (*d* values = score; bill -0.89 = 1; tail 2.8 = 2; total of 3) and plumage & soft parts (difference in bill colour, black versus cream = 2; difference in rump colour, pale rufous versus black = 3; total of 5).

### ***Rhyticeros (plicatus) mendanae***

Hartert (1924) first described the type of *R. (p.) mendanae* from the series of specimens kept at the Natural History Museum at Tring brought back from the Solomon islands (A.S. Meek collection), and designated a type from Guadacanal Island (No. 3065), based on a comparison with *R. p. ruficollis* skins from Waigeo and New Guinea. It was considered distinctive from the other two subspecies of *R. plicatus* by its paler colouration, being brownish-golden buff instead of chestnut. Specimens from the Northern Moluccas and New Guinea (*ruficollis*) were pale chestnut and with larger bare throat-patches, and the nominate form *plicatus* from Seram and Ambon was

true chestnut. Elliott (1882) had separated *R. undulatus* and *R. subruficollis*, and treated them as distinct species from *R. plicatus*. However, various past treatments consider these two species to be conspecific to *R. plicatus*, and Kemp (2001) acknowledges them forming a ‘Superspecies’.

Mayr (1934) later separated other populations from Northern Melanesia based on a significant series of skins collected during the Whitney South Sea Expedition, describing distinct subspecies from the Bismarck Archipelago (*R. p. dampieri*) and West Solomons Islands (*R. p. harterti*). Populations from eastern New Guinea (*R. p. jungei*) were likewise separated from *ruficollis* on the basis of size (Mayr 1937). Peters (1945) recognized these six subspecies and was followed by Sanft (1960). However, the range, size and colouration of these subspecies overlap and probably do not warrant recognition (Kemp 1988, 1995). Kemp (2001) subsumed them all into a single monotypic species, since variation between them was largely clinal, with notable overlaps in size and colour. Even *R. narcondami* has been considered conspecific to *plicatus*, as it only differs significantly in size and details of bill colour.

Putting aside existing subspecies available for *R. plicatus* and starting from a clean slate provided by Kemp (2001), we focus on the results of our mtDNA Cytb phylogeny (Fig. S1, see supplementary data; Gonzalez et al., in prep, see Chapter 5) basing our analysis on the clustering of geo-isolates of *R. plicatus* into subclades. *Rhyticeros plicatus* is paraphyletic, with the northern Melanesia clade (*mendanae*, *harterti* and *dampieri*) nested within another clade formed by North Moluccan birds, and forming sister clades with *R. narcondami* and the Seram-New Guinea clade. Taking this phylogeny into account, we compared geo-isolates based on these groups, comparing skins from Northern Melanesia with those from North Moluccas and New Guinea. However, the lack of sufficient adult male skins measured for *R. p. dampieri* from the Bismarck Archipelago prompted us to remove it from the analysis altogether and concentrate on *harterti* and *mendanae*.

The form *mendanae* differs from *plicatus* in having the bill pale yellowish white with a reddish maroon base (not dark maroon to dusky brown base) (1); head and neck pale golden-yellow to cream (not deep/dark to golden rufous) (3); bare throat patch smaller (1); and moderately longer tail (Western population, tail length  $278.5 \pm 12.65$ ,  $n=10$ ; Solomon Islands,  $236.5 \pm 17.41$ ,  $n=21$ ;  $\sigma=-2.709$ ) (2), total score 7 for plumage, bare parts and biometrics. We note a difference in

vocalization based on recordings available from Xeno-Canto Asia, with *plicatus* having harsh raucous grunts and honks, with a deep tone and resonant voice, while *mendanae* has a similar call but more raspier and trumpet-like (1), total score of 8. Our results indicate that combined birds from the Solomon islands (*mendanae* and *harterti*) differ substantially from birds from North Moluccas (*ruficollis*) and from those found in mainland New Guinea and Seram (*plicatus*, *ruficollis* and *jungei*). We propose resurrecting *R. plicatus mendanae* as a valid taxon and elevating it to species status, with priority of *mendanae* from East Solomon Islands (Hartert 1924) over closely related *harterti* (Mayr 1934) from West Solomon Islands. We also validate the subspecific difference between *R. plicatus harterti* and *R. plicatus mendanae*, given a phenotypic score of 4 and 2.99% genetic distance. Therefore, we regard *R. mendanae* as a polytypic species with *harterti* as a subspecies. The status of *R. plicatus dampieri* remains unresolved; not until sufficient skin samples become available can we make a robust analysis of phenotypic differentiation.

A comparison between *Rhyticeros (plicatus) harterti* and *R. plicatus* from New Guinea (combination of *R. plicatus ruficollis* from West Papua and *R. plicatus jungei* from East New Guinea) indicated that the difference between these two geo-isolates were sufficient to distinguish as species (analysis not shown in tables). Thus providing support to our proposal to include *harterti* in the split of *mendanae* as a distinct species. The total score for biometrics and plumage/soft parts was 7.0, and no score for vocalisation has been added since calls were unavailable for *harterti*. Phenotypic scores were based from Biometrics (*d* values = score: tail -2.64 = 2; bill 0.3 = 1; total of 3) and Plumage/soft parts (difference in bill-base colour, maroon versus dusky brown = 2; difference in head colour, golden rufous versus rufous = 2).

## CHAPTER 6

### References

- BirdLife International 2000. *Threatened Birds of the World*. Lynx Edicions and BirdLife International, Barcelona and Cambridge, UK.
- Delacour, J. & Mayr, E. 1946. *Birds of the Philippines*. The Macmillan Company, New York.
- Elliott, D.G. 1882. *A monograph of the Bucerotidae, or family of the hornbills*. Taylor and Francis, London.
- duPont, J.E. 1971. Philippine Birds. Monograph Ser 2. Delaware Museum of Natural History, Greenville, DE.
- Hachisuka, M. 1934. Notes on birds from the Philippine Islands and Borneo. *Tori* **8**: 220–222.
- Lua, H.K. and S. Nakkuntod (1998) Hornbills in the Singapore Zoological Reference Collection, pp 52–71 in: P. Poonswad, ed., *The Asian Hornbills: Ecology and Conservation*, Bangkok: National Centre for Genetic Engineering and Biotechnology.
- Mayr 1937. Birds collected during the Whitney South Sea Expedition. *American Museum Novitates* **939**: 1–25.
- McGregor, R.C.I. 1909-10. *A manual of Philippine birds*. Bureau of Printing, Manila.
- Peters, J.L. 1945. *Check-list of birds of the world*, Vol. 5. Harvard University Press, Cambridge MA.
- Steere, J.B. 1890. *A list of the Birds and Mammals collected by the Steere Expedition to the Philippines, with Localities and with brief preliminary Descriptions of supposed new Species*. Courier Printers, Ann Arbor, MI.
- Tweeddale, A. 1877. Reports on the collection of birds made during the Voyage of H.M.S. ‘Challenger’ – No. 11. On the birds of the Philippine Islands. *Proceedings of the Zoological Society of London* **1877**: 535–551.





**Figure S6.1:** Bayesian cladogram for 78 taxa of hornbills (Bucerotidae) based on mtDNA Cytb sequences (Gonzalez *et al.*, in prep, see Chapter 5). Nodes with  $\geq 70\%$  ML bootstrap values (BS) and  $\geq 90\%$  Bayesian posterior probabilities (PP) were considered moderate to strongly supported, and are indicated by black circles. Numbers on nodes indicate BS values  $< 70\%$  and PP  $< 90$ .

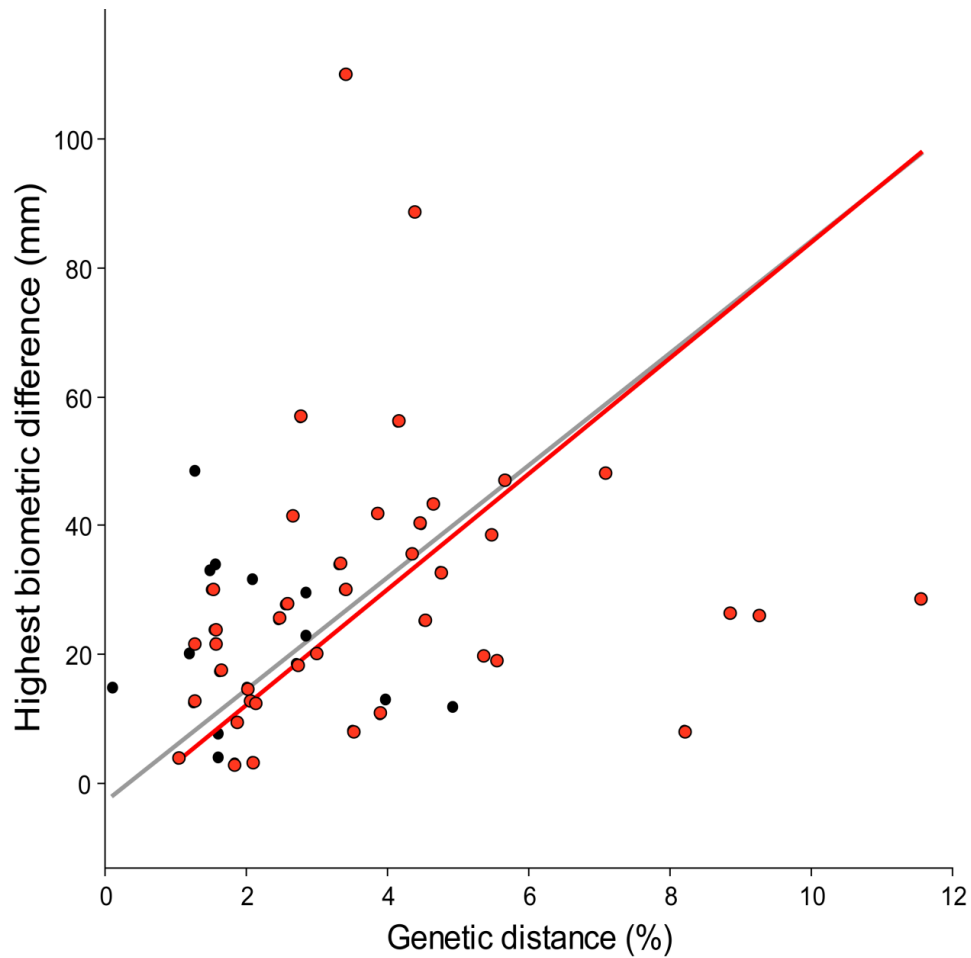


**Figure S6.2:** Comparison of linear regression models between highest biometric differences and genetic distance for 54 hornbill pairs (grey solid line), and 41 pairs with sample sizes less than six individuals excluded (red solid line). Data was based on the greatest difference between means of 10 morphological measurements, and corrected pairwise distances using mtDNA Cytb sequences. Black dots represent pairs with sample sizes  $< 6$ , and red dots represent pairs with sample sizes  $> 6$ .

## CHAPTER 6

**Table S6.1:** Morphological measurements taken from museum skins of adult hornbills based on biometrics of body, bill and casque from Lua & Nakkuntod (1998).

| Measurement (mm)                       | Details                                                                     |
|----------------------------------------|-----------------------------------------------------------------------------|
| Tarsus length <sup>a</sup>             | bend of foot to first tarsometatarsal scale                                 |
| Wing length <sup>a</sup>               | un-stretched wing chord, bend of wing to tip of longest primary             |
| Tail length <sup>a</sup>               | Tip of longest tail feather to insertion at pygostyle                       |
| Terminal band length CTF <sup>a</sup>  | Tip of central tail feather (CTF) to edge of proximal band                  |
| Subterminal band length of CTF         | Tip of central tail feather to edge of distal band                          |
| Pre-subterminal band length of CTF     | Tip of central tail feather to edge of 2 <sup>nd</sup> distal band (if any) |
| Terminal band length of OTF            | Tip of outer tail feather (OTF) to edge of proximal band                    |
| Subterminal band length of OTF         | Tip of outer tail feather to edge of distal band                            |
| Pre-subterminal band length of OTF     | Tip of outer tail feather to edge of 2 <sup>nd</sup> distal band (if any)   |
| Bill-casque length (a)                 | Bill tip to rear edge of casque                                             |
| Bill length (b) <sup>a</sup>           | Bill tip to nostril (at distal edge of nares)                               |
| Exposed bill length (c )               | Bill tip to front base of casque                                            |
| Casque length (d) <sup>a</sup>         | Front edge of casque to rear asque                                          |
| Front casque height (e)                | High point of front casque to base of casque                                |
| Bill tip to front casque (f)           | Bill tip to high point of front casque                                      |
| Gape length (g)                        | Bill tip to base of bill at cutting edge of upper bill                      |
| Bill-casque height (h)                 | Topmost edge of casque to bottom edge of lower bill                         |
| Middle casque height (h') <sup>a</sup> | Topmost edge at middle of casque to groove of nostril                       |
| Bill height (bh=h-h') <sup>a</sup>     | Difference of bill-casque height and middle casque height                   |
| Rear casque height (r)                 | Topmost edge of rear casque to base of skull                                |
| Casque width (w) <sup>a</sup>          | Width between nostrils                                                      |
| Bill width (w') <sup>a</sup>           | Width of bill at cutting edge of upper bill                                 |

<sup>a</sup> Biometrics included in the morphological analysis for calculating phenotypic scores (Table S6.4).



# CHAPTER 6

**Table S6.2:** List of hornbill skins from different museum collections examined in this study, with details of the museum registry and geographic origin.

| Museum    | Registry            | Taxa                                | Geo-isolate     | Geographic origin                       |
|-----------|---------------------|-------------------------------------|-----------------|-----------------------------------------|
| OUMNH     | B05361              | <i>Aceros corrugatus corrugatus</i> | Borneo          | Lawas, British North Borneo             |
| OUMNH     | B05362              | <i>Aceros corrugatus corrugatus</i> | Borneo          | British North Borneo                    |
| OUMNH     | B05363              | <i>Aceros corrugatus corrugatus</i> | Borneo          | Miri, Baram River, Sarawak              |
| OUMNH     | B05360              | <i>Aceros corrugatus corrugatus</i> | Borneo          | Lawas, British North Borneo             |
| NHM Tring | 88.10.30.76         | <i>Aceros corrugatus corrugatus</i> | Borneo          | Lawas River, NW Borneo                  |
| NHM Tring | 89.7.31.7           | <i>Aceros corrugatus corrugatus</i> | Borneo          | Baram Dist., Sarawak, NW Borneo         |
| NHM Tring | 1900.9.1.8          | <i>Aceros corrugatus corrugatus</i> | Borneo          | Baram Dist., Sarawak, NW Borneo         |
| NHM Tring | 78.5.3.7            | <i>Aceros corrugatus corrugatus</i> | Borneo          | Lawas River, NW Borneo                  |
| NHM Tring | 83.9.20.43          | <i>Aceros corrugatus corrugatus</i> | Borneo          | Lawas River, NW Borneo                  |
| NHM Tring | 1900.9.1.9          | <i>Aceros corrugatus corrugatus</i> | Borneo          | Baram Dist., Sarawak, NW Borneo         |
| ZRC NUS   | 3.11107             | <i>Aceros corrugatus corrugatus</i> | Borneo          | Baram Dist., Sarawak, NW Borneo         |
| ZRC NUS   | 3.11109             | <i>Aceros corrugatus corrugatus</i> | Borneo          | Borneo                                  |
| NHM Tring | 94.2.2.3            | <i>Aceros corrugatus corrugatus</i> | Borneo          | Mt Kalulonh, Sarawak 2000ft             |
| NHM Tring | 83.9.20.41          | <i>Aceros corrugatus corrugatus</i> | Borneo          | Lawas                                   |
| NHM Tring | 88.10.30.225        | <i>Aceros corrugatus rugosus</i>    | Malay Peninsula | Malacca                                 |
| NHM Tring | 87.9.1.270          | <i>Aceros corrugatus rugosus</i>    | Malay Peninsula | Malacca                                 |
| ZRC NUS   | 3.11106             | <i>Aceros corrugatus rugosus</i>    | Malay Peninsula | Malacca                                 |
| ZRC NUS   | no number           | <i>Aceros corrugatus rugosus</i>    | Malay Peninsula | Peninsular Malaysia                     |
| ZRC NUS   | 3.11175             | <i>Aceros corrugatus rugosus</i>    | Malay Peninsula | Singapore                               |
| NHM Tring | no tag, no registry | <i>Aceros corrugatus rugosus</i>    | Malay Peninsula | no tag                                  |
| ZRC NUS   | 3.11110.            | <i>Aceros corrugatus rugosus</i>    | Sumatra         | Siak                                    |
| ZRC NUS   | 3.11111             | <i>Aceros corrugatus rugosus</i>    | Sumatra         | Pulo Payong, Salap Rupert, East Sumatra |
| MSU-NSM   | 56487               | <i>Aceros leucocephalus</i>         | Dinagat/Siargao | Loreto, Dinagat Is., Surigao del Norte  |
| NHM Tring | 88.10.30.80         | <i>Aceros leucocephalus</i>         | Mindanao        | Surigao, Mindanao                       |
| NHM Tring | 88.10.30.85         | <i>Aceros leucocephalus</i>         | NE Mindanao     | Butuan, N Mindanao                      |
| UPLBMNH   | 2113                | <i>Aceros leucocephalus</i>         | NE Mindanao     | Cabadbaran, Mt Hilonghilong, Agusan     |
| NMP       | 0-7205              | <i>Aceros leucocephalus</i>         | NE Mindanao     | Mt Kapiagan, Misamis Oriental           |
| NMP       | 0-10280             | <i>Aceros leucocephalus</i>         | NE Mindanao     | Mt Kitanglad, Malaybalay, Bukidnon      |
| MSU-CSM   | C0019               | <i>Aceros leucocephalus</i>         | NE Mindanao     | Agusan del Sur                          |
| MSU-NSM   | 58345               | <i>Aceros leucocephalus</i>         | NE Mindanao     | Malibato, Opol, Misamis Oriental        |
| MSU-NSM   | 50775               | <i>Aceros leucocephalus</i>         | NE Mindanao     | Malibato, Opol, Misamis Oriental        |
| MSU-NSM   | 50776               | <i>Aceros leucocephalus</i>         | NE Mindanao     | Tu-od, Manticao, Misamis Oriental       |
| MSU-NSM   | 50771               | <i>Aceros leucocephalus</i>         | NE Mindanao     | Tu-od, Manticao, Misamis Oriental       |
| NHM Tring | 1905.11.26.238      | <i>Aceros leucocephalus</i>         | SE Mindanao     | Daliaoum, SE Mindanao                   |
| NHM Tring | 96.4.15.90          | <i>Aceros leucocephalus</i>         | SE Mindanao     | Ayala, Mindanao                         |
| NHM Tring | 1969.40.84          | <i>Aceros leucocephalus</i>         | SE Mindanao     | Piso, SE Mindanao                       |
| NHM Tring | 1969.27.188         | <i>Aceros leucocephalus</i>         | SE Mindanao     | Mt Apo, Mindanao                        |
| NHM Tring | 1969.28.48          | <i>Aceros leucocephalus</i>         | SE Mindanao     | Piso, SE Mindanao                       |
| UPLBMNH   | 2121                | <i>Aceros leucocephalus</i>         | SE Mindanao     | Datal-bukay, Mt Tuduk, Glan, Cotabato   |
| UPLBMNH   | 2116                | <i>Aceros leucocephalus</i>         | SE Mindanao     | Kibawalan, Malalag, Davao Prov          |
| UPLBMNH   | 2112                | <i>Aceros leucocephalus</i>         | SE Mindanao     | Kibawalan, Malalag, Davao Prov          |
| NMP       | 0-3354              | <i>Aceros leucocephalus</i>         | SE Mindanao     | Limot, Mati, Davao                      |

## CHAPTER 6

|           |                   |                                       |                 |                                                |
|-----------|-------------------|---------------------------------------|-----------------|------------------------------------------------|
| UPLBMNH   | 2123              | <i>Aceros leucocephalus</i>           | SE Mindanao     | Kablon, Matutum, Tupi, Cotabato                |
| UPLBMNH   | 2120              | <i>Aceros leucocephalus</i>           | SE Mindanao     | Tugal, Mt Tuduk, Glan, Cotabato                |
| NMP       | 0-4578            | <i>Aceros leucocephalus</i>           | SE Mindanao     | Cotabato                                       |
| UPLBMNH   | 2109              | <i>Aceros leucocephalus</i>           | W Mindanao      | Mt Malindang, Zamboanga del Norte              |
| UPLBMNH   | 2126              | <i>Aceros leucocephalus</i>           | W Mindanao      | Bayabao, Piagayungan Mts., Lanao del Sur       |
| UPLBMNH   | 2106              | <i>Aceros leucocephalus</i>           | W Mindanao      | Sigayan, Katipunan, Zamboanga del Norte        |
| UPLBMNH   | 2105              | <i>Aceros leucocephalus</i>           | W Mindanao      | Situbo, Katipunan, Zamboanga del Norte         |
| UPLBMNH   | 2107              | <i>Aceros leucocephalus</i>           | W Mindanao      | Masawan, Mt Malindang, Zamboanga del Norte     |
| UPLBMNH   | 2108              | <i>Aceros leucocephalus</i>           | W Mindanao      | Masawan, Mt Malindang, Zamboanga del Norte     |
| NMP       | 0-1916            | <i>Aceros leucocephalus</i>           | W Mindanao      | Siglayan, Katipunan, Zamboanga                 |
| SU-NHM    | number unrecorded | <i>Aceros leucocephalus</i>           | W Mindanao      | Sigayan, Katipunan, Zamboanga del Norte        |
| SU-NHM    | number unrecorded | <i>Aceros leucocephalus</i>           | W Mindanao      | Labao, Dabiak, Zamboanga                       |
| SU-NHM    | number unrecorded | <i>Aceros leucocephalus</i>           | W Mindanao      | Matam, Katipunan, Zamboanga                    |
| MSU-NSM   | 49272             | <i>Aceros leucocephalus</i>           | W Mindanao      | Mt Sugarloaf, Midsulip, Zamboanga del Sur      |
| MSU-NSM   | 49280             | <i>Aceros leucocephalus</i>           | W Mindanao      | Mt Sugarloaf, Midsalip, Zamboanga del Sur      |
| NHM Tring | B330.27.3.96.471  | <i>Aceros waldeni</i>                 | Negros          | Mt Canlaon, Negros                             |
| UPLBMNH   | 2103              | <i>Aceros waldeni</i>                 | Negros          | Lake Balinasayao, Negros Oriental              |
| NMP       | 0-3989            | <i>Aceros waldeni</i>                 | Negros          | Inubungan, Sta Catalina, Negros Oriental       |
| NMP       | 0-3797            | <i>Aceros waldeni</i>                 | Negros          | Naliong, Tolong, Sta Catalina, Negros Oriental |
| UPD       | 5579              | <i>Aceros waldeni</i>                 | Negros          | Lake Balinasayao, Negros Oriental              |
| NHM Tring | 90.9.9.6          | <i>Aceros waldeni</i>                 | Panay           | Concepcion, Panay                              |
| NHM Tring | 96.4.15.101       | <i>Aceros waldeni</i>                 | Panay           | Concepcion, Panay                              |
| NMP       | 0-5092            | <i>Aceros waldeni</i>                 | Panay           | Capiz, Capiz                                   |
| NHM Tring | 96.4.15.98        | <i>Aceros waldeni</i>                 | Guimaras        | Guimaras                                       |
| NHM Tring | 1904.7.24.1       | <i>Anorrhinus austeni</i>             | Northern India  | Inner hill ranges of Margherita, Upper Assam   |
| NMNH      | 263762            | <i>Anorrhinus austeni</i>             | Northern India  | Upper Assam                                    |
| NHM Tring | 80.11.3369        | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Malacca                                        |
| NHM Tring | 80.10.30.143      | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Malacca                                        |
| NHM Tring | 87.9.1.274        | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Malacca                                        |
| NHM Tring | 1906.7.23.357     | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Gunong Tahan, Malay Peninsula                  |
| NHM Tring | 80.1.1.3372       | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Malacca                                        |
| NHM Tring | 80.1.1.3232       | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Malacca                                        |
| NHM Tring | 87.9.1.170        | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Bankaoon, Malewoon, Tenasserim, Burma          |
| NHM Tring | no registry       | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Malacca                                        |
| NHM Tring | 94.2.3.35         | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Johor                                          |
| UMZC      | 25/Buc/2/a/4      | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Tahan River, Pahang                            |
| ZRC NUS   | 3.10981           | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Chong, Trang, P. Thailand                      |
| ZRC NUS   | 3.10980.          | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Khao Ram, Thailand                             |
| ZRC NUS   | 3.10986           | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | ginting Bidei, Selangor, Peninsular Malaysia   |
| ZRC NUS   | 3.10980.          | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Peninsular Malaysia                            |
| NHM Tring | no registry       | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Malacca                                        |
| ZRC NUS   | 3.10991           | <i>Anorrhinus galeritus carinatus</i> | Malay Peninsula | Pulai, Johor, Peninsular Malaysia              |
| ZRC NUS   | 3.10987           | <i>Anorrhinus galeritus galeritus</i> | Sumatra         | Penjalin I., Coast of Sumatra                  |
| ZRC NUS   | 3.11003           | <i>Anorrhinus galeritus galeritus</i> | Sumatra         | Siolah Daras, Korinchi, Sumatra                |
| NHM Tring | 88.10.30.142      | <i>Anorrhinus galeritus galeritus</i> | Sumatra         | Lampung, SE Sumatra                            |
| NHM Tring | 1920.6.29.98      | <i>Anorrhinus galeritus galeritus</i> | Sumatra         | Siolak Daras, Korinchi, Sumatra                |

## CHAPTER 6

|           |                |                                              |                 |                                     |
|-----------|----------------|----------------------------------------------|-----------------|-------------------------------------|
| NHM Tring | 58.4.27.7      | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Sumatra                             |
| NHM Tring | 82.7.24.12     | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | West coast Sumatra                  |
| NHM Tring | 1939.6.20.4    | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Lebong, Tandai, Sumatra             |
| ZRC NUS   | 3.010987       | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Penjalin I., Coast of Sumatra       |
| ZRC NUS   | 3.11004        | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Timbang Serdang, Sumatra            |
| ZRC NUS   | 3.10987        | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Penjalin I., Coast of Sumatra       |
| ZRC NUS   | 3.11003        | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Siolak Daras, Korinchi, Sumatra     |
| NHM Tring | 88.10.30.142   | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Lamong, SE Sumatra                  |
| NHM Tring | 1920.6.29.98   | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Siolak Daras, Korinchi, Sumatra     |
| NHM Tring | 58.4.27.7      | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Sumatra                             |
| NHM Tring | 82.7.24.12     | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | West coast Sumatra                  |
| NHM Tring | 1939.6.20.4    | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Lebong, Tandai, Sumatra             |
| ZRC NUS   | 3.010987       | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Penjalin I., Coast of Sumatra       |
| ZRC NUS   | 3.11004        | <i>Anorhinus galeritus galeritus</i>         | Sumatra         | Timbang Serdang, Sumatra            |
| OUMNH     | B05359         | <i>Anorhinus galeritus minor</i>             | Borneo          | Mt Salikan, Baram River, Sarawak    |
| NHM Tring | 1935.10.22.164 | <i>Anorhinus galeritus minor</i>             | Borneo          | Mt. Dulit, Sarawak                  |
| NHM Tring | 1935.10.22.172 | <i>Anorhinus galeritus minor</i>             | Borneo          | Mt. Dulit, Sarawak                  |
| NHM Tring | 1935.10.22.167 | <i>Anorhinus galeritus minor</i>             | Borneo          | Mt. Dulit, Sarawak                  |
| NHM Tring | 1935.10.22.166 | <i>Anorhinus galeritus minor</i>             | Borneo          | Mt. Dulit, Sarawak                  |
| NHM Tring | 88.10.30.141   | <i>Anorhinus galeritus minor</i>             | Borneo          | Lawas river, NW Borneo              |
| NHM Tring | 1935.10.22.165 | <i>Anorhinus galeritus minor</i>             | Borneo          | Mt. Dulit, Sarawak                  |
| NHM Tring | 78.5.3.8       | <i>Anorhinus galeritus minor</i>             | Borneo          | Lawas river, NW Borneo              |
| NHM Tring | 1927.4.18.81   | <i>Anorhinus galeritus minor</i>             | Borneo          | Gunong Sidong, Samarahan, Sarawak   |
| UMZC      | 25/Buc/2/a/7   | <i>Anorhinus galeritus minor</i>             | Borneo          | Sarawak                             |
| ZRC NUS   | 3.11001        | <i>Anorhinus galeritus minor</i>             | Borneo          | Anyut Paku, Saribas, Sarawak        |
| ZRC NUS   | 3.10993        | <i>Anorhinus galeritus minor</i>             | Borneo          | Baram, Sarawak                      |
| NHM Tring | 89.7.1.11      | <i>Anorhinus galeritus minor</i>             | Borneo          | Benkoka, Sabah                      |
| OUMNH     | B05358         | <i>Anorhinus galeritus minor</i>             | Borneo          | British North Borneo                |
| OUMNH     | B05356         | <i>Anorhinus galeritus minor</i>             | Borneo          | Lumbicdan, British North Borneo     |
| OUMNH     | B05357         | <i>Anorhinus galeritus minor</i>             | Borneo          | British North Borneo                |
| ZRC NUS   | 3.10994        | <i>Anorhinus galeritus minor</i>             | Borneo          | Baram, Sarawak                      |
| ZRC NUS   | 3.10995        | <i>Anorhinus galeritus minor</i>             | Borneo          | Baram, Sarawak                      |
| NHM Tring | 87.9.1.162     | <i>Anorhinus tickelli</i>                    | West Indo-China | Thoungyah, Brit. Burma              |
| NHM Tring | 87.9.1.158     | <i>Anorhinus tickelli</i>                    | West Indo-China | Thoungyeen river, Tenasserim, Burma |
| NHM Tring | 87.9.1.159     | <i>Anorhinus tickelli</i>                    | West Indo-China | Thoungyeen river, Tenasserim, Burma |
| NHM Tring | no registry    | <i>Anorhinus tickelli</i>                    | West Indo-China | Kau Rargit, Thorongyah, Brit. Burma |
| NHM Tring | 83.4.5.5       | <i>Anorhinus tickelli</i>                    | West Indo-China | Houmdraw valley, Tenasserim, Burma  |
| NHM Tring | 1924.12.22.201 | <i>Anorhinus tickelli</i>                    | West Indo-China | 28 miles E of Um Pang, Thailand     |
| NHM Tring | 87.9.1.160     | <i>Anorhinus tickelli</i>                    | West Indo-China | Thoungyah, Brit. Burma              |
| NHM Tring | 1915.12.21.4   | <i>Anorhinus tickelli</i>                    | West Indo-China | Sai Yoke, Thailand                  |
| NHM Tring | 1924.12.22.202 | <i>Anorhinus tickelli</i>                    | West Indo-China | 28 miles E of Um Pang, Thailand     |
| NHM Tring | 87.9.1.165     | <i>Anorhinus tickelli</i>                    | West Indo-China | Thoungyah, Brit. Burma              |
| NHM Tring | 87.9.1.169     | <i>Anorhinus tickelli</i>                    | West Indo-China | Thoungyah, Brit. Burma              |
| NMNH      | 264356         | <i>Anorhinus tickelli</i>                    | West Indo-China | Tenasserim                          |
| OUMNH     | B05577         | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Dehra Dun, Siwaliks, North India    |
| NHM Tring | 87.9.1.118     | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Dehra Dhoon                         |

## CHAPTER 6

|           |               |                                              |                 |                                              |
|-----------|---------------|----------------------------------------------|-----------------|----------------------------------------------|
| NHM Tring | 87.9.1.116    | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Dehra Dhoon                                  |
| NHM Tring | 87.9.1.114    | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Dehra Dhoon                                  |
| NHM Tring | 87.9.1.110    | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Dehra Dhoon                                  |
| NHM Tring | 87.9.1.120    | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Dehra Dhoon                                  |
| NHM Tring | 87.9.1.107    | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Dehra Dhoon                                  |
| NHM Tring | 95.7-14.693   | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Hill Tippera, Assam                          |
| NHM Tring | 95.7.14.696   | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Miril Hills, Assam                           |
| OUMNH     | B05568        | <i>Anthracoceros albirostris albirostris</i> | Northern India  | Dehra Dun, Siwaliks, North India             |
| NHM Tring | 1928.6.26.259 | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Ile de Hon-Thom, Cochin, China               |
| NHM Tring | 1928.6.26.254 | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Sambor, Cochin, China                        |
| NHM Tring | 97.9.1.49     | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Tahkamen, Siam                               |
| NHM Tring | 62.9.22.22    | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Siam                                         |
| NHM Tring | 89.5.23.18    | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Meklong, Siam                                |
| NHM Tring | 1955.1.2062   | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Lat Bua Kao E. Siam                          |
| NHM Tring | 1955.1.2058   | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Sriracha, SE Siam                            |
| NHM Tring | 1955.1.2057   | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Chantabun district, SE Siam                  |
| NHM Tring | 97.9.1.50     | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | near Kabin, Siam                             |
| NHM Tring | 87.9.1.78     | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Karope, Tavoy, Moulesin, Brit Burma          |
| ZRC NUS   | 3.11012       | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Pak Jong, SE Thailand                        |
| ZRC NUS   | 3.11013       | <i>Anthracoceros albirostris albirostris</i> | Indo-China      | Hat Sanuk, nr. Lak Island, Rajburi, Thailand |
| UMZC      | 25/Buc/3/a/9  | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | Kelantan, Malaysia                           |
| NHM Tring | 1906.7.23.356 | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | Kuala Tembeling, Pahang                      |
| NHM Tring | 1905.2.1.116  | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | Kampong Mabek, Patani                        |
| NHM Tring | 88.10.30.33   | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | Singapore                                    |
| NHM Tring | no registry   | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | Malacca                                      |
| ZRC NUS   | 3.11021       | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | S Tembeling, Pahang                          |
| ZRC NUS   | 3.11022       | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | S Tembeling, Pahang                          |
| ZRC NUS   | 3.11058       | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | Udang R. Teratau I., P. Thailand             |
| ZRC NUS   | 3.11062       | <i>Anthracoceros albirostris convexus</i>    | Malay Peninsula | Udang R. Teratau I., P. Thailand             |
| OUMNH     | B05376        | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Lawas, British North Borneo                  |
| OUMNH     | B05375        | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Labuan, British North Borneo                 |
| NHM Tring | 1901.1.31.56  | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Lawas River, N.W. Borneo                     |
| NHM Tring | 1908.12.16.52 | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Lamag, Kelengkin, NE Borneo                  |
| NHM Tring | 83.9.20.36    | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Labuan, British North Borneo                 |
| NHM Tring | 73.5.12.2429  | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Sarawak                                      |
| NHM Tring | 74.12.1.103   | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Labuan, British North Borneo                 |
| NHM Tring | 78.5.3.11     | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Lawas River, N.W. Borneo                     |
| NHM Tring | 1956.60.149   | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Kampong Meliau, N Borneo                     |
| NHM Tring | 1901.1.31.56  | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Lawas River, N.W. Borneo                     |
| ZRC NUS   | 3.11036       | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Baram, Sarawak                               |
| ZRC NUS   | 3.11037       | <i>Anthracoceros albirostris convexus</i>    | Borneo          | Baram, Sarawak                               |
| OUMNH     | B05373        | <i>Anthracoceros albirostris convexus</i>    | Sumatra         | Sumatra                                      |
| NHM Tring | 88.10.30.29   | <i>Anthracoceros albirostris convexus</i>    | Sumatra         | Piedjaunjoun                                 |
| NHM Tring | 88.10.30.27   | <i>Anthracoceros albirostris convexus</i>    | Sumatra         | Piedjaunjoun                                 |
| NHM Tring | 88.10.30.28   | <i>Anthracoceros albirostris convexus</i>    | Sumatra         | Pidjoeunjarug                                |
| NHM Tring | 73.5.12.2421  | <i>Anthracoceros albirostris convexus</i>    | Sumatra         | Sumatra                                      |

## CHAPTER 6

|           |                  |                                           |                 |                                                                       |
|-----------|------------------|-------------------------------------------|-----------------|-----------------------------------------------------------------------|
| NHM Tring | 83.2.19.6        | <i>Anthracoceros albirostris convexus</i> | Sumatra         | Rakal Lit in R. Rawas, Sumatra                                        |
| ZRC NUS   | 3.11052          | <i>Anthracoceros albirostris convexus</i> | Sumatra         | Merak I., Riau Is., Sumatra                                           |
| ZRC NUS   | 3.11048          | <i>Anthracoceros albirostris convexus</i> | Sumatra         | Singkep I., Riau Is., Sumatra                                         |
| NHM Tring | 88.10.30.26      | <i>Anthracoceros albirostris convexus</i> | Java            | Java                                                                  |
| NHM Tring | 80.1.1.1840      | <i>Anthracoceros albirostris convexus</i> | Java            | Java                                                                  |
| NHM Tring | 73.5.12.2432     | <i>Anthracoceros albirostris convexus</i> | Java            | E. Java                                                               |
| ZRC NUS   | 3.11053          | <i>Anthracoceros albirostris convexus</i> | Java            | Tamansari, Java                                                       |
| UMZC      | 25/Buc/3/a/16    | <i>Anthracoceros coronatus</i>            | Southern India  | Sultanpore, India                                                     |
| NHM Tring | 1925.12.23.154   | <i>Anthracoceros coronatus</i>            | Southern India  | Sirtsi, Kanara, Bombay                                                |
| NHM Tring | no registry      | <i>Anthracoceros coronatus</i>            | Southern India  | no tag                                                                |
| NHM Tring | 84.7.28.8        | <i>Anthracoceros coronatus</i>            | Southern India  | Muddin, Mysore                                                        |
| NHM Tring | 1925.12.23.1494  | <i>Anthracoceros coronatus</i>            | Southern India  | Kanara, Bombay Pres.                                                  |
| NHM Tring | 87.9.1.55        | <i>Anthracoceros coronatus</i>            | Southern India  | Deorukh, South Koukan                                                 |
| NHM Tring | 87.9.1.53        | <i>Anthracoceros coronatus</i>            | Southern India  | Ratuagiri, South Bombay                                               |
| NHM Tring | 87.9.1.42        | <i>Anthracoceros coronatus</i>            | Southern India  | Raipur, Central Prov                                                  |
| NHM Tring | 87.9.1.52        | <i>Anthracoceros coronatus</i>            | Central India   | Seoni, Central Province                                               |
| NHM Tring | 87.9.1.49        | <i>Anthracoceros coronatus</i>            | Central India   | Seoni, Central Province                                               |
| NHM Tring | 87.9.1.43        | <i>Anthracoceros coronatus</i>            | Central India   | Raipur, Central Province                                              |
| NHM Tring | 98.12.12.385     | <i>Anthracoceros coronatus</i>            | Central India   | Belaspur Dist, Central Prov                                           |
| NHM Tring | 87.9.1.51        | <i>Anthracoceros coronatus</i>            | Central India   | Seoni, Central Province                                               |
| NHM Tring | 87.9.1.44        | <i>Anthracoceros coronatus</i>            | Central India   | Raipur, Central Province                                              |
| NHM Tring | 1948.57.16       | <i>Anthracoceros coronatus</i>            | Sri Lanka       | Nilgala near Bibile, Uva Province 800 ft                              |
| NHM Tring | 97.12.13.1       | <i>Anthracoceros coronatus</i>            | Sri Lanka       | Trimcmalee, Ceylon                                                    |
| NHM Tring | 1949 Whl.1.16519 | <i>Anthracoceros coronatus</i>            | Sri Lanka       | Ellagalla Anicut Tissamaharama, S.P. Ceylon                           |
| NHM Tring | 88.10.30.23      | <i>Anthracoceros coronatus</i>            | Sri Lanka       | Ceylon                                                                |
| ZRC NUS   | 3.11136          | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Baram, Sarawak                                                        |
| OUMNH     | B05370           | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Baram, Sarawak                                                        |
| UMZC      | 25/Buc/3/c/1     | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Mt Dulit, Baram Dist, Sarawak, Malaysia                               |
| NHM Tring | 1927.4.18.84     | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Entawa, Samarahan, Sarawak                                            |
| NHM Tring | 1935.10.22-171   | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | R. Tinjar, Mt Dulit, Sarawak                                          |
| NHM Tring | 88.10.30.60      | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Lawas River, NW Borneo                                                |
| NHM Tring | 89.7.31.8        | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Baram, Sarawak                                                        |
| ZRC NUS   | 3.11136          | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Baram, Sarawak                                                        |
| ZRC NUS   | 3.11135          | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Kabayam, Sabah                                                        |
| NHM Tring | 1956.60.147      | <i>Anthracoceros malayanus deminutus</i>  | Borneo          | Kampong, Meliau at junction of Meliau R with Labuk R central N Borneo |
| OUMNH     | B05369           | <i>Anthracoceros malayanus malayanus</i>  | Sundaland       | Unknown                                                               |
| ZRC NUS   | 3.11134          | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Mawai Johore                                                          |
| NHM Tring | 88.10.30.70      | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Malacca                                                               |
| NHM Tring | 80.1.1.3230      | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Malacca                                                               |
| NHM Tring | 73.5.12.2427     | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Malacca                                                               |
| NHM Tring | no number        | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Wellesby Province                                                     |
| NHM Tring | no number        | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Malacca                                                               |
| NHM Tring | no number        | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Malacca                                                               |
| ZRC NUS   | 3.11066          | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Pulai, Johor                                                          |
| ZRC NUS   | 3.11067          | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Pulai, Johor                                                          |
| NHM Tring | 87.9.1.172       | <i>Anthracoceros malayanus malayanus</i>  | Malay Peninsula | Malacca                                                               |

## CHAPTER 6

|           |                |                                          |                 |                                             |
|-----------|----------------|------------------------------------------|-----------------|---------------------------------------------|
| NHM Tring | 88.10.30.65    | <i>Anthracoceros malayanus malayanus</i> | Sumatra         | Mount Faso, Sumatra                         |
| NHM Tring | 88.10.30.62    | <i>Anthracoceros malayanus malayanus</i> | Sumatra         | Siedjaenjoeng, Sumatra                      |
| NHM Tring | 1921.10.24.1   | <i>Anthracoceros malayanus malayanus</i> | Sumatra         | Siak River, Sumatra                         |
| NHM Tring | Y3.5.12.7433   | <i>Anthracoceros malayanus malayanus</i> | Sumatra         | Sumatra                                     |
| UPLBMNH   | 2129 (2131)    | <i>Anthracoceros marchei</i>             | N Palawan       | Tarabanan, Concepcion, Puerto Princesa      |
| NMP       | 0-14646        | <i>Anthracoceros marchei</i>             | N Palawan       | Dicabaitot, Linapacan Island                |
| NMP       | 0-18033        | <i>Anthracoceros marchei</i>             | N Palawan       | Langen Island, El Nido                      |
| NMP       | 0-16137        | <i>Anthracoceros marchei</i>             | N Palawan       | Langongan, Roxas                            |
| SU-NHM    | no registry    | <i>Anthracoceros marchei</i>             | N Palawan       | Babuyan, Puerto Princesa                    |
| NHM Tring | 96.4-4.24.1663 | <i>Anthracoceros marchei</i>             | S Palawan       | Taguso, Palawan                             |
| NMP       | 0-13094        | <i>Anthracoceros marchei</i>             | S Palawan       | Mt Borangbato, Brooke's Pt                  |
| NMP       | 0-4993         | <i>Anthracoceros marchei</i>             | S Palawan       | Aborlan, Palawan                            |
| NMP       | no registry    | <i>Anthracoceros marchei</i>             | Palawan         | Palawan                                     |
| NMNH      | 468428         | <i>Anthracoceros marchei</i>             | Palawan         | Palawan                                     |
| NMP       | 0-1010         | <i>Anthracoceros marchei</i>             | Calamianes      | Siuk, Culion Island                         |
| NMP       | 0-15296        | <i>Anthracoceros marchei</i>             | Calamianes      | San Pedro, Culion                           |
| NMP       | 0-6820         | <i>Anthracoceros marchei</i>             | Balabac         | Balabac, Palawan                            |
| NMP       | 0-6821         | <i>Anthracoceros marchei</i>             | Balabac         | Balabac, Palawan                            |
| NHM Tring | 1955.1.2078    | <i>Berenicomis comatus</i>               | Indo-China      | Khao Luang, Nakhon, Srithamerat, Thailand   |
| NHM Tring | 87.9.1.190     | <i>Berenicomis comatus</i>               | Indo-China      | Bankasoon, Malewon Dist., Tenasserim, Burma |
| NHM Tring | 87.9.1.161     | <i>Berenicomis comatus</i>               | Indo-China      | Bankasoon, Malewon Dist., Tenasserim, Burma |
| ZRC NUS   | 3.11080.       | <i>Berenicomis comatus</i>               | Indo-China      | Khao Ram, Thailand                          |
| NHM Tring | 87.9.1.194     | <i>Berenicomis comatus</i>               | Indo-China      | Bankasoon, Malewon Dist., Tenasserim, Burma |
| NHM Tring | 87.9.1.193     | <i>Berenicomis comatus</i>               | Indo-China      | Bankasoon, Malewon Dist., Tenasserim, Burma |
| NHM Tring | 87.9.1.195     | <i>Berenicomis comatus</i>               | Indo-China      | Bankasoon, Malewon Dist., Tenasserim, Burma |
| NHM Tring | no registry    | <i>Berenicomis comatus</i>               | Malay Peninsula | Malacca                                     |
| NHM Tring | 89.7.1.50      | <i>Berenicomis comatus</i>               | Malay Peninsula | Benkoka                                     |
| ZRC NUS   | 3.11082        | <i>Berenicomis comatus</i>               | Malay Peninsula | Pahang, Bentong, Malaysia                   |
| NHM Tring | 1927.4.18.83   | <i>Berenicomis comatus</i>               | Borneo          | Gunong Sidong, Samarahan, Sarawak           |
| NHM Tring | 1935.10.22.162 | <i>Berenicomis comatus</i>               | Borneo          | Mt. Dulit, Sarawak                          |
| NHM Tring | 1935.10.22.163 | <i>Berenicomis comatus</i>               | Borneo          | Mt. Dulit, Sarawak                          |
| ZRC NUS   | 3.11084        | <i>Berenicomis comatus</i>               | Borneo          | Gunong Sidang, Samarahan                    |
| NHM Tring | 82.7.24.12     | <i>Berenicomis comatus</i>               | Sumatra         | W. Coast, Sumatra                           |
| ZRC NUS   | 3.11122        | <i>Buceros bicornis bicornis</i>         | Malay Peninsula | Chong Trang, P. Thailand                    |
| ZRC NUS   | 3.11121        | <i>Buceros bicornis bicornis</i>         | Malay Peninsula | Klong Tun Sai, Phuket, Thailand             |
| ZRC NUS   | 3.11132        | <i>Buceros bicornis bicornis</i>         | Sumatra         | Sumatra                                     |
| NHM Tring | y3.3.12.2635   | <i>Buceros bicornis bicornis</i>         | Sumatra         | Sumatra                                     |
| NHM Tring | 80.1.1.1836    | <i>Buceros bicornis bicornis</i>         | Sumatra         | Sumatra                                     |
| NHM Tring | no registry    | <i>Buceros bicornis bicornis</i>         | Malay Peninsula | Malacca                                     |
| ZRC NUS   | 3.11091        | <i>Buceros bicornis bicornis</i>         | Malay Peninsula | Cheras, Selangor                            |
| OUMNH     | B14077         | <i>Buceros bicornis homrai</i>           | Northern India  | North India                                 |
| OUMNH     | B14076         | <i>Buceros bicornis homrai</i>           | Northern India  | North India                                 |
| UMZC      | 25/Buc/5/a/5   | <i>Buceros bicornis homrai</i>           | Northern India  | Darjeeling                                  |
| NHM Tring | 87.9.1.10      | <i>Buceros bicornis homrai</i>           | Northern India  | Sikhim                                      |
| NHM Tring | 95.7-10.1      | <i>Buceros bicornis homrai</i>           | Northern India  | Bhutan, 1500 ft                             |
| NHM Tring | 87.9.1.15      | <i>Buceros bicornis homrai</i>           | Northern India  | Darjeeling                                  |

## CHAPTER 6

|           |                |                                       |                |                                                     |
|-----------|----------------|---------------------------------------|----------------|-----------------------------------------------------|
| NHM Tring | 87.9.1.18      | <i>Buceros bicornis homrai</i>        | Northern India | Darjeeling                                          |
| NHM Tring | 87.9.1.6       | <i>Buceros bicornis homrai</i>        | Northern India | Sikhim                                              |
| NHM Tring | 87.9.1.16      | <i>Buceros bicornis homrai</i>        | Northern India | Dilkoushah, Cochar, NE India                        |
| NHM Tring | 88.10.30.213   | <i>Buceros bicornis homrai</i>        | Northern India | N Khasia Hills                                      |
| NHM Tring | 88.10.30.212   | <i>Buceros bicornis homrai</i>        | Northern India | Naga Hills, India                                   |
| NHM Tring | 87.9.1.4       | <i>Buceros bicornis homrai</i>        | Northern India | Native Sikhim                                       |
| NHM Tring | 87.9.1.7       | <i>Buceros bicornis homrai</i>        | Northern India | Sikhim                                              |
| NHM Tring | 87.9.1.67      | <i>Buceros bicornis homrai</i>        | Southern India | Ratuagiri (Deuzukh)                                 |
| NHM Tring | 87.9.1.20      | <i>Buceros bicornis homrai</i>        | Southern India | Mynall, S. Travancori, India                        |
| NHM Tring | 1915.10.14.11  | <i>Buceros bicornis homrai</i>        | Indo-China     | Koh Kha J, SE Siam                                  |
| NHM Tring | 1924.12.22.203 | <i>Buceros bicornis homrai</i>        | Indo-China     | 25 miles SW of Kempenpes, in Um Pang, Siam          |
| NHM Tring | 88.10.30.217   | <i>Buceros bicornis homrai</i>        | Indo-China     | Mong, W. Tonghoo                                    |
| NHM Tring | 1955.1.2067    | <i>Buceros bicornis homrai</i>        | Indo-China     | Nong Khor near Sriracha, SE Siam                    |
| NHM Tring | 1955.1.2068    | <i>Buceros bicornis homrai</i>        | Indo-China     | Nong Khor near Sriracha, SE Siam                    |
| NHM Tring | 1955.1.2066    | <i>Buceros bicornis homrai</i>        | Indo-China     | Koh Chang, off SE Siam                              |
| NHM Tring | 1927.6.5.314   | <i>Buceros bicornis homrai</i>        | Indo-China     | Djiring, S. Annam (Vietnam)                         |
| NHM Tring | 87.9.1.26      | <i>Buceros bicornis homrai</i>        | Indo-China     | Bankasoon, Maliwoon, Tenasserim, B. Burma           |
| NHM Tring | 87.9.1.22      | <i>Buceros bicornis homrai</i>        | Indo-China     | Pahpoon, Salivean Dist, Tenasserim Prov, Brit Burma |
| NHM Tring | 88.10.30.9     | <i>Buceros hydrocorax basilanicus</i> | Basilan        | Basilan                                             |
| NMNH      | 191463         | <i>Buceros hydrocorax basilanicus</i> | Basilan        | Isabella                                            |
| NMNH      | 200800         | <i>Buceros hydrocorax basilanicus</i> | Basilan        | Basilan                                             |
| NMNH      | 315019         | <i>Buceros hydrocorax basilanicus</i> | Basilan        | Isabella                                            |
| NHM Tring | 88.10.30.9     | <i>Buceros hydrocorax basilanicus</i> | Basilan        | Basilan                                             |
| NMNH      | 191463         | <i>Buceros hydrocorax basilanicus</i> | Basilan        | Isabella                                            |
| UPLBMNH   | 2132           | <i>Buceros hydrocorax hydrocorax</i>  | North Luzon    | Sawa, Mt Cetaceo, Sierra Madre, Cagayan             |
| NMP       | 0-7698         | <i>Buceros hydrocorax hydrocorax</i>  | North Luzon    | Bo. Dimalasud, San Mariano, Isabela                 |
| ERDB      | no tag         | <i>Buceros hydrocorax hydrocorax</i>  | North Luzon    | Callao Caves National Park                          |
| ERDB      | no tag         | <i>Buceros hydrocorax hydrocorax</i>  | North Luzon    | Callao Caves National Park                          |
| NHM Tring | 96.4.15.113    | <i>Buceros hydrocorax hydrocorax</i>  | Central Luzon  | Near Manilla                                        |
| NHM Tring | 88.10.30.4     | <i>Buceros hydrocorax hydrocorax</i>  | Central Luzon  | Monte Alban, Rizal Prov., Luzon                     |
| NHM Tring | 88.10.30.1     | <i>Buceros hydrocorax hydrocorax</i>  | Central Luzon  | Bulacan Prov., Luzon (Bilukan)                      |
| NHM Tring | 88.10.30.5     | <i>Buceros hydrocorax hydrocorax</i>  | Central Luzon  | Monte Alban, Rizal Prov., Luzon                     |
| UPD       | 43             | <i>Buceros hydrocorax hydrocorax</i>  | Central Luzon  | Mt Sampaloc, Tanay, Rizal                           |
| UPLBMNH   | 2133           | <i>Buceros hydrocorax hydrocorax</i>  | South Luzon    | Mt Bulusan, Bulusan, Sorsogon, Luzon                |
| UPLBMNH   | 2381           | <i>Buceros hydrocorax hydrocorax</i>  | South Luzon    | San Pascual, Gonzaga                                |
| UPD       | 343            | <i>Buceros hydrocorax hydrocorax</i>  | Marinduque     | Naampias, Nangka, Torrijos                          |
| NHM Tring | 88.10.30.7     | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Surigao, Mindanao                                   |
| NHM Tring | 88.10.30.6     | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Surigao, Mindanao                                   |
| NHM Tring | 88.10.30.11    | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Butuan, Mindanao                                    |
| UPLBMNH   | 2070           | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Cabadbaran, Mt Hilonghilong, Agusan                 |
| UPLBMNH   | 2160           | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Siwod, Mt Hilonghilong, Agusan Prov                 |
| UPLBMNH   | 2161           | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Siwod, Mt Hilonghilong, Agusan Prov                 |
| NMP       | 0-7201         | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Mt Kapiagan, Misamis Oriental                       |
| SU-NHM    | 3860           | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Mt Hilong-hilong                                    |
| MSU-NSM   | 50787          | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Mahayahay, Mainit, Iligan City, Lanao del Norte     |
| NMP       | 0-11117        | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao    | Musuan, Bukidnon                                    |

## CHAPTER 6

|           |                      |                                       |                 |                                                    |
|-----------|----------------------|---------------------------------------|-----------------|----------------------------------------------------|
| UPLBMNH   | 2171                 | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Tugal, Mt Tudok, Glan, Cotabato                    |
| UPLBMNH   | 2168                 | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Balisong, Mt Matutum, Tupi, Cotabato               |
| UPLBMNH   | 2163                 | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Kibawalan, Malalag, Davao                          |
| NMP       | 0-544                | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Mt Apo, SE Mindanao                                |
| NMP       | 0-3351               | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Tauantauan, Mati, Davao                            |
| NMP       | 0-543                | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | E slope of Mt McKinley, Davao                      |
| NMP       | 0-1096               | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Todaya, Mt Apo                                     |
| NMP       | 0-3350               | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Tauantauan, Mati, Davao                            |
| UPLBMNH   | 2173                 | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Datal-Bucay, Mt Tudok, Glan, Cotabato              |
| UPLBMNH   | 2172                 | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Datal-Bucay, Mt Tudok, Glan, Cotabato              |
| NMP       | 0-4575               | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Cotabato, Mindanao                                 |
| NHM Tring | 88.10.30.13          | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Zamboanga, Mindanao                                |
| UPLBMNH   | 2148                 | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Diway, Dabiak, Zamboanga del Norte                 |
| UPD       | 43                   | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Mt Matutum, Cotabato                               |
| MSU-NSM   | 58346                | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Mahayhay, Mainit, Iligan City, Lanao del Norte     |
| UPLBMNH   | 2143 A               | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Loreto, Dinagat Is., Surigao del Norte             |
| MSU-CSM   | C0015                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Loreto, Surigao del Norte                          |
| MSU-NSM   | 56498                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Kambinlio, Loreto, Dinagat, Surigao del Norte      |
| MSU-NSM   | 56495                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Balitbiton, Loreto, Dinagat Is., Surigao del Norte |
| MSU-NSM   | 56494                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Plaridel, Albor, Dinagat Is., Surigao del Norte    |
| MSU-NSM   | no number            | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Plaridel, Albor, Dinagat Is., Surigao del Norte    |
| MSU-NSM   | 56499                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Plaridel, Albor, Dinagat Is., Surigao del Norte    |
| MSU-NSM   | 56491                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Kambinlio, Loreto, Dinagat, Surigao del Norte      |
| NHM Tring | 88.10.30.10          | <i>Buceros hydrocorax mindanensis</i> | Basilan         | Basilan                                            |
| NHM Tring | 96.4.15.111          | <i>Buceros hydrocorax mindanensis</i> | Basilan         | Basilan                                            |
| NHM Tring | 80.11.18.36          | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Pasonanca, Zamboanga, Mindanao                     |
| NMP       | 0-4576               | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Cotabato, Mindanao                                 |
| SU-NHM    | number unrecorded    | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Sigayan, Katipunan, Zamboanga                      |
| MSU-NSM   | 53287                | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Mt. Piayagayungan, Lanao del Sur                   |
| MSU-NSM   | 49298                | <i>Buceros hydrocorax mindanensis</i> | W Mindanao      | Mt Sugarloaf, Midsalip, Zamboanga del Sur          |
| NHM Tring | 88.10.30.8           | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao     | Surigao, Mindanao                                  |
| NMP       | 0-850?               | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao     | Misamis                                            |
| MSU-NSM   | no number            | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao     | Diatagon, Lianga, Surigao del Sur                  |
| MSU-NSM   | 50791                | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao     | Tu-od, Manticao, Misamis Oriental                  |
| MSU-NSM   | 50790                | <i>Buceros hydrocorax mindanensis</i> | NE Mindanao     | Tagbalogo, Na-awan, Misamis Oriental               |
| NHM Tring | 1905.11.26.237       | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Davao, SE Mindanao                                 |
| NHM Tring | 1969.40.85           | <i>Buceros hydrocorax mindanensis</i> | Mindanao        | Davao, SE Mindanao                                 |
| NHM Tring | 1905.11.26.153 (148) | <i>Buceros hydrocorax mindanensis</i> | Mindanao        | Mt Apo, SE Mindanao                                |
| NHM Tring | 1969.28.49           | <i>Buceros hydrocorax mindanensis</i> | Mindanao        | Davao, SE Mindanao                                 |
| UPLBMNH   | 2169                 | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Kibawalan, Malalag, Davao                          |
| MGR       | no registry          | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Sarangani Province                                 |
| MGR       | no registry          | <i>Buceros hydrocorax mindanensis</i> | SE Mindanao     | Sarangani Province                                 |
| MSU-NSM   | 56501                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Kambinlio, Loreto, Dinagat, Surigao del Norte      |
| MSU-NSM   | 56503                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Plaridel, Albor, Dinagat Is., Surigao del Norte    |
| MSU-NSM   | 56500                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Loreto, Dinagat Is., Surigao del Norte             |
| MSU-NSM   | 56504                | <i>Buceros hydrocorax mindanensis</i> | Dinagat/Siargao | Plaridel, Albor, Dinagat Is., Surigao del Norte    |



## CHAPTER 6

|           |                   |                                        |                 |                                                                                             |
|-----------|-------------------|----------------------------------------|-----------------|---------------------------------------------------------------------------------------------|
| MSU-NSM   | 56490             | <i>Buceros hydrocorax mindanensis</i>  | Dinagat/Siargao | Kambinlio, Loreto, Dinagat, Surigao del Norte                                               |
| NHM Tring | 96.4.15.103       | <i>Buceros hydrocorax semigaleatus</i> | Samar           | Catbalogan, Samar                                                                           |
| UPLBMNH   | 2147              | <i>Buceros hydrocorax semigaleatus</i> | Samar           | Matuginao, Samar                                                                            |
| NMP       | 0-11119           | <i>Buceros hydrocorax semigaleatus</i> | Samar           | Buluan, Calbiga, Northern Samar                                                             |
| NMP       | 0-11121           | <i>Buceros hydrocorax semigaleatus</i> | Samar           | Buluan, Calbiga, Northern Samar                                                             |
| NMP       | 0-11120           | <i>Buceros hydrocorax semigaleatus</i> | Samar           | Buluan, Calbiga, Northern Samar                                                             |
| NMP       | 0-2123            | <i>Buceros hydrocorax semigaleatus</i> | Samar           | Oras, Samar                                                                                 |
| NMP       | not tag           | <i>Buceros hydrocorax semigaleatus</i> | Samar           | Tipawala, Llorente, Samar                                                                   |
| NMP       | 0-11123           | <i>Buceros hydrocorax semigaleatus</i> | Samar           | San Rafael, Taft, Samar                                                                     |
| NHM Tring | 88.10.30.18       | <i>Buceros hydrocorax semigaleatus</i> | Leyte           | South Leyte                                                                                 |
| NHM Tring | 78.5.20.86        | <i>Buceros hydrocorax semigaleatus</i> | Leyte           | South Leyte                                                                                 |
| NHM Tring | 88.10.30.17       | <i>Buceros hydrocorax semigaleatus</i> | Leyte           | South Leyte                                                                                 |
| UPLBMNH   | 2141              | <i>Buceros hydrocorax semigaleatus</i> | Leyte           | Paniniklan, Mt Kabolantian, Mahaplag, Leyte                                                 |
| UPLBMNH   | 2136              | <i>Buceros hydrocorax semigaleatus</i> | Bohol           | Duao, Sierra Bullones, Bohol                                                                |
| UPLBMNH   | 2135              | <i>Buceros hydrocorax semigaleatus</i> | Bohol           | Duao, Sierra Bullones, Bohol                                                                |
| UPLBMNH   | 2134              | <i>Buceros hydrocorax semigaleatus</i> | Bohol           | Cantab, Sierra Bullones, Bohol                                                              |
| SU-NHM    | number unrecorded | <i>Buceros hydrocorax semigaleatus</i> | Bohol           | Cantab, Sierra Bullones, Bohol                                                              |
| NHM Tring | 88.10.30.19       | <i>Buceros hydrocorax semigaleatus</i> | Panaon          | Panaon                                                                                      |
| UMZC      | 25/Buc/5/c/6      | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Mt Kalulong, Sarawak                                                                        |
| UMZC      | 25/Buc/5/c/9      | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | no specific loc                                                                             |
| NHM Tring | 1956.60.150       | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Primary forests near Kampong Meliau junction<br>Meliau River with Labuk River, North Borneo |
| NHM Tring | 1956.60.615       | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Mt Magdalena NE Tawau, SE N Borneo                                                          |
| NHM Tring | 1969.32.17        | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Mt Mulu, Sarawak, Borneo                                                                    |
| NHM Tring | 1935.10.22.170    | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Mt Dulit, 3600 ft, Sarawak                                                                  |
| NHM Tring | 88.10.30.210      | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Lawas River, North Borneo                                                                   |
| NHM Tring | 83.9.20.42        | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Lawas River, North Borneo                                                                   |
| NHM Tring | 1935.10.22.169    | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Mt Dulit, 1400 ft, Sarawak                                                                  |
| NHM Tring | 1935.10.22.177    | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Mt Dulit, Baram District Sarawak                                                            |
| NHM Tring | 1900.9.1.15       | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Baram District Sarawak                                                                      |
| UMZC      | 25/Buc/5/c/8      | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Mt Dulit?                                                                                   |
| UMZC      | 25/Buc/5/c/2      | <i>Buceros rhinoceros borneoensis</i>  | Borneo          | Mt Dulit?                                                                                   |
| UMZC      | 25/Buc/5/a/2      | <i>Buceros rhinoceros rhinoceros</i>   | Malay Peninsula | Bisarat in Jalor                                                                            |
| NHM Tring | no registry       | <i>Buceros rhinoceros rhinoceros</i>   | Malay Peninsula | Malacca                                                                                     |
| NHM Tring | 87.9.1.268        | <i>Buceros rhinoceros rhinoceros</i>   | Malay Peninsula | Johore, Malaysia                                                                            |
| NHM Tring | 88.10.30.209      | <i>Buceros rhinoceros rhinoceros</i>   | Malay Peninsula | Malacca                                                                                     |
| NHM Tring | MC 2178           | <i>Buceros rhinoceros rhinoceros</i>   | Malay Peninsula | Kuala Lumpar, Selangor                                                                      |
| OUMNH     | B05379            | <i>Buceros rhinoceros rhinoceros</i>   | Sundaland       | Sunda                                                                                       |
| OUMNH     | B05381            | <i>Buceros rhinoceros rhinoceros</i>   | Sundaland       | Sunda                                                                                       |
| NHM Tring | 88.10.30.207      | <i>Buceros rhinoceros rhinoceros</i>   | Sumatra         | Sidjawaajacug, Sumatra                                                                      |
| UMZC      | 25/Buc/5/c/3      | <i>Buceros rhinoceros rhinoceros</i>   | Sumatra         | Sumatra                                                                                     |
| NHM Tring | 82.2.19.7         | <i>Buceros rhinoceros rhinoceros</i>   | Sumatra         | Mocara Mengkoe lem, Rawas River, Sumatra                                                    |
| NHM Tring | 83.2.19.8         | <i>Buceros rhinoceros rhinoceros</i>   | Sumatra         | Taudjong Ning, Sumatra 800 ft                                                               |
| NHM Tring | 73.5.12.2443      | <i>Buceros rhinoceros rhinoceros</i>   | Sumatra         | Sumatra                                                                                     |
| NHM Tring | 88.10.30.205      | <i>Buceros rhinoceros rhinoceros</i>   | Sumatra         | Sidjoeuajorug, Sumatra                                                                      |
| NHM Tring | 88.10.30.208      | <i>Buceros rhinoceros rhinoceros</i>   | Sumatra         | Mt Sago, Sumatra                                                                            |
| NHM Tring | 80.1.1.1844       | <i>Buceros rhinoceros rhinoceros</i>   | Sumatra         | Sumatra                                                                                     |

## CHAPTER 6

|           |                   |                                      |                |                                                 |
|-----------|-------------------|--------------------------------------|----------------|-------------------------------------------------|
| NHM Tring | 88.10.30.206      | <i>Buceros rhinoceros rhinoceros</i> | Sumatra        | Sumatra                                         |
| NHM Tring | y3.5.12.2436      | <i>Buceros rhinoceros sylvestris</i> | Java           | East Java                                       |
| NHM Tring | 97.1-3.1          | <i>Buceros rhinoceros sylvestris</i> | Java           | Java, Indonesia                                 |
| NHM Tring | 57.6.10.8         | <i>Buceros rhinoceros sylvestris</i> | Java           | Captive from HongKong                           |
| NHM Tring | no registry       | <i>Buceros rhinoceros sylvestris</i> | Java           | Indian archipelago                              |
| NHM Tring | 82.3.24.13        | <i>Oryceros gingalensis</i>          | Sri Lanka      | MP Galla, Sri Lanka                             |
| NHM Tring | 1928.6.25.14      | <i>Oryceros gingalensis</i>          | Sri Lanka      | Tellula, Sri Lanka                              |
| NHM Tring | 87.9.1.183        | <i>Oryceros gingalensis</i>          | Sri Lanka      | Pullam N.P., Sri Lanka                          |
| NHM Tring | 1955.3.261        | <i>Oryceros gingalensis</i>          | Sri Lanka      | Medawachchiya, Sri Lanka                        |
| NHM Tring | 1940.12.3.158     | <i>Oryceros gingalensis</i>          | Sri Lanka      | Kalauwa, N.C.P., Sri Lanka                      |
| NHM Tring | 1916.9.20.537     | <i>Oryceros gingalensis</i>          | Sri Lanka      | Cocoawatte, Sri Lanka                           |
| NHM Tring | 87.9.1.184        | <i>Oryceros gingalensis</i>          | Sri Lanka      | Pullam N.P., Sri Lanka                          |
| NHM Tring | 1924.11.10.11     | <i>Oryceros gingalensis</i>          | Sri Lanka      | Rasagalla, Balangoda, Ratnapura, Sri Lanka      |
| NHM Tring | 1946.28.251       | <i>Oryceros gingalensis</i>          | Sri Lanka      | Illapaikande, near Colombo, Sri Lanka           |
| NHM Tring | 1940.12.3.161     | <i>Oryceros gingalensis</i>          | Sri Lanka      | Nilgala Dist, Uva, Sri Lanka                    |
| NHM Tring | 1925.12.23.1408   | <i>Oryceros griseus</i>              | Western India  | Siddapur, Kanara, India                         |
| NHM Tring | 1916.9.26.7       | <i>Oryceros griseus</i>              | Western India  | Oneichardi esate, Travancore, India             |
| NHM Tring | 81.5.1.4037       | <i>Oryceros griseus</i>              | Western India  | no locality                                     |
| NHM Tring | 87.9.1.177        | <i>Oryceros griseus</i>              | Western India  | Khandala, India                                 |
| NHM Tring | 243.a             | <i>Oryceros griseus</i>              | Western India  | Malabar                                         |
| NHM Tring | 1925.12.23.1407   | <i>Oryceros griseus</i>              | Western India  | Kumta, N. Kanara, India                         |
| NHM Tring | 87.9.1.180        | <i>Oryceros griseus</i>              | Western India  | Bramagherries, India                            |
| NHM Tring | 87.9.1.179        | <i>Oryceros griseus</i>              | Western India  | Wynaad                                          |
| NHM Tring | 87.9.1.181        | <i>Oryceros griseus</i>              | Western India  | Bramagherries, India                            |
| NHM Tring | 87.9.1.182        | <i>Oryceros griseus</i>              | Western India  | Mynall, India                                   |
| NHM Tring | 88.10.30.194      | <i>Oryceros griseus</i>              | Western India  | Khandala, India                                 |
| NHM Tring | 1925.12.23.151    | <i>Oryceros griseus</i>              | Western India  | Hebbati, Sirsi, Kanara, India                   |
| UMZC      | 25/Buc/12/i/1     | <i>Oryceros griseus</i>              | Southern India | Madras                                          |
| NHM Tring | 88.10.30.110      | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Butuan, Mindanao                                |
| UPLBMNH   | 2075              | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Cabadbaran, Mt Hilonghilong, Agusan             |
| UPLBMNH   | 2089              | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Camp Dunque, Tu-od, Manticao, Misamis Oriental  |
| UPLBMNH   | 2079              | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Cabadbaran, Mt Hilonghilong, Agusan             |
| UPLBMNH   | 2078              | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Cabadbaran, Mt Hilonghilong, Agusan             |
| NMP       | 0-3262            | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Mt Hilong-hilong, Cabadbaran, Agusan            |
| NMP       | 0-6635            | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Anahan, Gingoog City, Misamis Oriental          |
| MSU-CSM   | C0011-B           | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Mahayahay, Mainit, Iligan City, Lanao del Norte |
| MSU-NSM   | 50742             | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Tu-od, Manticao, Misamis Oriental               |
| SU-NHM    | number unrecorded | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Olango Spring, Lanao                            |
| MSU-NSM   | 53258             | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Saronayan, Lumba-Bayabao, Lanao del Sur         |
| MSU-NSM   | 50754             | <i>Penelopides affinis affinis</i>   | NE Mindanao    | Mahayahay, Mainit, Iligan City, Lanao del Norte |
| NHM Tring | 1969.28.47        | <i>Penelopides affinis affinis</i>   | SE Mindanao    | Piso, Mindanao                                  |
| NHM Tring | 1969.28.45        | <i>Penelopides affinis affinis</i>   | SE Mindanao    | Davao, SE Mindanao                              |
| NHM Tring | 96.4.15.82        | <i>Penelopides affinis affinis</i>   | SE Mindanao    | Ayala, Mindanao                                 |
| NHM Tring | 1905.11.26.152    | <i>Penelopides affinis affinis</i>   | SE Mindanao    | Piso, Mindanao                                  |
| NHM Tring | 1969.28.44        | <i>Penelopides affinis affinis</i>   | SE Mindanao    | Davao, SE Mindanao                              |
| UPLBMNH   | 1996              | <i>Penelopides affinis affinis</i>   | SE Mindanao    | Kibawalan, Malalag, Davao                       |

## CHAPTER 6

|           |                |                                    |                 |                                                   |
|-----------|----------------|------------------------------------|-----------------|---------------------------------------------------|
| UPLBMNH   | 2084           | <i>Penelopides affinis affinis</i> | SE Mindanao     | Kibawalan, Malalag, Davao                         |
| UPLBMNH   | 2081           | <i>Penelopides affinis affinis</i> | SE Mindanao     | Talagutong, Malita, Davao Prov.                   |
| UPLBMNH   | 2088           | <i>Penelopides affinis affinis</i> | SE Mindanao     | Datal-Bucay, Mt Tuduk, Glan, Cotabato             |
| UPLBMNH   | 2083           | <i>Penelopides affinis affinis</i> | SE Mindanao     | Balisong, Mt Matutum, Tupi, Cotabato              |
| UPLBMNH   | 2087           | <i>Penelopides affinis affinis</i> | SE Mindanao     | Datal-Bucay, Mt Tuduk, Glan, Cotabato             |
| UPLBMNH   | 2086           | <i>Penelopides affinis affinis</i> | SE Mindanao     | Datal-Bucay, Mt Tuduk, Glan, Cotabato             |
| NMP       | 0-4577         | <i>Penelopides affinis affinis</i> | SE Mindanao     | Labay Buayan, Cotabato                            |
| NMP       | 0-13428        | <i>Penelopides affinis affinis</i> | SE Mindanao     | Mt Matutum, Tupi, Cotabato                        |
| NMP       | 13425          | <i>Penelopides affinis affinis</i> | SE Mindanao     | Mt Matutum, Tupi, Cotabato                        |
| MGR       | no registry    | <i>Penelopides affinis affinis</i> | SE Mindanao     | Davao, SE Mindanao                                |
| NHM Tring | 88.10.30.107   | <i>Penelopides affinis affinis</i> | W Mindanao      | Zamboanga, Mindanao                               |
| MSU-CSM   | 322            | <i>Penelopides affinis affinis</i> | W Mindanao      | Oroquieta City, Misamis Occidental                |
| NHM Tring | 1977.16.39     | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Kambinliw, Loreto, Surigao del Norte              |
| NHM Tring | 88.10.30.118   | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Dinagat                                           |
| UPLBMNH   | 2090           | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Osmena, Dapa, Surigao del Norte, Siargao Is.      |
| MSU-CSM   | 0267B          | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Kambinlio, Loreto, Surigao del Norte              |
| MSU-CSM   | 0267A          | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Kambinlio, Loreto, Surigao del Norte              |
| MSU-NSM   | 56473          | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Balitbiton, Loreto, Dinagat Is, Surigao del Norte |
| MSU-NSM   | 57436          | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Esperanza, Loreto, Surigao del Norte              |
| MSU-NSM   | 57441          | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Loreto, Dinagat Is., Surigao del Norte            |
| MSU-NSM   | 57440          | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Loreto, Surigao del Norte                         |
| MSU-NSM   | 57438          | <i>Penelopides affinis affinis</i> | Dinagat/Siargao | Loreto, Dinagat Is., Surigao del Norte            |
| NMP       | 0-6636         | <i>Penelopides affinis affinis</i> | NE Mindanao     | Anahan, Gingoog City, Misamis Oriental            |
| NHM Tring | 88.10.30.154   | <i>Penelopides affinis affinis</i> | NE Mindanao     | Surigao, Mindanao                                 |
| NHM Tring | no registry    | <i>Penelopides affinis affinis</i> | NE Mindanao     | Butuan, Mindanao                                  |
| UPLBMNH   | 2098           | <i>Penelopides affinis affinis</i> | NE Mindanao     | Cabadbaran, Mt Hilonghilong, Agusan               |
| UPLBMNH   | 2102           | <i>Penelopides affinis affinis</i> | NE Mindanao     | Tu-od, Manticao, Misamis Oriental                 |
| UPLBMNH   | 2092           | <i>Penelopides affinis affinis</i> | NE Mindanao     | Cabadbaran, Mt Hilonghilong, Agusan               |
| UPLBMNH   | 2094           | <i>Penelopides affinis affinis</i> | NE Mindanao     | Cabadbaran, Mt Hilonghilong, Agusan               |
| UPLBMNH   | 2099           | <i>Penelopides affinis affinis</i> | NE Mindanao     | Cabadbaran, Mt Hilonghilong, Agusan               |
| UPLBMNH   | 2095           | <i>Penelopides affinis affinis</i> | NE Mindanao     | Cabadbaran, Mt Hilonghilong, Agusan               |
| NMP       | 0-7202         | <i>Penelopides affinis affinis</i> | NE Mindanao     | Mt Kapiagan, Misamis Oriental                     |
| MSU-NSM   | 53261          | <i>Penelopides affinis affinis</i> | NE Mindanao     | Saronayan, Lumba-Bayabao, Lanao del Sur           |
| MSU-NSM   | 53262          | <i>Penelopides affinis affinis</i> | NE Mindanao     | Saronayan, Lumba-Bayabao, Lanao del Sur           |
| MSU-NSM   | 53263          | <i>Penelopides affinis affinis</i> | NE Mindanao     | Saronayan, Lumba-Bayabao, Lanao del Sur           |
| MSU-NSM   | 50760          | <i>Penelopides affinis affinis</i> | NE Mindanao     | Mahayahay, Mainit, Iligan City, Lanao del Norte   |
| NHM Tring | 1905.11.26.151 | <i>Penelopides affinis affinis</i> | SE Mindanao     | Davao, SE Mindanao                                |
| NHM Tring | 1969.28.46     | <i>Penelopides affinis affinis</i> | SE Mindanao     | Davao, SE Mindanao                                |
| NHM Tring | 96.4.15.84     | <i>Penelopides affinis affinis</i> | SE Mindanao     | Ayala, Mindanao                                   |
| UPLBMNH   | 2101           | <i>Penelopides affinis affinis</i> | SE Mindanao     | Kibawalan, Malalag, Davao                         |
| NMP       | 0-548          | <i>Penelopides affinis affinis</i> | SE Mindanao     | E slope Mt McKinley, Davao                        |
| NMP       | 0-554          | <i>Penelopides affinis affinis</i> | SE Mindanao     | Calian, Davao                                     |
| NMP       | 0-551          | <i>Penelopides affinis affinis</i> | SE Mindanao     | Todaya, Mt Apo, Davao                             |
| NMP       | 0-3352         | <i>Penelopides affinis affinis</i> | SE Mindanao     | Limot, Mati, Davao                                |
| UPLBMNH   | 2100           | <i>Penelopides affinis affinis</i> | SE Mindanao     | Datal-Bucay, Mt Tuduk, Glan, Cotabato             |
| NMP       | 0-552          | <i>Penelopides affinis affinis</i> | SE Mindanao     | Baronootan, Upi, Cotabato                         |

## CHAPTER 6

|           |                 |                                        |                 |                                                   |
|-----------|-----------------|----------------------------------------|-----------------|---------------------------------------------------|
| NMP       | 0-5812          | <i>Penelopides affinis affinis</i>     | SE Mindanao     | Kabakaw, Cotabato                                 |
| NMP       | 0-13429         | <i>Penelopides affinis affinis</i>     | SE Mindanao     | Mt Matutum, Tupi, Cotabato                        |
| NHM Tring | 88.10.30.155    | <i>Penelopides affinis affinis</i>     | W Mindanao      | Zamboanga, Mindanao                               |
| UPLBMNH   | 2091            | <i>Penelopides affinis affinis</i>     | W Mindanao      | Labao, Diabak, Zamboanga                          |
| MSU-NSM   | 49250           | <i>Penelopides affinis affinis</i>     | W Mindanao      | Mt Sugarloaf, Midsalip, Zamboanga del Sur         |
| MSU-NSM   | 49254           | <i>Penelopides affinis affinis</i>     | W Mindanao      | Mt Sugarloaf, Midsilap, Zamboanga del Sur         |
| NHM Tring | 88.10.30.153    | <i>Penelopides affinis affinis</i>     | Dinagat/Siargao | Dinagat                                           |
| MSU-NSM   | 56474           | <i>Penelopides affinis affinis</i>     | Dinagat/Siargao | Kambinlio, Loreto, Surigao del Norte              |
| MSU-CSM   | 151             | <i>Penelopides affinis affinis</i>     | Dinagat/Siargao | Dapa, Siargao, Surigao del Norte                  |
| MSU-NSM   | 56923           | <i>Penelopides affinis affinis</i>     | Dinagat/Siargao | Maasin, Pilar, Siargao Is., Surigao del Norte     |
| MSU-NSM   | no number       | <i>Penelopides affinis affinis</i>     | Dinagat/Siargao | Maasin, Pilar, Siargao Is., Surigao del Norte     |
| MSU-NSM   | 56477           | <i>Penelopides affinis affinis</i>     | Dinagat/Siargao | Lorete, Dinagat Is., Surigao del Norte            |
| MSU-NSM   | 57456           | <i>Penelopides affinis affinis</i>     | Dinagat/Siargao | Kambinlio, Loreto, Surigao del Norte              |
| MSU-NSM   | 56483           | <i>Penelopides affinis affinis</i>     | Dinagat/Siargao | Balitbiton, Loreto, Dinagat Is, Surigao del Norte |
| NHM Tring | 99.5.22.13      | <i>Penelopides affinis basilanica</i>  | Basilan         | Isabela Basilan                                   |
| NHM Tring | 88.10.30.113    | <i>Penelopides affinis basilanica</i>  | Basilan         | Basilan                                           |
| NMNH      | 315024          | <i>Penelopides affinis basilanica</i>  | Basilan         | Isabella                                          |
| NHM Tring | 88.10.30.146    | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Menado, Sulawesi                                  |
| NHM Tring | 46.7.16.27      | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NHM Tring | 43.2.23.10      | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NMNH      | 250842          | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NMNH      | 250841          | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NMNH      | 250209          | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NMNH      | 112658          | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NMNH      | 249199          | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NMNH      | 249741          | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NMNH      | 250843          | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NMNH      | 250845          | <i>Penelopides exarhatus exarhatus</i> | North Sulawesi  | Sulawesi                                          |
| NHM Tring | 58.12.2.87      | <i>Penelopides exarhatus sandfordi</i> | South Sulawesi  | Makassar, Sulawesi                                |
| UMZC      | 25/Buc/9/a/2    | <i>Penelopides exarhatus sandfordi</i> | South Sulawesi  | Makassar, Sulawesi                                |
| NHM Tring | 1939.12.9.3479  | <i>Penelopides exarhatus sandfordi</i> | South Sulawesi  | Sulawesi (with grooves)                           |
| NHM Tring | 58.12.2.87      | <i>Penelopides exarhatus sandfordi</i> | South Sulawesi  | Makassar, Sulawesi                                |
| UMZC      | 25/Buc/9/a/2    | <i>Penelopides exarhatus sandfordi</i> | South Sulawesi  | Makassar, Sulawesi                                |
| NHM Tring | 1939.12.9.3479  | <i>Penelopides exarhatus sandfordi</i> | South Sulawesi  | Sulawesi (with grooves)                           |
| NHM Tring | 332.97.5.13.118 | <i>Penelopides manillae manillae</i>   | N Luzon         | La Trinidad, Benguet, NW Luzon                    |
| UPLBMNH   | 2056            | <i>Penelopides manillae manillae</i>   | N Luzon         | Balaoi, Pagudpud, Ilocos Norte                    |
| NMP       | 0-160           | <i>Penelopides manillae manillae</i>   | N Luzon         | Massisiat, Abra                                   |
| NMP       | 0-7699          | <i>Penelopides manillae manillae</i>   | N Luzon         | San Mariano, Dibuhan, Isabela, Sierra Madre       |
| NMP       | 0-7700          | <i>Penelopides manillae manillae</i>   | N Luzon         | San Mariano, Isabela, Sierra Madre Mtns           |
| NMP       | 0-3490          | <i>Penelopides manillae manillae</i>   | N Luzon         | Mt Pinatubo, Villar, Zambales                     |
| NMP       | 0-159           | <i>Penelopides manillae manillae</i>   | N Luzon         | Massisiat, Abra                                   |
| NMP       | 0-158           | <i>Penelopides manillae manillae</i>   | N Luzon         | Massisiat, Abra                                   |
| SU-NHM    | no number       | <i>Penelopides manillae manillae</i>   | N Luzon         | Mt Cagua, Sierra Madre, Cagayan Prov.             |
| NSMNP     | wild            | <i>Penelopides manillae manillae</i>   | N Luzon         | Dikatuktukan, Alomanay, Palanan, Isabela          |
| NSMNP     | wild            | <i>Penelopides manillae manillae</i>   | N Luzon         | Dikangriyan, Centro East, Palanan, Isabela        |
| NHM Tring | 88.10.30.89     | <i>Penelopides manillae manillae</i>   | Central Luzon   | Near Manila                                       |

## CHAPTER 6

|           |             |                                      |               |                                                |
|-----------|-------------|--------------------------------------|---------------|------------------------------------------------|
| NHM Tring | 97.5.13.18  | <i>Penelopides manillae manillae</i> | Central Luzon | Mt Arayat, Pampanga, C Luzon                   |
| NHM Tring | 88.10.30.96 | <i>Penelopides manillae manillae</i> | Central Luzon | Monte Alban, Rizal, C Luzon                    |
| NHM Tring | 96.4.15.74  | <i>Penelopides manillae manillae</i> | Central Luzon | Near Manilla                                   |
| NMP       | 0-15363     | <i>Penelopides manillae manillae</i> | Central Luzon | Jalajala, Rizal                                |
| NMP       | 0-6335      | <i>Penelopides manillae manillae</i> | Central Luzon | Palacio, Balian, Pangil, Laguna                |
| NMP       | 0-8321      | <i>Penelopides manillae manillae</i> | Central Luzon | Dumagat, Pangil, Laguna                        |
| NMP       | 0-6392      | <i>Penelopides manillae manillae</i> | Central Luzon | Sitio Deman, Barrio Balian, Pangil, Laguna     |
| UPLBMNH   | 2057        | <i>Penelopides manillae manillae</i> | S Luzon       | Mt Isarog, Curry, Pili, Camarines Sur          |
| UPLBMNH   | 2080 A      | <i>Penelopides manillae manillae</i> | S Luzon       | Tugas, Matnog, Sorsogon                        |
| NMP       | 0-11870     | <i>Penelopides manillae manillae</i> | S Luzon       | Bo. San Pedro, Pang., Camarines Norte          |
| SU-NHM    | no number   | <i>Penelopides manillae manillae</i> | S Luzon       | Tugas, Matnog, Sorsogon                        |
| SU-NHM    | no number   | <i>Penelopides manillae manillae</i> | S Luzon       | Tugas, Matnog, Sorsogon                        |
| UPD       | 1502        | <i>Penelopides manillae manillae</i> | S Luzon       | Bicol National Park, Sooc, Lupi, Camarines Sur |
| UPD       | 1577        | <i>Penelopides manillae manillae</i> | S Luzon       | Bulusan National Park, Bulusan, Sorsogon       |
| NHM Tring | 96.4.15.77  | <i>Penelopides manillae manillae</i> | Marinduque    | Marinduque                                     |
| UPD       | 341         | <i>Penelopides manillae manillae</i> | Marinduque    | Mulawin, Dampulan, Torrijos                    |
| UPD       | 334         | <i>Penelopides manillae manillae</i> | Marinduque    | Labo, Sta Cruz                                 |
| UPD       | 336         | <i>Penelopides manillae manillae</i> | Marinduque    | Labo, Sta Cruz                                 |
| UPD       | 338         | <i>Penelopides manillae manillae</i> | Marinduque    | Makolapnit, Sta Cruz                           |
| UPD       | 333         | <i>Penelopides manillae manillae</i> | Marinduque    | Labo, Sta Cruz                                 |
| UPD       | 335         | <i>Penelopides manillae manillae</i> | Marinduque    | Labo, Sta Cruz                                 |
| NMP       | 14239       | <i>Penelopides manillae manillae</i> | Marinduque    | Sihi, Buenavista, Marinduque                   |
| NMP       | 0-14898     | <i>Penelopides manillae manillae</i> | Marinduque    | Sitio Tawang, Magalakot, Sta Cruz              |
| NMP       | 0-10541     | <i>Penelopides manillae manillae</i> | Catanduanes   | Kongkoyame, Caramoran, Catanduanes             |
| NMP       | 0-16582     | <i>Penelopides manillae manillae</i> | Catanduanes   | Camp 4, Catanduanes                            |
| NMP       | 0-10539     | <i>Penelopides manillae manillae</i> | Catanduanes   | Duguitoo, Virac, Catanduanes                   |
| NMP       | 0-16599     | <i>Penelopides manillae manillae</i> | Catanduanes   | Basecamp, Catanduanes                          |
| NMP       | 0-10540     | <i>Penelopides manillae manillae</i> | Catanduanes   | Duguitoo, Virac, Catanduanes                   |
| UPD       | PSO-0398    | <i>Penelopides manillae subnigra</i> | Polillo       | Polillo Island                                 |
| UPD       | PSO-0403    | <i>Penelopides manillae subnigra</i> | Polillo       | Polillo Island                                 |
| UPD       | PSO-0400    | <i>Penelopides manillae subnigra</i> | Polillo       | Polillo Island                                 |
| NMP       | 0-5292      | <i>Penelopides manillae subnigra</i> | Polillo       | Anibawan, Polillo Island                       |
| NMP       | 0-5294      | <i>Penelopides manillae subnigra</i> | Polillo       | Anibawan, Polillo Island                       |
| NMP       | 0-5297      | <i>Penelopides manillae subnigra</i> | Polillo       | Anibawan, Polillo Island                       |
| NMP       | 0-5295      | <i>Penelopides manillae subnigra</i> | Polillo       | Anibawan, Polillo Island                       |
| NMP       | 0-5291      | <i>Penelopides manillae subnigra</i> | Polillo       | Anibawan, Polillo Island                       |
| NMP       | 0-5298      | <i>Penelopides manillae subnigra</i> | Polillo       | Anibawan, Polillo Island                       |
| NHM Tring | 90.12.1.95  | <i>Penelopides mindorensis</i>       | E Mindoro     | Mindoro (mislablled Basilan)                   |
| NHM Tring | 88.10.30.95 | <i>Penelopides mindorensis</i>       | E Mindoro     | North Mindoro                                  |
| NHM Tring | 2969.29.125 | <i>Penelopides mindorensis</i>       | E Mindoro     | North Mindoro                                  |
| NHM Tring | 1969.29.122 | <i>Penelopides mindorensis</i>       | E Mindoro     | North Mindoro                                  |
| NHM Tring | 95.6.13.3   | <i>Penelopides mindorensis</i>       | E Mindoro     | North Mindoro                                  |
| NHM Tring | 1969.29.123 | <i>Penelopides mindorensis</i>       | E Mindoro     | North Mindoro                                  |
| NHM Tring | 1969.29.124 | <i>Penelopides mindorensis</i>       | E Mindoro     | North Mindoro                                  |
| NHM Tring | 95.6.13.2   | <i>Penelopides mindorensis</i>       | E Mindoro     | North Mindoro                                  |
| UPD       | 34          | <i>Penelopides mindorensis</i>       | E Mindoro     | Alcate, Victoria                               |

## CHAPTER 6

|           |                   |                                     |           |                                                |
|-----------|-------------------|-------------------------------------|-----------|------------------------------------------------|
| UPD       | 7386              | <i>Penelopides mindorensis</i>      | E Mindoro | Big Tabinay, Puerto Galera, Mindoro Oriental   |
| UPD       | PSO-0402          | <i>Penelopides mindorensis</i>      | E Mindoro | Big Tabinay, Puerto Galera, Mindoro Oriental   |
| NMP       | 0-1261            | <i>Penelopides mindorensis</i>      | E Mindoro | Bolinan, Naujan, Oriental Mindoro              |
| NMP       | 0-1264            | <i>Penelopides mindorensis</i>      | E Mindoro | Bolinan, Naujan, Oriental Mindoro              |
| NMP       | 0-9599            | <i>Penelopides mindorensis</i>      | W Mindoro | Ygmanukan, Paluan, Occidental Mindoro          |
| NMP       | 0-9601            | <i>Penelopides mindorensis</i>      | W Mindoro | Ygmanukan, Paluan, Occidental Mindoro          |
| NMP       | 0-9600            | <i>Penelopides mindorensis</i>      | W Mindoro | Ygmanukan, Paluan, Occidental Mindoro          |
| NMP       | 0-9605            | <i>Penelopides mindorensis</i>      | W Mindoro | Tilago, Sta Cruz, Occidental Mindoro           |
| NMP       | 0-3848            | <i>Penelopides mindorensis</i>      | W Mindoro | Abra de Ilog, Occidental Mindoro               |
| NMP       | 0-9602            | <i>Penelopides mindorensis</i>      | W Mindoro | W slope of Mt Calavite, Occidental Mindoro     |
| NMP       | 0-9598            | <i>Penelopides mindorensis</i>      | W Mindoro | W slope of Mt Calavite, Occidental Mindoro     |
| NMP       | 0-9597            | <i>Penelopides mindorensis</i>      | W Mindoro | W slope of Mt Calavite, Occidental Mindoro     |
| NHM Tring | B272.97.5.13.472  | <i>Penelopides panini panini</i>    | Negros    | Mt. Canlaon, Negros                            |
| NHM Tring | 96.4.15.64        | <i>Penelopides panini panini</i>    | Negros    | San Antonio, Negros                            |
| UPLBMNH   | 2063              | <i>Penelopides panini panini</i>    | Negros    | Sta Catalina, Bayawan, Negros Oriental         |
| UPLBMNH   | 2067              | <i>Penelopides panini panini</i>    | Negros    | Balanán, Siaton, Negros Oriental               |
| NMP       | 0-3247            | <i>Penelopides panini panini</i>    | Negros    | Inubangan, Tolong, Negros Oriental             |
| NMP       | 0-3988            | <i>Penelopides panini panini</i>    | Negros    | Inubangan, Sta Catalina, Negros Oriental       |
| SU-NHM    | number unrecorded | <i>Penelopides panini panini</i>    | Negros    | Balanán, Siaton, Negros Oriental               |
| SU-NHM    | number unrecorded | <i>Penelopides panini panini</i>    | Negros    | Paniabonan, Bais (Mabinay), Negros Oriental    |
| SU-NHM    | number unrecorded | <i>Penelopides panini panini</i>    | Negros    | Pamo-at, Amio, Negros                          |
| SU-NHM    | number unrecorded | <i>Penelopides panini panini</i>    | Negros    | Naliong, Sta Catalina, Negros Oriental         |
| SU-NHM    | number unrecorded | <i>Penelopides panini panini</i>    | Negros    | Balanán, Siaton, Negros Oriental               |
| SU-NHM    | number unrecorded | <i>Penelopides panini panini</i>    | Negros    | Balangbang, Tolong, Negros Oriental            |
| ERDB      | FZL 05            | <i>Penelopides panini panini</i>    | Negros    | ILCO Hinobaan, Negros Occidental               |
| NHM Tring | 96.4.15.67        | <i>Penelopides panini panini</i>    | Guimaras  | Guimaras                                       |
| NHM Tring | 88.10.30.99       | <i>Penelopides panini panini</i>    | Guimaras  | Guimaras                                       |
| NHM Tring | 88.10.30.100      | <i>Penelopides panini panini</i>    | Guimaras  | Guimaras                                       |
| UMZC      | 25/Buc/9/b/1      | <i>Penelopides panini panini</i>    | Guimaras  | Guimaras                                       |
| NHM Tring | 96.4.15.70        | <i>Penelopides panini panini</i>    | Masbate   | Masbate                                        |
| NMP       | 0-12802           | <i>Penelopides panini panini</i>    | Masbate   | Sitio Caiipi, Danao, San Jacinto               |
| NMNH      | 161143            | <i>Penelopides panini panini</i>    | Masbate   | Masbate                                        |
| NMNH      | 161144            | <i>Penelopides panini panini</i>    | Masbate   | Masbate                                        |
| NMNH      | 211390            | <i>Penelopides panini panini</i>    | Masbate   | Dumurug Point                                  |
| NMNH      | 211391            | <i>Penelopides panini panini</i>    | Masbate   | Dumurug Point                                  |
| NMNH      | 211392            | <i>Penelopides panini panini</i>    | Masbate   | Dumurug Point                                  |
| NMNH      | 211393            | <i>Penelopides panini panini</i>    | Masbate   | Dumurug Point                                  |
| DMNH      | 11381             | <i>Penelopides panini ticaensis</i> | Ticao     | San Jacinto, Ticao, Masbate, 200-400 ft.       |
| DMNH      | 11382             | <i>Penelopides panini ticaensis</i> | Ticao     | Sitio Calpi Danao, San Jacinto, Ticao, Masbate |
| NHM Tring | B673.97.5.13.492  | <i>Penelopides samarensis</i>       | Samar     | Paranas, Samar                                 |
| NHM Tring | 90.12.1.92        | <i>Penelopides samarensis</i>       | Samar     | Samar                                          |
| NMP       | 0-65-2124         | <i>Penelopides samarensis</i>       | Samar     | Sitio Cadapnan, Barrio Bantayan, Oras          |
| NMP       | 0-339-2126        | <i>Penelopides samarensis</i>       | Samar     | Tagaslian, Borongan, Samar                     |
| NMP       | 0-12255           | <i>Penelopides samarensis</i>       | Samar     | Casarawagan, Buswood, Basey                    |
| NMP       | 0-583             | <i>Penelopides samarensis</i>       | Samar     | Mt Nabaluto, Borak, Samar                      |
| NMP       | 0-12242           | <i>Penelopides samarensis</i>       | Samar     | Bulusan, Calbiga, Samar                        |

## CHAPTER 6

|           |                   |                                        |                 |                                             |
|-----------|-------------------|----------------------------------------|-----------------|---------------------------------------------|
| NMP       | 0-12229           | <i>Penelopides samarensis</i>          | Samar           | Bulusan, Calbiga, Samar                     |
| NMP       | 0-4020            | <i>Penelopides samarensis</i>          | Samar           | Baros Matuginao, Samar                      |
| NHM Tring | 88.10.30.114      | <i>Penelopides samarensis</i>          | Leyte           | Southern Leyte                              |
| NHM Tring | 88.10.30.115      | <i>Penelopides samarensis</i>          | Leyte           | Southern Leyte                              |
| NHM Tring | 88.10.30.105      | <i>Penelopides samarensis</i>          | Leyte           | Amparo, Leyte                               |
| UPLBMNH   | 2071              | <i>Penelopides samarensis</i>          | Leyte           | Paniniklan, Mt Kabalantian, Mahaplag, Leyte |
| NMP       | 0-16410           | <i>Penelopides samarensis</i>          | Leyte           | Mt Pangasungan, Visca, Baybay               |
| NMP       | 0-13563           | <i>Penelopides samarensis</i>          | Leyte           | Balinsasayao, Abuyog, Leyte                 |
| NMP       | 0-13565           | <i>Penelopides samarensis</i>          | Leyte           | Balinsasayao, Abuyog, Leyte                 |
| UPLBMNH   | 2127 A            | <i>Penelopides samarensis</i>          | Bohol           | Cantaub, Sierra Bullones, Bohol             |
| SU-NHM    | number unrecorded | <i>Penelopides samarensis</i>          | Bohol           | Sandayong, Sierra Bullones                  |
| SU-NHM    | number unrecorded | <i>Penelopides samarensis</i>          | Bohol           | Sandayong, Sierra Bullones                  |
| SU-NHM    | number unrecorded | <i>Penelopides samarensis</i>          | Bohol           | Sandayong, Sierra Bullones                  |
| SU-NHM    | number unrecorded | <i>Penelopides samarensis</i>          | Bohol           | Sandayong, Sierra Bullones                  |
| ERDB      | FZL 02            | <i>Penelopides samarensis</i>          | Bohol           | Bohol                                       |
| NHM       | 87.9.1.266        | <i>Rhinoplax vigil</i>                 | Indo-China      | Malewoon, Tenasserim, British Burma         |
| ZRC NUS   | 3.1114            | <i>Rhinoplax vigil</i>                 | Malay Peninsula | Kuala Leku, Tahan R. Pahang                 |
| NHM Tring | 1936.4.12.864     | <i>Rhinoplax vigil</i>                 | Malay Peninsula | Chang Kat, Mentri Perak, Malaysia           |
| NHM Tring | 88.10.30.204      | <i>Rhinoplax vigil</i>                 | Malay Peninsula | Malacca                                     |
| NHM Tring | 82.1.20.1423      | <i>Rhinoplax vigil</i>                 | Indo-China      | Malewoon, Tenasserim, Burma                 |
| NHM Tring | 87.9.1.272        | <i>Rhinoplax vigil</i>                 | Malay Peninsula | Malacca                                     |
| OUMNH     | B05388            | <i>Rhinoplax vigil</i>                 | Borneo          | Lawas, British North Borneo                 |
| OUMNH     | B05389            | <i>Rhinoplax vigil</i>                 | Borneo          | Lawas, British North Borneo                 |
| OUMNH     | B05387            | <i>Rhinoplax vigil</i>                 | Borneo          | Lawas, British North Borneo                 |
| ZRC NUS   | 3.11142           | <i>Rhinoplax vigil</i>                 | Borneo          | Gunong Poe, Sarawak                         |
| NHM Tring | no tag            | <i>Rhinoplax vigil</i>                 | Borneo          | Borneo drawer                               |
| NHM Tring | 1956.60.154       | <i>Rhinoplax vigil</i>                 | Borneo          | Merutai river, Mt Magdalena, North Borneo   |
| NHM Tring | no registry       | <i>Rhinoplax vigil</i>                 | Borneo          | Lamag, N Borneo                             |
| NHM Tring | 1956.60.153       | <i>Rhinoplax vigil</i>                 | Borneo          | Mt Magdalena, North Borneo                  |
| NHM Tring | 78.5.3.1          | <i>Rhinoplax vigil</i>                 | Borneo          | Sarawak, Lawas river, North Borneo          |
| NHM Tring | 1935.10.22.176    | <i>Rhinoplax vigil</i>                 | Borneo          | Mt Dulit, Sarawak, N Borneo                 |
| ZRC NUS   | 3.11143           | <i>Rhinoplax vigil</i>                 | Sumatra         | Timbang, Serdang                            |
| ZRC NUS   | 3.11145           | <i>Rhinoplax vigil</i>                 | Sumatra         | Sumatra                                     |
| NMNH      | 159448            | <i>Rhinoplax vigil</i>                 | Indo-China      | Lower Siam                                  |
| NMNH      | 182806            | <i>Rhinoplax vigil</i>                 | Malay Peninsula | Sungai Pelawan, Pahang                      |
| NMNH      | 15271             | <i>Rhinoplax vigil</i>                 | Malay Peninsula | Malacca                                     |
| NMNH      | 181699            | <i>Rhinoplax vigil</i>                 | Borneo          | E Borneo                                    |
| NMNH      | 180966            | <i>Rhinoplax vigil</i>                 | Sumatra         | W Sumatra                                   |
| NMNH      | 181249            | <i>Rhinoplax vigil</i>                 | Sumatra         | E Sumatra                                   |
| NMNH      | 181104            | <i>Rhinoplax vigil</i>                 | Sumatra         | E Sumatra                                   |
| NMH Tring | 1910.12.28.121    | <i>Rhycticeros plicatus plicatus</i>   | Ceram           | Wahai, Ceram, Southern Moluccas             |
| NMH Tring | Y3.5.12.2438      | <i>Rhycticeros plicatus plicatus</i>   | Ceram           | Ceram, Southern Moluccas                    |
| UMZC      | 25/Buc/1/g/1      | <i>Rhycticeros plicatus plicatus</i>   | Ceram           | Ceram                                       |
| NMH Tring | 1911.12.20.907    | <i>Rhycticeros plicatus ruficollis</i> | West New Guinea | Canoe camp, Setakwa river, New Guinea       |
| NMH Tring | 1911.12.20.902    | <i>Rhycticeros plicatus ruficollis</i> | West New Guinea | Launch camp, Setakwa river, New Guinea      |
| NMH Tring | 1911.12.20.891    | <i>Rhycticeros plicatus ruficollis</i> | West New Guinea | Parimau, Mimika river, New Guinea           |

## CHAPTER 6

|           |                |                                       |                      |                                               |
|-----------|----------------|---------------------------------------|----------------------|-----------------------------------------------|
| NMH Tring | 1911.12.20.898 | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | Wataikwa river, New Guinea                    |
| NMH Tring | 1911.12.20.900 | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | Wataikwa river, New Guinea                    |
| NMH Tring | 1911.12.20.896 | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | Kapare river, New Guinea                      |
| NMH Tring | 1911.12.20.893 | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | Kapare river, New Guinea                      |
| NMH Tring | 1911.12.20.890 | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | Mimika river, New Guinea                      |
| NMH Tring | 1911.12.20.904 | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | Launch camp, Setakwa river, New Guinea        |
| NMH Tring | no registry    | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | Launch camp, Setakwa river, New Guinea        |
| NMH Tring | no registry    | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | White water camp, Kafari river, New Guinea    |
| NMH Tring | no registry    | <i>Rhyticeros plicatus ruficollis</i> | West New Guinea      | White water camp, Kafari river, New Guinea    |
| NMH Tring | 1969.27.192    | <i>Rhyticeros plicatus ruficollis</i> | Gilolo               | South Halmahera                               |
| NMH Tring | 1969.27.193    | <i>Rhyticeros plicatus ruficollis</i> | Gilolo               | South Halmahera                               |
| NMH Tring | 1996.41.1848   | <i>Rhyticeros plicatus ruficollis</i> | Gilolo               | Gilolo Island, New Guinea                     |
| NMH Tring | Y3.5.12.180    | <i>Rhyticeros plicatus ruficollis</i> | Gilolo               | Morty Island, Gilolo                          |
| NMH Tring | 59.4.8.62      | <i>Rhyticeros plicatus ruficollis</i> | Gilolo               | Gilolo Island, New Guinea                     |
| NMH Tring | Y3.5.12.2440   | <i>Rhyticeros plicatus ruficollis</i> | Gilolo               | Gilolo Island, New Guinea                     |
| UMZC      | 25/Buc/1/g/2   | <i>Rhyticeros plicatus ruficollis</i> | Gilolo               | Butchian, Maluku                              |
| NMH Tring | 1900.2.8.14    | <i>Rhyticeros plicatus ruficollis</i> | Morotai              | Morotai Island, Moluccas                      |
| NMH Tring | 88.10.30.128   | <i>Rhyticeros plicatus ruficollis</i> | Mysol                | Waigama, Misool                               |
| NMH Tring | 1934.10.21.86  | <i>Rhyticeros plicatus ruficollis</i> | Waigeo               | Waigeo Island, Rajah Ampat group              |
| NMH Tring | 1969.27.190    | <i>Rhyticeros plicatus ruficollis</i> | Obi                  | Obi Island, Central Moluccas                  |
| NMH Tring | 1900.3.1.23    | <i>Rhyticeros plicatus ruficollis</i> | Obi                  | Obi Island, Central Moluccas                  |
| NMH Tring | 1996.41.1242   | <i>Rhyticeros narcondami</i>          | Narcondam            | Narcondam Island                              |
| NMH Tring | 1920.10.29.1   | <i>Rhyticeros narcondami</i>          | Narcondam            | Narcondam Island                              |
| UMZC      | 25/Buc/1/e/1   | <i>Rhyticeros narcondami</i>          | Narcondam            | Narcondam Island                              |
| NMH Tring | 87.1.17.17     | <i>Rhyticeros plicatus harterti</i>   | Solomon Islands      | Shortland Island                              |
| NMH Tring | 1939.12.9.3477 | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | New Guinea                                    |
| NMH Tring | 1921.12.30.23  | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Munum, Markham valley, Huon Gulf              |
| NMH Tring | 1908.5.27.19   | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Humbolt's Bay, New Guinea                     |
| NMH Tring | 79.3.6.11      | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Port Moresby, New Guinea                      |
| NMH Tring | 88.10.30.129   | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Southern New Guinea                           |
| NMH Tring | 1923.10.28.1   | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Elenagora, New Guinea                         |
| NMH Tring | 1921.12.30.23  | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Gabmetzung, Markham valley, Huon Gulf, NG     |
| NMH Tring | 1921.12.30.22  | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Dugamor, NE coast of New Guinea               |
| NMH Tring | 1921.12.30.24  | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Munum, Markham valley, Huon Gulf              |
| NMH Tring | 73.5.12.2189   | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | Morty Island                                  |
| NMH Tring | no registry    | <i>Rhyticeros plicatus jungei</i>     | East New Guinea      | no tag, but pale rufous suggests Eastern form |
| OUMNH     | B03983         | <i>Rhyticeros plicatus mendanae</i>   | East Solomon Islands | Betilonga cave, Guadalcanal                   |
| NMH Tring | 88.3.9.5       | <i>Rhyticeros plicatus mendanae</i>   | East Solomon Islands | Aola, Guadalcanal                             |
| NMH Tring | 1936.7.1.15    | <i>Rhyticeros plicatus mendanae</i>   | East Solomon Islands | Popanu, Guadalcanal                           |
| NMH Tring | 88.10.30.119   | <i>Rhyticeros subruficollis</i>       | Indo-China           | Katsiegs, Tenasserim, Burma                   |
| NMH Tring | 87.9.1.243     | <i>Rhyticeros subruficollis</i>       | Indo-China           | Amherst, Mandalay, Burma                      |
| NMH Tring | 87.9.1.239     | <i>Rhyticeros subruficollis</i>       | Indo-China           | Karope, Moulmein, Burma                       |
| NMH Tring | 87.9.1.245     | <i>Rhyticeros subruficollis</i>       | Indo-China           | South of Yea, Tenasserim, Burma               |
| NMH Tring | 87.9.1.241     | <i>Rhyticeros subruficollis</i>       | Indo-China           | Karope, Moulmein, Burma                       |
| NMH Tring | 87.9.1.246     | <i>Rhyticeros subruficollis</i>       | Indo-China           | Meeta Myo Lavoy, Tenasserim, Burma            |
| NMH Tring | 88.10.30.119B  | <i>Rhyticeros subruficollis</i>       | Indo-China           | Tonghoo, Burma                                |



## CHAPTER 6

|           |                |                                        |                 |                                          |
|-----------|----------------|----------------------------------------|-----------------|------------------------------------------|
| NMH Tring | 80.1.1.3373    | <i>Rhyticeros subruficollis</i>        | Indo-China      | Tenasserim (Helfer)                      |
| NMH Tring | 88.10.30.125   | <i>Rhyticeros subruficollis</i>        | Indo-China      | Tonghoo, Burma                           |
| NMH Tring | 82.1.20.1420   | <i>Rhyticeros subruficollis</i>        | Indo-China      | Myibbeyo, Pegu, Burma                    |
| NMH Tring | 93.7.2.1       | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Mt. Kalulong, Sarawak                    |
| NMH Tring | 93.7.2.2       | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Mt. Kalulong, Sarawak                    |
| NMH Tring | 92.8.25.42?    | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Mt Dulit, Baram River, Sarawak           |
| NMH Tring | 92.8.25.41     | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Mt Dulit, Baram River, Sarawak           |
| NMH Tring | 88.10.30.125   | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Mt Dulit, Baram River, Sarawak           |
| NMH Tring | 92.8.25.43     | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Mt Dulit, Baram River, Sarawak           |
| NMH Tring | 1935.10.22.178 | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Mt Dulit, Baram River, Sarawak           |
| NMH Tring | no registry    | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Gunung Ulu, Kalimantan                   |
| NMH Tring | 78.5.3.3       | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Lawas river, NW Borneo                   |
| OUMNH     | B05365         | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Northwest Borneo                         |
| ZRC NUS   | 3.11163        | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Borneo                                   |
| ZRC NUS   | 3.11161        | <i>Rhyticeros undulatus aequabilis</i> | Borneo          | Gunung Tenabo, Baram, Sarawak            |
| UMZC      | 25/Buc/1h/1    | <i>Rhyticeros undulatus ticehursti</i> | Northern India  | near Shillong, Assam                     |
| NHM Tring | 88.10.30.223   | <i>Rhyticeros undulatus ticehursti</i> | Northern India  | N Khasia Hills, Assam                    |
| NHM Tring | 87.9.1.224     | <i>Rhyticeros undulatus ticehursti</i> | Northern India  | Noouzaibaso, Manipur                     |
| NHM Tring | 87.9.1.226     | <i>Rhyticeros undulatus ticehursti</i> | Northern India  | Cochar, Assam                            |
| NHM Tring | 71.1.13.4      | <i>Rhyticeros undulatus ticehursti</i> | Northern India  | Asakin, N. Cochar Hills                  |
| NHM Tring | 87.9.1.238     | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Kohloun, Victoria Pt, Tenasserim, Burma  |
| NHM Tring | 82.1.20.1428   | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Malewoon, Tenasserim, B. Burma           |
| NHM Tring | 87.9.1.229     | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Pakchan, Tenasserim, B Burma             |
| NHM Tring | 87.9.1.244     | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | S of Yea, Jahdee, Tenasserim, B. Burma   |
| NHM Tring | 1969.41.1057   | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Bankasoon, Tenasserim, B Burma           |
| NHM Tring | 87.9.263       | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Bankasoon, Tenasserim, B Burma           |
| NHM Tring | 87.9.1.261     | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Bankasoon, Tenasserim, B Burma           |
| NHM Tring | 87.9.1.251     | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Bankasoon, Tenasserim, B Burma           |
| NHM Tring | 1955.1.2073    | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Khao Sabab near Chantabin, SE Siam       |
| NHM Tring | 1955.1.2079    | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | near Sriracha, SE Siam                   |
| ZRC NUS   | 3.11150.       | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Tapli, Ban Khlong Wan, Pakchan, Thailand |
| ZRC NUS   | 3.11147        | <i>Rhyticeros undulatus ticehursti</i> | Indo-China      | Cheingdao, nr. Cheingmai, Thailand       |
| NMH Tring | no registry    | <i>Rhyticeros undulatus undulatus</i>  | Malay Peninsula | Sinang near Kuala Lumpur, Malaysia       |
| NMH Tring | no registry    | <i>Rhyticeros undulatus undulatus</i>  | Malay Peninsula | Sinang near Kuala Lumpur, Malaysia       |
| NMH Tring | no registry    | <i>Rhyticeros undulatus undulatus</i>  | Malay Peninsula | Sinang near Kuala Lumpur, Malaysia       |
| NMH Tring | no registry    | <i>Rhyticeros undulatus undulatus</i>  | Malay Peninsula | Malacca                                  |
| NMH Tring | 87.9.1.275     | <i>Rhyticeros undulatus undulatus</i>  | Malay Peninsula | Malacca                                  |
| NMH Tring | 42.2.15.229    | <i>Rhyticeros undulatus undulatus</i>  | Malay Peninsula | Malacca                                  |
| NMH Tring | 88.10.30.123   | <i>Rhyticeros undulatus undulatus</i>  | Malay Peninsula | Malacca                                  |
| ZRC NUS   | 3.11159        | <i>Rhyticeros undulatus undulatus</i>  | Malay Peninsula | Wray's Camp, Gunong Tahan, Pahang        |
| ZRC NUS   | 3.11151        | <i>Rhyticeros undulatus undulatus</i>  | Indo-China      | Tasan, Chum Pawn, P. Thailand            |
| ZRC NUS   | 3.11153        | <i>Rhyticeros undulatus undulatus</i>  | Indo-China      | Klong Tun Sai, Phuket, Thailand          |
| ZRC NUS   | 3.11155        | <i>Rhyticeros undulatus undulatus</i>  | Indo-China      | Pasir Raja, Lontar is. SW Thailand       |
| OUMNH     | B05364         | <i>Rhyticeros undulatus undulatus</i>  | Sumatra         | Sumatra                                  |
| NMH Tring | 88.10.30.222   | <i>Rhyticeros undulatus undulatus</i>  | Sumatra         | Lamong, SE Sumatra                       |
| NMH Tring | 1920.6.29.96   | <i>Rhyticeros undulatus undulatus</i>  | Sumatra         | Sungei Kumbang, Korinchi                 |

## CHAPTER 6

|           |              |                                       |         |                             |
|-----------|--------------|---------------------------------------|---------|-----------------------------|
| NMH Tring | 88.10.30.124 | <i>Rhyticeros undulatus undulatus</i> | Sumatra | Lampung, SE Sumatra         |
| ZRC NUS   | 3.11165      | <i>Rhyticeros undulatus undulatus</i> | Sumatra | Singkep, Riau Is., Sumatra  |
| ZRC NUS   | 3.11164      | <i>Rhyticeros undulatus undulatus</i> | Sumatra | Kumbang R. Kerinci, Sumatra |
| ZRC NUS   | 3.11166      | <i>Rhyticeros undulatus undulatus</i> | Sumatra | Singkep, Riau Is., Sumatra  |
| ZRC NUS   | 3.11167      | <i>Rhyticeros undulatus undulatus</i> | Java    | Badjaelmati, E. Java        |

<sup>a</sup> NHM Tring: Natural History Museum, Tring, UK; DMNH: Delaware Museum of Natural History, Delaware, USA; OUMNH: Oxford University Museum of Natural History, Oxford, UK; UMZC: Cambridge University Museum of Zoology, Cambridge, UK; UPLBMNH: University of the Philippines Los Baños Museum of Natural History, Laguna, Philippines; NSMNP: Northern Sierra Madre Natural Park, Isabela, Philippines; MGR: Malagos Garden Resort, Davao City, Philippines; MSU-NSM: Mindanao State University Natural Science Museum, Iligan City, Philippines; MSU-CSM: Mindanao State University Aga Khan Museum, Marawi City, Philippines; NMP: National Museum of the Philippines, Manila, Philippines; UPD: University of the Philippines Diliman Institute of Biology Collection, Quezon City, Philippines; SU-NHM: Silliman University R.B. Gonzales Natural Science Museum, Dumaguete City, Philippines; NMNH: National Museum of Natural History-Smithsonian Institution, Washington D.C., USA; ERDB: Ecosystems Research & Development Bureau, Department of Environment & Natural Resources, Laguna, Philippines; ZRC-NUS: National University of Singapore-Raffles Museum Zoological Research Collection, Singapore.

**Table S6.3:** List of hornbill samples used in this study, with details of GenBank accession number of mtDNA Cytochrome *b* sequences, equivalent museum registry or source material and their geographic origin.

| <b>Taxa</b>                                    | <b>GenBank No.</b> | <b>Museum number <sup>a</sup></b> | <b>Geo-isolate</b> |
|------------------------------------------------|--------------------|-----------------------------------|--------------------|
| <i>Aceros nipalensis</i>                       | XXX                | NHM1f1                            | North India        |
| <i>Aceros nipalensis</i>                       | XXX                | NHM91202                          | North India        |
| <i>Aceros nipalensis</i>                       | GU257913           | -                                 | Thailand           |
| <i>Aceros nipalensis</i>                       | XXX                | NHM121827                         | IndoChina          |
| <i>Aceros nipalensis</i>                       | XXX                | NHM222200                         | IndoChina          |
| <i>Anorrhinus austeni</i>                      | XXX                | NHM7241                           | North India        |
| <i>Anorrhinus austeni</i>                      | XXX                | NHM7242                           | North India        |
| <i>Anorrhinus austeni</i>                      | GU257908           | -                                 | Thailand           |
| <i>Anorrhinus austeni</i>                      | XXX                | NHM51431                          | East IndoChina     |
| <i>Anorrhinus austeni</i>                      | XXX                | NHM?                              | East IndoChina     |
| <i>Anorrhinus galeritus</i>                    | XXX                | NHM91170                          | IndoChina          |
| <i>Anorrhinus galeritus</i>                    | GU257909           | -                                 | Thailand           |
| <i>Anorrhinus galeritus</i>                    | XXX                | OUM5356                           | Borneo             |
| <i>Anorrhinus galeritus</i>                    | XXX                | OUM5357                           | Borneo             |
| <i>Anorrhinus galeritus</i>                    | XXX                | OUM5358                           | Borneo             |
| <i>Anorrhinus galeritus</i>                    | XXX                | OUM5359                           | Borneo             |
| <i>Anorrhinus galeritus</i>                    | XXX                | UMZC2a6                           | Borneo             |
| <i>Anorrhinus galeritus</i>                    | XXX                | NUS3.11003                        | Sumatra            |
| <i>Anorrhinus galeritus</i>                    | XXX                | AMNH645268                        | Sumatra            |
| <i>Anorrhinus tickelli</i>                     | GU257907           | -                                 | Thailand           |
| <i>Anorrhinus tickelli</i>                     | XXX                | NHM22202                          | West IndoChina     |
| <i>Anorrhinus tickelli</i>                     | XXX                | NHM83454                          | West IndoChina     |
| <i>Anthraceroceros albirostris albirostris</i> | XXX                | OUM5577                           | North India        |
| <i>Anthraceroceros albirostris albirostris</i> | XXX                | NHM25878                          | North India        |
| <i>Anthraceroceros albirostris albirostris</i> | XXX                | OUM5568                           | North India        |
| <i>Anthraceroceros albirostris albirostris</i> | GU257911           | -                                 | Thailand           |
| <i>Anthraceroceros albirostris albirostris</i> | XXX                | NHM26260                          | IndoChina          |
| <i>Anthraceroceros albirostris albirostris</i> | AAU89190           | -                                 | Jurong Bird Park   |
| <i>Anthraceroceros albirostris convexus</i>    | XXX                | OUM5374                           | Malay Peninsula    |
| <i>Anthraceroceros albirostris convexus</i>    | GU257912           | -                                 | Singapore          |
| <i>Anthraceroceros albirostris convexus</i>    | XXX                | OUM5375                           | Borneo             |
| <i>Anthraceroceros albirostris convexus</i>    | XXX                | OUM5376                           | Borneo             |
| <i>Anthraceroceros albirostris convexus</i>    | XXX                | OUM5378                           | Borneo             |
| <i>Anthraceroceros albirostris convexus</i>    | XXX                | OUM5373                           | Sumatra            |
| <i>Anthraceroceros albirostris convexus</i>    | XXX                | OUM5377                           | Sumatra            |
| <i>Anthraceroceros albirostris convexus</i>    | XXX                | NHM3026                           | Java               |
| <i>Anthraceroceros coronatus</i>               | XXX                | NHM31494                          | India              |
| <i>Anthraceroceros coronatus</i>               | XXX                | OUM5371                           | India              |
| <i>Anthraceroceros coronatus</i>               | XXX                | NHM85716                          | Sri Lanka          |
| <i>Anthraceroceros malayanus</i>               | GU257910           | -                                 | Thailand           |
| <i>Anthraceroceros malayanus</i>               | XXX                | NHM23355                          | Malay Peninsula    |
| <i>Anthraceroceros malayanus</i>               | XXX                | OUM5369                           | Malay Peninsula    |
| <i>Anthraceroceros malayanus</i>               | XXX                | NHM60148                          | Borneo             |
| <i>Anthraceroceros malayanus</i>               | XXX                | OUM5370                           | Borneo             |
| <i>Anthraceroceros malayanus</i>               | XXX                | UMZC3c3                           | Borneo             |
| <i>Anthraceroceros malayanus</i>               | XXX                | NHM10241                          | Sumatra            |
| <i>Anthraceroceros malayanus</i>               | XXX                | ZSLAMA1                           | Malay Peninsula    |

## CHAPTER 6

|                                      |          |            |                  |
|--------------------------------------|----------|------------|------------------|
| <i>Anthracoceros marchei</i>         | XXX      | NMP010100  | Calamianes       |
| <i>Anthracoceros marchei</i>         | XXX      | NMP014884  | Calamianes       |
| <i>Anthracoceros marchei</i>         | XXX      | NMP015297  | Calamianes       |
| <i>Anthracoceros marchei</i>         | XXX      | NMP074950  | Calamianes       |
| <i>Anthracoceros marchei</i>         | XXX      | UPLB2128   | North Palawan    |
| <i>Anthracoceros marchei</i>         | XXX      | PAWBWRCP42 | Palawan          |
| <i>Anthracoceros marchei</i>         | XXX      | NHM15102   | North Palawan    |
| <i>Anthracoceros marchei</i>         | XXX      | NMP14646   | North Palawan    |
| <i>Anthracoceros marchei</i>         | XXX      | NMP18033   | North Palawan    |
| <i>Anthracoceros marchei</i>         | XXX      | UPLB2129   | North Palawan    |
| <i>Anthracoceros marchei</i>         | XXX      | NMP016137  | North Palawan    |
| <i>Anthracoceros marchei</i>         | XXX      | NMP04993   | South Palawan    |
| <i>Anthracoceros marchei</i>         | XXX      | MGRHB1     | Palawan          |
| <i>Anthracoceros marchei</i>         | XXX      | MGRP43     | Palawan          |
| <i>Anthracoceros marchei</i>         | XXX      | OpolAviP15 | Palawan          |
| <i>Anthracoceros marchei</i>         | XXX      | OpolAviP41 | Palawan          |
| <i>Anthracoceros marchei</i>         | XXX      | NMP06976   | South Palawan    |
| <i>Anthracoceros marchei</i>         | XXX      | NMP068200  | Balabac          |
| <i>Anthracoceros marchei</i>         | XXX      | NMP068210  | Balabac          |
| <i>Anthracoceros marchei</i>         | XXX      | DMNH37064  | Balabac          |
| <i>Anthracoceros montani</i>         | XXX      | AMNH802255 | Sulu archipelago |
| <i>Anthracoceros montani</i>         | XXX      | MSUSulu    | Sangasanga       |
| <i>Anthracoceros montani</i>         | XXX      | DMNH27718  | Sangasanga       |
| <i>Anthracoceros montani</i>         | XXX      | DMNH27720  | Sangasanga       |
| <i>Anthracoceros montani</i>         | XXX      | DMNH27721  | Batubatu         |
| <i>Anthracoceros montani</i>         | XXX      | NHM1638    | Batubatu         |
| <i>Berenicornis comatus</i>          | GU257917 | -          | Thailand         |
| <i>Berenicornis comatus</i>          | XXX      | NHM12079   | IndoChina        |
| <i>Berenicornis comatus</i>          | XXX      | NHM91161   | IndoChina        |
| <i>Berenicornis comatus</i>          | XXX      | NUS3.11082 | Malay Peninsula  |
| <i>Berenicornis comatus</i>          | XXX      | NHM22163   | Borneo           |
| <i>Berenicornis comatus</i>          | XXX      | NHM60617   | Borneo           |
| <i>Berenicornis comatus</i>          | XXX      | NUS3.11084 | Borneo           |
| <i>Berenicornis comatus</i>          | XXX      | NHM72412   | Sumatra          |
| <i>Berenicornis comatus</i>          | XXX      | AMNH644968 | Sumatra          |
| <i>Berenicornis comatus</i>          | XXX      | NUS3.11085 | Sumatra          |
| <i>Buceros bicornis</i>              | XXX      | OUM14076   | North India      |
| <i>Buceros bicornis</i>              | XXX      | OUM14077   | North India      |
| <i>Buceros bicornis</i>              | XXX      | UMZC5a5    | North India      |
| <i>Buceros bicornis</i>              | XXX      | NHM79167   | West India       |
| <i>Buceros bicornis</i>              | XXX      | NHM231493  | West India       |
| <i>Buceros bicornis</i>              | AF346955 | -          | Berlin Zoo       |
| <i>Buceros bicornis</i>              | GU257920 | -          | Thailand         |
| <i>Buceros bicornis</i>              | XXX      | UMZC5a1    | Malay Peninsula  |
| <i>Buceros bicornis</i>              | XXX      | NUS3.11132 | Sumatra          |
| <i>Buceros bicornis</i>              | XXX      | NEZSBBB1   | Southeast Asia   |
| <i>Buceros hydrocorax hydrocorax</i> | XXX      | NSMNPBJA   | North Luzon      |
| <i>Buceros hydrocorax hydrocorax</i> | XXX      | UPLB2132   | North Luzon      |
| <i>Buceros hydrocorax hydrocorax</i> | XXX      | PAWBWRCP47 | Luzon            |
| <i>Buceros hydrocorax hydrocorax</i> | XXX      | UPDIB43    | Central Luzon    |
| <i>Buceros hydrocorax hydrocorax</i> | XXX      | UPLB2133   | South Luzon      |
| <i>Buceros hydrocorax hydrocorax</i> | XXX      | UPDIB344   | Marinduque       |

# CHAPTER 6

|                                        |          |              |                    |
|----------------------------------------|----------|--------------|--------------------|
| <i>Buceros hydrocorax mindanensis</i>  | XXX      | OpolAviP10   | Mindanao           |
| <i>Buceros hydrocorax mindanensis</i>  | XXX      | UPLB2070     | Northeast Mindanao |
| <i>Buceros hydrocorax mindanensis</i>  | XXX      | MSUNSM56503  | Dinagat Siargao    |
| <i>Buceros hydrocorax mindanensis</i>  | XXX      | NFEFIP32     | Mindanao           |
| <i>Buceros hydrocorax mindanensis</i>  | XXX      | UPLB2171     | Southeast Mindanao |
| <i>Buceros hydrocorax mindanensis</i>  | XXX      | NHM10309     | Basilan            |
| <i>Buceros hydrocorax mindanensis</i>  | XXX      | NMP45111     | Basilan            |
| <i>Buceros hydrocorax semigaleatus</i> | XXX      | PAWBWRCP17   | East Visayas       |
| <i>Buceros hydrocorax semigaleatus</i> | XXX      | UPLB2140     | Leyte              |
| <i>Buceros hydrocorax semigaleatus</i> | XXX      | NHM103019    | Panaon             |
| <i>Buceros hydrocorax semigaleatus</i> | XXX      | UPLB2147     | Samar              |
| <i>Buceros hydrocorax semigaleatus</i> | XXX      | UPLB2136     | Bohol              |
| <i>Buceros rhinoceros borneoensis</i>  | XXX      | OUM5384      | Borneo             |
| <i>Buceros rhinoceros borneoensis</i>  | XXX      | UMZC5c6      | Borneo             |
| <i>Buceros rhinoceros rhinoceros</i>   | GU257919 | -            | Thailand           |
| <i>Buceros rhinoceros rhinoceros</i>   | XXX      | AMNH645025   | Malay Peninsula    |
| <i>Buceros rhinoceros rhinoceros</i>   | XXX      | NHMMCS21     | Malay Peninsula    |
| <i>Buceros rhinoceros rhinoceros</i>   | XXX      | NHM1030207   | Sumatra            |
| <i>Buceros rhinoceros silvestris</i>   | XXX      | AMNH122436   | Java               |
| <i>Buceros rhinoceros silvestris</i>   | AF346956 | -            | Java               |
| <i>Buceros rhinoceros silvestris</i>   | AF346957 | -            | Java               |
| <i>Buceros rhinoceros silvestris</i>   | XXX      | NEZSBRA1     | Java               |
| <i>Buceros rhinoceros silvestris</i>   | XXX      | NEZSBRA2     | Java               |
| <i>Aceros corrugatus</i>               | GU257916 | -            | Thailand           |
| <i>Aceros corrugatus</i>               | HM755883 | -            | Pacheco            |
| <i>Aceros corrugatus</i>               | XXX      | NUS3.11132   | Sumatra            |
| <i>Aceros corrugatus</i>               | XXX      | NUS3.11111   | Sumatra            |
| <i>Aceros corrugatus</i>               | XXX      | OUM5360      | Borneo             |
| <i>Aceros corrugatus</i>               | XXX      | OUM5362      | Borneo             |
| <i>Aceros corrugatus</i>               | XXX      | NEZSACA1     | Borneo             |
| <i>Aceros leucocephalus</i>            | XXX      | LWPRCP45     | Mindanao           |
| <i>Aceros leucocephalus</i>            | XXX      | OpolAviFP07  | Mindanao           |
| <i>Aceros leucocephalus</i>            | XXX      | OpolAviMP07  | Mindanao           |
| <i>Aceros leucocephalus</i>            | XXX      | NMP0205      | Northeast Mindanao |
| <i>Aceros leucocephalus</i>            | XXX      | UPLB2113     | Northeast Mindanao |
| <i>Aceros leucocephalus</i>            | XXX      | NHM15130     | Camiguin           |
| <i>Aceros leucocephalus</i>            | XXX      | MSUNSM56487  | Dinagat Siargao    |
| <i>Aceros leucocephalus</i>            | XXX      | MSUNSM56488  | Dinagat Siargao    |
| <i>Aceros leucocephalus</i>            | XXX      | NFEFIP05     | Mindanao           |
| <i>Aceros leucocephalus</i>            | XXX      | UPLB2112     | Southeast Mindanao |
| <i>Aceros leucocephalus</i>            | XXX      | UPLB2121     | Southeast Mindanao |
| <i>Aceros leucocephalus</i>            | XXX      | UPLB2109     | West Mindanao      |
| <i>Aceros leucocephalus</i>            | XXX      | UPLB2126     | West Mindanao      |
| <i>Aceros waldeni</i>                  | HQ834454 | -            | West Panay         |
| <i>Aceros waldeni</i>                  | HQ834455 | -            | West Panay         |
| <i>Aceros waldeni</i>                  | XXX      | MariitWCPP19 | East Panay         |
| <i>Aceros waldeni</i>                  | XXX      | MtBaloyP21   | East Panay         |
| <i>Aceros waldeni</i>                  | XXX      | NHM41598     | Guimaras           |
| <i>Aceros waldeni</i>                  | XXX      | NMP03939     | Negros             |
| <i>Aceros waldeni</i>                  | XXX      | UPLB2103     | Negros             |
| <i>Aceros waldeni</i>                  | XXX      | UPLB2104     | Negros             |
| <i>Oryzomys birostris</i>              | XXX      | AMNH257649   | North India        |

# CHAPTER 6

|                                        |          |              |                    |
|----------------------------------------|----------|--------------|--------------------|
| <i>Ocyrceros birostris</i>             | XXX      | NHM15902     | North India        |
| <i>Ocyrceros birostris</i>             | XXX      | OUM5558      | North India        |
| <i>Ocyrceros birostris</i>             | XXX      | NHM2002.22.1 | India              |
| <i>Ocyrceros birostris</i>             | XXX      | ZSLOBA1      | India              |
| <i>Ocyrceros birostris</i>             | XXX      | NHM16530     | S India            |
| <i>Ocyrceros gingalensis</i>           | XXX      | NHM123162    | Sri Lanka          |
| <i>Ocyrceros gingalensis</i>           | XXX      | NHM3159      | Sri Lanka          |
| <i>Ocyrceros griseus</i>               | XXX      | AMNH344282   | South India        |
| <i>Ocyrceros griseus</i>               | XXX      | NHM16521     | South India        |
| <i>Ocyrceros griseus</i>               | XXX      | UMZC12i1     | South India        |
| <i>Ocyrceros griseus</i>               | XXX      | UMZC12i2     | South India        |
| <i>Penelopides affinis affinis</i>     | XXX      | UPLB2089     | Northeast Mindanao |
| <i>Penelopides affinis affinis</i>     | XXX      | UPLB2090     | Dinagat Siargao    |
| <i>Penelopides affinis affinis</i>     | XXX      | PAWBWRCP22   | Mindanao           |
| <i>Penelopides affinis affinis</i>     | XXX      | UPLB2081     | Southeast Mindanao |
| <i>Penelopides affinis affinis</i>     | XXX      | MSUNSM53258  | West Mindanao      |
| <i>Penelopides affinis basilanicus</i> | XXX      | NHM52213     | Basilan            |
| <i>Penelopides exarhatus exarhatus</i> | XXX      | NHM30146     | North Sulawesi     |
| <i>Penelopides exarhatus exarhatus</i> | XXX      | NHM5122423   | North Sulawesi     |
| <i>Penelopides exarhatus exarhatus</i> | XXX      | UMZC9a1      | North Sulawesi     |
| <i>Penelopides exarhatus sanfordi</i>  | XXX      | NHM12287     | South Sulawesi     |
| <i>Penelopides exarhatus sanfordi</i>  | XXX      | ZSLPE1       | South Sulawesi     |
| <i>Penelopides manillae manillae</i>   | XXX      | DMNH70334    | Palau              |
| <i>Penelopides manillae manillae</i>   | XXX      | DMNH70335    | Palau              |
| <i>Penelopides manillae manillae</i>   | XXX      | NSMNPPMC01   | North Luzon        |
| <i>Penelopides manillae manillae</i>   | XXX      | NSMNPPMSA1   | North Luzon        |
| <i>Penelopides manillae manillae</i>   | XXX      | UPLB2056     | North Luzon        |
| <i>Penelopides manillae manillae</i>   | XXX      | UPLB2061     | Central Luzon      |
| <i>Penelopides manillae manillae</i>   | XXX      | NMP16625     | Catanduanes        |
| <i>Penelopides manillae manillae</i>   | XXX      | NMP16582     | Catanduanes        |
| <i>Penelopides manillae manillae</i>   | XXX      | UPLB2057     | South Luzon        |
| <i>Penelopides manillae manillae</i>   | XXX      | UPDIB341     | Marinduque         |
| <i>Penelopides manillae subniger</i>   | XXX      | PESPPMSE1    | Polillo            |
| <i>Penelopides manillae subniger</i>   | XXX      | PESPPMSE2    | Polillo            |
| <i>Penelopides manillae subniger</i>   | XXX      | UPDIBPSO0398 | Polillo            |
| <i>Penelopides manillae subniger</i>   | XXX      | UPDIBPSO0400 | Polillo            |
| <i>Penelopides manillae subniger</i>   | XXX      | UPDIBPSO0403 | Polillo            |
| <i>Penelopides mindorensis</i>         | XXX      | CWRCP12      | West Mindoro       |
| <i>Penelopides mindorensis</i>         | XXX      | NMP9600      | West Mindoro       |
| <i>Penelopides mindorensis</i>         | XXX      | NMP9601      | West Mindoro       |
| <i>Penelopides mindorensis</i>         | XXX      | NMP09599     | West Mindoro       |
| <i>Penelopides mindorensis</i>         | XXX      | UPDIBPSO0402 | East Mindoro       |
| <i>Penelopides mindorensis</i>         | XXX      | NMP01261     | East Mindoro       |
| <i>Penelopides mindorensis</i>         | XXX      | UPLB2143     | East Mindoro       |
| <i>Penelopides panini panini</i>       | HQ834451 | -            | Panay              |
| <i>Penelopides panini panini</i>       | HQ834459 | -            | Panay              |
| <i>Penelopides panini panini</i>       | XXX      | NMP06363     | Negros             |
| <i>Penelopides panini panini</i>       | XXX      | UPLB2062     | Negros             |
| <i>Penelopides panini panini</i>       | XXX      | NHM30100     | Guimaras           |
| <i>Penelopides panini panini</i>       | XXX      | UMZC9b1      | Guimaras           |
| <i>Penelopides panini panini</i>       | XXX      | NMP012802    | Masbate            |
| <i>Penelopides panini panini</i>       | XXX      | NHM41570     | Masbate            |

# CHAPTER 6

|                                     |          |            |                      |
|-------------------------------------|----------|------------|----------------------|
| <i>Penelopides panini ticaensis</i> | XXX      | DMNH11381  | Ticao                |
| <i>Penelopides panini ticaensis</i> | XXX      | DMNH11382  | Ticao                |
| <i>Penelopides panini ticaensis</i> | XXX      | MNH11384   | Ticao                |
| <i>Penelopides samarensis</i>       | XXX      | NHM13492   | Samar                |
| <i>Penelopides samarensis</i>       | XXX      | NMP12255   | Samar                |
| <i>Penelopides samarensis</i>       | XXX      | UPLB2073   | Leyte                |
| <i>Rhinoplax vigil</i>              | GU257918 | -          | Thailand             |
| <i>Rhinoplax vigil</i>              | XXX      | NHM201423  | IndoChina            |
| <i>Rhinoplax vigil</i>              | XXX      | NHM412864  | Malay Peninsula      |
| <i>Rhinoplax vigil</i>              | XXX      | NUS3.11140 | Malay Peninsula      |
| <i>Rhinoplax vigil</i>              | XXX      | AMNH644995 | Sumatra              |
| <i>Rhinoplax vigil</i>              | XXX      | NUS3.11143 | Sumatra              |
| <i>Rhinoplax vigil</i>              | XXX      | OUM5388    | Borneo               |
| <i>Rhinoplax vigil</i>              | XXX      | OUMN5387   | Borneo               |
| <i>Rhinoplax vigil</i>              | XXX      | OUMNH5389  | Borneo               |
| <i>Rhyticeros cassidix</i>          | AF346938 | -          | Sulawesi             |
| <i>Rhyticeros cassidix</i>          | XXX      | NHM30140   | Sulawesi             |
| <i>Rhyticeros cassidix</i>          | XXX      | NHM3218    | Sulawesi             |
| <i>Rhyticeros cassidix</i>          | XXX      | UMZC1a1    | Sulawesi             |
| <i>Rhyticeros everetti</i>          | XXX      | AMNH346740 | Sumba                |
| <i>Rhyticeros everetti</i>          | XXX      | AMNH346744 | Sumba                |
| <i>Rhyticeros everetti</i>          | XXX      | NHM12871   | Sumba                |
| <i>Rhyticeros narcondami</i>        | XXX      | NHM10291   | Narcondam            |
| <i>Rhyticeros narcondami</i>        | XXX      | NHM10292   | Narcondam            |
| <i>Rhyticeros narcondami</i>        | XXX      | UMZC1e1    | Narcondam            |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH645221 | Bismarck archipelago |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH645225 | Bismarck archipelago |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM32993   | Bismarck archipelago |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH645227 | Solomon Islands      |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH645226 | Solomon Islands      |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM11717   | Solomon Islands      |
| <i>Rhyticeros plicatus</i>          | XXX      | OUM3983    | Guadacanal           |
| <i>Rhyticeros plicatus</i>          | XXX      | OUM3984    | Guadacanal           |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM28121   | Ceram                |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM28122   | Ceram                |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM23023   | East New Guinea      |
| <i>Rhyticeros plicatus</i>          | XXX      | OUM5368    | East New Guinea      |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM20891   | West New Guinea      |
| <i>Rhyticeros plicatus</i>          | XXX      | OpolAviP13 | West New Guinea      |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH300745 | Waigen               |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH645190 | Waigen               |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH467670 | Gilolo               |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM22439   | Gilolo               |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM22440   | Gilolo               |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM2814    | Morotai              |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH645189 | Mysool               |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM30128   | Mysool               |
| <i>Rhyticeros plicatus</i>          | XXX      | AMNH645182 | Obi                  |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM27191   | Obi                  |
| <i>Rhyticeros plicatus</i>          | XXX      | NHM3123    | Obi                  |
| <i>Rhyticeros subruficollis</i>     | GU257914 | -          | Thailand             |
| <i>Rhyticeros subruficollis</i>     | XXX      | NHM30119   | IndoChina            |

## CHAPTER 6

|                                 |          |            |                 |
|---------------------------------|----------|------------|-----------------|
| <i>Rhyticeros subruficollis</i> | XXX      | NHM91243   | IndoChina       |
| <i>Rhyticeros undulatus</i>     | XXX      | NHM91224   | North India     |
| <i>Rhyticeros undulatus</i>     | XXX      | NUS3.11153 | IndoChina       |
| <i>Rhyticeros undulatus</i>     | GU257915 | -          | Thailand        |
| <i>Rhyticeros undulatus</i>     | XXX      | NHMFMSM228 | Malay Peninsula |
| <i>Rhyticeros undulatus</i>     | XXX      | OUM5364    | Sumatra         |
| <i>Rhyticeros undulatus</i>     | XXX      | NUS3.11166 | Sumatra         |
| <i>Rhyticeros undulatus</i>     | XXX      | NHM43216   | Borneo          |
| <i>Rhyticeros undulatus</i>     | XXX      | OUM5365    | Borneo          |
| <i>Rhyticeros undulatus</i>     | XXX      | OUM5366    | Borneo          |
| <i>Rhyticeros undulatus</i>     | XXX      | OUM5367    | Borneo          |
| <i>Rhyticeros undulatus</i>     | XXX      | AMNH576107 | Java            |
| <i>Rhyticeros undulatus</i>     | XXX      | NUS3.11167 | Java            |

<sup>a</sup>AMNH: American Museum of Natural History, New York, USA; NHM: Natural History Museum, Tring, UK; DMNH: Delaware Museum of Natural History, Delaware, USA; OUMNH: Oxford University Museum of Natural History, Oxford, UK; UMZC: Cambridge University Museum of Zoology, Cambridge, UK; UPLBMNH: University of the Philippines Los Baños Museum of Natural History, Laguna, Philippines; ZSL: Zoological Society of London, London, UK; NEZS: North of England Zoological Society, Cheshire, UK; PAWB WRC: Protected Areas and Wildlife Bureau Wildlife Rescue Centre, Manila, Philippines; NSMNP: Northern Sierra Madre Natural Park, Isabela, Philippines; LWPRC: Laguna Wildlife Park and Rescue Centre, Laguna, Philippines; WVSU-MWCP: West Visayas State University Mariit Wildlife Conservation Park, Iloilo, Philippines; MGR: Malagos Garden Resort, Davao City, Philippines; CWRC: Calintaan Wildlife Rescue Centre, Occidental Mindoro, Philippines; MSUNSM: Mindanao State University Natural Science Museum, Iligan City, Philippines; MSU Marawi: Mindanao State University Aga Khan Museum, Marawi City, Philippines; NMP: National Museum of the Philippines, Manila, Philippines; UPDIB: University of the Philippines Diliman Institute of Biology Collection, Quezon City, Philippines; Silliman University R.B. Gonzales Natural Science Museum, Dumaguete City, Philippines; NFEFI: Negros Forests and Ecological Foundation Inc. Biodiversity Conservation Centre, Bacolod City, Philippines; OpolAvi: Opol Aviary, Misamis Oriental, Philippines; PESP: Polillo Ecology Stewardship Project; NUS: National University of Singapore-Raffles Museum Zoological Research Collection.



# CHAPTER 6

**Table S6.4:** Phenotypic scores for 54 hornbill pairs analyzed for morphological divergence using a standard quantitative criteria (Tobias *et al.* 2010).

| Group      | Pair No. | Genus                | Species              | Subspecies        | Geo-isolate             | Biometrics <sup>b</sup> |        |       |             | Score <sup>a</sup> | Plumage <sup>c</sup><br>& bare parts | Vocalization | Behaviour<br>& Ecology | Geographical<br>relationship | TOTAL SCORE |
|------------|----------|----------------------|----------------------|-------------------|-------------------------|-------------------------|--------|-------|-------------|--------------------|--------------------------------------|--------------|------------------------|------------------------------|-------------|
|            |          |                      |                      |                   |                         | Minor                   | Medium | Major | Exceptional |                    |                                      |              |                        |                              |             |
| Species    | 1        | <i>Aceros</i>        | <i>leucocephalus</i> |                   | Mindanao                | 9                       | 1      | 0     | 0           | 3                  | 6                                    | 1            | 0                      | 0                            | 10          |
|            |          | <i>Aceros</i>        | <i>waldeni</i>       |                   | Negros-Panay            |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 2        | <i>Anorrhinus</i>    | <i>austeni</i>       |                   | East Indo-China         | 7                       | 1      | 0     | 0           | 3                  | 7                                    | 0            | 0                      | 0                            | 10          |
|            |          | <i>Anorrhinus</i>    | <i>tickelli</i>      |                   | West Indo-China         |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 3        | <i>Anthracoseros</i> | <i>albirostris</i>   |                   | India-Sundaland         | 6                       | 2      | 0     | 0           | 2                  | 4                                    | 1            | 0                      | 0                            | 7           |
|            |          | <i>Anthracoseros</i> | <i>coronatus</i>     |                   | India-Sri Lanka         |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 4        | <i>Ocyrceros</i>     | <i>griseus</i>       |                   | India                   | 7                       | 2      | 1     | 0           | 5                  | 6                                    | 1            | 1                      | 0                            | 13          |
|            |          | <i>Ocyrceros</i>     | <i>gingalensis</i>   |                   | Sri Lanka               |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 5        | <i>Penelopides</i>   | <i>affinis</i>       |                   | Mindanao                | 9                       | 0      | 0     | 0           | 2                  | 6                                    | 1            | 0                      | 0                            | 9           |
|            |          | <i>Penelopides</i>   | <i>manillae</i>      |                   | Greater Luzon           |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 6        | <i>Penelopides</i>   | <i>panini</i>        |                   | Negros-Panay            | 7                       | 2      | 0     | 0           | 3                  | 7                                    | 1            | 0                      | 0                            | 11          |
|            |          | <i>Penelopides</i>   | <i>manillae</i>      |                   | Greater Luzon           |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 7        | <i>Penelopides</i>   | <i>panini</i>        |                   | Negros-Panay            | 8                       | 1      | 0     | 0           | 3                  | 8                                    | 1            | 0                      | 0                            | 12          |
|            |          | <i>Penelopides</i>   | <i>mindorensis</i>   |                   | Mindoro                 |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 8        | <i>Penelopides</i>   | <i>panini</i>        |                   | Negros-Panay            | 5                       | 4      | 0     | 0           | 3                  | 7                                    | 1            | 0                      | 0                            | 11          |
|            |          | <i>Penelopides</i>   | <i>affinis</i>       |                   | Mindanao                |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 9        | <i>Penelopides</i>   | <i>samarensis</i>    |                   | East Visayas            | 9                       | 0      | 0     | 0           | 2                  | 5                                    | 0            | 0                      | 0                            | 7           |
|            |          | <i>Penelopides</i>   | <i>affinis</i>       |                   | Mindanao                |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 10       | <i>Rhyticeros</i>    | <i>narcondami</i>    |                   | Narcondam               | 1                       | 4      | 4     | 0           | 3                  | 3                                    | 1            | 0                      | 0                            | 7           |
|            |          | <i>Rhyticeros</i>    | <i>plicatus</i>      |                   | Wallacea-Melanesia      |                         |        |       |             |                    |                                      |              |                        |                              |             |
| Subspecies | 11       | <i>Rhyticeros</i>    | <i>subruficollis</i> |                   | Indo-China              | 6                       | 3      | 0     | 0           | 3                  | 6                                    | 1            | 0                      | 0                            | 10          |
|            |          | <i>Rhyticeros</i>    | <i>undulatus</i>     |                   | India-Sundaland         |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            | 12       | <i>Aceros</i>        | <i>corrugatus</i>    | <i>corrugatus</i> | Borneo                  | 10                      | 0      | 0     | 0           | 2                  | 0                                    | 0            | 0                      | 0                            | 2           |
|            |          | <i>Aceros</i>        | <i>corrugatus</i>    | <i>rugosus</i>    | Malay Peninsula-Sumatra |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            |          |                      |                      |                   |                         |                         |        |       |             |                    |                                      |              |                        |                              |             |
|            |          |                      |                      |                   |                         |                         |        |       |             |                    |                                      |              |                        |                              |             |

# CHAPTER 6

|    |                       |                    |                     |                         |   |   |   |   |   |   |   |   |   |   |
|----|-----------------------|--------------------|---------------------|-------------------------|---|---|---|---|---|---|---|---|---|---|
| 13 | <i>Anthracosceros</i> | <i>albirostris</i> | <i>albirostris</i>  | Indo-China              | 9 | 0 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 6 |
|    | <i>Anthracosceros</i> | <i>albirostris</i> | <i>convexus</i>     | Sundaland               |   |   |   |   |   |   |   |   |   |   |
| 14 | <i>Buceros</i>        | <i>bicornis</i>    | <i>homrai</i>       | India-IndoChina         | 7 | 3 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 |
|    | <i>Buceros</i>        | <i>bicornis</i>    | <i>bicornis</i>     | Sundaland               |   |   |   |   |   |   |   |   |   |   |
| 15 | <i>Buceros</i>        | <i>hydrocorax</i>  | <i>hydrocorax</i>   | Greater Luzon           | 7 | 1 | 0 | 0 | 2 | 7 | 0 | 0 | 0 | 9 |
|    | <i>Buceros</i>        | <i>hydrocorax</i>  | <i>mindanensis</i>  | Mindanao                |   |   |   |   |   |   |   |   |   |   |
| 16 | <i>Buceros</i>        | <i>hydrocorax</i>  | <i>hydrocorax</i>   | Greater Luzon           | 7 | 1 | 0 | 0 | 2 | 7 | 0 | 0 | 0 | 9 |
|    | <i>Buceros</i>        | <i>hydrocorax</i>  | <i>semigaleatus</i> | East Visayas            |   |   |   |   |   |   |   |   |   |   |
| 17 | <i>Buceros</i>        | <i>hydrocorax</i>  | <i>mindanensis</i>  | Mindanao                | 7 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
|    | <i>Buceros</i>        | <i>hydrocorax</i>  | <i>semigaleatus</i> | East Visayas            |   |   |   |   |   |   |   |   |   |   |
| 18 | <i>Buceros</i>        | <i>rhinoceros</i>  | <i>rhinoceros</i>   | Malay Peninsula-Sumatra | 8 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
|    | <i>Buceros</i>        | <i>rhinoceros</i>  | <i>borneoensis</i>  | Borneo                  |   |   |   |   |   |   |   |   |   |   |
| 19 | <i>Buceros</i>        | <i>rhinoceros</i>  | <i>rhinoceros</i>   | Malay Peninsula-Sumatra | 9 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
|    | <i>Buceros</i>        | <i>rhinoceros</i>  | <i>silvestris</i>   | Java                    |   |   |   |   |   |   |   |   |   |   |
| 20 | <i>Buceros</i>        | <i>rhinoceros</i>  | <i>borneoensis</i>  | Borneo                  | 9 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 3 |
|    | <i>Buceros</i>        | <i>rhinoceros</i>  | <i>silvestris</i>   | Java                    |   |   |   |   |   |   |   |   |   |   |
| 21 | <i>Penelopides</i>    | <i>affinis</i>     | <i>basilanicus</i>  | Basilan                 | 7 | 0 | 0 | 0 | 3 | 4 | 0 | 0 | 0 | 7 |
|    | <i>Penelopides</i>    | <i>affinis</i>     | <i>affinis</i>      | Mindanao                |   |   |   |   |   |   |   |   |   |   |
| 22 | <i>Penelopides</i>    | <i>exarhatus</i>   | <i>exarhatus</i>    | North Sulawesi          | 9 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 5 |
|    | <i>Penelopides</i>    | <i>exarhatus</i>   | <i>sandfordi</i>    | South Sulawesi          |   |   |   |   |   |   |   |   |   |   |
| 23 | <i>Penelopides</i>    | <i>manillae</i>    | <i>manillae</i>     | mainland Luzon          | 9 | 0 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 6 |
|    | <i>Penelopides</i>    | <i>manillae</i>    | <i>subnigra</i>     | Polillo                 |   |   |   |   |   |   |   |   |   |   |
| 24 | <i>Penelopides</i>    | <i>panini</i>      | <i>panini</i>       | Negros-Panay            | 7 | 1 | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 6 |
|    | <i>Penelopides</i>    | <i>panini</i>      | <i>ticaensis</i>    | Ticao                   |   |   |   |   |   |   |   |   |   |   |
| 25 | <i>Rhyticeros</i>     | <i>plicatus</i>    | <i>ruficollis</i>   | West NG-N Moluccas      | 9 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 4 |
|    | <i>Rhyticeros</i>     | <i>plicatus</i>    | <i>jungei</i>       | East New Guinea         |   |   |   |   |   |   |   |   |   |   |
| 26 | <i>Rhyticeros</i>     | <i>plicatus</i>    | <i>harterti</i>     | West Solomon Islands    | 3 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
|    | <i>Rhyticeros</i>     | <i>plicatus</i>    | <i>mendanae</i>     | East Solomon Islands    |   |   |   |   |   |   |   |   |   |   |

# CHAPTER 6

|          |    |                       |                   |                        |                         |   |   |   |   |   |   |   |   |   |   |
|----------|----|-----------------------|-------------------|------------------------|-------------------------|---|---|---|---|---|---|---|---|---|---|
| Subsumed | 27 | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>jungei</i>          | East New Guinea         | 8 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 4 |
|          |    | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ruficollis</i>      | West New Guinea         |   |   |   |   |   |   |   |   |   |   |
|          | 28 | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>undulatus</i>       | Sundaland               | 8 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|          |    | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>aequabilis</i>      | Borneo                  |   |   |   |   |   |   |   |   |   |   |
|          | 29 | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>carinatus</i>       | Malay Peninsula         | 7 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |
|          |    | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>minor</i>           | Borneo                  |   |   |   |   |   |   |   |   |   |   |
|          | 30 | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>galeritus</i>       | Sumatra                 | 8 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
|          |    | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>carinatus</i>       | Malay Peninsula         |   |   |   |   |   |   |   |   |   |   |
|          | 31 | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>carinatus</i>       | Malay Peninsula         | 9 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |
|          |    | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>ssp.</i>            | Borneo-Sumatra          |   |   |   |   |   |   |   |   |   |   |
|          | 32 | <i>Anthracosceros</i> | <i>malayanus</i>  | <i>deminutus</i>       | Borneo                  | 8 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|          |    | <i>Anthracosceros</i> | <i>malayanus</i>  | <i>malayanus</i>       | Malay Peninsula-Sumatra |   |   |   |   |   |   |   |   |   |   |
|          | 33 | <i>Buceros</i>        | <i>bicornis</i>   | <i>homrai</i>          | India                   | 6 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|          |    | <i>Buceros</i>        | <i>bicornis</i>   | <i>ssp.</i>            | IndoChina-Sundaland     |   |   |   |   |   |   |   |   |   |   |
|          | 34 | <i>Buceros</i>        | <i>hydrocorax</i> | <i>mindanensis</i>     | Mindanao                | 7 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|          |    | <i>Buceros</i>        | <i>hydrocorax</i> | <i>basilanicus</i>     | Basilan                 |   |   |   |   |   |   |   |   |   |   |
|          | 35 | <i>Buceros</i>        | <i>hydrocorax</i> | <i>basilanicus (F)</i> | Basilan                 | 7 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|          |    | <i>Buceros</i>        | <i>hydrocorax</i> | <i>mindanensis (F)</i> | Mindanao                |   |   |   |   |   |   |   |   |   |   |
|          | 36 | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ssp.</i>            | Solomon Islands         | 6 | 3 | 0 | 0 | 3 | 4 | 1 | 0 | 0 | 8 |
|          |    | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ruficollis</i>      | North Moluccas          |   |   |   |   |   |   |   |   |   |   |
|          | 37 | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ssp.</i>            | Seram-New Guinea        | 6 | 2 | 0 | 0 | 2 | 5 | 1 | 0 | 0 | 8 |
|          |    | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ssp.</i>            | Solomon Islands         |   |   |   |   |   |   |   |   |   |   |
|          | 38 | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>plicatus</i>        | Ceram                   | 8 | 1 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 5 |
|          |    | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ssp.</i>            | New Guinea              |   |   |   |   |   |   |   |   |   |   |
|          | 39 | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>ticehursti</i>      | India-IndoChina         | 8 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 3 |
|          |    | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>ssp.</i>            | Sundaland               |   |   |   |   |   |   |   |   |   |   |
|          | 40 | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>ticehursti</i>      | India-IndoChina         | 5 | 3 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
|          |    | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>aequabilis</i>      | Borneo                  |   |   |   |   |   |   |   |   |   |   |

# CHAPTER 6

|         |    |                      |                      |                    |                 |    |   |   |   |   |   |   |   |   |   |
|---------|----|----------------------|----------------------|--------------------|-----------------|----|---|---|---|---|---|---|---|---|---|
| Cryptic | 41 | <i>Aceros</i>        | <i>leucocephalus</i> |                    | West Mindanao   | 7  | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 3 |
|         |    | <i>Aceros</i>        | <i>leucocephalus</i> |                    | East Mindanao   |    |   |   |   |   |   |   |   |   |   |
|         | 42 | <i>Anthracoceros</i> | <i>coronatus</i>     |                    | India           | 10 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|         |    | <i>Anthracoceros</i> | <i>coronatus</i>     |                    | Sri Lanka       |    |   |   |   |   |   |   |   |   |   |
|         | 43 | <i>Anthracoceros</i> | <i>marchei</i>       |                    | Balabac         | 4  | 4 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|         |    | <i>Anthracoceros</i> | <i>marchei</i>       |                    | Palawan         |    |   |   |   |   |   |   |   |   |   |
|         | 44 | <i>Anthracoceros</i> | <i>marchei</i>       |                    | Calamianes      | 8  | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|         |    | <i>Anthracoceros</i> | <i>marchei</i>       |                    | Palawan         |    |   |   |   |   |   |   |   |   |   |
|         | 45 | <i>Berenicornis</i>  | <i>comatus</i>       |                    | Malay Peninsula | 8  | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
|         |    | <i>Berenicornis</i>  | <i>comatus</i>       |                    | Borneo-Sumatra  |    |   |   |   |   |   |   |   |   |   |
|         | 46 | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | West Mindanao   | 10 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 3 |
|         |    | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | East Mindanao   |    |   |   |   |   |   |   |   |   |   |
|         | 47 | <i>Penelopides</i>   | <i>manillae</i>      | <i>manillae</i>    | South Luzon     | 9  | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 1 | 5 |
|         |    | <i>Penelopides</i>   | <i>manillae</i>      | <i>manillae</i>    | North Luzon     |    |   |   |   |   |   |   |   |   |   |
|         | 48 | <i>Penelopides</i>   | <i>mindorensis</i>   |                    | West Mindoro    | 9  | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 1 | 5 |
|         |    | <i>Penelopides</i>   | <i>mindorensis</i>   |                    | East Mindoro    |    |   |   |   |   |   |   |   |   |   |
|         | 49 | <i>Rhinoplax</i>     | <i>vigil</i>         |                    | Malay Peninsula | 8  | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
|         |    | <i>Rhinoplax</i>     | <i>vigil</i>         |                    | Borneo-Sumatra  |    |   |   |   |   |   |   |   |   |   |
|         | 50 | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | Dinagat/Siargao | 8  | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
|         |    | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | East Mindanao   |    |   |   |   |   |   |   |   |   |   |
|         | 51 | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | Dinagat/Siargao | 8  | 1 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 6 |
|         |    | <i>Penelopides</i>   | <i>samarensis</i>    |                    | East Visayas    |    |   |   |   |   |   |   |   |   |   |
|         | 52 | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i> (F) | East Mindanao   | 7  | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 3 |
|         |    | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i> (F) | West Mindanao   |    |   |   |   |   |   |   |   |   |   |
|         | 53 | <i>Penelopides</i>   | <i>panini</i>        | <i>panini</i>      | Masbate         | 10 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 3 |
|         |    | <i>Penelopides</i>   | <i>panini</i>        | <i>panini</i>      | Negros-Panay    |    |   |   |   |   |   |   |   |   |   |
|         | 54 | <i>Penelopides</i>   | <i>panini</i>        | <i>panini</i>      | Masbate         | 4  | 2 | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 5 |
|         |    | <i>Penelopides</i>   | <i>panini</i>        | <i>ticaensis</i>   | Ticao           |    |   |   |   |   |   |   |   |   |   |

## CHAPTER 6

<sup>a</sup> Total score represents the two highest or lowest d values among the ten measurements

<sup>b</sup> Scoring system for Cohen's d value: 1=minor (0.2-2), 2=medium (2-5), 3=major (5-10), 4 =exceptional(>10)

<sup>c</sup> Total score is the sum of scores from three (3) plumage characters including bare parts

<sup>d</sup> Nucleotide divergence based on pairwise comparisons of mtDNA Cytochrome b truncated to 909 bp, calculated using ML best-fit model TN93+G

# CHAPTER 6

**Table S6.5:** Biometric analysis for 54 hornbill pairs analyzed for morphological differentiation using a standard quantitative criteria (Tobias *et al.* 2010).

| Pair No. | Mean    |       |        |         |        |        |        |        |        |        | Standard deviation |       |       |         |        |        |        |        |        |        |
|----------|---------|-------|--------|---------|--------|--------|--------|--------|--------|--------|--------------------|-------|-------|---------|--------|--------|--------|--------|--------|--------|
|          | TARSU S | WIN G | TAIL   | TER BND | BILL L | BILL W | BILL H | CASQ L | CASQ W | CASQ H | TARSU S            | WING  | TAIL  | TER BND | BILL L | BILL W | BILL H | CASQ L | CASQ W | CASQ H |
| 1        | 51.86   | 337.8 | 260.29 | 36.13   | 111.89 | 39.82  | 42.81  | 72.05  | 29.89  | 26.97  | 2.88               | 17.49 | 16.49 | 5.32    | 7.42   | 1.95   | 3.18   | 6.66   | 1.86   | 2.26   |
|          | 54.30   | 346.3 | 271.11 | 39.71   | 117.10 | 40.44  | 45.14  | 91.40  | 29.53  | 25.46  | 2.24               | 15.43 | 16.83 | 3.78    | 12.85  | 2.15   | 3.23   | 9.95   | 2.83   | 3.34   |
| 2        | 46.30   | 322.2 | 298.20 | 0.00    | 114.42 | 37.40  | 32.60  | 78.25  | 23.75  | 17.10  | 5.43               | 20.17 | 27.43 | 0.00    | 18.76  | 0.85   | 0.85   | 15.91  | 2.47   | 7.21   |
|          | 46.30   | 315.7 | 321.62 | 30.53   | 105.82 | 37.17  | 34.43  | 83.92  | 25.35  | 18.88  | 1.99               | 12.87 | 13.21 | 9.02    | 6.14   | 2.49   | 2.16   | 5.89   | 2.63   | 3.84   |
| 3        | 53.52   | 300.5 | 306.21 | 2.14    | 132.90 | 39.18  | 48.14  | 136.94 | 31.27  | 38.00  | 4.68               | 21.27 | 24.37 | 6.88    | 15.83  | 3.30   | 14.40  | 22.24  | 5.21   | 6.64   |
|          | 61.11   | 331.6 | 346.24 | 1.15    | 163.33 | 43.12  | 47.81  | 183.79 | 32.33  | 47.44  | 4.10               | 10.19 | 17.80 | 4.73    | 12.58  | 2.82   | 3.20   | 20.09  | 1.97   | 4.69   |
| 4        | 42.54   | 210.0 | 247.58 | 0.00    | 87.02  | 28.55  | 22.32  | 69.72  | 16.51  | 11.45  | 2.72               | 6.85  | 12.54 | 0.00    | 7.26   | 2.06   | 2.11   | 7.61   | 0.71   | 2.32   |
|          | 38.00   | 205.2 | 243.00 | 25.90   | 85.54  | 26.56  | 18.29  | 62.14  | 16.57  | 11.91  | 1.43               | 4.89  | 9.09  | 5.01    | 4.85   | 2.15   | 1.59   | 6.14   | 2.01   | 1.63   |
| 5        | 43.57   | 230.9 | 210.29 | 57.51   | 79.59  | 30.59  | 23.97  | 55.32  | 18.44  | 14.94  | 3.24               | 8.57  | 9.15  | 9.39    | 4.92   | 3.13   | 1.97   | 4.63   | 1.24   | 8.71   |
|          | 42.58   | 235.8 | 218.20 | 51.30   | 80.40  | 31.87  | 25.89  | 54.95  | 19.71  | 13.47  | 2.30               | 14.03 | 11.78 | 5.98    | 4.94   | 2.14   | 2.59   | 4.49   | 1.10   | 1.48   |
| 6        | 45.26   | 260.8 | 238.30 | 70.19   | 92.86  | 29.39  | 29.96  | 53.01  | 22.17  | 13.73  | 4.06               | 13.49 | 16.09 | 8.45    | 7.61   | 5.42   | 3.15   | 4.55   | 5.40   | 1.82   |
|          | 42.58   | 235.8 | 218.20 | 51.30   | 80.40  | 31.87  | 25.89  | 54.95  | 19.71  | 13.47  | 2.30               | 14.03 | 11.78 | 5.98    | 4.94   | 2.14   | 2.59   | 4.49   | 1.10   | 1.48   |
| 7        | 45.26   | 260.8 | 238.30 | 70.19   | 92.86  | 29.39  | 29.96  | 53.01  | 22.17  | 13.73  | 4.06               | 13.49 | 16.09 | 8.45    | 7.61   | 5.42   | 3.15   | 4.55   | 5.40   | 1.82   |
|          | 42.95   | 228.2 | 221.08 | 70.66   | 78.79  | 30.70  | 24.61  | 48.50  | 17.84  | 13.23  | 1.95               | 10.12 | 10.13 | 4.97    | 7.10   | 2.44   | 2.63   | 4.91   | 1.09   | 1.30   |
| 8        | 45.26   | 260.8 | 238.30 | 70.19   | 92.86  | 29.39  | 29.96  | 53.01  | 22.17  | 13.73  | 4.06               | 13.49 | 16.09 | 8.45    | 7.61   | 5.42   | 3.15   | 4.55   | 5.40   | 1.82   |
|          | 43.57   | 230.9 | 210.29 | 57.51   | 79.59  | 30.59  | 23.97  | 55.32  | 18.44  | 14.94  | 3.24               | 8.57  | 9.15  | 9.39    | 4.92   | 3.13   | 1.97   | 4.63   | 1.24   | 8.71   |
| 9        | 43.65   | 241.7 | 222.82 | 55.16   | 84.50  | 31.80  | 24.83  | 60.60  | 19.57  | 13.59  | 2.48               | 8.64  | 4.12  | 6.94    | 6.28   | 2.76   | 1.63   | 5.70   | 0.81   | 1.65   |
|          | 43.57   | 230.9 | 210.29 | 57.51   | 79.59  | 30.59  | 23.97  | 55.32  | 18.44  | 14.94  | 3.24               | 8.57  | 9.15  | 9.39    | 4.92   | 3.13   | 1.97   | 4.63   | 1.24   | 8.71   |
| 10       | 40.93   | 298.2 | 202.33 | 0.00    | 107.63 | 35.80  | 34.83  | 68.20  | 33.73  | 18.00  | 1.32               | 16.75 | 12.47 | 0.00    | 2.18   | 1.30   | 1.15   | 5.71   | 2.25   | 1.45   |
|          | 58.31   | 407.9 | 275.41 | 0.00    | 186.37 | 48.95  | 56.75  | 100.73 | 43.92  | 21.67  | 2.90               | 24.42 | 11.52 | 0.00    | 17.64  | 2.61   | 3.96   | 10.23  | 2.61   | 2.99   |
| 11       | 50.11   | 411.2 | 267.70 | 0.00    | 165.30 | 47.14  | 51.46  | 86.95  | 43.57  | 21.61  | 1.44               | 14.58 | 12.88 | 0.00    | 7.96   | 1.70   | 4.69   | 6.56   | 2.24   | 1.08   |
|          | 61.44   | 467.0 | 322.58 | 0.00    | 191.29 | 52.20  | 63.93  | 85.08  | 45.33  | 19.19  | 5.49               | 33.83 | 26.81 | 0.00    | 16.32  | 3.73   | 6.34   | 7.64   | 4.05   | 3.95   |
| 12       | 52.48   | 374.0 | 291.71 | 160.98  | 139.78 | 42.89  | 52.95  | 86.85  | 30.39  | 27.29  | 6.60               | 16.00 | 13.65 | 16.16   | 6.04   | 2.94   | 11.79  | 6.03   | 1.95   | 2.58   |
|          | 55.27   | 394.7 | 317.25 | 157.33  | 148.31 | 46.53  | 61.89  | 95.66  | 78.15  | 29.60  | 4.27               | 17.29 | 59.69 | 10.97   | 10.85  | 1.67   | 15.01  | 19.35  | 111.14 | 2.06   |

# CHAPTER 6

|    |       |       |        |       |        |       |       |        |       |       |      |       |       |       |       |       |       |       |       |       |
|----|-------|-------|--------|-------|--------|-------|-------|--------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 13 | 52.96 | 299.8 | 307.82 | 2.49  | 127.97 | 37.95 | 42.65 | 131.72 | 29.20 | 36.30 | 4.29 | 26.35 | 32.36 | 9.64  | 12.80 | 2.34  | 11.70 | 17.76 | 2.98  | 6.01  |
|    | 53.91 | 301.0 | 305.11 | 1.89  | 136.19 | 40.26 | 51.80 | 140.43 | 32.60 | 39.43 | 4.96 | 17.40 | 17.40 | 4.23  | 16.96 | 3.67  | 15.02 | 24.43 | 5.91  | 6.93  |
| 14 | 75.11 | 521.1 | 448.66 | 94.45 | 264.28 | 57.53 | 68.15 | 182.08 | 53.50 | 42.58 | 4.57 | 20.99 | 28.06 | 9.92  | 14.96 | 2.79  | 4.21  | 12.24 | 2.94  | 4.00  |
|    | 70.80 | 478.0 | 418.00 | 91.00 | 229.56 | 63.20 | 85.79 | 160.67 | 64.43 | 40.55 | 9.82 | 26.25 | 24.19 | 9.64  | 9.09  | 14.39 | 26.90 | 8.77  | 12.37 | 1.63  |
| 15 | 58.12 | 402.0 | 358.08 | 0.00  | 165.59 | 49.00 | 59.16 | 161.42 | 42.92 | 28.18 | 4.51 | 18.33 | 13.11 | 0.00  | 10.90 | 3.23  | 3.01  | 12.58 | 3.61  | 1.95  |
|    | 58.48 | 388.0 | 338.51 | 0.00  | 158.23 | 43.89 | 51.87 | 135.24 | 37.09 | 26.16 | 2.78 | 14.55 | 21.86 | 0.00  | 22.90 | 3.31  | 3.50  | 15.08 | 3.04  | 2.49  |
| 16 | 58.12 | 402.0 | 358.08 | 0.00  | 165.59 | 49.00 | 59.16 | 161.42 | 42.92 | 28.18 | 4.51 | 18.33 | 13.11 | 0.00  | 10.90 | 3.23  | 3.01  | 12.58 | 3.61  | 1.95  |
|    | 58.44 | 383.1 | 336.69 | 0.00  | 150.41 | 45.21 | 50.51 | 132.96 | 38.75 | 27.59 | 2.25 | 16.62 | 29.20 | 0.00  | 10.45 | 2.58  | 5.88  | 11.63 | 1.92  | 3.92  |
| 17 | 58.48 | 388.0 | 338.51 | 0.00  | 158.23 | 43.89 | 51.87 | 135.24 | 37.09 | 26.16 | 2.78 | 14.55 | 21.86 | 0.00  | 22.90 | 3.31  | 3.50  | 15.08 | 3.04  | 2.49  |
|    | 58.44 | 383.1 | 336.69 | 0.00  | 150.41 | 45.21 | 50.51 | 132.96 | 38.75 | 27.59 | 2.25 | 16.62 | 29.20 | 0.00  | 10.45 | 2.58  | 5.88  | 11.63 | 1.92  | 3.92  |
| 18 | 69.22 | 472.0 | 404.45 | 88.20 | 244.15 | 59.29 | 77.81 | 159.75 | 55.99 | 65.89 | 6.82 | 42.40 | 21.94 | 11.74 | 17.83 | 4.09  | 45.27 | 18.91 | 3.26  | 17.63 |
|    | 72.51 | 457.7 | 394.87 | 87.08 | 227.82 | 57.73 | 78.37 | 141.10 | 53.53 | 55.75 | 5.71 | 27.70 | 22.97 | 6.89  | 16.62 | 3.92  | 38.81 | 21.53 | 4.08  | 5.99  |
| 19 | 69.22 | 472.0 | 404.45 | 88.20 | 244.15 | 59.29 | 77.81 | 159.75 | 55.99 | 65.89 | 6.82 | 42.40 | 21.94 | 11.74 | 17.83 | 4.09  | 45.27 | 18.91 | 3.26  | 17.63 |
|    | 70.32 | 488.0 | 402.50 | 76.50 | 208.75 | 59.23 | 91.20 | 181.10 | 53.32 | 66.85 | 4.18 | 43.00 | 38.01 | 1.73  | 31.75 | 1.63  | 48.71 | 34.16 | 3.58  | 15.52 |
| 20 | 72.51 | 457.7 | 394.87 | 87.08 | 227.82 | 57.73 | 78.37 | 141.10 | 53.53 | 55.75 | 5.71 | 27.70 | 22.97 | 6.89  | 16.62 | 3.92  | 38.81 | 21.53 | 4.08  | 5.99  |
|    | 70.32 | 488.0 | 402.50 | 76.50 | 208.75 | 59.23 | 91.20 | 181.10 | 53.32 | 66.85 | 4.18 | 43.00 | 38.01 | 1.73  | 31.75 | 1.63  | 48.71 | 34.16 | 3.58  | 15.52 |
| 21 | 45.10 | 235.3 | 204.50 | 58.67 | 90.40  | 27.63 | 25.50 | 60.73  | 18.00 | 13.30 | 4.40 | 10.35 | 12.94 | 7.23  | 11.14 | 5.01  | 0.70  | 6.45  | 2.17  | 0.75  |
|    | 42.59 | 227.7 | 207.80 | 57.58 | 78.03  | 30.72 | 24.10 | 53.77  | 18.33 | 14.62 | 2.79 | 9.78  | 10.42 | 9.13  | 5.84  | 2.00  | 1.92  | 5.96  | 0.86  | 9.39  |
| 22 | 39.70 | 230.7 | 197.18 | 0.00  | 78.91  | 33.60 | 25.55 | 72.74  | 20.56 | 14.75 | 2.59 | 4.88  | 9.82  | 0.00  | 5.52  | 3.22  | 1.67  | 8.44  | 1.57  | 0.82  |
|    | 42.96 | 236.8 | 207.60 | 0.00  | 90.52  | 31.87 | 26.50 | 66.60  | 21.07 | 15.53 | 3.10 | 11.12 | 11.04 | 0.00  | 10.33 | 1.19  | 1.32  | 4.85  | 0.21  | 0.06  |
| 23 | 42.03 | 231.9 | 215.45 | 51.20 | 79.33  | 31.89 | 25.58 | 55.16  | 19.47 | 13.29 | 2.07 | 12.04 | 10.60 | 6.08  | 4.23  | 2.07  | 2.71  | 4.59  | 1.06  | 1.52  |
|    | 45.01 | 253.2 | 230.42 | 51.76 | 85.19  | 31.78 | 27.24 | 54.03  | 20.69 | 14.26 | 1.63 | 7.57  | 8.85  | 5.84  | 5.27  | 2.53  | 1.29  | 4.15  | 0.63  | 1.04  |
| 24 | 45.05 | 258.5 | 238.04 | 70.19 | 92.58  | 29.39 | 29.67 | 52.80  | 21.69 | 14.08 | 4.14 | 11.02 | 16.72 | 8.45  | 7.76  | 5.42  | 3.08  | 4.63  | 5.32  | 1.25  |
|    | 48.93 | 290.0 | 254.33 | -     | 105.27 | 28.25 | 33.50 | 55.65  | -     | 9.25  | 2.21 | 7.21  | 22.23 | -     | 15.95 | 0.78  | 1.70  | 3.04  | -     | 2.19  |
| 25 | 57.77 | 405.0 | 270.05 | 0.00  | 180.52 | 48.39 | 56.15 | 96.61  | 42.95 | 21.18 | 2.01 | 14.23 | 8.51  | 0.00  | 12.07 | 1.75  | 2.78  | 7.56  | 2.06  | 2.92  |
|    | 59.50 | 432.4 | 285.44 | 0.00  | 201.77 | 51.04 | 59.33 | 105.80 | 46.10 | 22.25 | 3.73 | 18.71 | 12.43 | 0.00  | 19.33 | 2.49  | 4.12  | 9.25  | 2.69  | 2.19  |
| 26 | 51.90 | 405.4 | 235.00 | 0.00  | 193.43 | 43.10 | 53.50 | 107.10 | 40.60 | 14.90 | -    | 21.40 | 24.48 | 0.00  | 15.14 | -     | -     | -     | -     | -     |
|    | 61.50 | 390.8 | 236.96 | 0.00  | 173.65 | 45.05 | 50.03 | 93.67  | 43.07 | 22.63 | 2.69 | 13.33 | 15.33 | 0.00  | 8.84  | 2.33  | 0.80  | 2.94  | 1.70  | 1.04  |

# CHAPTER 6

|    |       |       |        |        |        |       |       |        |       |       |      |       |       |       |       |      |       |       |      |      |
|----|-------|-------|--------|--------|--------|-------|-------|--------|-------|-------|------|-------|-------|-------|-------|------|-------|-------|------|------|
| 27 | 59.50 | 432.4 | 285.44 | 0.00   | 201.77 | 51.04 | 59.33 | 105.80 | 46.10 | 22.25 | 3.73 | 18.71 | 12.43 | 0.00  | 19.33 | 2.49 | 4.12  | 9.25  | 2.69 | 2.19 |
|    | 58.37 | 402.7 | 269.80 | 0.00   | 175.79 | 47.77 | 55.60 | 92.89  | 41.75 | 19.43 | 2.51 | 19.22 | 8.40  | 0.00  | 9.54  | 1.48 | 2.65  | 6.87  | 1.57 | 1.75 |
| 28 | 59.19 | 462.7 | 325.77 | 0.00   | 192.07 | 51.23 | 64.02 | 84.72  | 44.49 | 17.47 | 5.50 | 29.78 | 30.29 | 0.00  | 16.61 | 3.97 | 7.36  | 7.71  | 4.27 | 4.95 |
|    | 59.13 | 437.5 | 303.13 | 0.00   | 175.79 | 50.13 | 63.74 | 81.63  | 42.61 | 18.74 | 5.48 | 26.26 | 20.03 | 0.00  | 9.83  | 2.41 | 7.88  | 6.48  | 2.60 | 1.95 |
| 29 | 47.47 | 344.6 | 322.34 | 118.73 | 127.09 | 40.35 | 45.11 | 90.82  | 25.70 | 19.28 | 4.15 | 11.01 | 12.42 | 15.74 | 5.81  | 1.38 | 10.67 | 5.76  | 1.03 | 1.15 |
|    | 47.64 | 323.1 | 298.67 | 111.15 | 116.57 | 40.34 | 42.69 | 84.01  | 25.71 | 17.77 | 4.34 | 50.89 | 15.82 | 9.41  | 10.64 | 1.91 | 8.25  | 6.42  | 1.17 | 3.44 |
| 30 | 47.58 | 341.4 | 305.21 | 120.50 | 121.23 | 38.96 | 41.60 | 84.92  | 24.91 | 18.89 | 5.60 | 27.41 | 21.84 | 8.42  | 11.64 | 3.61 | 9.23  | 6.04  | 1.44 | 1.43 |
|    | 47.47 | 344.6 | 322.34 | 118.73 | 127.09 | 40.35 | 45.11 | 90.82  | 25.70 | 19.28 | 4.15 | 11.01 | 12.42 | 15.74 | 5.81  | 1.38 | 10.67 | 5.76  | 1.03 | 1.15 |
| 31 | 47.47 | 344.6 | 322.34 | 118.73 | 127.09 | 40.35 | 45.11 | 90.82  | 25.70 | 19.28 | 4.15 | 11.01 | 12.42 | 15.74 | 5.81  | 1.38 | 10.67 | 5.76  | 1.03 | 1.15 |
|    | 47.62 | 329.4 | 300.93 | 114.43 | 118.12 | 39.77 | 42.33 | 84.31  | 25.44 | 18.14 | 4.70 | 44.46 | 17.97 | 9.96  | 10.99 | 2.73 | 8.43  | 6.19  | 1.29 | 2.93 |
| 32 | 49.66 | 308.4 | 331.24 | 0.00   | 122.91 | 39.47 | 50.32 | 116.01 | 32.70 | 34.98 | 5.80 | 7.52  | 18.14 | 0.00  | 10.15 | 3.46 | 15.48 | 12.07 | 2.42 | 3.98 |
|    | 52.11 | 318.6 | 349.42 | 0.00   | 130.34 | 40.46 | 48.54 | 123.97 | 32.17 | 36.45 | 4.08 | 11.30 | 22.11 | 0.00  | 10.78 | 2.30 | 13.17 | 22.84 | 2.07 | 5.43 |
| 33 | 77.32 | 527.5 | 462.42 | 94.69  | 263.38 | 58.56 | 67.94 | 181.06 | 54.83 | 42.28 | 4.60 | 20.38 | 25.88 | 12.25 | 15.53 | 2.73 | 4.59  | 12.69 | 2.70 | 4.63 |
|    | 71.43 | 498.3 | 424.06 | 93.33  | 249.99 | 58.98 | 76.08 | 173.73 | 55.24 | 42.33 | 6.40 | 27.46 | 20.73 | 6.57  | 22.23 | 9.98 | 19.36 | 15.75 | 8.98 | 2.64 |
| 34 | 55.32 | 365.7 | 322.25 | 0.00   | 140.65 | 41.58 | 45.78 | 121.04 | 35.25 | 27.76 | 3.11 | 12.60 | 19.98 | 0.00  | 12.38 | 3.94 | 4.77  | 15.88 | 3.72 | 3.30 |
|    | 52.98 | 353.0 | 315.25 | 0.00   | 132.10 | 44.20 | 47.68 | 120.93 | 34.55 | 24.58 | 2.11 | 11.37 | 27.27 | 0.00  | 8.92  | 1.70 | 1.49  | 2.40  | 3.61 | 0.69 |
| 35 | 52.98 | 353.0 | 315.25 | 0.00   | 132.10 | 41.18 | 47.68 | 120.93 | 28.50 | 24.58 | 2.11 | 11.37 | 27.27 | 0.00  | 8.92  | 2.20 | 1.49  | 2.40  | 7.55 | 0.69 |
|    | 55.32 | 365.7 | 322.25 | 0.00   | 140.65 | 41.58 | 45.78 | 121.04 | 35.25 | 27.76 | 3.11 | 12.60 | 19.98 | 0.00  | 12.38 | 3.94 | 4.77  | 15.88 | 3.72 | 3.30 |
| 36 | 58.30 | 394.5 | 236.50 | 0.00   | 178.11 | 44.40 | 50.90 | 97.03  | 42.45 | 20.70 | 5.86 | 16.58 | 17.41 | 0.00  | 13.29 | 2.00 | 1.85  | 7.13  | 1.86 | 3.96 |
|    | 57.16 | 407.3 | 270.30 | 0.00   | 185.25 | 49.02 | 56.70 | 100.33 | 44.15 | 22.93 | 1.18 | 6.83  | 9.07  | 0.00  | 12.84 | 1.83 | 2.91  | 6.50  | 1.82 | 2.84 |
| 37 | 58.86 | 413.7 | 278.05 | 0.00   | 189.42 | 49.44 | 57.68 | 101.48 | 44.05 | 21.24 | 2.97 | 25.15 | 12.65 | 0.00  | 19.51 | 2.52 | 3.89  | 12.00 | 2.99 | 2.84 |
|    | 58.30 | 394.5 | 236.50 | 0.00   | 178.11 | 44.40 | 50.90 | 97.03  | 42.45 | 20.70 | 5.86 | 16.58 | 17.41 | 0.00  | 13.29 | 2.00 | 1.85  | 7.13  | 1.86 | 3.96 |
| 38 | 58.45 | 384.0 | 286.00 | 0.00   | 198.67 | 50.30 | 59.97 | 120.00 | 45.70 | 24.80 | 1.48 | 19.80 | 5.66  | 0.00  | 17.56 | 2.17 | 4.12  | 7.21  | 1.76 | 3.99 |
|    | 58.91 | 416.8 | 277.21 | 0.00   | 188.22 | 49.33 | 57.38 | 99.07  | 43.83 | 20.77 | 3.11 | 23.94 | 12.98 | 0.00  | 19.79 | 2.59 | 3.85  | 10.29 | 3.07 | 2.40 |
| 39 | 65.45 | 494.0 | 334.30 | 0.00   | 201.35 | 54.56 | 63.95 | 87.92  | 48.04 | 21.37 | 2.65 | 20.60 | 18.59 | 0.00  | 10.84 | 2.97 | 3.78  | 7.63  | 2.93 | 2.35 |
|    | 59.17 | 452.6 | 316.71 | 0.00   | 185.77 | 50.81 | 63.91 | 83.52  | 43.80 | 17.91 | 5.40 | 30.66 | 28.59 | 0.00  | 16.31 | 3.46 | 7.43  | 7.31  | 3.81 | 4.17 |
| 40 | 65.45 | 494.0 | 334.30 | 0.00   | 201.35 | 54.56 | 63.95 | 87.92  | 48.04 | 21.37 | 2.65 | 20.60 | 18.59 | 0.00  | 10.84 | 2.97 | 3.78  | 7.63  | 2.93 | 2.35 |
|    | 59.13 | 437.5 | 303.13 | 0.00   | 175.79 | 50.13 | 63.74 | 81.63  | 42.61 | 18.74 | 5.48 | 26.26 | 20.03 | 0.00  | 9.83  | 2.41 | 7.88  | 6.48  | 2.60 | 1.95 |



# CHAPTER 6

|    |       |       |        |       |        |       |       |        |       |       |      |       |       |       |       |      |       |       |      |       |
|----|-------|-------|--------|-------|--------|-------|-------|--------|-------|-------|------|-------|-------|-------|-------|------|-------|-------|------|-------|
| 41 | 52.55 | 344.8 | 264.33 | 35.13 | 113.61 | 39.80 | 43.60 | 71.25  | 30.11 | 27.42 | 2.07 | 9.09  | 16.48 | 3.74  | 7.87  | 2.02 | 2.57  | 3.44  | 1.84 | 1.22  |
|    | 51.50 | 334.2 | 258.17 | 36.66 | 111.00 | 39.83 | 42.40 | 72.47  | 29.78 | 26.73 | 3.21 | 19.80 | 16.46 | 5.99  | 7.19  | 1.96 | 3.44  | 7.88  | 1.90 | 2.64  |
| 42 | 60.64 | 330.8 | 347.86 | 1.39  | 160.07 | 43.10 | 47.64 | 183.16 | 32.24 | 47.72 | 4.39 | 10.44 | 18.79 | 5.21  | 12.12 | 3.02 | 3.57  | 21.71 | 2.15 | 4.91  |
|    | 63.27 | 335.3 | 338.67 | 0.00  | 174.75 | 43.20 | 48.43 | 186.00 | 32.65 | 46.45 | 0.93 | 9.87  | 11.59 | 0.00  | 6.08  | 2.34 | 1.40  | 15.34 | 1.35 | 4.35  |
| 43 | 49.85 | 260.5 | 241.00 | 0.00  | 94.80  | 29.60 | 36.85 | 81.95  | 27.55 | 22.65 | 3.04 | 0.71  | 1.41  | 0.00  | 3.11  | 2.26 | 1.48  | 0.35  | 0.21 | 0.49  |
|    | 49.61 | 285.0 | 249.92 | 0.00  | 118.76 | 38.40 | 40.87 | 130.14 | 31.48 | 27.58 | 2.90 | 11.79 | 10.92 | 0.00  | 10.48 | 2.78 | 3.97  | 21.15 | 3.45 | 3.75  |
| 44 | 48.90 | 283.0 | 251.50 | 0.00  | 108.95 | 36.70 | 43.90 | 113.50 | 30.60 | 24.85 | 0.42 | 2.83  | 4.95  | 0.00  | 14.35 | 1.84 | 5.52  | 26.87 | 0.42 | 1.06  |
|    | 49.75 | 285.4 | 249.60 | 0.00  | 120.72 | 38.74 | 40.26 | 133.47 | 31.65 | 28.13 | 3.18 | 12.96 | 11.93 | 0.00  | 9.26  | 2.89 | 3.66  | 19.81 | 3.79 | 3.89  |
| 45 | 61.01 | 378.3 | 472.20 | 22.56 | 127.84 | 43.96 | 35.25 | 98.63  | 31.03 | 25.38 | 3.59 | 19.29 | 18.52 | 29.60 | 21.04 | 3.16 | 5.83  | 21.29 | 2.92 | 3.19  |
|    | 60.32 | 362.2 | 438.40 | 13.34 | 122.70 | 41.68 | 36.00 | 94.38  | 29.42 | 22.46 | 6.60 | 23.33 | 49.04 | 29.83 | 18.80 | 2.81 | 3.00  | 9.47  | 3.73 | 1.21  |
| 46 | 44.35 | 235.0 | 217.00 | 60.00 | 81.00  | 32.25 | 25.10 | 60.55  | 18.30 | 12.95 | 2.76 | 11.31 |       |       | 1.98  | 5.73 | 0.28  | 4.03  | 0.14 | 0.78  |
|    | 42.96 | 229.9 | 209.48 | 57.26 | 79.94  | 30.94 | 24.12 | 54.29  | 18.27 | 15.57 | 2.91 | 9.48  | 9.78  | 9.90  | 4.89  | 1.84 | 1.92  | 4.40  | 0.73 | 10.79 |
| 47 | 42.30 | 232.9 | 214.76 | 51.76 | 79.04  | 31.65 | 25.81 | 55.30  | 19.67 | 13.29 | 2.03 | 12.98 | 10.02 | 5.99  | 4.57  | 2.09 | 2.08  | 5.19  | 1.10 | 1.63  |
|    | 41.31 | 229.4 | 217.27 | 49.74 | 80.07  | 32.67 | 24.97 | 54.78  | 18.84 | 13.29 | 2.09 | 9.12  | 12.32 | 6.35  | 3.20  | 1.91 | 4.01  | 2.53  | 0.60 | 1.27  |
| 48 | 42.44 | 229.1 | 219.38 | 68.75 | 78.49  | 31.21 | 25.23 | 47.54  | 17.71 | 12.96 | 1.95 | 12.55 | 11.31 | 4.95  | 5.55  | 2.41 | 0.95  | 2.42  | 0.79 | 1.08  |
|    | 43.27 | 227.7 | 222.13 | 71.84 | 78.98  | 30.38 | 24.20 | 49.09  | 17.92 | 13.41 | 1.96 | 8.83  | 9.66  | 4.79  | 8.13  | 2.51 | 3.31  | 5.97  | 1.26 | 1.44  |
| 49 | 66.90 | 470.7 | 533.94 | 60.49 | 159.80 | 57.62 | 63.93 | 100.96 | 50.54 | 46.88 | 6.40 | 31.25 | 132.6 | 7.15  | 12.74 | 5.16 | 7.51  | 6.14  | 3.08 | 6.03  |
|    | 65.21 | 485.4 | 622.34 | 66.93 | 167.61 | 59.33 | 64.86 | 101.54 | 50.90 | 48.56 | 5.88 | 24.16 | 194.6 | 10.09 | 8.85  | 2.45 | 16.34 | 6.61  | 2.84 | 5.97  |
| 50 | 44.01 | 232.5 | 210.80 | 57.64 | 77.21  | 30.76 | 22.87 | 55.51  | 18.50 | 14.09 | 3.31 | 5.84  | 8.05  | 9.73  | 5.38  | 2.61 | 2.10  | 3.41  | 1.89 | 0.64  |
|    | 42.96 | 229.8 | 209.48 | 57.26 | 79.94  | 30.94 | 24.12 | 54.29  | 18.27 | 15.57 | 2.91 | 9.48  | 9.78  | 9.90  | 4.89  | 1.84 | 1.92  | 4.40  | 0.73 | 10.79 |
| 51 | 44.01 | 232.5 | 210.80 | 57.64 | 77.21  | 30.76 | 22.87 | 55.51  | 18.50 | 14.09 | 3.31 | 5.84  | 8.05  | 9.73  | 5.38  | 2.61 | 2.10  | 3.41  | 1.89 | 0.64  |
|    | 43.65 | 241.7 | 222.82 | 55.16 | 84.50  | 31.80 | 24.83 | 60.60  | 19.57 | 13.59 | 2.48 | 8.64  | 4.12  | 6.94  | 6.28  | 2.76 | 1.63  | 5.70  | 0.81 | 1.65  |
| 52 | 40.32 | 214.6 | 201.08 | 53.37 | 69.83  | 29.53 | 23.08 | 47.11  | 17.18 | 12.48 | 2.57 | 6.22  | 9.38  | 7.38  | 4.14  | 1.73 | 1.54  | 3.62  | 0.76 | 1.44  |
|    | 40.70 | 216.8 | 197.25 | 57.03 | 66.90  | 28.18 | 22.78 | 47.70  | 16.80 | 12.18 | 0.24 | 6.40  | 12.84 | 11.03 | 2.91  | 2.01 | 2.08  | 3.14  | 0.40 | 0.59  |
| 53 | 48.30 | 268.4 | 231.63 | 74.20 | 97.13  | 31.46 | 31.38 | 52.48  | 20.25 | 13.65 | 6.13 | 6.39  | 23.01 | 10.03 | 5.98  | 3.51 | 2.85  | 3.22  | 0.86 | 1.82  |
|    | 43.52 | 253.9 | 241.06 | 68.30 | 90.44  | 31.94 | 28.87 | 52.95  | 18.84 | 14.29 | 1.21 | 9.64  | 12.55 | 7.17  | 7.71  | 1.93 | 2.92  | 5.25  | 0.75 | 0.88  |
| 54 | 48.30 | 268.4 | 231.63 | 74.20 | 97.13  | 31.46 | 31.38 | 52.48  | 20.25 | 13.65 | 6.13 | 6.39  | 23.01 | 10.03 | 5.98  | 3.51 | 2.85  | 3.22  | 0.86 | 1.82  |
|    | 48.93 | 290.0 | 254.33 | -     | 105.27 | 28.25 | 33.50 | 55.65  | -     | 9.25  | 2.21 | 7.21  | 22.23 | -     | 15.95 | 0.78 | 1.70  | 3.04  | -    | 2.19  |

# CHAPTER 6

| TARSU<br>S | WING | TAI<br>L | TER<br>BND | Sample size |           |           | CASQ<br>L | CASQ<br>W | CASQ<br>H | Total samples |     |      | Remarks/ references                      |
|------------|------|----------|------------|-------------|-----------|-----------|-----------|-----------|-----------|---------------|-----|------|------------------------------------------|
|            |      |          |            | BILL L      | BILL<br>W | BILL<br>H |           |           |           | Min           | Max | Mean |                                          |
| 35         | 35   | 35       | 35         | 35          | 35        | 35        | 35        | 35        | 35        | 35            | 35  | 35.0 |                                          |
| 9          | 9    | 9        | 9          | 9           | 9         | 9         | 9         | 9         | 9         | 9             | 9   | 9.0  |                                          |
| 5          | 5    | 5        | 2          | 5           | 2         | 2         | 2         | 2         | 2         | 2             | 5   | 3.1  | Kemp 1995; in part measured by N. Collar |
| 13         | 13   | 13       | 13         | 13          | 13        | 13        | 13        | 13        | 13        | 13            | 13  | 13.0 | in part measured by N. Collar            |
| 54         | 54   | 54       | 54         | 55          | 44        | 55        | 55        | 51        | 44        | 44            | 55  | 51.3 |                                          |
| 17         | 17   | 17       | 17         | 18          | 18        | 18        | 18        | 18        | 18        | 17            | 18  | 17.5 |                                          |
| 13         | 13   | 12       | 12         | 13          | 13        | 13        | 13        | 13        | 13        | 12            | 13  | 12.7 |                                          |
| 10         | 10   | 10       | 10         | 10          | 10        | 10        | 10        | 10        | 10        | 10            | 10  | 10.0 |                                          |
| 43         | 42   | 41       | 42         | 42          | 43        | 43        | 43        | 43        | 43        | 41            | 43  | 42.4 |                                          |
| 49         | 49   | 49       | 49         | 49          | 47        | 49        | 49        | 47        | 49        | 47            | 49  | 48.5 |                                          |
| 27         | 27   | 27       | 25         | 27          | 25        | 27        | 27        | 27        | 27        | 25            | 27  | 26.5 |                                          |
| 49         | 49   | 49       | 49         | 49          | 47        | 49        | 49        | 47        | 49        | 47            | 49  | 48.5 |                                          |
| 27         | 27   | 27       | 25         | 27          | 25        | 27        | 27        | 27        | 27        | 25            | 27  | 26.5 |                                          |
| 21         | 21   | 21       | 21         | 21          | 21        | 20        | 21        | 21        | 20        | 20            | 21  | 20.7 |                                          |
| 27         | 27   | 27       | 25         | 27          | 25        | 27        | 27        | 27        | 27        | 25            | 27  | 26.5 |                                          |
| 43         | 42   | 41       | 42         | 42          | 43        | 43        | 43        | 43        | 43        | 41            | 43  | 42.4 |                                          |
| 22         | 22   | 22       | 22         | 22          | 22        | 22        | 22        | 22        | 22        | 22            | 22  | 22.0 |                                          |
| 43         | 42   | 41       | 42         | 42          | 43        | 43        | 43        | 43        | 43        | 41            | 43  | 42.4 |                                          |
| 6          | 6    | 6        | 3          | 3           | 3         | 3         | 3         | 3         | 3         | 3             | 6   | 3.8  | Kemp 1995                                |
| 34         | 34   | 34       | 34         | 42          | 41        | 42        | 42        | 42        | 42        | 34            | 42  | 38.3 |                                          |
| 10         | 10   | 10       | 10         | 10          | 10        | 10        | 10        | 10        | 10        | 10            | 10  | 10.0 |                                          |
| 47         | 46   | 45       | 45         | 48          | 46        | 48        | 48        | 47        | 46        | 45            | 48  | 46.5 |                                          |
| 14         | 14   | 14       | 12         | 14          | 8         | 14        | 14        | 12        | 12        | 8             | 14  | 12.4 |                                          |
| 7          | 7    | 8        | 4          | 8           | 6         | 7         | 8         | 6         | 6         | 4             | 8   | 6.5  |                                          |
| 22         | 22   | 22       | 22         | 22          | 21        | 22        | 22        | 20        | 20        | 20            | 22  | 21.4 |                                          |

## CHAPTER 6

|    |    |    |    |    |    |    |    |    |    |    |    |      |                                                |
|----|----|----|----|----|----|----|----|----|----|----|----|------|------------------------------------------------|
| 32 | 32 | 32 | 32 | 33 | 23 | 33 | 33 | 31 | 24 | 23 | 33 | 29.8 |                                                |
| 22 | 22 | 22 | 22 | 25 | 25 | 25 | 25 | 25 | 25 | 22 | 25 | 23.6 |                                                |
| 7  | 6  | 7  | 4  | 7  | 7  | 7  | 7  | 4  | 4  | 4  | 7  | 5.8  |                                                |
| 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13.0 |                                                |
| 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35.0 |                                                |
| 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13.0 |                                                |
| 17 | 17 | 16 | 16 | 17 | 17 | 17 | 17 | 17 | 17 | 16 | 17 | 16.7 |                                                |
| 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35 | 35.0 |                                                |
| 17 | 17 | 16 | 16 | 17 | 17 | 17 | 17 | 17 | 17 | 16 | 17 | 16.7 |                                                |
| 18 | 18 | 18 | 15 | 19 | 17 | 19 | 19 | 16 | 16 | 15 | 19 | 17.3 |                                                |
| 15 | 15 | 15 | 12 | 16 | 16 | 16 | 16 | 13 | 13 | 12 | 16 | 14.5 |                                                |
| 18 | 18 | 18 | 15 | 19 | 17 | 19 | 19 | 16 | 16 | 15 | 19 | 17.3 |                                                |
| 6  | 6  | 6  | 4  | 6  | 4  | 6  | 6  | 6  | 4  | 4  | 6  | 5.3  |                                                |
| 15 | 15 | 15 | 12 | 16 | 16 | 16 | 16 | 13 | 13 | 12 | 16 | 14.5 |                                                |
| 6  | 6  | 6  | 4  | 6  | 4  | 6  | 6  | 6  | 4  | 4  | 6  | 5.3  |                                                |
| 6  | 6  | 6  | 3  | 3  | 3  | 3  | 3  | 3  | 3  | 3  | 6  | 3.8  | Kemp 1995; in part measured by J. Dean (NMNH)  |
| 38 | 37 | 35 | 37 | 38 | 38 | 38 | 38 | 38 | 38 | 35 | 38 | 37.3 |                                                |
| 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11.0 | Kemp 1995; in part measured by N. Collar       |
| 5  | 5  | 5  | 2  | 5  | 3  | 3  | 3  | 3  | 3  | 2  | 5  | 3.5  | Kemp 1995                                      |
| 40 | 40 | 40 | 40 | 40 | 38 | 40 | 40 | 38 | 40 | 38 | 40 | 39.5 |                                                |
| 9  | 9  | 9  | 9  | 9  | 9  | 9  | 9  | 9  | 9  | 9  | 9  | 9.0  |                                                |
| 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25.0 |                                                |
| 3  | 3  | 3  | -  | 3  | 2  | 2  | 2  | -  | 2  | 2  | 3  | 2.4  | Kemp 1995; in part measured by J. Woods (DMNH) |
| 20 | 20 | 20 | 20 | 24 | 24 | 24 | 24 | 24 | 24 | 20 | 24 | 22.2 |                                                |
| 9  | 9  | 9  | 9  | 11 | 11 | 11 | 11 | 11 | 11 | 9  | 11 | 10.1 |                                                |
| 1  | 7  | 7  | 7  | 7  | 1  | 1  | 1  | 1  | 1  | 1  | 7  | 3.2  | Mayr 1934                                      |

# CHAPTER 6

|    |    |    |    |    |    |    |    |    |    |    |    |      |                                               |
|----|----|----|----|----|----|----|----|----|----|----|----|------|-----------------------------------------------|
| 2  | 21 | 23 | 23 | 24 | 2  | 3  | 3  | 3  | 3  | 2  | 24 | 9.9  | Mayr 1934                                     |
| 9  | 9  | 9  | 9  | 11 | 11 | 11 | 11 | 11 | 11 | 9  | 11 | 10.1 |                                               |
| 10 | 10 | 10 | 10 | 12 | 12 | 12 | 12 | 12 | 12 | 10 | 12 | 11.1 |                                               |
| 18 | 18 | 18 | 18 | 19 | 18 | 19 | 19 | 19 | 19 | 18 | 19 | 18.5 |                                               |
| 12 | 12 | 12 | 12 | 12 | 11 | 12 | 12 | 11 | 10 | 10 | 12 | 11.5 |                                               |
| 16 | 16 | 16 | 11 | 16 | 11 | 16 | 16 | 11 | 11 | 11 | 16 | 13.7 |                                               |
| 17 | 17 | 17 | 13 | 18 | 10 | 18 | 18 | 14 | 14 | 10 | 18 | 15.1 |                                               |
| 9  | 9  | 9  | 7  | 9  | 7  | 9  | 9  | 7  | 7  | 7  | 9  | 8.1  |                                               |
| 16 | 16 | 16 | 11 | 16 | 11 | 16 | 16 | 11 | 11 | 11 | 16 | 13.7 |                                               |
| 16 | 16 | 16 | 11 | 16 | 11 | 16 | 16 | 11 | 11 | 11 | 16 | 13.7 |                                               |
| 26 | 26 | 26 | 20 | 27 | 17 | 27 | 27 | 21 | 21 | 17 | 27 | 23.2 |                                               |
| 10 | 9  | 9  | 9  | 10 | 10 | 10 | 10 | 8  | 8  | 8  | 10 | 9.2  |                                               |
| 15 | 14 | 14 | 14 | 15 | 12 | 15 | 15 | 15 | 13 | 12 | 15 | 14.0 |                                               |
| 13 | 13 | 13 | 13 | 16 | 16 | 16 | 16 | 16 | 16 | 13 | 16 | 14.6 |                                               |
| 16 | 15 | 16 | 12 | 16 | 16 | 16 | 16 | 14 | 14 | 12 | 16 | 14.8 |                                               |
| 20 | 20 | 20 | 20 | 22 | 22 | 22 | 22 | 22 | 22 | 20 | 22 | 21.1 |                                               |
| 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4.0  | Kemp 1995; in part measured by J. Dean (NMNH) |
| 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4.0  | in part measured by J. Dean (NMNH)            |
| 20 | 20 | 20 | 20 | 22 | 22 | 22 | 22 | 22 | 22 | 20 | 22 | 21.1 |                                               |
| 3  | 28 | 30 | 30 | 31 | 3  | 4  | 4  | 4  | 4  | 3  | 31 | 13.1 |                                               |
| 10 | 10 | 10 | 10 | 12 | 12 | 12 | 12 | 12 | 12 | 10 | 12 | 11.1 |                                               |
| 21 | 21 | 21 | 21 | 26 | 26 | 26 | 26 | 26 | 26 | 21 | 26 | 23.7 |                                               |
| 3  | 28 | 30 | 30 | 31 | 3  | 4  | 4  | 4  | 4  | 3  | 31 | 13.1 | Mayr 1934                                     |
| 2  | 2  | 2  | 2  | 3  | 3  | 3  | 3  | 3  | 3  | 2  | 3  | 2.5  |                                               |
| 19 | 19 | 19 | 19 | 23 | 23 | 23 | 23 | 23 | 23 | 19 | 23 | 21.2 |                                               |
| 17 | 16 | 15 | 15 | 17 | 17 | 17 | 17 | 17 | 17 | 15 | 17 | 16.4 |                                               |
| 30 | 30 | 30 | 30 | 31 | 29 | 31 | 31 | 30 | 29 | 29 | 31 | 30.0 |                                               |

## CHAPTER 6

|    |    |    |    |    |    |    |    |    |    |    |    |      |                               |
|----|----|----|----|----|----|----|----|----|----|----|----|------|-------------------------------|
| 17 | 16 | 15 | 15 | 17 | 17 | 17 | 17 | 17 | 17 | 15 | 17 | 16.4 |                               |
| 12 | 12 | 12 | 12 | 12 | 11 | 12 | 12 | 11 | 10 | 10 | 12 | 11.5 |                               |
| 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12.0 |                               |
| 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23 | 23.0 |                               |
| 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14.0 | in part measured by N. Collar |
| 3  | 3  | 3  | 3  | 4  | 4  | 4  | 4  | 4  | 4  | 3  | 4  | 3.5  |                               |
| 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2.0  |                               |
| 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12.0 | in part measured by N. Collar |
| 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2.0  |                               |
| 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10.0 |                               |
| 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10.0 |                               |
| 5  | 5  | 5  | 5  | 5  | 5  | 5  | 5  | 5  | 5  | 5  | 5  | 5.0  |                               |
| 2  | 2  | 1  | 1  | 2  | 2  | 2  | 2  | 2  | 2  | 1  | 2  | 1.7  |                               |
| 28 | 27 | 27 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 27 | 28 | 27.7 |                               |
| 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29.0 |                               |
| 11 | 11 | 11 | 11 | 11 | 9  | 11 | 11 | 9  | 11 | 9  | 11 | 10.5 |                               |
| 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8.0  |                               |
| 13 | 13 | 13 | 13 | 13 | 13 | 12 | 13 | 13 | 12 | 12 | 13 | 12.7 |                               |
| 9  | 9  | 9  | 8  | 9  | 9  | 9  | 9  | 9  | 9  | 8  | 9  | 8.8  | in part measured by N. Collar |
| 16 | 16 | 16 | 14 | 16 | 12 | 16 | 16 | 16 | 15 | 12 | 16 | 15.0 | in part measured by N. Collar |
| 10 | 10 | 10 | 10 | 9  | 10 | 10 | 10 | 10 | 10 | 9  | 10 | 9.8  |                               |
| 28 | 27 | 27 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 27 | 28 | 27.7 |                               |
| 10 | 10 | 10 | 10 | 9  | 10 | 10 | 10 | 10 | 10 | 9  | 10 | 9.8  |                               |
| 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22 | 22.0 |                               |
| 26 | 26 | 26 | 26 | 26 | 26 | 26 | 26 | 26 | 26 | 26 | 26 | 26.0 |                               |
| 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4  | 4.0  |                               |
| 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8  | 8.0  |                               |
| 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17.0 |                               |

CHAPTER 6

|   |   |   |   |   |   |   |   |   |   |   |   |     |                                        |
|---|---|---|---|---|---|---|---|---|---|---|---|-----|----------------------------------------|
| 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8.0 | in part measured by J. Dean (NMNH)     |
| 3 | 3 | 3 | - | 3 | 2 | 2 | 2 | - | 2 | 2 | 3 | 2.4 | Kemp 1995; measured by J. Woods (DMNH) |

---

# CHAPTER 6

| Cohen's $d^b$ |        |        |         |        |        |        |        |        |        | Difference |        |       |             | Score <sup>a</sup> |
|---------------|--------|--------|---------|--------|--------|--------|--------|--------|--------|------------|--------|-------|-------------|--------------------|
| TARSUS        | WING   | TAIL   | TER BND | BILL L | BILL W | BILL H | CASQ L | CASQ W | CASQ H | Minor      | Medium | Major | Exceptional |                    |
| -0.902        | -0.508 | -0.669 | -0.724  | -0.611 | -0.320 | -0.747 | -2.676 | 0.176  | 0.619  | 9          | 1      | 0     | 0           | 3                  |
| -             | 0.459  | -1.391 | -3.786  | 0.847  | 0.103  | -0.944 | -0.849 | -0.657 | -0.456 | 7          | 1      | 0     | 0           | 3                  |
| -1.690        | -1.635 | -1.764 | 0.156   | -2.041 | -1.262 | 0.027  | -2.184 | -0.234 | -1.559 | 6          | 2      | 0     | 0           | 2                  |
| 2.101         | 0.825  | 0.432  | -8.084  | 0.243  | 0.994  | 2.209  | 1.130  | -0.046 | -0.237 | 7          | 2      | 1     | 0           | 5                  |
| 0.360         | -0.420 | -0.750 | 0.811   | -0.167 | -0.488 | -0.835 | 0.082  | -1.093 | 0.247  | 9          | 0      | 0     | 0           | 2                  |
| 0.896         | 1.826  | 1.514  | 2.773   | 2.099  | -0.695 | 1.475  | -0.436 | 0.744  | 0.162  | 7          | 2      | 0     | 0           | 3                  |
| 0.712         | 2.740  | 1.273  | -0.068  | 1.944  | -0.309 | 1.858  | 0.977  | 1.073  | 0.313  | 8          | 1      | 0     | 0           | 3                  |
| 0.482         | 2.819  | 2.300  | 1.421   | 2.206  | -0.296 | 2.444  | -0.509 | 1.088  | -0.178 | 5          | 4      | 0     | 0           | 3                  |
| 0.027         | 1.274  | 1.633  | -0.277  | 0.922  | 0.410  | 0.467  | 1.071  | 1.026  | -0.192 | 9          | 0      | 0     | 0           | 2                  |
| -6.499        | -4.781 | -6.434 | -       | -4.675 | -5.252 | -5.793 | -3.307 | -4.015 | -1.280 | 1          | 4      | 4     | 0           | 3                  |
| -2.282        | -1.807 | -2.236 | -       | -1.730 | -1.482 | -2.079 | 0.255  | -0.471 | 0.679  | 6          | 3      | 0     | 0           | 3                  |
| -0.492        | -1.325 | -0.724 | 0.256   | -1.111 | -1.580 | -0.729 | -0.743 | -0.815 | -1.008 | 10         | 0      | 0     | 0           | 2                  |
| -0.205        | -0.053 | 0.113  | 0.088   | -0.542 | -0.759 | -0.675 | -0.403 | -0.696 | -0.491 | 9          | 0      | 0     | 0           | 2                  |

## CHAPTER 6

|        |        |        |        |        |        |        |        |        |        |   |   |   |   |   |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---|---|---|---|---|
| 0.728  | 2.024  | 1.166  | 0.364  | 2.564  | -0.848 | -1.445 | 1.901  | -2.278 | 0.553  | 7 | 3 | 0 | 0 | 4 |
| -0.111 | 0.914  | 1.002  | -      | 0.368  | 1.587  | 2.202  | 1.848  | 1.860  | 0.876  | 7 | 1 | 0 | 0 | 2 |
| -0.097 | 1.126  | 0.945  | -      | 1.476  | 1.365  | 1.842  | 2.445  | 1.555  | 0.191  | 7 | 1 | 0 | 0 | 2 |
| 0.016  | 0.329  | 0.077  | -      | 0.403  | -0.432 | 0.316  | 0.165  | -0.618 | -0.483 | 7 | 0 | 0 | 0 | 2 |
| -0.535 | 0.404  | 0.441  | 0.117  | 0.973  | 0.404  | -0.014 | 0.954  | 0.700  | 0.766  | 8 | 0 | 0 | 0 | 2 |
| -0.182 | -0.394 | 0.077  | 1.158  | 1.706  | 0.019  | -0.303 | -0.964 | 0.841  | -0.058 | 9 | 0 | 0 | 0 | 2 |
| 0.430  | -0.983 | -0.289 | 1.836  | 0.933  | -0.435 | -0.324 | -1.659 | 0.057  | -1.348 | 9 | 0 | 0 | 0 | 2 |
| 0.849  | 0.793  | -0.314 | 0.123  | 2.039  | -1.404 | 0.765  | 1.193  | -0.354 | -0.148 | 7 | 0 | 0 | 0 | 3 |
| -1.268 | -0.897 | -1.094 | -      | -1.717 | 0.629  | -0.631 | 0.834  | -0.378 | -1.121 | 9 | 0 | 0 | 0 | 2 |
| -1.523 | -1.911 | -1.481 | -0.093 | -1.354 | 0.053  | -0.672 | 0.254  | -1.243 | -0.679 | 9 | 0 | 0 | 0 | 2 |
| -1.001 | -3.031 | -0.983 | -      | -1.519 | 0.222  | -1.311 | -0.648 | -      | 3.856  | 7 | 1 | 0 | 0 | 4 |
| -0.682 | -1.812 | -1.622 | -      | -1.493 | -1.361 | -1.008 | -1.167 | -1.428 | -0.404 | 9 | 0 | 0 | 0 | 2 |
| -      | 0.973  | -0.114 | -      | 1.955  | -      | -      | -      | -      | -      | 3 | 0 | 0 | 0 | 2 |
| 0.380  | 1.657  | 1.576  | -      | 1.810  | 1.690  | 1.137  | 1.669  | 2.095  | 1.497  | 8 | 1 | 0 | 0 | 2 |



## CHAPTER 6

|        |        |        |        |        |        |        |        |        |        |   |   |   |   |   |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---|---|---|---|---|
| 0.013  | 0.919  | 0.877  | -      | 1.167  | 0.330  | 0.038  | 0.440  | 0.517  | -0.314 | 8 | 0 | 0 | 0 | 2 |
| -0.040 | 0.595  | 1.711  | 0.624  | 1.245  | 0.009  | 0.263  | 1.147  | -0.007 | 0.585  | 7 | 0 | 0 | 0 | 1 |
| 0.024  | -0.183 | -1.094 | 0.140  | -0.735 | -0.602 | -0.359 | -1.049 | -0.695 | -0.332 | 8 | 0 | 0 | 0 | 1 |
| -0.033 | 0.435  | 1.361  | 0.363  | 0.974  | 0.263  | 0.306  | 1.103  | 0.220  | 0.474  | 9 | 0 | 0 | 0 | 1 |
| -0.528 | -1.062 | -0.919 | -      | -0.735 | -0.360 | 0.131  | -0.429 | 0.255  | -0.315 | 8 | 0 | 0 | 0 | 2 |
| 1.077  | 1.236  | 1.716  | 0.142  | 0.721  | -0.058 | -0.597 | 0.530  | -0.067 | -0.015 | 6 | 0 | 0 | 0 | 2 |
| 0.816  | 1.062  | 0.346  | -      | 0.742  | -0.730 | -0.439 | 0.008  | 0.197  | 1.070  | 7 | 0 | 0 | 0 | 2 |
| -0.816 | -1.062 | -0.346 | -      | -0.742 | -0.112 | 0.439  | -0.008 | -1.602 | -1.070 | 7 | 0 | 0 | 0 | 2 |
| 0.456  | -0.892 | -2.190 | -      | -0.555 | -2.676 | -2.279 | -0.531 | -0.993 | -0.767 | 6 | 3 | 0 | 0 | 3 |
| 0.176  | 0.948  | 2.709  | -      | 0.702  | 2.099  | 1.883  | 0.398  | 0.572  | 0.187  | 6 | 2 | 0 | 0 | 2 |
| -0.157 | -1.452 | 0.728  | -      | 0.555  | 0.395  | 0.694  | 2.163  | 0.652  | 1.627  | 8 | 1 | 0 | 0 | 3 |
| 1.393  | 1.532  | 0.698  | -      | 1.087  | 1.164  | 0.005  | 0.605  | 1.231  | 0.980  | 8 | 0 | 0 | 0 | 1 |
| 1.619  | 2.534  | 1.684  | -      | 2.537  | 1.662  | 0.037  | 0.907  | 2.007  | 1.234  | 5 | 3 | 0 | 0 | 2 |
| 0.377  | 0.638  | 0.385  | -0.295 | 0.362  | -0.018 | 0.387  | -0.187 | 0.181  | 0.310  | 7 | 0 | 0 | 0 | 2 |

## CHAPTER 6

|        |        |        |        |        |        |        |        |        |        |    |   |   |   |   |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----|---|---|---|---|
| -0.681 | -0.471 | 0.544  | 0.306  | -1.386 | -0.036 | -0.256 | -0.146 | -0.217 | 0.281  | 10 | 0 | 0 | 0 | 2 |
| 0.090  | -2.343 | -0.921 | -      | -2.568 | -3.463 | -1.135 | -2.571 | -1.282 | -1.481 | 4  | 4 | 0 | 0 | 2 |
| -0.309 | -0.213 | 0.182  | -      | -1.304 | -0.798 | 1.026  | -1.061 | -0.320 | -0.970 | 8  | 0 | 0 | 0 | 2 |
| 0.157  | 0.839  | 1.161  | 0.334  | 0.271  | 0.801  | -0.157 | 0.247  | 0.543  | 1.147  | 8  | 0 | 0 | 0 | 1 |
| 0.495  | 0.566  | 0.798  | 0.287  | 0.227  | 0.646  | 0.538  | 1.475  | 0.046  | -0.256 | 10 | 0 | 0 | 0 | 2 |
| 0.499  | 0.298  | -0.242 | 0.341  | -0.248 | -0.510 | 0.316  | 0.114  | 0.835  | 0.001  | 9  | 0 | 0 | 0 | 2 |
| -0.447 | 0.150  | -0.281 | -0.670 | -0.071 | 0.356  | 0.407  | -0.329 | -0.199 | -0.358 | 9  | 0 | 0 | 0 | 2 |
| 0.291  | -0.571 | -0.525 | -0.737 | -0.785 | -0.470 | -0.070 | -0.095 | -0.127 | -0.293 | 8  | 0 | 0 | 0 | 2 |
| 0.358  | 0.322  | 0.145  | 0.040  | -0.561 | -0.088 | -0.655 | 0.299  | 0.209  | -0.162 | 8  | 0 | 0 | 0 | 2 |
| 0.137  | -1.199 | -2.216 | 0.325  | -1.247 | -0.396 | -1.133 | -1.027 | -0.891 | 0.362  | 8  | 1 | 0 | 0 | 3 |
| -0.161 | -0.367 | 0.404  | -0.482 | 0.753  | 0.797  | 0.197  | -0.172 | 0.537  | 0.232  | 7  | 0 | 0 | 0 | 2 |
| 1.410  | 1.721  | -0.598 | 0.755  | 0.965  | -0.198 | 0.901  | -0.104 | 1.876  | -0.536 | 10 | 0 | 0 | 0 | 2 |
| -0.127 | -3.632 | -1.099 | -      | -0.980 | 1.091  | -0.870 | -1.109 | -      | 2.633  | 4  | 2 | 0 | 0 | 4 |

<sup>a</sup> Total score represents the two highest or lowest d values among the ten measurements

<sup>b</sup> Scoring system for Cohen's d value: 1=minor (0.2-2), 2=medium (2-5), 3=major (5-10), 4 =exceptional(>10)

## CHAPTER 6

**Table S6.6:** Scoring for plumage characters and bare parts for 54 hornbill pairs analyzed for morphological divergence using standard quantitative criteria (Tobias *et al.* 2010).

| Pair No. | Character (bare parts) <sup>a</sup>                                                                      | Character (plumage) <sup>a</sup>                                                                                       | Score <sup>b</sup> |
|----------|----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|--------------------|
| 1        | bill-casque red, blue-black grooves at base of lower mandible (2), facial skin red-orange (2)            | black tipped white tail stained cream by oil (1), face & neck to upper breast white (2)                                | 6                  |
|          | bill-casque orange-red, base of lower mandible ridged, facial skin saffron-yellow                        | black tipped white tail stained rufous by oil, face & neck to upper breast rufous                                      |                    |
| 2        | both sexes with pale yellow bill (3), male with narrower, lower, less truncate casque (biometric)        | white cheeks and throat (2), female without or very narrow white tips to tail (2)                                      | 7                  |
|          | male with pale yellow bill, female with dusky brown bill                                                 | rufous brown cheeks and throat                                                                                         |                    |
| 3        | smaller blotch on casque (1), light blue & white facial skin (2)                                         | outer tail either all white or half black & white                                                                      | 4                  |
|          | larger blotch on casque, dark blue & pink facial skin                                                    | outer tail all white                                                                                                   |                    |
| 4        | bill-casque orange-yellow in both sexes with pale base (2) with oval nostrils (1), facial skin black (1) | paler plumage with pale eyebrow stripe (2), grey breast & rusty vent (2), white tips on outer tail (2)                 | 6                  |
|          | bill cream with dusky base with circular nostrils, but bill black in female, facial skin purple          | dark grey plumage with pale streaks on head, white breast & vent, white outer tail                                     |                    |
| 5        | bill horn-coloured with black base above, brown & yellow ridges below (2), facial skin blue (2)          | tail-coverts black (2), tail yellowish-brown with black base & tip (2)                                                 | 6                  |
|          | brown-black bill-casque with pale yellow & pink ridges, pale bill tip, facial skin pink                  | tail-coverts dark brown, tail dark brown with pale band across tail                                                    |                    |
| 6        | blackish maroon bill-casque with prominent red & yellow ridges (2), facial skin white (2)                | underparts rufous (3), tail-coverts rufous (2), tail reddish-yellow (2), olive-green gloss above                       | 7                  |
|          | brown-black bill-casque with pale yellow & pink ridges, pale bill tip, facial skin pink                  | underparts cream, tail-coverts & tail dark brown with pale band across tail                                            |                    |
| 7        | blackish maroon bill-casque with prominent red & yellow ridges (2), facial skin white (2)                | underparts rufous (3), tail-coverts rufous (2), tail reddish-yellow (2), olive-green gloss above, female all black (3) | 8                  |
|          | brown-black bill-casque with yellow ridges & tip, low casque, facial skin flesh-pink                     | underparts yellowish-white, tail-coverts & tail black with large beige-rufous patch                                    |                    |
| 8        | blackish maroon bill-casque with prominent red & yellow ridges (2), facial skin white (2)                | underparts rufous (3), tail-coverts rufous (2), tail reddish-yellow (1), olive-green gloss above                       | 7                  |
|          | bill horn-coloured with black base above, brown & yellow ridges below, facial skin blue                  | underparts cream, tail-coverts black, tail yellowish-brown with black base & tip                                       |                    |
| 9        | black patch at base of upper mandible, maroon-coloured bill tip (1)                                      | slightly larger, pale rufous rump (3), black base on tail minimal (1), more ochre on tail (1)                          | 5                  |
|          | black patch at base of upper mandible, horn-coloured bill tip                                            | black rump, black base on tail moderate, tail black with central ochre patch                                           |                    |
| 10       | bill yellowish-white with dark crimson base (2)                                                          | head and neck deep to golden rufous (1)                                                                                | 3                  |
|          | bill pale yellow with dusky brown base                                                                   | head and neck deep/dark to golden rufous                                                                               |                    |
| 11       | smaller bill with dark red base with ridges on mandibles (3) & dark bar on throat sac absent (2)         | crown and nape reddish to pale rufous with pale rufous wash on side of face (1)                                        | 6                  |
|          | larger bill with no red base, ridges on mandibles & dark bar on throat sac present                       | crown and nape dark rufous-brown, no pale rufous wash on side of face                                                  |                    |
| 12       |                                                                                                          | slightly smaller                                                                                                       | 0                  |

## CHAPTER 6

|    |                                                                                                                                                                                        |                                                                                                                                                                                         |   |
|----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| 13 | black patch on distal bill and casque and at base of bill & casque (1), black spot at eye (1)<br>slightly larger bill and casque with less black patches on bill, no black spot at eye | white tip on outer tail (2)<br>outer tail all white                                                                                                                                     | 4 |
| 14 |                                                                                                                                                                                        |                                                                                                                                                                                         | 0 |
| 15 | black based red bill-casque (3), circumorbital skin yellow (1), feet red-brown (1)<br>outer half of bill pale yellow, circumorbital skin yellow & black, feet more reddish             | juvenile with black band across central tail (3)<br>juvenile tail with black base and no clear central band                                                                             | 7 |
| 16 | black based red bill-casque (3), circumorbital skin yellow (1), feet red-brown (1)<br>juvenile tail with black base and no clear central band                                          | juvenile with black band having chestnut margins across central tail (3)                                                                                                                | 7 |
| 17 | bill-casque with distal projection (not biometric, 1)<br>no projection in casque and tip wedge shaped                                                                                  | juvenile with black band across central tail (1)<br>juvenile with black band having chestnut margins across central tail                                                                | 2 |
| 18 | large cylindrical casque with black base and slightly recurved tip (not biometric, 1)<br>cylindrical casque shorter & broader with more sharply recurved tip                           | black band across white tail (1)<br>slightly smaller, black band across white tail broader                                                                                              | 2 |
| 19 | large cylindrical casque with black base slight recurved tip (not biometric, 1)<br>cylindrical casque longer with non-recurved tip                                                     | black band across white tail (1)<br>black band across white tail broader                                                                                                                | 2 |
| 20 | cylindrical casque shorter & broader with more sharply recurved tip (not biometric, 1)<br>cylindrical casque longer with non-recurved tip                                              |                                                                                                                                                                                         | 1 |
| 21 | flesh patch at base of upper mandible and flesh-coloured bill tip (2)<br>black patch at base of upper mandible, horn-coloured bill tip                                                 | black rump, black base on tail very minimal and more rufous (2)<br>black rump, black base on tail moderate                                                                              | 4 |
| 22 | black at base of lower mandible with no yellow ridges (2), casque is brown (1)<br>pale cream yellow ridges across blackish lower mandible, casque is reddish-brown                     | juvenile without superciliary stripes<br>juvenile with some cream-coloured superciliary stripes                                                                                         | 3 |
| 23 | brown-black bill-casque with dark brown base (1)<br>larger bill & casque, base of bill whitish                                                                                         | dark brown upperparts and wings with slight sheen (2), pale band across black tail (1)<br>more blackish above with metallic sheen, pale tailband is broader                             | 4 |
| 24 |                                                                                                                                                                                        | underparts and tail-coverts rufous (1), tail reddish-yellow, olive-green gloss above (1)<br>underparts pale rufous wash, tail-coverts darker rufous, tail white, blue-green gloss above | 2 |
| 25 |                                                                                                                                                                                        | head and neck dark rufous to golden rufous (1)<br>head and neck golden rufous                                                                                                           | 1 |
| 26 |                                                                                                                                                                                        | head and neck golden rufous (2)<br>head and neck pale golden-yellow to cream                                                                                                            | 2 |

## CHAPTER 6

|    |                                                                                                             |                                                          |   |
|----|-------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|---|
| 27 |                                                                                                             | head and neck pale golden rufous (1)                     | 1 |
|    |                                                                                                             | head and neck dark rufous to golden rufous               |   |
| 28 | reduced development of ridges across base of mandibles, blue bar on throat basal                            |                                                          | 0 |
|    | reduced development of ridges across base of mandibles, blue bar on throat basal                            |                                                          |   |
| 29 |                                                                                                             | some rufous wash on throat & beak, darker underparts (1) | 1 |
|    |                                                                                                             | no rufous wash on throat & neck, pale underparts         |   |
| 30 |                                                                                                             |                                                          | 0 |
| 31 | casque truly carinate, rising with a curve from forehead (not biometric, 1)                                 |                                                          | 1 |
| 32 | bill and casque smaller than Sumatran population                                                            | slightly smaller wing and tail                           | 0 |
|    | Sumatran population with larger bill and casque                                                             |                                                          |   |
| 33 |                                                                                                             | larger continental form (homrai)                         | 0 |
|    |                                                                                                             | smaller Sundaland                                        |   |
| 34 |                                                                                                             |                                                          | 0 |
| 35 |                                                                                                             |                                                          | 0 |
| 36 | bill pale yellowish white with reddish maroon base, darker towards bill base; bare throat patch smaller (1) | head and neck golden-yellow to golden-rufous (2)         | 4 |
|    | bill yellow-white with dark maroon to dusky brown base, more blackish at bill base                          | head and neck pale rufous to golden rufous               |   |
| 37 | bill yellow-white with dark maroon to dusky brown base, more blackish at bill base (1)                      | head and neck deep/dark to golden rufous (3)             | 5 |
|    | bill pale yellowish white with reddish maroon base, darker towards bill base; bare throat patch smaller (1) | head and neck pale golden-yellow to cream                |   |
| 38 |                                                                                                             | head and neck very dark rufous (2)                       | 2 |
|    |                                                                                                             | head and neck dark to golden rufous                      |   |
| 39 | ridges across base of mandibles well defined (1), blue bar at middle of throat (1)                          |                                                          | 2 |
|    | reduced development of ridges across base of mandibles, blue bar on throat basal                            |                                                          |   |
| 40 | ridges across base of mandibles well defined (1), blue bar at middle of throat (1)                          |                                                          | 2 |

## CHAPTER 6

|    |                                                                                  |                                                                                           |   |
|----|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|---|
| 41 | reduced development of ridges across base of mandibles, blue bar on throat basal |                                                                                           | 0 |
| 42 |                                                                                  |                                                                                           | 0 |
| 43 | smaller bill and casque                                                          | slightly smaller wing                                                                     | 0 |
| 44 |                                                                                  |                                                                                           | 0 |
| 45 |                                                                                  |                                                                                           | 0 |
| 46 |                                                                                  |                                                                                           | 0 |
| 47 |                                                                                  | pale black-brown upperparts with buffy edges to feathers (2)                              | 2 |
| 48 | dark horn-coloured casque (1)                                                    | darker black-brown upperparts without buffy edges to quills, no light edges to primaries  | 2 |
|    | pale casque                                                                      | cream crown with fine black streaks & no ochre wash-black front (1), all black rump       |   |
| 49 |                                                                                  | ochre wash on crown with blackish front, black rump with some rufous feathers             | 0 |
| 50 |                                                                                  | small pale rump, black base on tail moderate (2)                                          | 2 |
|    |                                                                                  | black rump                                                                                |   |
| 51 | black patch at base of upper mandible, horn-coloured bill tip (1)                | small pale rump, black base on tail moderate (1), tail black with central ochre patch (1) | 3 |
|    | black patch at base of upper mandible, maroon-coloured bill tip                  | slightly larger, pale rufous rump, black base on tail minimal, more ochre on tail         |   |
| 52 |                                                                                  |                                                                                           | 0 |
| 53 |                                                                                  | pale rufous rump larger (1)                                                               | 1 |
|    |                                                                                  | darker rufous rump moderate                                                               |   |
| 54 |                                                                                  | underparts & rump pale rufous, tail reddish-yellow, olive-green gloss above (1)           | 1 |

## CHAPTER 6

---

underparts with pale rufous wash, rump darker rufous, tail white, blue-green gloss above

---

<sup>a</sup> Source: Plumage and bare parts based on examination of skins, photographs and literature descriptions, mainly Kemp 1995 and Kemp 2001.

<sup>b</sup> Total score is the sum of scores from three (3) plumage characters including bare parts

## CHAPTER 6

**Table S6.7:** Scoring for vocalization, behaviour, ecology and geographical relationship for 54 hornbill pairs analyzed for morphological divergence using a standard quantitative criteria (Tobias *et al.* 2010).

| Pair No. | Vocalisation                                                                                     | Score | Sources | Behaviour & Ecology                                              | Score | Geographical Relationship <sup>a</sup> | Score | Sources <sup>b</sup> |
|----------|--------------------------------------------------------------------------------------------------|-------|---------|------------------------------------------------------------------|-------|----------------------------------------|-------|----------------------|
| 1        | loud nasal note auuk repeated every 1.5 sec                                                      | 1     | 5       | strictly primary and secondary lowland forest                    | 0     | Allopatric                             | 0     | 3; 4                 |
|          | loud stuttering nasak au-au-au-auk lasting 0.5 sec at 1 sec interval                             |       |         | primary and secondary lowland and mid-montane forest             |       |                                        |       |                      |
| 2        | no difference, both loud plaintive scream                                                        | 0     | 6       | generally noisy with loud calls, prefers evergreen forest        | 0     | Allopatric                             | 0     | 3; 4                 |
|          |                                                                                                  |       |         | not very noisy, with soft calls, evergreen and deciduous forests |       |                                        |       |                      |
| 3        | loud shrill, nasal squeals and raucous chuckles, loud cackling deeper in tone and less modulated | 1     | 3       |                                                                  | 0     | Sympatric                              | 0     | 2; 4                 |
| 4        | loud harsh croaks & chuckles, raucous cackling                                                   | 1     | 3       | not known to be cooperative breeding                             | 1     | Allopatric                             | 0     | 3; 4                 |
|          | hard repeated ka, harsh laughing kaa repeated & accelerating                                     |       |         | suspected but unconfirmed cooperative breeder                    |       |                                        |       |                      |
| 5        | very high pitched staccato trumpeting call                                                       | 1     | 7       | strictly primary and secondary lowland forest                    | 0     | Allopatric                             | 0     | 3; 4                 |
|          | loud trumpeting toot-toot                                                                        |       |         | extends into disturbed forest, edges and tree plantations        |       |                                        |       |                      |
| 6        | high pitched trumpeting, nasal te-rik-tik-tik-tik & accelerating                                 | 1     | 3       | primary to secondary lowland and mid-montane forest              | 0     | Allopatric                             | 0     | 3; 4                 |
|          | loud trumpeting toot-toot                                                                        |       |         | extends into disturbed forest, edges and tree plantations        |       |                                        |       |                      |
| 7        | high pitched trumpeting, nasal te-rik-tik-tik-tik & accelerating                                 | 1     | 3       | primary to secondary lowland and mid-montane forest              | 0     | Allopatric                             | 0     | 3; 4                 |
|          | much deeper & less trumpet-like                                                                  |       |         | extends into gallery forest and mid-montane forest               |       |                                        |       |                      |
| 8        | high pitched trumpeting, nasal te-rik-tik-tik-tik & accelerating                                 | 1     | 3       | primary to secondary lowland and mid-montane forest              | 0     | Allopatric                             | 0     | 3; 4                 |
|          | more high pitched and staccato call                                                              |       |         |                                                                  |       |                                        |       |                      |
| 9        | slightly more staccato and harsh trumpeting call                                                 | 0     | 7       | strictly primary and secondary lowland forest                    | 0     | Allopatric                             | 0     | 3; 4                 |
|          | very high pitched staccato trumpeting call                                                       |       |         |                                                                  |       |                                        |       |                      |
| 10       | loud harsh ka-ka-ka-ka or kok-kok-kokok followed by a cackle                                     | 1     | 7       | restricted to island's evergreen and deciduous forest            | 0     | Allopatric                             | 0     | 3; 4                 |
|          | loud raucous grunts, honks continuously ka-ka-ka-ka in flight                                    |       |         | primary to secondary lowland, mid-montane and swamp forest       |       |                                        |       |                      |
| 11       | higher pitched call                                                                              | 1     | 6       | highly gregarious, tall evergreen forests in valleys and hills   | 0     | Sympatric                              | 0     | 3; 4                 |
|          |                                                                                                  |       |         | nomadic, forms large communal roost in foothills to mountains    |       |                                        |       |                      |
| 12       | no distinct difference known/no comparison made                                                  | 0     | 3       |                                                                  | 0     | Allopatric                             | 0     | 3; 4                 |



## CHAPTER 6

|    |                                                                                                                       |   |   |                                                                                                                         |   |                   |   |      |
|----|-----------------------------------------------------------------------------------------------------------------------|---|---|-------------------------------------------------------------------------------------------------------------------------|---|-------------------|---|------|
| 13 | no distinct difference, but some degree of disparity in tone or pitch<br>requires further analysis of call difference | 0 | 3 | deciduous & evergreen forest to coastal and cultivated areas<br>more confined to coastal & riverine forests and islands | 0 | Allopatric        | 0 | 2; 4 |
| 14 | no distinct difference known/no comparison made                                                                       | 0 | 3 |                                                                                                                         | 0 | Allopatric        | 0 | 3; 4 |
| 15 | no distinct difference, but some degree of disparity in tone or pitch<br>requires further analysis of call difference | 0 | 3 | strictly primary and secondary lowland forest<br>extends into mid-montane forests, known cooperative breeder            | 0 | Allopatric        | 0 | 3; 4 |
| 16 | no distinct difference, but some degree of disparity in tone or pitch<br>requires further analysis of call difference | 0 | 3 | strictly primary and secondary lowland forest<br>extends into mangrove forests                                          | 0 | Allopatric        | 0 | 3; 4 |
| 17 | no distinct difference known/no comparison made                                                                       | 0 | 3 | extends into mid-montane forests, known cooperative breeder<br>extends into mangrove forests                            | 0 | Allopatric        | 0 | 3; 4 |
| 18 | no distinct difference known/no comparison made                                                                       | 0 | 3 |                                                                                                                         | 0 | Allopatric        | 0 | 3; 4 |
| 19 | no distinct difference known/no comparison made                                                                       | 0 | 3 |                                                                                                                         | 0 | Allopatric        | 0 | 3; 4 |
| 20 | no distinct difference known/no comparison made                                                                       | 0 | 3 |                                                                                                                         | 0 | Allopatric        | 0 | 3; 4 |
| 21 | no distinct difference known/no comparison made                                                                       | 0 | 3 | strictly primary and secondary forest                                                                                   | 0 | Allopatric        | 0 | 3; 4 |
| 22 | no distinct difference, but some degree of disparity in tone or pitch<br>requires further analysis of call difference | 0 | 3 |                                                                                                                         | 0 | Allopatric        | 0 | 3; 4 |
| 23 | no distinct difference, but some degree of disparity in tone or pitch<br>requires further analysis of call difference | 0 | 7 | extends into disturbed forest, edges and tree plantations<br>extends into disturbed forest and coconut plantations      | 0 | Allopatric        | 0 | 3; 4 |
| 24 | no distinct difference known                                                                                          | 0 | 3 | strictly primary and secondary forest                                                                                   | 0 | Allopatric        | 0 | 3; 4 |
| 25 | no distinct difference known/no comparison made                                                                       | 0 | 3 |                                                                                                                         | 0 | Broad hybrid zone | 1 | 1; 4 |
| 26 | no distinct difference known/no comparison made                                                                       | 0 | 3 |                                                                                                                         | 0 | Allopatric        | 0 | 1; 4 |

## CHAPTER 6

|    |                                                                                                |   |   |                                                                                                    |   |                   |   |      |
|----|------------------------------------------------------------------------------------------------|---|---|----------------------------------------------------------------------------------------------------|---|-------------------|---|------|
| 27 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Broad hybrid zone | 1 | 1; 4 |
| 28 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 3; 4 |
| 29 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 3; 4 |
| 30 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 3; 4 |
| 31 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 3; 4 |
| 32 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 2; 4 |
| 33 | no distinct difference known/no comparison made                                                | 0 | 3 | Lowland evergreen and deciduous hill forests<br>extends to highland forest or coastal swamp forest | 0 | Allopatric        | 0 | 3; 4 |
| 34 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 3; 4 |
| 35 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 3; 4 |
| 36 | harsh raucous grunts and honks, deeper tone and resonant<br>call more raspier and trumpet-like | 1 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 1; 4 |
| 37 | harsh raucous grunts and honks, deeper tone and resonant<br>call more raspier and trumpet-like | 1 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 1; 4 |
| 38 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 1; 4 |
| 39 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 3; 4 |
| 40 | no distinct difference known/no comparison made                                                | 0 | 3 |                                                                                                    | 0 | Allopatric        | 0 | 3; 4 |

## CHAPTER 6

|    |                                                 |   |   |                                                                                                 |   |                   |   |      |
|----|-------------------------------------------------|---|---|-------------------------------------------------------------------------------------------------|---|-------------------|---|------|
| 41 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Broad hybrid zone | 1 | 3; 4 |
| 42 | no distinct difference known/no comparison made | 0 | 3 | evergreen, deciduous hill forest, bamboo forests & plantations<br>restricted mainly to lowlands | 0 | Allopatric        | 0 | 2; 4 |
| 43 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Allopatric        | 0 | 2; 4 |
| 44 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Allopatric        | 0 | 2; 4 |
| 45 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Allopatric        | 0 | 3; 4 |
| 46 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Broad hybrid zone | 1 | 3; 4 |
| 47 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Broad hybrid zone | 1 | 3; 4 |
| 48 | no distinct difference known/no comparison made | 0 | 3 | Lowland rainforest and gallery forest<br>Lowland rainforest and mid-montane forest              | 0 | Broad hybrid zone | 1 | 3; 4 |
| 49 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Allopatric        | 0 | 3; 4 |
| 50 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Allopatric        | 0 | 3; 4 |
| 51 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Allopatric        | 0 | 3; 4 |
| 52 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Broad hybrid zone | 1 | 3; 4 |
| 53 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Allopatric        | 0 | 3; 4 |
| 54 | no distinct difference known/no comparison made | 0 | 3 |                                                                                                 | 0 | Allopatric        | 0 | 3; 4 |

## CHAPTER 6

---

<sup>a</sup> Geographic relationship based on guidelines set by Helbig *et al.* (2002) and Tobias *et al.* (2010).

<sup>b</sup> Sources: 1: Mayr & Diamond 2001; Frith & Frith 1983; 3: Kemp 1995; 4: Kemp 2001; 5: Kennedy *et al.* 2000; 6: Robson 2009; 7: Xeno-Canto Asia (<http://www.xeno-canto.org/asia/>).

# CHAPTER 6

**Table S6.8:** Molecular divergence for 54 hornbill pairs used in this study, based on corrected pairwise distances of mtDNA Cytb sequences.

| Group      | Pair No. | Genus                | Species              | Subspecies        | Geo-isolate             | Molecular (nucleotide divergence) <sup>a</sup> |                       |                    | % divergence |
|------------|----------|----------------------|----------------------|-------------------|-------------------------|------------------------------------------------|-----------------------|--------------------|--------------|
|            |          |                      |                      |                   |                         | Number of pairs                                | Mean genetic distance | Standard deviation |              |
| Species    | 1        | <i>Aceros</i>        | <i>leucocephalus</i> |                   | Mindanao                | 104                                            | 0.054                 | 0.008              | 5.36         |
|            |          | <i>Aceros</i>        | <i>waldeni</i>       |                   | Negros-Panay            |                                                |                       |                    |              |
|            | 2        | <i>Anorrhinus</i>    | <i>austeni</i>       |                   | East Indo-China         | 15                                             | 0.029                 | 0.006              | 2.86         |
|            |          | <i>Anorrhinus</i>    | <i>tickelli</i>      |                   | West Indo-China         |                                                |                       |                    |              |
|            | 3        | <i>Anthracoseros</i> | <i>albirostris</i>   |                   | India-Sundaland         | 42                                             | 0.057                 | 0.009              | 5.68         |
|            |          | <i>Anthracoseros</i> | <i>coronatus</i>     |                   | India-Sri Lanka         |                                                |                       |                    |              |
|            | 4        | <i>Ocyeros</i>       | <i>griseus</i>       |                   | India                   | 8                                              | 0.093                 | 0.011              | 9.26         |
|            |          | <i>Ocyeros</i>       | <i>gingalensis</i>   |                   | Sri Lanka               |                                                |                       |                    |              |
|            | 5        | <i>Penelopides</i>   | <i>affinis</i>       |                   | Mindanao                | 50                                             | 0.035                 | 0.007              | 3.52         |
|            |          | <i>Penelopides</i>   | <i>manillae</i>      |                   | Greater Luzon           |                                                |                       |                    |              |
|            | 6        | <i>Penelopides</i>   | <i>panini</i>        |                   | Negros-Panay            | 60                                             | 0.045                 | 0.007              | 4.53         |
|            |          | <i>Penelopides</i>   | <i>manillae</i>      |                   | Greater Luzon           |                                                |                       |                    |              |
|            | 7        | <i>Penelopides</i>   | <i>panini</i>        |                   | Negros-Panay            | 42                                             | 0.047                 | 0.008              | 4.75         |
|            |          | <i>Penelopides</i>   | <i>mindorensis</i>   |                   | Mindoro                 |                                                |                       |                    |              |
|            | 8        | <i>Penelopides</i>   | <i>panini</i>        |                   | Negros-Panay            | 30                                             | 0.034                 | 0.006              | 3.40         |
|            |          | <i>Penelopides</i>   | <i>affinis</i>       |                   | Mindanao                |                                                |                       |                    |              |
|            | 9        | <i>Penelopides</i>   | <i>samarensis</i>    |                   | East Visayas            | 12                                             | 0.021                 | 0.005              | 2.06         |
|            |          | <i>Penelopides</i>   | <i>affinis</i>       |                   | Mindanao                |                                                |                       |                    |              |
|            | 10       | <i>Rhyticeros</i>    | <i>narcondami</i>    |                   | Narcondam               | 75                                             | 0.034                 | 0.006              | 3.40         |
|            |          | <i>Rhyticeros</i>    | <i>plicatus</i>      |                   | Wallacea-Melanesia      |                                                |                       |                    |              |
|            | 11       | <i>Rhyticeros</i>    | <i>subruficollis</i> |                   | Indo-China              | 36                                             | 0.042                 | 0.007              | 4.15         |
|            |          | <i>Rhyticeros</i>    | <i>undulatus</i>     |                   | India-Sundaland         |                                                |                       |                    |              |
| Subspecies | 12       | <i>Aceros</i>        | <i>corrugatus</i>    | <i>corrugatus</i> | Borneo                  | 12                                             | 0.071                 | 0.010              | 7.09         |
|            |          | <i>Aceros</i>        | <i>corrugatus</i>    | <i>rugosus</i>    | Malay Peninsula-Sumatra |                                                |                       |                    |              |

# CHAPTER 6

|    |                      |                    |                     |                         |    |       |       |       |
|----|----------------------|--------------------|---------------------|-------------------------|----|-------|-------|-------|
| 13 | <i>Anthracoceros</i> | <i>albirostris</i> | <i>albirostris</i>  | Indo-China              | 48 | 0.019 | 0.004 | 1.88  |
|    | <i>Anthracoceros</i> | <i>albirostris</i> | <i>convexus</i>     | Sundaland               |    |       |       |       |
| 14 | <i>Buceros</i>       | <i>bicornis</i>    | <i>homrai</i>       | India-IndoChina         | 17 | 0.046 | 0.007 | 4.65  |
|    | <i>Buceros</i>       | <i>bicornis</i>    | <i>bicornis</i>     | Sundaland               |    |       |       |       |
| 15 | <i>Buceros</i>       | <i>hydrocorax</i>  | <i>hydrocorax</i>   | Greater Luzon           | 42 | 0.089 | 0.011 | 8.85  |
|    | <i>Buceros</i>       | <i>hydrocorax</i>  | <i>mindanensis</i>  | Mindanao                |    |       |       |       |
| 16 | <i>Buceros</i>       | <i>hydrocorax</i>  | <i>hydrocorax</i>   | Greater Luzon           | 30 | 0.116 | 0.014 | 11.56 |
|    | <i>Buceros</i>       | <i>hydrocorax</i>  | <i>semigaleatus</i> | East Visayas            |    |       |       |       |
| 17 | <i>Buceros</i>       | <i>hydrocorax</i>  | <i>mindanensis</i>  | Mindanao                | 35 | 0.082 | 0.011 | 8.22  |
|    | <i>Buceros</i>       | <i>hydrocorax</i>  | <i>semigaleatus</i> | East Visayas            |    |       |       |       |
| 18 | <i>Buceros</i>       | <i>rhinoceros</i>  | <i>rhinoceros</i>   | Malay Peninsula-Sumatra | 8  | 0.056 | 0.008 | 5.56  |
|    | <i>Buceros</i>       | <i>rhinoceros</i>  | <i>borneoensis</i>  | Borneo                  |    |       |       |       |
| 19 | <i>Buceros</i>       | <i>rhinoceros</i>  | <i>rhinoceros</i>   | Malay Peninsula-Sumatra | 16 | 0.043 | 0.007 | 4.35  |
|    | <i>Buceros</i>       | <i>rhinoceros</i>  | <i>silvestris</i>   | Java                    |    |       |       |       |
| 20 | <i>Buceros</i>       | <i>rhinoceros</i>  | <i>borneoensis</i>  | Borneo                  | 8  | 0.045 | 0.007 | 4.48  |
|    | <i>Buceros</i>       | <i>rhinoceros</i>  | <i>silvestris</i>   | Java                    |    |       |       |       |
| 21 | <i>Penelopides</i>   | <i>affinis</i>     | <i>basilanicus</i>  | Basilan                 | 5  | 0.013 | 0.004 | 1.27  |
|    | <i>Penelopides</i>   | <i>affinis</i>     | <i>affinis</i>      | Mindanao                |    |       |       |       |
| 22 | <i>Penelopides</i>   | <i>exarhatus</i>   | <i>exarhatus</i>    | North Sulawesi          | 6  | 0.049 | 0.008 | 4.93  |
|    | <i>Penelopides</i>   | <i>exarhatus</i>   | <i>sandfordi</i>    | South Sulawesi          |    |       |       |       |
| 23 | <i>Penelopides</i>   | <i>manillae</i>    | <i>manillae</i>     | mainland Luzon          | 50 | 0.013 | 0.004 | 1.28  |
|    | <i>Penelopides</i>   | <i>manillae</i>    | <i>subnigra</i>     | Polillo                 |    |       |       |       |
| 24 | <i>Penelopides</i>   | <i>panini</i>      | <i>panini</i>       | Negros-Panay            | 18 | 0.021 | 0.005 | 2.10  |
|    | <i>Penelopides</i>   | <i>panini</i>      | <i>ticaensis</i>    | Ticao                   |    |       |       |       |
| 25 | <i>Rhyticeros</i>    | <i>plicatus</i>    | <i>ruficollis</i>   | West NG-N Moluccas      | 26 | 0.026 | 0.005 | 2.57  |
|    | <i>Rhyticeros</i>    | <i>plicatus</i>    | <i>jungei</i>       | East New Guinea         |    |       |       |       |
| 26 | <i>Rhyticeros</i>    | <i>plicatus</i>    | <i>harterti</i>     | West Solomon Islands    | 6  | 0.030 | 0.006 | 2.99  |
|    | <i>Rhyticeros</i>    | <i>plicatus</i>    | <i>mendanae</i>     | East Solomon Islands    |    |       |       |       |

# CHAPTER 6

|          |    |                       |                   |                        |                         |    |       |       |      |
|----------|----|-----------------------|-------------------|------------------------|-------------------------|----|-------|-------|------|
|          | 27 | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>jungei</i>          | East New Guinea         | 4  | 0.015 | 0.004 | 1.52 |
|          |    | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ruficollis</i>      | West New Guinea         |    |       |       |      |
|          | 28 | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>undulatus</i>       | Sundaland               | 30 | 0.025 | 0.005 | 2.46 |
|          |    | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>aequabilis</i>      | Borneo                  |    |       |       |      |
| Subsumed | 29 | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>carinatus</i>       | Malay Peninsula         | 10 | 0.016 | 0.004 | 1.56 |
|          |    | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>minor</i>           | Borneo                  |    |       |       |      |
|          | 30 | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>galeritus</i>       | Sumatra                 | 4  | 0.016 | 0.004 | 1.64 |
|          |    | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>carinatus</i>       | Malay Peninsula         |    |       |       |      |
|          | 31 | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>carinatus</i>       | Malay Peninsula         | 14 | 0.016 | 0.004 | 1.58 |
|          |    | <i>Anorrhinus</i>     | <i>galeritus</i>  | <i>ssp.</i>            | Borneo-Sumatra          |    |       |       |      |
|          | 32 | <i>Anthracosceros</i> | <i>malayanus</i>  | <i>deminutus</i>       | Borneo                  | 15 | 0.027 | 0.005 | 2.72 |
|          |    | <i>Anthracosceros</i> | <i>malayanus</i>  | <i>malayanus</i>       | Malay Peninsula-Sumatra |    |       |       |      |
|          | 33 | <i>Buceros</i>        | <i>bicornis</i>   | <i>homrai</i>          | India                   | 26 | 0.055 | 0.008 | 5.49 |
|          |    | <i>Buceros</i>        | <i>bicornis</i>   | <i>ssp.</i>            | IndoChina-Sundaland     |    |       |       |      |
|          | 34 | <i>Buceros</i>        | <i>hydrocorax</i> | <i>mindanensis</i>     | Mindanao                | 10 | 0.040 | 0.007 | 3.98 |
|          |    | <i>Buceros</i>        | <i>hydrocorax</i> | <i>basilanicus</i>     | Basilan                 |    |       |       |      |
|          | 35 | <i>Buceros</i>        | <i>hydrocorax</i> | <i>basilanicus (F)</i> | Basilan                 | 10 | 0.040 | 0.007 | 3.98 |
|          |    | <i>Buceros</i>        | <i>hydrocorax</i> | <i>mindanensis (F)</i> | Mindanao                |    |       |       |      |
|          | 36 | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ssp.</i>            | Solomon Islands         | 55 | 0.033 | 0.006 | 3.32 |
|          |    | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ruficollis</i>      | North Moluccas          |    |       |       |      |
|          | 37 | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ssp.</i>            | Seram-New Guinea        | 30 | 0.039 | 0.007 | 3.88 |
|          |    | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ssp.</i>            | Solomon Islands         |    |       |       |      |
|          | 38 | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>plicatus</i>        | Ceram                   | 8  | 0.015 | 0.004 | 1.49 |
|          |    | <i>Rhyticeros</i>     | <i>plicatus</i>   | <i>ssp.</i>            | New Guinea              |    |       |       |      |
|          | 39 | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>ticehursti</i>      | India-IndoChina         | 27 | 0.027 | 0.005 | 2.67 |
|          |    | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>ssp.</i>            | Sundaland               |    |       |       |      |
|          | 40 | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>ticehursti</i>      | India-IndoChina         | 11 | 0.028 | 0.005 | 2.78 |
|          |    | <i>Rhyticeros</i>     | <i>undulatus</i>  | <i>aequabilis</i>      | Borneo                  |    |       |       |      |

# CHAPTER 6

|         |    |                      |                      |                    |                 |    |       |       |      |
|---------|----|----------------------|----------------------|--------------------|-----------------|----|-------|-------|------|
| Cryptic | 41 | <i>Aceros</i>        | <i>leucocephalus</i> |                    | West Mindanao   | 23 | 0.039 | 0.007 | 3.90 |
|         |    | <i>Aceros</i>        | <i>leucocephalus</i> |                    | East Mindanao   |    |       |       |      |
|         | 42 | <i>Anthracoceros</i> | <i>coronatus</i>     |                    | India           | 2  | 0.001 | 0.001 | 0.11 |
|         |    | <i>Anthracoceros</i> | <i>coronatus</i>     |                    | Sri Lanka       |    |       |       |      |
|         | 43 | <i>Anthracoceros</i> | <i>marchei</i>       |                    | Balabac         | 51 | 0.013 | 0.004 | 1.28 |
|         |    | <i>Anthracoceros</i> | <i>marchei</i>       |                    | Palawan         |    |       |       |      |
|         | 44 | <i>Anthracoceros</i> | <i>marchei</i>       |                    | Calamianes      | 52 | 0.012 | 0.004 | 1.20 |
|         |    | <i>Anthracoceros</i> | <i>marchei</i>       |                    | Palawan         |    |       |       |      |
|         | 45 | <i>Berenicomis</i>   | <i>comatus</i>       |                    | Malay Peninsula | 48 | 0.016 | 0.004 | 1.57 |
|         |    | <i>Berenicomis</i>   | <i>comatus</i>       |                    | Borneo-Sumatra  |    |       |       |      |
|         | 46 | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | West Mindanao   | 4  | 0.016 | 0.004 | 1.62 |
|         |    | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | East Mindanao   |    |       |       |      |
|         | 47 | <i>Penelopides</i>   | <i>manillae</i>      | <i>manillae</i>    | South Luzon     | 24 | 0.010 | 0.003 | 1.04 |
|         |    | <i>Penelopides</i>   | <i>manillae</i>      | <i>manillae</i>    | North Luzon     |    |       |       |      |
|         | 48 | <i>Penelopides</i>   | <i>mindorensis</i>   |                    | West Mindoro    | 12 | 0.021 | 0.005 | 2.10 |
|         |    | <i>Penelopides</i>   | <i>mindorensis</i>   |                    | East Mindoro    |    |       |       |      |
|         | 49 | <i>Rhinoplax</i>     | <i>vigil</i>         |                    | Malay Peninsula | 20 | 0.044 | 0.007 | 4.40 |
|         |    | <i>Rhinoplax</i>     | <i>vigil</i>         |                    | Borneo-Sumatra  |    |       |       |      |
|         | 50 | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | Dinagat/Siargao | 3  | 0.002 | 0.001 | 1.85 |
|         |    | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | East Mindanao   |    |       |       |      |
|         | 51 | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i>     | Dinagat/Siargao | 3  | 0.021 | 0.005 | 2.13 |
|         |    | <i>Penelopides</i>   | <i>samarensis</i>    |                    | East Visayas    |    |       |       |      |
|         | 52 | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i> (F) | East Mindanao   | 4  | 0.016 | 0.004 | 1.62 |
|         |    | <i>Penelopides</i>   | <i>affinis</i>       | <i>affinis</i> (F) | West Mindanao   |    |       |       |      |
|         | 53 | <i>Penelopides</i>   | <i>panini</i>        | <i>panini</i>      | Masbate         | 12 | 0.020 | 0.005 | 2.02 |
|         |    | <i>Penelopides</i>   | <i>panini</i>        | <i>panini</i>      | Negros-Panay    |    |       |       |      |
|         | 54 | <i>Penelopides</i>   | <i>panini</i>        | <i>panini</i>      | Masbate         | 7  | 0.003 | 0.002 | 2.85 |
|         |    | <i>Penelopides</i>   | <i>panini</i>        | <i>ticaensis</i>   | Ticao           |    |       |       |      |



## CHAPTER 6

<sup>a</sup> Nucleotide divergence based on pairwise comparisons of mtDNA Cytochrome b truncated to 909 bp, calculated using ML best-fit model TN93+Γ+I substitution model in MEGA5 (Tamura et al. 2011)

## Chapter 7

### Discussion



*Proc. Zool. Soc. London, Tweeddale 1878 p. 279*

*The abundance of endemic genera on the Sunda shelf and the Philippines indicates two major divergences in Asian hornbills, first when they arrived in Asia, and second when they invaded the Sunda shelf. Confirmation of this scenario will have to await the day when we finally have a reliable evolutionary tree for hornbills based on the DNA of all the species*

M.F. Kinnaird and T.G. O'Brien, 2007, The ecology & conservation of Asian hornbills.

## Discussion

In this thesis I have examined the evolutionary and biogeographic importance of hornbills (Aves: Bucerotidae), using a combination of different approaches, from molecular phylogenetics to phylogeographic analysis. To achieve this, I have used a variety of field and museum methods, laboratory techniques and recently developed bioinformatics programs to collect, process and analyse data, ranging from sequencing DNA from historical samples to constructing calibrated molecular phylogenetic trees, and combining genetic and morphological analysis for species delimitation. In this final chapter, I summarise the salient results from the five data chapters, discuss points each has raised, and highlight the novel contributions made in the context of hornbill evolution. Here, I aim to summarise how the molecular phylogenies I constructed were able to test hypotheses and address various longstanding questions about the origin and diversification of this fascinating, charismatic but enigmatic group of birds from the Palaeotropics. I also enumerate several important gaps and issues that need to be addressed and improved in future analyses, alongside other recommendations for further research.

### Summary of principal findings:

In the first part of this thesis, I undertook a detailed phylogenetic analysis of the family Bucerotidae by constructing a complete species-level multi-locus molecular phylogeny of all 61 species of hornbills worldwide (Chapter 2), based largely on the nuclear loci adenylate kinase 1 intron 5 (AK1 intron 5, novel for 54 species) and mitochondrial DNA (mtDNA) cytochrome *b* (*cytb*, new for 23 species) sequenced largely from historical samples. This well-resolved tree served as the template for conducting comparative analyses in the succeeding chapters, and also formed the basis upon which I constructed a more comprehensive molecular phylogeny of hornbills based on *cytb* sequences of nearly all subspecies worldwide and representative geolocal isolates from Asia (Chapter 5, total taxa covered: 87 of 89 worldwide). A well-resolved

concatenated nuclear-mtDNA tree revealed novel taxonomic relations, including new arrangements of clades and genera (see section on taxonomic implications).

In Chapter 3, I used this concatenated tree to test hypotheses about the evolution of sociality and cooperative breeding (CB) in hornbills. Specifically, I used ancestral state reconstructions to trace the evolution of CB, and then conducted phylogenetic comparative analyses to assess the role of climate in promoting CB. My findings established that stable climatic conditions are a key predictor of CB in hornbills, and therefore challenge the view that cooperation between breeding individuals evolves as a bet-hedging strategy when environmental conditions are unstable (Jetz & Rubinstein, 2011; Rubinstein, 2011). I argue that, for frugivorous species, stable climates are linked to increased survival and decreased availability of vacant territories, thus encouraging young birds to delay dispersal and cooperate with kin (Chapter 3).

In Chapter 4, I used the same concatenated nuclear-mtDNA dataset to construct a dated phylogeny calibrated using multiple molecular and fossil anchor-points. Divergence time was used as a basis for identifying palaeo-tectonic and palaeo-climatic drivers of diversification. Dated trees were also compared to reconstructed chronograms of hornbill-dispersed fruits (HDFs) to test co-evolutionary interactions, and models of diversification. My findings indicated an association between a shift to frugivory and an Out-of-Africa model which led to the radiation of Asian hornbills. These events also marked shifts in the diversification rates of hornbills, thus highlighting the role of diet shift and dispersal in hornbill radiation. Moreover, I also used phylogenetic data from fruiting trees to show that bursts of range expansion and diversification in hornbills were congruent with similar patterns in their food plants.

Based on these initial findings, more detailed reconstructions of ancestral ranges were conducted on a dated phylogeny, which was then used to delineate the routes taken by hornbills for colonising Asia from Africa, and reaching the Philippines, Wallacea and Melanesia. To gain more resolution, I constructed a phylogeny based on numerous hornbill geo-isolates (including all species, almost all subspecies, and most regional and island populations). In turn, this

chronogram was used in a more complex ancestral area reconstruction involving eight optimal distributions, which provided a detailed analysis of the historical biogeography of the family. Aligned with palaeo-tectonic and palaeo-climatic events, my findings revealed the colonisation routes taken by hornbills from Africa to Melanesia, as they crossed several biogeographic barriers – Engler’s line, Isthmus of Kra, Huxley’s line, Wallace’s line and Lydekker’s line (Chapters 5).

Finally, in Chapter 6, I set out to resolve taxonomic issues using a combination of genotypic and phenotypic data to delimit species and highlight appropriate conservation units in Asian hornbills. I used the same *cytb* sequences of Asian geo-isolates (from Chapters 2 and 5) to calculate genetic divergence, following a standardised method involving best-fit models and truncating aligned sequences (Fregin *et al.*, 2012). In combination with genetic data, I calculated phenotypic scores from effect sizes of key morphometric data taken from 54 pairs of Asian hornbills representing species, subspecies and geo-isolates, alongside scores for differences in plumage, colour of bare parts, vocalisations, behaviour, ecology and geographic relationships, following a standardised scoring system (Tobias *et al.*, 2010). Initial findings indicate a positive correlation between genetic and phenotypic divergence, reinforcing the importance of both types of data as a basis for delineating species. Results of this scoring system confirmed 11 recent splits (based on a list of taxonomic treatments in Chapter 2) as valid species and elevated three additional taxa to species status, each of which merit conservation attention. This therefore brings the total of extant hornbill species to 64. Some subspecies immediately below the threshold represent incipient species, and may require further analysis, while some geo-isolates may represent cryptic taxa meriting formal description.

## Perspectives

### Molecular Phylogeny of the Family Bucerotidae

Construction of molecular phylogenetic trees is a powerful tool for understanding evolutionary and ecological processes (Page & Holmes, 1998) – particularly when analysed in combination with other methods. It not only provides a topological view of relationships, but also allows calculation of node and branch values that can be used in a variety of evolutionary analyses (Avice, 2000). This has proved especially useful in studying bird evolution, resulting in a barrage of molecular studies in recent years, springing from the premise that molecular data have advantages over rather plastic morphological characters (Newton, 2003; Price, 2008). I applied molecular phylogenetics in order to understand taxonomic relationships within the family Bucerotidae, and to identify the evolutionary and biogeographic processes that have facilitated their radiation in the Palaetropics. However, sampling contemporary material from hornbills proved difficult, and the use of captive birds is problematic when locality of origin is crucial for representing geo-isolates in the biogeographic analysis. As such, I placed emphasis on the use of historical samples in this thesis as a useful source of molecular data, both for nuclear loci and for mtDNA (Irestedt *et al.*, 2006; Lerner & Mindell, 2005).

Different phylogenetic methods (Maximum Parsimony, Maximum Likelihood, Bayesian) facilitate the construction of robust (well-sampled, multi-locus) trees, test the congruence of topologies and draw well-resolved nodes showing moderate to strong support (Lovette & Rubenstein, 2007; Muellner *et al.*, 2008). Congruent and well-resolved trees allowed better inference for tree topology and analysis of taxonomic relationships. Any analysis is limited by the completeness of the dataset and the appropriate methods used to achieve optimal resolution (Derryberry *et al.*, 2011). However, I am optimistic that my current complete species-level (and geo-isolate level) phylogenetic analysis of the Bucerotidae (Chapter 2 & 5) best represents the overall relationships within the family. The resulting trees help to resolve many

of the topological concerns highlighted by previous landmark molecular phylogenies of the Bucerotidae (Hübner *et al.*, 2003; Viseshakul *et al.*, 2011).

A complete gene tree (at both the species and geo-isolate level) was useful for modelling diversification rates, reconstructing ancestral states/areas and deciphering biogeographic patterns. A complete set of lineages across representative species or taxa with known distribution is ideal for biogeographic analysis, and this was my rationale for constructing the comprehensive *cytb* tree represented by a wide number of geo-isolates. There have been a growing number of avian studies with family-level molecular phylogenetic studies covering near-complete sampling of species, including whistlers Pachycephalidae (71%, 39 species, Jönsson *et al.*, 2010), cockatoos Cacatuidae (76%, 16 species, White *et al.*, 2011), starlings Sturnidae (80%, 91 species, Lovette & Rubenstein, 2007), pittas Pittidae (85%, 28 species, Irestedt *et al.*, 2006), bee-eaters Meropidae (92%, 23 species, Marks *et al.*, 2007), and most impressively ovenbirds Furnariidae (97%, 293 species, Derryberry *et al.*, 2011). Derryberry *et al.* (2011) emphasised the value of complete species-level trees in testing models for lineage and phenotypic diversification. A well-aligned sequence matrix and consensus tree of all species of hornbills sets the stage for a wide array of phylogenetic analyses, such as comparative trait analysis; ancestral state reconstructions; divergence time estimation; and testing diversification models (Fordyce, 2010; Fritz & Purvis, 2010; Zhou *et al.*, 2011).

### **Historical biogeography and radiation into Asia**

The evolutionary history of hornbills was described by dated phylogenetic trees (Chapter 4 & 5) based on well-resolved molecular phylogenies (Chapter 2 & 5). Here I emphasise the importance of using precise fossil ages based on reliable geological horizons when assigning calibrations. There is increasingly a preference for using Bayesian methods (BEAST, Drummond & Rambaut, 2007) over simplified linearised trees (MEGA, Tamura *et al.*, 2011), as the former allows multiple calibration points, thus applying a combination of molecular and fossil anchor-points. My findings using the species-level chronogram allowed a

relatively precise dating of the diet shift in hornbills, leading to the test of the frugivory-shift model. Node ages of frugivorous clades were well aligned with geological, climatic and floristic events (Hall 1998; Zachos *et al.*, 2001; Bush & Flenley 2007). This scenario can also be observed in the comprehensive *cytb* chronogram (Fig. 7.1 C) as the divergence ages coincide well with the palaeo-tectonic environment (Fig. 7.1 A) and palaeo-climatic changes (Fig. 7.1 B).

A shift in diversification at the inception of frugivory in hornbills can be observed along the lineage-through-time plot (Fig. 7.1 D), and this shift coincides with the Late Oligocene warming event (LOWE). The same pattern was observed in the species-level tree (Chapter 4). The frugivory-shift model was closely associated with the expansion of mega-thermal forest and the emergence of tropical fruits during key warming events (Zachos *et al.*, 2001). As also noted in Chapter 4, this shift occurred after the post-Eocene collisions of the Arabian Peninsula with Africa (Allen & Armstrong, 2008) and Indian subcontinent with Asia (Aitchison *et al.*, 2007), thus forming the Palaeotropical biotic interchange. This coincided with the emergence of large-seeded arillate dehiscent fruits (albeit adapted for dispersal by large frugivorous birds like hornbills and imperial-pigeons *Ducula*) – the Myristicaceae (Doyle *et al.*, 2004) and Meliaceae (*Amoora* section, Muellner *et al.*, 2008) as well as other fruits regarded as hornbill-dispersed (Burseraceae, Weeks *et al.*, 2005; Annonaceae, Yu *et al.*, 2011).

A combination of the factors leading to the frugivory-shift model (Chapter 4) are implicated in the environmental stability hypothesis (Chapter 3), which explains the development of cooperative breeding (CB) in frugivorous hornbills. Matching bars for preferred habitat, diet and social system (see Fig. 7.2 C, D & E) show this interrelation between diet shift and sociality. A link also appears between biogeography and sociality, wherein (1) the Palaeotropical biotic interchange facilitated dispersal from Africa to Asia (Chapter 4); (2) megathermal forest expansion allowed forest hornbills to spread (Bush & Flenley 2007); (3) a diet shift occurred, which built matching dispersal regimes with large-seeded hornbill-dispersed fruits (HDFs, such as some species of Meliaceae and Myristicaceae); and (4) this shift established the propensity for cooperative breeding. Sociality is manifested in frugivorous



hornbills due to environmental stability, underpinned by adaptation to humid forests and frugivory. Similar integrative phylogenetic testing can be applied to other hypotheses or other questions (e.g. Why is nest-sealing not present in the African *Bucorvus* clade?). This approach may also be useful in resolving other confounding issues, such as the disparity in the radiation of lineages (e.g. Why did the *Aceros* clade diversify successfully in Asia, but not *Berenicornis*?).

My findings also highlight the importance of Sundaland as the centre of the Asian hornbill radiation, and reveal its African connections (Hosner *et al.* 2010). Many species of birds and other organisms (including some HDFs) have similar disjunctions between Africa and Indo-Malaya (Muellner *et al.*, 2008; Zhou *et al.*, 2011). Most basal forms of Asian hornbills are Sundaic, including *Berenicornis comatus*, *Rhinoplax vigil* and *Anorrhinus galeritus* (as shown in the light blue bars that are basal to most clades, see Fig 7.2 A & B). It seems likely that ancestral frugivorous hornbills arrived in Sundaland and diversified, as these were easily influenced by the contraction of forest refugia in Sundaland (Kinnaird & O'Brien 2007). In turn, geological and floristic changes may have facilitated dispersal out of Sundaland, both westward to India and eastward and northward into the Philippine and Australo-Papuan archipelagos (Zhou *et al.*, 2011). Sundaland was probably the receiving end of the Palaeotropical biotic interchange (Chapter 4), which allowed species to cross from Africa to Asia during warming events. Moving eastward, the mid-Miocene Sulu-Sulawesi convergence that connected (to some degree) the Sunda and Sahul shelves allowed hornbills to disperse into the Philippines, Wallacea and Melanesia (Hall 1998, Lohman *et al.*, 2011). An eastward dispersal during the mid-Miocene from Sundaland to Melanesia supported similar dispersal scenarios for HDFs, which in turn postulated that dispersal agents (like hornbills) might have facilitated their gradual expansion in Southeast Asia (Muellner *et al.*, 2008; Zhou *et al.*, 2011).

My work also showed an “Out-of-Africa” and “Out-of-Asia” (eastward spreading) pattern of dispersal and colonisation. Hornbills were able to cross multiple biogeographic barriers in the course of their evolutionary history, namely (1) Engler’s line, from Africa to Asia

following two continental collisions (Africa–Arabia and India–Asia); (2) the Isthmus of Kra into Sundaland (although core Sundaland includes Indo-China, Hall *et al.*, 2011); (3) Huxley’s line to the Philippines; (4) Wallace’s line to Sulawesi and the Moluccan Islands (Wallacea); and (5) Lydekker’s line to Melanesia, all following two tectonic collisions (Sunda shelf with Philippine plate and Sahul shelf). Further validation of the “Out-of-Africa” model and “frugivory-shift model” results from the discovery of pre-Pleistocene bucerotine fossils. All current valid hornbill fossils were either mid-Miocene ground-hornbills (*Bucorvus brailloni* and *Euroceros bulgaricus*, Boev & Kovachev, 2007) or subfossils of extant taxa from Southeast Asia and Melanesia (e.g. *Rhinoplax vigil*, *Anorrhinus cf. tickelli*, *Rhyticeros* sp., Boev & Kovachev, 2007; Steadman, 2006).

Overall, historical biogeographic patterns vary between Asian and African hornbills, and these patterns reflect the broad differences shaping diversification of tropical species in continental Africa and insular Indo-Malaya (and Melanesia). Asian hornbill biogeography was influenced more by intermittent expansion and contraction of megathermal forests (refugia) within continental Asia and subsequent palaeo-tectonic changes that allowed dispersal from Sundaland to Melanesia (Kinnaird & O’Brien, 2007; Hall *et al.*, 2011). Once they reached Sundaland, their dispersal into adjacent archipelagos was facilitated by movement of plates (e.g. rotation of Philippine plate, northward migration of Sahul shelf), which bridged gaps between islands (Hall *et al.*, 2011). Hornbills crossed temporary land-bridges to colonise new insular areas or made short water-crossings presumably following the dispersal of HDFs and vice-versa (Muellner *et al.*, 2008; Yu *et al.*, 2011). By contrast, African hornbill biogeography was influenced more by ecological adaptations (Kemp & Crowe, 1985; Muñoz *et al.*, 2003), a response to shifting habitats due to expansion of deserts (aridification) and grasslands. However, a relict frugivorous clade (that includes *Ceratogymna* and *Bycanistes*) allied to Asian hornbills preferred humid forests and was mostly limited to Central Africa, and their speciation was influenced by separation of the Guinea-Congolian megathermal forest (Bush & Flenley, 2007) into cyclical forest refugia and/or adaptation to dry woodland (Chapter 5).

### **Social evolution in faunivorous and frugivorous hornbills**

In Chapter 3, I was able to show that cooperative breeding (CB) is likely to have been an ancestral trait in hornbills, and that the development to CB was facilitated by stable environmental conditions. Hornbills are long-lived, and therefore have a propensity for group living, constrained by their need for cavity-nests (a limited resource) and that a greater proportion are frugivorous (69%, 42 species) (Kemp, 2001a). However, CB may have developed differently among the hornbill clades, as in the case of the *Bucorvus* clade, the *Tockus* clade and the diverse Afro-Asian frugivore clade. Faunivory (with a bias to vertebrate prey) and large size in ground-hornbills may have been influenced by environmental uncertainty, which promotes CB behaviour. Ground-hornbills generally inhabit savannas and dry woodland in Africa, and have inherently low productivity and long life-spans (Kemp, 1995). On the other hand, faunivory (with a bias to insectivory) and small size in the *Tockus* clade did not develop CB behaviour despite being influenced by the similar unpredictable conditions and inhabiting the same savannas and dry woodland in Africa as ground-hornbills. In contrast, frugivory, small size and preference for humid forests encouraged CB behaviour in Afro-Asian forest hornbills.

This shift to frugivory and adaptation to stable humid forest environments apparently contributed to the evolution of sociality in hornbills. In Chapter 3, I followed the CB data given by Cockburn (2006) and Jetz & Rubenstein (2011), which allowed comparison with climatic data and tested the hypothesis that environmental variability promotes sociality (Jetz & Rubenstein 2011). I rejected this hypothesis in hornbills, and propose that the mechanisms driving cooperation may differ in frugivores and faunivores, as opposed to insectivores.

Overall, faunivorous clades of hornbills remained in Africa, and their distribution and degree of speciation was influenced by palaeo-climatic changes and vicariance events that produced habitat barriers, separating populations and inducing specialisation to distinct habitats. African hornbills occur in geologically stable areas with variable climatic conditions, dry

woodland-grassland habitats, and are predominantly faunivorous and non-cooperative, whereas Asian hornbills occur in geologically unstable (insular) areas with relatively stable climatic conditions, humid forest habitats, and are predominantly frugivorous and cooperative.

### **Conservation issues arising from this study**

The species-level molecular phylogeny supported recognition of some recently proposed splits, based on deep nodes observed between sister species or subclades (Chapter 2). This was evident in some African hornbills (*Bycanistes albotibialis*–*B. cylindricus*), but not for all (*Tockus erythrorhynchus* complex). Ideally, more stringent species limits analysis is needed, as has been done with Asian hornbills (Chapter 6). In line with the results of Chapter 6, I added the proposed changes in conservation status for elevated taxa, based on IUCN rules and criteria (BirdLife International, 2000; 2004; IUCN Standards & Petitions Subcommittee, 2010), particularly on reduced area of occupancy (see Table 7.2). I recommend a re-setting of conservation priorities to include new splits made in this thesis, and their apparent restricted ranges (see Fig 7.3).

There is a predominance of threatened hornbills in Asia, particularly insular Asia (see Fig. 7.2 and Table 7.2) and most evident in the Philippines, a condition which may be further increased with the elevation of two taxa (*B. hydrocorax* and *P. basilanicus*, Chapter 6). The Philippines already harbours five threatened species – half the hornbill species known from the country (Collar *et al.*, 1999; BirdLife International 2000; 2004). The re-classified subspecies *Buceros mindanensis semigaleatus* also requires further study to ensure that all differences with the nominate form are taken into account. Despite its high genetic divergence from the nominate form, its low phenotypic scores were insufficient to elevate it to species status. The same holds true for subspecies of *Buceros rhinoceros* (Chapter 6). Initial phylogeographic analysis of *B. rhinoceros* indicates that some degree of genetic divergence has been achieved (Lohman & Meier 2009), but phenotypic evidence is insufficient to alter its taxonomic status. Solomon Islands Hornbill *Rhyticeros mendanae* is now one of only two species of hornbills found in

Melanesia, being a split from the widespread and vagile *R. plicatus* (Chapter 6). Its inherent small population size in these islands may warrant at least a near-threatened status.

The newly split *P. basilanicus* is the most worrying case, as it has an extremely small range, being unique to Basilan Island off the southwest tip of Mindanao. Basilan is seriously deforested, and any remaining fragments of lowland evergreen rainforest occur in the central mountain range (ESSC, 1999; Gonzalez, 2007), which is heavily disturbed by on-going civil war. It is not known whether this species still persists due to lack of recent reports, but studies of its behaviour, ecology and conservation status are impossible owing to inherent risks from crossfire, terrorist attacks and pirates. Local human populations living in camps and armed with guns are, moreover, a significant danger to edible wildlife, so the prospects for *P. basilanicus* may be all the poorer. These same problems are faced by the poorly known and critically endangered Sulu Hornbill *Anthracoceros montani* from the adjacent Sulu archipelago (BirdLife International, 2000; 2004).

The majestic Luzon Rufous Hornbill *Buceros hydrocorax* is here recognised as a monotypic species, which has been split from the southern form *B. mindanensis*, and thus unique to Luzon and Marinduque islands. It probably is nearly extirpated on Marinduque, and populations on Luzon have declined considerably (Collar *et al.* 1999). Prior to the split, *B. hydrocorax* was already classified as near-threatened (BirdLife International, 2000; 2004). A recent population estimate of *B. hydrocorax* showed only 0.48 individuals per km<sup>2</sup> occurring in primary lowland evergreen rainforests, and 0.24 individuals per km<sup>2</sup> in selectively logged forests, and none in degraded forests (Gonzalez *et al.*, in prep., Influence of habitat degradation on population density of hornbills and hornbill-dispersed fruits in the Philippines, see p. 385). Only 24% of Luzon was forested in 1999 (approximately 25,297 km<sup>2</sup> of forest cover remaining, ESSC, 1999) (Gonzalez, 2007), and using this as a conservative estimate (assuming forest cover has not declined in the past 12 years), then perhaps some 6,000 to 12,000 *B. hydrocorax* remain on the island. Apparently even in the pristine forests of the Northern Sierra Madre Natural Park hunting of Rufous Hornbills continues (Persoon *et al.*, 2009; Gonzalez, 2011).

### **Comments on phylogenetic sampling and techniques**

Given the improved recovery of both mtDNA *cytb* and nuclear locus AK 1 intron 5 from fragmented historical DNA, I recommend the use of the designed internal markers on other taxa or geo-isolates not included in this thesis (e.g. *Bycanistes fistulator fistulator*). The use of specific primers designed for hornbills not only improved amplification of target genes, but also reduced risk from contamination, and more importantly reduced the possibility of amplifying non-target nucleotides (e.g. pseudogenes, Zuccon *et al.*, 2008). I had relative success in amplifying both genes from very old historical samples, including those collected more than 150 years ago from Indonesia and Malaysia by A.R. Wallace (c. 1854-1862, Wallace, 1869). My results indicate that historical samples kept in museums located in temperate countries using ideal storage facilities have less degraded DNA, regardless of age, whereas toe-pads from more recently collected skins but kept in museums in tropical countries have more degraded DNA, even for those facilities with relatively high standards. These issues need to be considered when conducting molecular studies on historical samples (Irestedt *et al.*, 2006). The potential uses of the internal primers extends to ancient DNA studies, as modern methods have improved extraction of DNA from Miocene fossils (thus possibly applicable to *Euroceros*, Boev & Kovachev, 2007) and subfossils (e.g. *Rhyticeros* from Lifou, Steadman, 2006). Molecular data from these fossils may help improve further phylogenetic reconstruction and add to our understanding of the evolutionary history of the Bucerotidae.

### ***What more do we need to know about hornbill evolution?***

This thesis has attempted to answer longstanding questions about hornbill evolution and diversification. Although it was able to provide answers to some of the crucial questions, it has its limitations, and in the quest to solve these issues it also inevitably raised new ones. I refer to these unanswered questions and novel issues as research gaps, thus providing new avenues for

further studies. I also include in this section other potential analyses that can be done on existing datasets from my thesis, both genetic and phenotypic.

- The use of representative nuclear (Ak1 intron 5) and mtDNA (*cytb*) was helpful in resolving topological uncertainties highlighted in previous molecular phylogenies (Hübner *et al.*, 2003; Viseshakul *et al.*, 2011). Therefore the use of additional loci may provide more resolution to persistent uncertainties in the phylogeny – such as the polyphyly of *Anthracoceros* and *Ocyceros* (Chapter 2). The use of multiple nuclear and mtDNA loci provided a highly resolved phylogeny for the Sturnidae and Mimidae (Lovette & Rubenstein, 2007), and this extended comparison of DNA sequences can be applied to the Bucerotidae.
- Emerging studies have now begun to compare entire genomes. Indeed, molecular systematics has come a long way from earlier studies, and comparative mitogenomics provide a new perspective into detailed relationships between birds. Of the now 64 species of hornbills, five have full sequences of mtDNA genome currently deposited in GenBank (Pacheco *et al.*, 2011; Sammler *et al.*, 2011). The use of *de novo* sequencing techniques (e.g. 454 pyrosequencing) may help hasten the sequencing of many samples.
- Improved resolution can also be achieved from other molecular markers such as microsatellites, and their potential for more detailed phylogeographic studies in hornbills has been tested (Lohman & Meier 2009). The implications for the study of insular biogeography, extra-pair paternity and population dynamics are clear. Currently, microsatellites are available for *Buceros*, *Aceros* (= *Cranobrontes*) and *Penelopides* (Chamutpong *et al.*, 2009; Sammler *et al.*, 2012).
- Why was the *Aceros* clade so successful a radiation in Asia, and why was *Berenicornis comatus* so unsuccessful? There seems to be a disparity between speciation events in Asian hornbill lineages, as the *Aceros* clade was able to radiate successfully from Indo-China to Sundaland, the Philippines, Wallacea and Melanesia. Similar continental radiation was observed in the *Anorrhinus* clade, and to some extent the *Rhinoplax* clade.

However, *B. comatus* appears to be the only surviving relict from the *Berenicornis* clade, and the story behind this unusual terminus remains untold.

- Tests for other drivers of diversification may be apparent given earlier attempts using the well-resolved molecular phylogeny (Chapter 4) (Derryberry *et al.*, 2011). The predominantly faunivorous *Tockus* clade was equally impressive in diversifying within Africa through various adaptations to different ecological niches (Chapter 5), but it was not deemed successful in speciation compared to Afro-Asian frugivores. Diversification increased rapidly after the split from the ground-hornbills, and the Bucorvinae is not as diverse as the Bucerotinae. Therefore, other distinguishing traits between typical hornbills and ground-hornbills can be used to test diversification models, and one possible focal trait would be the shift to nest-sealing and nest-sanitation.
- Biogeographical patterns can be studied further at a regional level, and given the comprehensive coverage of the *cytb* dataset of hornbill geo-isolates (Chapter 5), further studies can be implemented to look into colonisation routes in specific biogeographic regions (e.g. Wallacea, the Philippines). The Sulu arc is recognised as an important colonisation route for Philippine hornbills (Chapter 5), but I am also interested in testing other hypotheses associated with colonisation of the Philippines, including those postulated by Jones & Kennedy (2008) and Lohman *et al.* (2010).
- Another aspect of comparative phylogenetics was to look into co-evolutionary patterns between associated phylogenetic trees. Although an initial comparison between dated phylogenies of hornbills and hornbill-dispersed fruits was undertaken in Chapter 4, a more detailed tree-by-tree comparison may be needed to understand biotic interactions. This comparison can also be made using a constructed phylogenetic tree for feather lice, given the congruence between earlier hornbill clades (based on cladistics) and their ectoparasites (Kemp, 1995).
- Kemp (2001b) highlighted the need for delineating species in African hornbills. However, due to limited phenotypic data and incomplete sampling of DNA from geo-



isolates, this thesis did not address this issue directly. Therefore, I reiterate the use of a combined approach in evaluating species limits on this group of hornbills, using both standardised genotypic and phenotypic data. I strongly advocate the use of a complete phenotypic dataset (e.g. biometrics, vocalisations, plumage) with adequate sampling to avoid impacting effect sizes. Some African taxa warrant a detailed analysis, including recognised (or subsumed) subspecies of *Tropicranus albocristatus*, *Tockus nasutus*, *Tockus leucomelas* and *Bycanistes subcylindricus*.

- In both Asian and African hornbills, several taxa likely represent incipient species, and additional methods of scoring would be helpful in re-evaluation. Further work is needed to help resolve these borderline cases, such as the use of quantitative scoring for plumage characters (e.g. spectrophotometry of feathers) and vocalisations (e.g. temporal and spectral analysis of calls, Tobias *et al.*, 2010).
- I suggest additional applications of the well-resolved complete molecular phylogeny (Chapter 2) and chronogram (Chapter 4) of hornbills, which can be used in comparative analyses to investigate the evolution of various phenomena, such as the following: (1) nest-sealing behaviour; (2) sexual dimorphism and social system; (3) the divergence of hornbill vocalisations; (4) differences in plumage patterns; (5) variation in display behaviour (e.g. jousting, head-bobbing); (6) anting and dust-bathing behaviour; (7), use of coloured uropygial gland secretions; (8) inter-species cooperation (e.g. cooperation with mongoose, ants and monkeys); (9) gular pouch development; (10) the development of age-dimorphic plumage; (11) casque development; (12) terrestrial progression; and (13) scratching behaviour.
- Kinnaird & O'Brien (2007) postulated five different hypotheses concerning the evolution of the casque in hornbills. Some studies have provided evidence (Alexander *et al.*, 1994; Gamble, 2007; Kasambe *et al.*, 2011) of structure and function, and to some degree shed light on the evolution of this enigmatic but distinctive character. It

appears there is no single ancestry, but testing evolutionary hypotheses within a robust phylogenetic framework would be worthwhile.

## Overall conclusion

This thesis presented a comprehensive phylogenetic and biogeographic analysis of the family Bucerotidae, based on a coalescence of various comparative analyses and molecular methods. I provided the first complete multi-locus molecular phylogeny of hornbills and a more resolved comprehensive mitochondrial (*cytb*) phylogeny of nearly all subspecies and Asian geo-isolates. From these phylogenetic trees, I have been able to calibrate accurately for divergence time, reconstruct ancestral ranges, trace the evolution of traits and calculate genetic divergence. These dataset offered an analytical framework for a suite of studies, leading to the following key conclusions:

- Comparative analyses based on the hornbill phylogeny have allowed us to see beyond global patterns driving the evolution of cooperative breeding in this highly social group of birds. Here I found that environment stability (rather than climatic uncertainty) facilitated hornbills to cooperate, in combination with frugivory, territoriality and body size. Therefore patterns of social evolution and influence of environmental and biotic factors may vary between taxonomic groups. We need to consider the significance of unique systems, and that mixed global patterns may reflect taxon-dependent interactions between diet, climate and cooperation.
- Divergence estimates and ancestral reconstructions using the hornbill phylogeny have allowed us to see the value of innovation (such as a diet shift or the arrival in a new environment) in facilitating diversification – as evident in the frugivory-shift model. Revised estimates of the emergence of frugivory in hornbills underpin the expansion of

tropical floristic components (such as hornbill-dispersed fruits) in Asia, and this model coincides more clearly with palaeo-climatic and palaeo-tectonic events.

- These same divergence estimates have allowed the tracing of colonisation routes between Africa and Asia, providing evidence for the Palaeotropical biotic interchange. This model best describes how Late Oligocene fauna and flora followed the expansion of megathermal forests from East Africa across the narrowing Tethyan Seaway to the Arabia Peninsula and then India. This biotic interchange supports the dispersal events of other frugivores and vertebrate-dispersed fruits found in tropical Africa and Asia.
- Ancestral reconstructions using the hornbill phylogeny have also established colonisation routes across key biogeographic barriers from continental Asia to insular Southeast Asia. Divergence ages coincided with plate-tectonic events that allowed the gradual eastward spread of hornbills. Interestingly, these same dispersal patterns were congruent with the historical biogeography of hornbill-dispersed fruits found across Indo-Malaya and Melanesia, thus supporting the idea that hornbills facilitated their dispersal.

## References

- Aitchison, J.C., Ali, J.R., & Davis, A.M. (2007) When and where did India and Asia collide? *Journal of Geophysical Research*, **112**, No. B05423.
- Alexander, G.D., Houston, D. C., & Campbell, M. (1994) A possible acoustic function for the casque structure in hornbills (Aves: Bucerotidae). *Journal of Zoology*, **233**, 57–67.
- Allen, M.B., & Armstrong, H.A. (2008) Arabia-Eurasia collision and the forcing of mid-Cenozoic global cooling. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **265**, 52–58.
- Avice, J.C. (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.
- BirdLife International (2000) *Threatened Birds of the World*. Lynx Edicions and BirdLife International, Barcelona and Cambridge.
- BirdLife International. 2004. *Threatened Birds of the World 2004*. Cambridge: BirdLife International. CD-ROM.
- BirdLife International (2010) Data zone search for Bucerotidae and Bucorvidae (Hornbills), <http://www.birdlife.org/datazone/index.html>.
- Boev, Z., & Kovachev, D. (2007) *Euroceros bulgaricus* gen. nov., sp. nov. from Hadzhidimovo (SW Bulgaria) (Late Miocene) – the first European record of Hornbills (Aves: Coraciiformes). *Geobios*, **40**, 39–49.
- Bush, M.B., & Flenley, J.R. (2007) *Tropical Rainforest Responses to Climatic Change*. Springer-Praxis, UK.
- Chamutpong, S., Saito, D. S., Viseshakul, N., Nishiumi, I., Poonswad, P., & Ponglikitmongkol, M. (2009) Isolation and characterization of microsatellite markers from the great hornbill, *Buceros bicornis*. *Molecular Ecology Resources*, **9**, 591–3.
- Cockburn, A. (2006) Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society B*, **273**, 1375–1383.
- Collar, N.J., Mallari, N.A.D., & Tabaranza, B.R. Jr. (1999) *Threatened birds of the Philippines*. Bookmark Inc., Makati City, Philippines.
- Derryberry, E. P., Claramunt, S., Derryberry, G., Chesser, R. T., Cracraft, J., Aleixo, A., Pérez-Emán, J., Remsen, J. V. Jr., & Brumfield, R.T. (2011). Lineage diversification and morphological evolution in a large-scale continental radiation: the neotropical ovenbirds and woodcreepers (aves: Furnariidae). *Evolution*, **65**, 2973–86.

- Doyle, J.A., Sauquet, H., Scharaschkin, T., & Le Thomas, A. (2004) Phylogeny, molecular and fossil dating, and biogeographic history of Annonaceae and Myristicaceae (Magnoliales). *International Journal of Plant Sciences*, **165**, 55–67.
- Drummond, A. J., & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Environmental Sciences for Social Change, Inc. [ESSC] (1999) *Decline of the Philippine Forest*. The Bookmark, Inc., Makati City.
- Fregin, S., Haase, M., Olsson, U., & Alström, P. (2012) Pitfalls in comparisons of genetic distances: a case study of the avian family Acrocephalidae. *Molecular Phylogenetics and Evolution*, **62**, 319–28.
- Fritz, S. A., & Purvis, A. (2010) Selectivity in Mammalian Extinction Risk and Threat Types: a New Measure of Phylogenetic Signal Strength in Binary Traits. *Conservation Biology* **24**, 1042–1051.
- Fordyce, J.A. (2010) Host shifts and evolutionary radiations of butterflies. *Proceedings of the Royal Society of London B*, **277**, 3735–3743.
- Gamble, K.C. (2007) Internal anatomy of the hornbill casque described by radiography, contrast radiography, and computed tomography. *Journal of Avian Medical Surgery*, **21**, 38–49.
- Gill, F., & Donsker, D. (2012) International Ornithological Congress World Bird List (v 2.11) Available from: <http://www.worldbirdnames.org/names/> (accessed April 2011).
- Gonzalez, J.C.T. (2007) Fragmented forests and isolated islands: the distribution and status of Philippine endemic hornbills. In: Kemp, A.C. & M.I. Kemp (eds.) *The active management of hornbills and their habitats for conservation*, pp. 51–74. CD-ROM Proceedings of the 4th International Hornbill Conference, Mabula Game Lodge, South Africa. Naturalists & Nomads, Pretoria.
- Gonzalez, J.C.T. (2011) Enumerating the ethno-ornithological importance of Philippine hornbills. *The Raffles Bulletin of Zoology Supplement No. 24*: 149–161.
- Hall, R. (1998) The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall R, Holloway JD, *Biogeography and Geological Evolution of SE Asia*, pp. 99–131, Backhuys Publishers, Leiden.
- Hall, R., Cottam, M.A., & Wilson, M.E.J. (2011) *The SE Asian Gateway: History and Tectonics of the Australia-Asia Collision*. Special publication 355, The Geological Society, Bath.
- Hay, W.W., De Conto, R., Wold, C.N., Wilson, K.M., Voigt, S., Schulz, M., Wold-Rossby, A., Dullo, W.-C., Ronov, A.B., Balukhovsky, A.N., & Soeding, E. (1999) Alternative Global Cretaceous Paleogeography, in Barrera E, Johnson C (eds.), *The Evolution of Cretaceous Ocean/Climate Systems, Geological Society of America Special Paper*, **332**, pp. 1–47.

- Hosner, P.A., Sheldon, F.H., Lim, H.C., & Moyle, R.G. (2010) Phylogeny and biogeography of the Asian trogons (Aves: Trogoniformes) inferred from nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics Evolution*, **57**, 1219–1225.
- Hübner, V.S.M., Prinzing, R., & Wink, M. (2003) Neue Erkenntnisse zur Taxonomie der Hornvögel (Aves: Bucerotiformes) und ihre Bedeutung für die Zucht in Menschenobhut. *Zoologische Garten N.F.*, **73**, 396–401.
- Irestedt, M., Ohlson, J. I., Zuccon, D., Källersjö, M., & Ericson, P. G. P. (2006) Nuclear DNA from old collections of avian study skins reveals the evolutionary history of the Old World suboscines (Aves, Passeriformes). *Zoologica Scripta*, **35**(6), 567–580.
- IUCN Standards & Petitions Subcommittee (2010) *Guidelines for Using the IUCN Red List Categories and Criteria* (version 8.1). Prepared by the Standards and Petitions Subcommittee in March 2010. <http://intranet.iucn.org/webfiles/doc/SSC/RedList/RedListGuidelines.pdf>.
- Jetz, W. & Rubenstein, D.R. (2011) Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, **21**, 1–7.
- Jones, A.W., & Kennedy, R.S. (2008) Evolution in a tropical archipelago: comparative phylogeography of Philippine fauna and flora reveals complex patterns of colonization and diversification. *Biological Journal of the Linnean Society*, **95**, 620–639.
- Jönsson, K.A., Bowie R.C.K., Moyle, R.G. Christidis, L., Norman, J.A., Benz, B.W., & Fjeldså, J. (2010) Historical biogeography of an Indo-Pacific passerine bird family (Pachycephalidae): Different colonization patterns in the Indonesian and Melanesian archipelagos. *Journal of Biogeography*, **37**, 245–257.
- Kasambe, R., Charde, P., & Yosef, R. (2011) Aerial jousting and bill grappling in Indian Grey Hornbill (*Ocyrceros birostris*). *Acta Ethologica*, **14**, 13–15.
- Kemp, A.C. (1995) *Bird families of the world. The Hornbills: Bucerotiformes*. Oxford University Press, Oxford.
- Kemp, A.C. (2001a) Family Bucerotidae (Hornbills). In: de Hoyo, J., Elliott, A., & Sargatal, J., eds. *Handbook of the Birds of the World*. Vol. 6, Mousebirds to Hornbills. pp. 436–523, Lynx Edicions, Barcelona.
- Kemp, A.C. (2001b) The role of species limits and biology in the conservation of African hornbills. *Ostrich Supplement No.*, **15**, 200–204.
- Kemp, A.C., & Crowe, T.M. (1985) The systematics and zoogeography of Afrotropical hornbills (Aves: Bucerotidae). In: Schuchmann, K. (ed) *Proceedings of the International Symposium African Vertebrates*, Bonn., pp. 279–324, Bonn.
- Kinnaird, M.F., & O'Brien, T.G. (2007) *The ecology and conservation of Asian hornbills: farmers of the forest*. The University of Chicago Press, Chicago.

- Körsgen, S., Kantz, R. & Weihelt, M. (2011) planiglobe<sup>®</sup> online map creation. kk+w digitale-digitale kartografie GmbH. Kiel, Germany. [http://www.planiglobe.com/omc\\_set.html](http://www.planiglobe.com/omc_set.html). Accessed December 2011.
- Kreft, H., & Jetz, W. (2010) A framework for delineating biogeographic regions based on global species distributions. *Journal of Biogeography*, **37**, 2029–2053.
- Lerner, H.R., & Mindell, D.P. (2005) Phylogeny of eagles, Old World vultures, and other Accipitridae based on nuclear and mitochondrial DNA. *Molecular Phylogenetics and Evolution*, **37**, 327–346.
- Lohman, D.J., & Meier, R. (2009) Regional genetic diversity in Oriental Pied and Rhinoceros Hornbills Genetic studies on hornbills. Abstract of Papers, The 5th International Hornbill Conference, Singapore Botanic Gardens, 22nd–25th March 2009. Singapore
- Lohman, D. J., Ingram, K. K., Prawiradilaga, D. M., Winker, K., Sheldon, F. H., Moyle, R. G., Ng, P. K. L., Ong, P.S., Wang, L.K., Braile, T.M., Astuti, D. & Meier, R. (2010) Cryptic genetic diversity in ‘widespread’ Southeast Asian bird species suggests that Philippine avian endemism is gravely underestimated. *Biological Conservation*, **143**(8): 1885–1890.
- Lohman, D.J., de Bruyn, M., Page, T., von Rintelen, K., Hall, R., Ng, P.K.L., Shih, T.-H., Carvalho, G.R., & von Rintelen, T. (2011) Biogeography of the Indo-Australian Archipelago. *The Annual Review of Ecology, Evolution, and Systematics*, **42**, 205–26.
- Lovette, I. J., Arbogast, B. S., Curry, R. L., Zink, R. M., Botero, C. a, Sullivan, J. P., Talaba, A. L., Harris, R.B., Rubenstein, D.R., Ricklefs, R.E., & Bermingham, E. (2012) Phylogenetic relationships of the mockingbirds and thrashers (Aves: Mimidae). *Molecular Phylogenetics and Evolution*, **63**, 219–29.
- Marks, B. D., Weckstein, J. D., & Moyle, R. G. (2007) Molecular phylogenetics of the bee-eaters (Aves: Meropidae) based on nuclear and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **45**(1), 23–32.
- Muellner, A.N., Pannell, C.M., Coleman, A., & Chase, M.W. (2008) The origin and evolution of Indomalayan, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *Journal of Biogeography*, **35**, 1769–1789.
- Muñoz, A., Román, R., Real, R., Olivero, J., Márquez, A.L., Guerrero, J.C., Bárcena, S.B., & Vargas, J.M. (2003) Biogeographical zonation of African hornbills and their biotic and geographic characterizations, *Ostrich*, **74** (1), 39–47.
- Newton, I. (2003) *The Speciation and Biogeography of Birds*. Academic Press, London.
- Pacheco, M. A., Battistuzzi, F. U., Lentino, M., Aguilar, R. F., Kumar, S., & Escalante, A. A. (2011) Evolution of modern birds revealed by mitogenomics: timing the radiation and origin of major orders. *Molecular Biology and Evolution*, **28**(6), 1927–42.

- Page, R.D.M., & Holmes, E.C. (1998) *Molecular Evolution: A Phylogenetic Approach*. Blackwell Science Ltd, Oxford.
- Persoon, G., Masipiqueña, A., Van der Ploeg, J., Masipiqueña, M. & Van Weerd, M. (2009) Crossing Boundaries: Celebrating 20 years of environmental research in Cagayan Valley and Sierra Madre. CVPED, Cabagan, Philippines.
- Price, T.D. (2008) *Speciation in Birds*. Roberts & Company Publishers, Colorado.
- Rubenstein, D. R. (2011) Spatiotemporal environmental variation, risk aversion , and the evolution of cooperative breeding as a bet-hedging strategy. *Proceedings of the National Academy of Science*, **108**, 10816–10822
- Sammler, S., Bleidorn, C., & Tiedemann, R. (2011) Full mitochondrial genome sequences of two endemic Philippine hornbill species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. *BMC Genomics*, **12**, 35–45.
- Sammler, S., Havenstein, K., & Tiedemann, R. (2012) Fourteen new microsatellite markers for the Visayan tarictic hornbill (*Penelopides panini*) and their cross-species applicability among other endangered Philippine hornbills. *Conservation Genetics Resources*, **4**, 435–438.
- Sibley, C.G., & Ahlquist, J.E. (1990) *Phylogeny and Classification of Birds*. Yale University Press, New Haven, CT.
- Srikwan, S., & Woodruff, D.S. (1998) Molecular genetics and the conservation of hornbills in fragmented landscape. In: Poonswad, P. (ed.) *The Asian Hornbills: Ecology and Conservation. Thai Studies in Biodiversity No.2*, pp. 257–263. Bangkok, Thailand.
- Steadman, D.W. (2006) *Extinction and biogeography of tropical Pacific birds*. University of Chicago Press, Chicago, IL.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731–2739.
- Tobias, J.A., Seddon, N., Spottiswoode, C.N., Pilgrim, J.D., Fishpool, L.D.C., & Collar, N.J. (2010) Quantitative criteria for species delimitation. *Ibis*, **152** (4), 724–746.
- Viseshakul, N., Charoennitikul, W., Kitamura, S., Kemp, A.C., Thong-aree, S., Surapunpitak, Y., Poonswad, P., & Ponglikitmongkol, M. (2011) A phylogeny of frugivorous hornbills linked to the evolution of Indian plants within Asian rainforests. *Journal of Evolutionary Biology*, **24**(7), 1533–45.
- Wallace, A.R. (1869) *The Malay Archipelago; the land of the orang-utan, and the bird of paradise; a narrative of travel, with studies of man and nature*. McMillan & Co., London.



- Weeks, A., Daly, D.C., & Simpson, B.B. (2005) The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution*, **35**, 85–101.
- White, N. E., Phillips, M. J., Gilbert, M. T. P., Alfaro-Núñez, A., Willerslev, E., Mawson, P. R., Spencer, P. B. S., & Bunce, M. (2011). The evolutionary history of cockatoos (Aves: Psittaciformes: Cacatuidae). *Molecular Phylogenetics and Evolution*, **59**(3), 615–22.
- Yu, Y., Harris, A.J., & He, X.J. (2011) A Novel Bayesian Method for Reconstructing Geographic Ranges and Ancestral States on Phylogenies. *Systematic Biology*, Under Review. RASP (Reconstruct Ancestral State in Phylogenies) is available at <http://mnh.scu.edu.cn/soft/blog/RASP>.
- Zachos, J., Pagani, M., Sloan, L., Thomas, E. & Billups, K. (2001) Trends, rhythms, and aberration in global climate 65 Ma to present. *Science*, **292**, 686–693.
- Zhou, L., Su, Y.C.F., Thomas, D.C., & Saunders, R.M.K. (2011) ‘Out-of-Africa’ dispersal of tropical floras during the Miocene climatic optimum: evidence from *Uvaria* (Annonaceae). *Journal of Biogeography*, **39**, 322–335.
- Zuccon, D., Pasquet, E., & Ericson, P. G. P. (2008) Phylogenetic relationships among Palearctic-Oriental starlings and mynas (genera *Sturnus* and *Acridotheres* : Sturnidae). *Zoologica Scripta*, **37**, 469–481.

**Table 7.1:** Molecular phylogenies of hornbills (Aves: family Bucerotidae). A comparison of different phylogenetic trees based on the number of taxa covered, gene region used and similarities in recovered clades between topologies.

|                                                  | Sibley & Ahlquist, 1990 | Srikwan & Woodruff, 1998 | Hübner <i>et al.</i> , 2003 | Viseshakul <i>et al.</i> , 2011 | Gonzalez <i>et al.</i> , in prep., Chapter 2 | Gonzalez <i>et al.</i> , in prep., Chapter 5 |
|--------------------------------------------------|-------------------------|--------------------------|-----------------------------|---------------------------------|----------------------------------------------|----------------------------------------------|
| No. of taxa HBW <sup>a</sup><br>(54 sp/79 taxa)  | 17/17                   | 11/11                    | 19/25                       | 31/36                           | 54/61                                        | 54/78                                        |
| No. of taxa IOC <sup>a</sup> (61 sp/89 taxa)     | 17/17                   | 11/11                    | 22/25                       | 34/37                           | 61/61                                        | 61/87                                        |
| Gene region used                                 | N.A. <sup>b</sup>       | cytb                     | cytb                        | cytb                            | AK1 intron 5; cytb                           | cytb                                         |
| Bucerotidae monophyletic                         | Yes                     | Yes                      | Yes                         | Yes                             | Yes                                          | Yes                                          |
| Bucorvinae basal to Bucerotinae                  | Yes                     | Yes                      | Yes                         | Yes                             | Yes                                          | Yes                                          |
| <i>Tockus</i> clade monophyly <sup>c</sup>       | Yes                     | N.A.                     | Yes                         | Yes                             | Yes                                          | Yes                                          |
| <i>Berenicornis</i> clade monophyly <sup>c</sup> | No                      | Yes                      | Yes                         | Yes                             | Yes                                          | Yes                                          |

## CHAPTER 7

|                                                       |      |      |      |     |     |     |
|-------------------------------------------------------|------|------|------|-----|-----|-----|
| <i>Rhinoplax</i> clade monophyly <sup>c</sup>         | Yes  | Yes  | Yes  | Yes | Yes | Yes |
| <i>Anorrhinus</i> clade monophyly <sup>c</sup>        | No   | Yes  | Yes  | Yes | Yes | Yes |
| <i>Aceros</i> clade monophyly <sup>c</sup>            | N.A. | Yes  | Yes  | Yes | Yes | Yes |
| <i>Tockus</i> clade basal to frugivorous<br>hornbills | Yes  | N.A. | Yes  | Yes | Yes | Yes |
| <i>Tockus</i> clade split                             | Yes  | N.A. | Yes  | Yes | Yes | Yes |
| <i>Berenicornis</i> basal to African<br>forest        | No   | N.A. | No   | Yes | Yes | Yes |
| <i>Rhinoplax</i> basal to <i>Buceros</i>              | Yes  | N.A. | N.A. | Yes | Yes | Yes |
| <i>Anorrhinus</i> basal to<br><i>Anthracoceros</i>    | No   | Yes  | N.A. | Yes | Yes | Yes |
| <i>Anthracoceros</i> polyphyly                        | N.A. | Yes  | No   | No  | Yes | Yes |
| <i>Penelopides-Aceros</i>                             | N.A. | N.A. | Yes  | Yes | Yes | Yes |
| <i>Aceros</i> polyphyly                               | N.A. | N.A. | Yes  | Yes | Yes | Yes |

<sup>a</sup>Number of taxa based on two bird lists used in this thesis, HBW: *Handbook of the Birds of the World* (Kemp, 2001a); IOC: International Ornithological Congress World Bird List version 2.11 (Gill & Donsker, 2012).

<sup>b</sup>Gene region not specified; *cytb*: mitochondrial DNA cytochrome *b*; AK1 intron 5: nuclear gene adenylate kinase 1 intron 5; N.A.: not applicable.

<sup>c</sup>Clade based on major clades discussed in Chapter 2

# CHAPTER 7

**Table 7.2:** Taxonomic recommendations and conservation status of hornbills (Bucerotidae) based on proposed changes from Gonzalez *et al.* (in prep.) and this study.

| Clade                   | Current taxonomy (IOC 2.10)      | Taxonomic recommendation <sup>a</sup> | Status <sup>b</sup> |
|-------------------------|----------------------------------|---------------------------------------|---------------------|
| A - <i>Bucorvus</i>     | <i>Bucorvus abyssinicus</i>      | <i>Bucorvus abyssinicus</i>           | LC                  |
|                         | <i>Bucorvus leadbeateri</i>      | <i>Bucorvus leadbeateri</i>           | VU                  |
| B - <i>Tockus</i>       | <i>Tockus ruahae</i>             | <i>Tockus ruahae</i>                  | LC                  |
|                         | <i>Tockus kemp</i>               | <i>Tockus kemp</i>                    | LC                  |
|                         | <i>Tockus damarensis</i>         | <i>Tockus damarensis</i>              | LC                  |
|                         | <i>Tockus rufirostris</i>        | <i>Tockus rufirostris</i>             | LC                  |
|                         | <i>Tockus erythrorhynchus</i>    | <i>Tockus erythrorhynchus</i>         | LC                  |
|                         | <i>Tockus monteiri</i>           | <i>Tockus monteiri</i>                | LC                  |
|                         | <i>Tockus deckeni</i>            | <i>Tockus deckeni</i>                 | LC                  |
|                         | <i>Tockus jacksoni</i>           | <i>Tockus jacksoni</i>                | LC                  |
|                         | <i>Tockus leucomelas</i>         | <i>Tockus leucomelas</i>              | LC                  |
|                         | <i>Tockus flavirostris</i>       | <i>Tockus flavirostris</i>            | LC                  |
|                         | <i>Tockus bradfieldi</i>         | <i>Rhynchaceros bradfieldi</i>        | LC                  |
|                         | <i>Tockus alboterminatus</i>     | <i>Rhynchaceros alboterminatus</i>    | LC                  |
|                         | <i>Tockus fasciatus</i>          | <i>Rhynchaceros fasciatus</i>         | LC                  |
|                         | <i>Tockus hemprichii</i>         | <i>Rhynchaceros hemprichii</i>        | LC                  |
|                         | <i>Tockus nasutus</i>            | <i>Rhynchaceros nasutus</i>           | LC                  |
|                         | <i>Tockus camurus</i>            | <i>Rhynchaceros camurus</i>           | LC                  |
|                         | <i>Tockus pallidirostris</i>     | <i>Rhynchaceros pallidirostris</i>    | LC                  |
| C - <i>Berenicornis</i> | <i>Bycanistes fistulator</i>     | <i>Bycanistes fistulator</i>          | LC                  |
|                         | <i>Bycanistes bucinator</i>      | <i>Bycanistes bucinator</i>           | LC                  |
|                         | <i>Bycanistes cylindricus</i>    | <i>Bycanistes cylindricus</i>         | NT                  |
|                         | <i>Bycanistes albotibialis</i>   | <i>Bycanistes albotibialis</i>        | LC                  |
|                         | <i>Bycanistes subcylindricus</i> | <i>Bycanistes subcylindricus</i>      | LC                  |
|                         | <i>Bycanistes brevis</i>         | <i>Bycanistes brevis</i>              | LC                  |
|                         | <i>Ceratogymna atrata</i>        | <i>Ceratogymna atrata</i>             | LC                  |
|                         | <i>Ceratogymna elata</i>         | <i>Ceratogymna elata</i>              | NT                  |
|                         | <i>Tropicranus hartlaubi</i>     | <i>Tropicranus hartlaubi</i>          | LC                  |
|                         | <i>Tropicranus albocristatus</i> | <i>Tropicranus albocristatus</i>      | LC                  |
| D - <i>Buceros</i>      | <i>Buceros rhinoceros</i>        | <i>Buceros rhinoceros</i>             | NT                  |
|                         | <i>Buceros bicornis</i>          | <i>Buceros bicornis</i>               | NT                  |
|                         | <i>Buceros hydrocorax</i>        | <i>Buceros hydrocorax</i>             | VU <sup>c</sup>     |
|                         |                                  | <i>Buceros mindanensis</i>            | VU <sup>c</sup>     |
|                         | <i>Rhinoplax vigil</i>           | <i>Rhinoplax vigil</i>                | NT                  |
| E - <i>Anorrhinus</i>   | <i>Anthracosceros marchei</i>    | <i>Anthracosceros marchei</i>         | VU                  |

## CHAPTER 7

|                   |                                  |                                   |                 |
|-------------------|----------------------------------|-----------------------------------|-----------------|
|                   | <i>Anthracoceros albirostris</i> | <i>Anthracoceros albirostris</i>  | LC              |
|                   | <i>Anthracoceros coronatus</i>   | <i>Anthracoceros coronatus</i>    | NT              |
|                   | <i>Anthracoceros montani</i>     | <i>Anthracoceros montani</i>      | CR              |
|                   | <i>Anthracoceros malayanus</i>   | <i>Anthracoceros malayanus</i>    | NT              |
|                   | <i>Ocyrceros griseus</i>         | <i>Ocyrceros griseus</i>          | LC              |
|                   | <i>Ocyrceros gingalensis</i>     | <i>Ocyrceros gingalensis</i>      | LC              |
|                   | <i>Ocyrceros birostris</i>       | <i>Ocyrceros birostris</i>        | LC              |
|                   | <i>Anorrhinus tickelli</i>       | <i>Anorrhinus tickelli</i>        | NT              |
|                   | <i>Anorrhinus austeni</i>        | <i>Anorrhinus austeni</i>         | NT              |
|                   | <i>Anorrhinus galeritus</i>      | <i>Anorrhinus galeritus</i>       | LC              |
| F - <i>Aceros</i> | <i>Aceros nipalensis</i>         | <i>Aceros nipalensis</i>          | VU              |
|                   | <i>Rhyticeros plicatus</i>       | <i>Rhyticeros plicatus</i>        | LC              |
|                   |                                  | <i>Rhyticeros mendanae</i>        | VU <sup>c</sup> |
|                   | <i>Rhyticeros narcondami</i>     | <i>Rhyticeros narcondami</i>      | EN              |
|                   | <i>Rhyticeros undulatus</i>      | <i>Rhyticeros undulatus</i>       | LC              |
|                   | <i>Rhyticeros everetti</i>       | <i>Rhyticeros everetti</i>        | VU              |
|                   | <i>Rhyticeros subruficollis</i>  | <i>Rhyticeros subruficollis</i>   | VU              |
|                   | <i>Aceros cassidix</i>           | <i>Cranorrhinus cassidix</i>      | LC              |
|                   | <i>Aceros waldeni</i>            | <i>Cranobrontes waldeni</i>       | CR              |
|                   | <i>Aceros leucocephalus</i>      | <i>Cranobrontes leucocephalus</i> | NT              |
|                   | <i>Penelopides exarhatus</i>     | <i>Cranobrontes exarhatus</i>     | LC              |
|                   | <i>Aceros corrugatus</i>         | <i>Cranobrontes corrugatus</i>    | NT              |
|                   | <i>Penelopides manillae</i>      | <i>Penelopides manillae</i>       | LC              |
|                   | <i>Penelopides mindorensis</i>   | <i>Penelopides mindorensis</i>    | EN              |
|                   | <i>Penelopides affinis</i>       | <i>Penelopides affinis</i>        | LC              |
|                   |                                  | <i>Penelopides basilanicus</i>    | VU <sup>c</sup> |
|                   | <i>Penelopides samarensis</i>    | <i>Penelopides samarensis</i>     | LC              |
|                   | <i>Penelopides panini</i>        | <i>Penelopides panini</i>         | EN              |

<sup>a</sup>Taxonomic treatment recommended on the basis of new data present in this paper, to compare with recent International Ornithological Congress World Bird List version 2.11 (Gill & Donsker 2012).

<sup>b</sup>Conservation status of hornbills according to the IUCN Red List assessments: LC, Least Concern; VU, Vulnerable; EN, Endangered; CR, Critically Endangered; NT, Near-threatened; assignment to categories follows BirdLife International (2000; 2004), apart from *Bucorvus leadbeateri* which was upgraded to VU from LC (BirdLife International, 2010; [www.birdlife.org/action/science/species/global\\_species\\_programme/whats\\_new.html](http://www.birdlife.org/action/science/species/global_species_programme/whats_new.html)).

<sup>c</sup>Proposed conservation status based on small effective population following elevated species status, based on IUCN Standards and Petitions Subcommittee (2010).

## CHAPTER 7

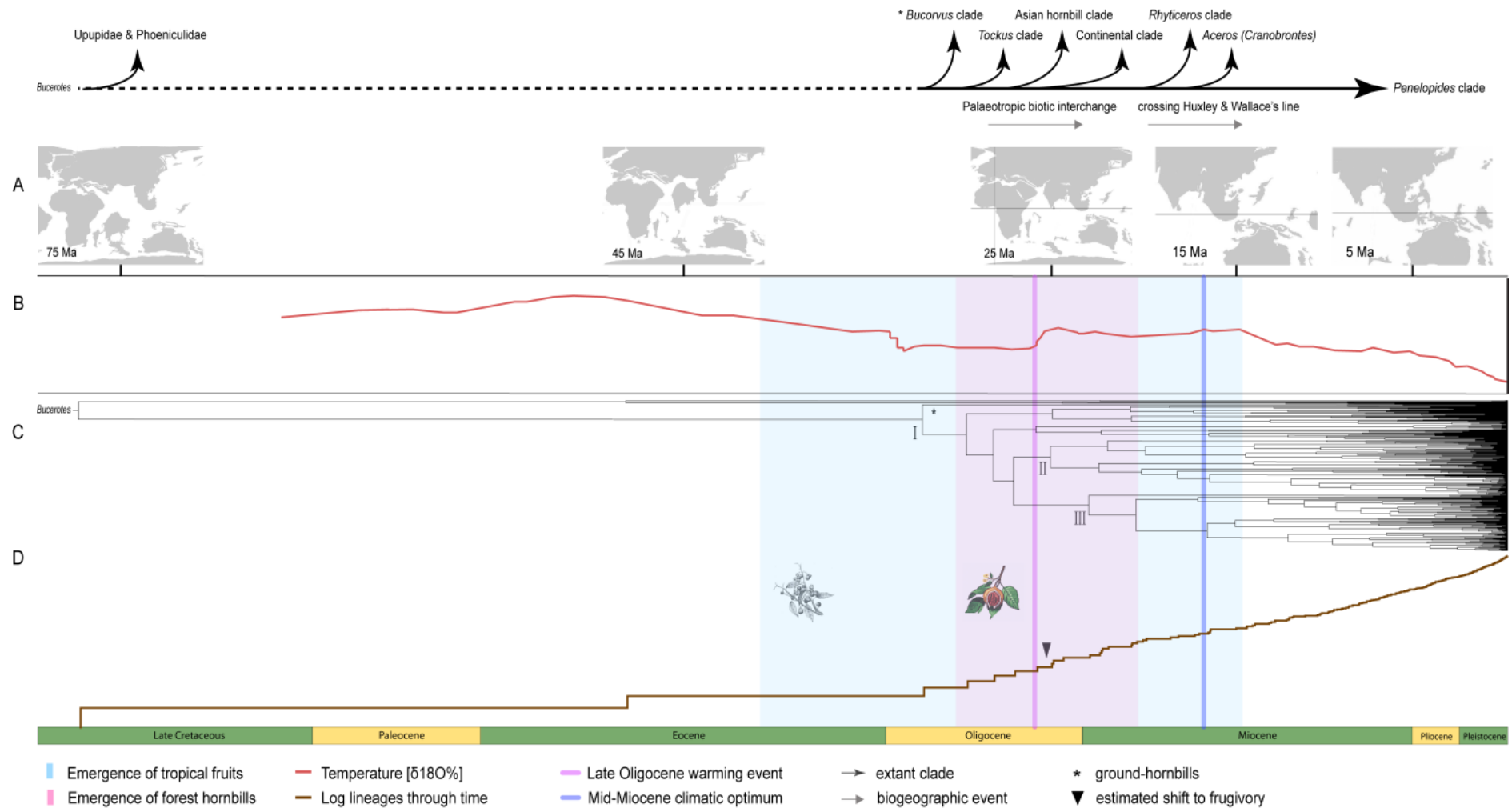
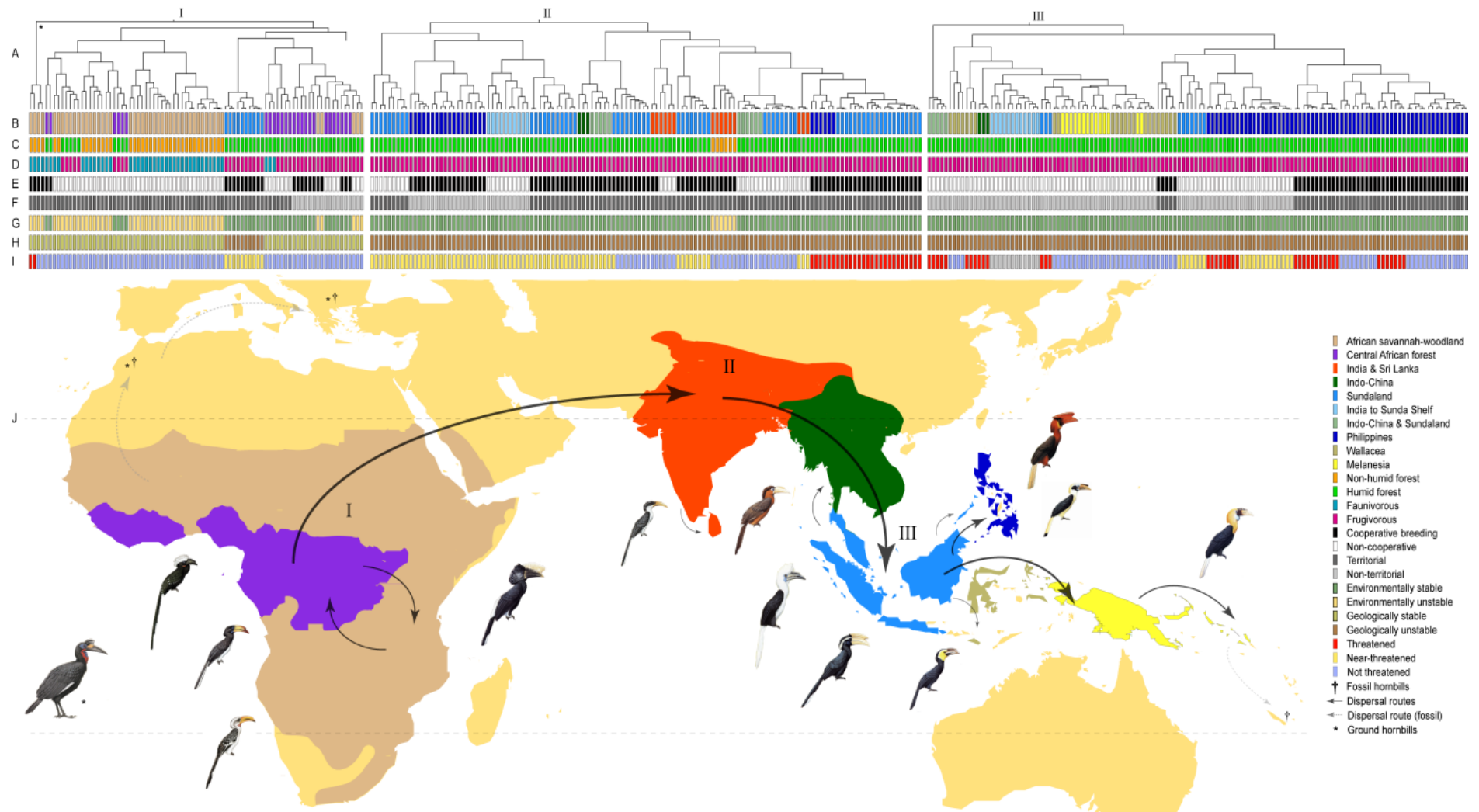


**Figure 7.1:** Evolutionary history of hornbills, described from key events marked along a timeline showing approximate palaeo-tectonic configuration (A) and climatic changes (B), based on a robust molecular chronogram of hornbill geo-isolates (mtDNA *cytb*) (C), with rate of diversification represented by a

## CHAPTER 7

lineage-through-time plot (D). Nodes on the *cytb* tree correspond to splits of extant clades along the *Bucerotes* lineage leading to the derived *Penelopides* clade (shown on top). Roman numbers at nodes of *cytb* tree refer to regional clades, represented by African hornbills (I), continental Asian hornbills (II), and insular Asian hornbills (III), which are equivalent clades shown in Fig. 7.2. Plate-tectonic maps were reconstructed in the on-line service ODSN-GEOMAR (Hay *et al.* 1999), and Palaeo-climatic data adapted from Zachos *et al.* (2001).

# CHAPTER 7



## CHAPTER 7

**Figure 7.2:** Biogeography and trait evolution in hornbills, described from a comparison of characters and distribution along a comprehensive molecular phylogeny of hornbill geo-isolates (mtDNA *cytb*) (A). Coloured bars indicate the corresponding biogeographic region (B), preferred habitat (C), predominant diet (D), social system (E), degree of territoriality (F), prevailing climatic conditions (G), palaeo-geologic history (H), and current conservation status (I). Map below showing approximate dispersal routes of extant and extinct hornbills across the Old World (J), with coloured codes for biogeographic regions. Roman numbers refer to regional clades, represented by African hornbills (I), continental Asian hornbills (II), and insular Asian hornbills (III) based on a full phylogeny shown in Fig 7.1. Map modified from Körsgen *et al.* (2011) and hornbill distribution based from Kemp (2001a). Biogeographic regions were based on Kreft & Jetz (2010). Illustrations of hornbills are reproduced from *Handbook of the Birds of the World* with the permission of Lynx Edicions.



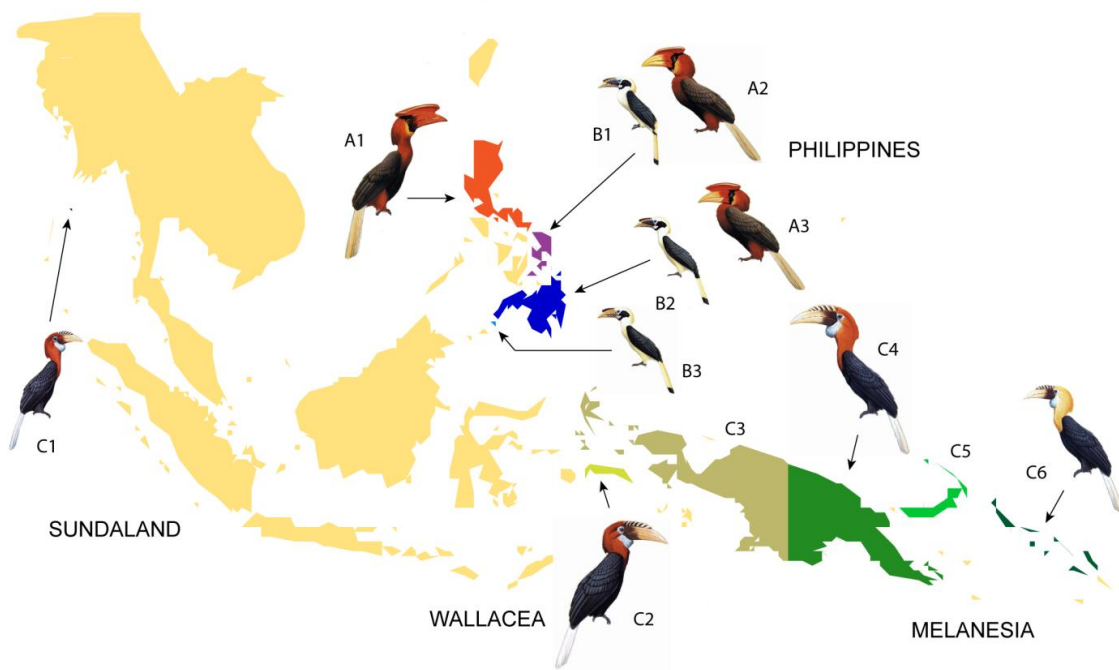
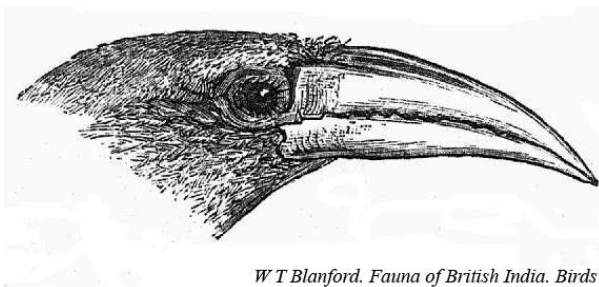


**Figure 7.3:** Distribution of three species complexes of hornbills from Southeast Asia and Melanesia, A: *Buceros hydrocorax* (1, *hydrocorax*; 2, *mindanensis semigaleatus*; 3, *mindanensis mindanensis*), B: *Penelopides affinis* (1, *samarensis*; 2, *affinis*; 3, *basilanicus*), and C: *Rhyticeros plicatus* (1, *narcondami*; 2, *plicatus plicatus*; 3, *plicatus ruficollis*; 4, *plicatus jungei*; 5, *plicatus dampieri*; 6, *mendanae*), illustrating the insular restriction of each taxon. Map modified from Körsgen *et al.* (2011) and hornbill distribution based on Kemp (2001a). Illustrations of hornbills are reproduced from *Handbook of the Birds of the World* with the permission of Lynx Edicions.

## Appendices

### Other papers on the Ecology and Ethno-ornithology of Philippine Hornbills



*W T Blanford. Fauna of British India. Birds*

*Although the extraordinary breeding habits of hornbills have excited comment for many years, little is actually recorded except that the female is walled up in a natural hole, where she is fed by the male through a slit and undergoes a complete moult*

Reginald Ernest Moreau, 1934, Breeding habits of Hornbills, *Nature* 134. Edward Grey Institute for Field Ornithology (1947-1966)

Other research papers completed and presented as a paper/poster while a DPhil student

## **Basic ecology of two sympatric species of Philippine hornbills**

### **Abstract**

Philippine hornbills are predominantly canopy frugivores that inhabit old growth lowland forests due to their need for large nesting trees and preference for fruits of climax tree species. In this chapter, I determined differences in resource use between two sympatric species of hornbills (*Buceros hydrocorax* and *Penelopides manillae*) inhabiting Northern Sierra Madre Natural Park in Luzon. I compared diet preferences and nest availability between three study sites for both hornbills, based on counts of large trees and nesting cavities. Large Dipterocarp trees with >1 m in diameter were counted in three 16-hectare plots within each site. Density of hornbill nests were estimated from actual counts found within the cumulative survey area of 16 km<sup>2</sup> with each cavity sampled for nest characters (i.e., cavity size) and contents (i.e., seeds). Presence of small seeds from palms & pioneer trees in Tarictic hornbill nests implies their ability to forage below the canopy and into disturbed forests. Nearly 40 species of seeds from rainforest plants were recorded in nest cavities, with the majority from large-seeded non-fig fruits (i.e., *Aglaia*, *Dysoxylum*, *Knema*, *Myristica*). This strengthens the importance of hornbills as key dispersal agents for the equally threatened old growth rainforest trees (*Myristica colinridsdalei*). Competition was limited between the two sympatric species, given their differences in resource use. Availability of suitable cavities was crucial in the persistence of both Rufous and Tarictic hornbills in old growth & disturbed forests on Luzon, regardless of quantity of large dipterocarps. Atypical low nest density of both Rufous and Tarictic hornbills in pristine forests of NSMNP raises a red-light on their conservation status, and therefore evokes urgent re-evaluation. Rufous hornbills appear to be vulnerable to habitat loss, being more specialised in nest choice, fruit and habitat preferences, whereas Tarictic hornbills are more adaptable and resilient, being able to use sub-optimal habitats and extend fruit choice.

## **Influence of habitat degradation on population density of hornbills and hornbill-dispersed fruits in the Philippines**

### **Abstract**

Obligate frugivores like Philippine hornbills may play an important role in forest dynamics, being key partners of fruit trees within the seed dispersal loop. Severe fragmentation of habitats may lead to disperser decline, which causes a disruption in this fragile loop. Philippine hornbills require large nesting trees and prefer fruits of climax tree species. Low tolerance to anthropogenic disturbance may restrict movements across fragmented habitats, limiting access to food resources and reducing breeding success. In this chapter, I tested the influence of limiting habitat quality through a comparison of hornbill densities across disturbance gradients with other canopy frugivores and the influence on density of hornbill-dispersed rainforest trees. Hornbills, pigeons and parrots were counted at 320 points on three study grids, representing (A) old growth (B) selectively-logged and (C) degraded lowland forests. Habitat was analysed based on tree counts of different size classes at each survey point within each grid in the Northern Sierra Madre Natural Park in Luzon, Philippines. Population density was calculated in DISTANCE 6.0, using different model functions. Avian frugivores responded differently to forest degradation, some persisting even with slight disturbance, unlike less resilient species. The decline of endemic frugivores corroborate the need to protect and restore old growth tropical lowland forests. Hornbills are generally less tolerant of forest fragmentation than other frugivores. Persistence of pigeons and parrots maybe due to ecotones, i.e., presence of figs and other pioneer fruit trees at gaps and forest edges. Smaller Tarictic hornbills can persist in degraded forests, unlike larger Rufous hornbills, which are more vulnerable to habitat alteration due to their dependence on large trees. Further analysis of densities of key hornbill-dispersed fruits (i.e., *Aglaia macrocarpa*) is necessary to evaluate the influence of fragmentation or the effectiveness of hornbills as dispersal agents across disturbance gradients.

Best Poster Award, 19<sup>th</sup> Annual Biodiversity Symposium of the Wildlife Conservation Society of the Philippines, April 2010, Legaspi City, Philippines,

## **Variation in population density of hornbills and other avian frugivores along a disturbance gradient**

**Juan-Carlos Gonzalez, Ben Sheldon and Joe Tobias**

*Edward Grey Institute for Field Ornithology, Department of Zoology, University of Oxford, Tinbergen Building, South Parks Road, Oxford OX1 3PS United Kingdom. E-mail: [juan.gonzalez@zoo.ox.ac.uk](mailto:juan.gonzalez@zoo.ox.ac.uk)*

Tropical rainforests in the Philippines suffer on-going degradation from both man-made perturbation and natural calamities. Canopy frugivores are key bio-indicators and their population densities can be used to test disturbance impacts. This paper aims to determine if forest disturbance has a deleterious effect on the population density of canopy frugivores. Hornbills, pigeons and parrots were counted in 320 points on three study grids at Northern Sierra Madre Natural Park in Luzon Island, representing old growth, selectively-logged and degraded lowland forests. Counts were analyzed with DISTANCE using different model functions. Degree of habitat change was based on counts of trees in three different size classes. Overall hornbill density decreased with greater forest disturbance. Population density of Rufous Hornbills dropped significantly at altered forests, while Tarictic Hornbills persisted in degraded forests. Most fruit-doves, brown-doves and parrots persisted in altered forests, while some showed increased densities in selectively-logged forest. Avian frugivores respond differently to forest disturbance and some can tolerate greater disturbance, unlike less resilient species. Apparently, smaller hornbills are more tolerable to degraded forests, while larger hornbills are vulnerable to habitat alternation. This is in common with other sympatric forest bucerotids in India and Africa. However, other variables must be considered to determine and test which factors influence density patterns the most. Given these variable reactions to disturbance, conservation strategies should be designed with these reactions into consideration.



Poster presented during the 6th International Hole-nesting Birds Conference, June 2010, Lady Margaret Hall, University of Oxford

## **Nesting behaviour and ecology of two sympatric species of Philippine hornbills**

**Juan-Carlos Gonzalez, Ben Sheldon and Joe Tobias**

*Edward Grey Institute for Field Ornithology, Department of Zoology, University of Oxford*

### **Summary**

Availability of suitable cavities was crucial in the persistence of both Rufous and Tarictic Hornbills in old growth & disturbed forests on Luzon, regardless of quantity of large dipterocarps. Atypical low nest density of both Rufous and Tarictic hornbills in pristine forests of NSMNP raises a red-light on their conservation status, and therefore evokes urgent re-evaluation. Presence of smaller seeds from palms & pioneer trees in Tarictic hornbill nests implies their ability to forage below the canopy and into disturbed forests. Nearly 40 species of rainforest plants were recorded in nest cavities, with the majority from large-seeded non-fig fruits. This strengthens the importance of hornbills as key dispersal agents for equally threatened old growth rainforest trees.



Paper presented during the 5<sup>th</sup> International Hornbill Conference, March 2009, Singapore Botanic Gardens, Singapore

## Enumerating the Ethno-ornithological Importance of Philippine Hornbills

THE RAFFLES BULLETIN OF ZOOLOGY 2011 Supplement No. 24: 149–161  
Date of Publication: 30 Mar.2011  
© National University of Singapore

### ENUMERATING THE ETHNO-ORNITHOLOGICAL IMPORTANCE OF PHILIPPINE HORBILLS

Juan Carlos T. Gonzalez

Edward Grey Institute for Field Ornithology, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS United Kingdom  
Animal Biology Division, Institute of Biological Sciences, University of the Philippines Los Baños, College, Laguna 4031 Philippines  
E-mail: juan.gonzalez@zoo.ox.ac.uk

**ABSTRACT.** – The ethno-biological value of hornbills in the Philippines are largely scattered in the local literature, and this paper represents a compilation of these local traditions and practices. Various ethno-linguistic groups emulate the significance of hornbills in their language and artifacts, which show similarities with other cultures in Asia and Africa. Importance of nine endemic species hornbills is well established among a number of different indigenous people in the Philippine islands. Their value varies from a basis for telling time or bringer of bad omen, to a source of food and local medicine. Among indigeous tribes of Northern Luzon, hornbills adorn headdresses (Yangnoh, Panglao), used as jewelry (Batling) and as basis for ceremonial dances (Tariktik). Hornbills are also found in several literary accounts such as fables, poems and songs, but are limited and require further research. Local beliefs are often patterned from behavior and have either negative or positive effects. Derivatives of vernacular names appear to be varied across major regional languages, from the onomatopoeic Tarictic to the widely used Kalaw. Official Filipino names for all 15 Philippine hornbills are proposed. The significance of these diverse uses and traditions may have implications to the conservation of these endemic Philippine hornbills.

This paper was presented at the 5<sup>th</sup> International Hornbill Conference jointly organised by the National Parks Board (Singapore) and the Hornbill Research Foundation (Thailand), in Singapore on 22<sup>nd</sup>–25<sup>th</sup> March 2009.

**KEY WORDS.** – Hornbills, Philippines, ethno-ornithology, local communities.

#### INTRODUCTION

There are scattered traces of hornbills in culture, in the Philippines and neighbouring parts of Southeast Asia. The publication by Maramba (1998), for example, contains photographs of these colourful adornments of the Ilonggot and Ifugao tribes taken by M. Yokoyama. Postcards have been produced showing tribal artifacts called Batling and Yangnoh that were adorned with beaks of the Rufous hornbill (*Buceros hydrocorax hydrocorax*). A webpage exists showing photographs of native dancers taken during a recent Ifugao festival. Each performer had worn the Yangnoh, and each had a real Rufous hornbill skull. Another webpage featured photos of a self-proclaimed Ifugao princess wearing another Yangnoh (normally worn by men), which was decorated with the skull of a Luzon Tarictic hornbill (*Penelopides manillae manillae*). These are merely fragments of the inimitable ties binding endemic hornbills with the traditions of the equally unique indigenous people of the Philippine islands.

A question exists whether these time-honoured practices of the Ifugao have had any influence on the decline of hornbills

in the Central Cordillera region of Luzon. Does this change from traditional use to commercial tourism have any future impacts on remaining hornbill populations? Recent surveys and birding reports have indicated far fewer sightings of hornbills from both Benguet and Mountain provinces, which are the domain of the Ifugao people. The Natural History Museum in Tring holds several skins of Rufous hornbills collected by John Whitehead in 1894 from La Trinidad, Benguet and Mount Data. Such localities no longer harbour any hornbills, but this is largely due to habitat loss. However, these historical accounts provide evidence of past associations between Ifugao tribes and hornbills.

This paper represents a compilation of human-hornbill associations noted from the Philippines, with particular emphasis on the traditions and beliefs of indigenous people, including the documentation of human-hornbill associations among indigenous tribes within the Northern Sierra Madre Natural Park.