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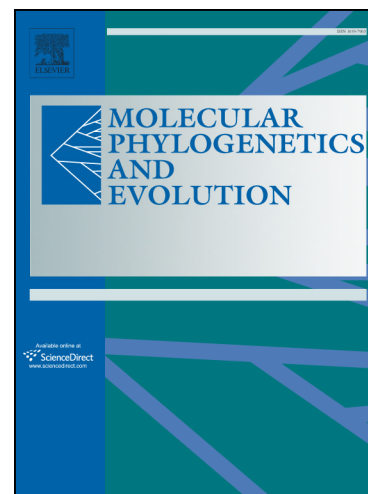
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# Is Amazonia a ‘museum’ for Neotropical trees? The evolution of the Brownea clade (Detarioideae, Leguminosae)

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## Highlights:

- The Brownea clade diversified gradually, following the ‘museum’ model
- The Brownea clade originated in the Eocene, and diversified throughout the Neogene
- The clade diversified mainly in Amazonia, with subsequent migrations across the Neotropics

## Abstract

The flora of the Neotropics is unmatched in its diversity, however the mechanisms by which diversity has accumulated are debated and largely unclear. The Brownea clade (Leguminosae) is a characteristic component of the Neotropical flora, and the species within it are diverse in their floral morphology, attracting a wide variety of pollinators. This investigation aimed to estimate species divergence times and infer relationships within the group, in order to test whether the Brownea clade followed the ‘cradle’ or ‘museum’ model of diversification, i.e. whether species evolved rapidly over a short time period, or gradually over many millions of years. We also aimed to trace the spatio-temporal evolution of the clade by estimating ancestral biogeographical patterns in the group. We used BEAST to build a dated phylogeny of 73 Brownea clade species using three molecular markers

(ITS, *trnK* and *psbA-trnH*), resulting in well-resolved phylogenetic relationships within the clade, as well as robust divergence time estimates from which we inferred diversification rates and ancestral biogeography. Our analyses revealed an Eocene origin for the group, after which the majority of diversification happened in Amazonia during the Miocene, most likely concurrent with climatic and geological changes caused by the rise of the Andes. We found no shifts in diversification rate over time, suggesting a gradual accumulation of lineages with low extinction rates. These results may help to understand why Amazonia is host to the highest diversity of tree species on Earth.

*Keywords: Diversification; Phylogenetics; Biogeography; Amazonia; Legumes*

## 1. Introduction

The tropical Americas are home to a great number of seed plant species (*ca.* 100,000 species (Antonelli and Sanmartín, 2011; Hughes, *et al*, 2013)), however the evolutionary and biogeographical forces which led to the formation of this superlative diversity are unclear for many groups. The time period over which this diversity has accumulated has been a topic of much debate, largely surrounding two theories. The first is the ‘museum’ model of diversification, whereby radiations dating from the Neogene or earlier have persisted in Amazonia due to climatic stability and niche conservatism, allowing many thousands of lineages to accumulate over the past 30 million years (Antonelli and Sanmartín, 2011). By contrast, the ‘cradle’ model of diversification suggests that geologically recent events, such as the orogeny of the Andes and the rise of the Panama isthmus, triggered large scale climatic, geographical and edaphic changes, resulting in rapid speciation due to reproductive isolation (Richardson, *et al*, 2001; Hughes and Eastwood, 2006). It is worth noting that these theories are not mutually exclusive, and indeed both are evident in different lineages, such as Bromeliaceae (Givnish, *et al*, 2011), Orchidaceae (Pérez- Escobar, Chomicki, *et al*, 2017; Pérez- Escobar, Gottschling, *et al*, 2017) and Annonaceae (Couvreur, *et al*, 2011). In addition to this, the interactions between speciation, extinction and geographical range fluctuation are also considered to be important in the accumulation of diversity (Jablonski, *et al*, 2017).

In the low-to-mid elevation forests of the Neotropics plant families show a bipartite pattern of diversity (Gentry, 1982). The first of these two major portions of Neotropical plant diversity includes the Andean-centred taxa, which are those lineages whose centre of diversity lies in North-western South America, within the foothills, valleys and montane zones of the Andes. The majority of the species in this portion are epiphytes and understory shrubs (Gentry and Dodson, 1987). The second portion includes the Amazonian-centred taxa, whose taxonomic diversity is highest in the Amazon basin. The majority of the species in this portion are canopy trees or lianas (Gentry, 1982; Prance, 1994). Several overlapping hypotheses are used to explain this biogeographical pattern (known as the ‘Gentry Pattern’ (Antonelli and Sanmartín, 2011)), the most prominent of which relates the rapid, punctuated rise of the Andes to the evolution of the Neotropical flora (Gentry, 1982). For the Amazonian-centred taxa it is well documented that the Andean orogeny drove much of the diversification of the modern flora (Hoorn, *et al*, 2010), as mountain building processes would have driven large scale hydrological and climatic changes throughout what is now Amazonia (Gregory-Wodzicki, 2000).

Around 50 Ma, during the Eocene, the lowland forests of northern South America were much more extensive than in modern times, with an area known as ‘pan-Amazonia’ stretching over much of South America unimpeded (Hoorn, *et al*, 2010). The Andean orogeny, beginning in earnest around the early Miocene (~23 Ma), would have caused the formation of the Pebas system, an enormous matrix of wetland and *terra firme* forest, and subsequently the Acre system (another vast area of wetlands in what is now western Amazonia) which could have driven speciation *in situ* through allopatry (Hoorn, *et al*, 2010; Antonelli and Sanmartín, 2011). During the Miocene, as the Andes rose, the drainage of the Amazon basin changed direction, resulting in a west-to-east flow; this would have gradually reduced the size of the Pebas and subsequent Acre wetlands until their disappearance. As well as this, fluvial regimes arising from the Andes would have deposited nutrient-rich sediments into the western Amazon basin, resulting in an ‘edaphic mosaic’ on a large scale (Hoorn, *et al*, 2010). This high edaphic heterogeneity was likely also a strong driver for local adaptation and concurrent speciation (Pennington and Lavin, 2016). As such, hydrological regimes, edaphic heterogeneity and dispersal

with subsequent diversification of taxa into novel niches (Erkens, *et al*, 2007) may help to explain the hyperdiversity of tree species found in the western Amazon (Valencia, *et al*, 2004).

Trees belonging to the Leguminosae (alternatively ‘Fabaceae’) are among the most common species found in Neotropical rainforests (Terborgh and Andresen, 1998). As such, they are an excellent system for studying the evolution of plant species in the Neotropics. The Brownea clade, which belongs to the legume subfamily Detarioideae (LPWG, *et al*, 2017) is made up of 111 species belonging to nine genera (Mackinder, 2005), which are all sub-canopy or canopy-level trees found in lowland Neotropical rainforests (Redden and Herendeen, 2006). While the majority of species in the Brownea clade occur in Amazonia, there are several species that are endemic to only one region, such as the Chocó-Darien moist forests or Central America. The seeds of most species within the clade are dispersed by explosively dehiscing woody pods, and as such many species are locally common, forming stands (Klitgaard, 1991), with relatively low dispersal. A few species, such as *Macrolobium acaciifolium*, are, however, very widely dispersed and are ‘hyperdominant’ in Amazonia (ter Steege, *et al*, 2013) due to their hydrophilic habitat preference and floating fruits. The Brownea clade is particularly diverse in floral morphology and attracts a wide variety of pollinators. Many species are adapted to attract vertebrate pollinators (mainly hummingbirds and bats) (Knudsen and Klitgaard, 1998; Fleming, *et al*, 2009), although exactly which species is visited by which pollinator is as yet unclear for most species. These factors make the Brownea clade an interesting system for testing evolutionary hypotheses relating to the diversification of Neotropical trees. However, inter-and-infrageneric relationships are as yet poorly resolved in the Brownea clade. Previous work has mainly focussed on resolving tribes within the traditionally circumscribed subfamily Caesalpinioideae (which included the Brownea clade (LPWG, 2017)) using plastid *trnL* and *matK* sequences (Bruneau, *et al*, 2001; Bruneau, *et al*, 2008), or focussed on several genera within the Brownea clade (namely *Paloue*, *Paloveopsis*, *Elizabetha* and *Heterostemon*) using plastid *trnL* sequences, nuclear ITS sequences and morphological data (Redden, *et al*, 2010). In order to study the patterns and rates of evolution within the Brownea clade a multilocus, dated phylogeny is required, from which phylogenetic patterns,

divergence time estimates and rates of evolution may be inferred. This will provide some insight into the evolution of the hyperdiverse tree flora of the Neotropics, especially within Amazonia.

This investigation aims to answer major questions surrounding the evolution of the Brownea clade, and by extension how a major portion of the Neotropical tree flora evolved:

- 1) Did the diversification of the Brownea clade follow the ‘museum’ or the ‘cradle’ model of lineage accumulation; that is, was diversification gradual, resulting in the accumulation of lineages over long time scales, or was diversification more recent and rapid?
- 2) Did the Brownea clade diversify under a specific regime of evolutionary rates, and were there any discrete shifts in diversification rates? In other words, did the majority of the lineages within the Brownea clade accumulate through bursts of rapid speciation?
- 3) Were ancestral species widely spread across many different regions, with subsequent diversification in Amazonia? Additionally, did diversification involve range shifts from Amazonia to the Andean region, or *vice versa*?

## 2. Materials and Methods

### 2.1 Taxon sampling, DNA sequencing and phylogenetic analyses

Our sampling represents all nine genera within the Brownea clade, comprising 73 of 111 species (excluding subspecies) and 109 accessions (Appendix 1A). A species list of the Brownea clade was compiled using the Plant List, Tropicos and generic monographs (Cowan, 1953; Klitgaard, 1991; Redden, Unpublished) in order to ensure taxonomically valid species were included in our analyses.

Analyses were run on 211 DNA sequences in total, belonging to three loci. DNA samples were extracted from 53 individuals representing 38 species in six genera from the Brownea clade, resulting in 77 DNA sequences. Accessions were obtained from herbarium and silica material collected from herbaria (AAU, E, K, NY, US). Sampling was targeted to represent as many species as possible, and species with broad ranges (i.e. those found in multiple biogeographical regions) were represented by

multiple accessions from throughout their range where possible. Forty-six additional sequences representing many species from the Guianas and generated using a similar laboratory protocol were obtained courtesy of Dr Karen Redden from the Smithsonian Institution. Eighty-eight sequences from species that were not represented by herbarium material or contributions from collaborators were downloaded from NCBI Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>).

Outgroup taxa were selected to represent the Amherstieae tribe within which the Brownea clade is nested (Bruneau, *et al*, 2008), as well as the larger subfamily containing the clade, the Detarioideae (LPWG, *et al*, 2017). In total, an additional 21 accessions from 21 outgroup species were used to root our phylogenetic analyses, taken from de la Estrella *et al.* (2017) (marked by an asterisk in Appendix 1A).

Geographical distribution data were collected using occurrences for all species in the Brownea clade collected from GBIF ([www.GBIF.org](http://www.GBIF.org)) and refined using OpenRefine (<http://openrefine.org/>). These were subsequently cross-referenced with the BIEN portal, which automatically refines data, checks the taxonomic validity of accessions and plots species distribution maps (<http://www.biendata.org/>). Distribution data for the genus *Macrolobium* were also checked using R.S. Cowan's monograph (Cowan, 1953), and data for the genera *Elizabetha*, *Paloue* and *Paloveopsis* were checked using Dr Karen Redden's unpublished monograph of the three genera. From these data sources, species were assigned to one or many of six biogeographical regions (Central America, Northern Andes, Northern Venezuela-Colombia, Amazonia (incl. Guiana shield), Cerrado and Atlantic forest/Coastal Brazil) using QGIS v.2.18.3 (QGIS Development Team, 2017), based on the phytogeographic regions delimited by Gentry (Gentry, 1982), and shown in Figure 1.

## 2.2 DNA extraction and sequencing

DNA extractions were carried out using 20 mg of dried leaf material with the CTAB method (Doyle and Doyle, 1987). The DNA concentration and purity of eluates were measured using 1.5 µl of eluate with a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Three target loci

were amplified using PCR: the nuclear ribosomal internal transcribed spacers (ITS) and two plastid markers (*trnK* and *psbA-trnH*). Complementary sequences were generated for all markers and were assembled, edited and aligned using the MAFFT algorithm (Kato, 2013) as implemented in Geneious v8.14 (<http://www.geneious.com>, (Kearse, *et al*, 2012)). Cycle sequencing reactions were performed using the BigDye Terminator cycle sequencing chemistry (v3.1; ABI, Warrington, Cheshire, UK), the same primers as for PCR, and following the manufacturer's protocol (except for ITS, for which 28 cycles were used instead of 26). PCR primers, amplification reactions and thermocycling conditions are detailed in Supplementary Information (Tables S1–S3).

### 2.3 Phylogenetic analyses and divergence dating

The combined matrix of the nuclear (ITS) and two plastid loci (*trnK* and *psbA-trnH*) contained 2,463 characters for 130 taxa in total. Model selection was conducted using the software JModelTest2 (Darriba, *et al*, 2012; Guindon and Gascuel, 2003) for the nuclear and plastid data sets separately. Model choice was based on the Akaike Information Criterion (AIC) (Posada and Buckley, 2004). Preliminary phylogenetic analyses were run on nuclear (ITS) and plastid (*trnK* and *psbA-trnH*) datasets separately using RAxML-HP2 (Stamatakis, *et al*, 2008) in the CIPRES web portal ((Miller, *et al*, 2010), <https://www.phylo.org/>) using 1000 bootstrap replicates. The resulting trees were used to compare topologies and node support, after which the nuclear and plastid datasets were combined for further analyses. Additionally, a combined matrix consisting of all 31 taxa for which there were no missing sequence data was run using the same RAxML framework to assess the effect of missing data on phylogenetic estimation.

Phylogenetic relationships and divergence time estimates were inferred from a concatenated matrix of all three markers (ITS, *trnK* and *psbA-trnH*) using the programme BEAST v.1.8.2 (Drummond, *et al*, 2012) on the CIPRES web portal. Multiple runs of BEAST were performed using several different tree models (including Yule, birth-death and birth-death (incomplete sampling)) in order to deduce which model best fitted the data. Tree models were chosen based on Bayes Factor comparisons



calculated using both the Path Sampling and the Stepping Stone sampling method in BEAST (Baele, *et al*, 2012). The birth-death (incomplete sampling) model was chosen for tree building, as it explained the data 4-13x better than the other two tree models. Dated trees were generated using a relaxed lognormal clock model, informed by coefficient of variation values taken from the BEAST log file (Drummond, *et al*, 2006). We used four calibration points to provide date estimates, which are outlined in Table 1.

Phylogenetic inference in BEAST comprised of four independent runs of 35 million generations (giving a total of 140 million generations), which were combined using Logcombiner v.1.8.2 with a burn-in level of 10%. Convergence of runs was assessed using effective sample size (ESS) of all parameters in Tracer v. 1.6 (Rambaut, *et al*, 2015) with a threshold value of 200. The final ultrametric trees were generated in TreeAnnotator v1.8.2 and visualised using Figtree (Rambaut, 2014). Additionally, a dated tree using a reduced dataset of 92 taxa and 2,449 characters from the three loci representing one tip per species was run using the same parameters as above for use in biogeographical analyses. Single accessions used to represent species were chosen by selecting those with the most complete molecular dataset where multiple accessions were present.

**Table 1:** Fossils and secondary calibration points used to generate a time-calibrated phylogenetic tree of the Brownea clade in BEAST v.18.2. Lognormal calibration priors were used for fossil ages to provide a minimum age estimate, whereas secondary calibrations were given normal priors, as uncertainty is distributed on both sides of the mean age (Ho and Phillips, 2009). Mean ages are shown for secondary calibrations, with 95% confidence intervals in parentheses. All ages in millions of years (Ma).

Calibration point	Age (Ma)	Prior distribution	Calibration type	Reference
<i>Aphanocalyx</i>	46	Lognormal	Fossil	(Herendeen and Jacobs, 2000)
<i>Crudia</i>	45	Lognormal	Fossil	(Herendeen and Dilcher, 1990)

Brownea clade	30.25 (38-22.5)	Normal	Secondary calibration	(de la Estrella, <i>et al</i> , 2017)
Detaroideae	65.5 (68-63)	Normal	Secondary calibration	(de la Estrella, <i>et al</i> , 2017)

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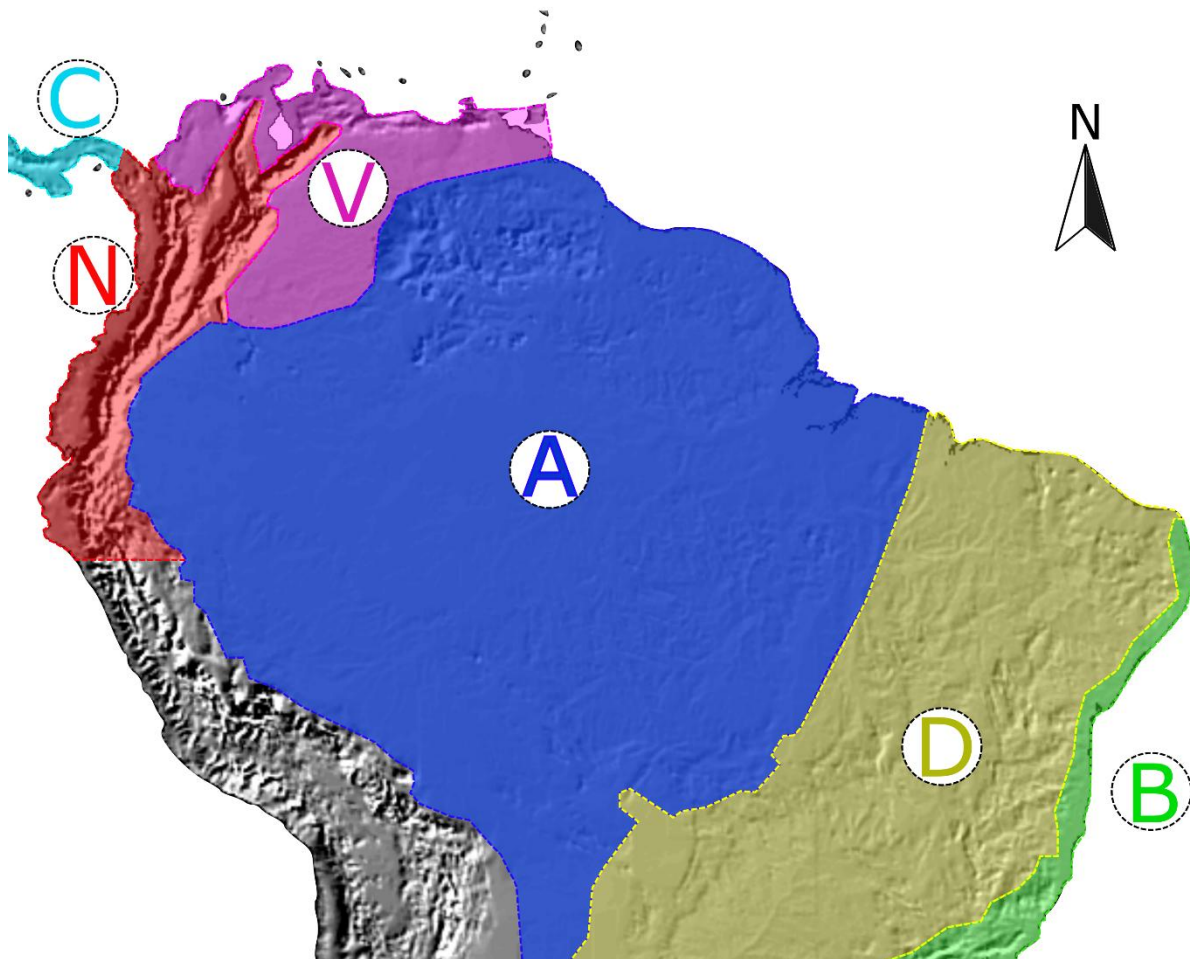
#### 2.4 Diversification rate analyses

Diversification rate analyses were performed on the full dataset containing nuclear and plastid loci from which evolutionary hypotheses were tested using multiple approaches. In order to test whether diversification in the Brownea clade followed the ‘museum’ or ‘cradle’ model, we tested the null hypothesis of constant diversification through time by comparing the fit of constant-rate and variable-rate models (both pure-birth and birth-death variants of each model type). This was estimated using a maximum-likelihood approach, with evolutionary rates inferred from the ultrametric BEAST tree with the function ‘fit\_bd()’ in the R package RPANDA v.1.3 (Morlon, *et al*, 2016). Starting values for each speciation and extinction parameter in RPANDA are outlined in Appendix 2. RPANDA can perform inference on incomplete phylogenetic datasets, and as such the sampling fraction for each genus was estimated from the species list compiled for sample selection (see Appendix 1B). The best model was then chosen using AICc and Akaike weights (Aw) (Burnham and Anderson, 2003), after which speciation and extinction rates were estimated for the entire Brownea clade. As well as this, in order to visually examine the pattern of lineage accumulation over time we used mean lineage through time (LTT) plots generated with the R package phytools v.0.6-44 (Revell, 2012; Revell, 16/04/2013), drawn with 95% confidence intervals generated using 10,000 of the converged BEAST trees. Under the museum hypothesis of diversification, we predict steady accumulation of species, possibly with low extinction rates, and constant diversification rates over time, whereas under the cradle hypothesis we predict a recent increase in diversification rates, with variable rates over time.

In order to further test for changes in the evolutionary rate regime under which the Brownea clade evolved we used BAMM 2.5.0, which tests for shifts in evolutionary rates across ultrametric trees. Post-run analyses and data visualizations were performed using the R package BAMMtools v.2.1.6 (Rabosky, *et al*, 2014). In order to minimize sources of error we accounted for missing taxa by including sampling fractions for every clade using the BAMM control file, and rate estimation priors were set using the function setBAMMpriors in BAMMtools. We ran our BAMM analyses using a birth-death model in order to account for missing taxa, which is not yet possible for the pure-birth process in BAMM 2.5.0. To check convergence of MCMC sampling, two runs of 50,000,000 generations were undertaken and their convergence checked using the posterior MCMC output and effective sample size, with a cut off value of 200. Although BAMM has received recent criticism due to prior sensitivity (Moore, *et al*, 2016), the latest version of BAMM addresses the problems raised by the authors (Rabosky, *et al*, 2017), and this sensitivity can be accounted for by testing diversification models using multiple ‘expected number of rate shift’ priors, a cautionary step which we performed. All methods which attempt to estimate extinction rate from dated phylogenies encounter the same intrinsic problems, as it is difficult to accurately re-construct extinction rates from a phylogeny containing no fossil taxa. This is because variation in speciation rates across clades can result in a tree topology that leads to underestimation of extinction rates (Rabosky, 2010). Due to this, extinction rates inferred from trees containing only extant taxa and with no corroborative fossil evidence should be treated as approximate estimates rather than definitive rates. Under the museum hypothesis of diversification, we predict that diversification regimes showing no shifts in rate over time will be favoured, whereas under the cradle hypothesis we would expect to find distinct shifts in diversification rates. This would reveal whether particular sub-clades experienced faster diversification and allow us to shed light on possible mechanisms to explain these shifts.

**Figure 1:** Phylogeographical regions of northern South America, adapted from Gentry (1982), based on areas of endemism, where areas are delimited by communities of species not found in other regions. C=Central America,

N=Northern Andes, V=Northern Venezuela-Colombia, A=Amazonia, D= Cerrado/Dry areas, B= Atlantic forest/Coastal Brazil.



### 2.5 Ancestral range estimation

In order to investigate the spatio-temporal regime of diversification in the Brownea clade we estimated ancestral ranges for the Brownea clade by comparing biogeographical models implemented in the R package BIOGEOBEARS v 0.2.1 (Matzke, 2013). We tested six likelihood-based models, all of which incorporate dispersal, extinction and range switching. These models are: DEC & DEC+J (Ree and Smith, 2008), which additionally account for vicariance and ‘subset’ sympatric speciation, DIVALIKE and DIVALIKE+J (Ronquist, 1997), which also account for vicariance, as well as BAYAREALIKE and BAYAREALIKE+J (Landis, *et al*, 2013), which additionally account for widespread sympatric speciation but not vicariance. For three of the models, the extra parameter ‘J’

represents founder-event speciation (i.e. rare long-distance dispersal events where the resultant population becomes genetically isolated and forms a new lineage). The model which was best described by the data was chosen using AICc and Akaike weights, as well as with likelihood ratio tests for nested models, after which we estimated ancestral ranges and range shifts across the *Brownea* clade.

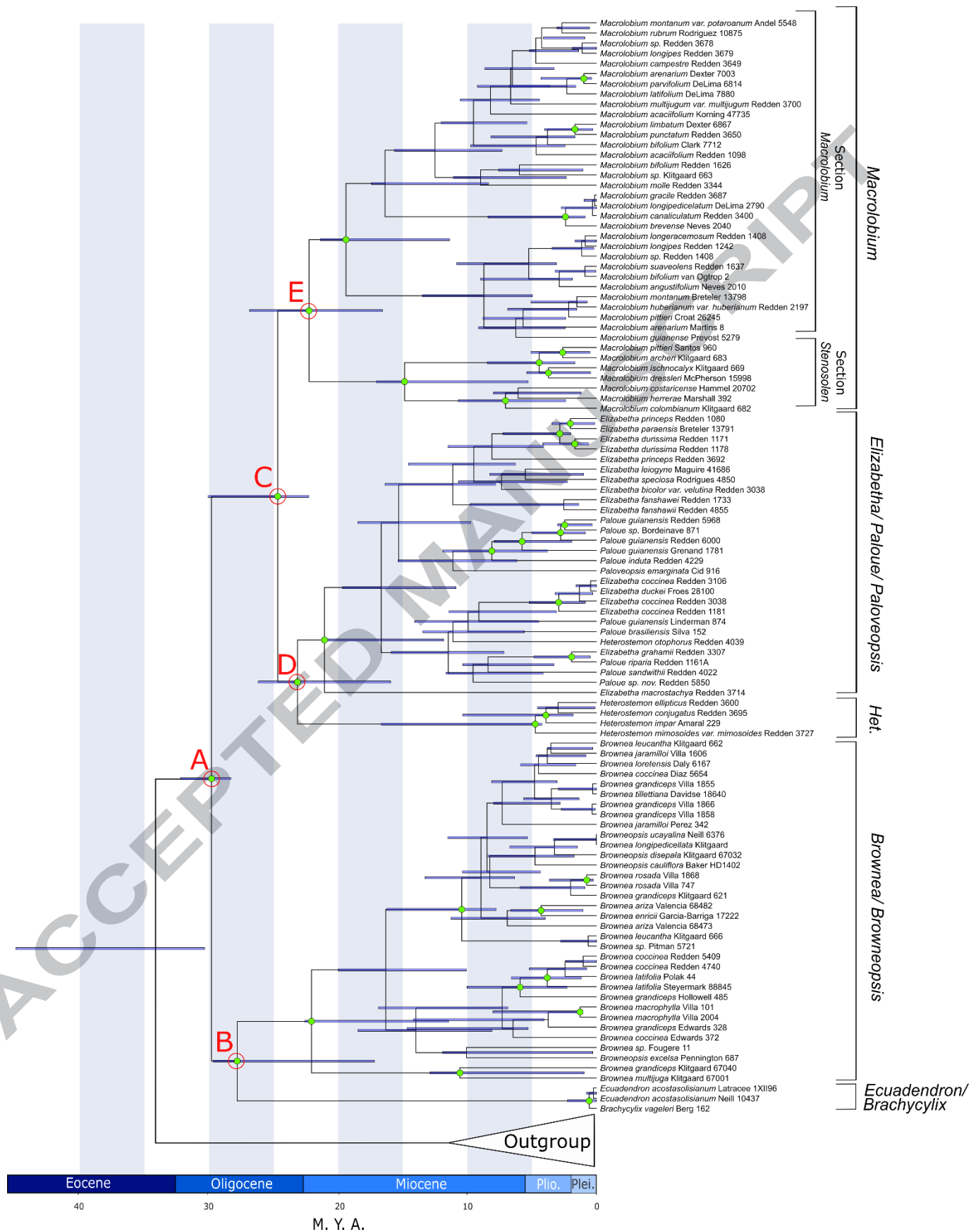
Species were assigned between one and six geographical areas, as defined in *Section 2.1*, based on their presence or absence in each area. Ancestral ranges were estimated using the best-fitting model and were plotted onto a single-species ultrametric tree with the function 'plot\_BioGeoBears\_results()'. The tree was constructed from a reduced dataset of all three loci for 92 taxa (see *Section 2.3*) representing a single accession per species, because one of the assumptions of the models implemented in BIOGEOBEARS is that each tip consists of an operational taxonomic unit (OTU) (i.e. there cannot be multiple accessions from a single species or population). The wide-ranging species *Brownea grandiceps* and *B. coccinea*, which contain multiple cryptic species or subpopulations, were represented with two accessions each from distinct geographical areas. The maximum number of range areas was set to six, as this was the total number of biogeographical areas used in the analysis. We predict an ancestrally 'Pan-Amazonian' range for the *Brownea* clade, showing diversification in Amazonia, and subsequently giving rise to dispersal and speciation events from East to West, towards the Andes.

### 3. Results

#### 3.1 Phylogenetic relationships and divergence time estimates

**Figure 2:** Fossil-calibrated maximum clade credibility tree of the *Brownea* clade, built from the concatenated ITS, *trnK* and *psba-trnH* sequence matrix of all *Brownea* clade species and outgroup taxa. Median node heights are shown for trees generated using a relaxed lognormal clock model (Drummond, *et al*, 2006), a birth-death (incomplete sampling) speciation tree model and four calibration points. Node bars show 95% highest posterior

density (HPD) for node ages. Posterior probabilities for each node are represented using coloured dots, with green dots showing a posterior probability  $>0.95$ , with lower support values not shown. Nodes of interest are labelled A-E and are discussed in the text. 'Het.' represents the genus *Heterostemon*, 'Plio.' and 'Plei.' represents the Pliocene and Pleistocene epochs, respectively. Shading on the background of the phylogeny represents 5 Ma time intervals.





Bayesian phylogenetic analyses reveal the *Brownea* clade to be well-supported and monophyletic, showing a posterior probability (PP) of 1 (Node A, shown in Figure 2). The ancestral *Brownea* clade lineage diverged from the rest of the of the Detarioideae subfamily around 34 million years ago (Ma), during the Eocene, and began to diversify around 30 Ma during the Oligocene. Subsequently, the *Ecuadendron/Brachycylix* subclade diverged from *Brownea/Browneopsis* subclade around 28 Ma, during the mid-Oligocene (Node B, PP=0.96); *Ecuadendron* and *Brachycylix* appear on a very long branch, with the divergence between the two genera only occurring around 0.6 Ma, during the Pleistocene (PP=1). Three additional nodes date to the Oligocene, the first being the split between the *Macrolobium* and *Heterostemon/Elizabetha/Paloue/Paloveopsis* subclades (Node C, ~25 Ma, PP=0.97), the split between *Heterostemon* and *Elizabetha/Paloue/Paloveopsis* (Node D, ~24 Ma, PP=0.99), and the split within *Macrolobium* between *M.* section *Stenosolen* and *M.* section *Macrolobium*, towards the end of the Oligocene (Node E, ~22 Ma, PP=0.99). All generic subclades within the tree are well supported (PP >0.95), however species within the *Elizabetha/Paloue/Paloveopsis* subclade are polyphyletic. Additionally, one species of *Heterostemon* (*H. otophorus*) appears within the subclade containing *Elizabetha/Paloue/Paloveopsis*, instead of with its congeners. It is also interesting to note that within the *Brownea/Browneopsis* subclade the two species *Brownea coccinea* and *B. grandiceps* appear to be polyphyletic, appearing in multiple subclades, and that the genus *Browneopsis* is nested within *Brownea*. Most crown groups in the *Brownea* clade appear to have diversified in the past 20 Ma, since the early Miocene. Deeper nodes have higher degrees of uncertainty associated with their divergence times as shown by the larger 95 % highest posterior probability densities (HPDs). Estimated divergence times for major nodes and their 95% HPDs are summarized in Supplementary Information (Table S4).

In the sequence matrix both nuclear and plastid loci were well-represented across all taxa. ITS was present for 92.79% of taxa, *trnK* was present for 73.87% of taxa and *psbA-trnH* was present for 32.43% of taxa. Preliminary analyses using RAxML on nuclear and plastid partitions revealed a well resolved tree for the nuclear partition (ITS) (Figure S5a & b) but a poorly resolved tree for the plastid partition (*trnK* and *psbA-trnH*). As such, we found no well-supported incongruence between the trees



built from the two data partitions, hence the datasets were combined for the BEAST analysis.

Furthermore, the combined dataset RAxML tree run using only taxa for which all three loci were present (Figure S5c) showed the same intra-clade relationships as both the combined BEAST tree (Figure 2) and the ITS RAxML tree (Figure S5a), with moderate to good support for most nodes (Bootstrap=70-100%).

### 3.2 Diversification rate analyses

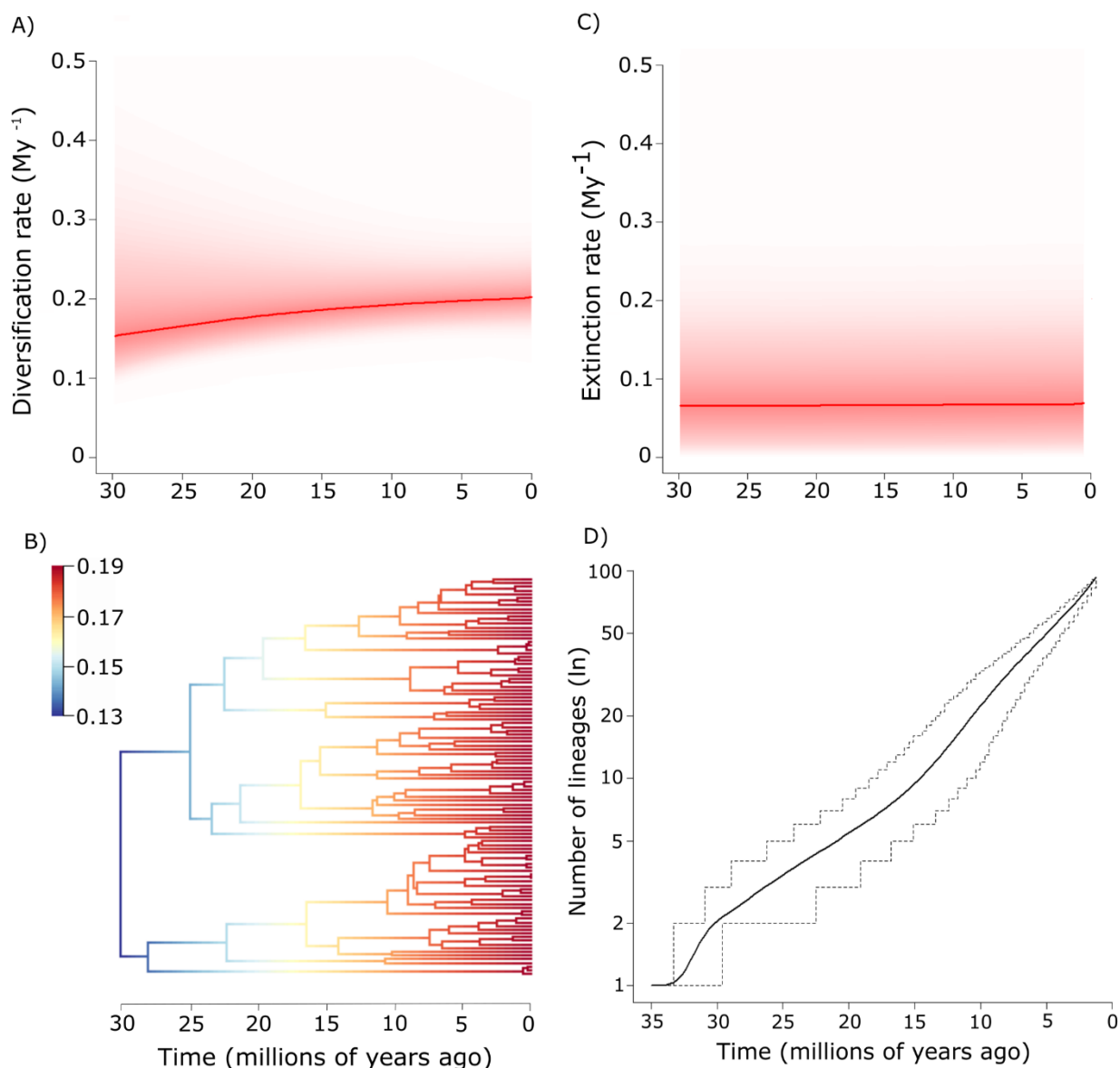
Inferred rates of diversification show no major rate shifts and support a relatively constant rate of diversification. Model testing using RPANDA did not allow the rejection of any of the next-best pure birth models (those with variable rates) when compared to the best-fitting pure birth model (pure birth with constant rates, AICc=632.578), due to very low  $\Delta\text{AICc}$  values ( $<1$ ). The same was true for the birth-death model with constant rates ( $\Delta\text{AICc} <1$ ). However, the best-fit pure birth model explained the data between 3-9 times better than the birth-death models with variable rates according to Akaike weights ( $A_w$ ), and as such we were able to reject them ( $\Delta\text{AICc} >2$ ). Model testing values and parameters for all models implemented in RPANDA are shown in Supplementary Information (Table S6).

BAMM analysis inferred no major shifts in diversification rates for the Brownea clade over the past 30 Ma, however there was a slight increase in diversification rate over time (Figure 3A & 3B).

Extinction rates appear to have remained consistently low over the majority of the evolutionary history of the Brownea clade (Figure 3C), and as such, the rate of lineage accumulation has followed more or less an exponential pattern (Figure 3D). This is consistent with a pattern of constant speciation and extinction rates. Log likelihood and configuration shift values for all BAMM runs had effective sample sizes of  $>30,000$  post-burnin, and MCMC outputs indicated that the runs converged. Multiple BAMM runs using different expected rate shift priors all gave the same result: there were no shifts in speciation or extinction rate within the Brownea clade, as shown by a posterior probability of 0.843 (this is further corroborated by the bar chart and phylorate plots shown in Supplementary

Information (Figures S7a and S7b)). Estimates of diversification rates generated by both RPANDA and BAMM are similar in magnitude, at  $0.171\text{My}^{-1}$  and  $0.201\text{My}^{-1}$  (with a posterior-probability interval of  $0.161 - 0.260\text{My}^{-1}$ ), respectively.

**Figure 3:** **A)** Net diversification rate through time in the Brownea clade, generated using BAMM 2.5.0 (and visualised using BAMMtools), with a birth-death speciation model based on the ultrametric tree using all three loci. Red shaded areas represent posterior probability distributions of rate estimates. **B)** Phylorate plot of the Brownea clade generated using BAMM 2.5.0. Diversification rates are coded on to each branch of the BEAST time-calibrated tree shown in Figure 1 according to colour, with red being the highest and blue being the lowest rates. Diversification rates are reported as rates per lineage per million years. **C)** Extinction rate through time for the Brownea clade, generated using BAMM 2.5.0, with a birth-death speciation model based on the ultrametric BEAST tree using all three loci. Red shaded areas represent posterior probability distributions of rate estimates. **D)** Lineage-through-time (LTT) plot of the Brownea clade plotted in phytools v.0.6-44. The plot is based on 10,000 converged trees generated in BEAST using all three loci, showing mean numbers of lineages (solid line) and the 95% CI (dashed lines).



### 3.3 Historical biogeography

Most of the diversification of the Brownea clade occurred in Amazonia, with subsequent dispersals into other biogeographical regions. AICc and Aw showed that the best-fitting models to our data were the two BAYAREALIKE models, and the best-fit BAYAREALIKE model (AICc= 297.870) explained the data 30x better than the next best model (DEC,  $\Delta$ AICc= 6.924). Likelihood-ratio tests showed that BAYAREALIKE+J was the most appropriate of the two BAYAREALIKE models ( $P=0.01$ ), suggesting that founder event speciation was important (see Supplementary Information (Table S8)). This also suggests that vicariance was less important than dispersal, extinction and founder-

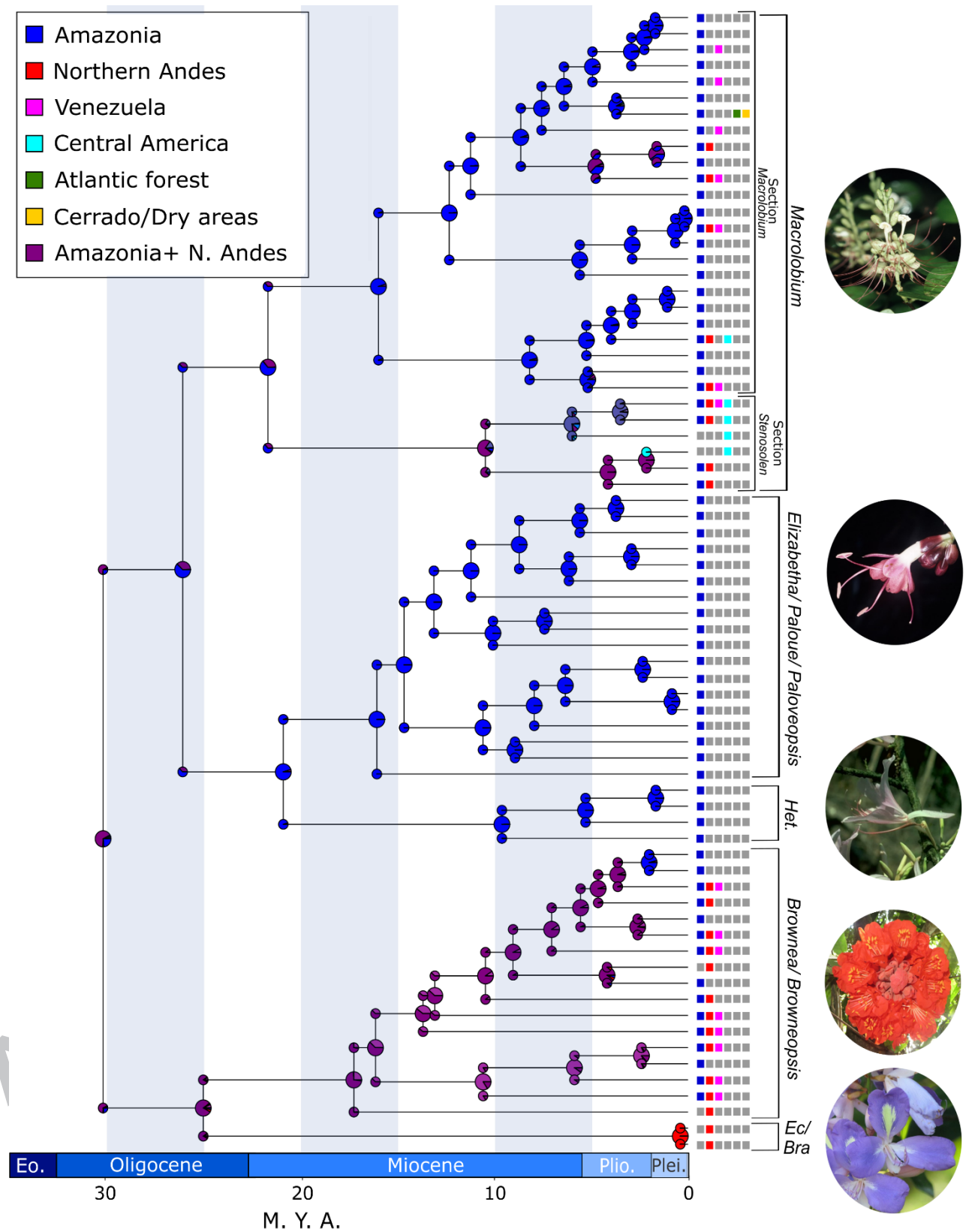
event speciation, because in contrast to the DEC and DIVA models, BAYAREALIKE+J does not contain parameters for vicariance, which implies diversification in Amazonia and subsequent dispersal and speciation events across the Neotropics.

Using the BAYAREALIKE+J model, we estimated a shared ancestral range of both Andean and Amazonian regions for the most recent common ancestor of the *Brownea* clade, with a subsequent split into one subclade with a shared Amazonian-Andean range and another that is mostly Amazonian (Figure 4). While both the most early-diverging genera (*Ecuadendron* and *Brachycylix*) in our study, as well as the most early-diverging lineages in *Brownea*, are found in the Andean regions, this is derived from an ancestor which we inferred had a shared Amazonian-Andean range. This broad range is shared among many of the descendent lineages within *Brownea* and *Browneopsis*, with few species being endemic to either West or East of the Andes in these genera. It is interesting to note that most species endemic to one region arose after relatively recent orogenic events in the Andes (between 12-7 Ma). Indeed, most diversification in the *Brownea* clade appears to have occurred after the initial rise of the Andes around 23 Ma.

The *Elizabetha*/*Paloue*/*Heterostemon* subclade has diversified into many lineages within Amazonia, and this appears to have occurred gradually since the Miocene, around 22 Ma. The genus *Macrolobium* also shows a primarily Amazonian distribution, however within *M.* section *Stenosolen*, many species are wide-ranging between the Andean and Amazonian regions, with two independent dispersals into Central America in the past 10 Ma. Finally, section *Macrolobium* shows a pattern of diversification resulting in many species within Amazonia (similar to the pattern found in *Paloue*, *Elizabetha* and *Heterostemon*) with subsequent dispersal to the Andean regions and beyond more recently (<10 Ma).

**Figure 4:** Biogeographical range estimation of the *Brownea* clade. Pie charts represent likelihoods of ranges derived from ancestral range estimations run on the reduced dataset BEAST tree, using the R package BIOGEOBEARS. The legend represents the six phytogeographical regions as defined by A. Gentry, as shown in Figure 1, as well as one shared ancestral range (Amazonia + Northern Andes). ‘*Ec/Bra*’ represents the monospecific genera *Brachycylix* and *Ecuadendron*, and ‘*Het.*’ represents the subclade containing species in the

genus *Heterostemon*. Images show inflorescences of species within the Brownea clade. Species shown are, from top to bottom, *Macrolobium microcalyx* and *Elizabetha speciosa* (both photographs by M. Hopkins), *Heterostemon mimosoides* (photograph by A. Gentry), *Brownea grandiceps* (photograph by R. J. Schley) and *Brachycylix vageleri* (photograph by A. Hay). ‘Eo.’, ‘Plio’ and ‘Plei’ represent the Eocene, Pliocene and Pleistocene epochs, respectively. Shading on the background of the phylogeny represents 5 Ma time intervals.



## 4. Discussion

### 4.1 Diversification of the *Brownea* clade

Bayesian phylogenetic analyses of both nuclear and plastid sequence data resulted in a monophyletic *Brownea* clade (PP= 1; Node A in Figure 2), which diverged from the rest of the Detarioideae subfamily around 34 Ma ago. Ancestral range estimations performed by de la Estrella *et al.* (2017) show that the *Brownea* clade most likely diverged from rainforest-dwelling Gondwanan ancestors, the closest relatives of which now live in the Paleotropics, with a centre of diversity in Africa. Generic subclades are well resolved in our dated phylogeny (PP >0.95), with the earliest divergences occurring between the ancestors of the genera *Ecuadendron*/*Brachycylix*/*Brownea*/*Browneopsis* and the rest of the clade around 30 Ma ago, with subsequent divergence between *Ecuadendron*/*Brachycylix* and *Brownea*/*Browneopsis* around 28 Ma ago, during the Oligocene (PP=0.96).

The earliest-branching stem lineage in the *Brownea* clade contains two monotypic, narrow range endemics found along the north-western foothills of the Andes - *Ecuadendron* and *Brachycylix* (Neill, 1998; Cowan, 1975). Both species have flowers held on long, pendent racemes, and as such appear to be bat-pollinated, which is common for many Detarioideae (Fleming, *et al.*, 2009). These two sister genera are sitting on a very long branch, and appear to have diverged very recently, only around 600,000 years ago (Fig. 2). It is quite likely that their morphological and ecological similarity is the result of a recent divergence driven by founder-event speciation after the Andean orogeny (Weir and Price, 2011), as *Brachycylix* and *Ecuadendron* are found east and west of the Western Andean Cordillera, respectively.

The genera *Brownea* and *Browneopsis*, which were previously circumscribed into separate genera by Klitgaard (1991), are not supported as monophyletic in our analyses (Figure 2). This is an unexpected result, as the two putative genera differ in stamen number, petal, leaf and pollen morphology, as well as phenology. However, resolution in the polyphyletic clade containing the two genera is probably low because of incomplete lineage sorting and hybridization, since complete monophyly with distinct

species boundaries is very uncommon in rainforest trees (Pennington and Lavin, 2016). Large effective population sizes, outcrossing through mutualist-facilitated pollination (Dick, *et al*, 2008) and retention of ancestral polymorphisms due to habitat stability mean that the time to coalescence for all genes in rainforest species is incredibly long, usually taking tens of millions of years (Pennington and Lavin, 2016). Additionally, the species *Brownea grandiceps* and *Brownea coccinea*, for which we sampled multiple accessions from across their ranges, appear in multiple sub-clades; both species are taxonomically well defined based on morphological characters, and both are made up of several subpopulations showing distinct, disparate geographical ranges (Klitgaard, 1991). While it is possible that the polyphyly of these two species is due to the presence of cryptic species, as yet indiscernible using solely morphological evidence, it is more likely that this pattern is due to the fact that there is a lack of informative molecular characters (indeed, the preliminary plastid RAxML tree showed very poor resolution (Figure SI5 b)). As such, these relationships should be interpreted critically.

It is possible that the amount of missing data in our matrices could have had a significant effect on the accuracy of our phylogenetic reconstruction. However, it has been shown that choosing an appropriate evolutionary model (e.g. the GTR site model) is more important for phylogenetic accuracy than the amount of missing data (Roure, *et al*, 2012; Wiens and Morrill, 2011). In addition to this, correctly partitioning different loci which evolve at different rates can help to improve the accuracy of phylogenetic inference (Wiens and Morrill, 2011). Indeed, in this investigation both nuclear and plastid partitions were used, with 92% and at least 73% of taxa represented in each partition, respectively. This was reflected by Figure S5c, which reconstructed relationships between all taxa for which there were no missing data, showing a topology congruent with that generated for all taxa (including those with missing data) by BEAST.

Within the well-supported subclade containing *Elizabetha*, *Paloue* and *Paloveopsis* (PP >0.95), the first two genera are paraphyletic (*Paloveopsis* is monotypic). This phylogenetic pattern reflects previous work (Redden, *et al*, 2010), and because these genera are morphologically similar they are being recombined into the genus *Paloue* (Redden, unpublished). Within *Macrolobium*, our analyses support the monophyly of *M.* sections *Stenosolen* and *Macrolobium* (Node E, PP >0.95; Fig. 2) as



described by R.S. Cowan based on biogeography and morphology in his seminal taxonomic monograph of *Macrobium* (Cowan, 1953) as well as in more recent work (Murphy, *et al*, 2018).

Both the subclades within *Macrobium* and the subclade containing *Elizabetha*/*Paloue*/*Paloveopsis* and *Heterostemon* diverged during the Oligocene, just after the first major orogenic acceleration within the Andes around 23 Ma. Indeed, most generic crown groups in the Brownea clade appear to have diversified in the past 23 Ma, since the late Oligocene (Figure 2), when the rising Andes were causing large-scale climatic, geological and hydrological changes in northern South America (Hoorn, *et al*, 2010; Antonelli and Sanmartín, 2011).

#### 4.2 'Museum' or 'cradle'?

Our analyses suggest that the Brownea clade underwent no major changes in diversification rate over its evolutionary history, coupled with very low rates of extinction over time. Bayesian Analysis of Macroevolutionary Mixtures (BAMM) inferred no shifts in diversification rates for the Brownea clade (Figs. 3A and 3B), although it did infer a very slight increase in diversification rate over time. BAMM provided evidence for a pattern of consistently low extinction rates, as demonstrated by the extinction rate through time plot (Figure 3C) and by the lineage-through-time (LTT) plot (Figure 3D), which suggested a more or less exponential pattern of lineage accumulation, congruent with a pattern of constant, low rates of extinction and constant rates of speciation.

Table S6 showed that while model testing in RPANDA did not allow the rejection of any of the next-best pure-birth models, or the birth-death model with constant rates, we were able to reject all birth-death models with variable rates. Despite this, AICc values indicate a slightly better fit for the pure birth models, especially the pure birth model with constant evolutionary rates. This model assumes that there was no extinction over the evolutionary history of a clade (or, more realistically, very low rates of extinction over time), which broadly agrees with the inferences made using BAMM.

We inferred similar estimates of speciation rate for the Brownea clade using both RPANDA and BAMM, at around  $0.2 \text{ My}^{-1}$  (see Section 3.2). The speciation rate of the Brownea clade (which is primarily Amazonian) is in stark contrast to the speciation rates inferred for Andean angiosperm

clades, such as Andean bellflowers (Lobelioideae, Campanulaceae) or Frailejóns (Espeletiinae, Asteraceae), which display rates of 1.12 and 1.01 My<sup>-1</sup>, respectively (Lagomarsino, *et al*, 2016; Madrinan, *et al*, 2013).

The BAMM results and LTT plot strongly suggest that the Brownea clade has followed the ‘museum’ model of diversification, due to the constant rates of speciation that our analyses inferred, the low and consistent extinction rates suggested by rate modelling, as well as the overall pattern of gradual lineage accumulation that is apparent within the group. This was also suggested by the RPANDA analyses, although other models of diversification were unable to be rejected. The ‘museum model’ may be explained by the climatic stability of the Neotropics, which has remained near the Equator for almost as long as it has existed, resulting in very low extinction rates, while speciation rates have remained almost constant over several million years (Mittelbach, *et al*, 2007). Indeed, the fact that the Brownea clade seem to have followed this pattern is in line with the predictions of Gentry; Andean-centred herbaceous lineages at mid-elevations have diversified very rapidly, but lowland tree lineages (most of whose phylogenetic diversity is centred in Amazonia, including the Brownea clade) evolved more gradually and diversified within Amazonia (Gentry, 1982). While estimation of extinction rates is notably problematic due to the possibility of unaccounted variation in speciation rates across clades (Rabosky, 2010), other studies have shown that extinction rates are estimated with acceptable precision for trees that are moderately sized (>100 tips), especially where sampling fractions are included in the model parameters (Beaulieu and O'Meara, 2015). Nevertheless, extinction rates should still be treated as approximate estimates.

As the Brownea clade continued to diversify, dispersal out of Amazonia and subsequent diversification of lineages may help to explain the slight increase in diversification rates inferred by our analyses. Events such as the continued rise of the Andean cordilleras and the closure of the Panama Isthmus could have provided novel ecological opportunities for ancestral Brownea clade lineages, followed by dispersal and diversification *in situ* (Pirie, *et al*, 2018; Erkens, *et al*, 2007), resulting in a maintained but very gradual increase in overall diversification rates through time. This

pattern of dispersal and diversification could also help to explain the current biogeographical patterns displayed by the species of the *Brownea* clade.

#### 4.3 Geography of speciation

Ancestral range constructions (Fig. 4; Table S7) suggest that large-scale vicariance was not important in the diversification of the *Brownea* clade. The fact that there are no strict Andean subclades is further evidence for this, as most species in the *Brownea* clade are Amazonian, and either share ranges between the Andean regions and Amazonia, or, as in a few species, dispersed to the Andean region secondarily. Ancestral range estimation (Fig. 4) shows a shared ancestral range of both Andean and Amazonian regions for the first node, which is the stem node for the entire *Brownea* clade.

Following this comes a divergence event between two subclades, one of which retained a shared Amazonian-Andean range and another which diversified almost solely in Amazonia, with a few secondary dispersals to the Andean regions. This suggests that the ancestors of the *Brownea* clade were found across northern South America in an era before the Andean orogeny, within the 'Pan-Amazonian' forests of the Eocene (Hoorn, *et al*, 2010). The acceleration of orogenic events of the Andes around 23 Ma ago (Hoorn, *et al*, 2010) would have begun to change the climate and geology of the Amazon and adjacent areas, resulting in the formation of the Pebas and Acre systems during the Miocene (Hoorn, *et al*, 2010; Antonelli and Sanmartín, 2011). This network of wetlands, rivers and *terra firme* forest would have isolated populations of *Brownea* clade stem lineages. This, coupled with hydrological changes causing the formation of hyperdiverse soil assemblages in Amazonia (Hoorn, *et al*, 2010), would have resulted in speciation over relatively long time scales (Antonelli and Sanmartín, 2011). This is particularly well demonstrated by in the subclade containing *Heterostemon*, *Elizabetha*, *Paloue* and *Paloveopsis*, which has diversified gradually in Amazonia over the past 20 Ma (Fig. 4).

This is in contrast to other Amazonian tree species, such as *Inga*, which shows a pattern of recent and rapid diversification (Richardson, *et al*, 2001).

*Ecuadendron* and *Brachycylix*, as well as a few lineages within *Brownea*, are endemic to the Northern Andes, and could result from very recent founder-event speciation in the Andean region, which would have occurred significantly after the formation of the Andes, as *Ecuadendron* and *Brachycylix* have

only diverged in the past million years or so (Table 2). It is also possible that this pattern of range evolution could have been caused by the extinction of wide-ranging species, leaving descendants only in the Andean regions, although our BAMM analysis suggested that extinction rates have been historically low and consistent for the Brownea clade (Figure 3C). The pattern of infrequent westward dispersal and concurrent speciation is also evident in *Macrolobium*, which mostly diversified in Amazonia, but which also includes species with broad ranges. Shifts to Central America in *M.* section *Stenosolen* are also evident, diverging from lineages that shared both Amazonian and Andean ranges. These Central American species are particularly interesting, because the inferred divergence dates for at least one species post-dates the closure of the Panama isthmus 3 Ma (O'Dea, *et al.*, 2016).

In the Brownea clade, species endemic to the Andean region almost always derive from ancestral lineages with shared Amazonian/ Andean ranges, or are derived from ancestors that dispersed to the Andean regions when the northern Andes were still a semi-permeable barrier, around 10 Ma ago (Fig. 4) (Hoorn, *et al.*, 2010). This pattern of infrequent shifts westward suggests that ancestrally wide-ranging species dispersed westwards, subsequently moving into regions that became isolated by the rise of the eastern and western Cordilleras around 5 Ma ago, leading to speciation due to geographical isolation, as has been demonstrated in other taxa (Weir and Price, 2011).

## Conclusions

Overall, our analyses suggest that the Brownea clade evolved gradually over a long period, mostly within Amazonia, with subsequent occurrences of founder-event speciation into the Northern Andes and Central America. This implies that some tree species in Amazonian forests may have been subject to low extinction rates, allowing lineages to accumulate over the long time period for which Amazonia has existed, followed by dispersal and speciation into other regions of the Neotropics. This, coupled with the climatic, geological and hydrological changes caused by the rise of the Andes may help to explain why the Amazonian tree flora is so diverse.

Our analyses are the first of their kind on the *Brownea* clade, and the first using a multilocus, dated phylogenetic approach. Future work will involve improving species-level resolution within the genera *Brownea* and *Browneopsis* by using phylogenomic methods on high-throughput sequencing data, as well as a speciation genomics study on two closely related, sympatric species of *Brownea* found in the Western Amazon which are divergent in floral morphology and pollination syndrome.

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## Appendix

### Appendix 1

#### A)

Sampled vouchers, collector information and collection localities for *Brownea* clade species (and *Amherstieae* outgroup species) used in this study. Sequence sources are shown for each locus, including Genbank accession numbers where appropriate. Dashes (-) indicate missing data in the sequence matrix. Also shown is the number of species that were sampled from each genus for the phylogenetic study. Herbaria from which accessions were collected are cited beneath the collector name and number. Outgroup taxa are marked with an asterisk (\*).

Species	Voucher	Collection locality	ITS	trnK	psbA-trnH
<i>Afzelia africana</i> Sm.*	Jongkind 2440 (WAG)	Ghana	KY306485	KX161926	-
<i>Annea afzelii</i> (Oliv.) Mackinder & Wieringa*	Andel 4244 (WAG)		KY306491	KX161933	-
<i>Anthonothea stipulacea</i> J. Léonard*	Walters 591 (WAG)	Gabon	KY306496	KX161940	-
<i>Aphanocalyx djumaensis</i> (De Wild.) J. Léonard*	Breteler 13056 (WAG)	Gabon	AF513655	EU361856	-
<i>Aphanocalyx ledermannii</i> (Harms) Wieringa*	Wieringa 1310 (WAG)	Gabon	AF513660	KX161942	-
<i>Aphanocalyx microphyllus</i> (Harms) Wieringa*	Breteler 13356 (WAG)	Ivory Coast	AF513662	KX161948	-
<i>Augouardia letestui</i> Pellegr.*	Wieringa 2898 (WAG)	Gabon	KY306499	KX161951	-
<i>Berlinia confusa</i> Hoyle*	Breteler 15455 (WAG)	Gabon	KY306506	EU361879	MG967502
<i>Bikinia grisea</i> Wieringa*	Wieringa 6210 (WAG)		KY306510	KX161967	-
<i>Copaifera mildbraedii</i> Harms*	Breteler 15025 (WAG)	Gabon	AY955814	EU361917	-
<i>Copaifera officinalis</i> (Jacq.) L.*	Fougère 27 (MT)	Singapore Botanic Garden (cultivated)	AY955816	EU361918	-
<i>Crudia choussyana</i> (Standl.) Standl.*	Hughes 1249 (MO)	El Salvador	KY306533	EU361921	-
<i>Crudia gabonensis</i> Pierre ex Harms*	Breteler 13770 (WAG)	Gabon	KY306534	KX162004	-
<i>Crudia klainei</i> Pierre & De Wild.*	Wieringa 2104 (WAG)	Kribi, Cameroon	KY306535	KX162006	-
<i>Dicymbe jenmanni</i> Sandwith*	Redden 2473	Guyana	KY306557	KX162048	-

	(US)				
<i>Gilbertiodendron diphylum</i>	Andel 3502				
(Harms) Estrella & Devesa*	(WAG)		KJ777211	KX162106	MG967504
<i>Oddoniodendron micranthum</i>	Wieringa 6165				
Baker f.*	(WAG)	Gabon	KY306632	KX162247	-
<i>Prioria platycarpa</i> (B.L. Burt)	Smith 7549				
Breteler*	(K)		AY955784	KX162280	-
<i>Schotia afra</i> (L.) Thunb.*	Hodgkiss 1				
	(BOL)	South Africa	AY955774	EU362037	GQ405086
<i>Tamarindus indica</i> L.*	Stevens	Montreal Botanical			
	(MT)	Garden	KY306655	EU362056	KJ426962
<i>Tetraberlinia bifoliolata</i>	Breteler 13081				
(Harms) Hauman*	(WAG)	Gabon	KY306662	KX162310	-
<i>Brachycylis vageleri</i> (Harms)	Berg 162				
R.S. Cowan	(US)	Colombia	FJ817504	-	-
<i>Brownea ariza</i> Benth.	Valencia 68482				
	(AAU)	Sucumbios, Ecuador	MG906825	-	MG967520
<i>Brownea ariza</i> Benth.	Valencia 68473				
	(AAU)	Sucumbios, Ecuador	MG906824	-	MG967528
<i>Brownea coccinea</i> Jacq.	Redden 4740				
	(AAU)	Guyana	FJ817505	MG967495	MG967517
<i>Brownea coccinea</i> Jacq.	Edwards 372				
	(K)	Venezuela	MG906827	-	MG967514
<i>Brownea coccinea</i> Jacq.	Diaz 5654				
	(US)	Bolivar, Venezuela	MG906826	MG967483	-
<i>Brownea coccinea</i> Jacq.	Redden 5409				
	(US)	Guyana	-	MG967496	MG967518
<i>Brownea enricii</i> L.M. Quiñones	Garcia-Barriga				
	17222	Boyaca, Colombia	MG906828	-	-

	(US)				
<i>Brownea grandiceps</i> Jacq.	Villa 1866 (Silica)	Ecuador	MG906834	MG967482	MG967525
<i>Brownea grandiceps</i> Jacq.	Villa 1858 (Silica)	Ecuador	MG906833	-	MG967527
<i>Brownea grandiceps</i> Jacq.	Villa 1855 (Silica)	Ecuador	-	MG967487	MG967519
<i>Brownea grandiceps</i> Jacq.	Klitgaard 621 (Silica)	Yasuni, Ecuador	MG906831	MG967478	MG967523
<i>Brownea grandiceps</i> Jacq.	Hollowell 485 (US)	Barima-Waini, Guyana	MG906830	MG967494	-
<i>Brownea grandiceps</i> Jacq.	Edwards 328 (NY)	Aragua, Venezuela	MG906829	MG967489	-
<i>Brownea grandiceps</i> Jacq.	Klitgaard 67040 (AAU)	Cuyabeno, Ecuador	MG906832	KX161989	-
<i>Brownea jaramilloi</i> A.J. Pérez & Klitg.	Villa 1606 (K)	Yasuni, Ecuador	MG906835	KF294052	MG967526
<i>Brownea jaramilloi</i> A.J. Pérez & Klitg.	Perez 3412 (K)	Yasuni, Ecuador	-	KF294051	-
<i>Brownea latifolia</i> Jacq.	Polak 44 (K)	Guyana	-	MG976726	MG967516
<i>Brownea latifolia</i> Jacq.	Steyermark 88845 (NY)	Bolivar, Venezuela	MG906836	KX161991	-
<i>Brownea leucantha</i> Jacq.	Klitgaard 666 (Silica)	Ecuador	KY306525	KX161992	-
<i>Brownea leucantha</i> Jacq.	Klitgaard 662 (Silica)	Yasuni, Ecuador	MG906837	MG967486	-
<i>Brownea longipedicellata</i> Huber	Klitgaard (Silica)	Ecuador	-	MG967485	-



<i>Brownea loretensis</i> Standl.	Daly 6167 (NY)	Loreto, Peru	MG906838	-	-
<i>Brownea macrophylla</i> hort. Ex Mast.	Villa 2004 (K)	Ecuador	MG906840	-	-
<i>Brownea macrophylla</i> hort. Ex Mast.	Villa 101 (AAU)	Ecuador	MG906839	-	-
<i>Brownea multijuga</i> Britton & Killip	Klitgaard 67001 (AAU)	Alluriquin, Ecuador	MG906841	EU361893	-
<i>Brownea</i> 'rosada' Jacq.	Villa 747 (Silica)	Yasuni, Ecuador	MG906843	-	MG967522
<i>Brownea</i> 'rosada' Jacq.	Villa 1868 (Silica)	Yasuni, Ecuador	MG906842	-	MG967521
<i>Brownea</i> sp. Jacq.	Pitman 5721 (US)	Ecuador	MG906845	KX161994	-
<i>Brownea</i> sp. Jacq.	Fougère 11 (MT)		MG906844	KX161993	-
<i>Brownea tillettiana</i> D. Velásquez & G. Agostini	Davidse 18640 (NY)	Perija, Venezuela	MG906846	-	-
<i>Browneopsis cauliflora</i> (Poepp.) Huber	Baker HD1402 (Silica)		-	MG967484	MG967529
<i>Browneopsis disepala</i> (Little) Klitg.	Klitgaard 67032 (Silica)	Rio Palenque, Ecuador	MG906847	KX161995	-
<i>Browneopsis excelsa</i> Pittier	Pennington 687 (K)	Rio Claro, Colombia	-	MG967492	MG967513
<i>Browneopsis ucayalina</i> Huber	Neill 6376 (AAU)	Ecuador	KY306527	MG967488	MG967524
<i>Ecuadendron Acosta-solisianum</i> D.A. Neill	Lewis 2876 (K)	Ecuador	KY306561	KX162067	-
<i>Ecuadendron Acosta-solisianum</i> D.A. Neill	Neill 10437 (K)	Ecuador	KY306562	EU361938	-

<i>Elizabetha bicolor</i> var. <i>velutina</i>	Prance 24350	Amazonas, Brazil	FJ817506	-	-
R.S. Cowan	(US)				
<i>Elizabetha coccinea</i> M.R.	Redden 3106	Guyana	MG906849	-	-
Schomb. Ex Benth.	(US)				
<i>Elizabetha coccinea</i> M.R.	Redden 1181	Guyana	KY306563	KX162068	-
Schomb. Ex Benth.	(US)				
<i>Elizabetha coccinea</i> var. <i>coccinea</i> M.R. Schomb. Ex Benth.	Redden 3038 (US)	Rupunini, Guyana	FJ817509	-	-
<i>Elizabetha duckei</i> Huber	Froes 28100 (US)	Brazil	FJ817511	-	-
<i>Elizabetha durissima</i> Ducke	Redden 1178 (K)	Guyana	FJ817513	MG967493	-
<i>Elizabetha durissima</i> Ducke	Redden 1171 (US)	Iwokrama, Guyana	FJ817514	EU361940	-
<i>Elizabetha fanshawei</i> R.S. Cowan	Redden 1733 (US)	Guyana	KY306564	KX162069	-
<i>Elizabetha fanshawei</i> R.S. Cowan	Redden 4855 (US)	Guyana	MG906850	KX538438	MG967503
<i>Elizabetha grahamiae</i> R.S. Cowan	Redden 3307 (US)	Guyana	KY306566	KX162071	MG967538
<i>Elizabetha leiogyne</i> Ducke	Maguire 41686 (US)	Venezuela	FJ817515	-	-
<i>Elizabetha macrostachya</i> Benth.	Redden 3714 (US)	Venezuela	FJ817516	KX162072	-
<i>Elizabetha paraensis</i> Ducke	Breteler 13791 (MO)	Guyana	KY306567	EU361941	-
<i>Elizabetha princeps</i> R.H. Schomb. Ex Benth.	Redden 1080 (US)	Guyana	FJ817520	EU361942	-
<i>Elizabetha princeps</i> R.H.	Redden 3692	Venezuela	FJ817519	KX162073	-

Schomb. Ex Benth.	(US)				
<i>Elizabetha speciosa</i> Ducke	Rodrigues 4850	Brazil	FJ817521	-	-
	(US)				
<i>Heterostemon conjugatus</i>	Redden 3695	Venezuela	FJ817522	EU361968	MG967505
Spruce ex Benth.	(US)				
<i>Heterostemon ellipticus</i> Mart.	Redden 3600	Venezuela	FJ817523	-	-
Ex Benth.	(US)				
<i>Heterostemon impar</i> Spruce ex Benth.	Amaral 229	Amazonas, Brazil	FJ817524	MG967491	-
	(US)				
<i>Heterostemon mimosoides</i> var. <i>mimosoides</i> Desf.	Redden 3727	Venezuela	FJ817528	KX162179	MG967506
	(US)				
<i>Heterostemon otophorus</i>	Redden 4039	Guyana	FJ817531	KX538456	MG967536
Sandwith	(US)				
<i>Macrolobium acaciifolium</i>	Korning 47735	Yasuni, Ecuador	KY306616	KX162225	MG967535
(Benth.) Benth.	(NY)				
<i>Macrolobium acaciifolium</i>	Redden 1098	Guyana	MG906851	KX162226	-
(Benth.) Benth.	(US)				
<i>Macrolobium angustifolium</i>	Neves 2010	Brazil	MF987609	MG967479	MG967500
(Benth.) R.S. Cowan	(Silica)				
<i>Macrolobium archeri</i> R.S. Cowan	Klitgaard 683	Napo, Ecuador	KY306617	KX162227	MG967512
	(Silica)				
<i>Macrolobium arenarium</i> Ducke	Dexter 7003	Amazonas, Brazil	MF987610	-	-
	(Silica)				
<i>Macrolobium arenarium</i> Ducke	Martins 8	Amazonas, Brazil	MG906852	-	-
	(K)				
<i>Macrolobium bifolium</i> (Aubl.) Pers.	Van Ogtrop 2		MG906853	KX162229	-
	(WAG)				
<i>Macrolobium bifolium</i> (Aubl.) Pers.	Clark 7712	Guyana	KY306618	KX162228	-
	(US)				
<i>Macrolobium bifolium</i> (Aubl.)	Redden 1626	Guyana	FJ817498	-	-

Pers.	(US)				
<i>Macrolobium brevense</i> Ducke	Neves 2040 (K)	Brazil	MF987614	-	-
<i>Macrolobium campestre</i> Huber	Redden 3649 (US)	Venezuela	FJ817499	KX162230	MG967531
<i>Macrolobium canaliculatum</i> Spruce ex Benth.	Redden 3400 (US)	Venezuela	-	KX538460	MG967507
<i>Macrolobium colombianum</i> (Britton & Killip) Killip ex L. Uribe	Klitgaard 682 (K)	Napo, Ecuador	MG906854	MG967490	-
<i>Macrolobium costaricense</i> W.C. Burger	Hammel 20702 (K)	Heredia, Costa Rica	MG906855	-	-
<i>Macrolobium dressleri</i> R.S. Cowan	McPherson 15998 (US)	Panama	KY306619	KX162231	-
<i>Macrolobium gracile</i> Spruce ex Benth.	Redden 3687 (US)	Venezuela	FJ817500	KX162232	MG967508
<i>Macrolobium guianense</i> (Aubl.) Pulle	Prevost 5279 (US)		-	KX538478	-
<i>Macrolobium herrerae</i> Zarucchi	Marshall 392 (K)	Sinai, Guatemala	MG906856	-	-
<i>Macrolobium huberianum</i> var. <i>huberianum</i> Ducke	Redden 2197 (US)	Guyana	KY306620	KX162233	-
<i>Macrolobium ischnocalyx</i> Harms	Klitgaard 669 (K)	Napo, Ecuador	KY306621	EU361997	-
<i>Macrolobium latifolium</i> Vogel	De Lima 7880 (K)	Sao Paulo, Brazil	MF987623	-	-
<i>Macrolobium limbatum</i> Spruce ex Benth.	Dexter 6867 (E)	Amazonas, Brazil	MF987624	-	MG967534
<i>Macrolobium longeracemosum</i>	Redden 1408	Guyana	MG906857	MG967481	-

Amshoff	(E)				
<i>Macrolobium longipedicellatum</i>	De Lima 2790				
		Brazil	MG906858	-	-
Ducke	(K)				
<i>Macrolobium longipes</i> R.S.	Redden 1242				
		Mazaruni, Guyana	MG906859	MG967480	MG967501
Cowan	(K)				
<i>Macrolobium longipes</i> R.S.	Redden 3679				
		Venezuela	FJ817501	KX538479	MG967532
Cowan	(US)				
<i>Macrolobium molle</i> (Spruce ex Benth.) R.S. Cowan	Redden 3344				
		Venezuela	KY306622	KX162234	MG967509
<i>Macrolobium montanum</i> Ducke	Breteler 13798				
	(WAG)	Mabura Hill, Guyana	KY306623	KX162235	-
<i>Macrolobium montanum</i> var. <i>potaroanum</i> R.S. Cowan	Andel 5548				
	(K)	Suriname	MG906860	-	-
<i>Macrolobium multijugum</i> var. <i>multijugum</i> (D.C.) Benth.	Redden 3700				
	(US)	Venezuela	FJ817502	KX162236	MG967530
<i>Macrolobium parvifolium</i> (Huber) R.S. Cowan	De Lima 6814				
	(US)	Brazil	MG906861	-	-
<i>Macrolobium pittieri</i> (Rose) Schery	Santos 960				
	(K)	Amazonas, Brazil	MG906862	-	-
<i>Macrolobium pittieri</i> (Rose) Schery	Croat 26245				
	(US)	Colon, Panama	MF987637	-	-
<i>Macrolobium punctatum</i> Spruce ex Benth.	Redden 3650				
	(US)	Venezuela	KY306624	KX162237	-
<i>Macrolobium rubrum</i> R.S. Cowan	Rodriguez 10875				
	(K)	Amazonas, Brazil	MG906863	-	-
<i>Macrolobium</i> sp. Schreb.	Redden 3678				
	(US)	Venezuela	FJ817503	KX538463	MG967533
<i>Macrolobium</i> sp. Schreb.	Klitgaard 663				
	(K)	Napo, Ecuador	MG906864	KX162238	-

<i>Macrolobium suaveolens</i>	Redden 1637				
Spruce ex Benth.	(US)	Guyana	KY306625	KX162240	MG967499
<i>Paloue brasiliensis</i> Ducke	Silva 152				
	(US)	Brazil	FJ817534	-	-
<i>Paloue guianensis</i> Aubl.	Redden 5968				
	(US)	French Guiana	FJ817538	KX538486	MG967511
<i>Paloue guianensis</i> Aubl.	Redden 6000				
	(US)	Guyana	FJ817539	KX538488	MG967515
<i>Paloue guianensis</i> Aubl.	Lindeman 874				
	(U)	Guyana	MG906865	KX162250	-
<i>Paloue guianensis</i> Aubl.	Grenand 1781				
	(US)	French Guiana	FJ817535	-	-
<i>Paloue induta</i> Sandwith	Redden 4229				
	(US)	Guyana	FJ817543	KX538489	MG967510
<i>Paloue riparia</i> Pulle	Redden 1161A	Potaro-Siparuni,			
	(US)	Guyana	FJ817546	EU362016	-
<i>Paloue sandwithii</i> K.M. Redden	Redden 4022				
	(US)	Guyana	FJ817549	MG967497	MG967537
<i>Paloue</i> sp. Aubl.	Bordenave 871				
	(US)		FJ817533	KX162251	-
<i>Paloue</i> sp.nov Aubl.	Redden 5850				
	(US)	Guyana	MG906866	MG967498	MG967539
<i>Paloveopsis emarginata</i> R.S.	Cid 916				
Cowan	(US)	Brazil	FJ817532	-	-

**B)**

Species	Total species
sampld	in genus

<i>Brachycylis</i>	1	1
<i>Brownea</i>	14	27
<i>Browneopsis</i>	4	6
<i>Ecuadendron</i>	1	1
<i>Elizabetha</i>	11	12
<i>Paloue</i>	5	5
<i>Paloveopsis</i>	1	1
<i>Heterostemon</i>	5	7
<i>Macrolobium</i>	31	53

## Appendix 2:

Initial speciation ( $\lambda$ ) and extinction ( $\mu$ ) parameters used to start Maximum Likelihood estimation of speciation and extinction rates in RPANDA v.1.3 (Morlon, *et al*, 2016), showing both constant rate and exponentially varying initial rate values.

	Constant birth	Constant death	Variable birth (Exponential)	Variable death (Exponential)
<b>Pure birth</b>	0.4	0	0.4, -0.05	0
<b>birth-death</b>	0.4	0.05	0.4, -0.05	0.1, 0.05

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- Amazonia
- Northern Andes
- Venezuela
- Central America
- Atlantic forest
- Cerrado/Dry areas
- Amazonia+ N. Andes

