SYSTEMATICS OF THE STICTOCARDIA CLADE

(*IPOMOEA* L., CONVOLVULACEAE)

Alex Sumadijaya
Department of Plant Sciences
Wolfson College, The University of Oxford

Thesis submitted for the degree of Doctor of Philosophy
Hilary Term 2022

In the beginning, there was chaos, a gaping ignorance

Let there be monograph.

ACKNOWLEDGEMENT

For centuries, dreaming spires of churches and colleges have been silently witnessing the University of Oxford, an academic hub inhabited by great figures with brilliant minds. Experiencing years of living abroad has given me a whole lot of intellectual and personal lessons. I have encountered a wide range of concepts such as systematics, species, big genera especially *Ipomoea,* and become aware of disparity of specimen collection and deposition and taxonomic information, as well as access inequality across herbaria during the construction of a botanical monograph. All of those aspects have transformed my perspective as well as expanded my horizon on working with natural history collections. These thoughts conceivably shine my professional track to contribute some improvements in dealing with the taxonomic impediment in Indonesia after returning to my home country later.

Being a member of the Department of Plant Sciences - soon to be the Department of Biology – is such once in a lifetime privilege that expands my professional understanding of systematics. I'd like to express my deepest gratitude to Robert Scotland for tirelessly guiding me from the very beginning to the end of this long, winding, and rocky road. Robert forged me to walk on every inch of my intellectual progress, which truly change my past understanding of taxonomy. On board the Scotland's group, I have learnt that there's no easy way to carry out and navigate through the whole D.Phil. research before reaching fruitful results. This group literally shaped and polished every corner of my botanical knowledge. I gained an enormous amount of support from Pablo Muñoz-Rodriguez for lab work and discussion of molecular systematics, John Wood for discussions on history, herbarium specimens and morphology, Tom Carruthers and Tom Wells for

computational analyses and writing-related advice. I enjoyed all refreshing and random chats with them in pubs and during lunch breaks pre and post Brexit.

A special gratitude to Stephen Harris and Julie Hawkins as internal and external examiners respectively, who provide invaluable suggestions to improve my thesis. Their invaluable feedback and critics during viva have opened a new world in doing taxonomic study.

Stephen Harris and Serena Marner of OXF have provided a great support for loans and correspondence with herbaria in Europe and America. Rosemary Wise provided seven precise and beautiful state-of-the-art illustrations. Helen Prescott dealt with my issues on the nitty-gritty of lab procedures and reagents. Last but not least, Gem Toes-Crichton and Nick Kruger who have helped me with an outstanding long-term administrative support.

I express my gratitude to curators and staff of various herbaria who provided access to herbarium material, either physical specimens, leaf fragments, or digital images; George Ferguson (ARIZ), Robert Vogt and Katharina Rabe (B), Yovita Yesilur (BM), Barbara Kennedy (BISH), Atik Retnowati and Jaenudin (BO), Ann (BR), Gillian Brown (BRI), Tiana Rehman (BRIT), Stephan Rakotonandrasana (CNARP), Frank Zich (CNS), Shambel Alemu and Sebsebe Demissew (ETH), Marie Briggs (K), Ong Poh Teck and Syahida Emiza Binti Suhaimi (KEP), Maria Cristina Duarte (LISC), Serena Marner (OXF), Matthew Pace (NY), Marc Jeanson (P), Khanit Wangwasit (QBG), Yashica Singh (SANBI), Meghann Toner and Inggrid Lin (US),

I would never accomplish this stage without a great and constant support from my wife Ivana, my daughter Cleo, as well as the whole family in Indonesia.

I am also greatly indebted to a few numbers of Indonesian fellas in the University of Oxford and Oxford Brookes University for being with me through this life-changing chapter, for better or worse. The significant ones, Atika Fitria Soemartono, Shinta Marshalita Prayitno, Diah Savitri Pritadrajati, Wulan Pusparini, Team of Wahyu-Alex-Aish, Gilbert Sanjaya, Ahmad Mukhlis Firdaus, Muhammad Najib, Muhammad Syarif Romadhon, who talked and walked together in the darkness, emptiness and loneliness of graduate life, such a time worth spent together.

I took pleasure as one of the inhabitants of Wolfson College and Summertown House that became my cozy homes far from home, a comfy place to declutter my mind after daily dose of hard work. I thank the support from Disability Advisory Service staff who worked empathetically optimizing my slow-thinking, impaired brain that suffers catastrophic failure of logic in astronomical proportions.

Last but not least, this extensive academic journey is only possible to finish with full sponsorship from the Endowment Fund for Education, Ministry of Finance, Republic of Indonesia, which supported the whole four years and an extension period during the pandemic. Knowledge, network and experience gained in this D.Phil. programme will further help me understand and synthesize the megabiodiversity of the Indonesian flora.

Table of content

ABSTRACT x

List of Tables xi

List of Figures xiii

List of Appendices xvi

CHAPTER 1. INTRODUCTION 1

1.1. The current state of the world flora: Issues and methods 1

1.2. Monographs: Comprehensive taxonomic revisions 1

1.3. Accumulation of specimens: The building blocks of monographic work 2

1.4. Accelerating the pace of taxonomy for big genera 3

1.5. *Ipomoea* L. 4

1.6. Monographing *Stictocardia* 5

CHAPTER 2. TAXONOMY AND MORPHOLOGY OF *STICTOCARDIA* 11

2.1. BACKGROUND 11

2.1.1. Taxonomic history of *Stictocardia* in the context of *Ipomoea* 11

2.1.1.1. Hans (Johannes) Gottfried Hallier f. (1894-1911) 11

2.1.1.2. Simon Jan Van Ooststroom (1943-1953) 12

2.1.1.3. Bernard Verdcourt (1958-1990) 13

2.1.1.4. Daniel F. Austin (1975-2001) 14

2.1.1.5. Other taxonomic literature (1914-2017) 14

2.1.2. Taxonomic issues of *Stictocardia* 15

2.1.2.1 Inconsistent generic delimitation 15

2.1.2.2. Unclear species delimitation 16

2.2. MATERIALS AND METHODS 16

2.2.1. Literature study 16

2.2.2. Herbarium specimens 17

2.2.3. Examination of specimens 17

2.2.4. Measured quantitative morphological characters of indistinct species 21

2.2.5. Principal Component Analysis (PCA) 22

2.2.6. Univariate Statistics 23
2.2.7. Reciprocal illumination between morphology and phylogenetics analysis 25
2.2.8. Examination of the taxonomic value of black glands 27
2.3. RESULTS 28
2.3.1. Distinct vs indistinct species of *Stictocardia* 28
2.3.2. Overlapping quantitative characters of indistinct species 29
2.3.3. PCA of the indistinct species 31
2.3.4. Statistical test of the indistinct species 35
2.3.5. Re-evaluation of the study group 41
2.3.6. Diagnosis of the study group 42
2.3.7. Morphological evaluation of the polyphyly of *I. beraviensis* 42
2.4. DISCUSSION 47
2.4.1. Diagnostic characters of the clade Stictocardia 47
2.4.1.1. Cordate leaf base 47
2.4.1.2. Black glands 47
2.4.1.3. Sepals accrescent in fruit 51
2.4.1.4. Indehiscent fruit 52
2.4.1.5. Valved Endocarp 53
2.4.2. The delimitation of species within the clade Stictocardia 53
2.4.2.1. Species outside Africa and Madagascar 53
2.4.2.2. Species in Africa and Madagascar 55
2.4.3. Reciprocal illumination between morphology and phylogenetics of *I. beraviensis:* Poor sampling, incomplete story 56
2.4.4. Refuted PCA result of the indistinct species 59
2.4.5 Statistical test of the indistinct species prove inconclusive 60
2.4.6. Species recovered outside the clade Stictocardia 61
2.5. CONCLUSION 62

CHAPTER 3. PHYLOGENETIC STUDY OF *STICTOCARDIA* OF THE OLD-WORLD *IPOMOEA* 67
3.1. BACKGROUND 67
3.1.1. Phylogenetic and phylogenomic studies of Convolvulaceae and *Ipomoea* 67
3.1.2. Taxon and character sampling of *Stictocardia* 68
3.1.3. Outgroup comparison 69
3.1.4. DNA barcoding (Nuclear and Chloroplast) 69
3.1.5. Plastome and Genome Skimming 70
3.2. MATERIALS AND METHODS 71
3.2.1. DNA extraction 71
3.2.2. DNA amplifications of *nr*ITS 72
3.2.3. Gel electrophoresis 73
3.2.4. DNA cleaning and sequencing 73
3.2.5. Sanger Sequencing and data evaluation of the *nr*ITS 74
3.2.6. Multiple sequences alignment of the *nr*ITS data set 74
3.2.7. Phylogenetic analysis of the *nr*ITS data set 75
3.2.8. High-Throughput Sequencing (HTS) 76
3.2.9. Assembly of plastome genes and variable regions 76
3.2.10. Phylogenomic analysis of plastome genes and variable regions 77
3.3. RESULT 78
3.3.1. The genus *Stictocardia* and the clade Stictocardia 80
3.3.2. Groups and species composition across phylogenetic trees 83
3.4. DISCUSSION 84
3.4.1. Polyphyly of *Stictocardia* 84
3.4.2. Monophyly of Stictocardia 85
3.4.3. Unresolved sister taxa of Stictocardia 88
3.5. CONCLUSION 89

CHAPTER 4. TAXONOMIC REVISION OF STICTOCARDIA 99
4.1. The systematic context of this study 99
4.2. Species concept and delimitation 99
4.3. Ecology 100
4.4. Pollination syndrome 100
4.5. Geographical distribution 101
4.6. Number of specimens examined 102
4.7. Taxon names 102
4.8. Conservation assessment 103
4.9. Clade Description 104
4.10. Artificial identification key 105
4.11. Species account 106
4.11.1. *Ipomoea lutambensis* Schulze-Menz 106
4.11.2. *Ipomoea beraviensis* Vatke 109
4.11.3. *Ipomoea macalusoi* Mattei 117
4.11.4. *Ipomoea incompta* Hallier f. 119
4.11.5. *Ipomoea pringsheimiana* (Dammer) Rendle 122
4.11.6. *Ipomoea tiliifolia* (Desr.) Roem. & Schult. 126
4.11.7. *Ipomoea queenslandica* (Domin) J.R.I.Wood & Scotland 137
4.12. Taxa require further investigation 139
4.12.1. *Ipomoea neglecta* (Ooststr.) J.R.I.Wood & Scotland 139
4.12.2. *Ipomoea lombokii* J.R.I.Wood & Scotland, 140
4.13. Excluded species 140
4.13.1. *Ipomoea orientalis* J.R.I.Wood & Scotland 140
4.13.2. *Ipomoea sivarajanii* (Biju, Pushp. & P.Mathew) J.R.I.Wood & Scotland 141
4.13.3. *Ipomoea mojangensis* Vatke 142
4.13.4. *Ipomoea jucunda* Thwaites 142
4.14. Unresolved species 143
4.14.1. *Stictocardia multiflora* Hallier f 143
4.142. *Stictocardia nervosa* Hallier f. 143

CHAPTER 5. SUMMARY 147
5.1. Monographing *Stictocardia* 147
5.2. Literature (Chapter Two) 147
5.3. Herbarium specimens 148
5.4. Morphological study (Chapter Two) 149
5.5. Phylogenetic study of molecular sequence data (Chapter Three) 151
5.6. Monograph of Stictocardia, a contribution to the systematics of *Ipomoea* (Chapter Four) 152
5.7. Concluding remarks 153

ABSTRACT

Botanical monographs are comprehensive taxonomic accounts of a specific group of plants. This thesis reviews relevant taxonomic literature and investigates a subsumed genus – *Stictocardia* - nested within *Ipomoea* (Convolvulaceae), one of the species-rich genera of Angiosperms. A large number of specimens representing twelve out of 13 species of *Stictocardia*, both herbarium sheets and digital images from 35 herbaria, are the source of quantitative morphological data (115 samples) and leaf material for DNA sequencing (36 samples). Morphological characters and DNA sequence data are two lines of evidence that interact through reciprocal illumination within the scope of integrative taxonomy. Principal Component Analysis and Mann-Whitney test were conducted on several indistinct taxa to ensure rigorous hypothesis testing on species delimitation. Phylogenetic study consistently identifies seven species of *Stictocardia* in a clade named Stictocardia (non-italic) based on independent data sets - *nr*ITS and plastome - and various inference methods (MP, A-ML and BI). This clade of seven species is characterised by a set of homoplastic morphological characters. These seven species are monographed for the first-time including clade circumscription, identification key, distribution maps and a comprehensive taxonomic account (nomenclature, description, illustration, habitat, notes and specimens seen). Conservation assessments based upon Area of Occupancy (AOO) and Extent of Occurrence (EOO) analyses, were conducted to this clade. In addition, four species of *Stictocardia* are recovered outside of the clade Stictocardia and more closely related to other species of *Ipomoea*. Two species require further investigation to resolve their placements whether inside or outside the clade Stictocardia.

List of Tables

Table 2.1. List of taxa in *Stictocardia* (species and subspecies), with the protolog and correct name arranged by the year of publication 18

Table 2.2. Herbarium where the specimens of *Stictocardia* deposited arranged in alphabetical order and the host Institution 20

Table 2.3. Score sheet for character examination of individual specimens of indistinct species 23

Table 2.4. The change of study group from *Stictocardia* (a polyphyletic taxon) into Stictocardia (a clade) following the phylogenetic study 26

Table 2.5. The statistics of PCA result based on 115 specimens of *Stictocardia* and seven quantitative morphological characters. 34

Table 2.6. Table of statistics of primary peduncle length across five indistinct species of *Stictocardia* 35

Table 2.7. Table of statistics of pedicel length across five indistinct species of *Stictocardia* 35

Table 2.8. Table of statistics of the number of flowers in inflorescence across five indistinct species of *Stictocardia* 35

Table 2.9. Table of statistics of corolla length across five indistinct species of *Stictocardia* 35

Table 2.10. Table of statistics of sepal length across five indistinct species of *Stictocardia* 36

Table 2.11. Table of statistics of the number of lateral veins across five indistinct species of *Stictocardia* 36

Table 2.12. Results of the Mann-Whitney of grouping of indistinct species across quantitative characters 41

Table 3.1. The length of region and average percent coverage of PG (left) and VR (right) data sets 79

Table 3.2. Statistics of multiple sequences alignments across nuclear (ITS) and plastome (PG and VR) data sets. 79

Table 3.3. The monophyly support values of Stictocardia clade, groups, species, and unresolved sister taxon across data sets and inference methods 87

List of Figures

Figure 2.1. Diagram to illustrate measurements taken 22

Figure 2.2. Workflow of the taxonomic revision of Stictocardia based on corroboration of morphological and phylogenetic evidence 25

Figure 2.3. Global distribution map of indistinct species of *Stictocardia* 29

Figure 2.4. Overlapping quantitative characters of six colour-coded indistinct species 29-31

Figure 2.5. Distribution of 115 specimens of six colour coded indistinct *Stictocardia* species relative to PC1 and PC2 33

Figure 2.6. PCA's eigenvalues and eigenvectors using *princomp* function 34

Figure 2.7. Mean and Standard Error of Mean of primary peduncle length across indistinct species 36

Figure 2.8. Mean and Standard Error of Mean of pedicel length across indistinct species 37

Figure 2.9. Mean and Standard Error of Mean of the average number of flowers in inflorescence across indistinct species 37

Figure 2.10. Mean and Standard Error of Mean of sepal length across indistinct species 38

Figure 2.11. Mean and Standard Error of Mean of corolla length across indistinct species 38

Figure 2.12. Mean and Standard Error of mean of the number of lateral veins across indistinct species 39

Figure 2.13. Distribution of primary peduncle length of two groups of indistinct species 39

Figure 2.14. Distribution of the number of flowers in inflorescence of two groups of indistinct species 40

Figure 2.15. Distribution of sepal length of two groups of indistinct species 40

Figure 2.16. Distribution of corolla length of two groups of indistinct species 40

Figure 2.17 A-F. Box plots of selected quantitative morphological characters of *I. beraviensis* 43

Figure 2.18 Distribution of morphological characters measurement between two putative groups of I. *beraviensis* 45

Figure 2.19A. Inferred consensus *nr*ITS phylogeny of the OW *Ipomoea* focusses on Stictocardia with distribution of morphological characters 48

Figure 2.19B. Inferred consensus plastome phylogeny of the OW *Ipomoea* focusses on Stictocardia with distribution of morphological characters 49

Figure 2.20. Box plots of the range of average density of black glands on the abaxial surface of the leaf of six species of Stictocardia 51

Figure 3.1. Workflow for molecular systematics from DNA extraction of herbarium specimens to phylogenetic analyses 72

Figure 3.2A. Inferred *nr*ITS phylogeny of the OW *Ipomoea* focusses on Stictocardia (blue arrow). 81

Figure 3.2B. Inferred plastome phylogeny of the OW *Ipomoea* focusses on Stictocardia 82

Figure 4.1. Global distribution of Stictocardia 101

Figure 4.2. *Ipomoea lutambensis* Schulze-Menz. 108

Figure 4.3. Map of Africa with five Stictocardia species; *I. beraviensis, I. lutambensis, I. incompta, I. pringsheimiana,* and *I. macalusoi* 110

Figure 4.4. *Ipomoea beraviensis* Vatke 111

Figure 4.5. *Ipomoea macalusoi* Mattei. 118

Figure 4.6. *Ipomoea incompta* Hallier f. 121

Figure 4.7. *Ipomoea pringsheimiana* (Dammer) Rendle 124

Figure 4.8. *Ipomoea tiliifolia* (Desr.) Roem. & Schult. 129

Figure 4.9. The distribution map of *I. tiliifolia* and *I. queenslandica* 130

Figure 4.10. *Ipomoea queenslandica* (Domin) J.R.I.Wood & Scotland 138

List of Appendices

Appendix 1. List of the observed specimens (sheets or images) of *Stictocardia* species 155

Appendix 2. Definition of morphological characters states of Stictocardia species following Stearn (1973) 162

Appendix 3. The script used in the PCA in RStudio ver. 1.4.1103 using *princomp* function 164

Appendix 4. List of measured specimens of indistinct species (*I. tiliifolia, I. pringsheimiana, I. orientalis, I. neglecta, I. queenslandica,* and *I. sivarajanii*) and the distribution map 166

Appendix 5. Passport of samples represented in the ITS and plastome phylogeny and the distribution map 170

Appendix 6. Result of the Model Test run in MEGA X for Nuclear ITS, VR, and PG data sets 173

Appendix 7. Custom script used for assembling plastome genes and variable regions 174

Appendix 8. Custom script used for phylogenomic analysis of plastome genes and variable regions 176

Appendix 9.1-7. The conservation status of seven species of *Stictocardia* based on the extrapolation of georeferenced samples using AOO and EOO analyses 178-182

CHAPTER I. INTRODUCTION

1.1. The current state of the world flora: Issues and methods

The world's species inventory of flowering plants (Angiosperms) is far from complete. Botanists estimate that the total number of flowering plants ranges from 223,000 to 422,000 species [1-7]. Each estimate, however, has its own advantages and disadvantages. Methods that use lists of binomial names such as *Index Kewensis* [1] and checklists [7] are straightforward and simply tally names. Checklists, however, are too simplistic as the names are not necessarily accompanied by a comprehensive taxonomic description. The lack or absence of a comprehensive taxonomic description for many species prohibits an accurate comparison between taxa. Without accurate descriptive comparison - primarily of morphology - multiple names may associate with the same taxonomic entity, a phenomenon known as synonymy [8] that causes bias in taxonomy [9]. Unidentified synonyms across Angiosperms inevitably causes inflation in species number [10]. The level of synonymy may vary across estimates [3] and considerably from 9 to 88 % in tropical plant families and genera [11]. The issue of synonymy is addressed by comprehensive monographs and taxonomic revisions [2, 3, 11, 12]. Published monographs, however, merely cover a small portion of Angiosperms genera [13].

1.2. Monographs: Comprehensive taxonomic revisions

A monograph is a taxonomic revision of a plant group that incorporates all species across the entire distribution of the group [12, 14, 15]. The inclusion of all species of a plant group in a monographic study allows access to a comprehensive documentation of information [16] includes morphological variation that enables accurate species delimitation, recognises widespread specimen misidentification [17],

identifies synonymy [3, 12, 15], and leads to the discovery of new taxa [18, 19]. Monographs are therefore more holistic in scope and lead to the generation of more data compared to other revisionary studies (revisions, synopsis, and conspectus) [8, 12, 15, 16, 20, 21]. In addition, once a monograph has been completed, other taxonomic outputs such as Floras and checklists can be produced [20]. Nevertheless, this effort is complex, time-consuming, and expensive [22]. There is no fixed format of a monograph albeit Stuessy et al. [15] proposed to standardise its content and currently an R package available for monographic production [23],

1.3. Accumulation of specimens: The building blocks of monographic work

Monographic work relies on three key components: specimens, literature and funding [15]. Whilst these components are equally important, this chapter is focused on specimens because they are the building blocks of a monograph [24] and literature that rich of associated biodiversity information [25]. On a global scale, the number of specimens has exponentially increased [26] and doubled in the last 50 years [17]. This accumulation of specimens is beneficial for the construction of monographs because the inclusion of more samples is likely to cover more of the morphological variation of a given taxon [15, 27-31]. Accessing specimens is important for monographic studies. Further, digitization of specimens not only increases access to digital images with less time and cheaper price [32, 33]. However, these aspects demand a substantial amount of effort and can potentially slow the rate of monographic work. Massive numbers of specimens are double-edged sword as they cover more morphological variation which is a good thing but they can also present a formidable obstacle due to information overload. Prioritising complete specimens with flower and fruit that represent the overall morphological variation from the entire distribution range of a given species

can optimally balance between morphological representative and manage time constraint effectively.

1.4. Accelerating the pace of taxonomy for big genera

For species-rich taxa, there are at least two strategies that have been proposed to accelerate the pace of taxonomy, Foundation Monographs [34] and Turbo-taxonomy [35]. The latter approach - used mainly in entomology [35-38] and rarely in botany [39] – is dealing with poorly known taxa with high number of undescribed species and almost no concern on synonymy at all.

The idea of the Foundation Monograph (FM) was developed at the University of Oxford [34] and it focusses on four principal taxonomic components: species description, identification keys, DNA barcodes (phylogeny) and nomenclature [40]. These components were selected to maximise the effectiveness and efficiency of taxonomic investigation given time and funding constraints [40]. The advantage of this approach is demonstrated by the fact that it has enabled the completion of a monograph for *Convolvulus*, a genus of more than 190 species, in a relatively short time [40] and a monograph of 425 species of *Ipomoea* in the New World [41]. These outputs demonstrate that FM is a potential tool for tackling the taxonomy of species-rich taxa in tropical areas.

Here, species-rich taxa are treated identically to big genera, which are defined as Angiosperm genera of more than 500 species and worldwide distributions [42]. The number of big genera is relatively small proportionally (57) [42], of the total number of Angiosperms genera recognised by either Stevens (13,208) [43], Brummitt (13,560) [44], or The Plant List (17,020) [45]. Although the number of big genera is small, revising them is far from easy. Until mid-1960s only less than half of them were either

partially and regionally revised by specialists [13]. Slow progress in revising big genera is likely to be caused by the considerable effort required to compile relevant literature, gather and observe specimens, and evaluate species delimitations of all taxa under investigation [15, 42, 46].

1.5. *Ipomoea* L.

Ipomoea L. [47] (Convolvulaceae Juss.) [48] is a big genus and under investigation in the ongoing monographic work at the University of Oxford. Initially, a handful of species of *Ipomoea* were recognised by Linnaeus in *Hortus Cliffortianus* (1737) [49], *Hortus Upsalensis* (1748) [50], and *Species Plantarum* (1753) [47]. Further taxonomic treatments by Grisebach in 1864 [51], House in 1908 [52], and Wilkin in 1999 [53], increased the number of *Ipomoea* species by the inclusion - either fully or partially - of the previously known genera, *Batatas* Choisy*, Calonyction* Choisy*, Exogonium* Choisy*, Quamoclit* Mill*.,* and *Pharbitis* Choisy [53].

Recent estimates for the number of species of *Ipomoea* range from 600 to 700 [54-57]. The number is still growing steadily, primarily due to recent discoveries from South America [41, 58-63]. To date, there is no comprehensive global monograph of *Ipomoea.* In the past, *Ipomoea* has been revised regionally, particularly in limited number of countries in the New World [13, 52, 64], and the recent one [41] at a continental scale. In the Old World, the genus has never been studied comprehensively and has merely been treated in accounts of regional flora and species discovery [65-74]. In addition, there are several scattered taxonomic revisions of infrageneric ranks within *Ipomoea* [75-78]. Taken together, these studies indicate that our taxonomic knowledge of *Ipomoea* is partial and incomplete.

Phylogenetic studies of Convolvulaceae or Ipomoeeae [57, 79-81] have suggested that *Ipomoea* is not a monophyletic genus because several other genera- *Argyreia* Lour., *Astripomoea* A. Meeuse, *Blinkworthia* Choisy, *Lepistemon* Blume, *Lepistemonopsis* Dammer, *Paralepistemon* Lejoly & Lisowski, *Rivea* Choisy, *Stictocardia* Hall. f., and *Turbina* Raf.- are nested within it. However, these studies are characterised by limited taxon and character sampling. A more recent study of *Ipomoea* [82] - the one that I involved in- that comprised far denser taxon and character sampling has confirmed the placement of these genera within the monophyletic *Ipomoea,* or *Ipomoea s.l.*

Taxonomically, several of the nested genera - *Turbina* [83-85], *Lepistemon* [86], *Argyreia* [87], and *Stictocardia* [88-91] - have been partially or regionally revised. The remaining genera - *Rivea, Lepistemonopsis, Paralepistemon, Astripomoea,* and *Blinkworthia* - are only recorded in regional floras [51, 68, 70, 92] and yet to be critically examined. Plant of the World Online/POWO [93] of Kew lists more taxonomic literature of these genera, but not all are accessible in this study. Therefore, further taxonomic studies of these putative genera are necessary to achieve a comprehensive understanding of the expanded *Ipomoea.*

1.6. Monographing *Stictocardia*

Taking advantage of the existing dense taxon and character sampling of the recently published *Ipomoea* phylogeny [82], this study is focused on *Stictocardia,* one of the poorly known taxa nested within the Old-World *Ipomoea. Stictocardia* is selected as the focus of this investigation for two reasons:

1. It is a taxon with no comprehensive study that evaluates generic and species-level delimitation, maps global distribution, or infers the

phylogenetic relationship among the species. Thus it fits with one of the criteria for monographic study, a taxon with major knowledge gap [94].

2. It has a small number of species, which is manageable for a time-constrained D.Phil. project [15] which provide unique challenges in dealing with all basic problems in plant systematics [95].

This study compiles and updates the present knowledge of *Stictocardia* as well as evaluating its delimitation and species composition within *Ipomoea*. Therefore, the output of this study is a taxonomic monograph that focuses on *Stictocardia* as a subset of *Ipomoea*.

List of references

1. Govaerts, R., *How many species of seed plants are there?* Taxon, 2001. **50**: p. 1085-1090.
2. Thorne, R.F., *How many species of seed plants are there?* Taxon, 2002. **51**: p. 511-512.
3. Scotland, R.W. and A.H. Wortley, *How many species of seed plants are there?* Taxon, 2003. **52**: p. 101-104.
4. Christenhusz, M.J.M. and J.W. Byng, *The number of known plants species in the world and its annual increase.* Phytotaxa, 2016. **261**: p. 201-217.
5. Mora, C., D.P. Tittensor, S. Adl, A.G.B. Simpson, and B. Worm, *How many species are there on Earth and in the ocean?* PLOS Biology, 2011. **9**: p. e1001127 (8 pages).
6. Lughadha, E.N., R. Govaerts, I. Belyaeva, N. Black, H. Lindon, R. Allkin, R.E. Magill, and N. Nicolson, *Counting counts: Revised estimates of numbers of accepted species of flowering plants, seed plants, vascular plants and land plants with a review of other recent estimates.* Phytotaxa, 2016. **272**: p. 82-88.
7. Bramwell, D., *How many plant species are there.* Plant Talk, 2002. **28**: p. 32-34.
8. Judd, W.S., C.S. Campbell, E.A. Kellogg, P.F. Stevens, and M.J. Donoghue, *Plant Systematics: A Phylogenetic Approach*. 3rd ed. 2007, Sunderland: Sinauer Association.
9. Alroy, J., *How many named species are valid?* PNAS, 2002. **99**: p. 3706-3711.
10. Karremans, A.P., I.F. Chinchilla, G. Rojas-Alvarado, M. Cedeño-Fonseca, A. Damián, and G. Léotard, *A reappraisal of Neotropical Vanilla: With a note on taxonomic inflation and the importance of alpha taxonomy in biological studies.* Lankesteriana, 2020. **20**: p. 395–497.
11. Wortley, A.H. and R.W. Scotland, *Synonymy, sampling and seed plant numbers.* Taxon, 2004. **53**: p. 478-480.
12. Stace, C.A., *Plant Taxonomy and Biosystematics*. 1980, Baltimore: University Park Press.
13. Kent, D.H., *Index to botanical monographs: A guide to monographs and taxonomic papers relating to phanerogams and vascular cryptogams found growing wild in the British Isles*. 1967, London: Academic Press.
14. Simpson, M.G., *Plant Systematics*. 2nd ed. 2010, Amsterdam: Academic Press.
15. Marhold, K., T. Stuessy, M. Agababian, D. Agosti, M.H. Alford, A. Crespo, J.V. Crisci, L.J. Dorr, Z. Ferencova, and D. Frodin, *The future of botanical monography: Report from an international workshop, 12–16 March 2012, Smolenice, Slovak Republic.* Taxon, 2013. **62**: p. 4-20.

16. Stuessy , T., *The importance of revisionary studies in plant systematics.* Sida, 1975. **6**: p. 104-113.
17. Goodwin, Z.A., D.J. Harris, D. Filer, J.R.I. Wood, and R.W. Scotland, *Widespread mistaken identity in tropical plant collections.* Current Biology, 2015. **25**: p. R1066-R1067.
18. Bebber, D.P., M.A. Carine, J.R.I. Wood, A.H. Wortley, D.J. Harris, G.T. Prance, G. Davidse, J. Paige, T.D. Pennington, and N.K.B. Robson, *Herbaria are a major frontier for species discovery.* PNAS, 2010. **107**: p. 22169-22171.
19. Thomas, W., *Conservation and monographic research on the flora of Tropical America.* Biodiversity & Conservation, 2004. **8**: p. 1007-1015.
20. Stuessy, T.F., *The role of creative monography in the biodiversity crisis.* Taxon, 1993. **42**: p. 313-321.
21. Hernández-Ledesma, P., W.G. Berendsohn, T. Borsch, S.V. Mering, H. Akhani, S. Arias, I. Castañeda-Noa, U. Eggli, R. Eriksson, H. Flores-Olvera, S. Fuentes-Bazán, G. Kadereit, C. Klak, N. Korotkova, R. Nyffeler, G. Ocampo, H. Ochoterena, B. Oxelman, R.K. Rabeler, A. Sanchez, B.O. Schlumpberger, and P. Uotila, *A taxonomic backbone for the global synthesis of species diversity in the Angiosperm order Caryophyllales.* Willdenowia, 2015. **45**: p. 281-383.
22. Thomas, W.W. and B.M. Thiers, *From manuscript to the public: Modes of monographic publication*, in *Monographic Plant Systematics: Fundamental Assessment of Plant Biodiversity*, T.F. Stuessy and H.W. Lack, Editors. 2011, ARG Gantner Verlag K. G.: Ruggell. p. 193-204.
23. Reginato, M., *monographaR: an R package to facilitate the production of plant taxonomic monographs.* Brittonia, 2016. **68**: p. 212-216.
24. Thomas, W.W., R.C. Forzza, F.A. Michelangeli, A.M. Giulietti, and P.M. Leitman, *Large-scale monographs and floras: The sum of local floristic research.* Plant Ecology & Diversity, 2012. **5**: p. 217-223.
25. Miller, S., L.N. Barrow, S.M. Ehlman, J.A. Goodheart, S. Greiman, H. Lutz, T. Misiewicz, S.M. Smith, M. Tan, C.J. Thawley, J. Cook, and J. Light, *Building natural history collections for the twenty-first century and beyond.* BioScience, 2020. **70**: p. 674-687.
26. Penn, M.G., S. Cafferty, and M.A. Carine, *Mapping the history of botanical collectors: Spatial patterns, diversity, and uniqueness through time.* Systematics and Biodiversity, 2018. **16**: p. 1-13.
27. Thiers, B.M., *The World's Herbaria 2016: A summary report based on data from Index Herbariorum*. 2017, William and Lynda Steere Herbarium, New York Botanical Garden Herbarium: New York.
28. Thiers, B.M., *The World's herbaria 2017: A summary report based on data from Index Herbariorum*. 2018, William and Lynda Steere Herbarium, The New York Botanical Garden: New York.
29. Thiers, B.M., *The World's herbaria 2018: A summary report based on data from Index Herbariorum*. 2019, William and Lynda Steere Herbarium, The New York Botanical Garden: New York.
30. Thiers, B.M., *The World's herbaria 2019: A summary report based on data from Index Herbariorum*. 2020, William and Lynda Steere Herbarium, The New York Botanical Garden: New York.
31. Thiers, B.M., *The World's herbaria 2020: A summary report based on data from Index Herbariorum*. 2021, William and Lynda Steere Herbarium, The New York Botanical Garden: New York.
32. Pearson, K.D., *Rapid enhancement of biodiversity occurrence records using unconventional specimen data.* Biodiversity and Conservation, 2018. **27**: p. 3007-3018.
33. Rogers, N., *Museum drawers go digital.* Science, 2016: p. 762-765.
34. Scotland, R.W. and J.R.I. Wood, *Accelerating the pace of taxonomy.* Trends in Ecology & Evolution, 2012. **27**: p. 415-416.

35. Riedel, A., K. Sagata, Y.R. Suhardjono, R. Tänzler, and M. Balke, *Integrative taxonomy on the fast track-towards more sustainability in biodiversity research.* Frontiers in Zoology, 2013. **10**: p. 15-23.
36. Butcher, B.A., M.A. Smith, M.J. Sharkey, and D.L.J. Quicke, *A turbo-taxonomic study of Thai Aleiodes (Aleiodes) and Aleiodes (Arcaleiodes)(Hymenoptera: Braconidae: Rogadinae) based largely on COI barcoded specimens, with rapid descriptions of 179 new species.* Zootaxa, 2012. **3457**: p. 1-232.
37. Fernandez-Triana, J., *Braconidae (Hymenoptera) parasitoid wasps as a model group for turbo taxonomy approaches: Lessons from the past and recommendations for the future.* ARPHA Preprints, 2021. **1**: p. 1-20.
38. Summers, M.M., I.I. Al-Hakim, and G.W. Rouse, *Turbo-taxonomy: 21 new species of Myzostomida (Annelida).* Zootaxa, 2014. **3873**: p. 301–344.
39. Lücking, R., M. Dal Forno, B. Moncada, L.F. Coca, L.Y. Vargas-Mendoza, A. Aptroot, L.J. Arias, B. Besal, F. Bungartz, and D.M. Cabrera-Amaya, *Turbo-taxonomy to assemble a megadiverse lichen genus: Seventy new species of Cora (Basidiomycota: Agaricales: Hygrophoraceae), honouring David Leslie Hawksworth's seventieth birthday.* Fungal Diversity, 2017. **84**: p. 139-207.
40. Wood, J.R., B.R. Williams, T.C. Mitchell, M.A. Carine, D.J. Harris, and R.W. Scotland, *A foundation monograph of Convolvulus L. (Convolvulaceae).* PhytoKeys, 2015. **51**: p. 1-282.
41. Wood, J.R.I., P. Muñoz-Rodríguez, B.R.M. Williams, and R.W. Scotland, *A foundation monograph of Ipomoea (Convolvulaceae) in the New World.* PhytoKeys, 2020. **143**: p. 1-823.
42. Frodin, D.G., *History and concepts of big plant genera.* Taxon, 2004. **53**: p. 753-776.
43. Stevens, P.F. *Angiosperm Phylogeny Website, version 14.* 2001 onwards [cited 2021 9th June]; Available from: http://www.mobot.org/MOBOT/Research/APweb/.
44. Brummitt, R.K., *Vascular plant families and genera*. 1992, Kew: Royal Botanic Gardens Kew.
45. List, T.P. *The Plant List v 1.1.* 2013 [cited 2017 28th December]; Available from: http://www.theplantlist.org/.
46. Parnell, J.A.N., L.A. Craven, and E. Biffin, *Matters of scale: Dealing with one of the largest genera of angiosperms*, in *Reconstructing the Tree of Life: Taxonomy and Systematics of Species Rich Taxa*, T.R. Hodkinson and J.A.N. Parnell, Editors. 2007, CRC Press: Boca Raton. p. 251-273
47. Linnaeus, C., *Species Plantarum*. Vol. 1. 1753, Holmiae: Laurentii Salvii.
48. de Jussieu, A.L., *Genera Plantarum Secundum Ordines Naturales Disposita*. 1789, Parisiis: Apud Viduam Herrisant.
49. Linnaeus, C., *Hortus Cliffortianus*. 1737: Amstelaedami.
50. Linnaeus, C., *Hortus Upsaliensis*. 1748, Stockholmiae: Laurentii Salvii.
51. Grisebach, A.H.R., *Convolvulaceae*. Flora of the British West Indian Islands. 1864, London: Lovell, Reeve & Co.
52. House, H.D., *The North American species of the genus Ipomoea.* Annals of the New York Academy of Sciences., 1908. **18**: p. 181-263.
53. Wilkin, P., *A revision of the Infrageneric classification of the genus Ipomoea L. and the related genera in the tribe Ipomeeae (Convolvulaceae)*. 1993, University of Reading.
54. de Dalla Torre, C.G. and H. Harms, *Genera Siphonogamarum ad Systema Englerianum Conscripta*. 1900, Lipsiae: Sumptibus Guilelmi Engelmann.
55. Willis, J.C., *Dictionary of the Flowering Plants and Ferns*. 4th ed. 1919, Cambridge: Cambridge University Press.
56. Mabberley, D.J., *The Plant-Book: A Portable Dictionary of the Vascular Plants*. 1997, Cambridge: Cambridge University Press.
57. Miller, R.E., M.D. Rausher, and P.S. Manos, *Phylogenetic systematics of Ipomoea (Convolvulaceae) based on ITS and waxy sequences.* Systematic Botany, 1999. **24**: p. 209-227.

58. Wood, J.R.I., R.D. de Arrúa, G.D. de Rojas, and R.W. Scotland, *Two overlooked species of Ipomoea L. (Convolvulaceae) from Paraguay.* Kew Bulletin, 2016. **71**: p. 1-6.
59. Wood, J.R.I., C. Urbanetz, and R.W. Scotland, *Ipomoea pantanalensis, a new species of Ipomoea L. (Convolvulaceae) from the Pantanal, Brazil.* Kew Bulletin, 2016. **71**: p. 1-3.
60. Wood, J.R.I., P. Muñoz-Rodríguez, R. Degen, and R.W. Scotland, *New species of Ipomoea (Convolvulaceae) from South America.* PhytoKeys, 2017. **88**: p. 1-38.
61. Wood, J.R.I. and R.W. Scotland, *Misapplied names, synonyms and new species of Ipomoea (Convolvulaceae) from South America.* Kew Bulletin, 2017. **72**: p. 1-26.
62. Wood, J.R.I. and R.W. Scotland, *Notes on Ipomoea (Convolvulaceae) from the Amazonian periphery.* Kew Bulletin, 2017. **72**: p. 1-18.
63. Wood, J.R.I., L.V. Vasconcelos, R. Simão-Bianchini, and R.W. Scotland, *New species of Ipomoea (Convolvulaceae) from Bahia.* Kew Bulletin, 2017. **72**: p. 1-20.
64. Matuda, E., *El género Ipomoea en México.* Anales del Instituto de Biologia 1965. **34**: p. 85-145.
65. Van Ooststroom, S.J., *The Convolvulaceae of Malaysia IV (with Indices).* Blumea, 1943. **5**: p. 339-411.
66. van Ooststroom, S.J. and R.D. Hoogland, *Convolvulaceae*, in *Flora Malesiana*, C.G.G.J. van Steenis, Editor. 1953, Noordhoff-Kolff N.V.: Djakarta. p. 389-512.
67. Backer, C.A. and R.C. Bakhuizen van den Brink Jr., *Flora of Java (Spermatophytes only)*. Vol. 2. 1965, Groningen: Noordhoff.
68. Verdcourt, B., *Convolvulaceae*, in *Flora of Tropical East Africa*, C.E. Hubbard and E. Milne-Redhead, Editors. 1963, Crown Agents for Oversea Governments & Administration: London. p. 1-161.
69. Meeuse, A.D.J., *The South African Convolvulaceae.* Bothalia, 1957. **6**: p. 643-792.
70. Meeuse, A.D.J. and W.G. Welman. Flora of Southern Africa: Convolvulaceae. Vol. 28. 2000, Pretoria: National Botanical Institute.
71. Johnson, R.W., *Four new species of Ipomoea L. (Convolvulaceae) from Australia.* Austrobaileya, 1986. **2**: p. 217-223.
72. Johnson, R.W., *New species and subspecies of Ipomoea L. (Convolvulaceae) from Northern Australia and a key to the Australian species.* Austrobaileya, 2012. **8**: p. 699-723.
73. Lejoly, J. and S. Lisowski, *The genera Merremia and Ipomoea (Convolvulaceae) in the flora of Central Africa (Zaire, Rwanda, Burundi).* Fragmenta Floristica et Geobotanica, 1992. **37**: p. 21-125.
74. Thulin, M., *Notes on Convolvulus, Astripomoea, Ipomoea and Merremia (Convolvulaceae) from the horn of Africa.* Nordic Journal of Botany, 2003. **23**: p. 629-640.
75. McDonald, J.A., *Revision of Ipomoea series Tyrianthinae (Convolvulaceae).* Lundellia, 2001. **4**: p. 76-93.
76. McDonald, A., *Revision of Ipomoea section Exogonium (Choisy) Griseb (Convolvulaceae).* Brenesia 1987. **28**: p. 41-87.
77. McDonald, J.A., *Revision of Ipomoea section Leptocallis (Convolvulaceae).* Harvard Papers in Botany, 1995. **1**: p. 97-122.
78. McPherson, G., *Studies in Ipomoea (Convolvulaceae) I: The Arborescens group.* Annals of the Missouri Botanical Garden, 1981. **68**: p. 527-545.
79. Manos, P.S., R.E. Miller, and P. Wilkin, *Phylogenetic analysis of Ipomoea, Argyreia, Stictocardia, and Turbina suggests a generalized model of morphological evolution in morning glories.* Systematic Botany, 2001. **26**: p. 585-602.
80. Eserman, L.A., G.P. Tiley, R.L. Jarret, J.H. Leebens-Mack, and R.E. Miller, *Phylogenetics and diversification of morning glories (tribe Ipomoeeae, Convolvulaceae) based on whole plastome sequences.* American Journal of Botany, 2014. **101**: p. 92-103.
81. Stefanović, S., D.F. Austin, and R.G. Olmstead, *Classification of Convolvulaceae: A phylogenetic approach.* Systematic Botany, 2003. **28**: p. 791-806.

82. Muñoz-Rodríguez, P., T. Carruthers, J.R.I. Wood, B.R.M. Williams, K. Weitemier, B. Kronmiller, Z.A. Goodwin, A. Sumadijaya, D. Harris, D. Filler, M.A. Rausher, S. Kelly, A. Liston, and R.W. Scotland, *A taxonomic monograph of Ipomoea integrated across phylogenetic scales.* Nature Plants, 2019. **5**: p. 1136-1144.
83. Austin, D.F. and G.W. Staples, *Further notes on Turbina and Merremia: Typification and taxonomy of the Neotropical Convolvulaceae.* Journal of the Arnold Arboretum, 1986. **67**: p. 263-264.
84. Austin, D.F. and G.W. Staples, *A revision of the Neotropical species of Turbina Raf. (Convolvulaceae).* Bulletin of the Torrey Botanical Club, 1991. **118**: p. 265-280.
85. Blume, C.L., *Bijdragen tot de flora van Nederlandsch-Indië*. Vol. 13. 1825, Batavia: Ter Lands Drukkerij.
86. Staples, G.W., *A synopsis of Lepistemon (Convolvulaceae) in Australasia.* Kew Bulletin, 2007. **62**: p. 223-232.
87. Staples, G.W. and P. Traiperm, *A nomenclatural review of Argyreia (Convolvulaceae).* Taxon, 2017. **66**: p. 445-477.
88. Austin, D.F. and S. Demissew, *Unique fruits and generic status of Stictocardia (Convolvulaceae).* Kew Bulletin, 1997. **52**: p. 161-169.
89. Austin, D.F. and E. Eich, *Synopsis of Stictocardia with another Madagascan species, S. mojangensis (Convolvulaceae).* Willdenowia, 2001. **31**: p. 79-85.
90. Gunn, C.R., *Notes on Stictocardia campanulata (L.) Merrill and S. jucunda (Thw.) CR Gunn (Convolvulaceae).* Brittonia, 1972. **24**: p. 169-176.
91. Verdcourt, B., *A new subgenus of Stictocardia (Convolvulaceae).* Kew Bulletin, 1990. **45**: p. 583-585.
92. Garcia, M.A. and S. Demissew, *Convolvulaceae*, in *Flora of Somalia*, M. Thulin, Editor. 2006, Royal Botanic Gardens, Kew,: Kew. p. 221-258.
93. POWO. *Plants of the World Online.* 2022 [cited 2022 3rd January]; Available from: http://www.plantsoftheworldonline.org/.
94. Grace, O.M., O. Pérez-Escobar, A., E.J. Lucas, M.S. Vorontsova, G.P. Lewis, B.E. Walker, L.G. Lohmann, S. Knapp, P. Wilkie, and T. Sarkinen, *Botanical monography in the Anthropocene.* Trends in Plant Science, 2021. **26**: p. 433-441.
95. Stuessy, T.F., *Importance of the botanical monograph*, in *Monographic Plant Systematics: Fundamental Assessment of Plant Biodiversity*, T.F. Stuessy and H.W. Lack, Editors. 2011, ARG Gantner Verlag K. G.: Ruggell. p. 7-14.

CHAPTER 2. TAXONOMY AND MORPHOLOGY OF *STICTOCARDIA*

2.1. BACKGROUND

2.1.1. Taxonomic history of *Stictocardia* in the context of *Ipomoea*

In this chapter, the taxonomic and morphological history of *Stictocardia* is provided. *Stictocardia* was described by Hallier f. in 1893 [1] after recognising three species with a specific set of characters in Convolvulaceae as explained in the subchapter below [2]. Since then, the number of species assigned to *Stictocardia* has slowly increased, by studies covering areas such as Africa [1, 3-7], Madagascar [8], Malesia [9, 10], Sri Lanka [11], India [12], and Australia [13]. Taxonomic estimates of the number of species of *Stictocardia* range from 6 to 12 [7, 8, 10, 12-16], plus several infraspecific taxa [7, 17]. Throughout history, four botanists had made substantial contributions to the taxonomy of *Stictocardia.* Their substantial works (year under parentheses) on *Stictocardia* that covered species description, important morphological characters, discussion of taxa delimitation, identification key, and distribution range are summarised below.

2.1.1.1. Hans (Johannes) Gottfried Hallier f. (1894-1911)

According to Wilkin [18], Hallier had a comprehensive knowledge of Convolvulaceae based on a published taxonomic treatment of this family in Africa [1] and his preliminary account for the monograph of Convolvulaceae [3]. His broad surveys of the morphological and anatomical characters of many genera of Convolvulaceae led him to recognise a group characterised by spiny pollen (subfamily Echinoconieae) [19]. He also split Echinoconieae into two groups, *ie.* Argyreieae and Ipomoeeae based on the state of the mature fruit [20], indehiscence and dehiscence, respectively. In Argyreieae, Hallier recognised several species - *Rivea tiliaefolia*

(Desr.) Choisy*, R. tiliaefolia* var*. multiflora* (Klotzch manuscripts in B) and *Ipomoea beraviensis* Vatke – that possessed a set of distinctive characters, *i.e.* black glands on the lower surface of leaf, and sepals accrescent in fruit [2]. He therefore transferred these three taxa to a newly described genus, *Stictocardia,* and treated them as three separate species. Etymologically, *Stictocardia* refers to the occurrence of distinctive dot-like black glands (*stictos*) on the lower surface of the cordate-shaped (*cardios*) leaf [1, 11].

Hallier recognised nine species of *Stictocardia*: *S. tiliifolia* (Desr.) Hallier f.; *S. beraviensis* (Vatke) Hallier f.; *S. multiflora* Hallier f.; *S. laxiflora* (Baker) Hallier f.; *S. pringsheimiana* (Dammer) Hallier f.; *S. nervosa* Hallier f.; *S. pulchra* (Bl.) Hallier f.; *S. incomta* (Hallier f.) Hallier f.*,* (later was corrected by Verdcourt into *S. incompta*); and *S. woodii* (N.E.Br.) Hallier f. [1, 3]. He transfered eight out of nine species above were from other genera; *Rivea* [1], *Ipomoea* [1, 3, 21], or *Argyreia* [3].

The first two decades after the publication of *Stictocardia*, Hallier focused his effort on determining the species that belonged to *Stictocardia*. He did not, however, elaborate on how to distinguish between species. Therefore, delimitations between species remained unclear for decades after the publication of the genus due to the absence of identification key.

2.1.1.2. Simon Jan van Ooststroom (1943-1953)

Van Ooststroom spent time and focussed on Convolvulaceae in Malesia [9, 10, 22], a floristic region from the south of the Isthmus of Kra in Asia to Papua New Guinea and Bismarck Archipelago [23]. However, he referred to the region as Malaysia, which is derived from Malay, the widespread trading language in this area [23]. This is not to be confused with the present-day country of Malaysia which is included in this region.

Here, he recorded the presence of three new species of *Stictocardia* - *S. cordatosepala* Ooststr., *S. discolor* Ooststr., and *S. neglecta* Ooststr – in addition to the widespread *S. tiliifolia*. He assembled the first identification key of *Stictocardia* – despite merely restricted to Malesia [9]. He treated *S. pulchra* as a synonym of *S. discolor* [9, 10], see Chapter Four for further detail.

2.1.1.3. Bernard Verdcourt (1958-1990)

After Hallier, Verdcourt was the botanist with the most comprehensive knowledge of *Stictocardia* in Africa. He maintained a scientific correspondence with van Ooststroom who worked in Malesia [6] and was likely to have been able to access Southeast Asian material deposited at the Leiden herbarium (L) back then. Verdcourt published six taxonomic accounts of African *Stictocardia*, discussed below, including the transfers of two species of *Ipomoea* - *I. lutambensis* Schulze-Menz and *I. macalusoi* Mattei - into *Stictocardia* [4, 6].

Having observed more specimens of *Stictocardia* than two previous experts, Verdcourt evaluated the status of several species that Hallier previously delimited. He sank three species - *S. pringsheimiana*, *S. laxiflora,* and *S. woodii* - as synonyms of *S. beraviensis* [24]. Later, he treated *S. laxiflora* as a distinct species and considered *S. woodii* a variant of this species [24]. He also stated that the status of *S. multiflora* is unknown as the type specimen was likely to have been destroyed in Berlin herbarium (B) in the World War II [6, 25]. Given his broad knowledge of *Stictocardia*, Verdcourt later assembled an identification key for this genus in the *Flora of Tropical East Africa* [7].

In the early 1990s Verdcourt also published the first infra-generic classification of *Stictocardia* that splits this genus into two subgenera. He placed *S. incompta*, a

morphologically distinct species with velvety hair covering the entire surface of the plant and deltoid calyx with parallel vertical ridges, in the subgenus *Madoadoa,* and the remaining species in the subgenus *Stictocardia* [26].

2.1.1.4. Daniel F. Austin (1975-2001)

Austin clarified a taxonomic issue between two problematic species, *S. campanulata* Merr. and *S. tiliifolia*, and treated the former as a species of *Ipomoea* [27]. Austin and his coworkers later identified a new, unique character of *Stictocardia*, *ie,* valved endocarp, amended the generic circumscription [16] and transferred *I. mojangensis* Vatke into *Stictocardia* [8] solely based on the presence of this character. Their work generated the first global distribution maps and identification key of *Stictocardia* [8].

2.1.1.5. Other taxonomic literature (1914-2017)

Five taxonomic works published by a number of botanists, individually or with co-workers, have contributed to the knowledge accumulation of *Stictocardia*. Merrill [28] published the first record of *Stictocardia* (*S. campanulate* Merr.) in the Pacific from Guam, whilst Johnson transferred one species from *Argyreia* in Australia [13]. In South Asia, two endemic species were recorded. *Stictocardia jucunda* (Thwaites) Gunn was transferred from *I. jucunda* Thwaites in Sri Lanka [11], whereas *S. sivarajanii* Biju, Push. & P. Mathew was discovered in India [12].

Wilkin was the first botanist working on Ipomoeeae taxonomy to recognise *Stictocardia* as a section of *Ipomoea* [18]. He placed *Stictocardia* as one of four sections in the subgenus *Florifera* and also split *Stictocardia* into three subsections: *Madoadoa* (*S. incompta*), *Stictocardia* (*S. tiliifolia*), and *Pilosisepalae* (*S. beraviensis, S. laxiflora, S. macalusoi, S. neglecta, S. discolor,* and *S. cordatosepala*) [18]. He did

not include *S. jucunda,* likely because the characters of this species deviate from the characteristics of *Stictocardia sensu* Hallier [1]*, i.e.,* dehiscent fruit, ellipsoid capsule, and villous, elongated seed.

2.1.2. Taxonomic issues in *Stictocardia*

The accumulation of taxonomic studies on *Stictocardia* by different generations of specialists who have worked in different regions of the world has resulted in two main taxonomic issues, inconsistent generic delimitation and unclear species delimitation, as discussed below.

2.1.2.1. Inconsistent generic delimitation

The generic circumscription of *Stictocardia* has changed over time [7, 10, 29] due to the accumulation of specimens and species. It has been expanded from the initial generic concept of Hallier's [1] by the addition of previously unobserved character to the generic diagnosis, *i.e.* valved endocarp [8, 16].

Austin and Sebsebe [16] stated that the presence of a valved endocarp diagnoses *Stictocardia.* They further dismissed a combination of diagnostic characters such as sepals accrescent in fruit, indehiscent fruit, and black glands on the surface of cordate shaped leaves, because individually these characters also appear in other species of *Ipomoea* [16]. The valved endocarp, on the other hand, is a derived character and considered autapomorphic of *Stictocardia* [30]. The absence of this character in Hallier's original description is noteworthy. This evaluation, however, lacked complete taxon sampling since it was based merely on six specimens of five species (*I. tiliifolia, I. beraviensis, I. macalusoi, I. incompta,* and *I. lutambensis*) distributed in Africa, Indonesia, and Maui but did not include species from other areas such as continental Asia and America.

2.1.2.2. Unclear species delimitation

The absence of taxonomic synthesis of *Stictocardia* implies that the boundaries between species have not been evaluated critically. Thus the species circumscriptions are potentially problematic. For example, the morphological delimitation of *S. tiliifolia*, a globally widespread species, is potentially overlap with a number of regional and endemic species within its range. In addition, several identification keys for Africa [7], Southeast Asia [9, 10], Australia [13], and India [12], merely focus on species that occur within their areas, thus lacking a comparative morphological assesment of all species. The most recent identification key covers the entire distribution [8] but relies on a limited number of specimens per species.

2.2. MATERIALS AND METHODS

2.2.1. Literature study

This study began by investigating all accessible protologues and literature of *Stictocardia* (Table 2.1) that describe taxa from Africa [1, 3, 4, 6, 7, 26, 31-33], Madagascar [8], Malesia [9, 34], Australia [13], India [12] Sri Lanka [11], and the Pacific [17, 28]. Accessible floras in Africa, the surrounding areas [35-39], and Asia [10, 40-42] were also consulted to include morphological variation of all species and obtain data on their geographical distribution. Other published studies, such as identification keys [8], taxonomic notes [16, 27], revisions and checklists [15, 43-46], were also consulted.

2.2.2. Herbarium specimens

This study used 705 specimens from 35 herbaria. Three hundred and eleven herbarium specimens were loaned from 13 herbaria, *i.e.* BM, BR, BRI, E, FHO, G, K, Naturalis (L and WAG), LISC, MO, NH, P, and US. Additionally, remaining 311 specimens were in digital fomat and downloaded from 26 herbaria (AK, B, BISH, BM, BRIP, BRIT, CANB, CNS, E, FTG, GH, HBG, IBK, IBSC, K, Naturalis, M, MO, NY, NBG, NZFRI, P, QBG, S, TAI, and US) that provide accessible online portals. The remaining herbaria (BO, CNARP, ETH, KEP, NH, QBG, and UZL), however, are not equipped with online search portals. These herbaria sent images of the relevant specimens following email correspondence. Codes of the herbaria follow the acronyms from *Index Herbariorum* [47] (Table 2.2). All specimens and images of individual species are listed in Appendix 1.

2.2.3. Examination of specimens

This morphological study aims to evaluate diagnostic characters and record variation within and between the species under study. Observations were made of all accessible specimens using both herbarium sheets and digital images. In this preliminary assessment, the names on determination slips were ignored regardless of the latest identification (Table 2.1) and specimens were grouped into putative species based on the variation of characters.

Recognition of putative species was not always straightforward. Some species were easily recognised due to the possession of diagnostic qualitative characters and were therefore unambiguously treated as distinct species. Qualitative characters states are defined in Appendix 2 following Stearn [48] and Eckel [49]. The remaining indistinct

Table 2.1. List of species and subspecies of *Stictocardia* with the protolog and the correct name arranged by the year of publication,

Species/ Subspecies	Author(s)	Year	Protolog and page	Correct name
Stictocardia multiflora	Hallier f.	1894	*Botanische Jahrbücher für Systematik* 18 159—160	Unresolved*
Stictocardia beraviensis	(Vatke) Hallier f.	1894	*Botanische Jahrbücher für Systematik* 18 159--160	*Ipomoea beraviensis* Vatke
Stictocardia tiliifolia	(Desr.) Hallier f.	1894	*Botanische Jahrbücher für Systematik* 18 159--160	*Ipomoea tiliifolia* (Desr.) Roem. & Schult.
Stictocardia pulchra	(Bl.) Hallier f.	1897	*Bulletin de l'Herbier Boissier* 5: 380	*Ipomoea orientalis* J.R.I.Wood & Scotland
Stictocardia laxiflora	(Baker) Hallier f.	1898	*Bulletin de l'Herbier Boissier* 6: 548	*Ipomoea buchananii* Baker*
Stictocardia woodii	(N.E.Br.) Hallier f.	1898	*Bulletin de l'Herbier Boissier* 6: 548.	*Ipomoea buchananii var. woodII* (N.E.Br.) J.R.I. Wood & Scotland
Stictocardia pringsheimiana	(Dammer) Hallier f.	1898	*Bulletin de l'Herbier Boissier* 6: 548.	*Ipomoea buchananii* Baker*
Stictocardia nervosa	Hallier f.	1898	*Bulletin de l'Herbier Boissier* 6: 548.	Unresolved*
Stictocardia incomta	(Hallier f.) Hallier f.	1911	*Mededelingen Herbarium Leiden* 26	*Ipomoea incompta* Hallier f.
Stictocardia campanulata	Merr.	1914	*Philippine Journal of Science* 6: 133-134	*Ipomoea tiliifolia* (Desr.) Roem. & Schult.
Stictocardia cordatosepala	Ooststr.	1943	*Blumea* 5: 348—352	*Ipomoea lombokii* J.R.I.Wood & Scotland

Stictocardia discolor	Ooststr.	1943	*Blumea* 5: 348—352	*Ipomoea orientalis* J.R.I.Wood & Scotland
Stictocardia neglecta	Ooststr.	1943	*Blumea* 5: 348—352	*Ipomoea neglecta* (Ooststr.) J.R.I.Wood & Scotland
Stictocardia lutambensis	(Schulze-Menz) Verdc.	1958	*Kew Bulletin* 16: 188	*Ipomoea lutambensis* Schulze-Menz
Stictocardia macalusoi	(Mattei) Verdc.	1961	*Kew Bulletin* 15: 6	*Ipomoea macalusoi* Mattei
Stictocardia laxiflora var. woodii	Verdc.	1963	*Kew Bulletin* 27: 173	*Ipomoea buchananii var. woodii* (N.E.Br.) J.R.I. Wood & Scotland
Stictocardia jucunda	(Thwaites) Gunn	1972	*Brittonia* 24 (2): 169—176	*Ipomoea jucunda* Thwaites
Stictocardia sivarajanii	Biju, Push. & P. Mathew	1999	*Novon* 9 (2): 147-149.	*Ipomoea sivarajanii* (Biju, Pushp. & P.Mathew) J.R.I.Wood & Scotland
Stictocardia mojangensis	(Vatke) Austin & Eich	2001	*Willdenowia* 31(1): 80	*Ipomoea mojangensis* Vatke
Stictocardia queenslandica	R.W. Johnson	2004	*Austrobaileya* 6 (4): 631—635	*Ipomoea queenslandica* (Domin) J.R.I.Wood & Scotland
Stictocardia tiliifolia subsp. marquesensis	Staples & Butaud	2016	*Phytologia* 98 (3): 203-206.	*Ipomoea tiliifolia subsp. marquesensis* (Staples & Butaud) J.R.I.Wood & Scotland

Correct names from Muñoz-Rodríguez [45] represent the species delimitation with several notes marked by asterisks (*). *Stictocardia multiflora* is unresolved as the type specimen was destroyed in B [24]. *Stictocardia nervosa* is a *nomen nudum* [3]. *Ipomoea buchananii* Baker is a later homonym of *I. buchananii* Choisy [50].

Table 2.2. Herbarium codes in alphabetical order and the host Institution

Herbarium code	Institution
AK	Auckland War Memorial Museum
B	Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin
BISH	Department of Natural Sciences, Bishop Museum
BO	Research Centre for Biology, Indonesian Institute of Sciences
BM	The Natural History Museum, London
BR	Meise Botanic Garden
BRIP	Department of Agriculture and Fisheries of Queensland
BRIT	Botanical Research Institute of Texas
CANB	Centre for Australian National Biodiversity Research, Australian National Herbarium
CNARP	Centre National d' Application de Recherches Pharmaceutiques of Madagascar
CNS	Australian Tropical Herbarium, Queensland
E	Royal Botanic Garden Edinburgh
ETH	Department of Plant Biology and Biodiversity, Addis Ababa University
FHO	Daubeny Herbarium, University of Oxford
FTG	Fairchild Tropical Botanic Garden
G	Conservatoire et Jardin botaniques de la Ville de Genève
GH	Harvard University
HBG	Department of Biology, University of Hamburg
IBK	Guangxi Institute of Botany
IBSC	The Chinese Academy of Sciences, South China Botanical Garden
K	Royal Botanic Gardens Kew
KEP	Forest Research Institute Malaysia
L, WAG, and U	Nationaal Herbarium Nederland, Naturalis
LISC	Instituto de Investigação Científica Tropical
M	Staatliche Naturwissenschaftliche Sammlungen Bayerns (SNSB)
MO	Missouri Botanical Garden
NH	South African National Biodiversity Institute
NY	The New York Botanical Garden
NZFRI	New Zealand Forest Research Institute Limited (Scion)
P	Muséum National d'Histoire Naturelle
QBG	Queen Sirikit Botanic Garden, The Botanical Garden Organization
S	Swedish Museum of Natural History
TAI	College of Life Science, National Taiwan University

US	United States National Herbarium, Smithsonian Institution
UZL	Biological Sciences Department, University of Zambia

species were problematic without obvious diagnostic characters and were the focus of further quantitative morphological assessment. A score sheet was developed to record variation of the morphological characters of individual specimens of the indistinct species as shown in Table 2.3. Characters listed in the score sheet were a combination of characters previously considered important for species delimitation in *Ipomoea* [7, 8] and my own observations.

2.2.4. Measured quantitative morphological characters of indistinct species

Observations were carried out to study the morphological variation of the indistinct species. Definitions of these morphological characters follow Harris and Harris [51]. The measurements were taken from the apex to the base of a given organ using a ruler with resolution to the millimetre unit. The measured organs are illustrated in Figure 2.1 and described as follows. Primary peduncle length: The length of the elongated structure from the stem at the base of the inflorescence to the first node of the inflorescence. Pedicel length: The length of the elongated structure from the uppermost node to the base of the flower (calyx). Sepal length: The length of the structure of outer sepal from to the base to the apex. Corolla length: The length of the corolla ideally during anthesis, not in bud or when developing, from the tip of the calyx to the tip of corolla. Lateral vein: The number of major lateral veins extending from the costa, counted from the first costa at the leaf base to the last one near the leaf tip. The number of flowers in the inflorescence. Secondary peduncle length: The length of the elongated structure from the first node of the inflorescence to the next node (usually the base of pedicel).

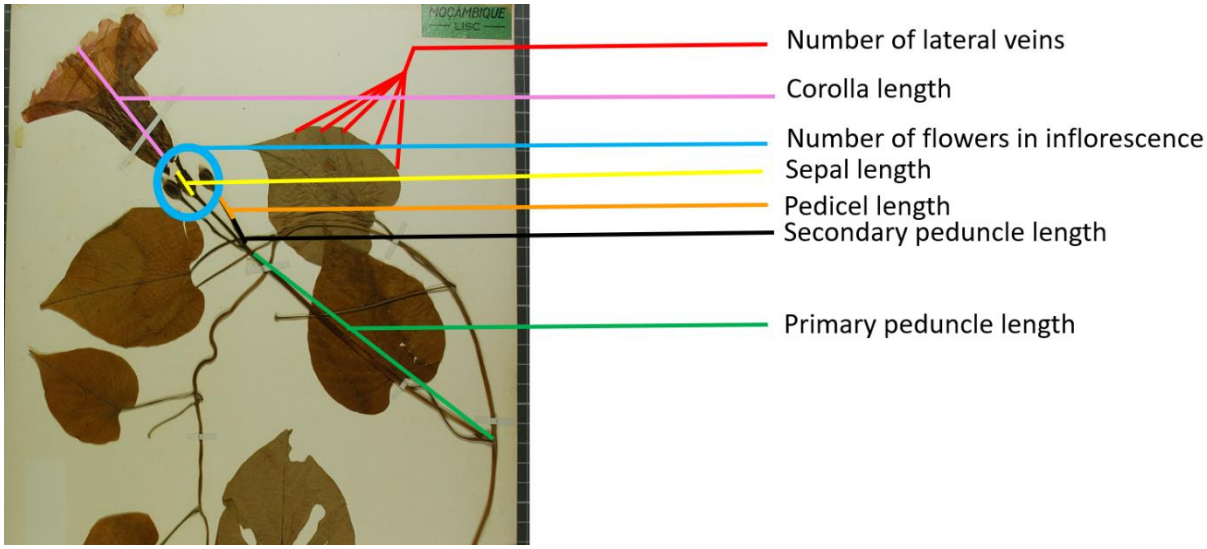


Figure 2.1 Diagram to illustrate measurements taken.

Observations of the macroscopic and microscopic characters (surface of organs) used a binocular microscope (Nikon SMZ 1000) with 8-80× magnification. A conventional ruler and digital callipers (TESA RS 374-092) were used for measuring quantitative characters such as length and width of organs. Measurements of the digital specimens were conducted using Image Processing and Analysis in Java (Image J) [52]. Fragmented and incomplete (sterile) specimens were excluded.

2.2.5. Principal Component Analysis (PCA)

The indistinct species were visualized using Principal Component Analysis (PCA), a multivariate analysis technique, to identify gaps in the characters variation. PCA reduces multivariate (multidimensional) data into uncorrelated multiple components that explain the variation [53]. This analysis is important to process and visualize the variation between specimens and detecting outliers and finding patterns [54]. PCA was executed in RStudio ver. 1. 4.1103 [55] using *princomp* function and visualised with ggfortify, a package from CRAN [56]. The whole process followed the script in Appendix 3. Seven quantitative morphological characters (Table 2.3, asterisks) were analysed. These characters were chosen as they were variable

between individual specimens and have been considered taxonomically informative in delimiting species boundaries in other studies [7, 8].

Table 2.3. Score sheet for character examination of individual specimens of indistinct species

Collector & Number Location	Character states
Stem surface	glabrous/puberulent/villous
Leaf width (cm)	
Leaf length (cm)	
Leaf apex	acute/acuminate/obtuse
Leaf adaxial surface	glabrous/puberulent/villous
Leaf abaxial surface	glabrous/puberulent/villous
***Lateral vein**	
***Number of Flower in inflorescence**	
***Primary peduncle length (mm)**	
***Secondary peduncle length (mm)**	
***Pedicel length (mm)**	
Outer sepal surface	glabrous/puberulent/villous
Outer sepal apex	acute/obtuse/rounded
***Sepal length (mm)**	
Corolla shape	funnelform/campanulate/salverform
***Corolla length (mm)**	
Corolla surface	glabrous/puberulent/villous
Corolla colour	

Asterisk are quantitative characters that were included in PCA.

2.2.6. Univariate Statistics

A univariate analysis was also conducted to test individual quantitative morphological characters independently. This approach uses the variation of mean values, which is reflected by Standard Error of Means (SEM) [57] across indistinct species, in this case, samples of individual taxa based on previously identified names. The SEM estimates how close the sample mean is to the population mean [58].

The overlapping SEM across the indistinct species would suggest a lack of discrete morphological boundaries between the putative species. In contrast, non-overlapping SEM would mark the occurrence of putatively indistinct species. Non-

overlapping SEM creates gaps between sets of indistinct species that allows the identification of more than a single taxon. The next step was to evaluate the number of taxonomic groups that could be recognised based on the SEM across all indistinct species based on individual quantitative characters. *Ipomoea sivarajanii* was excluded as it is only represented by a single specimen. A total of 89 specimens of the indistinct species were included in this step after excluding some of the non-georeferenced specimens (Appendix 4).

After generating a range of SEM across the indistinct species based on individual quantitative characters, a further step was taken to exclude data sets with too few samples. Two of the characters - pedicel length and the number of lateral veins on a leaf - were discarded as they had very few samples (<5) that was set arbitrarily here. Four of the quantitative characters were selected (primary peduncle length, the number of flowers in inflorescence, sepal length, and corolla length) as they have an adequate number of samples (>5) for a statistical test.

A non-parametric test was selected as it is a universal method and is recommended for dealing with a range of data, such as a normal distribution with a few outliers [59]. Mann-Whitney [60], one of the non-parametric tests, was selected over Kruskal Wallis [61] as each individual data set only has two groups to compare. The Mann-Whitney test was conducted in Microsoft ® Excel ® 2019 to evaluate groups of individual data sets whether they are significantly different to each other or not [62]. Species groups identified by this test were compared to the results of phylogenetic analysis (Chapter Three).

2.2.7. Reciprocal illumination between morphology and phylogenetics analysis

This study utilizes reciprocal illumination between morphology (Chapter Two) and phylogenetics (Chapter Three), as depicted in Figure 2.2, a modified workflow from Muñoz-Rodriguez et al. [45]. Reciprocal illumination is an interactive process of checking, correcting, and rechecking taxonomic hypothesis against new data [63] - or a synthesis - in this context between morphological and molecular analysis and results - as explained in Scotland and Wood [64].

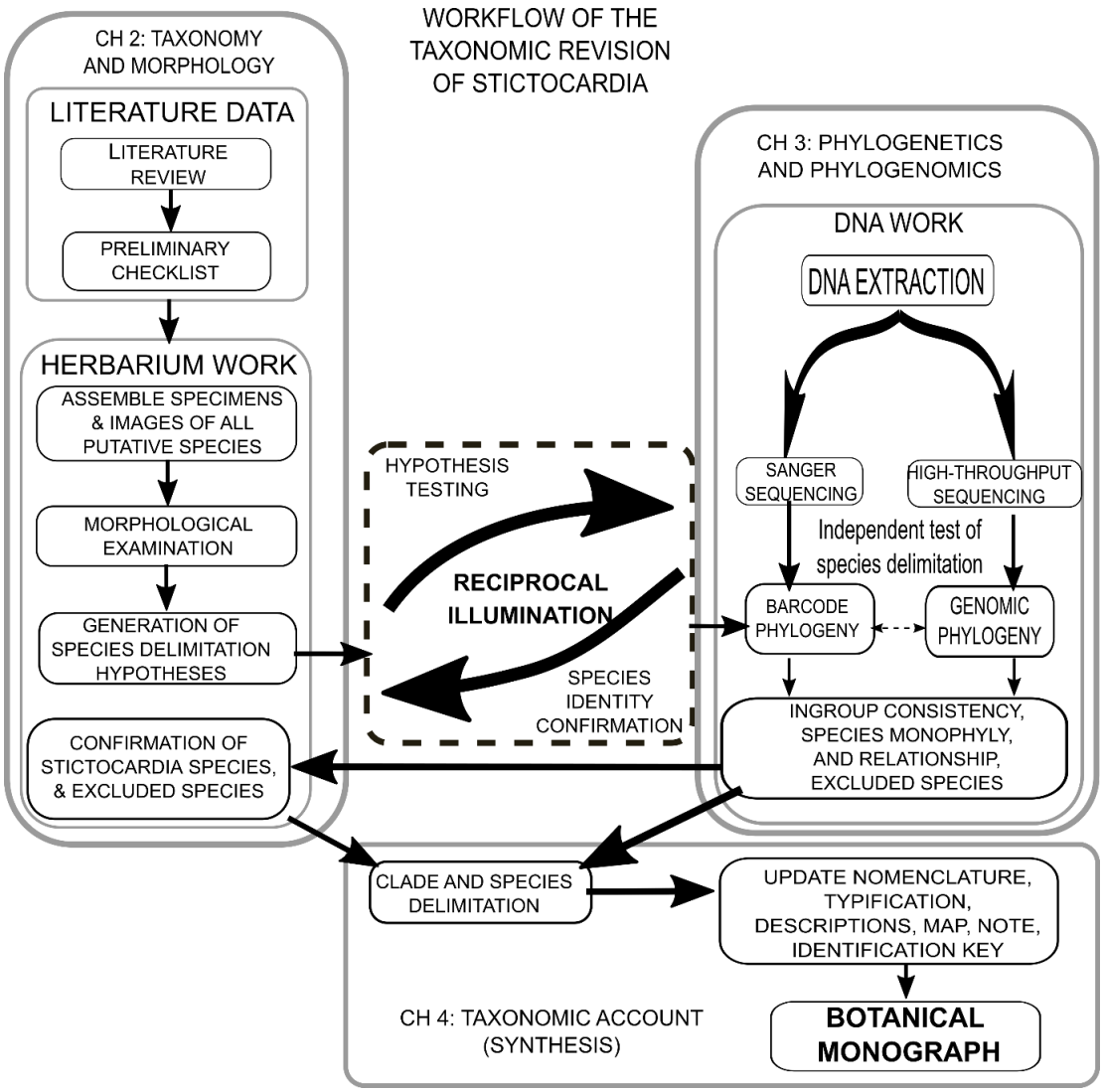


Figure 2.2. The workflow of the study that focusses on the taxonomic revision of Stictocardia within the OW *Ipomoea* based on corroboration of morphological and phylogenetic evidence. The study on morphology (left greybox/Chapter Two) and phylogenetics (right grey box/Chapter Three) - each comprises black boxes that represent various stages - are running in parallel and closely interacting to each other through reciprocal illumination (centre dashed line box) to synthesise a botanical monograph (bottom grey box/Chapter Four).

The morphological examination was running in parallel with the phylogenetic study of molecular sequence data. This involved using the phylogenetic hypotheses generated by the DNA sequence data of multiple specimens, if possible, of each species to test hypotheses of species delimitation based on morphology. During this period, the phylogenetic study demonstrated that *Stictocardia* is polyphyletic but identifies a clade of seven species, *Stictocardia sensu stricto* (*s.s.*), hereafter referred as Stictocardia (non-italic) (Table 2.4). Thereafter, the focus of this study concentrated on the delimitation of these seven species in this clade.

Table 2.4. The change of study group from *Stictocardia* (a polyphyletic taxon) into Stictocardia (a clade) following the result of phylogenetic study

Stictocardia (the polyphyletic taxon)	**Stictocardia** (the clade)
Ipomoea beraviensis	*Ipomoea beraviensis*
Ipomoea tiliifolia	*Ipomoea tiliifolia*
Ipomoea buchananii	*Ipomoea buchananii**
Ipomoea incompta	*Ipomoea incompta*
Ipomoea lutambensis	*Ipomoea lutambensis*
Ipomoea macalusoi	*Ipomoea macalusoi*
Ipomoea queenslandica	*Ipomoea queenslandica*
Ipomoea tiliifolia subsp*. marquesensis*	Synonym of *Ipomoea tiliifolia*
Ipomoea buchananii var*. woodii*	Synonym of *Ipomoea buchananii**
Ipomoea lombokii	Require more data
Ipomoea orientalis	Excluded
Ipomoea neglecta	Require more data
Ipomoea jucunda	Excluded
Ipomoea sivarajanii	Excluded
Ipomoea mojangensis	Excluded

**Ipomoea buchananii* is a synonym of *I. pringsheimiana*, see Chapter Four. Two subspecies*, I. tiliifolia* subsp*. marquesensis* and *Ipomoea buchananii* var*. woodii* are treated as synonym of their respective species due to their morphological similarity and geographical range.

The morphology of *I. beraviensis*, one of the species within the clade Stictocardia, have been re-evaluated. Samples of this species are recovered in two independent, non-closely related clades in phylogenetic analysis of molecular sequence data in Chapter Three. Specimens collected in East Africa form a clade independent of specimens collected in West Africa and Madagascar.

Unfortunately, these putative groups of *I. beraviensis* suffer very minimum, if not poor sampling. Therefore, box plots are the best option to explore the individual morphological characters because the small sampling size is not sufficient for a statistical analysis as elaborated later.

2.2.8. Examination of the taxonomic value of black glands

The presence of black glands on leaves was the basis for the name *Stictocardia*. The density of black glands, among other features, was suggested to be significant for separating a limited number of species according to Olaranont et al. [65]. Therefore, I conducted an assessment to test the taxonomic value of this feature for species of the clade Stictocardia. The observations were carried out using a binocular microscope (Nikon SMZ 1000) under the maximum magnification (80×). The total number of black glands within an observation field of the microscope were counted from three different relatively intact leaves of a given specimen. The number of black glands of an individual specimen is the average of multiple counts across random parts of the abaxial leaf surface. Subsequently, the lowest and highest average counts were gathered to obtain the range of the number of black glands of each species.

2.3. RESULTS

2.3.1. Distinct vs indistinct species of *Stictocardia*

From thirteen species of *Stictocardia*, six species are distinct and easily recognised based on their morphological features. The whole plant of *I. incompta* except the corolla is covered with villous white hairs. *Ipomoea jucunda* possesses salverform corolla, dehiscent fruit, ellipsoid capsule, and elongated seeds with silky hairs. *Ipomoea mojangensis* has a very distinctive arrangement of the non-accrescent sepals. *Ipomoea lutambensis* possesses thin, membranous leaves and partially exposed exocarp on mature fruit. Two species - *I. macalusoi* and *I. beraviensis* – are very similar to each other, but the former has less flowers in the inflorescence, larger sepals and a more expanded corolla limb than the latter.

Ipomoea lombokii is not included in the morphological analysis as no physical specimens were accessible to confirm the identity of this species. However, references [9, 10] described this species as morphologically distinct due to the cordate-shaped base of the sepals. This character, however, was not observed on digital images of the type specimens.

Six *Stictocardia* species are indistinct and do not have diagnosable morphological characters. One of these species - *I. tiliifolia* - is globally widespread and contains extensive variation for the traits that overlap with other indistinct species, see discussion. The remaining indistinct species are either regionally distributed such as *I. pringsheimiana* in East Africa, or geographically restricted as well as endemic outside of Africa *i.e., I. queenslandica* (Australia), *I. neglecta, I. orientalis* (Indonesia) and *I. sivarajanii* (India) as seen in Figure 2.3. This finding suggested that these indistinct species may represent regional and local variation of the widespread species *I. tiliifolia.*

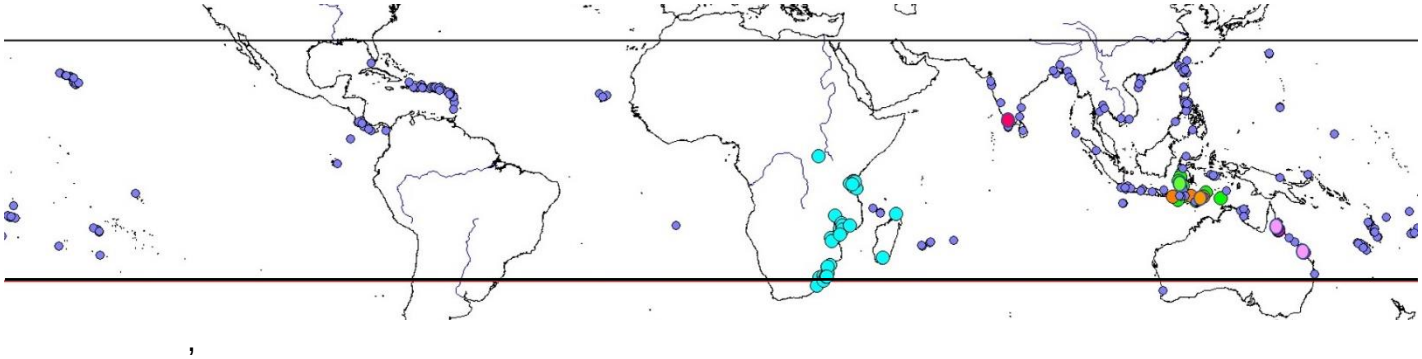
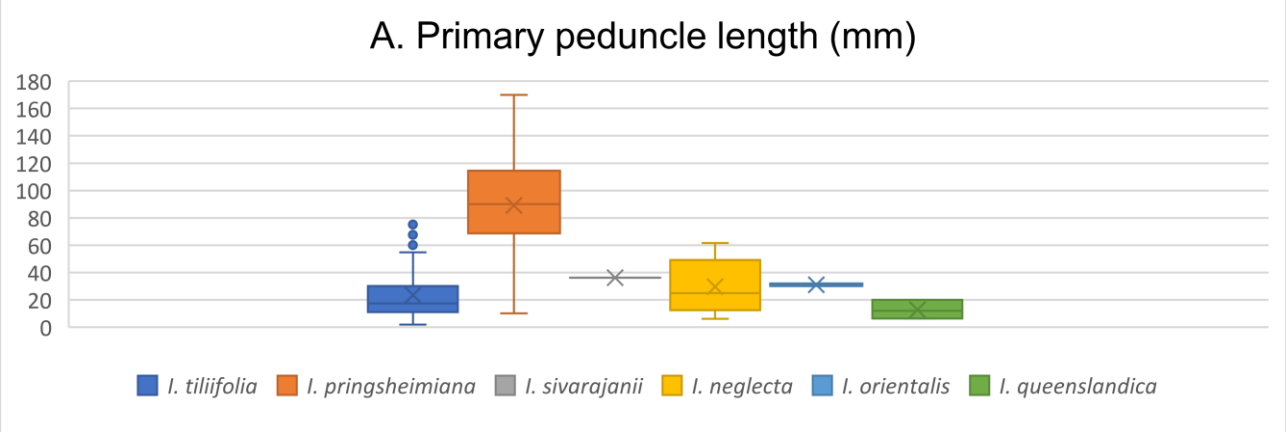
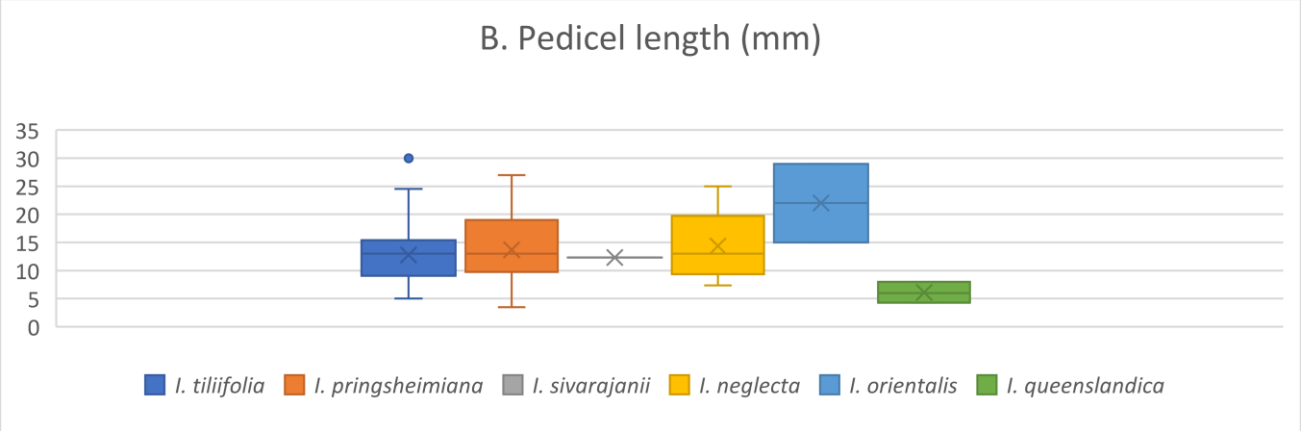


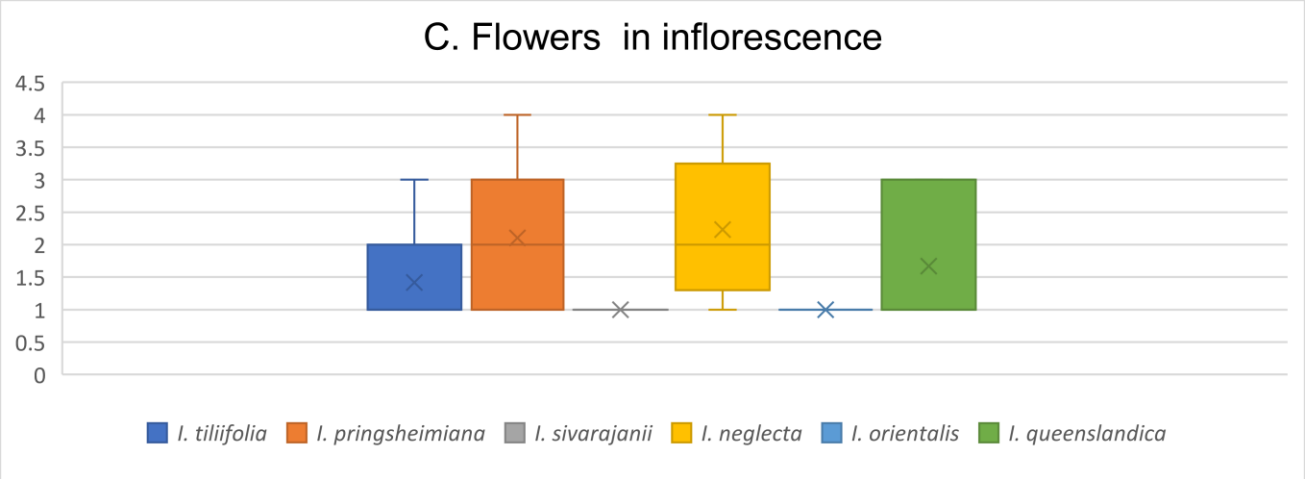
Figure 2.3. The global distribution map of indistinct species of *Stictocardia*. Note that *I. tiliifolia* (smaller purple circles) is globally widespread and all the remaining species (in larger coloured circles) - *I. pringsheimiana* (cyan), *I. sivarajanii* (red), *I. orientalis* (orange), *I. neglecta* (green) and *I. queenslandica* (pink) - are within the range of *I. tiliifolia.* Top and bottom lines refer to 30°N and 30°S respecticely.

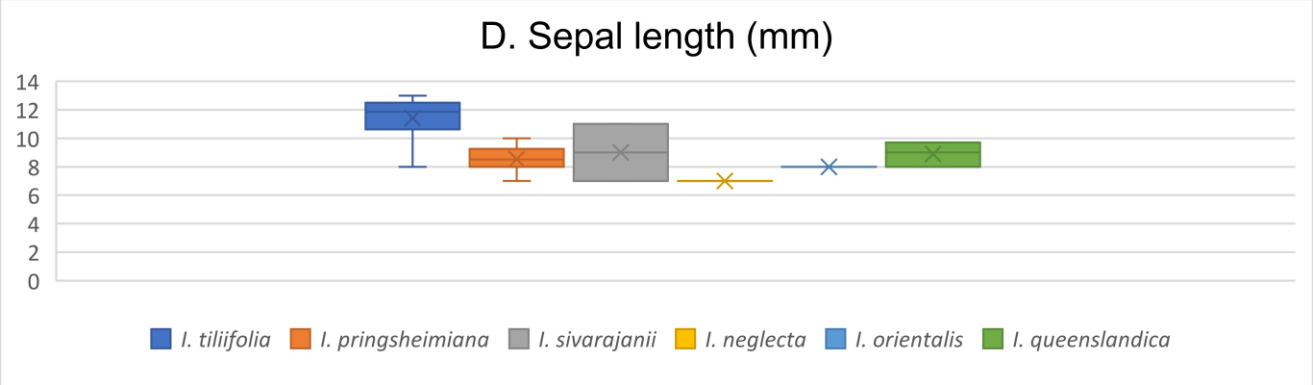
2.3.2. Overlapping quantitative characters of indistinct species

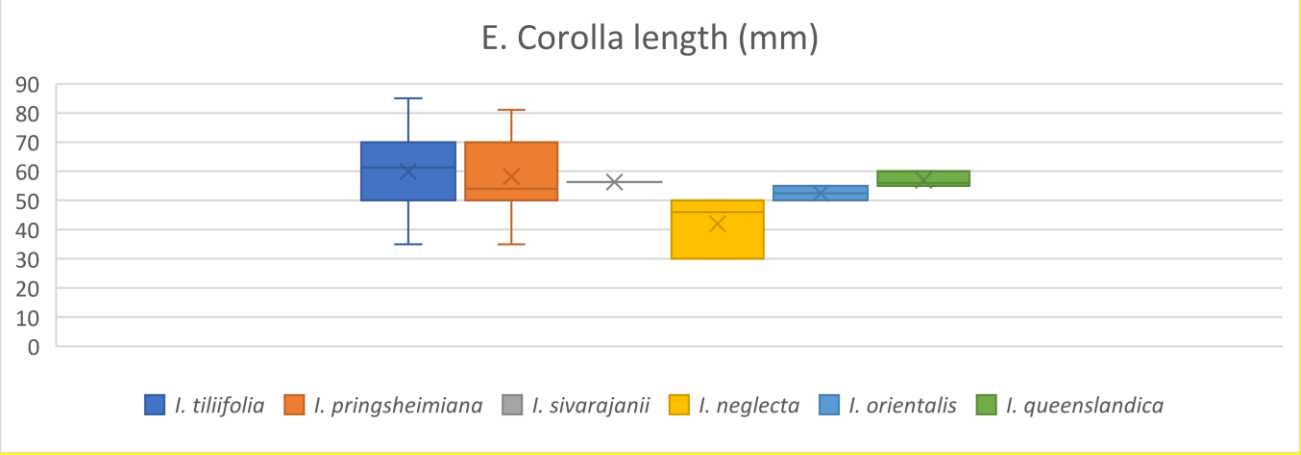
Measurements of quantitative characters demonstrate that the ranges between indistinct species are overlapping (Figure 2.4 A-F). The range of morphological characters of *I. tiliifolia* - species with the most morphological variability, the widest geographical distribution and represented by the greatest number of specimens - is noteworthy because this species not only overlaps with all the indistinct species but also has outliers in some quantitative characters, primary peduncle length, pedicel length and number of lateral veins (Figure 24.A, B and F).




B. Pedicel length (mm)
35
30
25
20
15
10
5
0
I. tiliifolia
I. pringsheimiana
I. sivarajanii
I. neglecta
I. orientalis
I. queenslandica


C. Flowers in inflorescence
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
I. tiliifolia
I. pringsheimiana
I. sivarajanii
I. neglecta
I. orientalis
I. queenslandica


D. Sepal length (mm)
14
12
10
8
6
4
2
0
I. tiliifolia
I. pringsheimiana
I. sivarajanii
I. neglecta
I. orientalis
I. queenslandica


E. Corolla length (mm)
90
80
70
60
50
40
30
20
10
0
I. tiliifolia
I. pringsheimiana
I. sivarajanii
I. neglecta
I. orientalis
I. queenslandica

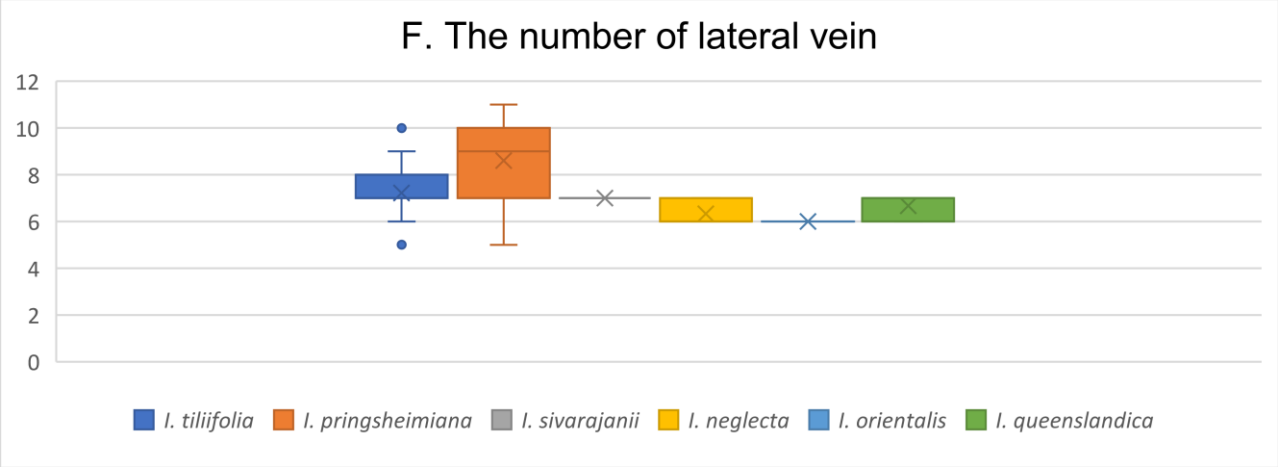


Figure 2.4 Overlapping quantitative characters of six colour-coded indistinct species with a focus on **A.** Primary peduncle length, **B**. Pedicel length**, C.** Number of flowers in the inflorescence, **D.** Sepal length, **E.** Corolla length, and **F.** The number of lateral veins. Distance of the bottom and top whiskers to boxes represent the lowest and highest 25% of the measured data, respectively, boxes represent distribution of 50% of the samples, lines and X within boxes represent median and mean, respectively and dots represent outliers

2.3.3. PCA of the indistinct species

Principal Component Analysis (PCA), was used to study the complexity of all measured characters. Results of the PCA based on measurements and observations of 115 specimens from six indistinct species are shown in Figure 2.5 and 2.6. Table 2.5 presents the summary of PCA statistics and shows the proportion and accumulation of variance as well as the eigenvalues and eigenvectors of the seven principal components (PC). Principal Components - PC1 (X-axis) and PC2 (Y-axis) - cover 84.75% and 9.31% of the variation, respectively. As these two principal components accumulate 94.06% of the total variation, the remaining components (PC3 to PC7) can be ignored (Table 2.6).

Figure 2.5 shows that *I. tiliifolia* and *I. pringsheimiana* occupy significant morphospace, reflecting the broad range of morphological variation in these two species. All the remaining indistinct species are either overlapping or nested within the morphospace of *I. tiliifolia. Ipomoea pringsheimiana* also overlaps with all other indistinct species with the exception of *I. queenslandica.* It should be noted that the

number of sampled specimens per species is largely uneven, 77 specimens of *I. tiliifolia*, 26 of *I. pringsheimiana*, six of *I. neglecta*, three of *I. queenslandica*, two of *I. orientalis*, and one of *I. sivarajanii*. Despite this low number of samples for the majority of putative species, the PCA result demonstrates that there are no clear gaps between these indistinct species and they may reflect one putative taxonomic entity.

Figure 2.6 shows the PCA result focussed on individual morphological characters, represented as eigenvalues (length of red arrows) and eigenvectors (direction of red arrows) relative to PC1 and PC2 based on the quantitative data set. Primary peduncle length and corolla length are two strongly influencing characters to PC1 and PC2, respectively. Despite their strong influence, these two characters do not correlate to each other as the angle between them is almost perpendicular. The rest of the characters have weak influence to PC1 and PC2 as the eigenvalues are very low at the centre of the axis. The statistics of these characters relative to PCs are shown in the Table 2.5.

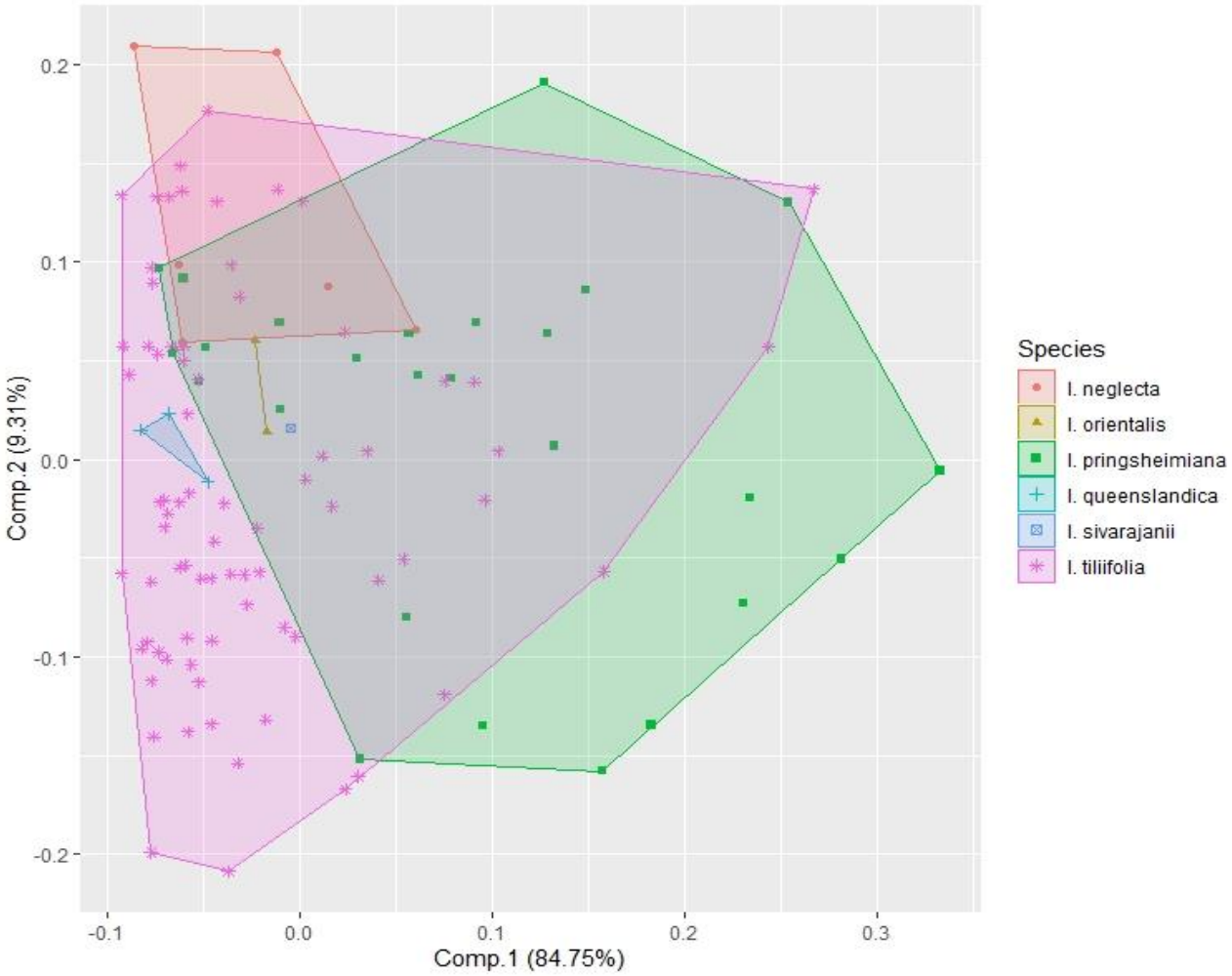


Figure 2.5 shows the distribution of 115 specimens of six indistinct colour coded *Stictocardia* species relative to PC1 and PC2. The distribution of the globally widespread *I. tiliifolia* with broad morphological variation occupies significant morphospace and overlaps with all other species.

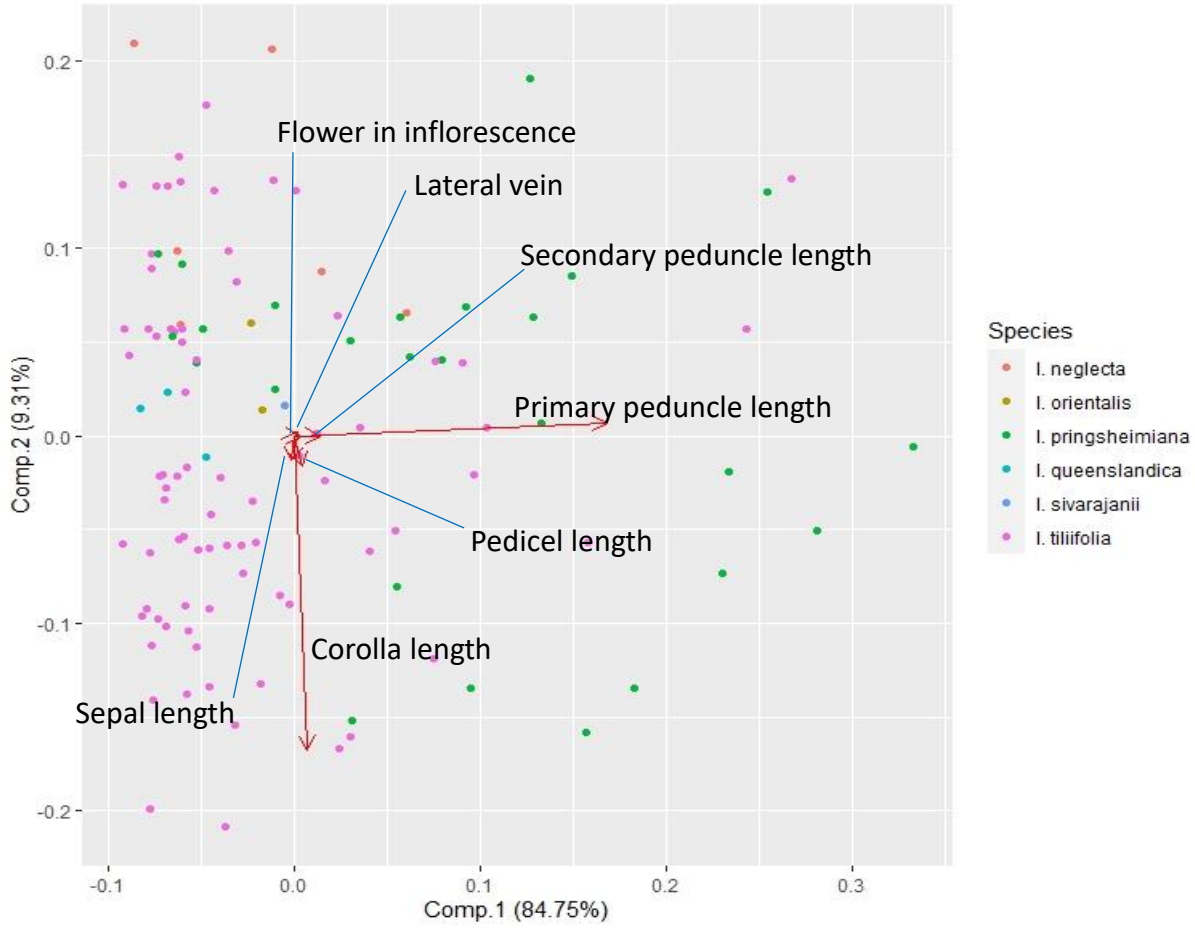


Figure 2.6. PCA's eigenvalues and eigenvectors using *princomp* function

Table 2.5. The statistics of the PCA result based on 115 specimens and seven quantitative morphological characters

	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6	Comp. 7
Standard deviation	36.9	12.2	7.41	5.08	3.07	2.04	0.899
Proportion of Variance	0.847	0.093	0.034	0.016	0.005	0.002	0.0005
Cumulative Proportion	0.847	0.941	0.975	0.991	0.997	0.999	1
Primary_peduncle_length	0.995						
Pedicel_length			-0.991				
Flower_in_inflorescence						-0.123	-0.989
Sepal_length					0.12	0.98	-0.128
Secondary_peduncle_length				-0.989			
Corolla_length		-0.991					
Lateral_vein					0.992	-0.121	

The upper section of this table contains the statistical summary of the PCA, whereas the lower section enumerates the value of individual morphological characters on a given PC (Principal Component) from PC1 to PC7. Values, either positively or negatively correlated, on the lower section depict the significance of a given character to a given PC.

2.3.4. Statistical test of the indistinct species

Statistics of the Mean and SEM of the indistinct species across individual quantitative characters is presented in Table 2.6-2.11. The number of samples of individual species is lower than PCA; *I. tiliifolia* (46)*, I. pringsheimiana* (23)*, I. neglecta* (12)*, I. orientalis* (6) and *I. queenslandica* (2). Most values exhibit overlapping ranges (bordered by dashed red lines) of SEM of the indistinct species across individual quantitative characters.

Table 2.6. Statistics table of primary peduncle length across five indistinct species of *Stictocardia*

	I. tiliifolia	*I. pringsheimiana*	*I. neglecta*	*I. orientalis*	*I. queenslandica*
Mean	28.3720779	89.0192308	29.53333333	31	12.76667
Standard Error of Mean	3.12782647	8.24931143	8.746110247	1	3.973384

Table 2.7. Statistics table of pedicel length across five indistinct species of *Stictocardia*

	I. tiliifolia	*I. pringsheimiana*	*I. neglecta*	*I. orientalis*	*I. queenslandica*
Mean	13.5435	13.712	14.392	22	6.1
Standard Error of Mean	8.04692	6.0186	6.5773	9.899	1.85

Table 2.8. Statistics table of the number of flowers in inflorescence across five indistinct species of *Stictocardia*

	I. tiliifolia	*I. pringsheimiana*	*I. neglecta*	*I. orientalis*	*I. queenslandica*
Mean	1.485714	2.384615385	2.233333333	1	1.666667
Standard Error of Mean	0.821698	1.42343031	1.098483804	0	1.154701

Table 2.9. Statistics table of corolla length across five indistinct species of *Stictocardia*

	I. tiliifolia	*I. pringsheimiana*	*I. neglecta*	*I. orientalis*	*I. queenslandica*
Mean	59.96753	58.18077	42	52.5	57
Standard Error of Mean	11.96421	12.59816	9.486833	3.535534	2.645751

Table 2.10. Statistics table of sepal length across five indistinct species of *Stictocardia*

	I. tiliifolia	*I. pringsheimiana*	*I. neglecta*	*I.orientalis*	*I. queenslandica*
Mean	11.91753247	9.519231	9.95	9.5	8.566667
Standard Error of Mean	1.897882585	2.39433	2.686075	0.707107	1.40119

Table 2.11. Statistics table of the number of lateral veins across five indistinct species of *Stictocardia*

	I. tiliifolia	*I. pringsheimiana*	*I. neglecta*	*I. orientalis*	*I. queenslandica*
Mean	5.623377	7.615385	6.333333	3	6.666667
Standard Error of Mean	3.141567	3.299417	0.516398	4.242641	0.57735

Comparison between individual quantitative characters in Figures 2.7 to 2.12 demonstrates that these putative species are very similar with overlapping values for these characters. However, all individual characters can distinguish gaps in the variation between two or three groups of indistinct species, albeit each of these groups have different species composition (Figure 2.7 to 2.12).

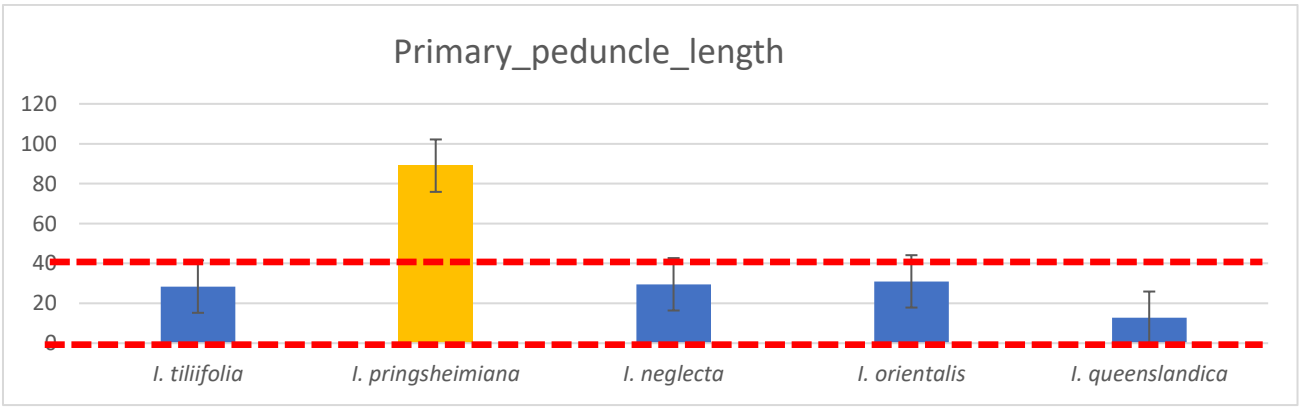


Figure 2.7. Mean and Standard Error of Mean (vertical lines with top and bottom whiskers) of primary peduncle length across five indistinct species. Group one (blue) includes *I. tiliifolia, I. neglecta, I. orientalis,* and *I. queenslandica. w*hereas group two (orange) includes *I. pringsheimiana*. Dashed red lines indicate the upper and lower limits (range) of the group one's Standard Error of Means that does not overlap with group two.

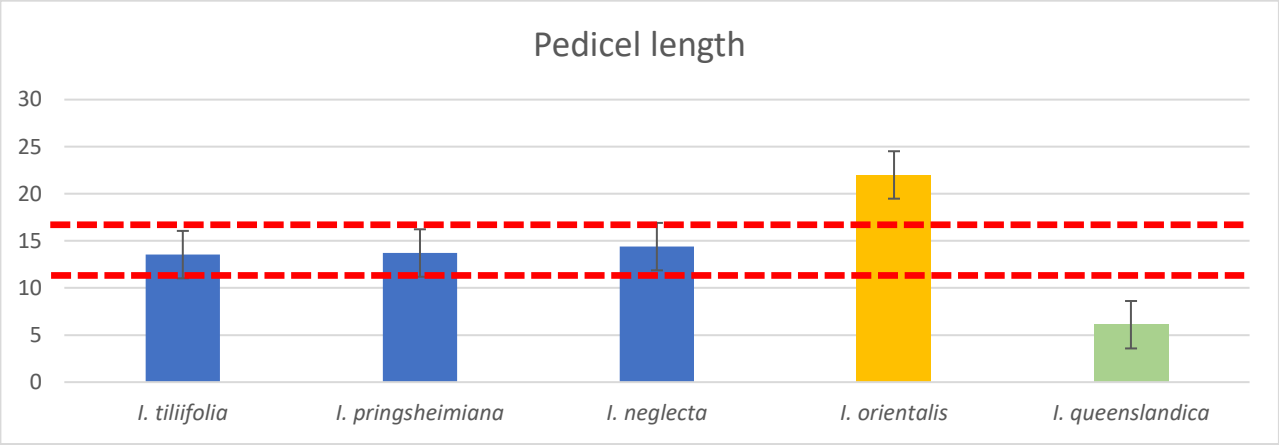


Figure 2.8. Mean and Standard Error of Mean (vertical lines with top and bottom whiskers) of pedicel length across five indistinct species. Group one (blue) includes *I. tiliifolia, I. pringsheimiana,* and *I. neglecta, g*roup two (orange) includes *I. orientalis*, whereas group three (green) includes *I. queenslandica.* Dashed red lines indicate the upper and lower limits (range) of the group one's Standard Error of Means that do not overlap with group two and three.

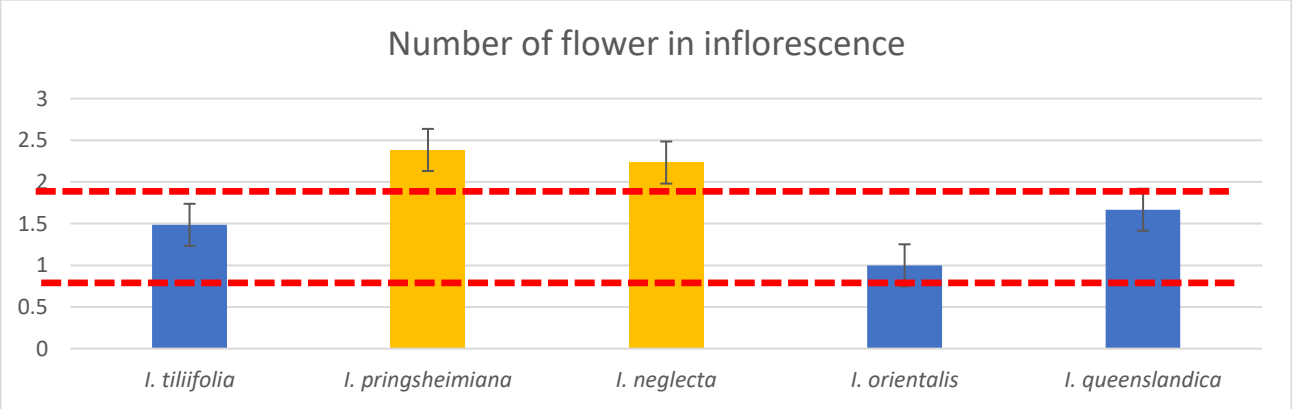


Figure 2.9. Mean and Standard Error of Mean (vertical lines with top and bottom whiskers) of the average number of flowers in inflorescence across indistinct species. Group one (blue) includes *I. tiliifolia, I. orientalis,* and *I. queenslandica,* whereas *g*roup two (orange) includes *I. pringsheimiana* and *I. neglecta.* Dashed red lines indicate the upper and lower limits (range) of the group one's Standard Error of Means that does not overlap with group two.

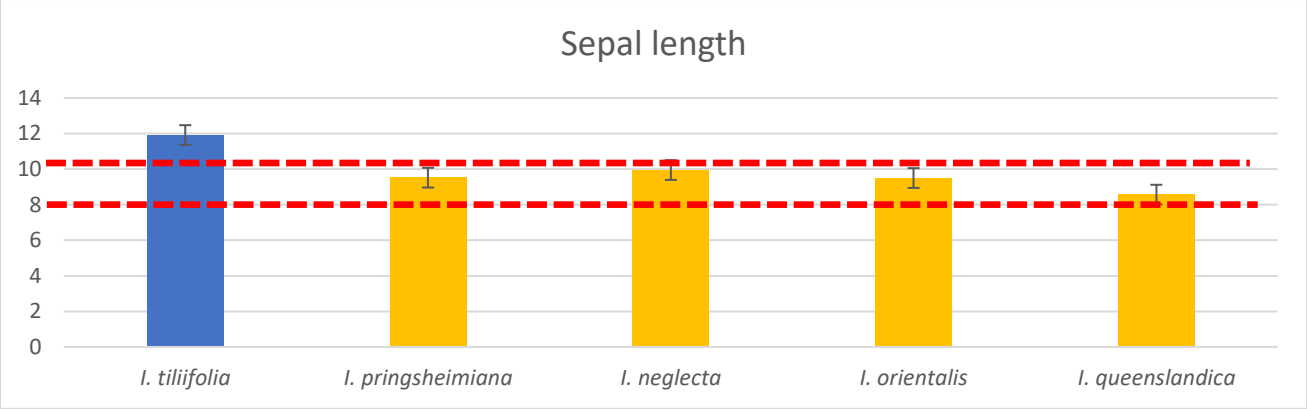


Figure 2.10. Mean and Standard Error of Mean (vertical lines with top and bottom whiskers) of sepal length across five indistinct species. Group one (blue) includes*I. tiliifolia. w*hereas group two (orange) includes *I. pringsheimiana, I. neglecta, I. orientalis,* and *I. queenslandica.* Dashed red lines indicate the upper and lower limits (range) of the group two's Standard Error of Means that does not overlap with group one.

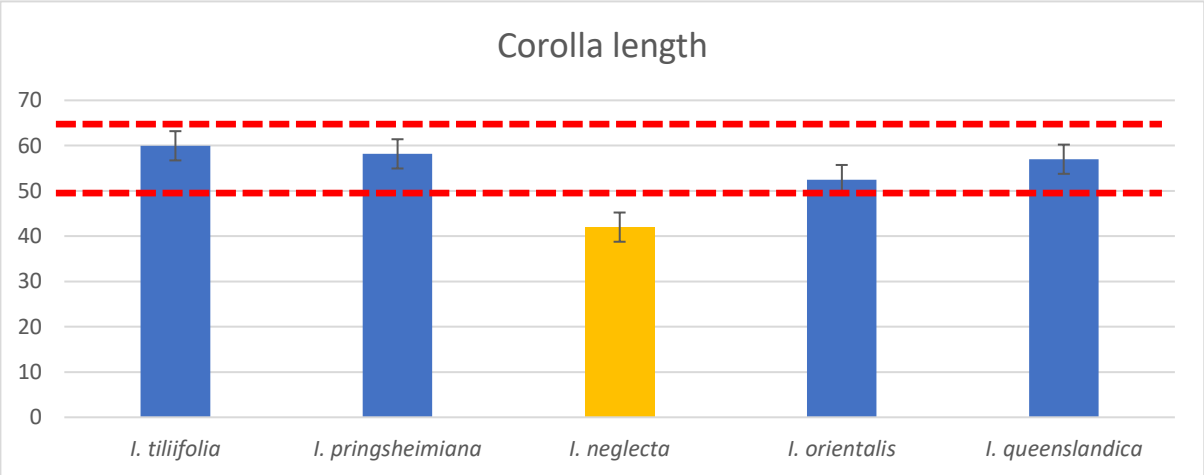


Figure 2.11. Mean and Standard Error of Mean (vertical lines with top and bottom whiskers) of corolla length across five indistinct species. Group one (blue) includes *I. tiliifolia, I. pringsheimiana, I. orientalis,* and *I. queenslandica.* whereas group two (orange) includes *I. neglecta.* Dashed red lines indicate the upper and lower limits (range) of the group one's Standard Error of Means that does not overlap with group two.

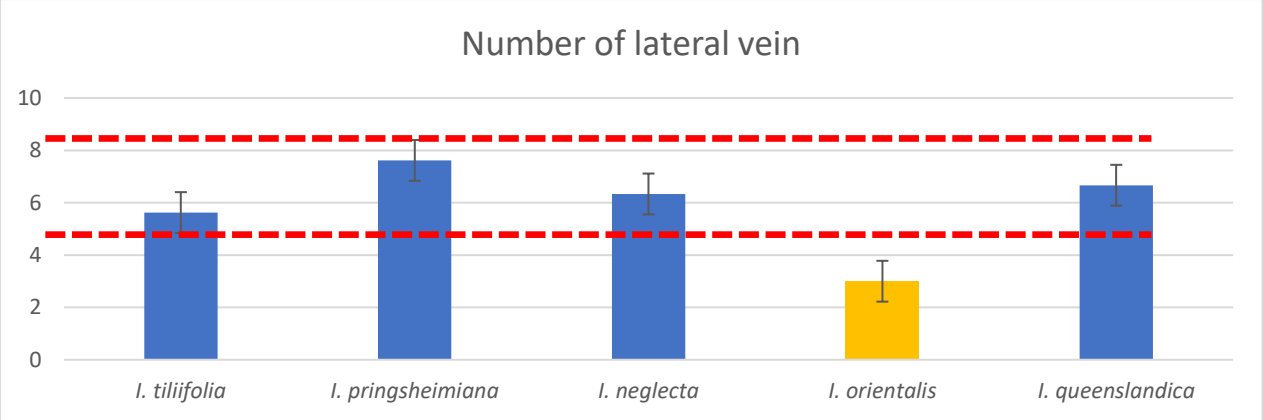


Figure 2.12. Mean and Standard Error of Mean (vertical lines with top and bottom whiskers) of the number of lateral veins across five indistinct species. Group one (blue) includes *I. tiliifolia, I. pringsheimiana, I. neglecta,* and *I. queenslandica* whereas group two (orange) includes *I. orientalis.* Dashed red lines indicate the upper and lower limits (range) of the group one's Standard Error of Means that does not overlap with group two.

The distribution for four quantitative characters, *i.e.* length of the primary peduncle, number of flowers per inflorescence, length of sepals and corolla, as they are distinguished by SEM are shown in Figures 2.13-2.16. The distribution of these data does not follow a Gaussian curve, which is justification to use a non-parametric analysis.

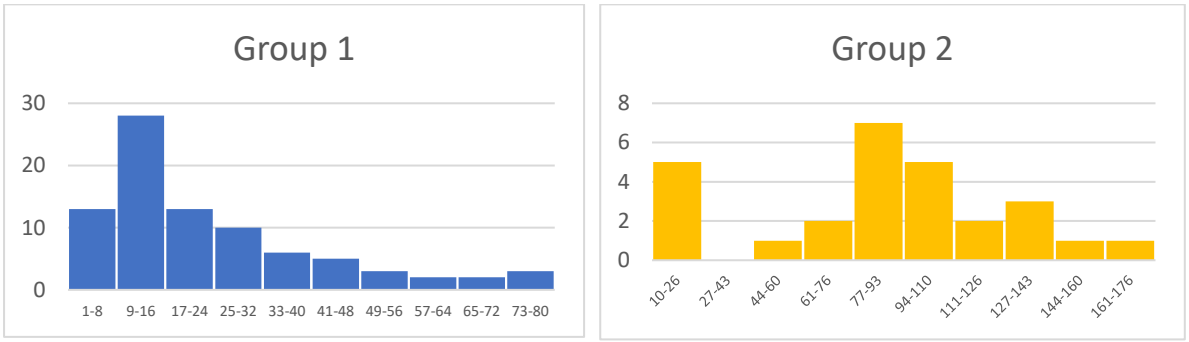


Figure 2.13. shows the distribution of primary peduncle length of two groups of indistinct species. Group one (left) comprises *I. tiliifolia, I. neglecta, I. orientalis* and *I. queenslandica*, whereas group two (right) comprises *I. pringsheimiana.* Number of bins is 10, homogenised across individual quantitative characters. Two numbers separated by a comma on the x-axis refers to the range of values in millimetres. The y-axis is the number of specimens.

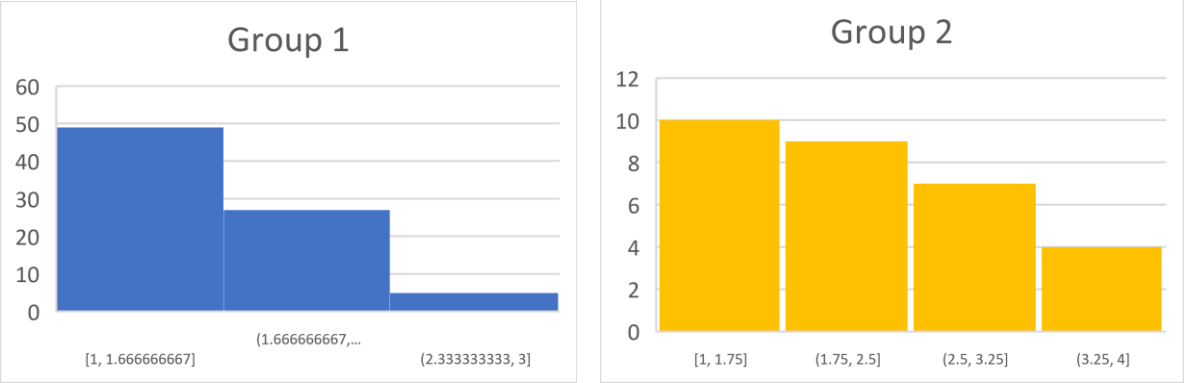


Figure 2.14. above shows the distribution of the number of flowers in the inflorescence of two groups of indistinct species. Group one (left) comprises *I. tiliifolia, I. orientalis,* and *I. queenslandica*, whereas group two (right) comprises *I. pringsheimiana* and *I. neglecta.* Number of bins is three (left) and four (right) to show the distribution of the measured character. Two numbers separated by a comma on the x-axis refers to the range of values in millimetres. The y-axis is the number of specimens.

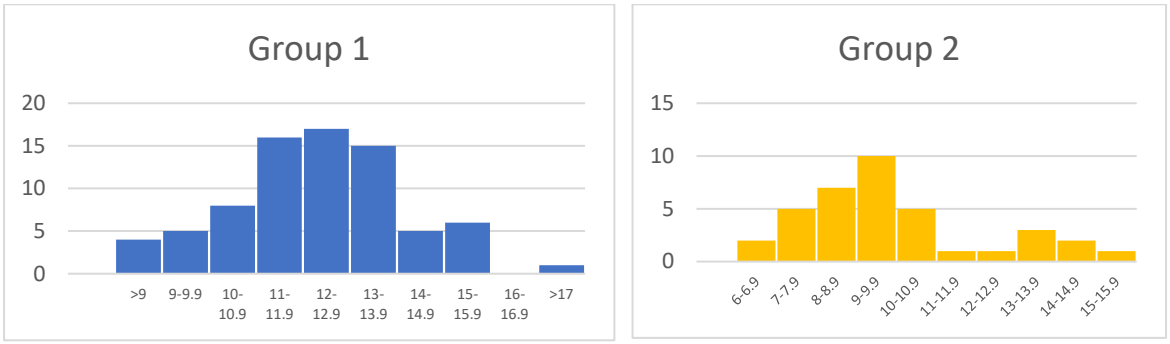


Figure 2.15. shows the distribution of sepal length of two groups of indistinct species. Group one (left) comprises *tiliifolia,* whereas group two (right) comprises *I. pringsheimiana, I. orientalis, I. queenslandica*, and *I. neglecta.* Number of bins is 10, homogenised across individual quantitative characters. Two numbers separated by a comma on the x-axis refers to the range of values in millimetres. The y-axis is the number of specimens.

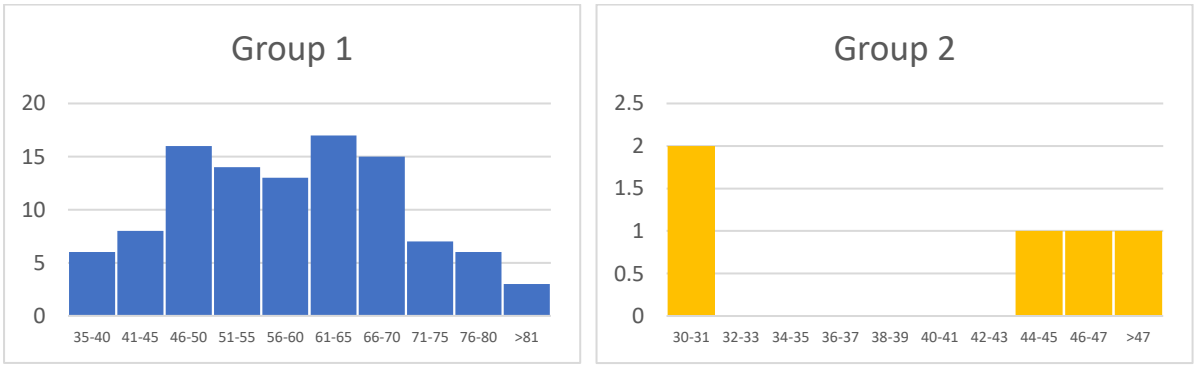


Figure 2.16. shows the distribution of corolla length of two groups of indistinct species. Group one (left) comprises *tiliifolia, I. orientalis I. queenslandica*, and *I. pringsheimiana* whereas group two (right) comprises *I. neglecta.* Number of bins is 10, homogenised across individual quantitative characters. Two numbers separated by a comma on the x-axis refers to the range of values in millimetres. The y-axis is the number of specimens.

The Mann-Whitney test demonstrates that the primary peduncle length and the number of flowers in the inflorescence can distinguish gaps in the variation between various indistinct species as their p values are < 0.05 (Table 2.12.). Two characters - sepal and corolla length – do not distinguish species groups as their p values >0.05 (=1). Pedicel length was omitted from this test because there are three groups appear based on SEM calculation. Number of lateral veins was also omitted.

Table 2.12. Results of the Mann-Whitney test for the grouping of indistinct species across quantitative characters

Character	Rank Sum		Total sample		μw	бw	z	p- value	Mann-Whitney Conclusion
	Group 1	Group 2	Group 1	Group 2					
Primary peduncle length	4241	2314.5	88	26	5060	148.08	-5.53	1.56237E-08	Significant
Flower in inflorescence	3535	3020.5	82	32	4715	158.58	-7.44	4.87178E-14	Significant
Sepal length	5261	1294	77	37	4427.5	165.24	5.04	1	Not significant
Corolla length	6561	108.5	105	6	5880	76.68	8.87	1	Not significant

μw is the mean and бw is the standard deviation. The z value is a component that determines the p-value

2.3.5. Re-evaluation of the study group

In conjunction with the study of morphology and the six indistinct species, I was also inferring phylogenies using molecular sequence data to test the monophyly of *Stictocardia.* These analyses (see Chapter Three) demonstrated that *Stictocardia* is polyphyletic. In addition, this study also finds that there are seven species of *Stictocardia* recovered in a monophyletic group, subsequently referred as the clade Stictocardia. Four of them - *I. incompta, I. beraviensis, I. macalusoi*, and *I. lutambensis* - are distinct species with clear morphological boundaries, whereas the remaining three - *I. tiliifolia, I. queenslandica* and *I. pringsheimiana* - are three of the indistinct species that could not be distinguished on the basis of morphology. However, phylogenetic analysis of multiple accessions of these species demonstrated that each

of these three morphologically indistinct species were monophyletic (see Chapter Three). The morphological variation of these three species is discussed below relative to monophyly, geography and overlapping characters.

2.3.6. Diagnosis of the study group

Stictocardia is characterized by a combination of characters; cordate-shaped leaf base, black glands on the lower leaf surface, sepals accrescent in fruit, indehiscent fruit, and valved endocarp. These characters, however, also appear in species of *Stictocardia* that are outside of Stictocardia. This finding indicates that these characters individually are homoplastic and not unique to Stictocardia.

2.3.7. Morphological evaluation of the polyphyly of *I. beraviensis*

The phylogenetic study in Chapter Three later shows that multiple samples of *I. beraviensis* collected from regions in Africa and Madagascar are not recovered as a monophyletic group. This phenomenon hints an initial impression that there are more than one group, possibly cryptic as indicated by two non-related lineages, of *I. beraviensis* in Africa. One is a putative widespread group that was collected in Liberia, Ghana and Madagascar, whereas the other is the one that restricts to the Eastern side of Africa (Ethiopia, Kenya and DR Congo) as seen in Appendix 5. This inference of two putative groups is therefore a hypothesis to be tested by revisiting the morphological data thoroughly.

The hypothesis testing, however, is constrained by the availability of the very limited number of samples in each putative group. Considering the situation, the logical option is limited to data sets visualization using box plots - as seen in the Figure 2.17 A to F - that exhibits the measurements of six quantitative morphological characters *i.e*.; primary peduncle length, pedicel length, the number of flowers in inflorescence, sepal length, corolla length and the number of lateral veins,

respectively. These morphological characters demonstrate clearly that, individually and collectively, they overlap between putative (Eastern vs Widespread) groups and do not discriminate *I. beraviensis* into two taxonomic entities. To date, there is no useful diagnostic character or combinations of characters identified to separate these putative taxonomic entities.

As mentioned earlier, the data sets suffer very limited samples per group, thus the distribution of samples does not follow the Gaussian curve (Figure 2.18). One of the data sets (corolla length) is only represented by two specimens from the Eastern group because of the incomplete flower part in a specimen (*Gardner* 1103), thus the analysis of this character even has fewer samples (2). Therefore, the only available statistical analysis that fits with the situation is a non-parametric one, the Mann-Whitney test as seen in Table 2.13, which can accommodate fewer than 30 samples. However, the use of statistical analysis based on a very few numbers of samples poses risk as the result and conclusion may not represent the accurate depiction of the situation as discussed later.

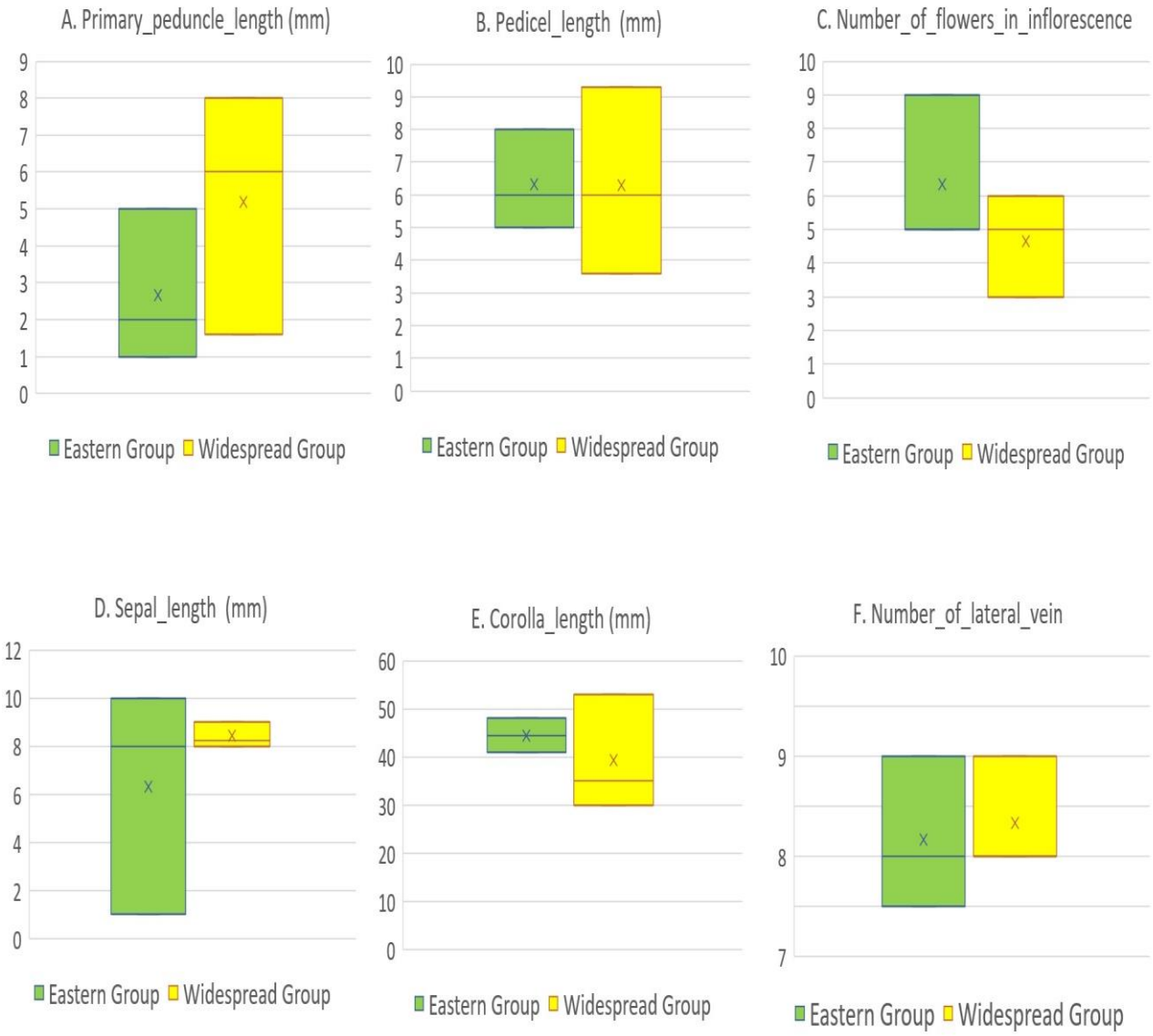


Figure 2.17 A-F. shows the box plots of comparative measurements of selected quantitative morphological characters and altitudinal range of *I. beraviensis* between colour coded geographical areas in Africa. Overlapping characters, individually and collectively, occur between putative groups (Widespread/yellow and Eastern/green) demonstrating no clear morphological distinction can be clearly drawn among specimens from these areas.

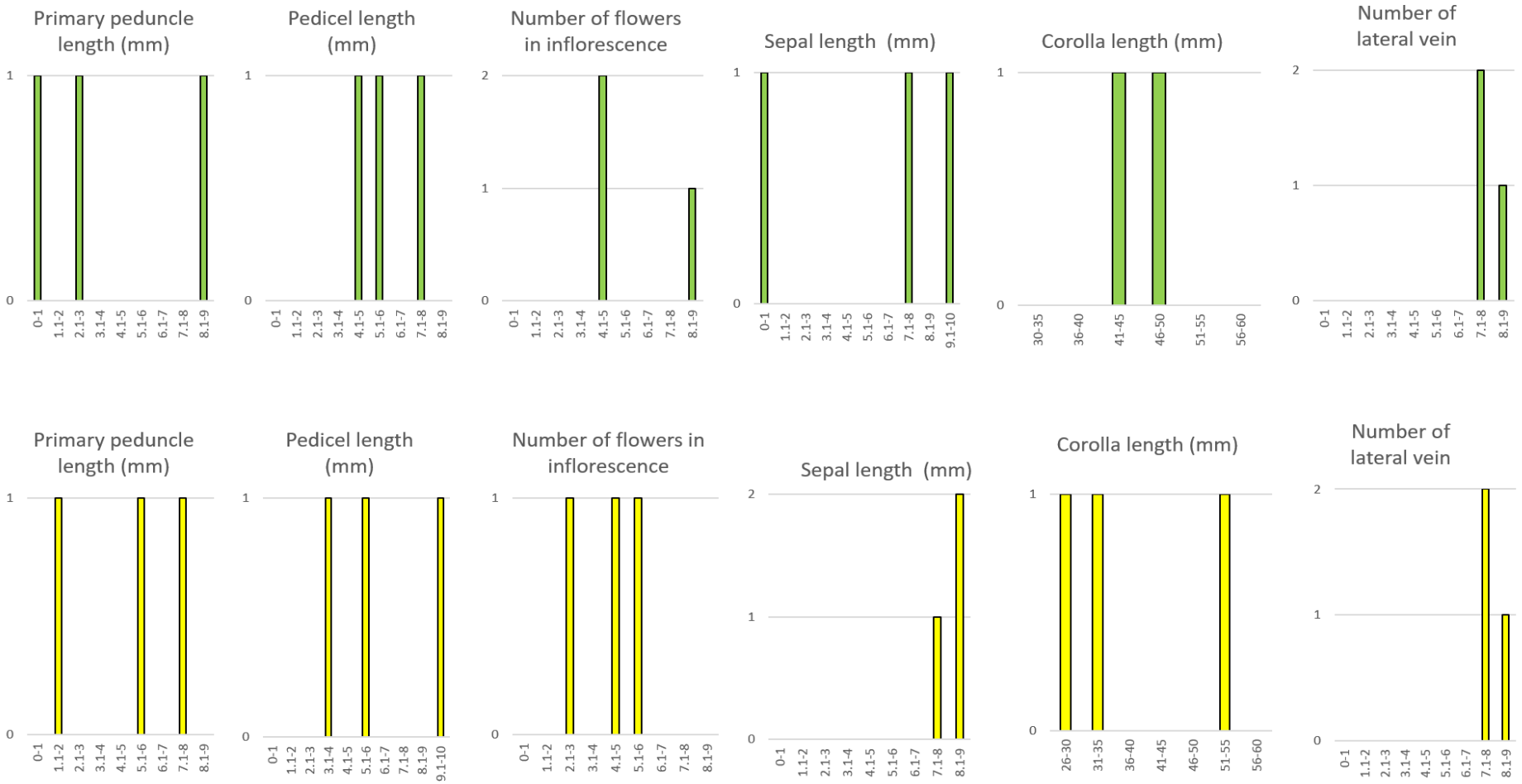


Figure 2.18. shows the distribution of six morphological characters measurement between two (Eastern/top vs. Widespread/bottom) putative groups of I. *beraviensis*. The x-axis refers to the interval that was set arbitrarily to display the distribution of measurement. The y-axis refers to the number of individuals measured on a given interval.

Table 2.13. Results of the Mann-Whitney test for the two putative groups within *I. beravienis* across moprhological characters

	Rank Sum		Total sample		µw	Ϭw	z	p- value	Conclusion
	Group 1	Group 2	Group 1	Group 2					
Primary peduncle length	9.5	11.5	3	3	104.5	2.291	-40.589	0	significant
Pedicel length	10.5	10.5	3	3	115.5	2.291	-45.826	0	significant
Number of flowers in inflorescence	12	9	3	3	132	2.291	-53.682	0	significant
Sepal length	9.5	11.5	3	3	104.5	2.291	-40.589	0	significant
Corolla length	13	8	2	3	143	1.732	-77.942	0	significant
Number of lateral veins	9.5	11.5	3	3	71.875	2.291	-31.369	2.7E-216	significant

µw is the mean and Ϭw is the standard deviation. The Z value is c component that determines the p-value.

2.4. DISCUSSION

This discussion covers the morphology of Stictocardia and individual species within it. PCA results are briefly discussed relative to species-level sampling. Lastly, there is a short discussion on the species excluded from Stictocardia.

2.4.1. Diagnostic characters of the clade Stictocardia

Stictocardia is morphologically diagnosed by a combination of characters: cordate leaf base, black glands, sepals accrescent in fruit, indehiscent fruit, and valved endocarp. Individually, these characters are not unique to *Stictocardia* and have evolved several times independently in Convolvulaceae, except valved endocarp [14, 30], but see discussion below. The distribution of three of these characters, sepals accrescent in fruit, valved endocarp, and black glands, are mapped relative to two phylogenies, *nr*ITS (Figure 2.19A) and plastome (Figure 2.19B). These characters, are not unique to the clade Stictocardia. These characters are discussed below.

2.4.1.1. Cordate leaf base

Cordate leaf base is not only common but also homoplastic in many species of *Ipomoea* [45]. This fact emphasises the trend of parallelism and convergence within *Ipomoea* [30]. Therefore, the presence of cordate leaf base is not taxonomically informative for *Ipomoea* as a whole but can, with caution, be diagnostic of particular collections of species at a more restrictive level within *Ipomoea*.

2.4.1.2. Black glands

Hallier described scattered black glands across the abaxial leaf surface of *Stictocardia*. These glands, coupled with sepals accrescent in fruit (see below), are the main reasons why Hallier [1] separated and described *Stictocardia* as a distinct

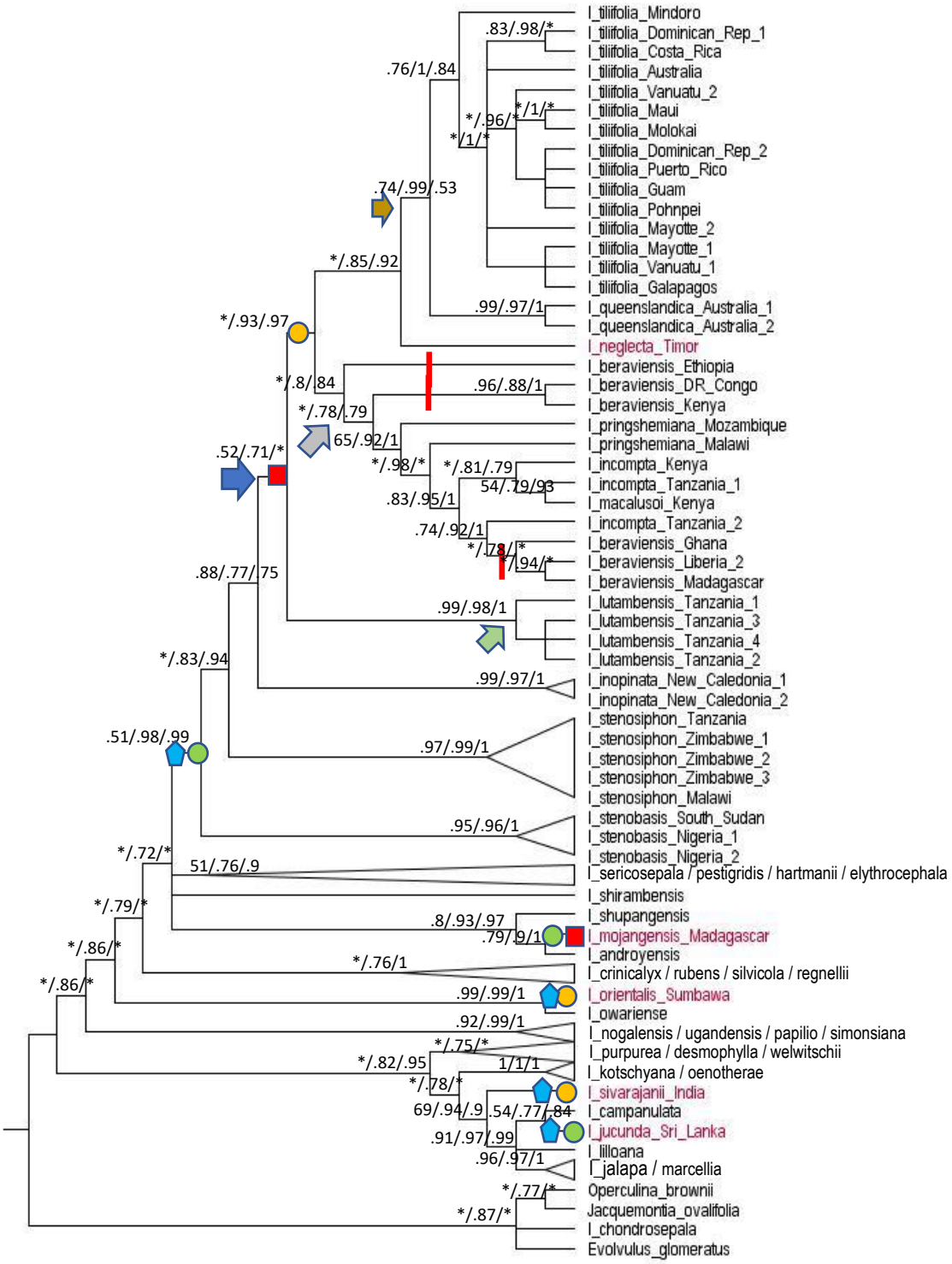


Figure 2.19A. Inferred *nr*ITS phylogeny of the OW *Ipomoea* focused on the clade Stictocardia (blue arrow). Red coloured species refer to *Stictocardia* species recovered outside Stictocardia with the exception of *I. neglecta*. Some clades are collapsed for simplicity and named with a group of *Ipomoea* species it comprised. Numbers on nodes refer to the bootstrap support (in decimal) of Maximum Parsimony/Approximate-Maximum Likelihood/Bayesian Inference. Asterisks represent support less than 0.5. Some multi specimens species are unresolved as monophyletic groups, *i.e*. *I. beraviensis, I. pringsheimiana,* and *I. incompta*. Red lines mark *I. beraviensis* as this species appears polyphyletic in the plastome phylogeny (see Chapter Three). Red squares show the species with a valved endocarp. Blue pentagons mark the presence of black glands on the lower side of leaves. Character states of the sepals accrescent in fruit are either fully covering exocarp species (yellow circles) or partially exposed exocarp (green circles).

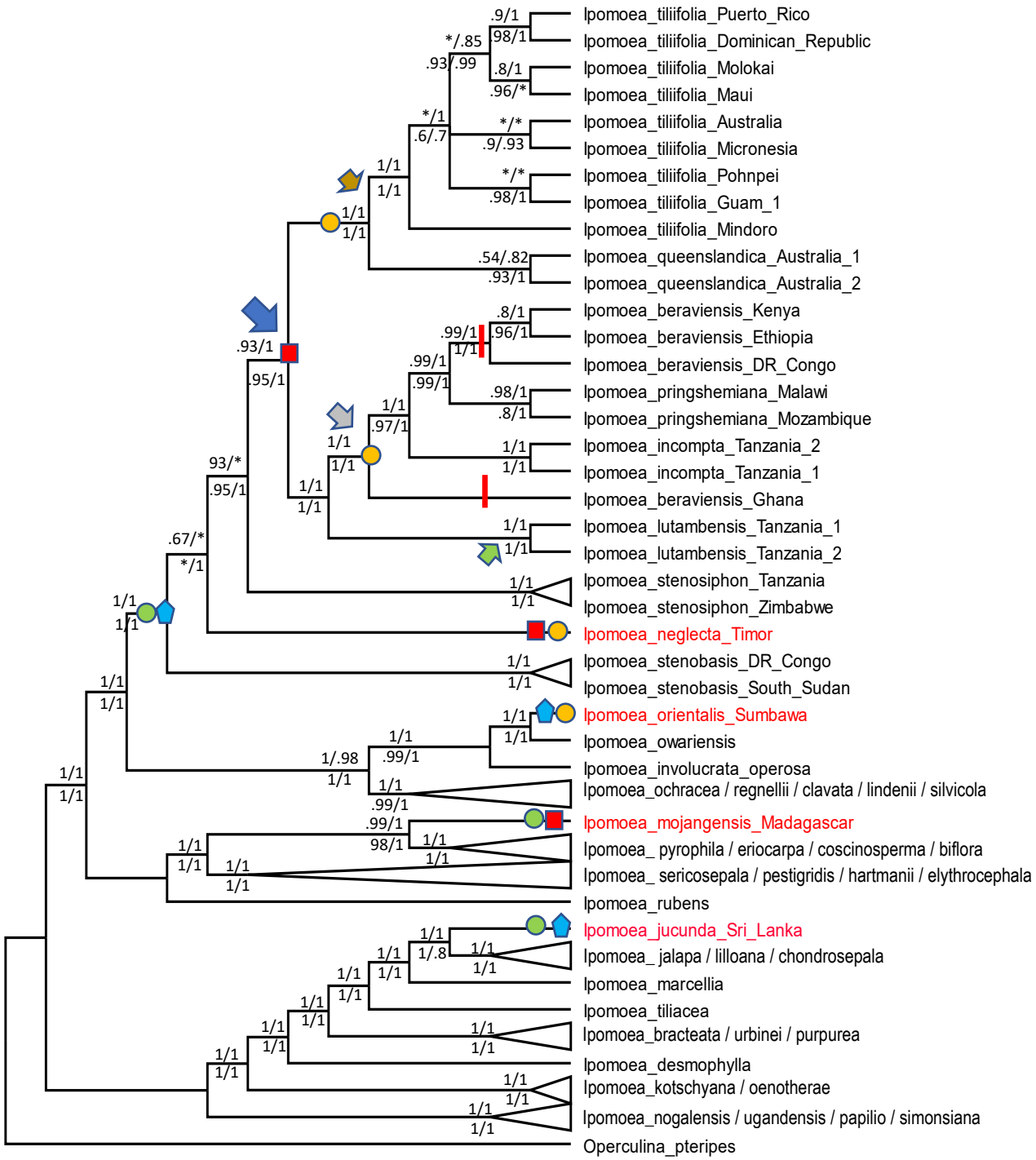


Figure 2.19B. Inferred consensus plastome phylogeny of the OW *Ipomoea* focused on Stictocardia (blue arrow) with distribution of diagnostic morphological characters. Red coloured species refer to *Stictocardia* species recovered outside Stictocardia. Some clades are collapsed for simplicity and named with a group of *Ipomoea* species it comprised. Numbers on nodes refer to the bootstrap support (in decimal) of the 28 plastome genes (above) and 26 variable regions of plastome (below) with Maximum Likelihood/Bayesian inference. Only *I. beraviensis* (red lines) is unresolved as a monophyletic group. Red squares show the species with valved endocarp, with exception in *I. pringsheimiana* due to no detailed observation on fruit. Blue pentagons mark the presence of black glands on the lower side of leaves. Character states of the sepals accrescent in fruit are either fully covering exocarp species (yellow circles) or partially exposed exocarp (green circles).

genus within Convolvulaceae [2]. Further observation has shown that black glands also occur on petioles, sepals, and corolla.

A study on leaf anatomy of *Stictocardia* found that these glands are dark - not always black - due to oxidation of tannin within the gland [65]. Anatomically, these glands are multicellular - either capitate or peltate - glandular trichomes that are suggested to deter foraging insects and conserve water [65]. Olaranont et al. [65] also suggested that the density of black glands is a taxonomically informative character between species. However, the study only included two species, *I. tiliifolia* and *I. beraviensis*, and the number of samples examined within each species was not reported. Therefore, that study did not provide comprehensive information on the practicality of this character for species delimitation in Stictocardia.

This study compares black gland density within and between species of Stictocardia as shown in the Figure 2.20. The average density of black glands varies widely between individual leaves and individual specimens as discussed below. The range of average density of black glands also overlaps between species.

The range of black gland average density varies within species. Some species like *I. pringsheimiana* and *I. beraviensis* have wide range of gland density as seen in the Figure 2.20. In contrast, low specimen sampling occurred on *I. macalusoi* and *I. queenslandica*. Thus, it is must be taken into account that the range of these two species is not represented properly. There also seems no clear association between the density of black glands relative to geography, habitat or altitude between species. Thus, gland density does not clearly discriminate species nor useful to indicate environmental preference.

In addition, black glands is a homoplastic character because it is not only appears on Stictocardia but also on a more inclusive group that comprises *I. stenobasis, I., stenosiphon,* and *I. inopinata* as well as not related species *e.g. I. orientalis, I. neglecta, I. sivarajanii* and *I. jucunda* (Figure 2.19A and 2.19B)

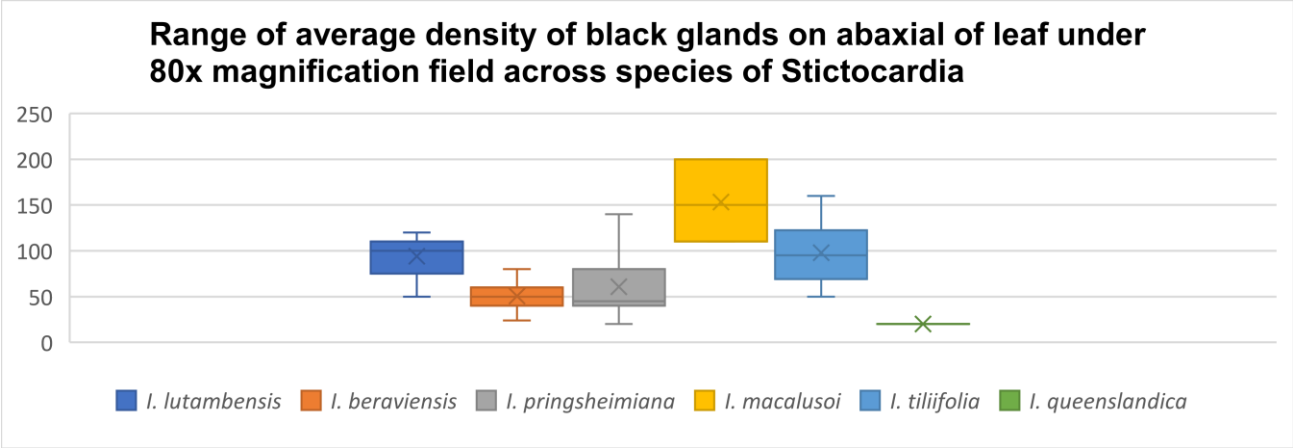


Figure 2.20. shows the box plots of the range of average density of black glands on the abaxial surface of the leaf of six species of Stictocardia. *Ipomoea incompta* is omitted. The number of samples are biased towards *I. beraviensis* (18) and *I. tiliifolia* (14), with only a few specimens for the remaining species. Distance of the bottom and top whiskers to boxes represent the lowest and highest 25% of the measured data, respectively, boxes represent distribution of 50% of the samples, lines and X within boxes represent median and mean, respectively. Variation occurs between individuals within species and the number of black glands overlaps between species.

2.4.1.3. Sepals accrescent in fruit

Sepals accrescent in fruit are only clearly observed at the fruiting stage and cannot be observed on the majority (73.4 %) of 653 specimens of Stictocardia as they bear no fruits. At fruit maturity, accrescent sepals can either enclose and cover the exocarp completely or reflexed and therefore partially expose the exocarp. This study also finds that this character has two states useful for species delimitation. *Ipomoea lutambensis* is the only species of Stictocardia with reflexed sepals that partially expose the exocarp. All the remaining species of Stictocardia possess accrescent sepals fully covering the exocarp. Outside of the clade, reflexed sepals are also found at more inclusive group that incorporates *I. stenosiphon*, *I. stenobasis,* as well as

Stictocardia species of *I. mojangensis* and *I. jucunda* (Figure 19.B). The distribution of this character state across the phylogenies hints that this trait is homoplastic.

The sepals become woody and larger to enclose the fruit has been mentioned in several older references [7, 9, 12, 13, 36]. This shows that fruit development alters the function of the sepals from bud protection to facilitating dispersal. At fruit maturity, the thin woody accrescent sepal structure can float as has been tested from loose fruit of herbarium specimens. Floating fruit facilitates water dispersal has been observed in *Ipomoea indica* [66].

The fruit of *Ipomoea tillifolia* has the largest accrescent sepals (up to 2.7 cm in diameter) in Stictocardia. This enables the fruit to float easily [16, 67], thus dispersed by ocean currents as it has been collected from a number of islands in the three oceans; Pacific, Atlantic and Indian. *Ipomoea tiliifolia* and the other five species of Stictocardia also occur around inland waters such as rivers and lakes. Evidence of water dispersal, however, remains unknown for *I. queenslandica* that occurs in limestone areas with no information on sustainable surface water based on specimen labels.

2.4.1.4. Indehiscent fruit

Hallier described indehiscent fruit as a diagnostic character of *Stictocardia*, although Austin later revised it to tardily dehiscent [27]. Austin's statement lacks supporting evidence since, to my knowledge, no observed fruit specimens are tardily dehiscent. However, his statement is logical for the purpose of seed germination and plant regeneration.

The fully developed fruit is not frequently observed on herbarium material and was only present in 174 (26.4%) out of 653 examined *Stictocardia* specimens. The

number of fruiting specimens is higher on the widespread, most collected species, *I. tiliifolia* (133 specimens; 76.4%). For the remaining species, the number of specimens with fruit varies between 1 (*I. macalusoi* and *I. lutambensis*) and 24 (*I. beraviensis*). Two specimens of *I. pringsheimiana* bears fruit thus the first record for this species as previously unknown in the *Flora of Tropical East Africa* [24] and Madagascar [37]. Self-incompatibility has been reported in cultivated *I. tiliifolia* that bears no fruit [68].

2.4.1.5. Valved Endocarp

Stictocardia possesses a small, valve-like membrane which is part of the endocarp, and useful for seed release [11]. In the early literature, the structure of the endocarp was often not mentioned [1, 7, 12]. This is most likely because it is hidden under the exocarp. Austin and Demissew [16] brought the valved endocarp to attention and treated it as the only diagnostic character of *Stictocardia.* Austin and Eich [8] used this character as the sole justification to transfer *I. mojangensis* into *Stictocardia* and ignoring all characters described by Hallier [1]. However, the next chapter (Chapter Three) demonstrate that this character appears multiple times in phylogenies.

2.4.2. The delimitation of species within the clade Stictocardia

Reciprocal illumination allows the evaluation of the study group after the phylogenetic study in Chapter Three. Seven *Stictocardia* species that consistently recovered in a clade are discussed below in terms of morphology.

2.4.2.1. Species outside Africa and Madagascar

Two species, *I. queenslandica* and *I. tiliifolia*, are reciprocally monophyletic thus sister to each other. *Ipomoea queenslandica* is an endemic species to Queensland, Australia. It is a poorly known species with limited specimens (10) and only deposited in four herbaria, see Chapter Four. It was described as *Argyreia queenslandica* Domin

in Queensland, Australia prior to the transfer to *Stictocardia*. Initially, I interpreted this species as an indistinct species because its characters overlap with the widespread species *I. tiliifolia.* Further observation, however, revealed that *I. queenslandica* possesses at least two distinct morphological characters that do not appear in all specimens. First, the dwarf habit that appears on six mature specimens with flowers and fruits (*Clarkson* 3025, *Clarkson* 6837, *Clarkson* 6860, *Conn Campo* 1347, *Domin* 7919, and *Johnson* 4043, all deposited at BRI). The dwarf habit may be caused by the fact that this species inhabits limestone areas in Queensland. Second, it is also notable that persistent cotyledons are present on two flowering specimens (*Clarkson* 6837, *Clarkson* 6860). Altogether, these characters coupled with endemism and monophyly from the sequence data (Chapter Three) distinguish *I. queenslandica* from *I. tiliifolia*.

Ipomoea tiliifolia is the only species of Stictocardia that has the global distribution. Hallier [1] transferred this species to *Stictocardia* from *Rivea* and listed several specimens from different parts of the world which indicated its widespread distribution. This study accessed 318 specimens and images deposited across 26 herbaria.

Merrill recorded *S. tiliifolia* in Guam [28] but mistakenly treated it as *S. campanulata.* His background as a historian of botany likely led him to use the earliest name published. His treatment was adopted by a number of botanists such as Vollesen (1947), Gunn (1971), Chao (1977), and Green (1985), as seen in determination slips on multiple specimens. Van Ooststroom adopted Merrill's idea for a short while (determination slip dated 1935), but later changed and recognised *I. tiliifolia* in his taxonomic treatments [9, 10]. Other botanists such as Boorsum Walkes (1953), Powell (1971), and Smith (1971) also recognised *S. tiliifolia* and not *S.*

campanulata. These two opinions created confusion for decades between these two taxa. Austin et al. [27] resolved the issue between *I. tiliifolia* and *I. campanulata* by identifying the diagnostic characters - lateral vein, sepal size, corolla margin and seed surface - and reexamining the type specimens of both species.

Ipomoea tiliifolia is highly variable in morphological characters as seen in Figure 2.4. However, distribution of *I. tiliifolia* is geographically distinct from *I. pringsheimiana*, which occurs exclusively in Africa and Madagascar. Additionally, these two species are recovered in different groups (Figure 2.19A) and are sister to different species (Figure 2.19B).

2.4.2.2. Species in Africa and Madagascar

There are five species of Stictocardia in Africa and Madagascar explained as follow. *Ipomoea pringsheimiana* comprises 30 specimens deposited in 13 herbaria. Verdcourt [4] sunk *Ipomoea pringsheimiana* (=*S. laxiflora*) as a subspecies of *I. beraviensis* until he later changed his mind after observing more specimens and elevated it as a distinct species [24] and recognised it in the *Flora of Tropical East Africa* [7].

Ipomoea lutambensis is diagnosed by completely glabrous leaves, partially exposed exocarp and glabrous seed. This species is an endemic of Tanzania with five collected specimens and multiple duplicates in eight herbaria, see Chapter Four.

Ipomoea incompta is a species with 23 specimens in twelve herbaria - see Chapter Four - with very distinct characters. The whole plant except corolla is covered by shaggy hairs. The sepals have filamentous outgrowths and parallel ridges on the lower half that increase in size following fruit maturity. The apex of the deltoid sepals of this species are acuminate while the rest of Stictocardia species are not acuminate.

Ipomoea macalusoi is known only from seven specimens in five herbaria, see Chapter Four. It was initially ranked as an infraspecific taxon of *I. tiliifolia* by Verdcourt [5]. Only later, he erected this taxon as a separate species after careful observation and communication with Van Ooststroom [6]. *Ipomoea macalusoi* has a restricted range on the East coast of Africa. This species is also commonly cultivated including in Mauritius and this was interpreted by earlier experts that it was a hybrid [6]. *Ipomoea macalusoi* and *I. beraviensis* share very short primary peduncle, but the former has longer sepals (>1 cm) and broadly funnel-shaped corolla, whereas the latter has shorter sepals (< 1 cm, rarely 1.2 cm) with narrowly funnel-shaped corolla.

Ipomoea beraviensis is one of the very first species transferred into *Stictocardia* [1]. This species comprises 247 specimens deposited in 16 herbaria, see Chapter Four. In the past, a number of species were synonymised under this species before they were placed as separate taxa [4, 7, 24]. This indicated that the diagnosability between these taxa was unclear. In this study, the diagnostic characters of *I. beraviensis* are sessile cymose inflorescence or often very short peduncle, longer pedicel, and multiple (up to 20) flowers in inflorescence.

2.4.3. Reciprocal illumination between morphology and phylogenetics of *I. beraviensis:* Poor sampling, incomplete story

Reciprocal illumination allows the evaluation of preliminary result based on different types of data sets. All the quantitative morphological characters of *I. beraviensis* in the Figure 2.17 A-F evidently demonstrate that the two putative groups are inseparable despite phylogenetically distinct. Further discussion of the figures is necessary to understand the nature of the data sets.

Normally, a box plot comprises a box (interquartile range/IQR, 50% of the data) and whiskers (range of upper and lower extremes, each 1.5x of the IQR) that

allow the exploration of the data sets in finer details. In extreme case like this, however, there are merely boxes with means (x symbols) and medians (horizontal lines within box) inclusive without whiskers, let alone outliers, in all data sets (Figure 2.17 A-F). This is the strong indication of very small sample size of groups under study that causes a number of issues as described below:

1. The range of the boxes, means and medians vary across individual morphological characters reflecting the variation within the data sets (Figure 2.17A-F). Noteworthy cases are the medians that are in conjunction with the end of the boxes as illustrated by the number of flowers in inflorescence (Figure 2.17C) and the number of lateral veins (Figure 2.17F). The medians are skewed and overlap with the low value at bottom of the boxes and indicate very limited, if not poor sampling.

2. With merely maximum of three samples on each putative group, the data sets fail to fulfil normal/Gaussian distribution (Figure 2.18). Therefore, a non-parametric statistical analysis, the Mann-Whitney test that accommodates <30 samples, is the only option for dealing with this situation. One approach of the test is using the mean rank and standard deviation as seen in the Table 2.13. This approach allows statistical analysis in comparing groups with minimum samples despite have to be proceeded very cautiously in this case. The test results ended in almost zero (the number of lateral veins) or zeroes (the remaining characters) that seem decisively reject the null hypothesis of that *I. beraviensis* a single taxonomic entity.

However, considering the small sample size that leads to poor and barely skewed distribution data across individual data sets (Figure 2.18), a cautionary action has to be taken. Different degrees of skewed distributions of two groups may exacerbate small differences in variances and produce large deviation to the type I

error [69] that leads to incorrect conclusion of a statistical analysis. It is misleading to conclude the significance results ($p<0.05$), which identical with type I error or false positive, that hinges on poor and barely skewed data sets. Thus, it remains preliminary to judge the presence of two putative groups in this case.

The other approach to do the Mann-Whitney test is using rank, middle term and U value. This approach, however, is not appropriate for hypothesis testing because the number of samples of the data sets ($n1=3$ and $n2=3$ or 2) have undefined values for the significance level (alpha value) =0.05. It only has a half or less of the group of indistinct species with the lowest number of samples (Table 2.12).

3. In a rare case, tied sum of rank (10.5) appears in both putative groups (Table 2.13) of the pedicle length despite differences in data distribution (Figure 2.18). Tied observation has a strong bias toward rejection of the null hypothesis, the result shown in Table 2.13. Therefore, the best strategy in dealing with the ties is by omitting them for achieving a more rigorous statistical confidence and minimizing the type I error [70], which is a false positive to accept the alternative hypothesis.

4. In the strictest sense, the Mann-Whitney test assumes no difference in the shape of data distribution between groups under study. The combination of different distribution shapes with small sample size (Figure 2.18) between putative groups under study violate the assumption the Mann-Whitney test and compromises the test result. In this case, therefore, it is a poor method in comparing the putative groups. Only the number of lateral veins that have similar data distribution between putative groups. The significant result of this character ($p<0.05$) splits *I. beraviensis* into two putative groups (Table 2.13) despite hinges on low sampling size that has been discussed above.

With the overwhelming amount of evidence above, both of the box plots and the artefact of Mann-Whitney test clearly shows flaws in conducting statistical analysis with poor sampling and skewed data. The data interpretations above are fallen short because the use of statistical analysis to the very little amount of variation covered by the very small number of samples is questionable. This case demonstrates that more sequenced samples of *I. beraviensis* are required in the future, thus, more samples are available for statistical analysis based on morphological characters. The case of I. *beraviensis* polyphyly shows that the Mann-Whitney test is not a silver bullet in solving all issues related to the non-parametric statistics despite considered extremely flexible [71].

2.4.4. Refuted PCA result of the indistinct species

Initially, PCA was carried out to explore the morphological data to possibly illuminate the relationship between characters and boundaries between species [53, 54]. This analysis was carried out on six indistinct species with overlapping morphological variation in the initial *Stictocardia* study group. These species were *I. pringsheimiana, I. queenslandica*, *I. neglecta, I. orientalis,* and *I. sivarajanii* that were found overlap with or within *I. tiliifolia* (Figure 2.5). Given that these six species do not form a monophyletic group (Figures 2.24A and 2.24B), the result of the PCA is inconclusive as it does not test -either accept or reject- the null hypothesis of a single taxonomic entity of all indistinct species due to their morphologically overlapping characters. Moreover, PCA has to be approached carefully as the may mislead the conclusion unless a restrictive set of criteria are met [53]. Having said that, these analyses have provided a simplified method to explore character variation and issues related to species delimitation.

2.4.5. Statistical test of the indistinct species prove inconclusive

A specific approach such as univariate analysis was required to test the hypothesis that was left unresolved by PCA. The Mann-Whitney test (Table 2.12) does not provide conclusive support that all indistinct species form a single taxonomic entity. After the exclusion of *I. sivarajanii* due to the inadequate number of samples, this test shows variable results across different quantitative characters. Primary peduncle length and number of flowers in the inflorescence support the separation of five indistinct species into two groups (Table 2.12), albeit different group composition in each case.

Primary peduncle length supports *I. pringsheimiana* as statistically distinguishable from the remaining indistinct species – *I. tiliifolia, I, neglecta, I. orientalis* and *I. queenslandica* (Table 2.12, Figure 2.7) in a group. This separation of *I. pringsheimiana* is congruent with geography as it is the only species that occurs in Africa and Madagascar (see Chapter Four), where the other indistinct species are absent.

The second character that distinguishes between indistinct species is the number of flowers in the inflorescence. In this case, the group of *I. pringsheimiana* and *I. neglecta* are distinguished from the group of *I. tiliifolia, I. orientalis* and *I. queenslandica* (Table 2.12, Figure 2.9). Neither geography, phylogeny nor overall morphology corroborate this hypothesis of grouping.

This study has demonstrated that univariate analysis is a useful technique to explore quantitative variation in some degree. Univariate analysis of individual characters demonstrates that this method is more appropriate than PCA in discriminating groups because it evaluates each and every quantitative character.

More sampling of *I. tiliifolia* may reduce the distance between outliers and the main body of measurements as more specimens could potentially fill existing gaps in the variation.

2.4.6. Species recovered outside the clade Stictocardia

The phylogenetic study in Chapter Three will show that *Stictocardia* is not a monophyletic group. The investigation, therefore, has been refocussed from the genus *Stictocardia* to the clade Stictocardia. Five *Stictocardia* species - *I. sivarajanii*, *I. jucunda, I. neglecta, I. orientalis* and *I. mojangensis* - are not part of Stictocardia and thus excluded from further analysis and discussion. These species are briefly discussed in Chapter Four.

The establishment of *Stictocardia* in the past was an artefact of partial and pragmatic morphological taxonomic assessments. The description of *Stictocardia* was expanded over time due to 1. The accumulation of specimens with various morphological characters, 2. Piecemeal taxonomic studies by different specialists that were carried out on a regional basis, and 3. Specialists weighed the diagnostic characters of this taxon differently [1, 3-6, 8-10, 16, 24, 27]. These factors led to a higher number of species and an expanded circumscription of the genus over time. However, this expanded circumscription has led to the current state of taxonomic instability since it deviates from the original description of this group by Hallier [1].

2.5. CONCLUSION

This study began as a taxonomic revision of a small segregate genus with approximately 13 species nested within *Ipomoea* [8, 13, 45]. To facilitate the study, loans of specimens were requested from thirteen herbaria and digital images were gathered from 35 herbaria. Protologues of all species and other relevant literature were assembled and consulted to summarise the taxonomic progress of *Stictocardia*. Two simultaneous studies - focussed on morphology and phylogenetics - were conducted and integrated via reciprocal illumination to address the taxonomic issues of *Stictocardia*.

Several species emerged from the exercise on morphology - *I. lutambensis, I. incompta, I. beraviensis, I. macalusoi, I. jucunda*, and *I. mojangensis* - as clear-cut and easily identifiable due to either unique morphological characters or particular combinations of characters. In contrast, six species were indistinct from one another due to largely overlapping morphological variation. A list of quantitative characters was drawn up as a score sheet for recording observations on a portion of specimens of these indistinct species.

Quantitative morphological characters were subjected to multivariate and univariate analyses, PCA and Mann-Whitney test respectively. The combination of these analyses was carried out to ascertain discrete gaps in the variation among indistinct species. They provide different views of the morphological data. PCA demonstrated that all indistinct species overlapped as a single taxonomic entity whereas Mann-Whitney test, was inconclusive due to contrasting results among individual quantitative characters that either support or reject the existence of multiple groups. These results were superseded by the phylogenetic study in Chapter Three.

In addition, *I. beraviensis* is treated as a single species with wide morphological range across Africa and Madagascar, albeit non-monophyletic nature. Reciprocal illumination between molecular and morphological studies do not deliver a robust conclusion of the presence multiple putative groups due to the small sample size. Inclusion of more specimens from across Africa and Madagascar is required to unravel the comprehensive picture of *I. beraviensis* taxonomy.

The phylogenetic analyses (Chapter Three) of molecular sequence data demonstrates that *Stictocardia* is polyphyletic which meant that the focus of the study was reduced in size to accommodate these findings. The phylogenetic analyses inferred a clade of seven species subsequently referred as Stictocardia. Seven species; *I. lutambensis, I. incompta, I. beraviensis, I. macalusoi, I pringsheimiana*, *I. tiliifolia,* and *I. queenslandica* form the basis of the taxonomic revision in Chapter Four. Thus, exclusion of several species - *I. mojangensis*, *I. jucunda, I. sivarajanii*, *I. neglecta,* and *I. orientalis* – from the taxonomic treatment is necessary [69-71].

List of references

1. Hallier, H., *Convolvulaceae Africanae*, in *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, A. Engler, Editor. 1893, Verlag Vos Wilhelm Engelmann: Leipzig. p. 80-160.
2. Wood, J.R.I., P. Muñoz-Rodríguez, B.R.M. Williams, and R.W. Scotland, *A foundation monograph of Ipomoea (Convolvulaceae) in the New World.* PhytoKeys, 2020. **143**: p. 1-823.
3. Hallier, H., *Bausteine zu einer Monographie der Convolvulaceen.* Bulletin de L'Herbier Boissier 1897. **5**: p. 366–387.
4. Verdcourt, B., *Notes from the East African herbarium: VI.* Kew Bulletin, 1958. **13**: p. 185-197.
5. Verdcourt, B., *Some notes on Tropical African Convolvulaceae.* Webbia, 1958. **13**: p. 321-330.
6. Verdcourt, B., *Notes from the East African herbarium: XII.* Kew Bulletin, 1961. **15**: p. 1-18.
7. Verdcourt, B., *Convolvulaceae*, in *Flora of Tropical East Africa*, C.E. Hubbard and E. Milne-Redhead, Editors. 1963, Crown Agents for Oversea Governments & Administration: London. p. 1-161.
8. Austin, D.F. and E. Eich, *Synopsis of Stictocardia with another Madagascan species, S. mojangensis (Convolvulaceae).* Willdenowia, 2001. **31**: p. 79-85.
9. Van Ooststroom, S.J., *The Convolvulaceae of Malaysia IV (with Indices).* Blumea, 1943. **5**: p. 339-411.
10. van Ooststroom, S.J. and R.D. Hoogland, *Convolvulaceae*, in *Flora Malesiana*, C.G.G.J. van Steenis, Editor. 1953, Noordhoff-Kolff N.V.: Djakarta. p. 389-512.

11. Gunn, C.R., *Notes on Stictocardia campanulata (L.) Merrill and S. jucunda (Thw.) CR Gunn (Convolvulaceae).* Brittonia, 1972. **24**: p. 169-176.
12. Biju, S.D., P. Pushpangadan, and P. Mathew, *Stictocardia sivarajanii, a new species of Convolvulaceae from Kerala, India.* Novon, 1999. **9**: p. 147-149.
13. Johnson, R.W., *Stictocardia Hallier f. (Convolvulaceae) in Queensland.* Austrobaileya, 2004. **6**: p. 631-637.
14. Austin, D.F., *Typification of the New World subdivisions of Ipomoea L. (Convolvulaceae).* Taxon, 1975. **24**: p. 107-110.
15. Simões, A.R., H. Silva, and P. Silveira, *The Convolvulaceae of Timor with special reference to East Timor.* Blumea, 2011. **56**: p. 49-72.
16. Austin, D.F. and S. Demissew, *Unique fruits and generic status of Stictocardia (Convolvulaceae).* Kew Bulletin, 1997. **52**: p. 161-169.
17. Staples, G.W. and J.F. Butaud, *A new endemic subspecies of Stictocardia (Convolvulaceae) from the Marquesas Islands, French Polynesia.* Phytologia, 2016. **98**: p. 203-206.
18. Wilkin, P., *A revision of the Infrageneric classification of the genus Ipomoea L. and the related genera in the tribe Ipomeeae (Convolvulaceae)*. 1993, University of Reading.
19. Hallier, H., *Versuch einer natürlichen Gliederung der Convolvulaceen auf morphologischer und anatomischer Grundlage.* Botanische Jahrbücher Für Systematik, Pflanzengeschichte Und Pflanzengeographie, 1893. **17**: p. 453-591.
20. Choisy, J.D., *Convolvulaceae*, in *Prodromus Systematis Naturalis Regni Vegetabilis*, A. De Candolle, Editor. 1845, Sumptibus Fortin, Masson et Sociorum: Parisiis. p. 323-465.
21. Hallier, H., *Ueber Phanerogamen von unsicherer oder unrichtiger Stellung.* Mededeelingen van 's Rijks Herbarium, 1910. **1**: p. 1-41.
22. van Ooststroom, S.J., *The Convolvulaceae of New Guinea.* Nova Guinea, 1955. **6**: p. 1-32.
23. van Steenis, C.G.G.J., *Flora Malesiana. Series I. Spermatophyta*. Noordhoff Kolff N. V., ed. C.G.G.J. van Steenis. Vol. 1. 1950, Djakarta: Noordhoff Kollf N.V.
24. Verdcourt, B., *Tropical African Plants: XXVII.* Kew Bulletin, 1963. **27**: p. 161-181.
25. Merrill, E.D., *Destruction of the Berlin herbarium.* Science, 1943. **98**: p. 490-491.
26. Verdcourt, B., *A new subgenus of Stictocardia (Convolvulaceae).* Kew Bulletin, 1990. **45**: p. 583-585.
27. Austin, D.F., D.A. Powell, and D.H. Nicolson, *Stictocardia tiliifolia (Convolvulaceae) re-evaluated.* Brittonia, 1978. **30**: p. 195-198.
28. Merrill, E.D., *The plants of Guam.* Philippine Journal of Science, Botany, 1914. **9**: p. 151-152.
29. Meeuse, A.D.J. and W.G. Welman. Flora of Southern Africa: Convolvulaceae. Vol. 28. 2000, Pretoria: National Botanical Institute.
30. Austin, D.F., *Parallel and convergent evolution in the Convolvulaceae*, in *Biodiversity and Taxonomy of Tropical Flowering Plants*, P. Mathews and M. Sivadasan, Editors. 1998, Mentor Books: Calicut. p. 234-247.
31. Brenan, J.P.M., *More new plants from the Idanre Hills, Nigeria.* Kew Bulletin, 1950. **5**: p. 227-232.
32. Kuntze, O., *Revisio Generum Plantarum: Vascularium Omnium Atque Cellularium Multarum Secundum Leges Nomenclaturae Internationales cum Enumeratione Plantarum Exoticarum in Itinere Mundi Collectarum*. Vol. 1. 1893, Leipzig: Arthur Felix.
33. von Mueller, F., *Systematic Census of Australian Plants with Chronologic, Literary, and Geographic Annotations*. Vol. 1. 1889, Melbourne: M'Carron, Bird & Company.
34. Blume, C.L., *Bijdragen tot de flora van Nederlandsch-Indië*. Vol. 13. 1825, Batavia: Ter Lands Drukkerij.
35. Garcia, M.A. and S. Demissew, *Convolvulaceae*, in *Flora of Somalia*, M. Thulin, Editor. 2006, Royal Botanic Gardens, Kew,: Kew. p. 221-258.
36. Meeuse, A.D.J., *The South African Convolvulaceae.* Bothalia, 1957. **6**: p. 643-792.

37. Deroin, T., *Famille Convolvulaceae*, in *Flore de Madagascar et des Comores*, P. Morat, Editor. 2001, Muséum National d'Histoire Naturelle: Paris. p. 11-287.
38. Goncalves, M.L., *Flora Zambesiaca, Convolvulaceae*, E. Launert, Editor. 1987, Flora Zambesiaca Managing Committee: London. p. 9–129.
39. Andrews, F.W., *Convolvulaceae*, in *The Flowering Plants of the Sudan. Vol. 3. (Compositae-Gramineae)*. 1956, T. Buncle & Co.,Ltd: Arbroath. p. 102-125.
40. Backer, C.A. and R.C. Bakhuizen van den Brink Jr., *Flora of Java (Spermatophytes only)*. Vol. 2. 1965, Groningen: Noordhoff.
41. Fang, R.-C. and G.W. Staples, *Convolvulaceae*, in *Flora of China*, Z.Y. Wu and P.H. Raven, Editors. 1995, Science Press: Beijing. p. 271-325.
42. Austin, D.F., *Convolvulaceae*. A Revised Handbook to the Flora of Ceylon. Vol. 1. 1980. 288-363.
43. Staples, G.W., B.N. Songkhla, C. Khunwasi, and P. Traiperm, *Annotated checklist of Thai Convolvulaceae.* Thai Forest Bulletin (Botany), 2005. **33**: p. 171-184.
44. Austin, D.F. and G.W. Staples, *Further notes on Turbina and Merremia: Typification and taxonomy of the Neotropical Convolvulaceae.* Journal of the Arnold Arboretum, 1986. **67**: p. 263-264.
45. Muñoz-Rodríguez, P., T. Carruthers, J.R.I. Wood, B.R.M. Williams, K. Weitemier, B. Kronmiller, Z.A. Goodwin, A. Sumadijaya, D. Harris, D. Filler, M.A. Rausher, S. Kelly, A. Liston, and R.W. Scotland, *A taxonomic monograph of Ipomoea integrated across phylogenetic scales.* Nature Plants, 2019. **5**: p. 1136-1144.
46. Staples, G.W., *A synopsis of Lepistemon (Convolvulaceae) in Australasia.* Kew Bulletin, 2007. **62**: p. 223-232.
47. Thiers, B. *Index Herbariorum: A global directory of public herbaria and associated staff*. 2021 [cited 2021 5th June]; Available from: http://sweetgum.nybg.org/science/ih/.
48. Stearn, W.T., *Botanical Latin*. 2nd ed. 1973, Portland: Timber Press.
49. Eckel, P. *A Grammatical Dictionary of Botanical Latin*. 2010 [cited 2022 2nd February]; Available from: http://www.mobot.org/mobot/LatinDict/search.aspx.
50. Choisy, J.D., *Memoires de la Société de physique et d'histoire naturelle*. 1833, Imprimerie A. L. Vignier, Rue de Rhone: Geneve.
51. Harris, J.G. and M.W. Harris, *Plant Identification Terminology: An Illustrated Glossary*. 1994, Payson: Spring Lake Publishing
52. Abràmoff, M.D., P.J. Magalhães, and S.J. Ram, *Image processing with ImageJ.* Biophotonics International, 2004. **11**: p. 36-42.
53. Parsons, K.J., W.J. Cooper, and R.C. Albertson, *Limits of principal components analysis for producing a common trait space: Implications for inferring selection, contingency, and chance in evolution.* PLOS One, 2009. **4**: p. e7957 (4 pages).
54. Bro, R. and A.K. Smilde, *Principal Component Analysis.* Analytical Methods, 2014. **6**: p. 2812-2831.
55. Team, R., *RStudio: Integrated Development for R. RStudio, PBC*. 2020: Boston.
56. Tang, Y., M. Horikoshi, and W.-H. Li, *ggfortify: Unified interface to visualize statistical results of popular R packages.* The R Journal, 2016. **8**: p. 474.
57. Streiner, D.L., *Maintaining standards: Differences between the standard deviation and standard error, and when to use each.* The Canadian Journal of Psychiatry, 1996. **41**: p. 498-502.
58. Andrade, C., *Understanding the difference between standard deviation and standard error of the mean, and knowing when to use which.* Indian Journal of Psychological Medicine, 2020. **42**: p. 409-410.
59. Cleophas, T.J. and A.H. Zwinderman, *Paired continuous data (Paired T-test, Wilcoxon signed rank test)*, in *Clinical Data Analysis on a Pocket Calculator*, T.J. Cleophas and A.H. Zwinderman, Editors. 2016, Springer: Heidelberg. p. 31-36.

60. Mann, H.B. and D.R. Whitney, *On a test of whether one of two random variables is stochastically larger than the other.* The Annals of Mathematical Statistics, 1947. **18**: p. 50-60.
61. Kruskal, W.H. and W.A. Wallis, *Use of ranks in one-criterion variance analysis.* Journal of the American Statistical Association, 1952. **47**: p. 583-621.
62. Cleophas, T.J. and A.H. Zwinderman, *Unpaired Continuous Data (Unpaired T-Test, Mann-Whitney)*, in *Clinical Data Analysis on a Pocket Calculator*, T.J. Cleophas and A.H. Zwinderman, Editors. 2016, Springer: Heidelberg. p. 37-43.
63. Sluys, R., *The unappreciated, fundamentally analytical nature of taxonomy and the implications for the inventory of biodiversity.* Biodiversity and Conservation, 2013. **22**: p. 1095-1105.
64. Scotland, R.W. and J.R.I. Wood, *Accelerating the pace of taxonomy.* Trends in Ecology & Evolution, 2012. **27**: p. 415-416.
65. Olaranont, Y., F.W. Stauffer, P. Traiperm, and G.W. Staples, *Investigation of the black dots on leaves of Stictocardia species (Convolvulaceae) using anatomical and histochemical analyses.* Flora, 2018. **249**: p. 133-142.
66. Guppy, H.B., *Observations of a naturalist in the Pacific between 1896 and 1899: Plant-dispersal*. Vol. 2. 1906, London: Macmillan and Company.
67. Muñoz-Rodríguez, P., T. Carruthers, J.R.I. Wood, B.R.M. Williams, K. Weitemier, B. Kronmiller, D. Ellis, N.L. Anglin, L. Longway, S.A. Harris, M.D. Rausher, S. Kelly, A. Liston, and R.W. Scotland, *Reconciling conflicting phylogenies in the origin of sweet potato and dispersal to Polynesia.* Current Biology, 2018. **28**: p. 1246-1256.
68. Aswani, K. and M. Sabu, *Pollination biology of Stictocardia tiliifolia (Desr.) Hall.f. (Convolvulaceae).* The International Journal of Plant Reproductive Biology, 2012. **4**: p. 5-8.
69. Fagerland, M.W. and L. Sandvik, *The Wilcoxon-Mann-Whitney test under scrutiny.* Statistics in Medicine, 2009. **28**: p. 1487-1497.
70. McGee, M., *Case for omitting tied observations in the two-sample T-test and the Wilcoxon-Mann-Whitney test.* PLOS One, 2018. **13**: p. e0200837 (19 pages).
71. Moczko, J.A., *Do we always correctly interpret the results of statistical nonparametric tests.* Przeglad Lekarski, 2014. **71**: p. 654-658.

CHAPTER 3. PHYLOGENETIC STUDY OF *STICTOCARDIA* OF THE OLD-WORLD *IPOMOEA*

3.1. BACKGROUND

3.1.1. Phylogenetic and phylogenomic studies of Convolvulaceae and *Ipomoea*

Previous phylogenetic studies of Convolvulaceae and Ipomoeeae based on morphological and molecular [1-6] data have concluded that *Ipomoea,* as traditionally understood, is a polyphyletic group, with nine smaller genera nested within it. An expanded *Ipomoea* that includes all segregated genera would allow the recognition of a monophyletic group and this has been recently proposed [5, 7, 8]. These studies used several nuclear (*nr*ITS, waxy) and chloroplast (*rbc*L, *atp*B, *psb*E-J operon, and *trn*L-*trn*F intron/spacer) DNA markers, as well as whole chloroplast genome (plastome) and putative single-copy nuclear DNA regions [1, 2, 4, 5, 9].

Stictocardia is currently subsumed under *Ipomoea*. *Stictocardia* has never been the primary target of a phylogenetic investigation and only a limited number of species have been sampled previously in such studies [1-3, 7, 9]. These studies did not sample across *Stictocardia* species diversity but rather focussed on a few readily available species. The bias of taxon sampling only covered several species such as the widespread *Ipomoea tiliifolia,* the Tanzanian endemic *I. lutambensis, the* morphologically extreme *I. incompta,* and the widely distributed in Africa *I. beraviensis*. Other geographically restricted and poorly studied species in Indonesia, Australia, Sri Lanka or India have never been sampled. Importantly, the incorporated species were often represented by a single specimen, thus their monophyly remains untested by

molecular sequence data. In summary, the monophyly and evolutionary relationships between species of *Stictocardia*, now treated as part of *Ipomoea,* are mostly unknown.

The work by Muñoz-Rodriguez et al. [5] is the first study that provides a more comprehensive phylogeny of the Old World (OW) clade of *Ipomoea*, a well-supported clade that includes almost half of the species in the genus. That study, using both extensive taxon sampling and several specimens of the same species, suggested that *Stictocardia* species may not form a monophyletic group within *Ipomoea*, although it only included a few species of *Stictocardia*, with some species missing. Therefore, the aim of this thesis was to use a more comprehensive taxon sampling, with multiple specimens per species, to fully understand the status and delimitation of *Stictocardia* within *Ipomoea.* This thesis presents phylogenetic analyses using DNA barcode and High-Throughput Sequencing similar to those by Muñoz-Rodriguez et al. [5] but using a more comprehensive taxon and specimen sampling.

3.1.2. Taxon and character sampling of *Stictocardia*

Adequate taxon and character sampling are essential for phylogenetic reconstruction [10, 11]. Comprehensive taxon sampling, for instance, has been linked with increased accuracy [12-14] and reduced error [15] as well as improved resolution of deep branches [16].

In addition, sampling of multiple specimens of a given species is also important to investigate species monophyly [17] and whether there has been sufficient time for speciation to occur [2]. Monophyly is commonly adopted as the main principle in different versions of the Phylogenetic Species Concept [18, 19] combined with diagnosability [20].

3.1.3. Outgroup comparison

Selection of an outgroup is an important step in any phylogenetic study [21-23]. Ideally, the selection of outgroups is based on previous knowledge of all the closest relatives of the group under study, known as the ingroup [24]. The ongoing monographic study of *Ipomoea* in the University of Oxford incorporates dense taxon sampling across the whole of *Ipomoea* (as outgroup), which allows testing the monophyly of *Stictocardia* (as ingroup) rigorously by incorporating a broad range of other putative outgroup taxa across *Ipomoea*.

3.1.4. DNA barcoding (Nuclear and Chloroplast)

Our knowledge of plant diversity and species relationships has been revolutionised by the advent of DNA barcoding technology [25-29] as a reliable, cost-effective, and accessible tool to assess biodiversity [30-34]. Barcoding utilises DNA barcodes, standardised short (400—800 bp) DNA segments, despite sequence of some regions are longer, that are primarily used for species identification [35, 36]. Barcode data has been claimed to be valuable for addressing taxonomic issues such as testing taxonomic hypotheses, accelerating species discovery [29, 37] and detect past errors that lead inaccurate identification [38]. On the other hand, choosing and refining the correct barcode(s) can create a challenge for species delimitation [39]. Therefore, a combination of barcodes has been used as a common strategy to yield the best discriminatory power [40-42].

Nuclear (*nr*)ITS, one of the most frequently used DNA barcodes for plant and fungi [28, 33, 34, 43, 44], is used in systematic studies at different hierarchical levels [45-49] and it seems that its advantages outweigh the limitations [41, 42, 44, 50]. In

addition, even a short fragment of *nr*ITS, ITS2, can discriminate species boundaries [51] when recovery of the full *nr*ITS sequence is difficult to achieve [39, 52-55].

Apart from nuclear regions, several chloroplast regions such as *rbc*L, *mat*K, *trn*H-*psb*A or *trnL-trnF* have been proposed as standard barcodes and frequently utilised to infer plant phylogenies and discriminate between species [32, 39, 40, 56]. In addition, a series of studies have been published to identify a number of variable slow and fast evolving regions of the chloroplast for phylogenetic studies [57-59]. More recently, the trend shows that the whole chloroplast genome (plastome) slowly replaced individual chloroplast markers in phylogenetic analyses [60].

3.1.5. Plastome and Genome Skimming

Currently, more and more plastomes are being assembled from various plant species and used for phylogenetic studies [1, 5, 60-63]. Plastome is treated as a single locus in such studies [64-66]. This genome is characterised by a highly conserved gene order, absence of recombination and low levels of nucleotide substitution that reflects the slow mutation rate [67, 68]. The plastome is traditionally known for maternal inheritance although there is evidence of biparental and paternal inheritance in many species [68, 69]. In Angiosperms, the plastome is commonly represented as a circular structure with a large single copy, a small single copy and two inverted repeats [58, 70], although at least one of these inverted repeats are missing in several plant groups [68]. Recently, the assumption of the circularity of the chloroplast genome has been challenged as a misconception that has a potential to cause inaccurate reconstruction of phylogeny [71].

Sufficient data to assemble the chloroplast genome can be retrieved through genome skimming [72], a shallow-sequencing technique of high-throughput

sequencing that targets high copy fractions in the genome [30, 73, 74]. Genomic-scale sequencing yields large numbers of short reads, thus it is most advantageous to cope with DNA extracted from herbarium specimens in which the DNA is typically fragmentary in nature [52, 54, 55, 75, 76].

Contemporary phylogenetic studies often use a combination of nuclear and either chloroplast regions or whole plastome [64, 65, 77-79]. This provides a comprehensive picture of nuclear and organelle evolution including the observed discordance between them [80-83].

3.2. MATERIALS AND METHODS

Plant material for this thesis was obtained from herbarium specimens and followed the workflow in Figure 3.1. Most herbarium specimens of *Stictocardia* studied are often poorly preserved and were collected more than 40 years ago. Thus, selection of samples for DNA extractions was determined by the colour [84] and age of the specimens [53] —although there are cases of successful extractions from older and darker specimens [52, 84].

3.2.1. DNA extraction

Approximately 0.5 cm^2 of leaf material was used of the specimens listed in Appendix 6. This material was disrupted by TissueLyser (QIAGEN) at the frequency of 1/25 or smaller for 2 minutes because non-sufficient tissue disruption may lead to low DNA yield [85]. Initially, a number of DNA extractions were conducted using a hybrid of CTAB + silica binding protocols [54]. Later on during the project, DNEasy® Plant Mini Kit (QIAGEN) was used to avoid CTAB and RNA contamination and obtain as much pure DNA as possible [85]. At the end of the process, DNA diluted in 60 µl water or AE buffer was stored in a -20°C freezer. The content of the DNA in the

aliquots was measured by Spectrophotometry using Nanodrop ND -1000. Samples with higher nanodrop values (Q unit >50 µg/µl) and lower contamination levels (1.8—2.0) were prioritised for DNA amplification.

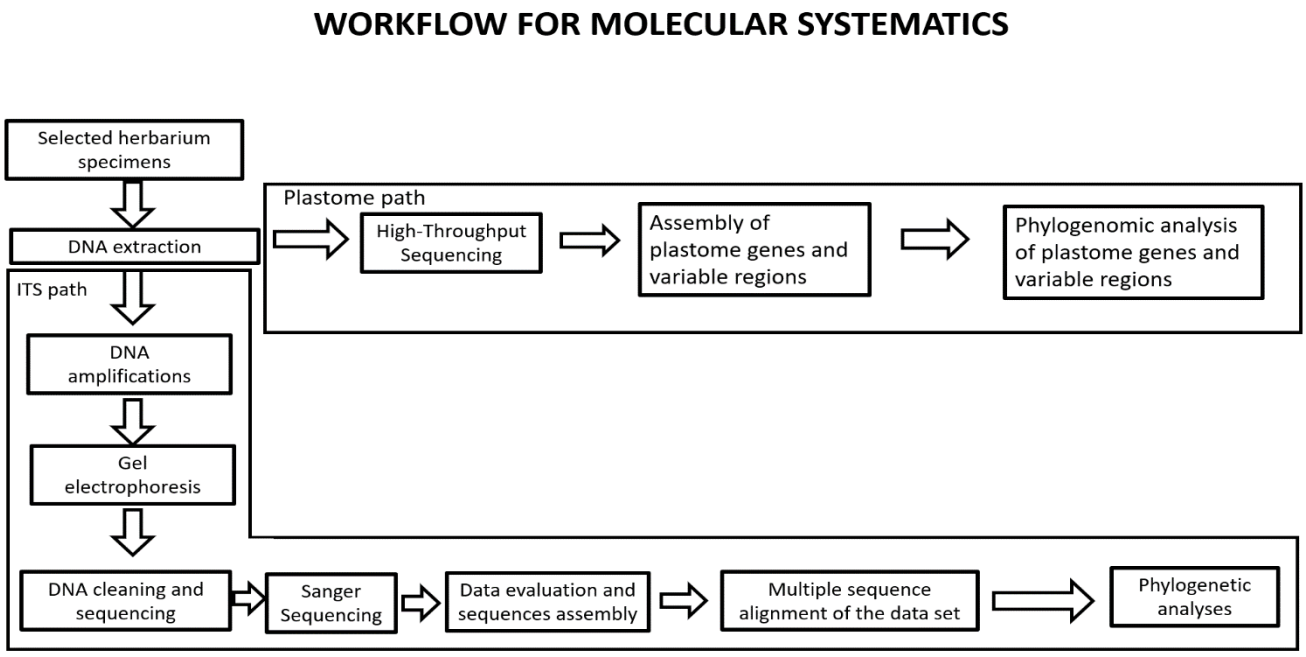


Figure 3.1. Workflow for molecular systematics from DNA extraction of herbarium specimens to phylogenetic analyses. There are two paths involved here that run independently,each with different stages *i.e*., Sanger sequencing for *nr*ITS and HTS for plastome.

3.2.2. DNA amplifications of *nr*ITS

For *nr*ITS amplification, 15 µl cocktail was prepared by mixing 6.3 µl Milli-Q, 3 µl QIAGEN® PCR Buffer 10X, 0.7 µl $MgCl_2$ 25 mM, 0.5 µl dNTPs, 2 µl Betaine, 0.3 µl forward primer AB101/3, 0.3 µl reverse primer AB102, 0.4 µl Taq DNA polymerase and 1.5 µl DNA sample. DNA of a living specimen of *I. batatas* growing in the greenhouse (1.5 µl) was used as a positive control. Meanwhile, 1.5 µl Milli-Q serves as a negative control to detect possible contamination. The *nr*ITS amplifications followed White et al. [86].

3.2.3. Gel electrophoresis

Agarose gels (1.5%) were made by adding 1x TAE to 1.5 g of agarose powder (Invitrogen) up to 100 ml. The suspension was boiled for 2 minutes at the highest temperature setting of a microwave. Four µl of Ethidium Bromide (Bio-Rad) or SYBR Safe was added to the liquid, mixed, and then poured into a plastic container with a 12 or 18-well gel comb. Following solidification, the gel was removed to a FlowGen water bath. Four µl of each amplification sample was pippetted into individual well. The electrophoresis ran at 130 volts for 15 minutes. The result of amplification and amplicons quality was observed under UV illumination using Visionworks 3.0.0. Wells with bright band indicated successful amplification. The remaining volume of the successful amplification was cleaned at the next phase.

3.2.4. DNA cleaning and sequencing

Sixty µl 20% PEG/2.5M NaCl was added to the samples inside the tubes. After a quick vortex and 30 minutes of incubation at room temperature, the samples were centrifuged at 4°C, 2750 g for 1 hour. Next, the supernatant was discarded by centrifugation at 500 g for 1 minute. The remaining pellet was washed with 150 µl cold 70% Ethanol and centrifuged at 2750 g for ten minutes. The tubes were positioned upside down and centrifuged at 500 g for 1 minute to completely drain the excess liquid. The washing and spinning steps were repeated once to ensure the purity of the DNA product.

Cocktail for 50 reactions of DNA re-amplification was prepared by mixing 93.8 µl Milli-Q, 93.8 µl 5x Buffer, 12.5 µl BigDye Terminator v3.1 (ABI), and 200 µl of diluted 1:15 of either forward or reverse primers. Eight µl of cocktail was dispensed into each tube, and two µl of purified DNA sample was added. The tubes were mixed and spun

at 500 g for 1 minute to settle the aliquot at the bottom of the tubes. Re-amplification was conducted for 30 cycles, consisting of denaturation at 96°C for ten seconds, annealing at 50°C for 5 seconds, and elongation at 60°C for 2 minutes. After the re-amplification finished, a volume of 15 µl sterile dH_2O was pipetted to each tube. Fifty-two µl mixture of seven ml 95% EtOH and 280 µl 5M NaOAc pH 5.2 was dispensed into each tube. After a quick vortex, the samples were incubated at room temperature for 45 minutes. Following centrifugation at 500 g for 1 minute, the samples were washed with 150 µl 70% Ethanol and spun at 2750 g for ten minutes to remove excess liquid before long-term storage at -20°C freezer**.**

3.2.5. Sanger Sequencing and data evaluation of the *nr*ITS

Ten µl of cleaned DNA product of each sample were prepared and sent in batches to Source BioScience (https://www.sourcebioscience.com/) at Oxford, UK to carry out Sanger sequencing. The generated chromatogram data were sent back in ab1 format. Quality of the chromatograms from Source BioScience was assessed in Geneious Prime [87]. Poorly aligned sections at the beginning (ambiguous and unsynchronised peaks) and the end (flat peaks) of sequences were discarded. Subsequently, the presence of multiple adjacent peaks in a single nucleotide position were carefully checked by visual inspection and manually edited, if necessary. The same software [87] was also used to align 5' end and 3' end of each sequence.

3.2.6. Multiple sequences alignment of the *nr*ITS data set

The evaluated sequences were incorporated to the existing *nr*ITS data set that already existed for *Ipomoea* [5]. The alignment has 36 specimens representing twelve species of *Stictocardia*. In addition, 74 other species of *Ipomoea* were also incorporated to test the monophyly of *Stictocardia*, 12 from the New World clade and

62 from the Old-World clade as defined in the previous study. Three species from different genera, *Evolvulus, Operculina* and *Jacquemontia*, were included to root the tree.

The updated ITS data set was aligned using MAFFT [88]. The aligned data set was checked by visual inspection to ensure no anomaly or ambiguous position. The overall data set was trimmed at the beginning and the end of the alignment to optimally balance the number of nucleotides in the alignment. Partial sequences were omitted from the alignment for uniformity and minimise missing data.

3.2.7. Phylogenetic analysis of the *nr*ITS data set

The *nr*ITS data set was analysed using three inference methods, Maximum Parsimony (MP), Approximate-Maximum Likelihood (A-ML) and Bayesian Inference (BI). In MP, the analysis was executed in PAUP [89] or MEGA X [90] using heuristic search, with gaps treated as missing data, stepwise addition with 100 pseudo-replicates and TBR branch swapping. Bootstrap replicates were conducted with 'fast' stepwise addition, retaining groups with >50% frequency from 100 replicates. For A-ML and BI, the selected model followed the result of the Model test [91] in MEGA X as seen in Appendix 7. The best model fits for analysis is the one with the lowest BIC (Bayesian Information Criterion) value [90]. A-ML was run using FastTree 2 with GTR + Gamma model in Geneious Prime [87]. For Bayesian Analysis, MrBayes in CIPRES [92, 93] was used via a plug-in within Geneious Prime [87]. This analysis also used the GTR + Gamma model and run for 10,000,000 generations with 2,000,000 burn-in, sampling trees every 100,000 generations. Posterior support values are presented in the phylogenies as percentages.

3.2.8. High-Throughput Sequencing

In parallel with Sanger sequencing, selected DNA extracts were sent to the Center of Genome Research and Biocomputing/CGRB (https://cgrb.oregonstate.edu/core) at Oregon State University, United States for high-throughput sequencing. The selected 29 specimens included rare species, geographically widespread collections of the same species, and representatives of as many species as possible given the available DNA of the required quality and quantity. CGRB conducted genome skimming for generating whole plastome sequence using Illumina HiSeq 3000.

3.2.9. Assembly of plastome genes and variable regions

All the steps in this section were conducted by Pablo Muñoz-Rodriguez D.Phil. Paired-end Illumina sequence reads (150 bp) were obtained using Illumina HiSeq 3000 at the CGRB. Sequence files were filtered using default parameters in Trimmomatic [94] and read quality was checked using FastQC [95]. All reads of a given sample were mapped to a reference nuclear genome, a bacteria genome, a reference *phix* virus genome and a mitochondrial genome using BBMap v.38.80 [96] to remove non-plastome reads from the data set. The remaining reads were mapped to a full plastome reference sequence [97] - deposited on GenBank (NC_026703.1) individually. The assemblies were processed using Samtools [98], Picard Tools [99] and GATK [100] to edit the alignments, remove duplicate reads and realign the assemblies around indels, respectively. The custom script for the whole process is available in Appendix 8. In parallel, a second assembly using only a number of chloroplast regions - instead of the whole genome - was conducted (see details below). The aim of this alternative strategy was to overcome the effect on the

phylogenetic analysis of poorly assembled regions near the edges of the inverted repeats.

3.2.10. Phylogenomic analysis of plastome genes and variable regions

The steps in this paragraph were conducted by Pablo Muñoz-Rodriguez D. Phil. The sequences were aligned using MAFFT v.7.310 [101, 102] and poorly aligned regions were removed using default parameters (all gaps) in Gblocks [103, 104]. Subsequently, Maximum Likelihood phylogenies were inferred using IQTREE [105] with automatic model inference. In the analyses of genes and of variable regions, regions were aligned individually and subsequently concatenated for phylogenetic analysis using AMAS [106].

Subsequently, I inferred genomic phylogenies from three independent data sets: one using whole plastome data (as a single long molecule), the second one using 28 chloroplast genes and the third one using the 26 most variable regions in the plastome as identified by Shaw et al. [57-59].

Concatenated alignments were analysed using FastTree for A-ML. For BI, the analysis used GTR + Gamma following the result of Model Test and run for 10,000,000 generations with 2,000,000 burn-in and sampled tree every 100,000 generations. The data sets comprise 25 specimens of ten ingroup species and 58 outgroup species, which are *Operculina pteripes* (as root), ten *Ipomoea* species of the NW clade and 47 *Ipomoea* species across the OW clade [5] to test the monophyly of the study group rigorously.

The results of the three analyses using different data sets are largely congruent, although the 28 plastome genes (PG) and 26 variable regions (VR) are preferred for

the reasons explained above. The custom script used for the whole process is seen in Appendix 9.

3.3. RESULT

Results of the nuclear and plastome data sets are presented here. From the nuclear genome, the *nr*ITS data set was analysed. Meanwhile, the plastome has two data sets; one including 28 genes and the other including the 26 most variable regions (Table 3.1). The ITS alignment is 748 bp long that includes 115 sequences, 38 from *Stictocardia* and 77 outgroups (Table 3.2). Plastome data sets include more data (64,895 and 47,893 bp for genes and variable regions, respectively) but have a more limited taxon and specimen sampling (Table 3.2) than *nr*ITS. The length of the individual genes ranges from 402 bp (*atp*E) to 4206 bp (*rpo*C2), whilst individual variable regions range from 438 bp (VR 14) to 4561 bp (VR 7) as seen in Table 3.1. Average coverage for most plastome regions is 100 % except a small number of them (*ndh*D*, rbc*L*,* and two variable regions VR8/ *trn*C GCA - *psb*M and VR18/*ndh*C - *tnr*V UAC).

Nuclear ITS and plastome phylogenies are presented in Figures 3.2A and 3.2B, respectively. The Maximum Parsimony analysis of *nrITS* data retrieved the most parsimonious tree with 1831 steps, with CI=0.405, RI= 0.611, RC= 0.247, and HI=0.595. MP analysis could not be conducted for the chloroplast genes and variable regions data sets as the computer run out of memory while using PAUP [89] or stopped because of the computational burden in MEGA X [90]. The likelihood values (lnL) across data sets for A-ML and BI analyses are shown in Appendix 7.

Table 3.1. The length of region and average percent coverage of PG (left) and VR (right) data sets

PG			VR		
Gene	**Length (bp)**	**Percent Cover**	**Variable region**	**Length (bp)**	**Percent Cover**
***atp*A**	1524	100	**VR1 (*trn*H GUG - *trn*K UUU)**	1598	100
***atp*B**	1473	100	**VR2 (*trn*K UUU - *trn*K UUU)**	2611	100
***atp*E**	402	100	**VR4 (*rps*16 - *trn*Q UUG)**	2026	91.5
***atp*F**	1260	100	**VR5 (*trn*S GCU - *trn*G UUC)**	1562	100
***ccs*A**	954	100	**VR6 (*atp*H - *atp*I)**	2105	100
***cem*A**	690	100	**VR7 (*rpo*B - *trn*C GCA)**	4561	100
***clp*P**	1166	100	**VR8 (*trn*C GCA - *psb*M)**	2214	99.7
***ndh*B**	2198	100	**VR9 (*psb*M - *trn*D GUC)**	837	100
***ndh*D**	1512	99.8	**VR10 (*trn*D - *trn*T GGU)**	1067	100
***ndh*G**	531	100	**VR11 (*trn*T - *psb*D)**	2444	100
***ndh*H**	1182	100	**VR12 (*trn*S UGA - *trnf*M CAU)**	1327	100
***ndh*I**	504	100	**VR13 (*trn*S GGA - *rps*4)**	1008	100
***ndh*K**	894	100	**VR14 (*rps*4 - *trn*T UGU)**	438	100
***pet*B**	1403	100	**VR15 (*trn*T UGU - *trn*L UAA)**	950	100
***pet*D**	525	100	**VR16 (*trn*L UAA - *trn*F GAA)**	872	100
***psa*A**	2253	100	**VR17 (*trn*F GAA - *ndh*J)**	1125	100
***psa*B**	2205	100	**VR18 (*ndh*C - *tnr*V UAC)**	1278	99.5
***psb*C**	1422	100	**VR19 (*acc*D - *psa*I)**	2372	100
***rbc*L**	1464	99.2	**VR20 (*pet*A - *psb*J)**	1954	100
***rpl*2**	816	100	**VR21 (*psb*E - *pet*L)**	1475	100
***rpl*22**	471	100	**VR22 (*rp*L20 - *rps*12)**	1278	100
***rpo*A**	1014	100	**VR23 (*psb*B - *psb*H)**	2382	100
***rpo*C2**	4206	100	**VR24 (*rpl*36 - *rpL*14)**	1594	100
***rps*11**	417	100	**VR26 (*ndh*F- *rpl*32)**	2798	100
***rps*3**	657	100	**VR27 (*rpl*32 - *trn*L UAG)**	1161	100
***rps*7**	468	100	**VR28 (NC *ndh*A - NC *ndh*A)**	1441	100
***trn*V-UAC**	659	100			
***ycf*4**	555	100			

Table 3.2. Statistics of multiple sequences alignments across nuclear (ITS) and plastome (PG and VR) data sets

	Nuclear ITS	**PG**	**VR**
No of specimens	115	83	83
***Stictocardia* specimens**	38	25	25
Outgroup specimens	77	58	58
Alignment length (bp)	748	64835	47893
No variable sites	406	4842	6181
Proportion variable sites	0.543	0.075	0.129
Parsimony informative sites	289	2018	2658
Proportion parsimony informative sites	0.386	0.031	0.055

3.3.1. The genus *Stictocardia* and the clade Stictocardia

The phylogenies based on *nr*ITS and plastome (Figure 3.2A and 3.2B) refute the monophyly of *Stictocardia.* Seven species of *Stictocardia* form a clade, which comprises seven species, *i.e. I. tiliifolia, I. queenslandica, I. lutambensis, I. beraviensis, I. macalusoi, I. pringsheimiana* and *I. incompta* (Table 3.3). Given that this clade consists exclusively of species of *Stictocardia*, it is reasonable to name this group *Stictocardia sensu stricto* (*s.s.*) or Stictocardia (non-italic), The remaining five species of *Stictocardia* are more closely related to multiple species of *Ipomoea* than to species inside Stictocardia. This study confirms that *Stictocardia* is a polyphyletic group as suggested by Muñoz-Rodriguez et al. [5]. In light of this result the ingroup had to be modified to satisfy the requirement that taxonomic accounts should be based on monophyletic groups [107-110].

The phylogenetic results indicate that at least two options are possible relative to the scale of a taxonomic account. One possibility is to include all species of *Stictocardia* and thus expand the study group by incorporating a high number of species of *Ipomoea* across the phylogeny. This option, however, requires sampling of all the potentially related *Ipomoea* species, and due to lack of relevant material and time constraints this seemed unrealistic.

In contrast, the second possibility is to focus on the monophyletic group that includes seven species of *Stictocardia* in the clade Stictocardia. This option, therefore, is realistic and justifiable considering the consistent species composition of this clade across phylogenetic inference of nuclear and chloroplast data. Five species are thus excluded from Stictocardia, *i.e. I. orientalis, I. neglecta, I. sivarajanii, I. jucunda* and *I. mojangensis. Ipomoea neglecta* is conservatively treated outside of Stictocardia because it is recovered outside of the clade in the plastome phylogeny (Figure 3.2B).

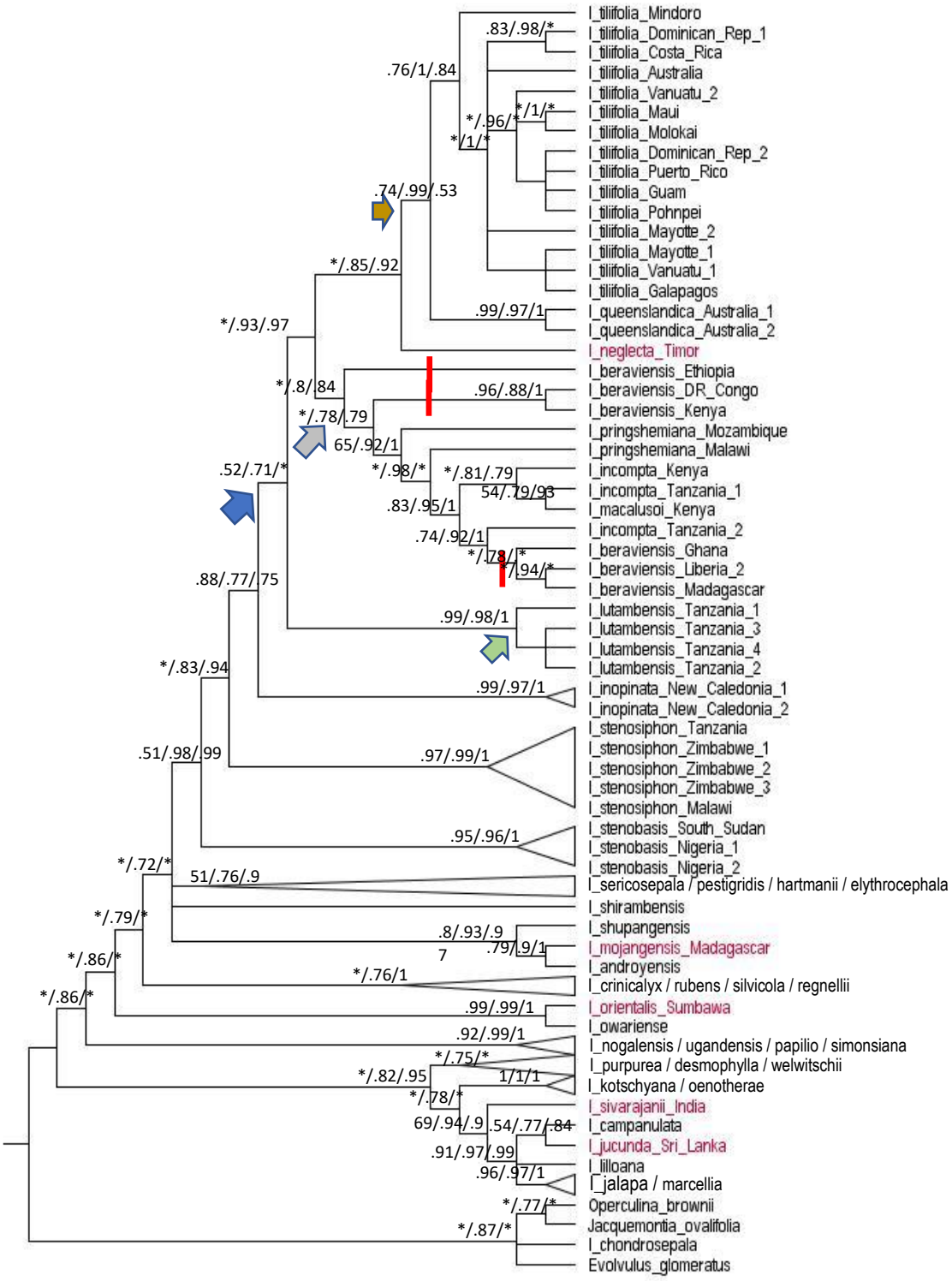


Figure 3.2A. Inferred *nr*ITS phylogeny of the OW *Ipomoea* focusses on Stictocardia (blue arrow). Stictocardia comprises three groups (smaller arrows), Tiliifolia (brown), Beraviensis (grey) and Lutambensis (green). Red coloured species refer to *Stictocardia* species recovered outside Stictocardia. *Ipomoea neglecta* is treated outside Stictocardia based on plastome phylogeny (see Figure 3.2B). Some clades are collapsed for simplicity and named by a group of *Ipomoea* species it comprised. Numbers on nodes refer to the bootstrap support percentage (in decimal) of Maximum Parsimony/Approximate-Maximum Likelihood/Bayesian Inference. Asterisks represent support less than 0.5. Some species are unresolved as monophyletic groups *i.e.*, *I. beraviensis, I. pringsheimiana,* and *I. incompta*. Red lines mark *I. beraviensis* as this species appears polyphyletic in the plastome phylogeny (see Figure 3.2B).

Figure 3.2B. Inferred plastome phylogeny of the OW *Ipomoea* focusses on Stictocardia (blue arrow). Stictocardia comprises three groups (smaller arrows), Tiliifolia (brown), Beraviensis (grey) and Lutambensis (green). Red coloured species refer to *Stictocardia* species recovered outside Stictocardia. Some clades are collapsed for simplicity and named by a group of *Ipomoea* species it comprises. Numbers on nodes refer to the bootstrap support percentage (in decimal) of A-ML (left) and posterior percentage of BI (right) of 28 plastome genes/PG (top) and 26 variable regions/VR (bottom). Only *I. beraviensis* (red lines) unresolved as a monophyletic group in Stictocardia.

3.3.2. Groups and species composition across phylogenetic trees

The plastome (PG and VR) phylogenies provide better resolution than the *nr*ITS phylogeny, presumably because they possess more characters and variable sites than the *nr*ITS tree. Several studies have shown that plastome provides useful phylogenetic information [111-113] and powerful at species level discrimination [114]. Despite the discordance, phylogenies based on the *nr*ITS and plastome data sets (Figure 3.2A and 3.2B) consistently recover three groups within Stictocardia with strong support. These groups are named based on a single occurring species / monospecific (Lutambensis/green arrow/*nr*ITS MP=0.99; A-ML=0.98; BI=1; PG and VR ML/BI=1), globally widespread species (Tiliifolia/brown arrow/*nr*ITS MP=0.74; A-ML=0.99; BI=0.53; PG and VR ML/BI=1), and regionally widespread species in Africa (Beraviensis/grey arrow/*nr*ITS A-ML=0.79; BI=0.84; PG and VR ML/BI=1).

The three groups show congruence on species composition although the relationship between them differs between the nuclear and the plastome trees. Composition wise, the Tiliifolia group includes two species *I. tiliifolia* and *I. queenslandica*, the Beraviensis group has four species, *I. beraviensis, I. macalusoi, I. pringsheimiana* and *I. incompta*, and the Lutambensis group is exclusively of *I. lutambensis*.

In the *nr*ITS tree (Figure 3.2A) several species are unresolved. Only three species are recovered as monophyletic, *i.e. I. lutambensis*, *I. tiliifolia* and *I. queenslandica* (Table 3.3). The remaining species are members of the Beraviensis group, *i.e. I. beraviensis, I. pringsheimiana,* and *I. incompta*, that are unresolved as monophyletic groups reflecting the limited amount of data that this single marker contains. However, this region also shows strong geographical affinity for species

grouping. The monophyly of *Ipomoea macalusoi* could not be tested as it is represented by a single specimen.

The topology of the plastome tree, in contrast, has five of the seven species as monophyletic (Table 3.3). *Ipomoea beraviensis* recovers as two separate entities, which will be discussed below. *Ipomoea macalusoi* is not included here given the poor quality of the single sample sequenced.

3.4. DISCUSSION

This is the first phylogenetic study focussed on *Stictocardia* within the context of *Ipomoea,* which is discussed below.

3.4.1. Polyphyly of *Stictocardia*

Inclusion of multiple species of *Ipomoea* outside the study group is necessary to test the monophyly of the ingroup rigorously, as mentioned by Barriel and Tally [115] and Maddison et al. [116]. Incorporation of multiple species is necessary to break long branches and avoid Long Branch Attraction (LBA) [117], which is an accumulation of homoplastic changes between distantly related taxa that attract each other when taxon sampling is sparse [118]. LBA can produce inaccurate [119] or misleading results [12, 22, 120], in this case compromising the test of monophyly of *Stictocardia* within *Ipomoea*.

Incorporation of the multiple species of *Ipomoea* in the phylogenetic study of *Stictocardia* demonstrates that this taxon is polyphyletic. The non-monophyletic status of *Stictocardia* rejects previous infra-generic classifications based on morphology [121, 122]. The phylogenies inferred in this thesis (Figure 3.2A and 3.2B) place five endemic, non-African species of *Stictocardia* - *I. neglecta, I. mojangensis, I. orientalis, I. sivarajanii,* and *I. jucunda* - outside of the clade Stictocardia. Individually, each one

of these species is more closely related to another species of *Ipomoea* than to the seven species of Stictocardia, which is now the focus of this taxonomic revision. However, the monophyly of these excluded *Stictocardia* species remains untested as they were represented merely by a single specimen each. Thus, this may affect LBA to occur between these species and distantly related species of *Ipomoea* that accumulates homoplastic changes.

3.4.2. Monophyly of Stictocardia

As a clade, the monophyly of Stictocardia is strongly supported regardless of the data set analysed and the methods of phylogenetic inference, as seen in Figure 3.2A and 3.2B. The recovery of this clade is relatively consistent of seven species across phylogenies within this and the previous study [5] that have justified to focus the taxonomic revision on this monophyletic group.

Five species of Stictocardia are in Africa and Madagascar; *I. lutambensis*, *I. beraviensis, I. pringsheimiana, I. incompta* and *I. macalusoi.* In the plastome phylogeny, these five species are within two clades/groups that sister to each other and suggest the presence of a clade that exclusively in Africa and Madagascar. *Ipomoea lutambensis* is endemic to Tanzania and its monophyly has been suggested by Muñoz-Rodriguez et al. [5]. The relationship of this species to the remaining species in Stictocardia is inconclusive as its placement differs between *nr*ITS and plastome phylogenies (Figure 3.2A and 3.2B). In the *nr*ITS phylogeny, *I. lutambensis* appears basal and is sister to all other species in the clade. Meanwhile, in the plastome tree, it is sister to all species in Africa, a pattern similar to the *nr*ITS topology in Muñoz-Rodríguez et al. [5].

This discordance between nuclear and organelle phylogenies here evidently reflects the independent evolutionary trajectories between them [123]. Cyto-nuclear discordance has also been observed in a number of studies across plant families and in *Ipomoea* [65, 81, 124]. More data is needed to confirm the correct placement of *I. lutambensis* within Stictocardia.

In the *nr*ITS tree (Figure 3.2A), three species - *I. beraviensis, I. pringsheimiana, I. incompta* - are unresolved as monophyletic groups (Table 3.3.). Despite *nr*ITS being a universal DNA barcode marker with a number of advantages [33, 42, 44, 50], it may not be informative [125] for delimitation of these species.

In the *nr*ITS tree (Figure 3.2A), *I. beraviensis* is represented by multiple specimens from Western and Eastern Africa plus Madagascar and unresolved as a monophyletic group. A single specimen from Madagascar is nested within the Western African group although it is geographically closer to the Eastern African group. In contrast*, I. beraviensis* is recovered as a polyphyletic group in the plastome phylogeny (Figure 3.2B), with two distantly related taxa, the Western Africa and Eastern Africa. Unfortunately, no sample from Madagascar was included in this analysis. In the plastome phylogeny, the only specimen from Western Africa is sister to all other species in Africa except *I. lutambensis*. Meanwhile, *I. beraviensis* in Eastern Africa is sister to *I. pringsheimiana* and as a group they are sister to *I. incompta* (Figure 3.2B and Table 3.3). *Ipomoea incompta* and *I. pringsheimiana* are resolved as monophyletic groups in the plastome tree (Figure 3.2B and Table 3.3).

Considering the existence of two entities that are separated geographically, *I. beraviensis* demands further study by extending sampling of multiple specimens from unsampled areas to shed light on its evolutionary history. This is necessary to get a comprehensive picture and establishes a species-level taxonomic decision rigorously.

Table 3.3. The monophyly support values of Stictocardia clade, groups, species, and unresolved sister taxon across data sets and inference methods

	***nr*ITS**			**PG**		**VR**	
	MP	**A-ML**	**BI**	**A-ML**	**BI**	**A-ML**	**BI**
Stictocardia	0.52	0.71	UR	0.93	1	0.95	1
Tiliifolia group	0.74	0.99	0.53	1	1	1	1
Beraviensis group	*	0.79	0.84	1	1	1	1
Lutambensis group	0.99	0.98	1	1	1	1	1
I. tiliifolia	0.76	1	0.84	1	1	1	1
I. pringsheimiana	*	UR	UR	0.98	1	0.8	1
I. incompta	UR	UR	UR	1	1	1	1
I. queenslandica	0.99	0.97	1	0.54	0.82	0.93	1
I. macalusoi	untested	untested	untested	NA	NA	NA	NA
I. beraviensis West Africa	*	0.78	NM	untested	untested	untested	untested
I. beraviensis East Africa	*	NM	NM	0.99	1	1	1
Unresolved sister taxon							
I. inopinata	0.88	0.77	0.75	NA	NA	NA	NA
I. neglecta + *I. stenobasis*	NA	NA	NA	NA	0.84	NA	NA
I. stenosiphon	NA	NA	NA	0.93	NA	0.95	1

This table has three parts. The top one highlights the support values for clade, Stictocardia and its groups, the centre on species delimitation and group - for the case of *I. beraviensis*- and the bottom one for the unresolved sister taxon. MP refers to Maximum Parsimony, A-ML refers to Approximate -Maximum Likelihood and BI refers to Bayesian Inference. NM's refer the Non-monophyletic group. Asterisks (*) refer to the support values <.5. Untested signifies the species or group that are not tested for monophyly. NA's signify Not Applicable.

It is counter-productive to drawing erroneous conclusions based on insufficient data [126, 127].

East Africa has the highest species richness of Stictocardia. This is in contrast with Gunn [128] and Simoes [129], who previously suggested that Malesia or Australasia were the centres of diversity of *Stictocardia*. Their suggestions were likely driven by the high number of endemic species of *Stictocardia* in these areas.

Outside of Africa and Madagascar, there are two reciprocally monophyletic species, *I. tiliifolia* and *I. queenslandica* (Table 3.3), recovered in both trees (Figure 3.2A and 3.2B). *Ipomoea queenslandica* is a poorly known endemic species from Queensland, Australia [130]. In contrast, *I. tiliifolia* is commonly found in tropical coastal habitats as well as inland habitats except in Africa and Madagascar. Interestingly, this species is neither recorded in *Flora of Tropical East Africa* [131], *Flora Zambesiaca* [132], *Flora of Somalia* [133], nor *Flore de Madagascar et des Comores* for Madagascar [134]. In the *nr*ITS and plastome trees (Figure 3.2A and Figure 3.2B), most of *I. tiliifolia* specimens were collected from a number of islands and countries in three oceans, the Pacific and nearby sea (Mindoro, Guam, Vanuatu, Federation of Micronesia, Molokai, Maui, Galapagos), the Atlantic and nearby sea (Dominican Republic, Puerto Rico) and the Indian (Mayotte), which is nearby Africa and Madagascar, that indicates the dispersal of this species is likely mediated by ocean currents.

3.4.3. Unresolved sister taxa of Stictocardia

The sister taxon of Stictocardia remains inconclusive since multiple inferences from different data sets provide conflicting results. The *nr*ITS tree (Figure 3.2A), with denser taxon sampling, places *I. inopinata* sister to Stictocardia. This species is an endemic species in New Caledonia [135] and geographically distant from Africa, where

most species of Stictocardia occurred. Meanwhile, the plastome phylogeny (Figure 3.2B) with less taxon sampling identifies *I. stenosiphon* - a species that occurs in Africa in which distribution overlaps with five species of Stictocardia - as the sister taxon in most analyses. The sister taxon of Stictocardia*,* therefore*,* remains unresolved due to incomplete taxon sampling of outgroup across the OW clade of *Ipomoea*. Comprehensive taxon sampling of the OW clade in the future is required to determine the sister taxon of Stictocardia as well as test the consistency of species composition in this clade.

3.5. CONCLUSION

This study is part of the global scale monographic study of *Ipomoea* [5, 136, 137] that has been conducted at the University of Oxford since 2015. The foundational work of Ipomoeeae by Wilkin [7, 122], the dense taxon and character sampling investigation of Muñoz-Rodriguez et al. [5] coupled with other studies [1-4, 6, 7] have demonstrated that *Ipomoea* as traditionally delimited is a polyphyletic group which includes multiple smaller genera *i.e.*, *Argyreia, Astripomoea, Blinkworthia, Lepistemon, Lepistemonopsis, Paralepistemon, Rivea, Stictocardia,* and *Turbina*. Among these subsumed genera, *Stictocardia* was a poorly known taxon that has been minimally sampled in most phylogenetic studies [1-3, 7, 9, 138]. Thus, until now delimitation of *Stictocardia* within *Ipomoea* remained untested as this group has never been globally monographed in the past.

The phylogenetic study of *Stictocardia* presented in this thesis extracted DNA from the selected herbarium specimens deposited in K, BM, BR, Naturalis (L, WAG), MO, LISC, NY, OXF, and US. This process generated multiple data sets – nuclear (ITS) and plastome (28 genes and 26 variable regions) that were analysed by several

inference methods, namely MP, A-ML and BI. The phylogenetic analyses have confirmed the polyphyly of *Stictocardia* as hinted by Muñoz-Rodríguez et al. [5].

Corroboration of nuclear and plastome data sets also confirms for the first time that seven species of *Stictocardia* are recovered in a morphologically diagnosed clade known as Stictocardia. Seven species of Stictocardia are placed in three consistently recovered groups, *i.e.* the African endemic Lutambensis (*I. lutambensis*), Beraviensis (*I. beraviensis, I. macalusoi, I. incompta* and *I. pringsheimiana*) that occurs in Africa and Madagascar, and Tiliifolia (*I. tiliifolia* and *I. queenslandica*), the one outside of Africa and Madagascar. The monophyly of four species – *I. tiliifolia, I. incompta, I pringsheimiana,* and *I. queenslandica* - is confirmed for the first time. Monophyly of *Ipomoea macalusoi* could not be tested as it is only represented by a single specimen in the *nr*ITS tree. This species is treated as a good species based on morphology (Chapter Two) and placed in the Beraviensis group following Muñoz-Rodriguez et al. [5, 139]. Another species - *Ipomoea beraviensis* - is consistently unresolved as a monophyletic group based on the *nr*ITS and plastome phylogenies. Specimens collected in Eastern and Western Africa seem to form different lineages. However, the decision to treat *I. beraviensis* as a single species stems from the rigorous morphological assessment coupled with statistical test of multiple specimens across Africa (Chapter Two). Future phylogenetic studies require more specimens of *I. beraviensis* from unsampled regions across Africa to satisfactorily address this issue. Morphological features of these seven species have been discussed in Chapter Two and also included in Chapter Four.

The remaining species - *I. mojangensis, I. sivarajanii, I. jucunda, I. neglecta, I. orientalis* - were recovered outside Stictocardia. These species were excluded from

the monographic study to ensure the focus of the study recovers a monophyletic group.

List of references

1. Eserman, L.A., G.P. Tiley, R.L. Jarret, J.H. Leebens-Mack, and R.E. Miller, *Phylogenetics and diversification of morning glories (tribe Ipomoeeae, Convolvulaceae) based on whole plastome sequences.* American Journal of Botany, 2014. **101**: p. 92-103.
2. Manos, P.S., R.E. Miller, and P. Wilkin, *Phylogenetic analysis of Ipomoea, Argyreia, Stictocardia, and Turbina suggests a generalized model of morphological evolution in morning glories.* Systematic Botany, 2001. **26**: p. 585-602.
3. Miller, R.E., T.R. Buckley, and P.S. Manos, *An examination of the monophyly of morning glory taxa using Bayesian phylogenetic inference.* Systematic Biology, 2002. **51**: p. 740-753.
4. Miller, R.E., M.D. Rausher, and P.S. Manos, *Phylogenetic systematics of Ipomoea (Convolvulaceae) based on ITS and waxy sequences.* Systematic Botany, 1999. **24**: p. 209-227.
5. Muñoz-Rodríguez, P., T. Carruthers, J.R.I. Wood, B.R.M. Williams, K. Weitemier, B. Kronmiller, Z.A. Goodwin, A. Sumadijaya, D. Harris, D. Filler, M.A. Rausher, S. Kelly, A. Liston, and R.W. Scotland, *A taxonomic monograph of Ipomoea integrated across phylogenetic scales.* Nature Plants, 2019. **5**: p. 1136-1144.
6. Stefanovic, S. and R.G. Olmstead, *Down the slippery slope: Plastid genome evolution in Convolvulaceae.* Journal of Molecular Evolution, 2005. **61**: p. 292-305.
7. Wilkin, P., *A morphological cladistic analysis of the Ipomoeeae (Convolvulaceae).* Kew Bulletin, 1999. **54**: p. 853-876.
8. Simões, A.R. and G.W. Staples, *Dissolution of Convolvulaceae tribe Merremieae and a new classification of the constituent genera.* Botanical Journal of the Linnean Society, 2017. **183**: p. 561-586.
9. Stefanović, S., L. Krueger, and R.G. Olmstead, *Monophyly of the Convolvulaceae and circumscription of their major lineages based on DNA sequences of multiple chloroplast loci.* American Journal of Botany, 2002. **89**: p. 1510-1522.
10. Cummings, M.P. and A. Meyer, *Magic bullets and golden rules: Data sampling in molecular phylogenetics.* Zoology, 2005. **108**: p. 329-336.
11. Nabhan, A.R. and I.N. Sarkar, *The impact of taxon sampling on phylogenetic inference: A review of two decades of controversy.* Briefings in Bioinformatics, 2011. **13**: p. 122-134.
12. Heath, T.A., S.M. Hedtke, and D.M. Hillis, *Taxon sampling and the accuracy of phylogenetic analyses.* Journal of Systematic Evolution, 2008. **46**: p. 239-257.
13. Pollock, D.D., D.J. Zwickl, J.A. McGuire, and D.M. Hillis, *Increased taxon sampling is advantageous for phylogenetic inference.* Systematic Biology, 2002. **51**: p. 664-671.
14. Wiens, J.J., *Missing data, incomplete taxa, and phylogenetic accuracy.* Systematic Biology, 2003. **52**: p. 528-538.
15. Zwickl, D.J. and D.M. Hillis, *Increased taxon sampling greatly reduces phylogenetic error.* Systematic Biology, 2002. **51**: p. 588-598.
16. Tamashiro, R.A., N.D. White, M.J. Braun, B.C. Faircloth, E.L. Braun, and R.T. Kimball, *What are the roles of taxon sampling and model fit in tests of cyto-nuclear discordance using avian mitogenomic data?* Molecular Phylogenetics and Evolution, 2019. **130**: p. 132-142.
17. Jackson, A.P., C.A. Machado, N. Robbins, and E.A. Herre, *Multi-locus phylogenetic analysis of Neotropical figs does not support co-speciation with the pollinators: The importance of systematic scale in fig/wasp cophylogenetic studies.* Symbiosis, 2008. **45**: p. 57-72.

18. Cracraft, J., *Species concepts and speciation analysis*, in *Current Ornithology*, R.F. Johnston, Editor. 1983, Springer: Boston. p. 159-187.
19. Donoghue, M.J., *A critique of the Biological Species Concept and recommendations for a phylogenetic alternative.* The Bryologist, 1985. **88**: p. 172-181.
20. Backlund, A. and K. Bremer, *To be or not to be: Principles of classification and monotypic plant families.* Taxon, 1998. **47**: p. 391-400.
21. Lyons-Weiler, J., G.A. Hoelzer, and R.J. Tausch, *Optimal outgroup analysis.* Biological Journal of the Linnean Society, 1998. **64**: p. 493-511.
22. Graham, S.W., R.G. Olmstead, and S.C.H. Barrett, *Rooting phylogenetic trees with distant outgroups: A case study from the Commelinoid Monocots.* Molecular Biology and Evolution, 2002. **19**: p. 1769-1781.
23. Dececchi, T.A., G.M. Narbonne, C. Greentree, and M. Laflamme, *Phylogenetic relationships among the Rangeomorpha: The importance of outgroup selection and implications for their diversification.* Canadian Journal of Earth Sciences, 2018. **55**: p. 1223-1239.
24. Platnick, N.I. and W.J. Gertsch, *The suborders of spiders: A cladistic analysis (Arachnida, Araneae).* American Museum Novitate, 1976. **2607**: p. 1-15.
25. APG, *An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III.* Botanical Journal of the Linnean Society, 2009. **161**: p. 105-121.
26. Chase, M.W., M.J.M. Christenhusz, M.F. Fay, J.W. Byng, W.S. Judd, D.E. Soltis, D.J. Mabberley, A.N. Sennikov, P.S. Soltis, and P.F. Stevens, *An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV.* Botanical Journal of the Linnean Society, 2016. **181**: p. 1-20.
27. APG, *An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II.* Botanical Journal of the Linnean Society, 2003. **141**: p. 399-436.
28. Kress, W.J., *Plant DNA barcodes: Applications today and in the future.* Journal of Systematics and Evolution, 2017. **55**: p. 291--307.
29. Savolainen, V., R.S. Cowan, A.P. Vogler, G.K. Roderick, and R. Lane, *Towards writing the encyclopedia of life: An introduction to DNA barcoding.* Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 2005. **360**: p. 1805-1811.
30. Hollingsworth, P.M., D.-Z. Li, M. van der Bank, and A.D. Twyford, *Telling plant species apart with DNA: From barcodes to genomes.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2016. **371**: p. 1-9.
31. Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard, *Biological identifications through DNA barcodes.* Proceedings of the Royal Society B: Biological Sciences, 2003. **270**: p. 313--321.
32. Coissac, E., P.M. Hollingsworth, S. Lavergne, and P. Taberlet, *From barcodes to genomes: Extending the concept of DNA barcoding.* Molecular Ecology, 2016. **25**: p. 1423-1428.
33. Chase, M.W., N. Salamin, M. Wilkinson, J.M. Dunwell, R.P. Kesanakurthi, N. Haider, and V. Savolainen, *Land plants and DNA barcodes: Short-term and long-term goals.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2005. **360**: p. 1889-1895.
34. Kress, W.J., K.J. Wurdack, E.A. Zimmer, L.A. Weigt, and D.H. Janzen, *Use of DNA barcodes to identify flowering plants.* PNAS, 2005. **102**: p. 8369-8374.
35. Rubinoff, D., S. Cameron, and K. Will, *Are plant DNA barcodes a search for the holy grail?* Trends in Ecology & Evolution, 2006. **21**: p. 1-2.
36. Kress, W.J. and D.L. Erickson, *DNA barcodes: Genes, genomics, and bioinformatics.* PNAS, 2008. **105**: p. 2761-2762.
37. Hebert, P.D.N. and T.R. Gregory, *The promise of DNA barcoding for taxonomy.* Systematic Biology, 2005. **54**: p. 852-859.
38. Dexter, K.G., T.D. Pennington, and C.W. Cunningham, *Using DNA to assess errors in tropical tree identifications: How often are ecologists wrong and when does it matter?* Ecological Monographs, 2010. **80**: p. 267-286.

39. Hollingsworth, P.M., S.W. Graham, and D.P. Little, *Choosing and using a plant DNA barcode.* PLOS One, 2011. **6**: p. e19254 (13 pages).
40. Chase, M.W., R.S. Cowan, P.M. Hollingsworth, C. van den Berg, S. Madriñán, G. Petersen, O. Seberg, T. Jørgsensen, K.M. Cameron, and M.A. Carine, *A proposal for a standardised protocol to barcode all land plants.* Taxon, 2007. **56**: p. 295-299.
41. Li, D.-Z., L.-M. Gao, H.-T. Li, H. Wang, X.-J. Ge, J.-Q. Liu, Z.-D. Chen, S.-L. Zhou, and S.-L. Chen, *Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants.* PNAS, 2011. **108**: p. 19641-19646.
42. Hollingsworth, P.M., *Refining the DNA barcode for land plants.* PNAS, 2011. **108**: p. 19451-19452.
43. Seifert, K.A., *Progress towards DNA barcoding of Fungi.* Molecular Ecology Resources, 2009. **9**: p. 83-89.
44. Schoch, C.L., K.A. Seifert, S. Huhndorf, V. Robert, J.L. Spouge, C.A. Levesque, W. Chen, and F.B. Consortium, *Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi.* PNAS, 2012. **109**: p. 6241-6246.
45. Arca, M., D.D. Hinsinger, C. Cruaud, A. Tillier, J. Bousquet, and N. Frascaria-Lacoste, *Deciduous trees and the application of universal DNA barcodes: A case study on the circumpolar Fraxinus.* PLOS One, 2012. **7**: p. e34089 (15 pages).
46. Roy, S., A. Tyagi, V. Shukla, A. Kumar, U.M. Singh, L.B. Chaudhary, B. Datt, S.K. Bag, P.K. Singh, and N.K. Nair, *Universal plant DNA barcode loci may not work in complex groups: A case study with Indian Berberis species.* PLOS One, 2010. **5**: p. e13674 (14 pages).
47. Martínez-Fort, J., M. León, and M.P. Donat-Torres, *A new subspecies of Peucedanum officinale L. subsp. album (Apiaceae) from the eastern part of the Iberian Peninsula.* PhytoKeys, 2019. **131**: p. 37-55.
48. Song, J., H. Yao, Y. Li, X. Li, Y. Lin, C. Liu, J. Han, C. Xie, and S. Chen, *Authentication of the family Polygonaceae in Chinese pharmacopoeia by DNA barcoding technique.* Journal of Ethnopharmacology, 2009. **124**: p. 434-439.
49. Vellinga, E.C., *Genera in the family Agaricaceae: Evidence from nrITS and nrLSU sequences.* Mycological Research, 2004. **108**: p. 354-377.
50. Alvarez, I. and J.F. Wendel, *Ribosomal ITS sequences and plant phylogenetic inference.* Molecular Phylogenetics and Evolution, 2003. **29**: p. 417-434.
51. Chen, S., H. Yao, J. Han, C. Liu, J. Song, L. Shi, Y. Zhu, X. Ma, T. Gao, and X. Pang, *Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species.* PLOS One, 2010. **5**: p. e8613 (8 pages).
52. Andreasen, K., M. Manktelow, and S.G. Razafimandimbison, *Successful DNA amplification of a more than 200-year-old herbarium specimen: Recovering genetic material from the Linnaean era.* Taxon, 2009. **58**: p. 959-962.
53. Erkens, R.H.J., H. Cross, J.W. Maas, K. Hoenselaar, and L.W. Chatrou, *Assessment of age and greenness of herbarium specimens as predictors for successful extraction and amplification of DNA.* Blumea, 2008. **53**: p. 407-428.
54. Särkinen, T., M. Staats, J.E. Richardson, R.S. Cowan, and F.T. Bakker, *How to open the treasure chest? Optimising DNA extraction from herbarium specimens.* PLOS One, 2012. **7**: p. e43808 (9 pages).
55. Staats, M., A. Cuenca, J.E. Richardson, R. Vrielink-van Ginkel, G. Petersen, O. Seberg, and F.T. Bakker, *DNA damage in plant herbarium tissue.* PLOS One, 2011. **6**: p. e28448 (9 pages).
56. Hollingsworth, P.M., L.L. Forrest, J.L. Spouge, M. Hajibabaei, S. Ratnasingham, M. van der Bank, M.W. Chase, R.S. Cowan, D.L. Erickson, A.J. Fazekas, S.W. Graham, K.E. James, K.-J. Kim, W.J. Kress, H. Schneider, J. van AlphenStahl, S.C.H. Barrett, C. van den Berg, D. Bogarin, K.S. Burgess, K.M. Cameron, M.A. Carine, J. Chacón, A. Clark, J.J. Clarkson, F. Conrad, D.S. Devey, C.S. Ford, T.A.J. Hedderson, M.L. Hollingsworth, B.C. Husband, L.J. Kelly, P.R.

Kesanakurti, J.S. Kim, Y.-D. Kim, R. Lahaye, H.-L. Lee, D.G. Long, S. Madriñán, O. Maurin, I. Meusnier, S.G. Newmaster, C.-W. Park, D.M. Percy, G. Petersen, J.E. Richardson, G.A. Salazar, V. Savolainen, O. Seberg, M.J. Wilkinson, D.-K. Yi, and D.P. Little, *A DNA barcode for land plants.* PNAS, 2009. **106**: p. 12794-12797.

57. Shaw, J., E.B. Lickey, J.T. Beck, S.B. Farmer, W. Liu, J. Miller, K.C. Siripun, C.T. Winder, E.E. Schilling, and R.L. Small, *The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis.* American Journal of Botany, 2005. **92**: p. 142-166.
58. Shaw, J., E.B. Lickey, E.E. Schilling, and R.L. Small, *Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in Angiosperms: The tortoise and the hare III.* American Journal of Botany, 2007. **94**: p. 275-288.
59. Shaw, J., H.L. Shafer, O.R. Leonard, M.J. Kovach, M. Schorr, and A.B. Morris, *Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in Angiosperms: The tortoise and the hare IV.* American Journal of Botany, 2014. **101**: p. 1987-2004.
60. Gitzendanner, M.A., P.S. Soltis, T.-S. Yi, D.-Z. Li, and D.E. Soltis, *Plastome phylogenetics: 30 years of inferences into plant evolution.* Advances in Botanical Research, 2018. **85**: p. 293-313.
61. Moore, M.J., P.S. Soltis, C.D. Bell, J.G. Burleigh, and D.E. Soltis, *Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots.* PNAS, 2010. **107**: p. 4623-4628.
62. Bakker, F.T., D. Lei, J. Yu, S. Mohammadin, Z. Wei, S. van de Kerke, B. Gravendeel, M. Nieuwenhuis, M. Staats, and D.E. Alquezar-Planas, *Herbarium genomics: Plastome sequence assembly from a range of herbarium specimens using an iterative organelle genome assembly pipeline.* Biological Journal of the Linnean Society, 2015. **117**: p. 33-43.
63. Givnish, T.J., M. Ames, J.R. McNeal, M.R. McKain, P.R. Steele, C.W. Depamphilis, S.W. Graham, J.C. Pires, D.W. Stevenson, and W.B. Zomlefer, *Assembling the tree of the monocotyledons: Plastome sequence phylogeny and evolution of Poales.* Annals of the Missouri Botanical Garden, 2010. **97**: p. 584-616.
64. Kim, S.-T. and M.J. Donoghue, *Incongruence between cpDNA and nrITS trees indicates extensive hybridization within Eupersicaria (Polygonaceae).* American Journal of Botany, 2008. **95**: p. 1122-1135.
65. Bruun-Lund, S., W.L. Clement, F. Kjellberg, and N. Rønsted, *First plastid phylogenomic study reveals potential cyto-nuclear discordance in the evolutionary history of Ficus L.(Moraceae).* Molecular Phylogenetics and Evolution, 2017. **109**: p. 93-104.
66. Doyle, J.J., *Gene trees and species trees: Molecular systematics as one-character taxonomy.* Systematic Botany, 1992. **17**: p. 144--163.
67. Twyford, A.D. and R.W. Ness, *Strategies for complete plastid genome sequencing.* Molecular Ecology Resources, 2017. **17**: p. 858-868.
68. Jansen, R.K. and T.A. Ruhlman, *Plastid genomes of seed plants*, in *Genomics of chloroplasts and mitochondria*, R. Bock and V. Knoop, Editors. 2012, Springer: Dordrecht. p. 103-126.
69. Hansen, A.K., L.K. Escobar, L.E. Gilbert, and R.K. Jansen, *Paternal, maternal, and biparental inheritance of the chloroplast genome in Passiflora (Passifloraceae): Implications for phylogenetic studies.* American Journal of Botany, 2007. **94**: p. 42-46.
70. Heldt, H.-W. and B. Piechulla, *Plant Biochemistry*. 4th ed. 2010, London: Academic Press.
71. Gonçalves, D.J.P., R.K. Jansen, T.A. Ruhlman, and J.R. Mandel, *Under the rug: Abandoning persistent misconceptions that obfuscate organelle evolution.* Molecular Phylogenetics and Evolution, 2020. **151**: p. 106903 (4 pages).
72. Dodsworth, S.A., *Genome skimming for phylogenomics*. 2015, Queen Mary University.
73. Straub, S.C.K., M. Parks, K. Weitemier, M. Fishbein, R.C. Cronn, and A. Liston, *Navigating the tip of the genomic iceberg: Next-generation sequencing for plant systematics.* American Journal of Botany, 2012. **99**: p. 349-364.

74. Dodsworth, S., *Genome skimming for next-generation biodiversity analysis.* Trends in Plant Science, 2015. **20**: p. 525-527.
75. Zeng, C.-X., P.M. Hollingsworth, J. Yang, Z.-S. He, Z.-R. Zhang, D.-Z. Li, and J.-B. Yang, *Genome skimming herbarium specimens for DNA barcoding and phylogenomics.* Plant Methods, 2018. **14**: p. 1-14.
76. Nevill, P.G., X. Zhong, J. Tonti-Filippini, M. Byrne, M. Hislop, K. Thiele, S. van Leeuwen, L.M. Boykin, and I. Small, *Large scale genome skimming from herbarium material for accurate plant identification and phylogenomics.* Plant Methods, 2020. **16**: p. 1-8.
77. Lee, C.-S. and S.R. Downie, *Phylogenetic relationships within Cicuta (Apiaceae tribe Oenantheae) inferred from nuclear rDNA ITS and cpDNA sequence data.* Canadian Journal of Botany, 2006. **84**: p. 453-468.
78. Kramina, T.E., G.V. Degtjareva, T.H. Samigullin, C.M. Valiejo-Roman, J. Kirkbride, J. H., S. Volis, T. Deng, and D.D. Sokoloff, *Phylogeny of Lotus (Leguminosae: Loteae): Partial incongruence between nrITS, nrETS and plastid markers and biogeographic implications.* Taxon, 2016. **65**: p. 997-1018.
79. Folk, R.A., J.R. Mandel, and J.V. Freudenstein, *Ancestral gene flow and parallel organellar genome capture result in extreme phylogenomic discord in a lineage of Angiosperms.* Systematic Biology, 2016. **66**: p. 320--337.
80. Degnan, J.H. and N.A. Rosenberg, *Gene tree discordance, phylogenetic inference and the multispecies coalescent.* Trends in Ecology & Evolution, 2009. **24**: p. 332-340.
81. Rose, J.P., C.A.P. Toledo, E.M. Lemmon, A.R. Lemmon, and K.J. Sytsma, *Out of sight, out of mind: Widespread nuclear and plastid-nuclear discordance in the flowering plant genus Polemonium (Polemoniaceae) suggests widespread historical gene flow despite limited nuclear signal.* Systematic Biology, 2020. **70**: p. 162-180.
82. Hedtke, S.M. and D.M. Hillis, *The potential role of androgenesis in cytoplasmic–nuclear phylogenetic discordance.* Systematic Biology, 2010. **60**: p. 87-96.
83. Hu, H., I.A. Al-Shehbaz, Y. Sun, G. Hao, Q. Wang, and J. Liu, *Species delimitation in Orychophragmus (Brassicaceae) based on chloroplast and nuclear DNA barcodes.* Taxon, 2015. **64**: p. 714-726.
84. Jankowiak, K., K. Buczkowska, and Z. Szweykowska-Kulinska, *Successful extraction of DNA from 100-year-old herbarium specimens of the liverwort Bazzania trilobata.* Taxon, 2005. **54**: p. 335-336.
85. Drábková, L., J. Kirschner, and Ĉ. Vlček, *Comparison of seven DNA extraction and amplification protocols in historical herbarium specimens of Juncaceae.* Plant Molecular Biology Reporter, 2002. **20**: p. 161-175.
86. White, T.J., T. Bruns, S.J.W.T. Lee, and J.L. Taylor, *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*, in *PCR Protocols: A Guide to Methods and Applications*, M. Innis, Gelfand, D., Sninsky, J, White, T., Editor. 1990, Academic Press: San Diego. p. 315-322.
87. Team, B.D. *Geneious Prime 2019.1.1*. 2019; Available from: https://www.geneious.com/prime/.
88. Pais, F.S.-M., P.d.C. Ruy, G. Oliveira, and R.S. Coimbra, *Assessing the efficiency of multiple sequence alignment programs.* Algorithms for Molecular Biology, 2014. **9**: p. 1-8.
89. Swofford, D.L., *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. 2002, Sunderland: Sinauer Associates.
90. Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura, *MEGA X: Molecular evolutionary genetics analysis across computing platforms.* Molecular Biology and Evolution, 2018. **35**: p. 1547-1549.
91. Posada, D. and K.A. Crandall, *Modeltest: Testing the model of DNA substitution.* Bioinformatics, 1998. **14**: p. 817-818.

92. Miller, M.A., W. Pfeiffer, and T. Schwartz. *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. in *Gateway Computing Environments Workshop*. 2010.
93. Miller, M.A., W. Pfeiffer, and T. Schwartz. *The CIPRES science gateway: A community resource for phylogenetic analyses*. in *Proceedings of the 2011 TeraGrid Conference: Extreme digital discovery*. 2011.
94. Bolger, A.M., M. Lohse, and B. Usadel, *Trimmomatic: A flexible trimmer for Illumina sequence data.* Bioinformatics, 2014. **30**: p. 2114-2120.
95. Brown, J.M., M. Pirrung, and L.A. McCue, *FQC Dashboard: Integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool.* Bioinformatics, 2017. **33**: p. 3137-3139.
96. Bushnell, B. *BBMap: A fast, accurate, splice-aware aligner*. 2014; Available from: https://www.osti.gov/servlets/purl/1241166.
97. Yan, L., X. Lai, X. Li, C. Wei, X. Tan, and Y. Zhang, *Analyses of the complete genome and gene expression of chloroplast of sweet potato [Ipomoea batata].* PLOS One, 2015. **10**: p. e0124083 (25 pages).
98. Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin, *The Sequence Alignment/Map format and SAMtools.* Bioinformatics, 2009. **25**: p. 2078-2079.
99. Team, P. *Picard*. 2021; Available from: https://broadinstitute.github.io/picard/.
100. McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, and M. Daly, *The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data.* Genome Research, 2010. **20**: p. 1297-1303.
101. Katoh, K., K. Misawa, K.I. Kuma, and T. Miyata, *MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform.* Nucleic Acids Research, 2002. **30**: p. 3059-3066.
102. Katoh, K. and D.M. Standley, *MAFFT multiple sequence alignment software version 7: Improvements in performance and usability.* Molecular Biology and Evolution, 2013. **30**: p. 772-780.
103. Castresana, J., *Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis.* Molecular Biology and Evolution, 2000. **17**: p. 540-552.
104. Talavera, G. and J. Castresana, *Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments.* Systematic Biology, 2007. **56**: p. 564-577.
105. Nguyen, L.-T., H.A. Schmidt, A. Von Haeseler, and B.Q. Minh, *IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies.* Molecular Biology and Evolution, 2015. **32**: p. 268-274.
106. Borowiec, M.L., *AMAS: A fast tool for alignment manipulation and computing of summary statistics.* PeerJ, 2016. **4**: p. e1660 (10 pages).
107. Mackinder, B.A. and R.T. Pennington, *Monograph of Berlinia (Leguminosae).* Systematic Botany Monographs, 2011. **91**: p. 1-117.
108. Tessler, M., *A monograph of Hymenodon (Orthodontiaceae).* The Bryologist, 2012. **115**: p. 493-517.
109. du Preez, B., L.L. Dreyer, C.H. Stirton, and A.M. Muasya, *A monograph of the genus Polhillia (Genisteae: Fabaceae).* South African Journal of Botany, 2021. **138**: p. 156-183.
110. Ji, Y., *A monograph of Paris (Melanthiaceae): Morphology, Biology, Systematics and Taxonomy* 2021, Beijing: Springer.
111. Daniell, H., C.-S. Lin, M. Yu, and W.-J. Chang, *Chloroplast genomes: Diversity, evolution, and applications in genetic engineering.* Genome Biology, 2016. **17**: p. 1-129.
112. Li, L., Y. Hu, M. He, B. Zhang, W. Wu, P. Cai, D. Huo, and Y. Hong, *Comparative chloroplast genomes: Insights into the evolution of the chloroplast genome of Camellia sinensis and the phylogeny of Camellia.* BMC Genomics, 2021. **22**: p. 1-22.

113. Jansen, R.K., Z. Cai, L.A. Raubeson, H. Daniell, C.W. Depamphilis, J. Leebens-Mack, K.F. Müller, M. Guisinger-Bellian, R.C. Haberle, and A.K. Hansen, *Analysis of 81 genes from 64 plastid genomes resolves relationships in Angiosperms and identifies genome-scale evolutionary patterns.* PNAS, 2007. **104**: p. 19369-19374.
114. Pyron, R.A., *Post-molecular systematics and the future of phylogenetics.* Trends in Ecology & Evolution, 2015. **30**: p. 384-389.
115. Barriel, V. and P. Tassy, *Rooting with multiple outgroups: Consensus versus parsimony.* Cladistics, 1998. **14**: p. 193-200.
116. Maddison, W.P., M.J. Donoghue, and D.R. Maddison, *Outgroup analysis and parsimony.* Systematic Biology, 1984. **33**: p. 83-103.
117. Bergsten, J., *A review of long-branch attraction.* Cladistics, 2005. **21**: p. 163-193.
118. Felsenstein, J., *Inferring phylogenies*. Vol. 2. 2004, Sunderland: Sinauer Associates.
119. Boussau, B., Z. Walton, J.A. Delgado, F. Collantes, L. Beani, I.J. Stewart, S.A. Cameron, J.B. Whitfield, J.S. Johnston, P.W.H. Holland, D. Bachtrog, J. Kathirithamby, and J.P. Huelsenbeck, *Strepsiptera, Phylogenomics and the long branch attraction problem.* PLOS One, 2014. **9**: p. e107709 (9 pages).
120. Felsenstein, J., *Cases in which parsimony or compatibility methods will be positively misleading.* Systematic Zoology, 1978. **27**: p. 401-410.
121. Verdcourt, B., *A new subgenus of Stictocardia (Convolvulaceae).* Kew Bulletin, 1990. **45**: p. 583-585.
122. Wilkin, P., *A revision of the Infrageneric classification of the genus Ipomoea L. and the related genera in the tribe Ipomeeae (Convolvulaceae)*. 1993, University of Reading.
123. Zhang, Y.-X., C.-X. Zeng, and D.-Z. Li, *Complex evolution in Arundinarieae (Poaceae: Bambusoideae): Incongruence between plastid and nuclear GBSSI gene phylogenies.* Molecular Phylogenetics and Evolution, 2012. **63**: p. 777-797.
124. Soltis, D.E. and R.K. Kuzoff, *Discordance between nuclear and chloroplast phylogenies in the Heuchera group (Saxifragaceae).* Evolution, 1995. **49**: p. 727-742.
125. Yong, Z.M., S.C. Yun, H. Gang, Y.X. Lin, L.C. Ye, and Z.G. Hua, *A preliminary analysis of phylogenetic relationships in Cymbidium (Orchidaceae) based on nrITS sequence data.* Journal of Integrative Plant Biology, 2002. **44**: p. 588-592.
126. Henderson, A., *Traditional morphometrics in plant systematics and its role in palm systematics.* Botanical Journal of the Linnean Society, 2006. **151**: p. 103-111.
127. Zachos, F.E., M. Apollonio, E.V. Bärmann, M. Festa-Bianchet, U. Göhlich, J.C. Habel, E. Haring, L. Kruckenhauser, S. Lovari, A.D. McDevitt, C. Pertoldi, G.E. Rössner, M.R. Sánchez-Villagra, M. Scandura, and F. Suchentrunk, *Species inflation and taxonomic artefacts—A critical comment on recent trends in mammalian classification.* Mammalian Biology, 2013. **78**: p. 1-6.
128. Gunn, C.R., *Notes on Stictocardia campanulata (L.) Merrill and S. jucunda (Thw.) CR Gunn (Convolvulaceae).* Brittonia, 1972. **24**: p. 169-176.
129. Simões, A.R., H. Silva, and P. Silveira, *The Convolvulaceae of Timor with special reference to East Timor.* Blumea, 2011. **56**: p. 49-72.
130. Johnson, R.W., *Stictocardia Hallier f. (Convolvulaceae) in Queensland.* Austrobaileya, 2004. **6**: p. 631-637.
131. Verdcourt, B., *Convolvulaceae*, in *Flora of Tropical East Africa*, C.E. Hubbard and E. Milne-Redhead, Editors. 1963, Crown Agents for Oversea Governments & Administration: London. p. 1-161.
132. Goncalves, M.L., *Flora Zambesiaca, Convolvulaceae*, E. Launert, Editor. 1987, Flora Zambesiaca Managing Committee: London. p. 9–129.
133. Garcia, M.A. and S. Demissew, *Convolvulaceae*, in *Flora of Somalia*, M. Thulin, Editor. 2006, Royal Botanic Gardens, Kew,: Kew. p. 221-258.

134. Deroin, T., *Famille Convolvulaceae*, in *Flore de Madagascar et des Comores*, P. Morat, Editor. 2001, Muséum National d'Histoire Naturelle: Paris. p. 11-287.
135. Heine, H., *Convolvulacees*, in *Flore de la Nouvelle-Calédonie et Dépendances*, J.F. Leroy and H.S. Mackee, Editors. 1984, Museum National D'Histoire Naturelle: Paris. p. 1-91.
136. Wood, J.R.I., P. Muñoz-Rodríguez, B.R.M. Williams, and R.W. Scotland, *A foundation monograph of Ipomoea (Convolvulaceae) in the New World.* PhytoKeys, 2020. **143**: p. 1-823.
137. Wood, J.R.I., M.A. Carine, D. Harris, P. Wilkin, B. Williams, and R.W. Scotland, *Ipomoea (Convolvulaceae) in Bolivia.* Kew Bulletin, 2015. **70**: p. 1-124.
138. Simoes, A.R., A. Culham, and M.A. Carine, *Resolving the unresolved tribe: A molecular phylogenetic framework for the Merremieae (Convolvulaceae).* Botanical Journal of the Linnean Society, 2015. **179**: p. 374-387.
139. Muñoz-Rodriguez, P., *Systematic studies of the sweet potato and its wild relatives*. 2018, University of Oxford.

CHAPTER 4. TAXONOMIC REVISION OF *STICTOCARDIA*

4.1. The systematic context of this study

This study has been conducted within the context of an expanded monophyletic *Ipomoea* that includes nine previously recognised segregate genera, one of which is *Stictocardia*. *Stictocardia* was selected for purely pragmatic reasons as it is a poorly known, problematic taxon that comprises a small number of species and has never been critically revised. In Chapter Three, phylogenetic studies demonstrated that *Stictocardia,* as traditionally understood, is a polyphyletic group and was rejected for monographic study. Instead, study was focussed on the clade Stictocardia that comprises seven species belonging to the genus *Stictocardia*. The aim of this study is to prepare a revision of *Stictocardia* that addresses major taxonomic issues such as species delimitation and the relationships between species.

4.2. Species concept and delimitation

All previous substantial taxonomic studies of *Stictocardia,* such as Hallier [1-3], van Ooststroom [4, 5], Verdcourt [6-11], Gunn [12], Austin, individually or with colleagues [13-15], and Biju et al. [16] relied heavily on morphological characters to delimit species. Chapter Two and Three, however, have revealed that morphological characters do not provide robust predictive value in the study of this group. The use of ecological information to support morphology was hinted by Verdcourt in his taxonomic assessment of *I. macalusoi* [9] but he and other past specialists provided no explicit adoption of various species concept [17] other than an intuitive assessment of morphology. This D.Phil. study, therefore, aims to test using molecular sequence data the validity of the species hypothesized and delimited using morphological traits, *i.e.* an individual species is largely determined by its corroboration with the results of

phylogenetic analysis of DNA sequence data taking account that species are heuristic [18].

4.3. Ecology

Based on label information, Stictocardia occupies a range of ecosystem types, both in open and shaded habitats. The specimens were recorded from coastal, mangrove, and swamp forest, grassland, savanna, secondary forest, dry forest, rain forest, riverine forest, forest margins, and limestone habitats. In coastal areas, only two species are present, *I. tiliifolia,* a widespread species and *I. macalusoi,* which is restricted to Eastern Africa and Mauritius. The remaining species occupy more inland areas (*I. queenslandica* at 300-400 m asl and *I. lutambensis* at 100—300 m asl). Some species occupy a broad elevation range such as *I. incompta* from 30 m to 1,030 m asl, *I. pringsheimiana* from 660 m to 1,300 m asl and *I. beraviensis* from 50 m to 1,800 m asl.

4.4. Pollination syndrome

Corolla colour is relatively well-preserved on herbarium specimens although it slowly fades over time. As no field work was conducted in this study, colour information is extracted from data on labels, although it is only recorded on 269 (39%) out of the 653 examined specimens (herbarium sheets and images) and strongly biased toward *I. beraviensis* (135 specimens/51%) and *I. tiliifolia* (79 specimens /30%)

Austin and Sebsebe [13] stated that the shape and colour of the corolla are likely the result of the co-evolution of plants and their pollinators although they also argued that colour alone is not reliable in predicting the pollinator. They speculated that birds, moths and perhaps bees have been involved in selecting corolla traits in *Stictocardia* and further listed potential groups of pollinators for each species [13].

John Wood (personal communication) expressed the view that corolla and stamen length are related to pollinators. Funnel-shaped corollas with unequal, included stamens are bee-pollinated whereas hypocrateriform and subcylindrical corollas have subequal, exerted stamens and appear to be bird-pollinated. However, birds have been observed to visit flowers of *I. macalusoi* [15] that possesses broad funnel-shape corolla. The only study that focuses on pollination is the work by Aswani and Sabu [19]. This study recorded that animals, both nectar-hunting birds (*Nectarinia asiatica* and *N. zeylanica*) and insects are the pollinators of ornamental *I. tiliifolia* [19]. No other species of pollinator are recorded as visitors to the flowers of *Stictocardia***.**

4.5. Geographical distribution

Stictocardia is distributed from 27 °N to 32.28 °S, most records being from tropical areas as shown in Figure 4.1. This map is more comprehensive than the two previous global maps of *Stictocardia* [13, 15] as they were based on a smaller number of specimens. Five species of Stictocardia occur in Africa (Figure 4.3) concentrated in the Eastern part, hence, this area has the highest species richness.

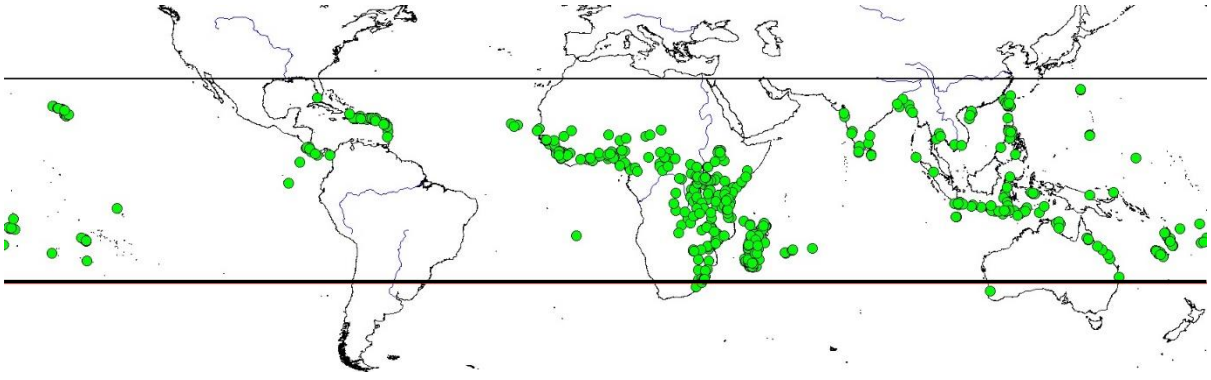


Figure 4.1. Global distribution of Stictocardia is tropical. Each dot represents a geolocated individual specimen. Multiple specimens with similar or adjacent localities are on the top of each other and appear as a single dot. The horizontal line is 30°N and 30°S.

4.6. Number of specimens examined

Biological and taxonomic information is not evenly available for all species of Stictocardia*. Ipomoea tiliifolia* is the most widespread species with the most collected specimens (318), followed by *I. beraviensis* (247). These two species comprises 86.5% of the 653 specimens in the study group. Inevitably, the most collected and widespread species, *I.tiliifolia,* has more associated information than the remaining species in terms of morphological variation and associated with a high level of synonymy.

The number of specimens examined by previous experts in Stictocardia, such as Hallier (24), van Ooststroom (69), Verdcourt (42), and Austin (22) were limited. These numbers are derived from the identification slips and specimens cited in their publications. This signifies that it is unlikely that any of these experts observed the entire breadth of morphological variation within Stictocardia. In contrast, some 80 specimens, mostly of *I. tiliifolia*, listed in the literature [7, 9, 10, 12, 16, 20-23] were not seen in this study and consequently some morphological variation may have not been observed.

4.7. Taxon names

Stictocardia taxa at specific and infraspecific rank appear in various botanical portals such as the International Plant Names Index (IPNI) [24], Tropicos [25], The Plant List (TPL) [26] and WCSP-Convolvulaceae [27]. WCSP has the most complete list of 24 names, followed by TPL that lists 18, whilst IPNI and Tropicos list 16. WCSP-Convolvulaceae has 16 accepted and eight non-accepted names without further discussion. In TPL, the names are given different taxonomic statuses as accepted (eight), synonym (two), or unresolved (eight) with medium and low confidence levels,

because of no prior monograph. The status of all African taxa is accepted as they have appeared in a number of taxonomic studies and regional floras [7-11, 28-30]. Meanwhile, the Australian endemic species, *I. queenslandica*, is treated as unresolved as it has only been minimally scrutinised in a few taxonomic studies [31, 32]. The status of species excluded from Stictocardia are unresolved as they are rarely discussed in taxonomic studies or in floras and checklists [4, 5, 33]. Leipzig Catalogue of Vascular Plant LCVP [34] does not list endemic species outside Africa. Hence the significant number of synonyms and unresolved names highlight the importance of a monograph.

4.8. Conservation assessment

The seven species of Stictocardia were assessed according to the IUCN criteria following default setting in GEOCAT editor [35]. Two analyses in GEOCAT are AOO (Area of Occupancy) and EOO (Extent of Occurrence). The former calculates area in which a species actually occurs whereas the later captures the overall geographic spread of the localities at which a species occurs by including the discontinuities in habitat and occupancy [36]. AOO exhibits a more conservative approach than EOO in assessing the status of a species, see Appendix 9.

For species with fewer than 15 georeferenced specimens - *I. queenslandica, I. macalusoi* and *I. lutambensis* - which is the recommended minimum number for the assessment [37], the assessment should be considered preliminary.

For the other species with adequate numbers of specimens, the result of AOO and EOO show contrasting results for *I. beraviensis, I. pringsheimiana* and *I. incompta.* Results of individual species assessments are presented in the following taxonomic account.

4.9. Clade Description

Slender erect herb or robust perennial herbaceous climbers, turning woody and becoming twining lianas or woody creepers up to 12 m long, scrambling over vegetation; *stems* terete, glabrous, puberulent, or villous with white hairs. *Leaves* petiolate, 3—20 × 3—18 cm, ovate, suborbicular or, rarely, subrenifom; *apex* acute, acuminate or obtuse; *margin* entire; *base* shallowly to deeply cordate, both surfaces glabrous, puberulent, or villous; adaxially darker green, abaxially paler green, somewhat glaucous with black dots, *lateral nerves* 6—11; *petioles* 1—14 cm*,* slender, glabrous, puberulent, or villous. *Inflorescences* of 1—21 flowers in axillary (compound) cymes; *primary peduncles* 0—20 cm, slender, glabrous, puberulent or villous; *secondary peduncles* 0—2.8 cm; *bracteoles* 5.5—12.5 × 2.6— 6 mm, paired, oblong or lanceolate, glabrous or villous, caducous; *pedicels* 2—30 mm, slender, glabrous, puberulent or villous; *calyx* accrescent to 2.7 cm in fruit; *outer* s*epals* 5—30 × 4—17 mm, ovate, suborbicular, or semi deltoid, glabrous, puberulent or villous; *apex* rounded, obtuse or truncate*; base* rounded; *middle sepal* equal or slightly larger *c.* 14 × 12 mm, ovate to suborbiculate; *corolla* 2.5—8 cm long, funnelform or campanulate, glabrous, colour variable, crimson, maroon, scarlet, pink, purple, the centre yellow, orange or darker purple, midpetaline bands puberulent, sometimes with black glands and white hairs apically, limb 2.2—5 cm wide; *stamens* 5*,* equal or unequal, included, white; filaments glabrous with pilose base, *long filaments* 3—5 cm; *short filaments c.* 3 cm, *anthers* linear, white, cream, yellow or pale brown; *style* 3—5 cm; included, *stigma* biglobose, pink or purple, *style* white, *ovary* four locular with one ovule per cell. *Capsules* 10—25 × 5—25 mm*,* ellipsoid, compressed globose or ovoid triangular, indehiscent, glabrous, brown, usually enclosed by accrescent sepals; *endocarp* with

partially covering valve, *seeds* 4, 5 –13.2 x 3 –11.4 mm, ellipsoid, globose, or subglobose, glabrous or densely puberulent.

4.10. Artificial identification key

This key facilitates the identification of fertile herbarium specimens.

1a. Plant covered with dense shaggy hairs; sepals deltoid with a prominent apical tail-like appendage, lower part muricate and ridged ……..… *I. incompta*

1b. Plant not with dense shaggy hairs, glabrous or puberulent; sepals suborbicular or ovate, smooth or puberulent ……..…….…….2

2a. Slender perennial herb <50 cm tall, flowering specimens often with persistent cotyledons; flowers solitary (Australia)……..……..… *I. queenslandica*

2b. Vigorous herbaceous or woody twiner or climber; flowering specimens never with persistent cotyledons; flowers solitary or in cymes ……..…….. 3

3a. Inflorescence compact, usually with >6 flowers; primary peduncles short, <1.1 cm long; corolla bright crimson ……..… 4

3b. Inflorescence lax, often few-flowered; primary peduncles usually >1.1 cm long, or occasionally shorter; corolla usually white or pink…….…….……... 5

4a. Plant usually puberulent; sepals 5—10 mm long at anthesis; corolla narrowly funnel-shaped (widespread in tropical Africa and Madagascar) …..……. *I. beraviensis*

4b. Plant mostly glabrous; sepals 10—12 mm long at anthesis; corolla broadly funnel-shaped (coastal sand dunes in Somalia) ……..…… *I. macalusoi*

5a. Leaves and stem completely glabrous; leaves finely acuminate; developed fruit partially exposed exocarp, seeds glabrous…… *I. lutambensis*

5b. Leaves and stem usually puberulent; leaves acute to very shortly acuminate, developed fruit does not exposed exocarp, seeds not glabrous…….…… 6

6a. Sepals at anthesis 7—10 mm long; fruit rarely found, accrescent, Tropical Africa and Madagascar. ……*I. pringsheimiana*

6b. Sepals at anthesis 8—13 mm long, very strongly accrescent in fruit to 27 mm; common near coastal areas except in Africa and Madagascar……*I.. tiliifolia*

4.11. Species account

4.11.1. *Ipomoea lutambensis* Schulze-Menz., Notizbl. Bot. Gart. Berlin–Dahlem 14: 11 (1938). [38].

Stictocardia lutambensis (Schulze-Menz.) Verdc., Kew Bull. 13: 188 (1958). [8]. Type: TANZANIA, Lindi District, Lake Lutamba, *Schlieben* 6181 (holotype B, possibly destroyed; isotypes BM-000930385!, BR-888606 image!, HBG-505571 image!, LISC-011328!, P-00434119 image!, S11-40453 image!).

Description. Perennial herbaceous climber, 2—4 m, all parts glabrous; *stems* terete. *Leaves* petiolate, 3—11 × 3.5–9 cm, ovate, *apex* long-acuminate, *margin* entire, *base* cordate to strongly cordate, thin and membraneous in texture, adaxially dark green, abaxially pale green with sparse sessile black glands, *lateral nerves* 6—7; *petioles* 2—8 cm, slender. *Inflorescence* of 2—4 flowers in axillary cymes; *primary peduncles* 1.8—5.5 cm, elongate, slender; *secondary peduncles* 7—28 mm, robust; *bracteoles* caducous, not observed; *pedicels* 1—3 cm; *outer sepals* 5—8 × 5—10 mm at anthesis, accrescent to 20 × 20 mm in fruit, ovate, suborbicular, *apex* obtuse*; middle sepal c.* 5 × 7 mm, suborbicular, a*pex* obtuse; *corolla* 4.8— 6.2 cm long, funnelform, dark purple with slightly darker centre, midpetaline bands puberulent in bud, glabrescent at maturity, darker, limb 22—31 mm wide; *androecium* and *gynoecium* unknown.

Capsule 10—18 × 6—10 mm, ovoid, indehiscent; *seeds* 5 × 5 mm, globose, glabrous, dark reddish brown.

Illustration. Figure 4.2.

Habitat. Seasonally moist lowland forest from 100 to 300 m asl.

Note. This species was described from Lake Lutamba and has also been recorded from other localities in Tanzania [7]. The conservation status of this species is “Indeterminate” [39] but the literature used [7] to support this categorization does not provide detailed justification except endemism. The current assessment from five georeferenced specimens shows that this species is Endangered based on the AOO analysis and Vulnerable based on EOO analysis. (Appendix 10.1-7)

Specimens seen. **(Figure 4.3). TANZANIA. Lindi:** Lake Lutamba, *Schlieben* 6181 (BM, BR, HBG, LISC, P, S). **Morogoro:** Kingupira, *Vollesen* 2619 (K); *Vollesen* 3497 (K). Uluguru Mts, *Bruce* 1036 (BM, K). **Mtwara:** Masasi, *Bidgood et al.* 2000 (K, MO).

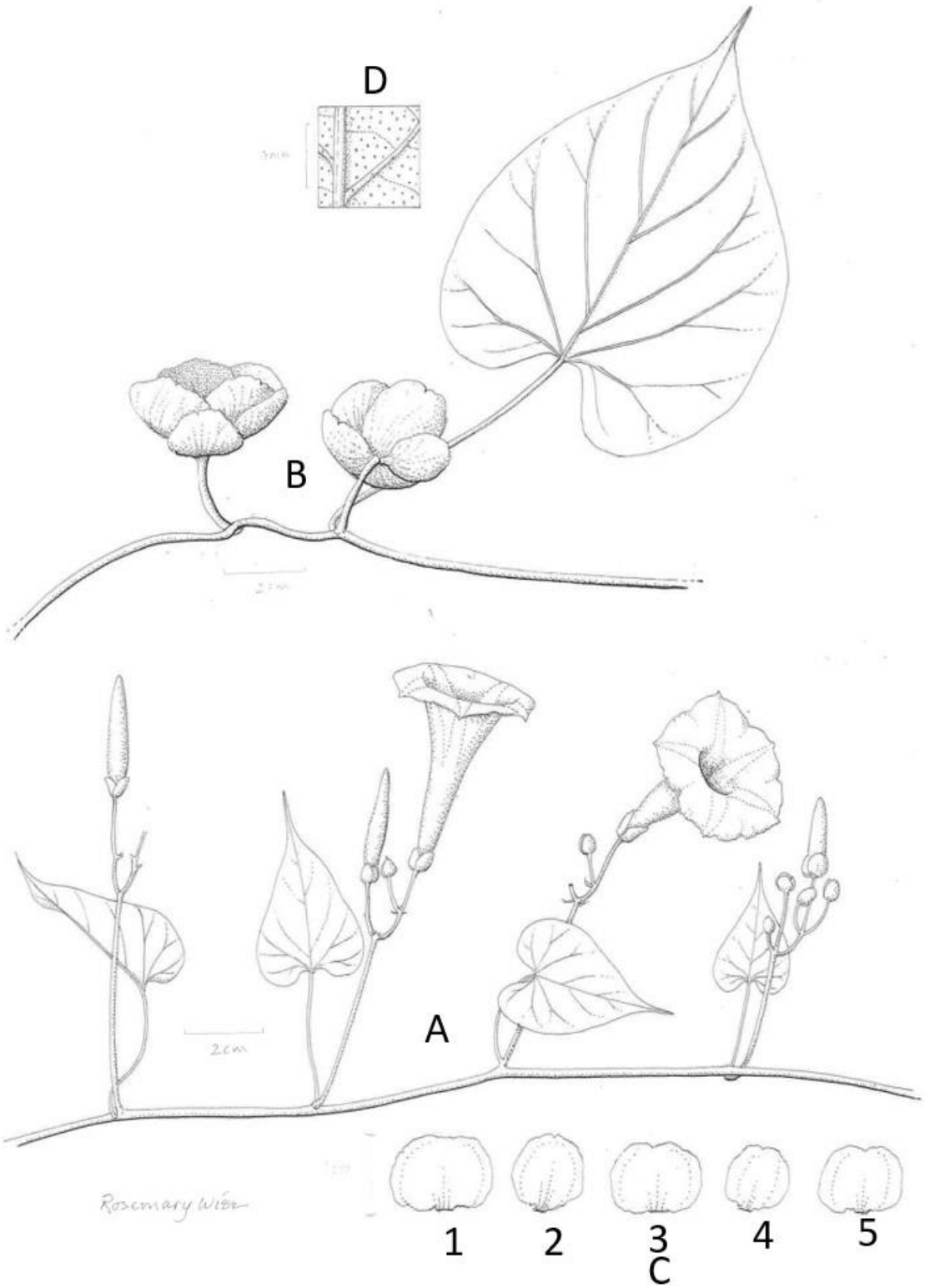


Figure 4.2. *Ipomoea lutambensis* Schulze-Menz. **A.** Habit while flowering**, B.** Habit while fruiting, **C.** Sepals (**1**) 1st outer sepal, (**2**) 2nd outer sepal, (**3**) middle sepal, (**4**) 1st inner sepal, (**5**) 2nd inner sepal, **D.** Abaxial leaf surface, Drawn by Rosemary Wise from *Schlieben* 6181 (**A, C**) and Vollesen 2619 (**B, D**).

4.11.2. *Ipomoea beraviensis* Vatke, Linnaea 43(7): 514 (1882). [40].

Stictocardia beraviensis (Vatke) Hallier f., Bot. Jahrb. Syst. 18: 159 (1893). [1]. Type: MADAGASCAR, Beravi interior, Gebirge, *Hildebrandt* 3094 (holotype B destroyed; isotypes K-000097044!, BM-001253007 image!, HUH-00054662 image!, L-0004255 image!)

Ipomoea hierniana Rendle. J. Bot. 39: 58. (1901). [41]. Type: CAMEROON, Bipinde, Urwaldgebiet, *Zenker* 1614 (holotype BM-000930386 image!; isotypes K-000097051 image!, P-03548451 image!, P-03548453 image!).

Description. Perennial, robust climber, scrambling over vegetation, or woody creeper 10—12 m; *stems* terete, glabrous. *Leaves* petiolate, 4—20 × 3.8—12 cm, ovate or suborbicular, *apex* acute, acuminate or obtuse; *margin* entire; *base* deeply or shallowly cordate, both surfaces glabrous or puberulent, adaxially dark green, abaxially light green, with black glands; *lateral nerves* 9—11; *petioles* 3–10 cm, slender. *Inflorescences* of multiple–21 flowers in compact, axillary compound cymes; *primary peduncles* 1—11 mm, glabrous or puberulent; *secondary peduncles* 1—10 mm; *bracteoles* caducous, not observed; *pedicels* 2—13 mm, glabrous or puberulent, slender; *calyx* fleshy, pale green turning dark brown in fruit; *outer* s*epals* 6.5—9.5 mm at anthesis, accrescent to 15 mm, ovate or slightly oval, glabrous or puberulent, *apex* obtuse or truncate; *middle* s*epal* equal or slightly larger, suborbiculate; *apex* obtuse or truncate; *corolla* 2.5—6.5 cm long, narrowly funnelform, glabrous, colour variable, crimson, pink, dark red, maroon, or, rarely violet, base orange, yellow or scarlet, limb 2—3 cm wide; *stamens* 5, included, unequal, *anthers* white, cream yellow or pale brown; *filaments* pale yellow; *stigma* pink or pink-purple, *style* white, *ovary* four locular with one ovule per cell. *Capsule* 1—1.5 cm, globose, indehiscent, glabrous; *seeds* 6 × 3—4 mm, ellipsoid, densely puberulent.

Illustration. Figure 4.4.

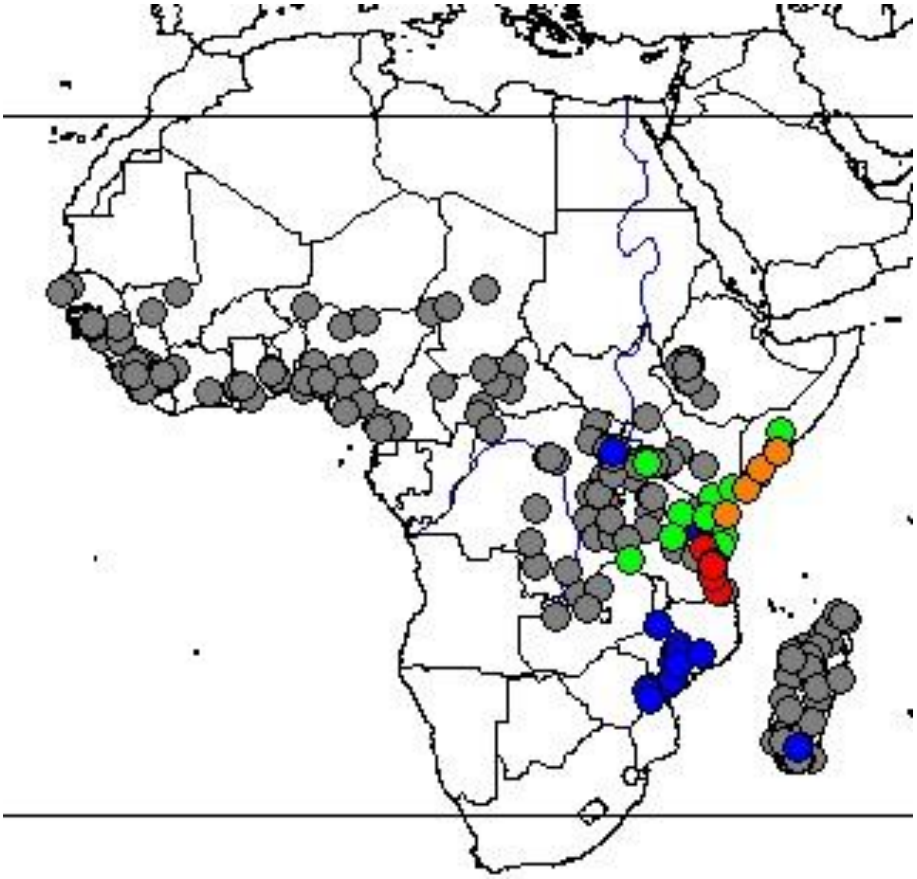


Figure 4.3. Map of Africa, Madagascar and Mauritius showing five Stictocardia species; a widespread species *I. beraviensis* (grey), an endemic of Tanzania *I. lutambensis* (red), regionally distributed species *I. incompta* (green), *I. pringsheimiana* (blue) and *I. macalusoi* (orange). The presence of *I. macalusoi* in Mauritius is the result of cultivation probably as an ornamental. The black horizontal lines are 30°N (top) and 30°S (bottom).

Habitat. Open habitats on a variety of well-drained soils including savannah and several types of forest (dry, evergreen, riverine, humid) from 50 m to 1,800 m asl.

Note. *Ipomoea beraviensis* is the most widespread species in Africa and Madagascar. Examination of African and Madagascar specimens (Chapter Two) shows plants from

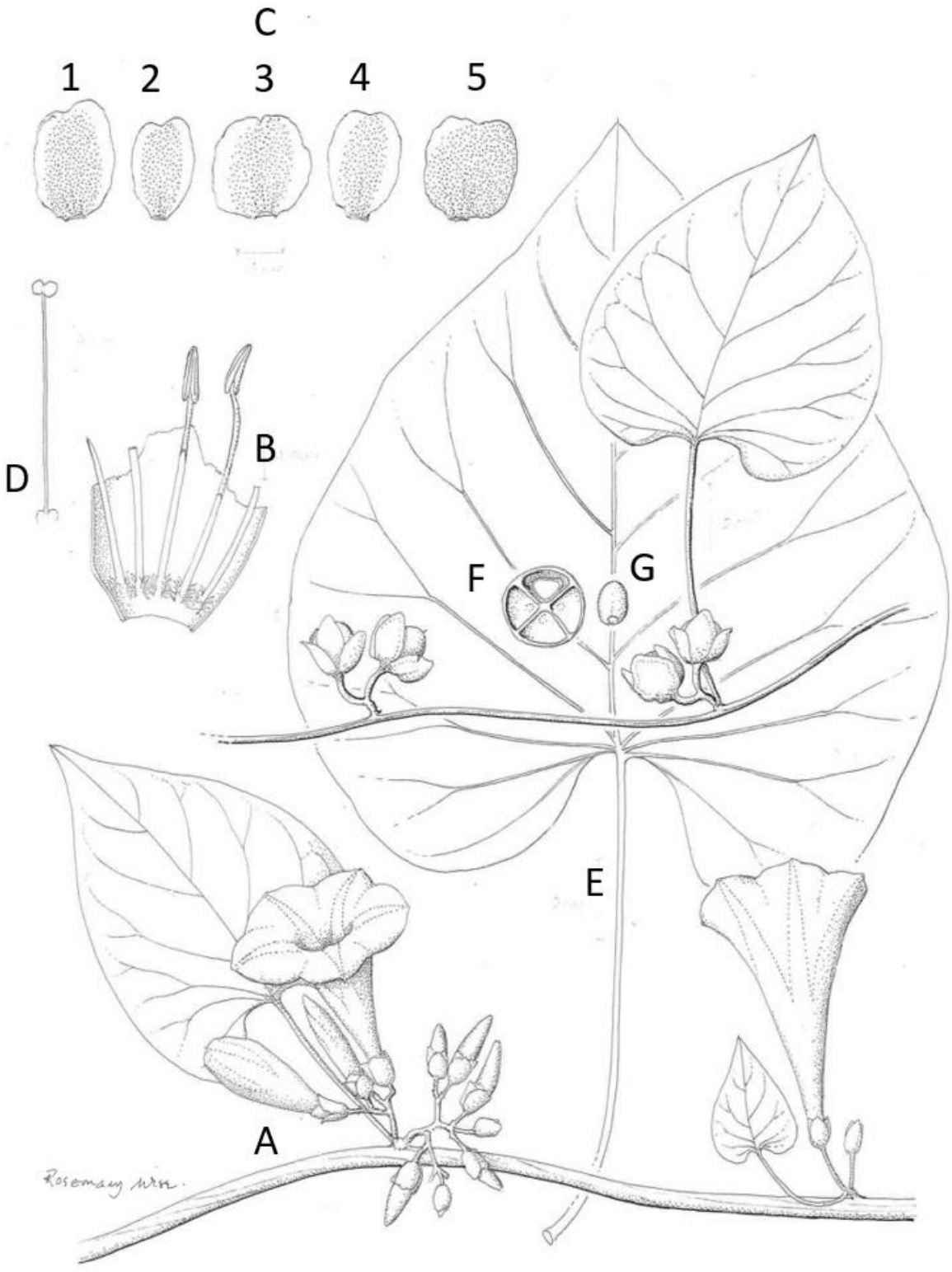


Figure 4.4. *Ipomoea beraviensis* Vatke. **A.** Habit while flowering, **B.** Habit while fruiting**, C** Sepals (**1**) st outer sepal, (**2**) 2nd outer sepal, (**3**) middle sepal, (**4**) 1st inner sepal, (**5**) 2nd inner sepal, **D.** Stamens ith anthers removed, **E.** Leaf, **F.** Fruit, longitudinal section, **G.** Seed. Drawn by Rosemary Wise from *Schatz Lowry* 1441 (**A, C, D**) and *Fay* 6279 (**B, E, F, G**).

Western Africa and Madagascar have shorter primary peduncles or subsessile inflorescences, and relatively short, narrow corollas with fewer flowers. In contrast, Eastern African plants have longer, distinct, stout primary peduncles with longer and broader corollas and more flowers. However, the characters (length of primary peduncle, pedicel, sepal and corolla) overlap, do not show clear morphological gaps across the range of the species and statistically inconclusive (see Chapter Two). Therefore, this species is conservatively treated as a single taxonomic entity based on morphological evidence despite appearing polyphyletic in the phylogenetic study (Chapter Three). This species is cultivated as an ornamental plant under the popular name Hawaiian Sunset Vine due to its colourful corolla. The previous conservation status of this species was Least Concern as it is considered not threatened [42]. The current assessment of 129 georeferenced specimens shows that the status of this species is Vulnerable based on the AOO analysis and Least Concern based on EOO analysis

Specimens seen. (Figure 4.3). BURUNDI. Bubanza: Kihanga, *Lewalle* 3723 (K). **Ngozi:** Ruyaga, *Kassner* 3173 (BR, BM, K). **CAMEROON. Bipinde**: *Urwaldgebiet, Zenker* 1614 (BM, HBG, K, L, P, S, WAG). **Centre:** Mbalmayo, *Jacques Felix* 9157 (K, P). **Yoko**: Ndong, *Biholong* 404 (P). **CENTRAL AFRICAN REPUBLIC.** *Chevalier* s.n., October 1902 (BR); *Leg. ign*. s.n., October 1902 (BR); *Leg. ign*. s.n., May 1908 (P). **Bamingui-Bangoran:** Koumbala, *Fay* 6279 (MO). **Bangui:** *Breyne* 1254 (BR); *Leg. ign*. 373, 13 December 1891 (P). **Haute-Kotto:** *Testu* 4294 (BM, BR, K); Yalinga**,** *Testu* 4239 (BM, P). **Kwilu:** Boukoko, *Tisserant* 583-1354 (P). **Obangui**: *Laurent* s.n., 19 December 1903 (BR); *Leg. ign.* 17β, 26 January (P). **Ouaka**: Bambari, *Tisserant* s.n. (P). **CHAD. Bangui**: Sibut, *Chevalier* 10719 (P); *Chevalier* 10819 (P). **Bol**: Ngouri: *Chevalier* s.n., 1902 (L, P). **Ombella**: Boma, *Chevalier* 5860 (P). **DEMOCRATIC**

REPUBLIC OF THE CONGO. *Deschamps* 1891 (BR); *de Witte* 8387 (WAG). *de Witte* 12897 (BR); *Gille* 169 (BR); *Jean Louis* 7812 (BR). **Haut-Katanga:** Ganza, *Haui* 6975 (BR); Kapolowe, *Malaisse* 6981 (BR); Musoshi, *Schmitz* 3559 (BR). **Haut-Uele**: Bombombi, *Gerard* 770 (BR, WAG); Garamba, *de Saeger* 1520 (BR, K, L). **Ituri:** Irimba, *de Wilde (d' Estmael)* 144 (BR); Maitatu*, Lisowski* 43603 (BR). **Kafubu**: Kiniama, *Schmitz* 2837 (BR, WAG). **Katanga**: Kasenga, *Robyns* et al. 1928 (B, BR, BM). **Kivu**: *Guy* s.n., 15 February 1936 (P). **Kwango**: Dembo, *Lescrauwaet* 307 (BR). **Lomami**: Butahu, *de Witte* 11858 (BR); Lukoma, *Delvaux* 391 (BR); Kaswabilenga, Parc National De L'Upemba, *de Witte* 3978 (BR). **Kwilu:** Ibele, *Dervulf* 505 (BR); Luisa, *Liben* 3313 (BR, WAG). **Lualaba**: Katela, *Wilde* 742 (BR); Kolwezi, *Holmes* 1462 (FHO). **Maniema:** Kabingwa, *Verdick* 454 (BR); Kasali, *de Witte* 14230 (BR); Kibangula, *Leonard* 5761 (BR). **Nord Kivu**: Beni: *Germain* 5449 (BR); *Lebrun* 4310 (BM, BR, K); Mutsora, *de Witte* 9887 (BR); Mwenda, *de Witte* 13021 (BR); Pinga, *Gutzwiller* 3123 (BR); Rumangabo, *Germain* 1435 (BR, WAG); Rutshuru, *de Witte* 14155 (BR); *Breyne* 3881 (BR); Talias, *de Wilde* (*d' Estmael*) 503/3 (BR); *de Witte* 11811 (BR); *de Witte* 13149 (BR). Watahinga, *de Witte* 8387 (BR). **Orientale**: Mambasa, *Ewango* and *Asani* 2437 (MO). **Tanganyika:** Moba, *de Witte* 318 (BR); Songo-Matumbi: *Seret* 313 (BR). **Tshopo:** Kisangani**,** *Mandago* 2479 (BR); Yangambi, *Gilbert* 7821 (BR); *Jean Louis* 1241 (B, BR); *Jean Louis* 8852 (BR, FHO); *Jean Louis* 11498 (P); *Jean Louis* 12852 (BR); *Jean Louis* 16447 (BR); *Jean Louis* 16849 (BR). **South Kivu**: Kabare, *Robyns* 2503 (BR, K). **Ubangi**: *Lebrun* 1730 (BR, K). **ETHIOPIA.** *Erskine* s.n., 1935 (K); *Leg*. *ign*. 1323, 27 August 1980 (ETH). **Ilubabor:** Ilubabor, *Friis* 1758 (K); *Meyer* 8928 (K, WAG); *Sebsebe* et al. 5525 (ETH). **Kefa**: Mezan Tefari, *Friis* 3970 (ETH, K); *Friis* et al. 7062 (ETH, K). **Oromiya**: Didessa, *Friis* et al. 6031 (ETH, K); *Friis* et al. 9099 (K, WAG); Lekemti, *De Wilde* 8896 (BR, K,

MO, P, WAG). **SNNPR:** Gamu Gofa, *Gilbert* et al. 254 (K, MO, WAG); Gojeb, *Mooney* 8623 (ETH, K). **GHANA.** *Leg ign.* 759 (K-undigitized). **Ashanti***:* Bobiri, *Hepper* et al. 7571 (K); Owab, *Lyor* 53 (FHO); *Lyor* 2722 (FHO); Tano-Ofin: *Jongkind* 1918 (BR, MO, P, WAG). **Bono**: Awisa, *Irvine* 2064 (K). **IVORY COAST.** *Jacques Felix* 1276 (P). **Lacs:** Anoumaba**,** *Chevalier* 22411 (P). **Touba:** Zala**,** *Kruif* 599 (BR, MO, WAG). **KENYA.** *Jarrett* 316 (K). **Kagera**: Minziro, *Willan* 154 (K). **Kakamega**: Bukura, *McDonald* 1093 (K). **Meru**: Meru**,** *Gardner* 3150 (K). **Nyanza:** Nyanza**,** *Moon* 6 (K). **West Pokot:** *Kirk* 358 (K); Cheranangi, *Gardner* 1103 (FHO); Mwina, *Honoré* 3451 (BR, K). **LIBERIA. Gbarpolu:** Gbanka**,** *Linder* 1232 (K). **Lofa:** Lofa, *Jongkind* 6817 (WAG); Vonjama, *Baldwin* Jr., 10037 (K). **Nimba**: Ganta, *Harley* s.n. November 1932 (K); Yekepa: *Jacques George* 24255 (MO). **MADAGASCAR.** *Bosser* 9186 (P); *Cours* 24276 (P); *Greve* 143 (P); *Homolle* 1514 (P); *Service Forester* 5 (P); *Service Forester* 24276 (P); *Toma* s.n., 23 July 1937 (P). **Androy:** Ambovombe, *Decary* 8609 (P); *Decary* 8822 (P); *Decary* 8943 (P); Beloha, *Bosser* 14052 (P); *Bosser* 16052 (CNARP); *Decary* 9910 (P); *Morat* 3940 (CNARP, MO, P); Tanandava, *Peltier* 5503. **Anosi**: Bekily, *Seyrig* 814 (P). **Antanarivo:** Miarinarivo, Gebirge, *Hildebrandt* 3094 (BM, GH, K, L); Tsimbazaza, *Montagnac* 1 (CNARP, P). **Atsimo-Andrefana**: Sakaraha, *Bosser* 19406 (CNARP, MO, P); *Humbert* 29629 (P); *Leg. ign*. 7386, 22 June 1955 (P); *Mabberley* 1000 (CNARP, K). **Atsinanana:** *Ranirison* 585 (P); Brickaville, *Cours* 4625 (P); Toamasina: *Phillipson* 1943 (BR, CNARP, MO, P); Berivotra, *Peltier* 5348 (P). **Betsiboka:** Andriba, *Perrier* 680 (P); Maevatanana, *Leg. ign*. 446, 2 September 1835 (P); *Schwabe* et al. s.n., October-December 1959 (B). **Boeny**: Ambalabongo, *Peltier* 5977 (P); Amborovy, *Peltier* 5287 (CNARP, P); Ampijoroa, *Boiteau* 3015 (P); Ankarafantsika, *Ramamonjisoa* 2032 (CNARP, P); Namoroka, *Service Forester* 17 (P); Soalala, *Decary* 7780 (P); *Jaeger* 4109 (P);

Keraudren 940 (P); *Rakotosoa* 4109 (P); *Ranoriamiera Surveyland* 6396 (P). **Fianarantsoa**: Ambalavao, *Rakotosoa* 11993 (P). **Ihorombe**: Ihosy, *Bosser* 17534. **Mahajanga**: Ambalakida, *Bosser* 9685 (P); *Labat* 3646 (MO, P); *Luino* 095 (P); *Rakotoarisoa* 446 (P); *Bosser* 5851 (P); Ankarafantsika, *Peltier* 3282 (CNARP, P); Antsingy, *Decary* 7947 (P); Beronono, *Harizo* 1065 (MO, P); Majunga, *Schatz Lowry* 1441 (CNARP, K, MO, P); Marovoay, *Bosser* 8346 (P); Tsaratanana, *Decary* 14402 (CNARP, P). **Menabe**: Ambahivahibe, *Leg. ign*. 6542, 10 June 1944 (P). **North Madagascar:** *Baron* 6172 (BM, P); *Baron* 6434 (K). **Sofia**: Andranosamonta, *Decary* 2232 (K, MO, P); Befandriana, *Humbert* 29743 (P). **Toliara:** *Randriantafika* 190 (K); Anadabolava, *Humbert* 12527 (P); Antevamena, *Sussman* 103 (MO); Beza Mahafaly, *Phillipson* 1759 (BR, CNARP, K, MO, P); Mahatomotsy, Gebirge, *Groeninckx* et al. 298 (BG, K, MO, P, TAN); Sakamena, *Poisson* 422 (P); Zombitsy, *Morat* 585 (CNARP, P); *Morat* 961 (P); *Morat* 976 (P); *Peltier* 5815 (P). **Sava**: Iharaña, *Aubriot* 201 (P). **MALI. Kayes:** Kita, *Jaeger* 3628 (G, K, P, WAG); *Jaeger* 5566 (K, P). **NIGERIA.** *Chester* 207 (FHO); *Fosters* 366 (K). **Cross River**: Oban**,** *Talbot* 801 (BM). **Delta**: Idumuje, *Thomas* 2122 (K). **Enugu**: Udi, *Smith* 20 (K). **Gombe**: Mada, *Hepburn* 87 (K). **Kogi**: Lokoja, *Carsons* s.n. 18 September 1908 (K). **Lagos:** Lagos, *Miller* 55 (K). **Ogun**: Abeokuta**,** *Onochie* 8129 (FHO); *Onochie* 35253 (K); **Ondo:** Idanre, *Keay et Onochie* 20240 (FHO, B); *Onochie* 34350 (K); *Orekeke* 26881 (K). **Onita:** *Thomas* 1889 (K). **Oyo**: Ibadan, *Jones* 7338 (BM). **Rivers**: Degema, *Talbot* s.n., 1916 (BM). **REPUBLIC OF GUINEA. Kindia**: Daramagnaky, *Molmou* 74 (K). **Mamou:** Sokotoro, *Pobeguin* 1843 (P). **REPUBLIC OF GUINEA-BISSAU.** *Chevalier* 20389 (P); *Fora* 21 (BR). **Bafata**: Bafata**,** *Leg. ign*. 2873, 28 January 1951 (WAG); *Leg. ign*. 3800, 24 November 1955 (BR, MO, P, WAG); *Leg. ign*. 3747, 3 December 1955 (MO, WAG). **Dalaba**: Fouta Djallon, *Chevalier* 18669 (P). **Faranah:** Kouria**,** *Chevalier* 14874 (P).

Nzerekore: Nimba Mts., *Jongkind* 8042 (BR, MO, P, WAG). **Labe:** Yambering, *Chevalier* 34421 (P). **Oio:** Contubo, *Leg. ign.* 2873, 28 January 1951 (K). **SENEGAL. Dakar:** Niayes**,** *Adam* 759 (P); Sangalkam, *Berhaut* 1031 (BR, P); *Jacques George* 1452 **(**MO). **SIERRA LEONE. Southern Province:** Mamboma**,** *Deighton* 3378 (K); Njala, *Deywith* 2570 (K). **Tingi:** Tingi, *Leg. ign*. 3648, 24 November 1955 (BR, MO, P, WAG); *Morton* and *Gledhill* 3218 (K; MO). **Eastern Province**: Joru, *Pyne* 42 (K, P). **SOMALIA.** *Rink* s.n., December 1873 (K). **Lower Shabeelle:** Brawa Yare, *Bally* 9375 (K). **SUDAN. Dandan:** *Friis et Vollesen* 751 (BR, K). **TANZANIA.** *Bally* 4005 (K); *Burtt* 2498 (K); *Burtt* 3469 (K); *Koritschemer* 2181 (K); *Swymerton* 1130 (BM). **Kagera**: Mabuye, *Festo* 1387 (BRIT, K, MO). **Katavi**: Mpanda, *Harley* 9368 (K). **Kigoma:** Kakombe, *Newbould Harley* 4256 (BR, K); Gombe National Park, *Mbago* 793 (MO). **Mboga:** Lesse, *Bequaert* 3013 (BR, K). **Mtwara**: Mwongo, *Tanner* 570 (B, BR, K). **Mwanza**: Magu, *Kayombo* et al. 3276 (BRIT, MO); *Semsei* 132/FH 2563 (K). **Tanga**: Usambara**,** *Peter* 51732 (B). **TOGO. Mpoti**: *Schäfer* et al. 7689 (B, K, MO, MPU, WAG). **Plateaux**: Ogou**,** *Ern et al.* 2551 (B, P). **UGANDA.** *Laurent* s.n., 22 December 1903 (BR). **Central Region**: Buyaya, *Maitland* 1181 (K); Kyagwe, *Eggeling* 517 (K); *Eggeling* 881 (FHO); Kajansi**,** *Chandler* 1184 (K); Mabira, *Loveridge* 37 (K); Mulange, *Dummer* 4365 (BM, K); Masindi, *Bagshawe* 869 (BM). **Eastern district:** Busoga, *Snowden* 403 (BM). **Western region**: Bwamba, *Hansford* 148 (K); *Longfield* 115 (BM); *Thomas* 1507 (BR, K); Kabanga Mutandi, *Osimiaston* 1363 (K). Kibale, *Eiilu* 104 (K); Mpanga, *Bagshawe Camb* 1180 (BM); Semliki, *Brasnett* 7 (K). **ZAMBIA. Copperbelt:** Chingola, *Fanshawe* 3281 (BR).

4.11.3. *Ipomoea macalusoi* Mattei, Boll. Reale Orto Bot. Colon. Palermo 7: 106 (1908). [43].

Stictocardia tiliifolia (Desr.) subsp. *macalusoi* (Mattei) Verdc., Webbia 13: 322 (1958). [10], ≡ *Stictocardia macalusoi* (Mattei) Verdc., Kew Bull. 15: 6 (1961). [9]. Type: SOMALIA, Goscia, Giumbo, *Macaluso* 80 (PAL image! photocopy at K).

Description. Vigorous perennial twining liana up to 4 m; *stems* terete, glabrous. *Leaves* petiolate, 4—14 × 3—11 cm, ovate, *apex* acute; *margin* entire; *base* cordate, both surfaces glabrous, abaxially black dotted; *lateral nerves* 6—8; *petioles* 2—4 cm, slender, glabrous or slightly puberulent. *Inflorescences* of 2—5 flowers in axillary cymes; *primary peduncles* 1—6 mm, glabrous; *secondary peduncles* 2—3 mm; *bracteoles* caducous, not observed; *pedicels* 3—7.8 mm, slender, glabrous; *outer sepals* 10—12 × 8 – 9 mm, accrescent to 20 × 15 mm, ovate, glabrous; *apex* obtuse; *middle sepal* slightly larger, broadly oblong-elliptic; *corolla* 3.1—5 cm long, broadly funnelform, glabrous, bright red or crimson, centre yellow, limb 2—5 cm wide; *stamens* 5, unequal, included, hairy at the base; *long filaments* 3.5—4 cm; *short filaments* 3 cm; *anthers* yellow; *style* 5 cm, *ovary* not observed. *Capsule* 1.5 × 1 cm, ellipsoid, indehiscent, glabrous, pale brown; *seeds* 8 x 6 mm, ellipsoid, densely puberulent.

Illustration. Figure 4.5.

Habitat: Bushland and forest near coast and around seasonal lakes from 170 to 250 m asl. It is also common in disturbed places around cultivation.

Note. This species has only been collected a few of times and was treated as a subspecies of *I. tiliifolia* [10] leading Van Ooststroom to cite the presence of *I. tiliifolia* in Africa without further discussion [4, 7]. Some specimens are cultivated, such as *Robertson* 6704! and *Jex Blake* in CM 11240 (not seen), both from Mombasa, Kenya

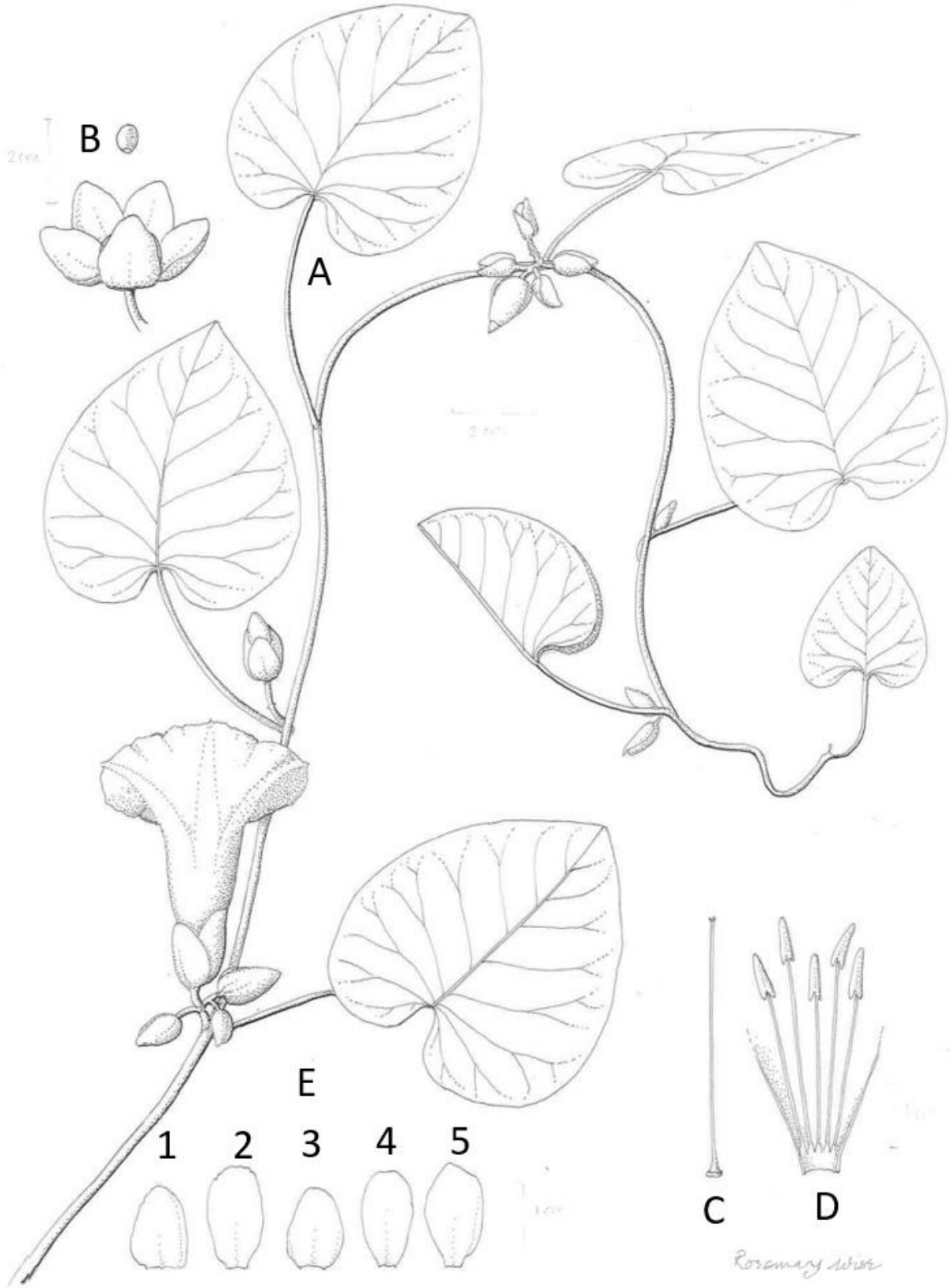


Figure 4.5. *Ipomoea macalusoi* Mattei. **A**. Habit, **B.** Fruit with seed, **C.** Style, **D**. Stamens, **E.** Sepals, (**1**) 1st outer sepal, (**2**) 2nd outer sepal, (**3**) middle sepal, (**4**) 1st inner sepal, (**5**) 2nd inner sepal. Drawn by Rosemary Wise from *Robertson* 6704.

[7]. Outside Africa, it occurs in cultivation on Mauritius (*Vaughan* 10808!) [7] as well as in Sri Lanka (*Austin & Austin* 6209, *Balakhrisnan* 998, both not seen) [22]. No conservation assessment had been conducted for this species. The current AOO and EOO analyses based on very limited four georeferenced specimens shows that the status of this species is Endangered**.**

Specimens seen (Figure 4.3): **KENYA. Lamu:** Kiwayu**,** *Luke* 5482 (K). **Mombasa**: Likoni, *Robertson* 6704 (K, MO, US). **MAURITIUS.** *Vaughan* 10808 (L). **SOMALIA.** *Bavazzano* 386 (K). **Banaadir:** Mogadishu, *Macaluso* 80 (K). **Lower Shabeelle:** Baraawe-Mudun**,** *Rose* 961 (K).

4.11.4. *Ipomoea incompta* Hallier f., Bot. Jahrb. Syst. 18: 151 (1894). [1].

Stictocardia incompta (Hallier f.) Hallier f., Meded. Herb. Leiden 1: 26 (1910). [2]. Type: TANZANIA, Zanzibar, Kokotuni, *Stuhlmann* 110 (holotype HBG 505542 image!; isotype HBG-505543 image!).

Ipomoea althoffiana Dammer, Pflanzenw. Ost-Afr. C.: 333 (1895). [44]. Type: TANZANIA, E. Usambara, Mts, Lutindi, *Holst* 3447 (holotype B destroyed; isotype K 000097049!).

Description. Perennial woody climber up to *c.* 5 m, all parts villous with eglandular white hairs; *stems* terete, ascending. *Leaves* petiolate, 8—11 × 7—8 cm, ovate to oblong, *apex* acute to acuminate, *base* weakly cordate (young) to strongly cordate (mature), *margin* entire, adaxially darker, abaxially paler, *lateral nerves* 6—7; *petioles* 2—8 cm. *Inflorescence* of 3—6 flowers in axillary compound cymes; *primary peduncles* 5—20 mm; *secondary peduncles* 15—28 mm; *bracteoles* 5.5 — 12.5 × 2.6— 6 mm, paired, oblong, pilose, caducous; *pedicels* 5—30 mm; *outer sepals* 21—30 × 9—17 mm, somewhat accrescent in fruit, ovate to semi deltoid, muricate in lower one-third to half, ornamented with up to five parallel vertical ridges in the lower half to

two-thirds, *apex* acute, *base* rounded; *middle sepal* smaller, 12—21 × 7—12 mm, *apex* obtuse; *corolla* 6—7.5 cm long, funnelform, glabrous, pinkish-mauve, midpetaline bands puberulent with black glands, paler, limb *c.* 4 cm wide; *stamens* 5, included, more or less equal; *filaments* 3.1—3.5 cm, glabrous, *base* pilose; *anthers* linear, 5.6 × 0.6 mm; *style* 3.1—4 cm. *Capsule* 13—17 × 8—15 mm, subglobose, indehiscent; *seeds* 13.2 × 11.4 mm, ovoid triangular, dark brown, puberulent.

Illustration. Figure 4.6.

Habitat. On steep rocky slopes in a wide range of forest types (mangrove woodland, riverine and rain forest), but mostly in open areas such as bushland, forest clearings and river margins from 30—1,030 m asl.

Note. *Ipomoea incompta* is the most distinctive species of Stictocardia with hair covering the whole plant except its corolla. Verdcourt noted this distinction [45] and put this species in a separate subgenus, *Madoadoa* Verdc. as well as correcting an old, meaningless specific epithet '*incomta*' [6]. His placement of this species was followed by Wilkin who raised *Madoadoa* to sectional rank [46] in his thesis. The majority of specimens were collected in coastal areas (Figure 4.4) and do not differ from four specimens collected further inland, thus confirming Verdcourt's decision to treat this species as a single taxonomic entity [6]. The recovery of this species within Stictocardia makes this clade very diverse in terms of its morphology. This species has never been assessed for the conservation status. Extrapolation of 24 georeferenced specimens demonstrates that the status of this species is Endangered based on the AOO analysis and Least Concern based on EOO.

Specimens seen. (Figure 4.3) **KENYA. Kilifi**: Arabuko, *Graham* 1915 (B, FHO). **Makueni:** Kibwezi, *Luke* 14331 (K); Tsavo, *Bally* 13401 (G). **Taita Taveta**: Msau,

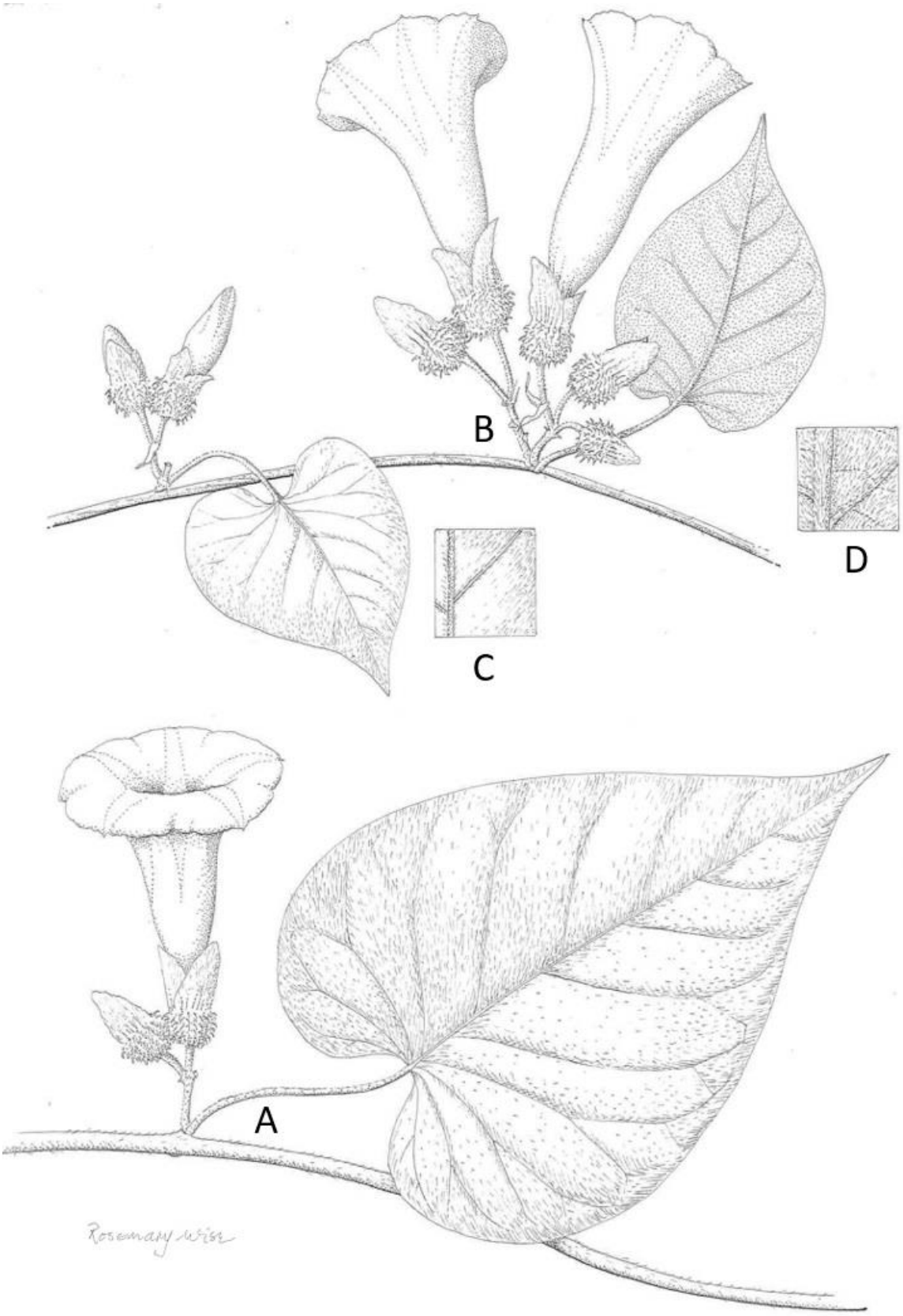


Figure 4.6. *Ipomoea incompta* Hallier f. **A** and **B.** Habit, **C.** Adaxial leaf surface, **D.** Abaxial leaf surface. Drawn by Rosemary Wise from *Faulkner* 2192 (**A**) and Faulkner 4152 (**B, C, D**).

Kabuye et al. 613 (US). **Tana**: Baomo, *Robertson* 6494 (MO). **TANZANIA. Kilimanjaro:** Same**,** *Semsei* 2117 (FHO). **Kitui**: Katuka, *Faulkner* 4152 (MO). **Manyara:** Mbulu, *Greenway Kirrika* 10983 (BR). **Pwani**: Pugu, *Harris* et al. 5845 (MO); *Wingfield* 1672 (MO). **Tanga**: Amboni, *Faulkner* 4650 (K); *Peter* 51750 (B); Lushoto, *Richards* 15330 (K); Maweni, *Harris* et *Harris* 3481 (WAG); Steinbruch, *Faulkner* 2013 (BR); *Faulkner* 2192 (B). **Usambara**, *Baagoe et al.* 226 (WAG); *Holst* 3447; Lutindi Mts, *Holst* 4337 (HBG, K); Ndolwa forest, *Semsei* 3080 (BR). **Zanzibar**: Kokotuni, *Stuhlmann* 110 (HBG). **SOMALIA. Buur Heybo:** Buur Akhaba**,** *Kilian* and *Lobin* 6837 (B, WAG). **UNKNOWN**: *Archbold* 2384 (LISC); *Taylor* 137 (BM).

4.11.5. *Ipomoea pringsheimiana* (Dammer) Rendle, Flora of Tropical Africa 4 (2): 185 (1905) [47].

Rivea pringsheimiana Dammer, Pflanzenwelt Ost-Afr. C: 334 (1895) [44], ≡ *Stictocardia pringsheimiana* (Dammer) Hallier f., Bull. Herb. Bois. 6: 548 (1898) [3]. Type: MOZAMBIQUE, Usambara, Bulua, Gonya Mountain 5000 ft. *Holst* 4337 (isotypes: K-000097050!, M - 0109980).

Argyreia laxiflora Baker, Bull. Misc. Inform. Kew 1894: 67 (1894). [48], ≡ *Stictocardia laxiflora* (Baker) Hallier f., Bull. Herb. Bois. 6: 548 (1898). [3], non *Ipomoea laxiflora* H.J. Chowdhery & Debta. Indian J. Forest. 32: 120 (2009). [49]. Type: MALAWI. Shiri Highlands, Zambesia, December 1881. *Buchanan* 388 (holotype: K-000097048!, isotype: BM!, undigitized) [4, 5].

Ipomoea buchananii Baker, Bull. Misc. Inform. Kew 1894: 73 (1894). [48], nom. Illeg., non *Ipomoea buchananii* Choisy, Mém. Soc. Phys. Genève 6: 474 (1833). Type:

MALAWI. Nyasaland 1891. *Buchanan* 319 (holotype K-000097046!, isotype: BM undigitized!).

Ipomoea woodii N.E. Br., Bull. Misc. Inform. Kew 1894: 101 (1894). [50], ≡ *Stictocardia woodii* (N.E.Br.) Hallier f. Bull. Herb. Bois. 6: 548 (1898).[3], ≡ *Stictocardia laxiflora* var. *woodii* (N.E.Br.) Verdc. Kew. Bull. 27(1): 173 (1963). [11], ≡ *Ipomoea buchananii* var. *woodii* (N.E.Br.) J.R.I.Wood & Scotland Nat. Plants 5 (11): 1136-1144. Suppl. Mat. 35. (2019). [51]. Type: SOUTH AFRICA. KwaZulu Natal, cultivated in Durban Botanic Gardens from plants collected in Zululand. *Medley-Wood*, 4146, 4864 (syntypes K-000097042! and K-000097043!).

Description. Perennial, shrubby woody climber up to 6 m; *stems* terete, glabrous, puberulent; *Leaves* petiolate, 6—13.3 × 6—12.5 cm, ovate or suborbicular; *apex* acute to acuminate; *margin* entire; *base* shallow to deeply cordate; both surfaces glabrous or puberulent, abaxially black dotted; *lateral nerves* 8—11; *petioles* 4—14 cm*,* slender. *Inflorescences* of 1—4, rarely with more flowers in very lax axillary cymes; *primary peduncles* 1.1—20 cm, glabrous, puberulent; *secondary peduncles* 5–17 mm; *bracteoles* lanceolate, caducous; *pedicels* 6—21 mm, slender, glabrous or puberulent; *outer sepals* 7—10 mm, ovate, glabrous, puberulent, sometimes puberulent in lower half and glabrous in upper half; *apex* acute obtuse; *middle sepal* equal; ovate, *apex* obtuse or rounded*; corolla* 5.3—8 cm long, funnelform or campanuliform, glabrous, pink, pale purple, white or pinkish-mauve with darker midpetaline bands, limb 4.2 cm wide; *stamens* 5, included, unequal*,* white; *long filaments* 3—5 cm; *short filaments* 3 cm; *anthers* white; *style* 5 cm, included; C*apsule* globose, *c.* 1.5 cm, indehiscent; *seed* unknown.

Illustration. Figure 4.7.

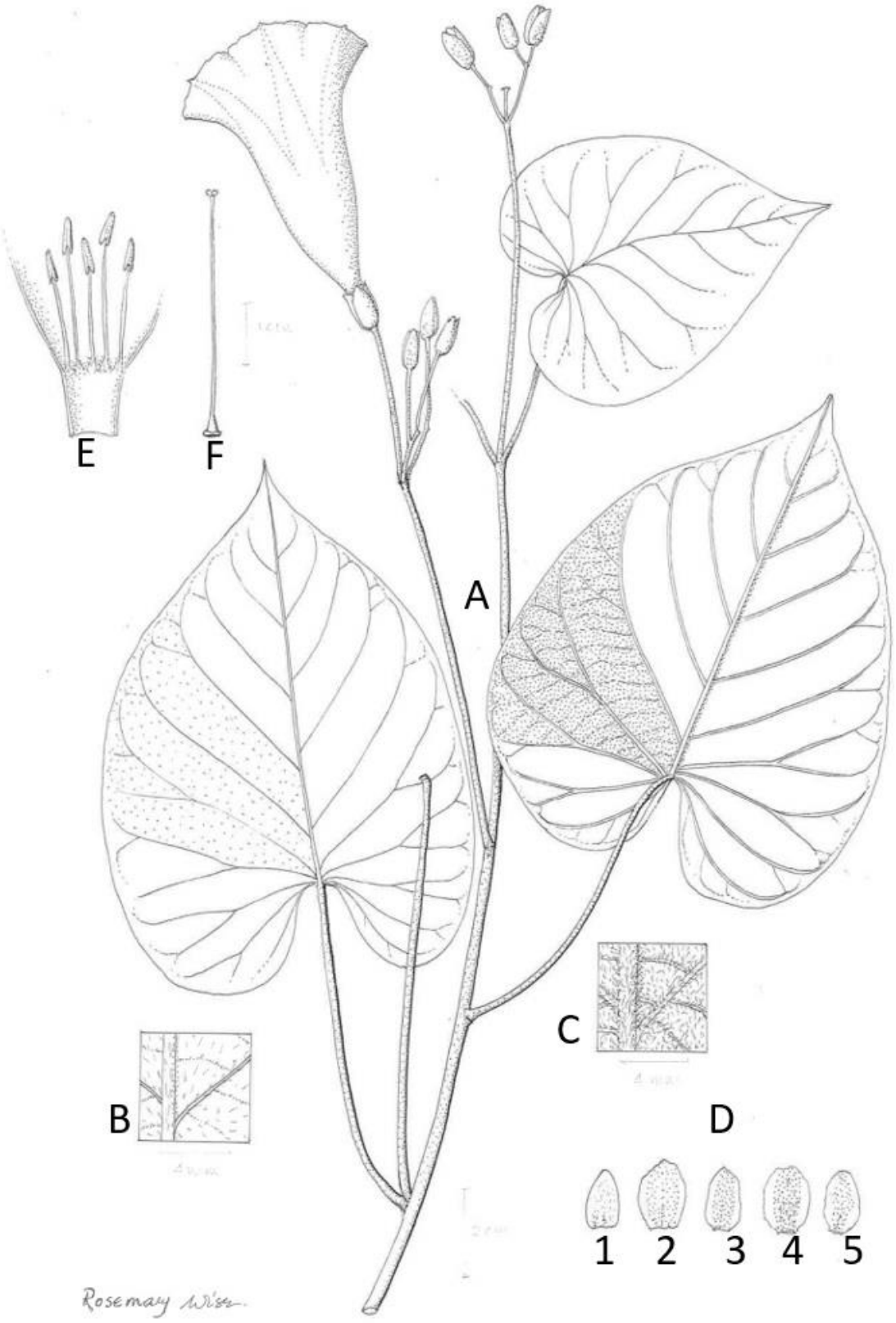


Figure 4.7. *Ipomoea pringsheimiana* (Dammer) Rendle. **A**. Habit, **B**, Leaf adaxial, **C**. Leaf abaxial, **D**. Sepals, (**1**) 1st outer sepal, (**2**) 2nd outer sepal, (**3**) middle sepal, (**4**) 1st inner sepal, (**5**) 2nd inner sepal, **E.** Stamens, **F.** Style. Drawn by Rosemary Wise from *Brummitt et al*. 15562.

Habitat. Open places in evergreen forest, such as abandoned clearings, forest margins, roadsides, riverine forest and rocky places from 660—1,300 m asl.

Note. *Ipomoea buchananii* Baker [51] is an illegitimate name as it is a later homonym of *I. buchananii* Choisy. This species is distributed from eastern to southern Africa (Tanzania, Mozambique and South Africa) and Madagascar. Several references report the fruit as unknown [7, 28, 52, 53]. However, there are two fruiting specimens with accrescent sepals (*Lisowski* 40804 and *Seyrig* 483), the latter identified by Verdcourt as *S. laxiflora* = *I. pringsheimiana* in 1969, after his account the *Flora of Tropical East Africa* [7] was published. The seed is unknown as the two specimens above are only accessible by images. The conservation status of *I. pringsheimiana* in South Africa was *Least Concern* [54]. The current assessment of 38 specimens conclude that the status of this species is Endangered based on the AOO analysis and Least Concern based on EOO analysis.

Ipomoea woodii [51] is treated under this species following Verdcourt's treatment as it is morphologically similar differing only in the smaller leaves length (<8 cm), shorter peduncles (<1.5 cm) and a smaller corolla length (<7 cm) [11]. Previously, Verdcourt treated this taxon as a synonym of *I. beraviensis* as he noted intermediates after comparing specimens [8]. He later changed his decision because the inflorescence of this taxon has fewer flowers and the corolla shape differs from that of *I. beraviensis*. More than half the collections are of cultivated plants from Durban Botanic Gardens. We were, unfortunately, unable to generate DNA sequences of *I. woodii,* so its treated as a synonym is provisional, being based on morphological evidence only.

Specimens seen (Figure 4.3). DEMOCRATIC REPUBLIC OF THE CONGO. Ituri: Mt. Hoyo, *Lisowski* 40804 (BR). **MADAGASCAR. Anosi**: Bekily, *Seyrig* 483 (P). **Diana**: Ambilobe, *Humbert* 25502 (P). **MALAWI. Southern region:** Chiradzulu, *Brummitt* 9828 (LISC); Mlanji, *Buchanan* 319 (BM); *Buchanan* 388 (K); *Buchanan* 682 (BM, K); *Leg. ign.* (K-undigitised); *Richards* 16773 (BR); Zomba: *Blackmore* et al. 1357 (BM, LISC); *Brummitt* et al. 15562 (LISC); *Masiye* 59 (BR, MO). **MOZAMBIQUE.** *Drummond* 10347 (MO). **Gaza:** Massingire, *Torre* 5322 (K, LISC). **Manica:** Chimoio, *Chase* 6877 (BM, BR); *Garcia* 523 (LISC); Garuso, *Gilliland* (BM). **Sofala:** Cheringoma, *Barbosa* 1119 (LISC); *Torre* 4141 (LISC, MO). **Zambesia:** Ile, *Torre* 5586 (LISC). **TANZANIA. Katavi**: Mpanda, *Mgaza* 150 (K). **Tanga:** Amani, *Peter* 51693 (B); Manga, *Peter* 51676 (MO); Usambara, *Greenway* 1670 (FHO, K); *Peter* 51627 (B, BR, MO); *Peter* 51628 (B, BR, MO); *Peter* 51684 (B, U); *Peter et al*. 51734 (B); *Peter et al.* 51737 (B); *Peter* 51876 (B, BR, MO). **ZIMBABWE. Manicaland:** Burma, *Chase* 6505 (K); Chimanimani**,** *Drummond* 10216 (K). **MOZAMBIQUE.** Magombera**,** *Luke* 9551 (K). **Maputo,** Delagua bay**,** *Junod* 439 (BR). **Sofala:** Cheringoma, *Barbossa* 1696 (LISC). **SOUTH AFRICA. Berea:** Botanic Gardens Durban, *Leg. ign*. 11579 (NH-0011579-0); *Leg. ign*. 13064 18 May 1911 (NH-0013064-0); *Medley-Wood* 4779 (NH); *Medley-Wood* 4806 (Z); *Medley*-Wood 4864 (K); *Medley-Wood* 10065 (NH). **Natal:** Durban, *Govender* 2930 (NH); Gwalaweni, *Strey* 4648 (NH). **Zululand:** *Medley-Wood*, 4146 (K); Ngoye, *Medley-Wood* 10354 (NH).

4.11.6. *Ipomoea tiliifolia* (Desr.) Roem. & Schult., Syst. Veg. 4: 229. (1819). [55]. *Convolvulus tiliifolius* ("*tiliaefolius*") Lam., Encycl. 3: 544. (1792). [56], ≡ *Rivea tiliifolia* (Desr.) Choisy, Mem. Soc. Phys. Geneve 6: 407. (1834). [57], ≡ *Amphione tiliifolia* (Desr.) Raf., Fl. Tellur. 4: 79. 1836 (1838). [58], ≡ *Argyreia tiliifolia* (Desr.) Wight, Icon. Pl. Ind. 4 (2): 12, t 1358. (1848). [59], ≡ *Stictocardia tiliifolia* (Desr.) Hallier f., Bot.

Jahrb. Syst. 18: 159. 1894. [1]. Type**.** MAURICE (MAURITIUS) *Commerson s.n.* (holotype P-LAM-00357542 (not seen), isotypes G-00135053 image!, MPU-016603 image!).

Ipomoea benghalensis Roem. & Schult., Syst. Veg. 4: 229. (1819). [55]. Type: INDIA*. Heyne* s.n. (location unknown, possibly destroyed in B).

Convolvulus gangeticus Roxb., Fl. Ind., 2: 46, (1824). [60], nom. illeg., non *Convolvulus gangeticus* L. (1756)*.,* ≡ *Ipomoea gangetica* (Roxb.) Sweet, Hortus Brit., 2nd ed: 288. (1830). [61]. Type: INDIA. River Ganges, lectotype: Roxburgh Icon no. 1793 (K, not seen) [20].

Ipomoea pulchra Blume, Bijdr. Fl. Ned. Ind. 716. (1826*).* [62], ≡ *Stictocardia pulchra* (Bl.) Hallier f. Bull. Herb. Boiss v. 5 s.380. (1897). [3], Type: INDONESIA. Moluccas, *C.L. Blume* 297 (holotype: L-0004259 image!).

Convolvulus melanostictus Schltdl., Linnaea 6: 737. (1831). [63]. Type: U.S. VIRGIN ISLANDS. St Thomas, *C. Ehrenberg* s.n. (holotype HAL-0037540 image!).

Stictocardia campanulata sensu Merrill*.* Philipp. J. Sci., Botany 9: 151-152 (1914). [64], nom. illeg., non *Ipomoea campanulata* L. Sp. Pl. 1:160. (1753).[65]. Type: *Adamboe* Rheed. Hortus Mal II p. 115 t. 56.

Stictocardia tiliifolia subsp. *marquesensis* Staples & Butaud, Phytologia 98 (3): 204 (2016). [66]. ≡ *Ipomoea tiliifolia* subsp. *marquesensis* (Staples & Butaud) J.R.I.Wood & Scotland. Nat. Plants 5 (11): 1136-1144. (2019). Suppl. Mat. 35 [51]. Type: FRENCH POLYNESIA, Marquesas Islands, Eiao, Tetiaenui plateau, dans les parages, *Thibault* 1066 (holotype PAP-001844, not seen; isotype P-04399830 image!).

Description. Perennial twiner scrambling over bushes; *stems* 5 –12 m long, herbaceous, turning woody when old, terete, glabrous or puberulent. *Leaves* petiolate,

3—15 × 5.5—18 cm, ovate or suborbicular, rarely subrenifom; *apex* acute, acuminate or obtuse; *margin* entire; *base* cordate, both surfaces glabrous to puberulent, adaxially dull green, abaxially subglaucous with black dots; *lateral nerves* 7—10; *petioles* 3—10 cm, slender, glabrous or puberulent, reddish. *Inflorescence* of 1—3 flowers in axillary cymes; *primary peduncles* 0.6—7.5 cm, glabrous or puberulent; *secondary peduncles* 3—14 mm, glabrous or puberulent; *bracteoles* lanceolate, caducous; *pedicels* 5—30 mm, slender, glabrous or puberulent; *outer sepals c.* 8—13 × 6—10 mm at anthesis, strongly accrescent, reaching up to 27 × 27 mm; ovate or suborbicular, glabrous or puberulent, *apex* rounded, obtuse or truncate; *middle sepal* ovate, *corolla* 4.5—7.3 cm long, funnelform or campanuliform, glabrous, pink to purple or occasionally white, midpetaline bands apically with white hairs, limb 2—4 cm wide; *stamens* 5, *filaments* 3—5 cm, more or less equal, included; *anthers* white; *style* 5 cm, included. *Capsules* 1.2—2.5 × 1.6—2.5 cm, compressed globose, indehiscent, glabrous, pale yellowish to green or dark brown; *seeds* 4, 1 x 0.8 cm, ellipsoid, densely puberulent, dark brown.

Illustration. Figure 4.8.

Habitat. In a wide range of coastal habitats including open and scrubby places as well as rain forest, river floodplain, forest margins, lava fields, grassland, and disturbed roadsides. It is very rarely found in high elevation up to 900 m asl (*Lorzing* 14763).

Note. *Ipomoea tiliifolia* is the most widespread species of Stictocardia. It has a large number of basionyms and types from different regions (Mauritius, India, Java, Marquesas, and St. Thomas) of the world. Figure 4.9. shows that it grows near tropical sea coasts but is absent from the continent of Africa [7, 28, 29, 53]. *Welwitsch* 6213 labelled ‘Iter Angolense’ was collected in Cape Verde presumably on his voyage to/from Angola and not on the African continent. Wood et al. [20] listed specimens

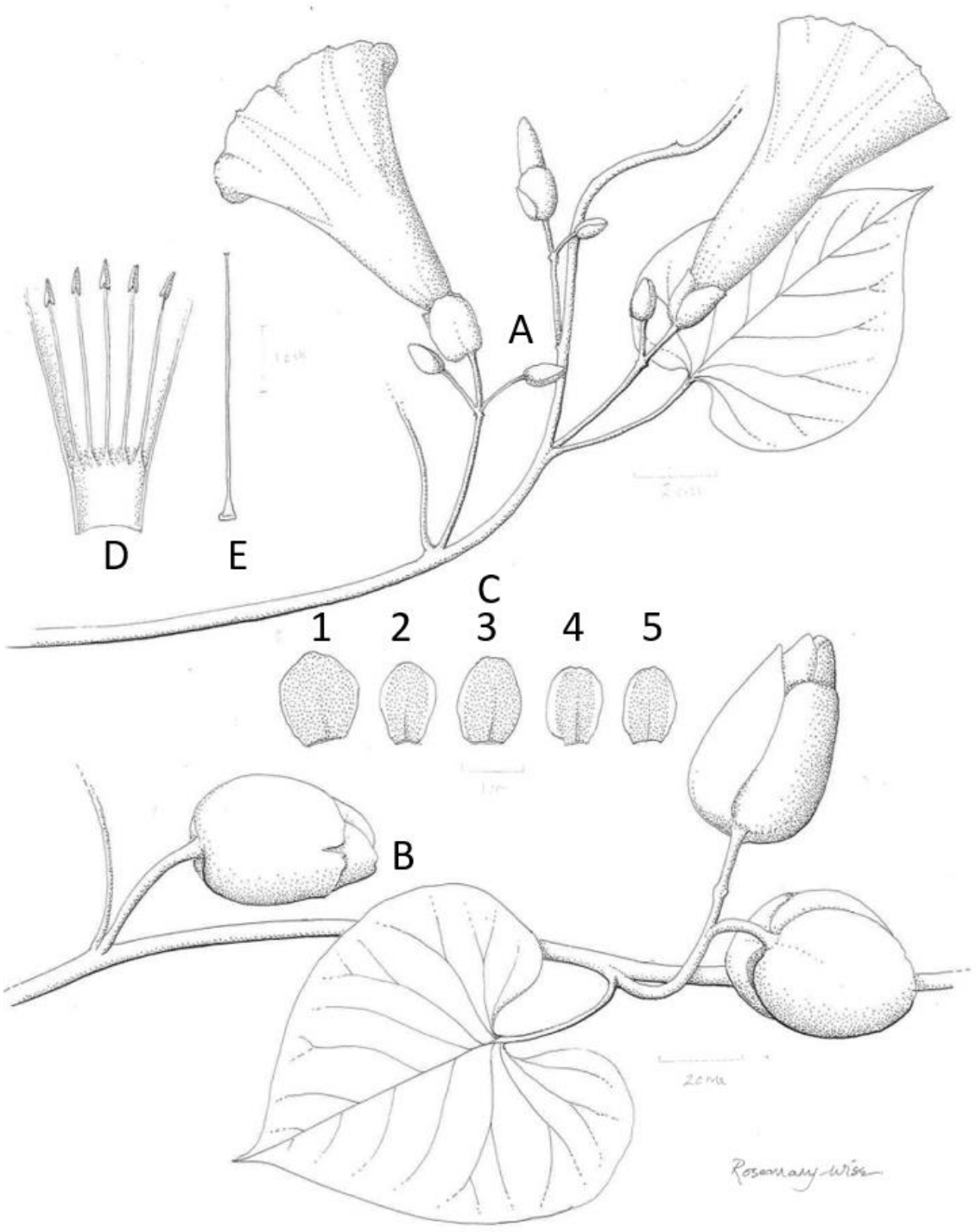


Figure 4.8. *Ipomoea tiliifolia* (Desr.) Roem. & Schult. **A**. Habit while flowering, **B**. Habit while fruiting. **C.** Sepals, (**1**) 1st outer sepal, (**2**) 2nd outer sepal, (**3**) middle sepal, (**4**) 1st inner sepal, (**5**) 2nd inner sepal, **D.** Stamens, **E**. Style. Drawn by Rosemary Wise from *Sulit* 17032 (**A, C, D, E**) and *Barthelat* 436 (**B**)**.**

from other countries - Ecuador, Nicaragua, Guatemala, and Cuba - in the New World, which are unseen in this study.

This species was wrongly named *Ipomoea campanulata* in a number of publications [12, 14, 64] for decades following Merrill [64]. *Ipomoea campanulata* is, in fact, a quite different species as described in Chapter Two.

Recently, a subspecies of *I. tiliifolia* [67] was described from the Marquesas [66]. This taxon is distinguished by the white corolla and dense hairs over all vegetative parts [66]. However, in the absence of a DNA sequence, the status of this taxon cannot be confirmed. However, it is treated as *I. tiliifolia,* since the observed differences seem unlikely to justify recognition at subspecific rank. It has not been included in the *Flora of the Marquesas Islands* [68].

This species has been evaluated multiple times for its conservation status at both national and global scales [42, 69-71]. The most recent assessment of *I. tiliifolia* was *Least Concern* under the IUCN Red List category as it has a widespread distribution and seems not facing major threats [72]. The status was reasonable albeit this species currently has no data on population size, trends, utilisation, and threats [72].

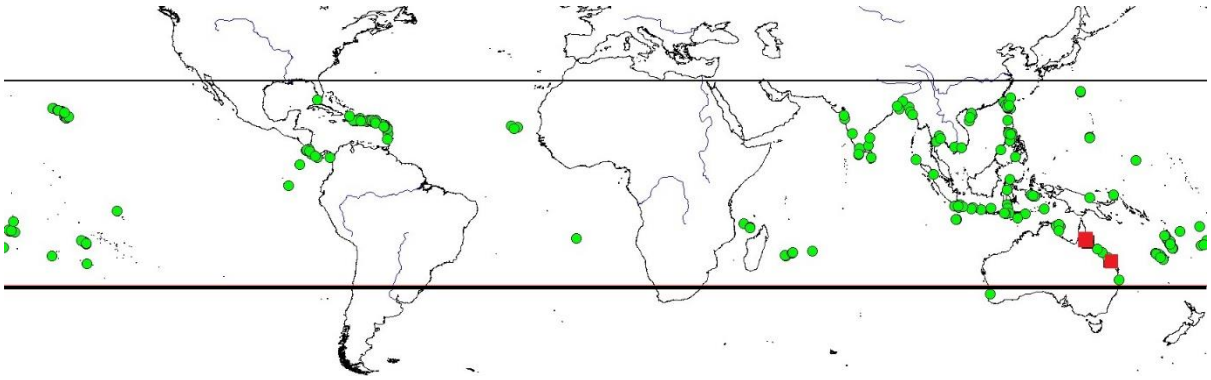


Figure 4.9. Distribution of *I. tiliifolia* (green circles) and *I. queenslandica* (red squares) based on georeferenced specimens. Black horizontal line is 30°N and 30°S.

Specimens seen

AMERICA

ANTIGUA AND BARBUDA. St. John: Galley bay, *Box* 1299 (BM, US); *Rose* 3383 (NY, US). **CONTINENTAL UNITED STATES. Florida Keys:** Monroe**,** *Small Mosier* 6002 (K, U). **COSTA RICA. Alajuela:** Los Loros, *Brenes* 22047 (NY). **Puntarenas**: Cabo Blanco, *Chavarria* 341 (MO); *Fernandez* and *Shallenger* 1330 (MO); Playa Balsita, *Quesada et al.* 832 (BM, MO); Playa Organos, *Hammel Garita* 19308 (MO); Peninsula de Nicoya, *Alvarado* 110 (MO); Ballena, *Hammel* 20619 (MO); Playa Espadilla, *Wilkin* 121 (BM). **Isla de Coco:** Rio Sucio, *Quesada* 1103 (BM, K, MO); Playa Blanca: *Valerio* 445 (BR). **DOMINICAN REPUBLIC.** *Imay* 230 (K). **Barahona**: *Turckheim* 2776 (BR, NY). **La Altagracia**: Bayahibe, *Liogier* 24805 (NY); Boca de Yuma*, Liogier* 22936 (NY). **Santo Domingo***: Rose* 4326 (NY); *Wright et al*. 390 (US); Boca Chica, *Liogier* 26073 (NY); La Caleta, *Ekman* 10929 (K, S). **La Romana:** Catalina island, *Liogier* 27392 (NY). **Peravia**: Jovero, *Albott* 2549 (US); *Albott* 2561 (US). **St. Patrick**: *Whitefoord* 5400 (BM, US). **Samana:** Samana Peninsula, *Liogier* 14397 (NY, P). **ECUADOR. Galapagos Islands:** St. Cruz, *van der Werff* 1282a (K, MO, NY, U). **FRENCH OVERSEAS TERRITORY. Antilles**: St. Claude, *Stehle* 233 (P). **Guadeloupe**: *Gallo* 3134 (US); Port Louis, *Questel* 4039 (US); Mamelles, *Raynal* 15952 (P); Pirogue, *Proctor* 20264 (BM, US). **Martinique**: *Belanger* 573 (P); *Duss* 2476 (NY, US); *Hahn* 628 (P); *Leg. ign.* s.n., July 1820 (P); Fort Desaix, *Mouret* 380 (P); Le Francois, *Duss* 3503 (NY); Precheur, *Duss* 1883 (US); St. Lucia, *Plee* s.n., January 1821 (P-04039428). **GUATEMALA.** *O'Donell* s.n., June 1953 (K). **HAITI. Nord:** Bayeux, *Ekman* 2923 (S, US). **Nord Ouest**: Anse a Foleur, *Zanoni* 33582 (NY). **NETHERLANDS OVERSEAS TERRITORY. St. Eustatius:** *Suringar* s.n., 16 April 1895 (L). **PANAMA.** *Duchassaing* s.n., 1851 (P-04039446). **Darien**: Jaque, *Sytsma*

and *D'Arcy* 3457 (MO). **San Jose:** Playa Grande**,** *Johnston* 1011 (MO). **PUERTO RICO.** *Drake* 9768 (P); *Woodbury* s.n. Aug 1969 (NY). **Arecibo***: Liogier* 33759 (NY). **Cabo Rojo:** Guanajibo Barrio, *Sintenis* 822 (US). **Humacao:** Cayo Santiago, *Axelrod et al.* 3326 (NY). **Dorado:** *Woodbury* s.n., 3 December 1960 (NY). **Quebradillas:** Quebradillas, *Sargent* B152 (US); *Stevenson* 5886 (US). **San Juan:** Fort Jeronimo, *Britton* 6638 (NY). **Vieques**: Santa Maria, *Shafer* 2583 (NY, US). **SAINT KITTS AND NEVIS. Saint George Basseterre**: Nag's Head, *Proctor* 18512 (US). **ST. VINCENT AND GRENADINES.** *Hooker* 27 (K). **UK OVERSEAS TERRITORY. Guiana island:** White bay**,** *Proctor* 43448 (NY). **Montserrat:** Balham river, *Proctor* 18931 (BM, US); Brochiners, *Shafer* 225 (NY); Plymouth, *Shafer* 64 (NY, US). **U.S. VIRGIN ISLAND: St. John,** Cruz Bay, *Acevedo-Rodriguez* 2463 (NY); *Acevedo-Rodríguez et al*. 4008 (NY); Center Line Road, *Acevedo-Rodríguez* 2898 (NY). **Tortola**: Fishlock 275 (K, NY). **St. Croix island:** Ricksecker 162 (MO, NY, US). **St. Thomas**: Britton 271 (NY); *Leg. ign*. s.n. (P-04039435). **Virgin Gorda:** Fishlock 282 (NY).

AFRICA

CAPE VERDE. *Leg. ign*. s.n. (L-2744536); *Lowe* s.n., 25 May 1875 (BM, K, NY); *Welwitsch* 6213 (BM, K). **Maio**: Mt. Batalha, *Kilian* et *Leyens* 2991 (B, MO). **Sao Vicente**: *Moseley* s.n., 1873 (K); *Smith* 1610 (K, NY). **COMOROS. Anjouan:** *Hildebrandt* 1628 (BM, K, P). **FRENCH OVERSEAS TERRITORY. Mayotte**: Accoua, *Barthelat* 436 (MO); Chiconi, *Barthelat* 929 (MO, P); Chirongui, *Barthelat* 1643 (MO, P); Sada, *Barthelat* 949 (P). **Reunion:** *Boivin* s.n., 1 June 1851 (P-04039504); La Marine, *Cadet* 5616 (P)**. MAURITIUS.** *Bouton* s.n. 1839 (G-00135069); *Commerson* s.n. (G00135053, P-00622214, 00622215, 00622216, 00622217). **Flacq:** Bramsthan, *Duljeet* 11220 (L). **Rodriguez:** *Balfour* s.n. (BM); *Cadet* 2738 (P); Sainte Anne, *Cadet*

5998 (P); Saint Denis, *Cadet and Figier* 6764 (P). **UK OVERSEAS TERRITORY. St. Helena***: Lady Dalhousie* s.n. (K-undigitised).

ASIA

AUSTRALIAN OVERSEAS TERRITORY. Christmas island: *Leg. ign.* s.n., October 1904 (SING); *Powell* 232 A (K); *Ridley* 150 (K). **BANGLADESH. Chittagong:** *Hooker et Thomson* s.n. (L-2749887); *Hooker et Thomson* 4859 (P); Teknaf, *Rahman et al.* 4240 (K). **Dacca:** *Blanco* s.n. (BM-undigitized); *Jabbar* 56-771 (K). **Rangpur Division**: Sundarban**,** *Hooker* et *Thomson* s.n. (K 2017/00132108); *Prain* s.n., 21 February 1900 (BM). **CHINA. Guangdong:** *Southroad* 00886 (IBK, IBSC, PE); *Zhou Yuanrui* 100145 (IBK). **Hainan**: *Zuo et Chen* 23016 (IBSC); *Li* 3086 (IBSC); *Li et Xing* 4744 (IBSC); *Zuo et al.* 1683 (IBSC); Yaichow, *Tso* 23016 (BP, P). **Taiwan:** *Genkei Masamune* 3871 (TAI); *Matuda* 786 (TAI) *Sasaki* s.n. (TAI- 093276); Bankinseng Mt, *Henry* 1671 (K); Koahsiung, Meilong, *Huang* 15939 (TAI); Pingtung Hsien, *Huang* 9158 (MO, TAI); *Kao* 9724 (TAI); *Wang* 2907 (TAI); *Wang* 02016 (L); *Wang et al.* W03088 (PE); Lantao*, Huang* 9305 (TAI); *Hsu* 468 (TAI); *Wang* 3987 (MO); Tainan, *Huang* 4408 (TAI); Taitung Hsien, *Yang et Liu* 14740 (KUN, MO); *Yang et Huang* 15741 (MO); South Cape, *Henry* 199A (P); *Kudo et. Suzuki* 15830 (TAI); Syamari, *Suzuki-Tokio* 6121 (NAS); Taihoku, *Tata* 1655 (TAI). **INDIA.** *Edgeworth* 447 (K); *Wight* 2247 (K); *Lambert* s.n. (BR-0000013308636); *Rottler* s.n., January 1798 (K); *Wallich* 1340A (G). **Andhra Pradesh:** Coromandel*, Mace* s.n. (P-04039485). **Karnataka:** Mangalore**,** *Steudel* s.n. (P); *Leg. ign.* s.n. in Hohenacker 1947 (K). **Kolkata***: Gaudichaud* 14 (P); *Leg*. *ign*. s.n., Cult. in hort. Bot Cal. (G); *Wight* 1948 (BO, L, P). **Ganges:** *Leg*. *ign*. 352, December 1817 (SING-0075944). **Tamil Nadu**: Chennai, *Wallich* 1247 (BM); Anaimalai, *Beddome* 5530 (BM); Coimbatore, *Fisher* 568 (K). **Maharashtra:** Mahim, *Patel* 2042 (BRI); Mumbai, *Roux* s.n. (G-00134790); Tiruchi*, Rajamani* THT 70 (K). **Kerala**:

Shimoga, *Biju* 15344 (ARIZ). **Nicobar Islands**: *Kurz* 26056 (BM). **INDONESIA. Ambon:** *Blume* 297 (L-0004259); Hila, *Reinwardt* 81 (L); *Reinwardt* 1462 (L). **Flores**: Nangalili, *Schmutz* 1669 (L); Reo, *Kostermans* 22212 (L). **Java**: Banten, *Borssum Waalkes* 154 (L); *Vogdt* 154 (L); Bogor, *Hallier* 218 (L); Cimahi, *van Slooten* 632 (BO); Karang Hawu, *Docters van Leeuwen* 14192 (BO); Pasuruan, *Backer* 3914 (BO); Priok, *Harreveld* 5077 (L); Sunda strait, *Stauton Macartney* s.n.,1873 (BM); **Nusa Laut**: *Reinwardt* s.n., July 1821 (L); *Reinwardt* 63 (L). **Kangean:** Sepanjang, *Backer* 28986 (BO). **Lombok**: *Elbert* 2478 (L); *Zollinger* 1563 (P). **Panaitan**: Tanjung Parat, *Borssum Waalkes* 697 (BO, K, L); *Borssum Waalkes* 764 (K); *Borssum Walkes* 1951 (SING). **Rinca:** *Suakov* 144 (BO). **Seram**: Tahalupu, *Kornassi* 1338 (BO). **Sulawesi:** *Leg*. *ign*. 82 (L-2744574); *Teijsmann* s.n. (BO-1724208); Kalao, *Docters van Leeuwen* 1526 (U); Pakamisang, *Rachmat* 311 (BO, L); Tanah Jampea, *Docters van Leeuwen* 1595 (BO, U); Tojambu, *Kjellberg* 1862 (BO). **Sumatra**: Perapat, Tongging, *Lorzing* 14763 (BO). **Timor:** *Riedel* s.n., June 1884 (K); Kupang, *Brown* s.n., April 1803 (BM); *Kooy* 1395 (L). **JAPAN. Bonin islands:** Nagahama, *Furuse* 11241 (K, L, PE); *Yang* 1124 (TAI); Motochi, *Leg. ign.* 8217, 20 December 1920 (K); *Wilson* 8213 (BM). **MALAYSIA. Kedah:** Pulau Timun, Henderson 29113 (K); **Sarawak:** *Native collector* 2348, February-June 1914 (BM). **MYANMAR. Rakhine:** Akyab, *Vogt* BU 259 (K). **PAPUA NEW GUINEA. Bismarck Archipelago:** Ugana, *Peekel* 10 (K, L); *Peekel* 161 (BO); *Peekel et al.* 1213 (B). **THAILAND.** *Haniff* 7469 (K). **Bangkok**: *Kerr* 3917 (BM, K); *Kerr* 3922 (BM). **Prachuan Khiri Khan:** Chalak**,** *Collins* 1345 (K); Pran, *Put* 2450 (BM, K). **Sriracha**: *Put* 468 (BM, K). **THE PHILIPPINES. Alabat**: *Merrill* 10452 (BM, P). **Bicol**: Albay, *Cuming* 1026 (K); *Cuming* 1087 (BM, K); Bauang, *Elmer* 5584 (K, P). **Bulacan**: *Ramos* 2028 (BM, BRI). **Calabarzon**: Los Banos, *Loher* 4150 (K); Calauan, *Mcgregor* 12385 (BM, K, L, P). **Ilocos**: Bangui, *Ramos* 2761 (BO, P). **Luzon:** Malabon,

Loher 4136 (K). **Mindanao**: Zamboaga, Pasanan, *Frake* 36247 (L). **Mindoro**: Manaul, *Sulit* 17032 (BM, BR, K, L). **Palawan***: McGregor* 45946 (BRI). **Polillo**: Karlagan, *Fox* 9166 (L). **Samboang:** *Perrottet* s.n. (P-04039468). **Tayabas:** *Whitford* 827 (P). **VIETNAM. Chau Doc:** *Pierre* s.n., December 1867 (P); *Pierre* s.n., August 1869 (P, BO). **Baria**: *Pierre* s.n., August 1866 (P). **SRI LANKA.** *CV* 2548 (BM-001253027); *Leschenault* s.n. (P-04039491).

AUSTRALIA

AUSTRALIA. Northern Territory: Goromuru River, *Cowie* 6659 (BRI); Refuge Bay, Elcho island, *Dunlop* 3913 (L); Bennet Bay, *Dunlop* 7350 (BRI). **Queensland:** Budekin, *Ford* 02382 (B); Burleigh Heads National Park, *Foster* 40510 (BRI); Cromarty, *Blake* 8318 (BRI); Gloucester Island, Mount Bertha, *Batianoff* 9403318 (BRI); Howard Point, *Batianoff* 11706 (BRI, P); Low isles, *Cull* 2839 (BRI); Ramingining, *Cowie Mangion* (BRI). **Western Australia**: Rockingham, *Mueller* s.n., 1865 (L); *Mueller* (L-2744546).

PACIFIC

COOK ISLAND: Rarotonga, *Cheeseman* 612 (AK, K). **FEDERATED STATES OF MICRONESIA. Guam:** *Nelson* 259 (BO); *Thompson* 93 (BO, K, L); Road to Dan Dan NASA radio tracking station, *Fosberg* 59740 (L, NY); Barrigada, *Fosberg* 35287 (BISH, L); Tumon Bay, Gogna Beach, *Fosberg* 43496 (BISH). **NEW CALEDONIA. North Province**: Diahot valley, *Balansa* 3353 (P); Koumac, *MacKee* 21946 (P). **Nehoue**: *MacKee* 16896 (P); *MacKee* 35001 (L, P); Plateau de la Montagne des Sources, *Blanchon* 196 (P). **Tipindje**: *MacKee* 23941 (L, P). **Santo**: *Blanchon* 849 (P); Vallée Koumac, *MacKee* 25228 (P, SING); *MacKee* 41490 (P). **South Province**: Bourail, *Balansa* 1056 (P); *Schmid* 1994 (P); Nouméa: *Balansa* 543 (P). **FIJI. Cuou**: Sigatoka, *Greenwood* 306 (K). **Savusavu:** *Seemann* 334 (K); Vanua Levu, *Leg. ign.* s.n., 1898

(K-undigitized). **FRENCH POLYNESIA. Marquesas.** *Butaud et Girardi* 2688 (BISH). **Pohnpei:** Nihwel, *Raynor* 125 (NY, US). **Society islands**: *Banks Solander* s.n. (BM); Moorea, *Sachet Bare* 2335 (BM, K, MO, NY). **Tahiti island:** *Setchell et Parks* 238 (BISH); Papeete, *Florence* 8313 (P). **Tubuai**: Hanareho. *Halle* 6789 (P). **SAMOA.** *Powell* 258 (K). **Manu'a:** Ofu, *Garber* 999 (L). **Namu'a island**: *Whistler et al.* W4559 (B). **Nu'utele island**: Vini, *Ogle* 3842 (AK). **Suisega**: *Whistler* 3065 (K). **Tutuila island**: *Setchell* 248 (BM); *Whistler* 2719 (B, BISH, K). **Upolu**: Lalonaea, *Cox* 148 (K). **Windward island**: Petite Coulibrie, *Whitefoord* 6056 (BM, US). **TONGA. Vavau:** *Crosby* 127 (K); Tu'anikivale, *Soakai* 774 (K); Leimatua, *Yuncker* 16052 (BISH, BM, U). **UNITED STATES in the PACIFIC**. **Hawaii:** *Hillebrand* 1983 (US); Halepuaa, *Renee Kubo* 24 (US); Kailua Kona, *Greenwell* et al. 19378 (B); Kau, Punaluu, *Faurie* 1041 (BM, P); *Faurie* 1043 (P); *Herbst* 5834 (US); *Hillebrand* 394 (K); Puna, Wahaula**,** *Degener et al.* 32417 (B). **Kauai**: Koloa, Lawai Valley, *Lorence* 7204 (MO, US). **Maui:** *Forbes* 1908 (US); *Imada* 2004-45 (US); *Le Roy Topping* 6001 (US); *Pearsall* s.n., 26 December 1955 (P-04039452); *Tam* 22038 (B, L, US). **Molokai:** *Degener et al.* 5989 (B); *Fosberg* 77a (US); *Forbes* 354 (P; US); *Hillebrand* s.n., December 1890 (BM-undigitized); *Rock* s.n., March 1910 (K-undigitized, L-2744549, US- 01232880). **Oahu:** *Mann Brigham* 42 (US); Koolaupoko, Kailua, *Staples* 1400 (SING). **VANUATU. Epi**: Filakara, *Curry* 1009 (L). **Erromango:** *Bevaridge* 19395 (NZFRI); Cook Bay, *Cabalion* 3095 (P). **Sanma**, Malao, *MacKee* 24255 (K); Matantas, *Curry* 1086 (K). **Shefa:** Efate**,** *Austin et Austin* 7466 (MO); Epi, *Curry* 1199 (K); Mt. Barnier, *Cabalion* 2307 (P); Port Vila, *Cabalion* 2714 (K, P); *Bourdyn* 772 (P); Vate, *Bourdyn* 142 (K, P).

UNCERTAIN LOCATION.

Dombey s.n. (P-04039477); *Helfer* 5864 (K); *Hooker* 174 (K); *Hooker* 1340 (K); *Hooker* 2845 (K); *Kuntze* 568 (NY); *Leg*. *ign*. 158, December 1887 (P-04039459); *Leg. ign*.

628 (BM-undigitized); *Leg. ign*. s.n. (BM-undigitized); *Martius* s.n. (BR-BR0000013308643); *Pueran* s.n., 1824. (G-00134803); Bengal, *Kurz* 12-66 (BO); Aryadaka *Clarke* 34533 (BM); Either Madagascar or Mauritius, *Bojer* s.n. (G-00135045); Either Andaman Islands or Tenaserrim, *Gaudichaud Beaupre* s.n., 1830 (G).

4.11.7. *Ipomoea queenslandica* (Domin) J.R.I. Wood & Scotland, Nat. Plants 5 (11): 1136-1144. (2019). Suppl. Mat. 35. [51].

Argyreia queenslandica Domin, Biblioth. Bot. 89: 533. (1928). [32], ≡ *Stictocardia queenslandica* (Domin) R. W. Johnson, Austrobaileya 6 (4): 631—637. (2004). [31]. Type: AUSTRALIA, Nord-Queensland, am fusse eines Karsthugels bei Mungana, *Domin* 7919 February 1910 (holotype: PR-530529 image!).

Description Perennial, erect herb, becoming a slender twining leafless vine; *stems* terete, glabrous. *Leaves* petiolate, 3.8—11 × 3.8—10.5 cm, ovate or suborbicular; *apex* acute or acuminate; *margin* entire; *base* shallowly to deeply cordate, both surfaces glabrous, adaxially darker green, abaxially paler green, subglaucous with black dots, veins tinged red; *lateral nerves* 6—8; *petioles* 5.5—11.5 cm*,* slender, glabrous. *Inflorescences* of 1–(rarely) 3 flowers in axillary cymes; *primary peduncles* 6—20 mm, glabrous; *secondary peduncles* 5 mm; *bracteoles* lanceolate, caducous; *pedicels* 4—8 mm, slender, glabrous; *outer* s*epals* 8—10 mm, ovate, glabrous; *apex* rounded or obtuse*; middle sepal* slightly larger; *corolla* 5.5 – 6 cm long, funnelform, glabrous, pink, purple with darker midpetaline bands, limb 4.2 cm wide; *stamens* unequal, included, white; *long filaments* 3—5 cm; *short filaments* 3 cm; *anthers* white; *style* 5 cm; included. *Capsule* 11–14 mm diam.*,* compressed globose, indehiscent, glabrous, brown; *seeds* 4, 10 x 8 mm, ellipsoid, tomentose, brown.

Illustration. Figure 4.10.

Figure 4.10. *Ipomoea queenslandica* (Domin) J.R.I. Wood & Scotland. **A.** Dwarf habit with persistent cotyledon and immature flower, **B**. Habit with fully opened corolla. Drawn by Rosemary Wise from *Clarkson* 6837 (**A**) and *Conn Campo* 1347 (**B**)

Habitat. Monsoon forest, on loam and limestone from 300 to 400 m asl.

Note. Some specimens of *I. queenslandica* possess the unique character of persistent, deeply bilobed cotyledons that remain at anthesis. This character is very useful for species discrimination. It grows on limestone substrate that might explain the dwarf habit. This species is not included in the Census of the Queensland Flora 2014 and 2017 [73, 74] despite being an endemic species. No conservation assessment had been conducted for this species. The current assessment of ten georeferenced specimens conclude the status is Endangered based on both AOO and EOO analyses.

Specimens seen. (Figure 4.10). AUSTRALIA. Queensland: Atherton, *Gray* 07551 (BRI); *Hyland* 15759 (BRI); Chillagoe, *Clarkson* 3025 (BRI); *Clarkson* 3789 (BRI); *Clarkson* 6837 (BRI, K); *Clarkson* 6860 (BRI); Mungana, *Conn Campo* 1347 (BRI); *Domin* 7919 (PR); *Ford* 02383 (BRI); *Johnson* 4043 (ATH, BRI).

4.12. Taxa require further investigation

The placement of two species in *Stictocardia, Ipomoea lombokii* and *I. neglecta*, is uncertain due to the absence or incongruence of molecular evidence. More DNA sequence data are needed to clarify the status of these species, which are discussed below.

4.12.1. *Ipomoea neglecta* (Ooststr.) J.R.I.Wood & Scotland, Nat. Plants 5 (11): 1136-1144. (2019). Suppl. Mat. 35. [51], ≡ *Stictocardia neglecta* Ooststr., Blumea 5: 348 (1943). [4]. Type: INDONESIA, Lombok, *Walsh* 373 (isotypes BM- 000929016!, L-0171358 image!).

Ipomoea neglecta cannot be placed certainly in Stictocardia because of the incongruence between *nr*ITS and plastome phylogeny data (see Chapter Three). See the discussion under *I. orientalis*.

4.12.2. *Ipomoea lombokii* J.R.I.Wood & Scotland, Nat. Plants 5 (11): 1136-1144. (2019). Suppl. Mat. 35. [51], ≡ *Stictocardia cordatosepala* Ooststr., Blumea 5: 351 (1943). [4]., non *I. cordatosepala* Britten. Type: INDONESIA, Lombok, *Elbert* 2011 (holotype L- 0004256 image! and L-0004257 image!).

This species is only known from type specimens, which are inaccessible. As there is no compelling morphological evidence for the acceptance of this species and in the absence of molecular data, the taxonomic status of this taxon is uncertain.

4.13. Excluded species

Phylogenetic studies show that the following four species are recovered outside Stictocardia, thus lying outside the scope of this revision. The history of each species is discussed briefly to explain the reason why they were placed within the genus *Stictocardia* in the past.

4.13.1. *Ipomoea orientalis* J.R.I.Wood & Scotland, Nat. Plants 5 (11): 1136-1144. (2019). Suppl. Mat. 35. [51], ≡ *Ipomoea pulchra* Bl. var. *foliis acuminatis cum mucrone, pedunculis petiole longioribus* Bl. Bijdr. 716 (1825). [4, 62], non *I. pulchra* Bl. Bijdr. 716 (1825). [62] ≡ *Stictocardia discolor* Ooststr., Blumea 5: 350 (1943). [4]. Type: INDONESIA, Timor, *Reinwardt* 1350 (holotype: L-0004258!).

Van Ooststroom elevated the unnamed variant of *Ipomoea pulchra* Bl. to species level and named it *Stictocardia discolor* (= *I. orientalis*). The type specimens of /. *pulchra* is now currently placed as one of the synonyms of *I. tiliifolia* [4]. He treated *I. orientalis* and *I. neglecta* as two distinct species [4, 5]. However, there is a case that

He identified three specimens of *Kostermans* 18075 in L that has the surface characteristic and shape of leaf both the apex and base. of *I. neglecta* as *I. cf. discolor* = *I. orientalis.* His opinion was followed by Staples who has seen more of the duplicates at BO, K and SING. Unfortunately, they did not published anything to clarify the similarity between these species. The morphological study, in fact, can not separate these two species as they are indistinctive to each other and to the widespread species, *I. tiliifolia.* Despite the morphologically overlapping characters, the shape of base and apex of the leaves, *I. orientalis* is phylogenetically distinct from *I. neglecta* (Chapter Three) so here is accepted as a distinct species.

4.13.2. *Ipomoea sivarajanii* (Biju, Pushp. & P.Mathew) J.R.I.Wood & Scotland, Nat. Plants 5 (11): 1136-1144. (2019). Suppl. Mat. 35. [51], ≡ *Stictocardia sivarajanii* Biju, Pushp. & P.Mathew, Novon 9: 147 (1999). [16]. Type: INDIA, Kerala, Rajamala, shola edge, 28 September 1996, *Biju* 40182 ARIZ! (holotype: K, isotypes: CALI, MH, TBGT, US all not seen).

Ipomoea sivarajanii was described from a very limited number of specimens in India. This species was examined by Verdcourt (correspondence letters in K) who forwarded the specimens to Austin at ARIZ for further study. Austin confirmed its novelty [16] but did not specify further details. Interestingly, a few years later, Austin and Eich [13], did not include this species in the first global identification key of *Stictocardia.* This may be because this species does not possess the distinctive characters of the genus, as proposed by Austin and Demissew few years earlier [15].

4.13.3. *Ipomoea mojangensis* Vatke, Linnaea 43: 515 (1882). [40], ≡ *Stictocardia mojangensis* (Vatke) D.F.Austin & E.Eich., Willdenowia 31(1): 80 (2001) [13]. Type: MADAGASCAR, env. de Majunga, *Hildebrandt* 3410 (holotype B not seen, isotype P not seen, M-0109958 image!).

Ipomoea mojangensis was included in *Stictocardia* by Austin and Eich [13] based on the presence of a recently recognised diagnostic character, the valved endocarp [15] that was presumably autopomorphy for *Stictocardia* [75]. This character, however, evolves more than once as shown in the *Ipomoea* phylogenies (Chapter Two). The inclusion of this species in *Stictocardia*, however, contradicts most characters previously recognised by Hallier [1], as this species does not possess black glands and accrescent sepals in fruit. Earlier study [51] has shown that this species is more closely related to the remaining species of *Ipomoea* thus indicated the polyphyly of *Stictocardia*.

4.13.4. *Ipomoea jucunda* Thwaites, Enum. Pl. Zey.: 211 (1864). [76], ≡ *Stictocardia jucunda* (Thwaites) C.R.Gunn, Brittonia 24(2): 174 (1972). [12]. Type: SRI LANKA *Thwaites* 3448 (BM-001014518 image!, HUH-00054660 image!, L- 2744553!).

Ipomoea jucunda is endemic to Sri Lanka and only known from the type specimen. Gunn [12] placed this species in *Stictocardia* because of the presence of black glands on the abaxial leaf surface. This species however, also possesses the characters such as elongate sepals, dehiscent fruit, and hairy seeds that are not found in *Stictocardia*.

4.14. Unresolved species

4.14.1. *Stictocardia multiflora* Hallier f. Bot. Jahrb. Syst.18: 160 (1893). [1], ≡ *Rivea tiliaefolia* var. *multiflora* Klotzsch mss. in B [1]. Type: MOZAMBIQUE, Cabanira (Cabaceira), *Peters*, August 1847 (holotype: B, destroyed).

This species was referred to *Stictocardia* by Hallier [1] but no specimen survives. The protologue is insufficient to allow further identification or taxon placement.

4.14.2. *Stictocardia nervosa* Hallier f. Bull. Herb. Bois. 6: 548 (1898). [3].

This unresolved species was mentioned once from the Congo area by Hallier [3] but without description or reference. Thus, it is a nomen nudum.

List of References

1. Hallier, H., *Convolvulaceae Africanae*, in *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, A. Engler, Editor. 1893, Verlag Vos Wilhelm Engelmann: Leipzig. p. 80-160.
2. Hallier, H., *Ueber Phanerogamen von unsicherer oder unrichtiger Stellung.* Mededeelingen van 's Rijks Herbarium, 1910. **1**: p. 1-41.
3. Hallier, H., *Bausteine zu einer Monographie der Convolvulaceen.* Bulletin de L'Herbier Boissier 1897. **5**: p. 366–387.
4. Van Ooststroom, S.J., *The Convolvulaceae of Malaysia IV (with Indices).* Blumea, 1943. **5**: p. 339-411.
5. van Ooststroom, S.J. and R.D. Hoogland, *Convolvulaceae*, in *Flora Malesiana*, C.G.G.J. van Steenis, Editor. 1953, Noordhoff-Kolff N.V.: Djakarta. p. 389-512.
6. Verdcourt, B., *A new subgenus of Stictocardia (Convolvulaceae).* Kew Bulletin, 1990. **45**: p. 583-585.
7. Verdcourt, B., *Convolvulaceae*, in *Flora of Tropical East Africa*, C.E. Hubbard and E. Milne-Redhead, Editors. 1963, Crown Agents for Oversea Governments & Administration: London. p. 1-161.
8. Verdcourt, B., *Notes from the East African herbarium: VI.* Kew Bulletin, 1958. **13**: p. 185-197.
9. Verdcourt, B., *Notes from the East African herbarium: XII.* Kew Bulletin, 1961. **15**: p. 1-18.
10. Verdcourt, B., *Some notes on Tropical African Convolvulaceae.* Webbia, 1958. **13**: p. 321-330.
11. Verdcourt, B., *Tropical African Plants: XXVII.* Kew Bulletin, 1963. **27**: p. 161-181.
12. Gunn, C.R., *Notes on Stictocardia campanulata (L.) Merrill and S. jucunda (Thw.) CR Gunn (Convolvulaceae).* Brittonia, 1972. **24**: p. 169-176.
13. Austin, D.F. and E. Eich, *Synopsis of Stictocardia with another Madagascan species, S. mojangensis (Convolvulaceae).* Willdenowia, 2001. **31**: p. 79-85.
14. Austin, D.F., D.A. Powell, and D.H. Nicolson, *Stictocardia tiliifolia (Convolvulaceae) re-evaluated.* Brittonia, 1978. **30**: p. 195-198.

15. Austin, D.F. and S. Demissew, *Unique fruits and generic status of Stictocardia (Convolvulaceae).* Kew Bulletin, 1997. **52**: p. 161-169.
16. Biju, S.D., P. Pushpangadan, and P. Mathew, *Stictocardia sivarajanii, a new species of Convolvulaceae from Kerala, India.* Novon, 1999. **9**: p. 147-149.
17. Mayden, R.L., *A hierarchy of species concepts: The denouement in the saga of the species problem*, in *Species: The Unit of Biodiversity*, M.F. Claridge, H.A. Dawah, and M.R. Wilson, Editors. 1997, Chapman and Hall: London. p. 381-424.
18. Wells, T., T. Carruthers, P.R. Munoz, A. Sumadijaya, J.R.I. Wood, and R.W. Scotland, *Species as a heuristic: Reconciling theory and practice.* Systematic Biology, 2021: p. 1-11.
19. Aswani, K. and M. Sabu, *Pollination biology of Stictocardia tiliifolia (Desr.) Hall.f. (Convolvulaceae).* The International Journal of Plant Reproductive Biology, 2012. **4**: p. 5-8.
20. Wood, J.R.I., P. Muñoz-Rodríguez, B.R.M. Williams, and R.W. Scotland, *A foundation monograph of Ipomoea (Convolvulaceae) in the New World.* PhytoKeys, 2020. **143**: p. 1-823.
21. Woodson, R.E., R.W. Schery, and D.F. Austin, *Flora of Panama. Part IX. Family 164. Convolvulaceae.* Annals of the Missouri Botanical Garden, 1975. **62**: p. 157-224.
22. Austin, D.F., *Convolvulaceae*. A Revised Handbook to the Flora of Ceylon. Vol. 1. 1980. 288-363.
23. Biju, S.D., *Taxonomic and morphologic studies on the family Convolculaceae of Southern Peninsular India*. 1997, University of Calicut.
24. The Royal Botanic Gardens Kew, H.U.H.a.L., Australian National Botanic Gardens. *IPNI International Plant Names Index*. 2020 [cited 2020 10th December]; Available from: http://www.ipni.org.
25. Tropicos.org. *Tropicos version 3.3.2*. 2021 [cited 2021 31st January]; Available from: http://www.tropicos.org.
26. List, T.P. *The Plant List v 1.1.* 2013 [cited 2017 28th December]; Available from: http://www.theplantlist.org/.
27. Staples, G.W. *World Checklist of Convolvulaceae*. 2021 [cited 2021 2nd May]; Available from: https://wcsp.science.kew.org/qsearch.do.
28. Goncalves, M.L., *Flora Zambesiaca, Convolvulaceae*, E. Launert, Editor. 1987, Flora Zambesiaca Managing Committee: London. p. 9–129.
29. Garcia, M.A. and S. Demissew, *Convolvulaceae*, in *Flora of Somalia*, M. Thulin, Editor. 2006, Royal Botanic Gardens, Kew,: Kew. p. 221-258.
30. Demissew, S., *Convolvulaceae*, in *Flora of Ethiopia and Eritrea: Gentianaceae to Cyclocheilaceae*, I. Hedberg, Kelbessa., E., Edwards, S., Demissew, S., Persson, E., Editor. 2006, The National Herbarium and Uppsala, The Department of Systematic Botany: Uppsala.
31. Johnson, R.W., *Stictocardia Hallier f. (Convolvulaceae) in Queensland.* Austrobaileya, 2004. **6**: p. 631-637.
32. Domin, K., *Biblioteca Botanica*. Vol. 89. 1928.
33. Simões, A.R., H. Silva, and P. Silveira, *The Convolvulaceae of Timor with special reference to East Timor.* Blumea, 2011. **56**: p. 49-72.
34. Freiberg, M., M. Winter, A. Gentile, A. Zizka, A. Muellner-Riehl, A. Weigelt, and C. Wirth, *LCVP, The Leipzig Catalogue of Vascular Plants, a new taxonomic reference list for all known vascular plants.* Scientific Data, 2020. **7**: p. 1-7.
35. Bachman, S. and J. Moat, *GeoCAT: An open source tool for rapid Red List assessments.* BGjournal, 2012. **9**: p. 11-13.
36. Gaston, K.J. and R.A. Fuller, *The sizes of species' geographic ranges.* Journal of Applied Ecology, 2009. **46**: p. 1-9.
37. Rivers, M.C., L. Taylor, N.A. Brummitt, T.R. Meagher, D.L. Roberts, and E.N. Lughadha, *How many herbarium specimens are needed to detect threatened species?* Biological Conservation, 2011. **144**: p. 2541-2547.

38. Mildbraed, J., *Neue und seltene Arten aus Ostafrika (Tanganyika-Territ. Mandat) leg. H. J. Schlieben, XIII.* Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem, 1938. **14**: p. 94-112.
39. Walter, K.S. and H.J. Gillett, *1997 IUCN Red List of Threatened Plants*. 1998, Cambridge: IUCN- The World Conservation Union.
40. Vatke, W., *Plantas in itinere africano ab J. M. Hidebrandt collectas determinare pergit.* Linnaea, 1882. **43**: p. 507-541.
41. Rendle, A.B., *Notes on African Convolvulaceae.* Journal of Botany, British and Foreign, 1901. **39** p. 12-22.
42. International., B.G.C. *ThreatSearch online database*. 2017 [cited 2021 3rd February]; Available from: https://tools.bgci.org/threat_search.php.
43. Mattei, G.E., *Contribuzioni alla flora della Somalia Italiana.* Bollettino R. Orto Botanico E Giardino Coloniale, 1908. **7**: p. 85--112.
44. Engler, A., *Die Pflanzenwelt Ost-Afrikas und der Nachbargebiete*. Vol. 3. 1895, Berlin: Geog. Verlagshandlung Dietrich Reimer.
45. Verdcourt, B., *Corrections and additions to the 'Flora of Tropical East Africa-Convolvulaceae': I.* Kew Bulletin, 1967. **21**: p. 83-92.
46. Wilkin, P., *A revision of the Infrageneric classification of the genus Ipomoea L. and the related genera in the tribe Ipomeeae (Convolvulaceae)*. 1993, University of Reading.
47. Baker, J.G. and A.B. Rendle, *Convolvulaceae*, in *Flora of Tropical Africa*, W.T. Thiselton-Dyer, Editor. 1906, Lovell and Reeve: London. p. 62-206.
48. Baker, J.G., *Diagnoses Africanae II. Convolvulaceae.* Bulletin of Miscellaneous Information Kew, 1894. **86**: p. 67-74.
49. Chowdhery, H.J. and M.R. Debta, *A new species of Ipomoea L. (Convolvulaceae) from India.* Indian Journal of Forestry, 2009. **32**: p. 119-121.
50. Anonymous, *Decades Kewenses. Plantarum Novarum in Herbario Horti Regii Conservatarum. Decas VIII.* Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew, 1894. **87**: p. 99-102.
51. Muñoz-Rodríguez, P., T. Carruthers, J.R.I. Wood, B.R.M. Williams, K. Weitemier, B. Kronmiller, Z.A. Goodwin, A. Sumadijaya, D. Harris, D. Filler, M.A. Rausher, S. Kelly, A. Liston, and R.W. Scotland, *A taxonomic monograph of Ipomoea integrated across phylogenetic scales.* Nature Plants, 2019. **5**: p. 1136-1144.
52. Meeuse, A.D.J. and W.G. Welman. Flora of Southern Africa: Convolvulaceae. Vol. 28. 2000, Pretoria: National Botanical Institute.
53. Deroin, T., *Famille Convolvulaceae*, in *Flore de Madagascar et des Comores*, P. Morat, Editor. 2001, Muséum National d'Histoire Naturelle: Paris. p. 11-287.
54. Victor, J.E., *Stictocardia laxiflora (Baker) Hallier f.* , in *National Assessment: Red List of South African Plants*. 2005.
55. Roemer, J.J. and J.A. Schultes, *Caroli a Linné Systema Vegetabilium Secundum*. Vol. 4. 1819.
56. Desrousseaux, L.A.J., *Convolvulaceae*, in *Encyclopédie Méthodique: Botanique*, J.B.P.A.M. Lamarck and J.L.M. Poiret, Editors. 1789: Paris. p. 539–568.
57. Choisy, J.D., *Convolvulaceae*, in *Prodromus Systematis Naturalis Regni Vegetabilis*, A. De Candolle, Editor. 1845, Sumptibus Fortin, Masson et Sociorum: Parisiis. p. 323-465.
58. Rafinesque, C.S., *Flora Telluriana*. 1836, Philadelphia: H. Probasco.
59. Wight, R., *Icones plantarum Indiae Orientalis*. Vol. 6. 1853, Madras: American Mission Press.
60. Roxburgh, W., *Flora Indica*. Vol. 2. 1832, Serampore: M. Thacker and Co.
61. Sweet, R., *Hortus Britannicus, Part 2*. 1826, London: James Ridgway.
62. Blume, C.L., *Bijdragen tot de flora van Nederlandsch-Indië*. Vol. 13. 1825, Batavia: Ter Lands Drukkerij.
63. Schlechtendal, D., *Florula Insulae Sti. Thomae Indiae Occidentalis.* Linnaea 1831. **6**: p. 722–772.

64. Merrill, E.D., *The plants of Guam.* Philippine Journal of Science, Botany, 1914. **9**: p. 151-152.
65. Linnaeus, C., *Species Plantarum*. Vol. 1. 1753, Holmiae: Laurentii Salvii.
66. Staples, G.W. and J.F. Butaud, *A new endemic subspecies of Stictocardia (Convolvulaceae) from the Marquesas Islands, French Polynesia.* Phytologia, 2016. **98**: p. 203-206.
67. Sachet, M.-H., *Flora of the Marquesas, 1: Ericaceae-Convolvulacae.* Smithsonian Contributions to Botany, 1975.
68. Lorence, D.H. and W.L. Wagner, *Flora of the Marquesas Islands: Dicots*. Vol. 2. 2020, Kalaheo: National Tropical Botanical Garden.
69. IUCN, C.F., C.B.N. Federation, and M.N.d.H. Naturelle *La Liste Rouge des Especes Menacees en France-Flore Vasculaire de Mayotte*. 2014.
70. IUCN, C.F., C.B.N. Federation, and M.N.d.H. Naturelle *La Liste Rouge des Especes Menacees en France-Flore Vasculaire de la Reunion*. 2014.
71. Ministry, o.E., *The National Red List 2012 of Sri Lanka: Conservation status of the fauna and flora*. 2012, Colombo: Karunarathne and Sons Pvt.
72. Patzelt, A. and S.G. Knees. *Stictocardia tiliifolia*. The IUCN Red List of Threatened Species 2016. 2016 [cited 2017 28th December].
73. Bostock, P. and A. Holland, *Introduction to the Census of the Queensland Flora 2014 ver 1.1.* 2014, Brisbane: Herbarium Queensland, Department of Science, Information Technology, Innovation and the Arts.
74. Bostock, P. and A. Holland, *Introduction to the Census of the Queensland Flora 2017 ver 1.0*. 2017, Brisbane: Herbarium Queensland Department of Science, Information Technology, Innovation and the Arts.
75. Austin, D.F., *Parallel and convergent evolution in the Convolvulaceae*, in *Biodiversity and Taxonomy of Tropical Flowering Plants*, P. Mathews and M. Sivadasan, Editors. 1998, Mentor Books: Calicut. p. 234-247.
76. Thwaites, G.H.K., *Enumeratio plantarum Zeylaniae*. 1864, London: Dulau and Co.

CHAPTER 5. SUMMARY

5.1. Monographing *Stictocardia*

Monographing species-rich genera, such as *Ipomoea*, is a challenge in terms of a number of variables including the volume of specimens and literature, geographical scope and sheer scale of the task [1, 2]. *Ipomoea* comprises nine subsumed genera [3-5], none of which are particularly well understood. Within the framework of integrative monographic taxonomy, an ongoing study at the University of Oxford has laid a foundation to understand the systematics of *Ipomoea* by generating several densely sampled phylogenies based on considerable molecular and morphological evidence as well as species level taxonomic treatments [1, 5].

The taxonomy of a small group of 13 species of *Stictocardia*, which is one of the subsumed genera within *Ipomoea,* is particularly poorly known. Revising a group of this size fits within the time constraint of a D.Phil. programme [6]. This investigation of *Stictocardia* aimed to test its circumscription as well as to fully understand species delimitation and phylogenetic relationships. The output of this thesis is a taxonomic monograph based on the study of the two foundational components; literature and herbarium specimens [7].

5.2. Literature (Chapter Two)

This study began with a review of the taxonomic history of *Stictocardia*. I assembled all the accessible and relevant yet scattered literature in various journals and floras including the substantial ones such as Hallier [8-10], van Ooststroom [11, 12], Verdcourt [13-18], and Austin, either personally or with colleagues [19-22]. Altogether, they cover more than two centuries of sporadic and piecemeal of taxonomic publications starting in 1792 [23], including the publication of *Stictocardia*

in 1893 [8], to the most recent phylogenetic considerations in 2019 [5] and 2020 [1]. This literature provides a wide range of information, not exclusively focussed on taxonomy and phylogeny but also pollination [24], chemistry and anatomy [25], and conservation [26].

The literature study revealed a complex history of this genus and charted the increasing geographical coverage and the species number over time. These developments in turn led to an expanding generic concept. Initially, taxonomic accounts mainly occurred in Africa [8-10], Southeast Asia [11, 12], reappeared again in Africa [13-17], before covered Sri Lanka [27], India [28], and Australia [29]. These studies incorporated more species within *Stictocardia* through transfers from different genera (*Rivea*, *Argyreia* and *Ipomoea*) [8-11, 21, 27, 29] as well as newly discovered species [11, 28]. The addition of new species and the incorporation a new supposedly diagnostic characters [20] changed the generic concept to something quite different from Hallier's original generic description [8]. This has created a situation that is unclear to the generic limits of *Stictocardia* and its constituent species.

5.3. Herbarium specimens

Herbarium specimens allow the documentation of morphological features of plants as well as providing biological and ecological data from labels. Specimens have also been the source of leaf material to extract DNA, a valuable resource for molecular systematics. The study of morphology and molecular phylogenetics in combination proves to be a novel and useful approach in *Ipomoea* taxonomy [5] and this study is the first attempt to integrate morphological and molecular data to better understand *Stictocardia* in the context of *Ipomoea.*

5.4. Morphological study (Chapter Two)

Seven hundred and five herbarium specimens (sheets and images) of *Stictocardia* from 35 herbaria have been observed and studied to yield a comprehensive sample of morphological variation of all putative species across their entire geographical distribution. Morphological data facilitated the revision of species boundaries and the generation of species hypotheses to be tested against the results of phylogenetic analysis of molecular sequence data. The initial morphological assessment recognised two categories of species, distinct and indistinct one. Six distinct species – *I. beraviensis, I. macalusoi, I. lutambensis, I. incompta, I. jucunda,* and *I. mojangensis* – are readily recognised by unique qualitative morphological characters. All the remaining species – *I. tiliifolia, I. neglecta, I. orientalis, I. pringshemiana, I. sivarajanii* and *I. queenslandica* – are indistinct as they do not possess a unique qualitative character but overlapping quantitative characters.

These six indistinct species with overlapping quantitative characters were subjected to further morphological assessment, Principal Component Analysis or PCA (a multivariate analysis) and Mann-Whitney test (a non -parametric, univariate analysis). PCA was used to visualise whether these species form discrete clusters based on measured characters. It showed that these species formed a continuum of morphological variation and could not be clearly separated into distinct groups, the result that must be taken cautiously without strict criteria. The next step was testing the grouping of species by Mann-Whitney test with fewer species and less samples that statistically better. Standard Error of Means (SEM) was used to determine whether the indistinct species individually were good species or a single taxonomic entity due to overlapping characters. Surprisingly, SEM demonstrate that the

indistinct species are separated into multiple groups with different species compositions. These groups were subjected to Mann-Whitney test to assess their significance. This test, however, was inconclusive because of contradictory results between individual morphological characters.

The results of PCA and Mann-Whitney test, however, was superseded by the results of phylogenetic analysis of molecular sequence data that demonstrated several *Stictocardia* species are more closely related to species of *Ipomoea* and therefore outside the revised study group that recovers as a monophyletic group.

Subsequently, the morphological study was refocussed on seven species of *Stictocardia* that form a clade in the molecular phylogenies, explained in the subchapter below. This clade possesses a combination of morphological features including cordate-shaped leaf base, black glands on the lower leaf surface, sepals accrescent in fruit, indehiscent fruit, and valved endocarp.

Morphological evaluation is also conducted to *I. beraviensis* through reciprocal illumination. Based on molecular study, this species recovers as a non-monophyletic taxonomic entity, thus suggesting the presence of more than one putative group, one in Eastern Africa and other is widespread in Africa and Madagascar. Box plots of six morphological characters demonstrate that there is no identified diagnostic character or combined characters to split *I. beraviensis* into two putative groups. The result of non-parametric statistical analysis, the Mann-Whitney test, is compromised as the samples are available in very limited number. More samples collected from across Africa and Madagascar are required to sort this issue in the future.

This study further evaluated the suggestion that the density of black glands on the leaf surface is a significant character for species delimitation [25]. This investigation revealed that the character to be taxonomically uninformative.

5.5. Phylogenetic study of molecular sequence data (Chapter Three)

All accessible species of *Stictocardia* were subjected to phylogenetic inference that tests delimitation of the genus, species monophyly and construct phylogenetic relationships. Corroboration of phylogenies based on different data sets (*nr*ITS and plastome) and inference methods (MP, A-ML and BI) recovered a monophyletic group, the clade Stictocardia - that consistently comprises seven species; *I. lutambensis, I. beraviensis, I. macalusoi, I. incompta. I. pringshemiana*, *I. tiliifolia* and *I. queenslandica*. The sister group of these seven species remains unresolved and will require denser taxon sampling within the OW *Ipomoea*.

This phylogenetic study demonstrated for the first time that *I. tiliifolia, I. queenslandica*, *I. incompta* and *I. pringshemiana* are monophyletic according to the plastome analyses. Plastome analysis also clarifies the phylogenetic relationships between most species of Stictocardia. *Ipomoea beraviensis, distinct in terms of morphology,* is shown to be polyphyletic by molecular sequence data. This species, provisionally accepted in the taxonomic account given the current morphology-based evidence (Chapter Two).

There are multiple species previously recognised within *Stictocardia i.e*., *I. mojangensis, I. sivarajanii, I. jucunda,* and *I. orientalis* that are more closely related to species of *Ipomoea*. Phylogenetic analysis has refuted the traditional genus *Stictocardia* as it is recovered as a polyphyletic group.

5.6. Monograph of Stictocardia, a contribution to the systematics of *Ipomoea* (Chapter Four)

The monograph of Stictocardia comprises seven species of *Stictocardia.* A description of this clade and an identification key are provided for taxon delimitation and species recognition. The seven species - *I. lutambensis, I. beraviensis, I. macalusoi, I. incompta, I. pringshemiana*, *I. tiliifolia* and *I. queenslandica* – are critically discussed in this taxonomic revision. The taxonomic account consists of a comprehensive synonymy, species descriptions, illustrations, habitat types, notes, distribution ranges and lists of specimens seen.

This monograph demonstrates that the majority of species are concentrated in Eastern Africa (Kenya and Tanzania) *i.e., I. beraviensis, I. incompta, I. pringsheimiana, I. lutambensis* and *I. macalusoi*. On the other hand, *I. tiliifolia* is a globally widespread species and primarily found in coastal areas worldwide except Africa and Madagascar, therefore implies the dispersal mechanism by ocean currents, which has been supported by a floating experiment of a fruit from a specimen in the lab. This species and its sister species, *I. queenslandica,* occur outside of Africa.

A conservation assessment of all seven species of Stictocardia was also conducted. However, the result is very preliminary on some species - *I. queenslandica, I. macalusoi* and *I. lutambensis*, - that only have fewer than 15 georeferenced specimens. At the moment, the status of these three species is Data Deficient, regardless the result of AOO and EOO analysis. Again, this situation illustrates that more georeferenced specimens are needed for further better assessment. Nevertheless, the assessment of those species, however imperfect,

served as a base line for further study. The assessments of the remaining species, *ie. I. pringsheimiana*, *I. beraviensis* and *I. incompta* are more robust because these species have more than 15 georeferenced specimens. The assessment of the widespread species *I. tiliifolia,* however, likely to be least concern as this species is tropically widespread except in Africa and Madagascar.

5.7. Concluding remarks

Initially, the goal of this study was to monograph a poorly known and taxonomically problematic, pantropical genus of *c.* 13 species, *Stictocardia,* which is subsumed under *Ipomoea.* This study evaluates and analyses the latest molecular data of *Stictocardia* and confirms that this taxon is polyphyletic. The output of this study is a monograph accompanied by a conservation assessment of seven species of *Stictocardia*, which is a subset of the Old-World *Ipomoea*.

List of references

1. Wood, J.R.I., P. Muñoz-Rodríguez, B.R.M. Williams, and R.W. Scotland, *A foundation monograph of Ipomoea (Convolvulaceae) in the New World.* PhytoKeys, 2020. **143**: p. 1-823.
2. Scotland, R.W. and J.R.I. Wood, *Accelerating the pace of taxonomy.* Trends in Ecology & Evolution, 2012. **27**: p. 415-416.
3. Wilkin, P., *A revision of the Infrageneric classification of the genus Ipomoea L. and the related genera in the tribe Ipomeeae (Convolvulaceae)*. 1993, University of Reading.
4. Manos, P.S., R.E. Miller, and P. Wilkin, *Phylogenetic analysis of Ipomoea, Argyreia, Stictocardia, and Turbina suggests a generalized model of morphological evolution in morning glories.* Systematic Botany, 2001. **26**: p. 585-602.
5. Muñoz-Rodríguez, P., T. Carruthers, J.R.I. Wood, B.R.M. Williams, K. Weitemier, B. Kronmiller, Z.A. Goodwin, A. Sumadijaya, D. Harris, D. Filler, M.A. Rausher, S. Kelly, A. Liston, and R.W. Scotland, *A taxonomic monograph of Ipomoea integrated across phylogenetic scales.* Nature Plants, 2019. **5**: p. 1136-1144.
6. Stuessy , T., *The importance of revisionary studies in plant systematics.* Sida, 1975. **6**: p. 104-113.
7. Marhold, K., T. Stuessy, M. Agababian, D. Agosti, M.H. Alford, A. Crespo, J.V. Crisci, L.J. Dorr, Z. Ferencova, and D. Frodin, *The future of botanical monography: Report from an international workshop, 12–16 March 2012, Smolenice, Slovak Republic.* Taxon, 2013. **62**: p. 4-20.
8. Hallier, H., *Convolvulaceae Africanae*, in *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*, A. Engler, Editor. 1893, Verlag Vos Wilhelm Engelmann: Leipzig. p. 80-160.

9. Hallier, H., *Bausteine zu einer Monographie der Convolvulaceen.* Bulletin de L'Herbier Boissier 1897. **5**: p. 366–387.
10. Hallier, H., Mededeelingen van 's Rijks Herbarium, 1910: p. 26.
11. Van Ooststroom, S.J., *The Convolvulaceae of Malaysia IV (with Indices).* Blumea, 1943. **5**: p. 339-411.
12. van Ooststroom, S.J. and R.D. Hoogland, *Convolvulaceae*, in *Flora Malesiana*, C.G.G.J. van Steenis, Editor. 1953, Noordhoff-Kolff N.V.: Djakarta. p. 389-512.
13. Verdcourt, B., *Notes from the East African herbarium: VI.* Kew Bulletin, 1958. **13**: p. 185-197.
14. Verdcourt, B., *Some notes on Tropical African Convolvulaceae.* Webbia, 1958. **13**: p. 321-330.
15. Verdcourt, B., *Notes from the East African herbarium: XII.* Kew Bulletin, 1961. **15**: p. 1-18.
16. Verdcourt, B., *Convolvulaceae*, in *Flora of Tropical East Africa*, C.E. Hubbard and E. Milne-Redhead, Editors. 1963, Crown Agents for Oversea Governments & Administration: London. p. 1-161.
17. Verdcourt, B., *Tropical African Plants: XXVII.* Kew Bulletin, 1963. **27**: p. 161-181.
18. Verdcourt, B., *A new subgenus of Stictocardia (Convolvulaceae).* Kew Bulletin, 1990. **45**: p. 583-585.
19. Austin, D.F., D.A. Powell, and D.H. Nicolson, *Stictocardia tiliifolia (Convolvulaceae) re-evaluated.* Brittonia, 1978. **30**: p. 195-198.
20. Austin, D.F. and S. Demissew, *Unique fruits and generic status of Stictocardia (Convolvulaceae).* Kew Bulletin, 1997. **52**: p. 161-169.
21. Austin, D.F. and E. Eich, *Synopsis of Stictocardia with another Madagascan species, S. mojangensis (Convolvulaceae).* Willdenowia, 2001. **31**: p. 79-85.
22. Woodson, R.E., R.W. Schery, and D.F. Austin, *Flora of Panama. Part IX. Family 164. Convolvulaceae.* Annals of the Missouri Botanical Garden, 1975. **62**: p. 157-224.
23. Desrousseaux, L.A.J., *Convolvulaceae*, in *Encyclopédie Méthodique: Botanique*, J.B.P.A.M. Lamarck and J.L.M. Poiret, Editors. 1789: Paris. p. 539–568.
24. Aswani, K. and M. Sabu, *Pollination biology of Stictocardia tiliifolia (Desr.) Hall.f. (Convolvulaceae).* The International Journal of Plant Reproductive Biology, 2012. **4**: p. 5-8.
25. Olaranont, Y., F.W. Stauffer, P. Traiperm, and G.W. Staples, *Investigation of the black dots on leaves of Stictocardia species (Convolvulaceae) using anatomical and histochemical analyses.* Flora, 2018. **249**: p. 133-142.
26. Patzelt, A. and S.G. Knees. *Stictocardia tiliifolia*. The IUCN Red List of Threatened Species 2016. 2016 [cited 2017 28th December].
27. Gunn, C.R., *Notes on Stictocardia campanulata (L.) Merrill and S. jucunda (Thw.) CR Gunn (Convolvulaceae).* Brittonia, 1972. **24**: p. 169-176.
28. Biju, S.D., P. Pushpangadan, and P. Mathew, *Stictocardia sivarajanii, a new species of Convolvulaceae from Kerala, India.* Novon, 1999. **9**: p. 147-149.
29. Johnson, R.W., *Stictocardia Hallier f. (Convolvulaceae) in Queensland.* Austrobaileya, 2004. **6**: p. 631-637.

Appendix 1. List of the observed specimens (sheets or images) of *Stictocardia* species

Ipomoea lutambensis: **TANZANIA. Lindi:** Lake Lutamba, *Schlieben* 6181 (BM, BR, HBG, LISC, P, S). **Morogoro:** Kingupira, *Vollesen* 2619 (K); *Vollesen* 3497 (K). Uluguru Mts, *Bruce* 1036 (BM, K). **Mtwara:** Masasi, *Bidgood et al.* 2000 (K, MO).

Ipomoea beraviensis: **BURUNDI. Bubanza**: Kihanga, *Lewalle* 3723 (K). **Ngozi:** Ruyaga, *Kassner* 3173 (BR, BM, K). **CAMEROON. Bipinde**: *Urwaldgebiet, Zenker* 1614 (BM, HBG, K, L, P, S, WAG). **Centre:** Mbalmayo, *Jacques Felix* 9157 (K, P). **Yoko**: Ndong, *Biholong* 404 (P). **CENTRAL AFRICAN REPUBLIC.** *Chevalier* s.n., October 1902 (BR); *Leg. ign*. s.n., October 1902 (BR); *Leg. ign*. s.n., May 1908 (P). **Bamingui-Bangoran:** Koumbala, *Fay* 6279 (MO). **Bangui:** *Breyne* 1254 (BR); *Leg. ign*. 373, 13 December 1891 (P). **Haute-Kotto:** *Testu* 4294 (BM, BR, K); Yalinga**,** *Testu* 4239 (BM, P). **Kwilu:** Boukoko, *Tisserant* 583-1354 (P). **Obangui**: *Laurent* s.n., 19 December 1903 (BR); *Leg. ign.* 17β, 26 January (P). **Ouaka**: Bambari, *Tisserant* s.n. (P). **CHAD. Bangui**: Sibut, *Chevalier* 10719 (P); *Chevalier* 10819 (P). **Bol**: Ngouri: *Chevalier* s.n., 1902 (L, P). **Ombella**: Boma, *Chevalier* 5860 (P). **DEMOCRATIC REPUBLIC OF THE CONGO.** *Deschamps* 1891 (BR); *de Witte* 8387 (WAG). *de Witte* 12897 (BR); *Gille* 169 (BR); *Jean Louis* 7812 (BR). **Haut-Katanga:** Ganza, *Haui* 6975 (BR); Kapolowe, *Malaisse* 6981 (BR); Musoshi, *Schmitz* 3559 (BR). **Haut-Uele**: Bombombi, *Gerard* 770 (BR, WAG); Garamba, *de Saeger* 1520 (BR, K, L). **Ituri:** Irimba, *de Wilde (d' Estmael)* 144 (BR); Maitatu*, Lisowski* 43603 (BR). **Kafubu**: Kiniama, *Schmitz* 2837 (BR, WAG). **Katanga**: Kasenga, *Robyns* et al. 1928 (B, BR, BM). **Kivu**: *Guy* s.n., 15 February 1936 (P). **Kwango**: Dembo, *Lescrauwaet* 307 (BR). **Lomami**: Butahu, *de Witte* 11858 (BR); Lukoma, *Delvaux* 391 (BR); Kaswabilenga, Parc National De L'Upemba, *de Witte* 3978 (BR). **Kwilu:** Ibele, *Dervulf* 505 (BR); Luisa, *Liben* 3313 (BR, WAG). **Lualaba**: Katela, *Wilde* 742 (BR); Kolwezi, *Holmes* 1462 (FHO). **Maniema:** Kabingwa, *Verdick* 454 (BR); Kasali, *de Witte* 14230 (BR); Kibangula, *Leonard* 5761 (BR). **Nord Kivu**: Beni: *Germain* 5449 (BR); *Lebrun* 4310 (BM, BR, K); Mutsora, *de Witte* 9887 (BR); Mwenda, *de Witte* 13021 (BR); Pinga, *Gutzwiller* 3123 (BR); Rumangabo, *Germain* 1435 (BR, WAG); Rutshuru, *de Witte* 14155 (BR); *Breyne* 3881 (BR); Talias, *de Wilde* (*d' Estmael*) 503/3 (BR); *de Witte* 11811 (BR); *de Witte* 13149 (BR). Watahinga, *de Witte* 8387 (BR). **Orientale**: Mambasa, *Ewango* and *Asani* 2437 (MO). **Tanganyika:** Moba, *de Witte* 318 (BR); Songo-Matumbi: *Seret* 313 (BR). **Tshopo:** Kisangani**,** *Mandago* 2479 (BR); Yangambi, *Gilbert* 7821 (BR); *Jean Louis* 1241 (B, BR); *Jean Louis* 8852 (BR, FHO); *Jean Louis* 11498 (P); *Jean Louis* 12852 (BR); *Jean Louis* 16447 (BR); *Jean Louis* 16849 (BR). **South Kivu**: Kabare, *Robyns* 2503 (BR, K). **Ubangi**: *Lebrun* 1730 (BR, K). **ETHIOPIA.** *Erskine* s.n., 1935 (K); *Leg*. *ign*. 1323, 27 August 1980 (ETH). **Ilubabor:** Ilubabor, *Friis* 1758 (K); *Meyer* 8928 (K, WAG); *Sebsebe* et al. 5525 (ETH). **Kefa**: Mezan Tefari, *Friis* 3970 (ETH, K); *Friis* et al. 7062 (ETH, K). **Oromiya**: Didessa, *Friis* et al. 6031 (ETH, K); *Friis* et al. 9099 (K, WAG); Lekemti, *De Wilde* 8896 (BR, K, MO, P, WAG). **SNNPR:** Gamu Gofa, *Gilbert* et al. 254 (K, MO, WAG); Gojeb, *Mooney* 8623 (ETH, K). **GHANA.** *Leg ign.* 759 (K-undigitized). **Ashanti***:* Bobiri, *Hepper* et al. 7571 (K); Owab, *Lyor* 53 (FHO); *Lyor* 2722 (FHO); Tano-Ofin: *Jongkind* 1918 (BR, MO, P, WAG). **Bono**: Awisa, *Irvine* 2064 (K). **IVORY COAST.** *Jacques Felix* 1276 (P). **Lacs:** Anoumaba**,** *Chevalier* 22411 (P). **Touba:** Zala**,** *Kruif* 599 (BR, MO, WAG). **KENYA.** *Jarrett* 316 (K). **Kagera**: Minziro, *Willan* 154 (K). **Kakamega**: Bukura, *McDonald* 1093 (K). **Meru**: Meru**,** *Gardner* 3150 (K). **Nyanza:** Nyanza**,** *Moon* 6 (K). **West Pokot:** *Kirk* 358 (K);

Cheranangi, *Gardner* 1103 (FHO); Mwina, *Honoré* 3451 (BR, K). **LIBERIA. Gbarpolu:** Gbanka**,** *Linder* 1232 (K). **Lofa:** Lofa, *Jongkind* 6817 (WAG); Vonjama, *Baldwin* Jr., 10037 (K). **Nimba**: Ganta, *Harley* s.n. November 1932 (K); Yekepa: *Jacques George* 24255 (MO). **MADAGASCAR.** *Bosser* 9186 (P); *Cours* 24276 (P); *Greve* 143 (P); *Homolle* 1514 (P); *Service Forester* 5 (P); *Service Forester* 24276 (P); *Toma* s.n., 23 July 1937 (P). **Androy:** Ambovombe, *Decary* 8609 (P); *Decary* 8822 (P); *Decary* 8943 (P); Beloha, *Bosser* 14052 (P); *Bosser* 16052 (CNARP); *Decary* 9910 (P); *Morat* 3940 (CNARP, MO, P); Tanandava, *Peltier* 5503. **Anosi**: Bekily, *Seyrig* 814 (P). **Antanarivo:** Miarinarivo, Gebirge, *Hildebrandt* 3094 (BM, GH, K, L), Tsimbazaza, *Montagnac* 1 (CNARP, P). **Atsimo-Andrefana**: Sakaraha, *Bosser* 19406 (CNARP, MO, P); *Humbert* 29629 (P); *Leg. ign*. 7386, 22 June 1955 (P); *Mabberley* 1000 (CNARP, K). **Atsinanana:** *Ranirison* 585 (P); Brickaville, *Cours* 4625 (P); Toamasina: *Phillipson* 1943 (BR, CNARP, MO, P); Berivotra, *Peltier* 5348 (P). **Betsiboka:** Andriba, *Perrier* 680 (P); Maevatanana, *Leg. ign*. 446, 2 September 1835 (P); *Schwabe* et al. s.n., October-December 1959 (B). **Boeny**: Ambalabongo, *Peltier* 5977 (P); Amborovy, *Peltier* 5287 (CNARP, P); Ampijoroa, *Boiteau* 3015 (P); Ankarafantsika, *Ramamonjisoa* 2032 (CNARP, P); Namoroka, *Service Forester* 17 (P); Soalala, *Decary* 7780 (P); *Jaeger* 4109 (P); *Keraudren* 940 (P); *Rakotosoa* 4109 (P); *Ranoriamiera Surveyland* 6396 (P). **Fianarantsoa**: Ambalavao, *Rakotosoa* 11993 (P). **Ihorombe**: Ihosy, *Bosser* 17534 (P). **Mahajanga**: Ambalakida, *Bosser* 9685 (P); *Labat* 3646 (MO, P); *Luino* 095 (P); *Rakotoarisoa* 446 (P); *Bosser* 5851 (P); Ankarafantsika, *Peltier* 3282 (CNARP, P); Antsingy, *Decary* 7947 (P); Beronono, *Harizo* 1065 (MO, P); Majunga, *Schatz Lowry* 1441 (CNARP, K, MO, P); Marovoay, *Bosser* 8346 (P); Tsaratanana, *Decary* 14402 (CNARP, P). **Menabe**: Ambahivahibe, *Leg. ign*. 6542, 10 June 1944 (P). **North Madagascar:** *Baron* 6172 (BM, P); *Baron* 6434 (K). **Sofia**: Andranosamonta, *Decary* 2232 (K, MO, P); Befandriana, *Humbert* 29743 (P). **Toliara:** *Randriantafika* 190 (K); Anadabolava, *Humbert* 12527 (P); Antevamena, *Sussman* 103 (MO); Beza Mahafaly, *Phillipson* 1759 (BR, CNARP, K, MO, P); Mahatomotsy, Gebirge, *Groeninckx* et al. 298 (BG, K, MO, P, TAN); Sakamena, *Poisson* 422 (P); Zombitsy, *Morat* 585 (CNARP, P); *Morat* 961 (P); *Morat* 976 (P); *Peltier* 5815 (P). **Sava**: Iharaña, *Aubriot* 201 (P). **MALI. Kayes:** Kita, *Jaeger* 3628 (G, K, P, WAG); *Jaeger* 5566 (K, P). **NIGERIA.** *Chester* 207 (FHO); *Fosters* 366 (K). **Cross River**: Oban**,** *Talbot* 801 (BM). **Delta**: Idumuje, *Thomas* 2122 (K). **Enugu**: Udi, *Smith* 20 (K). **Gombe**: Mada, *Hepburn* 87 (K). **Kogi**: Lokoja, *Carsons* s.n. 18 September 1908 (K). **Lagos:** Lagos, *Miller* 55 (K). **Ogun**: Abeokuta**,** *Onochie* 8129 (FHO); *Onochie* 35253 (K); **Ondo:** Idanre, *Keay et Onochie* 20240 (FHO, B); *Onochie* 34350 (K); *Orekeke* 26881 (K). **Onita:** *Thomas* 1889 (K). **Oyo**: Ibadan, *Jones* 7338 (BM). **Rivers**: Degema, *Talbot* s.n., 1916 (BM). **REPUBLIC OF GUINEA. Kindia**: Daramagnaky, *Molmou* 74 (K). **Mamou:** Sokotoro, *Pobeguin* 1843 (P). **REPUBLIC OF GUINEA-BISSAU.** *Chevalier* 20389 (P); *Fora* 21 (BR). **Bafata**: Bafata**,** *Leg. ign*. 2873, 28 January 1951 (WAG); *Leg. ign*. 3800, 24 November 1955 (BR, MO, P, WAG); *Leg. ign*. 3747, 3 December 1955 (MO, WAG). **Dalaba**: Fouta Djallon, *Chevalier* 18669 (P). **Faranah:** Kouria**,** *Chevalier* 14874 (P). **Nzerekore**: Nimba Mts., *Jongkind* 8042 (BR, MO, P, WAG). **Labe:** Yambering, *Chevalier* 34421 (P). **Oio:** Contubo, *Leg. ign.* 2873, 28 January 1951 (K). **SENEGAL. Dakar:** Niayes**,** *Adam* 759 (P); Sangalkam, *Berhaut* 1031 (BR, P); *Jacques George* 1452 **(**MO). **SIERRA LEONE. Southern Province:** Mamboma**,** *Deighton* 3378 (K); Njala, *Deywith* 2570 (K). **Tingi:** Tingi, *Leg. ign*. 3648, 24 November 1955 (BR, MO, P, WAG); *Morton* and *Gledhill* 3218 (K; MO). **Eastern Province**: Joru, *Pyne* 42 (K, P). **SOMALIA.** *Rink* s.n., December 1873 (K). **Lower Shabeelle:** Brawa Yare, *Bally*

9375 (K). **SUDAN. Dandan:** *Friis et Vollesen* 751 (BR, K). **TANZANIA.** *Bally* 4005 (K); *Burtt* 2498 (K); *Burtt* 3469 (K); *Koritschemer* 2181 (K); *Swymerton* 1130 (BM). **Kagera**: Mabuye, *Festo* 1387 (BRIT, K, MO). **Katavi**: Mpanda, *Harley* 9368 (K). **Kigoma:** Kakombe, *Newbould Harley* 4256 (BR, K); Gombe National Park, *Mbago* 793 (MO). **Mboga:** Lesse, *Bequaert* 3013 (BR, K). **Mtwara**: Mwongo, *Tanner* 570 (B, BR, K). **Mwanza**: Magu, *Kayombo* et al. 3276 (BRIT, MO); *Semsei* 132/FH 2563 (K). **Tanga**: Usambara**,** *Peter* 51732 (B). **TOGO. Mpoti**: *Schäfer* et al. 7689 (B, K, MO, MPU, WAG). **Plateaux**: Ogou**,** *Ern et al.* 2551 (B, P). **UGANDA.** *Laurent* s.n., 22 December 1903 (BR). **Central Region**: Buyaya, *Maitland* 1181 (K); Kyagwe, *Eggeling* 517 (K); *Eggeling* 881 (FHO); Kajansi**,** *Chandler* 1184 (K); Mabira, *Loveridge* 37 (K); Mulange, *Dummer* 4365 (BM, K); Masindi, *Bagshawe* 869 (BM). **Eastern district:** Busoga, *Snowden* 403 (BM). **Western region**: Bwamba, *Hansford* 148 (K); *Longfield* 115 (BM); *Thomas* 1507 (BR, K); Kabanga Mutandi, *Osimiaston* 1363 (K). Kibale, *Eiilu* 104 (K); Mpanga, *Bagshawe Camb* 1180 (BM); Semliki, *Brasnett* 7 (K). **ZAMBIA. Copperbelt:** Chingola, *Fanshawe* 3281 (BR).

Ipomoea pringsheimiana*: DEMOCRATIC REPUBLIC OF THE CONGO. Ituri**: Mt. Hoyo, *Lisowski* 40804 (BR). **MADAGASCAR. Anosi**: Bekily, *Seyrig* 483 (P). **Diana**: Ambilobe, *Humbert* 25502 (P). **MALAWI. Southern region:** Chiradzulu, *Brummitt* 9828 (LISC); Mlanji, *Buchanan* 319 (BM); *Buchanan* 388 (K); *Buchanan* 682 (BM, K); *Leg. ign.* (K-undigitised); *Richards* 16773 (BR); Zomba: *Blackmore* et al. 1357 (BM, LISC); *Brummitt* et al. 15562 (LISC); *Masiye* 59 (BR, MO). **MOZAMBIQUE.** *Drummond* 10347 (MO). *Magombera**, *Luke* 9551 (K). **Gaza:** Massingire, *Torre* 5322 (K, LISC). **Manica:** Chimoio, *Chase* 6877 (BM, BR); *Garcia* 523 (LISC); Garuso, *Gilliland* (BM). **Sofala:** Cheringoma, *Barbosa* 1119 (LISC); *Barbossa* 1696 (LISC); *Torre* 4141 (LISC, MO). **Maputo,** Delagua bay**,** *Junod* 439 (BR). **Zambesia:** Ile, *Torre* 5586 (LISC). **TANZANIA. Katavi**: Mpanda, *Mgaza* 150 (K). **Tanga:** Amani, *Peter* 51693 (B); Manga, *Peter* 51676 (MO); Usambara, *Greenway* 1670 (FHO, K); *Peter* 51627 (B, BR, MO); *Peter* 51628 (B, BR, MO); *Peter* 51684 (B, U); *Peter et al*. 51734 (B); *Peter et al.* 51737 (B); *Peter* 51876 (B, BR, MO). **ZIMBABWE. Manicaland:** Burma, *Chase* 6505 (K); Chimanimani**,** *Drummond* 10216 (K). **SOUTH AFRICA. Berea:** Botanic Gardens Durban, *Leg. ign*. 11579 (NH-0011579-0); *Leg. ign*. 13064 18 May 1911 (NH-0013064-0); *Medley-Wood* 4779 (NH); *Medley-Wood* 4806 (Z); *Medley*-Wood 4864 (K); *Medley-Wood* 10065 (NH). **Natal:** Durban, *Govender* 2930 (NH); Gwalaweni, *Strey* 4648 (NH). **Zululand:** *Medley-Wood*, 4146 (K); Ngoye, *Medley-Wood* 10354 (NH).

***Ipomoea macalusoi*: KENYA. Lamu:** Kiwayu**,** *Luke* 5482 (K). **Mombasa**: Likoni, *Robertson* 6704 (K, MO, US). **MAURITIUS.** *Vaughan* 10808 (L). **SOMALIA.** *Bavazzano* 386 (K). **Banaadir:** Mogadishu, *Macaluso* 80 (K). **Lower Shabeelle:** Baraawe-Mudun**,** *Rose* 961 (K).

***Ipomoea incompta*: KENYA. Kilifi**: Arabuko, *Graham* 1915 (B, FHO). **Makueni:** Kibwezi, *Luke* 14331 (K); Tsavo, *Bally* 13401 (G). **Taita Taveta**: Msau, *Kabuye et al.* 613 (US). **Tana**: Baomo, *Robertson* 6494 (MO). **TANZANIA. Kilimanjaro:** Same**,** *Semsei* 2117 (FHO). **Kitui**: Katuka, *Faulkner* 4152 (MO). **Manyara:** Mbulu, *Greenway Kirrika* 10983 (BR). **Pwani**: Pugu, *Harris* et al. 5845 (MO); *Wingfield* 1672 (MO). **Tanga**: Amboni, *Faulkner* 4650 (K); *Peter* 51750 (B); Lushoto, *Richards* 15330 (K); Maweni, *Harris* et *Harris* 3481 (WAG); Steinbruch, *Faulkner* 2013 (BR); *Faulkner* 2192 (B). **Usambara**, *Baagoe et al.* 226 (WAG); *Holst* 3447; Lutindi Mts, *Holst* 4337 (HBG, K); Ndolwa forest, *Semsei* 3080 (BR). **Zanzibar**: Kokotuni, *Stuhlmann* 110

(HBG). **SOMALIA. Buur Heybo:** Buur Akhaba**,** *Kilian* and *Lobin* 6837 (B, WAG). **UNKNOWN**: *Archbold* 2384 (LISC); *Taylor* 137 (BM).

Ipomoea queenslandica: **AUSTRALIA. Queensland:** Atherton, *Gray* 07551 (BRI); *Hyland* 15759 (BRI); Chillagoe, *Clarkson* 3025 (BRI); *Clarkson* 3789 (BRI); *Clarkson* 6837 (BRI, K); *Clarkson* 6860 (BRI); Mungana, *Conn Campo* 1347 (BRI); *Domin* 7919 (PR); *Ford* 02383 (BRI); *Johnson* 4043 (ATH, BRI).

Ipomoea lombokii: **INDONESIA. Lombok**: *Zollinger* 1563 (P); *Elbert* 1890(L); *Elbert*, 2011b (L); *Leg. ign*. sn (L)

Ipomoea neglecta: **INDONESIA. Timor:** *Hombron* 1841 (P); *Bloembergen* 1420 (BO); *Bloembergen* 3480 (BO); *Schmutz* 2294 (L); *Sauveur* 117 (BO, L); *Zippelius* 198 (L); *Walsh* 107 (B); *Jonker* 290 (U); *Teysmann* sn. (BO); *Walsh* 373 (BM); *Friedberg* 144c (L). **Selayar***: Zollinger* 180 (L); *Eyma* 326 (BO, L); *Zollinger* 3327 (P, G); **Sulawesi:** *Teysmann* 12171 (BO, L); **Flores***: Afriastini* 1572 (BO); **Wetar**: *Treub* 546 (BO).

Ipomoea orientalis: **INDONESIA. Timor:** *Reinwardt* 1350 (L); *Kooy* 292 (L); *Reinwardt* 44 (L). **Sumbawa**: *Kostermans* 18705 (SING, BO, K, G, L, P); *Leg. ign.* 296 (L). **Flores**: *Schmutz* 172 (L).

Ipomoea mojangensis**: MADAGASCAR.** *Afzelius* 1912 (P); *Humbert* 11329 (P); *Humbert* 11524 (P); *Humbert* 2328 (K, MO, P); *Humbert* 5532 (P); *Decary* 14423 (P); *Decary* 15788 (P); *Seyrig* 101A (P); *Seyrig* 719 (P); *White* sn. (BM); *Peltier* 2565 (P); *Allorge* 569 (P); *Bardot Vaucoulon* 490 (P); *Du Puy* 2467 (K), *Allorge* 2379 (P); *Labat* 2288 (P); *Phillipson* 2242 (P); *Rabesandratana* 4143 (P); *Sauther* 32 (P); *Bardot Vaucoulon* 1726 (P); *Véné* 1741 (P);

Ipomoea sivarajanii**: INDIA.** *Biju* 22013 (ARIZ); *Biju* 25924 (ARIZ)

Ipomoea tiliifolia**:**
AMERICA
ANTIGUA AND BARBUDA. St. John: Galley bay, *Box* 1299 (BM, US); *Rose* 3383 (NY, US). **CONTINENTAL UNITED STATES. Florida Keys:** Monroe**,** *Small Mosier* 6002 (K, U). **COSTA RICA. Alajuela:** Los Loros, *Brenes* 22047 (NY). **Puntarenas**: Cabo Blanco, *Chavarria* 341 (MO); *Fernandez* and *Shallenger* 1330 (MO); Playa Balsita, *Quesada et al.* 832 (BM, MO); Playa Organos, *Hammel Garita* 19308 (MO); Peninsula de Nicoya, *Alvarado* 110 (MO); Ballena, *Hammel* 20619 (MO); Playa Espadilla, *Wilkin* 121 (BM). **Isla de Coco:** Rio Sucio, *Quesada* 1103 (BM, K, MO); Playa Blanca: *Valerio* 445 (BR). **DOMINICAN REPUBLIC.** *Imay* 230 (K). **Barahona**: *Turckheim* 2776 (BR, NY). **La Altagracia**: Bayahibe, *Liogier* 24805 (NY); Boca de Yuma*, Liogier* 22936 (NY). **Santo Domingo***: Rose* 4326 (NY); *Wright et al*. 390 (US); Boca Chica, *Liogier* 26073 (NY); La Caleta, *Ekman* 10929 (K, S). **La Romana:** Catalina island, *Liogier* 27392 (NY). **Peravia**: Jovero, *Albott* 2549 (US); *Albott* 2561 (US). **St. Patrick**: *Whitefoord* 5400 (BM, US).**Samana:** Samana Peninsula, *Liogier* 14397 (NY, P). **ECUADOR. Galapagos Islands:** St. Cruz, *van der Werff* 1282a (K, MO, NY, U). **FRENCH OVERSEAS TERRITORY. Antilles**: St. Claude, *Stehle* 233 (P). **Guadeloupe**: *Gallo* 3134 (US); Port Louis, *Questel* 4039 (US); Mamelles, *Raynal* 15952 (P); Pirogue, *Proctor* 20264 (BM, US). **Martinique**: *Belanger* 573 (P); *Duss* 2476 (NY, US); *Hahn* 628 (P); *Leg. ign.* s.n., July 1820 (P); Fort Desaix, *Mouret* 380 (P); Le Francois, *Duss* 3503 (NY); Precheur, *Duss* 1883 (US); St. Lucia,

Plee s.n., January 1821 (P-04039428). **GUATEMALA.** *O'Donell* s.n., June 1953 (K). **HAITI. Nord:** Bayeux, *Ekman* 2923 (S, US). **Nord Ouest**: Anse a Foleur, *Zanoni* 33582 (NY). **NETHERLANDS OVERSEAS TERRITORY. St. Eustatius:** *Suringar* s.n., 16 April 1895 (L). **PANAMA.** *Duchassaing* s.n., 1851 (P-04039446). **Darien**: Jaque, *Sytsma* and *D'Arcy* 3457 (MO). **San Jose:** Playa Grande**,** *Johnston* 1011 (MO). **PUERTO RICO.** *Drake* 9768 (P); *Woodbury* s.n. Aug 1969 (NY). **Arecibo***: Liogier* 33759 (NY). **Cabo Rojo:** Guanajibo Barrio, *Sintenis* 822 (US). **Humacao:** Cayo Santiago, *Axelrod et al.* 3326 (NY). **Dorado:** *Woodbury* s.n., 3 December 1960 (NY). **Quebradillas:** Quebradillas, *Sargent* B152 (US); *Stevenson* 5886 (US). **San Juan:** Fort Jeronimo, *Britton* 6638 (NY). **Vieques**: Santa Maria, *Shafer* 2583 (NY, US). **SAINT KITTS AND NEVIS. Saint George Basseterre**: Nag's Head, *Proctor* 18512 (US). **ST. VINCENT AND GRENADINES.** *Hooker* 27 (K). **UK OVERSEAS TERRITORY. Guiana island:** White bay**,** *Proctor* 43448 (NY). **Montserrat:** Balham river, *Proctor* 18931 (BM, US); Brochiners, *Shafer* 225 (NY); Plymouth, *Shafer* 64 (NY, US). **U.S. VIRGIN ISLAND: St. John,** Cruz Bay, *Acevedo-Rodriguez* 2463 (NY); *Acevedo-Rodríguez et al*. 4008 (NY); Center Line Road, *Acevedo-Rodríguez* 2898 (NY). **Tortola**: Fishlock 275 (K, NY). **St. Croix island:** Ricksecker 162 (MO, NY, US). **St. Thomas**: Britton 271 (NY); *Leg. ign*. s.n. (P-04039435). **Virgin Gorda:** Fishlock 282 (NY).

AFRICA

CAPE VERDE. *Leg. ign*. s.n. (L-2744536); *Lowe* s.n., 25 May 1875 (BM, K, NY); *Welwitsch* 6213 (BM, K). **Maio**: Mt. Batalha, *Kilian* et *Leyens* 2991 (B, MO). **Sao Vicente**: *Moseley* s.n., 1873 (K); *Smith* 1610 (K, NY). **COMOROS. Anjouan:** *Hildebrandt* 1628 (BM, K, P). **FRENCH OVERSEAS TERRITORY. Mayotte**: Accoua, *Barthelat* 436 (MO); Chiconi, *Barthelat* 929 (MO, P); Chirongui, *Barthelat* 1643 (MO, P); Sada, *Barthelat* 949 (P). **Reunion:** *Boivin* s.n., 1 June 1851 (P-04039504); La Marine, *Cadet* 5616 (P)**. MAURITIUS.** *Bouton* s.n. 1839 (G-00135069); *Commerson* s.n. (G00135053, P-00622214, 00622215, 00622216, 00622217). **Flacq:** Bramsthan, *Duljeet* 11220 (L). **Rodriguez:** *Balfour* s.n. (BM); *Cadet* 2738 (P); Sainte Anne, *Cadet* 5998 (P); Saint Denis, *Cadet and Figier* 6764 (P). **UK OVERSEAS TERRITORY. St. Helena***: Lady Dalhousie* s.n. (K-undigitised).

ASIA

AUSTRALIAN OVERSEAS TERRITORY. Christmas island: *Leg. ign.* s.n., October 1904 (SING); *Powell* 232 A (K); *Ridley* 150 (K). **BANGLADESH. Chittagong:** *Hooker et Thomson* s.n. (L-2749887); *Hooker et Thomson* 4859 (P); Teknaf, *Rahman et al.* 4240 (K). **Dacca:** *Blanco* s.n. (BM-undigitized); *Jabbar* 56-771 (K). **Rangpur Division**: Sundarban**,** *Hooker* et *Thomson* s.n. (K 2017/00132108); *Prain* s.n., 21 February 1900 (BM). **CHINA. Guangdong:** *Southroad* 00886 (IBK, IBSC, PE); *Zhou Yuanrui* 100145 (IBK). **Hainan**: *Zuo et Chen* 23016 (IBSC); *Li* 3086 (IBSC); *Li et Xing* 4744 (IBSC); *Zuo et al.* 1683 (IBSC); Yaichow, *Tso* 23016 (BP, P). **Taiwan:** *Genkei Masamune* 3871 (TAI); *Matuda* 786 (TAI) *Sasaki* s.n. (TAI- 093276); Bankinseng Mt, *Henry* 1671 (K); Koahsiung, Meilong, *Huang* 15939 (TAI); Pingtung Hsien, *Huang* 9158 (MO, TAI); *Kao* 9724 (TAI); *Wang* 2907 (TAI); *Wang* 02016 (L); *Wang et al.* W03088 (PE); Lantao*, Huang* 9305 (TAI); *Hsu* 468 (TAI); *Wang* 3987 (MO); Tainan, *Huang* 4408 (TAI); Taitung Hsien, *Yang et Liu* 14740 (KUN, MO); *Yang et Huang* 15741 (MO); South Cape, *Henry* 199A (P); *Kudo et. Suzuki* 15830 (TAI); Syamari, *Suzuki-Tokio* 6121 (NAS); Taihoku, *Tata* 1655 (TAI). **INDIA.** *Edgeworth* 447 (K); *Wight* 2247 (K); *Lambert* s.n. (BR-0000013308636); *Rottler* s.n., January 1798 (K); *Wallich* 1340A (G). **Andhra Pradesh:** Coromandel*, Mace* s.n. (P-04039485). **Karnataka:** Mangalore**,** *Steudel* s.n. (P); *Leg. ign.* s.n. in Hohenacker

1947 (K). **Kolkata***: Gaudichaud* 14 (P); *Leg*. *ign*. s.n., Cult. in hort. Bot Cal. (G); *Wight* 1948 (BO, L, P). **Ganges:** *Leg*. *ign*. 352, December 1817 (SING-0075944). **Tamil Nadu**: Chennai, *Wallich* 1247 (BM); Anaimalai, *Beddome* 5530 (BM); Coimbatore, *Fisher* 568 (K). **Maharashtra:** Mahim, *Patel* 2042 (BRI); Mumbai, *Roux* s.n. (G-00134790); Tiruchi*, Rajamani* THT 70 (K). **Kerala**: Shimoga, *Biju* 15344 (ARIZ). **Nicobar Islands**: *Kurz* 26056 (BM). **INDONESIA. Ambon:** *Blume* 297 (L-0004259); Hila, *Reinwardt* 81 (L); *Reinwardt* 1462 (L). **Flores**: Nangalili, *Schmutz* 1669 (L); Reo, *Kostermans* 22212 (L). **Java**: Banten, *Borssum Waalkes* 154 (L); *Vogdt* 154 (L); Bogor, *Hallier* 218 (L); Cimahi, *van Slooten* 632 (BO); Karang Hawu, *Docters van Leeuwen* 14192 (BO); Pasuruan, *Backer* 3914 (BO); Priok, *Harreveld* 5077 (L); Sunda strait, *Stauton Macartney* s.n.,1873 (BM); **Nusa Laut**: *Reinwardt* s.n., July 1821 (L); *Reinwardt* 63 (L). **Kangean:** Sepanjang, *Backer* 28986 (BO). **Lombok**: *Elbert* 2478 (L); *Zollinger* 1563 (P). **Panaitan**: Tanjung Parat, *Borssum Waalkes* 697 (BO, K, L); *Borssum Waalkes* 764 (K); *Borssum Walkes* 1951 (SING). **Rinca:** *Suakov* 144 (BO). **Seram**: Tahalupu, *Kornassi* 1338 (BO). **Sulawesi:** *Leg*. *ign*. 82 (L-2744574); *Teijsmann* s.n. (BO-1724208); Kalao, *Docters van Leeuwen* 1526 (U); Pakamisang, *Rachmat* 311 (BO, L); Tanah Jampea, *Docters van Leeuwen* 1595 (BO, U); Tojambu, *Kjellberg* 1862 (BO). **Sumatra**: Perapat, Tongging, *Lorzing* 14763 (BO). **Timor:** *Riedel* s.n., June 1884 (K); Kupang, *Brown* s.n., April 1803 (BM); *Kooy* 1395 (L). **JAPAN. Bonin islands:** Nagahama, *Furuse* 11241 (K, L, PE); *Yang* 1124 (TAI); Motochi, *Leg. ign.* 8217, 20 December 1920 (K); *Wilson* 8213 (BM). **MALAYSIA. Kedah:** Pulau Timun, Henderson 29113 (K); **Sarawak:** *Native collector* 2348, February-June 1914 (BM). **MYANMAR. Rakhine:** Akyab, *Vogt* BU 259 (K). **PAPUA NEW GUINEA. Bismarck Archipelago:** Ugana, *Peekel* 10 (K, L); *Peekel* 161 (BO); *Peekel et al.* 1213 (B). **THAILAND.** *Haniff* 7469 (K). **Bangkok**: *Kerr* 3917 (BM, K); *Kerr* 3922 (BM). **Prachuan Khiri Khan:** Chalak**,** *Collins* 1345 (K); Pran, *Put* 2450 (BM, K). **Sriracha**: *Put* 468 (BM, K). **THE PHILIPPINES. Alabat**: *Merrill* 10452 (BM, P). **Bicol**: Albay, *Cuming* 1026 (K); *Cuming* 1087 (BM, K); Bauang, *Elmer* 5584 (K, P). **Bulacan**: *Ramos* 2028 (BM, BRI). **Calabarzon**: Los Banos, *Loher* 4150 (K); Calauan, *Mcgregor* 12385 (BM, K, L, P). **Ilocos**: Bangui, *Ramos* 2761 (BO, P). **Luzon:** Malabon, *Loher* 4136 (K). **Mindanao**: Zamboaga, Pasanan, *Frake* 36247 (L). **Mindoro**: Manaul, *Sulit* 17032 (BM, BR, K, L). **Palawan***: McGregor* 45946 (BRI). **Polillo**: Karlagan, *Fox* 9166 (L). **Samboang:** *Perrottet* s.n. (P-04039468). **Tayabas:** *Whitford* 827 (P). **VIETNAM. Chau Doc:** *Pierre* s.n., December 1867 (P); *Pierre* s.n., August 1869 (P, BO). **Baria**: *Pierre* s.n., August 1866 (P). **SRI LANKA.** *CV* 2548 (BM-001253027); *Leschenault* s.n. (P-04039491).

AUSTRALIA

AUSTRALIA. Northern Territory: Goromuru River, *Cowie* 6659 (BRI); Refuge Bay, Elcho island, *Dunlop* 3913 (L); Bennet Bay, *Dunlop* 7350 (BRI). **Queensland:** Budekin, *Ford* 02382 (B); Burleigh Heads National Park, *Foster* 40510 (BRI); Cromarty, *Blake* 8318 (BRI); Gloucester Island, Mount Bertha, *Batianoff* 9403318 (BRI); Howard Point, *Batianoff* 11706 (BRI, P); Low isles, *Cull* 2839 (BRI); Ramingining, *Cowie Mangion* (BRI). **Western Australia**: Rockingham, *Mueller* s.n., 1865 (L); *Mueller* (L-2744546).

PACIFIC

COOK ISLAND: Rarotonga, *Cheeseman* 612 (AK, K). **FEDERATED STATES OF MICRONESIA. Guam:** *Nelson* 259 (BO); *Thompson* 93 (BO, K, L); Road to Dan Dan NASA radio tracking station, *Fosberg* 59740 (L, NY); Barrigada, *Fosberg* 35287 (BISH, L); Tumon Bay, Gogna Beach, *Fosberg* 43496 (BISH). **NEW CALEDONIA. North Province**: Diahot valley, *Balansa* 3353 (P); Koumac, *MacKee* 21946 (P).

Nehoue: *MacKee* 16896 (P); *MacKee* 35001 (L, P); Plateau de la Montagne des Sources, *Blanchon* 196 (P). **Tipindje**: *MacKee* 23941 (L, P). **Santo**: *Blanchon* 849 (P); Vallée Koumac, *MacKee* 25228 (P, SING); *MacKee* 41490 (P). **South Province**: Bourail, *Balansa* 1056 (P); *Schmid* 1994 (P); Nouméa: *Balansa* 543 (P). **FIJI. Cuou**: Sigatoka, *Greenwood* 306 (K). **Savusavu:** *Seemann* 334 (K); Vanua Levu, *Leg. ign.* s.n., 1898 (K-undigitized). **FRENCH POLYNESIA. Marquesas.** *Butaud et Girardi* 2688 (BISH). **Pohnpei:** Nihwel, *Raynor* 125 (NY, US). **Society islands**: *Banks Solander* s.n. (BM); Moorea, *Sachet Bare* 2335 (BM, K, MO, NY). **Tahiti island:** *Setchell et Parks* 238 (BISH); Papeete, *Florence* 8313 (P). **Tubuai**: Hanareho. *Halle* 6789 (P). **SAMOA.** *Powell* 258 (K). **Manu'a:** Ofu, *Garber* 999 (L). **Namu'a island**: *Whistler et al.* W4559 (B). **Nu'utele island**: Vini, *Ogle* 3842 (AK). **Suisega**: *Whistler* 3065 (K). **Tutuila island**: *Setchell* 248 (BM); *Whistler* 2719 (B, BISH, K). **Upolu**: Lalonaea, *Cox* 148 (K). **Windward island**: Petite Coulibrie, *Whitefoord* 6056 (BM, US). **TONGA. Vavau:** *Crosby* 127 (K); Tu'anikivale, *Soakai* 774 (K); Leimatua, *Yuncker* 16052 (BISH, BM, U). **UNITED STATES in the PACIFIC**. **Hawaii:** *Hillebrand* 1983 (US); Halepuaa, *Renee Kubo* 24 (US); Kailua Kona, *Greenwell* et al. 19378 (B); Kau, Punaluu, *Faurie* 1041 (BM, P); *Faurie* 1043 (P); *Herbst* 5834 (US); *Hillebrand* 394 (K); Puna, Wahaula**,** *Degener et al.* 32417 (B). **Kauai**: Koloa, Lawai Valley, *Lorence* 7204 (MO, US). **Maui:** *Forbes* 1908 (US); *Imada* 2004-45 (US); *Le Roy Topping* 6001 (US); *Pearsall* s.n., 26 December 1955 (P-04039452); *Tam* 22038 (B, L, US). **Molokai:** *Degener et al.* 5989 (B); *Fosberg* 77a (US); *Forbes* 354 (P; US); *Hillebrand* s.n., December 1890 (BM-undigitized); *Rock* s.n., March 1910 (K-undigitized, L-2744549, US- 01232880). **Oahu:** *Mann Brigham* 42 (US); Koolaupoko, Kailua, *Staples* 1400 (SING). **VANUATU. Epi**: Filakara, *Curry* 1009 (L). **Erromango:** *Bevaridge* 19395 (NZFRI); Cook Bay, *Cabalion* 3095 (P). **Sanma**, Malao, *MacKee* 24255 (K); Matantas, *Curry* 1086 (K). **Shefa:** Efate**,** *Austin et Austin* 7466 (MO); Epi, *Curry* 1199 (K); Mt. Barnier, *Cabalion* 2307 (P); Port Vila, *Cabalion* 2714 (K, P); *Bourdyn* 772 (P); Vate, *Bourdyn* 142 (K, P).

UNCERTAIN LOCATION.

Dombey s.n. (P-04039477); *Helfer* 5864 (K); *Hooker* 174 (K); *Hooker* 1340 (K); *Hooker* 2845 (K); *Kuntze* 568 (NY); *Leg*. *ign*. 158, December 1887 (P-04039459); *Leg. ign*. 628 (BM-undigitized); *Leg. ign*. s.n. (BM-undigitized); *Martius* s.n. (BR-BR0000013308643); *Pueran* s.n., 1824. (G-00134803); Bengal, *Kurz* 12-66 (BO); Aryadaka *Clarke* 34533 (BM); Either Madagascar or Mauritius, *Bojer* s.n. (G-00135045); Either Andaman Islands or Tenaserrim, *Gaudichaud Beaupre* s.n., 1830 (G).

Appendix 2. Definition of morphological characters states of Stictocardia species following Stearn (1973) and Eckel (2010)

I. Shape of Organ (leaf, sepal)

Ellipsoid: Solid with elliptical figure.

Globose: Spherical, globular, round like a ball.

Lanceolate: Narrowly elliptical, tapered from the base to each end.

Linear: Narrow, short, with the two margins parallel.

Ovate: Oblong, elliptical, broadest at the lower end

Ovoid: Egg solid shaped broader at the base and gradually tapered above.

Suborbicular: Almost perfectly circular.

Subreniform: Resembling kidney-shaped.

II. Surface of organ (leaf, sepal, stem)

Glabrous: Not hairy or pubescent, lacking hairs, scales or other indument.

Glaucous: Covered with fine bloom of white or whitish, grayish or bluish overcast.

Puberulent: Covered with minute hairs or very fine down, short soft hair.

Villous: Covered with long, soft hairs.

III. Apex of organ (leaf, sepal)

Acuminate: Long-pointed, tapered to a slender point; having a gradually diminishing point or apex, making angle less than 90°

Acute: pointed acutely, sharply, keenly, pungently; narrowed gradually, with terminal angle less than 90° but greater than 45°.

Obtuse: Blunt.

Truncate: Ending very abruptly as if cut straight across with an apex squared at the end.

IV. Margin of organ (leaf)

Entire: Straight, without irregularity nor teeth.

V. Base of organ (leaf, sepal)

Cordate: Heart-shaped, with two equal rounded lobes at the base.

Semi deltoid: Solid, transverse section of which has almost a triangular outline.

VI. Shape of Corolla

Campanulate: Tube shapes like a bell, or cup-shaped with a broadened rim.

Funnelform: Tube shapes obconical, gradually enlarging upwards into the limb, the whole resembles a funnel.

Salverform: Tube shapes long and slender with the flat limb.

VII. Miscellaneous

Caducous: Falling off, dropping off early, detached upon maturity.

Terete: Round-headed.

Appendix 3. The script used in the PCA in RStudio ver. 1.4.1103 using *princomp* function

```
file.name <- read.csv ("file.name.csv")
file.name
        #Replace NA with 0
file.name[is.na(file.name)] <- 0
file.name
d<- file.name[,7:13]
pc <-princomp (d)
summary(pc)
plot(pc)
sumplot (pc, type="l")
biplot (pc)
dim (d)
attributes (pc)
pc$loadings
pc$scores
str(file.name)
summary(file.name)
library (ggfortify)
df <- file.name[c(7:13)]
autoplot(pc)
autoplot(pc, type = "n")
        #species distribution
autoplot(princomp(df))
autoplot(princomp(df), data = file.name, colour = 'Species')
autoplot(princomp(df), data = file.name, colour = 'Species', label = TRUE, label.size =
3)
autoplot(princomp(df), data = file.name, colour = 'Species', shape = FALSE, label.size
= 3)
autoplot(princomp(df), data = file.name, colour = 'Species', loadings = TRUE)
```

```
          #loadings
autoplot(princomp(df), data = file.name, colour = 'Species',
         loadings = TRUE, loadings.colour = 'blue',
         loadings.label = TRUE, loadings.label.size = 5)

          #loadings
autoplot(princomp(df), loadings = TRUE, loadings.colour = 'blue',
         loadings.label = TRUE, loadings.label.size = 5)

          #comprehensive
autoplot(princomp(df), data = file.name, colour = 'Species',
         loadings = TRUE, loadings.colour = 'blue',
         loadings.label = TRUE, loadings.label.size = 5,
         shape='Species', frame=T)

          #frame
autoplot(princomp(df), data = file.name, colour = 'Species',
                  shape='Species', frame=T
```

Appendix 4. List of measured specimens of indistinct species (*I. tiliifolia, I. pringsheimiana, I. orientalis, I. neglecta, I. queenslandica,* and *I. sivarajanii*) and the distribution map

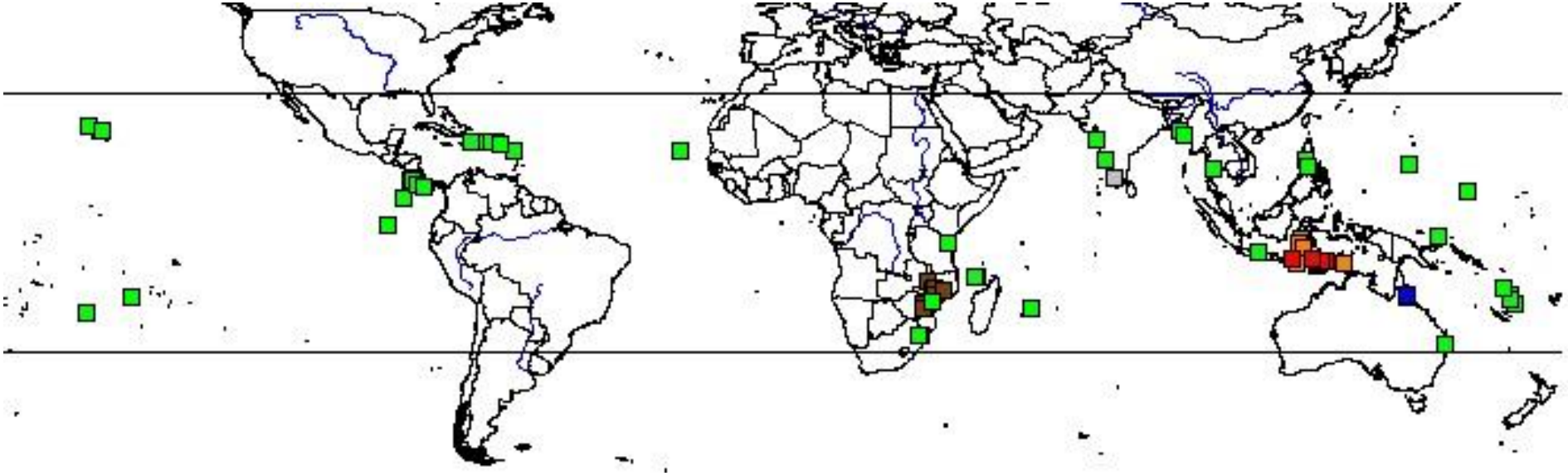
Collector(s) number	Deposited herbaria	Country	Major area and locality	Altitue (m)	Year of collection	Latitude	Longitude
I. tiliifolia							
Axelrod 10071	US	Puerto Rico	Humacao, Cayo Santiago	1	1997	18.16°N	65.736°W
Cuming 1087	BM, K	Philippines	Luzon			12.8797°N	121.774°E
Curry 1009	L	Vanuatu	Epi, Filakara	0	1993	16.816°S	168.4°E
Curry 1086	K	Vanuatu	Port-Vila	5	1993	17.733°S	168.316°E
Curry 1199	K	Vanuatu	Santo Matantas	10	1993	15.166°S	166.95°E
Duljeet 11220	L	Mauritius	Flacq, Bramthan	45	1964	20.198°S	57.729°E
Fernandez Shallenger 1330	MO	Costa Rica	Cabo Blanco, Nikoya peninsula, Orillas de Playa Balsita	200	1993	9.583°N	85.116°W
Forster 40510	BRI	Australia	Queensland, Burleigh Heads National Park	10	2013	28.1°S	153.45°E
Fosberg 59740	NY	The United States in Micronesia	Guam, Marianas Islands. Road in to Dan Dan NASA Radio Tracking Station	85	1980	13.273°N	144.748°E
Fosberg 35287	BISH, L	The United States in Micronesia	Guam, Barrigada	65	1953	13.468°N	144.798°E
Barthelat 1643	MO, P	French overseas territory	Mayotte, Gande Terre-Accoua, Tsimkoura		2006	12.936°S	45.138°E
Gallo 3134	US	French overseas territory	Guadeluope, Moulin de Valleral	30	1967	16.265°N	61.551°W
Hammel 19308	MO	Costa Rica	Puntarenas, Nicoya peninsula, Paquera a playa organos	30	1993	9.816°N	84.9°W
Hammel 20619	MO	Costa Rica	Puntarenas, Canton de Osa, Ballena, Punta Dominica	20	1996	9.225°N	83.827°W
Henry 1671	K	Taiwan	Formosa, Mt. Bankingseng		1895	Unknown	Unknown
Hitchcock 15064	US	The United States	Molokai, Pukoo		1916	21.076°N	156.795°W
Imada 2004-45	US	The United States	Maui, Waihee	6	2004	20.938°N	156.5115°W
Johnston 1011	MO	Panama	San jose island, Playa grande	0	1946	8.089°N	82.353°W
Barthelat 929	MO, P	French overseas territory	Mayotte, Grande Terre-Accoua, Sohoa beach		2002	12.833°S	45.11°E
Kilian 2991	B, MO	Cape Verde	Maio, Batalha	200	1994	16.538°N	23.0418°W
Loigier 26073	NY	Dominica	Boca Chica		1976	18.46°N	69.595°W
Loigier Woodbury 33759	NY	Puerto Rico	Arecibo	0	1983	18.472°N	66.715°W
Lorence 7204	MO, US	The United States	Hawaii, Kauai, Koloa district	30	1992	21.921°N	159.509°W
Mackee 24255	K	Vanuatu	New Hebrides, Espiritu santo		1971	15.25°SN	166.833°E
Mcgregor 12385	BM, K, L, P	Philippines	Luzon, Laguna prov. Calauan		1910	14.145°N	121.315°E

Patel 2042	BRI	India	Mahim Sea Shore [Pahelgadh]		1957	19.031°N	72.826°E
Peekel 10	K, L	Papua New Guinea	New Ireland, Ligong, Ugana		1939	3.316°S	151.775°E
Put 2450	K, BM	Thailand	Pran		1929	12.384°N	99.901°E
Quesada 1103	K, BM, MO	Costa Rica	Puntarenas, isla del coco, Bahia chatan, Rio Sucio	20	1994	5.544°N	87.055°W
Quesada Segura Solis 832	MO, BM	Costa Rica	Peninsula de Osa, Playa san josecito	30	1993	8.616°N	83.733°W
Rahman et al 4240	K	Bangladesh	Teknaf		1999	20.928°N	92.246°E
Raynor 125	NY, US	Federated states of Micronesia	Pohnpei, Nihwel, Temwen		2002	6.848°N	158.323°E
Reinwardt 1462	L	Indonesia	Java		1821	6.993°S	110.42°E
Ricksecker 162	NY, US, MO	The United States	Virgin island, St. Croix		1895	17.735°N	64.747°W
Rock sn	US, K, L	The United States	Molokai, Mapulehu		1910	21.09°N	156.817°W
Sachet 2335	NY, K, MO, BM	French Polynesia	Society Islands. Moorea. Maatea, S. side of island, Valley of Paoroa		1976	17.55°S	149.8°W
Brenes 22047	NY	Costa Rica	Alajuela, Costa del Pacifica (Los Loros).		1937	10.023°N	84.205°W
Sulit 17032	BM, K, BR, L	Philippines	Mindoro, Mansalay		1952	12.516°N	121.4°E
Thompson 93	L, K, BO	The United States in Micronesia	Guam		1913	13.444°N	144.793°E
Turckheim 2776	NY, BR	Dominica	Santo Domingo, Barahona		1909	18.213°N	71.099°W
Van der Werff 1282A	U, K, MO, NY	Equador	Galapagos, Santa Cruz	65	1974	0.639°S	90.3372°W
Vogt 259	K	Myanmar	Myoma, Akyab	30	1952	20.132°	92.8725°E
Cabalion 2049	P	Vanuatu	Emomango, Ipota	5	1983	18.857°S	169.285°E
Chavarria 341	MO	Costa Rica	Cabo Blanco	20	1991	9.583°N	85.1°W
Cheeseman 612	AK, K	New Zealand	Cook island, Rarotonga	1		21.234°S	159.775°E
Clarke 34533	BM	India	Bengal, Aryadaha		1884	Unknown	Unknown
I. pringsheimiana							
Barbossa 1119	LISC	Mozambique	Cheringoma, Inhamitonga		1948	18.218°S	35.16°E
Drummond 10216	K	Zimbabwe	Chimanimani		1973	19.916°S	33.016°E
Drummond 10347	MO	Mozambique	Manica E sofala		1974	19°S	34°E
Dummer 1044	K	Uganda	Kimvafro	1300	1914	Unknown	Unknown
Garcia 523	LISC	Mozambique	Chimoio (Chimaia), serra de garuzo		1948	19.116°S	33.483°E
Greenway 1670	K, FHO	Tanzania	Usambara, Amani, ampandrandava	1000	1929	5.063°S	38.649°E
Masiye 59	MO, BR	Malawi	Salisbury, Mt. Zomba		1978	15.43°S	35.418°E
Torre 5322	K, LISC	Mozambique	Massingire, Morrumbala		1943	16.983°S	35.633°E

Torre 5586	LISC	Mozambique	Ile, Zambesi	1000	1943	15.981°S	37.535°E
Blackmore Brumit 1357	LISC, BM	Malawi	Old naisy, Chivunde Valley		1980	15.43°N	25.918°E
Brummit Masiye 15562	LISC	Malawi	Mponda, Mt. Zomba	1140	1980	13.556°S	33.766°E
Brummitt Banda 9828	LISC	Malawi	Southern Region, Chiradzulu District. Foot of Chiradzulu mountain, south-west side, near Lisao Forestry Depot;	1250	1970	15.7525°S	35.215°E
Chase 6505	K	Zimbabwe	Untabi, Burma Valley	660	1957	19.183°S	32.783°E
Chase 6877	BM, BR	Mozambique	Ghimoio, Bandula	670	1958	19.011°S	33.143°E
Worthington 3781	NH	South Africa	Natal, Zululand	600	1906	28.841°S	31.686°E
Mgaya 150	K	Tanzania	Tanganyika		1957	5.158°	38.8025°E
Barbosa 1696	LISC	Mozambique	Cheringoma, Durundi,Inhamitonga		1948	18.218°S	35.16°E
Junod sn 1893	BR	Mozambique	plantes de Delagoa Bay		1893	26.043°S	32.756°E
Strey 4680	K	South Africa	Natal Mt. Lebombo, Gwalaweni forest		1963	26.25°S	32°E
wood 4864/4146	K	South Africa	Natal, Zulu	5	1889	28°S	32°E
I. orientalis							
kooy 292	L	Indonesia	Timor, Bone	750	1967	8.8742°S	125.7275°E
Kostermans 18705	SING, BO, K, G, L, P	Indonesia	Sumbawa, Mt. Batulante	500	1961	8.7381°S	118.1171°E
leg ign 296	L	Indonesia	Timor			9.233°S	124.933°E
Reindwardt 1350	L	Indonesia	Timor, Jujula			9.233°S	124.933°E
Reinwardt 44	L	Indonesia	Timor			9.233°S	124.933°E
Schmutz 172	L	Indonesia	Flores, Kumang	1000	1966	8.61°S	122.731
I. neglecta							
Afriastini 1572	BO	Indonesia	Flores, Ile Mandiri, Larantuka	150	1984	8.657°S	121.0794°E
Walsh 107	B	Indonesia	South Central Timor	600	1929	9.35°S	129.985°E
Walsh 373	BM	Indonesia	South Central Timor, Ofoe	600		9.35°S	129.985°E
Zollinger 3327	P, G	Indonesia	Selayar		1847	6.0957°S	120.504°E
Bloembergen 3480	BO	Indonesia	Timor, batoe Napi, Kapan	950	1939	9.728°S	124.283°E
Eyma 326	L, BO	Indonesia	Celebes, Rapang, Bulawayo, Poetjoek	20	1937	3.85°S	119.966°E
Freidberg 144	L	Indonesia	Timor, Abis	600	1966	9.63°S	119.255°E
Hombron sn	P	Indonesia	Timor, Coepang		1841	9.916°S	123.833°E
Jonker 290	U	Indonesia	Timor			9.233°S	124.933°E
Sauveur 117	BO, L	Indonesia	Timor, Kumlol		1961	9.614°S	124.328°E

Schmutz 2294	L	Indonesia	Timor, Oetulu	700	1968	9.517°S	124.374°E
Teiysmann 12171	BO, L	Indonesia	Sulawesi, Pangkajene			4.849°S	119.588°E
I. queenslandica							
Canpo 1347	BRI	Australia	Mungana	300	1983	17.066°S	144.366°E
Clarkson 6837	BRI, K	Australia	Queensland Cook District, Chillagoe, Royal Arch Tower	400	1982	17.183°S	144.483°E
I sivarajanii							
Biju 15344	ARIZ	India	Karnataka, Shimoga		1991	14.05°N	75.16°E
Biju 22013	ARIZ	India	Kerala, Idukki	1219	1994	10°N	77°E

I. sivarajanii was included in the PCA but not included in the univariate analysis (the mann-Whitney test)



The figure shows the distribution of measured specimens (boxes) of indistinct species across the globe. The species are colour-coded as follow; *I. tiliifolia* (green), *I. queenslandica* (dark blue), *I. sivarajanii* (grey), *I. orientalis* (red), *I. neglecta* (orange). Two horizontal black lines refer to 30°N (top) and 30°S (bottom)

Appendix 5. Passport of samples represented in the ITS and plastome phylogeny and the distribution map

Species and region	Collector(s) and number	Deposited Herbaria	Country	Major region/island and locality	Year of Collection	Latitude	Longitude	ITS phylogeny	Plastome phylogeny
*I. beraviensis*_DR_Congo	P.R.O. Bally 6506	K	DR Congo	Ruwenzori	1938	0.48°N	29.849°E	+	+
*I. beraviensis*_Kenya	H.M. Gardner 1103	FHO	Kenya	Cherangani hill	1937	1.251°N	35.442°E	+	+
*I. beraviensis*_ Ethiopia	I. Friis, G. Awake, F. Rasmussen, Vollesen 1758	K	Ethiopia	Illubabor	1972	8.45°N	35.616°E	+	+
*I. beraviensis*_ Ghana	C.H.H. Jongkind & D.K. Abbiw1918	WAG, MO, BR, P	Ghana	Tano Ofin Forest Reserve, Ashanti	1994	6.775°N	2.123°W	+	+
*I. beraviensis*_Liberia_2	P. Adames 795	K,P	Liberia	Dolo farm road	1964	6.553°N	10.392°W	+	
*I. beraviensis*_Madagascar	Phillipson 1759	K, P, MO, CNARP, BR	Madagascar	Tulear- Befioky, Beza Mahafaly-Parcelle	1987	23.65S	44.63°E	+	
*I. pringsheimiana*_Malawi	R.K. Brumitt, M.J. Masiye, E.J. Tawakali 15562	LISC	Malawi	Mponda, Mt. Zomba	1980	13.556°S	33.766°E	+	+
*I. pringsheimiana*_Mozambique_1	R.B. Drummond 10347	MO	Mozambique	Manica E sofala	1974	19°S	34°E	+	+
*I. incompta*_Tanzania 2	H. Faulkner 2192	B, K, BR	Tanzania	Tanga, Steinbruch	1958	5.1°S	39.016°E	+	+
*I. incompta*_Tanzania_1	H. Faulkner 4152	MO, K	Tanzania	Katuka	1968	8.116°S	31.516°E	+	+
*I. incompta*_Kenya	Luke 14331	K	Kenya	Makueni, Kibwezi	NA	2.7605°S	385425°E	+	
*I. jucunda*_SriLanka	G.H.K.Thwaites 3448	GH, BM, L	Sri Lanka	NA	NA	7.873°N	80.771°E	+	+
*I. lutambensis*_Tanzania_1	E.M. Bruce 1036	K,BRI, BM	Tanzania	Tanganyika, Ulugutus, Kimbosa	1935	6.255°S	29.51°E	+	+
*I. lutambensis*_tanzania_4	K. Vollesen 3497	K	Tanzania	Kingupira	1976	8.5°S	38.566°E	+	
*I. lutambensis*_Tanzania_2	K. Vollesen 2619	K	Tanzania	Kingupira	1975	8.333°S	38.466°E	+	+
*I. lutambensis*_Tanzania_3	S. Bidgood, R. Abdallah, K. Vollesen 2000	BR, MO, K	Tanzania	T8. Masasi Distr.: 8 Km NE of Masasi, Masasi Hill,	1991	10.666°S	38.833°E	+	
*I. macalusoi*_Kenya	S.A. Robertson 6704	K, US, MO	Kenya	Mombasa, Likony Conway	1992	4.0833°S	39.666°E	+	
*I. mojangensis*_Madagascar	D.J. Du Puy, S.Comtet 2467	K	Madagascar	Manombo, Tulear	1993	22.881°S	43.556°E	+	+
*I. neglecta*_Flores	P.E. Schmutz 2294	L	Indonesia	Timor, Oetulu	1968	9.517°S	124.374°E	+	+
*I. orientalis*_Sumbawa	A.J.G.H. Kostermans 18705	SING, BO, K, G, L, P	Indonesia	Sumbawa, Mt. Batulante	1961	8.7381°S	118.117°E	+	+
*I. queenslandica*_ Australia_1	B.J. Conn, J.De Campo 1347	BRI	Australia	Mungana	1983	17.066°S	144.366°E	+	+
*I. queenslandica*_Australia_2	J.R. Clarkson 6837	BRI, K	Australia	Queensland Cook District, Chillagoe, Royal Arch Tower	1982	17.183°S	144.483°E	+	+
*I. sivarajanii*_India	S.D. Biju 22013	ARIZ	India	Kerala, Idukki	1994	10°N	77°E	+	
*I. tiliifolia*_Puerto Rico	F. Axelrod 10071	US	Puerto Rico	Humacao, Cayo Santiago#	1997	18.16°N	65.7366°W	+	+

*I. tiliifolia*_Dominican Republic_1	C. Whitefoord 6056	BM, US	Dominica	Petite Coulibrie Windward	1988	15.383°N	61.25°W	+	
*I. tiliifolia*_Australia_1	P.I. Foster 40510	BRI	Australia	Queensland, Burleigh Heads National Park	2013	28.1°S	153.45°E	+	+
*I. tiliifolia*_Guam_1	F.R. Fosberg 59740	NY	The Federated States in Micronesia	Marianas Islands, Guam, Road in to Dan Dan NASA Radio Tracking Station	1980	13.273°N	144.748°E	+	+
I. *tiliifolia*_Guam 2	Fosberg 35287	BISH, L	The Federated States in Micronesia	Guam, Barrigada	1953	13.468°N	144.798°E	+	
*I. tiliifolia*_Mayotte 2	F. Barthelat, M. Mchangama, B.A. Sifari 436	MO	French overseas territory	Mayotte, Grande Terre-Accoua	2001	12.936°S	45.138°E	+	
*I. tiliifolia*_Mayotte 1	F. Barthelat, M. Mchangama, B.A. Sifari 929	MO, P	French overseas territory	Mayotte, Grande Terre-Accoua, Sohoa beach	2002	12.833°S	45.1105°E	+	
*I. tiliifolia*_Molokai	A.S. Hitchcock 15064	US	The United States	Molokai, Pukoo	1916	21.076°N	156.795°W	+	+
*I. tiliifolia*_Galapagos	H.H. van der Werff 1282a	U, K, MO, NY	Equador	Galapagos, Santa Cruz	1974	0.6394°S	90.337°W	+	
*I. tiliifolia*_Maui	C. Imada, T. Erickson, R. Ganske, S. Fisher, P. Levin, J. Kamaunu 2004-45	US	The United States	Maui, Waihee	2004	20.938°N	156.511°W	+	+
*I. tiliifolia*_Dominican _Republic_2	A. Liogier, P. Liogier 26073	NY	Dominican Republic	Boca Chica	1976	18.46°N	69.595°W	+	+
*I. tiliifolia*_Vanuatu1/2?	P. Curry_1086	K	Vanuatu	Port-Vila	1993	17.733°S	168.316°E	+	
*I. tiliifolia*_Costa_Rica	P. Wilkin, S.B. Jennings 121	BM	Costa Rica	Puntarenas, Manuel Antonio National Park, South end of Playa Espadilla	1989	9.383°N	84.15°W	+	
*I. tiliifolia*_Pohnpei	P. Raynor 125	NY, US	The Federated States in Micronesia	Pohnpei, Nihwel	2002	6.848°N	158.323°E		+
*I. tiliifolia*_Micronesia	Thompson 93	BO, K, L	The Federated States in Micronesia		1913	134.443°N	144.7937°E	+	+
*I. tiliifolia*_Mindoro	M.D. Sulit 17032	BM, K, BR, L	Philippines	Mindoro, Mansalay	1952	12.516°N	121.4°E	+	+

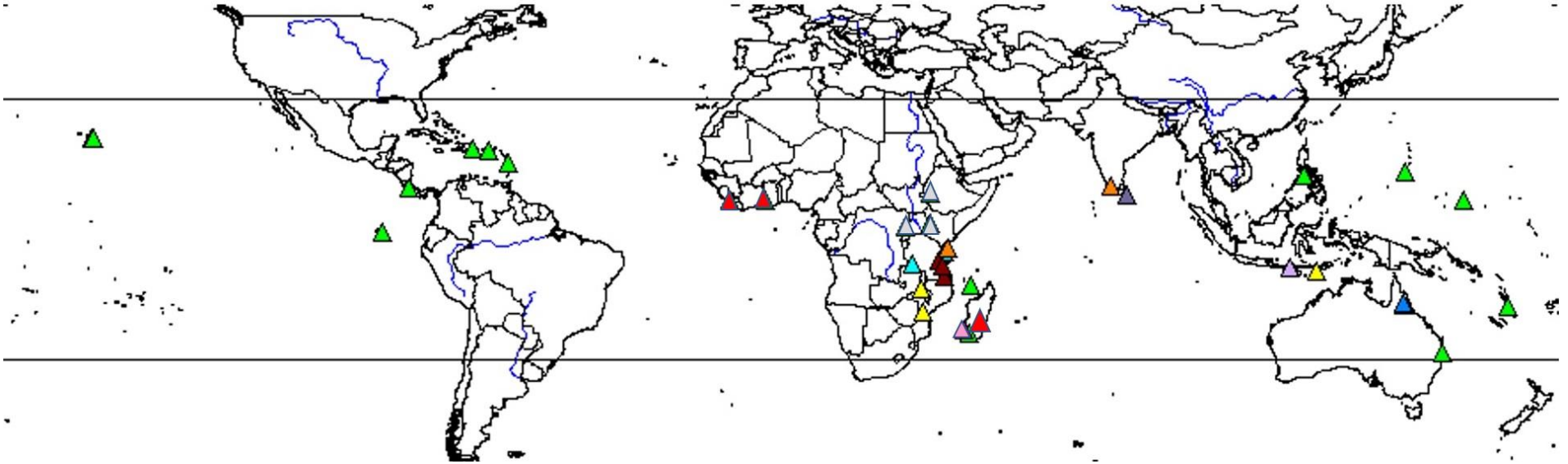


Figure above shows the distribution of *Stictocardia* specimens extracted for DNA as triangles across the globe. Species are colour coded and mostly occur in Africa and Madagascar, *I. beraviensis* (red: Widespread putative group; grey: Eastern putative group), *I. lutambensis* (brown) *I. macalusoi* (orange), *I. pringsheimiana* (yellow), *I. incompta* (cyan), *I. mojangensis* (pink). Meanwhile, in the rest of the world, *I. tiliifolia* (green), *I. queenslandica* (blue), *I. sivarajanii* (orange), *I. jucunda* (dark purple) *I. orientalis* (light purple), *I. neglecta* (yellow). Two horizontal black lines refer to 30°N (top) and 30°S (bottom).

Appendix 6. Result of the Model Test run in MEGA X [88] for Nuclear ITS (left), VR (middle), and PG (right) data sets

Nuclear ITS			
Model	**BIC**	**AICc**	**lnL**
GTR+G+I	22685	20507.6	-10016
K2+G+I	22696.3	20583.2	-10060.8
K2+G	22705.1	20601.1	-10070.8
TN93+G+I	22711.7	20561.9	-10046.2
GTR+G	22712.6	20544.4	-10035.4
HKY+G	22757	20625.5	-10080
HKY+G+I	22764.7	20624.1	-10078.3
T92+G+I	22796	20673.8	-10105.1
T92+G	22808.9	20695.8	-10117.1
JC+G+I	23048.5	20944.6	-10242.6
JC+G	23057.2	20962.5	-10252.5
GTR+I	23234.3	21066.1	-10296.3
TN93+I	23323.8	21183.2	-10357.8
K2+I	23324.6	21220.7	-10380.6
T92+I	23403.3	21290.2	-10414.4
JC+I	23660.6	21565.8	-10554.2
GTR	24596.1	22437.1	-10982.8
K2	24721.6	22626.9	-11084.7
T92	24822.8	22718.9	-11129.7
JC	25046.3	22960.7	-11252.7
TN93+G	45466.1	43325.4	-21429
HKY+I	68761.6	66630.1	-33082.3
HKY	70201.6	68079.3	-33807.9
TN93	115371	113240	-56387.2

VR			
Model	**BIC**	**AICc**	**lnL**
GTR+G	270312	268055	-133855
GTR+G+I	270327	268057	-133855
T92+G	270762	268583.4	-134126
T92+G+I	270781	268589.7	-134128
GTR+I	274333	272075.7	-135866
T92+I	274846	272668	-136168
K2+G	275369	273203.5	-136437
K2+G+I	275390	273212.1	-136440
JC+G	275681	273529.1	-136601
JC+G+I	275696	273531.1	-136601
GTR	275729	273484.8	-136571
T92	276258	274093.4	-136882
K2+I	279628	277462.9	-138566
JC+I	279973	277820.9	-138746
K2	281110	278957.9	-139315
JC	281442	279302.8	-139488
TN93+I	319774	317556.4	-158609
HKY+I	319777	317572.7	-158618
TN93+G+I	1E+06	1316023	-657841
TN93	1E+06	1319608	-659636
HKY	1E+06	1319670	-659668
HKY+G+I	1E+06	1338790	-669226
HKY+G	2E+06	1498196	-748930
TN93+G	2E+06	1498197	-748929

PG			
Model	**BIC**	**AICc**	**lnL**
GTR+G	288397	286078.8	-142867
GTR+G+I	288404	286072.2	-142863
T92+G	288848	286611	-143139
T92+G+I	288866	286615.5	-143141
GTR+I	290984	288665.6	-144161
T92+I	291453	289215.6	-144442
K2+G	292073	289849.2	-144760
K2+G+I	292085	289847.9	-144758
GTR	292306	290001.2	-144830
T92	292789	290564.8	-145117
JC+G	293138	290927.7	-145300
JC+G+I	293145	290921.1	-145296
K2+I	294764	292540.2	-146105
JC+I	295838	293627.7	-146650
K2	296124	293913.3	-146793
JC	297194	294997	-147336
HKY+I	450615	448351.2	-224008
TN93+I	450622	448344.2	-224003
TN93+G+I	516402	514110.8	-256885
TN93+G	539170	536892.5	-268277
HKY+G+I	584691	582412.9	-291037
HKY+G	607476	605211.6	-302438
HKY	633537	631286.7	-315476
TN93	679031	676767	-338216

GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor; BIC: Bayesian Information Criterion; AIC: Akaike Information Criterion, corrected; lnL: Maximum Likelihood value

Appendix 7. Custom script used for assembling plastome genes and variable regions

```
#!/bin/bash

#Clear screen and present the program
clear
tput cup 3 0
echo 'IPOMOEA CHLOROPLAST REGIONS ASSEMBLY PIPELINE. PABLO MR, 2020 -
University of Oxford'; echo; sleep 1

for d in Ipomoea_*; do
# 1) Map reads to reference genome #

        IN2=$d/Reads/in2.fq;
# 2) Align raw reads ('query sequences') to reference probes with BBMap ###
        if test -e $IN2; then
        ~/../../home/pablo/Software/BBMap/bbmap.sh
ref=./Reference_genomes/cp_genome_cds.fasta in=$d/Reads/in1.fq in2=$d/Reads/in2.fq
out=$d/Chloroplast_genome/cds_assembly.sam outm=$d/Reads/cp_cds_reads1.fq
outm2=$d/Reads/cp_cds_reads2.fq touppercase=t ambiguous=toss
statsfile=$d/Chloroplast_genome/cds_assembly.stats local=t;
        else
        ~/../../home/pablo/Software/BBMap/bbmap.sh
ref=./Reference_genomes/cp_genome_cds.fasta in=$d/Reads/in1.fq.gz
in2=$d/Reads/in2.fq.gz out=$d/Chloroplast_genome/cds_assembly.sam
outm=$d/Reads/cp_cds_reads1.fq outm2=$d/Reads/cp_cds_reads2.fq touppercase=t
ambiguous=toss statsfile=$d/Chloroplast_genome/cds_assembly.stats local=t;
        fi
        echo 'INFO: Reads successfully mapped to reference'

# 3) Prepare file for GATK #
        echo 'INFO: Preparing file for GATK...'
        java -jar ~/../../home/pablo/Software/Picard/picard.jar CreateSequenceDictionary
R=Reference_genomes/cp_genome_cds.fasta O=Reference_genomes/cp_genome_cds.dict
        java -XX:ParallelGCThreads=80 -jar ~/../../home/pablo/Software/Picard/picard.jar
AddOrReplaceReadGroups I=$d/Chloroplast_genome/cds_assembly.sam
O=$d/Chloroplast_genome/cds_assembly2.sam RGLB=1 RGPL=Illumina RGPU=unit1
RGSM=20;
        java -XX:ParallelGCThreads=80 -jar ~/../../home/pablo/Software/Picard/picard.jar
SortSam I=$d/Chloroplast_genome/cds_assembly2.sam
O=$d/Chloroplast_genome/cds_assembly3.sam SO=coordinate;
        samtools faidx Reference_genomes/cp_genome_cds.fasta;
        echo; sleep 1

# 4) Convert SAM file to BAM format #
        echo 'Converting SAM file to BAM format';
        samtools view --threads 80 -h -b -F 4 $d/Chloroplast_genome/cds_assembly3.sam >
$d/Chloroplast_genome/cds_temp1.bam;
        # order sequences in the BAM file by name #
        samtools sort -@ 80 -n -o $d/Chloroplast_genome/cds_temp2.bam
$d/Chloroplast_genome/cds_temp1.bam;
        # add ms and MC tags for markdup #
        samtools fixmate -@ 80 -m $d/Chloroplast_genome/cds_temp2.bam
$d/Chloroplast_genome/cds_temp3.bam;
```

```
	# order sequences in the BAM file by position #
	samtools sort -@ 80 $d/Chloroplast_genome/cds_temp3.bam -o
$d/Chloroplast_genome/cds_temp4.bam
	# mark duplicates #
	samtools markdup -@ 80 $d/Chloroplast_genome/cds_temp4.bam
$d/Chloroplast_genome/cds_temp5.bam

# 5) Index the genome assembly #
	echo 'Indexing the assembly';
	samtools index $d/Chloroplast_genome/cds_temp5.bam;
	echo 'Assembly completed!'

# 6) Realign around indels using GATK #
	echo 'Realigning the assembly around indels';
	/usr/lib/jvm/java-8-openjdk-amd64/jre/bin/java -jar
~/../../home/pablo/Software/GATK3/GenomeAnalysisTK.jar -T RealignerTargetCreator -R
./Reference_genomes/cp_genome_cds.fasta -I $d/Chloroplast_genome/cds_temp5.bam -o
$d/Chloroplast_genome/index.list;
	# Step 4) Realign
	/usr/lib/jvm/java-8-openjdk-amd64/jre/bin/java -jar
~/../../home/pablo/Software/GATK3/GenomeAnalysisTK.jar -T IndelRealigner -R
./Reference_genomes/cp_genome_cds.fasta -targetIntervals
$d/Chloroplast_genome/index.list -I $d/Chloroplast_genome/cds_temp5.bam -o
$d/Chloroplast_genome/cds_assembly.bam;

	samtools index $d/Chloroplast_genome/cds_assembly.bam;
	rm *temp*;
done
```

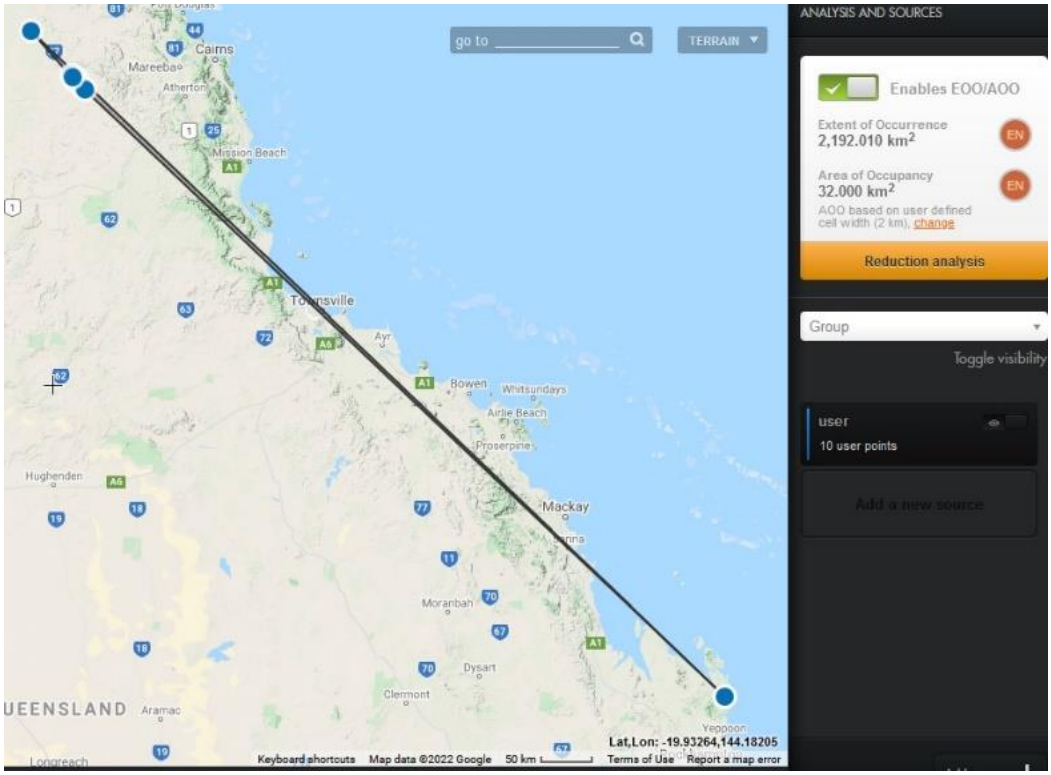
Appendix 8. Custom script for phylogenomic analysis of plastome genes and variable regions

```
#!/bin/bash

#Clear screen and present the program
clear
tput cup 3 0
echo 'PHYLOGENETIC ANALYSIS OF IPOMOEA CHLOROPLAST REGIONS. PABLO MR, 2021 - University of Oxford'; echo; sleep 1
echo 'DEPENDENCIES: perl, python, seqkit, Sequence-Cleaner, MAFFT v.7.3.1, GBlocks, IQTree, Astral III, AMAS, GNU parallel'; echo; sleep 1
echo 'Make sure you modify the paths to the different programmes and files according to their location in your machine'; echo; sleep 1
echo 'I advise to run (unhide) one step at a time and visually explore the result rather than running the entire script in one go'; echo; sleep 5

### 1) EDIT THE FILES BEFORE PHYLOGENETIC ANALYSIS

### 2) Remove leading and trailing Ns

#for f in *fasta; do

        ### Remove leading and trailing Ns ###
#       seqkit -is replace -p "^n+|n+$" -r "" $f > "${f%.fasta}_clean.fasta"

        ### Replace original files for clean files ###
#       rm $f;
#       mv "${f%.fasta}_clean.fasta" $f;

#done

### 3) Remove sequences shorter than 300 bp or with more than 10% missing data (Ns)

#sequence_cleaner --query ./ --output_directory ./ -ml 300 -mn 10 --keep_all_duplicates

#for f in clean*; do
#       rename 's/.fasta/_clean.fasta/g' $f;
#       rename 's/clean_//g' $f
#done

#zip 00_2_cp_regions_edited.zip clean*fasta

### 4) ALIGN SEQUENCES USING MAFFT (default parameters, adjust direction) ###

# Important: modify the number of threads depending on the nodes available in your machine #

#for f in *.fasta; do
#       mafft --thread 40 --adjustdirection --auto $f > "${f%.fasta}_mafft.fasta";
#done

#zip 01_1_cp_original_alignments.zip *mafft.fasta

#parallel 'mafft --thread 10 --adjustdirection --auto {} > "{}_mafft.fasta"' ::: *fasta
```

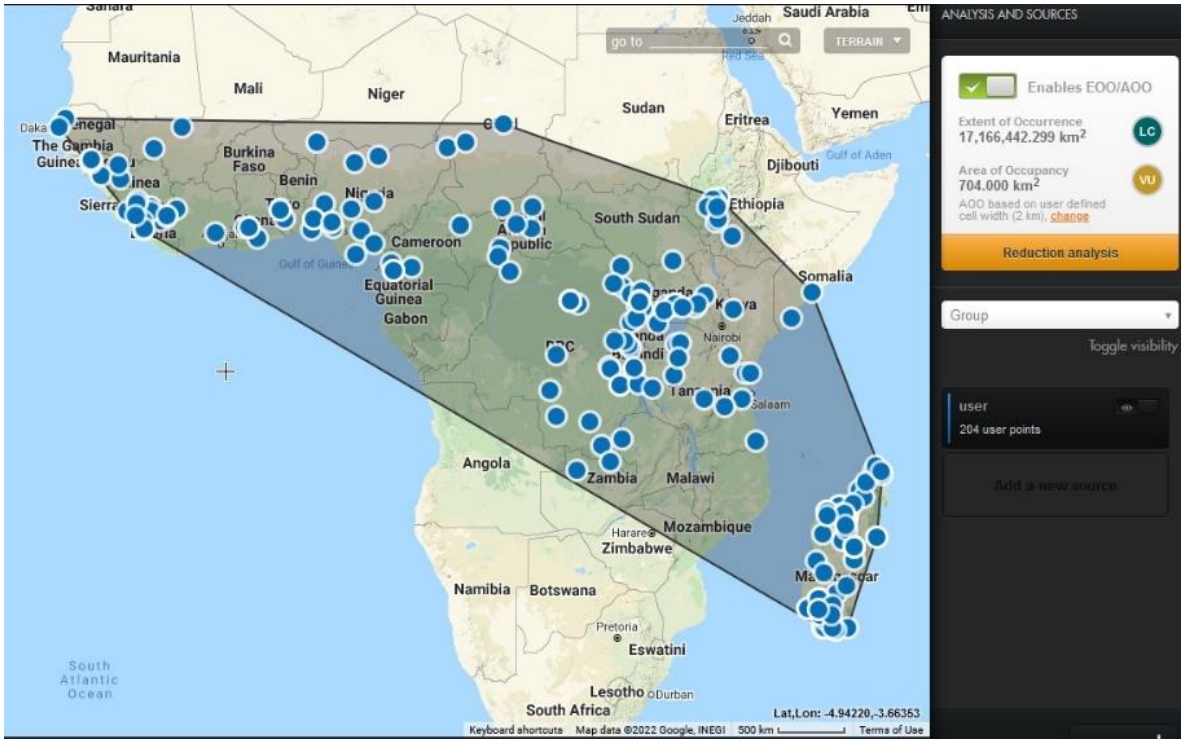
```
#for f in *mafft.fasta; do
#	~/../../home/pablo/Software/Gblocks/Gblocks $f -t=d -b5=h;
#	rm *htm;
#done

#zip 01_2_cp_edited_alignments.zip *mafft.fasta-gb
#rm *mafft.fasta

### 5) INFER MAXIMUM LIKELIHOOD SPECIES TREE USING CONCATENATED
ALIGNMENTS ###

# 3A) Concatenate sequences
python ~/../home/pablo/Software/AMAS/amas/AMAS.py concat -i *mafft.fasta-gb -f fasta -d
dna -u fasta -y nexus

# 3B) ML analysis with IQTree

iqtree -s concatenated.out -sp partitions.txt -nt 40 -czb -o "Operculina_pteripes_MM_2409" -
minsup 0.5 -m TESTMERGE -bb 1000 -alrt 1000 -rclusterf 5 -rcluster-max 5000

echo 'Process complete
```

Appendix 9.1-7. The conservation status of seven species of the clade Stictocardia based on the extrapolation of the georeferenced samples using AOO and EOO.

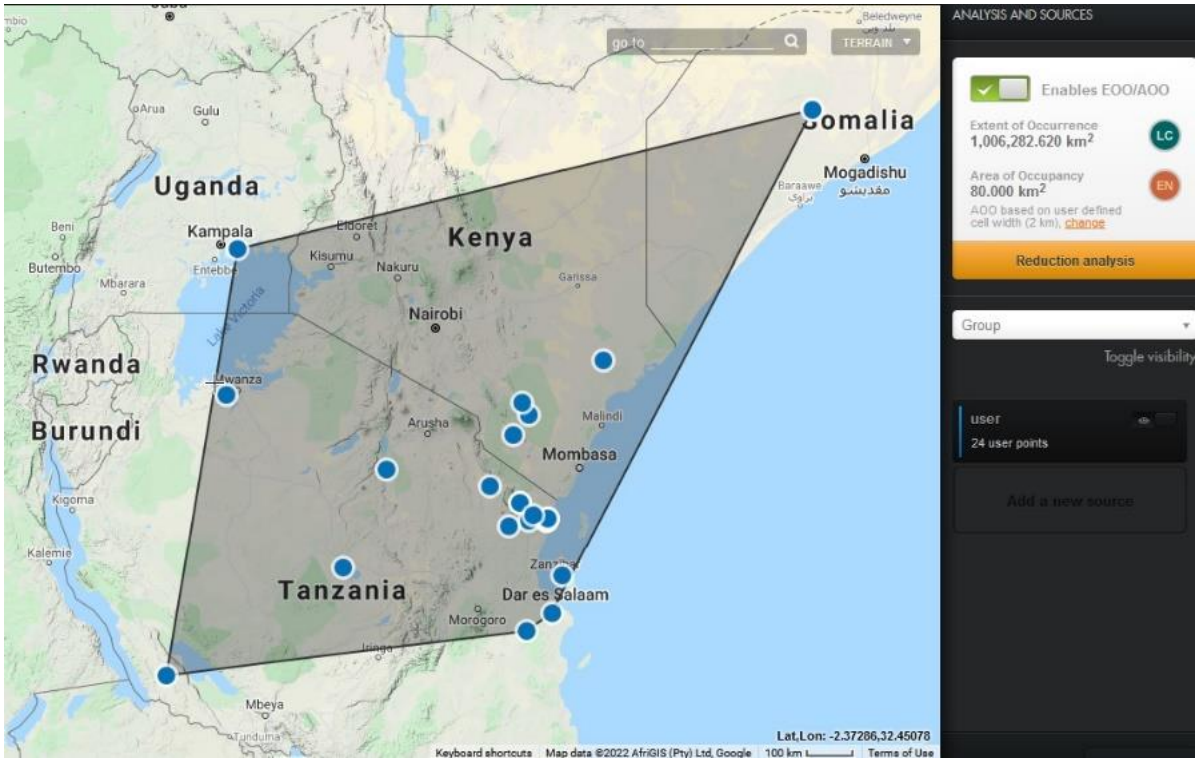
Appendix 9.1. *Ipomoea tiliifolia* is the only species that has LC status (not shown on the right panel). The AOO and EOO analyses cannot accurately set the perimeter of this species due to its worldwide distribution, except in Africa and Madagascar.



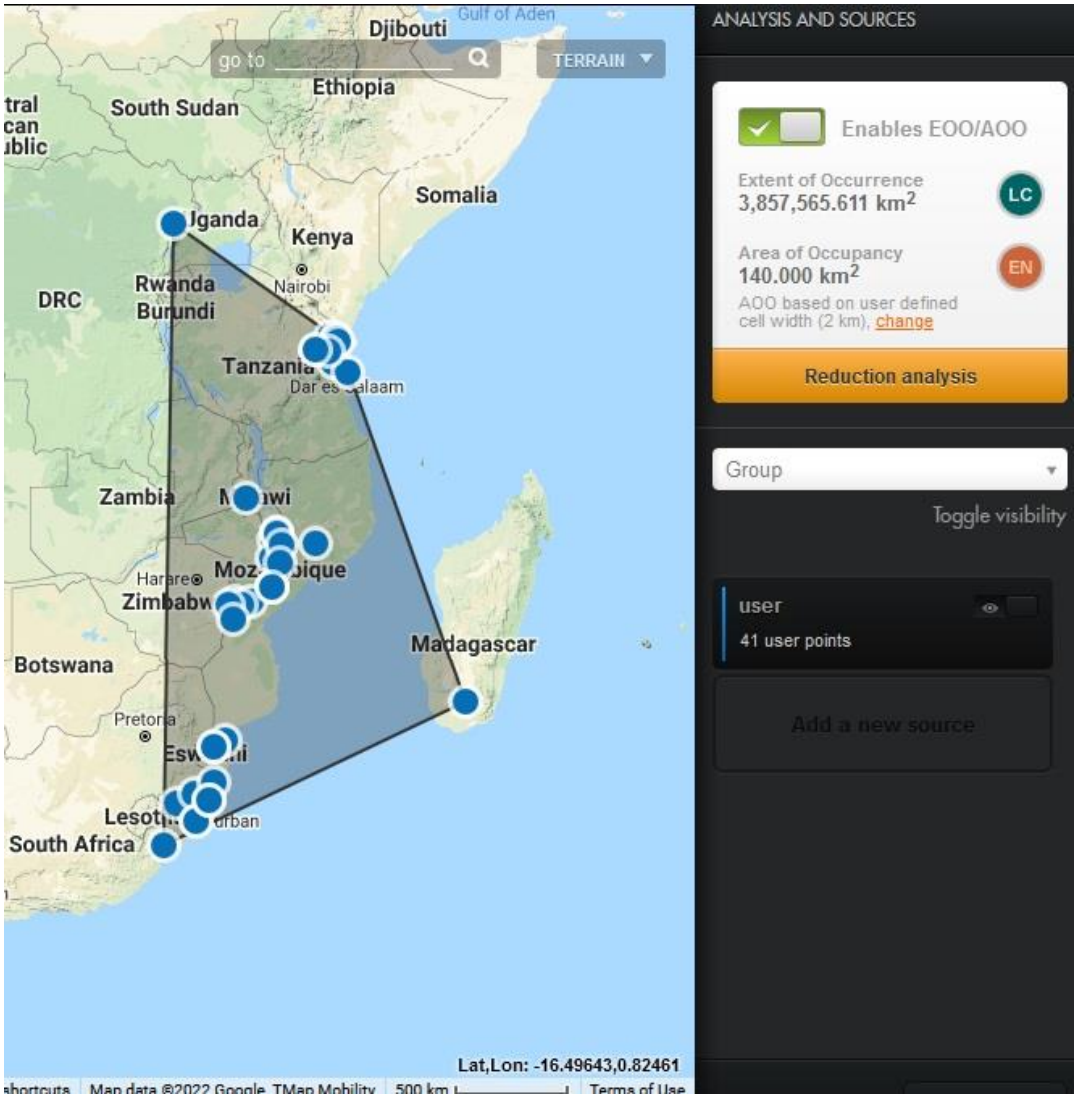
Appendix 9.2. *Ipomoea queenslandica* is an Australian endemic has limited (ten) georeferenced specimens, some are almost overlapping. The right panel shows that the status of this species is Endangered (En) based on the AOO and EOO analyses.



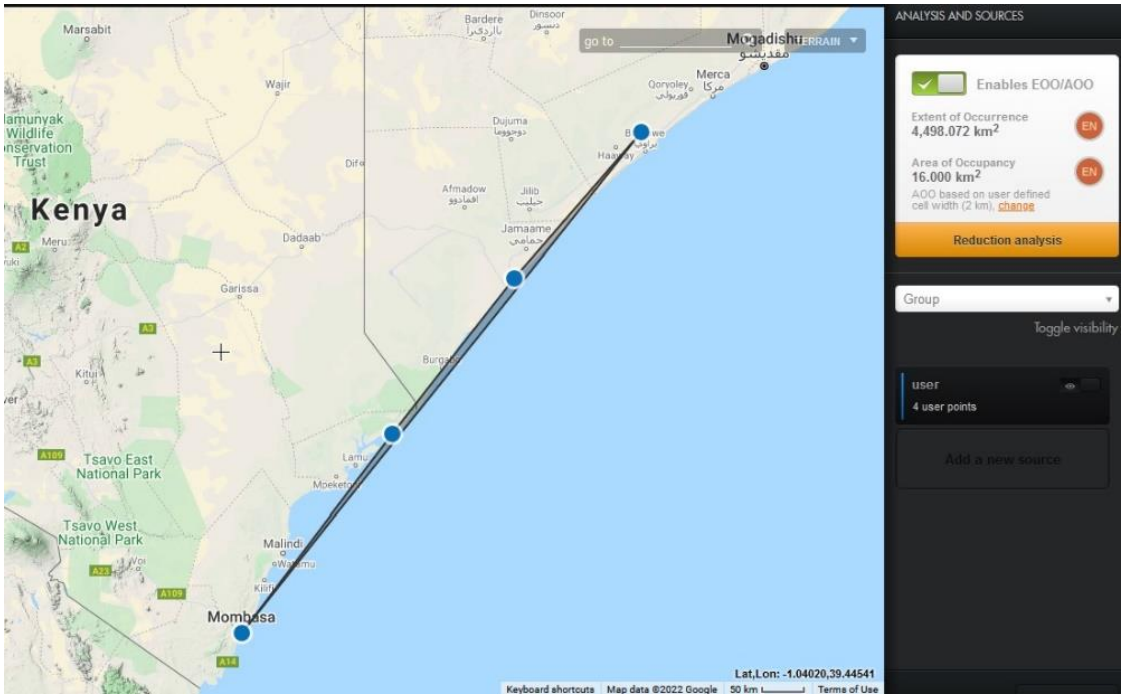
Appendix 9.3. *Ipomoea beraviensis* is a widespread species in Africa and Madagascar with 129 georeferenced specimens. The right panel shows that the status of this species is Vulnerable (Vu) based on the AOO analysis and Least Concern (LC) based on EOO analysis.



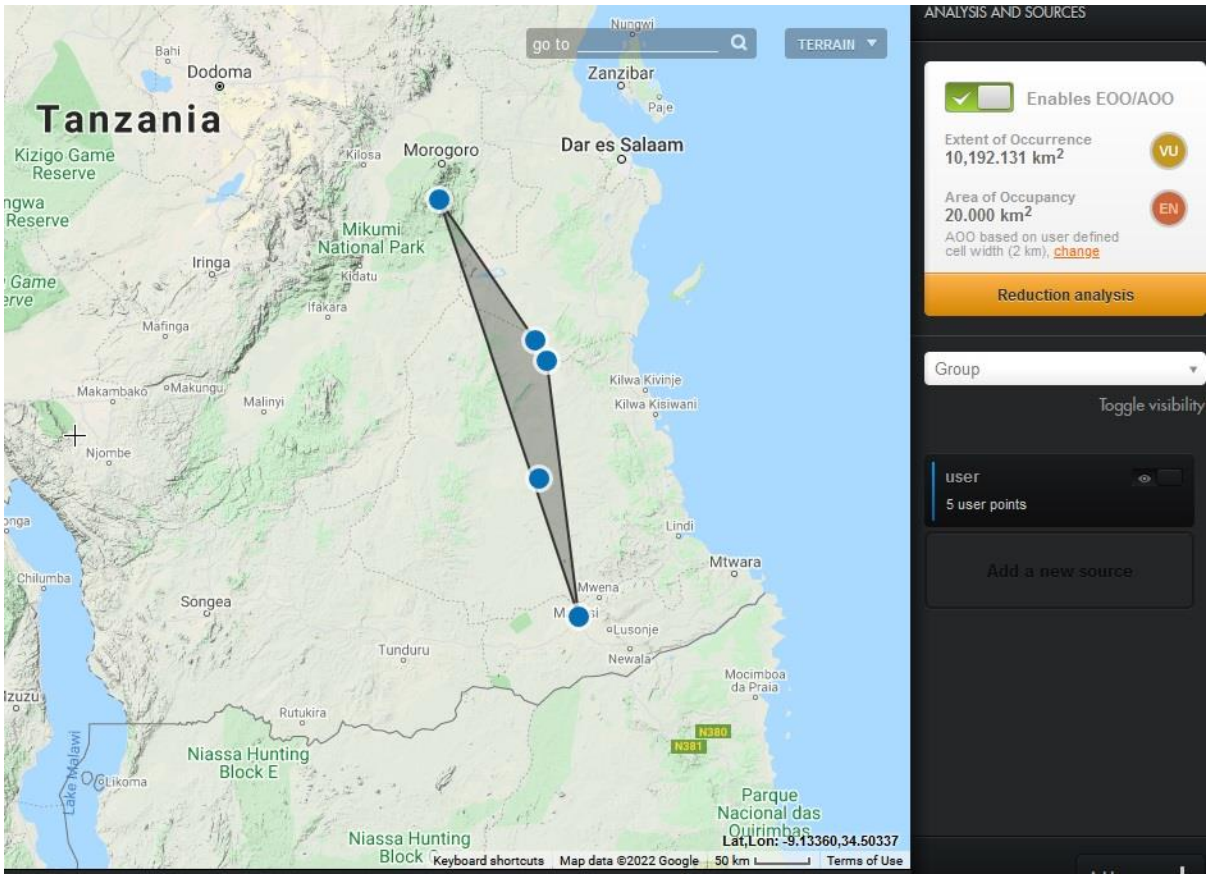
Appendix 9.4. *Ipomoea incompta* is a common species in Eastern Africa with 24 georeferenced specimens. The right panel shows that the status of this species is Endangered (En) based on the AOO analysis and Least Concern (LC) based on the EOO analysis.



Appendix 9.5. *Ipomoea pringsheimiana* is a common species in Eastern to Southern Africa with 38 georeferenced specimens. The right panel shows that the status of this species is Endangered (En) based on the AOO analysis and Least Concern (LC) based on the EOO analysis.



Appendix 9.6. *Ipomoea macalusoi* is a species in the East coast of Africa with restricted distribution four georeferenced specimens. A specimen (*Vaughan* 10808) in Mauritius is not included here as the specimen is introduced there. The right panel shows that the status of this species is Endangered (En) based on the AOO and EOO analyses.



Appendix 9.7. *Ipomoea lutambensis* is an endemic species in Tanzania with five georeferenced specimens. The right panel shows that the status of this species is Endangered (En) based on the AOO analysis and Vulnerable (Vu) based on the EOO analysis.