

Spring 2015

Molecular Phylogenetics and Historical Biogeography of the Tribe Chiococceae (Rubiaceae)

Sushil Kumar Paudyal
Old Dominion University

Follow this and additional works at: https://digitalcommons.odu.edu/biology_etds

Part of the [Bioinformatics Commons](#), [Botany Commons](#), and the [Plant Biology Commons](#)

Recommended Citation

Paudyal, Sushil K.. "Molecular Phylogenetics and Historical Biogeography of the Tribe Chiococceae (Rubiaceae)" (2015). Doctor of Philosophy (PhD), dissertation, Biological Sciences, Old Dominion University, DOI: 10.25777/v377-hr74
https://digitalcommons.odu.edu/biology_etds/72

This Dissertation is brought to you for free and open access by the Biological Sciences at ODU Digital Commons. It has been accepted for inclusion in Biological Sciences Theses & Dissertations by an authorized administrator of ODU Digital Commons. For more information, please contact digitalcommons@odu.edu.

**MOLECULAR PHYLOGENETICS AND HISTORICAL BIOGEOGRAPHY OF
THE TRIBE CHIOCOCCEAE (RUBIACEAE)**

by


Sushil Kumar Paudyal
B.Sc. December 1996, Tribhuvan University, Nepal
M.Sc. August 2000, Tribhuvan University, Nepal

A Dissertation Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirements of the Degree of

DOCTOR OF PHILOSOPHY

ECOLOGICAL SCIENCES

OLD DOMINION UNIVERSITY
May 2015

Approved by: 

Lytton J. Musselman (Director)

Rebecca D. Bray (Member)

Piero G. Delprete (Member)

Tatyana A. Lobova (Member)

ABSTRACT

MOLECULAR PHYLOGENETICS AND HISTORICAL BIOGEOGRAPHY OF THE CHIOCOCCEAE (RUBIACEAE)

Sushil Kumar Paudyal
Old Dominion University, 2015
Director: Dr. Lytton J. Musselman

Chiococceae are a monophyletic assemblage of morphologically very diverse groups of plants ranging in habit from subshrubs to shrubs to tall trees exhibiting an astonishing variation in shapes and sizes of corolla, and kinds of fruits and seeds. They are primarily distributed in the Neotropics but also occur in the West Pacific islands; thus exhibiting amphi-Pacific tropical disjunction. This study addresses the phylogenetic relationships and biogeography of the Chiococceae using molecular DNA sequence data, and presents novel data on the tribal and generic delimitations, intergeneric relationships, and the origin and dispersal of this group.

In the most recent tribal delimitations within subfamily Cinchonoideae, *Strumpfia*, a monotypic genus with historically uncertain tribal affiliation, is included in tribe Chiococceae despite distinctly differing morphologically from the rest of the genera in Chiococceae. Based on intertribal genetic divergences in the subfamily Cinchonoideae, analyzed in this study, coupled with morphological and palynological data, is transferred to a new monotypic tribe Strumpfiae; concurrently tribe Chiococceae is re-delimited to include 29 genera.

This study presents the most comprehensive molecular phylogeny, to date, of the Chiococceae that includes 126 species and 27 genera and enables better understanding of

taxonomic affinities and evolutionary relationships within the tribe. Based on the phylogenies generated by analyzing molecular sequence data of two nuclear (ETS, ITS) and two chloroplast (*petD*, *trnL-F*) regions using Bayesian inference and maximum parsimony frameworks, a total of nine new taxonomic changes are proposed- generic recognition for five new genera, and synonymization and new combinations for three genera (*Ceuthocarpus*, *Morierina*, and *Phyllacanthus*) and two species of *Chiococca* (*C. plowmanii* and *C. naiguatensis*).

Historical events of origin, diversification and disjunction in Chiococceae were inferred with the help of molecular dating analysis using BEAST and ancestral area reconstruction using S-DIVA and BBM. Results indicate that tribe Chiococceae originated in Mexico in the Eocene and through subsequent dispersal, vicariance, and extinction events dispersed to the current distribution in the Neotropics. Multiple dispersal events to the Caribbean and back to Mexico and Central America are inferred. Two Mid-Miocene long-distance dispersal events from the Greater Antilles, one to the New Caledonia and another to other islands of the West Pacific, resulted in the amphipacific tropical disjunction in the Chiococceae.

This dissertation is dedicated to three great souls, who left for eternity during my PhD program, and without whose blessings I would not have been what I am today-

Indramani Paudyal, my father;

John B. Tyson, sponsor and my High School Headmaster; and

Timothy J. Motley, my PhD Advisor.

May their souls rest in peace.

ACKNOWLEDGEMENTS

A great many people have shared their time and expertise to assist me during this project and I heartily thank them all. I am greatly indebted to the late Tim Motley, my major advisor, not only for his assistance, training and mentorship, but also for believing in me and giving me the opportunity of a lifetime. I am grateful to Lytton Musselman for his guidance over the years; more importantly for accepting to be my advisor upon untimely demise of Tim Motley. Piero Delprete provided great mentorship and I cannot thank him enough for invaluable support while writing my dissertation. I am thankful to Rebecca Bray and Tatyana Lobova for serving on my committee, and being there for me during my low times, encouraging me and boosting my morale.

I thank Wayne Hynes, Chair, and other Biological Sciences faculty, staff and students who have provided assistance and have been part of my graduate life. Qing-Feng Wang, Jay Bolin, Mohammad Al-Zein, Marcus Jones, and Suman Neupane have been instrumental at various stages of my graduate program. I thank fellow coworkers- David Ingersoll, Hunter Shanks, Kenny Parker, Peter Schafran, Tim Hammer, Tony Mathews, and Rachel Jabaily for their assistance and camaraderie. I am also grateful for the assistance of curators of various herbaria and the staff of ODU Inter library loan services.

I gratefully acknowledge the financial support from ODU in the form of University fellowships and Graduate assistantships, the Marshall scholarship, Nick Savage scholarship, and the Stiffler endowment, without which this work would not have been possible. I also take this opportunity to thank all my friends, colleagues, and family that have always supported me. Finally, I thank my wife Samjhana and daughter Shreya for their patience and putting up with me.

TABLE OF CONTENTS

	Page
LIST OF TABLES	ix
LIST OF FIGURES	x
 Chapter	
1. INTRODUCTION	1
SPECIFIC AIMS	6
2. TRIBAL POSITION OF <i>STRUMPFIA</i> AND RE-DELIMITATION OF THE TRIBE CHIOCOCCEAE	7
INTRODUCTION	7
MATERIALS AND METHODS	10
TAXON SAMPLING	10
SEQUENCING	10
PHYLOGENETIC ANALYSES	11
RESULTS	12
DISCUSSION	19
TAXONOMIC TREATMENT	21
3. PHYLOGENETIC RELATIONSHIPS OF THE TRIBE CHIOCOCCEAE AND NEW GENERIC DELIMITATIONS	23
INTRODUCTION	23
TAXONOMIC HISTORY OF CHIOCOCCEAE	24
MATERIALS AND METHODS	30
TAXON SAMPLING	30
SEQUENCING	30
PHYLOGENETIC ANALYSES	31
RESULTS	34
SEQUENCES AND DATASETS	34
PHYLOGENETIC ANALYSES	36
INCONSISTENCIES AMONG TREES FROM DIFFERENT ANALYSES	38
DISCUSSION	39
GENERAL TOPOLOGY OF THE PHYLOGENY	40
NAMING AND DELIMITATING THE TRIBE	41
THE <i>COUTAPORTLA-LORENCEA</i> CLADE	42
THE <i>EXOSTEMA-SOLENANDRA-COUTAREA-HINTONIA</i> CLADE	45
THE <i>CATESBAEA-PORTLANDIA</i> CLADE	53

	Page
THE CHIOCOCCEAE S.S., CUBAN ENDEMIC, AND PACIFIC GENERA	58
TAXONOMIC TREATMENT	71
4. BIOGEOGRAPHY OF TRIBE CHIOCOCCEAE: ORIGIN, DIVERSIFICATION AND DISJUNCT DISTRIBUTION	78
INTRODUCTION	78
MATERIALS AND METHODS	86
TAXON SAMPLING	86
PHYLOGENETIC ANALYSIS	87
ESTIMATION OF DIVERGENCE TIMES	90
BIOGEOGRAPHICAL ANALYSES	91
RESULTS	92
PHYLOGENETIC ANALYSIS	92
DIVERGENCE TIME ESTIMATE	93
BIOGEOGRAPHICAL ANALYSIS	100
DISCUSSION	107
GENERAL TOPOLOGY OF THE PHYLOGENETIC TREES	107
DIVERGENCE TIME ESTIMATE	108
HISTORICAL BIOGEOGRAPHY	110
AMPHI-PACIFIC TROPICAL DISJUNCTION	116
5. SUMMARY	121
LITERATURE CITED	125
APPENDICES	
A. TAXA USED IN THE STUDY WITH GENBANK ACCESSION AND VOUCHER INFORMATION	144
B. TAXA USED IN MOLECULAR PHYLOGENY OF CHIOCOCCEAE	146
C. PREVIOUSLY PUBLISHED SEQUENCES USED IN THIS STUDY	149
D. DISTRIBUTION OF TAXA USED IN THE BIOGEOGRAPHY STUDY	150
E. TREE NOT INCLUDED IN THE BIOGEOGRAPHY CHAPTER	153
VITA	155

LIST OF TABLES

Table	Page
1. Genetic variation among pairs of tribes within the subfamily Cinchonoideae, as calculated by total branch lengths (base pairs) between tribal pairings	17
2. Characters distinguishing <i>Strumpfia</i> from members of Chiococceae	18
3. Primers used for amplification and sequencing of DNA regions and the PCR conditions used to amplify DNA	33
4. Statistics for the DNA regions used in the study	35
5. Species diversity and world distribution of the tribe Chiococceae	88

LIST OF FIGURES

Figure	Page
1. Majority rule consensus tree (detailed) retrieved from the maximum parsimony analyses of <i>trnL-F</i> data showing all the taxa used in the study	13
2. Majority rule consensus tree (simplified to the scope of this study) retrieved from the Maximum Parsimony analyses of <i>trnL-F</i> data	15
3. Majority rule consensus tree of the tribe Chiococceae	37
4. Detailed phylogeny of the <i>Coutaportla-Lorencea</i> clade	44
5. Detailed phylogeny of the <i>Exostema-Solenandra-Coutarea-Hintonia</i> clade	46
6. Detailed phylogeny of the <i>Catesbaea-Portlandia</i> clade	54
7. Detailed phylogeny of the Chiococceae s. s., Cuban endemics, and Pacific genera	60
8. World distribution of Chiococceae	80
9. Maximum clade credibility tree from the BEAST analysis	94
10. Graphical results of the ancestral area reconstructions using S-DIVA analysis.....	102

CHAPTER 1

INTRODUCTION

The Rubiaceae (coffee family) is the fourth largest family of flowering plants, with approximately 650 genera and over 13,600 species (Delprete 2004; Delprete and Jardim 2012; Govaerts et al. 2014). The family is very diverse morphologically, consisting of small herbs, shrubs, lianas, and trees; mostly pantropical, and secondarily distributed throughout most terrestrial habitats worldwide. The Rubiaceae is a well-defined natural group whose diagnostic characters include (although with many exceptions): simple, opposite (or whorled), entire leaves, interpetiolar (or intrapetiolar) stipules, and sympetalous, epigynous flowers (Verdcourt 1958; Bremekamp 1966; Robbrecht 1988; Delprete 2004; Delprete and Jardim 2012).

Currently, the family is divided into three subfamilies and more than 40 tribes (Delprete 2004; Bremer and Eriksson 2009; Delprete and Jardim 2012). The family classification has undergone various rearrangements over time, as historical classifications treated flower, fruit and seed characters as the taxonomically most significant. However, due to the enormous variation of these characters, now shown to be evolutionarily plastic, classifications at subfamilial and tribal levels have gone through several modifications. Different authors divided the family in a varying numbers of subdivisions, ranging from eight subfamilies and 41 tribes (Bremekamp 1966) to four subfamilies and 44 tribes (Robbrecht 1988) to only two subfamilies and 41 tribes (Robbrecht and Manen 2006); subfamilial and tribal delimitations also varied considerably.

During the last three decades, molecular data, analyzed with modern phylogenetic tools, have been utilized extensively to understand the evolutionary relationships within the family. Various studies using molecular data (Bremer and Jansen 1991; Bremer et al. 1995; Bremer 1996; Andersson and Rova 1999; Bremer et al. 1999; Andreasen and Bremer 2000; Bremer and Manen 2000; Rova et al. 2002; Delprete 2004; Robbrecht and Manen 2006, Bremer 2009; Bremer and Eriksson 2009) have expanded our understanding of the evolutionary relationships within the family. Molecular data have indicated that flower, fruit, and seed characters are more plastic than previously thought, and are highly variable even among closely related genera. In a recent phylogenetic study using molecular data, Bremer & Eriksson (2009) showed support for dividing Rubiaceae into 44 tribes and three subfamilies: Rubioideae, Ixoroideae, and Cinchonoideae.

The Cinchonoideae is the smallest of the three subfamilies, with 120 genera and ca. 1600 species (Ixoroideae: ca. 4500 spp., Rubioideae: ca. 7500 spp.; Delprete, 2014). It has been variously circumscribed over the years (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988). Tribal delimitations within Rubiaceae and in particular the subfamily Cinchonoideae have also undergone various changes over time (Verdcourt 1958; Bremekamp 1966; Robbrecht 1988; Bremer and Thulin 1998; Rova 1999; Razafimandimbison and Bremer 2002; Rova et al. 2002; Andersson and Antonelli 2005; Razafimandimbison and Bremer 2006; Robbrecht and Manen 2006; Bremer and Eriksson 2009). Recently, in the most comprehensive molecular phylogenetic study to date, Manns & Bremer (2010) delimited nine monophyletic tribes within Cinchonoideae, while the two genera, *Chione* DC. and *Wondersong* D.W.Taylor (as "*Colleteria* D.W.Taylor" *nom. illeg.*), were not placed in any of the tribes. In addition, they delimited the tribe

Chiococceae to 27 genera consisting primarily of the genera previously grouped in the Catesbaeeae-Chiococceae complex by Motley et al. (2005). The essential difference between the two circumscriptions was that Manns & Bremer (2010) included *Strumpfia* Jacq. in the tribe while Motley et al. (2005) purposely excluded it from the complex.

The tribal affiliation of *Strumpfia* has historically been dubious, with various authors placing it in differing tribes (Guettardaceae, Candolle 1830; Ixoreae, Hooker 1873) or unable to place it in any of the tribes (Bremekamp 1966; Bridson and Robbrecht 1985; Robbrecht 1988, Igersheim 1993, Puff et al. 1995). Although molecular phylogenetic studies (Bremer et al. 1995; Rova 1999; Rova et al. 2002; Motley et al. 2005) have placed *Strumpfia* close to the genera of the tribes Chiococceae and Catesbaeeae (both *sensu* Hooker 1873), opinions vary greatly on whether it should be included within the Chiococceae (Bremer and Eriksson 2009; Manns and Bremer 2010) or be delimited as a sister tribe (Motley et al. 2005). Hence it is essential to establish the tribal position of *Strumpfia* and delimitation of Chiococceae.

Chiococceae includes ca. 29 genera and more than 200 species (Motley et al. 2005; Manns and Bremer 2010, Govaerts et al. 2014). In this section, the name “Chiococceae” is used to represent the group of all the genera that, in most recent studies, have been called clade C4 by Rova (1999) and Rova et al. (2002), Catesbaeeae-Chiococceae-Complex by Motley et al. (2005), Catesbaeeae-Chiococceae-*Exostema* complex by Huysmans et al. (1999) and Robbrecht & Manen (2006), and Chiococceae (excluding *Strumpfia*) by Bremer and Eriksson (2009), Manns and Bremer (2010) and Manns et al. (2012).

Chiococceae is a very diverse group of plants that range in habit from vine- like subshrubs, shrubs, or treelets to tall trees and show extreme variation in shapes and sizes of flowers, and fruit types and seeds (Motley et al. 2005). Members of this tribe have flowers with corolla tubes ranging from about 3 mm to as long as 27 cm. Fruits vary from capsular to drupaceous and baccate. Similarly, seeds can be winged, flattened, or globose. Extreme variations in floral characters within a single genus are also common; for example, *Catesbaea spinosa* has long funnel-shaped corolla tubes up to 15 cm long while *Catesbaea parviflora* has short campanulate corollas that are only 6 mm long (Britton and Millspaugh 1920; Delprete 1996a). Similarly, *Exostema* has terminal flowers with long narrow corolla tubes up to 21 cm long in *Exostema longiflorum*, while *Exostema nitens* has axillary flowers with short (1-4 cm) corolla tubes (McDowell 1996). There is no single synapomorphy that can be used to distinguish Chiococceae and only a combination of two homoplasious characters can delimit this tribe-- stamen inserted near or at the base of the corolla tube, and spinulose pollen (Motley et al. 2005). Owing mainly to the extreme morphological diversity, tribal delimitations of the genera now included in Chiococceae have historically been ambiguous, often being delimited in more than one tribe.

Molecular studies (Bremer and Jansen 1991; Rova et al. 2002; Motley et al. 2005; Bremer & Eriksson, 2009; Manns & Bremer, 2010) have now established the monophyly of Chiococceae. Two major molecular phylogenetic studies of Chiococceae are those of Motley et al. (2005) and Manns and Bremer (2010); the former had the most extensive sampling of the ingroup taxa and the latter used the most DNA markers (six) in their analysis. However, both studies were unable to fully resolve the intergeneric

relationships, mainly due to poorly supported basal nodes and inadequate sampling in larger genera. Various genera (*Catesbaea*, *Chiococca*, *Exostema*, and *Thiollierea*), as currently circumscribed, are shown to be non-monophyletic. Hence evolutionary relationships within this group are yet to be fully understood; many intergeneric relationships are still ambiguous and remain subject of further systematic and taxonomic research.

Chiococceae is predominantly distributed in the Neotropics with its highest diversity in the Greater Antilles. In the Neotropics, its distribution ranges from South Florida, Bahamas, Lesser Antilles to Mexico, Central America and the South America. In addition, ca. 26 species are distributed in the islands of the West Pacific, ranging from the Philippines, Marianas to Melanesia, and the Tonga, with no species in the whole of the Pacific plate (Motley et al. 2005; Manns et al. 2012; Govaerts et al. 2014). This kind of amphi-Pacific tropical disjunction between the West Pacific genera and Neotropical genera is a rare distribution pattern in the family Rubiaceae.

Biogeography of the Chiococceae has been discussed in only a few previous studies. Motley et al. (2005) suggested that Chiococceae possibly originated in the Greater Antilles and reached the West Pacific via one or two independent long distance dispersal events, most probably wind-dispersed. Based on molecular dating and dispersal vicariance analysis of the whole subfamily Cinchonoideae, Manns et al. (2012) suggested that this group originated in Central America and dispersed to the Caribbean and South America; they did not discuss the disjunction in distribution. Biogeographical inferences in the above studies were restricted mainly due to lack of a well-resolved phylogeny and limited taxa sampling; both are essential in historical biogeographical reconstructions

using phylogenies and molecular dating (Motley et al. 2005; Milne 2006; Antonelli et al. 2009; Ali et al. 2012).

As discussed above, many previous studies have dealt with the phylogenetic relationships and biogeographical history of the Chiococceae taxa, mostly as part of subfamily or family level studies, except for a study by Motley et al. (2005). In spite of the most extensive taxa sampling thus far, Motley et al. (2005) were unable to resolve many of the relationships within the group. One of the goals of this research is to present a comprehensive phylogeny of the Chiococceae by expanding the ingroup taxa sampling. An adequately sampled and well-resolved phylogeny could then be used to understand various evolutionary relationships and the historical biogeography of this morphologically diverse group of plants.

Specific aims

- 1) To establish tribal position of *Strumpfia* and to delimit the tribe Chiococceae by analyzing genetic divergence among different tribes within the subfamily Cinchonoideae in addition to morphological and palynological evidence;
- 2) To obtain a comprehensive molecular phylogeny of the tribe Chiococceae by expanding taxa sampling and using four DNA markers; and
- 3) To reconstruct an historical biogeography of the tribe Chiococceae using molecular dating and statistical dispersal-vicariance analyses.

CHAPTER 2
TRIBAL POSITION OF *STRUMPFIA* AND RE-DILIMITATION OF THE TRIBE
CHIOCOCCEAE ^a

INTRODUCTION

Strumpfia maritima Jacq. is a morphologically unique member of the Rubiaceae. It is a dwarf shrub (sometimes forming thickets) with ericoid, broom-like branches that occurs in littoral habitats and rocky crevices. It is distributed in the Caribbean region, ranging from southern Florida, the Bahamas, the Antilles, and Central America to the Caribbean coast of Venezuela (Igersheim 1993; Rogers 2005). *Strumpfia* is a monotypic genus that was first described by Jacquin (1760, pp. 8, 28). The tribal affiliation of *Strumpfia* has historically remained dubious mainly due to its unique set of morphological characters that render difficult the association of this genus to any known taxa of the family. Because of this, different authors variably placed *Strumpfia* in different tribes or as an *incertae sedis* genus. Candolle (1830) placed *Strumpfia* in the tribe Guettardeae (as “Guettardaceae”) along with *Erithalis* P. Browne (currently placed in Chiococceae), *Chione* (currently placed in the subfamily Cinchonoideae, but not included in any tribe), and *Guettarda* L. *Strumpfia* was then transferred to the tribe Ixoreae by Hooker (1873) along with *Phyllomelia* Griseb., *Pavetta* L., and *Coffea* L. (the former is currently placed in the tribe Rondeletieae while the latter two are placed in the subfamily Ixoroideae).

^a This chapter was published in *Systematic Botany* 39 (4): 1197-1203.

However, Bremekamp (1966) excluded *Strumpfia* and *Phyllomelia* from the tribe Ixoreae (subfamily Ixoroideae) and mentioned that the taxonomic position of these two genera was uncertain. The palynological studies by Bridson and Robbrecht (1985) could not provide sufficient information for a precise position for *Strumpfia* and they only concluded that it is not a member of the tribe Pavetteae. Robbrecht (1988, 1993) followed Hooker and Bremekamp and listed *Strumpfia* among the “*genera incertae sedis*”.

Igersheim (1993) carried out the most comprehensive morphological and anatomical study of *Strumpfia* to date; however, he could not assign it to a subfamily. Although he indicated the possibility of a monotypic tribe to include *Strumpfia*, he could only conclude that *Strumpfia* is “hidden” amongst the Neotropical Rubiaceae. Puff et al. (1995), in an overview of Rubiaceae genera with united stamens, wrote that *Strumpfia* “stands out among all other Rubiaceae with fusions or agglutinations in the androecium in that it is the only known taxon in which all five anthers are united into a tube by means of a discrete cell layer (a kind of “super epidermis”)” (Puff et al. 1995, p. 368).

Rova (1999) and Rova et al. (2002), with molecular phylogenies using *rps16* and *trnL-F* sequences, respectively, showed that *Strumpfia* is closely related to the genera of tribes Chiococceae, Catesbaeeae (both sensu Hooker 1873), and some former members of the tribes Condamineae and Cinchoneae. They specifically pointed out the isolated position of *Strumpfia* within the subfamily Cinchonoideae and stressed the need to treat *Strumpfia* as sister taxon to the Chiococceae. Bremer et al. (1999) had also earlier placed *Strumpfia* in the subfamily Cinchonoideae and sister to the Chiococceae (albeit no discussion on its placement). Other phylogenetic studies of the Rubiaceae using molecular DNA sequence data (Motley et al. 2005; Robbrecht and Manen 2006; Bremer

and Eriksson 2009) have shown *Strumpfia* to be closely related to the genera of the Chiococceae. In the most recent tribal delimitations within the subfamily Cinchonoideae, based on molecular phylogenetic analyses, Manns and Bremer (2010) place *Strumpfia* as sister to the rest of the Chiococceae and they preferred to include it in the tribe Chiococceae. Although they considered *Strumpfia* as sister to the rest of the Chiococceae, Rova (1999), Rova et al. (2002) and Motley et al. (2005) preferred not to include it in the Chiococceae mainly due to the multiple morphological and palynological differences, and pointed out that by doing so it would create a group not supported by a single synapomorphy. For clarity of discussion, the name Chiococceae in this paper to accommodate the genera that were previously grouped as the C4 clade by Rova (1999) and Rova et al. (2002), the Catesbaeeae-Chiococceae-Complex by Motley et al. (2005), the Catesbaeeae-Chiococceae-*Exostema* complex by Huysmans et al. (1999) and Robbrecht and Manen (2006), and more recently as the tribe Chiococceae (excluding *Strumpfia*) by Manns and Bremer (2010).

Thus, the affinities of *Strumpfia* within Rubiaceae have long remained uncertain, previously due to its unique morphology and palynology, and recently due to different tribal delimitations based on molecular phylogenies, that have hindered a consensual taxonomic placement. Morphologically, *Strumpfia* not only differs distinctly from the rest of the genera in Chiococceae, it is also the only genus within Rubiaceae that has all five anthers united by a cell layer forming a tube (Puff et al. 1995). *Strumpfia* species also has a hairy nectar disc that surrounds the base of the style, a character uncommon in the family (Igersheim 1993; Piesschaert et al. 2001). The aim of this paper is to revisit the inclusion of *Strumpfia* in the Chiococceae *sensu* Manns and Bremer (2010), by analyzing

the genetic sequence divergence among different tribes within the subfamily Cinchonoideae as additional evidence, in conjunction with morphological and palynological characters.

MATERIALS AND METHODS

Taxon Sampling

A total of 131 taxa representing nine tribes of subfamily Cinchonoideae and four outgroup taxa from the subfamily Ixoroideae were included in the molecular analyses. The focus of sampling was to include the maximum number of taxa from the tribe Chiococceae, and in total, 50 taxa including four different accessions of *Strumpfia*. We used sequences from more than one accession from different locations in order to ascertain the taxonomic position of *Strumpfia* by eliminating potential genetic changes in one accession. In total 35 sequences were newly generated during this study, and 96 sequences were obtained from GenBank. A complete list of taxa sampled and literature citations for previously published sequences are presented in Appendix A.

Sequencing

Sequences of most taxa of the Chiococceae were generated in our lab from leaf material dried in silica gel or from herbarium specimens. DNA extraction, amplification and sequencing primarily followed the procedures of Jabaily et al. (2012). The plastid DNA region (*trnL* intron and *trnL-F* intergenic spacer) was amplified utilizing external primers “c” and “f” of Taberlet et al. (1991) and the PCR conditions consisted of an initial denaturation for 1 min at 72°C, followed by 32 cycles of 94°C for 50 sec, 50°C for

90 sec, and 72°C for 50 sec, followed by a final extension phase of 7 min at 72°C. Upon consideration of available DNA sequences and difficulty in aligning sequences of more variable nuclear regions, we chose to use the more conserved plastid *trnL-F* region for the phylogenetic analyses. The non-coding *trnL-F* region has been shown to be useful in resolving phylogenetic relationships among tribes and higher taxonomic levels (Bayer and Starr 1998; Bremer et al. 2002; Rova et al. 2002; Borsch et al. 2003).

Phylogenetic analyses

DNA sequences were manually edited using Sequencher v. 4.8 (Gene Codes, Ann Arbor, Michigan), and initially aligned using MAFFT v. 6 (Kato and Toh 2008) followed by visual alignment using MacClade version 4.08a (Maddison and Maddison 2005) and Mesquite version 2.72 (Maddison and Maddison 2009). Indels were treated as missing data.

We analyzed the aligned dataset using both Bayesian inference (BI) and maximum parsimony (MP) analyses. Maximum parsimony searches were performed using PAUP*4.0b10 (Swofford 2002) with 1,000 random addition replicates, 10 trees held at each step in stepwise-addition, tree-bisection-reconnection (TBR) branch swapping, and multiple parsimonious trees (MULTREES) option off. BI was performed using MrBayes 3.1 (Ronquist and Huelsenbeck 2003) using the general time reversal model (GTR) with a gamma distribution of substitution rates of nucleotides (evaluated using JModeltest v. 0.1; Posada 2008) for 10,000,000 generations with trees sampled every 1,000 generations and the first 25% of the trees discarded.

RESULTS

The data matrix included 1,090 bp when all the sequences were aligned, 229 of which were parsimony informative. The MP analysis generated a single tree of 631 steps (CI = 0.70, RI = 0.91). A phylogram illustrating the relative branch lengths and bp changes is shown in Fig. 1. The majority rule consensus generated from BI had a similar tribal-level topology to that of the MP analysis with all the phylogenetic relationships among tribes showing congruency. However, there exist incongruencies at five intergeneric relationships in three tribes, where trees from BI show polytomy for the relationships resolved in a MP tree. The incongruent nodes are marked in Fig. 1 with asterisks, but not discussed here as it is beyond the scope of this paper. The posterior probability values for tribal relationships are mapped on the MP tree (Fig. 2).

The ingroup taxa were grouped into ten clades that included all nine tribes described by Manns and Bremer (2010) and a separate clade with *Chione* DC. and *Colleteria* D.W.Taylor (*nom. illeg.*, recently renamed *Wondersong* W.D.Taylor), which have not yet been assigned to any tribe. Although the tree backbone is not well resolved and forms a polytomy, positions of terminal clades in our results corroborate with most of the intertribal relationships established by Manns and Bremer (2010). For example, the tribal pairs Hillieae-Hamelieae, Rondeletieae-Guettardeae, and Naucleaeae-Hymenodictyeae are supported in our study, but the tribes Isertieae and Cinchoneae did not form a sister relationship. Four different accessions of *Strumpfia* (three accessions from Puerto Rico and one from Dominican Republic) were used in the analyses and all four were retrieved in a clade that is sister to the clade containing the remaining genera of the tribe Chiococceae.

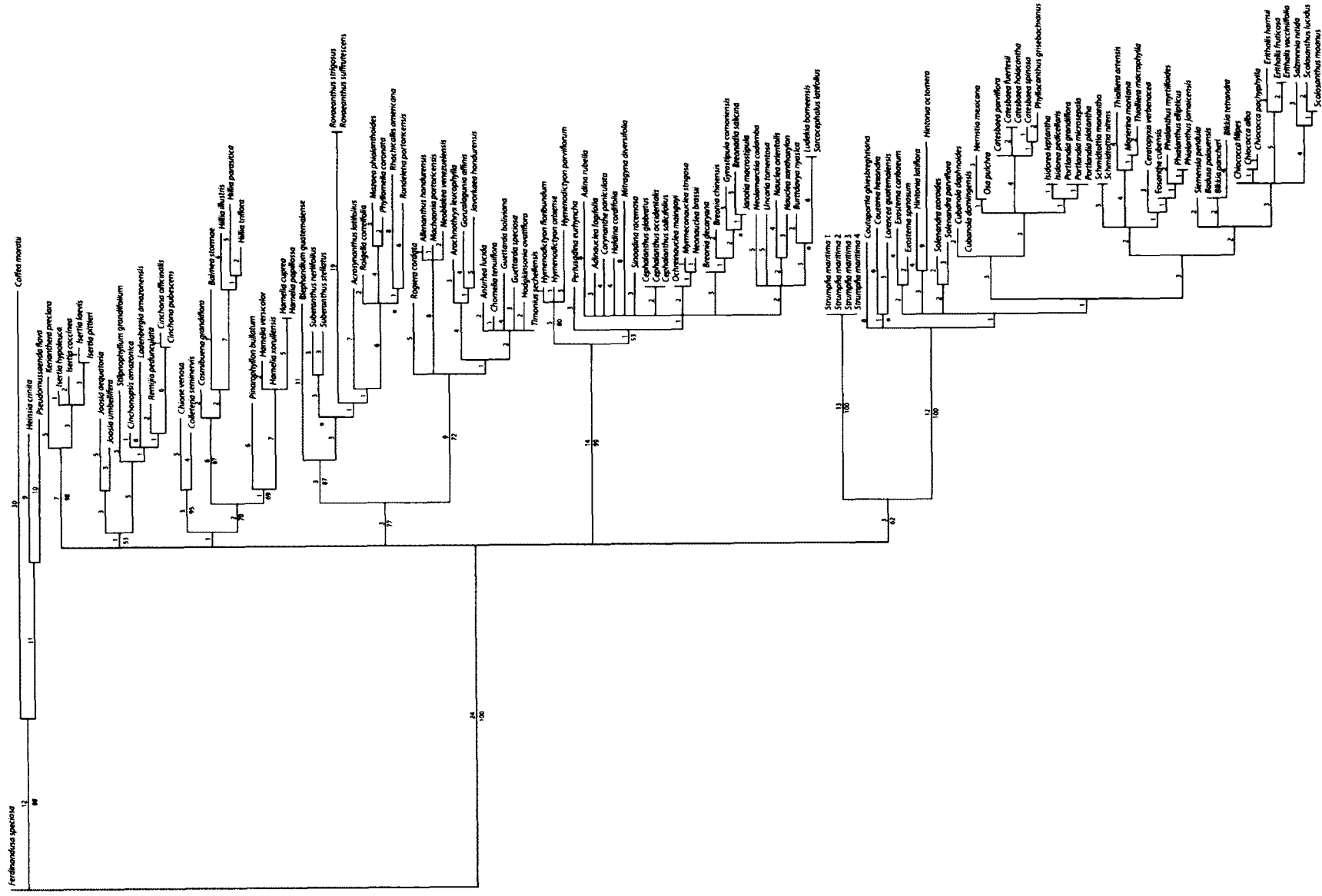


Fig. 1 Majority rule consensus tree (detailed) retrieved from the Maximum Parsimony analyses of *trnL-F* data showing all the taxa used in the study. The phylogram shows the branch lengths (changes in base pair) above each branch and parsimony bootstrap values below the branch (bootstrap values shown only for the tribal level clades).

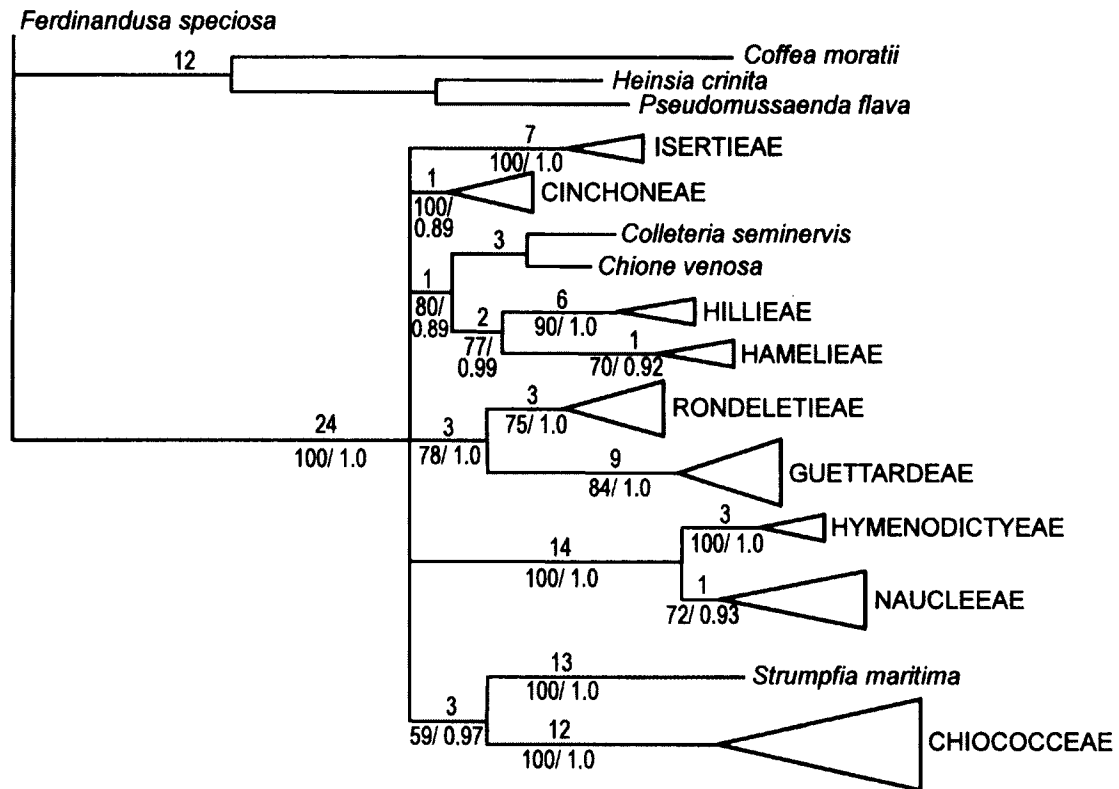


Fig. 2 Majority rule consensus tree (simplified to the scope of this study) retrieved from the Maximum Parsimony analyses of *trnL-F* data. The parsimony bootstrap values obtained from separate parsimony analyses and posterior probability values obtained from Bayesian analyses are indicated on the phylogram. Numbers above the branches represent the branch length (changes in base pair), and numbers below the branch represent parsimony bootstrap values and posterior probability values respectively.

The ingroup taxa were grouped into ten clades that included all nine tribes described by Manns and Bremer (2010) and a separate clade with *Chione* and *Colleteria* (now *Wondersong*), which have not yet been assigned to any tribe. Although the tree backbone is not well resolved and forms a polytomy, positions of terminal clades in our results corroborate with most of the intertribal relationships established by Manns and Bremer (2010). For example, the tribal pairs Hillieae-Hamelieae, Rondeletieae-Guettardeae, and Naucleae-Hymenodictyeae are supported in our study, but the tribes Isertieae and Cinchoneae did not form a sister relationship. Four different accessions of *Strumpfia* (three accessions from Puerto Rico and one from Dominican Republic) were used in the analyses and all four were retrieved in a clade that is sister to the clade containing the remaining genera of the tribe Chiococceae.

Intertribal genetic variation was evaluated by calculating the total branch lengths (bp changes) between each pair of the nine tribes delimited by Manns and Bremer (2010). The pair-wise comparisons are presented in Fig. 1. Similarly, genetic variation between *Strumpfia* and the rest of Chiococceae was calculated. Of the three pairs of sister tribes, Naucleae and Hymenodictyeae had the fewest differences separating them, with only four bp changes, while Rondeletieae and Guettardeae had the most, with 12 bp changes, and the Hamelieae and Hillieae had seven bp changes. Notably, *Strumpfia* is separated from the rest of Chiococceae by 25 bp.

Table 1. Genetic variation among pairs of tribes within the subfamily Cinchonoideae, as calculated by total branch lengths (base pairs) between tribal pairings. Values for sister tribes are in bold.

	Strumpfieae	Rondeletieae	Naucleaeae	Isertieae	Hymenodictyeae	Hillieae	Hamelieae	Guettardeae	Cinchoneae
Chiococceae	25	21	30	22	32	24	19	27	16
Cinchoneae	17	7	16	8	18	10	5	13	
Guettardeae	28	12	27	19	29	21	16		
Hamelieae	20	10	19	11	21	7			
Hillieae	25	15	24	16	26				
Hymenodictyeae	33	23	4	24					
Isertieae	23	13	22						
Naucleaeae	31	21							
Rondeletieae	22								

Table 2. Characters distinguishing *Strumpfia* from members of Chiococceae (Bridson and Robbrecht 1985; Igersheim 1993; Piesschaert et al. 2001; Rova et al. 2002; Motley et al. 2005; Rogers 2005)

Character	<i>Strumpfia</i>	Chiococceae (excluding <i>Strumpfia</i>)
Flowers	Protogynous, buzz-pollinated	Protandrous, not buzz-pollinated
Corolla aestivation	Quincuncial	Imbricate, imbricate-induplicate or valvate
Anthers	Fused to form a synandrium	Free
Pollen exine	Verrucose and smooth at poles (not spinulose)	Spinulose
Fruit	Drupaceous	Drupaceous, baccaceous, or capsular
Pyrenes	Plurilocular	Absent or, when present, unilocular

DISCUSSION

In their wide delimitation of the tribe Chiococceae, Manns and Bremer (2010) considered the presence of spinulose pollen as the sole synapomorphy of the tribe. However, it is interesting to note that *Strumpfia*, which they included in the tribe Chiococceae, does not have spinulose pollen, unlike all other members of Chiococceae; the pollen of *Strumpfia* has a verrucose, perforated exine, without suprategal elements (Bridson and Robbrecht 1985; Igersheim 1993; Robbrecht and Manen 2006) (Table 2).

Motley et al. (2005) stated that a combination of two morphological homoplasious characters would best define Catesbaeeae-Chiococceae complex: 1) the presence of spinulose pollen and 2) anthers attached at the base of the corolla tube. However, neither of these characters is unique to this group. Spinulose pollen occurs in other groups in the Rubiaceae (e.g., Spermacocceae), and anther attachment at the base of the corolla tube is a character also found in *Chione* and the tribe Hamelieae. Bremer and Eriksson (2009) considered basal stamen insertion as a rare character state in the family, and a synapomorphy for including *Strumpfia* in the Chiococceae in spite of acknowledging that *Strumpfia* does have many unique morphological characters lacking in other members of the Chiococceae. Manns and Bremer (2010) also agreed that considering spinulose (echinate) pollen as the sole identified synapomorphy of Chiococceae would leave *Strumpfia* excluded from the tribe, which would conversely support the conclusion of Motley et al. (2005) that *Strumpfia* should be considered a monotypic tribe sister to amended Chiococceae.

Strumpfia, however, has been shown to be either closely associated or sister to remaining genera of Chiococceae in many different molecular analyses (Bremer et al.

1999; Rova 1999; Rova et al. 2002; Motley et al. 2005; Robbrecht and Manen 2006; Bremer and Eriksson 2009; Manns and Bremer 2010). In spite of the unique morphology discussed earlier in this paper and the detailed work of Igersheim (1993), the recent phylogenetic placement of taxa into other tribes, and the reluctance of some authors to create monotypic tribes, had kept *Strumpfia* from being treated as its own tribe. However, increasingly more monogeneric tribes have recently been proposed in the Rubiaceae (e.g., Andreasen and Bremer 2000).

Our results support the sister relationship of *Strumpfia* and Chiococceae. However, in the MP analyses the relationship is weakly supported (bootstrap support=62) corroborating the results of Rova (1999) and Rova et al. (2002). Additionally, the results of Motley et al. (2005) were unable to fully retrieve *Strumpfia* as the sole sister group to Chiococceae, a possible reason for them suggesting a monotypic tribe for *Strumpfia*. Phylograms retrieved from MP analyses of the *trnL-F* data indicate abundant genetic divergence between *Strumpfia* and Chiococceae. The number of bp changes separating *Strumpfia* from the rest of Chiococceae is 25, while a number of other well established and widely accepted sister tribes have fewer bp changes between them. For example, there are 12 bp changes between the Guettardeae and the Rondeletieae, and seven bp changes in the Hamelieae-Hillieae pair. Although our results do not place the Isertieae and Cinchoneae as sister tribes as established by Manns and Bremer (2010), there are only eight bp changes between them. This comparison of genetic variation among different tribes provided us with additional evidence in support of morphological distinctness of *Strumpfia* from the rest of Chiococceae (as shown in Table 2) further

supporting some of the previous authors' suggestion that it is best to transfer *Strumpfia* to a monotypic tribe.

In conclusion, the molecular data (Fig. 1) and its morphological distinctness (Table 2) clearly support the exclusion of *Strumpfia* from the tribe Chiococceae.

Therefore, following the above conclusion, the monotypic tribe Strumpfieae is described, to accommodate *Strumpfia*, and the tribe Chiococceae is re-delimited, along with generic additions, synonymies, and confirmations of several genera to belong to this tribe.

Taxonomic treatment

Strumpfieae Delprete & Motley, trib. nov. Type genus: *Strumpfia* Jacq.

Shrubs or subshrubs (often forming thicket, on the rocks of ocean spray); leaves ternate, congested at tip of branches; leaf blades with calcium oxalate druses (raphides absent); stipules interpetiolar, triangular. Inflorescences axillary, racemose, few-flowered. Flowers (4)5(6)-merous, protogynous, buzz-pollinated; ovary bicarpellate with two erect, anatropous ovules and a partial septum within each locule; nectar disc hairy, surrounding the base of the style; corolla lobes with quincuncial aestivation; stamens united into a tube, opening by an common apical pore; pollen grains 3(4)-colporate, with verrucose (minutely perforate at poles) exine. Fruit drupaceous; pyrenes plurilocular.

Genus included: *Strumpfia* Jacq.

Tribe Chiococceae

Subshrubs, shrubs, treelets or tall trees; leaves opposite (rarely ternate), not congested at tip of branches; with calcium oxalate druses (raphides absent); stipules interpetiolar,

triangular. Inflorescences axillary, rarely terminal or subterminal, racemose, paniculate, corymbose (cluster of flowers on thorns in *Scolosanthus*), reduced cymes, or rarely uniflorous. Flowers 4-6(-8)-merous, protandrous, not buzz-pollinated; ovary bicarpellate, bilocular (5-20 cells in *Erithalis*), with one pendulous ovule per locule, or few or many ovules horizontal or descendingly imbricate, and a complete septum within each locule; nectar disc not hairy, variably surrounding the base of the style; corolla lobes narrowly imbricate or rarely valvate, tube induplicate or not; stamens inserted at base of tube or on disk, sometimes basally adnate and forming a minute tube, or free at base, anthers free, dorsifixed around the middle or at base, dehiscing by longitudinal slit; pollen grains 3(4)-colporate, with echinate exine. Fruit drupaceous, baccaceous, or capsular; pyrenes (when present), unilocular.

Genera included: *Badusa* A. Gray, *Bikkia* Reinw., *Catesbaea* L. (incl. *Phyllacanthus* Hook. f.), *Ceratopyxis* Hook. f., *Ceuthocarpus* Aiello, *Chiococca* P. Browne (incl. *Asemnantha* Hook. f.), *Coutaportla* Urb., *Coutarea* Aubl., *Cubanola* Aiello, *Eosanthe* Urb., *Erithalis* P. Browne, *Exostema* (Pers.) Rich., *Hintonia* Bullock, *Isidorea* A. Rich. ex DC., *Lorencea* Borhidi, *Morierina* Vieill., *Nernstia* Urb., *Osa* Aiello, *Phialanthus* Griseb., *Portlandia* P. Browne, *Salzmannia* DC., *Schmidtottia* Urb., *Scolosanthus* Vahl, *Shaferocharis* Urb., *Siemensia* Urb., *Solenandra* Hook.f., *Thogsennia* Aiello, and *Thiollierea* Montrouz.

Note: The following changes were made in the tribe Chiococceae (sensu Manns and Bremer 2010) in this paper: *Asemnantha* Hook. f. is treated as a synonym of *Chiococca*; and *Shaferocharis* Urb., *Thiollierea* Montrouz., *Ceuthocarpus* Aiello, and *Thogsennia* Aiello are confirmed to belong to this tribe.

CHAPTER 3

PHYLOGENETIC RELATIONSHIPS OF THE TRIBE CHIOCOCCEAE AND NEW GENERIC DELIMITATIONS

INTRODUCTION

The Chiococceae *sensu* Paudyal et al. (2014), is a monophyletic group positioned in the subfamily Cinchonoideae, which includes ca. 29 genera and more than 200 species (Motley et al. 2005; Negron-Ortiz 2005; Borhidi 2007; Borhidi 2008; Iturralde 2008; Borhidi et al. 2009; Manns and Bremer 2010; Taylor and Lorence 2010; Barrabé et al. 2011; Alejandro et al. 2014). They are primarily distributed in the Neotropics where nearly 90 % species occur; the remaining species occur in the islands of the western Pacific Ocean, and members of this tribe are entirely absent in the vast Pacific plate (Motley et al. 2005; Manns et al. 2012). This intriguing biogeographic disjunction between the western Pacific genera and the Neotropical genera has generated significant interest in this group, and necessitates further understanding of the phylogenetic relationships within this tribe.

The Chiococceae *sensu* Paudyal et al. (2014) constitute a morphologically very diverse group that range in habit from subshrubs, vine-like or erect shrubs to treelets and tall trees. Members of this group have flowers with corolla tubes ranging in length from about 3 mm (e.g., *Erithalis* P. Browne) to as long as 27 cm (e.g., *Osa* Aiello); and fruits that vary from capsular, drupaceous to baccate; and seeds that could be winged, flattened, or globose. Astonishing variation in floral characters within a single generic group is also not uncommon; for example, in *Catesbaea* L., here shown to be a monophyletic genus

(including *Phyllacanthus* Hook. f.), *Catesbaea spinosa* L. has long funnel-shaped corolla tubes up to 15 cm long, and at the other extreme, *Catesbaea parviflora* Sw. has short campanulate corollas 6 mm long (Britton and Millspaugh 1920; Delprete 1996a).

Similarly, the genus *Exostema* (Pers.) Rich., as traditionally delimited (here shown to be paraphyletic), has terminal inflorescences and flowers with corolla tubes 13- 21 cm long (e.g., *E. longiflorum* Roem. and Schult.) as well as axillary inflorescences and flowers with corolla tubes 1-4 cm long (e.g., *E. nitens* Urb.) (McDowell 1995). There is no single synapomorphy to distinguish the Chiococceae *sensu* Paudyal et al. (2014) and only a combination of two homoplasious characters can be used to define this group: 1) stamens inserted near or at the base of corolla tube or on a disc, and 2) presence of spinulose pollen (Motley et al. 2005). However, neither of these two characters is restricted to this group. Basal stamen insertion, although a rare character state in the Rubiaceae, is also found in other groups within the Cinchonoideae, for example, in the tribe Hamelieae, in the genus *Chione* (Manns and Bremer 2010), and within Ixoroideae (*Neobertiera* Wernham, tribe Sipaneeae; Delprete in press). Other groups within Rubiaceae also have spinulose pollen (e. g. tribe Spermacocceae; Dessein et al. 2002).

Taxonomic history of Chiococceae

Tribal delimitations of the genera currently included within the Chiococceae *sensu* Paudyal et al. (2014) have historically remained unclear mainly due to the extreme morphological diversity and plasticity of flower, fruit and seed characters of the taxa. Different authors have variously placed these genera in different tribes ever since the family Rubiaceae was established by Jussieu (1789); five genera (*Catesbaea*, *Chiococca*

P. Browne, *Coutarea* Aubl., *Erithalis*, *Portlandia* P. Browne) of the Chiococceae were listed among a total of 80 genera for the family. Two important publications in the early 19th century placed the genera currently included in the tribe (only 10 genera were described up to that time) into six different tribes. *Coutarea*, *Exostema*, *Isidorea* A.Rich. ex DC. and *Portlandia* were included in the newly described tribe Cinchoneae by Richard (1830) while Candolle (1830) placed them in two separate tribes: Hedyotideae (*Bikkia* Reinw., *Isidorea*, *Portlandia*) and Cinchoneae (*Coutarea*, *Exostema*). *Erithalis* and *Scolosanthus* Vahl were included in the tribe Guettardeae (as “Guettardaceae”) by Richard (1830), while Candolle (1830) placed *Erithalis* in the Guettardeae and *Scolosanthus* together with *Chiococca* in the Coffeae (as “Coffeaceae”). Later, Hooker (1873) placed the genera of Chiococceae into four tribes: 1) Chiococceae (*Asemnantha* Hook. f., *Ceratopyxis* Hook. f., *Chiococca*, *Erithalis*, *Phialanthus* Griseb., *Salzmannia* DC., *Scolosanthus*), 2) Catesbaeeae (*Catesbaea*, *Phyllacanthus* Hook. f.), 3) Condamineae (*Bikkia*, *Isidorea*, *Morierina* Vieill., *Portlandia*), and 4) Cinchoneae (*Badusa* A. Gray, *Coutarea*, *Exostema*, *Solenandra* Hook. f.). He divided the Rubiaceae into three series based on number of ovules per locule; series A with species having many ovules per locule, series B with species having two ovules per locule, and series C with species having one ovule per locule. Tribe Chiococceae was placed in series C while other three were placed in series A. In series A, tribe Catesbaeeae included genera with fleshy fruits with large compressed seeds, while the species with dry capsular fruits with winged seeds were included in Cinchoneae and those with dry capsular fruits and unwinged seeds were included within Condamineae. Schumann (1891) generally followed Hooker (1873) in assigning tribal delimitations of the genera of Chiococceae

but he did not recognize the tribe Catesbaeeae and instead placed *Catesbaea* and *Phyllacanthus* in the Gardenieae. Verdcourt (1958) recognized Hooker's four tribes, including the Catesbaeeae, while in Bremekamp (1966), there is no mention of the Catesbaeeae. Aiello (1979) conducted a thorough morphological study of the genera of the *Portlandia* complex (all the taxa that had ever been placed within the genus *Portlandia*) and pointed that all those taxa could not be placed in a single tribe. She showed closer association between *Cubanola* Aiello, *Isidorea*, *Osa*, *Portlandia*, and *Thogsennia* Aiello, and transferred them to the tribe Condamineae based on their horizontal seed arrangement. She also moved *Siemensia* Urb. to the tribe Hedyotideae (subfamily Rubioideae) based on the presence of raphides, multicellular, uniseriate hairs and numerous tiny seeds, and *Coutarea* to the tribe Cinchoneae based on its winged, vertical seeds. However, she was unable to suggest tribal placement for *Ceuthocarpus* Aiello, *Coutaportla* Urb., *Hintonia* Bullock, *Nernstia* Urb. (*Cigarrilla* Aiello), and *Schmidtottia* Urb. Also in the comprehensive survey of Rubiaceae taxa, Robbrecht (1988) still placed the genera of Chiococceae *sensu* Paudyal et al. (2014) into the tribes Chiococceae, Cinchoneae, Condamineae, and Hedyotideae, while he transferred *Badusa* (previously in the Cinchoneae) and he also placed *Ceuthocarpus* and *Nernstia* to the Condamineae. Interestingly, considering its imbricate seeds, Aiello (1979) had earlier stated that *Nernstia* (as "*Cigarrilla* Aiello") did not belong to the Condamineae, but should instead be placed in Cinchoneae or Hedyotideae. Robbrecht (1988) designated the Catesbaeeae as *tribus incertae*, while he treated *Coutaportla*, *Eosanthe* Urb., *Hintonia* and *Schmidtottia* as *genera incertae sedis*. Later, with a phylogenetic analysis using

morphological data, Andersson and Persson (1991) retrieved *Coutarea* and *Exostema* as closely related with *Portlandia*.

In one of the earliest molecular phylogenies of Rubiaceae using molecular data, Bremer and Jansen (1991) first detected the monophyly of the Chiococceae. They showed, for the first time, that five genera that had thus far been placed in tribes Chiococceae (*Chiococca* and *Erithalis*), Catesbaeeae (*Catesbaea*) and Cinchoneae (*Coutarea* and *Exostema*) formed a monophyletic clade. Bremer (1992) later expanded the work of Bremer and Jansen (1991) with supplementary morphological data and additional taxon sampling and pointed out that there was no support for distinction of clades corresponding to previously delimited tribes Chiococceae, Cinchoneae and Condamineae. Following this conclusion, and based on a number of morphological characters, most notably inserted stamens that form a ring at the corolla base, she amended tribe Chiococceae to include 22 genera formerly placed in Condamineae subtribe Portlandiinae, Cinchoneae, and Catesbaeeae along with the genera in the tribe Chiococceae (all except *Phialanthus*). Since then she has used the name “Chiococceae” for this tribe. Subsequent phylogenies using molecular data (Bremer et al. 1995; Bremer 1996; Bremer et al. 1999), though with scant sampling, clearly supported the delimitation for the tribe Chiococceae by Bremer (1992). Delprete (1996b), with a phylogenetic analysis using morphological data, also found the genera of the Chiococceae in a monophyletic assemblage. However, the distinction in key fruit, flower, and pollen characters within the group showed corroboration with the retrieved clades, and therefore he subdivided the complex into Catesbaeeae, Chiococceae, and *Exostema* group. *Exostema*, as traditionally delimited, is a highly polymorphic taxon (and later shown to

be paraphyletic), and this was reflected in the difficulties in coding the morphological characters used in the phylogenetic analysis of Delprete (1996a). Despite the phylogeny showing similarity to the amended Chiococceae (per Bremer 1992 and Bremer et al. 1995), Rova (1999) preferred using the name “Catesbaeeae” to include the genera of Chiococceae. Ochoterena-Booth (2000) also followed Rova (1999). Rova et al. (2002) obtained very similar phylogeny to that of Rova (1999). However, Rova et al. (2002) did not use “Catesbaeeae” to name the tribe, and used instead the name “Chiococceae” in their discussion.

Motley et al. (2005) presented the most extensive molecular phylogeny focusing on the intergeneric and intrageneric relationships within the Chiococceae *sensu* Paudyal et al. (2014) using DNA sequence data from one chloroplast (*trnL-F* intron and spacer) and one nuclear (ITS) region. Although it was the most expanded sampling thus far, with 23 genera and 59 species, Motley et al. (2005) could not fully define many of the relationships owing to poor branch support and polytomies. However, they were able to identify two major clades, one with 12 genera including seven members of Chiococceae s. s., and the other with six genera that had been earlier classified within Catesbaeeae by Delprete (1996b). This data justified their use of the name Catesbaeeae-Chiococceae Complex (CCC) to stress the existence of two formerly recognized tribes in this monophyletic alliance. Robbrecht and Manen (2006) also named this group the Catesbaeeae-Chiococceae-*Exostema* complex in their study of the whole family using the super tree approach. More recently, molecular phylogenies by Bremer and Eriksson (2009) and Manns and Bremer (2010) also discussed taxonomic relationships within Chiococceae. In the phylogeny of the subfamily Cinchonoideae by Manns and Bremer

(2010), the intergeneric relationships within Chiococceae generally corroborated the relationships of Motley et al. (2005). In the same study, Manns and Bremer (2010) delimited tribe Chiococceae by including *Strumpfia* together with 26 other genera of the CCC supporting the conclusions of Bremer and Eriksson (2009). However, they listed *Ceuthocarpus* and *Thogsennia* as tentatively included within the tribe and did not include *Shaferocharis* Urb., although these three taxa were previously included within the group by Motley et al. (2005). Instead they included *Eosanthe* that was not included by Motley et al. (2005). The inclusion of *Strumpfia* within tribe Chiococceae has since been refuted by Paudyal et al. (2014), and placed in a monotypic tribe Strumpfieae; the Chiococceae was re-delimited with 29 genera (inclusive of the members of CCC).

While molecular data (Bremer and Jansen 1991; Bremer et al. 1995; Bremer et al. 1999; Rova et al. 2002; Motley et al. 2005; Bremer and Eriksson 2009; Manns and Bremer 2010; Paudyal et al. 2014) have now fully established that the Chiococceae is a monophyletic group, the intergeneric relationships within this group are still not fully understood and remain a subject of further systematic and taxonomic research. Understanding the intergeneric relationships may also resolve the long-standing confusion on whether we should name this monophyletic alliance a complex (Motley et al. 2005) in recognition of formerly recognized groups or with no such distinctions (Bremer 1992).

By increasing the species sampling and using additional chloroplast and nuclear DNA markers, the primary aim of this study is to produce well resolved phylogenies to gain better understanding of the taxonomy and evolution of the group. The specific objectives of this study are to: 1) re-examine the phylogenetic relationships among the

genera of the Chiococceae *sensu* Paudyal et al. (2014), 2) test the monophyly of the larger genera that were not adequately sampled in previous studies, 3) test infra-generic relationships of the genera that have been shown to be paraphyletic and polyphyletic in recent studies, 4) test the validity of the merging and segregating of certain generic complexes as suggested in recent studies, 5) test the phylogenetic placement of the previously untested genus *Ceuthorcarpus* using molecular data.

MATERIALS AND METHODS

Taxon sampling

Our taxon sampling included a total of 157 accessions from 126 species, doubling the number of species included in Motley et al. (2005), and representing 27 genera within Chiococceae and five accessions of outgroup taxa. Considering the uncertainties in inter- and intra- generic relationships as obtained in previous phylogenies of the Chiococceae, our focus was to include the maximum number of species within the large genera. For the species that were considered to hold significant taxonomic positions and monotypic genera, we used more than one accession, from different locations where possible. A list of all the species sampled with voucher details is presented in Appendix B.

Sequencing

Sequences were generated from fresh leaves dried in silica gel or from herbarium specimens. For some taxa used in this study, sequence data for some of the DNA regions were obtained from GenBank. GenBank accession numbers of the previously published sequences are listed in Appendix C with literature citation. The DNA was extracted from

leaves dried in silica gel or from herbarium specimens using the Qiagen DNeasy Plant Mini kit (Qiagen, Valencia, CA, USA) with some modifications to the manufacturer's protocols. 30 ul beta mercaptoethanol and 30 ul Protinease K (for herbarium specimens) were added to each tube along with AP1 buffer and incubated at 42⁰C on a rocker bed for 12--24 hours, before the next step that required adding AP2 buffer and incubating in ice for 5 minutes. Manufacturer's protocols were followed from that step onwards.

The DNA was amplified using Polymerase Chain Reaction (PCR) run in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA), with each PCR reaction prepared in 25 ul volumes with 12.5 ul Promega Go taq DNA polymerase (Promega, Madison, WI, USA), 1.25 ul DMSO, 0.25 ul BSA (Bovine Serum Albumin), 1 ul each of two 10 uM/L primers, 8 ul autoclaved DI water, and 1 ul of genomic DNA. Amplification of ITS, *trnL-F*, and *petD* regions utilized standard primers and PCR conditions while ETS region was amplified using the touchdown procedure. Details of primers used and PCR conditions are presented in Table 3.

Amplified PCR products were purified using QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocols. Purified PCR products were dehydrated in a Speed Vac concentrator (Mandel Scientific Company Inc., Guelph, Canada) and sequenced at Macrogen Sequencing Services (Seoul, Korea).

Phylogenetic analysis

The Sequences obtained from Macrogen Sequencing Services were manually edited using Sequencer version 4.8 (Gene Codes, Ann Arbor, Michigan, USA). Edited sequences were initially aligned using online alignment software, PRANK

(<http://www.ebi.ac.uk/goldman-srv/webprank>; Loytynoja and Goldman 2005) followed by visual alignment using MacClade version 4.08 (Maddison and Maddison 2005) and Mesquite version 2.71 (Maddison and Maddison 2009). Indels were treated as missing data. Since sequences of all the taxa were not of equal lengths and the actual nucleotide base pairs sequenced varied slightly among taxa, only nucleotide base pair positions that were complementary to most taxa were included in the analysis.

We analyzed the aligned dataset using both Bayesian inference (BI) analysis and maximum parsimony (MP). Analyses were performed separately for each of the four DNA regions, as well as for the concatenated datasets (chloroplast and nuclear separately and all four regions together). The heuristic searches were performed using PAUP*4.0b10 (Swofford 2002) with 1000 random addition replicates, with tree-bisection-reconnection (TBR) branch swapping, and multiple parsimonious trees (MULTREES) option off. Bayesian inference analyses were performed using MrBayes 3.2 (Ronquist et al. 2012). Analyses of four individual DNA regions as well as combined data sets (two nuclear regions, two chloroplast regions, and all 4 regions combined) were performed. Each DNA region was considered as individual partition. The best model of molecular evolution for each DNA region was evaluated with Bayesian Information Criterion (BIC) using the program JModeltest version 2.1.3 (Darriba et al. 2012). The DNA regions, ITS and *petD* datasets used the general time reversal model (GTR), while the Hasegawa-Kishino-Yano model (HKY) was used for the DNA regions ETS and *trnL-F* (intron and spacer). Substitution rates of nucleotides in all regions were gamma distribution. Each analysis was run for 10,000,000 generations with trees sampled every 1000 generations; 25% of trees were discarded.

Table 3. Primers used for amplification and sequencing of DNA regions and the PCR conditions used to amplify DNA. References of primers are denoted by superscripts following the primer name: ^a Baldwin and Markos (1998), ^b Negron-Ortiz and Watson (2002), ^c Nickrent et al. (1994), ^d Lohne and Borsch (2005), ^e Taberlet et al. (1991); * denotes addition of 4 sec in each consecutive cycle.

DNA region	Primer	Primer sequence (5'—3')	PCR conditions
ETS	18s-igs ^a	GAGACAAGCATATGACTACTGGCAGGATCA ACCAG	1 min at 97°C + 40 x [50 sec at 97°C + 50 sec at 53°C + *20 sec at 72°C] + 7 min at 72°C + ∞ at 4°C
	ETS-Erit-F ^b	CTTGTATGGGTTGGTTGGA	
ITS	18S 1830 ^c	AACAAGGTTTCCGTAGGTGA	50 sec at 97°C + 30 x [50 sec at 97°C + 50 sec at 53°C + 1 min 50 sec at 72°C] + 7 min at 72°C + ∞ at 4°C
	26S 25 ^c	TATGCTTAAAYTCAGCGGGT	
<i>petD</i>	PIpetB1365 ^d	TTGACYCGTTTTTATAGTTTAC	1 min at 95°C + 37 x [1 min at 95°C + 90 sec at 50°C + 90 sec at 72°C] + 7 min at 72°C + ∞ at 4°C
	PIpetD738 ^d	AATTTAGCYCTTAATACAGG	
<i>trnL-F</i>	Primer "c" ^e	CGAAATCGGTAGACGCTACG	1 min at 72°C + 32 x [50 sec at 94°C + 90 sec at 50°C + 50 sec at 72°C] + 7 min at 72°C + ∞ at 4°C
	Primer "f" ^e	ATTTGAACTGGTGACACGAG	

RESULTS

Sequences and datasets

Sequence data were generated from four different DNA regions: two nuclear, ITS, ETS, and two from chloroplast, *trnL-F* and *petD*. Sequences from ETS and *petD* regions were rarely used in previous phylogenetic studies involving members of Chlococeae. A total of 483 sequences from 153 accessions were newly generated during this study. A complete list of all the taxa included in this study are presented in Appendix B. The complete aligned dataset is comprised of 3556 characters. The sequences of the ingroup taxa generally aligned well. However, the *petD* region contained a number of long insertions and also had inversions at two positions that were corrected. To maintain uniformity in the overall length of sequences in the dataset of each region, certain characters at the two ends each dataset were excluded from the analyses. The combined dataset used in the analyses consisted of 3311 characters (ETS: 491, ITS: 688, *petD*: 1135, *trnL-F*: 997) of which 971 (29%) were variable and 730 (22%) were potentially parsimony informative. The nuclear ETS region was most parsimony informative (49%) while chloroplast *petD* had the lowest variation (10%). The summary of the DNA dataset is presented in Table 4.

Table 4. Statistics for the DNA regions used in the study. (^a includes 29 new sequences published together with results of Chapter 2 of this dissertation; Paudyal et al. 2014).

	ETS	ITS	<i>petD</i>	<i>trnL-F</i>	nrDNA (ETS + ITS)	cpDNA (<i>petD</i> + <i>trnL-F</i>)	Combined (4 regions)
Model of evolution	HKY + G	GTR + G	GTR + G	HKY + G	-	-	-
Number of taxa included in analyses	155	148	143	149	157	154	157
Number of new sequences generated	153	109	143	78	-	-	483
Sequences used from previous studies	2	39	0	71 ^a	-	-	112 ^a
Length of aligned matrices (bp)	522	751	1194	1089	1273	2283	3556
Length included in analyses (bp)	491	688	1135	997	1179	2132	3311
Total variable characters	287	294	186	204	581	390	971
Constant characters	204	394	949	793	598	1742	2340
Parsimony informative characters	239	251	112	128	490	240	730
% Parsimony informative characters	48.7	36.5	9.9	12.8	41.6	11.3	22.0

Phylogenetic analyses

The Bayesian analyses based on separate and combined datasets generated majority rule consensus trees that illustrate the phylogenetic relationships within the Chiococceae. The overall topology of the phylogenetic trees generated from the Bayesian analyses of the chloroplast and the nuclear datasets (combined and as separate partitions) are mostly congruent with only a few unresolved or poorly supported nodes, but not contradicting the overall phylogenetic relationships. Phylogenetic relationships are reported here based on the 50% majority rule consensus tree generated from the analysis of combined dataset that shows posterior probability (PP) values greater than 0.50 next to each node although only the values above 0.90 are considered supported (Manns and Bremer 2010). A simplified phylogenetic tree is presented in Fig. 3 and detailed phylogeny of individual clades is presented along with discussions of each clade.

Monophyly of Chiococceae is further confirmed here with 27 genera forming a highly supported clade in all of the analyses (PP = 1.0, BS = 100). *Strumpfia maritima* Jacq. is sister to the rest of the group (PP = 1.0, BS = 50). However, in analyses of separate datasets (trees not shown here), the position of *Strumpfia* is also found unresolved as a polytomy with other outgroups (in case of ETS) and poorly supported as sister to the rest of Chiococceae (in the case of *petD*, PP = 0.79).

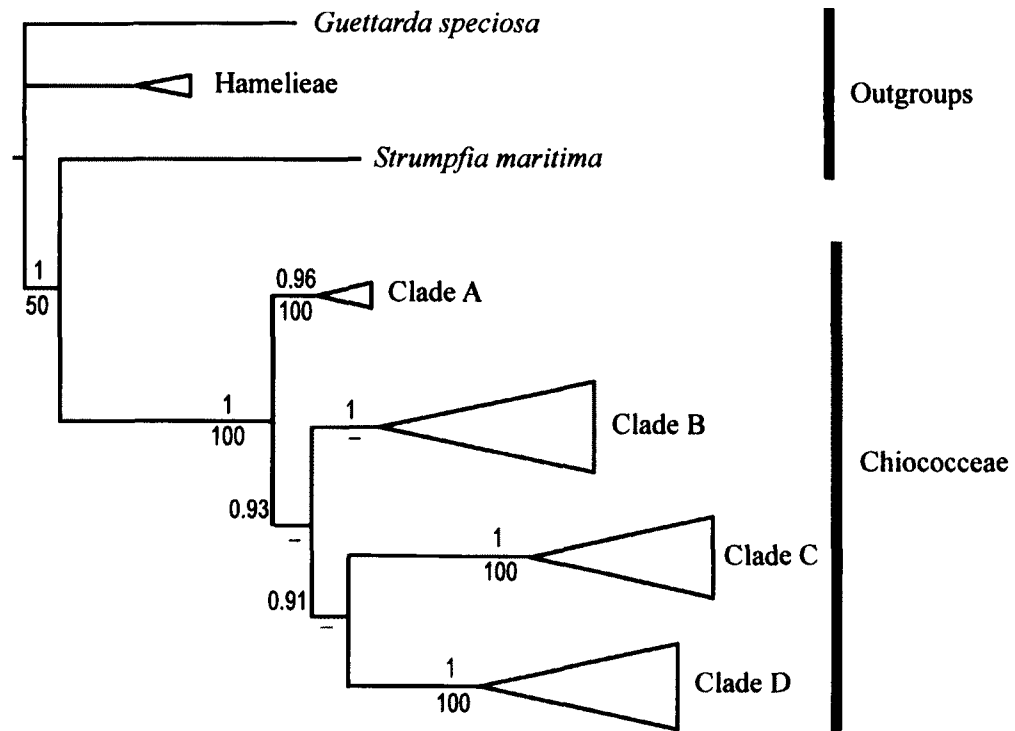


Fig. 3 Majority rule consensus tree of the tribe Chiococceae. 50% majority rule consensus tree retrieved from the Bayesian analyses of combined dataset (ETS, ITS, *petD*, and *trnL-F*) is simplified to show relationship between major clades. The parsimony bootstrap values obtained from separate parsimony analyses are also indicated. Numbers above the branches represent the Bayesian posterior probability values, and numbers below the branches represent parsimony bootstrap values.

The Chiococceae is resolved into four well-supported clades (Fig. 3). The first clade, Clade A, comprises two genera, *Coutaportla* and *Lorencea* Borhidi, well resolved as sister to each other (PP = 0.96). Clade B includes *Coutarea*, *Exostema*, *Hintonia*, and *Solenandra* forming five well-supported subclades (PP \geq 0.99). *Coutarea* and *Exostema* as currently circumscribed are not monophyletic. Clade C comprises *Catesbaea* (with *Phyllacanthus* nested within) and five genera of the *Portlandia* complex (*Cubanola*, *Isidorea*, *Nernstia*, *Osa*, *Portlandia*) with well-resolved intergeneric relationships (PP \geq 0.99). The fourth clade (labeled D) comprises 14 genera that include genera of tribe Chiococceae s. s. (*Ceratopyxis*, *Chiococca*, *Erithalis*, *Phialanthus*, *Salzmannia*, *Scolosanthus*), the Pacific genera (*Badusa*, *Bikkia*, *Morierina*, *Thiollierea* Montrouz), and four of the Cuban endemics (*Ceuthocarpus*, *Eosanthe*, *Siemensia*, *Schmidtottia*). Clade D resolved into ten well-supported subclades. *Badusa*, *Bikkia*, *Chiococca*, *Phialanthus*, and *Schmidtottia* as currently circumscribed are not monophyletic. Using analyses of combined chloroplast and nuclear datasets, clades C and D resolve as sister to each other (PP = 0.91); clade B is sister to clades C and D together (PP = 0.93).

Inconsistencies among trees from different analyses

Phylogenetic trees generated from Parsimony analysis and Bayesian analyses of the chloroplast and nuclear data separately resolved some nodes differently than from Bayesian analysis using a combined dataset. However such incongruences are in most cases not well supported and do not contradict the overall phylogenetic relationships. In the nuclear tree, all four clades are well supported (PP = 1.0); however, clade A is aligned as sister to clade C, despite a low support (PP = 0.78). In the Parsimony tree, Clade A is

also placed together with clades C and D but with poor support (BS = 56), thus essentially resulting in an unresolved backbone forming a polytomy of clade A, clade C, clade D, and subclades within clade B. Similarly, in the trees generated from only the chloroplast data (Bayesian analysis) and the Parsimony analysis, clade B is not resolved as a single clade; however, its five subclades are still well-supported (PP \geq 0.95, BS \geq 96), but formed an unresolved grade with a larger clade of clades C and D combined. *Coutaportla* and *Lorencea* of clade A do not resolve as one clade in the phylogeny using the chloroplast dataset, but still show a sister relationship with a well-supported clade of all other genera (clades B, C and D combined; PP = 1.0). Phylogenetic relationships will primarily be discussed based on the majority rule consensus tree generated from the combined dataset. Inconsistencies among different analyses will also be discussed where applicable.

DISCUSSION

The present study has been successful in generating highly resolved phylogenies that will enable us to better understand phylogenetic relationships within Chiococceae *sensu* Paudyal et al. (2014). The present study is the most comprehensive phylogenetic analysis of the tribe Chiococceae *sensu* Paudyal et al. (2014) in terms of ingroup taxa sampling, with 126 species sampled from 27 genera. We were unable to successfully amplify and sequence DNA of the genera *Shaferocharis* and *Thogsennia*, due to the unavailability of recent collections; both genera have not been included in any of the previous molecular phylogenies. To the best of our knowledge, 55 species and one genus, *Ceuthocarpus*, have not been previously included in any of the earlier molecular

phylogenetic studies; 24 out of the 55 species added in the present study are Cuban endemics. Among the earlier studies, Motley et al. (2005) had the most extensive sampling of 59 species from 23 genera and later Manns and Bremer (2010) added three genera (*Eosanthe*, *Lorencea* and *Nernstia*) and six species from their sampling of 41 species from 25 genera in their study. However, a total of eight taxa included in previous studies- three species of the genus *Erithalis* included in Negron-Ortiz and Watson (2002), three species of *Exostema* included in McDowell et al. (2003), and one species each of *Coutarea* and *Phialanthus* included in Robbrecht and Manen (2006), were missing in those two studies. In addition, previous molecular phylogenies have included less than ten species that are endemic to Cuba; now a total of 33 Cuban endemics are included in the phylogeny.

ETS markers had not been used in previous phylogenetic studies of Chiococceae, except in the phylogeny of *Erithalis* by Negron-Ortiz and Watson (2002). The use of ETS markers greatly helped to obtain well-resolved phylogenies. While discussing phylogenetic relationships, comparisons will primarily focus on previous molecular phylogenies.

General topology of the phylogeny

The analyses of the combined nuclear and chloroplast datasets generated a majority rule consensus tree with very well-supported clades (more than 80% of the total nodes have $PP \geq 0.9$), and most of the less-supported clades present at the infra-generic level. The genera of the Chiococceae *sensu* Paudyal et al. (2014) were resolved as a monophyletic group, with four distinct, well-supported clades. Considerable

morphological diversification can be seen within these clades. The general topology of the tree shows some similarity with the results of Motley et al. (2005), Robbrecht and Manen (2006), and Manns and Bremer (2010) in that they also retrieved two clades within the monophyletic alliance that corroborate with the clades C and D in our results. Delprete (1996a; 1996b) also recognized two clades within the complex, although there is considerable difference in the taxa groupings suggested in his study. However, our topology disagrees considerably with the results of some other phylogenetic studies that also included large taxa sampling (Bremer 1992; Rova 1999; Rova et al. 2002; Bremer and Eriksson 2009) in that they retrieved a mosaic of generic relationships within the alliance rather than discrete clades. Intergeneric relationships within each of the four clades are discussed below.

Naming and delimiting the tribe

Our results clearly support the existence of distinct, well resolved clades within the monophyletic group, which in a broad sense corresponds to the group retrieved by Motley et al. (2005) and Robbrecht and Manen (2006), based on molecular data, but contradict with the groups proposed by Delprete (1996a), based on morphological data. Although our results show that there are distinct clades that include genera of *Chiococceae* s. s., *Catesbaeae* (both *sensu* Hooker), and *Exostema* (*sensu* McDowell 1996) in different clades, there is a lack of direct corroboration of those clades with previously delimited tribes. Hence, the term “complex” is dismissed in favor of “*Chiococceae sensu* Paudyal et al. (2014)” for naming the tribe.

Morphological studies (Bridson and Robbrecht 1985; Igersheim 1993; Puff et al. 1995) were unable to ascertain any tribal affiliation of *Strumpfia*. Molecular data were more helpful, and showed that this genus is closely related to the Chiococceae (Bremer et al. 1999; Rova 1999). But there have been differing views on whether to include *Strumpfia* in the Chiococceae or treat it as a sister taxon. *Strumpfia* was included in the Chiococceae by Bremer and Eriksson (2009) and Manns and Bremer (2010), while Rova et al. (2002), Motley et al. (2005), and Robbrecht and Manen (2006) maintained it as a sister taxon to the Chiococceae. Recently, citing numerous morphological differences and supporting molecular data, Paudyal et al. (2014) included *Strumpfia* in the monotypic Strumpfiaceae, as sister tribe of the Chiococceae *sensu* Paudyal et al. (2014). Our results from combined dataset placed *Strumpfia* sister to the rest of Chiococceae as shown in most of the previous studies. Furthermore, the analyses using the ETS dataset separately placed *Strumpfia* in an unresolved grade together with other outgroups (*Hamelia* Jacq., *Syringantha* Standl., and *Guettarda* L.), thus further supporting the exclusion of *Strumpfia* from the tribe Chiococceae *sensu* Manns and Bremer (2010). Hence our results here further reiterate our previous delimitation of the tribe Chiococceae *sensu* Paudyal et al. (2014).

The Coutaportla-Lorencea clade

Coutaportla and *Lorencea*, genera endemic to Mexico and Central America, were resolved in Clade A (Fig. 4), which is strongly supported as sister to the remainder of the Chiococceae (PP = 1.0). Although the analyses of the combined dataset placed *Coutaportla* and *Lorencea* in one strongly supported clade (PP = 0.98), these two genera

are not positioned in a single clade in the separate analyses of chloroplast data. However, also in the chloroplast data all the other genera are resolved as one strongly supported clade, which is in turn sister to *Coutaportia* and *Lorencea* forming a trichotomy. Earlier, Rova et al. (2002) showed similar relationships (although they did not state the support values) and in the results of *trnL-F* data, Motley et al. (2005) also placed *Coutaportia* sister to all the other genera, although with relatively low support. These phylogenetic relationships suggest *Coutaportia* and *Lorencea* to be the basal lineage within Chiococceae and supports Manns et al. (2012) assertion that the Chiococceae were distributed from Central America (as the center of origin of the tribe) to the Caribbean islands and to South America. However, Central America is geologically much younger than the Caribbean islands.

Coutaportia was separated from *Portlandia* by Urban (1923), and later Borhidi (2003) transferred *Coutaportia guatemalensis* (Standl.) Lorence to the new genus *Lorencea*. Association of these two genera with *Portlandia* as suggested by Aiello (1979), Delprete (1996b), and Ochoterena-Booth (2000) is not supported by our results. These two genera were instead retrieved as sister taxa to the rest of the Chiococceae genera. Although the two species of *Coutaportia* and *Lorencea* form a single clade in our combined and nuclear datasets, they are not resolved in a single clade in chloroplast datasets. Therefore, our results do not reject the segregation of *Lorencea* as a monotypic genus. However, opinions greatly differ on separating or merging monotypic taxa.

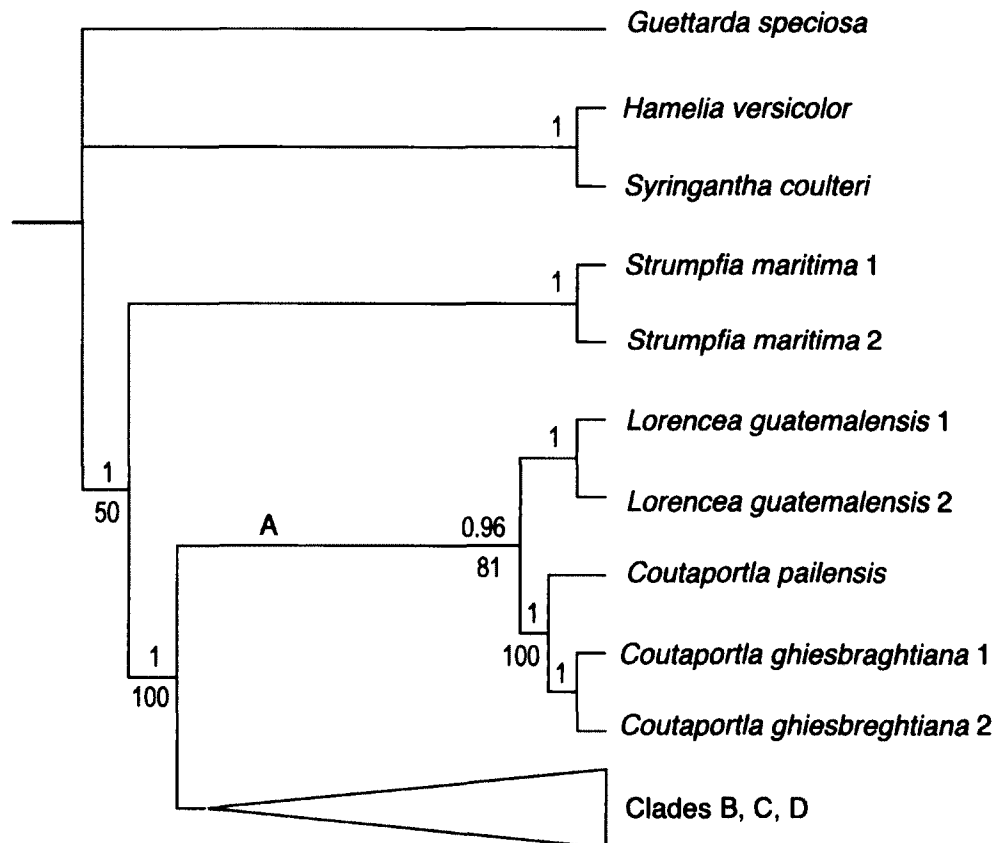


Fig. 4 Detailed phylogeny of the *Coutaportla-Lorencea* clade. This is a section (clade A, Fig. 3) of the 50% majority rule consensus tree of the tribe Chiococceae retrieved from the Bayesian inference analyses of combined dataset (ETS, ITS, *petD*, and *trnL-F*). Number above each branch represents posterior probability value and the number below the branch represents the parsimony bootstrap value.

The Exostema-Solenandra-Coutarea-Hintonia clade

Clade B is strongly supported, and comprises all the capsular, wing-seeded genera of the Chiococceae *sensu* Paudyal et al. (2014), namely *Coutarea*, *Exostema*, *Hintonia*, and *Solenandra*, forming a monophyletic group (Fig. 5) not detected in previous phylogenies (Bremer 1992; Rova et al. 2002; Motley et al. 2005; Robbrecht and Manen 2006; Bremer and Eriksson 2009; Manns and Bremer 2010; Manns et al. 2012). Our results clearly establish the monophyly of *Hintonia* and its close relationship with *Exostema sensu* McDowell (1996) and *Coutarea*, and not with other members of *Portlandia* complex, as indicated in some morphology-based studies (Aiello 1979; Delprete 1996b; Ochoterena-Booth 2000). However, *Coutarea* and *Exostema* are not resolved as monophyletic. Previous phylogenies, mostly lacking extensive sampling, placed these genera as an unresolved grade either intermixed (Bremer 1992; Bremer 1996; Delprete 1996a; Bremer and Eriksson 2009; Manns et al. 2012), or basal (Bremer et al. 1995; Motley et al. 2005; Robbrecht and Manen 2006; Manns and Bremer 2010) to other genera of Chiococceae.

The taxonomic relationships among *Coutarea*, *Hintonia* and *Exostema* have been variously interpreted by different authors. *Coutarea* was described as a monotypic genus by Aublet (1775), using material that he collected in French Guiana. He described it as *Coutarea speciosa* Aubl., a synonym of *Coutarea hexandra* (Jacq.) K. Schum., which occurs throughout the Neotropics. Several authors later included numerous species in *Coutarea*, which were later transferred to other genera, or synonymized with other species. Recently, Taylor and Lorence (2010) recognized five species of *Coutarea*.

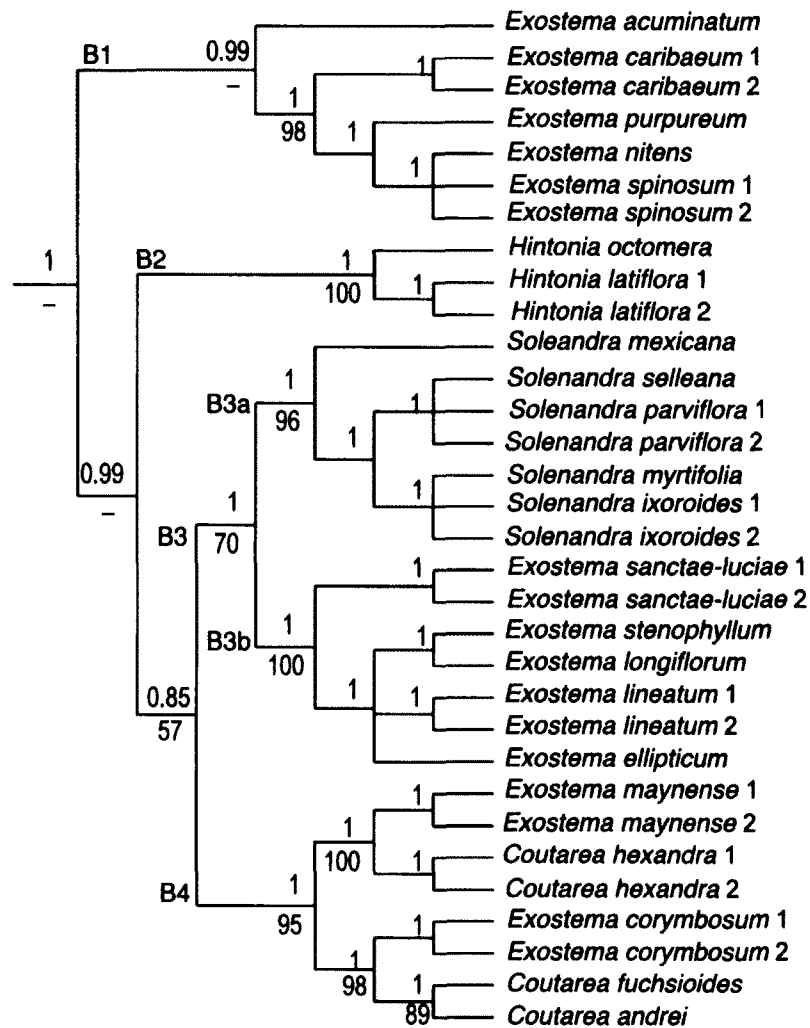


Fig. 5 Detailed phylogeny of the *Exostema-Solenandra-Coutarea-Hintonia* clade. This is a section (clade B, Fig. 3) of the 50% majority rule consensus tree of the tribe Chiococceae retrieved from the Bayesian inference analyses of combined dataset (ETS, ITS, *petD*, and *trnL-F*). Parsimony bootstrap values obtained from separate maximum parsimony analyses are also indicated. Number above each branch represents posterior probability value and the number below the branch represents the parsimony bootstrap value. Parsimony bootstrap value is given only for the basal nodes and nodes with taxonomic implications that are discussed in this paper.

Although *Coutarea alba* Griseb. is sometimes treated as a distinct species (Taylor and Lorence 2010) or treated as a synonym of *Coutarea hexandra* (Govaerts et al. 2014), we prefer to follow Taylor and Lorence (2010). *Coutarea alba* is distinguished from *Coutarea hexandra* in having inflorescences on lateral short shoots, smaller capsules with numerous circular lenticels (vs. inflorescences terminal, larger capsules with elliptic lenticels or without lenticels), and occur in dry vegetation of South America (vs. occurring in wet forests, ranging from Mexico to Argentina). Hooker (1873) placed *Coutarea* and *Exostema* together in the tribe Cinchoneae, because of their winged seeds.

Hintonia was segregated from *Coutarea* by Bullock (1935) because of the cylindrical capsules (vs. laterally compressed in *Coutarea*), and seeds basipetal-imbricate (vs. vertical in *Coutarea*). Ochoterena-Booth (2000) confirmed this distinction. In addition, *Hintonia* was placed in the Condamineae, together with *Portlandia*, despite the fact that *Hintonia* has winged seeds while *Coutarea* remained in Cinchoneae. Aiello (1979) regarded both *Coutarea* and *Hintonia* as members of the *Portlandia* complex. Considering the presence of winged seeds, Robbrecht (1988, 1993) removed *Hintonia* from the Condamineae, and treated it as *incertae sedis*. Based on a phylogeny using morphological characters, Andersson and Persson (1991) removed *Coutarea* and *Exostema* from the Cinchoneae and returned them to the Condamineae, stating their close association with *Portlandia* instead of their newly delimited Cinchoneae. In the morphology based phylogenies, Bremer (1992) and Delprete (1996b) retrieved *Coutarea* and *Hintonia* as sister genera, more closely related to *Portlandia* than to *Exostema*. However, Bremer (1992) placed all three genera in the amended Chiococceae, while

Delprete (1996b) placed *Exostema* in a separate group and included *Coutarea* and *Hintonia* in the *Portlandia* group of the tribe Catesbaeeae.

Hintonia is currently recognized as a genus of three species occurring in Mexico and Central America (Ochoterena-Booth 2000). *Hintonia latiflora* Bullock (with two accessions) and *Hintonia octomera* Bullock were included in our study. In the present phylogenies the genus is showed to be monophyletic, and was positioned as a sister taxon to the clade including *Coutarea* and the two South American species of *Exostema* (see below for further discussion of this clade).

Our results further reiterate that *Exostema*, as currently circumscribed, is not monophyletic, corroborating most of the previous molecular phylogenies (McDowell et al. 2003; Motley et al. 2005; Robbrecht and Manen 2006; Bremer and Eriksson 2009; Manns and Bremer 2010; Manns et al. 2012). However, contrary to previous molecular phylogenies, in our results, all species of *Exostema* except the two South American species, *Exostema maynense* Poepp. & Endl. and *Exostema corymbosum* Spreng., are resolved into two well-supported clades (subclade B1 and B3b), partially supporting the groupings of McDowell (1996) and McDowell and Bremer (1998). In the phylogenies of McDowell and Bremer (1998), *Exostema* (*sensu* McDowell 1996) was monophyletic, although there is high probability that this erroneous result arose by the use of *Coutarea* as the only outgroup, the absence of additional ingroup genera, and the close association of *Coutarea hexandra* with South American *Exostema* species, as later shown by McDowell et al. (2003), Motley et al. (2005), and also by the present study. Our results resolved these three groups (subclades B1, B3a, and B3b) in a similar way to those resolved by McDowell and Bremer (1998, Fig. 7); however, our results do not support

their conclusion that South American species are the basal lineage of *Exostema*. On the other hand, our results support Motley et al. (2005) suggestion to elevate the three sections of *Exostema* to generic level (with the exclusion of the two South American species), but we disagree with their suggestion to elevate *Exostema acuminatum* Urb. to generic level (see below).

Subclade B1 comprises all the species of *Exostema* with axillary inflorescences (McDowell 1996). For the first time, all axillary-flowered *Exostema* species have been retrieved in a well-supported clade using molecular data. Previous molecular phylogenies (McDowell et al. 2003; Motley et al. 2005; Manns and Bremer 2010, Manns et al. 2012) were unable to resolve *Exostema acuminatum* together with other axillary flowered *Exostema* species; instead *Exostema acuminatum* was often placed closer to the *Catesbaea-Portlandia* lineage. Our results agree with morphology-based delimitation of section *Exostema* as delimited by McDowell (1996). In consideration of *Exostema* being retrieved as polyphyletic and that *Exostema caribaeum* Roem. and Schult., the type species of *Exostema*, is resolved within clade B1, we conclude that *Exostema* s. s. should include only the eight species positioned in *Exostema* section *Exostema* as delimited by McDowell (1996), distributed in Cuba and Hispaniola (although *Exostema caribaeum* extends to other Antilles, Mexico and other parts of Central America).

Solenandra, a genus of 12 species endemic to Cuba, Hispaniola, and Mexico, was resurrected by Borhidi (2002); where he transferred all the species of *Exostema* section *Brachyantha* (sensu Borhidi and Fernandez-Zequeira 1989) except *Exostema corymbosum*. The latter is here transferred to a separate, monotypic genus in this study. *Solenandra* is characterized by terminal inflorescences and corolla tubes 1--3 cm long. In

our study, *Solenandra*, as delimited by Borhidi (2002) formed a well-supported monophyletic clade (subclade B3a). The monophyly of *Solenandra* was retrieved in some previous studies (McDowell and Bremer 1998; McDowell et al. 2003; Manns and Bremer 2010; Manns et al. 2012), but *Solenandra selleana* (Urb. & Ekman) Borhidi, not included in those phylogenies, was found to be nested within the clade of terminal flowered *Exostema* (clade B3b in the present study) by Motley et al. (2005). However, in the present study *Solenandra selleana* is positioned with the rest of the *Solenandra* species.

Subclade B3b comprises five terminal-flowered *Exostema* species with flowers 4--21 cm long (McDowell 1996), and generally corresponds to Section *Pitonia* as delimited by McDowell (1996), excluding the South American species *Exostema maynense*.

Subclade B3b is sister to *Solenandra* clade (which are also terminal-flowered). However, *Solenandra* have short corolla tubes (1--3 cm long). Species in subclade B3b can be morphologically distinguished from *Solenandra* by having flowers 4--21 cm long (vs. 1--3 cm long in *Solenandra*), white corollas that turn pink to maroon after anthesis (vs. turning pale yellow), and acropetal or centripetal seed alignment (vs. basipetal). Because Subclade B3b is positioned as sister clade to the *Solenandra* clade, and because of the morphological differences stated above, all the species recognized by McDowell (1996) in Section *Pitonia* (except *Exostema maynense*, see discussion below) are transferred to the newly described genus, Genus 1. The necessary new combinations will be published separately from this dissertation.

The two South American species of *Exostema*, *Exostema maynense* and *Exostema corymbosum*, form a strongly supported monophyletic group together with the *Coutarea* species (subclade B4). McDowell et al. (2003) also detected a close

relationship of the South American species of *Exostema* (*sensu* McDowell 1996) with *Coutarea*, albeit only *Coutarea hexandra* was included in their phylogeny. The association of *Coutarea* and South American *Exostema* species is also supported by similarity in fruit and seed morphology. The capsules are strongly laterally compressed with a narrow septum, and the seeds are perpendicular to the septum and acro-basipetally aligned (McDowell 1996). Interestingly, neither the two *Exostema* species nor the three *Coutarea* species included in our analyses hold sister relationships with another species from the same genus, as traditionally delimited. *Exostema maynense* was found as a sister taxon to *Coutarea hexandra*, a relationship also recovered by McDowell et al. (2003). *Exostema maynense* is the only *Exostema* species with six-merous flowers, and its association with *Coutarea hexandra*, also with six-merous flowers, has been also discussed by McDowell (1996). However, *Coutarea hexandra* has asymmetrical, dorsally inflated, campanulate corollas, while *Exostema maynense* has narrowly tubular, actinomorphic corollas, with long-narrow lobes reflexed at anthesis. The other two species of *Coutarea*, *Coutarea andrei* Standl. and *Coutarea fuchsioides* C.M.Taylor, form a well-supported clade, which in turn is sister to *Exostema corymbosum*; all three are shrubs distributed in dry areas at higher elevations of the Andes, suggesting a recent (ca. 10 my) evolutionary radiation corresponding with the Andean uplift. Due to similar general aspect and very similar capsules and seeds, *Coutarea andrei*, *Coutarea coutaportloides* C.M.Taylor (not included in this study) and *Coutarea fuchsioides* are known to be confused with *Exostema corymbosum* (Taylor and Lorence 2010). However, the three *Coutarea* species differ from *Exostema corymbosum* in having flowers on short-shoots and entire stipules. *Exostema corymbosum* and *Coutarea coutaportloides* show

great similarity in the size and shape of their corollas (hypocrateriform), but in case of *Exostema corymbosum* the lobes are equal to or longer than the tube (Taylor and Lorence 2010). Also, the corollas of the three species of *Coutarea* are tubular to funnel-form, with five to seven lobes, while in *Exostema corymbosum* the corollas are salverform, and with five lobes. In the phylogenies produced by Rova et al. (2002) and Robbrecht and Manen (2006), *Coutarea andrei* and *Coutarea hexandra* were positioned in one clade as sister taxa, a relationship not supported by our results, possible due to more extensive sampling. Most previous studies did not include either of the South American *Exostema* species in their phylogeny. This is the first phylogenetic study that includes both species of South American *Exostema* species, and more than one species of *Coutarea* in the same study (three species of *Coutarea*; two accessions each of *Coutarea hexandra*, *Exostema corymbosum*, and *Exostema maynense*), thereby providing a better understanding of phylogenetic relationships. In conclusion, *Exostema corymbosum* and *Exostema maynense* do not belong to *Exostema*, as already suggested by Rova (1999), and this is further substantiated by our results. Following these results, three new genera are here proposed, in order to accommodate the species of this group.

Exostema corymbosum is transferred to a new genus, Genus 2, which can be distinguished from other *Exostema* species by having salverform corollas with the corolla tube shorter than the lobes, strongly laterally compressed capsules (vs. slightly laterally compressed), trapezoidal placenta, and acrobasiptal seeds (vs. hemi-ellipsoidal, lanceolate or linear placenta, with basiptal, acropetal or centripetal seed insertion), and it is found in the high elevations of the Andes, at 1000--2800 m altitude. *Exostema maynense* is transferred to another new genus, Genus 3, which can be distinguished from

Exostema species in having six-merous flowers (vs. four- or five-merous), calyx lobes broad and long with obtuse tip (vs. calyx lobes short, with acute tips), strongly flattened capsules (vs. slightly flattened), and in being a large tree found in the lowland forests of the western Amazon basin. Following these results, the Andean shrubby species of *Coutarea* are here segregated from *Coutarea* and are transferred to a new genus, Genus 4, which can be distinguished from *Coutarea* in having actinomorphic (vs. zygomorphic), pink to red corollas (vs. white to pink to purple), anthers partly or completely inserted (vs. exserted), and is found in dry vegetation at high elevations of the Andes above 1900 m altitude. The necessary new combinations will be published separately from this dissertation.

The Catesbaea-Portlandia clade

Clade C is strongly supported as monophyletic (Fig. 6), and it comprises *Catesbaea*, *Cubanola*, *Isidorea*, *Nernstia*, *Osa*, *Phyllacanthus*, and *Portlandia*. Based on a morphological phylogeny, Delprete (1996a, 1996b) included these seven genera into expanded tribe Catesbaeeae, along with other eight genera of the Chiococceae (*Bikkia*, *Ceuthocarpus*, *Coutaportla*, *Coutarea*, *Hintonia*, *Schmidtottia*, *Siemensia*, and *Thogsennia*). *Thogsennia*, not included in this study, is an extremely rare, monotypic genus, known only from a few old collections. Recent collection projects were unable to find it in its natural environment suggesting that it is probably extirpated. These genera were also resolved as one clade in some of the previous studies (Rova et al. 2002; Motley et al. 2005; Robbrecht and Manen 2006; Manns and Bremer 2010; Manns et al. 2012).

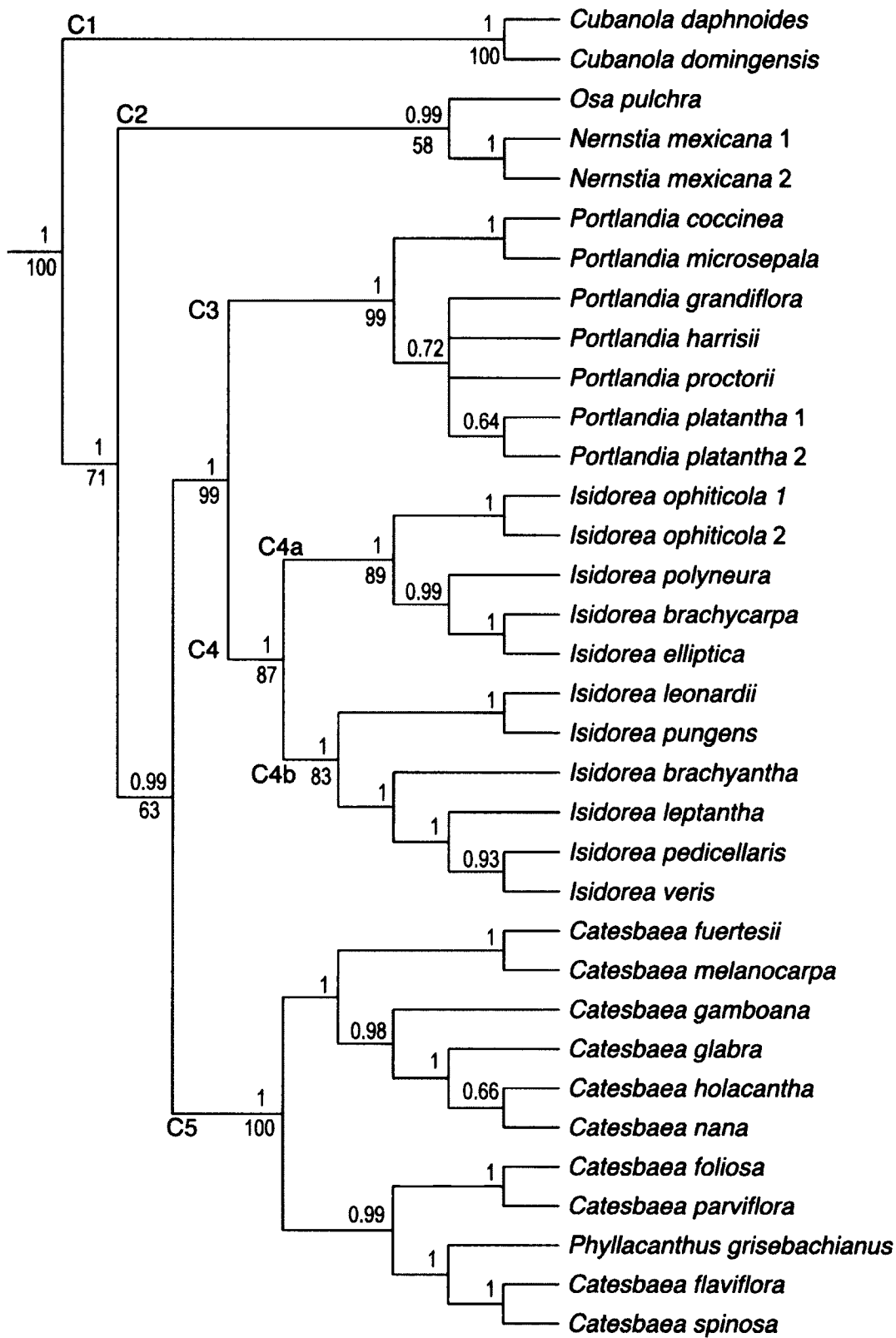


Fig. 6 Detailed phylogeny of the *Catesbaea-Portlandia* clade. This is a section (clade C, Fig. 3) of the 50% majority rule consensus tree of the tribe Chiococceae retrieved from the Bayesian inference analyses of combined dataset (ETS, ITS, *petD*, and *trnL-F*). Parsimony bootstrap values obtained from separate maximum parsimony analyses are also indicated. Number above each branch represents posterior probability value and the number below the branch represents the parsimony bootstrap value. Parsimony bootstrap value is given only for the basal nodes and nodes with taxonomic implications that are discussed in this paper.

Aiello (1979) segregated *Osa* from *Hintonia*, and *Cubanola* and *Nernstia* (as “*Cigarrilla* Aiello”) from *Portlandia* based mainly on the placentation and seed characters. She distinguished *Osa* from *Hintonia* as having large wingless seeds (vs. small winged seeds) with tuberculate testa (vs. reticulate) and persistent funicle (vs. non-persistent), a long trumpet-shaped corolla (vs. funnelform), and long leaves with attenuate apex (vs. short leaves with acute to acuminate apex). Similarly, Aiello (1979) distinguished *Cubanola* from *Portlandia* in having reticulate to foveate seeds (vs. tuberculate in *Portlandia*) and no persistent funicle (vs. persistent), loculicidally and septicidally dehiscent capsule (vs. loculicidally dehiscent from above), placenta which is circular in cross section (vs. linear and adnate to septum), and thin leaves (vs. coriaceous).

Nernstia is distinguished from *Portlandia* in having colliculate seeds (vs. tuberculate) with acropetally imbricate arrangement and no persistent funicle (vs. persistent), and large spongy placenta (vs. linear and adnate to septum). These segregations are supported by our results. The monotypic genera *Nernstia*, endemic to Mexico, and *Osa*, endemic to Central America, are resolved in a strongly supported clade (subclade C2), sister to the *Catesbaea-Portlandia-Isidorea* clade. *Cubanola*, a genus with two species endemic to Cuba and Hispaniola, is placed sister to all the other genera of the clade. These relationships are very similar to the molecular phylogenies of Motley et al. (2005), and Manns and Bremer (2010), although *Nernstia* was not included in the former, while in the latter the *Nernstia-Osa* clade was placed sister to *Catesbaea* (no support values stated). Even though both *Nernstia* and *Osa* were missing in their studies, Robbrecht and Manen (2006) and Bremer and Eriksson (2009) also had the remaining five genera resolved as one clade, although with varying relationships within the clade.

Portlandia (subclade C3), as currently circumscribed, is a genus of six species endemic to Jamaica (Delprete and Motley 2003). Aiello (1979) studied *Portlandia* and associated taxa and re-circumscribed *Portlandia* to include only the species endemic to the island of Jamaica from what was previously delimited as a genus of over 20 species. *Portlandia* includes species with broadly triangular stipules, and coriaceous, non-pungent leaves. Delprete and Motley (2003), based on molecular and morphological data, elevated one of the varieties to species level, adding to a total of six species, which in the present study formed a strongly supported monophyletic clade (subclade B2a), sister to the *Isidorea* clade. Sister relationship of *Portlandia* and *Isidorea* was found also in previous studies (Delprete 1996b; Rova et al. 2002; Delprete and Motley 2003; Motley et al. 2005; Robbrecht and Manen 2006; Manns and Bremer 2010), while such relationship was not fully resolved in some others (Bremer 1992; Bremer and Eriksson 2009).

Isidorea (subclade C4), a genus with over 15 species endemic to Cuba and Hispaniola, was found to be monophyletic in previous studies, although it was not adequately sampled. It differs from *Portlandia* in having stiff, pungent, coriaceous leaves and stipules divided at the base into two parts, looking like four, apically pungent stipules per node (Aiello 1979). We were able to include ten species of *Isidorea* in our study, six of which were not included in previous phylogenies. Our results further support the monophyly of *Isidorea*; this clade is divided into two subclades, one with the species from Cuba (subclade C4a), and the other with species from Dominican Republic (subclade C4b). The Cuban species of *Isidorea* had not been previously included in any molecular studies. In addition, in the results from the chloroplast dataset *Isidorea* clades are retrieved in a trichotomy with the *Portlandia* clade.

Catesbaea, a genus of about 16 species occurring in the Bahamas, Florida Keys, and the Greater and Lesser Antilles, as currently circumscribed, is not monophyletic. In our analyses, *Catesbaea* formed a strongly supported clade with *Phyllacanthus* nested within it (subclade C5), corroborating with many previous molecular phylogenies (Rova et al. 2002; Motley et al. 2005; Robbrecht and Manen 2006; Manns and Bremer 2010; Manns et al. 2012). *Phyllacanthus* was segregated from *Catesbaea* by Hooker (1871) because of its large, laterally flattened, triangular thorns, and uniseriate ovules. Aside from these characters, the two genera are morphologically very similar (Delprete 1996). Although the analysis using combined data resolved a different relationship, based on their *trnL-F* results and morphological similarity with *Catesbaea flaviflora* Urb., with flowers almost identical to those of *Phyllacanthus grisebachianus* Hook. f., Motley et al. (2005) suggested returning *Phyllacanthus* to *Catesbaea*. While delimiting the tribe Chiococceae, Manns and Bremer (2010) also treated *Phyllacanthus* as included in *Catesbaea*. Present results further support these suggestions and we propose to return *Phyllacanthus grisebachianus* to *Catesbaea*, using the original binomial *C. phyllacantha* Griseb.

The Chiococceae s. s., Cuban endemics, and Pacific genera

Clade D is strongly supported as monophyletic (Fig. 7) and is comprised of 14 genera, namely *Badusa*, *Bikkia*, *Ceratopyxis*, *Ceuthocarpus*, *Chiococca*, *Eosanthe*, *Erithalis*, *Morierina*, *Phialanthus*, *Salzmannia*, *Schmidtottia*, *Scolosanthus*, *Siemensia*, and *Thiollireea*. These genera are grouped together into five strongly supported subclades (Subclades D1--D5). Several genera of this clade are endemic to Cuba (*Ceratopyxis*,

Ceuthocarpus, *Eosanthe*, *Schmidtottia*, *Siemensia*), some others occur in Cuba and in the other Antilles (*Erithalis*, *Phialanthus*, *Scolosanthus*), two are widespread in the Neotropics (*Chiococca*, *Salzmannia*), and four occur in the Pacific region (*Badusa*, *Bikkia*, *Morierina*, *Thiollierea*). Corolla shapes and fruit types within this clade vary greatly, showing no distinct pattern in the evolution of such characters, hence support for the evolutionary plastic nature of these characters. Although the tree generated from the combined analyses resolves the backbone relationships among the five subclades (Subclades D1--D5), such relationships are not well supported (PP < 0.7) and, in essence, the five subclades within clade D form an unresolved grade. However, clade D is well supported as monophyletic clade (PP = 1, BS = 100) and each of the subclades are highly supported (PP = 1.0, BS ≥ 90).

The genera mentioned above grouped as a monophyletic alliance in most of the previous molecular studies (Rova et al. 2002; Motley et al. 2005; Robbrecht and Manen 2006; Manns and Bremer 2010; Manns et al. 2012); however, they did not form a monophyletic group in Bremer and Eriksson (2009). The genera of the previously delimited tribe Chiococceae s. s. (Hooker 1873: 105) are retrieved as two separate clades: Clade D5, with *Chiococca* (incl. *Asemnantha*), *Erithalis*, *Salzmannia*, and *Scolosanthus*, and Clade D2, with *Phialanthus*, *Ceratopyxis*, *Ceuthocarpus*, *Eosanthe*, and *Schmidtottia* (the last four endemic to Cuba).

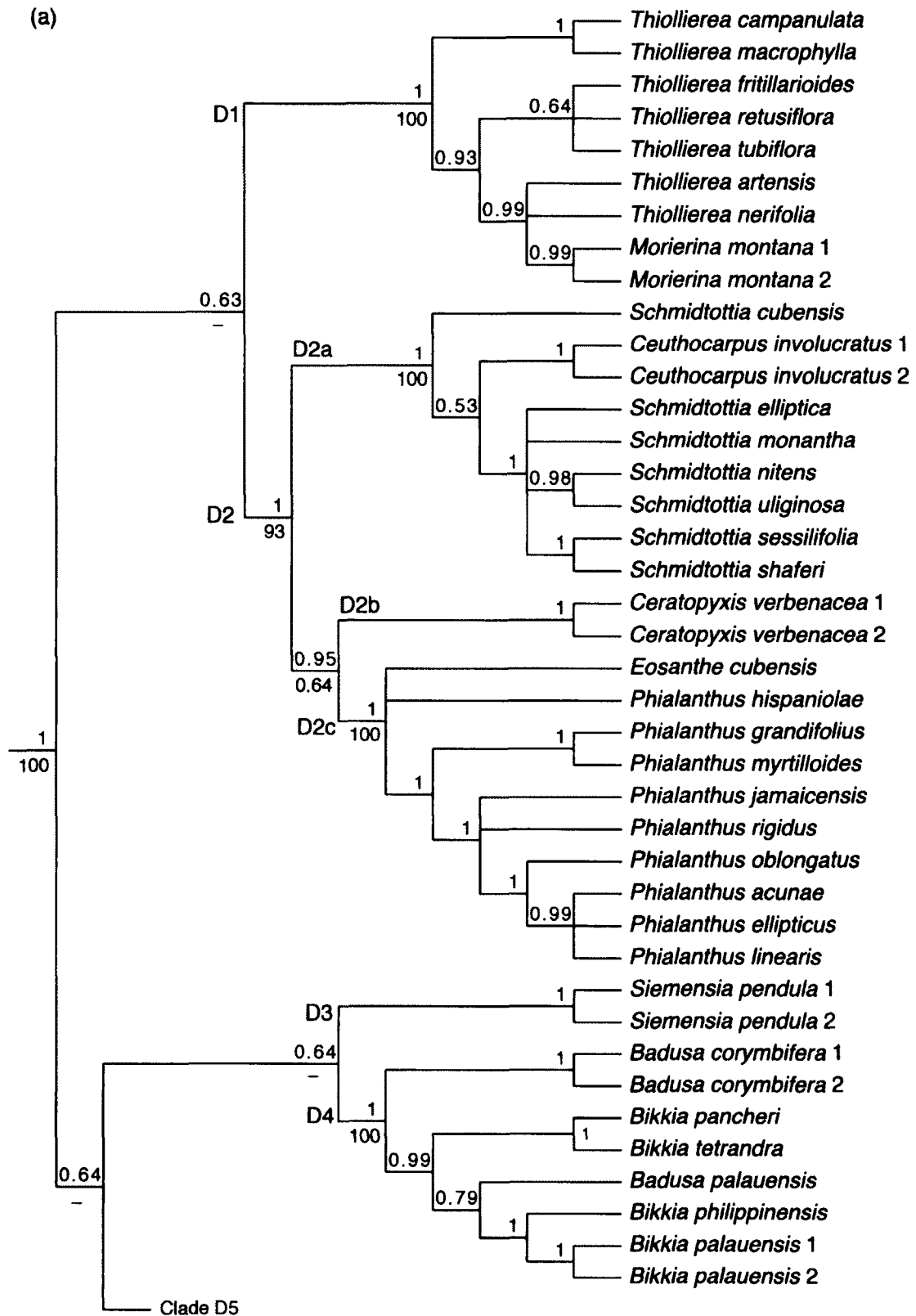


Figure 7 continued.

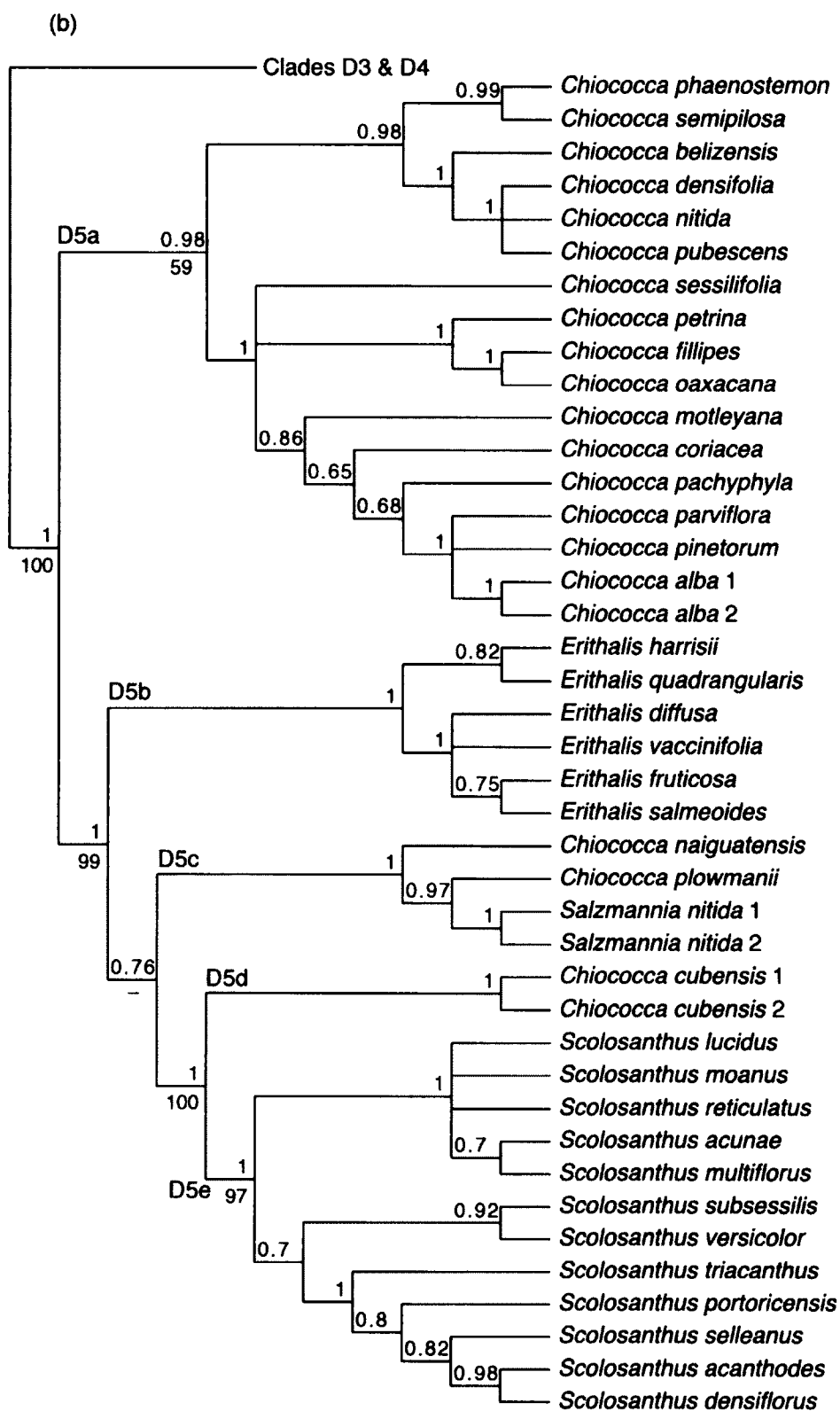


Fig. 7 Detailed phylogeny of the Chiococceae s. s., Cuban endemics, and Pacific genera.

This is a section (clade D, Fig. 3) of the 50% majority rule consensus tree of the tribe Chiococceae retrieved from the Bayesian inference analyses of combined dataset (ETS, ITS, *petD*, and *trnL-F*). Parsimony bootstrap values obtained from separate maximum parsimony analyses are also indicated. Number above each branch represents posterior probability value and the number below the branch represents the parsimony bootstrap value. Parsimony bootstrap value is given only for the basal nodes and nodes with taxonomic implications that are discussed in this paper.

The four genera from the western Pacific islands, *Badusa*, *Bikkia*, *Morierina*, and *Thiollierea*, in our analyses are found in two separate clades (Subclades D1 and D4). *Morierina* and *Thiollierea*, endemic to New Caledonia, form a highly supported monophyletic clade (subclade D1) with the morphologically very distinct *Morierina* nested within *Thiollierea*. *Morierina montana* Vieill. is a large tree with narrow, tubular flowers, found in forested area, while the species of *Thiollierea* are shrubs with large, colorful, campanulate flowers, growing in scrub coastal vegetation on ultrabasic soils (Motley et al. 2005). Considering their morphological similarity, mostly because of their long-narrow corolla tube with long-reflexed lobes, Bremer (1992) and Delprete (1996) placed *Morierina* sister to *Exostema*. Motley et al. (2005) for the first time included *Morierina* in molecular phylogenies, and found it nested in the *Thiollierea* clade. The genus *Thiollierea* was recently resurrected by Barrabé et al. (2011) to include ten New Caledonian endemic species that were previously placed in *Bikkia*. They amended *Thiollierea* mostly based on the results of Motley et al. (2005) and morphological data presented in Barrabé et al. (2011). The main character they used to distinguish *Thiollierea* from *Bikkia* s. s. is that the anthers twist at anthesis in *Thiollierea*. Other characteristics of *Thiollierea* used to differentiate it from *Bikkia* s. s. include sheathing, truncate stipules (vs. free, acuminate), flat ovules (vs. globose), drooping inflorescence (vs. erect), flat or round seeds (vs. angular or diamond shaped). Despite acknowledging the fact that *Morierina* in molecular phylogenies is nested within *Thiollierea*, Barrabé et al. (2011) refrained from adequately addressing the phylogenetic position of *Morierina* in relation to the phylogenetic delimitation of *Thiollierea*, as shown in Motley et al. (2005). We were unable to include in our study the second species of *Morierina*, *Morierina*

propinqua Brongn. & Gris, which is probably extinct. However, we feel that *Morierina* is another example of extreme morphological variation within a single genus, present in this tribe, which most probably is a result of a shift in ecological niche and pollinator syndrome, as suggested by Motley et al. (2005). Although only one species was included in our analysis, both *Morierina* species are here proposed to be transferred to *Thiollierea*, based on the morphological similarities of these two species.

Our results resolved the four Cuban endemics *Ceuthocarpus*, *Ceratopyxis*, *Eosanthe*, and *Schmidtottia*, and the West Indian genus *Phialanthus* in a strongly supported monophyletic clade (subclade D2). This further supports the same relationships retrieved in some recent molecular studies (Rova et al. 2002; Motley et al. 2005; Bremer and Eriksson 2009; Manns and Bremer 2010), although it contradicts the phylogenies produced by Robbrecht and Manen (2006), where *Eosanthe* was placed differently. None of the recent publications included all the genera in same study, and the large genera *Phialanthus* and *Schmidtottia* had very limited sampling, and therefore they did not have a significant resolution.

Schmidtottia was segregated from *Portlandia* by Urban (1923) because of its terminal inflorescence (vs. lateral), sheathing, truncate stipules (vs. interpetiolar, triangular), septicidal capsules (vs. loculicidal), and oval -- obovate placenta (vs. linear). Although Robbrecht (1988, 1993) considered its tribal affiliation uncertain, *Schmidtottia* was still considered closer to *Portlandia* (Aiello 1979; Bremer 1992; Delprete 1996) until Rova et al. (2002) placed it in a clade with the genera of Chiococceae s. s., which was also supported by later molecular phylogenies (Motley et al. 2005; Manns and Bremer 2010; Manns et al. 2012). However, only one species of *Schmidtottia* (*Schmidtottia*

sessilifolia Urb.) was included in those molecular studies. Seven species are included in the present study and the results show that *Schmidtottia*, as currently circumscribed, is not monophyletic and forms a strongly supported monophyletic clade with *Ceuthorcarpus* nested within (subclade D2a). *Ceuthorcarpus involucratus* (Wernham) Aiello was originally described as *Portlandia involucrata* by Wernham (1913), and was transferred to *Schmidtottia* by Alain (1959). Later, Aiello (1979) segregated this taxon from *Schmidtottia* and transferred it to the new genus *Ceuthorcarpus*, because of its distinctive involucre bracts surrounding the ovary and persistent on the fruit. *Ceuthorcarpus* is here included in a molecular phylogeny for the first time, and its segregation from *Schmidtottia* is not supported by our results. In the analyses of nrETS datasets separately, *Ceuthorcarpus* and *Schmidtottia cubensis* formed a clade sister to the rest of *Schmidtottia* (although not in the same position in the analyses of other datasets). The nrETS phylogeny supports the morphological similarity with *Schmidtottia cubensis* suggested by Aiello (1979), because of the terminal solitary flowers and two or three leaves per node. Because *Ceuthorcarpus* in our analyses is nested within *Schmidtottia*, it is here returned to this genus, in agreement with Alain (1959), and the binomial *Schmidtottia involucrata* (Wernham) Alain is already available.

The placement of *Ceratopyxis*, another Cuban endemic monotypic genus, as sister to the *Phialanthus-Eosanthe* clade is highly supported by our results. *Ceratopyxis* has been placed sister to *Phialanthus* in most previous studies, except by Manns and Bremer (2010), who placed *Schmidtottia* sister to *Phialanthus* with no support value stated.

Phialanthus, a genus of about 20 species occurring in the Bahamas, and the Greater and Lesser Antilles, was positioned within the Chiococceae s. s. by Hooker

(1873). Bremer (1992) excluded it from the amended Chiococceae citing the presence of free filaments, but later Rova et al. (2002) showed that it is closely related to other members of the Chiococceae. In Motley et al. (2005) *Phialanthus* was supported to be monophyletic; however, this was not shown in Robbrecht and Manen's (2006) results as the two species of *Phialanthus* were not resolved together. *Phialanthus stillans* Griseb. (not included in the present study nor in Motley et al. 2005) was found on a clade with *Eosanthe*, while *P. grandifolius* Alain was found on a clade with *Ceratopyxis* and *Schmidtottia* (also in Motley et al. 2005). We included nine species of *Phialanthus* in the present study. In both combined and separate analyses of chloroplast and nuclear datasets, *Phialanthus* is retrieved as non-monophyletic. Eight species of *Phialanthus* are found on one clade forming a trichotomy with *Eosanthe* and *Phialanthus hispaniolae* Alain & R.G. Gracia as sisters within a highly supported monophyletic clade (Subclade D2c). Although including only one species of *Phialanthus* in their phylogeny, Manns and Bremer (2010) also placed *Eosanthe* sister to *Phialanthus*. Even though Delprete (1999a; 1999b), based on morphological observations, could not ascertain tribal affiliation of *Eosanthe* at the time, he pointed out that *Eosanthe* is similar to *Phialanthus*. The sheathing stipules, axillary inflorescence, persistent four-lobed calyx, filaments not connate to the corolla tube, and the two-seeded indehiscent fruits of *Eosanthe* resemble those of *Phialanthus*. He also discussed other morphological characters (resinous branches, thick coriaceous leaves, foliose calyx lobes, ridged corolla tube, and linear-oblong anthers) that showed *Eosanthe* also to be similar to *Schmidtottia*; however, he stated that inflorescence and fruits differed between the two (solitary axillary flowers and two-seeded pseudosamaras in *Eosanthe* vs. terminal few flowered inflorescence and

many seeded capsules in *Schmidtottia*). Our results are in agreement with these morphological observations, but unfortunately we were able to include sequence data of *Eosanthe* from only two regions in our analyses. Sequence data from the remaining two regions coupled with additional taxa sampling of *Phialanthus* will help to better resolve the *Eosanthe-Phialanthus* relationships. Although our results position *Eosanthe cubensis* Urb. together with *Phialanthus* species in a strongly supported clade (subclade D2c; PP = 1.0, BS = 100), considering the limitation in sequence data of *Eosanthe* and also because its fruits are narrowly winged pseudo-samaras, which is a fruit type unique within the tribe, we prefer to keep *Eosanthe* separated from *Phialanthus*.

Our results are unable to fully ascertain the phylogenetic position of *Siemensia*, a monotypic genus endemic to western Cuba. However, our phylogenies contradict some earlier morphological studies (Aiello 1979; Delprete 1996) and positioned *Siemensia* as associated with *Portlandia*, from which it was segregated by Urban (1923). In the combined analyses, *Siemensia* was placed on a clade sister to the *Badusa-Bikkia* clade, although not strongly supported. It is strongly supported as sister to the *Thiollierea-Morierina* clade in the phylogenies using the nuclear dataset, while in the phylogenies using chloroplast datasets it was resolved in a trichotomy with the *Badusa-Bikkia* clade and *Chiococca-Scolosanthus* clade as sister clades. The parsimony analyses also placed *Siemensia* as sister to *Thiollierea-Morierina* clade but with relatively poor support (BS = 66), which in essence places *Siemensia* together with Pacific genera in an unresolved grade basal to the other genera in the clade. The same contrasting relationships were retrieved by Motley et al. (2005) in separate analyses of *trnL-F* and ITS sequences. Other studies placed *Siemensia* closer to *Chiococca-Scolosanthus* (Manns and Bremer 2010; no

support value stated) or simply within the Chiococceae s. s.-Cuban endemics-Pacific genera clade (Rova et al. 2002; Robbrecht and Manen 2006).

The widespread genera of the western Pacific islands, *Badusa* and *Bikkia* s. s. (including only the species remaining after the resurrection of *Thiollierea*) are resolved as a strongly supported clade (subclade D4), but neither genera are retrieved as monophyletic. This result contradicts the earlier phylogenies of Motley et al. (2005) and Manns and Bremer (2010), where the two genera were found as sister taxa, albeit with inadequate sampling in the latter study where only one species per genus was included. In the previous study, too, the analyses of *trnL-F* data separately retrieved an unresolved grade of *Bikkia* and *Badusa* species in a clade while the sister relationship was poorly supported in the analysis of ITS data. In her study using morphological data where she included all three species of *Badusa*, Ochoterena-Booth (2000: 147) also could not fully establish monophyly of the genus. *Badusa* is morphologically distinct from *Bikkia* in having short-tubular 5-merous flowers and fusiform capsules, while the species of *Bikkia* have large funnel-shaped, 4-merous flowers and subcylindrical, costate capsules (Fosberg et al. 1993; Motley et al. 2005). *Badusa* was earlier treated as associated with *Exostema* and *Morierina* based on certain morphological characters (e.g. tubular flowers with narrowly oblong imbricate corolla lobes, anthers basally attached to filaments, and dorsoventrally flattened seeds) (Ridsdale 1982; Delprete 1996a). The resolving of a monophyletic clade with species of these two genera with distinguishing floral morphology is another example of morphological specialization seen in Pacific islands, as in the case of *Thiollierea* and *Morierina*.

Chiococca is a genus of over 20 species occurring throughout the Neotropics, with the center of diversity in Mexico, Central America and the Caribbean Region. Previously, Motley et al. (2005) and Manns and Bremer (2010) pointed out that *Chiococca* is paraphyletic with *Asemnantha* nested in it. Borhidi (2011), based on these results, synonymized *Asemnantha* with *Chiococca* and proposed the new name *C. motleyana* Borhidi. Our results, as expected, support this merging. In addition, by increasing taxa sampling, we were able to retrieve newer relationships within *Chiococca*. In our analyses, we included 17 species of this genus, while previous studies only included six species (including *Chiococca motleyana*). Our results show that *Chiococca* (including *Asemnantha*), as currently circumscribed, is not monophyletic, but is retrieved within a well-supported monophyletic group (subclade D5) together with *Erithalis*, *Salzmannia*, and *Scolosanthus*. All but three species of *Chiococca* (*Chiococca cubensis* Urb., *Chiococca naiguatensis* Steyerm., *Chiococca plowmanii* Delprete) are resolved as a well-supported clade (subclade D5a) in the combined and nuclear trees generated from Bayesian analysis, although in the chloroplast tree, the species in subclade D5a are not resolved as one clade and instead form an unresolved grade with other subclades (D5b--D5e). However, in the parsimony tree, all species in subclade D5a resolve as one clade. In some previous studies (Motley et al. 2005; Manns and Bremer 2010), these four genera were also found in one clade, but not in other studies (Rova et al. 2002; Robbrecht and Manen 2006; Bremer and Eriksson 2009). Within this clade, *Erithalis* is resolved as a monophyletic genus (subclade D5b), corroborating previous results (Negron-Ortiz and Watson 2002; Negron-Ortiz and Watson 2003; Motley et al. 2005; Manns and Bremer 2010).

Two other species of *Chiococca*, *Chiococca plowmanii* (from coastal dunes of Brazil) and *Chiococca naiguatensis* (from coastal cordillera of Venezuela) form a strongly supported clade with the monotypic genus *Salzmannia* (from coastal dunes of Brazil, sympatric with *Chiococca plowmanii*). Motley et al. (2005) found that, despite the morphological similarities between *Salzmannia* and *Chiococca motleyana* (as “*Asemnantha*”), *Salzmannia* was placed closer to the genera with geographical proximity (as *Scolosanthus* and *Erithalis* are from the Greater and Lesser Antilles). In our results too, *Salzmannia* is placed sister to two South American *Chiococca* species (subclade D5c), while the other 14 species of *Chiococca* form a monophyletic clade (subclade D5a). Therefore, we propose to transfer *Chiococca plowmanii* and *Chiococca naiguatensis* to *Salzmannia*.

Scolosanthus is a genus of over 20 species occurring in the Bahamas, and Greater and Lesser Antilles, and we were able to include 12 of them (nine of which were not included in previous studies) in our analyses. Our results further reiterate the monophyly of *Scolosanthus*, as already suggested by Motley et al. (2005). However, *Chiococca cubensis*, endemic to Cuba, is placed as a sister taxon to *Scolosanthus* (subclade D5d) in the combined and nuclear trees as well as in the parsimony tree, while in the phylogeny using the chloroplast dataset it is nested within the *Scolosanthus* clade. *Chiococca cubensis* is morphologically distinct from *Scolosanthus* in having valvate corolla (vs. imbricate corolla) and not having spines (vs. bifurcate or trifurcate spines); in addition, it is distinguished from other species of *Chiococca* in having flowers with a corolla that is purple-brown outside and yellow inside (vs. white, cream-white to pale yellow

throughout in *Chiococca*). Due to distinctions mentioned above, *Chiococca cubensis* is here transferred to the new genus, Genus 5, which is below described.

Taxonomic treatment

According to the present results, following genera are recognized in the tribe

Chiococceae: *Badusa* A. Gray (3 spp.), *Bikkia* Reinw. (11 spp.), *Catesbaea* L. (incl. *Phyllacanthus* Hook. f., 17 spp.), *Ceratopyxis* Hook f. (1 spp.), *Chiococca* P. Browne (19 spp.), *Coutaportia* Urb. (2 spp.), *Coutarea* Aubl. (2 spp.), *Cubanola* Aiello (2 spp.), *Eosanthe* Urb. (1 spp.), *Erithalis* (8 spp.), *Exostema* (Pers.) Rich. (8 spp.), *Hintonia* Bullock (3 spp.), *Isidorea* A.Rich. ex DC. (17 spp.), *Lorencea* Borhidi (1 spp.), *Nernstia* Urb. (1 spp.), *Osa* Aiello (1 spp.), *Phialanthus* Griseb. (22 spp.), *Portlandia* P. Browne (6 spp.), *Salzmannia* DC. (3 spp.), *Schmidtottia* Urb. (incl. *Ceuthocarpus* Aiello, 16 spp.), *Scolosanthus* Vahl (20 spp.), *Shaferocharis* Urb. (3 spp.), *Siemensia* Urb. (1 spp.), *Solenandra* Hook. f. (12 spp.), *Thogsennia* Aiello (1 spp.), *Thiollierea* Montrouz. (incl. *Morierina* Vieill., 12 spp.), Genus 1 (10 spp.), Genus 2 (1 spp.), Genus 3 (1 spp.), Genus 4 (3 spp.), Genus 5 (1 spp.); new synonymies, new genera, and new combinations are presented below.

All the new generic descriptions and new combinations will be formally published separately from this dissertation. Hence new nomenclature is not presented in this dissertation.

Genus 1, gen. nov.

Subshrubs, shrubs, or small trees, up to 15 m tall; stem terete, flat or widened wide below nodes. Stipules interpetiolar, moderately or evidently sheathing at the base, often keeled, triangular, flat, lobes entire (may split upon stem expansion), mostly obtuse or also acutely triangular. Leaves opposite, subsessile to petiolate; blades round to linear, apex commonly acuminate or round. Inflorescences terminal, inserted at distal nodes, or terminal on lateral shoots in distal nodes, cymose, paniculate, usually multiflorous. Flowers perfect, 5-merous, 4--21 cm long, fragrant, homostylous. Calyx campanulate or short-tubular; tube extremely reduced or absent; lobes triangular, subulate, digitate, or deltate, acuminate, commonly shorter than hypanthium. Corolla infundibular, white at anthesis and turning pink to maroon after anthesis, glabrous; tube narrowly cylindrical, longer than lobes, 1.2--16 cm long; lobes narrow, 1.5--5 cm long, narrowly imbricate. Stamens exserted; filaments straight, inserted at the base of corolla tube, glabrous or subglabrous; anthers linear, basifixed. Style filiform, exserted, style branches clavate to capitate. Fruit a woody capsule, subcylindrical to cylindrical or oblanceolate, apically truncate, crowned by the persistent calyx lobes, bilocular, basipetally septicidal, placenta linear, narrowly ellipsoid to lanceolate. Seeds acropetally or centripetally aligned, 6--400 per locule, light tan to brown, elliptic, ovate, oblong, triangular, flattened or polygonal, vertically imbricate, wing entire, dissected or lacking.

Diagnosis: Genus 1 is similar to *Solenandra* because of the terminal inflorescences and narrowly cylindrical corolla tube; the former can be distinguished from the latter by the corollas white during anthesis that turn pink to maroon after

anthesis (vs. turning pale yellow after anthesis), flowers 4--21 cm long (vs. 1--3 cm long), and seeds acropetally or centripetally aligned (vs. basipetal in *Solenandra*).

Distribution: Genus 1 is a genus with 10 species ranging from Cuba, Jamaica to the Lesser Antilles (St. Vincent), growing mostly on serpentine or limestone substrates in moist areas near streams or on rocks in streams of the forests at 0--1100(--1800) m altitude.

Genus 2, gen. nov.

Shrubs or small trees, to 10 m tall; branches terete, widened below nodes, conspicuous white lenticels. Stipules interpetiolar, free at base, lobes triangular, colleters absent or inconspicuous. Leaves opposite or ternate, petiolate; blades narrowly to broadly elliptic, apex acute to acuminate, base acute, round or cuneate. Inflorescence terminal, compound cyme, 10--50-flowered, lateral branches subtended by leaf-like bracts. Flowers perfect, 5-merous, 2.5 -- 3 cm long, very fragrant. Calyx finely strigose pubescent; hypanthium broadly elliptic, 2.5--3 mm long, 2--2.5 mm wide; tube brief to 1 mm long; lobes 5, narrowly triangular to digitate, equal or longer than hypanthium. Corolla infundibular, salverform, 1.6--2.7 cm long, lightly to densely strigose-pubescent, white during anthesis, turning pale yellow with age; tube narrowly cylindrical, 8--13 mm long; lobes narrow, 8--14 mm long. Stamen exserted; filaments at the base of the corolla tube, pubescent at basal portion; anthers linear, basifixed. Style linear, exserted. Fruit capsular, basipetally septicidal, obpyriform to rotundate, strongly compressed laterally, septum narrow, placenta trapezoidal. Seeds vertically imbricate, acrobasispetal arrangement, oval to ovate, winged; wing margin entire.

Diagnosis: Genus 2 is similar to *Solenandra* in having terminal, multiflorous inflorescences, and corollas with a short, narrowly-cylindrical tube, turning pale yellow with age; it differs from *Solenandra* in having acrobasipetal seed arrangement, trapezoidal placenta and strongly laterally compressed capsules (vs. basipetal seed arrangement, hemi-ellipsoidal placenta and slightly compressed capsules in *Solenandra*).

Distribution: Genus 2 is a monotypic genus known from open places and shrublands of the Andes in Peru, at 1000 -- 2800 m altitude, on slopes along streams in both moist and dry areas.

Genus 3, gen. nov.

Tree, 7--30 m tall; branches terete or laterally compressed, wider below nodes. Stipules interpetiolar, sheathing, 4--6 mm long, lobes obtuse, glabrous, colleters as a basal fringe. Leaves opposite, petiolate; blades elliptic to ovate, apex acuminate, base rounded to briefly acute. Inflorescence terminal, in upper axils on lateral shoots, compound cyme, many-flowered; bracts subtending secondary branches, leaf-like, elliptic. Flowers perfect, 6-merous, 10--12.5 cm long, fragrant. Calyx obconical, 4--6 mm long, deeply lobed; tube 1--2 mm long; lobes broad, apex obtuse, tip mucronate, shorter than hypanthium. Corolla infundibular, white at anthesis, turning pink to maroon with age; tube narrowly cylindrical, 5.5--6.5 cm long; lobes 6, 4.5--5.5 cm long, narrow, narrowly imbricate in bud. Stamens exserted; filaments inserted at the base of corolla tube, pubescent at basal portion; anthers linear, basifixed. Style exserted, filiform, clavate to subcapitate. Fruit a woody capsule, laterally compressed, obovate in outline, basipetally septicidal, septum narrow, placenta trapezoidal. Seeds many, vertically

imbricate, acrobasipetally aligned, light brown, dorsoventrally flattened, winged; wing concentric, elliptic to oblong in outline, entire.

Diagnosis: Genus 3 is similar to Genus 1 in having terminal inflorescence, with narrowly cylindrical corolla tube, corollas that turn pink to maroon with age; the former differs from the latter in having 6-merous flowers (vs. 5-merous), laterally compressed capsules (vs. not flattened), and seeds acrobasipetally arranged (vs. acropetally or centripetally arranged).

Genus 3 is also similar to Genus 2 in having terminal inflorescence, laterally compressed capsule and acrobasipetal seed arrangement; the former differs from the latter in that it is large tree (vs. shrub), 6-merous flowers (vs. 5-merous), and corolla that turn pink to maroon with age (vs. turning pale yellow), and by being a tall tree that grows in the lowland or at small elevation in the western Amazon Basin (vs. shrub growing in open places and shrublands of the Andes, at 1000 -- 2800 m altitude).

Distribution: A monotypic genus known from western Amazonian lowlands and uplands and eastern slopes of the Andes from Bolivia to Ecuador, at 120--500 (--1100) m altitude, in swampy and upland forests.

Genus 4, gen. nov.

Shrubs; branches laterally compressed or terete, puberulent to glabrous, with lateral short shoots. Stipules interpetiolar, shortly fused, broadly triangular to ovate, puberulent to glabrescent, persistent. Leaves opposite, subsessile to petiolate; blades lanceolate to ovate, base acuneate to truncate or cordate, apex acute to obtuse, glabrous. Inflorescence terminal or axillary, at stem apices or uppermost leaf axils, subfasciculate,

subumbellate or cymose, 1--4-flowered; bracts linear to elliptic or foliaceous, sometimes with glandular margins. Flowers perfect, 5--7-merous, actinomorphic. Calyx deeply lobed; lobes 5--7, narrowly triangular to spatulate or subulate to lanceolate. Corolla tubular to funnelform or slightly inflated, pink to red, glabrous; lobes 5--7, obtuse to rounded, imbricate in bud. Stamens 5--7, included or partially exerted; filaments inserted at base of corolla, glabrous, sometimes puberulent at basal portion; anthers narrowly oblong. Style glabrous, style branches 2, ovate to oblong. Fruit capsular, laterally flattened, ellipsoid to obovate in outline, septicidal from apex, crowned by the persistent calyx lobes, placentation axile. Seed flattened, winged, elliptic, oblong or suborbicular in outline.

Diagnosis: Genus 4 is similar to *Coutarea* in having septicidal capsules, winged seeds, axile placentation, and tubular to broadly funnelform corolla. The former differs from the latter in having actinomorphic (vs. zygomorphic in *Coutarea*), pink to red corollas (vs. white, pink, violet to purple), anthers included or partially exerted (vs. exerted), and by occurring in dry vegetation at high elevations of the Andes above 1900 m altitude (vs. distributed throughout the Neotropics from lowland to low elevations).

Distribution: Genus 4 is a genus of three species occurring in the Andean region of Ecuador and Peru above 1900 m altitude in the dry forests, scrub vegetation, with limestone substrates.

Genus 5, gen. nov.

Shrub; branches scandent, glabrous, terete; young branches slightly resinous; basal internodes may be compressed below nodes. Stipules short, basally connate,

persistent. Leaves opposite, petiolate; blades chartaceous to coriaceous, ovate to oblong. Inflorescence axillary, paniculate, pedunculate, many-flowered; bracts small, lanceolate or triangular. Calyx tube narrowly obovoid, margin undulate or denticulate. Corolla funnelform, 4-merous, purple-brown outside, yellow inside; tube 10--11 mm gradually tapering to the base; lobes short, one fourth the length of the tube, ovate, obtuse at tip, narrowly imbricate in bud valvate. Stamens included; filaments adnate to the base corolla tube, lower half pubescent; anthers linear. Style filiform, apex slightly thickened, longer than stamen, obsolete bilobate. Fruit drupaceous, obovoid, with two pyrenes.

Diagnosis: Genus 5 is distinguished from *Chiococca* in having corollas purple-brown outside and yellow inside (vs. white, cream-white to pale yellow throughout in *Chiococca*).

Distribution: Genus 5 is known from thickets and pinelands of Oriente, Cuba.

CHAPTER 4

BIOGEOGRAPHY OF TRIBE CHIOCOCCEAE: ORIGIN, DIVERSIFICATION AND DISJUNCT DISTRIBUTION

INTRODUCTION

Chiococceae sensu Paudyal et al. (2014) is primarily a Neotropical tribe with its highest diversity in the Greater Antilles, ranging from Mexico, Central America, and in the Caribbean region from southern Florida, the Bahamas, the Lesser Antilles, with several species widespread throughout South America (including a few local, endemic species). In addition, three genera of the tribe, *Badusa*, *Bikkia*, and *Thiollierea* (including *Morierina*), are distributed in the West Pacific islands ranging from the Philippines, Marianas to the Melanesia and all the way to Tonga (Motley et al. 2005; Govaerts et al. 2014). While *Badusa* and *Bikkia* are widely distributed, *Thiollierea* is endemic to New Caledonia. Over 70% of the species diversity of *Chiococceae* occurs in the Greater Antilles, nearly 12% of the species are distributed in the West Pacific islands, another 15% in Mexico, Central America and South America and the remaining species are found in other islands in the Caribbean region (Motley et al. 2005; Govaerts 2014). World distribution of tribe *Chiococceae* is presented in Fig. 8.

Trans-oceanic dispersals in plants have been known for a long time, and various authors (Raven 1972; Thorne 1972; Wen 1999; McCarthy 2003; Givnish and Renner 2004; Milne 2006) have discussed examples of major trans-oceanic disjunctions in the plant world. “Amphi-Pacific tropical” distribution patterns primarily include the discontinuous range of the plant groups found both in tropical America as well as the

tropical lands of the western borders of the Pacific basin (Thorne 1972). Many studies deal with amphi-Pacific tropical disjunctions in plants at family or lower taxonomic levels (Thorne 1972; Van der Hammen and Cleef 1983; Tan 1998; Heads 1999; Qian 1999; Fritsch 2001; Howarth et al. 2003; Chung et al. 2005; Heads 2010; Li et al. 2011; Woo et al. 2011; Li and Wen 2013, Fritsch et al. 2014). In total there are over 100 genera and higher taxa of flowering plants that have amphi-Pacific tropical distribution (Thorne 1972; Fritsch et al. 2014)

Rubiaceae is one of the largest plant families, with ca. 13600 species distributed in all continents (Govaerts et al. 2014). Previous studies (Negron-Ortiz and Watson 2002; McDowell et al. 2003; Nie et al. 2005; Achille et al. 2006; Wikstrom et al. 2010; Manns et al. 2012; Huang et al. 2013; Nie et al. 2013; Tosh et al. 2013) discussed the biogeographic histories of trans-oceanic disjunctions and trans-oceanic dispersals in some of the Rubiaceae taxa. Chiococceae is an example of amphi-Pacific tropical disjunction between the Neotropical and the West Pacific genera. Those species in the West Pacific islands occur only to the west of the Andesite line, which corresponds with the edge of the Pacific plate. With no species distributed on the Pacific plate (although *Bikkia tetrandra* ranges eastwards up to Niue Island), Chiococceae has a very interesting amphi-Pacific tropical disjunction between the South Pacific and the American taxa. To our knowledge, the only other group in Rubiaceae that shares a similar amphi-Pacific tropical disjunction is the genus *Augusta* (Delprete 1997; Kirkbride 1997; Motley et al. 2005). Thus, understanding the biogeographic history of Chiococceae may enable us to clarify many evolutionary events, not only within this tribe, but hopefully also in other groups.

(a)



(b)

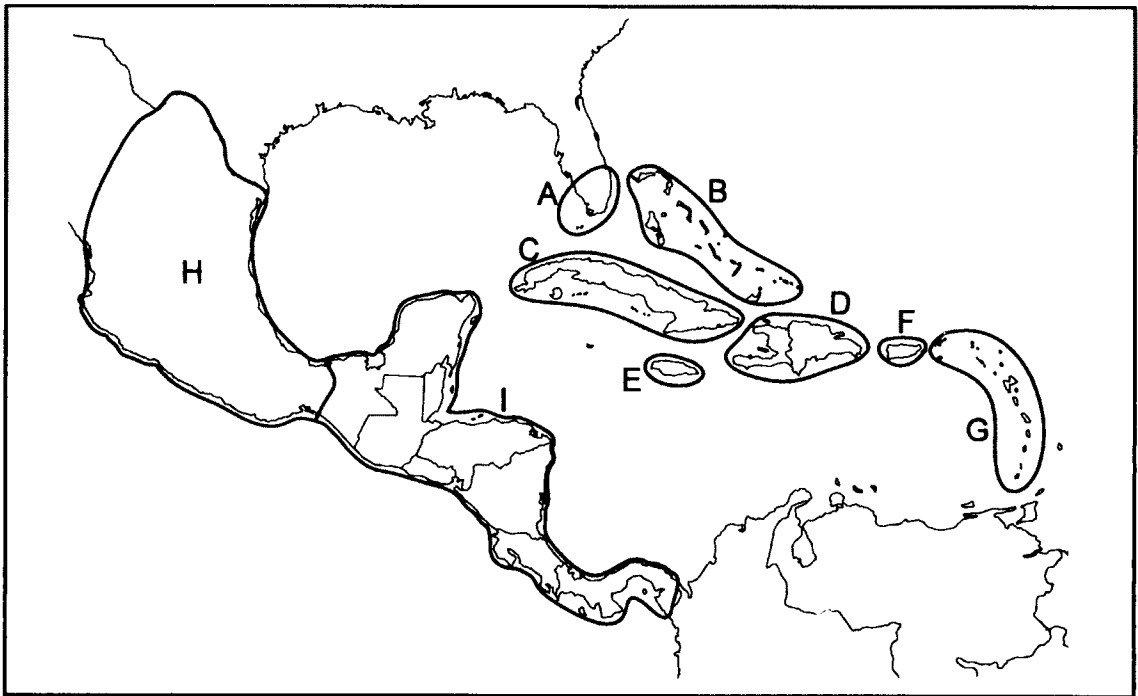


Fig. 8 continued.

(c)



Fig. 8 World distribution of Chiococceae. (a) distribution shown in world map; (b) Distribution in Mexico, Central America and the Caribbean islands; (c) Distribution in South America. Geographic regions are indicated by capital letters; A: Florida Keys and Continental USA, B: Bahamas and adjoining islands, C: Cuba, D: Hispaniola, E: Jamaica, F: Puerto Rico, G: Lesser Antilles, H: Northern and Central Mexico, I: Southern Mexico and Central America, J: Atlantic coastal region, K: Orinoco-Amazon basin, L: Amazon Piedmont region, M: Andean region, N: Western Pacific Islands except New Caledonia, O: New Caledonia.

One of the more common explanations for trans-oceanic disjunctions, especially between continents of the southern hemisphere, is commonly attributed to the existence of the Gondwana super continent, and its subsequent break up. Vicariance as a result of the break up and subsequent movement of landmasses to the present day positions would have led to the disjunction in distribution of many extant taxa. In the past, vicariance was mainly sought as the explanation for intercontinental disjunctions (Zhou et al. 2006). However, with advanced techniques to generate and analyze molecular data, molecular dating has enabled us to make hypotheses and estimate an age on a divergence event on a phylogeny; and in turn have also shown that many plant groups with southern intercontinental disjunction may have actually diverged at much more recent times than what was previously considered to have arisen as a result of Gondwanan vicariance. Molecular dating therefore plays an important role towards the understanding of ancestral areas, as phylogenies using extant plant taxa alone may give misleading results (Milne 2006) in hypothesizing the historical biogeography of certain groups. The Gondwana continental break up occurred during the Late Cretaceous to Early Eocene (54--49 Mya), and New Caledonia fully separated from Australia around 65 Mya (Morley 2003; Neall and Trewick 2008). Recent molecular dating analyses have estimated the divergence time of the tribe Chiococceae differently (19.2 Mya, Antonelli et al. 2009; 34.4 Mya, Bremer and Eriksson 2009; 43.1 Mya, Manns et al. 2012). Although there is a considerable difference in dating estimations, these estimated divergence times of the tribe, as well as inference to a more recent evolution of the West Pacific genera, indicate that the West Pacific-Neotropical disjunction of the Chiococceae is not a result of Gondwanan vicariance.

Long distance dispersal (LDD) and dispersal via land bridges have been suggested as alternate hypotheses of trans-oceanic dispersals that may have resulted in the disjunction in distribution of extant plant groups. LDD can occur when a single propagule is carried across a barrier by water, wind, or some animal vector and successfully establishes itself into a new population. With known examples of birds accidentally travelling long distances beyond their migratory ranges across the Atlantic ocean (Milne 2006), it can be expected that rare events of successful establishment of a seed carried between continents would have resulted in disjunctions in many of the later diverged plant groups. However, the distance between tropical America and the South Pacific seems too large for any migratory birds to be the dispersal vector within the tribe.

As stated earlier, the tribe Chiococceae is predominantly Neotropical in distribution and has the highest endemism and diversity in the Greater Antilles. Based on the species richness of the Chiococceae, Motley et al. (2005) considered the Greater Antilles as the center of origin for the tribe. However, it may not be appropriate to consider the most species-rich region as the center of origin of a group, primarily because the higher diversity could be a result of lower extinction rates, or higher speciation rates (Milne 2006). The Caribbean region is one of the hotspots of endemism that may have resulted due its close proximity to the continental Neotropics to its south, west and north (Santiago-Valentin and Oldmstead 2004). The complex geological and environmental history of the Caribbean Region (Iturralde-Vinent and McAfee 1999) has led to debates on whether vicariance or dispersal was vital in establishing the current distribution patterns of plants and animals in this region (e.g., Fritsch and McDowell 2003; Ali 2012).

Only a few studies have attempted to provide an explanation for the biogeography of the Chiococceae taxa. Motley et al. (2005) had the largest number of taxa sampling until then, and their study focused on the Chiococceae. However, they were unable to provide definitive conclusions about the origin and dispersal of the group mostly due to the lack of resolution among major lineages in their phylogenetic tree, and, because of this, they did not perform any biogeographical analysis. However, from their preliminary results, Motley et al. (2005) concluded a Neotropical origin of the tribe, with one or two long distance dispersals to the West Pacific. Recently, in the ancestral area reconstruction of the subfamily Cinchonoideae using molecular dating and dispersal vicariance analysis, Manns et al. (2012) inferred Central America as the center of origin of the Chiococceae and dispersal to the Caribbean during the Oligocene-Early Miocene. Despite incomplete sampling of the Chiococceae taxa (probably due to the scope of the study), their results inferred back dispersals to Central America and additional dispersal events, at later times, from Central America to the Caribbean islands and to South America. Earlier in a study of the genus *Erithalis*, Negron-Ortiz and Watson (2003) suggested the Greater Antilles, in particular Jamaica, as the most recent ancestral area of the genus, which differed from their own earlier assertion (Negron-Ortiz and Watson 2002) that *Erithalis* originated from Central America. In both studies, they suggested that *Erithalis* colonization of the Caribbean occurred relatively recently by a combination of vicariance and dispersal; possibly by water but mostly by birds as the fruits of *Erithalis* are small drupes eaten by birds.

Based on phylogenetic analyses, using molecular and morphological datasets, McDowell and Bremer (1998) suggested that *Exostema* (*sensu* McDowell 1996)

originated in South America. Their conclusion was primarily based on the placing of the two South American species (*Exostema corymbosum* and *Exostema maynense*) basal to Caribbean species, which most probably was the result of using the closely related species, *Coutarea hexandra*, as the outgroup in their phylogenetic analyses. However, upon using more outgroups in their phylogeny, McDowell et al. (2003) could not support the South American origin hypothesis, and could only conclude that the distribution of Caribbean species primarily involved wind dispersal. However, *Exostema*, as delimited by McDowell (1996), was later shown to be polyphyletic (Motley et al. 2005; Manns and Bremer 2010), and two groups of species have since then been segregated as three new genera; the genus *Exostema*, as newly delimited, is restricted to the Caribbean region (S. Paudyal unpubl. data).

Historical biogeographical reconstructions using phylogenies and molecular dating play a very important role in constructing evolutionary hypotheses about the geographical distribution of plant groups (Milne 2006; Ali et al. 2012). To do so, it is essential to have a phylogeny with well-resolved nodes in order to determine the area of origin (Motley et al. 2005), and direct fossil calibration in molecular dating (Milne 2006). It is also very important to have adequate taxa sampling in molecular dating as error in inference methods increases with the distance of a node from the calibration point, thus not accurately dating nodes that are phylogenetically not close to fossil calibration (Antonelli et al. 2009; Milne 2009). Taxa sampling was greatly expanded during our work on the molecular phylogeny of the Chiococceae (S. Paudyal unpubl. data; chapter 3), and we obtained a highly resolved and well supported phylogeny of the tribe. This data provided essential components for understanding evolutionary history of the group.

The primary aim of this study is to reconstruct the historical biogeography of the tribe Chiococceae using molecular dating and statistical dispersal-vicariance analyses. More specifically, our goal is to address the following questions: 1) When and where did the Chiococceae originate? 2) What are the major dispersal events within the tribe and when did they occur? 3) How did the amphi-Pacific tropical disjunction in Chiococceae arise? 4) Are previous hypotheses on origin and dispersal of Chiococceae taxa, in particular *Exostema* and *Erithalis*, supported?

MATERIALS AND METHODS

Taxon sampling

In this study, sampling generally follows earlier study on the molecular phylogeny of the tribe Chiococceae (S. Paudyal unpubl. data; chapter 3) in which all the phylogenetic relationships within the tribe were discussed and also proposed new generic delimitations. For this study, primarily the same molecular dataset was used. However, to suit the scope of this paper, only one accession per species were included in the present analyses. A total of 126 species were included in the analyses and 27 out of the 29 genera of the Chiococceae *sensu* Paudyal et al. (2014) were sampled. The two east Cuban endemic genera *Shaferocharis* and *Thogsennia* were not included due to unavailability of any molecular sequence data since no recent collections of either genus was made. A list of genera of the Chiococceae, their distribution and number of species sampled in this study is presented in Table 5. For all sequences used in analyses, the details of voucher information, and distribution are provided in Appendix D.

Phylogenetic analysis

DNA sequence data from two nuclear (ETS and ITS) and two chloroplast regions (*petD* and *trnL-F* intron and spacer) were used for the present analyses. To align the sequences, online alignment software, PRANK (Loytynoja and Goldman 2005) was used followed by visual alignment using MacClade version 4.08 (Maddison and Maddison 2005) and Mesquite version 3.01 (Maddison and Maddison 2014). The sequences of all four regions were concatenated and those datasets were used for all the analyses with each partition indicated appropriately. Concatenated datasets were analyzed using Markov chain Monte Carlo (MCMC) methods within a Bayesian framework to obtain general topology and posterior probability of trees. Bayesian inference analyses were performed using MrBayes 3.2 (Ronquist et al. 2012) online in CIPRES Science gateway (Miller et al. 2010). The best-fit model of nucleotide substitution for each of the four partitions was evaluated with Bayesian Information Criterion (BIC) using the program jModelTest version 2.1.3 (Darriba et al. 2012). The models were Hasegawa-Kishino-Yano model (HKY) for ETS and *trnL-F* regions and general time reversal model (GTR) for ITS and *petD* regions; all four regions having gamma distribution as substitution rates of nucleotides in all regions. The MCMC chains were run for 10 million generations with trees sampled every 1000 generations and 25% of trees discarded. Remaining trees were used to construct a 50% majority rule consensus tree with posterior probability distribution.

Table 5. Species diversity and world distribution of the tribe Chiococceae. For each genus, total number of species and the number included in this study are listed along with the number of species of the genus in each geographic region (Fig. 8). A: Florida Keys and Continental USA, B: Bahamas, C: Cuba, D: Hispaniola, E: Jamaica, F: Puerto Rico, G: Lesser Antilles, H: Northern and Central Mexico, I: Southern Mexico and Central America, J: Atlantic coastal region, K: Orinoco-Amazon basin, L: Amazon Piedmont region, M: Andean region, N: Western Pacific Islands except New Caledonia, O: New Caledonia.

Genera (sensu Paudyal et al. 2014)	Total number of species	Species included in this study	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
<i>Badusa</i> A. Gray	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
<i>Bikkia</i> Reinw.	11	4	-	-	-	-	-	-	-	-	-	-	-	-	-	11	-
<i>Catesbaea</i> L.	16	10	1	3	9	6	1	2	2	-	-	-	-	-	-	-	-
<i>Ceratopyxis</i> Hook.f.	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ceuthocarpus</i> Aiello	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chiococca</i> P. Browne	23	17	2	4	2	1	1	1	1	11	13	5	9	1	3	-	-
<i>Coutaportia</i> Urb.	2	2	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
<i>Coutarea</i> Aubl.	5	3	-	-	-	-	-	-	-	1	1	1	1	1	3	-	-
<i>Cubanola</i> Aiello	2	2	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
<i>Eosanthe</i> Urb.	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Erithalis</i> P. Browne	8	6	1	5	3	4	4	2	4	1	1	-	2	-	-	-	-
<i>Exostema</i> (Pers.) Rich.	20	12	1	1	10	11	3	1	2	1	1	-	1	1	1	-	-
<i>Hintonia</i> Bullock	3	2	-	-	-	-	-	-	-	2	3	-	-	-	-	-	-
<i>Isidorea</i> A. Rich. ex DC.	17	10	-	-	9	8	-	-	-	-	-	-	-	-	-	-	-
<i>Lorencea</i> Borhidi	1	1	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-

Table 5 continued.

Genera (sensu Paudyal et al. 2014)	Total number of species	Species included in this study	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
<i>Morierina</i> Vieill.	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
<i>Nernstia</i> Urb.	1	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
<i>Osa</i> Aiello	1	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
<i>Phialanthus</i> Griseb.	22	9	1	1	17	2	3	1	-	-	-	-	-	-	-	-	-
<i>Phyllacanthus</i> Hook.	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Portlandia</i> P. Browne	6	6	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-
<i>Salzmannia</i> DC.	1	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
<i>Schmidtottia</i> Urb.	15	7	-	-	15	-	-	-	-	-	-	-	-	-	-	-	-
<i>Scolosanthus</i> Vahl	20	12	-	1	14	8	2	2	1	-	-	-	-	-	-	-	-
<i>Shaferocharis</i> Urb.	3	0	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
<i>Siemensia</i> Urb.	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Solenandra</i> Hook.f.	12	5	-	-	11	2	-	-	-	1	1	-	-	-	-	-	-
<i>Thogsennia</i> Aiello	1	0	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Thiollierea</i> Montrouz	10	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Total	210	126	6	15	100	43	20	9	10	21	22	7	13	3	7	14	12

Estimation of divergence times

Divergence times were estimated using Bayesian framework as implemented in BEAST 1.8.0 package (Bayesian evolutionary analysis sampling trees; Drummond et al. 2007) online in CIPRES Science gateway (Miller et al. 2010). The concatenated dataset partitioned into four partitions (ETS, ITS, *petD*, and *trnL-F*) and the best models of molecular evolution as stated above were used. Analyses were run for 50 million generations, sampling every 5 thousand generations. The output was evaluated using Tracer 1.6 (Rambaut & Drummond 2014) for adequate effective sample size (ESS) values. The output trees were combined in TreeAnnotator version 1.8.0 (part of BEAST package) with 25% of trees discarded as burn-in and posterior probability set at 90% to retrieve a maximum clade credibility tree.

BEAST analysis was performed using fossils to calibrate the lineages within the phylogeny against geological time. Two fossils were used to constrain ages of nodes within the phylogeny. *Exostema precaribaeum* is a fossil from the Miocene and is considered closest to *Exostema caribaeum*, an extant species and also the type species for the genus. It has been estimated to be from any time between 5.1 Mya to 30 Mya (Graham 2010). This fossil was used to constrain the basal node of the *Exostema* clade that includes *Exostema caribaeum*. Another fossil used for calibration was also from the Miocene. Leaf fossil of *Chiococca* from Mint Canyon flora is not associated with any extant *Chiococca* species. The estimated age of this fossil is between 4.1 Mya and 25 Mya (Graham 2010). This fossil has not been associated with any particular extant species of *Chiococca*. *Chiococca* was found to be polyphyletic in our molecular phylogenetic study and new generic combinations were proposed (S. Paudyal unpubl.

data; chapter 3). Considering this taxonomic ambiguity, this fossil was used to constrain the basal node of the clade that includes all extant species that were previously included in the genus. In dating analysis using fossil calibration, minimum age of a dated fossil is assigned to a node (Milne 2006); this in turn will offer the minimum age of each node. Since more definitive ages have not yet been determined for the Chiococceae fossils, the lowest age from the given range was considered as the minimum possible age of the fossil. Hence, to constrain the nodes, 5.1 Mya and 4.1 Mya were used as the minimum ages of the fossils of *Exostema precaribaeum* and *Chiococca* respectively.

Biogeographical analyses

To infer ancestral areas and historical biogeography of the tribe Chiococceae, two analytical methods were used, statistical dispersal-vicariance analysis (S-DIVA), and Bayesian binary MCMC analysis (BBM), as implemented in the software RASP v. 3.0 (Reconstruct Ancestral State in Phylogenies; Yu et al. 2014). To account for potential skewed output due to taxa distributed throughout the Neotropics, separate analyses were also performed, where the very widely distributed taxa were excluded from the dataset.

Trees generated from the phylogenetic analysis were used in the S-DIVA and BBM analyses. A total of 10,000 binary trees obtained from the MCMC output were used for the analyses. To generate a condensed tree, 1000 random trees were used. The number of maximum areas in each node was set at 2. A single tree with possible ancestral areas was obtained. BBM analysis was performed with five million MCMC generations with trees sampled every 1000 generations using settings set at default values as given in the RASP software.

The geographic distribution of the tribe Chiococceae was divided into 15 areas. These divisions were based on the occurrence of endemic species and primarily followed Motley et al. (2005). Considering the greater diversity of Chiococceae in the Caribbean islands, larger islands and island groups were treated as separate geographical areas, as centers of endemism, for a refined understanding of the distribution routes within the Caribbean region. South America was divided into four regions in view of a number of new genera proposed (S. Paudyal unpubl. data; chapter 3). The distribution data for each taxon were assigned based on the distributions reported in the World checklist of Rubiaceae (Govaerts et al. 2014) and locality information in herbarium collections.

RESULTS

Phylogenetic analysis

The aligned matrix is comprised of 3556 characters, 262 of which were excluded in order to maintain uniformity in the lengths of sequences in the dataset analyzed. A total of 3294 characters (ETS: 491, ITS: 688, petD: 1135, trnL-F: 980) were included in the analyses, of which 2342 characters were constant and 952 characters were variable. The final data matrix consisted of 635 characters (19%) that were parsimony informative.

The general topology and support values of the phylogenetic trees generated from Bayesian analysis carried out in MrBayes and BEAST are highly similar. In the 50% majority rule consensus tree generated from Bayesian analysis of the concatenated dataset using MrBayes (phylogenetic tree in Appendix E), Chiococceae is resolved into four highly supported major clades (PP \geq 0.95). More than 80% of all nodes in the tree are well supported (PP \geq 0.9). Of the less-supported nodes, two-thirds are at intrageneric

level while the remaining few represent intergeneric relationships. The phylogenetic relationships in the output of BEAST analyses show some incongruency at basal nodes. The basal *Coutaportla-Lorencea* clade (Clade A, Appendix E), is placed sister to clade C in the BEAST tree, albeit with very weak support (PP = 0.39). In fact, all basal nodes between four major clades from Bayesian analysis are weakly supported in the maximum clade credibility tree from BEAST analysis (PP < 0.65). Any inconsistencies in the phylogenetic relationships between the two analyses, which may have potential implications on historical biogeography, are discussed later while discussing historical biogeography.

Divergence time estimate

The maximum clade credibility tree generated from BEAST analysis and the estimated divergence times are presented in Fig. 9. Except for the incongruences noted earlier, maximum clade credibility tree generated from BEAST analysis was generally similar to the 50% majority rule consensus tree generated from the Bayesian analysis. Most nodes were well resolved and aptly supported. According to our results, Chiococceae originated in the Eocene when it diverged from sister tribe Strumpfieae, ca. 47.8 Mya. However, the Chiococceae crown group divergence occurred much later, in the Late Oligocene-Early Miocene, and by the end of Miocene most genera had already diverged. Two separate divergence events of the West Pacific genera occurred in the Mid Miocene at around 10.1 Mya and 15.4 Mya. Among the Neotropical species, speciation within the island groups occurred more recently than within the continental taxa, mostly in the last four million years.

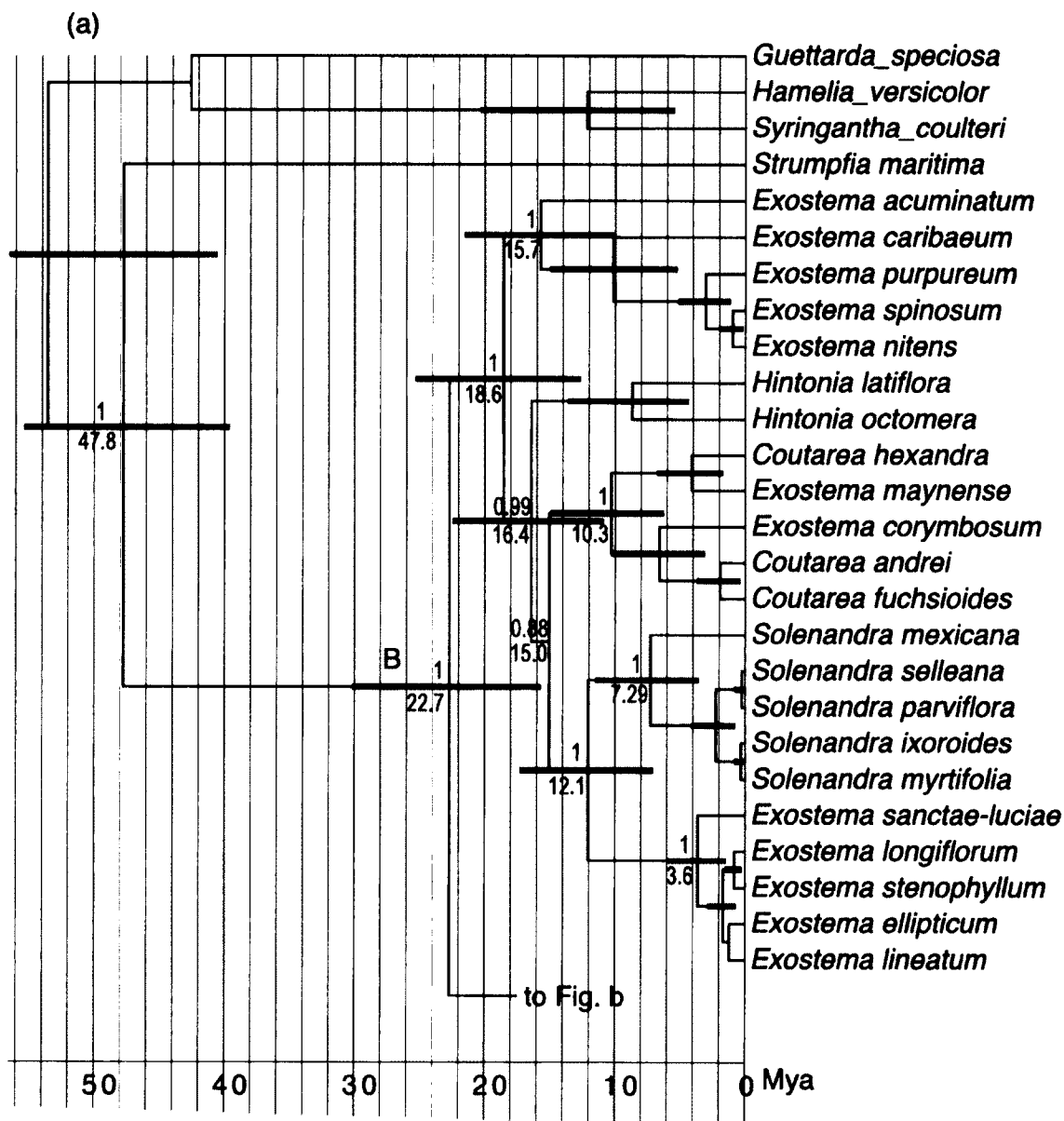


Fig. 9 continued.

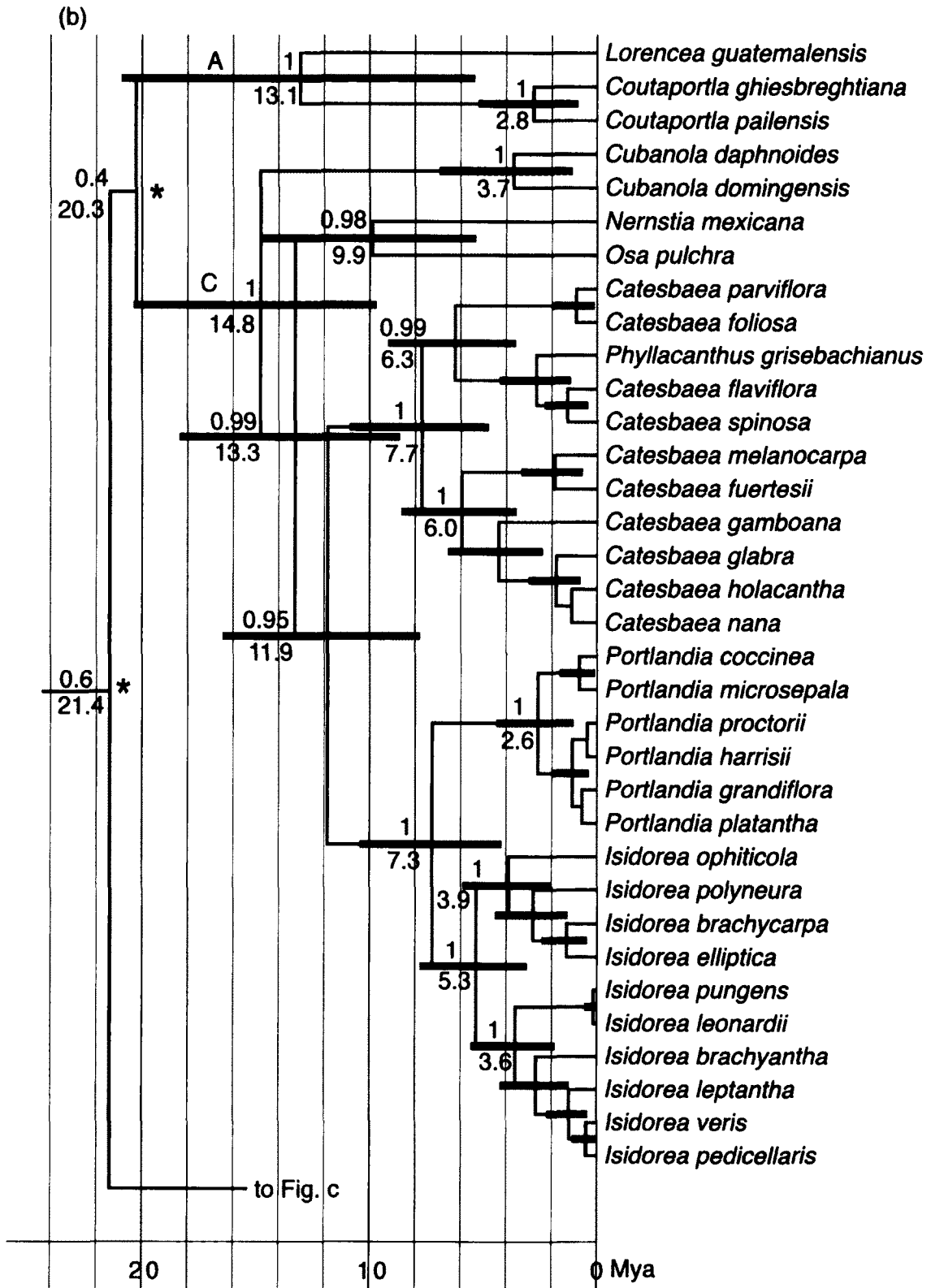


Fig. 9 continued.

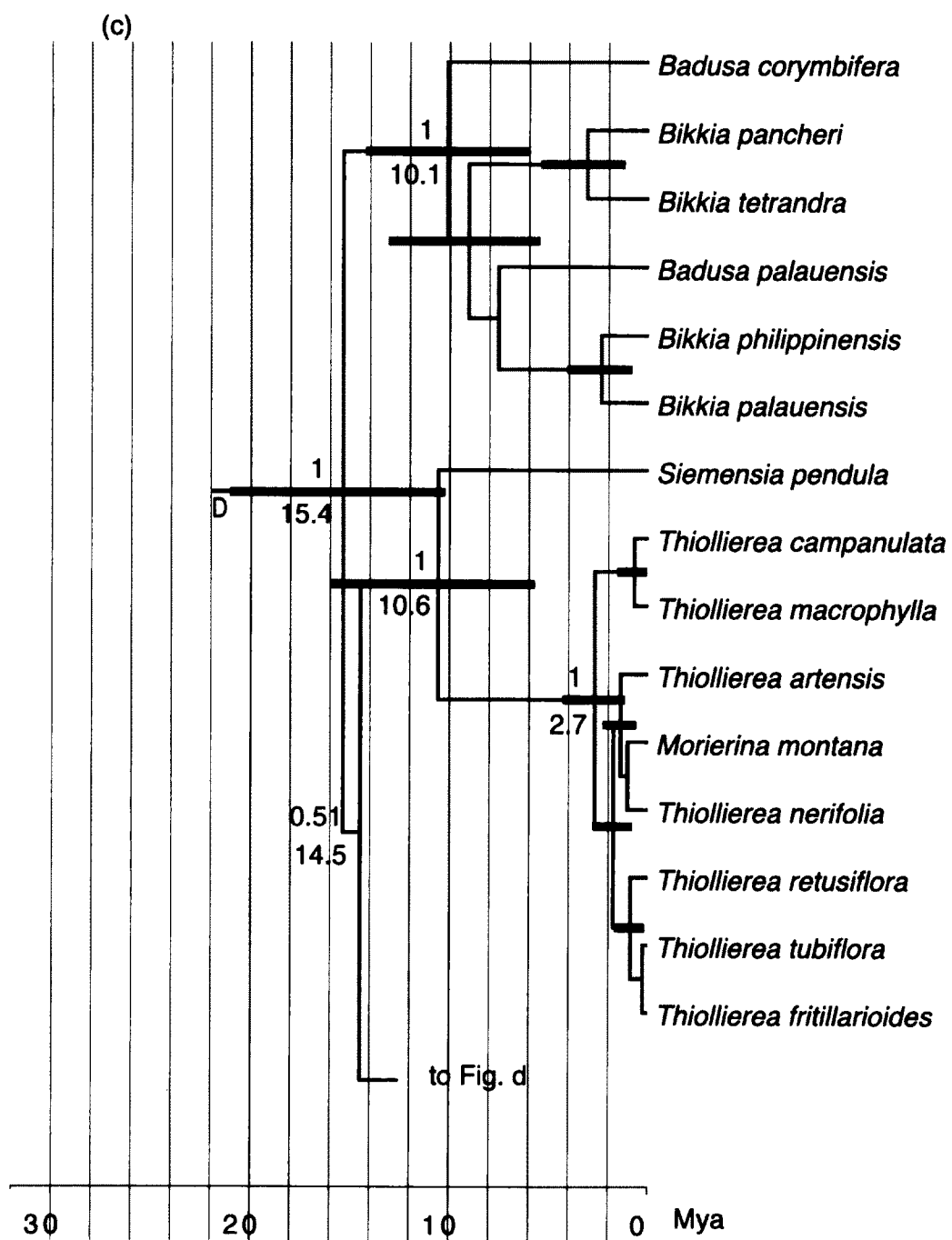


Fig. 9 continued.

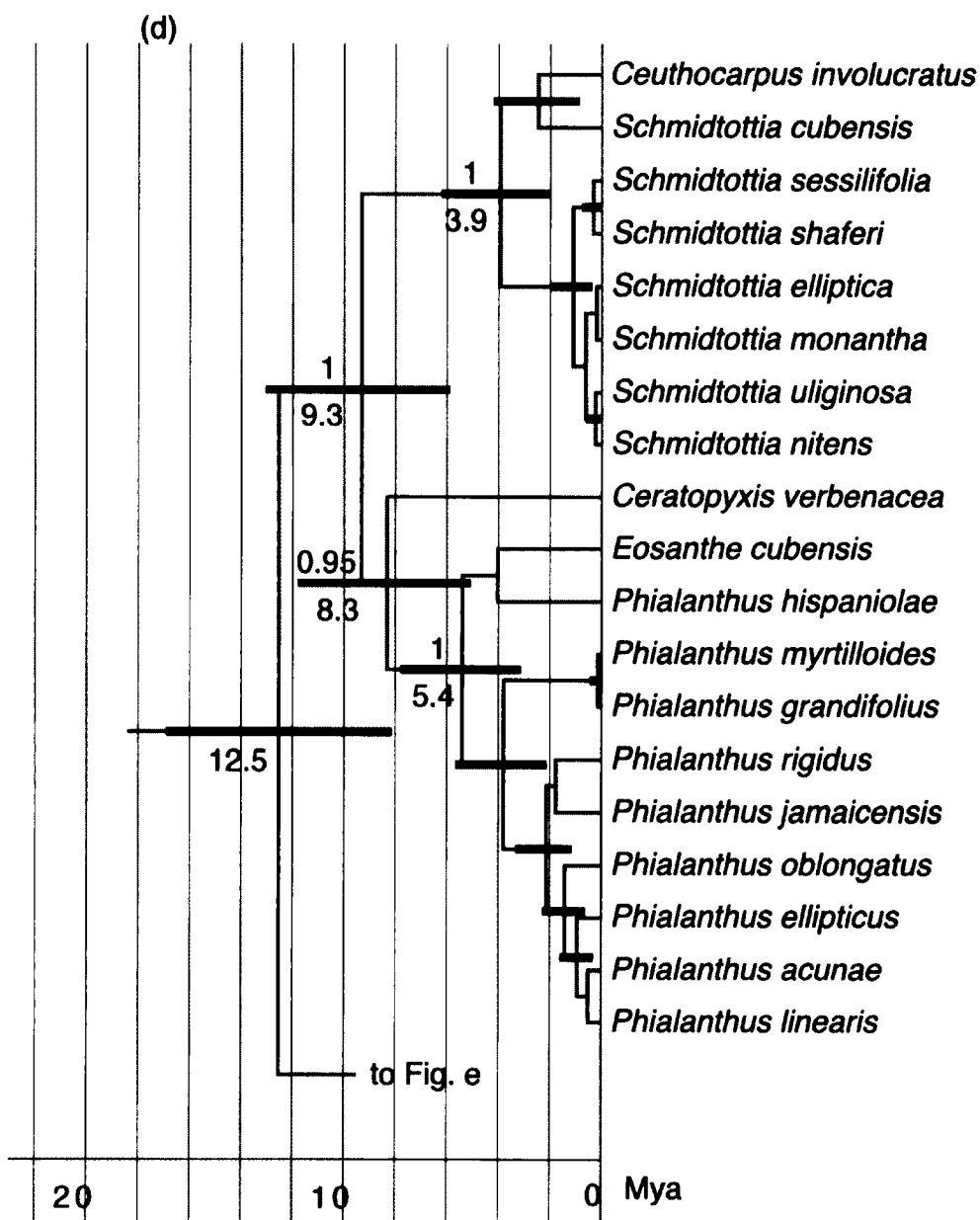


Fig. 9 continued.

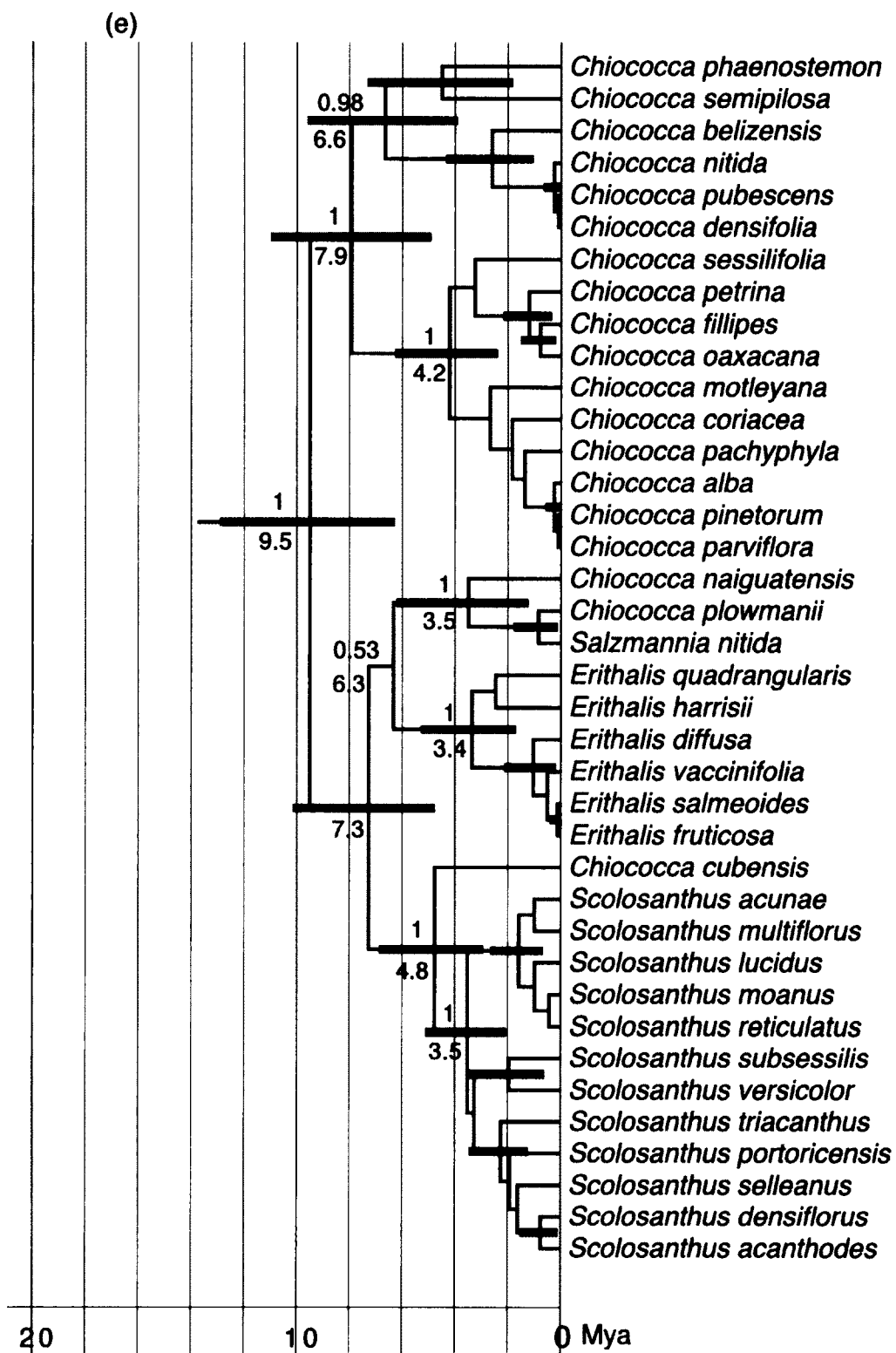


Fig. 9 Maximum clade credibility tree from the BEAST analysis. The 95% HPDs of age estimates are shown as node bars only for the nodes with posterior probabilities above 90%. Posterior probability values and estimated mean age are mapped for the major nodes of biogeographic implications. The number above each branch represents posterior probability value and the number below the branch represents estimated mean age of nodes.

Biogeographical analysis

The results of the S-DIVA and BMM analyses suggest a complex biogeographical history of the Chiococceae. Dispersal, vicariance, and extinction events have all played important role in establishing the current distribution. The results of S-DIVA and BBM analyses as implemented in RASP are presented in Fig. 10. Ancestral area reconstructions in S-DIVA and BBM analyses generally showed congruency. However, the relative probability values differed. Out of the 128 ingroup nodes in the tree, 115 nodes had ancestral area reconstruction supported by more than 50% probability and 83 nodes had ancestral area reconstructions supported by at least 90% probability in S-DIVA analysis, while in the BBM analysis, 109 and 78 nodes had more than 50% and 90% probabilities respectively. Out of a total of 128 nodes optimized, S-DIVA postulated 88 key nodes associated with biogeographical events, while the BBM analysis identified 63 nodes. At node 261, Chiococceae diverges from its sister tribe Strumpfieae. The ancestral area at node 261 is Mexico with marginal probability support of 100% in S-DIVA and 52% in BBM analyses. Both analyses suggest that the ancestors of Chiococceae originated in the Mexican region.

All the members of the tribe Chiococceae diverged from node 260 with the possible ancestral area as Mexico and Cuba with a marginal probability of 100% in S-DIVA analysis; the BBM analysis, however, postulates 64% probability of Mexico. Our results also suggest 8 dispersal events at this node. *Coutaportla* and *Lorencea* are the descendants of the Mexican lineage at node 260 and the second lineage dispersed to Cuba. Subsequently the Cuban lineage diversified extensively and spread in the Caribbean Region, along with multiple back dispersals to Mexico. Back dispersal to

Mexico and Central America occurred at various times throughout the Miocene, and is reconstructed for the ancestors of *Chiococca* (~9.5 Mya), *Hintonia* (~16.5 Mya), *Nernstia-Osa* (~13.3 Mya) and one species of *Solenandra* (*S. mexicana*; ~7.3 Mya). Dispersal of *Chiococca* species to South America occurred very recently via Central America around 2.6 Mya (Fig. 10 node 222). However, our results suggest that ancestors of *Salzmannia* (node 253) and most probably also the ancestors of *Coutarea* and two species of *Exostema* (node 154) reached South America directly from the Caribbean islands (node 253) through two independent introductions at around 6.3 Mya and 15.4 Mya respectively. Additionally, our results suggest two independent trans-oceanic dispersals (nodes 212 and 218) to the West Pacific from Cuba.

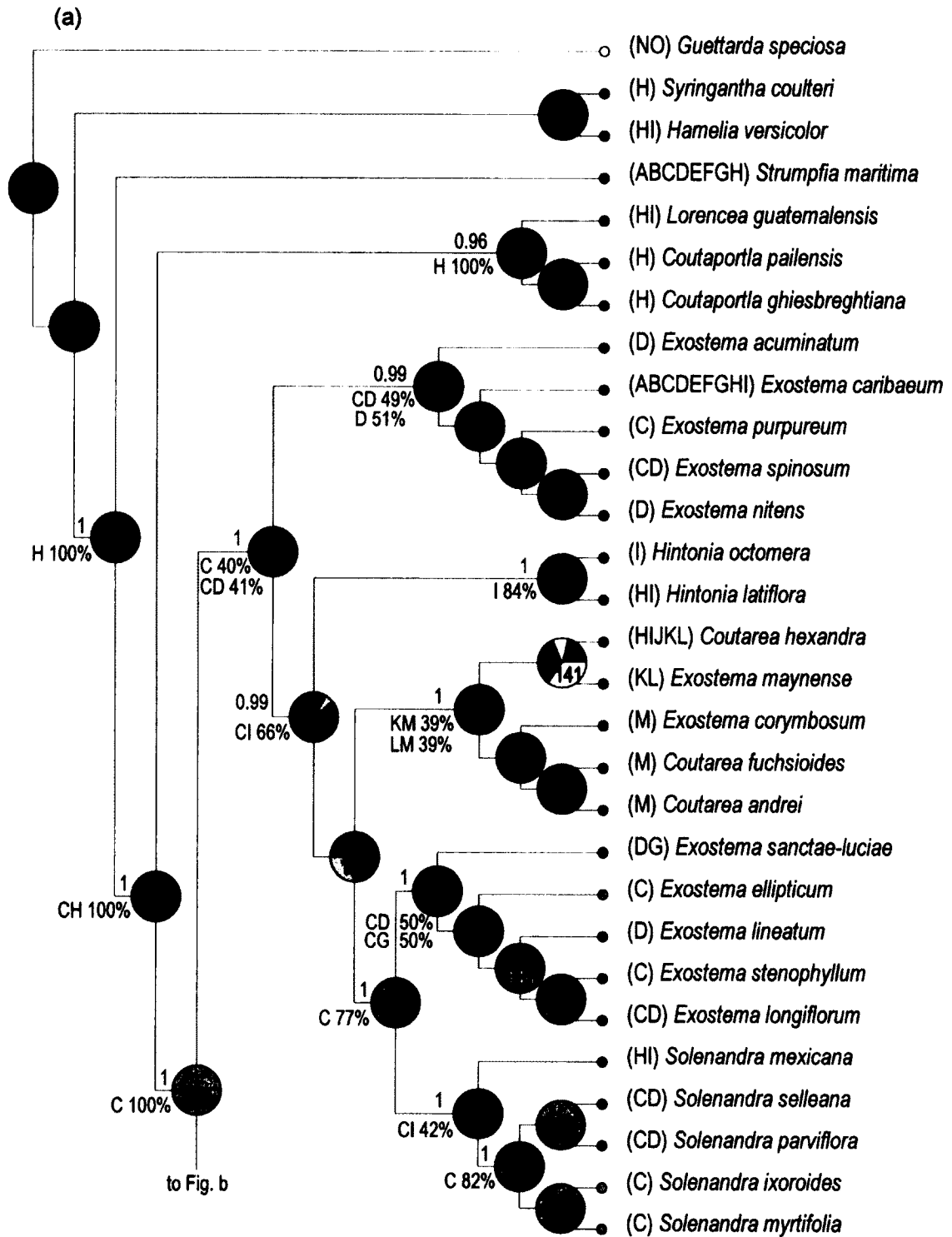


Fig. 10 continued.

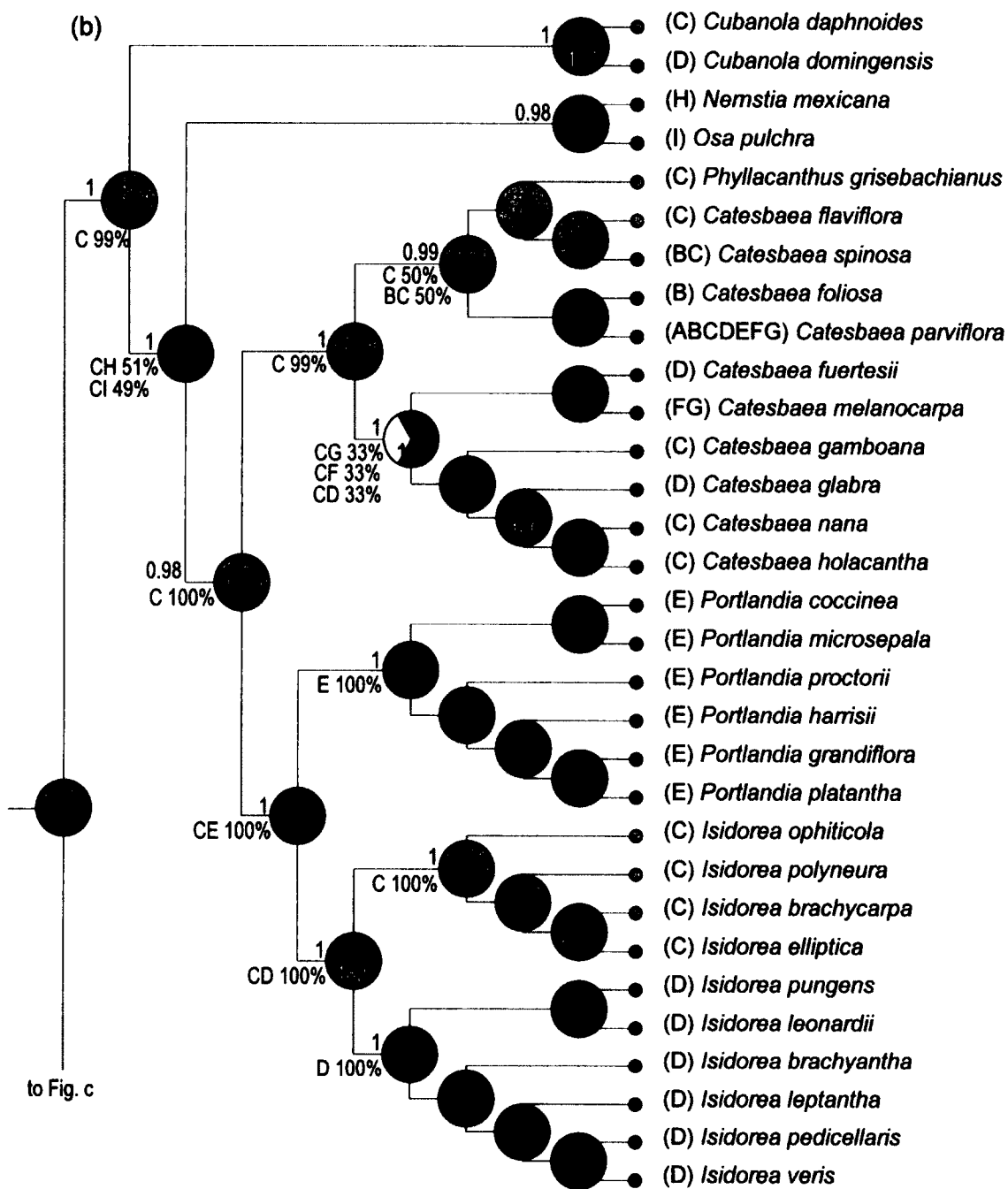


Fig. 10 continued.

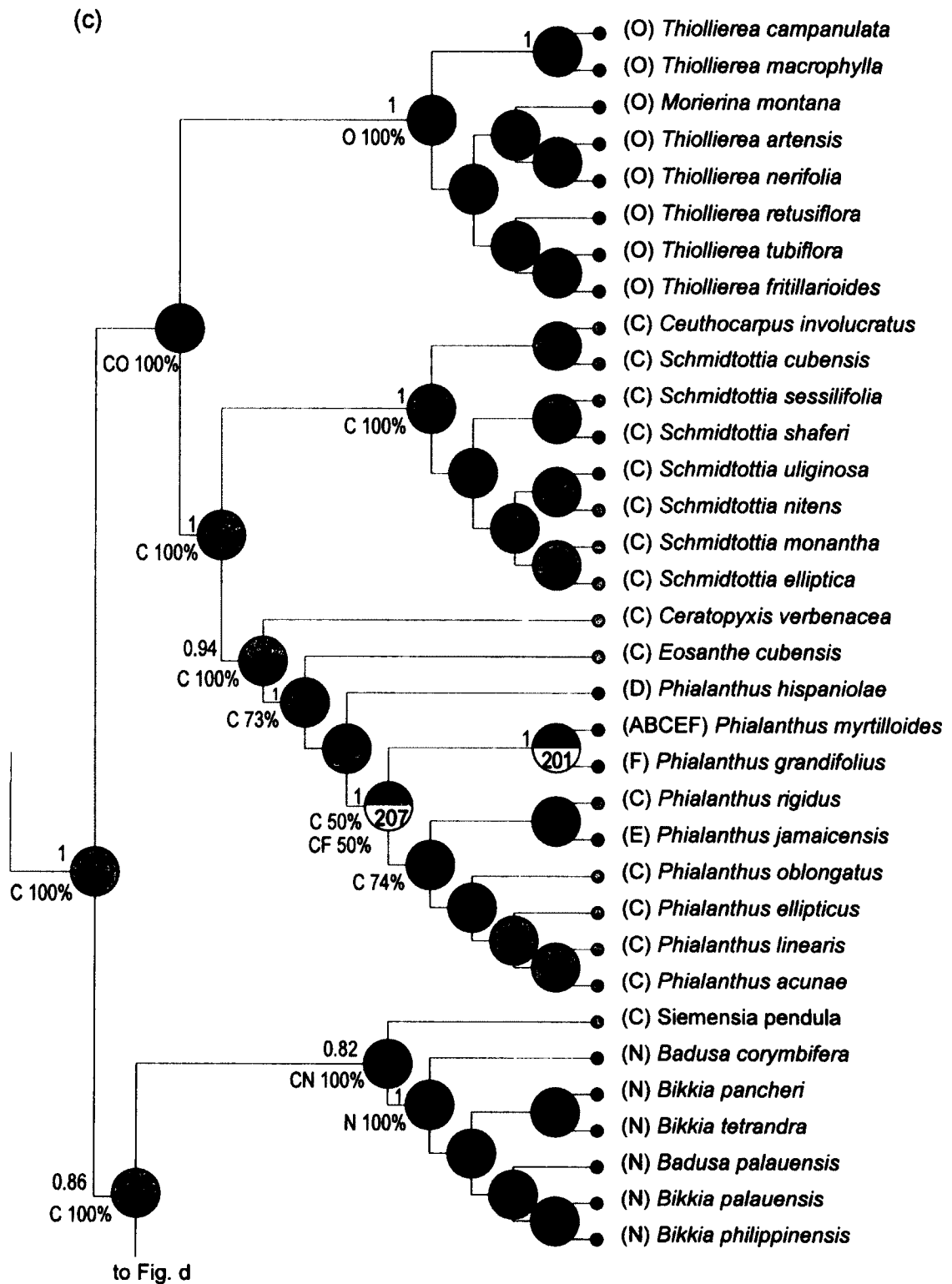
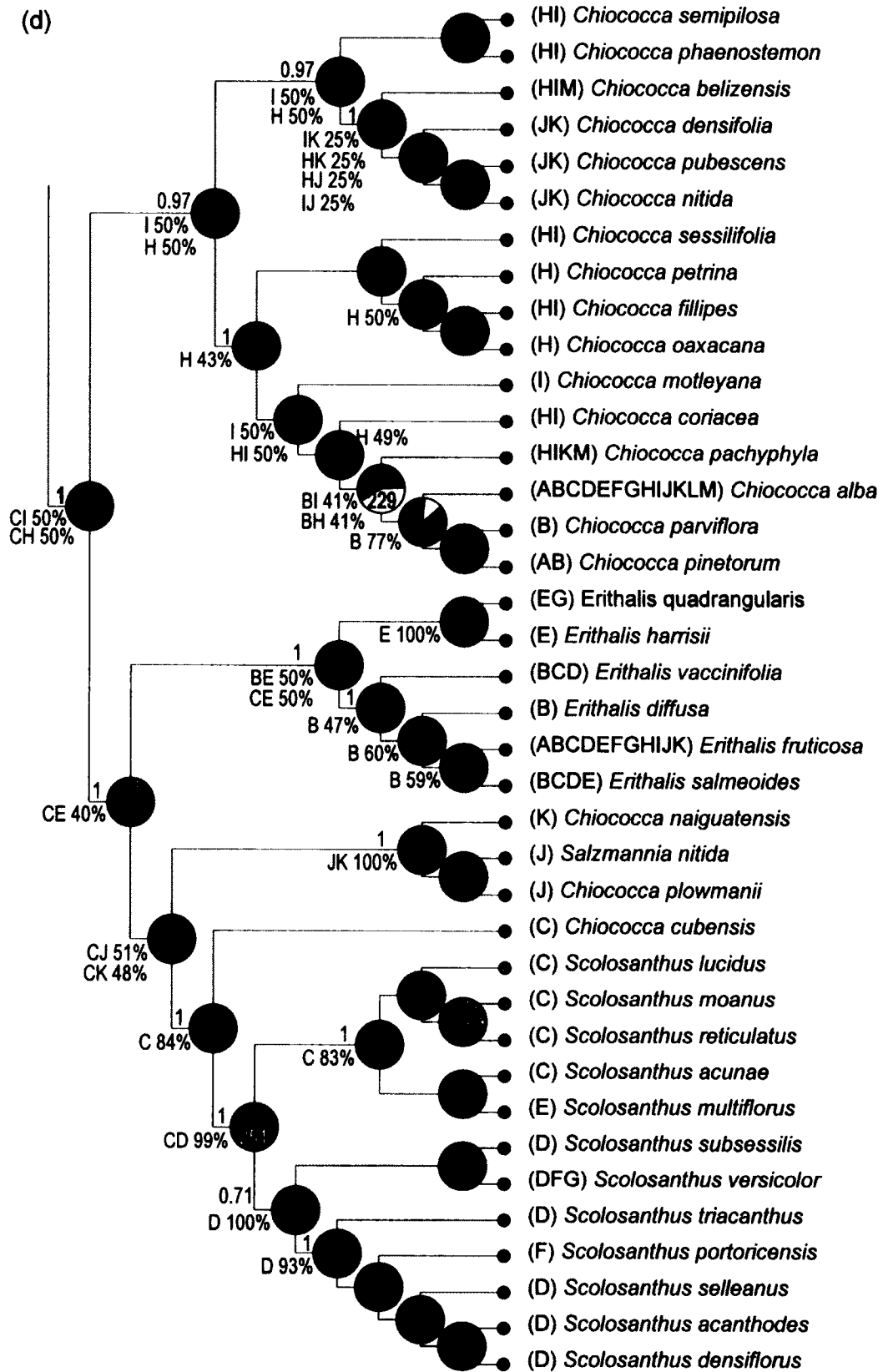


Fig. 10 continued.



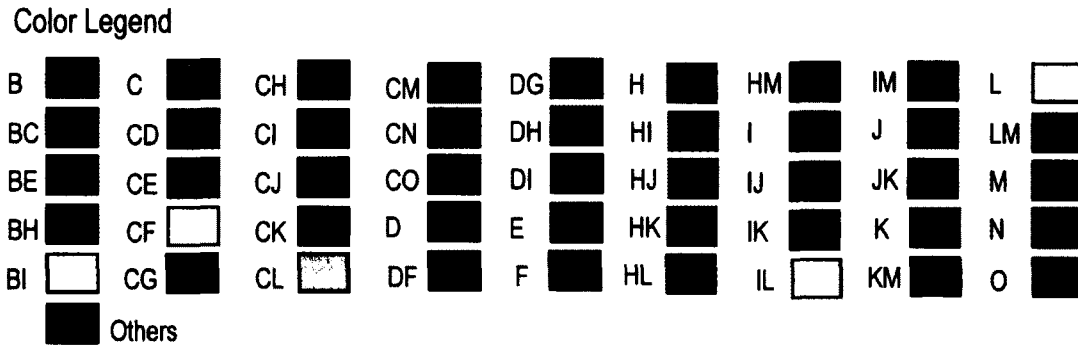


Fig. 10 Graphical results of the ancestral area reconstructions using S-DIVA analysis. Pie charts in each node show the probabilities of ancestral ranges. Number within each pie chart is the number of that node. Bayesian posterior probability values (above the branch) and probability value of the most probable ancestral area (below the branches) are noted for the biogeographically important nodes and for the nodes discussed in the text. Color key for the possible ancestral ranges. Uppercase letters in parentheses next to the species name indicate the current distribution (Fig. 8). A: Florida Keys and Continental USA, B: Bahamas, C: Cuba, D: Hispaniola, E: Jamaica, F: Puerto Rico, G: Lesser Antilles, H: Northern and Central Mexico, I: Southern Mexico and Central America, J: Atlantic coastal region, K: Orinoco-Amazon basin, L: Amazon Piedmont region, M: Andean region, N: Western Pacific Islands except New Caledonia, O: New Caledonia.

DISCUSSION

Historical biogeographical reconstructions using phylogenies and molecular dating play a very important role in illuminating the evolutionary history of plant groups (Milne 2006; Ali et al. 2012). In plant groups like the Rubiaceae, where fossil data are limited (Manns et al. 2012), it is essential to have adequate taxa sampling and well-resolved phylogenies to date nodes that are not close to fossil calibration (Motley et al. 2005; Antonelli et al. 2009; Milne 2009). With greatly expanded taxa sampling and a well-resolved molecular phylogeny, this study has been successful in generating ancestral area reconstructions that enable us to better understand the historical biogeography of the Chiococceae. Several biogeographic inferences can be made from the ancestral area reconstructions and the molecular dating analyses.

General topology of the phylogenetic trees

The 50% majority-rule consensus tree generated from Bayesian analyses is very well resolved ($PP \geq 0.9$ in more than 80% nodes). All genera of the Chiococceae are resolved in four major clades and the basal nodes are well resolved. Out of the only five nodes not well supported at generic level, three nodes involve the West Pacific genera. The tree topology is similar to our earlier phylogenetic analyses (S. Paudyal unpubl. data; chapter 3) with some differences in support values, which may be due to changed dataset and nature of analysis. The crown group relationships corroborate in general with previous studies (Motley et al. 2005, Manns and Bremer 2010, Manns et al. 2012). The maximum clade credibility tree generated from BEAST analysis is also well resolved. However, the *Coutaportla-Lorencea* clade, which is resolved in a basal position in the

Bayesian analysis (clade A, Fig. 9), is placed sister to clade C. Since inter-clade relationships at basal nodes are very weakly supported (PP = 0.39; PP = 0.64), in essence, the four clades form a polytomy. Polytomies may occasionally indicate multiple speciation events occurring simultaneously when geological changes (e.g., sea level changes, glaciation) isolate several populations of a widespread species and initiate divergence ultimately resulting in speciation (Walsh et al. 1999). These four clades (A--D) are estimated to have diverged from each other within a very short time range of less than 3 Myr and coincide with the Oligocene inundation of the Caribbean. The crown group taxa relationships within each clade corroborate with the tree generated from Bayesian analysis. Thus the tree topology generated from BEAST analysis does not contradict the phylogenetic relationships within the tribe Chiococceae and all the taxonomic implications that have been discussed in our earlier paper (S. Paudyal unpubl. data; chapter 3). Further discussion based on the current phylogeny is not presented here and is beyond the scope of this study.

Divergence time estimate

Bremer and Eriksson (2009) and Manns et al. (2012) are the only two molecular dating studies that have included a considerable number of Chiococceae taxa in their analyses. In the two studies, divergence time of the Chiococceae is estimated at around 34.4 Mya and 43.1 Mya, respectively. Since *Strumpfia* (now in a monotypic tribe sister to the Chiococceae) was included within tribe Chiococceae in both studies, the divergence times stated in those studies are for the clade inclusive of *Strumpfia*. However, Manns et al. (2012) referred the crown node age as the estimated divergence time of the tribe

Chiococceae. Thus the divergence time of the tribe (43.1 Mya) estimated in Manns et al. (2012) would be the time at which *Strumpfia* split from the rest of the Chiococceae and the crown node age for the tribe Chiococceae sensu Paudyal et al. (2014) would then be inferred as 31 Mya. Similarly in Bremer and Eriksson (2009), the estimated crown node age of 27.6 Mya is inferred as the age at which Chiococceae and Strumpfieae split. The estimated stem age or the time at which sister lineages split is considered as the time of divergence in this paper.

The time of divergence and the crown node age of the Chiococceae were estimated differently in our study. According to the present study, the Chiococceae and the sister tribe Strumpfieae diverged from each other during the Eocene at an estimated mean age of 47.8 Mya. This age is older than those estimated in both previous studies. Our results indicate that further diversification of the crown group occurred much later in the Late Oligocene-Early Miocene at around 22.7 Mya; while the crown node age was inferred to be earlier in the two previous studies. Interestingly, despite the very limited sampling of Chiococceae taxa, inferred time of divergence (~ 45 Mya) and crown node age (~ 19 Mya) of Chiococceae in Antonelli et al. (2009) are closer to our results. The alternating submersions and emersions of the Caribbean islands in the Oligocene (Buskirk 1985) may have been a factor in recent diversification of the crown groups. The four major clades in the Chiococceae are not well resolved at the basal node in the BEAST analysis, and our results indicate that all clades diverged around the same time. This suggests rapid diversification of the Chiococceae taxa once favorable conditions were prevalent. Since fossil calibration points represent the minimum age of a calibrated node, all other nodes also represent minimum ages of the divergence of diversification

event (Milne 2006). Thus all the time estimates from our results are the minimum ages of the particular divergence event. McDowell's (1995) statement that the genus *Exostema* (*sensu* McDowell 1996) was already in Greater Antilles by mid-Miocene (ca. 15 Mya) is supported by our results. Our results also indicate divergence time of the clade comprising all species of *Exostema* (*sensu* McDowell 1996) to be 22.7 Mya and the crown node age of 18.5 Mya indicates that the group diversified in the Greater Antilles before 15 Mya.

Inadequate or erroneously biased sampling, distantly placed fossil calibration, and unresolved nodes in the phylogeny are some of the major factors that reduce the accuracy in divergence time estimation. This study has the most comprehensive taxa sampling of the Chiococceae thus far, and used two ingroup fossil calibrations in the molecular dating analysis, hence more reliable estimated times of divergence and diversification of taxa within the tribe.

Historical Biogeography

Ancestral area reconstruction and molecular dating analyses indicate that the tribe Chiococceae originated in Mexico and through subsequent dispersal, vicariance and extinction events dispersed to reach the current distribution in the Caribbean, Central and South America, and the West Pacific. Chiococceae diverged from sister tribe Strumpfieae during the Eocene, but its distribution may not have expanded to the Caribbean islands until early Miocene when the Oligocene inundation of the Caribbean receded (Buskirk 1985), re-emerging much of the island landmasses. Members of the Chiococceae may have spread in the Caribbean during the Late Eocene-Early Oligocene when the above-

water land was at its maximum, due to massive uplift (Iturralde-Vinent and MacPhee 1999). It is also possible that major extinction occurred as a result of sea level rise leaving behind relict populations that ultimately diverged as four major lineages within Chiococceae. Our results indicate that most extant species in Chiococceae are result of speciation in the last 5 million years during the Pliocene--Pleistocene. Rapid radiation inferred in many genera in the last three million years may be the result of environmental pressure due to extreme sea level fluctuations in the Caribbean during the Quaternary (McDowell 1996). Except *Chiococca*, all other species-rich genera in Chiococceae are primarily Caribbean in distribution. In the Bayesian analysis, Mexican-Central American clade of two genera, *Lorencea* and *Coutaportla* (clade A), is resolved at basal node as sister to all the remaining Chiococceae. *Lorencea* is currently distributed in southern Mexico (Chiapas, Veracruz) and extends to Guatemala and Honduras, while *Coutaportla* is restricted to Mexico. Manns et al. (2012) suggested that Chiococceae spread to the Caribbean from Central America. Mexico and Central America were grouped together as one geographical unit in Manns and Bremer (2012), thus in essence their conclusion on the origin of Chiococceae is supported by the present results.

The ancestors of the remaining Chiococceae taxa dispersed to the Caribbean in the Early Miocene and immediately diverged into three major lineages (clades B, C, and D). The basal node of four clades is not well resolved in the BEAST analysis, suggesting a rapid divergence of three lineages. Clade A is associated with clade C, albeit with negligible support. Results infer that four major lineages diverged from each other within a time interval of about 2.5 million years. This may be a result of extinction and rapid diversification due to changing environments (Manns et al. 2012) and was probably the

beginning of separation of the Greater Antilles magmatic arc (Graham 2003; Santiago-Valentin and Olmstead 2004). A higher number of Cuba-Hispaniola endemics has been attributed to this connection between East Cuba, North and Central Hispaniola, and Puerto Rico (Graham 2003). Occurrence of high species diversity of Chiococceae in the Greater Antilles, in particular the intermixed distribution of extant taxa in the same lineage, is most probably due to the vicariance as a result of the splitting of the arc during the Late Miocene.

Our results indicate that one of the major lineages (clade B) comprised of *Exostema* (*sensu* McDowell 1996), *Coutarea*, and *Hintonia* dispersed in the Greater Antilles during the Early Miocene by 18.5 Mya. Based on phylogenetic analyses, using molecular and morphological datasets, McDowell and Bremer (1998) suggested South America as the origin of the *Exostema* (*sensu* McDowell 1996). But, their conclusion was primarily based on their phylogenies, which positioned the two South American species basal to Caribbean species; this erroneous result most probably was due to the use of a closely related species, *Coutarea hexandra*, as the outgroup, and to the lack of additional Chiococceae genera in the ingroup. Our results do not support a South American origin for *Exostema* (*sensu* McDowell 1996), but indicate that this lineage originated in the Greater Antilles. S-DIVA analysis postulates ancestral area at basal Node 156 (Fig. 10) as Cuba-Hispaniola with 41% and Cuba with 40% probability. This suggests that the ancestors reached the Greater Antilles when eastern Cuba, North-Central Hispaniola, and Puerto Rico were still connected (Graham 2003). Vicariance certainly played a role in the diversification of this lineage as the islands separated in Late Miocene (Santiago-Valentin and Olmstead 2004). McDowell's (1995) assertion that *Exostema* was established in the

Caribbean by 15 Mya is supported by our results. Three independent dispersals out of the Greater Antilles to the Continental America are indicated in this lineage. An early back dispersal of *Hintonia* to Mexico-Central America around 16.5 Mya and a much later dispersal of *Solenandra mexicana* back to Mexico at around 7.3 Mya were suggested. Both dispersal and vicariance events are postulated in the divergence of their ancestors. Our results suggest that dispersal and another vicariance event occurred (Fig. 10 node 154) when the ancestors of the South American *Exostema* and *Coutarea* diverged from the Greater Antilles around 15.4 Mya when the Orinoco-Amazon basin of South America was heavily flooded. Further diversification of this lineage possibly occurred with the cessation of flooding of the Lake Pebas and uplifting of the Eastern Andean Cordilleras around 11-7 Mya (Antonelli et al. 2009). Our results estimate the crown node age at around 10.3 Mya, and the S-DIVA analysis postulates vicariance at node 144 (Fig. 10) leading to divergence of the high altitude Andean taxa.

Another lineage (clade C) diversified in the Caribbean slightly later than the *Exostema* s. l. lineage at around 14.8 Mya. Our results suggest a Mid Miocene divergence of the ancestors of *Osa* and *Nernstia* to Central America, and also support Manns et al. (2012)'s suggestion that dispersal was followed by vicariance (Fig. 10 node 185). *Catesbaea* is primarily distributed in Cuba and Hispaniola with some species extended to the Bahamas, Puerto Rico and the Lesser Antilles, most probably dispersed by birds or animals that eat their fleshy fruits (Motley et al. 2005). *Portlandia*, a Jamaican endemic of six species, diversified in Jamaica more recently in the Pleistocene when maximum uplift and faulting of Jamaica occurred (Buskirk 1985). *Isidorea* has two distinct clades of Cuban and Hispaniola species that had diverged by the end of Miocene. S-DIVA

analysis postulate a vicariance event at node 182 (Fig. 10); this probably indicates the separation of Hispaniola and Eastern Cuba although the estimated time of divergence (~ 5.3 Mya) looks a bit far-fetched.

The third lineage (clade D) is comprised of the West Pacific and Neotropical genera exhibiting an interesting biogeographical disjunction. Both S-DIVA and BBM analyses postulate the ancestors of this lineage also originated in Cuba with 100 % and 95% probability respectively (Fig. 10, node 257). Dispersal of the West Pacific lineages occurred during the Mid Miocene most probably by long distance dispersal (Motley et al. 2005). Our results support two independent dispersal events, one to New Caledonia and the other to the other islands of the West Pacific. The dispersal to New Caledonia is relatively more recent (~ 10.6 Mya) than the dispersal to the other West Pacific islands (~ 15.4 Mya). The remaining taxa of this lineage, except *Chiococca* and *Salzmannia*, diversified in the Caribbean islands. Generally tropical plants with fleshy fruits have higher diversity than those with non-fleshy fruits in the same lineage (Smith 2001) due to the dispersal advantage by endozoochory. *Chiococca*, one of the most species-rich genera in the Chiococceae, is also the most widely distributed genus in the tribe most probably because of the small drupaceous fruits that are eaten by birds (Motley et al. 2005). Our results indicate that ancestors of the *Chiococca* upon reaching the Mexico-Central America region, diverged into two lineages around 7.9 Mya (Fig. 10 node 233); one spread towards south reaching South America after the rise of the Isthmus of Panama, while the other diversified in Mexico and Central America and also back to the Caribbean islands very recently in the Pleistocene. Ancestors of *Salzmannia* probably reached South America a little earlier than the Chiococceae species. Our results indicate that *Salzmannia*

probably reached South America from the Greater Antilles (Fig. 10 node 253).

Salzmannia has colorful drupaceous fruits, dispersed by birds.

Schmidtottia, *Phialanthus*, and *Scolosanthus* are other large genera in this lineage; the first is a Cuban endemic and the latter two are distributed in the Greater and Lesser Antilles. Our results indicate that the Cuban and Hispaniola lineages in *Scolosanthus* diverged about 3.5 Mya. This date is well after the two islands separated in late Miocene, but a vicariance event postulated for this divergence (Fig 4 node 251) suggests that the lineage may have actually diverged earlier than inferred by the dating analysis.

Erithalis is another genus in Chiococceae with a wide distribution range in the Antilles; one species, *Erithalis fruticosa* extends all the way from Mexico to South America and into Florida, along the coasts of the Caribbean Region. Our results indicate that the ancestors of *Erithalis* originated in the Greater Antilles, and most probably in Cuba (Fig 4 node 254), and one of the first islands to be colonized was Jamaica (Fig 4 node 238). This is in support of Negron-Ortiz and Watson (2003), who also suggested Jamaica as the ancestral area of *Erithalis*. The fruits of *Erithalis fruticosa* make up the diet of white-crowned pigeons (Bancroft and Bowman 1994) and are also known to float in water (Negron-Ortiz and Watson 2003), which suggests that *Erithalis* colonization of the Neotropics is mainly a result of endozoochory, but hydrochory may have played some role in local distribution (the viability of the seeds in fruits floating in sea water has never been tested).

Amphi-Pacific tropical disjunction

While discussing the major disjunctions in seed plants, Thorne (1972) listed a total of 89 genera, 4 tribes or subtribes, 3 or 4 subfamilies and 8-10 families of flowering plants as having amphi-Pacific tropical distribution. That list has since expanded to include 100 genera and higher groups (Fritsch et al. 2014). This distribution pattern primarily includes taxa that are found in tropical America and tropical areas of Asia-Australia on the western borders of the Pacific basin (Thorne 1972; Qian 1999). However, many plants exhibiting amphi-Pacific tropical disjunction also extend their distributions farther west and occasionally may reach all the way to Madagascar and even tropical eastern Africa; some may only reach Polynesia, or as far as Australasia. There are some groups with some members distributed beyond tropical amphi-Pacific (Thorne 1972).

The tribe Chiococceae is a rare example in the Rubiaceae that has amphi-Pacific tropical disjunction, with 28 genera in the Neotropics (S. Paudyal unpubl. data; chapter 3) with the remaining three genera distributed in the islands of the West Pacific and no member in the vast Pacific plate. *Thiollierea* (12 species; including *Morierina*) is endemic to New Caledonia, while *Badusa* (3 species) and *Bikkia* (11 species) are distributed in the Pacific islands to the west of the Andesite line from the Philippines, Marianas to Melanesia and Tonga (Govaerts et al. 2014). To our knowledge, the only other Rubiaceae lineage that shares a similar disjunction between the Neotropics and the tropical West Pacific islands is the genus *Augusta* that has one species each in Fiji, New Caledonia, Brazil and Central America (Delprete 1997).

Different studies in recent years have addressed amphi-Pacific disjunction in various plant groups (*Abrotanella*, Heads 1999; *Coriaria*, Yokoyama et al. 2000; *Retrophyllum*, Herbert et al. 2000; *Styrax*, Fritsch 2001; *Oreomyrrhis*, Chung et al. 2005; Coreopsidae, Mort et al. 2008; Moutabeeae, Abbott 2009; *Persea* group, Li et al. 2011; Coronanthereae, Woo et al. 2011; *Jovellana*, Nylinder et al. 2012; *Dendropanax*, Li and Wen 2013; Symplocaceae, Fritsch et al. 2014). However, only a few groups exhibit an exact distribution to that of Chiococceae (i.e., distributed only in the Neotropics and the West Pacific islands) while many others extend farther to southeast and eastern Asia or to Australia and New Zealand. Tribe Moutabeeae (Polygalaceae), with five genera, has two genera distributed in the West Pacific (*Balgoya* from New Caledonia; *Eriandra* from New Guinea and the Solomon Islands) and the remaining three genera are in the Neotropics (Abbott 2009; Heads 2010). *Retrophyllum* (Podocarpaceae), a genus of five species, has two species endemic to New Caledonia (*R. minus* and *R. comptonii*), one species (*R. vitiense*) distributed in the West Pacific islands, and two species (*R. piresii* and *R. rospigliosii*) in tropical South America. Two genera of iguanas, *Brachylophus* and *Lapitiguana* distributed in the Neotropics, Galapagos Islands, and in the West Pacific islands of Fiji and Tonga (Pregill and Steadman 2004), and the Loliginid squid genus, *Sepioteuthis*, distributed in Indonesia, New Zealand, Australia and the Caribbean (Anderson 2000) are examples of terrestrial and aquatic animals exhibiting a very similar disjunction to that of the Chiococceae.

In the Chiococceae, molecular phylogenies and dating and ancestral area reconstruction analyses indicate two separate dispersal events from the Neotropics to the West Pacific occurred. Although basal nodes inferring the divergence times are weakly

supported, the New Caledonian and the remaining West Pacific taxa are resolved as two separate and strongly supported clades, thus supporting the hypothesis of two independent dispersal events. Our results indicate that ancestors of both West Pacific lineages diverged from the Greater Antilles around 15 Mya. This divergence age is too recent to support a possible Gondwanan vicariance hypothesis to explain the disjunction between the West Pacific and Neotropical Chiococceae. The last Gondwana continental connection, New Caledonia break off from Australia, is estimated at about 65 Mya (Neall and Trewick 2008). Any role of the Trans-Pacific Land Bridge from the Jurassic-Cretaceous (Morley 2003) in this dispersal is also not supported. An alternative Gondwana vicariance hypothesis was also evaluated to explain amphi-Pacific disjunctions (McCarthy 2003; McCarthy et al. 2007), which considers the juxtaposition of Australia, New Zealand, and East Asian islands along the western edge of South America. This hypothesis may support a vicariance origin of many amphi-Pacific disjunctions. However, with the divergence time of the West Pacific Chiococceae taxa being much more recent than the estimated age of South Pacific seafloor (~40 Myr; McCarthy et al. 2007), even this alternate Gondwana hypothesis does not support the disjunction in Chiococceae.

Alternatively, ancestors of both lineages would have reached the West Pacific islands and New Caledonia by Long Distance Dispersal (LDD). Birds have been recorded to accidentally travel long distances across Atlantic Ocean (Milne 2006). So any fruit or seed adaptation that facilitates its adherence to a bird or an animal vector (internal or external) would enhance the success of such dispersal. Alternatively, anemochory and hydrochory are also commonly known methods to disperse plants over long distances.

The capsular fruits with tiny seeds of the West Pacific genera of Chiococceae are well adapted for anemochory. Our results show that *Siemensia* from western Cuba is closely associated with the West Pacific genera. Capsular fruits and wind-dispersed seeds are present in *Siemensia* and all Pacific taxa, which suggest anemochory was important in the dispersal across Pacific. Motley et al. (2005) suggested anemochory as long-distance dispersal to the West Pacific. Their conclusion was based on the fact that all the genera present in the West Pacific have capsular fruits releasing small, wind-dispersed seeds, and the trade winds are all going westwards from the Caribbean Region to the West Pacific. Ectozoochory, although not very realistic considering the length of the Pacific, may also be possible in this group when seeds get accidentally attached to birds.

Nylinder et al. (2012) has discussed the potential of ocean water currents to transport seeds and even entire plants as they get displaced in little islands of debris during earthquakes, landslides or flooding and raft long distances. They consider the possibility of Humboldt Current playing a role in the dispersal of *Jovellana* from the coasts of South America to New Zealand. Chen et al. (2013) have also suggested rafting as a rare but possible LDD of hinged-teeth snakes from tropical South Asia to the Neotropics; rafting on debris during extreme weather events has also been suggested by Keppel et al. (2009) as a possible mode of LDD. At the time when the West Pacific taxa diverged, the Central American seaway was still open, prior to the uplift of Isthmus of Panama. At that time, there existed active exchange between the Caribbean and the eastern Pacific mainly due to the Easterly trade winds driving surface currents westwards (Morelock et al. 2015).

In light of the above discussion, it can at least be concluded that the ancestors of the West Pacific genera reached the West Pacific islands via two independent LDD events, most likely occurring via the tiny seeds being carried by high altitude air currents to the other side of the Pacific. However, the possibility of dispersal by rafting first in Caribbean-East Pacific currents followed by warm equatorial currents also cannot be denied. And with everything considered, the LDD most likely has occurred via the tiny wind-dispersed seeds of their related taxa from both sides of the Pacific.

CHAPTER 5

SUMMARY

This study examined the phylogenetic relationships and historical biogeography in the tribe Chiococceae. With an expanded taxa sampling and use of molecular sequence data from multiple DNA regions it was possible to present a comprehensive phylogeny of the tribe, which enabled development of robust hypotheses on the origin and dispersal of this tribe, revisit earlier tribal and generic delimitations and propose various taxonomic changes.

Tribal delimitations of Chiococceae genera have historically remained unclear mainly due to the extreme morphological diversity and plasticity of flower, fruit and seed characters of the taxa. Furthermore, recent studies have not been consensual in the tribal affinity of the genus *Strumpfia*; researchers have disagreed about the inclusion of *Strumpfia* within the tribe Chiococceae. Due to its unique morphology and palynology, affinities of *Strumpfia* within the Rubiaceae have also long remained uncertain. It is the only genus in the family which has all five anthers united into a tube. With the help of molecular data generated in this study, genetic sequence divergences between different tribes within the subfamily Cinchonoideae were analyzed. Coupled with morphological and palynological data, *Strumpfia* was transferred to a new monotypic tribe Strumpfieae. Tribe Chiococceae was then re-delimited to include only 29 genera.

This study presents the most comprehensive phylogeny of the tribe Chiococceae (*sensu* Paudyal et al. 2014). Sampling was expanded to include 126 species from 27 genera. The most extensive sampling thus far included 59 species from 23 genera. Fifty-five species and one genus, *Ceuthocarpus*, in this study were included for the first time in

a molecular phylogeny. A total of 33 species endemic to Cuba were included in this study, while less than ten were included in previous molecular phylogenies.

This study was successful in generating highly resolved phylogenies by analyzing molecular sequence data of two nuclear (ETS, ITS) and two chloroplast (petD, trnL-F) regions using Bayesian inference and maximum parsimony frameworks. Expanded sampling and use of ETS markers helped to obtain a well-resolved phylogeny. Although phylogenetic trees generated from maximum parsimony and Bayesian analyses of chloroplast and nuclear datasets showed some incongruences, such relationships were in most cases not well supported and did not contradict the overall phylogenetic relationships inferred by the results of concatenated dataset presented in this study.

With the help of the molecular phylogeny from this study, many intergeneric and infra-generic relationships are now well resolved and we have a better understanding of evolutionary relationships between Chiococceae taxa. This study addresses all the taxonomic relationships inferred by the phylogeny. In addition to *Catesbaea*, *Chiococca*, *Exostema* and *Thiollierea* that are shown to be not monophyletic in previous studies, five other genera, *Badusa*, *Bikkia*, *Coutarea*, *Phialanthus*, and *Schmidtottia*, as currently circumscribed, are also not resolved as monophyletic in the present study. In addition, a total of nine taxonomic changes at the generic level are proposed. These include five new generic delimitations. Also, the genera *Ceuthocarpus*, *Morierina* and *Phyllacanthus* are merged into *Schmidtottia*, *Thiollierea*, and *Catesbaea* respectively; and two species of *Chiococca* are transferred to genus *Salzmannia*. However, further research with better taxon sampling is necessary to understand the *Badusa-Bikkia*, and *Phialanthus-Eosanthe* taxonomic relationships.

The comprehensive phylogeny generated during this study facilitated the reconstruction of the historical biogeography of the Chiococceae. Lacking adequate taxon sampling and well-resolved phylogeny, previous studies were unable to address this issue. Historical events of origin, diversification and disjunction in Chiococceae were inferred with the help of molecular dating analysis using BEAST and ancestral area reconstruction using S-DIVA and BBM. These data indicate that the tribe Chiococceae originated in Mexico in the Eocene and through subsequent dispersal, vicariance and extinction events dispersed to reach the current distribution in the Caribbean, Central and South America, and the West Pacific. Diversification in the Caribbean occurred later in the Late Oligocene-Early Miocene, possibly after the environmental conditions became more favorable with the end of major Oligocene-inundation. Multiple dispersals to the Caribbean and back dispersals are inferred. Dispersals to Central America occurred both from Mexico as well as via the Caribbean. Similarly, introduction to the South American occurred via Central America as well as directly from the Caribbean islands. Currently, all the species-rich genera (except *Chiococca*) are distributed in the islands; the rapid radiation inferred in the last three million years possibly occurred by the environmental pressure such as extreme sea level fluctuations in the Caribbean during the Quaternary. An earlier hypothesis that *Exostema* originated in South America was not supported by this study; instead a Greater Antillean origin is inferred. However, Jamaica as the ancestral area of *Erithalis* is supported.

The tribe Chiococceae is a rare example, in the family Rubiaceae, of amphipacific tropical disjunction, with 28 genera occurring in the Neotropics and three genera, *Badusa*, *Bikkia* and *Thiolloierea*, in the West Pacific islands. A short review of amphipacific

Pacific tropical disjunctions similar to that of *Chiococceae* is presented in this study.

Molecular dating and the ancestral area reconstruction analyses indicate that two separate dispersal events from the Neotropics, in particular from the Greater Antilles to the West Pacific occurred around 15 Mya, leading to the disjunction in distribution. The divergence times inferred in this study are too recent to support the vicariance hypotheses explaining disjunctions. This study indicates LDD as the plausible explanation for the amphi-Pacific disjunction in this tribe. LDD events most probably occurred as high altitude air currents carried the tiny wind-dispersed seeds across the Pacific, or less likely the seeds rafted across the Pacific with the Caribbean-East Pacific and the warm equatorial water currents.

LITERATURE CITED

- Abbott, J. R. 2009. *Phylogeny of the Polygalaceae and a revision of Badiera*. Ph. D. dissertation. Gainesville, Florida: University of Florida.
- Achille, F., T. J. Motley, P. P. Lowry II, and J. Jérémie et al. 2006. Polyphyly in *Guettarda* L. (Rubiaceae, Guettardeae) based on nrDNA ITS sequence data. *Annals of the Missouri Botanical Garden* 93: 103–121.
- Aiello, A. 1979. A reexamination of *Portlandia* (Rubiaceae) and associated taxa. *Journal of Arnold Arboretum* 60: 38–123.
- Alain, H. 1959. Novedades en la flora Cubana, X. *Contribuciones Ocasionales del Museo de Historia Natural del Colegio "de La Salle"* 17:10.
- Alejandro, G. J. D., L. A. R. Santos, H. W. C. Hsu, M. S. S. Mejillano, P. J. R. Santor, and V. B. Amoroso. 2014. Molecular phylogeny of the genus *Bikkia* (Rubiaceae) including a new endemic Philippine inland forest species *Bikkia montoyae*. *Philippine Science Letters* 7: 88–96.
- Alejandro, G. D., S. G. Razafimandimbison, and S. Liede-Schumann. 2005. Polyphyly of *Mussaenda* inferred from ITS and *trnT-F* data and its implication for generic limits in Mussaendeae (Rubiaceae). *American Journal of Botany* 92: 544–557.
- Ali, J. R. 2012. Colonizing the Caribbean: is the GAARlandia land-bridge hypothesis gaining a foothold? *Journal of Biogeography* 39: 431–433.
- Anderson, F. E. 2000. Phylogeny and historical biogeography of the loliginid squids (Mollusca, Cephalopoda) based on mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 15: 191–214.

- Andersson, L. and A. Antonelli. 2005. Phylogeny of the tribe Cinchoneae (Rubiaceae), its position in Cinchonoideae, and description of a new genus, *Ciliosemina*. *Taxon* 54: 17–28.
- Andersson, L. and J. H. E. Rova. 1999. The *rps16* intron and phylogeny of the Rubioideae (Rubiaceae). *Plant Systematics and Evolution* 214: 161–186.
- Andersson, L. and C. Persson. 1991. Circumscription of the tribe Cinchoneae (Rubiaceae)- a cladistic approach. *Plant Systematics and Evolution* 178: 65–94.
- Andreasen, K. and B. Bremer. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology, nuclear and chloroplast DNA data. *American Journal of Botany* 87: 1731–1748.
- Antonelli, A., J. A. A. Nylander, C. Persson, and I. Sanmartin. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences of the USA* 106: 9749–9754.
- Aublet, J. B. C. F. 1775. *Histoire des plantes de la Guiane Française*. Paris: P. F. Didot.
- Baldwin, B. G. and S. Markos. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463.
- Bancroft, G. T. and R. Bowman. 1994. Temporal patterns in diet of nestling White-crowned Pigeons, implications for conservation of frugivorous columbids. *The Auk* 111: 844–852.
- Barrabé, L., A. Mouly, P. P. Lowry, and J. Munzinger. 2011. Reinstatement of the endemic New Caledonian genus *Thiollierea* Montrouz. (Rubiaceae) necessitated by the polyphyly of *Bikkia* Reinz. as currently circumscribed. *Adansonia* 33: 115–

134.

- Bayer, R. J. and J. R. Starr. 1998. Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the *trnL* intron and *trnL/F* intergeneric spacer. *Annals of the Missouri Botanical Garden* 85: 242–256.
- Borhidi, A. 2002. Revalidacion del genero *Solenandra* Hook. f. (Rubiaceae). *Acta Botanica Hungarica* 44: 223–231.
- Borhidi, A. 2003. *Lorencea*, gen. nov. (Condamineae, Rubiaceae) de Guatemala y Mexico. *Acta Botanica Hungarica* 45:13–21.
- Borhidi, A. 2007. Una especie nueva del género *Chiococca* P. Browne (Rubiaceae) de Guatemala. *Acta Botanica Hungarica* 49: 63–64.
- Borhidi, A. 2008. Taxa et combinationes novae florum Antillanae. *Acta Botanica Hungarica* 50: 275–280.
- Borhidi, A. 2011. Estudios sobre rubiáceas mexicanas XXXV - *Chiococca motleyana* nomen novum. *Acta Botanica Hungarica* 53: 267–268.
- Borhidi, A. and M. Fernandez-Zequeira. 1989. El genero *Exostema* L.C. Rich (Rubiaceae) en Cuba. *Acta Botanica Hungarica* 35: 287–307.
- Borhidi, A., M. Fernandez-Zequeira, and R. Oviedo-Prieto. 2009. Species combinationesque nonnullae novae florum Cubanae. *Acta Botanica Hungarica* 51: 273–282.
- Borsch, T., K. W. Hilu, D. Quandt, V. Wilde, C. Neinhuis, and W. Barthlott. 2003. Non-coding plastid *trnT-trnF* sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* 16: 558–576.

- Bremekamp, C. E. B. 1966. Remarks on the position, the delimitation, and the subdivision of the Rubiaceae. *Acta Botanica Neerlandica* 15: 1–33.
- Bremer, B. 1992. Phylogeny of the Rubiaceae (Chiococceae) based on molecular and morphological data- useful approaches for classification and comparative ecology. *Annals of Missouri Botanical Garden* 79: 380–387.
- Bremer, B. 1996. Combined and separate analyses of morphological and molecular data in the plant family Rubiaceae. *Cladistics* 12: 21–40.
- Bremer, B. 2009. A review of molecular phylogenetic studies of Rubiaceae. *Annals of the Missouri Botanical Garden* 96: 4–26.
- Bremer, B. and M. Thulin. 1998. Collapse of Isertieae, re-establishment of Mussaendeae, and a new genus of Sabiceae (Rubiaceae): phylogenetic relationships based on *rbcL* data. *Plant Systematics and Evolution* 211: 71–92.
- Bremer, B. and R. K. Jansen. 1991. Comparative restriction site mapping of chloroplast DNA implies new phylogenetic relationships within the Rubiaceae. *American Journal of Botany* 78: 198–213.
- Bremer, B. and T. Eriksson. 2009. Time tree of Rubiaceae-phylogeny and dating the family, subfamilies, and tribes. *International Journal of Plant Sciences* 170: 766–793.
- Bremer, B., K. Andreassen, and D. Olsson. 1995. Subfamilial and tribal relationships in the Rubiaceae based on *rbcL* sequence data. *Annals of the Missouri Botanical Garden* 82: 383–397.
- Bremer, B., K. Bremer, N. Heidari, P. Erixon, R. G. Olmstead, A. A. Anderberg, M. Kallersjo, and E. Barkhordarian. 2002. Phylogenetics of asteroids based on 3

- coding and 3 non-coding chloroplast DNA markers and utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* 24: 274–301.
- Bremer, B., R. K. Jansen, B. Oxelman, M. Backlund, H. Lantz, and K.-J. Kim. 1999. More characters or more taxa for a robust phylogeny, a case study from the Coffee family (Rubiaceae). *Systematic Biology* 48: 413–435.
- Bridson, D. M. and E. Robbrecht. 1985. Further notes on the tribe Pavetteae (Rubiaceae). *Bulletin du Jardin Botanique National de Belgique* 55: 83–115.
- Britton, N. L., and C. F. Millspaugh. 1920. *The Bahama Flora*. New York: N. L. Britton & C. F. Millspaugh.
- Bullock, A. A. 1935. Tabula 3295. *Hooker's Icones Plantarum* 33: 1–7. London: Dulau & Co., Ltd.
- Buskirk, R. 1985. Zoogeographic patterns and tectonic history of Jamaica and the northern Caribbean. *Journal of Biogeography* 12: 445–461.
- Candolle, A. P. de. 1830. Rubiaceae. Pp. 341–622 in *Prodromus* vol. 4. Paris: Treuttel and Würtz.
- Chen, X., S. Huang, P. Guo, G. R. Colli, A. N. M. de Oca, L. J. Vitt, R. A. Pyron, F. T. Burbrink. 2013. Understanding the formation of ancient intertropical disjunct distributions using Asian and Neotropical hinged-teeth snakes (Sibynophis and Scaphiodontophis: Serpentes: Columridae). *Molecular Phylogenetics and Evolution* 66: 254–261.
- Chung, K.-F., H.I. Peng, S. R. Downie, K. Spalik, and B. A. Schaal. 2005. Molecular systematics of the Trans-Pacific alpine genus *Oreomyrrhis* (Apiaceae),

- phylogenetic affinities and biogeographic implications. *American Journal of Botany* 92: 2054–2071.
- Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.
- Delprete, P. G. 1996a. *Revision of the tribe Condamineeae (sensu Hooker, 1873) (Rubiaceae)*. Ph. D. dissertation. Austin, Texas: University of Texas at Austin.
- Delprete, P. G. 1996b. Evaluation of the tribes Chiococceae, Condamineeae, and Catesbaeeae (Rubiaceae) based on morphological characters. *Opera Botanica Belgica* 7: 165–192.
- Delprete, P. G. 1997. Revision and typification of Brazilian *Augusta* (Rubiaceae, Rondeletieae) with ecological observations on the riverine vegetation of the Cerrado and Atlantic forests. *Brittonia* 49: 487–497.
- Delprete, P. G. 1999a. The Cuban endemic genera *Ariadne*, *Mazaea*, *Acunaeanthus*, *Phyllomelia* (Rondeletieae), and *Eosanthe* (Rubiaceae). *Brittonia* 51: 217–230.
- Delprete, P. G. 1999b. Rondeletieae (Rubiaceae): Part I (*Rustia*, *Tresanthera*, *Condaminea*, *Picardaea*, *Pogonopus*, *Chimarrhis*, *Dioicodendron*, *Molopanthera*, *Dolichodelphys*, and *Parachimarrhis*). *Flora Neotropica Monographs*, vol. 77, pp. 1–226. New York: New York Botanical Garden Press.
- Delprete, P. G. 2004. Rubiaceae. Pp. 328–333 in *Flowering plant families of the American tropics*, ed. N. P. Smith et al. New York: Princeton University Press/New York Botanical Garden Press.
- Delprete, P. G. In press. Revision of *Neobertiera* (Rubiaceae, Sipaneeae) with observations on distyly, and three new species from the Guianas. *Phytotaxa*.

- Delprete, P. G. and J. G. Jardim. 2012. Systematics, taxonomy and floristics of Brazilian Rubiaceae: an overview about the current status and future challenges. *Rodriguésia* 63: 101–128.
- Delprete, P. G. and T. J. Motley. 2003. *Portlandia proctorii* (Rubiaceae, Catesbaeeae), a new combination for a narrow endemic Jamaican species. *Brittonia* 55: 230–236.
- Dessein, S., S. Huysmans, E. Robbrecht, and E. Smets. 2002. Pollen of African *Spermacoce* species (Rubiaceae) morphology and evolutionary aspects. *Grana* 41: 69–89.
- Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Phylogenetics and Evolution* 29: 1969–1973.
- Fosberg, F. R., M. H. Sachet, and R. L. Oliver. 1993. Bignoniaceae- Rubiaceae. *Flora of Micronesia* vol. 5. Washington D.C.: Smithsonian Institution Press.
- Fritsch, P. W. 2001. Phylogeny and biogeography of the flowering plant genus *Styrax* (Styraceae) based on chloroplast DNA restriction sites and DNA sequences of the internal transcribed spacer region. *Molecular Phylogenetics and Evolution* 19: 387–408.
- Fritsch, P. W. and T. D. McDowell. 2003. Biogeography and phylogeny of Caribbean plants, introduction. *Systematic Botany* 28: 376–377.
- Fritsch, P. W., S. R. Manchester, R. D. Stone, B. C. Cruz, and F. Almeda. 2014. Northern hemisphere origins of the amphi-Pacific tropical plant family Symplocaceae. *Journal of Biogeography* 42: 891-901.

- Givnish, T. J. and S. S. Renner. 2004. Tropical intercontinental disjunctions, Gondwana breakup, immigration from the Boreotropics, and transoceanic dispersal. *International Journal of Plant Sciences* 165: S1–S6.
- Givnish, T. J., K. C. Millan, T. M. Evans, J. C. Hall, J. C. Pires, P. E. Berry, and K. J. Sytsma. Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American-African disjunctions in Rapteaceae and Bromeliaceae based on *ndh F* sequence data. *International Journal of Plant Sciences* 165: S35–S54.
- González, F. A. and P. J. Rubdall. 2010. Flower and fruit characters in the early-divergent lamiid family Metteniusaceae, with particular reference to the evolution of pseudomonometry. *American Journal of Botany* 97: 191–206.
- Govaerts, R., L. Andersson, D. Bridson, A. Davis, E. Robbrecht, M. Ruhsam, I. Schanzer, and B. Sonke. 2014. World Checklist of Rubiaceae. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; <http://apps.kew.org/wcsp/> Last consulted on 15 November 2014.
- Graham, A. 2003. Geohistory models and Cenozoic paleoenvironments of the Caribbean region. *Systematic Botany* 28: 378–386.
- Graham, A. 2010. *Late Cretaceous and Cenozoic history of Latin American vegetation and terrestrial environment*. St. Louis, MO: Missouri Garden Press.
- Heads, M. 1999. Vicariance biogeography and terrane tectonics in the South Pacific, analysis of the genus *Abrotanella* (Compositae). *Biological Journal of the Linnean Society* 67: 391–432.

- Heads, M. 2010. The endemic plant families and the palms of New Caledonia, a biogeographical analysis. *Journal of Biogeography* 37: 1239–1250.
- Herbert, J., P. M. Hollingsworth, M. F. Gardner, R. R. Mill, P. I. Thomas, and T. Jaffre. 2000. Conservation genetics and phylogenetics of New Caledonian *Retrophyllum* (Podocarpaceae) species. *New Zealand Journal of Botany* 40: 175–188.
- Hooker, J. D. 1873. Ordo LXXXIV. Rubiaceae. Pp. 7–151 in *Genera Plantarum*, vol. 2, ed. G. Bentham and J. D. Hooker. London: Lovell and Co.
- Howarth, D. G., M. G. Gustafsson, D. A. Baum, and T. J. Motley. 2003. Phylogenetics of the genus *Scaevola* (Goodeniaceae), implication for dispersal patterns across the Pacific basin and colonization of the Hawaiian islands. *American Journal of Botany* 90: 915–923.
- Huang, W.-P., H. Sun, T. Deng, S. G. Razafimandibison, Z.-L. Nie, and J. Wen. 2013. Molecular phylogenetics and biogeography of the eastern Asian-eastern North American disjunct *Mitchella*, and its close relative *Damnacanthus* (Rubiaceae, Mitchelleae). *Botanical Journal of the Linnean Society* 171: 395–412.
- Huysmans, S., E. Robbrecht, P.G. Delprete, and E. Smets. 1999. Pollen morphological support for the Catesbaeae-Chiococceae-*Exostema*-complex (Rubiaceae). *Grana* 38: 325–338.
- Igersheim, A. 1993. The character states of the Caribbean monotypic endemic *Strumpfia* (Rubiaceae). *Nordic Journal of Botany* 13: 545–559.
- Iturralde-Vinent, M. A. and R. D. E. MacPhee. 1999. Paleogeography of the Caribbean region, implications for Cenozoic biogeography. *Bulletin of the American Museum of Natural History* 238: 1–95.

- Iturralde, R. B. 2008. Actualización de la lista de los generos endemicos cubanos espermatofitos. *Revista del Jardin Botanico Nacional* 29: 3–10.
- Jabaily, R. S., K. A. Shepherd, M. H. G. Gustafsson, L. W. Sage, Siegy L. Krauss, D. G. Howarth, and T. J. Motley. 2012. Systematics of Austral-Pacific family Goodeniaceae: establishing a taxonomic and evolutionary framework. *Taxon* 61: 419–436.
- Jacquin, N. J. 1760. *Enumeratio Systematica Plantarum*. Leiden: Theodorum Haak.
- Jussieu, A. L. de. 1789. Ordo II. Rubiaceae. Pp. 196–210 in *Genera Plantarum*. Paris: E. T. Barrois.
- Katoh, K. and H. Toh. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298.
- Keppel, G., A. J. Lowe, and H. P. Possingham. 2009. Changing perspectives on the biogeography of the tropical South Pacific, influences of dispersal, vicariance and extinction. *Journal of Biogeography* 36: 1035–1054.
- Kirkbride, J. H. 1997. Manipulus rubiacearum VI. *Brittonia* 49: 354–379.
- Li, L., J. Li, J. G. Rohwer, H. van der Werff, Z.-H. Wang, and H.-W. Li. 2011. Molecular phylogenetic analysis of the *Persea* group (Lauraceae) and its biogeographic implications on the evolution of tropical and subtropical Amphi-Pacific disjunctions. *American Journal of Botany* 98: 1520–1536.
- Li, R. and J. Wen. 2013. Phylogeny and biogeography of *Dendropanax* (Araliaceae), an Amphi-Pacific disjunct genus between tropical/subtropical Asia and the Neotropics. *Systematic Botany* 38: 536–551.
- Lohne, C. and T. Borsch. 2005. Molecular evolution and phylogenetic utility of the *petD*

- group II intron- a case study in basal angiosperms. *Molecular Biology and Evolution* 22: 317–332.
- Loytynoja, A. and N. Goldman. 2005. An algorithm for progressive multiple alignment of sequences with insertions. *Proceedings of the National Academy of Sciences* 102: 10557–10562.
- Maddison, D. R. and W. P. Maddison. 2005. MacClade 4: Analysis of phylogeny and character evolution. Version 4.08a. <http://macclade.org>.
- Maddison, W. P. and D. R. Maddison. 2009. Mesquite: a modular system for evolutionary analysis. Version 2.72. <http://mesquiteproject.org>.
- Maddison, W. P. and D. R. Maddison. 2014. Mesquite, a modular system for evolutionary analysis. Version 3.01 <http://mesquiteproject.org>.
- Manns, U. and B. Bremer. 2010. Towards a better understanding of intertribal relationships and stable tribal delimitations within Cinchonoideae s. s. (Rubiaceae). *Molecular Phylogenetics and Evolution* 56: 21–39.
- Manns, U., N. Wikstrom, C. M. Taylor, and B. Bremer. 2012. Biogeography of the predominantly Neotropical subfamily Cinchonoideae (Rubiaceae): into or out of America? *International Journal of Plant Sciences* 173: 261–286.
- Maurin, O., A. P. Davis, M. Chester, E. F. Mvungi, Y. J. Ally-Fakim, and M. F. Fay. 2007. Towards a phylogeny for *Coffea* (Rubiaceae) - identifying well-supported lineages based on nuclear and plastid DNA sequences. *Annals of Botany* 100: 1565–1583.

- McCarthy, D. 2003. The Trans-Pacific zipper effect, disjunct sister taxa and matching geological outlines that link the Pacific margins. *Journal of Biogeography* 30: 1545–1561.
- McCarthy, D., M. C. Ebach, J. J. Morrone, and L. R. Parenti. 2007. An alternative Gondwana: biota links South America, New Zealand and Australia. *Biogeografia* 2: 2–12.
- McDowell, T. 1995. A monograph of *Exostema* (Rubiaceae). Ph. D. dissertation. Durham, North Carolina: Duke University.
- McDowell, T. 1996. *Exostema* (Rubiaceae): taxonomic history, nomenclature, position and subgeneric classification. *Opera Botanica Belgica* 7: 277–295.
- McDowell, T. and B. Bremer. 1998. Phylogeny, diversity, and distribution in *Exostema* (Rubiaceae): implications of morphological and molecular analyses. *Plant Systematics and Evolution* 212: 215–246.
- McDowell, T., M. Volovsek, and P. Manos. 2003. Biogeography of *Exostema* (Rubiaceae) in the Caribbean region in light of molecular phylogenetic analyses. *Systematic Botany* 28: 431–441.
- Miller, M.A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp 1-8 in *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans: Institute of Electrical and Electronics Engineers.
- Milne, R. I. 2006. Northern hemisphere plant disjunctions, a window on Tertiary land bridges and climate change? *Annals of Botany* 98: 465–472.

- Milne, R. I. 2009. Effects of taxon sampling on molecular dating for within-genus divergence events, when deep fossils are used for calibration. *Journal of Systematics and Evolution* 47: 383–401.
- Morelock, J., R. Wilson, P. Hallock, and D. Hubbard. 2015. A paleoceanographic summary. Available at <http://geology.uprm.edu/Morelock/paleoceanog.htm>, retrieved on 28 January 2015.
- Morley, R. J. 2003. Interplate dispersal paths for megathermal angiosperms. *Perspectives in Plant Ecology, Evolution and Systematics* 6: 5–20.
- Mort, M. E., C. P. Randle, R. T. Kimball, M. Tadesse, and D. J. Crawford. 2008. Phylogeny of Coreopsidae (Asteraceae) inferred from nuclear and plastid DNA sequences. *Taxon* 57: 1–12.
- Motley, T. J., K. J. Wurdack, and P. G. Delprete. 2005. Molecular systematics of the Catesbaeeae-Chiococceae complex (Rubiaceae), flower and fruit evolution and biogeographic implications. *American Journal of Botany* 92: 316–329.
- Neall, V. E. and S. A. Trewick. 2008. The age and origin of the Pacific islands, a geological overview. *Philosophical Transactions of the Royal Society B* 363: 3293–3308.
- Negron-Ortiz, V. 2005. Taxonomic revision of the neotropical genus *Erithalis* (Rubiaceae, Chiococceae). *SIDA, Contributions to Botany* 21: 1565–1598.
- Negron-Ortiz, V. and L. E. Watson. 2002. Molecular phylogeny and biogeography of *Erithalis* (Rubiaceae), an endemic of the Caribbean Basin. *Plant Systematics and Evolution* 234: 71–83.
- Negron-Ortiz, V. and L. E. Watson. 2003. Hypotheses for the colonization of the

- Caribbean basin by two genera of the Rubiaceae: *Erithalis* and *Ernodea*. *Systematic Botany* 28: 442–451.
- Nickrent, D. L., K. P. Schuette, and E. M. Starr. 1994. A molecular phylogeny of *Arceuthobium* (Viscaceae) based on nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 81: 1149–1160.
- Nie, Z.-L., J. Wen, H. Sun, and B. Bartholomew. 2005. Monophyly of *Kelloggia* Torrey ex Benth. (Rubiaceae) and evolution of its intercontinental disjunction between western North America and eastern Asia. *American Journal of Botany* 92: 642–652.
- Nie, Z.-L., T. Deng, Y. Meng, H. Sun, and J. Wen. 2013. Post-Boreotropical dispersals explain pantropical disjunction in *Paderia* (Rubiaceae). *Annals of Botany* 111: 873–886.
- Nylinder, S., U. Swenson, C. Persson, S. B. Janssens, and B. Oxelman. 2012. A dated species-tree approach to the trans-Pacific disjunction of the genus *Jovellana* (Calceolariaceae, Lamiales). *Taxon* 61: 381–391.
- Ochoterena-Booth, H. 2000. Systematics of *Hintonia* Bullock and the *Portlandia* complex (Rubiaceae). Ph. D. dissertation. Ithaca, New York: Cornell University.
- Paudyal, S. K., P. G. Delprete, and T. J. Motley. 2014. Using molecular, morphological, and palynological evidence to transfer *Strumpfia maritima* to the monotypic tribe Strumpfieae (Cinchonoideae, Rubiaceae), and a re-delimitation of the tribe Chiococceae. *Systematic Botany* 39: 1197–1203.
- Piesschaert, F., S. Jansen, I. Jaimes, E. Robbrecht, and E. Smets. 2001. Morphology, anatomy, and taxonomic position of *Pagameopsis* (Rubiaceae - Rubioideae).

- Brittonia* 53: 490–504.
- Posada, D. 2008. jModelTest; Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.
- Pregill, G. K. and D. W. Steadman. 2004. South Pacific iguanas, human impacts and a new species. *Journal of Herpetology* 38: 15–21.
- Puff, C., A. Igersheim, R. Buchner, and U. Rohrhofer. 1995. The united stamens of Rubiaceae. Morphology, anatomy; their role in pollination ecology. *Annals of the Missouri Botanical Garden* 82: 357–382.
- Qian, H. 1999. Floristic analysis of vascular plant genera of North America north of Mexico, characteristics of phytogeography. *Journal of Biogeography* 26: 1307–1321.
- Rambaut, A., M. A. Suchard, D. Xie, and A. J. Drummond. 2014. Tracer Version 1.6, available from <http://beast.bio.ed.ac.uk/Tracer>.
- Raven, P. 1972. Plant species disjunctions, a summary. *Annals of the Missouri Botanical Garden* 59: 234–246.
- Razafimandimbison, S. G. and B. Bremer. 2002. Phylogeny and classification of Naucleae s. l. (Rubiaceae) inferred from molecular (ITS, *rbcL*, and *trnT-F*) and morphological data. *American Journal of Botany* 89: 1027–1041.
- Razafimandimbison, S. G. and B. Bremer. 2006. Taxonomic revision of the tribe Hymenodictyeae (Rubiaceae, Cinchonoideae). *Botanical Journal of the Linnean Society* 152: 331–386.
- Richard, A. 1830. *Memoire sur la famille Rubiacées, contenant les caractères des genres de cette famille et d'un grand nombre d'espèces nouvelles*. Paris: J. Tastu.

- [Reprinted in *Memoires de la Societ  d'Histoire Naturelle de Paris*, 5: 81–304. 1834].
- Ridsdale, C. E. 1982. A revision of *Badusa* (Rubiaceae, Condamineae, *Portlandia*). *Blumea* 28: 145–150.
- Robbrecht, E. 1988. Tropical woody Rubiaceae: charactersistic features and progressions, contributions to a new subfamilial classification. *Opera Botanica Belgica* 1: 1–271.
- Robbrecht, E. 1993. Supplement to the 1988 outline of the classification of the Rubiaceae, index to genera. *Opera Botanica Belgica* 6: 173–196.
- Robbrecht, E., and J. Manen. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms)- combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-F* and *atpB-rbcL* data, a new classification in two subfamilies, Cinchonoideae and Rubioideae. *Systematics and Geography of Plants* 76: 85–145.
- Rogers, G. K. 2005. The genera of Rubiaceae in the southeastern United States, Part II; subfamily Rubioideae, and subfamily Cinchonoideae revisited (*Chiococca*, *Erithalis*, and *Guettarda*). *Harvard Papers in Botany* 10: 1–5.
- Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Ronquist, F., M. Teslenko, P. van der Mark, D. Ayres, A. Darling, S. H hna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

- Rova, J. H. E. 1999. Rubiaceae phylogeny based on *rps16* sequence data. Pp. 1–29 in *The Condamineae-Rondeletieae-Sipaneeae complex (Rubiaceae)*. Ph. D. dissertation. Goteborg, Sweden: Göteborg University.
- Rova, J. H. E., L. Andersson, P. G. Delprete, and V. A. Albert. 2002. A *trnL-F* cpDNA sequence study of the Condamineae-Rondeletieae-Sipaneeae complex with implications on the phylogeny of the Rubiaceae. *American Journal of Botany* 89: 145–159.
- Rydin, C., S. G. Razafimandimbison, and B. Bremer. 2008. Rare and enigmatic genera (*Dunnia*, *Schizocolea*, *Colletoecema*), sisters to species-rich clades: phylogeny and aspects of conservation biology in the coffee family. *Molecular Phylogenetics and Evolution* 48: 74–83.
- Santiago-Valentin, E. and R. G. Oldmstead. 2004. Historical biogeography of Caribbean plants, introduction to current knowledge and possibilities from a phylogenetic perspective. *Taxon* 53: 299–319.
- Schumann, K. 1891. Rubiaceae. Pp. 1–156 in *Die Natürlichen Pflanzenfamilien*, vol. 4, part 4, eds. A. Engler and K. Prantl. Leipzig: Engelmann.
- Smith, J. F. 2001. High species diversity in fleshy-fruited tropical understory plants. *The American Naturalist* 157: 646–653.
- Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and Other Methods). Version 4. Sunderland: Sinauer Associates.
- Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

- Tan, B. C. 1998. Noteworthy disjunctive patterns of Malesian mosses. Pp. 235–241 in *Biogeography and Geological Evolution of SE Asia*, ed. R. Hall and J. D. Holloway. Leiden: Backhuys Publishers.
- Taylor, C. M. and D. H. Lorence. 2010. Rubiacearum Americanarum Magna Hama Pars XXII: notable new species of South American *Coutarea*, *Morinda*, *Patima*, and *Rosenbergiodendron*. *Novon* 20: 95–105.
- Thorne, R. F. 1972. Major disjunctions in the geographic ranges of seed plants. *The Quarterly Review of Biology* 47: 365–411.
- Tosh, J., S. Dessein, S. Buerki, I. Groeninckx, A. Mouly, B. Bremer, F. F. Smets, and P. De Block. 2013. Evolutionary history of the Afro-Madagascan *Ixora* species (Rubiaceae), species diversification and distribution of key morphological traits inferred from dated molecular phylogenetic trees. *Annals of Botany* 112: 1723–1742.
- Urban, I. 1923. *Symbolae antillanae seu fundamenta florae Indiae occidentalis*. vol. 9. Lipsiae: Fratres Borntraeger.
- Van der Hammen, T. and A. M. Cleef. 1983. *Trigonobalanus* and the tropical Amphipacific element in the north Andean forest. *Journal of Biogeography* 10: 437–440.
- Verdcourt, B. 1958. Remarks on the classification of the Rubiaceae. *Bulletin du Jardin Botanique de l'Etat (Bruxelles)* 28: 209–281.
- Walsh, H. E., M. G. Kidd, T. Moum, and V. L. Friesen. 1999. Polytomies and the power of phylogenetic inference. *Evolution* 53: 932–937.

- Wen, J. 1999. Evolution of Eastern Asian and Eastern North American disjunct distributions flowering plants. *Annual Review Ecology and Systematics* 30: 421–455.
- Wernham, H. F. 1913. New Rubiaceae from tropical America III. *Journal of Botany British and foreign* 51: 320–324
- Wikstrom, N., M. Avino, S. G. Razafimandimbison, and B. Bremer. 2010. Historical biogeography of the coffee family (Rubiaceae, Gentianales) in Madagascar: case studies from the tribes Knoxieae, Naucleaeae, Paederieae and Vanguerieae. *Journal of Biogeography* 37: 1094–1113.
- Woo, V. L., M. M. Funke, J. F. Smith, P. J. Lockhart, and P. J. Garnock-Jones. 2011. New world origins of Southwest Pacific Gesneriaceae, multiple movements across and within the South Pacific. *International Journal of Plant Sciences* 172: 434–457.
- Yokoyama, J., M. Suzuki, K. Iwatsuki, and M. Hasebe. 2000. Molecular phylogeny of Coriaria, with special emphasis on the disjunct distribution. *Molecular Phylogenetics and Evolution* 14: 11–19.
- Yu Y., A. J. Harris, and X. J. He. 2014. RASP (Reconstruct Ancestral State in Phylogenies) 3.0. Available at <http://mnh.scu.edu.cn/soft/blog/RASP>
- Zhou, Z., X. Yang, and Q. Yang. 2006. Land bridge and long-distance dispersal, old views, new evidence. *Chinese Science Bulletin* 51: 1030–1038.

APPENDIX A

TAXA USED IN THE STUDY WITH GENBANK ACCESSION AND VOUCHER INFORMATION

Voucher information is given only for newly generated sequences and includes: taxon, origin, voucher, herbarium, and GenBank accession number, respectively. New sequences generated for this study are indicated with asterisks (*). For sequences used in previous studies, only the GenBank accession numbers are given with respective publications denoted by superscripts following the GenBank accession numbers: ^a Rova et al. (2002), ^b Razafimandimbison and Bremer (2002), ^c Motley et al. (2005), ^d Manns and Bremer (2010), ^e Maurin et al. (2007), ^f Alejandro et al. (2005), ^g Bremer and Eriksson (2009), ^h Andersson and Antonelli (2005), ⁱ Wikstrom et al. (2010), ^j Rydin et al. (2008).

Acrosynanthus latifolius Standl. AF152751^a. *Adina rubella* Hance. AJ346910^b. *Adinauclea fagifolia* (Teijsm. & Binn. Ex Havil.) Ridsdale. AJ346911^b. *Allenanthus hondurensis* Standl. AF152734^a. *Arachnothryx leucophylla* (Kunth) Planch. AF152718^a. *Badusa palauensis* Valetton. AY763799^c. *Balmea stormiae* Martinez. GQ852464^d. *Bikkia pancheri* (Brogn.) Guillaumin. NEW CALEDONIA, Isle of Pines. Moiley 2547 (NY). KJ906562*. *Bikkia tetrandra* (L.f.) A. Rich. AY763805^c. *Blepharidium guatemalense* Standl. AF152735^a. *Breonadia salicina* (Vahl) Hepper & J.R.I. Wood. AJ346912^b. *Breonia chinensis* (Lam.) Capuron. AJ346913^b. *Breonia decaryana* Homolle. AJ346914^b. *Burttavya nyasica* Hoyle. AJ346918^b. *Catesbaea fuertesii* Urb. AY763807^c. *Catesbaea holacantha* C. Wright ex Griseb. CUBA, Prov. Guantanamo. *Delprete et al.* 8890 (NY). KJ906563*. *Catesbaea parviflora* Sw. USA, Florida, Big Pine Key. *Brumbach 9544* (NY). KJ906564*. *Catesbaea spinosa* L. AY763811^c. *Cephalanthus glabratus* (Spreng.) K. Schum. AJ346919^b. *Cephalanthus occidentalis* L. AJ346955^b. *Cephalanthus salicifolius* Bonpl. AJ346909^b. *Ceratopyxis verbenacea* (Griseb.) Hook. f. CUBA, Prov. Pinar del Rio. *Delprete et al.* 8904 (NY) KJ906565*. *Chiococca alba* (L.) Hitchc. CUBA, Prov. Holguin. *Delprete et al.* 8738 (NY). KJ906566*. *Chiococca filipes* Lundell. AY763814^c. *Chiococca pachyphylla* Wernham. EL SALVADOR, Santa Ana. *Linares 7363* (NY). KJ906567*. *Chione venosa* (Sw.) Urb. I. AM117352^g. *Chomelia tenuiflora* Benth. AF152729^a. *Cinchona officinalis* L. AY538450^h. *Cinchona pubescens* Vahl. AJ346963^b. *Cinchonopsis amazonica* (Standl.) L. Andersson. AY538452^h. *Coffea moratii* J.-F. Leroy ex A. P. Davis & Rakotonas. DQ153861^c. *Colleteria seminervis* (Urb. & Ekman) D. W. Taylor. GQ852484^d. *Corynanthe paniculata* Welw. AJ346923^b. *Cosmibuena grandiflora* (Ruiz & Pav.) Rusby. AF152686^a. *Coutaportia ghiesbreghtiana* (Baill.) Urb. MEXICO, Oaxaca, Coixtlahuaca. *Panero 4061* (NY). KJ906568*. *Coutarea hexandra* (Jacq.) K. Schum. BRAZIL, Bahia, Itaberaba. *Melo 4318* (NY). KJ906569*. *Cubanola daphnoides* (Graham) Aiello. CUBA, Prov. Holguin. *Delprete et al.* 8808 (NY). KJ906570*. *Cubanola domingensis* (Britton) Aiello. DOMINICAN REPUBLIC. *Acevedo-Rodriguez 8476* (US). KJ906571*. *Eosanthe cubensis* Urb. GQ852495^d. *Erithalis fruticosa* L. AY763824^c. *Erithalis harrisii* Urb. AY763823^c. *Erithalis vacciniifolia* (Griseb.) Wright Ex Sauv. AY763825^c. *Exostema caribaeum* (Jacq.) Schult. CUBA, Prov. Guantanamo. *Delprete et al.* 8892 (NY). KJ906572*. *Exostema spinosum* (Vavass.) Krug. & Urb. CUBA, Prov. Santiago. *Delprete et al.* 8896 (NY). KJ906573*. *Ferdinandusa speciosa* (Pohl) Pohl. EU145534ⁱ. *Gonzalagunia affinis* Standl. Ex Steyer. AJ847405^f. *Guettarda boliviana* Standl. AF152727^a. *Guettarda speciosa* L. FRENCH POLYNESIA, Bora Bora. *Motley 2040* (NY). KJ906574*. *Gyrostipula comorensis* Leroy. AJ346925^b. *Haldina cordifolia* (Roxb.) Ridsdale. AJ346956^b. *Hamelia cuprea* Griseb. AM117361^g. *Hamelia papillosa* Urb. GQ852511^d. *Hamelia versicolor* A. Gray. USA, Hawaii, living collection at National Tropical Botanical Garden, NTBG 980457001. KJ906575*. *Hamelia xorullensis* Kunth. MEXICO, Jalisco. *Ochoterena-Booth 220* (MEXU). KJ906576*. *Heinsia crinita* (Afzel.) G. Taylor. AJ847376^f. *Hillia illustris* (Vell.) K. Schum. GQ852565^d. *Hillia parasitica* Jacq. GQ852512^d. *Hillia triflora* (Oerst.) C. M. Taylor. AM117362^g. *Hintonia latiflora* (Sesse & Moc. ex DC.) Bullock. MEXICO, Puebla. *Ochoterena-Booth 182* (MEXU). KJ906577*. *Hintonia octomera* (Hemsl.) Bullock. MEXICO, Yucatan. *Ochoterena-Booth 171* (MEXU). KJ906578*. *Hodgkinsonia ovatiflora* F. Muell. AM117363^g. *Hymenodictyon floribundum* (Hochst. & Steud.) B. L. Rob. AY538454^h. *Hymenodictyon orixense* (Roxb.) Mabb. GQ852518^d. *Hymenodictyon parvifolium* Oliv. FN376382ⁱ. *Isertia coccinea* (Aubl.) J. F. Gmel. AF152689^a. *Isertia hypoleuca* Benth. AF152688^a. *Isertia laevis* (Triana) B.M. Boom. AY538456^h. *Isertia pittieri* (Standl.) Standl. AM117365^g. *Isidorea leptantha*

Urb. DOMINICAN REPUBLIC, Prov. La Altagracia. *Delprete & Clase 7590* (NY). KJ906579*. *Isidorea pedicellaris* Urb. & Ekman. DOMINICAN REPUBLIC, Prov. Espaillat. *Delprete & Clase 7628* (NY). KJ906580*. *Janotia macrostipula* (Capuron) Leroy. AJ346928^b. *Javorkaea hondurensis* (Donn.Sm.) Borhidi & Jarai-Koml. AF152716^a. *Joosia aequatoria* Steyerl. AY538457^h. *Joosia umbellifera* H. Karst. AY538458^h. *Kerianthera preclara* J. H. Kirkbr. AY538459^h. *Ladenbergia amazonensis* Ducke. AY538460^h. *Lorencea guatemalensis* (Standl.) Borhidi. MEXICO, Chiapas. *Mendez Ton 7548* (XAL). KJ906581*. *Ludkia borneensis* Ridsdale. AJ346962^b. *Machaonia portoricensis* Baill. AF152733^a. *Mazaea phialanthoides* (Griseb.) Krug & Urb. AF152749^a. *Mitragyna diversifolia* (Wall. ex G. Don) Havil. AJ346931^b. *Morierina montana* Vieill. AY763843^c. *Myrmeconuclea strigosa* (Korth.) Merr. AJ346934^b. *Nauclea orientalis* (L.) L. AJ346958^b. *Nauclea xanthoxylon* (A.Chev.) Aubrev. AJ346937^b. *Neoblakea venezuelensis* Standl. AF152732^a. *Neolamarckia cadamba* (Roxb.) Bosser. AJ346938^b. *Neonuclea brassii* S.Moore. AJ346939^b. *Nernstia mexicana* (Zucc. & Mart. ex DC.) Urb. MEXICO, San Luis Potosi. *Aiello & Medellin-Leal 1237* (NY). KJ906582*. *Ochreinauclea maingayi* (Hook.f.) Ridsdale. AJ346943^b. *Osa pulchra* (D. R. Simpson) Aiello. AY763844^c. *Pertusadina eurhyncha* (Miq.) Ridsdale. AJ346947^b. *Phialanthus ellipticus* Urb. AM117380^b. *Phialanthus jamaicensis* Urb. JAMAICA, Par. Trelawny. *Delprete et al. 7469* (NY). KJ906583*. *Phialanthus myrtilloides* Griseb. PUERTO RICO, Sabana Grande. *Axelrod 3985* (NY). KJ906584*. *Phyllacanthus grisebachianus* Hook. f. CUBA, Prov. Pinar del Rio. *Ekman 17432* (NY). KJ906585*. *Phyllomelia coronata* Griseb. AF152748^a. *Pinarophyllon bullatum* Standl. GQ852456^d. *Portlandia grandiflora* L. AY763850^c. *Portlandia microsepala* Urb. AY763852^c. *Portlandia platantha* Hook. f. AY763853^c. *Pseudomussaenda flava* Verdc. AJ847385^f. *Remijia pedunculata* (H. Karst.) Flueck. AY538473^h. *Rhachicallis americana* (Jacq.) Hittchc. AF152747^a. *Rogiera cordata* (Benth.) Planch. AF152715^a. *Roigella correifolia* (Griseb.) Borhidi & M. Fernández AF152746^a. *Rondeletia portoricensis* J. C. Krug & Urb. AF152743^a. *Rovaeanthus strigosus* (Benth.) Borhidi. GQ852550^d. *Rovaeanthus suffrutescens* (Brandege) Borhidi. GQ852551^d. *Salzmannia nitida* DC. BRAZIL, Bahia. *Jardim 580* (NY). KJ906586*. *Sarcocephalus latifolius* (Sm.) Bruce. AJ346960^b. *Schmidtottia monantha* Urb. CUBA, Prov. Guantanamo. *Delprete et al. 8855* (NY). KJ906587*. *Schmidtottia nitens* (Britton) Urb. CUBA, Prov. Guantanamo. *Delprete et al. 8860* (NY). KJ906588*. *Scolosanthus lucidus* Britton. CUBA, Prov. Holguin. *Delprete et al. 8775* (NY). KJ906589*. *Scolosanthus moanus* Borhidi & O. Muniz. CUBA, Prov. Holguin. *Delprete et al. 8829* (NY). KJ906590*. *Siemensia pendula* (C. Wright ex Griseb.) Urb. CUBA, Prov. Pinar del Rio. *Delprete et al. 8902* (NY). KJ906591*. *Sinoadina racemosa* (Siebold & Zucc.) Ridsdale. AJ346961^b. *Solenandra ixoroides* Hook. f. CUBA, Prov. Holguin. *Delprete et al. 8821* (NY). KJ906592*. *Solenandra parviflora* (A. Rich. ex Humb. & Bonpl.) Borhidi. DOMINICAN REPUBLIC, Bahoruco mountains. *Liogier 11125* (NY). KJ906593*. *Stenostomum lucidum* (Sw.) C.F. Gaertn. AM117348^g. *Stilpnophyllum grandifolium* L. Andersson. AY538476^h. *Strumpfia maritima* Jacq. 1. AF152714^a. *Strumpfia maritima* Jacq. 2. AY763866^c. *Strumpfia maritima* Jacq. 3. PUERTO RICO, Guanica State forest. *Alain 9144*. (NY). KJ906594*. *Strumpfia maritima* Jacq. 4. DOMINICAN REPUBLIC, Boca de Yuma. *Acevedo-Rodriguez 14123* (NY). KJ906595*. *Suberanthus neriifolius* (A.Rich.) Borhidi & M. Fernández Zeq. AF152737^a. *Suberanthus stellatus* (Griseb.) Borhidi & M. Fernandez Zeq. AF152736^a. *Thiollierea artensis* Montrouz. NEW CALEDONIA, Tiebaghie. *Cameron & Motley 2068* (NY). KJ906596*. *Thiollierea macrophylla* (Brongn.) Baum.-Bod. AY763801^c. *Timonius sechellensis* Summerh. AF152730^a. *Uncaria tomentosa* (Willd. ex Schult.) DC. AF152690^a.

APPENDIX B

TAXA USED IN MOLECULAR PHYLOGENY OF CHIOCOCCEAE

Voucher information includes: taxon, origin, voucher, and herbarium respectively.

Badusa corymbifera (G.Frost.) A.Gray 1. FIJI, Ovalau. *Smith 7438* (NY). *Badusa corymbifera* (G.Frost.) A.Gray 2. TONGA, Mount Talau. *Drake 265* (US). *Badusa palauensis* Valetton. PALAU, living collection at National Tropical Garden, NTBG980229. *Flynn 6354* (PTBG). *Bikkia palauensis* Valetton 1. PALAU, living collection at National Tropical Botanical Garden, NTBG980159. *Flynn 6415* (PTBG). *Bikkia palauensis* Valetton 2. PALAU, Koror island. *Fosberg 50623* (NY). *Bikkia pancheri* Guillaumin. NEW CALEDONIA, Isle of Pines. *Motley 2547* (NY). *Bikkia philippinensis* Valetton. PHILIPPINES, Dako Island. *Baete et al. BP01* (USTH). *Bikkia tetrandra* A.Gray. GUAM, Ritidian National Wildlife Refuge. *Motley 2451* (NY). *Catesbaea flaviflora* Urb. CUBA, Prov. Santiago de Cuba. *Delprete et al. 8899* (NY). *Catesbaea foliosa* Millsp. TURKS AND CAICOS ISLANDS, Parrot Cay. *Neis 193* (FTG). *Catesbaea fuertesii* Urb. DOMINICAN REPUBLIC, Prov. Peravia. *Delprete & Clase 7523* (NY). *Catesbaea gamboana* Urb. CUBA, Prov. Las Tunas, Jardin Botanico. *Delprete et al. 8901* (NY). *Catesbaea glabra* Urb. DOMINICAN REPUBLIC, Prov. Azua. *Delprete & Clase 7529* (NY). *Catesbaea holacantha* Griseb. CUBA, Prov. Guantanamo. *Delprete et al. 8890* (NY). *Catesbaea melanocarpa* Krug & Urb. USA, Florida, cultivated at Fairchild Tropical Garden FTG95986. *Catesbaea nana* Greenm. CUBA, North of Camaguey city. *Acuna 3890* (NY). *Catesbaea parviflora* Sw. USA, Big Pine Key. *Brumbach 9544* (NY). *Catesbaea spinosa* L. JAMAICA, Kingston, Hope Gardens. *Delprete et al. 7521* (NY). *Ceratopyxis verbenacea* Hook.f. 1. CUBA, Prov. Pinar del Río, Viñales. *Delprete et al. 8904* (NY). *Ceratopyxis verbenacea* Hook.f. 2. CUBA, Prov. Pinar del Río. *Rova et al. 2279* (GB). *Ceuthocarpus involucratus* (Wernham) Aiello 1. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8796* (NY). *Ceuthocarpus involucratus* (Wernham) Aiello 2. CUBA, Prov. Guantánamo, Baracoa. *Delprete et al. 8857* (NY). *Chiococca alba* (L.) Hitchc. 1. PUERTO RICO, Isabela. *Stevens et al. 234* (NY). *Chiococca alba* (L.) Hitchc. 2. DOMINICA, Par. St. Paul. *Hill 24108* (NY). *Chiococca belizensis* Lundell. COLOMBIA, Valle del Cauca. *Murphy 413* (MO). *Chiococca coriacea* M.Martens & Galeotti. MEXICO, Veracruz, coast of Oro. *Francisco 16657* (XAL). *Chiococca cubensis* Urb. 1. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8699* (NY). *Chiococca cubensis* Urb. 2. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8765* (NY). *Chiococca densifolia* Mart. BRAZIL, Distrito Federal. *Irwin 26404* (NY). *Chiococca fillipes* Lundell. MEXICO, Oaxaca. *Torres & Cadillo 2786* (NY). *Chiococca motleyana* Borhidi. MEXICO, Campache. *Martinez 28253* (NY). *Chiococca naiguatensis* Steyererm. VENEZUELA, Distrito Federal, Cerro Naiguata. *Steyermark 92128* (NY). *Chiococca nitida* Benth. BRAZIL, Para. *Davidse et al. 17833* (NY). *Chiococca oaxacana* Standl. MEXICO, Jalisco. *Anderson 5080* (MO). *Chiococca pachyphyla* Wernham. BELIZE, Cayo Distr. *Atha 1117* (NY). *Chiococca parviflora* Humb. & Bonpl. ex Roem. & Schult. BAHAMAS, Abaco National Park. *Freid 04089* (NY). *Chiococca petrina* Wiggins. MEXICO, Sonora. *Van Devender 98-1286* (NY). *Chiococca phaenostemon* Schitdl. HONDURAS, El Paraiso. *Davidse 2253* (NY). *Chiococca pinetorum* Britton ex Millsp. BAHAMAS, New Providence. *Britton 6532* (NY). *Chiococca plowmanii* Delprete. BRAZIL, Bahia. *Plowman 13948* (US). *Chiococca pubescens* Humb. & Bonpl. ex. Schult. BRAZIL, Bahia. *Anderson 37099* (NY). *Chiococca semipilosa* Standl. & Steyererm. NICARAGUA, Masaya. *Neill 2969* (MO). *Chiococca sessilifolia* Miranda. MEXICO, Chiapas. *Jimenez 907* (MO). *Coutaportla ghiesbraghtiana* (Baill.) Urb. 1. MEXICO, Hidalgo. *Lorence 4147* (MO). *Coutaportla ghiesbreghtiana* (Baill.) Urb. 2. MEXICO, Hidalgo, Cardonal. *Lorence 5042* (NY). *Coutaportla pailensis* Villarreal. MEXICO, Coahuila. *Villarreal 3063* (NY). *Coutarea andrei* Standl. ECUADOR, El Cisne. *Smith 225b* (MO). *Coutarea fuchsoides* C.M.Taylor. PERU, Antonio Raimondi. *Jara 55* (MO). *Coutarea hexandra* (Jacq.) K.Schum. 1. PARAGUAY, Cerro Pero. *Zardini & Aguayo 9554* (MO). *Coutarea hexandra* (Jacq.) K.Schum. 2. BRAZIL, Bahia. *Melo 4318* (NY). *Cubanola daphnoides* (Graham) Aiello. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8808* (NY). *Cubanola domingensis* (Britton) Aiello. DOMINICAN REPUBLIC, Prov. Altigracia. *Acevedo-Rodriguez 8476* (US). *Eosantha cubensis* Urb. CUBA, Prov. Oriente. *Ekman 15990* (S). *Erithalis diffusa* Correll. BAHAMAS, San Salvador. *Strittmatter s.n.* (MU). *Erithalis fruticosa* L. PUERTO RICO, Guanica state forest. *Gustafsson 280* (NY). *Erithalis harrisii* Urb. JAMAICA, Par. Trelawny. *Delprete et al. 7476* (NY). *Erithalis quadrangularis* Krug & Urb. JAMAICA, Manchester Parish. *Barcelona 1196* (MU). *Erithalis salmeoides* Correll. TURKS AND CAICOS ISLANDS, Pine Cay. *Raven 28205* (MO). *Erithalis vacciniifolia* (Griseb.)

C. Wright. DOMINICAN REPUBLIC, Prov. Pedernales. *Delprete & Clase 7551* (NY). *Exostema acuminatum* Urb. DOMINICAN REPUBLIC, living collection from NATIONAL TROPICAL GARDEN930070001. *McDowell 4410* (DUKE). *Exostema caribaeum* (Jacq.) Roem. & Schult. 1. JAMAICA, Par. St. Catherine. *Delprete et al. 7463* (NY). *Exostema caribaeum* (Jacq.) Roem. & Schult. 2. CUBA, Prov. Guantanamo, San Antonio del Sur. *Delprete et al. 8892* (NY). *Exostema corymbosum* Spreng. 1. PERU, Chota, Huambos. *Ferreya 8446* (US). *Exostema corymbosum* Spreng. 2. PERU, Chota. *Weigend 8613* (MO). *Exostema ellipticum* Griseb. DOMINICAN REPUBLIC, Prov. Azua. *Delprete & Clase 7543* (NY). *Exostema lineatum* Roem. & Schult. 1. DOMINICAN REPUBLIC, living collection from NATIONAL TROPICAL GARDEN960211. *McDowell 4353* (DUKE). *Exostema lineatum* Roem. & Schult. 2. DOMINICAN REPUBLIC, Prov. Barahona. *Delprete & Clase 7574* (NY). *Exostema longiflorum* Roem. & Schult. DOMINICAN REPUBLIC, living collection from NATIONAL TROPICAL GARDEN950031. *McDowell 4991* (DUKE). *Exostema maynense* Poepp. & Endl. 1. BOLIVIA, Santa Cruz. *Nee 44497* (US). *Exostema maynense* Poepp. & Endl. 2. BOLIVIA, Beni, Gral. Ballivian. *Guareco 283* (MO). *Exostema nitens* Urb. DOMINICAN REPUBLIC, living collection from NATIONAL TROPICAL GARDEN96027001. *McDowell 4414* (NY). *Exostema purpureum* Griseb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8661* (NY). *Exostema sanctae-luciae* (Kentish) Britten 1. DOMINICA, Dominica. *Higgins 126* (NY). *Exostema sanctae-luciae* (Kentish) Britten 2. DOMINICA, Morne Diablotins, near Syndicate. *Whiteford 3656* (US). *Exostema spinosum* Krug & Urb. 1. DOMINICAN REPUBLIC, Prov. Independencia. *Delprete & Clase 7576* (NY). *Exostema spinosum* Krug & Urb. 2. CUBA, Prov. Santiago de Cuba. *Delprete et al. 8896* (NY). *Exostema stenophyllum* Britton. CUBA, Oriente. *McDowell 4960* (MO). *Guettarda speciosa* L. FRENCH POLYNESIA, Bora Bora. *Motley 2040* (NY). *Hamelia versicolor* A.Gray. USA, Hawaii, cultivated at National Tropical Botanical Garden. *Hintonia latiflora* (Sessé & Moc. Ex DC.) Bullock 1. MEXICO, Puebla. *Ochoterena-Booth 182* (MEXU). *Hintonia latiflora* Bullock 2. MEXICO, Sonora. *Reina 98-2067* (NY). *Hintonia octomera* (Hemsl.) Bullock. MEXICO, Yucatan. *Ochoterena-Booth 171* (MEXU). *Isidorea brachyantha* Urb. DOMINICAN REPUBLIC, El Seibo. *Zanoni et al. 36220* (NY). *Isidorea brachycarpa* (Urb.) Aiello. CUBA, Prov. Guantánamo, Baracoa. *Delprete et al. 8852* (NY). *Isidorea elliptica* Alain. CUBA, Prov. Guantanamo, San Antonio del Sur. *Delprete et al. 8887* (NY). *Isidorea leonardii* Urb. DOMINICAN REPUBLIC, Azua. *Mejia et al. 1873* (NY). *Isidorea leptantha* Urb. DOMINICAN REPUBLIC, Prov. Independencia. *Delprete & Clase 7590* (NY). *Isidorea ophiticola* (Borhidi) Borhidi 1. CUBA, Prov. Oriente. *Carabia 3809* (NY). *Isidorea ophiticola* (Borhidi) Borhidi 2. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8670* (NY). *Isidorea pedicellaris* Urb. & Ekman. DOMINICAN REPUBLIC, Prov. Espaillat. *Delprete & Clase 7628* (NY). *Isidorea polyneura* (Urb.) Aiello. CUBA, Prov. Oriente. *Figueiras & Morton 2426* (NY). *Isidorea pungens* B.L.Rob. DOMINICAN REPUBLIC, Prov. Azua. *Delprete & Clase 7524* (NY). *Isidorea veris* Ekman ex Aiello & Borhidi. DOMINICAN REPUBLIC, Prov. Puerto Plata. *Delprete & Clase 7639* (NY). *Lorencea guatemalensis* (Standl.) Borhidi 1. MEXICO, Chiapas, Ocosingo. *Breedlove & Almeda 48414* (MO). *Lorencea guatemalensis* (Standl.) Borhidi 2. MEXICO, Chiapas, La Trinitaria. *Breedlove & Almeda 57623* (NY). *Morierina montana* Vieill. 1. NEW CALEDONIA, Riviere des Pirogues. *McPherson 6272* (MO). *Morierina montana* Vieill. 2. NEW CALEDONIA, Riviere Bleue. *Motley & Cameron 2203* (NY). *Nernstia mexicana* (Zucc. & Mart. ex DC.) Urb. 1. MEXICO, San Luis Potosi. *Aiello & Medellin-Leal 1237* (NY). *Nernstia mexicana* (Zucc. & Mart. ex DC.) Urb. 2. MEXICO, Hidalgo. *Lorence 4151* (XAL). *Osa pulchra* (D.R.Simpson) Aiello. COSTA RICA, Osa peninsula. *Hammel 18371* (MO). *Phialanthus acunae* Borhidi. CUBA, Prov. Oriente. *Marie-Victorin & Clement 21830* (US). *Phialanthus ellipticus* Urb. CUBA, Prov. Holguín, Sierra Nipe. *Morton & Acuna 2962* (US). *Phialanthus grandifolius* Alain. PUERTO RICO, Maricao state forest. *Liogier 35814* (NY). *Phialanthus hispaniolae* Alain & R.G.García. DOMINICAN REPUBLIC, Independencia. *Gracia et al. 4488* (NY). *Phialanthus jamaicensis* Urb. JAMAICA, Par. Trelawny. *Delprete et al. 7469* (NY). *Phialanthus linearis* Alain. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8827* (NY). *Phialanthus myrtilloides* Griseb. PUERTO RICO, Sabana Grande. *Axelrod 3985* (NY). *Phialanthus oblongatus* Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8655* (NY). *Phialanthus rigidus* Griseb. CUBA, Prov. Pinar del Río, La Palma. *Delprete et al. 8907* (NY). *Phyllacanthus grisebachianus* Hook.f. CUBA, Prov. Pinar del Río, Toscano. *Ekman 17432* (NY). *Portlandia coccinea* Sw. JAMAICA, Par. Trelawny. *Delprete et al. 7480* (NY). *Portlandia grandiflora* L. JAMAICA, Par. Trelawny. *Delprete et al. 7477* (NY). *Portlandia harrisii* Britton. JAMAICA, Par. Clarendon. *Delprete et al. 7484* (NY). *Portlandia microsepala* Urb. JAMAICA, Par. St. Ann. *Delprete et al. 7505* (NY). *Portlandia platantha* Hook.f. 1. JAMAICA, Par. St. Andrew. *Delprete et al. 7516* (NY). *Portlandia platantha* Hook.f. 2. JAMAICA, Par. St. Mary. *Delprete et al. 7510* (NY). *Portlandia proctorii*

(Aiello) Delprete. JAMAICA, Par. St. Catherine. *Delprete et al.* 7460 (NY). *Salzmannia nitida* DC. 1. BRAZIL, Bahia. *Jardin et al.* 580 (NY). *Salzmannia nitida* DC. 2. BRAZIL, Bahia. *Thomas et al.* 10294 (NY). *Schmidtottia cubensis* (Standl.) Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8722 (NY). *Schmidtottia elliptica* Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8838 (NY). *Schmidtottia monantha* Urb. CUBA, Prov. Guantánamo, Baracoa. *Delprete et al.* 8855 (NY). *Schmidtottia nitens* Urb. CUBA, Prov. Guantánamo, Baracoa. *Delprete et al.* 8860 (NY). *Schmidtottia sessilifolia* Urb. CUBA, Prov. Oriente. *Rova et al.* 2201 (GB). *Schmidtottia shaferi* (Standl.) Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8745 (NY). *Schmidtottia uliginosa* Urb. CUBA, Prov. Holguín, Moa. *Delprete et al.* 8871 (NY). *Scolosanthus acanthodes* Urb. DOMINICAN REPUBLIC, Prov. Puerto Plata. *Delprete & Clase* 7643 (NY). *Scolosanthus acunae* Borhidi & Muñiz. CUBA, Prov. Pinar del Río, La Palma. *Delprete et al.* 8918 (NY). *Scolosanthus densiflorus* Urb. DOMINICAN REPUBLIC, Santo Domingo. *Liogier & Liogier* 20476 (NY). *Scolosanthus lucidus* Britton. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8775 (NY). *Scolosanthus moanus* Borhidi & Muñiz. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8829 (NY). *Scolosanthus multiflorus* Krug & Urb. JAMAICA, Par. St. Ann. *Harris* 10374 (US). *Scolosanthus portoricensis* Borhidi. PUERTO RICO, Susua state forest. *Liogier* 9736 (NY). *Scolosanthus reticulatus* Borhidi. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8702 (NY). *Scolosanthus selleanus* Urb. & Ekman. DOMINICAN REPUBLIC, Prov. Peravia. *Acevedo-Rodriguez* 8503 (US). *Scolosanthus subsessilis* Alain. DOMINICAN REPUBLIC, Bonao. *Liogier* 17371 (NY). *Scolosanthus triacanthus* DC. DOMINICAN REPUBLIC, Prov. Puerto Plata. *Delprete & Clase* 7533 (NY). *Scolosanthus versicolor* Vahl. BRITISH VIRGIN ISLANDS, Virgin Gorda. *Acevedo-Rodriguez* 10501 (US). *Siemensia pendula* Urb. 1. CUBA, Prov. Pinar del Río, Viñales. *Delprete et al.* 8902 (NY). *Siemensia pendula* Urb. 2. CUBA, Prov. Pinar del Río. *Ekman* 17643 (NY). *Solenandra ixoroides* Hook.f. 1. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8821 (NY). *Solenandra ixoroides* Hook.f. 2. CUBA, living collection at NATIONAL TROPICAL GARDEN960209. *McDowell* 4913-17 (DUKE). *Solenandra mexicana* (A.Gray) Borhidi. MEXICO, Campeche. *Martinez* 28090 (FLAS). *Solenandra myrtifolia* (Griseb.) Borhidi. CUBA, Prov. Guantanamo. *Axelrod* 10421 (MO). *Solenandra parviflora* (Rich.) Borhidi 1. DOMINICAN REPUBLIC, Monicon, La Leonor. *McDowell* 4436 (US). *Solenandra parviflora* (Rich.) Borhidi 2. DOMINICAN REPUBLIC, Bahoruco mountains. *Liogier* 11125 (NY). *Solenandra selleana* (Urb. & Ekman) Borhidi. HAITI, Fonds Verrettes. *Holdridge* 1385 (US). *Strumpfia maritima* Jacq. 1. PUERTO RICO. *Gustafsson* 284 (NY). *Strumpfia maritima* Jacq. 2. DOMINICAN REPUBLIC, Boca de Yuma. *Acevedo-Rodriguez* 14123 (NY). *Syringantha coulteri* (Hook.f.) T.McDowell. MEXICO, Tamaulipas. *Henrickson* 19113 (US). *Thiolliera artensis* Montrouz. NEW CALEDONIA, Tiebaghie. *Cameron & Motley* 2068 (NY). *Thiolliera campanulata* (Brongn.) Baum.-Bod. NEW CALEDONIA, Fausse Yate. *Zirnik* 101 (US). *Thiolliera fritillarioides* (Schltr.) Baum.-Bod. NEW CALEDONIA, Tontouta. *Catala-Stucki* 122 (MO). *Thiolliera macrophylla* (Brongn.) Baum.-Bod. NEW CALEDONIA, Yate. *Cameron & Motley* 2034 (NY). *Thiolliera nerifolia* (Brongn.) Barrabé & Mouly. NEW CALEDONIA, Mont du Poum. *Veillon* 7761 (NOU). *Thiolliera retusiflora* (Brongn.) Barrabé & Mouly. NEW CALEDONIA, Canala. *McPherson* 5485 (MO). *Thiolliera tubiflora* (Brongn.) Barrabé & Mouly. NEW CALEDONIA, Dzumac. *Veillon* 7620 (NOU).

APPENDIX C

PREVIOUSLY PUBLISHED SEQUENCES USED IN THE STUDY

GenBank accession numbers of previously published DNA sequences used in the study. Literature citations are indicated by respective superscripts following each GenBank accession number: ^a Motley et al. (2005), ^b Paudyal et al. (2014), ^c Manns & Bremer (2010), ^d Negron-Ortiz and Watson (2002).

Badusa palauensis Valetton. AY763868^a, AY763799^a. *Bikkia palauensis* Valetton. AY763872^a, AY763803^a. *Bikkia pancheri* Guillaumin. KJ906562^b. *Bikkia tetrandra* A.Gray. AY763874^a, AY763805^a. *Catesbaea fuertesii* Urb. AY763876^a, AY763807^a. *Catesbaea holacantha* Griseb. KJ906563^b. *Catesbaea parviflora* Sw. KJ906564^b. *Catesbaea spinosa* L. AY763811^a. *Ceratopyxis verbenacea* Hook.f. KJ906565^b. *Ceratopyxis verbenacea* Hook.f. AY763881^a, AY763812^a. *Chiococca fillipes* Lundell. AY763883^a, AY763814^a. *Chiococca pachyphyla* Wernham. AY763884^a, AY763815^a. *Coutaportla ghiesbreghtiana* (Baill.) Urb. AY763889^a, AY763820^a. *Coutarea hexandra* (Jacq.) K.Schum. KJ906569^b. *Cubanola daphnoides* (Graham) Aiello. KJ906570^b. *Cubanola domingensis* (Britton) Aiello. KJ906571. *Eosanthe cubensis* Urb. GQ852127^c, GQ852495^c. *Erithalis diffusa* Correll. AF484187^d, AF483628^d. *Erithalis fruticosa* L. AY763892^a, AY763824^a. *Erithalis harrisii* Urb. AY763893^a, AY763823^a. *Erithalis quadrangularis* Krug & Urb. AF484201^d, AF483641^d. *Erithalis vacciniifolia* (Griseb.) C.Wright. AY763894^a, AY763825^a. *Exostema caribaeum* (Jacq.) Roem. & Schult. AY763897^a, AY763828^a, KJ906572^b. *Exostema ellipticum* Griseb. AY763900^a, AY763831^a. *Exostema lineatum* Roem. & Schult. AY763902^a, AY763833^a. *Exostema lineatum* Roem. & Schult. AY763901^a, AY763832^a. *Exostema longiflorum* Roem. & Schult. AY763903^a, AY763834^a. *Exostema spinosum* Krug & Urb. AY763899^a, AY763830^a, KJ906573^b. *Guettarda speciosa* L. KJ906574^b. *Hamelia versicolor* A.Gray. KJ906575^b; *Hintonia latiflora* Bullock. AY763905^a, AY763836^a, KJ906577^b. *Hintonia octomera* (Hemsl.) Bullock. KJ906578^b. *Isidorea leptantha* Urb. KJ906579^b. *Isidorea pedicellaris* Urb. & Ekman. KJ906580. *Isidorea pungens* B.L.Rob. AY763910^a, AY763840^a; *Isidorea veris* Ekman ex Aiello & Borhidi. AY763911^a, AY763842^a. *Morierina montana* Vieill. AY763912^a, AY763843^a. *Nernstia mexicana* (Zucc. & Mart. ex DC.) Urb. KJ906582^b. *Osa pulchra* (D.R.Simpson) Aiello. AY763913^a, AY763844^a. *Phialanthus grandifolius* Alain. AY763914^a, AY763845^a. *Phialanthus jamaicensis* Urb. KJ906583^b. *Phialanthus myrtilloides* Griseb. KJ906584^b. *Phyllacanthus grisebachianus* Hook.f. KJ906585. *Portlandia coccinea* Sw. AY763918^a, AY763849^a. *Portlandia grandiflora* L. AY763919^a, AY763850^a. *Portlandia harrisii* Britton. AY763920^a, AY763851^a. *Portlandia microsepala* Urb. AY763921^a, AY763852^a. *Portlandia platantha* Hook.f. AY763917^a, AY763922^a, AY763848^a, AY763853^a. *Portlandia proctorii* (Aiello) Delprete. AY763923^a, AY763854^a. *Salzmannia nitida* DC. AY763924^a, AY763855^a, KJ906586^b. *Schmidtottia monantha* Urb. KJ906587^b. *Schmidtottia nitens* Urb. KJ906588. *Schmidtottia sessilifolia* Urb. AY763925^a, AY763856^a. *Scolosanthus acanthodes* Urb. AY763926^a, AY763857^a. *Scolosanthus lucidus* Britton. KJ906589^b. *Scolosanthus moanus* Borhidi & Muñiz. KJ906590^b. *Scolosanthus triacanthus* DC. AY763929^a, AY763860^a. *Siemensia pendula* Urb. KJ906591. *Solenandra ixoroides* Hook.f. AY763931^a, AY763862^a, KJ906592^b. *Solenandra mexicana* (A.Gray) Borhidi. AY763932^a, AY763863^a. *Solenandra parviflora* (Rich.) Borhidi. KJ906593^b. *Strumpfia maritima* Jacq. AY763935^a, AY763866^a, KJ906595^b. *Thiolliera artensis* Montrouz. KJ906596^b. *Thiolliera macrophylla* (Brongn.) Baum.-Bod. AY763870^a, AY763801^a.

APPENDIX D

DISTRIBUTION OF TAXA USED IN THE BIOGEOGRAPHY STUDY

Distribution data of the taxa used in the study. Voucher information includes: taxon, origin, voucher, and herbarium respectively. For this study, world distribution is divided into 15 geographical regions (A: Florida Keys and Continental USA, B: Bahamas, C: Cuba, D: Hispaniola, E: Jamaica, F: Puerto Rico, G: Lesser Antilles, H: Northern and Central Mexico, I: Southern Mexico and Central America, J: Atlantic coastal region, K: Orinoco-Amazon basin, L: Amazon Piedmont region, M: Andean region, N: Western Pacific Islands except New Caledonia, O: New Caledonia, P: other areas)

Badusa corymbifera (G.Frost.) A.Gray. FIJI, Ovalau. *Smith 7438* (NY). N. *Badusa palauensis* Valetton. PALAU, living collection at NATIONAL TROPICAL GARDEN 980229. *Flynn 6354* (PTBG). N. *Bikkia palauensis* Valetton. PALAU, Koror island. *Fosberg 50623* (NY). N. *Bikkia pancheri* Guillaumin. NEW CALEDONIA, Isle of Pines. *Motley 2547* (NY). N. *Bikkia philippinensis* Valetton. PHILIPPINES, Dako Island. *Baleta et al. BP01* (USTH). N. *Bikkia tetrandra* A.Gray. GUAM, Ritidian National Wildlife Refuge. *Motley 2451* (NY). N. *Catesbaea flaviflora* Urb. CUBA, Prov. Santiago de Cuba. *Delprete et al. 8899* (NY). C. *Catesbaea foliosa* Millsp. TURKS AND CAICOS ISLANDS, Parrot Cay. *Neis 193* (FTG). B. *Catesbaea fuertesii* Urb. DOMINICAN REPUBLIC, Prov. Peravia. *Delprete & Clase 7523* (NY). D. *Catesbaea gamboana* Urb. CUBA, Prov. Las Tunas, Jardin Botanico. *Delprete et al. 8901* (NY). C. *Catesbaea glabra* Urb. DOMINICAN REPUBLIC, Prov. Azua. *Delprete & Clase 7529* (NY). D. *Catesbaea holacantha* Griseb. CUBA, Prov. Guantanamo. *Delprete et al. 8890* (NY). C. *Catesbaea melanocarpa* Krug & Urb. USA, Florida, cultivated at Fairchild Tropical Garden FTG95986. F, G. *Catesbaea nana* Greenm. CUBA, North of Camaguey city. *Acuna 3890* (NY). C. *Catesbaea parviflora* Sw. USA, Big Pine Key. *Brumbach 9544* (NY). A, B, C, D, E, F, G. *Catesbaea spinosa* L. JAMAICA, Kingston, Hope Gardens. *Delprete et al. 7521* (NY). B, C. *Ceratopyxis verbenacea* Hook.f. CUBA, Prov. Pinar del Río, Viñales. *Delprete et al. 8904* (NY). C. *Ceuthocarpus involucreatus* (Wernham) Aiello. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8796* (NY). C. *Chiococca alba* (L.) Hitchc. PUERTO RICO, Isabela. *Stevens et al. 234* (NY). A, B, C, D, E, F, G, H, I, J, K, L, M. *Chiococca belizensis* Lundell. COLOMBIA, Valle del Cauca. *Murphy 413* (MO). H, I, M. *Chiococca coriacea* M.Martens & Galeotti. MEXICO, Veracruz, coast of Oro. *Francisco 16657* (XAL). H, I. *Chiococca cubensis* Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8699* (NY). C. *Chiococca densifolia* Mart. BRAZIL, Distrito Federal. *Irwin 26404* (NY). J, K. *Chiococca fillipes* Lundell. MEXICO, Oaxaca. *Torres & Cadillo 2786* (NY). H, I. *Chiococca motleyana* Borhidi. MEXICO, Campache. *Martinez 28253* (NY). I. *Chiococca naiguatensis* Steyererm. VENEZUELA, Distrito Federal, Cerro Naiguata. *Steyermark 92128* (NY). K. *Chiococca nitida* Benth. BRAZIL, Para. *Davidse et al. 17833* (NY). J, K. *Chiococca oaxacana* Standl. MEXICO, Jalisco. *Anderson 5080* (MO). H. *Chiococca pachyphyla* Wernham. BELIZE, Cayo Distr. *Atha 1117* (NY). H, I, K, M. *Chiococca parviflora* Humb. & Bonpl. ex Roem. & Schult. BAHAMAS, Abaco National Park. *Freid 04089* (NY). B. *Chiococca petrina* Wiggins. MEXICO, Sonora. *Van Devender 98-1286* (NY). H. *Chiococca phaenostemon* Schitdl. HONDURAS, El Paraiso. *Davidse 2253* (NY). H, I. *Chiococca pinetorum* Britton ex Millsp. BAHAMAS, New Providence. *Britton 6532* (NY). A, B. *Chiococca plowmanii* Delprete. BRAZIL, Bahia. *Plowman 13948* (US). J. *Chiococca pubescens* Humb. & Bonpl. ex. Schult. BRAZIL, Bahia. *Anderson 37099* (NY). J, K. *Chiococca semipilosa* Standl. & Steyererm. NICARAGUA, Masaya. *Neill 2969* (MO). H, I. *Chiococca sessilifolia* Miranda. MEXICO, Chiapas. *Jimenez 907* (MO). H, I. *Coutaportla ghiesbreghtiana* (Baill.) Urb. MEXICO, Hidalgo, Cardonal. *Lorence 5042* (NY). H. *Coutaportla pailensis* Villarreal. MEXICO, Coahuila. *Villarreal 3063* (NY). H. *Coutarea andrei* Standl. ECUADOR, El Cisne. *Smith 225b* (MO). M. *Coutarea fuchsoides* C.M.Taylor. PERU, Antonio Raimondi. *Jara 55* (MO). M. *Coutarea hexandra* (Jacq.) K.Schum. BRAZIL, Bahia. *Melo 4318* (NY). H, I, J, K, L. *Cubanola daphnoides* (Graham) Aiello. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8808* (NY). C. *Cubanola domingensis* (Britton) Aiello. DOMINICAN REPUBLIC, Prov. Altigracia. *Acevedo-Rodriguez 8476* (US). D. *Eosanthus cubensis* Urb. CUBA, Prov. Oriente. *Ekman 15990* (S). C. *Erithalis diffusa* Correll. BAHAMAS, San Salvador. *Strittmatter s.n.* (MU). B. *Erithalis fruticosa* L. PUERTO RICO, Guanica state forest. *Gustafsson 280* (NY). A, B, C, D, E, F, G, H, I, J, K. *Erithalis harrisii* Urb. JAMAICA, Par. Trelawny. *Delprete et al. 7476* (NY). E. *Erithalis quadrangularis* Krug & Urb. JAMAICA, Manchester Parish. *Barcelona 1196* (MU). E, G. *Erithalis salmeoides* Correll. TURKS AND CAICOS ISLANDS, Pine Cay. *Raven 28205* (MO). B, C, D, E. *Erithalis vacciniifolia*

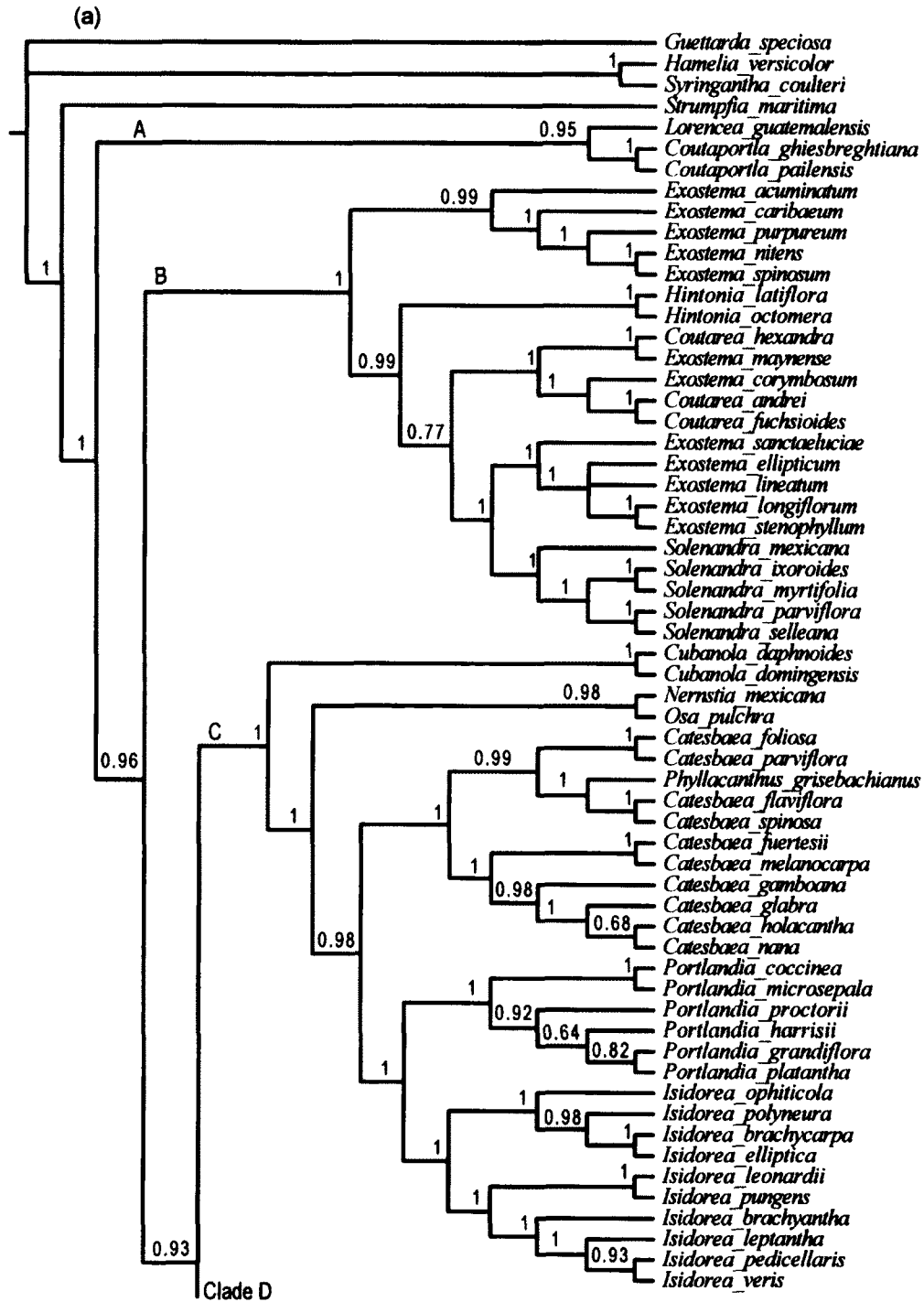
(Griseb.) C. Wright. DOMINICAN REPUBLIC, Prov. Pedernales. *Delprete & Clase 7551* (NY). B, C, D. *Exostema acuminatum* Urb. DOMINICAN REPUBLIC, living collection from NATIONAL TROPICAL GARDEN 930070001. *McDowell 4410* (DUKE). D. *Exostema caribaeum* (Jacq.) Roem. & Schult. CUBA, Prov. Guantanamo, San Antonio del Sur. *Delprete et al. 8892* (NY). A, B, C, D, E, F, G, H, I. *Exostema corymbosum* Spreng. PERU, Chota, Huambos. *Ferreyra 8446* (US). M. *Exostema ellipticum* Griseb. DOMINICAN REPUBLIC, Prov. Azua. *Delprete & Clase 7543* (NY). C. *Exostema lineatum* Roem. & Schult. DOMINICAN REPUBLIC, living collection at National Tropical Botanical Garden, NTBG 960211. *McDowell 4353* (DUKE). D. *Exostema longiflorum* Roem. & Schult. DOMINICAN REPUBLIC, living collection at National Tropical Botanical Garden, NTBG950031. *McDowell 4991* (DUKE). C, D. *Exostema maynense* Poepp. & Endl. BOLIVIA, Santa Cruz. *Nee 44497* (US). K, L. *Exostema nitens* Urb. DOMINICAN REPUBLIC, living collection at NTBG960207001. *McDowell 4414* (NY). D. *Exostema purpureum* Griseb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8661* (NY). C. *Exostema sanctae-luciae* (Kentish) Britten. DOMINICA, Morne Diablotins, near Syndicate. *Whiteford 3656* (US). D, G. *Exostema spinosum* Krug & Urb. CUBA, Prov. Santiago de Cuba. *Delprete et al. 8896* (NY). C, D. *Exostema stenophyllum* Britton. CUBA, Oriente. *McDowell 4960* (MO). C. *Guettarda speciosa* L. FRENCH POLYNESIA, Bora Bora. *Motley 2040* (NY). N, O, P. *Hamelia versicolor* A. Gray. USA, Hawaii, cultivated at National Tropical Botanical Garden. H, I. *Hintonia latiflora* (Sessé & Moc. Ex DC.) Bullock. MEXICO, Puebla. *Ochoterena-Booth 182* (MEXU). H, I. *Hintonia octomera* (Hemsl.) Bullock. MEXICO, Yucatan. *Ochoterena-Booth 171* (MEXU). I. *Isidorea brachyantha* Urb. DOMINICAN REPUBLIC, El Seibo. *Zanoni et al. 36220* (NY). D. *Isidorea brachycarpa* (Urb.) Aiello. CUBA, Prov. Guantánamo, Baracoa. *Delprete et al. 8852* (NY). C. *Isidorea elliptica* Alain. CUBA, Prov. Guantanamo, San Antonio del Sur. *Delprete et al. 8887* (NY). C. *Isidorea leonardii* Urb. DOMINICAN REPUBLIC, Azua. *Mejia et al. 1873* (NY). D. *Isidorea leptantha* Urb. DOMINICAN REPUBLIC, Prov. Independencia. *Delprete & Clase 7590* (NY). D. *Isidorea ophiticola* (Borhidi) Borhidi. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8670* (NY). C. *Isidorea pedicellaris* Urb. & Ekman. DOMINICAN REPUBLIC, Prov. Espaillat. *Delprete & Clase 7628* (NY). D. *Isidorea polyneura* (Urb.) Aiello. CUBA, Prov. Oriente. *Figueiras & Morton 2426* (NY). C. *Isidorea pungens* B.L. Rob. DOMINICAN REPUBLIC, Prov. Azua. *Delprete & Clase 7524* (NY). D. *Isidorea veris* Ekman ex Aiello & Borhidi. DOMINICAN REPUBLIC, Prov. Puerto Plata. *Delprete & Clase 7639* (NY). D. *Lorencea guatemalensis* (Standl.) Borhidi. MEXICO, Chiapas, La Trinitaria. *Breedlove & Almeda 57623* (NY). H, I. *Morierina montana* Vieill. NEW CALEDONIA, Riviere Bleue. *Molloy & Cameron 2203* (NY). O. *Nernstia mexicana* (Zucc. & Mart. ex DC.) Urb. MEXICO, San Luis Potosi. *Aiello & Medellin-Leal 1237* (NY). H. *Osa pulchra* (D.R. Simpson) Aiello. COSTA RICA, Osa peninsula. *Hammel 18371* (MO). I. *Phialanthus acunae* Borhidi. CUBA, Prov. Oriente. *Marie-Victorin & Clement 21830* (US). C. *Phialanthus ellipticus* Urb. CUBA, Prov. Holguín, Sierra Nipe. *Morton & Acuna 2962* (US). C. *Phialanthus grandifolius* Alain. PUERTO RICO, Maricao state forest. *Liogier 35814* (NY). F. *Phialanthus hispaniolae* Alain & R.G. García. DOMINICAN REPUBLIC, Independencia. *Gracia et al. 4488* (NY). D. *Phialanthus jamaicensis* Urb. JAMAICA, Par. Trelawny. *Delprete et al. 7469* (NY). E. *Phialanthus linearis* Alain. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8827* (NY). C. *Phialanthus myrtilloides* Griseb. PUERTO RICO, Sabana Grande. *Axelrod 3985* (NY). A, B, C, E, F. *Phialanthus oblongatus* Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8655* (NY). C. *Phialanthus rigidus* Griseb. CUBA, Prov. Pinar del Río, La Palma. *Delprete et al. 8907* (NY). C. *Phyllacanthus grisebachianus* Hook.f. CUBA, Prov. Pinar del Río, Toscano. *Ekman 17432* (NY). C. *Portlandia coccinea* Sw. JAMAICA, Par. Trelawny. *Delprete et al. 7480* (NY). E. *Portlandia grandiflora* L. JAMAICA, Par. Trelawny. *Delprete et al. 7477* (NY). E. *Portlandia harrisii* Britton. JAMAICA, Par. Clarendon. *Delprete et al. 7484* (NY). E. *Portlandia microsepala* Urb. JAMAICA, Par. St. Ann. *Delprete et al. 7505* (NY). E. *Portlandia platantha* Hook.f. JAMAICA, Par. St. Mary. *Delprete et al. 7510* (NY). E. *Portlandia proctorii* (Aiello) Delprete. JAMAICA, Par. St. Catherine. *Delprete et al. 7460* (NY). E. *Salzmannia nitida* DC. BRAZIL, Bahia. *Jardim et al. 580* (NY). J. *Schmidtottia cubensis* (Standl.) Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8722* (NY). C. *Schmidtottia elliptica* Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8838* (NY). C. *Schmidtottia monantha* Urb. CUBA, Prov. Guantánamo, Baracoa. *Delprete et al. 8855* (NY). C. *Schmidtottia nitens* Urb. CUBA, Prov. Guantánamo, Baracoa. *Delprete et al. 8860* (NY). C. *Schmidtottia sessilifolia* Urb. CUBA, Prov. Oriente. *Rova et al. 2201* (GB). C. *Schmidtottia shaferi* (Standl.) Urb. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al. 8745* (NY). C. *Schmidtottia uliginosa* Urb. CUBA, Prov. Holguín, Moa. *Delprete et al. 8871* (NY). C. *Scolosanthus acanthodes* Urb. DOMINICAN REPUBLIC, Prov. Puerto Plata. *Delprete & Clase 7643* (NY). D. *Scolosanthus acunae*

Borhidi & Muñiz. CUBA, Prov. Pinar del Río, La Palma. *Delprete et al.* 8918 (NY). C. *Scolosanthus densiflorus* Urb. DOMINICAN REPUBLIC, Santo Domingo. *Liogier & Liogier* 20476 (NY). D. *Scolosanthus lucidus* Britton. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8775 (NY). C. *Scolosanthus moanus* Borhidi & Muñiz. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8829 (NY). C. *Scolosanthus multiflorus* Krug & Urb. JAMAICA, Par. St. Ann. *Harris* 10374 (US). E. *Scolosanthus portoricensis* Borhidi. PUERTO RICO, Susua state forest. *Liogier* 9736 (NY). F. *Scolosanthus reticulatus* Borhidi. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8702 (NY). C. *Scolosanthus selleanus* Urb. & Ekman. DOMINICAN REPUBLIC, Prov. Peravia. *Acevedo-Rodriguez* 8503 (US). D. *Scolosanthus subsessilis* Alain. DOMINICAN REPUBLIC, Bonaó. *Liogier* 17371 (NY). D. *Scolosanthus triacanthus* DC. DOMINICAN REPUBLIC, Prov. Puerto Plata. *Delprete & Clase* 7533 (NY). D. *Scolosanthus versicolor* Vahl. BRITISH VIRGIN ISLANDS, Virgin Gorda. *Acevedo-Rodriguez* 10501 (US). D, F, G. *Siemensia pendula* Urb. CUBA, Prov. Pinar del Río, Viñales. *Delprete et al.* 8902 (NY). C. *Solenandra ixoroides* Hook.f. CUBA, Prov. Holguín, Sierra de Nipe. *Delprete et al.* 8821 (NY). C. *Solenandra mexicana* (A.Gray) Borhidi. MEXICO, Campeche. *Martinez* 28090 (FLAS). H, I. *Solenandra myrtifolia* (Griseb.) Borhidi. CUBA, Prov. Guantanamo. *Axelrod* 10421 (MO). C. *Solenandra parviflora* (Rich.) Borhidi. DOMINICAN REPUBLIC, Monicon, La Leonor. *McDowell* 4436 (US). C, D. *Solenandra selleana* (Urb. & Ekman) Borhidi. HAITI, Fonds Verrettes. *Holdridge* 1385 (US). C, D. *Strumpfia maritima* Jacq. DOMINICAN REPUBLIC, Boca de Yuma. *Acevedo-Rodriguez* 14123 (NY). A, B, C, D, E, F, G, H. *Syringantha coulteri* (Hook.f.) T.McDowell. MEXICO, Tamaulipas. *Henrickson* 19113 (US). H. *Thiolliera artensis* Montrouz. NEW CALEDONIA, Tiebaghie. *Cameron & Motley* 2068 (NY). O. *Thiolliera campanulata* (Brongn.) Baum.-Bod. NEW CALEDONIA, Fausse Yate. *Zirnik* 101 (US). O. *Thiolliera fritillarioides* (Schltr.) Baum.-Bod. NEW CALEDONIA, Tontouta. *Catala-Stucki* 122 (MO). O. *Thiolliera macrophylla* (Brongn.) Baum.-Bod. NEW CALEDONIA, Yate. *Cameron & Motley* 2034 (NY). O. *Thiolliera nerifolia* (Brongn.) Barrabé & Mouly. NEW CALEDONIA, Mont du Poum. *Veillon* 7761 (NOU). O. *Thiolliera retusiflora* (Brongn.) Barrabé & Mouly. NEW CALEDONIA, Canala. *McPherson* 5485 (MO). O. *Thiolliera tubiflora* (Brongn.) Barrabé & Mouly. NEW CALEDONIA, Dzumac. *Veillon* 7620 (NOU). O.

APPENDIX E

TREE NOT INCLUDED IN THE BIOGEOGRAPHY CHAPTER

The 50% majority rule consensus tree of the tribe Chiococceae retrieved from the Bayesian inference analyses of combined dataset (ETS, ITS, *petD*, and *trnL-F*).



VITA

Sushil Kumar Paudyal

Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529

Education:

Ph.D. Ecological Sciences. 2015. Old Dominion University, Norfolk, VA.

Advisor: The late Tim Motley; Committee members: Rebecca Bray, Piero Delprete, Tatyana Lobova, Lytton Musselman.

M. Sc. Botany. 2000. Tribhuvan University, Nepal. Advisor: Govind P. S. Ghimire.

Professional Experience:

- Graduate Teaching Assistant (2007--2013), ODU. Ethnobotany, Botany, Environmental Science, Human Biology, and General Biology for non-majors.
- Graduate Assistant (2011--2012): Kaplan orchid conservatory as Acting Conservator.
- Academic Coordinator & Instructor of Biology (2003--2007): Malpi Institute (Nepal).

Professional Organizations:

- Steering committee (2013--2015): ODU Preparing Future Faculty Program
- Curriculum committee (2013--2014): ODU Dept. of Biological Sciences
- Founder & President (2012--2013): ODU Student Chapter of the Botanical Society of America.

Publications:

- Paudyal, S. K., P. G. Delprete, T. J. Motley. 2014. *Systematic Botany* 39:1197-1203.
- Paudyal, S. K. and G. P. S. Ghimire. 2006. *Education and Development* 22: 98-105.
- *Abstracts*: Virginia Journal of Science 65: 43-44 (2014); Six abstracts in the conference proceedings of the Botanical Society of America annual meetings (2010, 2011, 2012, 2013); Program, Abstracts & Participants, 5th International Rubiaceae and Gentianales conference (2010): 47; Scripta Botanica Belgica 44: 56 (2008).