Made in the Americas:

How three lineages of flowering plants evolved and dispersed across the Neotropics and the World

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Abstract

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Bignoniaceae is a flowering plant family of ca. 850 species. The family has the highest species diversity in the Neotropics, but taxa are also found in tropical Africa and Asia, as well as the temperate zones in both the Northern and Southern hemisphere. This widespread distribution allowed me to ask questions about how three of these lineages- Tecomeae, Jacarandeae, and Crescentieae- achieved their current global, Neotropical, and Central American distributions, respectively. Using a combination of molecular phylogenetic and biogeographic methods, I inferred the evolutionary relationships of species in these groups, then used the resulting phylogenies, along with fossil calibrations and species distributions, to estimate divergence times, perform ancestral state reconstructions, and infer diversification rates to explore temporal and spatial patterns of evolution and dispersal. Tecomeae is inferred to have originated in the

Neotropics about 40 Ma, likely achieving its current distribution via a combination of overland and overwater dispersal aided by wind-dispersed seeds. Jacarandeae is estimated to have originated in the Neotropics also, achieving its current distribution by dispersing within and across the many biomes of the region over the past 23 Ma. Crescentieae, restricted to Central America and the West Indies, is unique in the family for having fleshy fruit that is likely adapted for dispersal by now extinct megafauna or water.

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To my family:
Greer, Andy, and Adrian

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CHAPTER 1: Around of the World in 40 Million Years: Phylogeny and Biogeography of Tecomeae (Bignoniaceae)

ABSTRACT

Aim

The Neotropics are the most species-rich region on Earth. Studying groups of Neotropical origin with a biogeographic approach could help us to understand how evolution in the Neotropics has influenced plant diversity worldwide. Tecomeae are a tribe of ca. 57 species in the Bignoniaceae with a putative Neotropical origin. Its pantropical distribution is remarkable in that it has achieved a similar distribution to its sister group in Bignoniaceae, which includes the rest of the family, except *Jacaranda* and the small tribe Tourrettieae, a clade of 750 spp. Our goal was to generate a dated phylogeny of the tribe to estimate divergence times and infer dispersal paths to better understand the underpinnings of this pantropical distribution.

Location

Global tropics, temperate South America, North America, and Asia

Taxon

Tribe Tecomeae (Bignoniaceae)

Methods

We generated a phylogeny of Tecomeae by gathering sequence data from chloroplast and nuclear markers for 63 taxa. Using the resulting phylogeny, fossil calibrations were used to determine divergence times to understand the temporal history of dispersal events. Ancestral states were reconstructed to infer biogeographic history.

Results

With a South American origin supported, the crown age of the tribe is estimated at ca. 40 Ma. Dispersal events were inferred from South America to the Old World and South America to North America in the Eocene-Oligocene, North America to Asia and Australia to South America in the Miocene. Two genera with species diversity in mountain ranges, *Tecoma* and *Incarvillea*, have crown ages that correspond to periods of major uplift.

Main Conclusions

We suggest intercontinental dispersal via land bridges, island hopping, and sweepstakes long distance dispersal from the Eocene to the present to explain the global distribution of Tecomeae.

INTRODUCTION

There are two major ways that plant groups can achieve intercontinental disjunct distributions. The first is the severing of a once widespread distribution by vicariance, and the second is dispersal from one landmass to another via rare events facilitated by short-lived land connections, island hopping, wind or water patterns, or migratory birds (Gillespie et al., 2012, Christenhusz & Chase 2013). Due to the complexity of Earth's geologic and climatic history as well as the difficulty of achieving a widespread distribution, it is likely that a combination of these two patterns must be invoked to explain the present distributions of widely distributed plant groups.

Distributions of widespread plant groups with stem or crown ages older than ca. 100 Ma may be explained by the breakup of Gondwana (Thorne, 1972, Raven & Axelrod, 1974). Relictual distributions of Thyrsopteridaceae and Lactoridaceae inferred from the fossil record appear to fit this vicariant hypothesis (Christenhusz & Chase 2013). The disjunct distribution of temperate *Nothofagus* (Nothofagaceae) was also once thought to be explained by this vicariant event (Barker et al., 2007; Knapp et al. 2005). However, Cook & Crisp (2005) have shown that while the stem age of Nothofagaceae is old enough to be Gondwanan in origin, the crown age of *Nothofagus* is too young for vicariance alone to explain its distribution and that dispersal must also be considered. Pantropical Ebenaceae is thought to share a similar story, its stem lineage originating in western Gondwana and subsequently splitting into *Diospyros* in Africa and *Lissocarpa* in the Neotropics following vicariance (Rose et al., 2018). Unlike its family, *Diospyros* is hypothesized to have achieved its own pantropical distribution with dispersal only (Duangjai et al., 2009), suggesting that the overall distribution of Ebenaceae is best explained by a combination of vicariance and dispersal. During the Eocene, a warmer global climate allowed tropical rainforests to migrate north, creating a boreotropical migration route likely used by groups like Annonaceae (Couvreur et al. 2011) and Rubiaceae (Antonelli et al. 2009) to geodisperse to other landmasses.

South America, once connected to proto-Africa in Western Gondwana, split from the landmass at ca. 100 Ma. This vicariance event created a major dispersal limitation for South American plant groups, leaving the continent in total isolation from other landmasses until it joined North America with the closure of the isthmus of Panama, possibly as recently as 5 Ma. (Raven and Axelrod, 1974; McLoughlin 2001, Morley, 2003; Cody et al, 2010; Bacon et al., 2015; Montes et al., 2015). However, this isolation cultivated the most diverse assemblage of plants on Earth, and many of these groups did manage to disperse to other continents during this period (Goldblatt, 1993; Burnham and Graham, 1999; Morley, 2003; Antonelli et al., 2011). Asteraceae (Funk et al. 2005), Bignoniaceae (Olmstead 2013), Malphigiaceae (Davis et al. 2002), and Solanaceae (Olmstead 2013, Dupin et al., 2016) are examples of groups that have achieved pantropical or global distributions from a Neotropical origin. It is estimated that stem and crown ages for these families are younger than the estimated breakup of Gondwana (~100 Ma), supporting the hypothesis that dispersal off the isolated continent, rather than vicariance, is the best explanation to explain these disjunct distributions. Bromeliaceae (Givnish et al. 2011), Melastomataceae (Renner & Meyer, 2001), and Verbenaceae (Lu-Irving & Olmstead, 2012) provide additional evidence of intercontinental transoceanic dispersal events out of South America. Therefore, to better understand the impact of the Neotropical flora on global diversity, more plant groups of Neotropical origin with disjunct distributions should be investigated in a phylogenetic and biogeographic context.

Tecomeae (Bignoniaceae) is a group with putative Neotropical origin and pantropical distribution (Olmstead et al. 2009; Olmstead, 2013). Containing only about 57 species, its distribution is remarkable in having achieved a similar pantropical distribution to its sister group in Bignoniaceae, a clade of 750 spp. excluding only *Jacaranda* and the small tribe Tourrettieae. While it is not surprising that large clades, like Tecomeae's sister group, achieve pantropical distributions, the ability of a comparatively species poor clade to do it begs further investigation. Therefore, inferring phylogenetic relationships, divergence times, and biogeographic history of Tecomeae will offer special insight into the biogeographic patterns of pantropically distributed groups from the New World.

Endlicher (1839) circumscribed tribe Tecomeae to include genera in Bignoniaceae with loculocidal fruit dehiscence, initially including *Catalpa, Chilopsis, Jacaranda, Spathodea, Tecoma*, and *Zeyheria*. Gentry (1992) followed suit, including additional genera that fit Endlicher's characterization of Tecomeae. However, early phylogenetic studies showed that this Tecomeae was paraphyletic, with all other groups in Bignoniaceae derived from within it (Spangler and Olmstead, 1999; Olmstead et al., 2009).

The most recent phylogenetic study by Olmstead et al. (2009) circumscribed a much-reduced Tecomeae containing 12 genera and approximately 55 species, with evidence suggesting *Argylia* as its sister. Those 12 genera include three New World genera, *Campsidium, Campsis,* and

Tecoma, African *Dinklageodoxa* (unsampled but suggested to belong based on lianoid habit), *Podranea* and *Tecomaria*, Australasian *Deplanchea*, *Lamiodendron*, *Neosepicaea*, *Pandorea*, and *Tecomanthe*, and Asian *Incarvillea*. Sampling in Olmstead et al. (2009) included 10 genera, but only 14 species. Besides that study, the only other work on Tecomeae has been Chen et al. (2005), who produced a nearly complete phylogeny of *Incarvillea* using one chloroplast locus and one nuclear locus.

Taxonomic sampling in this study includes the New World Andean genus *Tecoma* (7 spp.), North Temperate *Campsis* (2 spp., one in eastern North America, one in East Asia), and *Campsidium* (1 sp.) endemic to the Valdivian forests of Chile. In this study, we provide novel systematic evidence that the Meso-American desert endemic *Astianthus* (1 sp.) belongs to Tecomeae, bringing the number of genera to 13. Tecomeae's proposed sister group *Argylia* (12 spp.) also occurs in the New World, distributed in the southern Andes (12 spp.). We will test this proposed sister relationship in a systematic context. Old World genera sampled include African *Tecomaria* (2 spp.) and *Podranea* (2 spp.), Australasian *Deplanchea* (5 spp.), *Lamiodendron* (1 sp.), *Neosepicaea* (4 spp.), *Pandorea* (9 spp.), and *Tecomanthe* (5 spp.), and temperate Asian *Incarvillea* (16 spp.).

Our goals are to (1) generate a phylogeny of Tecomeae with increased taxonomic sampling throughout the tribe using both nuclear and chloroplast data, (2) estimate divergence times using fossil calibrations to understand the temporal history of divergence and dispersal events, and (3) reconstruct the biogeographic history of Tecomeae.

MATERIALS AND METHODS

Taxon Sampling

The dataset consisted of 76 accessions of Tecomeae and its putative sister group with taxonomic sampling as follows: *Argylia* (4 of 12 spp.), *Astianthus* (1/1), *Campsidium* (1/1), *Campsis* (2/2), *Deplanchea* (1/5), *Incarvillea* (13/16), *Lamiodendron* (1/1), *Neosepicaea* (1/4) *Pandorea* (5/9), *Podranea* (2/2), *Tecoma* (ca. 7/7), *Tecomanthe* (5/5), and *Tecomaria* (2/2). Multiple accessions representing infraspecific taxa or to accommodate wide geographic distributions were included for *Incarvillea* and *Tecoma*. Only *Dinklageodoxa*, of the proposed genera in Tecomeae, remains unsampled. Sequence data were obtained from a combination of field collections, herbarium tissue, and GenBank. Sampling of *Tecoma* covers the distribution of the genus throughout South America and includes named subspecies and varieties (Gentry, 1992, Wood, 2008, Sarkinen dissertation). Though not initially thought to belong in Tecomeae, *Astianthus* was determined to belong here based on data obtained for a broader study of Bignoniaceae. Outgroups included 15 species in Bignoniaceae, as well as *Sesamum indicum* (Pedaliaceae), *Buddleja davidii* (Scrophulariaceae), and *Antirrhinum majus* (Plantaginaceae) representing related families in the Lamiales. All voucher information for sampled taxa is listed in Appendix 1.1.

DNA Extraction, PCR, and Sequencing

DNA extraction from silica-dried or herbarium leaf tissue was performed with Qiagen DNeasy Plant Mini Kits (Qiagen, Valencia, CA). Five chloroplast regions- *ndhF*, *rpl32-trnL*, *trnLF, rbcL, matK*- were amplified via polymerase chain reaction (PCR) using primers developed in Olmstead & Sweere (1994), Taberlet et al. (1991), and Zuntini et al. (2013), respectively. ITS was amplified using the ITS4 and ITS5 primers (White et al., 1990), and TecF and TecR primers designed specifically for *Tecoma* to avoid contamination by fungi present in most of the silica-dried leaf samples (TecF: 5' CGG AAG GAT CAT TGT CGA A 3' and TecR: 5' AAG GCA CCT TTG GGT CAT TA 3'). ETS was amplified using the 18s-IGS primer of Baldwin & Markos (1998) with a custom primer used to amplify ETS in the Lamiales.

PCR for chloroplast loci was performed under the following general conditions: 94°C for 2m, following by 35 cycles of 94°C for 30s, 50°C for 30s, 72°C for 1.5-2.5 minutes, following by 72°C for 10 minutes. PCR for nuclear loci was performed at 94°C for 2 min, 30 cycles of 94°C for 30s, 51.4°C for 45s and 72°C for 1min, followed by a final extension of 72°C for 4 min. PCR products were purified prior to sequencing using either ExoSAP (Exonuclease I and Shrimp Alkaline Phosphatase) with 1U Exonuclease I (New England Biolabs) and 1U Shrimp Alkaline Phosphatase (US Biochemical Co., Amersham) per 5µL PCR product at 37°C for 30min followed by 80 °C for 15 min, or polyethylene glycol (PEG) precipitation followed by 70% ethanol washes.

Cycle sequencing reactions were performed using BigDye ver. 3.1 (Applied Biosystems Inc.) using a standard Applied Biosystems protocol. ETS products were sequenced on an 3130xl DNA Analyzer (Applied Biosystems) at Genewiz (Seattle, WA, USA), and ITS products were sequenced on a CEQ 8000 Genetic Analysis System (Beckman Coulter, UK), with chloroplast products sequenced on both.

Phylogenetic Analyses

Sequence editing and alignment was performed in Geneious 10.2.3 (Biomattters Ltd.) or Sequencher version 4.5 (Gene Codes, Ann Arbor, MI). Data were analyzed in both Maximum Likelihood and Bayesian frameworks in three ways: concatenated chloroplast loci (*ndhF*, *rpl*32-*trnL*, *trnLF*, *rbcL*, *matK*), concatenated nuclear loci (ITS, ETS), and a combined, partitioned chloroplast-nuclear dataset. Chloroplast and nuclear datasets were analyzed separately to detect any conflicting phylogenetic signal. The GTR+I+G model of evolution was used for all loci. (Abadi et al., 2019).

Phylogenetic reconstructions were performed in a Maximum Likelihood framework using RAxML 8.0.0 (Stamatakis, 2014) and a Bayesian framework using MrBayes 3.1.2 (Ronquist et al., 2012). Maximum likelihood analyses in RAxML 8.0.0 were run for 1000 rapid bootstraps with the GTRCAT model. Bayesian analyses used two replicate runs, each consisting of four chains sampled every 1000 generations. Analyses were assessed for convergence using Tracer 1.6 (Rambaut et al., 2018). Analyses were run until convergence diagnostics indicated that they had reached stationarity, usually around one-million MCMC generations. When summarizing consensus trees over all runs, the first 25% of sampled trees were discarded as burn-in.

Divergence Time Estimation

BEAST 1.10.4 (Suchard et al., 2018) was used to determine divergence times via fossil calibration using concatenated chloroplast data, ITS, and ETS. Trees and clock models were treated as linked, while substitution models were treated as unlinked. The GTR and gamma + invariable site heterogeneity model was used for each alignment. Taxa in Bignoniaceae and

Cuspidaria/*Fridericia*/*Tanaecium*/*Xylophragma* were constrained as monophyletic for fossil calibrations on those nodes. The following clades that were well supported in trees from the concatenated dataset also had monophyly constrained: *Argylia,* Tecomeae, *Campsis*/*Astianthus*, *Tecoma*, Old World Clade, African Clade, Australasian Clade, and *Incarvillea*. Rates were set to an uncorrelated relaxed clock with a lognormal distribution. The tree prior was set to birth-death with incomplete sampling.

Two fossils have been used in previous studies on Bignoniaceae to calibrate phylogenies. The first is a pollen grain from the Gatuncillo formation of Panama (Graham, 1985), which current generic taxonomy could place in several genera (*Cuspidaria* DC., *Fridericia* Mart., *Tanaecium* Sw., and *Xylophragma* Sprague.). This calibration was set on the MRCA of the *Cuspidaria*/*Fridericia*/*Tanacium*/*Xylophragma* clade using a lognormal distribution with an offset of 33.7 and a standard deviation and mean of 1.0. The other calibration point was determined by various fossils that are all about 50 Mya (e.g. Wehr & Hopkins, 1994; Wehr, 1995; Wehr & Manchester 1996; Wilf, 1997; Pigg & Wehr, 2002). This calibration was set on the crown node of Bignoniaceae using a lognormal distribution with a mean of 49 and a standard deviation of 1.0.

BEAST analysis was run for 1.0 x 10^8 generations, sampling every 1.0 x 10^4 trees. Run convergence was assessed using Tracer 1.7.1 (Rambaut et al., 2018). A maximum clade credibility tree was generated using TreeAnnotator 1.10.4, discarding the first 25% of trees sampled as burn-in.

Ancestral State Reconstruction

Ancestral state reconstruction was performed in BioGeoBEARS (Matzke, 2013a) using the calibrated BEAST maximum clade credibility tree with outgroup taxa removed. Biogeographic areas were coded to reflect the collection localities of each specimen (Table S1). Those areas were as follows: (1) North America; (2) South America, (3) Australasia, (4) Africa, and (5) temperate Asia.

Analyses were run for the following likelihood models: DEC, DIVALIKE, and BAYAREALIKE. DEC enables cladogenesis by both within area speciation and narrow vicariance, DIVALIKE favors cladogenesis by widespread vicariance, and BAYAREALIKE favors cladogenesis by within area speciation (Matzke, 2013b). We also ran these models enabling the J parameter, which enables cladogenesis by founder-event speciation. All models allow anagenesis by dispersal and extinction. The AIC criterion was used to compare fit of both nested and non-nested models to our data.

Time-stratified dispersal multiplier matrices were incorporated to account for changing distances between the coded regions over deep geological time. Time slices were set at 40-24, 24-10, and 10 Ma-present. The first slice at 40 Ma reflects the approximate root age of our tree, with outgroups removed, estimated by the BEAST analysis. The second time slice at 24 Ma represents a significant shift in plant dispersal between North and South America estimated by Bacon et al. (2015). The third time slice at 10 Ma represents the closure of the Isthmus of Panama, which is a conservative estimate based on recent studies (Cody et al., 2010; Bacon et al., 2015; Montes et al., 2015), as well as a second shift in plant dispersal between North and South America (Bacon et al., 2015). The dispersal multiplier matrices for each of these strata give the relative probability of dispersal between areas and are roughly scaled to represent the relative distance of the areas during each time slice, modeled after a matrix used in a study of similar scope (Appendix 1.2; Dupin et al., 2017).

RESULTS

Sequence Alignment

The chloroplast dataset consisted of 85 taxa. Outgroups for the 63 taxa in Tecomeae included four taxa in *Argylia*, 15 in greater Bignoniaceae, and *Sesamum indicum* (Pedaliaceae), *Buddleja davidii* (Scrophulariaceae), and *Anthirrinum majus* (Plantaginaceae). The total sequence length of the dataset was 6833 positions, containing concatenated sequences of 47 taxa for *ndh*F (2095 positions), 48 taxa for *rpl*32-*trn*L (1071 positions), 44 taxa for *trn*L-F (898 positions), 31 taxa for *rbc*L (1430 positions), and 19 taxa for *mat*K (1339 positions).

The nuclear dataset consisted of 58 taxa. Outgroups for the 46 taxa in Tecomeae included three taxa in *Argylia*, 8 in greater Bignoniaceae, and *Sesamum indicum* (Pedaliceae). The total sequence length of the dataset was 1088 positions, containing concatenated sequences of 15 taxa for ETS (479 positions) and 58 taxa for ITS (609 positions).

The full dataset was generated by concatenating the chloroplast and nuclear datasets. The resulting 85 taxa included the same ingroups and outgroups used in chloroplast dataset. The total sequence length of the dataset was 7921 positions. Due to the various sources of these sequences, the final alignment contained 46.9% missing data.

Phylogeny of Tecomeae

Phylogenetic analyses of the chloroplast dataset support a monophyletic Tecomeae sensu Olmstead (2009), containing *Astianthus*, *Campsidium*, *Campsis*, *Deplanchea*, *Incarvillea*, *Lamiodendron*, *Neosepicaea*, *Podranea*, *Pandorea*, *Tecoma*, *Tecomanthe*, and *Tecomaria* (Appendix 1.3, 1.4). The chloroplast data provide moderate support for a sister relationship between Tecomeae and *Argylia* (PP = 0.85, BS = 75). Three major clades are identified within Tecomeae: 1) *Astianthus* and *Campsis* form a clade that has strong support as sister to the rest of Tecomeae. 2) *Tecoma*, and 3) a large clade consisting of African, Asian, and Australasian species. Within the latter, which we will call the Old World clade, two subclades are supported: i) *Podranea* and *Tecomaria* (0.95, 68) and ii) *Deplanchea*, *Lamiodendron*, *Campsidium, Pandorea*, *Tecomanthe*, *Neosepicaea*, and *Incarvillea* (0.75, 46). The five species of *Pandorea* sampled are paraphyletic, with *Neosepicaea*, *Tecomanthe* and *Campsidium valdivianum* derived from within *Pandorea*.

Phylogenetic analyses of the comparatively smaller nuclear dataset result in less well supported trees than the chloroplast data (Appendix 1.5, 1.6). Neither monophyly of Tecomeae (0.89, 52) nor a sister relationship between *Argylia* and Tecomeae receive strong support (Appendix 1.5, 1.6). Instead, *Argylia* is sister to *Incarvillea* (0.85, 65), solidly placing *Argylia* within Tecomeae. Due to this novel sister relationship in the nuclear trees, monophyly of the Old World clade is not supported.

Relationships in the trees generated from the full dataset mirror those in the chloroplast trees, with Tecomeae supported as monophyletic (PP=1.0, BS=99; Fig. 1.1, Appendix 1.7, 1.8). A sister relationship between *Argylia* and Tecomeae is weakly supported (0.60, 69), reflecting uncertainty seen in the nuclear trees. Within Tecomeae, a clade containing *Astianthus* as sister to *Campsis* (0.98, 98) is sister to the rest of the tribe (1.0, 99). *Tecoma* is monophyletic (0.95, 98) and is supported as sister to a clade containing all Old World taxa, except *C. grandiflora* (0.95, 98). The Old World clade is monophyletic (0.94, 96) and consists of three clades: African genera *Podranea* and *Tecomaria* (0.84, 63), Australasian genera *Lamiodendron*, *Deplanchea*, *Pandorea*, *Campsidium*, *Neosepicaea*, and *Tecomanthe* (0.84, 73), and Asian *Incarvillea* (1.0, 100).

Divergence Time Estimation

Analysis in BEAST with two fossil calibrations on outgroups estimate the crown age of Tecomeae at 37.09 Ma (95% highest posterior density interval 31.64-41.78 Ma; Fig 1.2). The tribe diverged from its likely sister group, *Argylia*, at 40.79 Ma (95% HPD 36.3-45.04 Ma). Crown age estimates for the maximum clade credibility tree are summarized in Table 1.1. While many clades were constrained for monophyly in this analysis, the relationships between them are consistent with those inferred from the concatenated dataset in MrBayes and RAxML.

Ancestral State Reconstruction

When comparing model fit for biogeographic analyses in BioGeoBEARS, AIC likelihood tests of nested models determined that analyses enabling the J parameter were a better fit for our data than those without (Table 1.2). Among models enabling the J parameter, AIC and AICc likelihood tests determined that DEC+J and DIVALIKE+J were equally good fits for our data, while BAYAREALIKE + J had AIC and AICc values more than Δ2 from the second highest score (Table 1.2). Despite AIC rankings, the only differences among these results occur in reconstructions for the three deepest nodes and in three recent dispersal events. The inference for dispersal between SA and NA and Australasia and SA is one dispersal event in BAYAREALIKE, but two in DEC + J and DIVALIKE + J. Because two subsequent LDD events in the same lineage in a short amount of time are unlikely, BAYAREALIKE + J offers the most biologically likely scenario. Therefore, we will focus on the results of the BAYARELIKE + J reconstruction in our results and discussion (Table 1.1). The results of DEC + J and DIVALIKE + J are provided in the supplementary information provided with this article (Appendix 1.9-1.12).

BAYAREALIKE + J reconstructed a SA origin of Tecomeae with 96.4% likelihood (Fig. 1.3, Table 1.1). The first range splitting event is inferred in the late Eocene, with one lineage dispersing from South America to North America (96.1%), giving rise to the *Astianthus*/*Campsis* clade. This clade experiences its own splitting event in the Miocene, with one lineage dispersing from North America to temperate Asia, eventually giving rise to *Campsis grandiflora*. Another split is inferred in South America in the late Eocene, with one lineage giving rise to South American *Tecoma* (100%) and the other dispersing from South America to the Old World, with Africa (48%) and Asia (52%) receiving nearly equal probability. Within the Old World, there are further dispersal events inferred, with one lineage in the Himalayan region in Central/East Asia between the late Eocene and mid-Miocene that gave rise to *Incarvillea*, and another lineage that reached Australasia around the Eocene-Oligocene boundary, giving rise to taxa distributed across Sundaland to northern Australia. Lastly, the ancestor of *Campsidium valdivianum* dispersed to South America from Australasia sometime during the mid-Miocene.

DISCUSSION

Phylogenetic Implications

Our well resolved phylogeny provides a strong foundation for reconstructing the biogeographic history of Tecomeae (Fig. 1.1). Taxonomic sampling was greatly increased from 14 species in Tecomeae and two in *Argylia* in the Bignoniaceae phylogeny of Olmstead et al. (2009) to 41 species in Tecomeae and four in *Argylia* here. This dataset also includes multiple accessions of species in *Tecoma* and *Incarvillea* to represent recognized subspecies and widespread geographic distributions. The phylogeny supports three major clades in Tecomeae: North American and Asian *Astianthus*/*Campsis*, New World *Tecoma*, and an Old World clade containing African *Podranea* and *Tecomaria,* Australasian *Deplanchea, Lamiodendron, Neosepicaea, Pandorea*, *Campsidium*, and *Tecomanthe*, and Asian *Incarvillea*. Our phylogeny also supports the sister group relationship between of *Argylia* and Tecomeae, although with only modest support from the chloroplast and combined data.

Tecoma is strongly supported as monophyletic across all phylogenetic analyses. Gentry (1992) circumscribed the genus to include 14 species, including two species now classified as *Tecomaria*. Recent taxonomic treatments have not considered *Tecomaria* distinct from *Tecoma* (Gentry, 1992; Fischer et al., 2004) due to their morphological similarity. Gentry noted that in *Tecomaria* the anther thecae are fused to the connective, whereas in *Tecoma* they are divaricate and attached only at the tip of the filament, but did not think this warranted generic distinction. Our results show that these two genera are phylogenetically distinct and should be treated as separate genera, as previously suggested (Olmstead et al., 2009; Spangler and Olmstead, 1999). Furthermore, Wood (2008), in a revision of the Bolivian species, reduced several species recognized by Gentry to synonymy under *T. fulva* and *T. stans*, reducing the number of species recognized to seven. We recognize these seven species here.

In the phylogeny from our concatenated dataset (Fig. 1.1), five clades are supported in *Tecoma*. *Tecoma beckii* is strongly supported as sister to the rest of *Tecoma*. *Tecoma rosifolia* is another well supported clade that diverges early. The remaining species form three well-supported clades consisting mostly of taxa classified into two species complexes, *T. stans* and *T. fulva*, plus *T. castanifolia*, *T. tenuiflora* and *T. weberbaueriana*. Relationships among and within the latter three clades are poorly resolved. Gentry (1992) grouped species in *Tecoma* based on flower morphology differentiated into putative bee and hummingbird pollinated species. Of the taxa sampled here, *T. beckii*, *T. castanifolia*, *T. weberbaueriana*, and *T. stans* have yellow, campanulate corollas, whereas *T. fulva*, *T. rosifolia*, and *T. tenuiflora* have red, salverform corollas. One might expect species with similar floral morphology to be closely related. However, each of the three larger clades includes both floral morphs and the other early diverging lineages represent bee (*T. beckii*) and hummingbird (*T. rosifolia*) pollinated species.

Differing taxonomic treatments by Wood (2008) and Gentry (1992) reflect the complex patterns of morphological variation found in these complexes. The molecular data presented here further highlight this taxonomic uncertainty and suggest that neither the *T. stans* nor *T. fulva* complexes are monophyletic (Figs 1.1, Appendix 1.3-1.8). Because data collection was limited to only one chloroplast region (*rpl32-trnL*) and one nuclear region (ITS) for most taxa sampled in *Tecoma*, the non-monophyly of *T. fulva* and *T. stans* in our phylogenetic results could also be a consequence of limited data sampling, incomplete lineage sorting, and/or hybridization (Gentry,

1992) as a result of the young crown age of the clade (ca. 10 Ma; Table 1.1). More work is needed to resolve species delimitation in *Tecoma*.

In the tribal classification of Bignoniaceae presented by Olmstead et al. (2009), *Argylia* was one of two genera unassigned to a tribe or other suprageneric clade, receiving modest support in plastid trees (PP=0.85, BS=45%) as sister to Tecomeae. Our chloroplast results show very similar support for this relationship in the plastid tree (0.85, 75%; Appendix 1.3, 1.4). While lacking strong support, *Argylia* is estimated as sister to *Incarvillea* (0.51, 56) in our nuclear trees (Appendix 1.5,1.6). In our concatenated trees, *Argylia* receives similar support as sister to monophyletic Tecomeae to the plastid tree (0.6, 69; Fig 1.1, Appendix 1.7, 1.8). Despite this incongruence, the fact that *Argylia* is nested within Tecomeae in our nuclear analyses and falls out as sister to Tecomeae in our plastid and concatenated results, as well as in previous work, suggests that this sister relationship will persist with future studies. Continued low support for this relationship may be due to our limited sampling in the group, only including 4/12 spp. It seems that additional species-level sampling of *Argylia* is the next step to further resolving this relationship.

Historical Biogeography of Tecomeae

Historical biogeography suggests a South American (SA) origin of Tecomeae sensu Olmstead (2009) in the late Eocene (Fig. 1.3, Table 1.1). During this epoch, the landscape in SA was dominated by the Amazon rain forest, which appeared shortly after the Cretaceous-Paleogene extinction event at approximately 64 Ma (Dick & Pennington, 2019). Since all extant SA taxa in the tribe, as well as the proposed sister group *Argylia*, are distributed in the Andes, one might hypothesize an origin there. However, due to uncertainty around the crown age of Tecomeae (31.64 - 41.78 Ma), it is unclear whether the Andes were uplifting when ancestral taxa originated since Andean uplift did not begin until the middle Oligocene (Hoorn et al., 2010, Antonelli & Sanmartin 2011). Older crown age estimates would suggest that ancestral taxa in Tecomeae originated in non-montane biomes, dispersing into the mountain range following initial uplift, while younger crown age estimates would align with the timing of initial uplift. Climate at this time was characterized by a trend toward cooler conditions as measured by a 3% rise in O^{18} levels with much of the change occurring from the middle Eocene to early Oligocene (Zachos et al., 2001).

Astianthus*/*Campsis

One dispersal event was detected from South America (SA) to North America (NA) between the late Eocene and early Miocene, giving rise to the *Astianthus*/*Campsis* crown clade in the early Miocene at ca. 21.79 Ma (9.93 – 34.2 Ma HPD) (Fig 1.3, Table 1.1). Most likely occurring well before the estimated closure of the isthmus of Panama, long distance overwater dispersal must be invoked to explain this event. It is possible that this lineage was part of a wave of plant dispersal between SA and NA predating the closure of the isthmus at ca. 24 or 10 Ma (Bacon et al., 2015). Dispersal from SA to NA has been documented in Malphigiaceae (Davis et al., 2002), Solanaceae (Dupin et al., 2017; Olmstead, 2013), Verbenaceae (Olmstead, 2013), and other clades of Bignoniaceae (Grose & Olmstead, 2007; Olmstead et al., 2009; Olmstead, 2012).

The genus *Campsis* underwent further splitting at 5 Ma (1.13-11.08 Ma), resulting in an Eastern North America and East Asian disjunct distribution. Our date for the divergence of eastern

North American *C. radicans* and east Asian *C. grandiflora* is supported by previous estimates (3.62 ± 2.10 Ma; Xiang et al., 2000). This intercontinental disjunct has been explained by the fragmentation of a once widespread mixed mesophytic forest that occurred throughout the Northern Hemisphere, and dispersal between the two continents is likely to have occurred via the Bering Strait land bridge (Manchester, 1999; Xiang et al., 2000).

Tecoma

While the stem lineage of *Tecoma* is estimated to have originated in late Eocene-early Oligocene SA (ca. 35 Ma), the crown group of *Tecoma* did not begin to radiate until ca. 10 Ma (6.47-16.49 Ma) in the Miocene (Fig 1.3, Table 1.1). *T. beckii*, sister to the rest of *Tecoma*, is a narrow endemic in seasonally dry tropical forests (SDTF) of central Bolivia (Gentry, 1992, Wood, 2008). *T. rosifolia*, another early diverging lineage, is also endemic to SDTF in Peru. These distributions point to the origin of *Tecoma* in the Eastern Cordillera of the central Andes, the first section of the Andes to begin uplifting between 29 and 25 Ma, with the lineage diversifying in concert with a second burst of uplift between 10 and 6 Ma (Gregory-Wodzicki, 2000).

Tecoma fulva consists of six subspecies described by Wood (2008) that were previously recognized as species (Gentry, 1992), each restricted to narrow ranges in dry Andean valleys in Peru, Bolivia, and Argentina. The most widespread species, *Tecoma stans,* includes four varieties, two of which (*T. stans* var. *stans* and *T. stans* var. *velutina*) are widespread along the tropical mountains of the Americas, from Mexico to northern Argentina, a third (*T. stans* var. *sambucifolia*) is distributed from southern Ecuador to northern Bolivia, and the fourth (*T. stans* var. *angustata*) occupies the northernmost portion of the species distribution from Mexico into the SW USA. Three other species nested within these large *T. fulva* and *T. stans* complexes in our phylogeny are *T. castanifolia*, endemic to the dry part of coastal Ecuador, *T. weberbaueriana*, found in the dry coastal region of northwestern Peru (Gentry, 1992), and *T. tenuiflora* of the inter-Andean valleys of southern Bolivia and northern Argentina. Stem and crown ages for *Tecoma* align with those of legume clades endemic to SDTF, suggesting that the diversification of these groups coincided with the second phase of rapid Andean uplift that started around 9-10 Ma (Sarkinen et al., 2012). A dispersal event from South to North America occurred in *Tecoma* with the range expansion of *T. stans* into Central America and reaching to the SW USA and the Caribbean between ca. 2 Ma and the present (Fig. 1.3). The timing of this event implies dispersal via GABI, with most estimates supporting closure of the isthmus by 3 Ma (Bacon et al., 2015, Montes, at al. 2015). Subsequent overwater dispersal may explain the Caribbean distribution of *T. stans*.

The Old World Clade

Another dispersal event was inferred from SA to the Old World between 34.88 Ma (29.07-39.59) and 30.91 Ma (25.09-36.08) in the late Eocene to early Oligocene (Fig 1.3, Table 1.1), giving rise to three clades comprising species in Africa, Australasia, and temperate Asia.

Two dispersal paths into the Old World received nearly equal support: from South America to Africa (48%) and from South America to Asia (temperate Asia and Australasia) (52%). Regardless of whether dispersal occurred East or West off South America, both routes would require intercontinental overwater dispersal. Direct land connections between the African and S American plates were severed at the end of the Albian (96 Ma), fitting approximately with faunal evidence. Fossil pollen suggests that a series of islands acting as stepping-stones between the two continents existed from the Late Cretaceous possibly into the late Oligocene. Formed by hot spot volcanism, the Walvis Ridge is thought to have become submerged at ca. 35 Ma, but subsidence estimations for the Rio Grande Rise suggest that it may have been above sea level until the Oligocene (ca. 25 Ma) (Theide, 1997, Pennington & Dick, 2004, Katinas et al 2013). The inferred age of the Rio Grande Rise makes an island-hopping dispersal route between South America and Africa plausible for Tecomeae. Dispersal between South America and Africa following their split has been inferred in many plant groups, including Verbenaceae (Lu-Irving & Olmstead, 2012), Melastomataceae (Renner & Meyer, 2001) and Bromeliaceae (Givnish et al. 2011), with at least the latter two families predicted to have achieved this dispersal via wind (Renner, 2004).

A dispersal event from South America into continental Asia during the Eocene-Oligocene boundary could have occurred via either trans-Pacific dispersal or a boreotropical migration route through NA into Asia in the late Eocene. However, it is more likely that long distance dispersal explains this event based on current evidence. A different route into Asia may have existed via the SA-Antarctic land bridge from the late Cretaceous into the late Eocene, making subsequent island hopping into the sub-Antarctic and Oceania possible. However, this route ceased to exist at ca. 35 Ma with drops in temperature, the opening of the Drake Passage (McLoughlin, 2001), and the appearance of ice sheets on Antarctica just above the Eocene-Oligocene boundary (ca. 34 Ma) (Zachos et al., 2001). Older age estimates for this dispersal event in the late Eocene align with the last few million years that the Antarctic dispersal route was possible. The lack of fossil evidence of Bignoniaceae in Antarctica makes it difficult to implicate this route with confidence. Younger age estimates would rule out this route, leaving trans-Pacific dispersal the likely explanatory mechanism.

Dispersal within the Old World

The Eocene-Oligocene boundary was a time of remarkable tectonic activity in the Old World. From the crown age of the Old World clade (30.9 Ma; 25.09 - 36.08) to the present, the Indian subcontinent migrated northward and collided with continental Asia, spurring the uplift of the Qinghai-Tibetan Plateau that peaked in the mid-Miocene, and Australia migrated northward from Antarctica and collided with the Sunda shelf, triggering the formation and uplift of New Guinea in the late Miocene (Morley, 2018). The physical proximity of these intersecting regions likely facilitated dispersal between these areas, making almost any order of dispersal plausible. The most likely path inferred by our analyses estimate a mainly overland dispersal route from Africa through Asia into Australasia (Fig 1.3, Table 1.1). However, it is important to acknowledge that subsequent long distance dispersal events from Africa into Australasia, then into Asia are also possible, though are less likely based on our results. The reverse could have also happened—origin of the Old World clade in Australasia, followed by two dispersal events into Africa and continental Asia.

Assuming an African origin of Old World taxa in Tecomeae, there is a dispersal event inferred from Africa to Asia between 31 Ma (25.09-36.08) and 30 Ma (24.17-35.32). Dispersal between the two continents was possible by short dispersal over the Tethys Sea from Africa to Asia, or dispersal from Africa to Asia via India, which is thought to have had connections to Africa via the Kohistan-Ladakh Island Arc as it drifted northward toward Asia (Morley, 2018). Examples of plant groups that have dispersed from Africa to continental Asia are Annonaceae (Couvreur et al. 2011), Asteraceae (Funk et al. 2005), and *Begonia* (Rajbhandary et al., 2011). A reverse dispersal path from Asia to Africa may also have been possible via the same routes.

The lineage giving rise to *Incarvillea* originated in central Asia between 30 Ma (24.17 – 35.32) and 19 Ma (13.77 – 24.74) between the Oligocene and Miocene. The crown age of *Incarvillea* in the late Miocene (ca. 19 Ma) precedes the main uplift period of the Qinghai-Tibetan Plateau around 15 Ma (Morley, 2018). *Incarvillea arguta, I. emodi*, *I. olgae*, *I. semiretischenskia*, *I. sinensis var. sinensis, and I. sinensis var. przewalskii* are all found in eastern and central Asia. The remaining taxa sampled are endemic to the Himalaya-Hengduan mountain region and form a well-supported clade with a crown age of 4.66 Ma (2.46 – 7.35). This crown age aligns with the uplift of the Himalaya-Hengduan range during the early Pliocene, a biodiversity hotspot for Himalayan plant diversity (Hughes & Atchison, 2015). These eight species, assigned to subgenus *Pteroscleris* by Chen et al. (2005), are herbaceous, and the other species in the genus are suffruticose, supporting the notion of montane taxa as a group with several advanced characters. An herbaceous habit is a derived trait in Tecomeae, as well as Bignoniaceae, with Andean *Argylia* the only other genus that shares this trait. Curiously, this transition from woody to herbaceous growth habit is opposite to what has been documented in other clades that have shifted from lowland to montane habits (Hughes & Atchison, 2015). Furthermore, the *Incarvillea* clade experiences an increased substitution rate in our phylogeny (Figs 1.1, Appendix 1.3-1.8), a pattern that has been correlated with niche changes in other angiosperm groups (Smith & Donoghue, 2008, Smith & Bealieau, 2009).

A dispersal event into Australasia occurred between 31 Ma (25.09-36.08) and 28 Ma (21.77 – 33.98). From the Late Cretaceous to early Eocene, the core area of Sundaland, which included the present-day islands of Borneo, Sumatra, Java, and Bali, as well as the Malay peninsula, became an elevated and emergent extension of continental Asia (Kooyman et al. 2019). The collision of Australia (Sahel) with the Sunda shelf is implied by pollen evidence demonstrating floristic exchanges in the late Oligocene to early Miocene. The collision of Sunda and Sahel transformed New Guinea from an arc of isolated islands to a mountainous region with peaks reaching 5000 m by the late Miocene (Kooyman et al. 2019). Assuming that dispersal from continental Asia occurred overland to eastern Sunda during the Oligocene, one clade of Australasian taxa may have remained there, eventually giving rise to the *Deplanchea-Lamiodendron* clade, while another dispersed from there into proto-New Guinea or northern Australia, east of Wallace's line, eventually giving rise to the remainder of Australasian taxa in the tribe. This could explain the distribution of the first clade across the Sundaland region to Australia, and the distribution of the second clade restricted to areas east of Lydekker's Line (Van Welzen et al., 2001). It is possible that, following diversification in each region, extant taxa in these clades ultimately met in New Guinea and northern Australia, explaining the distribution of taxa we see today. This dispersal event could also have occurred from continental Asia to New Guinea, with only one clade experiencing subsequent range expansion into Sundaland. Additional species sampling in *Deplanchea* and *Pandorea* and subsequent biogeographic analyses at a finer geographic scale may help tell a clearer story of the dispersal routes of Australasian taxa in Tecomeae.

Dispersal back to SA

Last, but not least, there is one species nested in a clade of Australasian species that is distributed in the temperate forests of Patagonian South America: *Campsdium valdivianum*. This dispersal event from Australasia to SA occurred between 18 Ma (12.25 – 24.78) and 13 Ma (6.88 – 20.79) in the Miocene (Fig 1.3, Table 1.1). Dispersal events from Australia/New Zealand to SA have often been attributed to the strong westerly wind and ocean currents referred to as West Wind Drift and the Antarctic Circumpolar Current (Wagstaff et al, 2006, Winkworth et al., 2002). These were established around Antarctica after the opening of the Drake Passage between South America and Antarctica in the Oligocene (McLoughlin, 2001). Furthermore, the seeds of Tecomeae are adapted to wind dispersal, so a rare event of sweepstakes dispersal between Australia and South America is possible with the prevailing wind direction (Gentry, 1992). Examples of other plant groups that have achieved dispersal from Australasia to South America include *Oreobolus* (Cyperaceae), *Myosotis* (Boraginaceae), *Coriaria* (Coriaraceae), *Sophora* (Fabaceae), and *Hebe* (Scrophulariaceae) (Winkworth et al., 2002).

CONCLUSIONS

Phylogenetic analysis of our concatenated dataset inferred a monophyletic Tecomeae consisting of three well supported clades. Lineages in Tecomeae dispersed from South America to North America and to the Old World and back in a series of putative dispersal events from the Eocene to present. This study implicates possible intercontinental dispersal via land bridges, island hopping, and sweepstakes long distance dispersal to explain the pantropical distribution of Tecomeae. This is unique testimony that circumnavigating the globe in ca. 40 Ma is possible for a small clade of plants.

Overall, Tecomeae is an extraordinary group because understanding its evolutionary history and current distribution sheds light on the biogeographic patterns at play in both tropical and temperate regions. Specifically, this study required the examination of species diversity in both the Neotropics and Southeast Asia, the two most biodiverse regions on Earth (Antonelli & Sanmartin, 2011). Habitat heterogeneity defines the modern landscapes of both regions, but how this was achieved through deep time involved disparate geologic processes. The Neotropical flora evolved in situ, influenced mainly by endogenous changes to the landscape paired with large barriers to immigration and emigration for much of its existence. Conversely, the Southeast Asian flora literally came together through the movement of continental plates, forming a crossroads of Asian, Indian, and Australian floras. Africa has experienced the least of both scenarios, which may explain its low species diversity compared to other tropical regions in which this group is distributed.

Table 1.1 Node information for the Tecomeae phylogeny and the BioGeoBEARS ancestral state reconstruction. Posterior probability (PP) support values are from maximum clade credibility tree generated from the concatenated dataset in MrBayes and bootstrap support values are from the tree generated from the concatenated dataset in RAxML. Median crown and stem ages and 95% highest posterior distributions (HPD) are from the time calibrated tree generated in BEAST. Ancestral state probabilities for each coded region are from BioGeoBEARS under the BAYAREALIKE + J model. Coded regions are as follows: Africa (A), South America (S), Australasia (U), Africa (F), and Asia (A).

Clade	PP	Bootstrap	Median Crown Age (Ma)	95% HPD Crown Age	Median Stem Age (Ma)	95% HPD Stem Age	N	S	U	F	A
Bignoniaceae	0.66	83	49.72	47.71-51.6							
Calibration	0.96	92	34.15	33.75-35							
Tecomeae	1	99	37.09	31.64-41.78	40.79	36.3-45.04	0.024586	**0.964071**	0.000809	0.004803	0.00573
Argylia	1	100	8.81	3.69-16.28	40.79	36.3-45.04	7.55E-14	1	4.49E-17	2.75E-16	1.30E-15
Astianthus/*Campsis*	1	100	21.79	9.93-34.2	37.09	31.64-41.78	**0.960759**	4.09E-09	1.58E-14	2.85E-13	0.039241
Campsis	1	100	5	1.13-11.08	21.79	9.93-34.2	**0.997785**	8.72E-12	2.96E-17	2.84E-16	0.002215
Tecoma + Old World Clade	1	99	34.88	29.07 - 39.59	37.09	31.64 - 41.78	1.94E-06	**9.68E-01**	7.19E-05	4.34E-04	0.031195
Tecoma	0.95	98	10.46	6.47-16.49	34.88	29.07-39.59	2.18E-06	**0.999998**	1.52E-15	9.09E-15	2.55E-12
T. stans/*T. stans* var. *sambucifolia*	0.64	85	2.07	0.4-4.58	4.57	2.29-7.38	0.001	**0.999**	4.23E-20	4.14E-20	3.27E-19
Old World Clade	0.94	96	30.91	25.09-36.08	34.88	29.07-39.59	4.92E-12	0.000111	**0.158926**	**0.479776**	**0.361187**
African Clade	0.84	63	25.57	15.11-32.95	30.91	25.09-36.08	4.97E-13	7.30E-11	8.46E-11	**1**	5.69E-10
Asian Clade	0.41	40	29.99	24.17-35.32	30.91	25.09-36.08	3.16E-12	0.000102	**0.302506**	2.40E-09	**0.697392**
Australasian Clade	0.84	73	27.96	21.77-33.98	29.99	24.17-35.32	4.93E-14	9.42E-05	**0.999906**	3.86E-11	1.05E-10
Incarvillea	1	100	18.93	13.77-24.74	29.99	24.17-35.32	1.65E-14	6.71E-13	3.10E-11	6.76E-11	**1**
Campsidium/*P. jasminoides*	0.7	73	13.54	6.88-20.79	18.19	12.25-24.78	1.58E-16	0.000137	**0.999863**	2.98E-16	1.31E-15

Table 1.2 Results of AIC and AICc model comparison for time stratified analyses in BioGeoBEARS.

Model	**LnL**	**AIC**	**AICc**
DEC	-44.28	92.55	92.73
DEC+J	-36.15	78.3	78.67
DIVALIKE	-42.79	89.58	89.77
DIVALIKE+J	-36.9	79.79	80.17
BAYAREALIKE	-59.62	123.2	123.4
BAYAREALIKE+J	-38.41	82.82	83.19

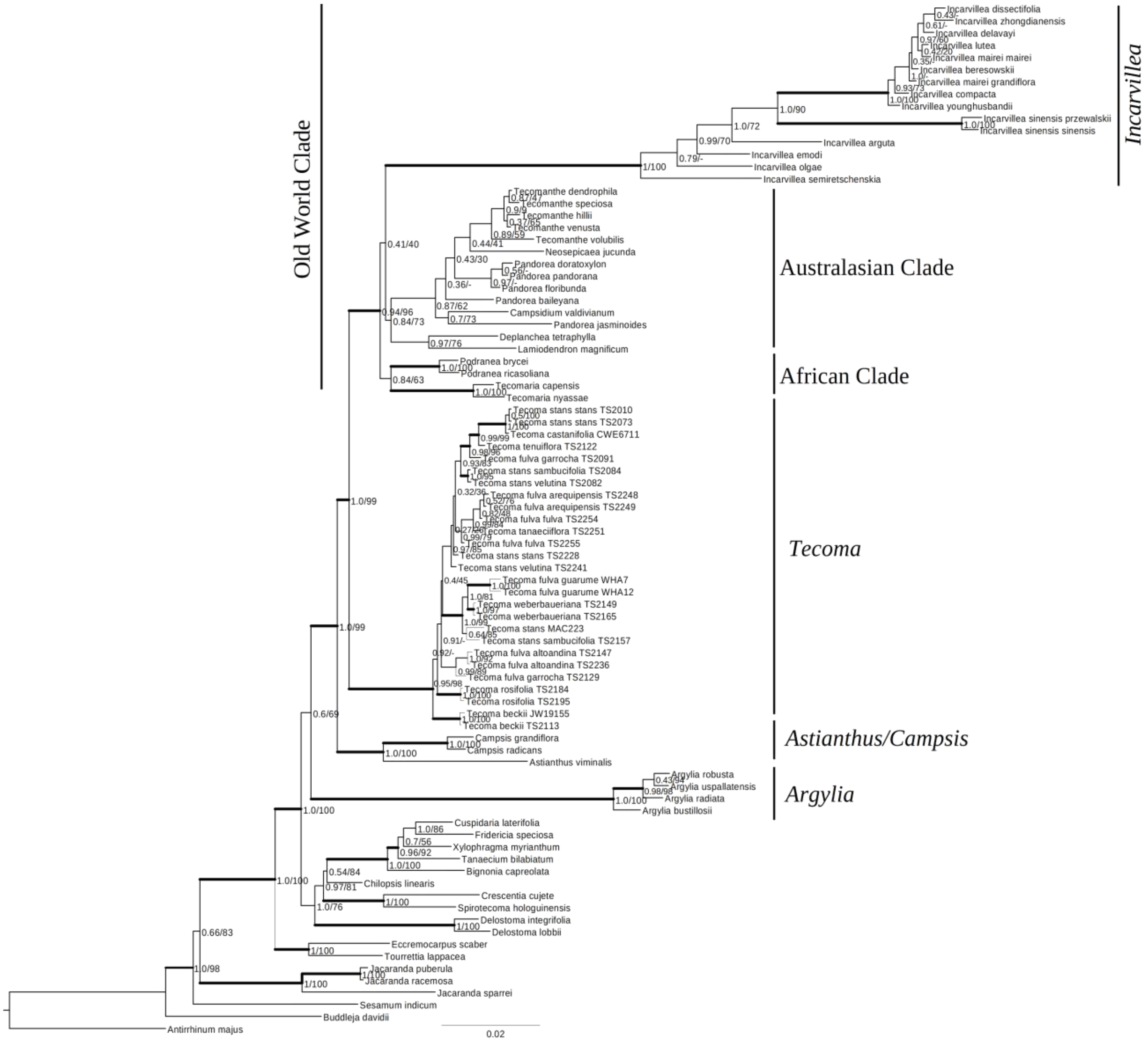

Fig 1.1 Maximum clade credibility Bayesian tree generated from the total concatenated dataset. Posterior probability and bootstrap support values are indicated at nodes. Nodes with both posterior probability values > 0.9 and bootstrap values > 90 are indicated with bolded branches leading to nodes. Relationships not present in the ML tree are indicated by dashes.

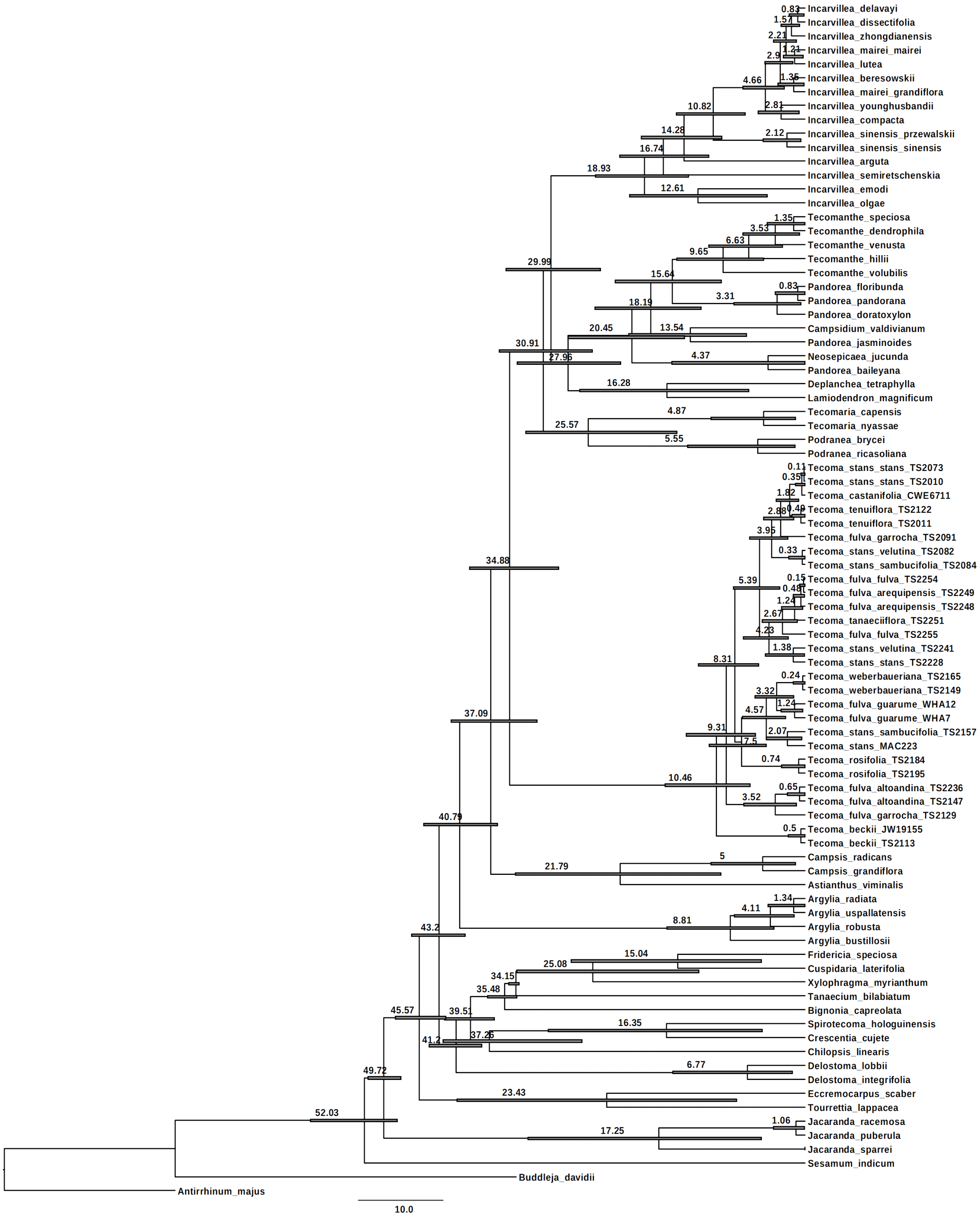

Figure 1.2 BEAST maximum clade credibility tree generated from the total concatenated dataset. Median age estimates and 95% HPD bars in millions of years are shown at nodes.

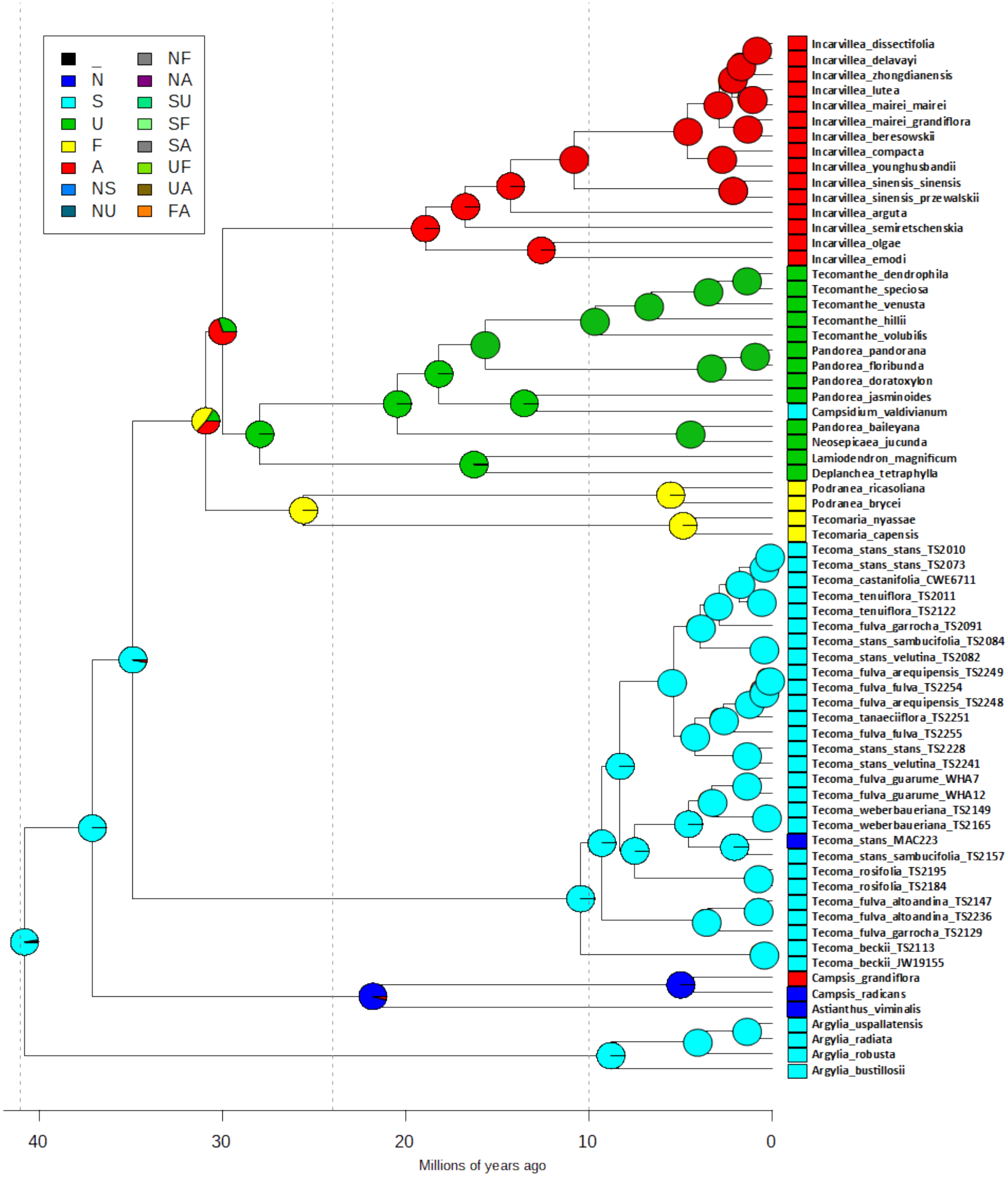

Figure 1.3 Historical biogeography of Tecomeae. Colored pie charts represent ancestral range probabilities at each node as estimated by the BAYAREALIKE + J model. Colors refer to geographic regions: South America (light blue), North America (dark blue), Australasia (green), Africa (yellow), Asia (red).

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CHAPTER 2: Phylogeny of the Neotropical tribe Jacarandeae (Bignoniaceae)

PREMISE: The tribe Jacarandeae includes *Jacaranda* (49 species) and *Digomphia* (3 species), two genera of trees and woody shrubs with Neotropical distribution. Jacarandeae is sister to the rest of the Bignoniaceae, but not much is known about interspecific and intergeneric relationships within this group.

METHODS: We reconstructed the phylogeny of Jacarandeae using chloroplast (*ndhF*, *rpl*32-*trnL*, *trnL-F*) and nuclear (ETS, PPR62) markers. Evolutionary relationships within Jacarandeae were inferred using Bayesian, Maximum Likelihood, and species tree approaches. The resulting phylogenetic framework was used as the basis to interpret the evolution of key morphological character states (i.e., stamen and calyx traits) and revise the infra-generic classification of the group.

RESULTS: *Jacaranda* and *Digomphia* belong to a well-supported clade, with *Digomphia* nested within *Jacaranda*. We propose the necessary taxonomic changes to recognize monophyletic taxa, including a broadly circumscribed *Jacaranda* divided into four sections: (1) *Jacaranda* sect. *Nematopogon*, species previously included in *Digomphia* and united by divided staminode apices and spathaceous calyces; (2) *Jacaranda* sect. *Copaia*, species with monothecal anthers and cupular calyces; (3) *Jacaranda* sect. *Jacaranda*, species with monothecal anthers and campanulate calyces; and (4) *Jacaranda* sect. *Dilobos*, species with dithecal anthers and cupular calyces, and including more than half of the species of the genus, all restricted to Brazil.

CONCLUSIONS: As circumscribed here, Jacarandeae includes only a broadly defined *Jacaranda* divided into four sections. Each section is defined by a unique combination of anther and calyx morphologies.

KEY WORDS: Bignoniaceae, *Digomphia*; *Jacaranda*; Jacarandeae; Neotropical flora; phylogeny; systematics.

INTRODUCTION

Tribe Jacarandeae Fenzl (Bignoniaceae) traditionally consisted of two genera (*Jacaranda* and *Digomphia*) and 50 species of trees and shrubs that are distributed across the New World tropics (Fenzl, 1841; Gentry, 1992b). *Jacaranda* Juss. contains 47 species, the majority of species in the tribe, and is distributed from southern Mexico to northern Argentina (Gentry, 1992a; Lohmann and Ulloa Ulloa, 2006 onward; Da Silva-Castro, 2017; Farias-Singer, 2019 onward). Members of this genus occupy a wide range of biomes and present a variety of growth habits, ranging from rainforest canopy trees to fire-adapted xylopodial subshrubs. For example, *Jacaranda copaia* (Aubl.) D. Don is a hyperdominant tree in lowland, wet tropical forests from Belize to Bolivia (ter Steege et al., 2013), while *Jacaranda rugosa* A.H. Gentry is endemic to the Caatingas of Pernambuco State, Brazil (Gentry, 1992b; Milet-Pinheiro and Schlindwein, 2009). *Digomphia* Benth., with only three species, consists of shrubs restricted to lowland Amazonia and the Guiana shield (Gentry, 1992a; Lohmann and Ulloa Ulloa, 2006 onward; Farias-Singer and Singer, 2007).

Jacaranda species are known to have an array of horticultural and ethnobotanical uses (Gentry, 1992a). The species of greatest horticultural importance is *Jacaranda mimosifolia* D. Don, perhaps the most widely planted subtropical tree worldwide (Gentry, 1992a). Described as a "long-lived pioneer species" (Scotti-Santaigne et al., 2013), *J. copaia* is a good candidate for reforestation projects due to its rapid growth in high-light environments and persistence in mature forests. The Siona Indians of Amazonian Ecuador use the capsular fruit of *J. copaia* as tools to shape pottery (Vickers and Plowman, 1984; Gentry, 1992a). Ethnomedically, species in *Jacaranda* have been used as treatment for venereal disease and ailments of the skin, stomach, and intestines (Gentry, 1992a). Jacaranone, a chemical constituent present in *Jacaranda*, has been shown to have anticancer and antimicrobial properties and was patented for antitumor activity (Ogura et al., 1977; Gachet and Schuhly, 2009).

Two genera within the Bignoniaceae were originally recognized based on fruit type (Linnaeus, 1753): *Bignonia* (including *Bignonia caerulea* L.) with dehiscent fruit and *Crescentia* L. with indehiscent fruit. Subsequent discoveries resulted in the splitting of these genera and the proposal for several new genera, including *Jacaranda*, which was described based on *B. caerulea* (Jussieu, 1789). De Candolle (1838) accepted many of the newly proposed genera, while recognizing Linnaeus' groups as tribes Crescentieae and Bignonieae, the latter containing two subgroups based on whether the fruit dehiscence was loculicidal or septicidal.

The tribe Jacarandeae was first circumscribed by Fenzl (1841), to include *Jacaranda, Distictis* Mart. ex Meisn., and *Platycarpum* Bonpl. Currently, *Distictis* is placed in tribe Bignonieae (Lohmann, 2006), while *Platycarpum* is currently included in Rubiaceae. Bentham (1846) initially described *Digomphia* as a new genus of Scrophulariaceae, but later placed it in Jacarandeae (Hooker, 1876). Hooker (1876) recognized four main tribes in the Bignoniaceae: Bignonieae, Crescentieae, Jacarandeae, and Tecomeae. He recircumscribed Jacarandeae to include *Jacaranda*, *Eccremocarpus* Ruiz and Pav., *Colea* Bojer ex Meisn., and *Parmentiera* DC., the last three of which would later be placed in tribes Eccremocarpeae (or Tourretieae), Coleeae, and Crescentieae, respectively (Hooker, 1876; Olmstead et al., 2009). Failing to recognize Jacarandeae, Bureau and Schumann (1896-1897) returned *Jacaranda* and *Digomphia* to Tecomeae. Gentry (1980, 1992b) adopted Schumann's classification, agreeing that Hooker's Jacarandeae was not a "natural group" and retained *Jacaranda* and *Digomphia* in Tecomeae.

The first molecular phylogeny of the Bignoniaceae recovered the sole *Jacaranda* species sampled as sister to all other Bignoniaceae (Spangler and Olmstead, 1999). A subsequent phylogeny of the family based on increased sampling and including four species of *Jacaranda*, corroborated the initial results (Olmstead et al., 2009). Based upon both these results, Olmstead et al. (2009) resurrected Jacarandeae, restricted to *Jacaranda* and *Digomphia*. More recent phylogenetic work provided additional support for the monophyly of the tribe, as well as to its sister-group relationship to the rest of the family (Appendix 2.1, 2.2, Farias-Singer et al., 2011). Despite that, our understanding of phylogenetic relationships among members of Jacarandeae remains poor.

Traits that distinguish Jacarandeae from the rest of Bignoniaceae include the elongated and glandular staminode, typically bipinnately compound leaves (occasionally simply compound, or even simple), and a broad, dehiscent, oblong to circular or elliptic fruit flattened perpendicular to the septum (Fig. 2.1). Furthermore, most taxa in Bignoniaceae have 2n = 40 chromosomes, but *Jacaranda* has 2n = 36 (Cordeiro et al., 2016).

Gentry (1992b) suggested that *Digomphia* should be recognized as its own genus based on differences in staminode apex and leaf architecture. *Digomphia* has simple or simply pinnate compound leaves, while most *Jacaranda* species have bipinnately compound leaves (Fig. 2.1; Gentry, 1992). The apices of *Digomphia* staminodia are bifurcating or trifurcating while staminodia of *Jacaranda* are entire (Fig. 2.1). The purpose of these showy staminodia is uncertain, but they seem to be multifunctional (Guimarães et al., 2008). In a pollination study of *J. rugosa*, staminode presence was associated with significantly higher pollinator visitation frequency compared to flowers with staminodia experimentally removed, suggesting that showy staminodia in this tribe are important for pollinator attraction (Milet-Pinheiro and Schlindwein, 2009). A pollination study of *Jacaranda oxyphylla* Cham. suggested that secretions from glandular trichomes of staminodia attract pollinators, and that the physical location of staminodia in flowers play a role in increased pollinator contact with reproductive organs (Guimarães et al., 2008b). Most species in Jacarandeae seem to be pollinated by euglossine bees (Gentry, 1974; Guimarães et al., 2008; Maues et al., 2008; Milet-Pinheiro and Schlindwein, 2009).

Endlicher (1839) described three sections within *Jacaranda* based on number of anther thecae and calyx morphology: (1) *Copaia* with one anther sac and tubular, truncate calyces; (2) *Hemilobos* with one anther sac and campanulate, five-lobed calyces; and (3) *Dilobos* with two anther sacs and campanulate calyces. Subsequent classifications (de Candolle, 1845; Gentry, 1992b) did not use calyx characters, recognizing sections only on the number of anther thecae, *Dilobos* and *Monolobos*, with nearly equal numbers of species in each group. Biogeographically, this classification segregates *Jacaranda* into two main areas of the neotropics; section *Monolobos* (hereafter sect. *Jacaranda*, as required by the ICN, Turland et al., 2018) characterized by a single anther sac, and widespread throughout Central America and Amazonia, and section *Dilobos*, characterized by two anther sacs and found throughout South America, with greatest diversity in eastern Brazil (Gentry, 1992b). The absence of one theca in *Jacaranda* is unique in Bignoniaceae and rare in the greater core Lamiales (Gentry, 1980; Judd et al., 2016).

The dichotomy based on the number of anther thecae is supported by differences in *Jacaranda* wood anatomy. Jacarandeae are distinguished from the rest of the family by the narrow vessels, paratracheal winged-aliform parenchyma, and non-storied rays (Pace et al., 2015). Within *Jacaranda*, section *Jacaranda* has wood anatomy marked by homocellular and uniseriate rays, while sect. *Dilobos* has heterocellular and multiseriate rays (Dos Santos and Miller, 1997). The only exception to this rule is found in *Jacaranda copaia*. Even though *J. copaia* was assigned to sect. *Jacaranda* due to the single anther sac, this species has a unique combination of anatomical traits that are intermediate between sections (Gentry, 1992b, Dos Santos and Miller 1997). Specifically, *J. copaia* has multiseriate rays of homocellular composition, and the widest vessels of any tree in Bignoniaceae (Pace et al., 2015).

Other clades in Bignoniaceae that have been studied extensively include Bignonieae (e.g., Lohmann, 2006; Lohmann et al., 2013; Lohmann and Taylor, 2014), Catalpeae (Li, 2008), Coleeae (Zjhra et al., 2004; Callmander et al., 2016), *Incarvillea* (Chen et al., 2005), and the *Tabebuia* alliance (Grose and Olmstead, 2007a, 2007b). Molecular phylogenetics provides strong support for the circumscription and monophyly of these clades and provides a foundation for the current study.

Understanding the evolutionary history of Jacarandeae requires knowledge of relationships among species within this tribe. The aims of this paper are to: (1) test the monophyly of Jacarandeae; (2) test the monophyly of the two genera recognized in the tribe, *Jacaranda* and *Digomphia*, as well as resolve relationships among species therein; and (3) revise the classification of tribe Jacarandeae. To address these goals, we sampled widely in *Jacaranda* and *Digomphia* and built a dataset comprising three chloroplast (*ndh*F, *rpl*32-*trn*L, *trn*L-F) and two nuclear (ETS, PPR62) markers, which was used to generate a phylogenetic framework for the tribe using Bayesian, Maximum Likelihood, and species tree approaches. Our phylogeny provides a foundation for future investigation of the origin and maintenance of species diversity within the group, and an improved understanding of Bignoniaceae systematics.

MATERIALS AND METHODS

Sampling

We sampled 35 of the 50 species recognized in the most comprehensive treatment of Jacarandeae to date (Gentry, 1992). This sampling strategy included all three species of *Digomphia*, and 32 out of 47 species of *Jacaranda* (including both subspecies of *J. copaia*). Furthermore, five species from related lineages within Bignoniaceae (*Bignonia capreolata* L., *Crescentia cujete* L., *Chilopsis linearis* (Cav.) Sweet, *Eccremocarpus scaber* Ruiz and Pav., *Tabebuia rigida* Urb.), and five species from closely related lineages within the Lamiales (*Paulownia tomentosa* (Thunb.) Steud., *Schlegelia fuscata* A.H. Gentry, *Schlegelia parviflora* (Oerst.) Monach., *Sesamum indicum* L., *Thunbergia alata* Bojer ex Sims) were also included as outgroups (Lohmann and Ulloa Ulloa, 2006 onward; Olmstead et al., 2009; Refulio and Olmstead, 2014; Appendix 1). Of the ingroup taxa, five were collected in the field, six were obtained from plants in cultivation, and the remaining samples were gathered from herbaria. Sampling of the ingroup includes previously published sequences for two taxa (Olmstead et al., 2009), with the remaining taxa representing new accessions.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from silica-dried material or herbarium tissue using either the CTAB method (modified from Doyle and Doyle, 1987) or DNeasy Kits (Qiagen, Valencia, California, USA). Three chloroplast regions (*ndh*F, *rpl*32-*trn*L, *trn*L-F) were amplified via polymerase chain reaction (PCR) using primers developed in Olmstead and Sweere (1994), Taberlet et al. (1991), and Zuntini et al. (2013), respectively. Two nuclear regions (ETS and PPR62) were amplified using the 18s-IGS primer of Baldwin and Markos (1998) with a custom primer used to amplify ETS in the Lamiales (Beardsley and Olmstead, 2002) and PPR62 using primers developed in Yuan et al. (2009, 2010). PCR was performed under the following general conditions: 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 30 sec, 50^{o}C for 30 sec, 72^{o}C for 1.5-2.5 min, followed by 72^{o}C for 10 min. Amplified PCR products were purified using 20% polyethylene glycol (PEG) precipitation and 70% ethanol washes prior to sequencing. Cycle sequencing reactions were performed using BigDye 3.1 (Applied Biosystems Inc., Foster City, California, USA) using a standard Applied Biosystems protocol. Products of sequencing reactions were purified by passing through Sephadex G-50 columns (Sigma-Aldrich, St. Louis, MO) or sodium acetate precipitation. An Applied Biosystems genetic analyzer was used to generate raw sequence data.

Phylogenetic analyses

Sequence chromatogram editing and manual alignments were performed in Geneious 10.2.3 (Kearse et al., 2012.). Data were analyzed in both Maximum Likelihood and Bayesian frameworks in four ways: (1) concatenated chloroplast loci (*ndh*F, *rpl*32-*trn*L, *trn*L-F); (2) each nuclear locus separately; (3) concatenated nuclear loci (ETS, PPR62); and (4) combined chloroplast-nuclear dataset partitioned. Chloroplast and nuclear datasets were analyzed separately to detect any conflicting phylogenetic signal.

Appropriate models of evolution for these data were determined using jModelTest 2.1.4 (Darriba et al., 2012) using the Akaike information criterion (AIC). Phylogenetic reconstructions were performed in a Maximum Likelihood framework using RAxML 8.0.0 (Stamatakis, 2014), Bayesian framework using MrBayes 3.1.2 (Ronquist et al., 2012), and Bayesian coalescent framework using *BEAST 2.4.7 (Bouckaert et al., 2014). One thousand bootstraps were performed with maximum likelihood.. All other parameters were kept at default values set in RAxML 8.0.0. Bayesian analysis used two replicate runs, each consisting of four chains sampled every 1000 generations. Analyses were assessed for convergence using Tracer 1.6 (Rambaut et al., 2014). Analyses were run until convergence diagnostics indicated that they had reached stationarity. When summarizing consensus trees over all runs, the first 25% of sampled trees were discarded as burn-in.

In *BEAST, the chloroplast loci were treated as a single organellular (haploid) locus and nuclear loci were treated as autosomal. Due to the degradation of herbarium specimen DNA, it was difficult to achieve complete sequencing coverage for all loci. Therefore, *BEAST was run twice— once including only taxa with full sequencing coverage for all loci and once including all taxa with ‘N’ inserted for missing data. The final analysis used a GTR model for all datasets, default speciation and clock models, and priors for mean population size and birth rate were set to gamma distributions with shape = 2. Runs were performed for 500 million generations, sampling every 5000 trees. TreeAnnotator 2.4.7 (Rambaut and Drummond, 2017) was used to summarize the sampled trees into single target trees, with the first 25% of trees sampled discarded as burn-in.

Morphological trait mapping

Stamen (monothecal or dithecal; staminodia entire or divided) and calyx (cupular, campanulate, or spathaceous; persistent or not persistent) morphology was assessed for each taxon (Gentry, 1992b, personal observation). Ancestral state reconstructions were performed in Mesquite 3.3 (Maddison and Maddison, 2017) on the maximum clade-credibility tree generated from the *BEAST tree using the “parsimony ancestral states” option.

RESULTS

Sequence data were derived from three cpDNA regions (*ndh*F, *rpl32-trn*L, *trn*L-F) and two nDNA regions (ETS, PPR62). Sequences were submitted to GenBank (Appendix 2.3). Due to difficulty in amplifying and sequencing target DNA regions extracted from herbarium specimens, about 20% of sequences from target loci were partial or missing from the final dataset.

For the nuclear dataset, new sequence data were generated for all 48 accessions included. After alignment, the total length of alignment for each dataset was 457 for ETS and 360 for PPR62, resulting in a combined nuclear dataset of 817 positions. While the separate ETS and PPR regions did not have the same sequencing coverage, tree topologies were in agreement with regards to the major clades uncovered.

Chloroplast sequences of *ndh*F and *trn*L-F for *Jacaranda arborea* Urb., *J. sparrei* A.H. Gentry, and outgroups were obtained from previous studies (Olmstead et al., 2009). Remaining sequences for *ndh*F and *trn*L-F, as well as all *rpl*32-*trn*L sequences, were newly generated for this study. After alignment, the total length of alignment for each dataset was 2001 for *ndh*F, 917 for *trn*L-F, and 1196 for *rpl*32-*trn*L, resulting in a combined chloroplast dataset of 4114 positions. When the nuclear regions were added to this dataset, the resulting concatenated dataset contained 4931 positions.

Appropriate models of evolution were evaluated with JModelTest 2.1.4 and the Akaike information criterion (AIC), which determined that the GTR+I+G model was the most likely fit for ETS and all chloroplast loci, while GTR+I was the most likely fit for the PPR62 dataset (Darriba et al., 2012).

Phylogenetic trees generated from nuclear and chloroplast datasets in both Bayesian and ML frameworks support a monophyletic Jacarandeae. In the nuclear tree (Fig. 2.2, Appendix 2.4, 2.5), four clades emerged, as follows: 1) a monophyletic *Digomphia* (PP = 1, BS = 100%); 2) all species from *Jacaranda* sect. *Jacaranda* (PP = 1, BS = 100%), except *J. copaia*; 3) *J. copaia* (including both subspecies) (PP = 1, BS = 100%); and 4) a monophyletic *Jacaranda* sect. *Dilobos* (PP = 1, BS = 100%). *Jacaranda* sect. *Jacaranda* appears as sister to *Digomphia*, although poorly supported (PP = 0.63, BS = 72%), while *Jacaranda* sect. *Dilobos* appears as sister to *J. copaia* and also is poorly supported (PP = 0.64, BS = 60%).

In contrast, differences were found in the tree that resulted from the analysis of the combined cpDNA dataset (Fig. 2.3, Appendix 2.6, 2.7). This topology includes six main clades: (1) a monophyletic *J. copaia* (PP = 1, BS = 100%); (2) all Caribbean species of *Jacaranda* sect. *Jacaranda* (PP = 0.98, BS = 85%); (3) the remaining species of *Jacaranda* sect. *Jacaranda* (PP = 1, BS = 71%); (4) *Digomphia laurifolia* Benth. and *Digomphia densicoma* (Mart. ex DC.) Pilg. (PP = 1, BS = 73%); (5) *Digomphia ceratophora* A.H. Gentry; and (6) *Jacaranda* sect. *Dilobos* (PP = 1, BS = 98%). Neither *Jacaranda* sect. *Jacaranda* nor *Digomphia* appear as monophyletic, while *Jacaranda* sect. *Dilobos* and *J. copaia* are monophyletic. The relationships among the six clades that emerge in the chloroplast tree are largely unresolved, with weak support for any nodes suggesting relationships among them in the optimal tree. The previous results of Farias-Singer et al. (2011) based on two chloroplast loci are consistent with the results obtained here (Appendices 2.1, 2.2).

Concatenated, partitioned analysis of the combined dataset analyzed in both Bayesian and ML frameworks (Fig. 2.4, Appendix 2.8, 2.9) recovered the same four clades found in the tree that resulted from the analyses of the nuclear dataset. Jacarandeae is again inferred to be monophyletic. There is low probability (PP = 0.7, BS = 44%) of a clade comprising *Jacaranda* sect. *Dilobos* and *J. copaia*. The relationships among this clade, *Jacaranda* sect. *Jacaranda* (except *J. copaia*), and *J. copaia* are unresolved (Fig. 2.4).

To unravel incongruence among the results of the three previously described analyses, a coalescent species tree was generated in *BEAST (Fig. 2.5). Like all previous analyses, the resulting tree strongly supported a monophyletic Jacarandeae (PP = 1) containing four clades: (1)

Digomphia (PP = 0.67); (2) *J.* copaia (PP = 1); (3) *Jacaranda* sect. *Jacaranda* (PP = 0.2); and (4) *Jacaranda* sect. *Dilobos* (PP = 1). Relationships among the four clades are unresolved. Unique to the *BEAST tree, *J. copaia* is weakly supported as sister to the rest of the tribe (PP = 0.4).

Morphological trait mapping on the *BEAST maximum clade credibility tree (Fig. 2.5) suggested two equally parsimonious hypotheses for the evolution of anther thecae in Jacarandeae: (1) a monothecal anther ancestral state led to the gain of a second functional anther sac twice, i.e., once on the branch to *Digomphia* and once on the branch to sect. *Dilobos*; and (2) a dithecal anther ancestral state preceded the loss of a functional anther sac twice, i.e., once on the branch to *J. copaia* and once on the branch to sect. *Jacaranda*. Undivided staminodia were inferred to be the ancestral condition in Jacarandeae, with divided staminodia evolving in *Digomphia*. Spathaceous calyces of *Digomphia* and campanulate calyxes of sect. *Jacaranda* are likely derived from cupular calyces, and the persistent calyces of sect. *Dilobos* are derived from deciduous calyces.

DISCUSSION

In this study, we used nuclear and chloroplast datasets to reconstruct the phylogeny of Jacarandeae (Figs. 2.2-2.5, Appendix 2.4-2.9). The trees provide a consistent picture of the phylogeny of the tribe, although differences were detected. Below we summarize phylogenetic relationships within the tribe and discuss the implication of those findings for morphological evolution and the classification of the group.

Phylogenetic relationships of tribe Jacarandeae

Our primary goal was to resolve the main clades of Jacarandeae via molecular phylogenetic methods and use them as a basis for a revised classification. Using concatenated sequence and coalescent approaches, the results support that: (1) Jacarandeae is monophyletic; (2) *Digomphia* is nested within *Jacaranda*; and (3) Jacarandeae consists of four well-supported clades. However, resolution of the relationships among clades is weak (Figs. 2.2-2.5). Previous classifications differed in their methods of grouping taxa in Jacarandeae. While Endlicher (1839) recognized three groups in Jacarandeae based on a combination of number of anther sacs and calyx morphology, de Candolle (1845) and Gentry (1992b) recognized two groups based only on number of anther sacs. *Digomphia* was not recognized in Jacarandeae until Hooker (1876), whose circumscription also included other Bignoniaceae taxa. Olmstead et al. (2009) resurrected Jacarandeae to contain only *Jacaranda* and *Digomphia* based on a hypothesis by Gentry (1992b) that *Digomphia* is derived from within *Jacaranda*. Our molecular phylogenetic results support the monophyly of *Digomphia* and *Jacaranda* sect. *Dilobos*. However, our results do not support the monophyly of *Jacaranda* sect. *Jacaranda*, because *J. copaia* forms a distinct lineage from the rest of sect. *Jacaranda* as reflected in Endlicher's (1839) classification. Jacarandeae's monophyly, *Digomphia*'s placement within *Jacaranda*, and paraphyly of *Jacaranda* sect. *Jacaranda* are consistent with the results of Farias-Singer et al. (2011).

Our analyses recovered a monophyletic *Digomphia* nested within *Jacaranda*, supporting the relationship posited by Gentry (1992b) and the most recent circumscription of Jacarandeae proposed by Olmstead et al. (2009). *Digomphia* has dithecal anthers, as seen in sect. *Dilobos*. Despite that, *Digomphia* shows a number of characters that are not found in any members of *Jacaranda* such as the deeply divided staminode and large, foliaceous calyx. A sister relationship between *Digomphia* and sect. *Dilobos* is weakly supported in trees generated from both *BEAST and the chloroplast dataset (Figs. 2.3, 2.5). In the tree that resulted from the analyses of the nuclear dataset, *Digomphia* receives modest support as sister to sect. *Jacaranda*. In the concatenated tree, the relationship between *Digomphia* and other major clades is unresolved. Dos Santos and Miller (1992) found the wide rays of *Digomphia* most similar to those of *J. copaia* but these taxa do not appear as closely related in our analyses. However, Dos Santos and Miller (1992) did not find any major anatomical differences between *Digomphia* and *Jacaranda* at the macroscopic or microscopic level, providing additional evidence that the genus belongs in *Jacaranda*. Given the morphological similarities between *Jacaranda* and *Digomphia* and lack of clear evidence of a monophyletic *Jacaranda* excluding *Digomphia*, the genus *Jacaranda* is best circumscribed broadly, including *Digomphia*.

We also found incongruence among gene trees and the species tree with regards to the monophyly of *Jacaranda* sect. *Jacaranda* (excluding *J. copaia*). This group receives low support in the species tree and is not monophyletic in the tree that resulted from the analyses of the chloroplast dataset, where it is split into two well-supported clades, one in South America and the other in the Caribbean.

Anther thecae evolution

The number of anther thecae present in *Jacaranda* species has been the traditional guide to classifying species within the genus. The major clades generated in the coalescent tree (Fig. 2.5) suggest two possible directions of thecal evolution: (1) the loss of one functional anther sac occurred twice from a typical dithecal anther, once in *J. copaia* and once in sect. *Jacaranda*; or

(2) a monothecal ancestral state reversed to dithecal anthers on the branch leading to *Digomphia* and sect. *Dilobos*. However, because monothecal anthers are unique to *Jacaranda* in the Bignoniaceae, and the gain of a functional trait once lost is relatively rare, scenario one seems more likely. Alternatively, it is possible that our optimal trees do not correctly reconstruct phylogenetic relationships within the group and the loss of one anther sac was a unique event without reversal.

Calyx evolution

As suggested by Endlicher (1839), calyx morphology can also be used as a guide to distinguish *Jacaranda* species in each of the four major clades uncovered. The major clades generated in the coalescent tree (Fig. 2.5) suggest that campanulate calyces in *Jacaranda* sect. *Jacaranda* and spathaceous calyces in *Digomphia* each evolved once from a cupular calyx ancestral state. Furthermore, calyx persistence evolves once on the branch leading to *Jacaranda* sect. *Dilobos* from non-persistent calyces as the ancestral state (Fig. 2.5).

Taxonomic treatment

These new molecular phylogenetic results warrant a new classification for the group. The tribe currently contains two genera, *Jacaranda* and *Digomphia*. The results presented in this study support a single, well-supported clade including both genera, comprised of four well-supported subclades. We resurrect *Jacaranda* sect. *Nematopogon* to house former *Digomphia* species and propose that the three sections of *Jacaranda* designated by Endlicher (1839) be resurrected. Names and diagnostic traits of the four sections are provided below:

I. *Jacaranda* Jussieu, Gen. Pl. 138. 1789. Type: *Jacaranda caerulea* (L.) Juss.

Leaves usually bipinnate, occasionally pinnate or simple. Inflorescence a few to many-flowered terminal or axillary thyrse or thyrsoid raceme. Flowers with short calyces; corolla blue or blue-purple to magenta, tubular campanulate above a narrow basal tube, the staminode elongate, exceeding the stamens. Fruit an oblong capsule flattened perpendicularly to the septum. Seeds winged, the wings membranaceous. Distribution: Belize, Guatemala, and the Antilles to northern Argentina.

1. *Jacaranda* sect. ***Copaia*** Endlicher. Gen. Pl. 711. 1839. Type: *Jacaranda copaia* (Aubl.) D. Don.

The clade containing the two subspecies of *Jacaranda copaia* (*J. copaia* subsp. *spectabilis* (Mart. ex DC.) A.H. Gentry and *J. copaia* subsp. *copaia*) receives strong support across all analyses. Phylogenetic trees generated from nuclear and concatenated datasets suggest a position sister to *Jacaranda* sect. *Dilobos*, while the species tree supports a position sister to the rest of the tribe.

The decision to recognize *Jacaranda* sect. *Copaia* is supported by morphology and distribution that sets it apart from the rest of the genus. *Jacaranda copaia* has monothecal anthers, but unlike all other species in *Jacaranda*, species in this section have unlobed, cupular calyces, a wood anatomy of multiseriate, homocellular rays intermediate between sections *Jacaranda* and *Dilobos*, and the largest wood vessels of any tree in the Bignoniaceae. *Jacaranda copaia* is also the only rainforest canopy tree in *Jacaranda* that reaches 45 m in height.

The decision to designate these two taxa as subspecies is supported by previous monographic and phylogeographic studies (Gentry, 1992b; Scotti-Saintagne et al., 2013). Based on their mainly allopatric distribution (except for a small region of range overlap in northern Brazil), Gentry hypothesized that it is likely that two species exist (Gentry, 1992b). Furthermore, microsatellite analyses of multiple populations of both subspecies revealed evidence of two divergent chloroplast lineages within *J. copaia*, corresponding to the ranges of the two subspecies. Nuclear microsatellites, however, suggest that nuclear variation between populations supports gene flow between these two divergent chloroplast lineages (Scotti-Saintagne et al., 2013). In this study, chloroplast analyses (Fig. 2.3) resulted in a paraphyletic *Jacaranda copaia* subsp. *spectabilis*, with our single accession of *Jacaranda copaia* subsp. *copaia* nested within the clade, while nuclear analyses (Fig. 2.2) placed the two accessions of *J. copaia* subsp. *spectabilis* together, sister to *J. copaia* subsp. *copaia*. Combined analyses showed the same result as the nuclear tree. These different outcomes lend further support for the population structuring described in Scotti-Saintagne et al. (2013), and our decision to keep them all as one species.

Jacaranda copaia subsp. *copaia* is characterized by elliptic or oblong-elliptic leaflets with distinct petioles, and capsule valves less than 7 cm wide. Distribution: Guianasregion of South America. *Jacaranda copaia* subsp. *spectabilis* is characterized by rhomboid-elliptic, sessile to subsessile leaflets with acute to acuminate tips, and capsule valves <6 cm. Distribution: widespread from Amazonia to Central America.

Ecologically, *J. copaia* is considered a “long-lived pioneer species” in lowland tropical forests, experiencing fast growth in light-rich environments, but persisting in mature forests (Scotti-

Saintagne et al., 2013). This species also tolerates drought and can therefore inhabit seasonally dry forests. The broad ecological tolerance of this species, particularly in its adaptations to xeric environments, contributes to its being one of the most abundant tree species in Amazonia (ter Steege et al., 2013), and could have favored expansion from Amazonia to Central America through a dry northern dispersal corridor along the Caribbean coast (Scotti-Saintagne et al., 2013).

2. *Jacaranda* sect. ***Jacaranda***. Type: *Jacaranda caerulea* (L.) Juss.

Trees derived from the analyses of the nuclear, concatenated, and species tree datasets lend strong support for the monophyly of this group, which contains most of the species formerly included in sect. *Monolobos*. The Antillean species of *Jacaranda* form a clade within section *Jacaranda* (Figs. 2.2-2.5). The remaining species include trees in sub-Amazonian dry areas and the xylopodial subshrub *J. decurrens* Cham.

The ca. 17 species in *Jacaranda* sect. *Jacaranda* are united by the combination of monothecal anthers and a campanulate, sometimes deeply lobed, calyx, as opposed to the cupular, minutely-toothed calyces of *Jacaranda* sect. *Copaia*. Distribution: widespread, ranging from Central and South America to the Antilles.

3. *Jacaranda* sect. ***Nematopogon*** A.P. de Candolle. Prodr. 9: 232. 1845. Type: *Jacaranda densicoma* Mart. ex DC. Basionym: *Nematopogon densicoma* (Mart. ex DC.) Bureau and K. Schum., Fl. Bras. 8(2): 396. 1897. Synonym: *Digomphia densicoma* (Mart. ex DC.) Pilg.

Jacaranda sect. *Nematopogon* consists of three species of the former genus *Digomphia*. There is strong support for the monophyly of sect. *Nematopogon* in the trees that resulted from the analyses of the nuclear and concatenated datasets, with modest support in the species tree. This section is distinguished morphologically from the rest of the tribe by the presence of deeply divided staminode apices and spathaceous calyces. It shares dithecal anthers with *Jacaranda* sect. *Dilobos*. There is disagreement among nuclear, chloroplast, and concatenated analyses regarding the position of this clade within the tribe. This clade emerges as sister to sect. *Dilobos* in both the chloroplast and species tree analyses, sister to sect. *Jacaranda* in the nuclear gene tree, and remains unresolved relative to the other sections in the combined concatenated tree. The simple leaved species *D. ceratophora* is sister to a clade that contains another simple leaved species (i.e., *D. laurifolia*), and a pinnately compound leaved species (i.e., *D. densicoma*), suggesting a loss of compound leaves twice in the genus.

a. *Jacaranda ceratophora* (A.H. Gentry) Ragsac, **comb. nov.** Basionym: *Digomphia ceratophora* A.H. Gentry, Mem. N.Y. Bot. Gard. 29: 270. 1978. Type: Venezuela. Amazonas: Cerro Yapacana, 150 m, *Maguire & Wurdack 34523* (holotype, NY; isotypes, P, VEN). *Jacaranda ceratophora* is characterized by 4-6 parted staminode apices and narrowly elliptic, simple leaves. Distribution: Below 200 m elevation in the white sand savannas of Amazonas, Venezuela and adjacent Colombia.

b. *Jacaranda densicoma* Mart. ex DC. Synonyms: *Digomphia densicoma* (Mart. ex DC.) Pilg.; *Nematopogon densicoma* (Mart. ex DC.) Bureau and K. Schum.

Jacaranda densicoma is the only species in *Jacaranda* sect. *Nematopogon* that has pinnately compound leaves. Distribution: Guayana highland region of northern Amazonian Brazil and southern Venezuela and Colombia.

c. *Jacaranda laurifolia* (Benth.) Ragsac, **comb. nov.** Basionym: *Digomphia laurifolia* Benth., Hooker's London J. Bot. 5: 364. 1846; Synonym: *Nematopogon laurifolius* (Benth.) Bureau and K. Schum., Fl. Bras. 8(2): 396. 1897. Type: Guyana. Roraima, *Schomburgk* 1049 (K).

Jacaranda laurifolia has bifurcate staminode apices, simple elliptic leaves, and acute fruit apices. Distribution: Above 450 m in the Guayana highlands of Venezuela, Guyana, and Brazil.

4. *Jacaranda* sect. ***Dilobos*** Endlicher**.** Gen. Pl. 712. 1839. Type: *Jacaranda jasminoides* (Thunberg) Sandwith. Basionym: *Bignonia jasminoides* Thunberg.

Jacaranda sect. *Dilobos* is the largest clade supported by all analyses. It consists of ca. 30 species united by the presence of dithecal anthers, undivided staminodia, and cupular, persistent calyces. The group contains species with a wide variety of growth habits across eastern South America, within which many species have endemic distributions (e.g., *J. rugosa* in campo rupestre and *J. jasminoides* in the Atlantic forest of Brazil). The lack of resolution within this section suggests that it is undergoing a recent and rapid radiation. Distribution: South America, concentrated in the Cerrado and Atlantic forest.

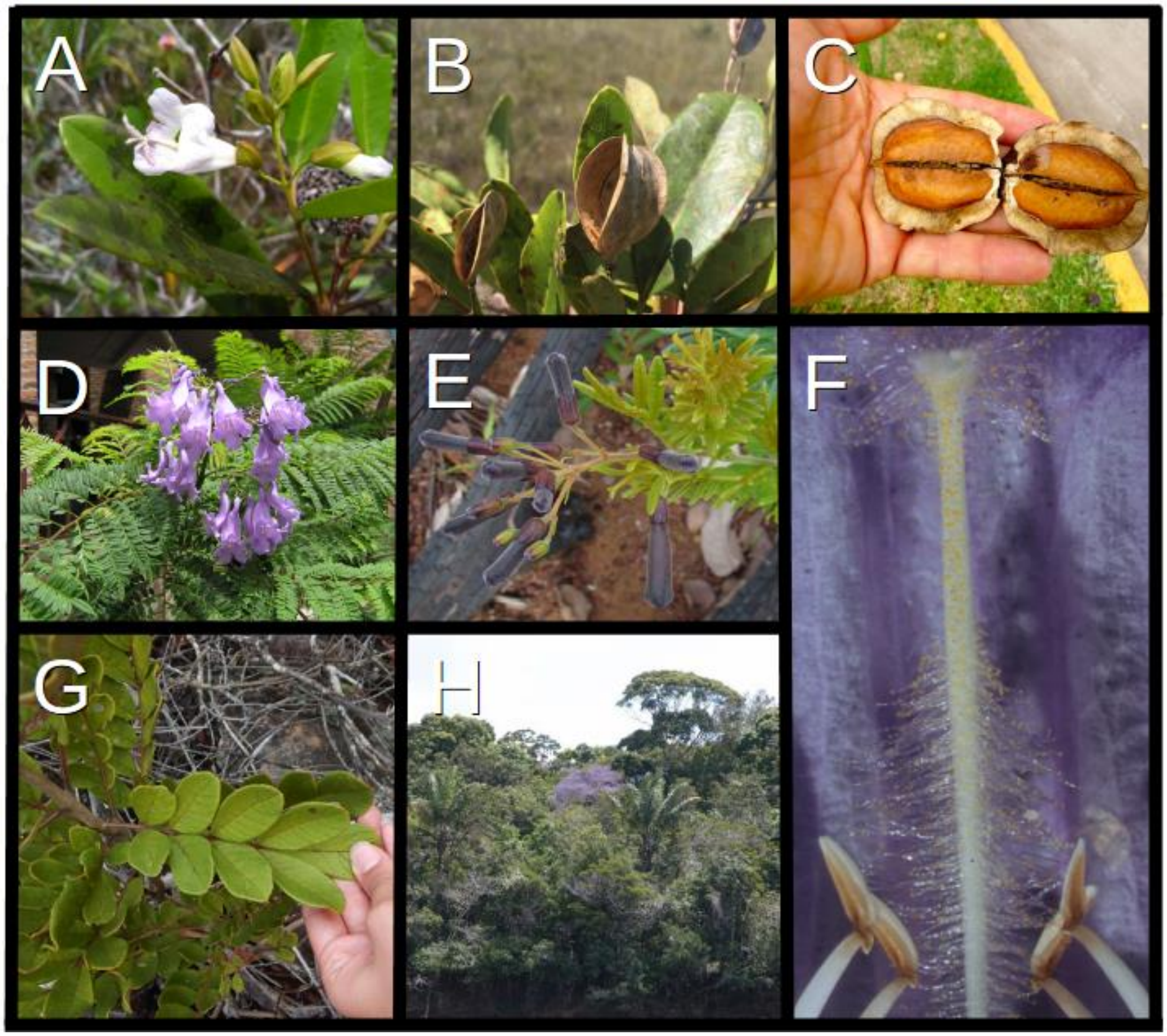

Figure 2.1 Reproductive and vegetative morphology of *Jacaranda* and *Digomphia*. (A) *Digomphia ceratophora* with a divided staminode apex, spathaceous calyx, and simple leaves. (B) *D. ceratophora* with round, dehiscent fruits. (C) *Jacaranda mimosifolia* with dehiscent fruit flattened perpendicular to the septum. (D) *J. mimosifolia* with a thyrse inflorescence and bipinnately compound leaves. (E) *Jacaranda ulei* with a thyrse inflorescence and cupular calyx. (F) *J. mimosifolia* with an entire staminode, monothecal anthers, and style. (G) *Jacaranda irwinii* with simply pinnate compound leaves. (H) *Jacaranda copaia*'s crown of purple inflorescences peeking through wet forest canopy near Inírida, Colombia. Photo credits: Mateo Fernandez (A), Richard G. Olmstead (B, C, D, H), Rosana Farias-Singer (E, F), and Audrey C. Ragsac (G).

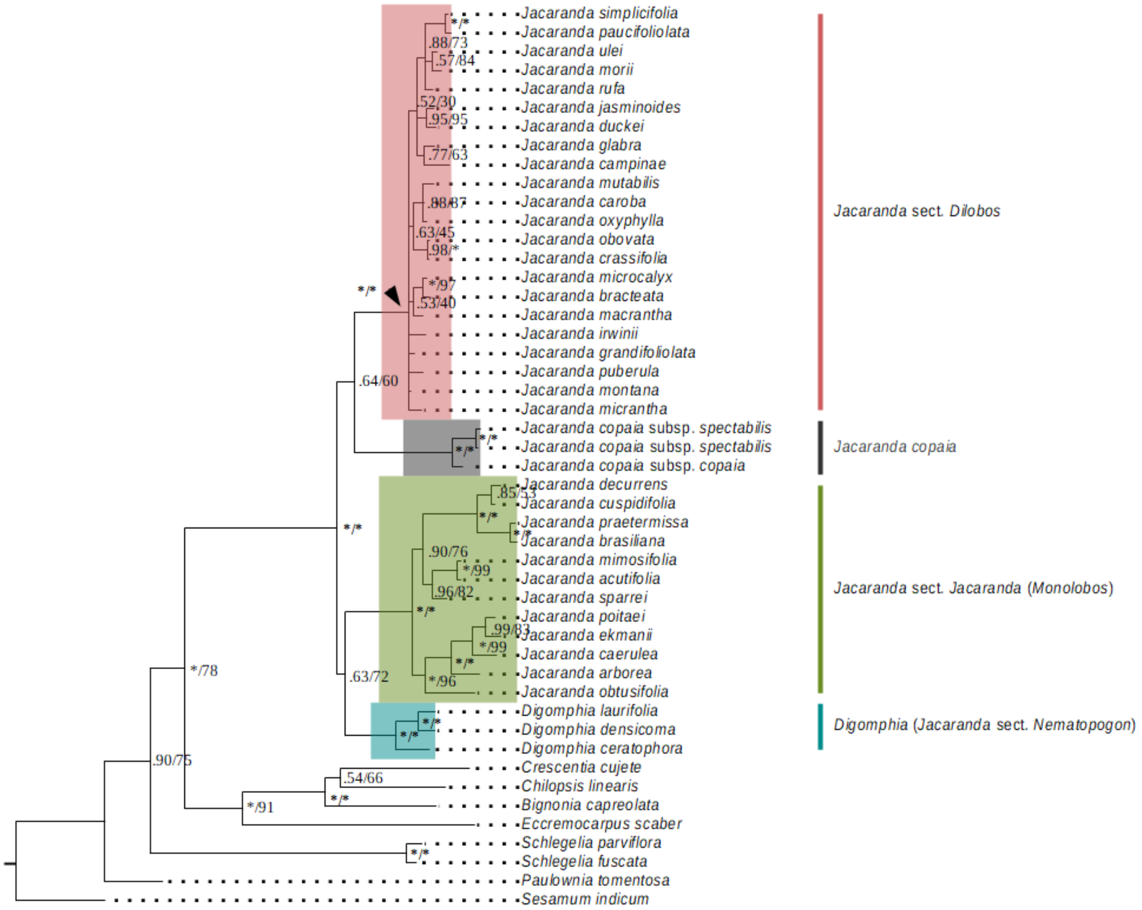

Figure 2.2 Bayesian majority rule consensus tree derived from the analysis of the concatenated nuclear dataset with both Bayesian posterior probability and ML bootstrap support values (* denotes a PP of 1 and bootstrap of 100%) indicated at nodes.

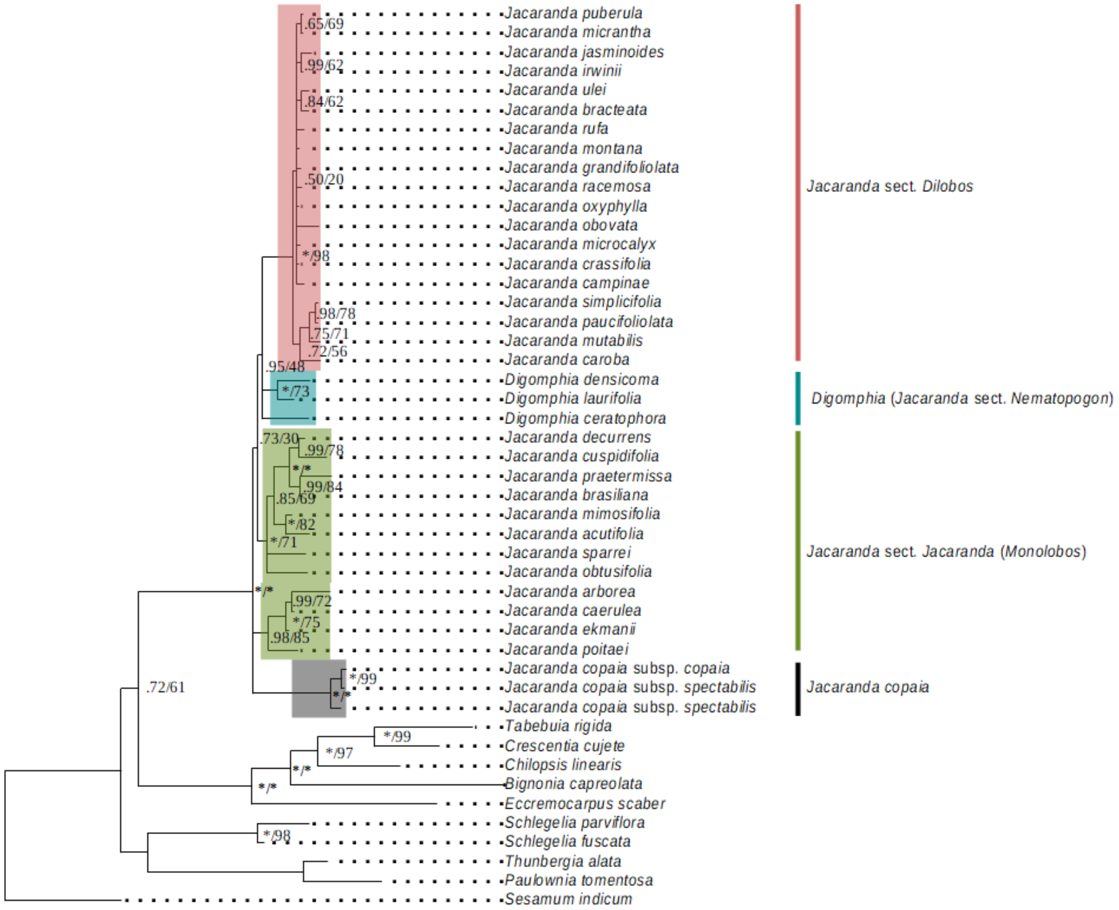

Figure 2.3 Bayesian majority rule consensus tree derived from the analysis of the concatenated chloroplast dataset with both Bayesian posterior probability and ML bootstrap support values (* denotes a PP of 1 and bootstrap of 100%) indicated at nodes.

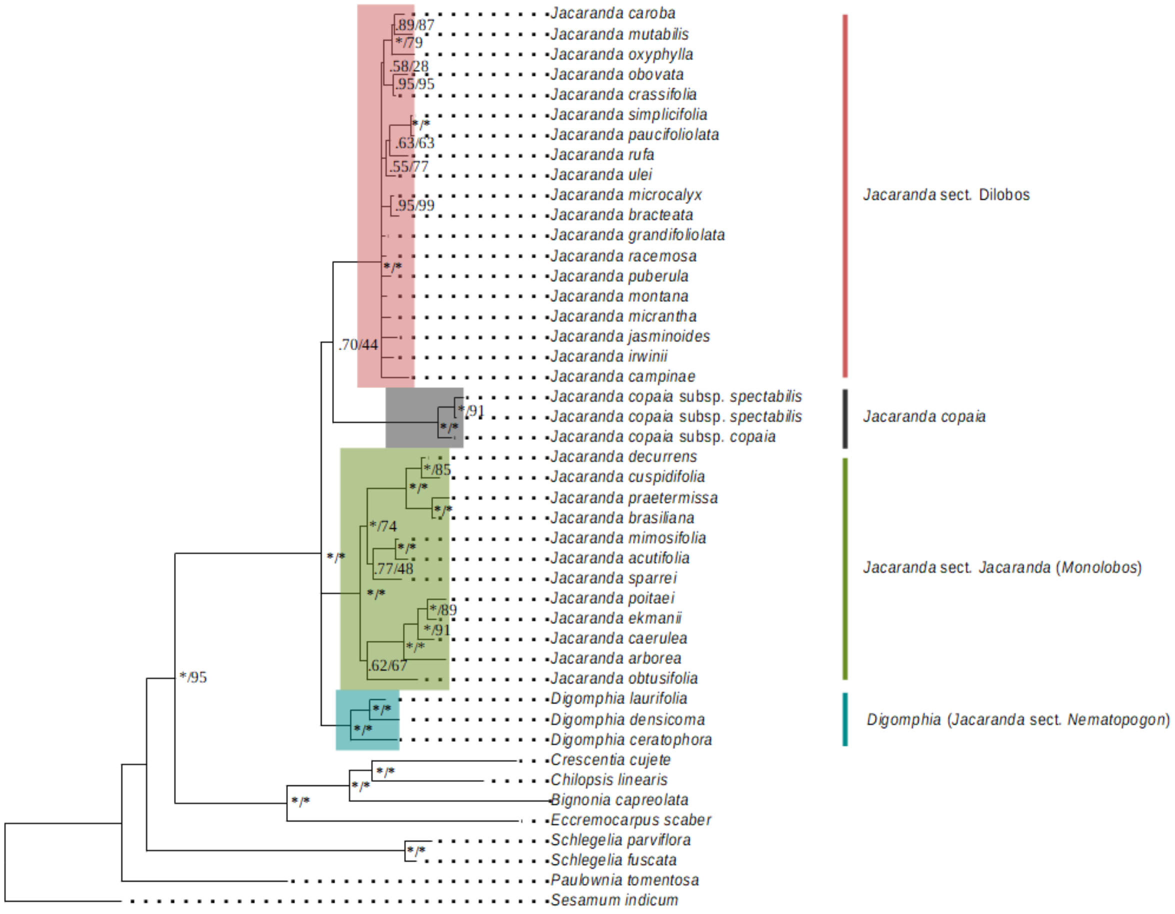

Figure 2.4 Bayesian majority rule consensus tree derived from the analysis of the concatenated nuclear and chloroplast datasets with both Bayesian posterior probability and ML bootstrap support values (* denotes a PP of 1 and bootstrap of 100%) indicated at nodes.

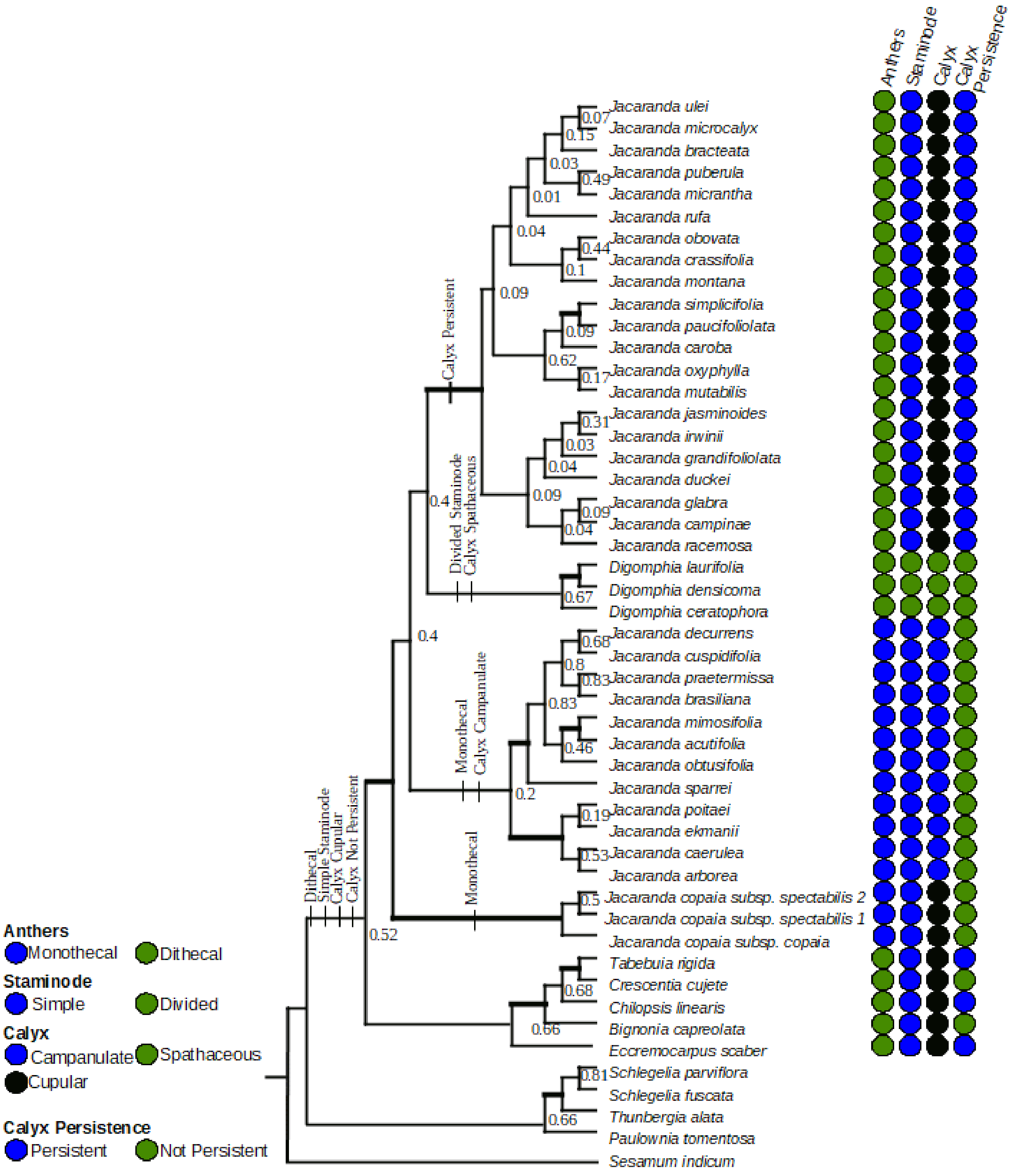

Figure 2.5 *BEAST maximum clade credibility species tree generated from chloroplast, ETS, and PPR62 loci. Weighted branches subtend clades receiving > 0.9 posterior probability; numbers indicate support for other nodes. Most parsimonious character histories for anther thecae, staminodia, calyces, and calyx persistence are mapped onto branches, with character states for each taxon indicated at tips.

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Chapter 3: Detecting patterns of biome conservatism and biome shift in Neotropical *Jacaranda* (Bignoniaceae)

ABSTRACT

Aim

The heterogenous Neotropical environment, with diversity in biome age, climate, and landscape evolution through deep time, is an ideal setting to study how different evolutionary patterns combine to generate the unparalleled amount of plant diversity present there today. The focus of this work is to explore the interplay of two evolutionary patterns - biome conservatism and biome shifting- and how they have impacted Neotropical plant diversification. Biome conservatism is the tendency of lineages to diversify within a habitat to which they are adapted, while biome shifting occurs when plants adapted to one biome disperse and adapt to a new biome with different climatic conditions and biotic factors.

Location

New World tropics

Time Period

Miocene to present

Taxa Studied

Jacaranda (Bignoniaceae)

Methods

To explore how biome shifting and biome shifting have influenced species diversification in *Jacaranda*, our goals were to (1) infer divergence times, (2) reconstruct biogeographic history, (3) detect shifts in diversification rate, (4) determine if shifts in diversification rate correspond with biome conservatism or biome shifts, and (5) explore how biome shifting and biome conservatism relate to climatic affinities of species.

Results

All four sections within *Jacaranda*, with a median crown age of 22.82 Ma, diverged at about the same time, making it difficult to resolve relationships between them. *Jacaranda* likely originated in the Amazon rain forest in the Miocene, dispersing into the West Indies, dry biomes of South America, and rain forests of Mesoamerica within the last 10 Ma.

Main Conclusions

Despite their close stem ages, sects. *Jacaranda* and *Dilobos* exhibit classic evidence of niche shifting early in their diversifications, followed by radiation within biomes associated with positive diversification rates. In contrast, sects. *Nematopogon* and *Copaia* show no evidence of niche shifting, only exhibiting patterns of relatively recent diversification within biomes.

INTRODUCTION

The Neotropics is widely considered the most species-rich area on Earth (Antonelli & Sanmartin, 2011). With estimates of 37% of all seed plant species found in the region, the complex combination of abiotic and biotic factors driving evolution across this heterogenous landscape makes pinpointing a single most important factor nearly impossible (Antonelli & Sanmartín, 2011). We focus on a pair of patterns that have been proposed to be important for plant evolution in the Neotropics: biome shifting and biome conservatism (Wiens & Donoghue, 2004, Donoghue, 2008). Biome conservatism is the tendency of lineages to diversify within a habitat to which they are adapted. Biome shifting is the opposite pattern-- plants adapted to one biome disperse and adapt to a new biome with different climatic conditions and biotic factors (Wiens & Donoghue, 2004)**.** In a phylogenetic context, lineages experiencing biome conservatism should consist of taxa restricted to a single biome, whereas lineages experiencing biome shifting should consist of taxa distributed in multiple biomes.

In the southern hemisphere, biome shifts are estimated to be far rarer than biome conservatism in plant lineages, with only 3.6% of 11,000 lineages shifting biomes either within landmasses or following transoceanic dispersal events (Crisp et al., 2009). It has been suggested that these rare biome shifting events can trigger shifts in diversification rate if the new biome has ample opportunity for adaptation and speciation and the lineage is able to adapt to these new conditions (Edwards & Donoghue, 2013, Koenen et al., 2013). In Fabaceae, one of the most species rich plant families, biome shifts have been associated with increased diversification rate in *Lupinus* following a shift from Mediterranean climate habitats into montane environments, *Mimosa* and *Calliandra* following shifts into tropical savanna and campo rupestre, and *Indigofera* following a shift into Fynbos vegetation (Koenen et al., 2013, Gagnon et al., 2019). Conversely, biome conservatism has also been documented in *Lupinus* (Hughes & Eastwood, 2006) in montane habitats and *Inga* in lowland rainforest (Dexter et al., 2017).

Species dispersal and successful establishment into new habitats depends on proximity of source biome, ecological niche availability, and abiotic and biotic barriers to dispersal. Once a biome shift has occurred, ecological pre-adaptation of the lineage and availability of open niches become important for species fitness in the new environment (Wiens & Donoghue, 2004). A species with a trait that is particularly advantageous in its new biome will have a higher fitness than a species that does not. Ease of adaptation coupled with a young biome with open niches, like newly uplifted mountains, can lead to increases in speciation rate that outpaces extinction rate (Simon et al., 2009). Conversely, older environments, like the Amazon rainforest, have less available niche space due to the long period of time that diversification has finely divided niches. This can result in high levels of inter- and intraspecific competition forming a barrier to new species establishment and, consequently, upticks in lineage diversification rate (Dexter et al., 2017). Therefore, establishment of species from another biome is likely more difficult in the Amazon.

The heterogenous Neotropical environment, with diversity in biome age, climate, and landscape evolution through deep time, is an ideal setting to study the interplay between biome conservatism and biome shifting relative to diversification rate. The Amazon rainforest is estimated to have originated in the Cretaceous (Pennington et al., 2004, Davis et al., 2005, Dick & Pennington, 2019), when it was characterized by interior alluvial and braided river deposits and peripheral marine embayments (Antonelli et al., 2008, Hoorn et al., 2010). From the late Miocene to present, the main uplift of the Andes along the western periphery of the Amazon basin not only created novel montane environments, but shifted the continental divide, changing the direction of water flow from westward to eastward. This drained an extensive wetland "Pebas" system-- the progenitor of Cretaceous Amazonian rivers and embayments-- into the Atlantic, creating the Amazon and Orinoco River basins as we know them today (Antonelli et al., 2008, Hoorn et al., 2010). Also during the Miocene, a global drop in atmospheric CO_2 levels influenced the creation of dry, open biomes defined by seasonal climates (Pennington et al., 2004). These include biomes in the "dry diagonal" of South America: closed-canopy seasonally dry tropical forests (SDTFs), grass-dominated and fire prone savanna (Cerrado), and frost prone Chaco (Pennington et al., 2004, Werneck, 2011). Dated phylogenies of Cerrado clades estimate an age for this biome at younger than 10 Ma, coinciding with the period of most rapid Andean uplift, with the youngest crown ages of Cerrado lineages at ca. 4 Ma (Simon et al., 2009). Most SDTF-centered lineages are inferred to have originated during the late Miocene and Pliocene, further supporting recent widespread aridification in the Neotropics (Pennington et al., 2004, Lavor et al., 2019). Conversely, the Atlantic Forest of Brazil is estimated to be as old as Amazonia with many faunal and floristic similarities implying a past biogeographic connection between these biomes (Oliveira-Filho & Fontes, 2000, Costa, 2003, Pennington et al., 2004, Batalha-Filho et al., 2013). The West Indies have biogeographic connections to Central and South American still under debate but are frequently included in studies of Neotropical groups (Iturralde-Vinent & MacPhee, 1999, Santiago-Valentin & Olmstead, 2004, Antonelli et al., 2018). Studies looking at the extent to which each individual pattern has impacted diversification rate in strictly Neotropical plant groups with widespread distributions will help us better understand the unprecedented species diversity we see today.

Jacaranda (Bignoniaceae) is a genus of ca. 50 species found throughout the Neotropics (Gentry, 1992; Olmstead et al., 2009, Ragsac et al, 2019). It is sister to the remaining Bignoniaceae, a family with high diversity in the Neotropics and representation worldwide (Olmstead et al., 2009, Olmstead, 2013). Species diversity in *Jacaranda* is highest in the Cerrado, but species are also distributed in Amazonia, the West Indies, Atlantic Forest, and other dry regions of South America. Some species, like *Jacaranda copaia*, are widespread, distributed throughout lowland tropical forests of South and Central America, while others are narrow endemics, like *Jacaranda grandifoliolata* in the restingas of southern Bahia, Brazil (Gentry, 1992). *Jacaranda* shares the ancestral trait of small, winged seeds dispersed by wind from dry, dehiscent fruit with most of the remaining Bignoniaceae. This widespread distribution and adaptation for dispersal makes *Jacaranda* an ideal system for addressing questions about the contribution of biome conservatism versus biome shifting to the evolution of a strictly Neotropical clade.

The most recent phylogeny of *Jacaranda* contains four well supported clades: *Jacaranda* sections *Jacaranda*, *Dilobos*, *Nematopogon*, and *Copaia* (Ragsac et al., 2019). The first two clades are the most species rich, with ca. 17 and ca. 30 species, respectively, and occur in multiple biomes. Species in *Jacaranda* sect. *Jacaranda* are distributed in the West Indies, Chaco, Seasonally Dry Tropical Forest, and Cerrado, while species in *Jacaranda* sect. *Dilobos* are distributed in Cerrado, Atlantic Forest, and Amazonia. The other two clades are species poor, with three species in *Jacaranda* sect *Nematopogon* and one species, *Jacaranda copaia* (containing two subspecies), in *Jacaranda* sect. *Copaia*. These two clades are each restricted to a single biome, with all species in both clades found in the Amazon rain forest. Not only do these phylogenetic patterns suggest dynamic biogeographic history and variable diversification rates across the *Jacaranda* phylogeny, but they also suggest that the climatic affinities of closely related species in the genus may not be predicted by ancestral traits.

To explore how biome shifting and biome shifting have influenced species diversification in *Jacaranda*, our goals are to (1) infer divergence times, (2) reconstruct biogeographic history, (3) detect shifts in diversification rate, (4) determine if shifts in diversification rate correspond with biome conservatism or biome shifts, and (5) explore how biome shifting and biome conservatism relate to climatic affinities of species.

MATERIALS AND METHODS

Taxonomic Sampling— The dataset consisted of 40 accessions of *Jacaranda*, including 39 species and both subspecies of *Jacaranda copaia*. The chloroplast dataset contained sequences for *ndh*F, *rpl*32-*trn*L, *trn*L-F, and *rbc*L. The nuclear dataset contained sequences for ITS, ETS, and PPR62. *Jacaranda glabra and Jacaranda intricata* were newly sequenced for this study since the publication of the *Jacaranda* phylogeny (Ragsac et al., 2019). PCR was performed using protocols described in Ragsac et al. (2019). *rbc*L-5F and *rbc*L-3R primers were used to amplify *rbc*L (Olmstead & Sweere, 1994). ITS4 and ITS5 primers were used to amplify ITS (White et al., 1990). Fifteen outgroup taxa were selected to enable fossil calibrations for divergence time estimation. Sequences were edited and aligned in Geneious Prime 2019.1.3 (Biomatters, Ltd.). Voucher information is listed in Appendix 3.1.

Phylogenetic Analyses—Phylogenetic analyses were run in both Bayesian and Maximum Likelihood frameworks using the total concatenated dataset. Bayesian analysis was performed in MrBayes 3.2.6 using two replicate runs and four chains for 1 million MCMC generations. Maximum likelihood analysis was performed in RAxML 8.2.4 for 1000 rapid bootstraps. Both analyses had sequence data partitioned as follows: (1) chloroplast loci, (2) ETS, (3) ITS, and (4) PPR62. All partitions were assigned the GTR+I+G model of evolution (Abadi et al., 2019).

Divergence Time Estimation--

Divergence time estimation was performed in BEAST v1.8.4 (Drummond et al., 2012). Sequence data were partitioned into concatenated chloroplast loci (*ndh*F, *rpl*32-trnL, *trn*L-F, and *rbc*L), ETS, ITS and PPR62. Substitution rates were set to GTR+G, clocks were simulated under the relaxed lognormal clock. Four clades with well-supported monophyly in the *Jacaranda* phylogeny were constrained: *Jacaranda* sect. *Copaia*, *Jacaranda* sect. *Nematopogon*, *Jacaranda* sect *Jacaranda*, and *Jacaranda* sect. *Dilobos* (Ragsac et al., 2019). Two replicate runs were performed for $5.0x10^7$ generations under the birth-death process, sampling every $1.0x10^3$ trees. Logs and tree files were combined using LogCombiner 1.8.4, and runs were assessed for convergence using Tracer 1.7.1. (Ramabut et al., 2018). The maximum clade credibility tree was generated using TreeAnnotator 1.10.4.

We used two fossils to calibrate the dated phylogeny. A pollen grain from the Gatuncillo formation of Panama (Graham, 1985) could be placed in several genera: *Cuspidaria* DC., *Fridericia* Mart., *Tanaecium* Sw., and *Xylophragma* Sprague. Therefore, this calibration was set on the *Cuspidaria*/*Fridericia*/*Tanacium*/*Xylophragma* clade using a lognormal distribution with an offset of 33.7 and a standard deviation and mean of 1.0. Various fossils identified as belonging to Bignoniaceae (e.g. Wehr & Hopkins, 1994; Wehr, 1995; Wehr & Manchester 1996; Wilf, 1997; Pigg & Wehr, 2002) were used to calibrate the crown node of Bignoniaceae. Because these fossils are about 50 Mya, the calibration was placed on the common ancestor of Bignoniaceae using a mean of 49 and a standard deviation of 1.0.

Ancestral State Reconstruction--

Taxa were coded according to their extant ranges, as determined from GBIF collection records, monographic descriptions (Gentry, 1992, gbif.org), and a map of Neotropical regions (Antonelli et al., 2018). Areas were coded as follows: Amazon (A), Atlantic Forest (F), Cerrado (C), West Indies (I), Dry Western South America (W), Chaco (H), and Mesoamerica (M). Amazon includes South American wet tropical forest outside of the Amazon basin, such as the Guianas region, but excludes the Atlantic Forest. Dry Western South America (W) encompasses species distributed in dry inter-Andean valleys. The dry biomes (C, W, H) are coded separately to reflect climatic differences between them. The tree was trimmed to include only ingroup taxa in *Jacaranda* to eliminate the influence of outgroups since the nearest relative to Bignoniaceae is not known, as well as only one accession of *J. copaia* subsp. *spectabilis*. Ancestral state reconstruction was performed using BioGeoBEARS (Matzke 2013a) for all possible models: DEC, DIVALIKE, and BAYAREALIKE. DEC models cladogenesis by both within area speciation and narrow vicariance, DIVALIKE favors cladogenesis by widespread vicariance, and BAYAREALIKE favors cladogenesis by within area speciation (Matzke, 2013b). All models allow anagenesis by extinction and dispersal. We also ran these models while enabling the J parameter, which models founder-event speciation. All runs were constrained using a time stratified model, areas allowed matrix adapted from Antonelli et al. (2018, Appendix 3.2, 3.3) that indicates biomes and regions that existed during each time slice, and a multiplier dispersal matrix indicating the physical proximity and dispersal likelihood between available biomes and regions. Time strata were placed at 23, 13, and 9 Ma. The slice between 23 Ma and 13 Ma encompasses the crown age of *Jacaranda*, the development of wetland conditions in western Amazonia, low elevation of the Andes, and the Panama arc remaining unconnected to South America. The slice between 13 Ma and 9 Ma incorporates the closure of the Central American Seaway, drainage of the Pebas system, and moderate uplift of the Andes. The slice between 9 Ma and the present represents the emergence and expansion of dry biomes in South America, rapid uplift in the Andes, and onset of the Amazon River (Antonelli et al., 2018). The best fitting model was determined using AIC comparisons of both nested and non-nested models, as well as consideration of the most likely biological scenarios.

Diversification Rate and Correlating Factors-- Shifts in diversification rate across the tree were detected using BAMM and the bammtools package in R, as well as MEDUSA in the geiger package in R. BAMM was run for 50 million generations sampling every 10,000, and diversification shifts were estimated after a 25% burn-in. Because we did not achieve complete sampling of *Jacaranda*, a table of sampling probabilities for each clade was included in both the BAMM and MEDUSA analyses (Appendix 3.4).

In order to test if biome or clade correlated with shifts in diversification rate, we used MuSSE (FitzJohn, 2012). Due to the high computational demand of increasingly complex models, four biome labels more inclusive than those used in ancestral state reconstructions were assigned as follows: Amazon, Atlantic Forest, Dry (including Cerrado, Chaco, and Seasonally Dry Tropical Forest), and West Indies. Furthermore, four clades were assigned: *Jacaranda* sect. *Copaia, Jacaranda* sect. *Jacaranda*, *Jacaranda* sect. *Nematopogon*, and *Jacaranda* sect. *Dilobos*. Sampling proportions for both biome and clade were included in the analysis to account for incomplete sampling (Appendix 3.4). Net diversification rate was estimated for each set of traits using a model allowing rates of speciation and extinction to vary depending on each character state for 10000 MCMC generations with the first 20% of samples discarded as burn-in.

Climate Data-- To see if climatic conditions experienced by each species are explained better by biome or clade, GPS coordinates for collections of all taxa were gathered from GBIF using the R package dismo. Coordinates were vetted and trimmed by hand to only include collections from native ranges, as determined by the monograph of *Jacaranda* and *Digomphia* (Gentry, 1992), thereby excluding horticultural specimens and anomalous accessions that are likely to be misidentified. Data for 19 climatic variables were gathered for each collection from Worldclim with 2.5 km resolution (Fick & Hijmans, 2017) using the rgdal (Bivand et al., 2017) and raster (Hijmans et al., 2015) packages in R. Principal component analysis (PCA) was performed on climate data relative to each taxon in a non-phylogenetic context in the R packages FactoMineR (Husson et al., 2018) and factoextra (Kassambara & Mundt, 2017).

RESULTS

Phylogenetic Analyses—The concatenated dataset consisted of 55 accessions with a total length of 9,007 positions. The *ndh*F alignment contained 49 accessions with a total sequence length of

2021 positions, *rpl*32-*trn*L contained 46 accessions at 1225 positions, *trn*L-F contained 46 accessions at 990 positions, *rbc*L contained 38 accessions at 1386 positions, *mat*K contained 5 accessions at 1749 positions, ETS contained 48 accessions at 457 positions, ITS contained 18 accessions at 717 positions, PPR62 consisted of 42 accessions at 360 positions. The total combined dataset contained 37.5% missing data.

The maximum clade credibility tree inferred by MrBayes (Appendix 3.5) using the concatenated dataset contains both a monophyletic Bignoniaceae (PP=0.92) and *Jacaranda* (1.0). Within *Jacaranda*, four main clades are supported: *Jacaranda* sect. *Copaia* (1.0), sect. *Dilobos* (1.0), sect. *Jacaranda* (1.0), and sect. *Nematopogon* (1.0). The maximum likelihood tree inferred by RAxML (Appendix 3.6) using the concatenated dataset gives strong bootstrap support for the same clades: Bignoniaceae (82%), *Jacaranda* (100), *Jacaranda* sect. *Copaia* (100), sect. *Dilobos* (100), sect. *Jacaranda* (100), sect. *Nematopogon* (100). While neither tree resolves all relationships among the four sections of *Jacaranda*, the Bayesian tree gives strong support for a sister relationship between sect. *Copaia* and sect. *Dilobos* (0.95) that is not supported in the Maximum Likelihood tree (22).

Divergence Times—

BEAST estimated the crown age of *Jacaranda* at 22.82 Ma (15.29 – 31.96 95% HPD), *Jacaranda* sect. *Copaia* at 3.86 Ma (1.4 – 7.64 Ma), sect. *Jacaranda* at 15.22 Ma (9.59 – 21.95), sect. *Nematopogon* at 8.94 Ma (4.06 – 15.35), and sect. *Dilobos* at 6.36 Ma (3.8 – 9.93) (Fig 3.1, Table 3.1). Within sect. *Jacaranda*, the age of the Caribbean clade is estimated at 7.6 Ma (3.83 – 12.72), Cerrado clade at 4.76 Ma (2.44 – 8.22), and dry biome clade at 7.61 Ma (2.99 – 12.9).

Constraining each of the four sections of *Jacaranda* as monophyletic in the BEAST analysis did not improve the resolution of relationships between them.

Ancestral State Reconstruction—The AIC model comparison determined that models enabling the J parameter are a better fit for our data. Among these three models, the DEC + J model of range evolution was ranked highest, DIVALIKE + J second highest, and BAYAREALIKE + J third (Table 3.2). Despite its ranking, BAYAREALIKE + J is likely the most appropriate model for investigating biome shifting and biome conservatism. Biome conservatism is defined by within area speciation, which BAYAREALIKE favors, and the J parameter models founder-event speciation, which is a proxy for biome shifting. Vicariance is not favored in this model, which is fitting because we do not have strong reason to believe that vicariance influenced range evolution in *Jacaranda*. Reconstructions and node information for DEC +J and DIVALIKE + J are provided in Appendices 3.7-3.10.

BAYAREALIKE + J inferred the Amazon (88.3%) as the ancestral biome of *Jacaranda* (Fig. 3.2, Table 3.1). Furthermore, all the ancestral states prior to ca. 10 Ma are estimated to be Amazon, including the ancestor of *Jacaranda* sect *Jacaranda*, with probability > 94% for each. The ancestral states for *Jacaranda* sect. *Nematopogon*, *Jacaranda* sect. *Dilobos*, and *Jacaranda* sect. *Copaia* are also inferred as Amazon, with probability >80% for each. The clade containing Caribbean taxa in Jacaranda sect. Jacaranda is reconstructed as originating in the West Indies (91.7%), while the clade containing Cerrado taxa in the same section is estimated as originating in the Cerrado (86.8%). Only the clade containing taxa distributed in Dry Western South America and Chaco has an uncertain ancestral state reconstruction, with a 61.8% chance of originating in the Amazon, a 9.3% chance of originated in Dry Western South America, and a 15.2% of originating in a distribution spanning the Amazon and Dry Western South America.

Diversification Rate--- Both BAMM (Fig. 3.3.1) and MEDUSA (Fig. 3.3.2) detect a shift in diversification rate at the base of the *Jacaranda* sect. *Dilobos* clade. MuSSE detected much higher mean net diversification rates in *Jacaranda* sect. *Jacaranda* (0.14) and *Jacaranda* sect. *Dilobos* (0.26) compared to *Jacaranda* sect. *Copaia* (-1.0) and *Jacaranda* sect. *Nematopogon* (-0.88) (Fig. 3.4.1). MuSSE did not, however, detect a difference in diversification rate among Amazon (0.06), West Indies (-0.16), Cerrado (0.13), and Atlantic Forest (-0.10) (Fig. 3.4.2).

Climate Data—A non-phylogenetic PCA of data for 19 climate variables for each taxon produced a PC1 explaining 51.9% of the variation in the data and a PC2 explaining 19.1% of the variation in the data (Appendix 3.11). Variables that influenced PC1 most were Minimum Temperature in the Coldest Month (9.4%), Mean Temperature in the Driest Quarter (8.9%), Mean Temperature in the Coldest Quarter (8.7%), Mean Annual Temperature (8.2%), and Precipitation in the Coldest Quarter (6.9%). Variables that influenced PC2 most were Precipitation in the Warmest Quarter (15.1%), Precipitation in the Driest Month (13%), Precipitation in the Driest Quarter (12.6%), Annual Precipitation (8.7%), and Maximum Temperature in the Warmest Month (6.9%) (Appendix 3.12). Biplots produced with taxon scores show well defined elliptic groupings based on biome assignment, but not based on clade assignment.

Overlap of ellipses placed around centroids for species in each biome (Fig. 3.5.1) suggests that species assigned to Atlantic Forest and West Indies have high climatic affinities with species assigned to the Amazon. Conversely, species assigned to Cerrado have high climatic affinities with species assigned to the Atlantic Forest, but not to the Amazon or West Indies. Species assigned to Amazon and Mesoamerica fall within the Amazon ellipsis. There were not enough species assigned to Chaco and SDTF for ellipses to be generated, but the species assigned to these biomes fall within the Atlantic Forest and Cerrado ellipsis.

Overlap of ellipses placed around centroids for species in each clade (Fig. 3.5.2) suggest that species assigned to *Jacaranda* sect. *Jacaranda* and *Jacaranda* sect. *Dilobos* do not cluster closely based on climatic affinities of species within them. Conversely, species in *Jacaranda* sect. *Nematopogon* and *Jacaranda* sect. *Copaia* do cluster closely based on clade assignment.

DISCUSSION

Historical Biogeography

Regardless of the biogeographic model invoked on our time-calibrated phylogeny (Figs. 3.1, 3.2, Appendix 3.7, 3.9), the ancestral biome of *Jacaranda* is inferred to be Amazon. A crown age of 22.82 Ma (15.29-31.96) supports this estimate because the Amazon rain forest had already existed for about 40 Ma, expansive dry biomes did not yet exist in tropical South America, and the Andes were still early in their uplift (Kurschner et al., 2007, Simon et al., 2009, Hoorn et al., 2010, Luebert & Weigand, 2014, Dick & Pennington, 2019).

While relationships among major clades are unresolved in the current *Jacaranda* phylogeny, our reconstruction estimates that *Jacaranda* did not experience any biome shifts until 7.6 Ma (3.83-12.72), when it shifted from Amazon into the West Indies. Since the four sections of *Jacaranda* are well supported, we can consider the evolution of each separately and construct a biogeographic story of the genus by comparing the patterns that emerge among them.

***Jacaranda* sect. *Jacaranda*—**

The Amazon (97.8%) is the inferred ancestral biome of *Jacaranda* sect. *Jacaranda*, with a crown age of 15.22 Ma (9.59-21.95) in the Miocene (Figs. 3.1, 3.2, Table 3.1). There was one transition from the Amazon into the West Indies in this clade, occurring between 15.22 Ma (9.59-21.95) and 7.6 Ma (3.83-12.72) in the late Miocene. A good comparison for this estimated dispersal event is the genus *Brunfelsia* (Solanaceae), which experienced a dispersal event from Brazil to the lesser Antilles between 15 and 4 Ma, with radiations in the greater-Antilles dated to the Late Miocene or Early Pliocene (Filipowicz & Renner, 2012). Therefore, a stem age of 15.22 Ma and a crown age of 7.6 Ma for the Caribbean clade of *Jacaranda* sect. *Jacaranda* is consistent with biogeographic estimates in *Brunfelsia*. Furthermore, the stem ages of Caribbean *Cestrum* (Solanaceae) and *Charianthus* (Melastomataceae) at 14.1 and 15.1, respectively, and crown ages of 9.1 (3.2-15.4) and 5.35 (1.2-13.5), also support a late Miocene dispersal event into the Caribbean in *Jacaranda* sect. *Jacaranda* (Abrahamczyk et al., 2015).

There are also up to four inferred dispersal events from Amazon into dry biomes. A shift into the Cerrado is estimated between 15.22 Ma (9.59-21.95) and 4.76 Ma (2.44-8.22). Between 7.61 Ma (2.99-12.9) and the present, up to two separate shifts into dry inter-Andean valleys and one shift into the Chaco are inferred. The crown age of these two dry adapted clades in the late

Miocene and early Pliocene align with the proposed ages of dry biomes in South America at less than 10 Ma (Simon et al., 2009). Shifts from Amazon to dry biomes have been documented in numerous groups, including Burseraceae (Protieae—Fine at al., 2014), Fabaceae (*Andira*—Simon et al., 2009), and an impressive number of times in Bignonieae (Lohmann et al., 2012), the most species rich tribe in Bignoniaceae.

Species in *Jacaranda* sect. *Jacaranda* appear to tolerate a range of temperatures accompanied by low precipitation (Fig 3.5.1). Low precipitation is a defining factor of dry biomes, but even *J. obtusifolia*, a species distributed in the Amazon, is on the lower precipitation extreme compared to other Amazonian species in *Jacaranda*. The ability of species in this clade to tolerate seasonal conditions could allude to evolutionary lability inherent in lineages able to undergo multiple biome shifts.

Jacaranda sect. Dilobos

The most species rich clade in *Jacaranda*, species in sect. *Dilobos* are distributed in the Amazon, Atlantic Forest, and Cerrado. The Amazon (94.9%) is inferred as the ancestral biome of this clade, with a crown age in the late Miocene at 6.36 Ma (3.80-9.93) (Figs. 3.1, 3.2, Table 3.1). Between this crown age and the present, there is at least one shift into each of the Atlantic Forest and Cerrado from the Amazon, and the possibility of a shift back into the Amazon from either of these biomes. Because species relationships in this section are unresolved, we are unable to infer any additional details about the timing and order of these dispersal events.

The Atlantic Forest stretches ca. 3000 km along the Brazilian coast and borders the Cerrado to the east (Oliveira-Filho & Fontes, 2000). We find similar climatic affinities of closely related *Jacaranda* species that have mutually exclusive distributions in these two biomes. Species distributed in the Atlantic Forest are adapted to a wide range of temperature and precipitation, while Cerrado species tolerate a narrower subset of temperatures within the Atlantic Forest conditions. Conversely, Amazonian distributed species experience far higher temperatures than species in either the Atlantic Forest or Cerrado. Like in sect. *Jacaranda*, the ability to tolerate a wide range of temperature and precipitation may define groups that are able to undergo biome shifting and subsequent diversification.

Jacaranda sect. *Dilobos* has the highest estimated diversification rate of all Jacaranda clades (Figs. 3.3, 3.4). The majority of species are distributed in the Cerrado, one of the last biomes to originate and expand in the Neotropics, likely within the past 5 Ma (Simon et al., 2009). Given the young age of this biome, one would expect high ecological opportunity coupled with low competition, resulting in adaptation and speciation with low rates of extinction. These predictions are supported by the positive diversification rate for this clade (Fig. 4a). However, the Cerrado itself was not associated with an increase in diversification rate in *Jacaranda* (Fig. 3.4.2), implying that additional factors must be invoked to explain it.

***Jacaranda* sect. *Nematopogon*--**

Consisting of three species with narrow distributions in the northern Amazon and Guiana Shield, *Jacaranda* sect. *Nematopogon* is inferred to have originated in the Amazon in the Miocene between 19.41 Ma (12.49-28.29) and 8.94 Ma (4.06-15.35) (Figs. 3.1, 3.2, Table 3.1). The Guiana Shield is home to many ancient South American endemic, relictual lineages, but this crown age suggests that *Jacaranda* did not colonize the region until relatively recently. The clade's early Miocene stem age and negative diversification rate (-0.88) may be explained by lack of ecological opportunity, high interspecific competition, high extinction rates, or dispersal barriers in the Amazon biome (Fig. 3.4.1, Table 3.1). While all species are found in the Guiana Shield, two species in this section, *Jacaranda ceratophora* and *Jacaranda laurifolia*, are restricted to the region, while *Jacaranda densicoma* is the only species to achieve a wider distribution into the northern Amazon.

Jacaranda ceratophora is an example of a lineage currently adapting to drier environments from a wet forest ancestor as illustrated by its affinity with white sand soils in the Guiana Shield. Fine et al. (2005) found that species in Protieae (Burseraceae) evolved white sand specialization multiple times from terre firme ancestors in the Amazon, an edaphic-driven pattern that likely promoted speciation in this tribe. This pattern could also be occurring in *Jacaranda* sect. *Nematopogon*, suggesting eventual adaptation to dry biomes. *Jacaranda campinae* in *Jacarand*a sect. *Dilobos* is also a white sand endemic (Gentry, 1992).

***Jacaranda* sect. *Copaia*--**

The stem of *Jacaranda* sect. *Copaia* is inferred to have originated in the Amazon between 22.82 (15.29-31.96) and 3.86 Ma (1.4-7.64) (Fig 2. 3.1, 3.2, Table 3.1). Between this Pliocene crown age estimate and the present, there is a dispersal event inferred from South America to Mesoamerica in *Jacaranda copaia* subsp. *copaia*, resulting in a large range spanning the Amazon and Mesoamerica. This was likely an overland dispersal event, as the isthmus of Panama is estimated to have been closed by this time (Cody et al., 2010, Bacon et al., 2015, Montes et al., 2015).

In *Jacaranda* sect. *Copaia, Jacaranda copaia*, despite being a single species, has the most widespread distribution of any species in the genus. Its two subspecies, *J. copaia* subsp. *copaia* and *J. copaia* subsp. *spectabilis*, occupy mostly allopatric distributions with some overlap at the edges of their ranges, with the former restricted mainly to the Guiana Shield and the latter widespread across Western Amazonia and Mesoamerica. As a result, this species exhibits a tremendous amount of genetic variation across its distribution (Scotti-Santiagne et al., 2013). It is possible that the young crown age of this lineage (3.86 Ma) implies that not enough time has passed for speciation to occur. Conversely, considering its stem age at 22.82 Ma, it is also possible that speciation in the Amazon has been difficult due to low availability of ecological niches, high interspecific competition, or a high rate of extinction in this lineage. *J. copaia* seems to be a classic example of a niche conserved species, embodying the notion that it is "easier to move than evolve" (Donoghue, 2008).

Jacaranda copaia is ecologically distinct from species in sect. *Nematopogon* in that the former is a rainforest canopy tree and the latter an understory shrub. Long-distance dispersal of wind-dispersed seeds in *J. copaia* is facilitated by flowers and fruits produced high in the canopy, while species in *Jacaranda* sect. *Nematopogon* produce flowers and fruit near the ground. This may explain the difference in distribution of these two clades otherwise similar in biome, climatic affinity, stem age, species diversity, and diversification rate.

High Diversification Rate and Correlating Factors

We did not find a correlation between a specific biome and increased diversification rate (Fig. 4b), but we did find a relationship between clade and diversification rate (Fig. 4a). Clades with higher diversification rates experienced more biome shifting than clades with lower diversification rates (Fig. 2). There is also no relationship between clade age and increased diversification rate. Of clades with high diversification rates, *Jacaranda* sect. *Dilobos* has a crown age of 6.36 Ma and stem age of 19.41 while, comparatively, the age of *Jacaranda* sect. *Jacaranda* is older with a crown age of 15.22 Ma and stem age of 21.97 Ma (Fig 3.1, Table 3.1). The same applies to clades with low diversification rates: the crown age of *Jacaranda* sect. *Copaia* is 3.86 Ma and the stem age is 22.86 Ma, while the crown age of *Jacaranda* sect. *Nematopogon* is 8.94 Ma with a stem age of 19.4 Ma. This supports the notion that biome shifting can lead to an increase in lineage diversification rate, but it is not because these lineages have had longer to evolve. Since there was no single biome associated with significant increases in diversification in our study, we can surmise that it does not matter which biome a clade shifts into, but how many biomes a lineage can shift into, that promotes diversification in *Jacaranda*. Because of their wind-dispersed seeds, it is also possible that dispersal within dry, open biomes is easier in *Jacaranda* than in closed canopy forest, allowing lineages in *Jacaranda* faster spatial and temporal access to new niches. Lack of competition in young biomes, like the Cerrado, would likely alleviate the effects of fitness trade-offs. Furthermore, an ease of fire adaptation has been documented in other immigrant Cerrado clades (Simon et al., 2009). Fire adaptations that have evolved in *Jacaranda* species in the Cerrado, include xylopodia, evolving independently in both *Jacaranda* sects. *Jacaranda* and *Dilobos* (Gentry, 1992). Xylopodial species include *J. decurrens* (*Jacaranda* sect. *Jacaranda*) and *J. intricata, J. oxyphylla, J. racemosa, J. rufa, J.*

simplicifolia, and *J. ulei* (*Jacaranda* sect. *Dilobos*) (Gentry, 1992, Ragsac et al. 2019). There is also an Amazonian species, *J. glabra*, that exhibits pachycaul growth, an adaptation to fire common in the Cerrado, but not in rain forests (Gentry, 1992, Simon et. al, 2009). The current distribution of *J. glabra* may represent a very recent dispersal event from the Cerrado or Atlantic Forest into the Amazon.

Species Poor/Low Diversification Rate Clades

Much focus is placed on Neotropical clades experiencing high diversification rates, but what does it mean when these lineages are closely related to comparatively species poor Neotropical clades? In *Jacaranda*, two clades are species poor: *Jacaranda* sect. *Nematopogon* and *Jacaranda* sect. *Copaia*. Both are distributed in wet lowland forests, with the former in the Amazon and Guiana Shield, and the latter widespread across the Amazon, Guiana Shield, and Mesoamerica. One might be tempted to implicate biome conservatism in the wet lowland rainforest distributions of species in these clades. One might also say that this pattern reflects the Amazon as a museum hypothesis, which says that most Amazonian lineages are old and have experienced biome conservatism and low levels of extinction rather than high levels of speciation (Antonelli & Sanmartin, 2011, Hughes et al., 2012). However, neither the crown age nor stem age of these lineages are old on the time scale of the Amazon rainforest, estimated to have originated in the mid-Cretaceous, making conclusions about either hypothesis difficult to support using these results (Davis et al., 2005, Pennington et al., 2004). It is also possible, due to the antiquity of the Amazon compared to those of dry biomes in South America, that niche space is more finely divided and occupied there than in younger biomes. This would limit the diversification rates of Amazonian adapted clades, as well as the shifting of lineages from surrounding biomes into Amazonia. A classic example of a group experiencing niche conservatism in the Amazon is *Inga* (Fabaceae), a pattern that may be explained by large primate-dispersed fruit and seeds that are not well suited to long distance dispersal in closed canopy forest (Dexter et al., 2017). In contrast, dispersal in sect. *Copaia* is facilitated by dehiscent fruit with small, wind-dispersed seeds produced high in the rain forest canopy, which may explain why it has been able to colonize an extensive area without diversification.

Climatic Affinities

When looking at climatic ranges of each species sampled, we found that species clustered based on biome assignment rather than clade assignment (Fig. 3.5). In a pattern of phylogenetic niche conservatism, species from a clade would be expected to be adapted to similar climatic conditions. However, we find that species in *Jacaranda* cluster based on climatic conditions experienced independent of evolutionary relationships. This illustrates the ability of some closely related species in this group to overcome evolutionary constraints and evolve into new environments. We also found that the climatic variables that explain variance most among *Jacaranda* species define seasonal climates—temperature in the coldest month and quarter for PC axis one, as well as precipitation in the driest month and quarter for PC axis two (Appendix 3.12). This suggests that, in *Jacaranda*, evolution into biomes with strong seasonality has had a strong influence on diversification of the genus.

CONCLUSIONS

The evolutionary patterns observed across the four sections of *Jacaranda* provide an excellent case study on the interplay between biome conservatism and biome shifting in a Neotropical group. All four sections diverged at about the same time, making it difficult to resolve relationships between them. Despite these close stem ages, sects. *Jacaranda* and *Dilobos* exhibit classic evidence of niche shifting early in their respective diversifications, followed by radiation within biomes. In contrast, sects. *Nematopogon* and *Copaia* show no evidence of niche shifting, only exhibiting patterns of relatively recent diversification within biomes. *Jacaranda* sect. *Copaia*, having spread throughout much of the Amazon rain forest and ecologically related Mesoamerica without speciating, provides strong evidence supporting the statement that it's "easier to move than evolve."

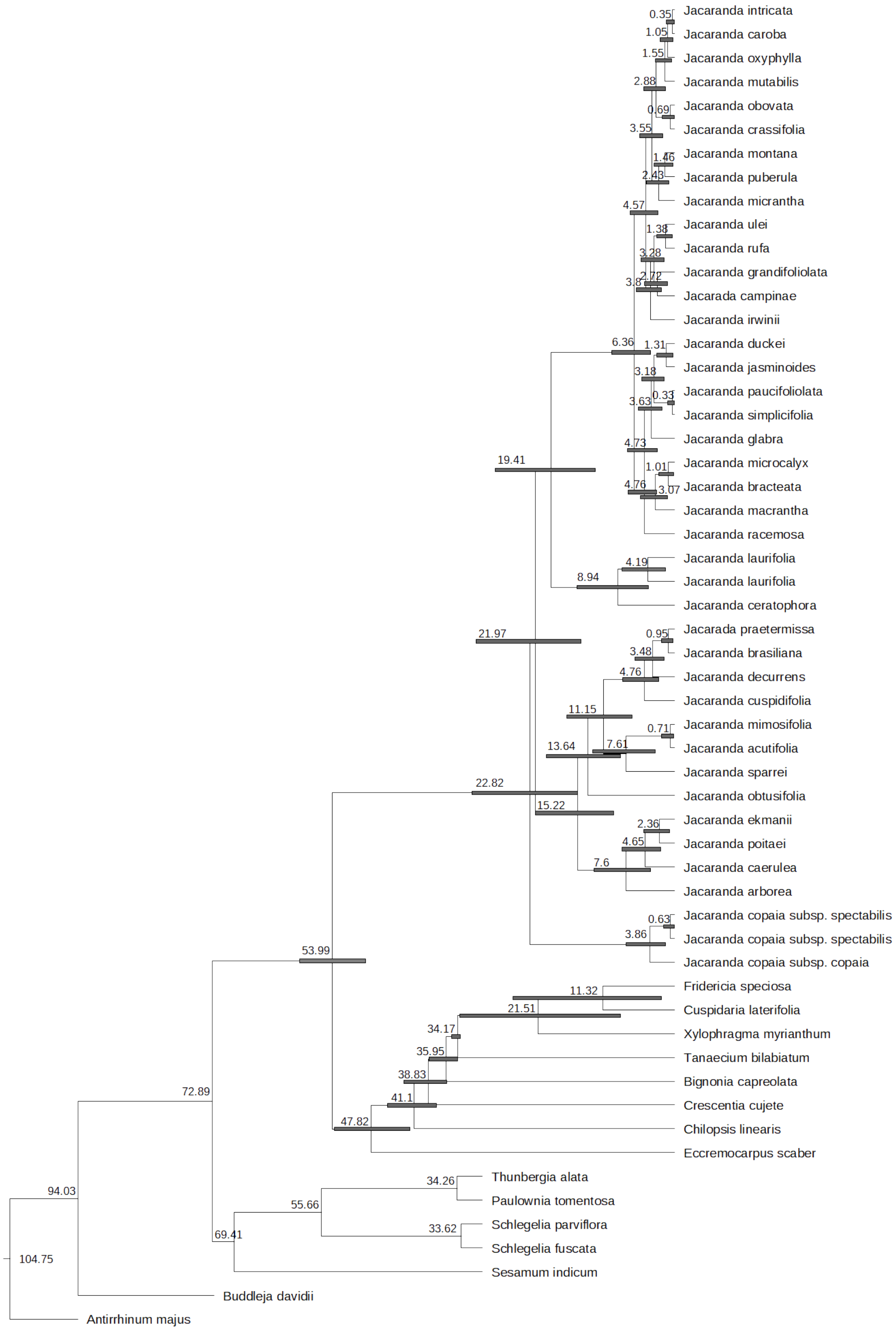

Figure 3.1 Time calibrated BEAST maximum clade credibility tree of *Jacaranda*. Values at nodes represented median age estimates. Bars at nodes represent highest posterior density intervals.

Table 3.1 Results of AIC and AICc model comparison for time stratified analyses in BioGeoBEARS.

	LnL	AIC	AICc
DEC	-81.14	166.3	166.6
DEC+J	-67.38	140.8	141.4
DIVALIKE	-81.48	167	167.3
DIVALIKE+J	-69.49	145	145.7
BAYAREALIKE	-93.67	191.3	191.7
BAYAREALIKE+J	-70.63	147.3	147.9

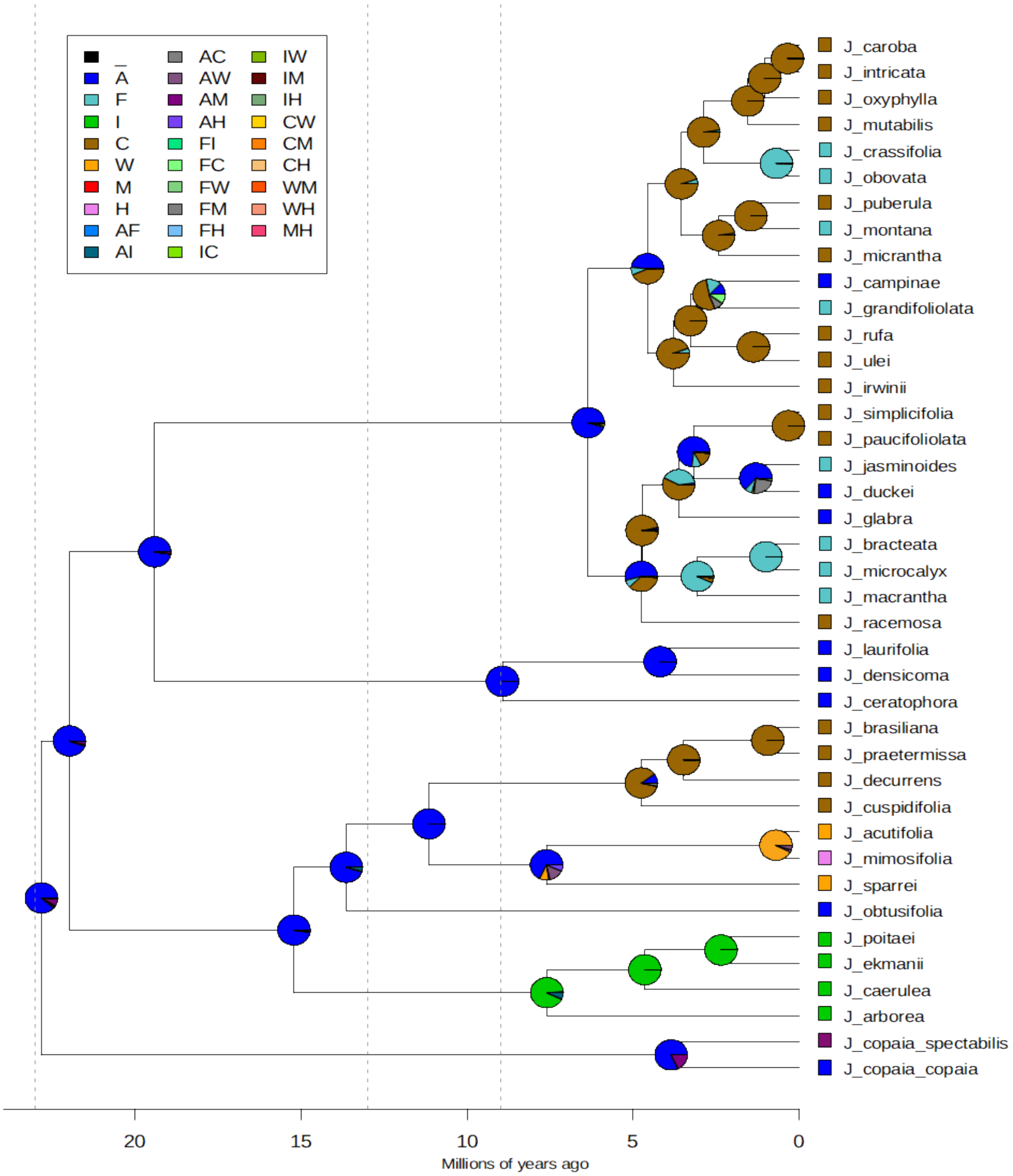

Figure 3.2 Ancestral state reconstruction of *Jacaranda* using the BAYAREALIKE + J model of range evolution. Areas were coded as follows: Amazon (A), Atlantic Forest (F), Cerrado (C), West Indies (I), Dry Western South America (W), Chaco (H), and Mesoamerica (M). Pie charts represent probabilities of ancestral biomes at each node. Labels at tips represent biomes assigned to each taxon prior to analysis.

Table 3.2 Node information for the *Jacaranda* phylogeny and the BioGeoBEARS ancestral state reconstruction. Posterior probability (PP) support values are from the maximum clade credibility tree generated in BEAST and MrBayes from analysis of the concatenated dataset, and bootstrap support values are from the tree generated in RAxML from analysis of the the concatenated dataset. Median crown ages and 95% highest posterior distributions (HPD) are from the time calibrated tree generated in BEAST. Ancestral state probabilities for each coded region are from BioGeoBEARS under the BAYAREALIKE + J model. Coded regions are as follows: Amazon (A), Atlantic Forest (F), West Indies (I), Cerrado (C), Dry Western South America (W), Mesoamerica (M), and Chaco (H).

Clade	PP (BEAST)	PP (MrBayes)	Bootstrap	Median Age (Ma)	95% HPD (Ma)	A	F	I	C	W	M	H	AW
Bignoniaceae	1	0.92	82	53.99	48.65-59.08								
Calibration 2	1	1	98	34.17	33.75-35.12								
Jacaranda	1	1	100	22.82	15.29-31.96	**0.882525**	0.005746462	0.000372	0	0	0.011118	0	0
Sect. *Jacaranda* + Sect. *Nematopogon* + Sect. *Dilobos*	0.3			21.97	14.77-31.23	**0.946356**	0.000601244	6.50E-05	0	0	2.30E-04	0	0
Sect. *Nematopogon* + Sect. *Dilobos*	0.6			19.41	12.49-28.29	**0.976835**	0.00050134	4.63E-07	0	0	6.88E-06	0	0
Sect. *Copaia*	1	1	100	3.86	1.4-7.64	**0.819336**	1.07E-06	1.39E-07	3.41E-08	3.59E-08	0.001178	3.22E-08	7.34E-05
Sect. *Jacaranda*	1	1	100	15.22	9.59-21.95	**0.977614**	0.000194621	0.001273	0	0	1.20E-04	0	0
Sect. *Nematopogon*	1	1	100	8.94	4.06-15.35	**0.998803**	1.04E-06	4.63E-09	3.01E-11	3.34E-11	1.97E-06	2.84E-11	2.19E-06
Sect. *Dilobos*	1	1	100	6.36	3.8-9.93	**0.948909**	0.01809663	8.02E-08	0.021907	2.72E-07	6.40E-07	1.92E-06	0.00037
Sect. *Jacaranda* 1 (*J. arborea* + *J. caerulea* + *J. ekmanii* + *J. poitaei*)	1	1	100	7.6	3.83-12.72	0.014069	1.18E-05	**0.916708**	8.30E-07	9.28E-07	2.38E-04	7.83E-07	6.15E-06
Sect. *Jacaranda* 2 (*J. acutifolia*, *J. mimosifolia*, *J. sparrei*)	0.99	1	79	7.61	2.99-12.9	**0.681148**	7.81E-05	5.63E-05	1.43E-05	**0.092892**	7.79E-06	**0.005439**	**0.152463**
Sect. *Jacaranda* 3 (*J. brasiliana*, *J. cuspidifolia*, *J. decurrens*, *J. praetermissa*)	1	1	100	4.76	2.44-8.22	0.098503	0.000253454	8.29E-06	**0.86798**	1.48E-05	1.91E-06	5.11E-05	0.00E+00

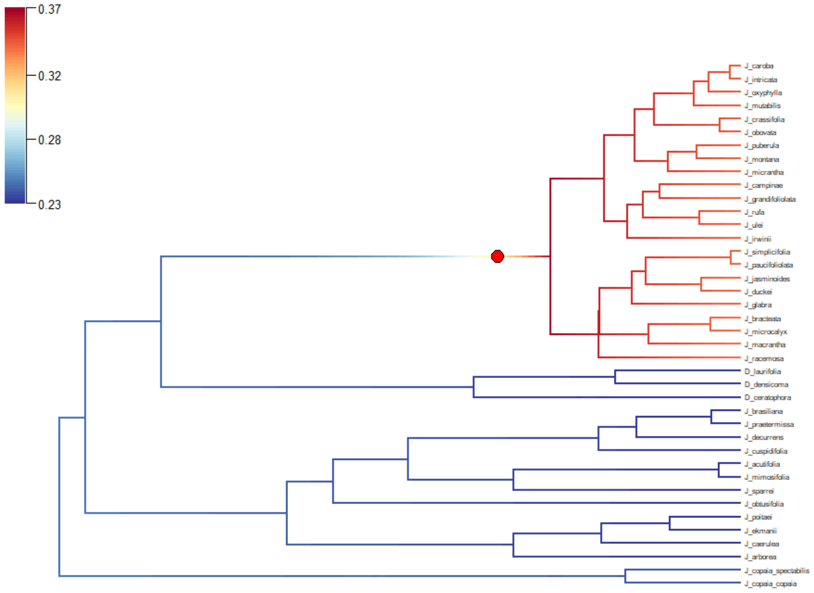

Figure 3.3a Shifts in diversification rate across the *Jacaranda* phylogeny estimated in BAMM.

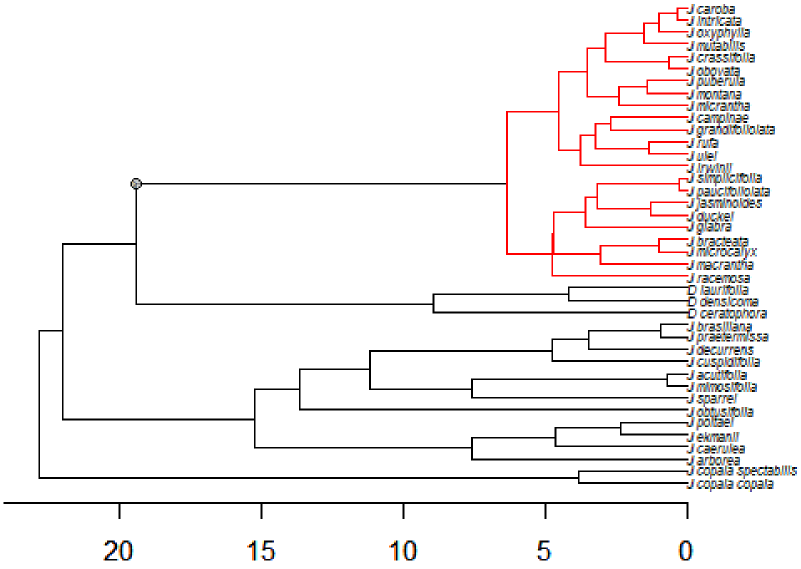

Figure 3.3b Shifts in diversification rate across the *Jacaranda* phylogeny estimated in MEDUSA.

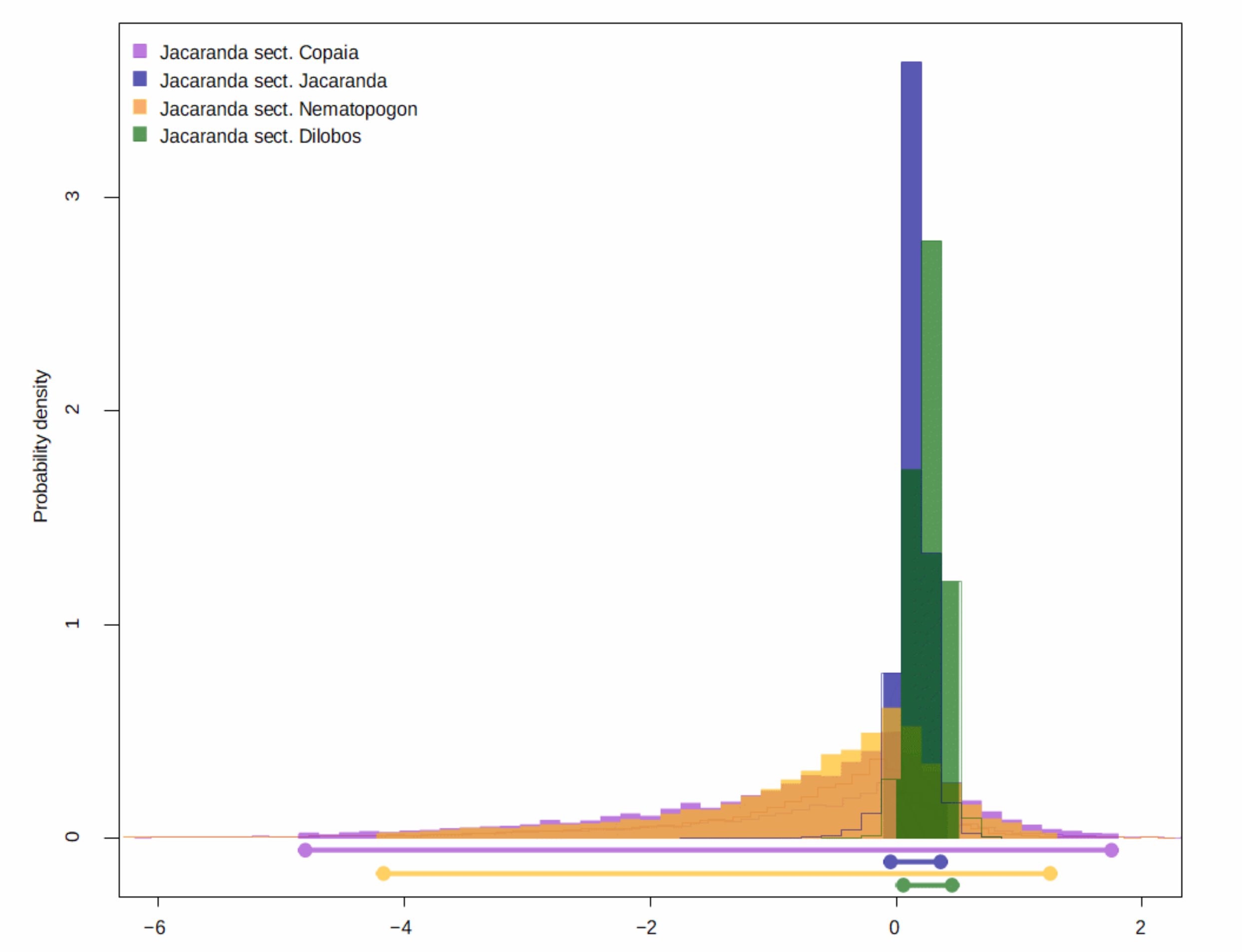

Figure 3.4a MuSSE results showing the net diversification rate of four *Jacaranda* clades: *Jacaranda* sect. *Copaia* (-0.996189), *Jacaranda* sect. *Jacaranda* (0.136093), *Jacaranda* sect. *Nematopogon* (-0.8753344), *Jacaranda* sect. *Dilobos* (0.2905065). Bars show 95% credibility intervals.

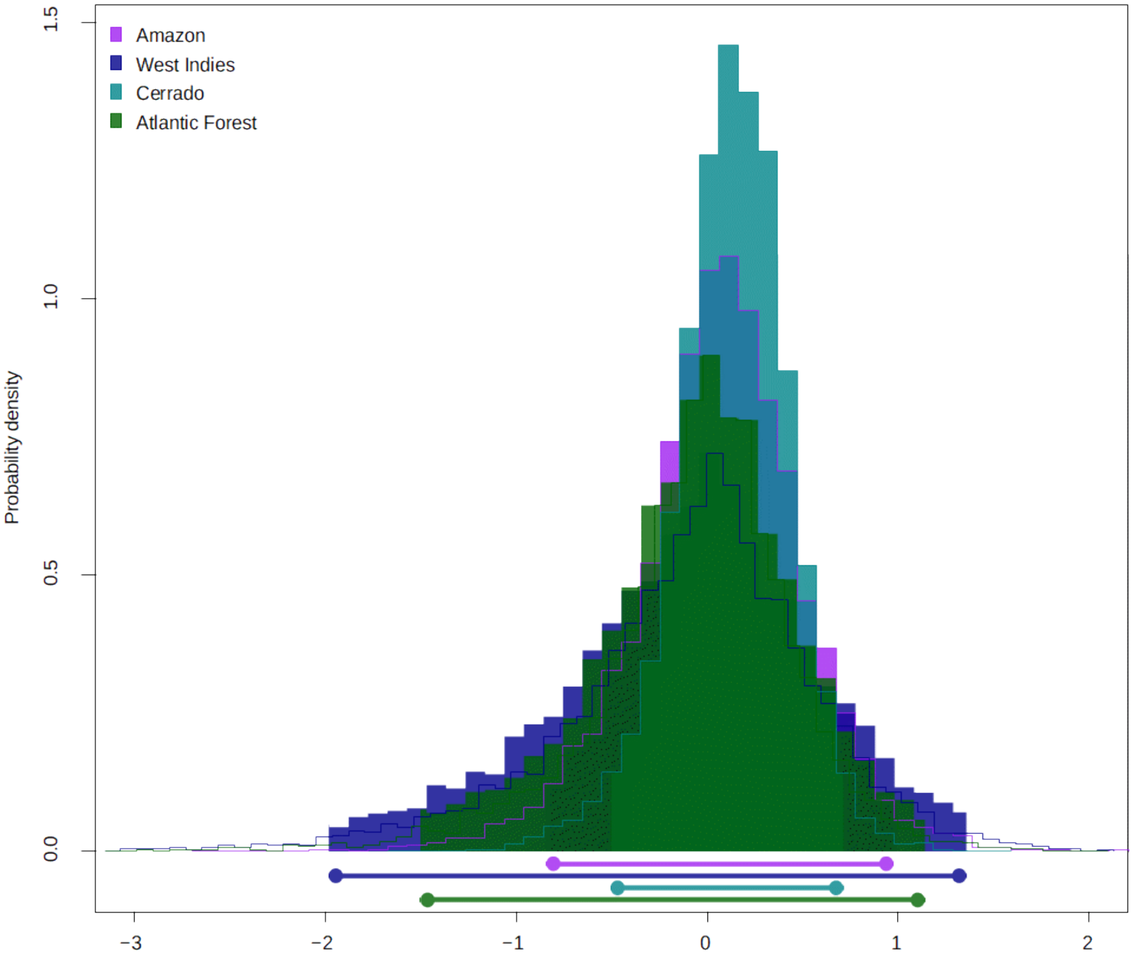

Figure 3.4b MuSSE results showing the net diversification rate of *Jacaranda* lineages in four biomes: Amazon (0.06140949), West Indies (-0.1580727), Cerrado (0.1287245), Atlantic (-0.0969718). Bars represent 95% credibility intervals.

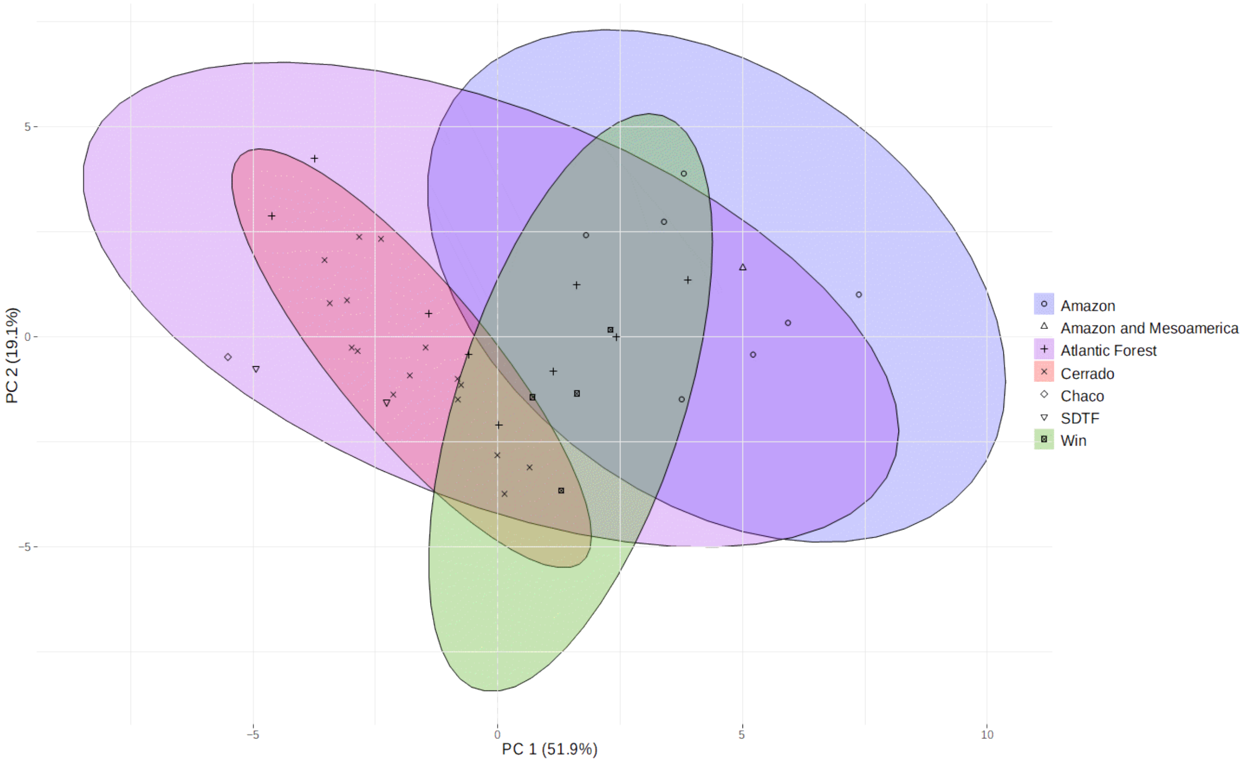

Figure 3.5a Principal component analysis of *Jacaranda* species and outgroups using 19 climatic variables. Species names are removed, and each species is now represented by a shape corresponding to biome assignment. Ellipses are drawn around the centroids of species assigned to Amazon, Atlantic Forest, Cerrado, and West Indies. Remaining biomes and outgroups do not have enough species represented to generate ellipses.

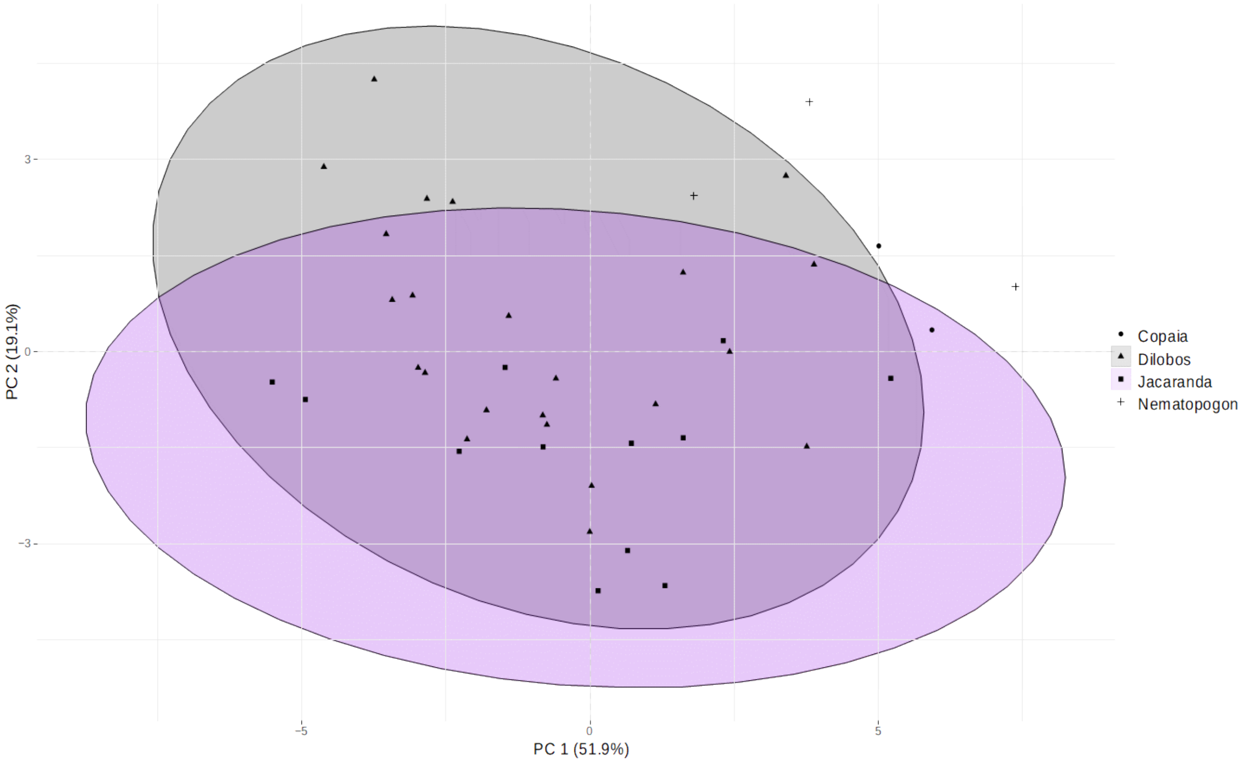

Figure 3.5b Principal component analysis of *Jacaranda* species and outgroups using 19 climatic variables. Species names are removed, and each species is now represented by a shape corresponding to clade assignment. Ellipses are drawn around the centroids of species in *Jacaranda* sect. *Jacaranda* and *Jacaranda* sect. *Dilobos*. Remaining clades and outgroups do not have enough species represented to generate ellipses.

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Chapter 4: Phylogeny and Systematics of Crescentieae (Bignoniaceae), a Neotropical Clade of Cauliflorous and Bat-Pollinated Trees

Abstract— The tribe Crescentieae includes *Amphitecna* (21 species), *Crescentia* (six species), and *Parmentiera* (10 species), three genera of understory trees with a center of diversity in Central America and a small number of species in the Antilles and northern South America. Species in Crescentieae are united by their fleshy, indehiscent fruit and cauliflorous, bat-pollinated flowers. The large fruits are presumed to have evolved to be mammal dispersed, although water dispersal is known to occur. To lay a foundation for examining morphological, ecological, and biogeographic patterns within the tribe, we inferred the phylogeny for Crescentieae using both chloroplast (*ndhF*, *trnL-F*) and nuclear markers (PepC, ITS). The most recent circumscription of Crescentieae, containing *Amphitecna*, *Crescentia*, and *Parmentiera* is confirmed by our phylogenetic results, as is a sister relationship with Antillean-endemic *Spirotecoma*. This relationship implies the evolution of fleshy, indehiscent fruit from dry, dehiscent fruit and bat pollination syndrome from insect pollination. Fruits and seeds from species in Crescentieae are consumed by humans, ungulates, birds, and even fish.

Keywords— *Amphitecna*, bat pollination, Bignoniaceae, Crescentieae, *Crescentia*, fruit dispersal, mammal dispersal, molecular phylogeny, *Parmentiera*

INTRODUCTION

Bignoniaceae Juss. are a family of approximately 850 species with a pantropical distribution (Fischer et al. 2004, Olmstead et al. 2009, Lohmann and Ulloa Ulloa, 2006 onward). The family is best known as trees and woody vines, with trumpet shaped flowers that usually form showy inflorescences (Lohmann and Hopkins, 1999). The fruits of most taxa are bilocular and dehiscent, releasing the winged seeds for dispersal by wind. However, three lineages have fruits that are uncharacteristic for the family; Coleeae Bojer ex Reveal, Crescentieae G. Don and *Kigelia* DC. have fruits that are indehiscent and adapted to dispersal by mammals. Historically, all of these taxa were united in tribe Crescentieae (Candolle, 1838; Bentham and Hooker, 1876; Gentry, 1980), or in a family Crescentiaceae (Seeman, 1860; Miers, 1861).

Besides fruits adapted for mammal dispersal, species in Coleeae, Crescentieae, and *Kigelia* share the characters of tree-like habit and cauliflory (Perrier de la Bathie 1938). A problem with the traditional concept of Crescentieae was that it had a very disjunct distribution, with the majority of the species being restricted to either Central America or Madagascar, and with one species (*Kigelia africana* (Lam.) Benth.) found on mainland Africa (Gentry 1976). To explain this distribution, one needed to invoke either widespread extinction or an unusual long-distance dispersal event. Gentry examined the morphology in depth and suggested that the African and Madagascan species formed one group, tribe Coleeae, and the Central American taxa comprised a second group, tribe Crescentieae, and that each evolved autochthonously (Gentry 1976). Gentry (1980) circumscribed Crescentieae to include 33 species in three genera: *Amphitecna* Miers, *Crescentia* L., and *Parmentiera* DC. With the subsequent addition of three new species of *Amphitecna* and one new *Parmentiera* (Gentry, 1982; Burger and Gentry, 2000; Ortiz-Rodriguez et al., 2016), Crescentieae now comprise 37 species.

The first molecular phylogeny of Bignoniaceae supported a monophyletic Crescentieae (*Crescentia*, *Amphitecna*), as well as a close relationship between the tribe and *Tabebuia* Gomes ex DC., which is widely distributed in the Neotropics (Spangler and Olmstead 1999). Spangler and Olmstead (1999) identified a second clade of taxa with indehiscent fruit comprised of *Kigelia* and *Ophiocolea* H. Perrier, which was consistent with Gentry's concept of Coleeae (Gentry, 1976). However, with greater sampling of African and Madagascan taxa, Zjhra et al. (2004) redefined Coleeae as species restricted to Madagascar and containing *Colea* Bojer ex Meisn., *Ophiocolea*, *Phyllarthron* DC., and *Rhodocolea* Baill., while excluding *Kigelia*, which belonged with other African species with dehiscent fruit. Grose and Olmstead (2007a) found support for a monophyletic Crescentieae containing *Amphitecna*, *Crescentia*, and *Parmentiera*, as well as its placement nested within *Tabebuia* as then circumscribed. Gentry (1976) suggested a close relationship between Crescentieae and *Tabebuia*, including the possibility that Crescentieae were derived from *Tabebuia*, based on shared palmately compound leaves and fruit characteristics. Molecular phylogenetic studies (Grose and Olmstead, 2007a; Olmstead et al., 2009) confirmed these relationships while also reinforcing that Coleeae and *Kigelia* diverged more distantly in the tree within the Paleotropical clade.

The 37 species in Crescentieae are mostly small understory trees (Gentry, 1980, Burger and Gentry, 2000, Ortiz-Rodriguez et al., 2016; Appendix 4.1, 4.2). Plants in Crescentieae are united by their fleshy, indehiscent fruit and cauliflorous flowers, often with white-green corollas (Fig.

4.1). *Parmentiera* is the only genus that contains species that become large trees. The 10 species in this genus are distinguished from other Crescentieae by elongate indehiscent fruit with a fibrous-fleshy core and lacking a hard shell, opposite leaves, reticulate, tricolpate pollen, and vestigial seed wings (Gentry and Tomb, 1979, Gentry 1980). Its large fruit are often yellow or purple in color, persist for a long time on the trunk and branches, and are the showiest of the three genera (S. Grose, pers. obs.). *Crescentia*, with six species, has sessile, simple leaves borne in fascicles along their branches, and branches only rarely, giving them a unique appearance. *Crescentia* flowers have off-white corollas with maroon penciling and 3 or 6-colpate pollen (Gentry and Tomb 1979, Gentry 1980). The largest genus of the three, *Amphitecna*, contains 21 species united by polyporate and finely reticulate pollen (Gentry, 1980, Ortiz-Rodriguez et al., 2016). Due to their simple, alternate leaves not in fascicles, species in *Amphitecna* are difficult to identify if not in flower (Gentry, 1980). When flowering, the fallen corollas are scattered on the ground under and around the tree (S. Grose, pers. obs.). They are not collected often and most of what is known about their distributions can be attributed to occasional coincidental collecting, as in many other tropical species (Funk, Zermoglio, and Nasir 1999; Funk and Richardson 2002). *Crescentia* and *Amphitecna* share pepo or calabash fruits spherical to ellipsoid in shape with pulpy cores containing unwinged seeds (Gentry 1980). The hard exocarps of *Crescentia cujete* L. fruit have been used to bail water out of native canoes for centuries, reported by Columbus as early as 13 October 1492—the day he arrived in the New World. Smaller exocarps are used to make maracas in much of Mexico and Central America (Gentry, 1992b).

All species in *Amphitecna* are restricted to Central America except for *A. latifolia* (Mill.) A.H. Gentry, which has a much larger distribution spanning from the Greater Antilles to coastal

Venezuela and Costa Rica to Ecuador. In *Crescentia*, *C. alata* Kunth and *C. cujete* are widespread throughout Central America and the West Indies, three species, *C. linearifolia* Miers, *C. mirabilis* Ekman ex Urb., and *C. portoricensis* Britton, are restricted to the West Indies, and one species, *C. amazonica* Ducke, occurs in northern South America as far south as the Upper Orinoco and Amazon basins. *Parmentiera* is mostly restricted to Central America, with the exception of *P. stenocarpa* Dugand & L.B.Sm. in northwest Colombia. Species in Crescentieae that are not widespread are usually restricted to small ranges, sometimes only known from one mountain (Gentry, 1980).

While there is variation in flower size and color among species, floral morphologies in Crescentieae fall into a suite of traits called the “chiropterophilic syndrome,” which includes muted colors, night flowering, cauliflory, and bell-shaped flowers (van der Pijl 1961, Faegri and van der Pijl 1966). There have been bat visitations recorded to species in all three genera in both Central America and Cuba, and *C. alata* and *C. cujete* pollen has been found in bat guano (de Carvalho 1961, Gentry 1974, 1976, 1980, Gardner 1977, Dobat and Peikert-Holle 1985, Clairmont et al. 2014, Thompson 2014). Means of dispersal is poorly understood in Crescentieae. Because the fruits are large, fleshy, and sweet, they are presumed to have evolved to be mammal dispersed, although water dispersal also occurs (Gentry, 1974, 1980, Thompson 2014). Even prehistoric gomphotheres have been suggested to be dispersers of *C. cujete* and other trees with large indehiscent fruits (Janzen and Martin, 1982).

Grose & Olmstead (2007a) found an unexpected relationship between Crescentieae and Antillean-endemic *Spirotecoma* (Baill.) Dalla Torre & Harms. Within the *Tabebuia* Alliance, *Spirotecoma* is the only group that shares ramiflorous inflorescences reduced to one or two flowers with Crescentieae. The fruits are linear, terete, and spirally coiled, with small-winged seeds embedded in pits in the enlarged septum. Otherwise, the fruit is typical for Bignoniaceae in that it is bilocular and dehiscent (Gentry, 1992a). Consisting of four species, three of which are restricted to Cuba, understanding the relationship of *Spirotecoma* to Crescentieae can lead to insights on the morphological evolution and biogeographic history of these closely related groups.

The goal of this paper is to reconstruct the phylogeny of Crescentieae and use species relationships as a guide to examine morphological, ecological, and biogeographic hypotheses within the tribe. Sequence data from two chloroplast loci, *ndh*F and the *trn*L-F, and two nuclear loci, the fourth intron of PepC and ITS, are used in the phylogenetic analyses.

MATERIALS AND METHODS

Taxonomic Sampling- A total of 30 accessions representing 28 spp. of Crescentieae were sampled, including seven species of *Parmentiera*, six species of *Crescentia*, and 15 species of *Amphitecna*. Two accessions were included for *C. cujete* and *A. breedlovei*. Collections, including silica gel preserved samples of fresh tissue, were made in Puerto Rico, Cuba, Panamá, Costa Rica, Nicaragua, México, and Guatemala, as well as at the U.C. Berkeley Botanic Gardens and Fairchild Tropical Botanic Gardens. Additional tissue was provided by Missouri Botanical Garden and Jardín Botánico Nacional de Cuba (Havana). Thirteen outgroups were included from a previous phylogenetic study of the *Tabebuia* alliance (Grose and Olmstead 2007a, b). Vouchers were deposited in herbaria in their country of origin or WTU (Appendix 4.1).

DNA extraction was performed using a modified CTAB protocol and amplified product was further purified using the Qiaquick kit (Qiagen Co., Valencia, California, USA) as described in Beardsley and Olmstead (2002) or polyethylene glycol (PEG) precipitation.

Initial PepC sequences were amplified using the primers published in Malcomber (2002). Later sequences were amplified using primers designed specifically for members of the *Tabebuia* alliance. Their sequences are: BignPPC 4xF 5'- ATTCAATTCTCGTCCTGGATGG -3' and BignPPC 5xR 5'- ATTSGCWGCCATCATTTCTNGC-3'. Sequences were amplified in 50 µl reactions containing: 11.9 µl of ddH2O, 2.5 µl Taq salts (Qiagen Co., Valencia, California, USA), 2.5 µl 25 mM Mg2+, 1.0 µl 5 µM Primer 1, 1.0 µl 5µM primer 2, 2.0 µl 100mM dNTP, 0.1 µl Taq Polymerase, and 4 µl genomic DNA. PCR conditions consisted of four repetitions of: 70^{o}C for 4min, 94^{o}C for 90 seconds, 56^{o}C for 60s, and 72^{o}C for 2 min, followed by 29 repetitions of 72^{o}C for 2 min., 54^{o}C for 60s, 72^{o}C for 90s, and a final elongation at 72^{o}C for 10 min. Products were visualized on a 1% agarose gel, and usually consisted of two bands, of approximately 800 and 500 bp. The larger band was excised and the DNA extracted by dissolving the agarose in 10M NaI then adding glass milk to separate the DNA. The glass milk solution was incubated on ice for 15 minutes, spun to collect the pellet, then washed with cold Gene Clean washing buffer (Qbiogene, Morgan Irvine, CA) and spun down three times. The pellets were then suspended in water and incubated in 95°C to release the DNA from the glass fragments. The water and glass milk was next spun to precipitate the glass, and the supernatant was pipetted off and placed in a clean 1.5 ml tube. The supernatant was spun and pulled off twice more to ensure removal of all the glass. Next, the samples were dried down for 12 minutes, or until the water was all evaporated, and resuspended in 5 µl water. This solution of DNA and water was used in the Topo-TA cloning protocol (Invitrogen Corp, USA). After cloning, a minimum of 8 clones were screened and six with inserts of the appropriate size were directly sequenced in each direction using the T3 and T7 primers.

For chloroplast loci and ITS, standard PCR methods were used to amplify the target regions, general *ndhF* primers and those modified for use were described in (Olmstead and Sweere, 1994; Olmstead and Reeves, 1995) and for *trnL-F* in Taberlet et al (1991) and Beardsley and Olmstead (2002). ITS sequences were amplified using the ITS4 (5' TCCTCCGCTTATTGATATGC 3') and ITS5 (5' GGAAGGAGAAGTCGTAACAAGG 3') primers modified for angiosperms from White et. al (1991). Products from these gene regions were then directly sequenced using DYEnamic ET chemistry (Amersham Biosciences, Piscataway, New Jersey, USA), in the Center for Comparative Genomics at the University of Washington, or by Genewiz (Seattle, WA, USA). Sequences were edited and aligned using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, Michigan, USA), Se-Al Carbon (A. Rambaut, University of Oxford, United Kingdom), or Geneious 10.2.3 (Biomatters Ltd.).

Twenty-six taxa in Crescentieae were included in the nuclear analyses: six species of *Parmentiera*, six species of *Crescentia*, and 14 species of *Amphitecna.* Outgroups were *Spirotecoma holguinensis, Tabebuia bullata, Roseodendron donnell-smithii*, *Cybistax antisyphilitica*, *Sparattosperma leucanthum*, and eight species in *Handroanthus*. Twenty-seven taxa in Crescentieae were included in the chloroplast analyses. The ingroup consisted of the same taxa as the nuclear dataset, with the addition of a second *C. cujete* specimen from Puerto

Rico, A. *kennedyi*, and *P. millspaughiana*, and excluding *C. amazonica*. All taxa used in the nuclear and chloroplast datasets were included in the combined dataset.

Appropriate substitution models were determined using JModelTest 2.1.4 (Darriba et al. 2012). Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist et al. 2012) and run until they reached stationarity, checked in Tracer 1.6 (Rambaut et al. 2014). Maximum Likelihood analyses were performed in RAxML 8.2.4 (Stamatakis 2014) with 1000 bootstrap replicates. For the combined dataset, loci were concatenated as chloroplast, PepC, and ITS. Coalescent analyses were performed in BEAST 2.6.0 using the *BEAST template (Bouckaert et al. 2014). *C. amazonica* was excluded from the *BEAST analysis due to a lack of chloroplast and PepC sequence data. Nuclear regions were treated as autosomal, whereas the concatenated chloroplast data was treated as haploid (Y or mitochondrial). Priors were set to a GTR substitution models, strict clock, Yule model, birthrate, and population mean were set to a Gamma distribution with a shape of 2. *BEAST analysis was run for $5.0\text{x}10^8$ generations, sampling every $1.0\text{x}10^4$ trees. Trees were summarized using TreeAnnotator 2.5.0 (Rambaut and Drummond 2017), with the first 25% of trees discarded as burn-in.

RESULTS

Datasets-- Of the 28 taxa in Crescentieae used in this study, 17 are used here for the first time (Grose & Olmstead, 2007a, Olmstead et al., 2009). Several taxa with very restricted distributions were not collected and therefore were unsampled in this study. The aligned length of the *ndhF* dataset is 2101 bp. Alignment was readily achieved with no gaps needed. The aligned length of *trnL-F* is 947 bp. Individual sequences of PepC ranged from 598-649 bp, with an aligned length

668 bp. ITS was sequenced for a subset of ten taxa. Individual sequences of ITS ranged from 442–655 bp, with an aligned length of 655 bp.

In Crescentieae, *A. kennedyi* and the wild collection of *A. breedlovei* did not amplify for PepC. Several of the outgroup taxa also did not amplify for PepC. As found in other taxa, two bands, one each of approximately 800 and 500 bp amplified for PepC (Malcomber, 2002; Lohmann, 2006). The smaller band was tested for phylogenetic utility, but was uninformative with respect to relationships. Within the larger band, two copies of PepC were found in most taxa. To ensure comparison of orthologs, an analysis was run on a dataset containing all sequenced copies. Each copy formed a clade. In one clade, there was no resolution among the species and branch lengths were very short, while in the other, there were longer branch lengths providing more resolution. Therefore, only sequences of the latter clade were included in the analysis.

Phylogenetic Analyses – JModelTest indicated that, under the Akaike criteria, the GTR+G model is the most appropriate for the combined dataset. Both Bayesian and Maximum Likelihood analyses of the nuclear dataset (Fig. 4.2) recover a clade consisting of *Spirotecoma holguinensis* and Crescentieae (PP = 1.0, BS = 100%). A monophyletic Crescentieae receives mixed support (0.52, 90%). *Amphitecna*, *Crescentia*, and *Parmentiera* are all supported as monophyletic (1.0, 97%; 1.0, 98%; 0.93, 89%). There is mixed support for a sister relationship between *Parmentiera* and a *Crescentia-Amphitecna* clade (0.52, 100%). A sister relationship between *Crescentia* and *Amphitecna* is strongly supported (1.0, 100%). Interspecific relationships within genera receive low support, resulting in conflict between Bayesian and ML trees (Appendix 4.3, 4.4).

The topologies generated by the chloroplast dataset (Fig. 4.3) are nearly identical in Bayesian and Maximum Likelihood frameworks (Appendix 4.5, 4.6), with exception of the interspecific relationships in *Amphitecna*. A clade consisting of *S. holguinensis* and Crescentieae receives mixed support across analyses (0.98, 71%). Support for a monophyletic Crescentieae is also weak (0.87, 66%). Only the monophyly of *Parmentiera* (1.0, 97%) receives strong support. *Crescentia* is not supported as monophyletic, and *Amphitecna* receives mixed support between analyses (0.99, 54%). Two *Crescentia* clades emerge: one with widespread species *C. alata* and *C. cujete* (Mexico) and one with all other *Crescentia* species, including the Antillean accession of *C. cujete*. However, the monophyly of *Amphitecna* + *Crescentia* is consistent with the nuclear tree, but with weak support (0.94, 52%).

In the tree generated from the combined dataset (Fig. 4.4), a clade containing *S. holguinensis* and Crescentieae receives strong support (1.0, 100%). Crescentieae is supported as monophyletic (0.97, 94%), and the monophyly of each genus in Crescentieae is also supported [*Amphitecna* (1.0, 94%), *Crescentia* (0.74, 72%), *Parmentiera* (1.0, 100%)]. A clade containing *Crescentia* and *Amphitecna* is also well supported (1.0, 100%). Interspecific relationships within genera receive low support, resulting in conflict between Bayesian and ML trees (Appendix 4.7, 4.8).

The maximum clade credibility *BEAST tree (Fig. 4.5) produces strong support for a clade containing *Spirotecoma* and Crescentieae (PP = 0.82). *Amphitecna, Crescentia,* and *Parmentiera* are each supported as monophyletic (1.0, 0.95, 1.0).

DISCUSSION

The most recent circumscription of Crescentieae, containing *Amphitecna*, *Crescentia*, and *Parmentiera* is confirmed by our phylogenetic results, as is its sister relationship with *Spirotecoma*. *Amphitecna* and *Parmentiera* are both strongly supported as monophyletic, while *Crescentia* receives strong support only in the species tree. Crescentieae is monophyletic in all analyses, receiving especially strong support in the combined analysis (Fig. 4.4) and species tree (Fig. 4.5).

Species relationships in *Parmentiera* vary across trees, but some relationships are evident. In trees from the combined-data and coalescent trees, *Parmentiera cereifera* Seem., *Parmentiera millspaughiana* L.O. Williams, *Parmentiera valerii* Standl., and *Parmentiera macrophylla* Standl form a clade (Figs. 4.4, 4.5). *Parmentiera cereifera* is restricted to limestone outcrops on the Atlantic side of the Panama Canal and adjacent central Panama. *Parmentiera macrophylla* is distributed from western Panama to southern Nicaragua. *Parmentiera valerii* is restricted to northern Costa Rica and *P. millspaughiana* occurs in dry thorn shrub forests of the Yucatan peninsula and the Colima State on the Pacific Coast of Mexico. The other group includes *Parmentiera aculeata* (Kunth) Seem and *Parmentiera parviflora* Lundell, which form a Mexican endemic clade in all trees. *Parmentiera trunciflora* Standl. & L.O. Williams (Nicaragua) is sister to one or the other of these clades in the combined-data and coalescent trees (Gentry, 1980).

Like *Parmentiera*, *Crescentia* also is divided into two clades (Fig. 4.4). The first contains widespread taxa *C. alata* and the mainland accession of *C. cujete*. The second contains taxa restricted to the Greater Antilles: *C. portoricensis, C. linearifolia, C. mirabilis,* and the Puerto

Rican accession of *C. cujete*. Two accessions of *C. cujete* were included in our dataset to encompass the wide geographic distribution of this species. Unexpectedly, these accessions come out separately with their geographic congeners. It is possible that an ancestral taxon with a wide distribution gave rise to the rest of the genus autochthonously, that two separate lineages have converged on the same morphology of *C. cujete*, or that chloroplast transfer occurred in one geographic region. Furthermore, *C. amazonica*, a species for which only nuclear data was available, receives low support as a close relative to the mainland accession of *C. cujete* and *C. alata* in trees from both nuclear and concatenated datasets. In the chloroplast tree, the two major clades do not form a monophyletic *Crescentia*. However, *Crescentia* receives strong support as monophyletic in both the nuclear and species trees and exhibits a unique combination of morphological traits, including 3 or 6-colpate pollen, fasciculate leaves, the smallest seeds of the tribe, and white-maroon corollas. (Gentry and Tomb, 1979; Gentry, 1980).

Amphitecna is strongly supported as monophyletic in both the combined (Fig. 4.4) and species trees (Fig. 4.5). However, there is little well-supported resolution among species. *Amphitecna* is the most species-rich clade in Crescentieae, and it seems likely that much more data will be needed to resolve species relationships.

The sister relationship between Crescentieae and *Spirotecoma* presents insights into the directionality of morphological and biogeographic evolution in this group. *Spirotecoma* has opposite, unifoliolate to digitate leaves with 3-7 leaflets, inflorescences in a few-flowered raceme or 1-2 individual flowers borne ramiflorously from leaf axils, tubular-campanulate flowers with reddish-purple to yellowish-brown corollas, dehiscent fruit in elongate capsules nearly round in cross-section, and winged seeds (Gentry, 1992a). It shares opposite and palmately compound leaves with *Parmentiera* and *Crescentia*. *Spirotecoma*'s ramiflorous inflorescences bear developmental similarity to the cauliflorous inflorescences of Crescentieae. Cauliflory follows ramiflory when the site of ramiflorous inflorescences does not become inactive after abcission of the spent inflorescence, the perennial inflorescences becoming situated on old branches as the plant ages (Endress, 2010). Furthermore, while the majority of species in *Spirotecoma* have yellow to red corollas, *S. spiralis* has greenish-purple to yellowish-brown corollas that are likely bat pollinated, a pollination syndrome shared by species in Crescentieae (Gentry, 1992). *Spirotecoma* does have a thickened, fleshy septum with seeds embedded in it. *Parmentiera* fruits also have a fibrous central core (Gentry, 1980), while *Amphitecna* and *Crescentia* have a more uniformly fleshy interior derived from placental tissue (S. Grose, pers. obs.).

An argument can be made, based on stronger phylogenetic support for the more inclusive clade of Crescentieae plus *Spirotecoma*, that *Spirotecoma* should be included in Crescentieae. However, indehiscent, fleshy fruits with vestigially winged or unwinged seeds traditionally defines Crescentieae. For this reason, *Spirotecoma*, with its dehiscent fruits and winged seeds characteristic of the rest of Bignoniaceae, has never been included in Crescentieae. The distribution of *Spirotecoma* is limited to Cuba and Hispaniola, sharing a range with several derived taxa in Crescentieae, including *A. latifolia*, and five species in *Crescentia*. However, *Spirotecoma* does not occur in Central America, which is the center of diversity and likely ancestral home for Crescentieae. Therefore, we retain Gentry's (1976) circumscription of Crescentieae, exclusive of *Spirotecoma*.

Gentry described the syndrome of bat pollination in Bignoniaceae as, "*Crescentia*-type," observing that several species of bats visit *C. alata* and *C. cujete*, and citing a "characteristic musky odor" and "night flowering, extremely copious production of nectar, an open rachitic branching pattern, and cauliflorous flowers" as adaptations that facilitate visits by bats (Gentry 1974). Thompson (2014) documented 366 nectivorous bats, from three species, over 25 nights of mist netting next to *C. cujete* trees in Western Mexico. The number of nectivorous bats was significantly and positively associated with the number of open flowers on *C. cujete* trees. Cauliflory may also be an adaptation for supporting large, heavy fruits, such as those in Crescentieae, *Theobroma cacao* (Malvaceae), or other large-fruited, caluliflorous species. Cauliflorous flowers have also been documented in *Kigelia*, Coleeae, and *Adenocalymma* Mart. Ex Meisn. (Bignonieae) in Bignoniaceae (Zjhra et al., 2004, Fonseca and Lohmann 2017), all of which have large fruits, with *Kigelia* dispersed by baboons, Coleeae by lemurs, and *Adenocalymma* by wind. The groups all have evidence of bat pollination, with the exception of Coleeae, which are pollinated mainly by insects and birds (Gentry 1974, Machado and Vogel, 2004, Zjhra, 2008).

Gentry (1974) documented water dispersal of both *C. alata* and *C. cujete*. During the rainy season in Guanacaste, Costa Rica, he noticed *C. alata* fruits floating in standing water of poorly drained savannas after heavy rain, with naturally dispersed juvenile plants relatively common along sandbars. Furthermore, *C. cujete* trees grow along the Caribbean coast of Costa Rica, where fruits with viable seeds cast up on the beach. A study of seed dispersal in flood plain forests of Amazonia found that *C. amazonica* fruit can float for up to 82 days (Kubitzki and Ziburski, 1994). Large individuals of the fish species *Colossoma macropomum* were able to crush the hard shells and devour the pulp together with seeds. They also found seeds removed from the intestines of fish to be viable.

Animal dispersal of the large, heavy indehiscent fruit of species in Crescentieae have piqued the imaginations of many, considering a lack of large native mammals in the Americas due to Pleistocene extinctions (Simpson, 1980, Lessa & Farina, 1996). Many organisms, ranging from monkeys to Gomphotheres and tapirs to agoutis, have been implicated as dispersers (Gentry, 1974, 1976; Janzen and Martin, 1982; Haber, Zuchowski, and Bello, 2000), but little observational evidence exists to confirm their effectiveness as dispersers. Janzen (1982a), explored the consumption and dispersal of *C. alata* fruit, finding that “once the hard hull is broken by a large dispersal agent, the seeds are largely protected from grinding by the agent’s molars by being small and embedded in a sweet, slippery, and messy matrix that is easily and eagerly swallowed with little effort and chewing.” Furthermore, Janzen (1982b) presented a herd of 17 horses with 160 *C. alata* fruits, and “they broke and ate the contents of all of them in an afternoon.” This behavior may serve as a proxy for the eating preferences of now extinct Pleistocene megafauna responsible for past seed dispersal in Crescentieae. Thompson (2014) found low levels of genetic structure in *C. cujete* populations in Western Mexico, the opposite of what one would expect with the extinction of a dispersal agent. Therefore, Thompson (2014) proposed high pollen-mediated gene flow facilitated by bat pollinators as a possible mechanism leading to the homogenization of genetic diversity in *C. cujete*. It is also possible that, because *C. cujete* trees are found along arroyos in Western Mexico, flooding during the rainy season promotes seed-mediated gene flow of fruit adapted for water dispersal. Seeds from *C. alata* fruits have been documented as feed supplements in rats and chickens (Janzen 1982a). The fruits and seeds of Crescentieae have also made it into the human diet. *Parmentiera aculeata* is a cultivated fruit tree in the Mayan region of Central America, and *P. stenocarpa* produces an edible fruit that has a flavor “apt for desserts or fruit juices.” (Gentry 1992b). *Crescentia cujete* seeds are also used to make “semilla de jícaro” a locally popular drink in Nicaragua (Gentry 1992b).

For a group with such charismatic morphology, fruits that are consumed by a diversity of animals, and a distribution that implies long distance dispersal over water, it is surprising how little is known about the ecology and biogeography of Crescentieae. Therefore, we hope that this systematic framework serves as a foundation for additional studies to resolve species relationships, reconstruct biogeographic history, and better understand the patterns driving the evolution of one of the most derived groups in Bignoniaceae.

Figure 4.1 Floral and fruit morphology of Crescentieae. (A) *Amphitecna tuxtlensis* A.H. Gentry, (B) *Crescentia cujete*, and (C) *Parmentiera valerii* flowers illustrating off-white to maroon corollas and cauliflorous habits. (D) *Amphitecna breedlovei* A.H. Gentry fruit, (E) *Crescentia alata* fruit, and (F) *Parmentiera aculeata* fruit. (G) *Amphitecna gentryi* fruit section demonstrating fleshy pulp and unwinged seeds. Photos: SOG.

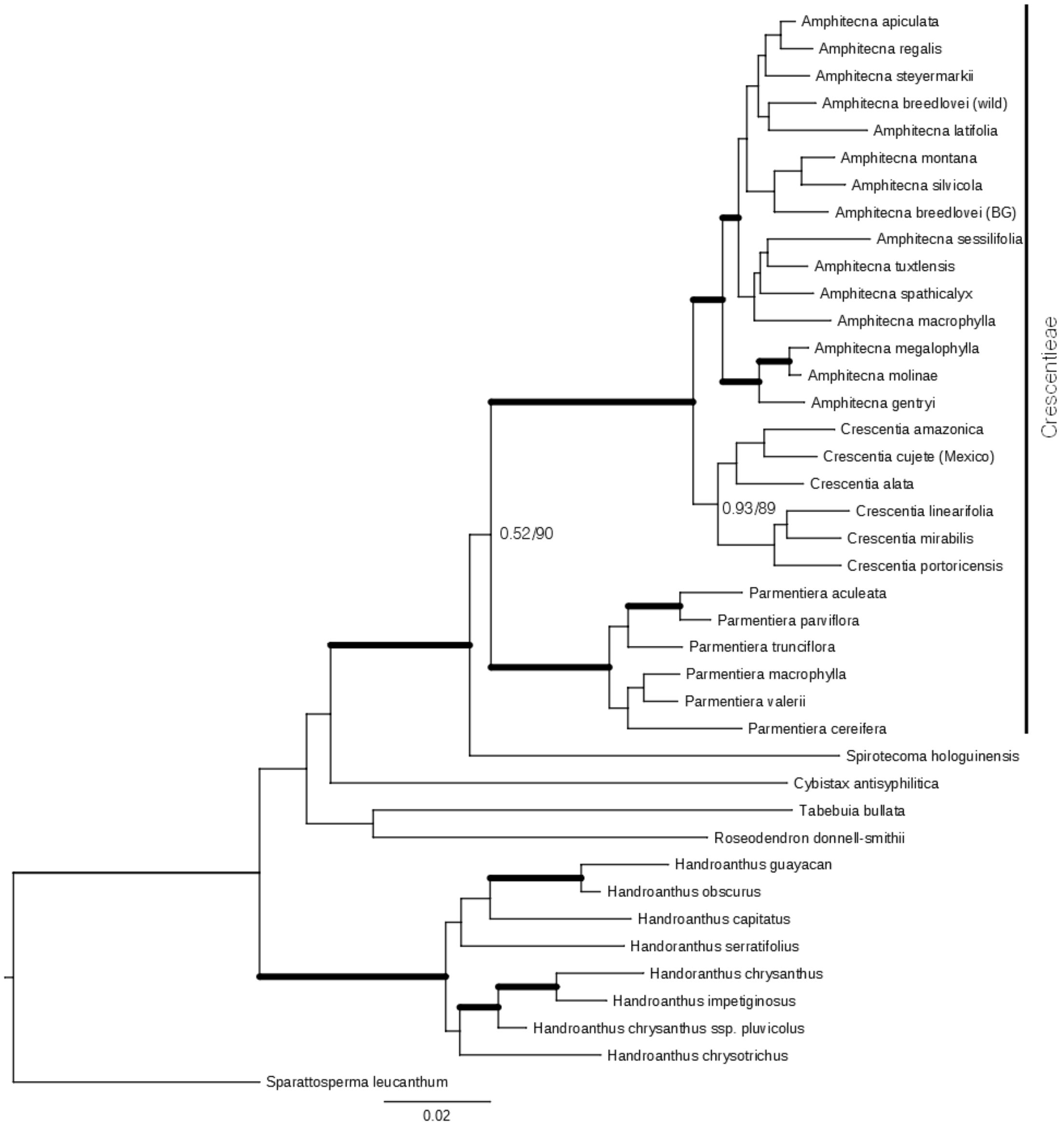

Figure 4.2 Bayesian majority rule consensus tree generated from analysis of the nuclear dataset. Branches leading to clades with both > 0.9 posterior probability and > 90% bootstrap support from ML analysis are bold. Other relationships in Crescentieae recovered in both Bayesian and ML analyses are indicated by posterior probability and bootstrap values in nodes.

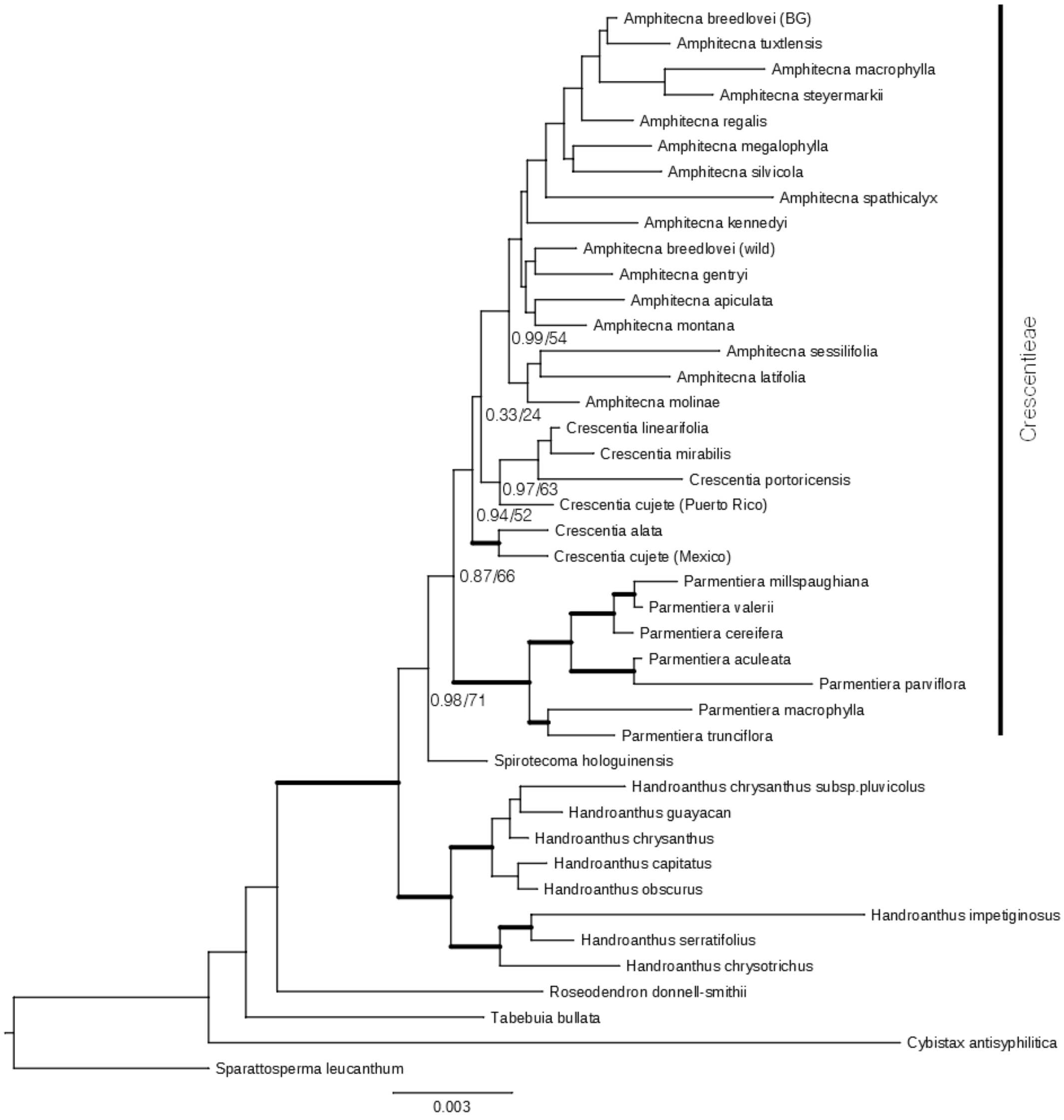

Figure 4.3 Bayesian majority rule consensus tree generated from analysis of the chloroplast dataset. Branches leading to clades with both > 0.9 posterior probability and > 90% bootstrap support from ML analysis are bold. Other relationships in Crescentieae recovered in both Bayesian and ML analyses are indicated by posterior probability and bootstrap values in nodes.

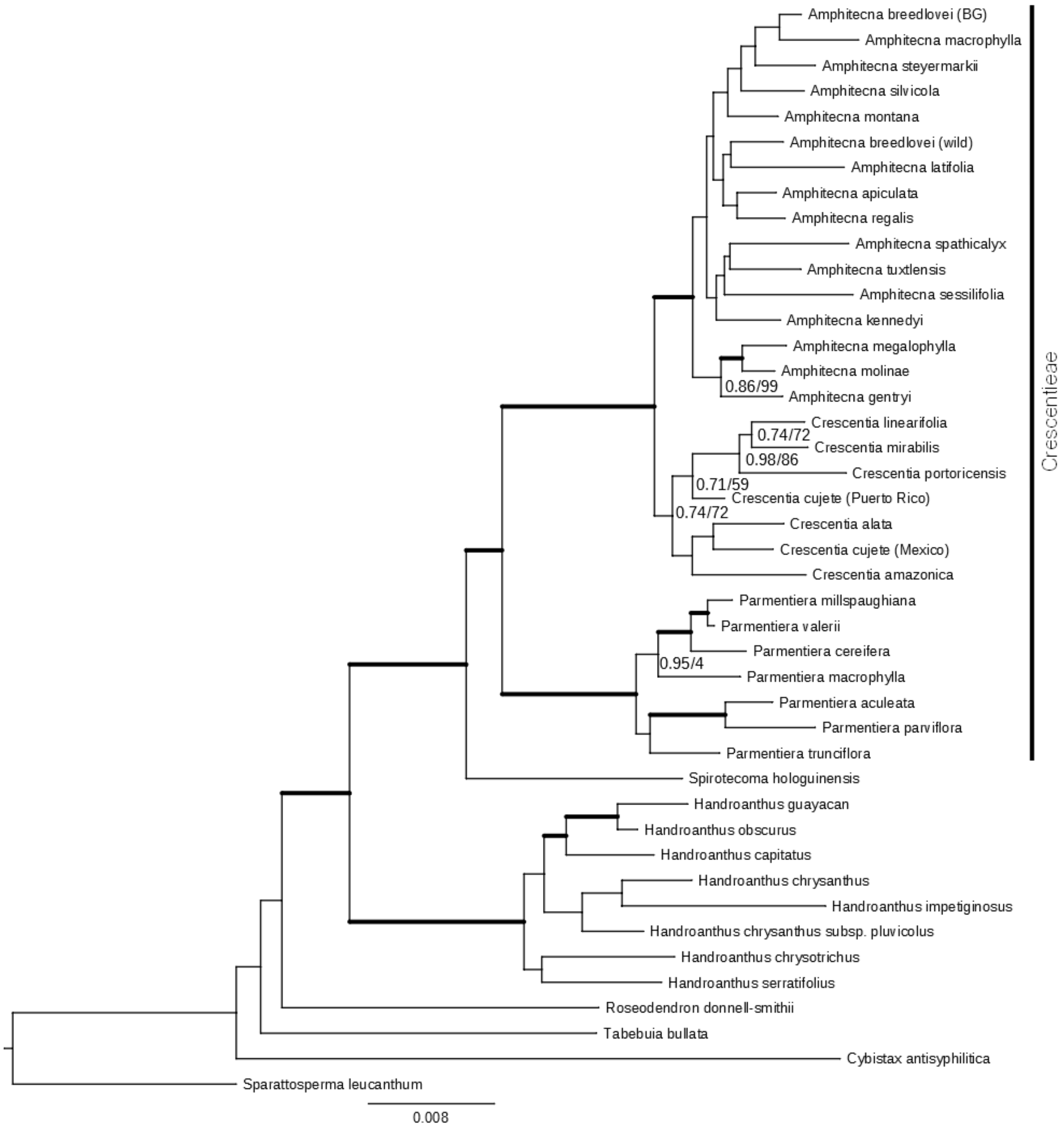

Figure 4.4 Bayesian majority rule consensus tree generated from analysis of the combined dataset. Branches leading to clades with both > 0.9 posterior probability and > 90% bootstrap support from ML analysis are bold. Other relationships in Crescentieae recovered in both Bayesian and ML analyses are indicated by posterior probability and bootstrap values in nodes.

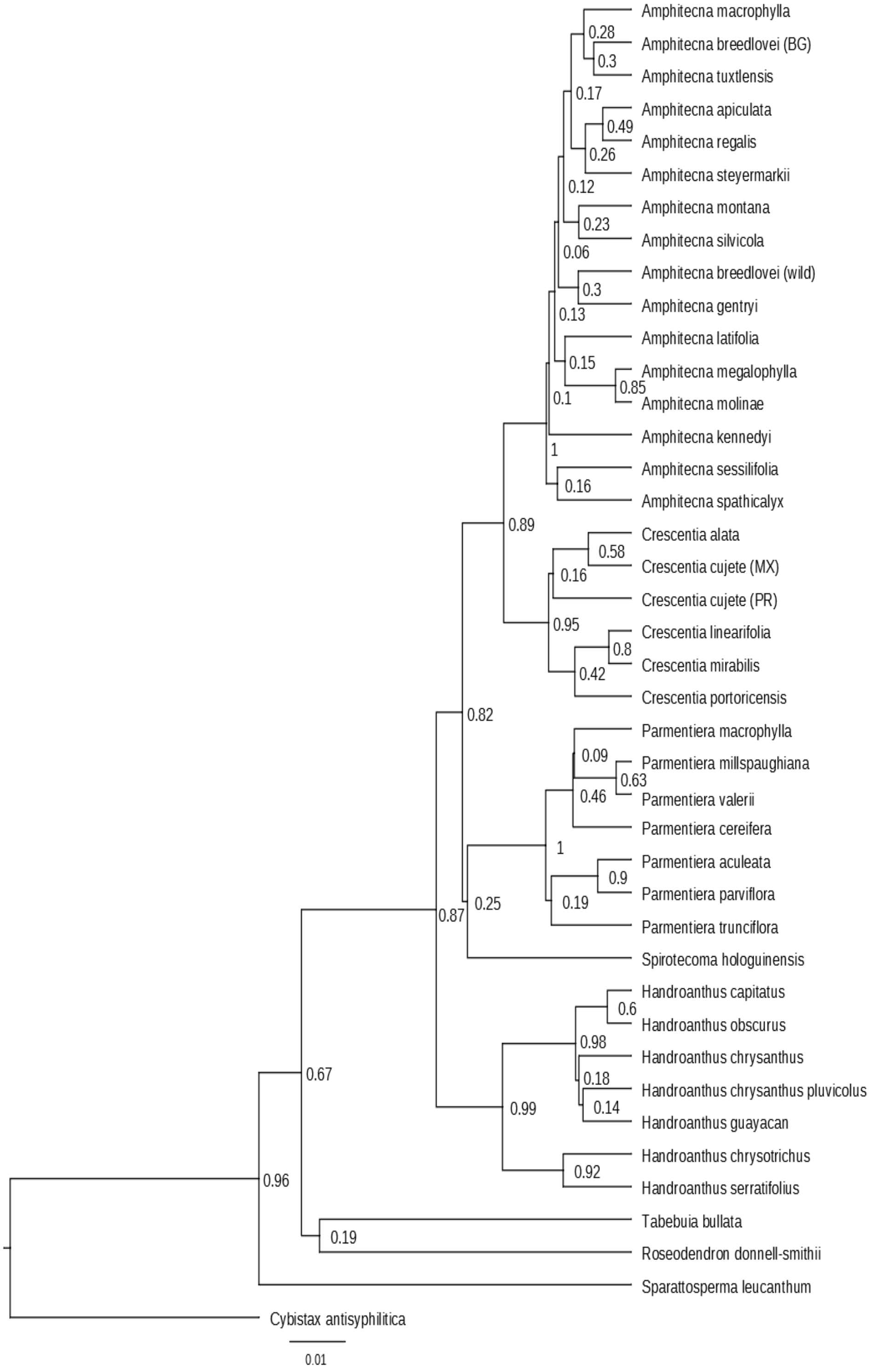

Figure 4.5 Maximum clade credibility *BEAST tree. All relationships recovered are represented by posterior probability values in the nodes.

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Appendix 1.1 Voucher information for sampled taxa. See Index Herbariorum (http://sweetgum.nybg.org/science/ih/) for herbarium information. X represents sequences to be submitted to GenBank.

Taxon	Locality	Collector and Collection Number	Genbank *ndh*F	Genbank *trn*L-F	Genbank *rbc*L	Genbank *mat*K	Genbank *rpl*32-*trn*L	Genbank ITS	Genbank ETS
Antirrhinum majus L.			L36392.1	AY591322.1		KX783633.1	X		
Argylia bustillosii Phil.	Argentina	J. Andrews & M. Broader s.n. (E)	FJ887852.1	FJ870016.1	FJ870007.1			X	X
Argylia radiata (L.) D. Don					X			X	
Argylia robusta Sandwith.	Argentina	J. Andrews & M. Broader s.n. (E)	FJ887853.1	FJ870017.1				X	X
Argylia uspallatensis DC.					X				X
Astianthus viminalis Kunth. (Baill.)	Mexico	Manzanero 89 (NY)	X	X	X			X	
Bignonia capreolata L.	RBG Kew, living accession #1980-3846	no voucher	FJ887855.1	FJ870021.1	HQ384884.1	HQ384518.1	X	X	X
Buddleja davidii Franch.			AF130143.1	AF380861.1	L14392.1	HQ384530.1			
Campsidium valdivianum (Phil.) W. Bull	Chile	Gardner, M & Knees, S G 4050 (E)	EF104999	EF105056					
Campsis grandiflora (Thunb.) K. Schum	USNA accession #72901	no voucher		X	AF190429.1	GQ434140.1	X	X	
Campsis radicans (L.) Seem.	Boulder, CO. In Cult.	RGO 92-193 (WTU)	AF130144.1	AY695865.1	AF156737.1	AF531775.1	X	X	X
Chilopsis linearis (Cav.) Sweet	RSA Bot Gardens, in cult.	no voucher	FJ887856.1	FJ870024.1	JN796935.1				X
Crescentia cujete L.	Puerto Rico	S.O. Grose 60 (WTU)	X	X	KJ082242.1	KJ012551.1		X	
Cuspidaria lateriflora (Mart.) DC.			DQ222575.1	KP757339.1			KP757339.1		
Deplanchea tetraphylla (R. Br.) F. Muell ex Steenis		R. A. Howard 19758 (GH)	EF105006	EF105064	KF496405.1				
Delostoma integrifolium D. Don	Peru	T.E. Sarkinen 2206 (FHO)	EF105004.1				X	X	
Delostoma lobbii Seem.		P. Hutchison & J.K. Wright 5465 (UC)	EF105005				X	X	X
Eccremocarpus scaber Ruiz. & Pav.	USA	RGO 07-155 (WTU)	AF102630.1	FJ870030.1	AF102646.1	HQ384523.1	X	X	X
Fridericia speciosa Mart.			DQ222584.1				KC914604.1		
Incarvillea arguta Royle. (Incarvillea diffusa)			FJ887864	AY695871.1	KX527169.1	KX526592.1	X	AY695847	X
Incarvillea beresowskii Batalin				AY695877				AY695854	
Incarvillea compacta Maxim.				AY695878				Y695855	
Incarvillea delavayi Bureau & Franch.			X	AY695883	X		X	AY695860	
Incarvillea dissectifolia Q.S. Zhao				AY695881				AY695858	
Incarvillea emodi (Royale ex Lindl.) Chaterjee				AY695881	JQ933368.1			AY695849.1	

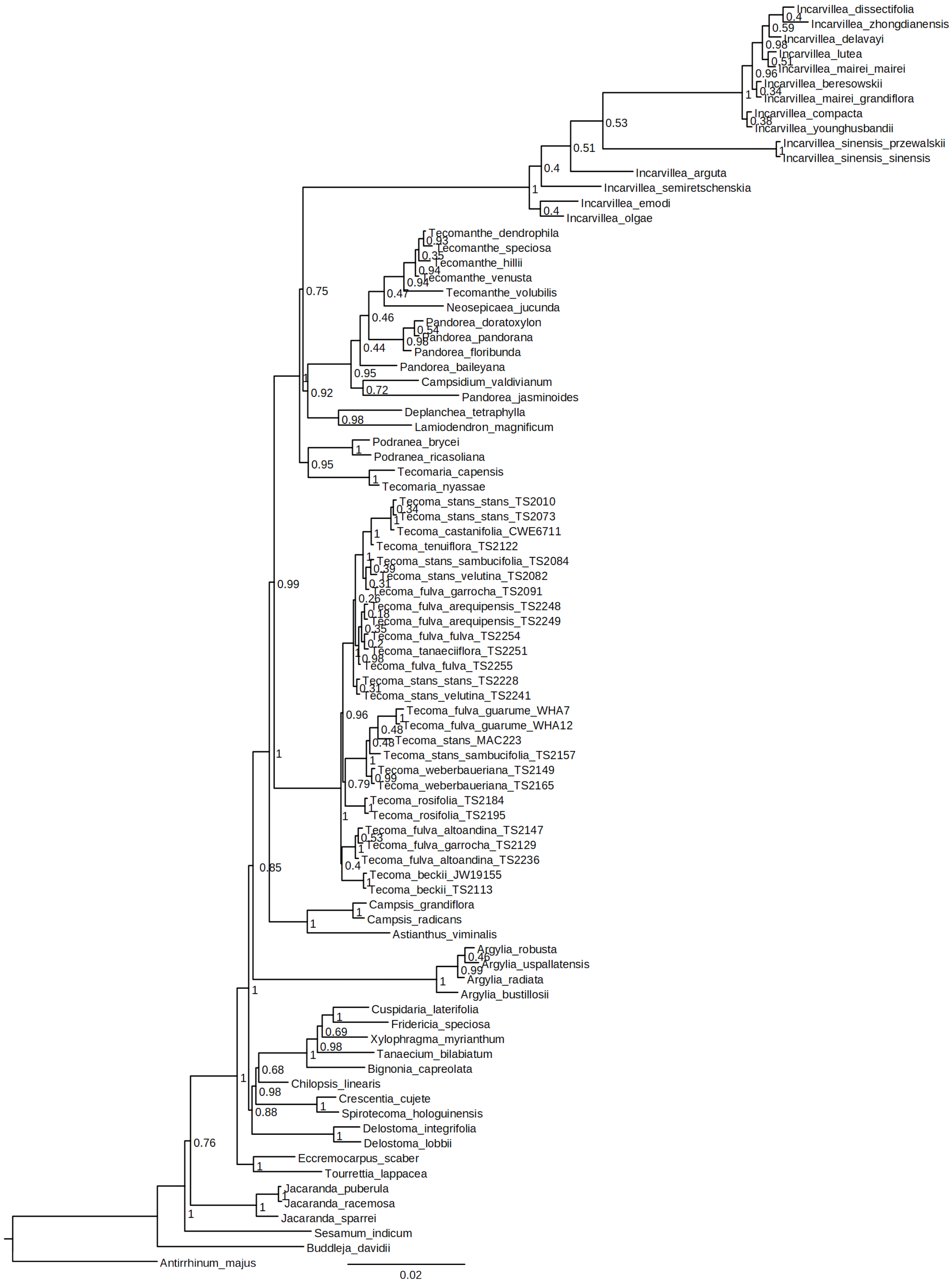
Incarvillea lutea Bureau & Franch.				AY695876		KX526593.1		AY695853	
Incarvillea mairei var. grandiflora (H. Lev.) Grierson				AY695879.1				AY695856	
Incarvillea mairei var mairei (H. Lev) Grierson			KX526864	AY695880	X	KX526594.1		AY695857	
Incarvillea olgae Regel				AY695869				AY695846	
Incarvillea semiretschenskia (B. Fedtsch) Grierson				AY695875				AY695852	
Incarvillea sinensis var przewalskii Lam.				AY695874				AY695851	
Incarvillea sinensis var sinensis Lam.				AY695873				AY695850	
Incarvillea younghusbandii Sprague				AY695884				AY695861	
Incarvillea zhongdianensis Grey-Wilson			FJ887865	FJ870038				AY695859	
Jacaranda puberula Cham.	Rio de Janeiro, Brazil	V.F. Mansono 07-406 (MO)	MN416937	MN445277		MG718684.1	X	X	X
Jacaranda racemosa Cham.	Minas Gerais, Brazil	V.C. Souza 8300 (MO)	MN416938	MN445278			X		
Jacaranda sparrei A.H. Gentry	Waimea, HI, USA, in cult.	H. Descimon s.n. (MO)	AF102631.1	FJ870040.1			X	X	X
Lamiodendron magnificum Steenis		Millar 1911 (GH)	EF105014	EF105073	X		X	X	X
Neosepicaea jucunda (F.Muell.) Steenis				KC428565.1					
Pandorea baileyana (Maiden & R.T. Baker) Steenis					KM895975.1	KM894810.1			
Pandorea doratoxylon (J.M. Black) J.M. Black			X					X	X
Pandorea floribunda (A. Cunn ex DC.) Guymer					KM895724.1	KM894612.1			
Pandorea jasminoides (Lindl.) K. Schum.			AF102636	FJ870049	AF102652.1	KM894818.1			
Pandorea pandorana (Andrews) Steenis			EF105016	EF105076	KF496377.1		X		
Podranea brycei (N.E.Br.) Sprague		AD Crook 29072 (NY)	X						X
Podranea ricasoliana (Tanfani) Sprague			AF102637	FJ870054.1	AF102653.1	MF349888.1			
Sesamum indicum L.			L36413.1	AF479010.1	HQ384882.1	AJ429340.1	JQ764617.1		
Spirotecoma hologuinensis (Britton) Alain	Cuba	no voucher	EF105024.1					X	X
Tanaecium bilabiatum (Sprague) L.G. Lohmann					MF786235.1		KP757333.1		
Tecoma beckii J.R.I. Wood	Bolivia	T.E. Sarkinen 2113 (FHO)	X				X	X	
Tecoma beckii J.R.I. Wood	Bolivia	Wood JRI 19155 (LPB)					X	X	
Tecoma castanifolia (D.Don) Melch	Ecuador	C. Ceron, W. Elisens, & E. Junak 6711 (AAU)					X		
Tecoma fulva subsp. *altoandina* J.R.I. Wood	Bolivia	T.E. Sarkinen 2147 (FHO)	X				X	X	
Tecoma fulva subsp. *altoandina* J.R.I. Wood	Peru	T.E. Sarkinen 2236 (FHO)					X	X	
Tecoma fulva subsp. *arequipensis* (Sprague) J.R.I. Wood	Peru	T.E. Sarkinen 2248 (FHO)					X	X	

Tecoma fulva subsp. *arequipensis* (Sprague) J.R.I. Wood	Peru	T.E. Sarkinen 2249 (FHO)					X	X	
Tecoma fulva subsp. *fulva* (Cav.) G. Don	Peru	T.E. Sarkinen 2255 (FHO)	X				X	X	
Tecoma fulva subsp. *fulva* (Cav.) G. Don	Peru	T.E. Sarkinen 2254 (FHO)					X	X	
Tecoma fulva subsp. *garrocha* (Hieron.) J.R.I. Wood	Bolivia	T.E. Sarkinen 2129 (FHO)	X				X	X	
Tecoma fulva subsp. *garrocha* (Hieron.) J.R.I. Wood	Bolivia	T.E. Sarkinen 2091 (FHO)					X	X	
Tecoma fulva subsp. *guarume* (DC.) J.R.I. Wood	Peru	O. Whaley 12 (K)					X	X	
Tecoma fulva subsp. *guarume* (DC.) J.R.I. Wood	Peru	O. Whaley 7 (K)					X	X	
Tecoma rosifolia Kunth.	Peru	T.E. Sarkinen 2184 (FHO)	X				X	X	
Tecoma rosifolia Kunth.	Peru	T.E. Sarkinen 2195 (FHO)					X	X	
Tecoma stans (L.) Juss. ex Kunth	Mexico	Macqueen 223 (FHO)	X	X			X	X	
Tecoma stans var. *sambucifolia* (Kunth) J.R.I. Wood	Bolivia	T.E. Sarkinen 2084 (FHO)					X	X	
Tecoma stans var. *sambucifolia* (Kunth) J.R.I. Wood	Peru	T.E. Sarkinen 2157 (FHO)					X	X	
Tecoma stans var. *stans* (L.) Juss. ex Kunth	Argentina	T.E. Sarkinen 2010 (FHO)					X	X	
Tecoma stans var. *stans* (L.) Juss. ex Kunth	Peru	T.E. Sarkinen 2228 (FHO)					X	X	
Tecoma stans var. *stans* (L.) Juss. ex Kunth	Bolivia	T.E. Sarkinen 2073 (FHO)					X	X	
Tecoma stans var. *velutina* DC.	Bolivia	T.E. Sarkinen 2082 (FHO)					X		
Tecoma stans var. *velutina* DC.	Peru	T.E. Sarkinen 2241 (FHO)					X		
Tecoma stans (L.) Juss. ex Kunth	Peru	T.E. Sarkinen 2226 (FHO)					X	X	
Tecoma tanaeciiflora (Kraenzl.) Sandwith.	Peru	T.E. Sarkinen 2250 (FHO)					X	X	
Tecoma tenuiflora (DC.) Fabris	Bolivia	T.E. Sarkinen 2122 (FHO)	X				X	X	
Tecoma tenuiflora (DC.) Fabris	Argentina	T.E. Sarkinen 2011 (FHO)					X	X	
Tecoma weberbaueriana (Kraenzl.) Melch.	Peru	T.E. Sarkinen 2165 (FHO)	X				X	X	
Tecoma weberbaueriana (Kraenzl.) Melch.	Peru	T.E. Sarkinen 2149 (FHO)					X	X	
Tecomanthe dendrophila (Blume) K. Schum.	Papua New Guinea	T Motley 2369 (NY)	X		JF738685.1				X
Tecomanthe hillii (F. Muell.) Steenis			EF105051	EF105113	KM895888.1	KM894740.1			X
Tecomanthe speciosa W.R.B. Oliv.					KT626789.1				
Tecomanthe venusta S. Moore					JQ933499.1				
Tecomanthe volubilis Gibbs.			FJ887873.1	FJ870060.1	X				
Tecomaria capensis (Thunb.) Spach	UC Berkeley Botanical Garden (50-1870)	No voucher	FJ887874.1		FJ870009.1	KX783791.1	X	X	X
Tecomaria nyassae (Oliv.) Baill.			X					X	X
Tourrettia lappacea (L'Her.) Willd.			FJ887876.1	FJ870063.1					

Xylophragma myrianthum (Cham.) Sprague			DQ222648.1	FJ870065.1			KP757334.1		

Appendix 1.2 Multiplier dispersal matrices for time slices used in time stratified BioGeoBEARS analyses.

10-0 Ma	N	S	U	F	A
N	1	1	0.1	0.1	0.2
S	1	1	0.1	0.1	0.2
A	0.1	0.1	1	0.7	0.7
A	0.1	0.1	0.7	1	1
A	0.2	0.2	0.7	1	1
24-10 Ma	N	S	U	F	A
N	1	0.5	0.1	0.4	0.5
S	0.5	1	0.1	0.5	0.2
A	0.1	0.1	1	0.3	0.5
A	0.4	0.1	0.3	1	1
A	0.5	0.2	0.5	1	1
40-24 Ma	N	S	U	F	A
N	1	0.2	0.3	0.7	1
S	0.2	1	0.1	0.1	0.1
A	0.3	0.1	1	0.3	0.3
A	0.7	0.1	0.3	1	0.7
A	0.8	0.1	0.3	0.7	1



Appendix 1.3 Bayesian majority rule consensus tree generated from analysis of the chloroplast dataset. Numbers at nodes indicate posterior probability support values.

Appendix 1.4 Maximum likelihood tree generated from analysis of the chloroplast dataset. Numbers at nodes indicate bootstrap support values.

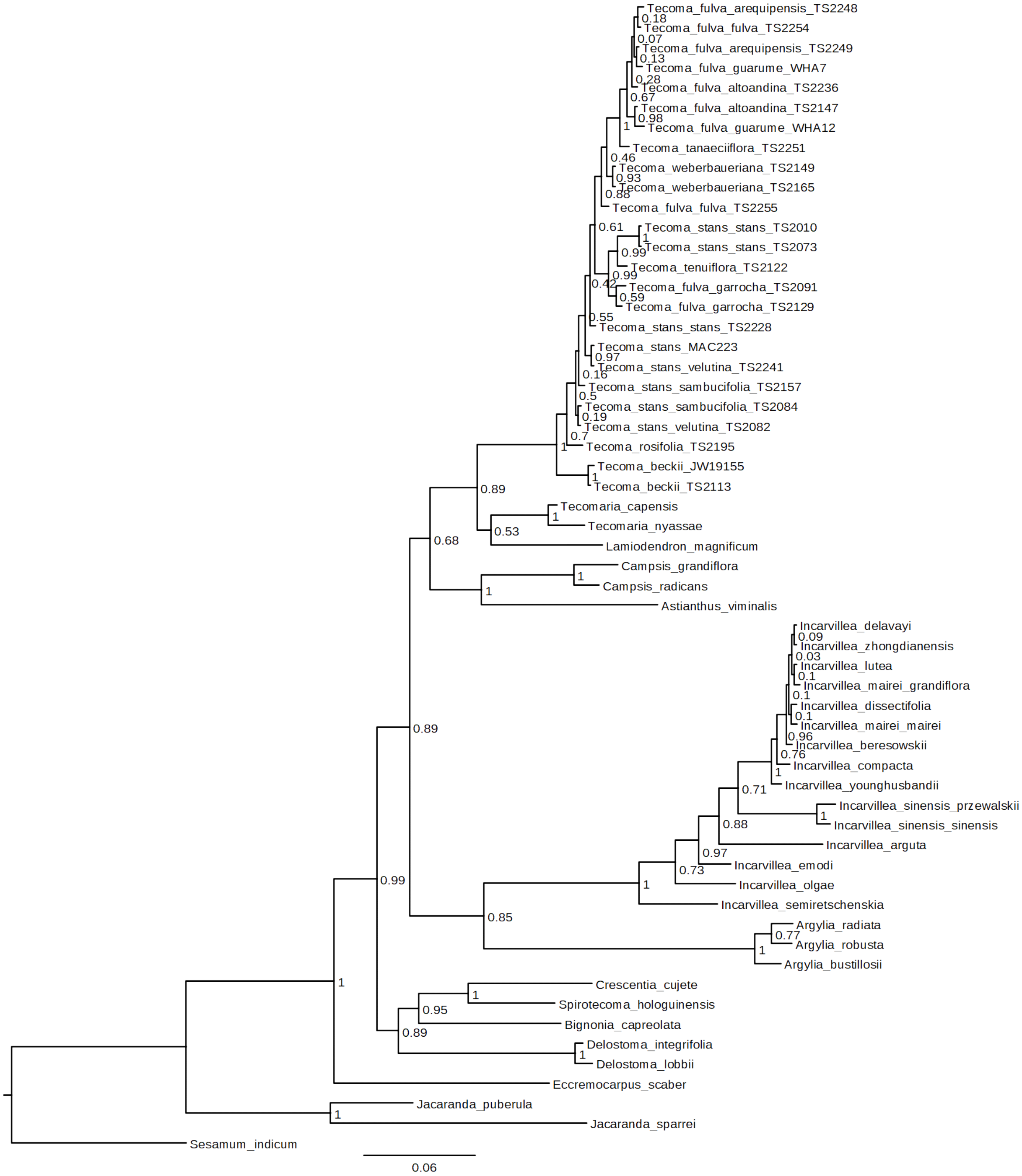
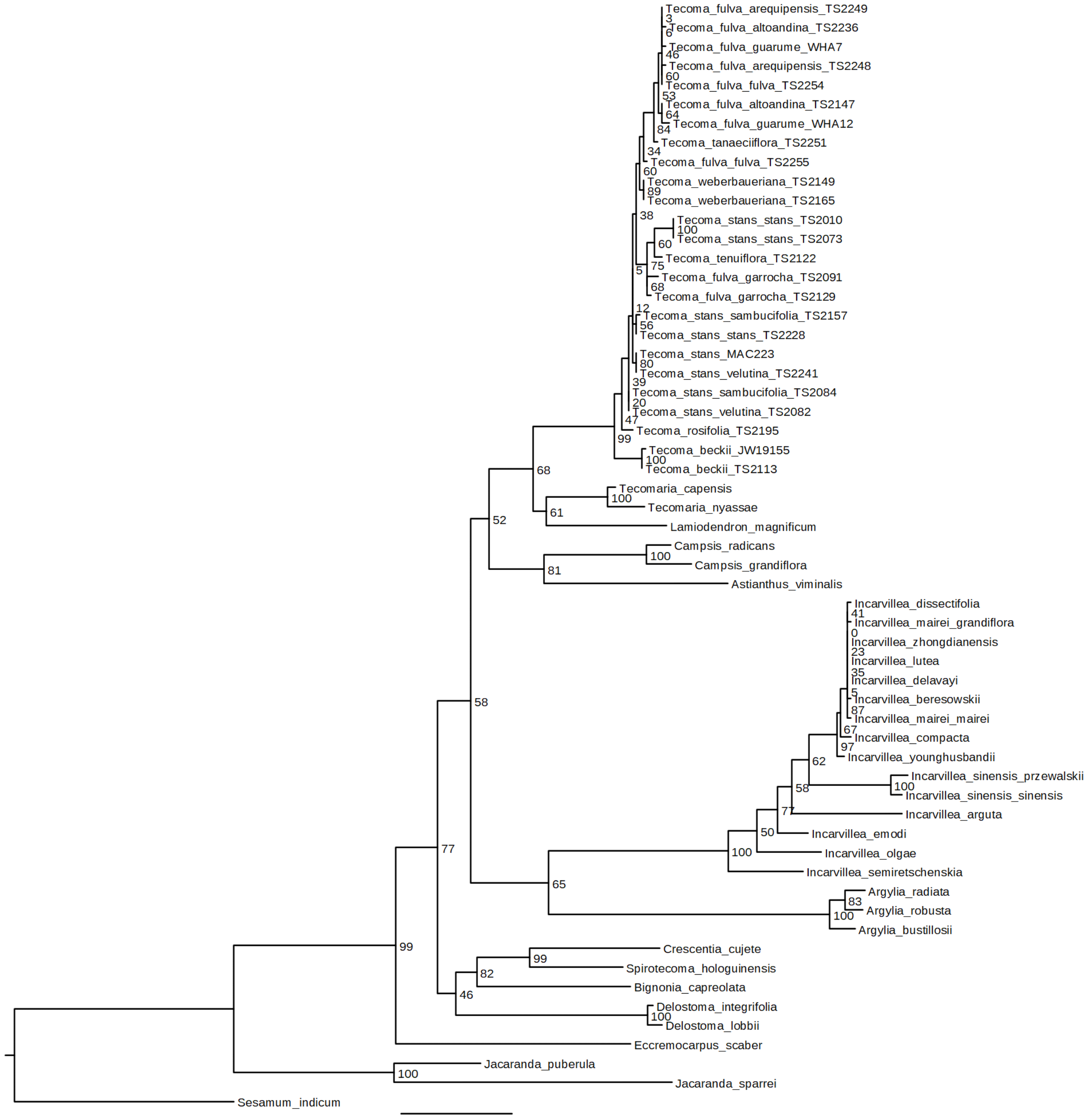


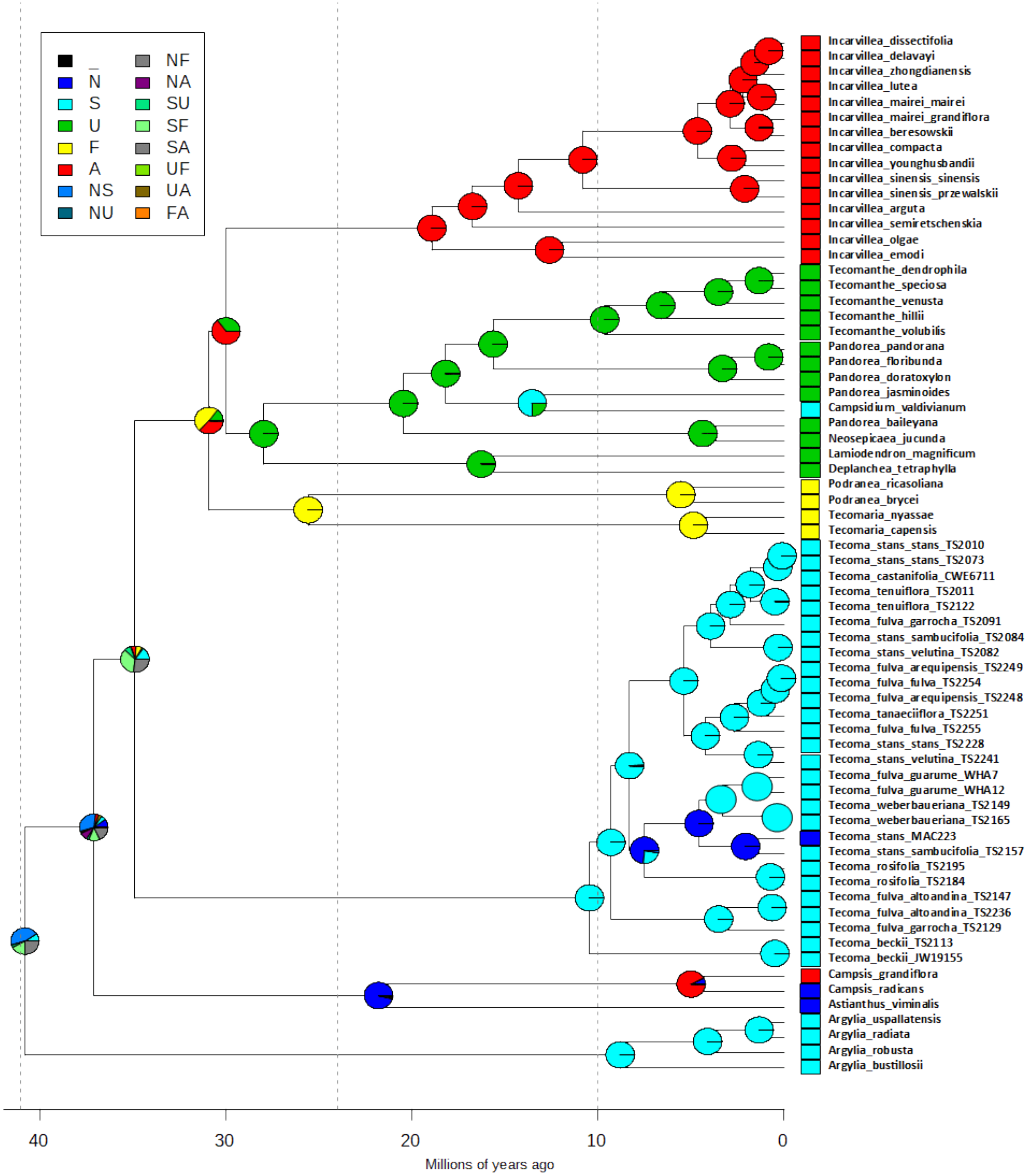
Figure 1.5 Bayesian maximum clade credibility tree generated from analysis of the nuclear dataset. Numbers at nodes indicate posterior probability support values.



Appendix 1.6 Maximum likelihood tree generated from analysis of the nuclear dataset. Numbers at nodes indicate bootstrap support values.

Appendix 1.7 Bayesian maximum clade credibility tree generated from analysis of the concatenated chloroplast and nuclear datasets. Numbers at nodes indicate posterior probability support values.

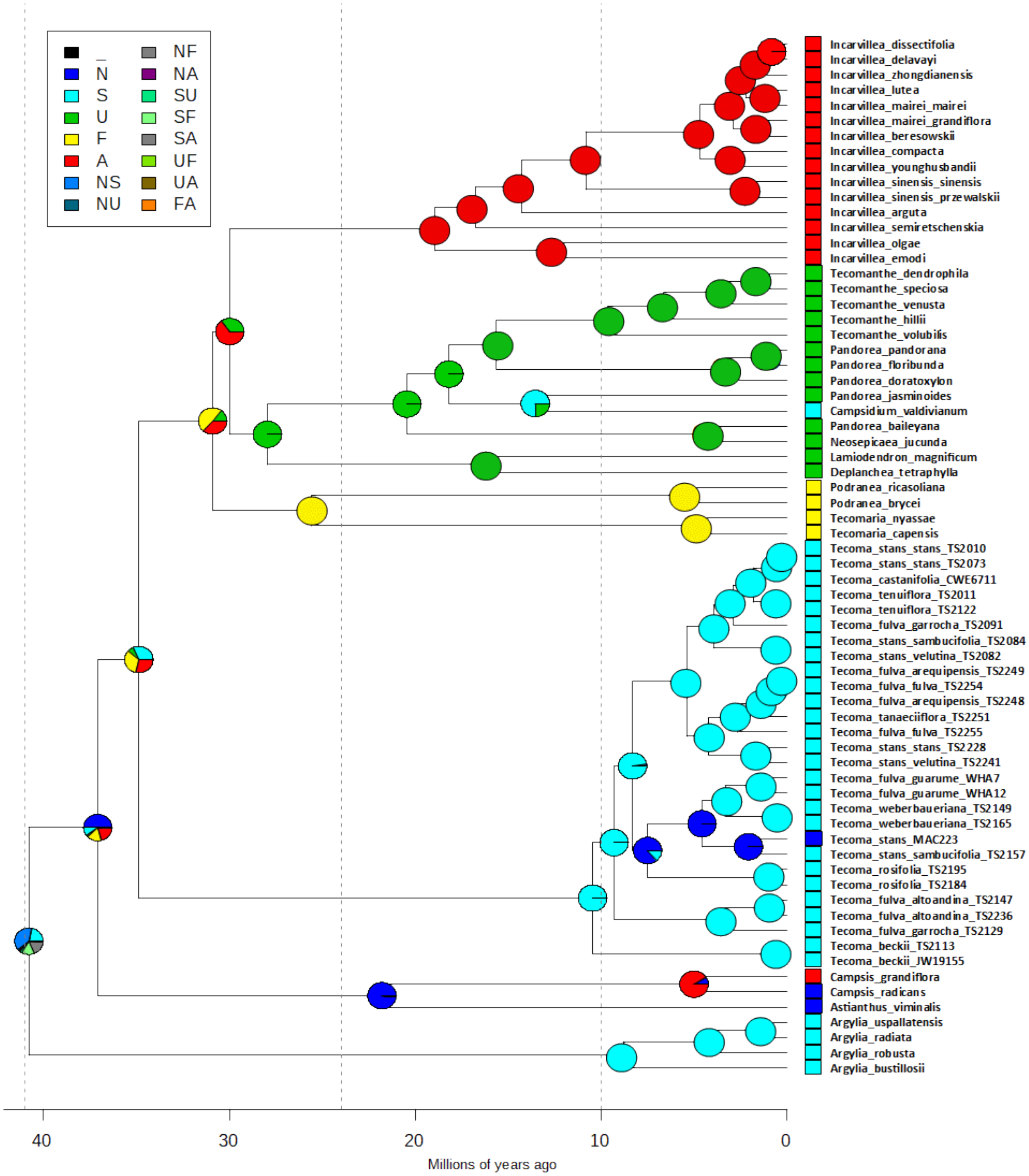
Appendix 1.8 Maximum likelihood tree generated from analysis of the concatenated chloroplast and nuclear datasets. Numbers at nodes indicate bootstrap support values.



Appendix 1.9 Historical biogeography of Tecomeae. Colored pie charts represent ancestral range probabilities at each node as estimated by the DEC + J model. Colors refer to geographic regions: South America (light blue), North America (dark blue), Australasia (green), Africa (yellow), Asia (red).

Appendix 1.10 Node information for the Tecomeae phylogeny and BioGeoBEARS ancestral state reconstruction. Posterior probability (PP) support values are from maximum clade credibility tree generated from the concatenated dataset in MrBayes and bootstrap support values are from the tree generated from the concatenated dataset in RAxML. Median crown and stem ages and 95% highest posterior distributions (HPD) are from the time calibrated tree generated in BEAST. Ancestral state probabilities for each coded region are from BioGeoBEARS under the DEC + J model. Coded regions are as follows: Africa (A), South America (S), Australasia (U), Africa (F), and Asia (A).

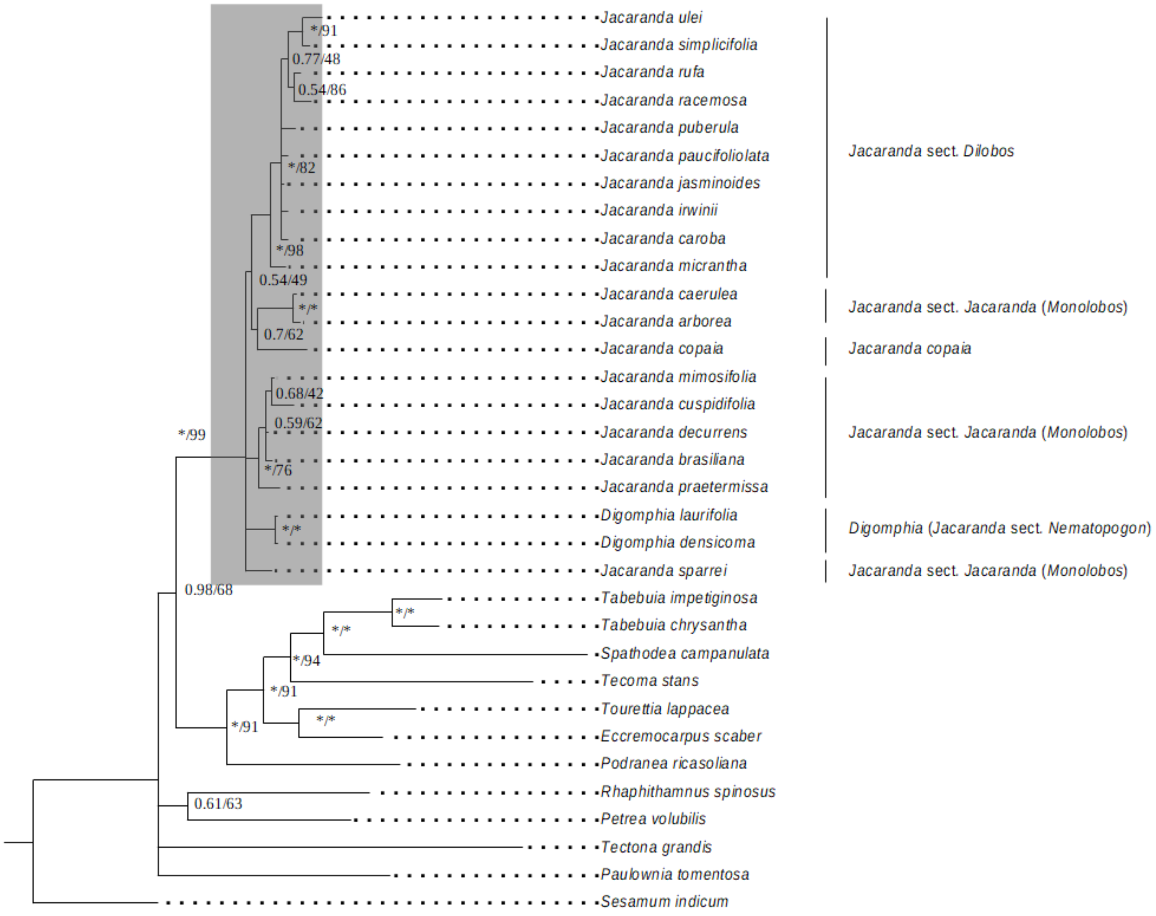
Clade	PP	Bootstrap	Median Crown Age (Ma)	95% HPD Crown Age	Median Stem Age (Ma)	95% HPD Stem Age	N	S	U	F	A
Bignoniaceae	0.66	83	49.72	47.71-51.6							
Calibration	0.96	92	34.15	33.75-35							
Tecomeae	1	99	37.09	31.64-41.78	40.79	36.3-45.04	0.002208	0.095208	7.00E-05	0.000429	0.000517
Argylia	1	100	8.81	3.69-16.28	40.79	36.3-45.04	2.96E-24	1	1.27E-26	2.29E-26	1.10E-25
Astianthus/*Campsis*	1	100	21.79	9.93-34.2	37.09	31.64-41.78	0.965467	1.30E-23	6.34E-25	8.06E-24	0.016594
Campsis	1	100	5	1.13-11.08	21.79	9.93-34.2	0.077289	4.92E-23	2.03E-24	1.08E-23	0.915072
Tecoma + Old World Clade	1	99	34.88	29.07 - 39.59	37.09	31.64 - 41.78	1.80E-08	0.156548	0.013617	0.068935	0.059472
Tecoma	0.95	98	10.46	6.47-16.49	34.88	29.07-39.59	7.04E-08	0.999857	3.14E-26	5.17E-26	2.31E-25
T. stans/*T. stans* var. *sambucifolia*	0.64	85	2.07	0.4-4.58	4.57	2.29-7.38	0.999947	3.63E-06	9.31E-31	8.88E-31	2.46E-29
Old World Clade	0.94	96	30.91	25.09-36.08	34.88	29.07-39.59	1.12E-22	4.46E-09	0.149022	0.485638	0.354494
African Clade	0.84	63	25.57	15.11-32.95	30.91	25.09-36.08	7.33E-24	1.13E-24	1.85E-24	1	9.35E-24
Asian Clade	0.41	40	29.99	24.17-35.32	30.91	25.09-36.08	7.85E-23	1.01E-08	0.351742	4.13E-23	0.644444
Australasian Clade	0.84	73	27.96	21.77-33.98	29.99	24.17-35.32	1.32E-24	2.70E-08	0.999367	1.29E-24	1.43E-24
Incarvillea	1	100	18.93	13.77-24.74	29.99	24.17-35.32	6.31E-25	6.04E-26	9.58E-25	2.19E-24	1
Campsidium/*P. jasminoides*	0.7	73	13.54	6.88-20.79	18.19	12.25-24.78	9.61E-23	0.750552	0.241844	3.15E-22	1.66E-21



Appendix 1.11 Historical biogeography of Tecomeae. Colored pie charts represent ancestral range probabilities at each node as estimated by the DIVALIKE + J model. Colors refer to geographic regions: South America (light blue), North America (dark blue), Australasia (green), Africa (yellow), Asia (red).

Appendix 1.12 Node information for the Tecomeae phylogeny and BioGeoBEARS ancestral state reconstruction. Posterior probability (PP) support values are from maximum clade credibility tree generated from the concatenated dataset in MrBayes and bootstrap support values are from the tree generated from the concatenated dataset in RAxML. Median crown and stem ages and 95% highest posterior distributions (HPD) are from the time calibrated tree generated in BEAST. Ancestral state probabilities for each coded region are from BioGeoBEARS under the DIVALIKE + J model. Coded regions are as follows: Africa (A), South America (S), Australasia (U), Africa (F), and Asia (A).

Clade	PP	Bootstrap	Median Crown Age (Ma)	95% HPD Crown Age	Median Stem Age (Ma)	95% HPD Stem Age	N	S	U	F	A
Bignoniaceae	0.66	83	49.72	47.71-51.6							
Calibration	0.96	92	34.15	33.75-35							
Tecomeae	1	99	37.09	31.64-41.78	40.79	36.3-45.04	0.0053	0.212101	0.000173	0.001034	0.001236
Argylia	1	100	8.81	3.69-16.28	40.79	36.3-45.04	1.61E-24	1	2.02E-26	2.03E-26	8.15E-26
Astianthus/*Campsis*	1	100	21.79	9.93-34.2	37.09	31.64-41.78	0.98793	8.88E-24	6.31E-25	5.51E-24	0.01207
Campsis	1	100	5	1.13-11.08	21.79	9.93-34.2	0.091176	3.86E-23	2.15E-24	9.78E-24	0.908824
Tecoma + Old World Clade	1	99	34.88	29.07 - 39.59	37.09	31.64 - 41.78	1.30E-07	0.317859	0.06466	0.334615	0.282863
Tecoma	0.95	98	10.46	6.47-16.49	34.88	29.07-39.59	2.17E-07	1	6.00E-26	1.01E-25	4.52E-25
T. stans/*T. stans* var. *sambucifolia*	0.64	85	2.07	0.4-4.58	4.57	2.29-7.38	0.999998	2.34E-06	7.39E-31	6.35E-31	2.19E-29
Old World Clade	0.94	96	30.91	25.09-36.08	34.88	29.07-39.59	4.73E-23	5.13E-09	0.148691	0.486676	0.364633
African Clade	0.84	63	25.57	15.11-32.95	30.91	25.09-36.08	6.45E-24	8.59E-25	1.66E-24	1	8.80E-24
Asian Clade	0.41	40	29.99	24.17-35.32	30.91	25.09-36.08	4.24E-23	1.11E-08	0.349226	2.29E-23	0.650774
Australasian Clade	0.84	73	27.96	21.77-33.98	29.99	24.17-35.32	7.74E-25	3.05E-08	1	7.41E-25	7.95E-25
Incarvillea	1	100	18.93	13.77-24.74	29.99	24.17-35.32	2.69E-25	2.14E-26	4.60E-25	1.14E-24	1
Campsidium/*P. jasminoides*	0.7	73	13.54	6.88-20.79	18.19	12.25-24.78	9.65E-23	0.754466	0.245534	3.19E-22	1.68E-21



Appendix 2.1.1 Bayesian majority rule consensus tree derived from the analysis of the concatenated chloroplast loci *ndhF* and *trnL-F* (Farias-Singer et al., 2011). The clade highlighted in gray is Jacarandeae. Bayesian posterior probability (PP) and ML bootstrap support (BS) values are indicated at nodes (* denotes a PP of 1 and bootstrap of 100%). Outgroups are taxa from other tribes of Bignoniaceae and families of Lamiales.

Molecular phylogeny of *Jacaranda* Juss. (Bignoniaceae)

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Introduction & Aims

According to phylogenetic analyses, *Jacaranda* may be one of the earliest-diverging lineages within Bignoniaceae. This genus is currently divided into two sections based on the number of thecae: *Dilobos*, whose anthers display two thecae and *Jacaranda* (= *Monolobos*) with just one theca. *Jacaranda* species range from subshrubs to large trees bearing pinnate or bipinnate leaves. Some species have a broad distribution while others are endemic or restricted to particular regions. The aim of this work was to study the phylogeny of *Jacaranda* through molecular tools as well as to infer the evolution of some morphological features within the genus.

A. *Jacaranda cuspidifolia* Mart. Ex DC. Leaves, flower and fruit. **B.** *J. macrantha* Cham. Dithecate anther, section *Dilobos*. **C.** *J mimosifolia* D. Don. Monothecate anther, section *Jacaranda*.

Molecular phylogeny of *Jacaranda* Juss. (Bignoniaceae)

Results

All used methods recovered the same tree topology. In order to achieve monophyly, the genus *Jacaranda* should include all *Digomphia* species, which were recovered as a well-supported clade. Concerning the traditional *Jacaranda* sections, sect. *Dilobos* is monophyletic and well-supported, but sect. *Jacaranda* is a polyphyletic group.

Phylogenetic tree obtained by Bayesian analysis.

Molecular phylogeny of *Jacaranda* Juss. (Bignoniaceae)

Methods

Twenty eight *Jacaranda* species representing the morphological diversity within the two sections were sampled. Species of *Cybistax*, *Digomphia*, *Handroanthus*, *Podranea*, *Spathodea*, *Tabebuia*, and *Tecoma* were used as out-groups. DNA extraction was made from silicagel-dehydrated leaf samples and herbarium vouchers. Sequences were obtained through polymerase chain reaction (PCR) for the following molecular markers: *rbcL*, *ndhF* and *trnL-trnF*. Phylogenetic analyses were performed through bayesian methods using MrBayes software, by ML and distance (with appropriate evolutionary models for each dataset), and MP in PAUP*software.

A. *Jacaranda decurrens* subsp. *symmetrifoliolata* Farias & Proença. **B.** *J. Oxyphyla* Cham. **C.** *J. Macrantha* Cham. **D.** *J. ulei* Bureau & K. Schum. Flowers.

Molecular phylogeny of *Jacaranda* Juss. (Bignoniaceae)

Conclusion

The *Dilobos* clade includes species which are typically found at the Cerrado, Atlantic Rain Forest and transitional regions. Species with a single theca are found in clades from Central America, Amazonia and Northern South America as well as in a species group from the Cerrado Biome. The species of *Digomphia* are Amazonian, mainly from highlands (Tepuis).

Therefore, the results suggest that the Cerrado Biome is a diversification region for the genus, since it includes species from different groups.

Distribuition of *Jacaranda*.

This study was conducted at the Laboratory of Molecular Evolution, Department of Genetics, Universidade Federal do Rio Grande do Sul. Funding was provided by CNPq (process 0151978/208 5), FAPERGS, PPGBM-UFRGS.

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Appendix 2.1.2 Digital poster by Farias-Singer et al. (2011) that initially described the Jacarandeae phylogeny. This work was presented at the International Botanical Congress XVIII in Melbourne, Australia.

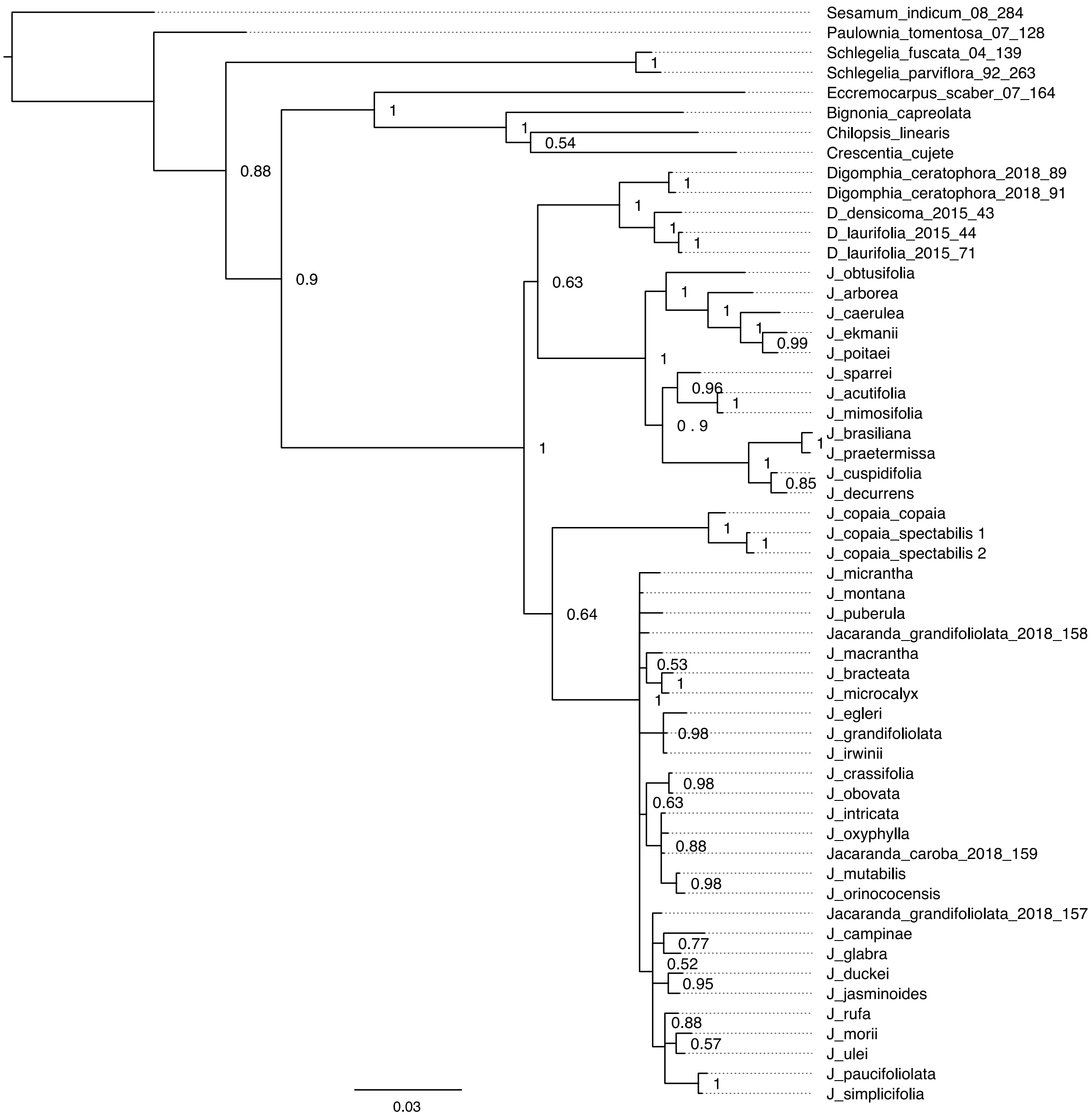
Appendix 2.2 Voucher information of samples used to generate tree in Appendix S1. See Index Herbariorum (http://sweetgum.nybg.org/science/ih/) for herbarium information.

Taxa	Collector and Collection Number	Genbank *ndhF*	Genbank *trnLF*
Digomphia densicoma (Mart. ex DC.) Pilg.	R. Farias-Singer 921 (UEC)	MN447899	MN445281
Digomphia laurifolia Benth.	R. Farias-Singer 920 (UEC)	MN447900	MN445282
Eccremocarpus scaber Ruiz & Pav.	M.W. Chase 2999 (K)	AF102630.1	FJ870030.1
Jacaranda arborea Urban	Olmstead 96-96 (WTU)	FJ887866.1	FJ870039.1
Jacaranda brasiliana (Lam.) Pers.	R. Farias-Singer 1018 (UB)	MN447901	MN445283
Jacaranda caerulea (L.) Juss.	P.R. Fantz 3982 (FTG)	MN447902	MN445284
Jacaranda caroba (Vell.) A. DC.	R. Farias-Singer *et al.* 730 (UEC)	MN447903	MN445285
Jacaranda copaia (Mart. ex A. DC.) A.H. Gentry	R. Farias-Singer 905 (UEC)	MN447904	MN445286
Jacaranda cuspidifolia Mart.	R. Farias-Singer 1030 (ICN)	MN447905	MN445287
Jacaranda decurrens Cham.	R. Farias-Singer 776 (UEC)	MN447906	MN445288
Jacaranda irwinii A.H. Gentry	V. Thode 232 (ICN)	MN447907	MN445289
Jacaranda jasminoides (Thunb.) Sandwith	R. Farias-Singer 755 (UEC)	MN447908	MN445290
Jacaranda micrantha Cham.	R. Farias-Singer 998 (ICN)	MN447909	MN445291
Jacaranda mimosifolia D. Don	R. Farias-Singer 999A (UEC)	MN447922	MN445292
Jacaranda mimosifolia D. Don	L. Lohman 262 (MO)	EF105012.1	EF105070.1
Jacaranda paucifoliolata Mart. ex A. DC.	R. Farias-Singer 756 (UB)	MN447910	MN445293
Jacaranda praetermissa Sandwith	C. Proença *et al.* 3268 (ICN)	MN447911	MN445294
Jacaranda puberula Cham.	R. Farias-Singer 994 (UB)	MN447912	MN445295
Jacaranda racemosa Cham.	C. Proença & S.A. Harris 3131 (ICN)	MN447913	MN445296
Jacaranda rufa Manso	R. Farias-Singer *et al.* 1016 (ICN)	MN447914	MN445297
Jacaranda simplicifolia K. Schum.	R. Farias-Singer 1034 (ICN)	MN447915	MN445298
Jacaranda sparrei A.H. Gentry	Descimon s.n. (MO)	AF102631.1	FJ870040.1
Jacaranda ulei Bureau & K. Schum.	R. Farias-Singer & V.C. Mendes 1006 (ICN)	MN447916	MN445299
Paulownia tomentosa Stued.		L36406.1	AF479005.1
Petrea volubilis L.		FJ887872.1	HM216655.1
Podranea ricasoliana (Tanfani) Sprague	R. Farias-Singer 1023 (ICN)	MN447917	MN445300
Rhaphithamnus spinosus (Juss.) Moldenke		L36409.1	FJ870056.1
Sesamum indicum L.		L36413.1	AF479010.1
Spathodea campanulata P. Beauv.	R. Farias-Singer 1024 (ICN)	MN447918	MN445301
Tabebuia chrysotricha (Mart. ex A. DC.) Standl.	R. Farias-Singer 1022 (ICN)	MN447919	MN445302
Tabebuia impetiginosa (Mart. ex DC.) Standl.	R. Farias-Singer 1021 (ICN)	MN447920	MN445303
Tecoma stans (L.) Juss. ex Kunth	R. Farias-Singer 1020A (ICN)	MN447921	MN445304
Tectona grandis L.f.		U78705.1	FJ870062.1
Tourrettia lappacea (L'Hér.) Willd		FJ887876.1	FJ870063.1

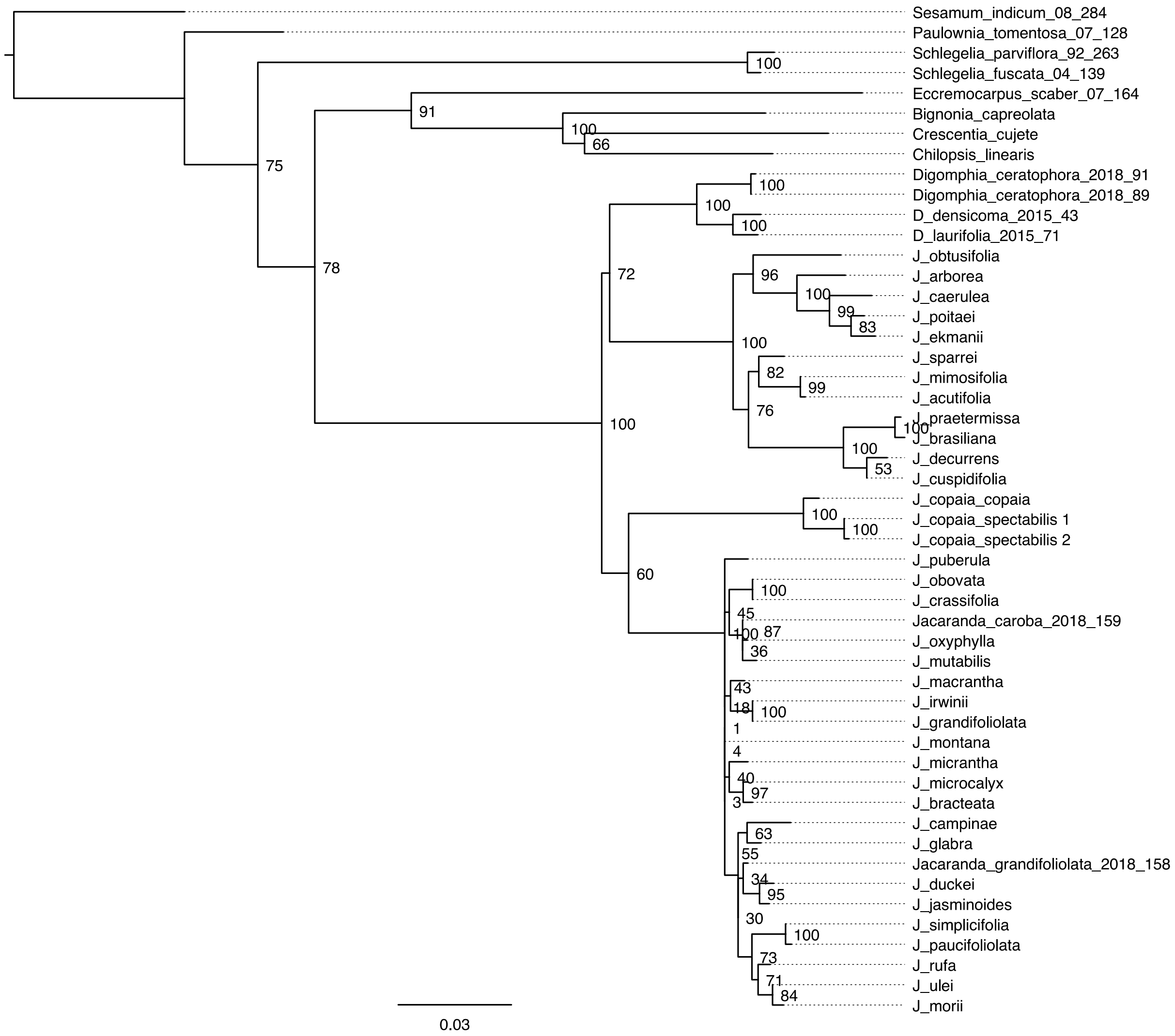
Appendix 2.3 Voucher information for sampled taxa. See Index Herbariorum (http://sweetgum.nybg.org/science/ih/) for herbarium information.

Taxa	Locality	Collector and Collection Number	Genbank *ndhF*	Genbank *rpl*32-*trn*L	Genbank *trn*L-F	Genbank ETS	Genbank PPR62
Bignonia capreolata L.	RBG Kew, living accession #1980-3846	no voucher	FJ887855.1	MN447857	FJ870021.1	MN445199	MN447816
Chilopsis linearis D. Don	RSA Bot Gardens, in cult.	no voucher	FJ887856.1	MN447858	FJ870024.1	MN445200	MN447817
Crescentia cujete L.	Puerto Rico	SOG PR 009 (WTU)	MN416908	MN447859	MN445244	MN445201	MN447817
Digomphia ceratophora A.H. Gentry	Guainia, Inirida, Colombia	AMB 616 (WTU)	MN416909	MN447864	MN445247	MN445209	MN447822
Digomphia densicoma (Mart. ex DC.) Pilg.	Amazonas, Brazil	Acevedo-Rodriguez 14674 (US)	MN416910	MN447865	MN445248	MN445207	MN447820
Digomphia laurifolia Benth.	Bolivar, Venezuela	Huber 11149 (NY)	MN416911	MN447866	MN445249	MN445208	MN447821
Eccremocarpus scaber Ruiz & Pav.	Seattle, WA, USA. escaped from cult.	RGO 07-155 (WTU)	MN416912	MN447860	MN445245	MN445202	MN447819
Jacaranda acutifolia Bonpl.	Santa Cruz, Bolivia	Nee 50718 (NY)	MN416913	MN447867	MN445250	MN445210	MN447823
Jacaranda arborea Urb.	Jardin Botanica Nacional, Havana, Cuba, in cult.	R. G. Olmstead 1996-96 (WTU)	FJ887866.1	-	FJ870039.1	MN445211	MN447824
Jacaranda bracteata Bureau & K. Schum.	Rio de Janeiro, Brazil	W. Bensen 39 (F)	MN416914	MN447868	MN445251	MN445212	MN447825
Jacaranda brasiliana (Lam.) Pers.	Bahia, Brazil	H. Irwin et al. 31597 (F)	MN416915	MN447869	MN445252	MN445213	MN447826
Jacaranda caerulea (L.) Juss.	Fairchild Tropical Botanic Gardens, USA, in cult.	T. Berman 2013-001 (FTG)	MN416916	MN447870	MN445253	MN445214	MN447827
Jacaranda campinae A.H. Gentry & Morawetz	Amazonas, Brazil	Ferreira 5816 (NY)	MN416917	MN447871	MN445254	MN445215	MN447828
Jacaranda caroba (Vell.) DC.	University of Sao Paulo, Sao Paulo, Brazil, in cult.	Living Acccession (SPF)	MN416942	MN447872	MN445255	MN445242	MN447852
Jacaranda copaia ssp. *copaia* (Aubl.) D. Don	Amapa, Brazil	Daly and Souza 3937 (NY)	MN416919	MN447874	MN445258	MN445217	MN447830
Jacaranda copaia ssp. *spectabilis* (Mart. ex A.P. DC.) A.H, Gentry	Roraima, Brazil	Hopkins et al. 620 (GH)	MN416918	MN447873	MN445257	MN445216	MN447829
Jacaranda copaia ssp. spectabilis (Mart. ex DC.) A.H. Gentry	Para, Brazil	Fonseca 408B (SPF)	MN416920	MN447875	MN445259	MN445218	MN447831
Jacaranda crassifolia Morawetz	Rio de Janeiro, Brazil	Gottsberger 11-12878 (MO)	MN416921	MN447876	MN445256	MN445219	MN447832
Jacaranda cuspidifolia Marti. ex DC.	Fairchild Tropical Botanic Gardens, USA, in cult.	T. Berman 2013-003 (FTG)	MN416922	MN447877	MN445260	MN445220	MN447833
Jacaranda decurrens Cham.	Sao Paulo, Brazil	Lombardi 6445 (NY)	MN416923	MN447878	MN445261	MN445221	MN447834
Jacaranda ekmanii Alain	Dominican Republic	Liogier (GH)	MN416924	MN447879	MN445262	MN445222	MN447835
Jacaranda glabra (DC.) Bureau & K. Schum.	Napo, Ecuador	D. Irvine 832 (F)	-	-	-	MN445223	
Jacaranda grandifoliolata A.H. Gentry	Bahia, Brazil	Fonseca 389 (SPF)	MN416943	MN447880	MN445263	MN445243	MN447853
Jacaranda irwinii A.H. Gentry	Bahia, Brazil	Fonseca 383 (SPF)	MN416925	MN447881	MN445264	MN445224	MN447837
Jacaranda jasminoides (Thunb.) Sandwith	Parnamirim, Brazil	Neto 3440 (NY)	MN416926	MN447882	MN445265	MN445225	MN447838
Jacaranda micrantha Cham.	Brazil	A. Kegler 383 (MO)	MN416927	MN447883	MN445266	MN445226	MN447839
Jacaranda microcalyx A.H. Gentry	Bahia, Brazil	Gentry & Zardini 49997 (MO)	MN416928	MN447884	MN445267	MN445227	MN447840
Jacaranda mimosifolia D. Don	Bahia, Brazil, in cult.	Fonseca 371 (SPF)	MN416929	MN447885	MN445268	MN445228	MN447841
Jacaranda montana Morawetz	Sao Paulo, Brazil	Kubitzki 81-27 (NY)	MN416930	MN447886	MN445269	MN445229	MN447842
Jacaranda mutabilis Hassl.	Paraguay	N. Soria 1785 (MO)	-	MN447887	MN445270	MN445230	MN447843

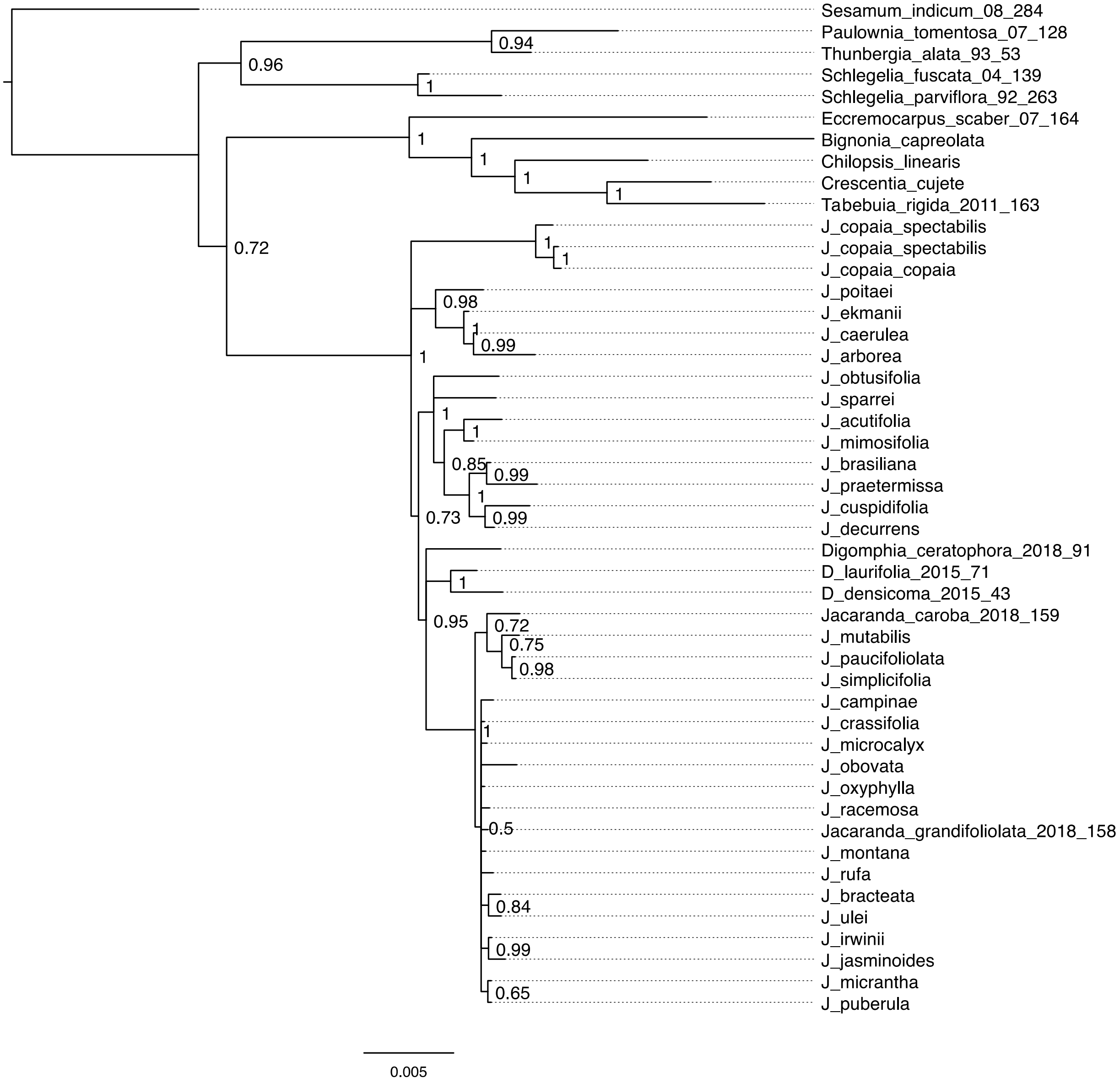
Jacaranda obovata Cham.	Minas Gerais, Brazil	R.M. Castro 610 (MO)	MN416931	MN447888	MN445271	MN445231	-
Jacaranda obtusifolia Bonpl.	Colombia	Frost 13-013 (WTU)	MN416932	MN447889	MN445272	MN445232	MN447844
Jacaranda oxyphylla Cham.	Parana, Brazil	Krapovickas & Cristobal 40766 (GH)	MN416933	MN447890	MN445273	MN445233	MN447845
Jacaranda paucifoliolata Mart. ex DC.	Minas Gerais, Brazil	V.C. Souza 10154 (MO)	MN416934	MN447891	MN445274	MN445234	-
Jacaranda poitaei Urb.	Peravia, Dominican Republic	Gentry & Zanoni 50507 (MO)	MN416935	-	MN445275	MN445235	-
Jacaranda praetermissa Sandwith.	Brazil	R.S. Albino 11474 (MO)	MN416936	MN447892	MN445276	MN445236	MN447846
Jacaranda puberula Cham.	Rio de Janeiro, Brazil	V.F. Mansono 07-406 (MO)	MN416937	MN447893	MN445277	MN445237	MN447847
Jacaranda racemosa Cham.	Minas Gerais, Brazil	V.C. Souza 8300 (MO)	MN416938	MN447894	MN445278		-
Jacaranda rufa Silva Manso	Bolivia: Vaca Diez, Beni	J.C. Solomon 7732 (MO)	MN416939	MN447895	MN445279	MN445238	MN447848
Jacaranda simplicifolia K. Schum. ex. Bureau & K. Schum.	Maranhao, Brazul	Rosario and Santos 1073 (NY)	MN416940	MN447896	-	MN445239	MN447849
Jacaranda sparrei A.H. Gentry	Waimea, HI, USA, in cult.	H. Descimon s.n. (MO)	AF102631.1	MN447897	FJ870040.1	MN445240	MN447850
Jacaranda ulei Bureau & K. Schum.	Para, Brazil	Fonseca 406 (SPF)	MN416941	MN447898	MN445280	MN445241	MN447851
Paulownia tomentosa Steud.	University of Washington Arboretum, Seattle, USA, in cult.	RGO 88-008 (WTU)	L36406.1	-	-	MN445203	MN447854
Schlegelia fuscata A.H. Gentry	Ecuador, Imbabura, Ibarra	John L. Clark 8578 (MO)	HQ384828.1	MN447861	-	MN445204	MN447855
Schlegelia parviflora (Oerst.) Monach.	Aragua, Venezuela	Gentry 14221 (MO)	L36410.1	-	MN445246	MN445205	-
Sesamum indicum L.	Côte d'Ivoire	G. A. Ambe 48 (MO)	L36413.1	MN447862	AF479010.1	MN445206	MN447856
Thunbergia alata Bojer x Sims	Denver Botanical Garden, in cult.	no voucher	-	MN447863	KT075029.1	-	-



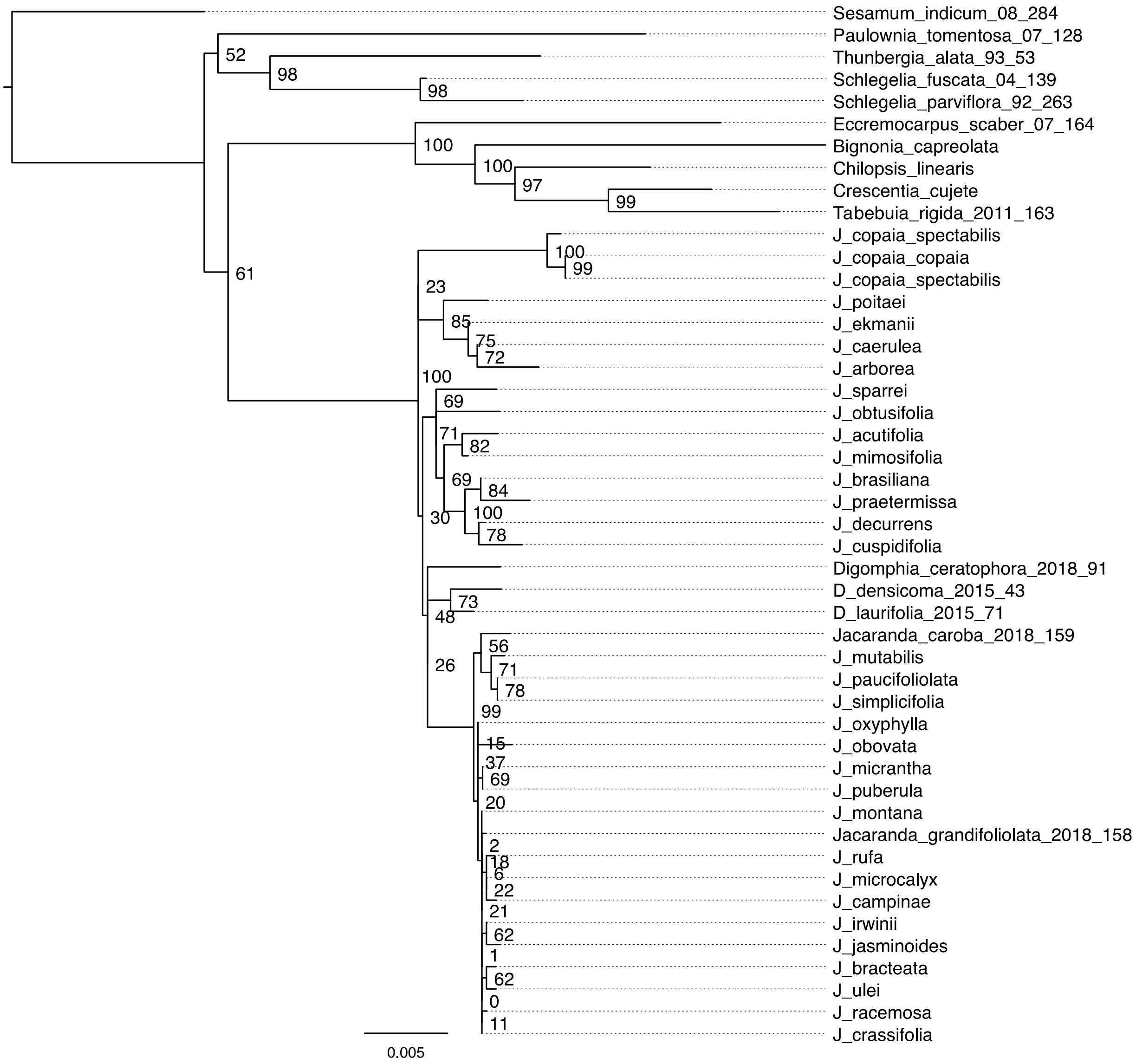
Appendix 2.4 Bayesian majority rule consensus tree derived from the analysis of the concatenated nuclear dataset. Numbers on nodes indicate posterior probabilities.



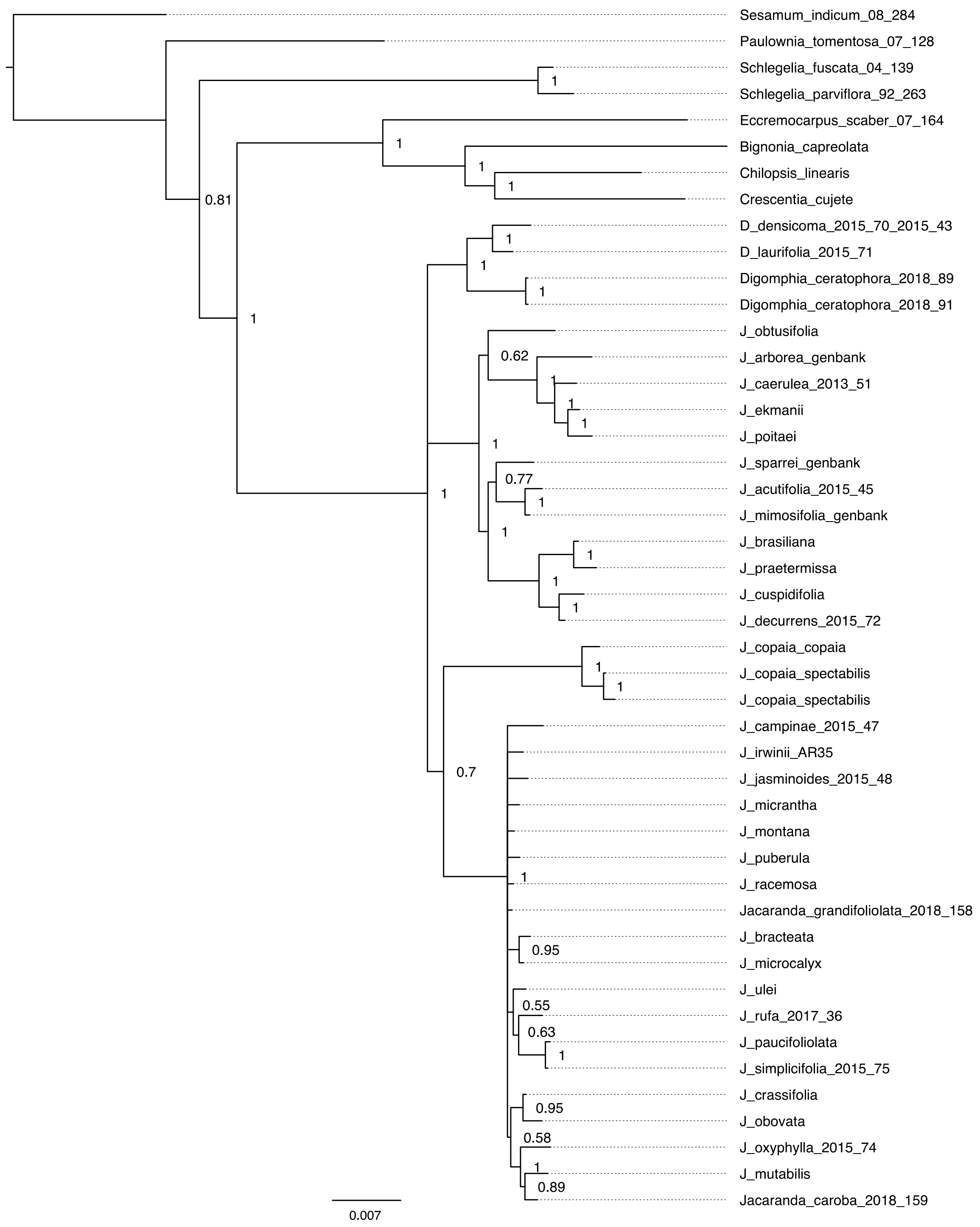
Appendix 2.5 Maximum likelihood tree derived from analysis of the concatenated nuclear dataset. Numbers on nodes indicate bootstrap values.



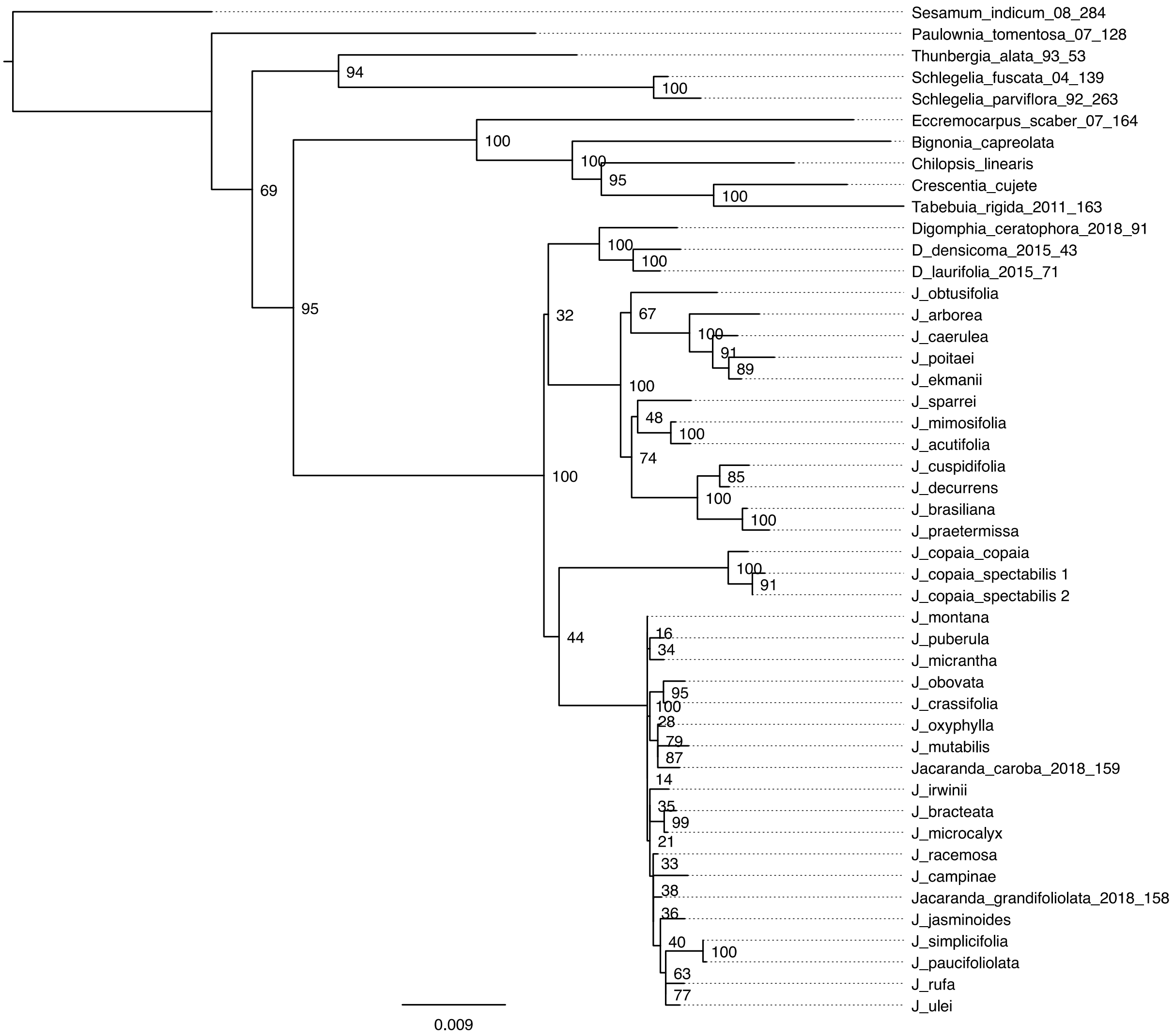
Appendix 2.6 Bayesian majority rule consensus tree derived from the analysis of the concatenated chloroplast dataset. Numbers on nodes indicate posterior probabilities.



Appendix 2.7 Maximum likelihood tree derived from analysis of the concatenated chloroplast dataset. Numbers on nodes indicate bootstrap values.



Appendix 2.8 Bayesian majority rule consensus tree derived from analysis of the concatenated nuclear and chloroplast datasets. Numbers on nodes indicate posterior probabilities.



Appendix 2.9 Maximum likelihood tree derived from analysis of the concatenated nuclear and chloroplast datasets. Numbers on nodes indicate bootstrap values.

Appendix 3.1 Voucher information for sampled taxa. See Index Herbariorum (http://sweetgum.nybg.org/science/ih/) for herbarium information. X represents sequences to be submitted to GenBank.

Taxa	**Locality**	**Collector and Collection Number**	**Genbank *ndhF***	**Genbank *rpl*32-*trn*L**	**Genbank *trn*L-F**	**Genbank rbcL**	**Genbank matK**	**Genbank ETS**	**Genbank ITS**	**Genbank PPR62**
Antirrhinum majus L.			L36392.1		AY591322.1		KX783633.1			
Bignonia capreolata L.	RBG Kew, UK, living acc. 1980-3846	no voucher	FJ887855.1	MN447857	FJ870021.1	HQ384884.1		MN445199	x	MN447816
Buddleja davidii Franch.			AF130143.1		AF380861.1	HQ384884.1	HQ384518.1			
Chilopsis linearis D. Don	RSA Bot Gardens, California, USA, in cult.	no voucher	FJ887856.1	MN447858	FJ870024.1	JN796935.1		MN445200	x	MN447817
Crescentia cujete L.	Puerto Rico	SOG PR 009 (WTU)	MN416908	MN447859	MN445244	KJ082242.1		MN445201	x	MN447817
Cuspidaria laterifolia (Mart.) DC.			DQ222575.1	KP757339.1	KP757339.1					
Digomphia ceratophora A.H. Gentry	Guainia, Inírida, Colombia	AMB 616 (WTU)	MN416909	MN447864	MN445247	x		MN445209		MN447822
Digomphia densicoma (Mart. ex DC.) Pilg.	Amazonas, Brazil	Acevedo-Rodriguez 14674 (US)	MN416910	MN447865	MN445248	x		MN445207	x	MN447820
Digomphia laurifolia Benth.	Bolivar, Venezuela	Huber 11149 (NY)	MN416911	MN447866	MN445249	MF786310.1		MN445208		MN447821
Eccremocarpus scaber Ruiz & Pav.	Seattle, Washington, USA. escaped from cult.	RGO 07-155 (WTU)	MN416912	MN447860	MN445245	AF102630.1		MN445202	x	MN447819
Fridericia speciosa Mart.			DQ222584.1	KC914604.1						
Jacaranda acutifolia Bonpl.	Santa Cruz, Bolivia	Nee 50718 (NY)	MN416913	MN447867	MN445250			MN445210	x	MN447823
Jacaranda arborea Urb.	Jardin Botanica Nacional, Havana, Cuba, in cult.	R. G. Olmstead 1996-96 (WTU)	FJ887866.1	-	FJ870039.1	FJ870008.1		MN445211		MN447824
Jacaranda bracteata Bureau & K. Schum.	Rio de Janeiro, Brazil	W. Bensen 39 (F)	MN416914	MN447868	MN445251	x		MN445212	x	MN447825
Jacaranda brasiliana (Lam.) Pers.	Bahia, Brazil	H. Irwin et al. 31597 (F)	MN416915	MN447869	MN445252	x		MN445213	x	MN447826
Jacaranda caerulea (L.) Juss.	Fairchild Tropical Botanic Gardens, Florida, USA, in cult.	T. Berman 2013-001 (FTG)	MN416916	MN447870	MN445253	x		MN445214	x	MN447827
Jacaranda campinae A.H. Gentry & Morawetz	Amazonas, Brazil	Ferreira 5816 (NY)	MN416917	MN447871	MN445254	x		MN445215		MN447828
Jacaranda caroba (Vell.) DC.	University of Sao Paulo, Sao Paulo, Brazil, in cult.	Living Acccession (SPF)	MN416942	MN447872	MN445255			MN445242		MN447852
Jacaranda copaia ssp. *copaia* (Aubl.) D. Don	Amapa, Brazil	Daly and Souza 3937 (NY)	MN416919	MN447874	MN445258	JQ626146.1	JQ626519.1	MN445217		MN447830
Jacaranda copaia ssp. *spectabilis* (Mart. ex A.P. DC.) A.H. Gentry	Roraima, Brazil	Hopkins et al. 620 (GH)	MN416918	MN447873	MN445257	x		MN445216	x	MN447829
Jacaranda copaia ssp. spectabilis (Mart. ex DC.) A.H. Gentry	Para, Brazil	Fonseca 408B (SPF)	MN416920	MN447875	MN445259			MN445218		MN447831

Jacaranda crassifolia Morawetz	Rio de Janeiro, Brazil	Gottsberger 11-12878 (MO)	MN416921	MN447876	MN445256			MN445219		MN447832
Jacaranda cuspidifolia Mart. ex DC.	Fairchild Tropical Botanic Gardens, Florida, USA, in cult.	T. Berman 2013-003 (FTG)	MN416922	MN447877	MN445260	JX856717.1		MN445220		MN447833
Jacaranda duckei Vattimo	Brazil	A. Amorim 970 (MO)				x		x		
Jacaranda decurrens Cham.	São Paulo, Brazil	Lombardi 6445 (NY)	MN416923	MN447878	MN445261	x		MN445221	x	MN447834
Jacaranda ekmanii Alain	Dominican Republic	Liogier (GH)	MN416924	MN447879	MN445262	x		MN445222		MN447835
Jacaranda glabra (DC.) Bureau & K. Schum.	Napo, Ecuador	D. Irvine 832 (F)	-	-	-	x		MN445223	x	
Jacaranda grandifoliolata A.H. Gentry	Bahia, Brazil	Fonseca 389 (SPF)	MN416943	MN447880	MN445263	x		MN445243		MN447853
Jacaranda intricata A.H.Gentry & Morawetz	Goias, Brazil	Morawetz 22-151280 (MO)								
Jacaranda irwinii A.H. Gentry	Bahia, Brazil	Fonseca 383 (SPF)	MN416925	MN447881	MN445264	x		MN445224		MN447837
Jacaranda jasminoides (Thunb.) Sandwith	Parnamirim, Brazil	Neto 3440 (NY)	MN416926	MN447882	MN445265			MN445225		MN447838
Jacaranda micrantha Cham.	Brazil	A. Kegler 383 (MO)	MN416927	MN447883	MN445266	MG718468.1	MG718974.1	MN445226		MN447839
Jacaranda microcalyx A.H. Gentry	Bahia, Brazil	Gentry & Zardini 49997 (MO)	MN416928	MN447884	MN445267			MN445227	x	MN447840
Jacaranda mimosifolia D. Don	Bahia, Brazil, in cult.	Fonseca 371 (SPF)	MN416929	MN447885	MN445268	HQ384888.1	AJ429328.1	MN445228		MN447841
Jacaranda montana Morawetz	São Paulo, Brazil	Kubitzki 81-27 (NY)	MN416930	MN447886	MN445269			MN445229		MN447842
Jacaranda mutabilis Hassl.	Paraguay	N. Soria 1785 (MO)	-	MN447887	MN445270	x		MN445230		MN447843
Jacaranda obovata Cham.	Minas Gerais, Brazil	R.M. Castro 610 (MO)	MN416931	MN447888	MN445271			MN445231		-
Jacaranda obtusifolia Bonpl.	Colombia	Frost 13-013 (WTU)	MN416932	MN447889	MN445272	x		MN445232		MN447844
Jacaranda oxyphylla Cham.	Parana, Brazil	Krapovickas & Cristobal 40766 (GH)	MN416933	MN447890	MN445273			MN445233	x	MN447845
Jacaranda paucifoliolata Mart. ex DC.	Minas Gerais, Brazil	V.C. Souza 10154 (MO)	MN416934	MN447891	MN445274			MN445234		-
Jacaranda poitaei Urb.	Peravia, Dominican Republic	Gentry & Zanoni 50507 (MO)	MN416935	-	MN445275	x		MN445235	x	-
Jacaranda praetermissa Sandwith.	Brazil	R.S. Albino 11474 (MO)	MN416936	MN447892	MN445276			MN445236		MN447846
Jacaranda puberula Cham.	Rio de Janeiro, Brazil	V.F. Mansono 07-406 (MO)	MN416937	MN447893	MN445277	x	MG718684.1	MN445237		MN447847
Jacaranda racemosa Cham.	Minas Gerais, Brazil	V.C. Souza 8300 (MO)	MN416938	MN447894	MN445278					-
Jacaranda rufa Silva Manso	Vaca Diez, Beni, Bolivia:	J.C. Solomon 7732 (MO)	MN416939	MN447895	MN445279	x		MN445238		MN447848
Jacaranda simplicifolia K. Schum. ex. Bureau & K. Schum.	Maranhao, Brazil	Rosario and Santos 1073 (NY)	MN416940	MN447896	-	x		MN445239	x	MN447849
Jacaranda sparrei A.H. Gentry	Waimea, Hawaii, USA, in cult.	H. Descimon s.n. (MO)	AF102631.1	MN447897	FJ870040.1	AF102647.1		MN445240		MN447850
Jacaranda ulei Bureau & K. Schum.	Para, Brazil	Fonseca 406 (SPF)	MN416941	MN447898	MN445280	x		MN445241		MN447851
Paulownia tomentosa Steud.	University of Washington Arboretum, Seattle, Washington, USA, in cult.	RGO 88-008 (WTU)	L36406.1	-	-			MN445203		MN447854

Schlegelia fuscata A.H. Gentry	Ibarra, Imbabura, Ecuador,	John L. Clark 8578 (MO)	HQ384828.1	MN447861	-			MN445204	x	MN447855
Schlegelia parviflora (Oerst.) Monach.	Aragua, Venezuela	Gentry 14221 (MO)	L36410.1	-	MN445246			MN445205	x	-
Sesamum indicum L.	Côte d'Ivoire	G. A. Ambe 48 (MO)	L36413.1	MN447862	AF479010.1			MN445206		MN447856
Tanaecium bilabiatum (Sprague) L.G. Lohmann				KP757333.1		MF786235.1				
Thunbergia alata Bojer x Sims	Denver Botanical Garden, Denver, Colorado, USA, in cult.	no voucher	-	MN447863	KT075029.1		HQ384512.1	-		-
Xylophragma myrianthum (Cham.) Sprague			DQ222648.1	KP757334.1	FJ870065.1					

Appendix 3.2 Areas allowed matrix used in BioGeoBEARS analysis. 1 represents regions or biomes present, while 0 represents regions or biomes not present.

9 Ma - present	A	F	I	C	W	M	H
A	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1
I	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1
W	1	1	1	1	1	1	1
M	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1
13 Ma to 9 Ma	A	F	I	C	W	M	H
A	1	1	1	0	0	1	0
F	1	1	1	0	0	1	0
I	1	1	1	0	0	1	0
C	0	0	0	0	0	0	0
W	0	0	0	0	0	0	0
M	1	1	1	0	0	1	0
H	0	0	0	0	0	0	0
23 Ma to 13 Ma	A	F	I	C	W	M	H
A	1	1	1	0	0	1	0
F	1	1	1	0	0	1	0
I	1	1	1	0	0	1	0
C	0	0	0	0	0	0	0
W	0	0	0	0	0	0	0
M	1	1	1	0	0	1	0
H	0	0	0	0	0	0	0

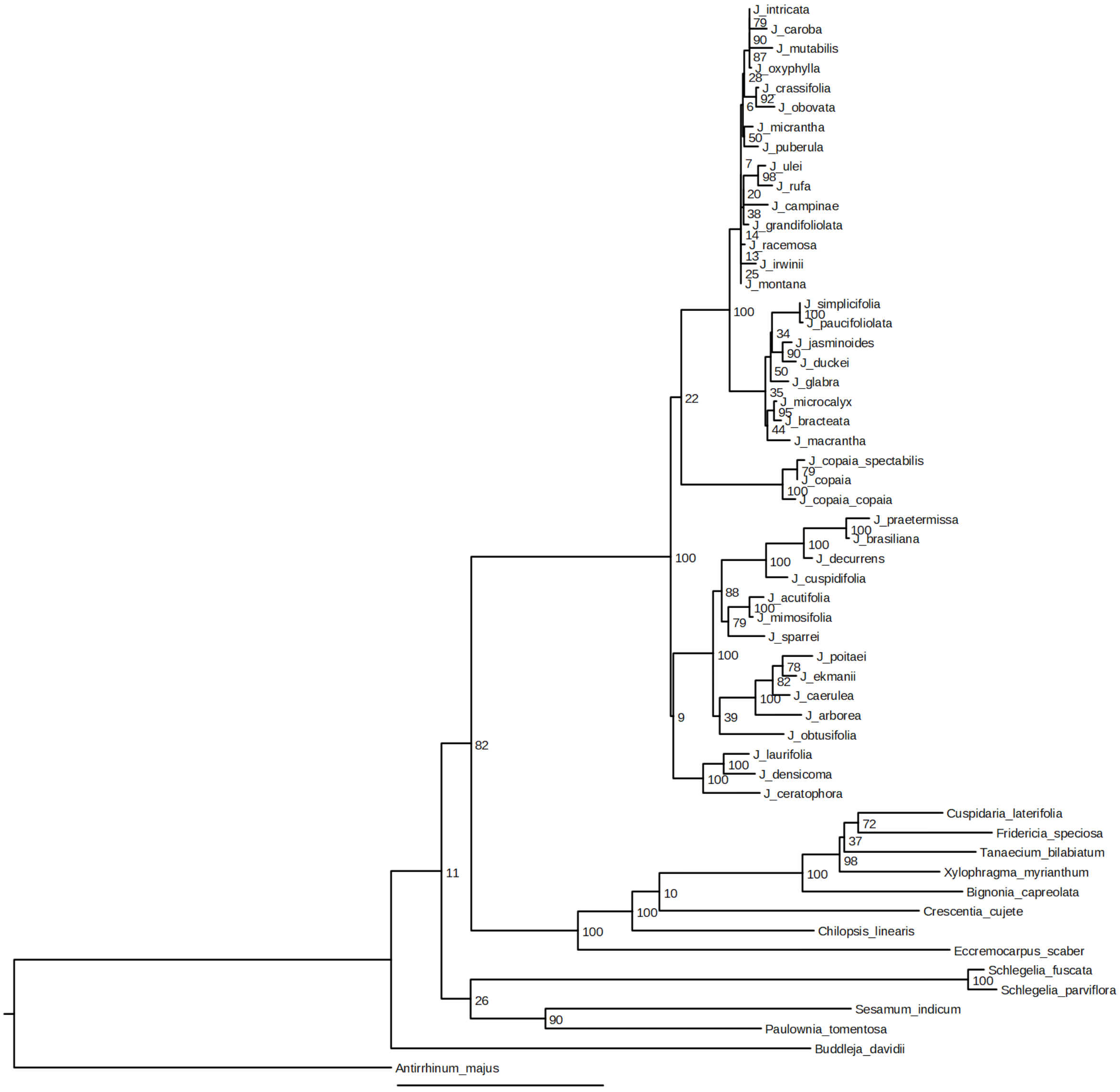
Appendix 3.3 Multiplier dispersal matrix used in BioGeoBEARS analysis. 1 represents high dispersal probability, 0.5 represents medium dispersal probability, and 0.1 represents low dispersal probability. 0 represents biomes not present at each time slice.

9 Ma - present	A	F	I	C	W	M	H
A	1	0.5	0.1	1	1	1	1
F	0.5	1	0.1	1	0.1	0.1	1
I	0.1	0.1	1	0.1	0.1	0.5	0.1
C	1	1	0.1	1	0.5	0.1	1
W	1	0.1	0.1	0.5	1	0.1	1
M	1	0.1	0.5	0.1	0.1	1	0.1
H	1	1	0.1	1	1	0.1	1
13 Ma to 9 Ma	A	F	I	C	W	M	H
A	1	1	0.1	0	0	0.5	0
F	1	1	0.1	0	0	0.1	0
I	0.1	0.1	1	0	0	0.5	0
C	0	0	0	0	0	0	0
W	0	0	0	0	0	0	0
M	0.5	0.1	0.5	0	0	1	0
H	0	0	0	0	0	0	0
23 Ma to 13 Ma	A	F	I	C	W	M	H
A	1	1	0.1	0	0	0.5	0
F	1	1	0.1	0	0	0.1	0
I	0.1	0.1	1	0	0	0.5	0
C	0	0	0	0	0	0	0
W	0	0	0	0	0	0	0
M	0.5	0.1	0.5	0	0	1	0
H	0	0	0	0	0	0	0

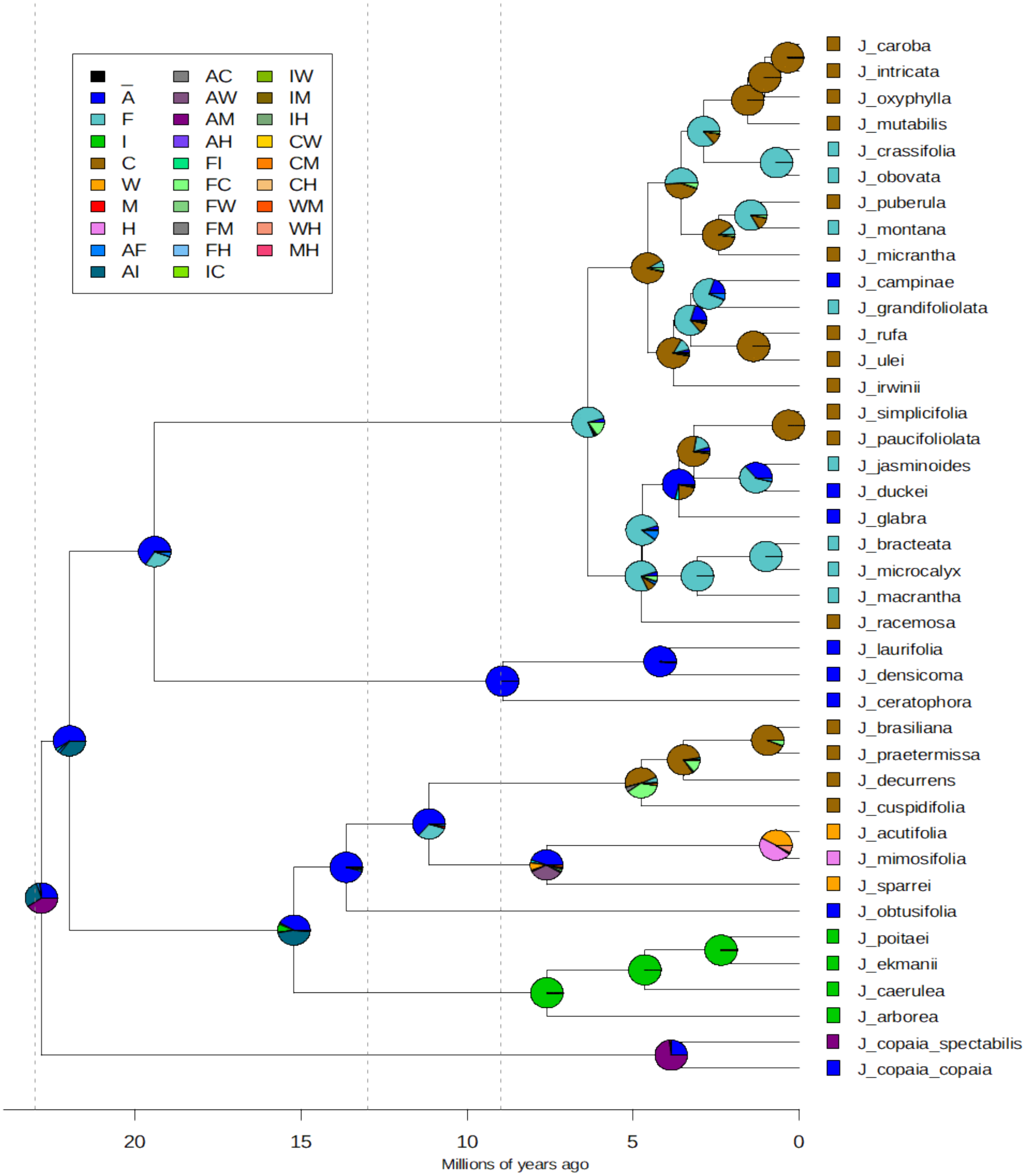
Appendix 3.4 Sampling information for *Jacaranda* species used in diversification analyses.

Clade	# Taxa Sampled	# Taxa Unsampled	Proportion Sampled
Jacaranda sect. *Copaia*	2	0	1
Jacaranda sect. *Jacaranda*	12	5	0.706
Jacaranda sect. *Nematopogon*	3	0	1
Jacaranda sect. *Dilobos*	23	8	0.742
Total	40	13	0.755
Biome			
Amazon	9	6	0.6
Atlantic Forest	8	1	0.89
Cerrado	19	4	0.83
West Indies	4	2	0.67
Total	40	13	

Appendix 3.5 Bayesian maximum clade credibility tree generated from analysis of the concatenated dataset. Values at nodes indicate posterior probability support values.



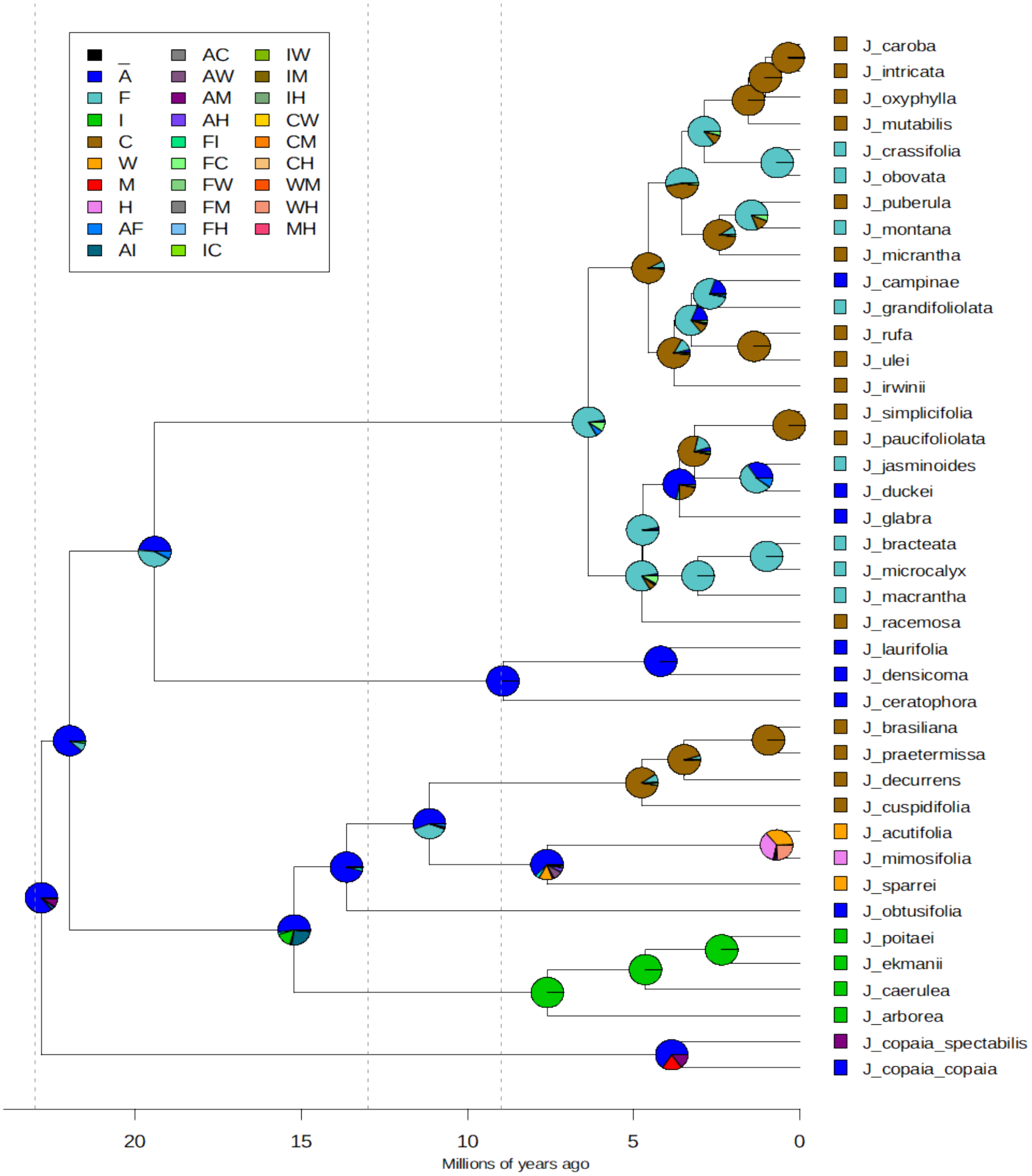
Appendix 3.6 Maximum likelihood tree generated from analysis of the concatenated dataset. Values at nodes indicate bootstrap support values.



Appendix 3.7 Ancestral state reconstruction of *Jacaranda* using the DEC + J model of range evolution. Areas were coded as follows: Amazon (A), Atlantic Forest (F), Cerrado (C), West Indies (I), Dry Western South America (W), Chaco (H), and Mesoamerica (M). Pie charts represent probabilities of ancestral biomes at each node. Labels at tips represent biomes assigned to each taxon prior to analysis.

Appendix 3.8 Node information for the *Jacaranda* phylogeny and the BioGeoBEARS ancestral state reconstruction. Posterior probability (PP) support values are from the maximum clade credibility tree generated in BEAST and MrBayes from analysis of the concatenated dataset, and bootstrap support values are from the tree generated in RAxML from analysis of the the concatenated dataset. Median crown ages and 95% highest posterior distributions (HPD) are from the time calibrated tree generated in BEAST. Ancestral state probabilities for each coded region are from BioGeoBEARS under the DEC + J model. Coded regions are as follows: Amazon (A), Atlantic Forest (F), West Indies (I), Cerrado (C), Dry Western South America (W), Mesoamerica (M), and Chaco (H).

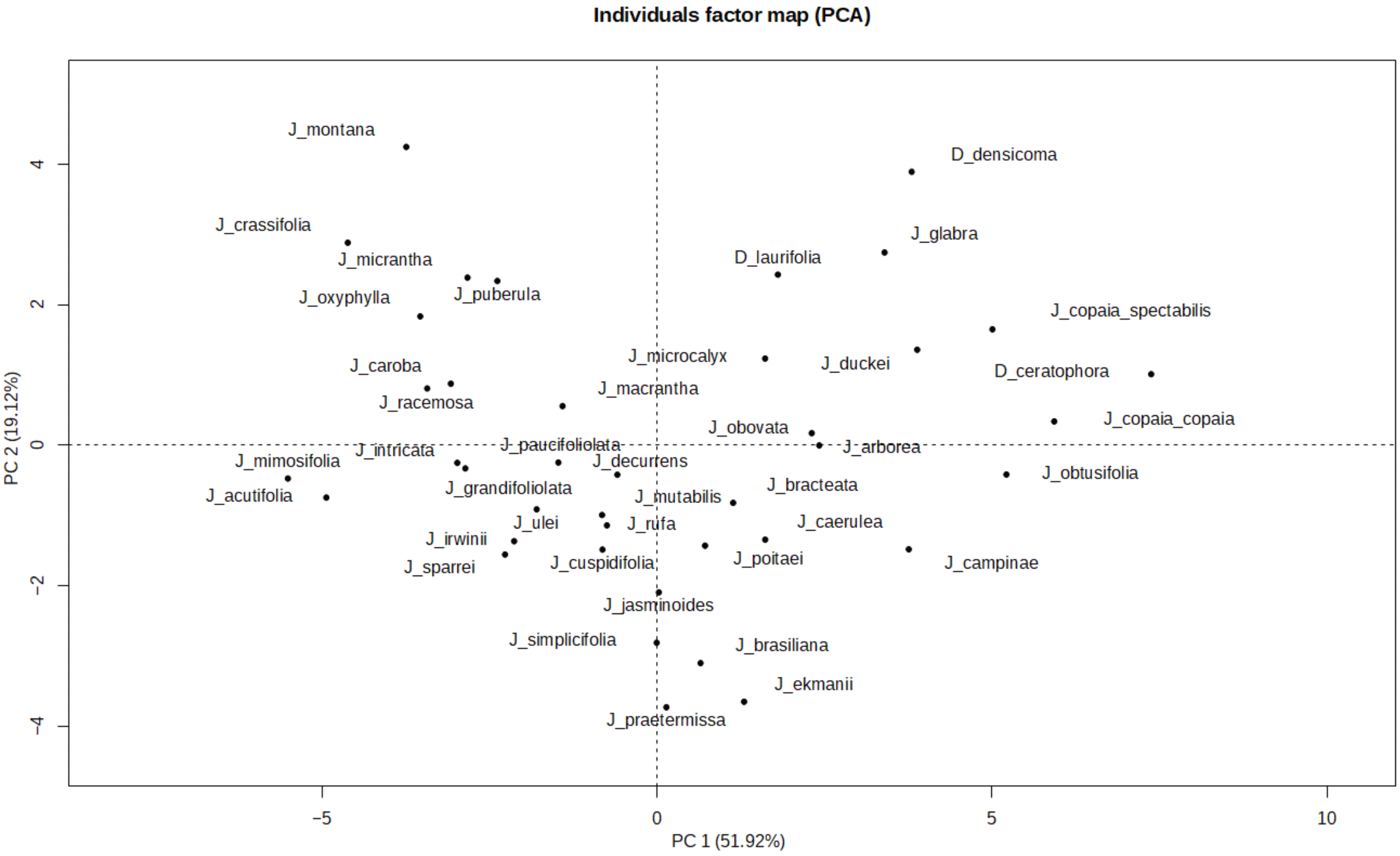
Clade	PP (BEAST)	PP (MrBayes)	Bootstrap	Median Crown Age (Ma)	95% HPD (Ma)	A	F	I	C	W	M	H
Bignoniaceae	1	0.92	82	53.99	48.65-59.08							
Calibration 2	1	1	98	34.17	33.75-35.12							
Jacaranda	1	1	100	22.82	15.29-31.96	0.263371	0.001355	0.000163	0	0	0.006407	0
Sect. *Jacaranda* + Sect. *Nematopogon* + Sect. *Dilobos*	0.3			21.97	14.77-31.23	0.58907	0.025019	0.00458	0	0	0.000454	0
Sect. *Nematopogon* + Sect. *Dilobos*	0.6			19.41	12.49-28.29	0.65734	0.287782	4.42E-05	0	0	0.000364	0
Sect. *Copaia*	1	1	100	3.86	1.4-7.64	0.246796	4.19E-06	9.07E-07	1.88E-08	1.16E-08	0.021716	1.77E-08
Sect. *Jacaranda*	1	1	100	15.22	9.59-21.95	0.417227	0.012598	0.092247	0	0	0.003571	0
Sect. *Nematopogon*	1	1	100	8.94	4.06-15.35	0.994743	6.11E-05	6.70E-07	5.21E-11	3.68E-11	8.22E-05	4.91E-11
Sect. *Dilobos*	1	1	100	6.36	3.8-9.93	0.042668	0.766518	9.43E-07	0.003499	1.52E-08	5.73E-06	2.46E-07
Sect. *Jacaranda* 1 (*J. arborea* + *J. caerulea* + *J. ekmanii* + *J. poitaei*)	1	1	100	7.6	3.83-12.72	6.22E-06	2.10E-05	0.99328	1.34E-09	5.38E-10	0.000218	1.26E-09
Sect. *Jacaranda* 2 (*remaining species)*	0.99			13.64	8.51-20.21	0.954865	0.022628	0.000183	0	0	0.003084	0
Sect. *Jacaranda* 3 (*remaining species* without *J. obtusifolia*)	1			11.15	6.73-16.98	0.636778	0.314307	0.002949	0	0	0.025445	0
Sect. *Jacaranda* 2 (*J. acutifolia*, *J. mimosifolia*, *J. sparrei*)	0.99	1	79	7.61	2.99-12.9	0.446001	0.02802	0.001459	1.87E-05	0.085446	0.004949	0.008262
Sect. *Jacaranda* 3 (*J. brasiliana*, *J. cuspidifolia*, *J. decurrens*, *J. praetermissa*)	1	1	100	4.76	2.44-8.22	0.006945	0.060096	7.33E-05	0.47768	2.19E-06	0.000433	2.87E-05



Appendix 3.9 Ancestral state reconstruction of *Jacaranda* using the DIVALIKE + J model of range evolution. Areas were coded as follows: Amazon (A), Atlantic Forest (F), Cerrado (C), West Indies (I), Dry Western South America (W), Chaco (H), and Mesoamerica (M). Pie charts represent probabilities of ancestral biomes at each node. Labels at tips represent biomes assigned to each taxon prior to analysis.

Appendix 3.10 Node information for the *Jacaranda* phylogeny and the BioGeoBEARS ancestral state reconstruction. Posterior probability (PP) support values are from the maximum clade credibility tree generated in BEAST and MrBayes from analysis of the concatenated dataset, and bootstrap support values are from the tree generated in RAxML from analysis of the the concatenated dataset. Median crown ages and 95% highest posterior distributions (HPD) are from the time calibrated tree generated in BEAST. Ancestral state probabilities for each coded region are from BioGeoBEARS under the DIVALIKE + J model. Coded regions are as follows: Amazon (A), Atlantic Forest (F), West Indies (I), Cerrado (C), Dry Western South America (W), Mesoamerica (M), and Chaco (H).

Clade	PP (BEAST)	PP (MrBayes)	Bootstrap	Median Age (Ma)	95% HPD (Ma)	A	F	I	C	W	M	H
Bignoniaceae	1	0.92	82	53.99	48.65-59.08							
Calibration 2	1	1	98	34.17	33.75-35.12							
Jacaranda	1	1	100	22.82	15.29-31.96	0.854946	0.00362	0.000211	0	0	0.006148	0
Sect. *Jacaranda* + Sect. *Nematopogon* + Sect. *Dilobos*	0.3			21.97	14.77-31.23	0.883231	0.088941	0.019597	0	0	0.001148	0
Sect. *Nematopogon* + Sect. *Dilobos*	0.6			19.41	12.49-28.29	0.479645	0.437893	5.79E-05	0	0	0.000574	0
Sect. *Copaia*	1	1	100	3.86	1.4-7.64	0.656554	4.58E-05	7.34E-06	1.97E-07	7.86E-08	0.19372	1.86E-07
Sect. *Jacaranda*	1	1	100	15.22	9.59-21.95	0.521059	0.026269	0.163377	0	0	0.004315	0
Sect. *Nematopogon*	1	1	100	8.94	4.06-15.35	0.999554	8.86E-05	8.82E-07	8.69E-11	5.46E-11	0.000116	8.20E-11
Sect. *Dilobos*	1	1	100	6.36	3.8-9.93	0.022889	0.805804	8.72E-07	0.004877	1.49E-08	6.34E-06	3.69E-07
Sect. *Jacaranda* 1 (*J. arborea* + *J. caerulea* + *J. ekmanii* + *J. poitaei*)	1	1	100	7.6	3.83-12.72	6.66E-06	3.42E-05	0.999612	2.68E-09	8.73E-10	0.000268	2.54E-09
Sect. *Jacaranda* 2 (*remaining species)*	0.99			13.64	8.51-20.21	0.955887	0.036009	0.000149	0	0	0.003113	0
Sect. *Jacaranda* 3 (*remaining species* without *J. obtusifolia*)	1			11.15	6.73-16.98	0.558218	0.38775	0.001687	0	0	0.017702	0
Sect. *Jacaranda* 2 (*J. acutifolia, J. mimosifolia, J. sparrei*)	0.99	1	79	7.61	2.99-12.9	0.627612	0.04045	0.001914	3.61E-05	0.133541	0.006572	0.012502
Sect. *Jacaranda* 3 (*J. brasiliana, J. cuspidifolia, J. decurrens, J. praetermissa*)	1	1	100	4.76	2.44-8.22	0.00892	0.08785	7.71E-05	0.875465	4.02E-06	0.000573	5.63E-05



Appendix 3.11 Principal component analysis of *Jacaranda* species and outgroups using 19 climatic variables.

Appendix 3.12 Climatic variable contributions to PC 1 and PC 2.

Variable	PC 1	PC 2
Mean Annual Temperature	8.200746964	3.920803
Mean Diurnal Range	3.971773663	1.986212
Isothermality	2.42287728	0.302826
Temperature Seasonality	4.388434943	1.154042
Max Temperature Warmest Month	5.427706082	6.918018
Min Temperature Coldest Month	9.409255752	1.217512
Annual Temperature Range	6.805653668	0.283699
Mean Temperature Wettest Quarter	5.794294633	3.853241
Mean Temperature Driest Quarter	8.874240608	3.151067
Mean Temperature Warmest Quarter	6.770215199	3.190772
Mean Temperature Coldest Quarter	8.728299906	3.507131
Annual Precipitation	5.182222128	8.703623
Precipitation Wettest Month	3.000001161	5.289315
Precipitation Driest Month	3.665643693	12.95529
Precipitation Seasonality	2.434722678	6.110383
Precipitation Wettest Quarter	2.932704363	5.593859
Precipitation Driest Quarter	4.137869218	12.61117
Precipitation Warmest Quarter	0.952450641	15.09535
Precipitation Coldest Quarter	6.900887419	4.155688

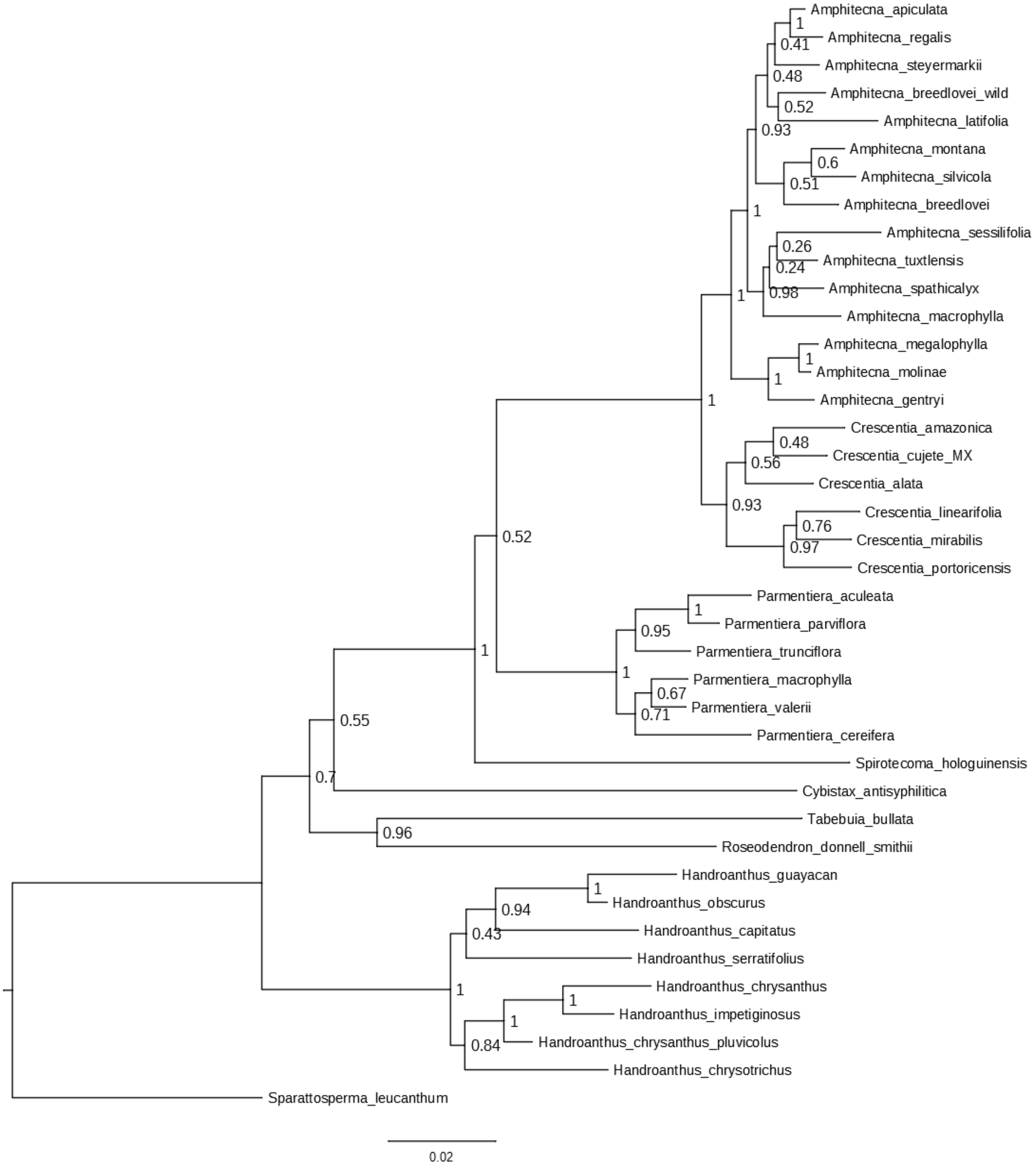
Appendix 4.1 Voucher information for sampled taxa. See Index Herbariorum (http://sweetgum.nybg.org/science/ih/) for herbarium information. X represents sequences to be submitted to GenBank.

Taxon	**Locality**	**Voucher**	***ndh*F**	***trn*L-*trn*F**	**PepC**	**ITS**
Amphitecna apiculata A.H. Gentry	México	SOG 176, WTU	X	X	X	X
Amphitecna breedlovei A.H. Gentry (upland)	México, Cult. Miranda BG, Santiago Las Tuxtlas	SOG 163, WTU	X	X		
Amphitecna breedlovei A. Gentry (lowland)	Guatemala	SOG 174. WTU	X	X	X	X
Amphitecna gentryi W.C. Burger	Costa Rica	MAB 2155, FLU	EF104996.1	EF105054.1	X	X
Amphitecna kennedyi (A.H. Gentry) A.H. Gentry	Costa Rica	SOG 133, WTU	X	X	X	
Amphitecna latifolia (Mill.) A.H. Gentry	Cuba	RGO 96-101, WTU	EF104997.1	EF175740.1	X	
Amphitecna macrophylla (Seem.) Miers ex. Baill.	México	SOG 182, WTU	X	X	X	
Amphitecna megalophylla (Don.Sm.) A.H. Gentry	Guatemala	SOG 173, WTU	X	X	X	
Amphitecna molinae L.O. Williams	Nicaragua	SOG 152, HULE	X	X	X	X
Amphitecna montana L.O. Williams	UC Berkeley	H. Forbes s.n., UC	FJ887850	FJ870014	X	X
Amphitecna regalis (Linden) A. Gentry	México	SOG 181, WTU	X	X	X	X
Amphitecna sessilifolia (Donn.Sm.) L.O. Williams (Specimen sampled identified as *A. haberi*, but is a synonym of *A. sessilifolia*)	Costa Rica	SOG 130, WTU	X	X	X	
Amphitecna silvicola L.O. Williams	México	SOG 166, WTU	X	X	X	
Amphitecna spathicalyx (A.H. Gentry) A.H. Gentry	Panamá	SOG 125, WTU	X	X	X	
Amphitecna steyermarkii (A.H. Gentry) A.H. Gentry	México	SOG 168, WTU	X	X	X	X
Amphitecna tuxtlensis A.H Gentry	México	SOG 160, WTU	EF104998.1	EF105055.1	X	X
Crescentia alata Kunth	UC Berkeley	None	FJ887856	FJ870025	X	X
Crescentia amazonica Ducke	Venezuela	Diaz 5096, NY				X
Crescentia cujete L	Puerto Rico	SOG 060, WTU	X	X		
Crescentia cujete L	México	SOG 157, WTU	X	X	X	X
Crescentia linearifolia Miers	Puerto Rico	SOG 058, WTU	EF105002.1	EF105059.1	X	X
Crescentia mirabilis Ekman ex Urb.	Cuba, Cult. Jardín Botanico Nacional, Havana	None	X	X	X	X
Crescentia portoricensis Britton	Puerto Rico	Gentry 50488, MO	AF102627	EF105060.1	X	X
Cybistax antisyphilitica (Mart.) Mart.	Bolivia	Nee and Bohs 51868	EF105003.1	EF105061.1	X	X
Handroanthus capitatus (Bureau and K. Schum.) Mattos	Guyana	KM Redden 1657, US	EF105045.1	EF105107.1	X	

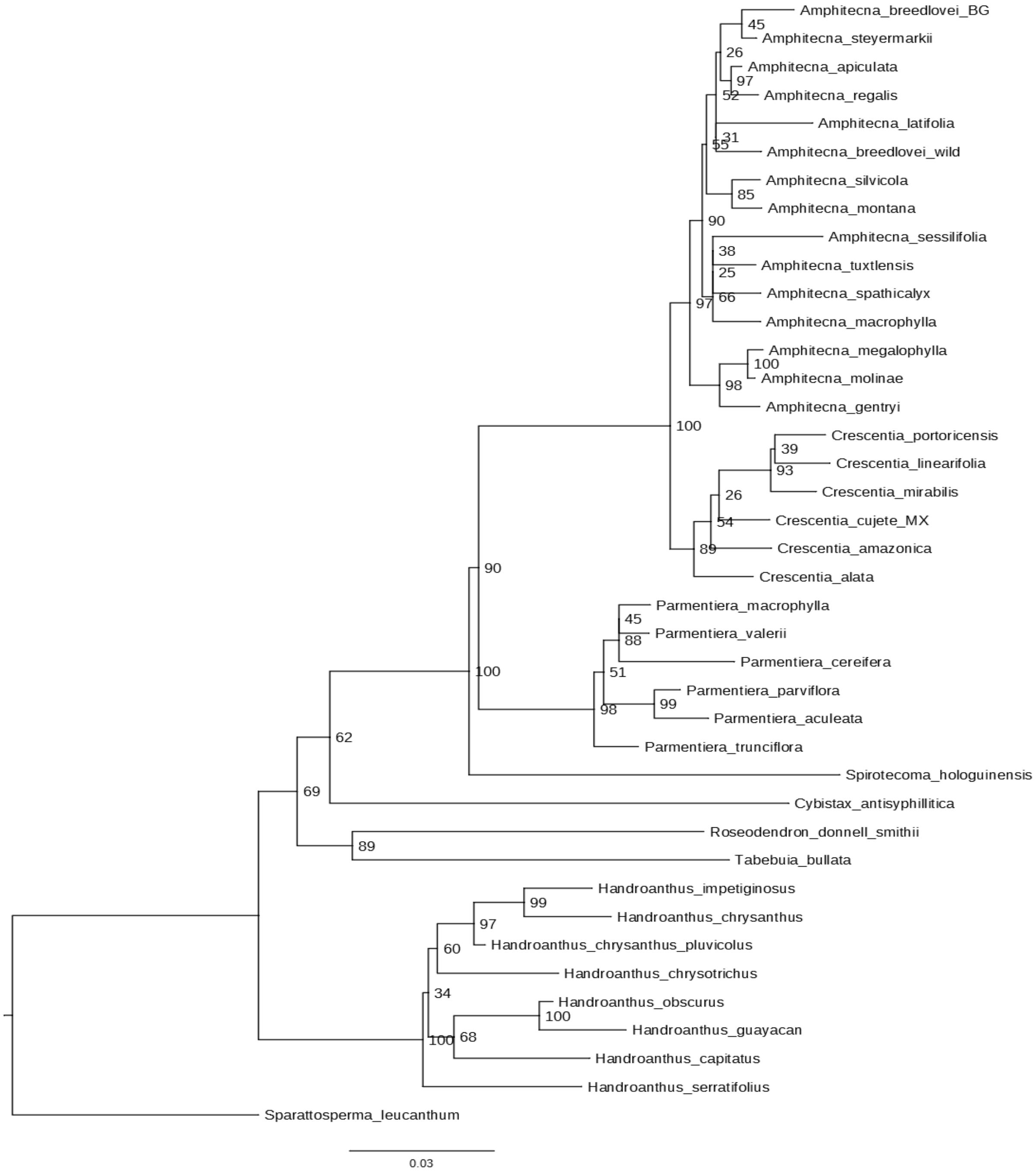
Handroanthus chrysanthus ssp. chrysanthus (Jacq.) S.O. Grose	México	SOG 164, WTU	EF105030.1	EF105090.1	X	X
Handroanthus chrysanthus ssp. pluvicolus (Jacq.) S.O. Grose	Ecuador	MAB 2512, FLU	EF105031.1	EF105091.1	X	
Handroanthus chrysotrichus (Mart. ex. DC) Mattos	Cult at UC Berkeley BG	UCBBG #85-0562	EF105032.1	EF105092.1	X	
Handroanthus guayacan (Seem.) S.O. Grose	Panamá	SOG 122, WTU	EF105033.1	EF105094.1	X	
Handroanthus impetiginosus (Mart. ex. DC) Mattos	Cult at UC Berkeley BG	None	EF105035.1	EF105097.1	X	
Handroanthus obscurus (Bureau and K.Schum.) Mattos	Guyana	HD Clarke 10977, US	EF105047.1	EF105109.1	X	
Handroanthus serratifolius (Vahl) S.O. Grose	Cult at Waimea BG	None	EF105043.1	EF105105.1	X	
Parmentiera aculeata (Kunth) Seem.	Cult Fairchild BG	FBG. #x4241A	X	X		
Parmentiera cereifera Seem.	Panamá; cult at Fairchild BG	FBG. Gard.#x 4183	X	X	X	
Parmentiera macrophylla Standl.	Panamá	SOG 126, WTU	EF105017.1	EF105077.1	X	X
Parmentiera millspaughiana L.O. Williams	Nicaragua	SOG 154, HULE	X	X		
Parmentiera parviflora Lundell	Guatemala	SOG 170, WTU	EF105018.1	EF105078.1	X	
Parmentiera trunciflora Standl. and L.O. Williams	Nicaragua	SOG 141, WTU	X	X	X	X
Parmentiera valerii Standl.	Costa Rica	SOG 135, WTU	X	X	X	X
Roseodendron donnell-smithii (Rose) Miranda	Cult. at Waimea BG	Waimea #89-P166	X	EF105093.1	X	
Sparattosperma leucanthum (Vell.) K. Schum.	Cult at Waimea BG	Waimea BG #87-5446	EF105022.1	EF105082.1	X	
Spirotecoma hologuinensis (Britton) Alain	Cuba	No Voucher	EF105024.1	EF105084.1	X	
Tabebuia bullata A.H. Gentry	Dominican Republic	SOG 77, WTU	X	X	X	

Appendix 4.2 Currently accepted species names and references in Crescentieae.

Taxon	Reference
Amphitecna apiculata A.H. Gentry	*Rhodora 79: 435 1977*
Amphitecna breedlovei A.H. Gentry	*Rhodora 79: 432 1977*
Amphitecna costata A.H. Gentry	*Rhodora 79: 431 1977*
Amphitecna donnell-smithii (Sprague) L.O. Williams	*Fieldiana, Bot. 36(4): 22 1973*
Amphitecna gentryi W.C. Burger	*Fieldiana, Bot. 36(4): 22 1973*
Amphitecna isthmica (A.H. Gentry) A.H. Gentry	*Taxon 25: 108 1976*
Amphitecna kennedyi (A.H. Gentry) A.H. Gentry	*Taxon 25: 108 1976*
Amphitecna latifolia (Mill.) A.H. Gentry	*Taxon 25: 108 1976*
Amphitecna loreae Ortiz-Rodr. & Burelo	*PhytoKeys 65: 15-23 2016*
Amphitecna lundellii A.H. Gentry	*Wrightia 7: 83 1982*
Amphitecna macrophylla (Seem.) Miers ex Baill.	*Rev. Hort. 54: 465 1882*
Amphitecna molinae L.O. Williams	*Fieldiana, Bot. 36(4): 22 1973*
Amphitecna montana L.O. Williams	*Fieldiana, Bot. 36(4): 22 1973*
Amphitecna parviflora A.H. Gentry	*Fl. Neotrop. Monogr. 25(1): 75 1980*
Amphitecna regalis (Linden) A.H. Gentry	*Fl. Neotrop. Monogr. 25(1): 76 1980*
Amphitecna sessilifolia (Donn.Sm.) L.O. Williams (includes *Amphitecna haberi* ined.)	*Fieldiana, Bot. 36(4): 25 1973*
Amphitecna silvicola L.O. Williams	*Fieldiana, Bot. 36(4): 25 1973*
Amphitecna spathicalyx (A.H. Gentry) A.H. Gentry	*Taxon 25: 108 1976*
Amphitecna steyermarkii (A.H. Gentry) A.H. Gentry	*Taxon 25: 108 1976*
Amphitecna tuxtlensis A.H. Gentry	*Fl. Neotrop. Monogr. 25(1): 81 1980*
Crescentia alata Kunth	*Nov. Gen. Sp. Pl. 3: 158 1818 publ. 1819*
Crescentia amazonica Ducke	*Arq. Inst. Biol. Veg. 4: 61 1938*
Crescentia cujete L.	*Sp. Pl. 2: 626 1753*
Crescentia linearifolia Miers	*Trans. Linn. Soc. London 26: 172 1868*
Crescentia mirabilis Ekman ex Urb.	*Repert. Spec. Nov. Regni Veg. 22: 88 1925*
Crescentia portoricensis Britton	*Bull. Torrey Bot. Club 43: 457 1916*
Parmentiera aculeata (Kunth) Seem.	*Bot. Voy. Herald 183 1854*
Parmentiera cereifera Seem.	*Bot. Voy. Herald 182 1854*
Parmentiera dressleri A.H. Gentry	*Wrightia 7(2): 85 1982*
Parmentiera macrophylla Standl.	*Publ. Field Columb. Mus., Bot. Ser. 4: 263 1929*
Parmentiera millspaughiana L.O. Williams	*Fieldiana, Bot. 36(4): 28 1973*
Parmentiera morii A.H. Gentry	*Fl. Neotrop. Monogr. 25(1): 105 1980*
Parmentiera parviflora Lundell	*Lloydia 3: 211 1940*
Parmentiera stenocarpa Dugand & L.B.Sm.	*Caldasia 7: 17 1955*
Parmentiera trunciflora Standl. & L.O. Williams	*Ceiba 3: 219 1953.*
Parmentiera valerii Standl.	*J. Wash. Acad. Sci. 17: 16 1927*



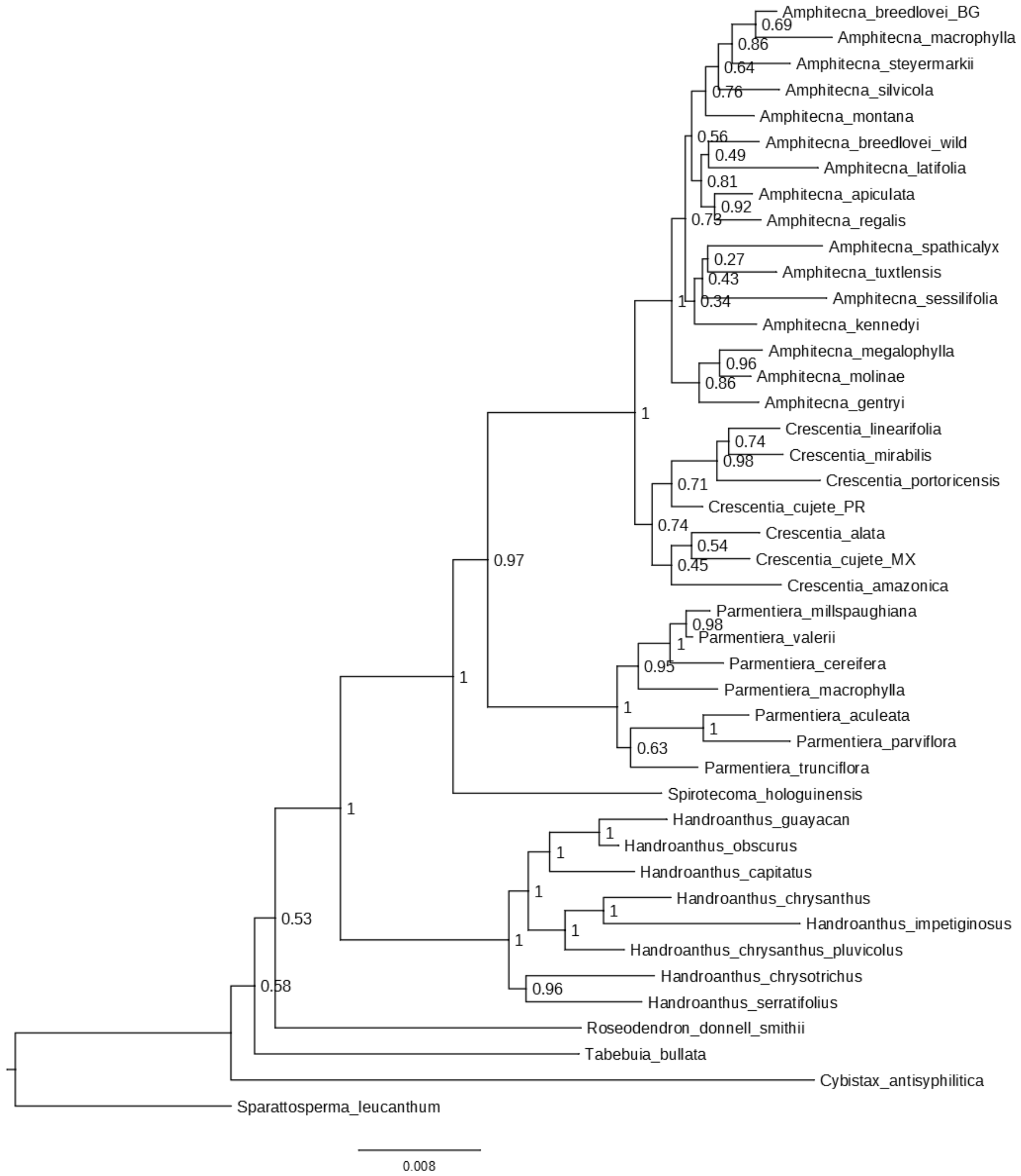
Appendix 4.3 Bayesian majority rule consensus tree generated from the nuclear dataset. Posterior probability support values are indicated at nodes.



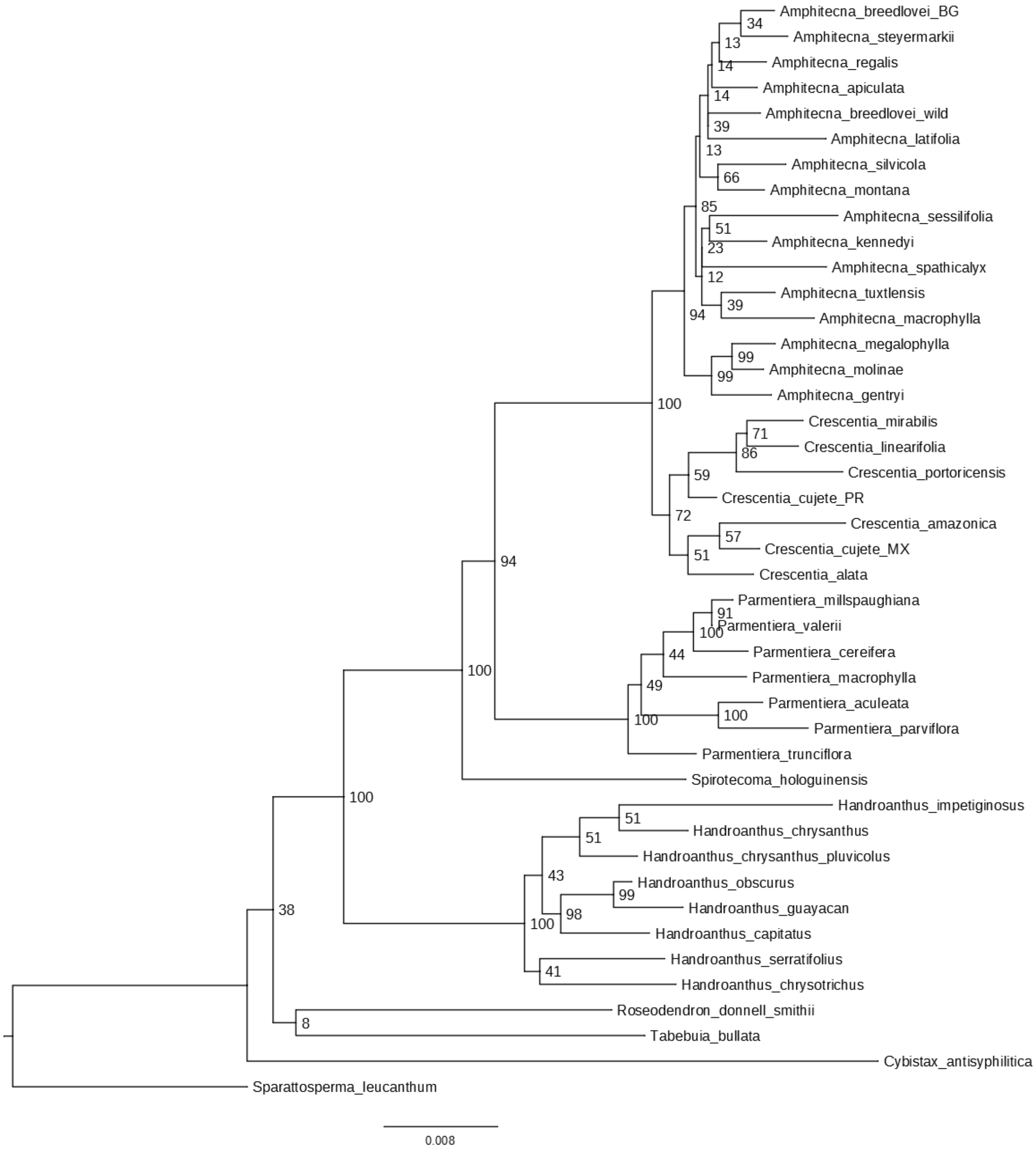
Appendix 4.4 Maximum likelihood tree generated from the nuclear dataset. Bootstrap support values are indicated at nodes.

Appendix 4.5 Bayesian majority rule consensus tree generated from the chloroplast dataset. Posterior probability support values are indicated at nodes.

Appendix 4.6 Maximum likelihood tree generated from the chloroplast dataset. Bootstrap support values are indicated at nodes.



Appendix 4.7 Bayesian majority rule consensus tree generated from the total combined dataset. Posterior probability support values are indicated at nodes.



Appendix 4.8 Maximum likelihood tree generated from the total combined dataset. Bootstrap support values are indicated at nodes.