A MOLECULAR PHYLOGENY AND CLASSIFICATION OF BIGNONIACEAE[1]

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Bignoniaceae are woody, trees, shrubs, and lianas found in all tropical floras of the world with lesser representation in temperate regions. Phylogenetic analyses of chloroplast sequences (*rbcL*, *ndhF*, *trnL-F*) were undertaken to infer evolutionary relationships in Bignoniaceae and to revise its classification. Eight clades are recognized as tribes (Bignonieae, Catalpeae, Coleeae, Crescentieae, Jacarandeae, Oroxyleae, Tecomeae, Tourrettieae); additional inclusive clades are named informally. Jacarandeae and Catalpeae are resurrected; the former is sister to the rest of the family, and the latter occupies an unresolved position within the "core" Bignoniaceae. Tribe Eccremocarpeae is included in Tourrettieae. Past classifications recognized a large Tecomeae, but this tribe is paraphyletic with respect to all other tribes. Here Tecomeae are reduced to a clade of approximately 12 genera with a worldwide distribution in both temperate and tropical ecosystems. Two large clades, Bignonieae and Crescentiina, account for over 80% of the species in the family. Coleeae and Crescentieae are each included in larger clades, the Paleotropical alliance and Tabebuia alliance, respectively; each alliance includes a grade of taxa assigned to the traditional Tecomeae. Parsimony inference suggests that the family originated in the neotropics, with at least five dispersal events leading to the Old World representatives.

Key words: Bignoniaceae; biogeography; chloroplast DNA; classification; molecular systematics; *ndhF*; phylogeny; *rbcL*; *trnL-F*.

This paper is dedicated to the memory of Al Gentry, Bignoniaceae scholar; without his tremendous energy and insights, this study would not have been possible.

Bignoniaceae are predominantly a neotropical family and are an important component of neotropical forests, with lesser contributions to African, Malagasy, and SE Asian tropical forests. The family includes 82 genera and 827 species (Lohmann and Ulloa, 2007). Approximately half of both genera and species belong to the New World endemic tribe Bignonieae, which comprise a major component of the neotropical liana flora. Most other species are woody shrubs and trees, including savannah and tropical forest canopy trees, although three groups have ad-

opted a herbaceous habit, mostly at high elevations in the Himalaya (*Incarvillea*) and the Andes (*Argylia*, *Tourrettia*). Several genera include species of horticultural importance in tropical and temperate regions, including *Bignonia*, *Campsis*, *Catalpa*, *Jacaranda*, *Spathodea*, and *Tabebuia*. Relatively few species have economic significance outside horticulture, but numerous species have been used by indigenous peoples for food, timber, containers, medicinal, and ritual purposes (Gentry, 1992c).

Bignoniaceae belong in Lamiales (Olmstead et al., 1993; Angiosperm Phylogeny Group, 2003), where their closest relatives are unclear (Olmstead et al., 2001; Oxelman et al., 2005). A group of small genera including *Schlegelia* often has been placed in Bignoniaceae (Armstrong, 1985) as tribe Schlegelieae (Gentry, 1980). However, phylogenetic evidence for this relationship is equivocal, with some studies finding a weakly supported sister group relationship with Bignoniaceae s.s. (e.g., Olmstead et al., 2001) and others placing Schlegelieae elsewhere, though nearby, on the phylogenetic tree (Spangler and Olmstead, 1999; Bremer et al., 2002; Oxelman et al., 2005). Without strong support for a clade comprising Bignoniaceae s.s. and Schlegelieae, it is appropriate to classify them separately (Reveal, 1996; APG II, 2003). *Paulownia* also is sometimes considered close to Bignoniaceae (Armstrong, 1985), but this relationship is rejected by all phylogenetic studies currently available (e.g., Spangler and Olmstead, 1999; Olmstead et al., 2001; Bremer et al., 2002). Hence, *Paulownia* also is placed in its own small family Paulowniaceae (Nakai, 1949; APG II, 2003).

The taxonomic history of the family was described in detail by Gentry (1980) and summarized by Spangler and Olmstead (1999). The most recent classification of Bignoniaceae by Fischer et al. (2004) recognized seven of the eight tribes proposed by Gentry (1980): Bignonieae, Coleeae, Crescentieae, Eccremocarpeae, Oroxyleae, Tecomeae, and Tourrettieae. Only Schlegelieae were excluded from this treatment.

Phylogenetic and monographic studies focusing on clades within Bignoniaceae have revised tribal and generic boundaries and species numbers for several groups, including Coleeae (Zjhra et al., 2004), Bignonieae (Lohmann, 2006, in press), *Incarvillea* (Chen et al., 2005), Crescentieae/*Tabebuia* s.l. (Grose and Olmstead, 2007a, b), and *Catalpa* (Li, 2008). Taking these recent studies into account, along with the two-volume monograph of neotropical Bignoniaceae (Gentry, 1980, 1992a), the portions of the family that remain most poorly known are the African and Asian groups, which account for approximately 29 genera and 115 species (Lohmann and Ulloa, 2007).

Previous phylogenetic studies have provided an outline of relationships in the family. However, these studies have lacked much detail due to limited sampling for many lineages. A preliminary study of the family using the chloroplast DNA (cpDNA) genes *rbcL* and *ndhF* (Spangler and Olmstead, 1999) found that the monophyly of Bignoniaceae itself was only weakly supported, with conflict between the two DNA regions with respect to its monophyly. The lack of strong molecular evidence for monophyly contrasts with the consistent recognition of Bignoniaceae in all previous classifications (apart from the debate over *Paulownia* and Schlegelieae). Bignoniaceae are easily recognized by a suite of vegetative and reproductive characters, including woody habit (except the herbaceous *Argylia*, *Incarvillea*, and *Tourrettia*), usually opposite and compound leaves, bilabiate flowers mostly with four stamens and a staminode, and usually dehiscent, capsular fruits splitting along two sutures and containing winged seeds (except the indehiscent Coleeae, Crescentieae, and *Kigelia*). Three further characters that provide more explicit evidence of monophyly with fewer exceptions are (1) reduced endosperm in the seeds; (2) bilamellate, sensitive stigmas; and (3) two placentae per carpel (Manning, 2000; Judd et al., 2002).

The study by Spangler and Olmstead (1999) suggested that tribes Bignonieae, Coleeae (represented by *Ophiocolea* and *Kigelia*), and Crescentieae were monophyletic, while Tecomeae were not. Coleeae and Crescentieae, once considered to be a single tribe on the basis of shared traits including fleshy indehiscent fruits (e.g., Bentham and Hooker, 1876), were found not to belong together, confirming Gentry's (1976) decision to keep them separate. Spangler and Olmstead (1999) included the monotypic tribe Eccremocarpeae and one member of the small tribe Oroxyleae, while the monotypic tribe Tourrettieae was not sampled at all. Subsequently, the following detailed studies of some groups have helped fill out portions of the tree.

A cpDNA study of Coleeae (Zjhra et al., 2004) found that the clade comprising the African genus *Kigelia* and the Malagasy Coleeae implied by the study of Spangler and Olmstead (1999) was an artifact of limited sampling. These results inferred that Bentham's Crescentieae (Bentham and Hooker, 1876) consists of three independent lineages with indehiscent fruits. In addition, this study indicated that Coleeae and *Kigelia* represent two distinct lineages that originated from a group of African genera assigned to Tecomeae (Zjhra et al., 2004). Further work on Coleeae using ITS sequences (M. L. Zjhra, unpublished data) has helped resolve species relationships within the clade.

Crescentieae sensu Gentry (1976) is a small group of trees from Central America and the Caribbean that were found to belong in a clade comprising New World members of Tecomeae (Grose and Olmstead, 2007a). This relationship is very similar to that encountered between Coleeae and the African Tecomeae. This previously unrecognized clade (Crescentieae, *Tabebuia*, and a few small genera of neotropical Tecomeae) is named the *Tabebuia* alliance (Grose and Olmstead, 2007a). This clade is characterized by palmately compound leaves (or a derivative unifoliate condition) and was recognized by Bentham and Hooker (1876) as the informal group Digitifolieae. Furthermore, Crescentieae are nested within the large neotropical genus *Tabebuia*, requiring that *Tabebuia* be split into three genera: *Handroanthus*, *Roseodendron*, and *Tabebuia* (Grose and Olmstead, 2007b).

The classification of tribe Bignonieae traditionally has consisted of a few large genera and many small ones, with many problematic generic delimitations (Lohmann, 2006). A combined cpDNA and nuclear DNA study of Bignonieae (Lohmann, 2006) with nearly one-third of the species sampled, sorted out many of the problems of generic delimitation and resulted in a revised classification of Bignonieae in which the 47 genera traditionally recognized in the tribe are reduced to 21 (Lohmann, in press).

Other, fine-scale, species-level phylogenetic studies in Bignoniaceae have been few. Chen et al., (2005) used a combined cpDNA (*trnL-F*) and ITS approach to understand species relationships in Chinese *Incarvillea*, and Li (2008) used *ndhF* and ITS to resolve relationships in the North American and Asian *Catalpa*.

This study integrates results from three studies that focused on major clades of Bignoniaceae (Bignonieae: Lohmann, 2006; Coleeae: Zjhra et al., 2004; Crescentieae and the *Tabebuia* alliance: Grose and Olmstead, 2007a), with data from representative sampling of the rest of the family to provide a more comprehensive picture of Bignoniaceae phylogeny. Sampling throughout the family was designed to provide sufficient detail to assess existing classifications and identify where changes are needed. Our goal is to identify the primary lineages of Bignoniaceae, especially those that will be important to future classifications. A good understanding of major lineages and relationships among them provides a basis for subsequent studies on the comparative biology of Bignoniaceae. These results also serve as basis to frame future studies of clades where additional work is needed.

MATERIALS AND METHODS

A total of 96 species of Bignoniaceae was included in this study (Appendix 1), along with a selection of 18 taxa from other clades of Lamiales. Our goal was to sample genus-level diversity, including multiple species of larger genera. Testing hypotheses of generic monophyly was largely beyond the scope of this study. A total of 65 of the approximately 82 genera of Bignoniaceae was included, along with two genera of Schlegelieae (*Exarata* and *Schlegelia*). DNA was obtained from fresh plant tissue; field-collected, silica-gel-dried tissue; and herbarium specimens.

Sequences were obtained by direct sequencing of PCR products following protocols described previously (Olmstead and Sweere, 1994; Olmstead and Reeves, 1995; Beardsley and Olmstead, 2002; Lohmann, 2006). Sequences were aligned by eye and adjusted manually (Simmons, 2004) using the sequence editor Se-Al 2.0 (Rambaut, 2002). Gaps shared by two or more taxa were coded as binary characters using simple gap coding (Graham et al., 2000; Simmons and Ochoterena, 2000).

Parsimony analyses were conducted on data sets of individual genes (results not shown) and a combined data set using the program PAUP* version 4.0b10 (Swofford, 2002) with 200 initial replicates using random-order-entry starting trees and tree-bsiection-reconnection (TBR) branch swapping with MULTREES on. All transformations were equally weighted. A second round of analysis was then conducted using 1000 starting trees and keeping only two trees per replicate, while using the strict consensus tree obtained from the first analysis as an inverse constraint to filter out trees compatible with that tree. These analyses allowed a more detailed search through the tree space for other

islands of equally parsimonious trees (Catalán et al., 1997). Bootstrap analyses were conducted using parsimony with 1000 bootstrap replicates using TBR branch swapping, but with MULTREES off (DeBry and Olmstead, 2000).

Bayesian Markov chain Monte Carlo (MCMC) analyses (Yang and Rannala, 1997) of the data were conducted with the data partitioned into five regions: *ndhF*, *trnL-F*, *rbcL*, *ndhF* gaps, and *trnL-F* gaps. The three nucleotide regions were analyzed using the program ModelTest version 3.6 (Posada and Crandall, 1998) to determine the most appropriate model of DNA substitution for each data partition using the Akaike information criterion (Akaike, 1974). The two regions representing gap presence/absence characters were given a simple model with only variable rates. The Bayesian analysis was run using the program MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003), with the MCMC algorithm running with five cold chains and one hot for one million generations. Two independent runs were performed. The burn-in was set at 10 000 generations, at which point the parameter values had stabilized, and samples were taken every 200 generations.

Because of the extensive missing data for *rbcL*, a maximum likelihood analysis (Felsenstein, 1973) was conducted using PAUP* 4.0b10 (Swofford, 2002) on a smaller data set consisting of the 39 taxa, including 22 Bignoniaceae, *Schlegelia*, and all other outgroups, for which all three sequence regions were obtained. A hierarchical likelihood ratio test, implemented in ModelTest 3.6 (Posada and Crandall, 1998), was used to determine the best sequence substitution model, and one of the most parsimonious trees was used to estimate starting parameters in an iterative approach in which the parameters were re-estimated following an initial heuristic search and the search repeated until no further change in parameters was obtained. Ten replicates using TBR branch swapping and random taxon addition were conducted at each iteration. Bootstrap analysis was conducted using the same parameters and 300 bootstrap replicates using TBR branch swapping with one tree saved per replicate.

RESULTS

Sequence data were derived from three cpDNA regions: *ndhF*, *trnL-F*, and *rbcL*. Sequences from prior studies were compiled with new sequences for this study (a total of 85 new sequences; see Appendix 1). Data for all three regions were available for all outgroups (except *rbcL* for *Exarata chocoensis*). Data for nearly all accessions were obtained for *ndhF* and *trnL-F*, although different accessions were used for a few species, to combine previously obtained results from different laboratories. In such cases, analyses of separate data sets confirmed similar placements for individual sequences. In a few instances, only *ndhF* (seven spp.) or *trnL-F* (two spp.) was available for some taxa, either because only a small amount of DNA was obtained from a herbarium specimen and PCR failed for one region or because only one gene was sequenced as part of another study and DNA was not available for this study. Sequences for *rbcL* were available only for 22 species of Bignoniaceae, along with *Schlegelia* and all outgroups. These included the species sampled by Spangler and Olmstead (1999) and were supplemented by three additional sequences to expand representation to include all major lineages of Bignoniaceae identified by the *ndhF* and *trnL-F* data.

The region of *ndhF* sequenced ranged from 2090 bp in *Incarvillea arguta* to 2110 bp in *Stizophyllum perforatum*. The total aligned length for *ndhF* was 2149 bp, which included 1108 variable and 665 parsimony-informative sites. Three parsimony-informative alignment gaps also were scored as characters. Sequences of *ndhF* were not obtained for two taxa (*Bignonia binata*, *Incarvillea mairei*); both of these represent additional species sampled for *trnL-F* in genera to confirm their placements. Efforts to amplify *ndhF* in *Incarvillea* were fully successful only in *I. arguta*, but failed routinely in several other species tried. A partial sequence was obtained for *I. zhongdianensis*. However, this sequence presented multiple frame-shift mutations and internal stop codons, suggesting that this is a

pseudogene. Pseudogenes of *ndhF* also are known from conifers (e.g., *Pinus* [Wakasugi et al., 1994], legumes [Palmer and Delwiche, 1998], Portulacaceae [Applequist and Wallace, 2001], and orchids [Neyland and Urbatsch, 1996]).

The *trnL-F* region ranged in length from 880 bp in *Melloa quadrivalvis* to ca. 980 bp in *Bignonia capreolata* (a short portion at the 3′ end of the region was not complete for this accession, thus the length was estimated). The total aligned length for *trnL-F* is 1293, but the last 40 bp of the alignment was excluded from the analysis, because data were missing from many accessions. The *trnL-F* data set includes 509 variable and 267 parsimony-informative characters and 52 shared alignment gaps that were scored as binary characters. Sequences were not obtained for seven taxa (*Amphitecna latifolia*, *Mansoa difficilis*, *Ophiocolea* sp., *Pachyptera aromatica*, *P. kerere*, *Rhigozum madagascariense*, *Tecomella undulata*). Five of these taxa represent additional species of their respective genera; the inclusion of the *ndhF* sequences helps confirm placement of those genera.

The *rbcL* data set consists of a 1402-bp section of the gene with no alignment gaps and includes 273 variable sites of which 132 are parsimony-informative (49 within Bignoniaceae). Sequences are available for all outgroups, except *Exarata*, and 22 ingroup taxa, all of which are represented in both the *ndhF* and *trnL-F* data sets.

Parsimony analyses of the individual *ndhF* and *trnL-F* data sets yielded trees that were highly consistent with each other (Appendices S2–S5; see Supplemental Data with the online version of this article). The results of the incongruence length difference test (Farris et al., 1994; as implemented in PAUP*) indicate that the two regions are not significantly different from two, similarly sized, random partitions of the combined data set ($P = 0.16$). However, there was one important difference between the two individual results. In the *ndhF* strict consensus tree, monophyly of Bignoniaceae is not obtained, with *Jacaranda* emerging from a polytomy that includes several outgroups and a clade consisting of the rest of Bignoniaceae (a monophyletic Bignoniaceae is found in some of the most parsimonious trees). In the *trnL*-F trees, Bignoniaceae are found to be monophyletic (79% bootstrap support), with a unique 6-bp direct repeat contributing to support for its monophyly. In addition, a unique 7-bp repeat marks the monophyly of all Bignoniaceae excluding *Jacaranda*. A separate analysis of *rbcL* also found a monophyletic Bignoniaceae (Spangler and Olmstead, 1999).

Parsimony analysis of the combined data set (Appendix S1; see Supplemental Data with the online version of this article) yielded 194 407 equally most parsimonious trees (length = 4992 steps; CI = 0.544; RI = 0.625). Despite the large number of equally most parsimonious trees, the resulting strict consensus tree (Fig. 1) is highly resolved, with most of the unresolved nodes in terminal groups. There is relatively little variation in branch lengths on the tree (Fig. 2), with the apparent exception of *Incarvillea*, which has somewhat greater branch lengths (Fig. 2), despite the availability of only a partial *ndhF* sequence for *I. zhongdianensis* and only a partial *trnL-F* sequence for *I. mairei*. The Bayesian majority rule consensus tree (not shown, but note Bayesian posterior probabilities on Fig. 1) is entirely consistent with the parsimony strict consensus tree within Bignoniaceae, but has fewer unresolved nodes. Only one of these nodes is resolved with a posterior probability (PP) greater than 0.90, uniting *Dolichandrone*, *Markhamia*, *Fernandoa*, *Heterophragma*, *Newbouldia*, *Kigelia*, and *Stereospermum* (PP = 0.96) as sister to Coleae. The parsimony strict consensus tree resolved two clades, one within Bignonieae and

Fridericia sp.
Fridericia pubescens
Xylophragma myrianthum
Fridericia mollissima
Cuspidaria convoluta
Tynanthus panurensis
Lundia cordata
Fridericia corallina
Tanaecium pyramidatum
Pachyptera aromatica
Pachyptera kerere
Anemopaegma parkeri
Mansoa difficilis
Mansoa verrucifera
Amphilophium bauhinioides
Amphilophium elongata
Bignonia capreolata
Bignonia aequinoctialis
Bignonia callistegioides
Bignonia binata
Dolichandra unguis-cati
Dolichandra quadrivalvis
Martinella obovata
Pleonotoma jasminifolia
Manaosella cordifolia
Stizophyllum perforatum
Adenocalymma cymbalum
Adenocalymma moringifolium
Perianthomega vellozoi

Bignonieae

Catalpa aff. *speciosa*
Macrocatalpa punctata

Catalpeae

Chilopsis linearis
Oroxylum indicum
Millingtonia hortensis

Oroxyleae

Amphitecna apiculata
Amphitecna montana
Amphitecna latifolia
Crescentia portoricensis
Crescentia alata
Parmentiera cereifera
Parmentiera macrophylla
Handroanthus serratifolius
Handroanthus chrysotrichus
Roseodendron donnell-smithii
Ekmanianthe actinophylla
Tabebuia rosea
Tabebuia heterophylla
Tabebuia lepidota
Tabebuia sauvallei
Cybistax antisyphilitica
Zeyheria tuberculosa
Godmania aesculifolia
Sparattosperma leucanthum

Crescentieae

Tabebuia alliance (Neotropics)

Colea sytsmae
Ophiocolea sp.
Ophiocolea floribunda
Phyllarthron articulatum
Rhodocolea racemosa

Coleeae

Dolichandrone spathacea
Markhamia platycalyx
Fernandoa madagascariensis
Heterophragma adenophyllum
Newbouldia laevis
Kigelia africana
Stereospermum nematocarpum
Stereospermum euphorioides
Radermachera frondosa
Tecomella undulata
Rhigozum madagascariense
Rhigozum obovatum
Catophractes alexandri
Spathodea campanulata

Paleotropical clade

Delostoma lobbii
Delostoma integrifolium
Campsidium valdiviana
Pandorea jasminoides
Pandorea pandorana
Tecomanthe volubilis
Tecomanthe hillii
Deplanchea tetraphylla
Lamiodendron magnificum
Tecomaria capensis
Podranea ricosoliana
Incarvillea arguta
Incarvillea mairei
Incarvillea zhongdianensis
Tecoma stans
Campsis radicans

Tecomeae

Argylia bustillosii
Argylia robusta
Eccremocarpus scaber
Tourrettia lappacea

Tourrettieae

Jacaranda arborea
Jacaranda mimosifolia
Jacaranda rufa
Jacaranda sparrei

Jacarandeae

Callicarpa dichotoma
Tectona grandis
Paulownia tomentosa — Paulowniaceae
Petrea volubilis
Rhaphithamnus spinosus
Barleria prionitus
Elytraria
Sesamum indicum
Schlegelia parviflora
Exarata chocoensis

Schlegeliaceae

Verbascum thapsus
Scrophularia californica
Buddleja davidii
Myoporum mauritianum
Digitalis purpurea
Antirrhinum majus
Nematanthus
Nyctanthes arbor-tristis

Outgroups

Crescentiina

"Core" Bignoniaceae

Bignoniaceae

Bootstrap / PP values on branches:
77/0.99, 100*, 79*, 92*, 60/0.88, 68/0.88, 58/0.93, 62/0.69, 46/0.92, 44/0.79, 87/0.99, 100*, 97*, 92*, 98*, 79*, 93*, 91*, 26/X, 100*, 43/0.70, 77/0.98, 100*, 100*, 100*, 100*, 48/0.63, 100*, 84*, 66/0.98, 83*, 100*, 100*, 100*, 46/0.69, 75/0.99, 84*, 100*, 76/0.99, 93*, 99*, 55*, 38/0.53, 96*, 76/0.97, 76/0.99, 100*, 96*, 99*, 76/0.74, 100*, 100*, 100*, 100*, 100*, 99*, 82*, 76/0.94, 100*, 63*, 83*, 96*, 57/0.95, 81*, 100*, 100*, 19/X, 57/0.74, 100*, 57/0.93, 100*, 100*, 91*, 45/0.85, 100*, 99*, 100*, 100*, 78*, 97*, 97*, 72*, 100*, 100*, 91*, 100*, 26/X, 75*, 75/0.90, 92*, 100*, 74*
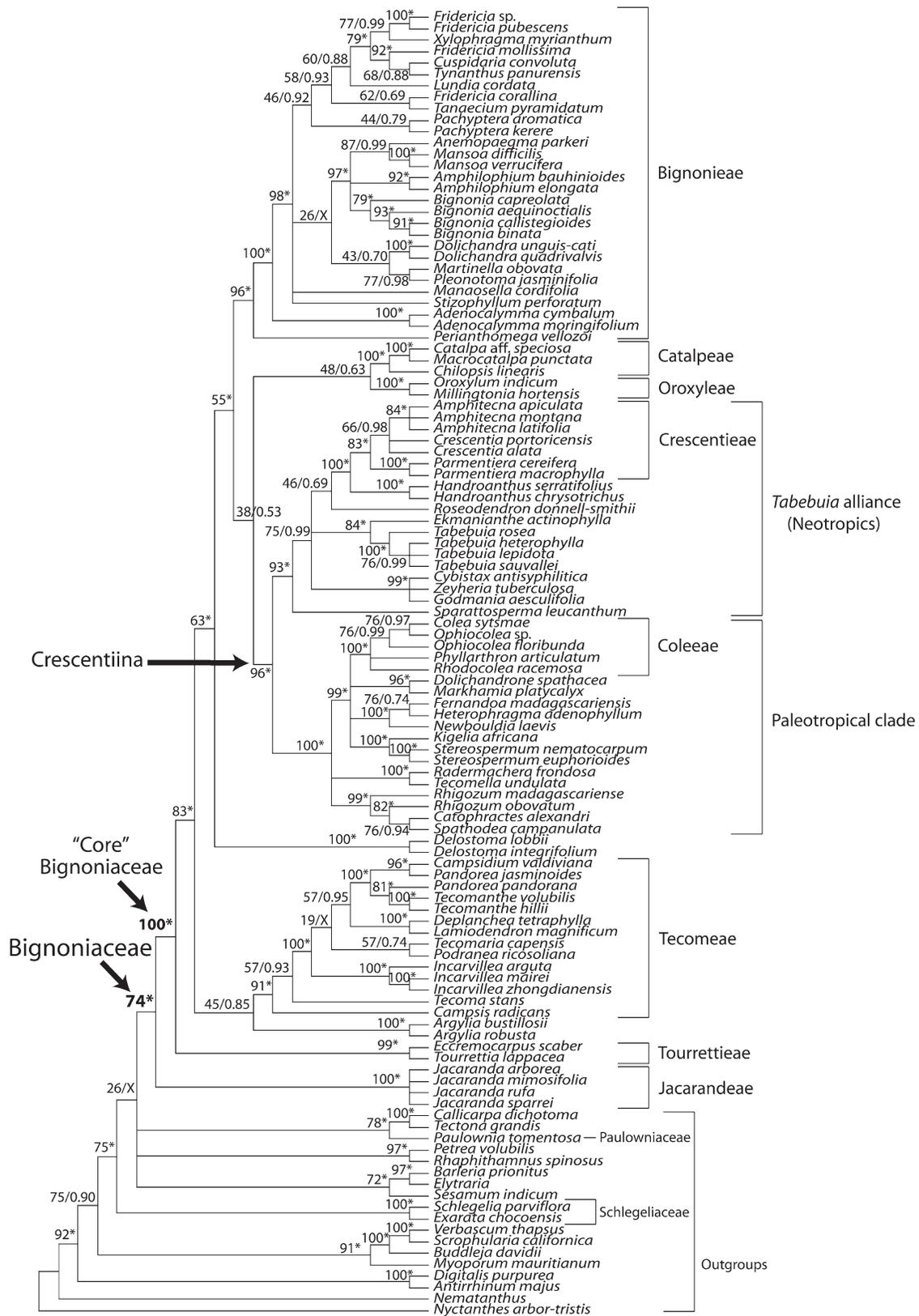
Fig. 1. Parsimony strict consensus tree based on combined analysis of *ndhF*, *trnL-F*, and *rbcL* sequences. Numbers on branches indicate bootstrap values based on the parsimony analysis (first number) and posterior probability (PP) values from the Bayesian analysis (* indicates PP of 1.0; "X" indicates clade not obtained in Bayesian majority rule tree).

one within Tecomeae, that were unresolved in the Bayesian analysis, but with low bootstrap support in both cases. The ML analysis of the 39-taxon data set yielded results (Fig. 3) that are almost entirely consistent with the parsimony and Bayesian analyses, with three and two conflicting nodes respectively, all of which are weakly supported in each analysis.

DISCUSSION

Previous studies (Bignonieae: Lohmann, 2006; Coleeae: Zjhra et al., 2004; Crescentieae and the *Tabebuia* alliance: Grose and Olmstead, 2007a) provide details of large portions of the phylogeny of Bignoniaceae. In each of these studies, greater taxon sampling and additional sequence data provide more detail on the phylogeny of those clades. Our results identify the primary lineages within Bignoniaceae, confirming and extending prior results (Spangler and Olmstead, 1999) and permitting a re-evaluation of the existing classifications of the family (revised in Fischer et al., 2004).

Our results (Figs. 1, 3) confirm the exclusion of *Paulownia* (as Paulowniaceae) and *Schlegelia* and *Exarata* (as Schlegeliaceae) from Bignoniaceae (Spangler and Olmstead, 1999). Paulowniaceae comprise a single genus and six species native to east Asia. Schlegeliaceae comprise four genera (*Exarata*, *Gibsoniothamnus*, *Schlegelia*, *Synapsis*) and approximately 25–35 species distributed from the Caribbean through Central America into northern South America (Fischer et al., 2004; Ulloa and Barringer, 2007). While there are many similarities between Bignoniaceae and Schlegeliaceae (e.g., woody trees, opposite leaves, bilateral floral symmetry, androecia typically including a staminode), Schlegeliaceae differ from typical Bignoniaceae by their simple leaves and berry-like indehiscent fruits (Gentry, 1980). Both these characters are found in derived clades within Bignoniaceae (e.g., both Coleeae and Crescentieae have these traits in some species), but indehiscent fruits in New World Bignoniaceae are rather large and leathery or woody and not the relatively small berries found in Schlegeliaceae. These characters in Schlegeliaceae now can be interpreted as nonhomologous with any occurrences within Bignoniaceae. Previous molecular systematic studies only included a single species of *Schlegelia* (e.g., Spangler and Olmstead, 1999), or *Schlegelia* and *Synapsis* (Wortley et al., 2007). We have included *Exarata chocoensis*, confirming its close relationship to *Schlegelia* (Gentry, 1992b) and providing additional support for the distinction of Schlegeliaceae from Bignoniaceae.

Monophyly of Bignoniaceae is supported by these results, albeit with modest bootstrap support in the parsimony analysis (74%) of the entire data set (Fig. 1) and ML analysis (74%) of the taxa for which all three gene regions were available (Fig. 3). However, Bayesian posterior probability for Bignoniaceae based on the entire data set is high (1.0). Two of the DNA regions used here, *rbcL* and *trnL-F*, identify a monophyletic Bignoniaceae when analyzed separately. However *ndhF*, which comprises the largest partition of the combined data, finds a monophyletic Bignoniaceae in some, but not all, most parsimonious trees. In the remaining *ndhF* trees, *Jacaranda* does not form a clade with the rest of the family. A 6-bp repeat in the *trnL-F* spacer region is found in all Bignoniaceae, including *Jacaranda*. This same repeat is absent in all outgroups. The repeat was scored as a single character in the parsimony and Bayesian analyses (though not considered in the ML analysis) and lends additional confidence in the monophyly of this clade. Despite the relatively modest

molecular support for Bignoniaceae, a series of morpho-anatomical traits are shared throughout the family. These include seeds winged and with reduced endosperm, two placentae per carpel, and bilamellate stigmas. The clade comprising all Bignoniaceae excluding *Jacaranda* receives 100% bootstrap support in both parsimony and ML analyses and a Bayesian PP of 1.0.

Clades of Bignoniaceae—Our main goal was to identify the primary clades within Bignoniaceae and to use them as basis for a revised classification. Prior studies (e.g., Spangler and Olmstead, 1999; Zjhra et al., 2004; Lohmann, 2006; Grose and Olmstead, 2007a) have shown that some groups recognized in previous classifications were monophyletic (e.g., tribes Bignonieae and Crescentieae), whereas others were not (e.g., tribes Coleeae and Tecomeae). Some adjustments have been made on the basis of these studies. For example, *Perianthomega* has been transferred from Tecomeae to Bignonieae (Lohmann, 2006, in press), and *Kigelia* has been excluded from Coleeae (Zjhra et al., 2004). We make further changes here where the evidence permits. Several of the large, prominent clades identified in this study lack evident diagnostic traits. Hence, we use informal names to refer to these clades to facilitate communication.

Jacarandeae Seem.—*Jacaranda* and the small genus *Digomphia* are included in Jacarandeae. This tribe is sister to the remaining Bignoniaceae, a novel result that had not been predicted by any prior classification. A similar result was obtained by Spangler and Olmstead (1999), who found a clade comprising *Jacaranda* and *Podranea* as sister to the rest of the family. In retrospect, the placement of *Podranea* with *Jacaranda* in that study was likely an artifact of taxonomic sampling because all three single-gene analyses in this study placed *Podranea* in a well-supported clade with *Tecoma* and other members of Tecomeae (Figs. 1, 3). Traditional classification of *Jacaranda* splits it into two subgenera, *Monolobos* and *Dilobos*, characterized by one or two anther thecae, respectively. Two species of each subgenus were included here, but no resolution among species was evident. Gentry (1992a) suggested that *Digomphia* is derived from within *Jacaranda*, but that hypothesis remains to be tested. There are a number of traits typical of Jacarandeae that set them apart from most other Bignoniaceae, including bipinnately compound leaves, an elongate staminode that often is divided and glandular, calyx lobes that are deeply divided, and a fruit that is circular in outline and flattened perpendicularly to the septum. Bentham and Hooker (1876) recognized tribe Jacarandeae, which included *Jacaranda* and *Digomphia*, but also included several other unrelated genera that have parietal or nearly parietal placentation, including taxa with indehiscent fruits where the septum has been lost secondarily. Recent classifications (Gentry, 1980; Fischer et al., 2004) place *Jacaranda* and *Digomphia* in a paraphyletic Tecomeae. We resurrect Jacarandeae in a narrow circumscription to include only *Jacaranda* and *Digomphia*. Jacarandeae include approximately 55 species that are widely distributed throughout the neotropics (Gentry, 1980).

Core Bignoniaceae—Bignoniaceae, excluding Jacarandeae, comprise a strongly supported clade with a relatively long subtending branch (Figs. 2, 3) suggesting a substantial temporal distance relative to the divergence of *Jacaranda* and other close outgroups. Whereas the molecular phylogenetic distinctiveness is clear, there are no evident morphological traits that unite this clade. It is possible that some of the traits that are distinctive of
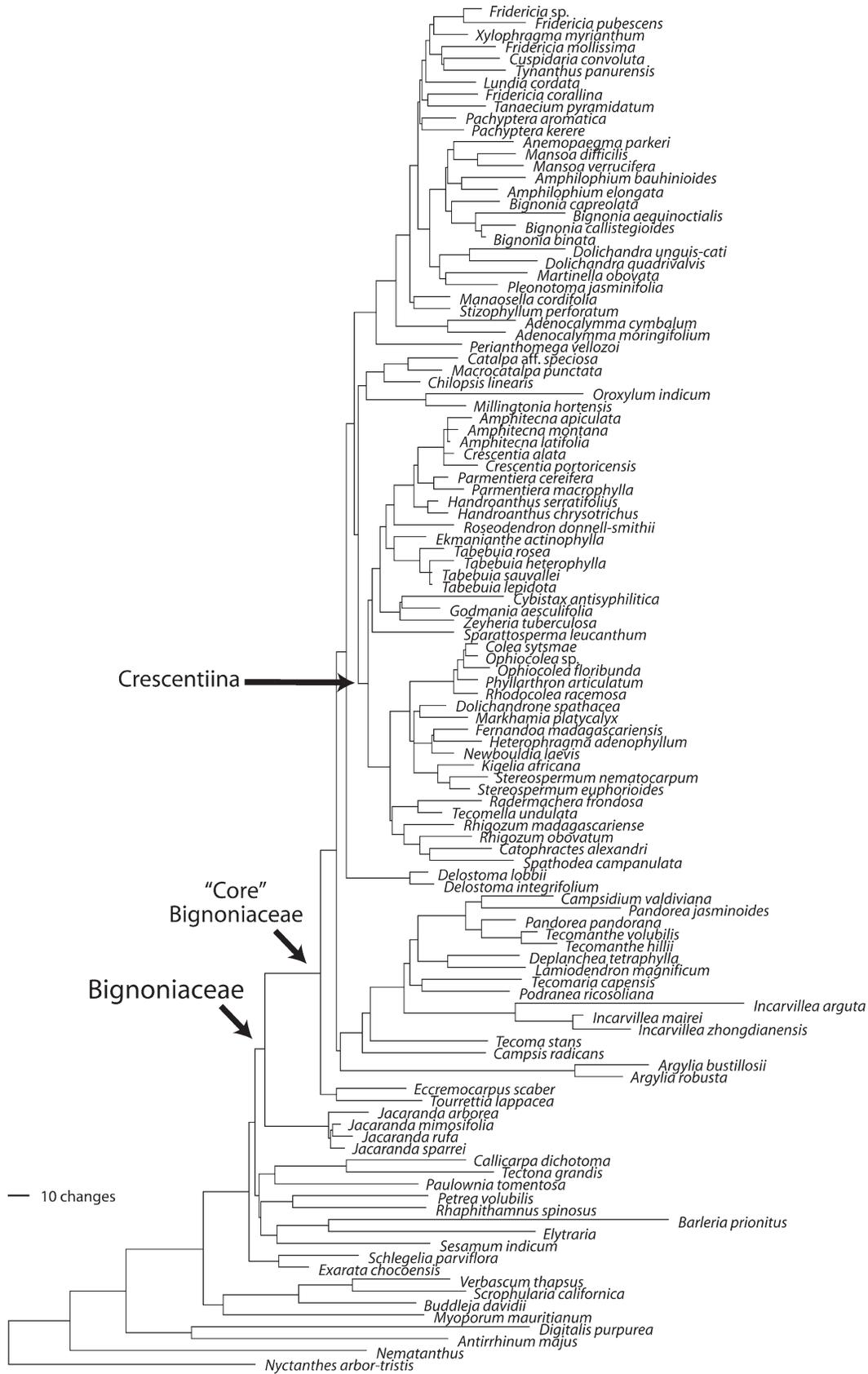
Fig. 2.   One of the most parsimonious trees with branch lengths proportional to number of inferred substitutions.

Jacarandeae represent ancestral traits for the family, thus leaving some family-level traits to be synapomorphies for the core Bignoniaceae. However, uncertainties regarding outgroup relationships and equivocal polarity of traits that might represent transformational series (e.g., singly vs. doubly compound leaves, reduced vs. elaborated staminodes) make such determinations impossible.

All the remaining genera in our study are assigned to tribes or to informally named clades, except *Argylia* and *Delostoma*, which do not belong to any well-supported clade within the core Bignoniaceae. *Argylia* is one of three herbaceous genera in Bignoniaceae (with *Incarvillea* and *Tourrettia*) and comprises a dozen species distributed in the southern Andes in high altitude and/or arid habitats in Peru, Chile, and Argentina. *Delostoma*, with four species, is distributed in the northern Andes from Peru to Venezuela. Gentry (1980, p. 43) remarked that "*Delostoma* has no obvious affinities with either Old or New World genera," a conclusion we confirm in this study.

Tourrettieae G. Don—The two small genera of subwoody to herbaceous vines distributed in the Andes that comprise this small clade have been assigned to separate monogeneric tribes in recent treatments of Bignoniaceae (Gentry, 1980; Fischer et al., 2004). In addition to the similar habit and distribution, both genera have doubly compound leaves (bipinnate to tripinnate in *Eccremocarpus* and biternate in *Tourrettia*) with branched tendrils derived from modified leaflets, and flowers lacking staminodes (Gentry, 1980; D'Arcy, 1997; Fischer et al., 2004). They differ in ovary structure (four locules in *Tourrettia* vs. a single locule in *Eccremocarpus*), fruit (*Tourrettia* has a bur-like fruit covered with hooked spines vs. a smooth capsule in *Eccremocarpus*), and inflorescence (a many-flowered terminal spike in *Tourrettia* vs. a few-flowered lateral raceme in *Eccremocarpus*). Given the strong support for their monophyly (99% bootstrap and 1.0 PP), it makes sense to eliminate the redundancy of monogeneric tribes and unite them into a single tribe Tourrettieae, thus recognizing their similarity and shared ancestry. Gentry (1980, p. 44) mentioned that "*Tourrettia* and *Eccremocarpus* are related to each other, but otherwise isolated in the family." He was correct on both counts.

Tourrettieae share some traits with Jacarandeae that set them apart from other Bignoniaceae. The fact that Tourrettieae, like Jacarandeae have doubly compound leaves, suggests that this may be the ancestral condition in Bignoniaceae, with a reduction to singly pinnate leaves in the common ancestor of the remainder of the family exclusive of these two basal clades. Also, both of these groups have similar pollen that is psilate and tricolpate (Gentry and Tomb, 1979); such pollen is found elsewhere in Bignoniaceae, but only in a few genera of Bignonieae, where common ancestry is unlikely to explain the similarity with these taxa. The absence of staminodes in Tourretieae and most outgroups to Bignoniaceae, in contrast to the long, elaborate staminodes of Jacarandeae and the smaller simple staminodes characteristic of most other Bignoniaceae makes interpretation of staminode evolution uncertain.

Tecomeae Endl.—Most recent treatments of the Bignoniaceae (e.g., Gentry, 1980; Fischer et al., 2004) recognize a large, diverse Tecomeae comprising all taxa with bilocular, dehiscent fruits that dehisce perpendicular to the septum. Taxa not assigned to Tecomeae were assigned to Bignonieae and Oroxyleae with septa parallel to the valves in fruit, Crescentieae and Coleeae with indehiscent fruits, and Eccremocarpeae and Tour-

rettieae with one and four locular fruits, respectively. Early phylogenetic studies showed that Tecomeae, thus circumscribed, was paraphyletic, with all other groups derived from within it (Spangler and Olmstead, 1999). Such a paraphyletic relationship was suggested by Gentry (1976) for specific cases (e.g., Coleeae and Crescentieae). Our results include much greater sampling of Tecomeae sensu lato and confirm the paraphyly of this group. Thus, we circumscribe a much-reduced Tecomeae. Here, several taxa are excluded from the former Tecomeae, including Jacarandeae (described earlier), Catalpeae, the *Tabebuia* alliance (an assemblage of New World genera centered on *Tabebuia* and forming a paraphyletic grade with respect to Crescentieae, discussed later), the paleotropical alliance (an assemblage of Old World genera with a corresponding paraphyletic relationship to Coleeae, see later), and two isolated genera (*Argylia* and *Delostoma*, see earlier). In analyses of the full data set, *Argylia* forms the sister group to Tecomeae, but support for this result is weak and equivocal in the ML analysis of the reduced data set, and thus we exclude it from Tecomeae.

As here circumscribed, Tecomeae comprises 12 genera and approximately 55 species, distributed worldwide. *Campsis* has one species distributed in each of temperate North America and East Asia, *Tecoma* (14 spp.) is entirely New World from southwestern United States to Andean South America, *Incarvillea* (17 spp.) is Himalayan, *Podranea* (2 sp.) is South African, *Deplanchea* (8 spp.), *Lamiodendron* (1 sp.), *Pandorea* (7 spp.) and *Tecomanthe* (5 spp.) are Australasian, and *Campsidium* (1 sp.) is distributed in southern South America (Lohmann and Ulloa, 2007). While cautioning against drawing firm conclusions from a small, widely distributed group, such as this, Tecomeae appear to be temperate or Andean (especially if *Argylia* is sister to this clade) New World in origin. Whereas speculation as to a mechanism for their widespread distribution is unwarranted at this time, their position as sister to most of the rest of Bignoniaceae suggests this is an old clade, thereby providing time for such a broad distribution to develop.

Fischer et al. (2004) follow Gentry's recommendation (Goldblatt and Gentry, 1979; Gentry, 1980) that *Tecomaria* should be included in *Tecoma*. However, our results indicate that *Tecomaria* is closer to *Podranea*, the only other African member of this clade. A handful of small genera from SE Asia (*Neosepicia*, *Pajanella*, *Pauldopia*, and *Santisukia*) that were not sampled in this study may belong in Tecomeae as circumscribed here. There is no apparent morphological synapomorphy for this clade, but a climbing habit is found in five genera, which is unusual outside of Bignonieae; the remaining genera are trees or shrubs (except the herbaceous *Incarvillea*). C-4 formyl iridoids uniformly characterize this group and are not found elsewhere in the Bignoniaceae, except in *Argylia*, where they co-occur with C-4 carboxyl and decarboxylated iridoids, which are found in other members of Bignoniaceae (von Poser et al., 2000). The presence in *Argylia* of C-4 formyl iridoids may lend support to the weak molecular evidence for a close relationship with Tecomeae.

Crescentiina—Two clades, one strictly paleotropical, the other neotropical, together form this well-supported monophyletic group (BS = 96%, PP = 1.0). The ending "ina" is used here to denote an unranked, informal clade name (see Kron, 1997). This clade contains approximately 34 genera and 300 species, thus making it comparable in size to Bignonieae. Each of the two included clades consists of a paraphyletic grade of taxa as-
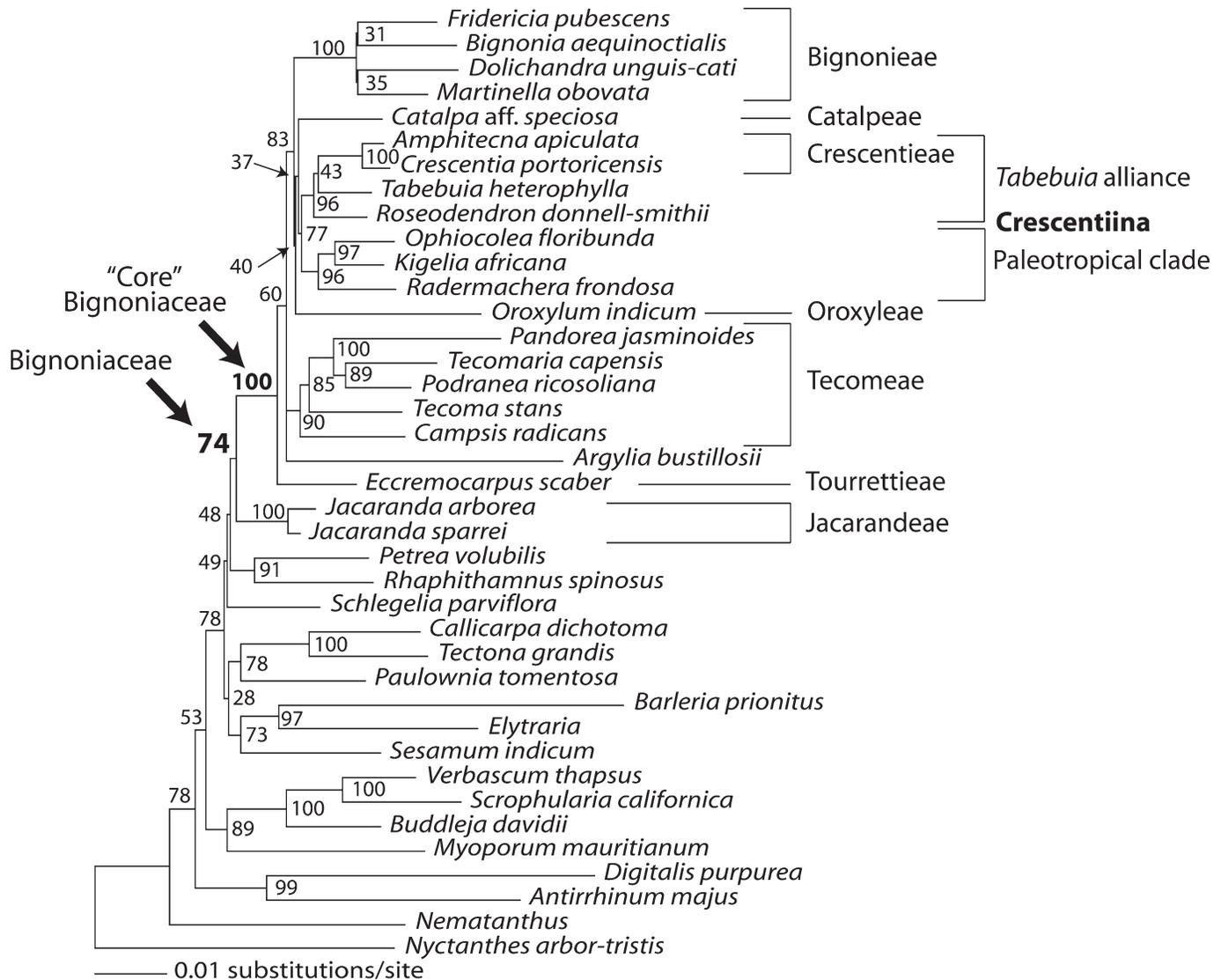
Fig. 3. Maximum likelihood (ML) tree from analysis of taxa with all three genes available; ML bootstrap values are indicated next to branches.

signed traditionally to Tecomeae with a clade defined by inde-hiscent fruits, Coleeae (paleotropics) and Crescentieae (neotropics) nested within them. Despite the strong evidence for monophyly of this group and the striking symmetry in the evolutionary trajectory of each clade within it (including the evolution of indehiscent fruits and reduced leaves in both the paleotropical and the neotropical clades), there are no evident morphological traits that diagnose the inclusive group. No prior classification recognized a group similar to this. All analyses (Figs. 1–3) found the small clades Catalpeae and Oroxyleae associated with this clade in an inclusive clade that is sister to Bignonieae. However, the support for the placement of Catalpeae and Oroxyleae with this group is very weak (parsimony bootstrap = 38%; PP = 0.53; ML bootstrap = 37%).

Paleotropical clade—This clade contains about 20 genera and approximately 150 species of trees (Lohmann and Ulloa, 2007). Coleeae (see later) is endemic to Madagascar and surrounding islands. The monotypic *Catophractes*, *Kigelia*, *New-*

bouldia, and *Spathodea* are African. *Heterophragma* (2 spp.) and *Radermachera* (17 spp.) are Asian. *Dolichandrone* (10 spp.) and *Markhamia* (5 spp.) have an African and Asian distribution. *Rhigozum* (7 spp.) is found in Africa and Madagascar. *Fernandoa* (15 spp.) and *Stereospermum* (23 spp.) are distributed in Africa, Madagascar, and Asia. Our results are consistent with earlier studies with less generic level sampling and sequence data (Spangler and Olmstead, 1999; Zjhra et al., 2004).

Our limited sampling of SE Asian species means that, based on geographic distribution, several representatives of Tecomeae s.l. may belong either to this clade or to Tecomeae s.s. as circumscribed earlier. Aside from a paleotropical distribution, all members of this clade share an arborescent habit distinct from the climbing habit shared by most members of Tecomeae s.s. Of the 20 genera likely to belong to the paleotropical clade (rather than Tecomeae), 16 of the genera were sampled. We predict that unsampled genera formerly assigned to Tecomeae s.l. that are lianas (Saharan *Dinklageodoxa* and New Guinean/Queensland *Neosepicaea*) will belong to Tecomeae, whereas

unsampled tree genera (Malagasy *Perichlaena*, Thai *Santisukia*, Indo-Malayan *Pajanelia*, and Asian *Pauldopia*) will belong to the paleotropical clade.

Coleeae Bojer—Of the 60 described species in this clade, 54 are endemic to Madagascar, and four are endemic to the surrounding Pacific Ocean islands. Perrier de la Bâthie (1938a, b) recognized five genera of Coleeae: *Phylloctenium*, *Phyllarthron*, *Colea*, *Ophiocolea*, and *Rhodocolea*. Molecular results confirm that the latter four genera are monophyletic, if *Phylloctenium* is included within *Phyllarthron* (Zjhra, 2003; Zjhra, et al., 2004; M. Zjhra, unpublished results). Species of Coleeae are found predominately in wet forests from sea level to the highest mountains, with fewer species in dry deciduous and spiny forests (Zjhra, 2006). Most species are rare and narrow endemics, with the majority threatened with extinction (Good et al., 2006). Originally, taxa now comprising the Coleeae were placed in tribe Crescentieae (de Candolle, 1838, 1845; Seemann, 1860; Baillon, 1887, 1888; Perrier de la Bâthie, 1938a, b), an otherwise neotropical group. The geographically disjunct Crescentieae and Coleeae share a number of characteristics not otherwise observed in the family: spines, phyllodes, simple leaves, cauliflory, and fleshy indehiscent fruits. Gentry (1976) distinguished Coleeae from Crescentieae based on geographic distance. This was confirmed by molecular data (Spangler and Olmstead, 1999; Zjhra et al., 2004).

Tabebuia alliance—The sister clade to the paleotropical clade is endemic to the neotropics and contains 14 genera, 11 of which are included in this study. The 147 species in this group are all trees or shrubs and share the trait of palmately compound leaves (except for a few species that present a reduction in leaflet number and appear simple leaved). This morphological characteristic led Bentham and Hooker (1876) to place them in an unranked group "Digitifolieae." This study corroborates the findings of a previous study (Grose and Olmstead, 2007a) in showing strong support (93% parsimony bootstrap and 100% PP; Fig. 1) for the *Tabebuia* alliance. This clade consists in large part of the genus *Tabebuia* s.l. However, Grose and Olmstead (2007a) showed that *Tabebuia* was a paraphyletic assemblage comprising three distinct clades. Thus, they split *Tabebuia* into three genera, *Handroanthus*, *Roseodendron*, and *Tabebuia* s.s. (Grose and Olmstead, 2007b). The *Tabebuia* alliance includes the small tropical South American genus *Sparattosperma* as sister group to a group of three clades that form an unresolved trichotomy (Fig. 1). *Handroanthus* and *Roseodendron* represent successive sister groups to Crescentieae (plus *Spirotecoma*) in one clade, *Ekmanianthe* and *Tabebuia* s.s. form the second clade, and *Cybistax*, *Godmania*, and *Zeyheria* form the third.

Three genera currently placed within this alliance, *Romeroa*, *Paratecoma*, and *Spirotecoma*, were not included in this study. *Spirotecoma* has been shown to be allied with *Crescentieae* (Grose and Olmstead, 2007a), but the other two are monotypic genera with restricted distributions. *Romeroa* is endemic to the Magdalena Valley of Colombia and, despite Gentry's hypothesis (Gentry, 1992a), its placement within this alliance is questionable based on its distinct morphology and anatomy (Gentry, 1992a; dos Santos and Miller, 1992). Conversely, placement of *Paratecoma* within this clade is more certain. It is endemic to the Rio Doce area of eastern Brazil and is now near extinction as the result of overlogging (Gentry, 1992a, b). The wood anatomy of *Paratecoma* was shown by dos Santos and Miller (1992) to be very similar to that of *Handroanthus*

(referred to in that study as the Lapachol group). This genus is unique within the *Tabebuia* alliance with a reduction to two functional stamens.

Crescentieae G. Don—This small tribe contains 35 species in three genera of trees endemic to Central America and the Greater Antilles. Crescentieae traditionally included all Bignoniaceae with indehiscent fruits, but is recognized here in the restricted sense as defined by Gentry (1976, 1980) to include only the neotropical species. Seven species representing all three genera were sampled for this study. However, a study with 27 species (Grose and Olmstead, in prep.) has shown that all three genera are monophyletic and *Spirotecoma* is most likely sister to Crescentieae, rather than in an unresolved position with respect to it (Fig. 1).

Crescentieae is one of the lineages that inspired Janzen and Martin (1982) to postulate the existence of a relict group of neotropical plants adapted to seed dispersal by the now extinct Pleistocene megafauna. This guild is composed of species with large, fleshy fruits containing a sweet pulp, that remain beneath the tree after falling, not apparently eaten by any nearby organisms. Members of Crescentieae fit this pattern. Very little is known about fruit dispersal in this group, and seedlings are rarely seen in nature (S. Grose, personal observerations). Members of Crescentieae are infrequently seen, due at least in part to their inconspicuous, understory habit. Therefore, little is known about the distribution and population density of species in this group.

Oroxyleae A. H. Gentry—This small clade of four genera and approximately six species of trees and lianas is Indomalaysian in distribution and characterized by fruits dehiscent parallel to the septum (septicidal), a condition otherwise found only in the neotropical Bignonieae. Apart from the geographic disjunction and the tree habit of some species, these species also lack the unusual phloem arms found in Bignonieae (Gentry, 1980). Gentry (1980) erected Oroxyleae to account for these species that otherwise had been assigned to Bignonieae and suggested that they bore closer relationship to Tecomeae. Our results provide weak evidence that they may be closest to Catalpeae (Fig. 1), with which they have little in common morphologically, or they are sister to a clade comprising Catalpeae and the *Tabebuia* alliance plus the paleotropical clade. The poor resolution in this part of the tree leaves open the possibility that they are, in fact, more closely related to Bignonieae and that the transformation in fruit dehiscence from loculocidal to septicidal is a synapomorphy for Bignonieae plus Oroxyleae. However, this hypothesis is neither supported nor rejected by our results. *Oroxylum* and some species of *Nyctocalos* have five stamens, an exception to the four typical of the family, which led Gentry (1980) to suggest that Oroxyleae may be near the root of Bignoniaceae, but this suggestion is not supported by our results.

Catalpeae DC. ex Meisn.—The earliest classifications of Bignoniaceae (e.g., de Candolle, 1838) recognized two tribes, Bignonieae (species with dehiscent fruits), and Crescentieae (species with indehiscent fruits). de Candolle (1838) first used the name Catalpeae for a subtribe within Bignonieae characterized by fruits dehiscing perpendicularly to the septum, but Tecomeae has priority at the rank of tribe. We resurrect Catalpeae as a small clade consisting of two or three genera and approximately 11 species. Catalpeae are best characterized by their simple leaves, a trait found in several other taxa of derived

position within larger clades. *Catalpa* s.l. has stamen number reduced to two, but *Chilopsis*, a monotypic genus from southwestern North America that is sister to *Catalpa* s.l., has the four stamens typical of Bignoniaceae. *Catalpa* s.l. consists of two clades (Li, 2008), *Macrocatalpa* (with four species in the Greater Antilles), and *Catalpa* (with two species in eastern North America and four in east Asia). *Macrocatalpa* usually is considered part of *Catalpa*.

Bignonieae Dumort.—This large clade contains 377 species and nearly half of the species in Bignoniaceae (Lohmann, in press). The tribe includes neotropical lianas and shrubs that are distributed through a variety of habitats, ranging from dry savannas to wet forests. Traditionally, 47 genera have been recognized in the tribe (see Fischer et al., 2004). However, a detailed phylogenetic study of Bignonieae that sampled one-third of all species indicated that the majority of the traditionally recognized genera are not monophyletic (Lohmann, 2006). A revised classification of Bignonieae, recognizing only monophyletic groups diagnosed by morphological synapomorphies, includes 21 genera (Lohmann, in press). Among the morphological synapomorphies of the tribe are leaflets modified into tendrils and an unusual wood anatomy with the phloem developing 4–32 discontinuous wedges in cross sections. The leaves are usually 2–3-foliolate, less often palmate, pinnate, biternately compound or simple. The fruits are septicidal capsules with the septum parallel to the fruit valves. Bignonieae have long been recognized as a monophyletic group (see Lohmann, in press). Indeed, the monophyly of Bignonieae is strongly supported in all analyses (parsimony bootstrap 96% and PP = 1.0); the only novelty in the circumscription of Bignonieae is the inclusion of *Perianthomega* (Lohmann, in press), which has been included previously in Tecomeae (Gentry, 1992a; Fischer et al., 2004). It is still unclear which are the closest relatives of Bignonieae. The poor resolution in this portion of the tree leaves open the possibility that Oroxyleae, Catalpeae, or the Crescentiina, or some combination of the three clades might be the closest relatives of Bignonieae. More data are needed to clarify the relationships among these lineages.

Historical biogeography of Bignoniaceae—Bignoniaceae are predominantly New World in distribution. Several of the basally branching lineages, including Jacarandeae, Tourrettieae, *Argylia*, and *Delostoma*, are strictly New World, as are Bignonieae and the *Tabebuia* alliance. Early-diverging branches in Catalpeae (i.e., *Chilopsis* and *Macrocatalpa*, which are paraphyletic to any Asian species of *Catalpa*) and in Tecomeae (*Campsis*, *Tecoma*) also are entirely, or predominantly, New World in distribution. A simple parsimony interpretation implies that Bignoniaceae are New World in origin. Three distinct clades are virtually entirely Old World in distribution: the paleotropical clade, Oroxyleae, and a large clade within Tecomeae (exclusive of basal branches *Campsis* and *Tecoma*). This Old World clade of Tecomeae has one New World species, *Campsidium valdivianum*, nested in a clade of otherwise Australasian taxa. Two small clades include Old World species in what are most likely groups of New World origin (*Catalpa* and *Campsis*). Thus, colonization of Old World ecosystems from the New World appears to have occurred on at least five occasions, including both temperate and tropical regions.

Conclusions—Bignoniaceae are an important element in tropical, woody floras worldwide and contribute, in particular,

to the woody liana flora of neotropical forests. By resurrecting tribes Catalpeae and Jacarandeae, restricting Tecomeae to a monophyletic group of genera, and combining Eccremocarpeae and Tourrettieae, we modify the existing classification of Bignoniaceae to be consistent with the phylogeny presented here. We give informal names to additional clades to enable communication about groups that are not part of the formal nomenclature. Identifying *Jacaranda* (with *Digompha* forming Jacarandeae) as sister to all other Bignoniaceae, followed by Tourrettieae (*Tourrettia* and *Eccremocarpus*) helps to provide insight into the character evolution and biogeographic history of the family by providing a context for its ancestral nodes. These results pave the way for analyses that should further clarify the evolutionary history of Bignoniaceae. The most critical remaining systematic and phylogenetic questions involve the Southeast Asian genera, which are relatively poorly collected and understudied. The sister group to Bignoniaceae also remains uncertain and provides a barrier to interpreting character evolution among early Bignoniaceae.

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Appendix 1. Taxa, geographic origins (where known, if from cultivation), vouchers, and GenBank accession numbers (*rbcL*, *ndhF*, *trnL-F*). Names in parentheses are names that were used in recent phylogenetic studies of Bignoniaceae (Spangler and Olmstead, 1999), Bignonieae (Lohman, 2006), *Tabebuia* (Grose and Olmstead, 2007a), and *Catalpa* (Li, 2008) to aid in comparison with those studies. Vouchers and GenBank accession numbers with an asterisk represent specimens that were sequenced for *ndhF* by Lohman (2006) and confirm the sequences used in this study. GenBank numbers in boldface indicate sequences used for the first time in this study. "na" indicates no sequence available for that accession.

Species; Geographic origin; *Voucher* (Herbarium); GenBank accessions: *rbcL*; *ndhF*; *trnL-F*.

Adenocalymma cymbalum (Cham.) Bureau & K. Schum.; Brazil: Minas Gerais; *J. Lombardi 2495* (BHCB, MO); na; DQ222528; **FJ870010**. **Adenocalymma moringifolium (DC.) L. G. Lohmann (= Memora moringifolia)**; Brazil: Amazonas; *Lohmann 19* (INPA, K, MG, MO, NY, R, SP, SPF, U); na; DQ222613; **FJ870011**. **Amphilophium bauhinioides (Bureau ex Baill.) L. G. Lohmann (= Glaziova bauhinioides)**; Brazil: Espirito Santo; *D. Folli 1654* (CVRD, MO); *Lohmann 655* (CVRD, MO)*; na; **FJ887849**/DQ222586*; **FJ870012**. **Amphilophium elongata (Vahl.) L. G. Lohmann (= Distictella elongata)**; Brazil: Minas Gerais; *J. Lombardi 2433* (BHCB, MO); na; DQ222578; **FJ870013**. **Amphitecna apiculata A. H. Gentry;** cult. At MBG, coll. by Cook; *R. Spangler B1* (COLO); AF102640; AF102624; AY500411/AY500431. **Amphitecna latifolia (Miller) A. H. Gentry;** Cuba; cult. at Jard. Bot. Nac., Cuba; *Olmstead 96-101* (WTU); na; EF104997; na. **Amphitecna montana L. O. Williams;** Mexico, UCB Bot. Gard. #76.2178; cult. from *Breedlove 42783*; *H. Forbes s.n.* (UC/JEPS); na; **FJ887850**; **FJ870014**. **Anemopaegma parkeri Sprague;** Brazil; cult in MBG from collection by A. Gentry; no voucher; na; **FJ887851**; **FJ870015**. **Argylia bustillosii Phil.;** Argentina; cult in RBGE #19951083; no voucher; **FJ870007**; **FJ887852**; **FJ870016**. **Argylia robusta Sandwith;** Argentina; cult in RBGE #19951084; no voucher; na; **FJ887853**; **FJ870017**. **Bignonia aequinoctialis L. (= Cydista aequinoctialis)**; cult in MBG from collection by A. Gentry; *R. Spangler B2* (MO); AF102645; AF102629; **FJ870018**. **Bignonia binata Thunb.;** Brazil; *D. Follii 1657* (CVRD, MO); na; na; **FJ870019**. **Bignonia callistegioides Cham. (= Clytostoma callistegioides)**; cult RBGK #1969-17435; no voucher; *Lohman 352* (MO)*; na; **FJ887854**/DQ222569*; **FJ870020**. **Bignonia capreolata L.;** cult RBGK #1980-3846; no voucher; *Lohmann 356* (MO)*; na; **FJ887855**/DQ222566*; **FJ870021**. **Campsidium valdiviana (Phil.) Bull;** Chile; cult RBGE #1988-0922; *M. Gardner & S. G. Knees 4050* (E); na; EF104999; EF105056. **Campsis radicans (L.) Bureau;** USA; *K.-J. Kim 12802* (TEX); AF102642; AF102626; **FJ870022**. **Catalpa aff. speciosa;** USA; in cult Colorado, Michigan; *ndhF*: *Olmstead 92-99* (WTU); *trnL-F*: *Olmstead 88-003* (WTU); *rbcL*: *C.W. dePamphilis s.n.* (COLO); L11679; L36397; **FJ870023**. **Catophractes alexandri D. Don;** Namibia: cult. in Huntington Bot. Gard. (Debra Folsom coll.); *Lavranos & Pehleman 21181* (HNT); na; EF105000; EF105057. **Chilopsis linearis Sweet;** USA; cult in Seattle, UW campus; *Olmstead 96-139* (WTU); na; **FJ887856**; **FJ870024**. **Colea sytsmae Zjhra;** Madagascar; *M. Zjhra 917* (WIS); na; EF105001; EF105058. **Crescentia alata Kunth;** Mexico, cult UCB Bot. Gard. #89.1516; Cult. from *J.A. Lomeli Sencion & V. Galindo s.n.*; no voucher; na; **FJ887857**; **FJ870025**. **Crescentia portoricensis Britton;** Puerto Rico; cult. In MBG from collection by A. Gentry; *A. Gentry & Zardini 50458* (MO); AF102643; AF102627; EF105060. **Cuspidaria convoluta (Vell.) A. H. Gentry;** Brazil: Sao Paulo; *Lohmann 713* (MO, SPF); na; DQ222573; **FJ870026**. **Cybistax antisyphilitica (Mart.) Mart.;** Bolivia; *M. Nee & L. Bohs 51868* (NY); na; EF105003; EF105061. **Delostoma integrifolium D. Don;** Ecuador; *M.A. Blanco 2155* (WTU); na; EF105004; EF105062. **Delostoma lobbii Seem.;** Peru; *P. Hutchison & J.K. Wright 5465* (UC); na; EF105005; EF105063. **Deplanchea tetraphylla (R. Br.) F. Muell.;** Australia; cult at James Cook University; *P. Gadek s.n.* (JCT); na; EF105006; EF105064. **Dolichandra quadrivalvis (Jacq.) L. G. Lohmann (= Melloa quadrivalvis)**; cult in MBG from collection by A. Gentry; no voucher; *Lohmann 353* (MO)*; na; **FJ887858**/DQ222607*; **FJ870027**. **Dolichandra unguis-cati (L.) L. G. Lohmann (= Macfadyena unguis-cti)**; cult in MBG from collection by A. Gentry; *R. Spangler B3* (MO); *L. G. Lohmann 2546* (BHCB, MO)*; AF102649; AF102633/DQ222595*; **FJ870028**. **Dolichandrone spathacea Seem.;** Malaysia; cult in Waimea BG #74S950; coll. by B. Stone; no voucher; na; **FJ887859**; **FJ870029**. **Eccremocarpus scaber Ruiz & Pav.;** cult. In RBGK #1988-132; *M.W. Chase 2999* (K); AF102646; AF102630; **FJ870030**. **Ekmanianthe actinophylla (Grieseb.) Urb.;** Cuba; *Olmstead 96-108* (WTU); na; EF105002; EF105065. **Fernandoa madagascariensis (Baker) A. H. Gentry;** cult RBGK #1990-2265; *M.W. Chase 5570* (K); na; EF105009; EF105067. **Fridericia corallina (Jacq.) L. G. Lohmann (= Arrabidaea corallina)**; cult MBG from collection by A. Gentry; no voucher; na; **FJ887861**; **FJ870032**. **Fridericia mollissima (Kunth) L. G. Lohmann (= Arribidaea mollissima)**; cult MBG from collection by A. Gentry; no voucher; na; **FJ887862**; **FJ870033**. **Fridericia pubescens L. (L. G. Lohmann) (= Arrabidaea pubescens)**; cult MBG from collection by A. Gentry; *Gentry 10234* (MO); *Lombardi 2529* (BHCP, MO)*; AF102641; AF102625/DQ222556*; **FJ870034**. **Fridericia sp.;** Brazil: Espirito Santo; *D. Folli 1658* (CVRD); na; **FJ887863**; **FJ870035**. **Godmania aesculifolia (HBK.) Standl.;** Costa Rica; *Grose 129* (WTU); na; EF105010; EF105068. **Handroanthus chrysotrichus (Mart. ex DC.) Mattos (= Tabebuia chrysotrichus)**; Brazil, UCB Bot. Gard. 85-0562; Cult. from *L. Anderson s.n.*; *H. Forbes s.n.* (UC/JEPS); na; EF105032; EF105092. **Handroanthus serratifolius (Vahl) S. Grose (= Tabebuia serratifolia)**; cult in MBG (material provide by G. dos Santos); no voucher; na; EF105043; EF105105. **Heterophragma adenophyllum Seem. ex Benth. & Hook.f.;** India; cult in Waimea #79S763; no voucher; na; EF105011; EF105069. **Incarvillea arguta Royle;** Nepal; cult in RBGE #19931281; *C. Grey-Wilson & Phillips s.n.* (E); na; **FJ887864**; **FJ870036**. **Incarvillea mairei (Levl.) Grierson;** China; cult in Seattle; no voucher; na; na; **FJ870037**. **Incarvillea zhongdianensis Grey-Wilson;** China; cult in Seattle; no voucher; na; **FJ887865**; FJ870038. **Jacaranda arborea Urban;** Cuba; cult in Jard. Bot. Nac. Cuba; *Olmstead 96-96* (WTU); **FJ870008**; **FJ887866**; **FJ870039**. **Jacaranda mimosifolia D. Don;** Brazil; *L. Lohmann 369* (MO); na; EF105012; EF105070. **Jacaranda rufa Manso;** Brazil; *L. Lohmann 262* (MO); na; EF105013; EF105071. **Jacaranda sparrei A. H. Gentry;** Ecuador; cult in Waimea #82S772; *H. Descimon s.n.* (MO); AF102647; AF102631; **FJ870040**. **Kigelia africana (Lam.) Benth.;** Kenya; cult in Waimea #74S980 coll by R.C.A. Rica; no voucher; AF102648; AF102632; EF105072. **Lamiodendron magnificum Steenis;** Papua New Guinea; *Takeuchi & Ama 16634* (GH); na; EF105014; EF105073. **Lundia cordata (Vell.) DC.;** Brazil, Espirito Santo; *Gentry et al. 59242* (MO); *L. G. Lohmann 652* (CVRD, MO)*; na; **FJ887867**/DQ222590*; **FJ870041**. **Macrocatalpa punctata Britton (= Catalpa macrocatalpa Ekman);** Cuba, Pinar del Rio; *Olmstead 96-131* (WTU); na; **FJ887868**; **FJ870042**. **Manaosella cordifolia (DC.) A. H. Gentry;** Brazil: Minas Gerais; *L. Lombardi 2546* (CVRD, MO); na; DQ222596; **FJ870043**. **Mansoa difficilis (Cham.) Bureau & K. Schum.;** Brazil, Espirito Santo; *D. Folli 1659* (CVRD, MO); *Lohmann 662* (CVRD, MO)*; na; **FJ895602**/DQ222598*; na. **Mansoa verrucifera (Schltdl.) A. H. Gentry;** cult in RBGE #19731250; no voucher; *L. G. Lohmann 612* (MO, MOL)*; na; **FJ895603**/DQ222604*; **FJ870044**. **Markhamia platycalyx Sprague;** Uganda; cult in Waimea #78P352; no voucher; na; AY500448; **FJ870045**. **Martinella obovata (Kunth) Bureau & K. Schum.;** French Guiana; cult in MBG from collection by A. Gentry; *Gentry & Zardini 50277* (MO); *L. G. Lohmann 126* (BBS, MO)*; L36444; L36402/DQ222606*; **FJ870046**. **Millingtonia hortensis L. f.;** Thailand; *Soejarto & Nantasan 6060*, (GH); na; **FJ887869**; **FJ870047**. **Newbouldia laevis Seem.;** cult in RBGE #19671901; no voucher; na; AY500449; EF105074. **Ophiocolea floribunda (Boj. ex Lindl.) H. Perrier;** Madagascar; *G.E. Schatz, W.D. Stevens & P.J. Rakotomalaza 3448* (MO); AF102650; AF102634; EF105075. **Ophiocolea sp.;** Madagascar; *M. Zjhra 939* (WIS); na; **FJ887870**; na. **Oroxylum indicum Vent.;** Sri Lanka; cult in Waimea #79S51; no voucher; AF102651; AF102635; **FJ870048**. **Pachyptera aromatica (Barb. Rodr.) L. G. Lohmann (= Leucocalantha aromatica)**; *Lohmann 28* (INPA, MO, SPF); na; DQ222589; na. **Pachyptera kerere (Aubl.) Sandwith (= Mansoa kerere)**; Brazil: Amazonas; *Lohmann 336* (MO, NY, SPF, UNIP); na; DQ222600; na. **Pandorea jasminoides (Lindley) K. Schumann;** cult in Matthaei Bot Gard; no voucher; AF102652; AF102636; **FJ870049**. **Pandorea pandorana (Andrews) van Steenis;** Australia, in cult. Austr. Nat. Bot. Gard.; *S. Kelchner 219* (CANB); na; EF105016; EF105076. **Parmentiera**

cereifera **Seem.;** cult in RBGK #1986-6001; *M.W. Chase 3947* (K); na; **FJ870050**; **FJ887871**. *Parmentiera macrophylla* **Standl.;** Costa Rica; *Grose 126* (WTU); na; EF105017; EF105077. *Perianthomega vellozoi* **Bureau;** Bolivia: Santa Cruz; *Nee 35808* (NY); na; DQ222619; **FJ870051**. *Phyllarthron articulatum* **Schum.;** Madagascar; *M. Zjhra 752* (WIS); na; AY500438; EF105079. *Pleonotoma jasminifolia* **(Kunth) Miers;** Brazil: Amazonas; *Lohmann 122* (INPA); na; DQ222625; **FJ870053**. *Podranea ricosoliana* **(Tanfani) Sprague;** S Africa; cult in Waimea #84P524; no voucher; AF102653; na; na. *Podranea ricosoliana* **(Tanfani) Sprague;** Minas Gerais: Belo Horizonte: cultivated plant; *Lombardi 2437* (BHCB, MO); na; DQ222628; **FJ870054**. *Radermachera frondosa* **Chun & H. C. How;** China; cult in Waimea #85S277; no voucher; AF 102654; AF 102638; **FJ870055**. *Rhigozum madagascariense* **Drake;** Madagascar; *S. Malcomber 1138* (MO?); na; AY500451; na. *Rhigozum obovatum* **Burch.;** S Africa; cult Kew #1957-1802; *M.W. Chase 3892* (K); na; EF105020; EF105080. *Rhodocolea racemosa* **(Lam.) H. Perrier;** Madagascar; *M. Zjhra 943* (WIS); na; AY500443; EF105081. *Roseodendron donnell-smithii* **(Rose) Miranda (= *Cybistax donnell-smithii, Tabebuia donnell-smithii*);** cult in Waimea #89P166; no voucher; AF102644; AF102628; EF105093. *Sparattosperma leucanthum* **(Vell.) K. Schum.;** cult in Waimea #87-5446; no voucher; na; EF105022; EF105082. *Spathodea campanulata* **Beauv.;** Africa; cult in Waimea #78P1079; no voucher; na; AY500452; EF105083. *Stereospermum euphorioides* **DC.;** Madagascar; *M. Zjhra 682* (WIS) appears to be same as *Phillipson 3923* (MO); na; AY500453; AY500429/AY500409. *Stereospermum nematocarpum* **DC.;** Madagascar; cult in RBGK #1990-2276; *M.W. Chase 3891* (K); na; EF105025; EF105085. *Stizophyllum perforatum* **(Cham.) Miers;** Brazil: Minas Gerais; *Lombardi 2431* (BHCP, MO); na; DQ222639; FJ870057. *Tabebuia heterophylla* **(DC.) Britton;** Florida; cult in MBG; coll by Gentry; no voucher; L36451; L36416; EF105096. *Tabebuia lepidota* **(HBK.) Britton;** Cuba, Pinar del Rio; *Olmstead 96-112a* (WTU); na; EF105036; EF105098. *Tabebuia rosea* **(Bertol.) DC.;** Mexico; *Grose 156* (WTU); na; EF105040; EF105102. *Tabebuia sauvellei* **Britton;** Cuba; *Olmstead 96-74* (WTU); na; EF105041; EF105103. *Tanaecium pyramidatum* **(Rich.) L. Lohmann (= *Paragonia pyramidata*);** Brazil: Amazonas; *Lohmann 274* (MO, NY, SPF, UNIP); na; DQ222618; **FJ870058**. *Tecoma stans* **Juss.;** *K.-J. Kim 12805* (TEX); AF102655; AF102639; **FJ870059**. *Tecomanthe hillii* **(F. Muell.) Steenis;** Australia in cult, Queensland; No Voucher; na; EF105051; EF105113. *Tecomanthe volubilis* **Gibbs;** cult in RBGE #19930930; *D. Mitchell & P. Smith 164* (E); na; **FJ887873**; **FJ870060**. *Tecomaria capensis* **(Thunb.) Spach;** S Africa; coll in cult in Mexico; *R.J. Barr 63-533 & W. E. Niles 261* (UC/JEPS); **FJ870009**; **FJ887874**; **FJ870061**. *Tecomella undulata* **Seem.;** Pakistan; *R. R. Stewart 23756* (GH); na; **FJ887875**; na. *Tourrettia lappacea* **(L'Hérit.) Willd.;** Ecuador; *Dobson & Wolfe 1088* (US); na; **FJ887876**; **FJ870063**. *Tynanthus panurensis* **(Bureau) Sandwith;** Brazil: Amazonas; *L. Procópio 14* (G, INPA, K,

MG, MO, NY, RB, SP, U, UB); na; DQ222644; **FJ870064**. *Xylophragma myrianthum* **(Cham.) Sprague;** Brazil: Espirito Santo; *Lohmann 649* (CVRD, MO); na; DQ222648; **FJ870065**. *Zeyheria tuberculosa* **Bureau;** Brazil; cult in MBG from collection by A. Gentry; no voucher; na; EF105053; EF105115.

OUTGROUPS. **Acanthaceae**—*Barleria prionitis* **L.**; Hedren et al. 1994; L01886; U12653; na. *Barleria prionitis* **L.;** McDade & Moody 1999; na; na; AF063118. *Elytraria crenata* **Vahl.;** R. Scotland; AF188127; U12657; na. *Elytraria imbricata* **(Vahl.) Pers.;** USA; *Arizona; McDade & Jenkins 1155* (ARIZ); na; na; AF061819. **Gesneriaceae**—*Nematanthus hirsutus* **(Mart.) Wiehler;** cult. in Marie Selby Bot Gard; no known voucher; L36446; L36404; na. *Nematanthus strigillosus* **(Mart.) H. E. Moore**; *Zimmer et al. 2002*; na; na; AY047148. **Lamiaceae**—*Callicarpa dichotoma* **Raeusch;** China; cult in Beal Bot Gard; *Olmstead 88-012* (WTU); L14393; L36395; AF363665. *Tectona grandis* **L.f.;** Asia; cult in Waimea Bot. Gard. #73P72; *R. Olmstead 92-277* (WTU); U28884; U78705; FJ870062. **Oleaceae**—*Nyctanthes arbor-tristis* **L.;** cult in RBGK #099-86.00993; no voucher; U28877; U78708; na. *Nyctanthes arbor-tristis* **L.;** Wallander & Albert 2000; na; na; AF231863. **Paulowniaceae**—*Paulownia tomentosa* **(Thunb.) Steud.;** cult in Washington Park Arb, Seattle; *Olmstead 88-008* (WTU); L6447; L36406; na. *Paulownia tomentosa* **(Thunb.) Steud.;** China; Bremer et al. 2002; na; na; AJ430926. **Pedaliaceae**—*Sesamum indicum* **L.;** cultivated; no voucher; L14408; L36413; na. *Sesamum indicum* **L.;** cultivated; McDade & Moody 1999; na; na; AF067067. **Schlegeliaceae**—*Exarata chocoensis* **A. H. Gentry;** Ecuador; *J. L. Clark 8836* (US); na; **FJ887860**; **FJ870031**. *Schlegelia parviflora* **(Oerst.) Monach.;** Venezuela; cult in MBG; *Gentry 14221* (MO); L36448; L36410; AJ608570. **Scrophulariaceae**—*Buddleja davidii* **Franch.;** China; cult in Seattle WA; *Olmstead 88-007* (WTU); L14392; L36394; na. *Buddleja davidii* **Franch.;** China; Wallich et al. unpubl; na; na; AF380861. *Myoporum mauritianum* **A. DC.;** cult in RBGK #1984-4220; Wallander & Albert 2000; no known voucher; L36445; L36403; AJ299257. *Scrophularia californica* **Cham. & Schldtl.;** USA; *C.W. dePamphilis s.n.* (PAC); L36449; L36411; na. *Scrophularia californica* **Cham. & Schldtl.;** USA; E. Freeman unpubl.; na; na; AF118802. *Verbascum thapsus* **L.;** cultivated; L36452; L36417; na. *Verbascum thapsus* **L.;** cultivated; Kornhall et al. 2001; na; na; AJ296522. **Verbenaceae**—*Petrea volubilis* **L.;** RBGK #326.75.03134; no voucher; U28880; **FJ887872**; **FJ870052**. *Rhaphithamnus spinosus* **(A. L. Juss.) Moldenke;** Kew 128-83.01596; no voucher; U32160; L36409; **FJ870056**. **Veronicaceae**—*Antirrhinum majus* **L.;** cultivated; *C.W. dePamphilis s.n.* (PAC); L11688; na; na. *Antirrhinum majus* **L.;** cultivated; L36413; na. *Antirrhinum majus* **L.;** cultivated; Bremer et al. 2002; na; na; AJ430929. *Digitalis purpurea* **L.;** cultivated; no voucher; L01902; na. *Digitalis purpurea* **L.;** cultivated; *K.-J. Kim 13943* (YNUH); na; AF130150; na. *Digitalis purpurea* **L.** cultivated**;** E. Freeman unpubl.; na; na; AF034871.