

Evolution of a Charismatic Neotropical Clade: Molecular Phylogeny of *Tabebuia* s. l., Crescentieae, and Allied Genera (Bignoniaceae)

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ABSTRACT. *Tabebuia* is one of the most commonly encountered genera of Bignoniaceae in the neotropics. Previous research has suggested that this genus may be paraphyletic and contain the tribe Crescentieae. Molecular sequence data of the chloroplast *trnL-F* and *ndhF* regions were used to reconstruct the phylogeny of *Tabebuia*, Crescentieae and related genera. A previously unrecognized clade of Neotropical, arboreal Bignoniaceae characterized by palmately compound leaves was identified and is referred to as the *Tabebuia* alliance. Within this group, *Tabebuia* is confirmed to be paraphyletic, since it includes Crescentieae, *Spirotecoma*, and *Eknmanianthe*. The position of *Zeyheria*, *Godmania*, and *Cybisstax* with relation to *Tabebuia* is equivocal. *Sparattosperma* is sister to the rest of the *Tabebuia* alliance. *Spirotecoma* is inferred to be sister to Crescentieae. The phylogeny of *Tabebuia* presented here corresponds to the species groups established by Gentry, while at the same time highlighting the need for taxonomic revisions. When considered on a biogeographic scale, the phylogeny indicates a minimum of four dispersal events from the mainland to the Greater Antilles.

KEYWORDS: Bignoniaceae, Biogeography, Crescentieae, molecular phylogeny, *Tabebuia*.

Tabebuia contains some of the most distinctive species of Neotropical trees. The most easily recognized species are tall trees with large, showy inflorescences that bloom when the trees have dropped all their leaves (Gentry 1992a; 1992b). Yet, *Tabebuia* is one of the most inscrutable genera of Bignoniaceae. It is the largest and most taxonomically complex genus in Bignoniaceae (approx. 100 spp; dos Santos and Miller 1992; Gentry 1992a) and has been placed historically in tribe Tecomeae, a paraphyletic assemblage of Bignoniaceae defined by having bilocular fruits that dehisce perpendicular to the septum (Gentry 1980; Spangler and Olmstead 1999).

In the most recent treatment of New World Tecomeae, Gentry (1992a) commented that *Tabebuia* was related to several genera, including *Sparattosperma*, *Godmania*, *Spirotecoma*, and *Cybisstax*. The character uniting these taxa is palmately compound leaves, a feature found rarely in Bignoniaceae as a whole, but most commonly in New World members of Tecomeae (Gentry 1992a).

Tabebuia is considered the most morphologically and anatomically diverse genus in Bignoniaceae (Gentry 1969, 1992a; dos Santos and Miller 1992). This genus has a convoluted nomenclatural history (Gentry 1969), implying that taxonomists saw much morphological diversity within this taxon that they were unable to partition satisfactorily. In his effort to partition that morphological diversity, Gentry (1992a) defined five groups that he considered natural. However, for the purposes of keying, he split two of them in to smaller groups, thus he enumerated a total of 10 species groups (Gentry 1992a).

Nineteenth century classifications recognized a tribe, Crescentieae, identified by indehiscent fruits (e.g., Bentham and Hooker 1876), in contrast to the dehiscent and mostly wind-dispersed fruits typical of the rest of Bignoniaceae. Gentry (1976) recognized that the geographically disjunct New and Old World representatives of this group were each more closely related to representatives of tribe Tecomeae in the same geographic region (Neotropics and Africa/Asia/Madagascar, respectively). This has been corroborated by subsequent molecular phylogenetic studies (Spangler and Olmstead 1999; Zjhra et al. 2004). Crescentieae, as it is recognized today (Gentry 1980), is endemic to Central America and the Caribbean basin and contains three genera and approximately 33 species, most of which are poorly known. The two other lineages in Bignoniaceae that share the condition of indehiscent fruits, Coleeae in Madagascar and *Kigelia* from continental Africa, are known to be mammal dispersed, while mammal dispersal in Crescentieae is only postulated based on the fruit morphology and circumstantial evidence (Gentry 1974; Janzen 1981; Janzen and Martin 1982).

Gentry speculated that Crescentieae were closely related to, or even derived from *Tabebuia*: "In the New World Crescentieae the probable evolutionary sequence leads from a *Tabebuia*-like species of Tecomeae through *Parmentiera* to *Amphitecna* and *Crescentia*" (Gentry 1976: 258). This suggestion has been born out in subsequent molecular phylogenetic studies, which show a close relationship between the two groups (Spangler and Olmstead 1999) with Crescentieae nested within *Tabebuia* (R.

Olmstead, unpubl.). Understanding the relationship between *Tabebuia* and Crescentieae and identifying the sister group to Crescentieae are valuable for inferring the transition from wind dispersed to putative mammal dispersed fruits and the evolution of the morphological innovations that accompany that shift. In order to better characterize these changes, a thorough phylogeny of these lineages is necessary. Furthermore, this study, in conjunction with studies of Coleeae and *Kigelia*, eventually will allow for comparative studies of the evolution of indehiscent, mammal dispersed fruit.

It is commonly assumed that wind-dispersed species have a wide distribution (Gentry 1976, 1979, 1980, 1983). Species distributions in Bignoniaceae generally support this (Gentry 1983, 1990, 1992a), with species of Crescentieae typically having narrow ranges, whereas many species of *Tabebuia* are widely distributed. However, two species of Crescentieae, *Amphitecna latifolia* and *Crescentia cujete*, are found naturally on both Caribbean Islands and the mainland. These species are thought to be secondarily water dispersed, and *C. cujete* may also be human dispersed. Gentry (1992a) described four of his 10 *Tabebuia* species groups (7–10) as being restricted to the Caribbean islands. He noted only one species of *Tabebuia* group three, *T. billbergii* var. *billbergii*, with a natural distribution in the West Indies. Thus, it appears that for over-water transport, the indehiscent-fruited Crescentieae are at least as capable of range expansion as their wind-dispersed relatives. A phylogenetic analysis will permit the assessment of the degree to which each of these dispersal modes has been capable of dispersal between continental and island localities.

The goals of this study were to 1) test monophyly of the palmate-leaved Bignoniaceae (exclusive of those trifoliolate leaved lianas in tribe Bignoniaceae that clearly are unrelated) and estimate their phylogeny, 2) test for monophyly of *Tabebuia*, 3) determine the sister group to Crescentieae and 4) interpret the biogeographic patterns in light of the phylogeny. To achieve these goals, a phylogeny was estimated using sequence data from two loci in the chloroplast genome, *ndhF* and the *trnL-F* intron/spacer. These regions have been used in previous studies to successfully estimate relationships at the familial, tribal and generic levels (Spangler and Olmstead 1999; Beardsley and Olmstead 2002; Sytsma et al. 2002; Zjhra et al 2004).

included. Species of *Tabebuia* were selected so as to represent the morphological diversity within the genus. Outgroups were included from a Bignoniaceae-wide phylogenetic study (R. Olmstead unpubl.). Vouchers were deposited in herbaria at the botanic gardens, herbaria in their country of origin, or WTU.

DNA extraction, Amplification, and Sequencing. DNA was extracted using a modified CTAB protocol (Doyle and Doyle 1987) and further purified using the Qiaquick kit (Qiagen Co., Valencia, California) as described in Beardsley and Olmstead (2002). Target regions were amplified using standard PCR methods for *ndhF* (Spangler and Olmstead 1999) and *trnL/F* (Taberlet et al. 1991; Beardsley and Olmstead 2002). Products from the two chloroplast regions were then directly sequenced using DYEnamic ET chemistry (Amersham Biosciences, Piscataway, New Jersey). Sequences were edited using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, Michigan) and aligned by eye using Se-Al Carbon (A. Rambaut, University of Oxford). For the *trnL/F* dataset, gap presence/absence was scored using simple gap coding (Graham et al. 2000; Simmons and Ochoterena 2000) and included as binary characters. Since both DNA regions are part of a single non-recombining chloroplast DNA molecule, they were combined for analysis. Overall, 8% of the data were coded as missing. The dataset was deposited in TreeBASE (study number S1798) and sequences were deposited in GenBank.

Phylogenetic analyses were performed under maximum parsimony (MP) and maximum likelihood (ML) using PAUP*4.0b10 (Swofford 2003) and Bayesian inference criteria using MrBayes 3.0 (Ronquist and Huelsenbeck 2003). For MP, heuristic searches were performed using the default settings except as indicated. Trees were found using 1000 random taxon addition replicates with multrees on. For each replicate, searching was stopped after the 100 shortest trees were found. These trees were swapped before moving onto the next replicate. Branch support was found using bootstrap values following the recommendation of DeBry and Olmstead (2000). Character history was traced using Mesquite 1.05 (Maddison and Maddison 2004).

ModelTest 3.6.1 (Posada and Crandall 1998) was used to determine the appropriate models to use for the combined dataset in the maximum likelihood analysis and for each gene individually in the Bayesian analyses. For maximum likelihood, the gap characters were excluded, and a heuristic search was done using the strict consensus of all most parsimonious trees as the starting tree, and all parameters input from ModelTest results.

Bayesian analyses were performed on the combined dataset using Mr. Bayes 3.0 (Ronquist and Huelsenbeck 2003) using prior probabilities based on the likelihood model identified in ModelTest 3.6.1, gap characters were included and given flat priors. Three independent searches were run. For each of these, the Markov Chain Monte Carlo algorithm was run with five cold and one hot chain for 3 million generations and sampled every 200 generations. The data were examined graphically to establish the burn-in limit, which was determined to be at 25000 generations (Ronquist and Huelsenbeck 2003). The trees prior to stationarity were discarded. The remaining trees were summarized as a majority rule consensus tree. The resulting support values on the branches are the posterior probabilities (p.p.) and were used to evaluate the support for the tree (Ronquist and Huelsenbeck 2003).

MATERIALS AND METHODS

Taxonomic Sampling. A total of 41 species representing the palmately leaved genera and 24 outgroup species were

RESULTS

Datasets. Of the 65 taxa used in this study, 54 are included in a phylogenetic analysis here for the

first time. The aligned length of the *ndhF* dataset is 2102 bp. Alignment was readily achieved by eye without any gaps. The aligned length of *trnL-F* is 1004 bp and it was also readily alignable by eye. Twenty-eight shared gaps were coded as presence/absence characters.

Hierarchical likelihood ratio tests performed with ModelTest indicated that, under the Akaike information criterion (Akaike 1974), the general time reversible + gamma model was the most appropriate for the combined dataset and *trnL-F* alone while TVM+G is more appropriate when only *ndhF* is considered.

Combined Analyses. The combined dataset has 3134 total characters, of which 2234 are constant. Of the 900 variable characters, 475 are parsimony-uninformative and 425 are parsimony informative. The analysis retained 1000 trees that were 1401 steps long. The strict consensus of these trees is presented here (Fig. 1). To ensure that no shorter trees existed, the strict consensus tree was loaded as a constraint tree, and the heuristic search rerun under identical parameters but with 10000 replicates retaining only those trees incompatible with the constraint tree (Catalán et al. 1997). No equal-length or shorter trees were found under the constrained search, indicating that the strict consensus tree is representative of the dataset even though not all optimal topologies were found. With the Maximum likelihood criterion, the combined dataset was analyzed with the GTR+G model. The most likely tree had an lnL -13315.47246 (Fig. 2).

For the Bayesian analysis, *ndhF* was analyzed under the TVM+G model while *trnL-F* with the GTR+G as indicated by ModelTest. However, the prior probabilities for each region were different, and the parameters for the search reflect those differences. The lnL values of the sampled trees ranged from -13988.492 to -3696.211. The consensus of these trees contained 41 clades with posterior probability (p.p.) ≥ 0.95 , of which 33 had p.p. of 1.0.

The results of all searches identified a clade of taxa including: *Sparattosperma*, *Cybistax*, *Zeyheria*, *Godmania*, *Tabebuia*, *Ekmanianthe*, *Spirotecoma*, and *Crescentieae* (figs. 1 and 2). This clade was supported by a bootstrap value of 74%, and a p.p. of 1.0. Within this group *Sparattosperma leucanthum* was sister to a clade comprising the rest of the taxa (65% bs; 1.0 p.p.). The rest of the clade comprised three major groups whose relationships to each other could not be resolved. The first of these groups was *Zeyheria*, *Godmania* and *Cybistax* (99% bs; 1.0 p.p.). The second was *Ekmanianthe* and one clade of *Tabebuia* that corresponds to Gentry's

groups 6–10, and will hereafter be referred to as *Tabebuia* group I (67% bs; 1.0 p.p.). The third group contained *T. donnell-smithii*, a second group of *Tabebuia* that corresponded to Gentry's groups 3–5 (hereafter referred to as *Tabebuia* group II), *Spirotecoma* and *Crescentieae* (52% bs; 0.91 p.p.). Within the resulting trees, the position of *Spirotecoma* shifted between being sister to *Crescentieae* to being nested within it.

DISCUSSION

This study focuses on a clade of New World Bignoniaceae identified by Spangler and Olmstead (1999) that includes the tribe *Crescentieae* and the large genus *Tabebuia* along with a handful of small genera, all assigned to the paraphyletic tribe *Tecomeae*. Most of the taxa in this group are characterized by palmately compound leaves, a trait that is unusual in Bignoniaceae. This clade is sister to an Old World clade comprised of the Madagascan endemic tribe *Coleeae* and related genera in *Tecomeae* that are distributed in Africa, Madagascar, and across south Asia to SE Asia (Paleotropical clade in Figs. 1, 2). *Coleeae* and several of the related genera in *Tecomeae* have been the subject of a molecular phylogenetic study (Zjhra et al. 2004). The results presented here extend the results of Zjhra et al. by placing two additional Old World *Tecomeae*, *Catophractes* (Africa) and *Heterophragma* (India and SE Asia), into the clade with *Coleeae* and related genera. This study also provides increased support for the monophyly of the group comprising these two sister clades (65% bs; 0.99 p.p.).

Monophyly of Palmate-Leaved Bignoniaceae. All analyses conducted support the monophyly of the Neotropical taxa with palmately compound leaves: *Sparattosperma*, *Cybistax*, *Zeyheria*, *Godmania*, *Tabebuia*, *Ekmanianthe*, *Spirotecoma*, and *Crescentieae*, herein called the *Tabebuia* alliance in recognition of the largest and most widely distributed genus in the group (Figs. 1, 2). Most of this group of taxa were recognized by Bentham and Hooker (1876) as an informal group, "Digitifolieae," within tribe *Tecomeae*, to contain American taxa having "seeds with hyaline wings that are rarely opaque and undivided, four perfect stamens and leaves simple or digitate." In *Digitifolieae* they placed *Tabebuia*, *Couralia* (= *Tabebuia*), *Delostoma*, and *Zeyheria*. Except for *Delostoma*, which these results show does not belong with *Digitifolieae* (Figs. 1, 2), this group, as they described it, is consistent with the phylogeny presented here. The other taxa included in the clade were not yet described in 1876 (*Cybistax*, *Godmania*, *Ekmanianthe*, *Sparattosperma*,

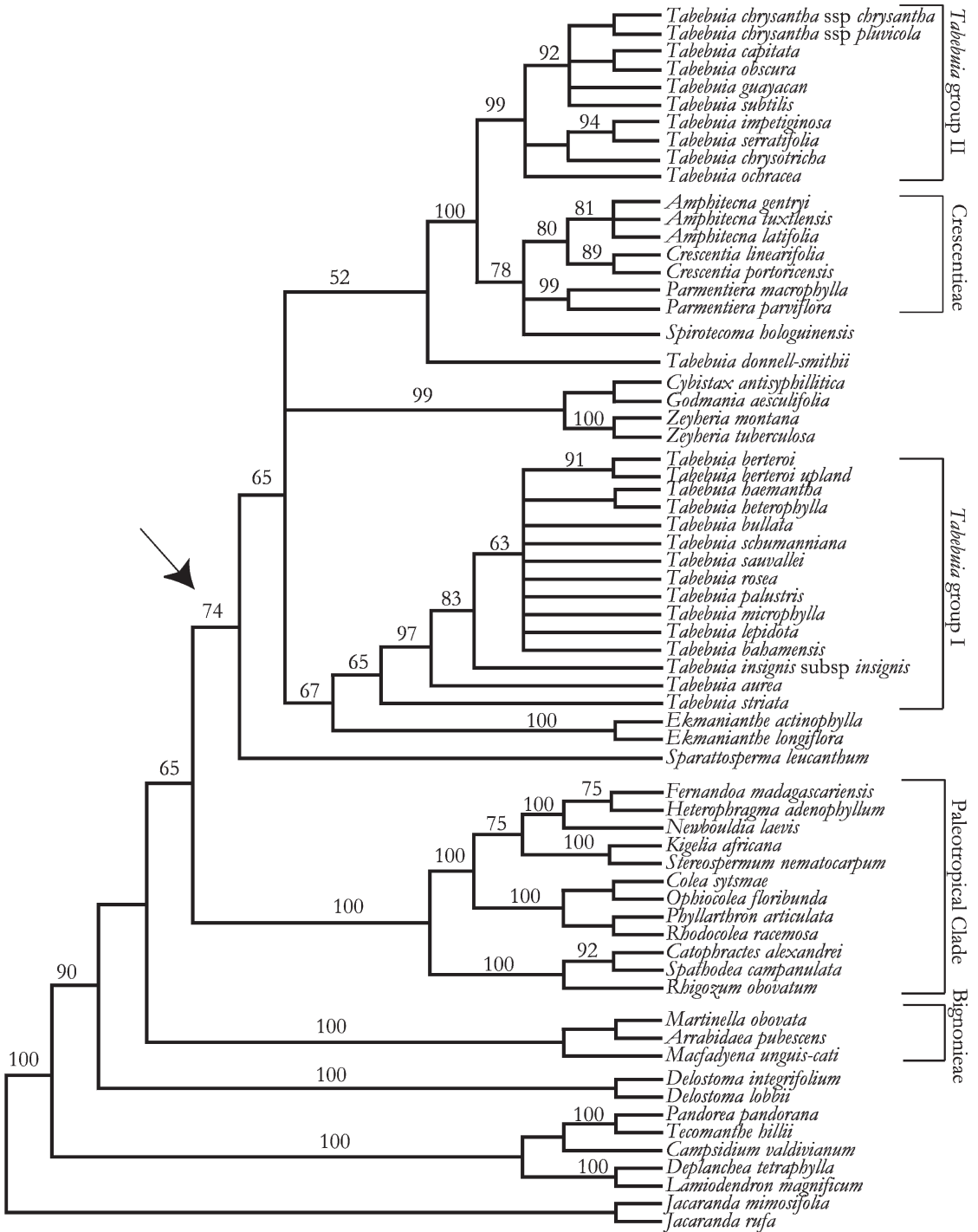


FIG. 1. Strict consensus of 1000 most parsimonious trees (L = 1401, CI = 0.76, RI = 0.81). The arrow indicates the branch leading to the *Tabebuia* alliance and the numbers above branches are bootstrap values greater than 50%.

Spirotecoma), or were assigned to Crescentieae on the basis of their indehiscent fruits.

Within the *Tabebuia* alliance, *Amphitecna* is the only genus without palmately compound leaves.

However, its derived position within the group suggests that this condition is a reduction to unifoliolate leaves. Several species in *Tabebuia* group I, including the type, *T. cassinoides*, also

have putatively unifoliolate leaves, but given current sampling, there is no evidence that they form a clade within the group, suggesting that the reduction to unifoliolate leaves has occurred independently multiple times. This corroborates Gentry's suggestion of multiple origins of unifoliolate leaves in *Tabebuia* (Gentry 1992a).

Leaf morphology is generally considered to be a highly plastic trait and not reliable for defining higher taxa. Within Bignoniaceae, most genera have pinnately compound leaves and may be quite variable even between closely related genera (e.g., Coleaeae; Gentry 1980). Bignoniae is the only other group with distinctive leaf morphology. Leaves of Bignoniaceae are trifoliolate (although modifications can be found), with the terminal leaflet often modified into a tendril for climbing (Gentry 1992a; Lohmann and Hopkins 1999). Leaf morphology is inconstant within Crescentieae and Coleaeae. Therefore, a key finding of this study is that of a large group of genera in Bignoniaceae united by leaf morphology – the *Tabebuia* alliance.

Monophyly of *Tabebuia*. These results show that *Tabebuia*, as currently circumscribed (Gentry 1992a), is not monophyletic. This genus formed three, well-defined groups (represented here by *Tabebuia* group I, *Tabebuia* group II, and *Tabebuia donnell-smithii*). These groups also are distinctive morphologically and merit recognition at the generic level (Grose and Olmstead 2007). *Tabebuia* group I is distinguished by its spathaceous calyx, indumentum of lepidote scales and generally pink to white flowers. *Tabebuia* group II contains the *Tabebuia* with cupular calices, indumentum of hairs and yellow or magenta flowers. *Tabebuia donnell-smithii* is distinctive in that its calyx while spathaceous is the same color and texture as the corolla, the indumentum is of gland tipped trichomes and the flowers are yellow. While not calling them sections, Gentry (1992a) recognized five “natural” species groups within *Tabebuia* based on morphology that correspond closely to the distinct lineages identified here. One group corresponds to *Tabebuia donnell-smithii* (and included another similar species, *T. chrysea*). Two of his other groups correspond to *Tabebuia* groups I and II recognized here. His remaining species groups two and five consisted of two and four species, respectively. He correctly predicted that his group four was closest to our *Tabebuia* group II and that his group two was not close to that group

(in the results presented here, it belongs in *Tabebuia* group I).

Gentry's view on the monophyly of *Tabebuia* is difficult to ascertain. While expressing his opposition to segregating a portion of *Tabebuia* as *Handroanthus* by Mattos (1970), he forcefully argued for *Tabebuia* as a “natural group” (Gentry 1972). Yet he commented that *Parmentiera* and, by extension, Crescentieae were derived from *Tabebuia* ancestors (Gentry 1974) and that *Spirotecoma* evolved directly from the Haitian species *T. conferta* (Gentry 1992a, p. 173). This ambiguity probably stems from the evolution of the word “natural” from a definition that would include paraphyletic groups in the mid 20th century to one that is synonymous with monophyletic group today.

Determining the sister group to Crescentieae. Crescentieae is an enigmatic tribe whose affinity to *Tabebuia* was suggested in other studies (Gentry 1974; Spangler and Olmstead 1999). This study confirms the close relationship between Crescentieae and *Tabebuia* (Spangler and Olmstead 1999) and prior suggestions that Crescentieae is derived from *Tabebuia* ancestors (R. Olmstead, unpubl.). Despite the obvious differences in fruits of Crescentieae and *Tabebuia*, which are putatively associated with dispersal syndrome, the fruit morphology of *Parmentiera* is very similar to that of juvenile fruits of *Tabebuia* (Gentry 1974, 1976). The pericarp wall is fleshy and rather thick, and does not dehisce, but the fruit is bilocular with two placentae per locule, and the seeds are winged (Gentry 1976, 1980). Gentry (1974) suggested that the mechanism driving evolution of an indehiscent fruit was monkeys eating the juvenile fruits, thereby destroying the seeds. He proposed a developmental shift in seed maturation relative to fruit maturation, so that the pericarp remains in an immature edible condition, while the seeds mature and become indigestible. Thus, one-time seed predators became agents of seed dispersal.

Another key finding of this study is the heretofore, unsuspected relationship between Crescentieae and the Antillean endemic *Spirotecoma*. *Spirotecoma* contains four species, three of which are endemic to Cuba. Gentry proposed a close relationship between *Spirotecoma* and *Tabebuia* related to a pollinator shift from bees to perching birds (Gentry 1992a p. 125). *Spirotecoma* also is visited by bats, but it is not known if they

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FIG. 2. The single tree resulting from the maximum likelihood search, -lnL 13315.47246. The arrow marks the branch leading to *Tabebuia* alliance. Numbers at branches indicate parsimony bootstrap values greater than 50% posterior probabilities. *Jacaranda* has been pruned from this tree.

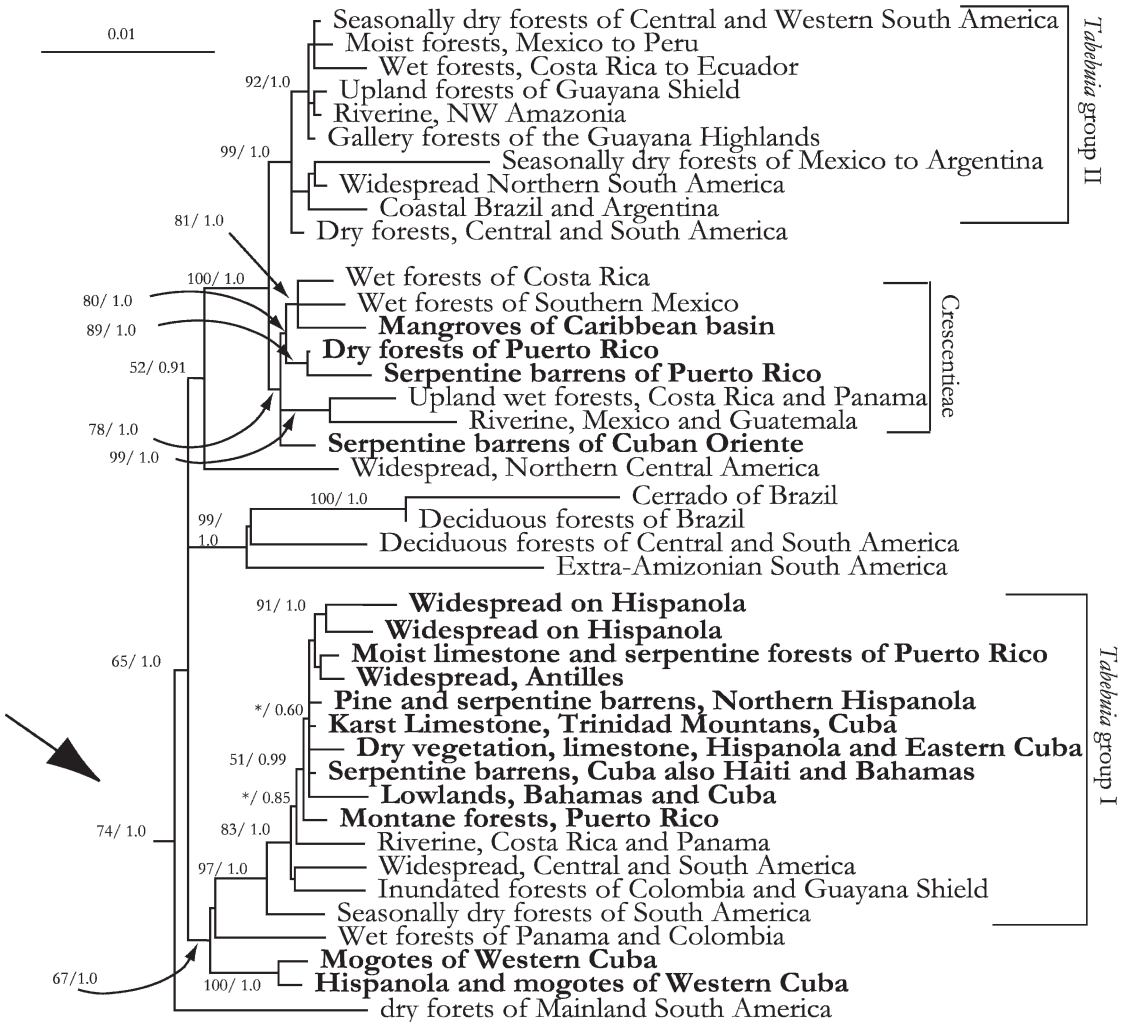


FIG. 3. Area cladogram of *Tabebuia* alliance based on ML tree (fig. 2). Taxa in bold have Antillean distribution.

are pollinators (Gutierrez et al 2005). *Spirotecoma* is the only lineage in the *Tabebuia* alliance to share with Crescentieae the character of inflorescences that are ramiflorous (borne directly on the branches), and are reduced to one or two flowers. The corolla lobes are curved into a shape that is reminiscent of corollas in Crescentieae. The fruit, however, is dehiscent and has a spiral twist (similar to that of *Godmania*), but the septum is thickened with pits into which the seed bodies fit. Putative bat pollination (also found in Crescentieae), combined with the inflorescence characters, corroborate the molecular data in allying this genus with Crescentieae. Thus, its placement near *Tabebuia* or Crescentieae was evident to traditional taxonomists, but its exact phylogenetic placement was not. The close relationship between *Spirotecoma* and Crescentieae is now evident, but its exact position is unresolved in the molecular phylogeny

presented here. However, the combination of morphological traits, including the plesiomorphic condition of dehiscent fruits, suggests it may be sister to Crescentieae. If *Spirotecoma* is sister to Crescentieae, its distribution raises questions regarding the geographic origin of Crescentieae (Antilles or mainland), and the relationship of Crescentieae with the geographical history of the Caribbean basin.

Biogeography. The known species distributions of Bignoniaceae suggest that dispersal in the family, in general, rarely crosses large bodies of water. Outside of the Caribbean, when Bignoniaceae occur naturally on islands, the islands are usually of continental origin, such as Madagascar, the Seychelles, Sri Lanka, Borneo, New Caledonia, the Philippines and Papua New Guinea (Zjhra et al. 2004; van Welzen et al. 2005). Only rarely are Bignoniaceae found naturally on islands of volca-

nic origin (e.g., the Comores and Mascarenes). Of these insular regions, the family is most diverse in Madagascar where several lineages of Bignoniaceae occur. Of these lineages, only Coleeae have been studied in depth in a phylogenetic context (Zjhra et al. 2004). The approximately 80 species of Coleeae represent an *in situ* radiation resulting from one colonization event. In the Coleeae, colonization by a wind-dispersed species was followed by changes in fruit morphology to exploit mammals, notably lemurs, as dispersal agents (Gentry 1976; Zjhra et al. 2004). In the New World, there was an island radiation in the Greater Antilles where the approximately 60 taxa are the result of a few colonization events. The radiation in *Tabebuia* proceeded without any change in dispersal agent. The near absence of native mammals in the Antilles, may have prevented a diversification of Crescentieae parallel to that of *Tabebuia*. Given the proximity of these areas to the mainland, one might expect to find greater representation of Bignoniaceae in these areas, yet this does not appear to be the case. The seeds of Bignoniaceae, including those of the *Tabebuia* alliance, are large for wind-dispersed taxa. It is likely that they cannot remain long enough in the wind column to successfully colonize islands except in rare, catastrophic events, such as Gentry postulated to explain the lone accession of *T. billbergii* (*Tabebuia* group II), a South American species of coastal forests, recorded from Cuba (Gentry 1992a, p. 153). Gentry (1979; 1983) postulated that the indehiscent nature of Crescentieae fruits in general and *Amphitecna* in particular allow them to use water as a dispersal agent.

The phylogeny presented here suggests at least four and possibly more independent colonizations of the Caribbean islands (figure 3). Caribbean endemics are sister to two clades with mixed mainland and Antillean distributions: *Ekmanianthe*, sister to *Tabebuia* group I, and *Spirotecoma*, sister to Crescentieae. In both cases the outgroups to each clade are continental in distribution. *Ekmanianthe* and the Antillean *Tabebuia* clade within *Tabebuia* group I can each be inferred to be separate colonization events. However, the poor resolution in Crescentieae makes inference of biogeographic events more equivocal. Within Crescentieae, *Amphitecna latifolia* is present in mangrove swamps around the Caribbean basin, including the Greater Antilles. It is the only species of *Amphitecna* found in the Caribbean and probably represents an independent colonization event. *Crescentia* has both Antillean and continental species (not included in this study) and likewise probably represents an independent colonization event. If *Spirotecoma* is

sister to Crescentieae, as seems likely, rather than nested within it in a position near one of the Antillean groups, it represents a fifth independent colonization event.

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LITERATURE CITED

- AKAIKE, H. 1974. A new look at the statistical model identification. *IEEE Transaction on Automatic Control* 19: 716–723.
- BEARDSLEY, P. M. and R. G. OLMSTEAD. 2002. Redefining Phrymaceae: The placement of *Mimulus*, tribe Mimuleae and *Phryma*. *American Journal of Botany* 89: 1093–1102.
- BENTHAM, G. and J. D. HOOKER. 1876. Bignoniaceae. Pp. 1026–1053 in *Genera Plantarum* vol. 2. London.
- CATALÁN, P., E. A. KELLOGG, and R. G. OLMSTEAD. 1997. Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndhF* gene sequences. *Molecular Phylogenetics and Evolution* 8: 150–166.
- DEBRY, R. W. and R. G. OLMSTEAD. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. *Systematic Biology* 49: 171–179.
- DOS SANTOS, G. and R. B. MILLER. 1992. Wood anatomy of Tecomeae. Pp. 336–358 in *Flora Neotropica Monograph*. vol. 25, ed. A. H. Gentry. New York: New York Botanical Garden.
- DOYLE, J. J. and J. L. DOYLE. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical Bulletin* 19: 11–15.
- GENTRY, A. H. 1969. *Tabebuia*: The tortuous history of a generic name (Bignon.). *Taxon* 18: 635.
- . 1972. *Handroanthus* (Bignoniaceae): A Critique. *Taxon* 21: 113–114.
- . 1974. Coevolutionary patterns in Central American Bignoniaceae. *Annals of the Missouri Botanical Garden* 61: 728–759.
- . 1976. Relationships of the Madagascar Bignoniaceae: a striking case of convergent evolution. *Plant Systematics and Evolution* 126: 255–256.
- . 1979. Distribution patterns of Neotropical Bignoniaceae: Some phytogeographic implications. Pp. 339–354 in K. Larsen and L. B. Holm-Nielsen. *Tropical Botany*. London: Academic Press.
- . 1980. *Bignoniaceae- Part I: Crescentieae and Tourrettieae*.

- Flora Neotropica Monograph* 25. New York: New York Botanical Garden.
- . 1983. Dispersal and distribution in Bignoniaceae. *Sonderbände enschftlichen Vereins in Hamburg* 7: 198–199.
- . 1990. Evolutionary patterns in Neotropical Bignoniaceae. *Memoirs of the New York Botanical Garden* 55: 118–129.
- . 1992a. Bignoniaceae— Part II (Tribe Tecomeae). *Flora Neotropica Monographs* 25.
- . 1992b. A synopsis of Bignoniaceae ethnobotany and economic botany. *Annals of the Missouri Botanical Garden* 79: 53–64.
- GRAHAM, S. W., P. A. REEVES, A. C. E. BURNS, and R. G. OLMSTEAD. 2000. Microstructural changes in noncoding chloroplast DNA: Interpretation, evolution, and utility of indels and inversions in basal angiosperm phylogenetic inference. *International Journal of Plant Sciences* 161(6 Suppl.): S83–S96.
- GROSE, S. O. and R. G. OLMSTEAD. 2007. Taxonomic revisions in the polyphyletic genus *Tabebuia* s. l. (Bignoniaceae). *Systematic Botany* 32: 660–670.
- GUTIERREZ, J., C. U. KOCH, and O. VON HELVERSON. 2005. *Chiropterophily in the Cuban flora*. [Abstract]. XVI International Botanical Congress, Vienna, Austria.
- JANZEN, D. H. 1981. Digestive seed predation by a Costa Rican Baird's Tapir. *Biotropica* 13: 59–63.
- and P. S. MARTIN. 1982. Neotropical anachronisms - the fruits the Gomphotheres ate. *Science* 215: 19–27.
- LOHMANN, L. G. and M. J. G. HOPKINS. 1999. Bignoniaceae. Pp. 608–623 in *Flora da Reserva Ducke: guia de identificação das plantas vasculares de uma floresta de terra-firme na Amazônia central*, eds. J. E. Ribero, M. J. G. Hopkins, A. Vicentini, C. A. Sothers, M. A. S. Costa, J. M. Brito, M. A. D. da Souza, L. H. P. Martins, L. G. Lohmann, P. A. C. L. Assuncao, E. C. Pereira, C. F. Silva, M. R. Mesquita e L. C. Procopio. Manaus: INPA-DFID.
- MADDISON, W. P. and D. R. MADDISON. 2004. Mesquite: a modular system for evolutionary analysis. v. 1.05. <http://Mesquiteproject.org>. [downloaded June 2005]
- MATTOS, J. R. 1970. *Handroanthus*, Um novo gênero para os "ipês" do Brasil. *Loefgrenia* 50(2): 1–4.
- POSADA, D. and K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- RONQUIST, F. and J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- SIMMONS, M. P. and H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.
- SPANGLER, R. E. and R. G. OLMSTEAD. 1999. Phylogenetic analysis of Bignoniaceae based on the cpDNA gene sequences *rbcl* and *ndhF*. *Annals of the Missouri Botanical Garden* 86: 33–46.
- SWOFFORD, D. L. 2003. PAUP* Phylogenetic analysis using parsimony (*and other methods). v. 4.0b10. Sunderland: Sinauer Associates.
- SYTSA, K. J., J. MORAWETZ, J. C. PIRES, M. NEPOKROEFF, E. CONTI, M. ZJHRA, J. C. HALL, and M. W. CHASE. 2002. Urticalean rosids: circumscription, rosid ancestry, and phylogenetics based on *rbcl*, *trnL-F*, and *ndhF* sequences. *American Journal of Botany* 89: 1531–1546.
- TABERLET, P., L. GIELLY, G. PAUTOU, and J. BOUVET. 1991. Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- VAN WELZEN, P. C., J. W. F. SILK, and J. ALAHUHTA. 2005. Plant distribution patterns and plate tectonics in Malesia. Pp. 199–217 in *Plant Diversity and Complexity Patterns: Local Regional and Global Dimensions*. Biologiske Skrifter vol. 55, eds. I. Friis and H. Balslev. Copenhagen: The Royal Danish Academy of Sciences and Letters.
- ZJHRA, M. L., K. J. SYTSA, and R. G. OLMSTEAD. 2004. Delimitation of Malagasy tribe Coleeae and implications for fruit evolution in Bignoniaceae inferred from a chloroplast DNA phylogeny. *Plant Systematics and Evolution* 245: 55–67.

APPENDIX 1. Species groups, taxa included, information, voucher and genbank numbers (*ndhF*, *trnL-trnF* respectively). Species and genus definitions are used following Gentry (1992a). All vouchers are deposited at WTU.

Tabebuia 01. *Tabebuia donnell-smithii* Rose, Waimea #89p166 (Olmstead and Reeves 1995), AF102628, EF105093.

Tabebuia 02. *Tabebuia aurea* (Manso) Benth. & Hook. f. ex. S. Moore, *SO Grose* 88, EF105027, EF105087.

Tabebuia 03. *Tabebuia guayacan* (Seem.) Hemsl., *SO Grose* 122, EF105033, EF105094. *Tabebuia serratifolia* (Vahl.) Nicholson, no voucher, EF105043, EF105105.

Tabebuia 04. *Tabebuia capitata* (Bur. & K. Schum.) Sandwith, *KM Redden* 1657, EF105045, EF105107. *Tabebuia chrysotricha* (Mart. ex. DC) Standl., UCBBG #85-0562, EF105032, EF105092. *Tabebuia chrysantha* (Jacq.) Nicholson ssp. *chrysantha*, *SO Grose* 164, EF105030, EF105091. *Tabebuia chrysantha* (Jacq.) Nicholson ssp. *pluvicola*, *MA Blanco* 2512, EF105031, EF105090. *Tabebuia ochracea* (Cham.) Standl., *G. dos Santos s.n.*, EF105038, EF105100. *Tabebuia subtilis* Sprague & Sandwith, *HD Clarke* 10929, EF105048, EF105110. *Tabebuia obscura* (Bur. & K. Schum.) Sandwith, *HD Clarke* 10977, EF105047, EF105109.

Tabebuia 05. *Tabebuia impetiginosa* (Mart. ex. DC) Standley, no voucher, EF105035, EF105097.

Tabebuia 06. *Tabebuia insignis* (Miq.) Sandwith ssp. *insignis*, *HD Clarke* 10989, EF105046, EF105108. *Tabebuia rosea* (Bert.) DC, *SO Grose* 156, EF105040, EF105102. *Tabebuia palustris* Hemsl., *SO Grose* 132, EF105039, EF105101. *Tabebuia striata* A. H. Gentry, *SO Grose* 128, EF105049, EF105111.

Tabebuia 07. *Tabebuia schumanniana* Urb., *SO Grose* 057, EF105042, EF105104. *Tabebuia microphylla* (Lam.) Urb., *SO Grose* 071, EF105037, EF105099.

Tabebuia 08. *Tabebuia acrophylla* (Urb.) Britton, *SO Grose* 077, EF105026, EF105086.

Tabebuia 09. *Tabebuia bahamensis* (Northrop) Britton, *SO Grose* 89, EF105028, EF105088. *Tabebuia berteroi* (DC) Britton, *SO Grose* 084, EF105050, EF105112. *Tabebuia berteroi* (DC) Britton, *SO Grose* 078, EF105029, EF105089. *Tabebuia heterophylla* (DC) Britton, no voucher cited (Spangler and Olmstead 1999), L36451, EF105096.

Tabebuia 10. *Tabebuia haemantha* (Bert. ex. Spreng.) DC, *SO Grose* 162, EF105034, EF105095. *Tabebuia lepidota* (HBK) Britton, *RG Olmstead* 96-93, EF105036, EF105098. *Tabebuia sawallei* Britton, *RG Olmstead* 96-74, EF105041, EF105103. *Tabebuia* sp., *SO Grose* 067, EF105044, EF105106.

Crescentieae. *Amphitecna gentryi* W.C. Burger, *MA Blanco* 2155, EF104996, EF105054. *Amphitecna latifolia* (Miller) A. H. Gentry, *RG Olmstead* 96-101, EF104997, EF175740. *Amphitecna tuxtlenis* A. H. Gentry, *SO Grose* 160, EF104998, EF105055. *Crescentia linearifolia* Miers, *SO Grose* 058, EF105002, EF105059. *Crescentia portoricensis* Britton, *Gentry and Zardini* 50458 (MO; Spangler and Olmstead 1999), AF102627, EF105060. *Parmentiera macrophylla* Standl., *SO Grose* 126, EF105017, EF105077. *Parmentiera parviflora* Lundell, *SO Grose* 170, EF105018, EF105078.

Tecomeae. *Cybistax antisiphilitica* (Mart.) Mart., *Nee and Bohs* 51868, EF105003, EF105061. *Ekmmanianthe actinophylla* (Griesb.) Urb., *No voucher*, EF105007, EF105065. *Ekmmanianthe longiflora* (Griesb.) Urb., *SO Grose* 073, EF105008, EF105066. *Godmania aesculifolia* (HBK) Standl., *SO Grose* 129, EF105010,

EF105068. *Sparattosperma leucanthum* (Vell.) K. Schum., Waimea BG #87s446, EF105022, EF105082. *Spirotecoma hologuinensis* (Britton) Alain, RG Olmstead 96-108, EF105024, EF105084. *Zeyheria montana* Mart., Carvalcanti 35092, EF105052, EF105114. *Zeyheria tuberculosa* (Vell.) Bur., No voucher, EF105053, EF105115.

Bignoniaceae. *Arrabidaea pubescens* (L.) A. H. Gentry, Gentry 10234 (MO; Spangler and Olmstead 1999), AF102627, EF175742. *Macfadyena unguis-cati* (L.) A. H. Gentry, Gentry s.n. (MO; Olmstead and Reeves 1995), AF102633, EF175741. *Martinella obovata* (Kunth) Bur. & K. Schum., Gentry & Zardini 50277 (MO; Spangler and Olmstead 1999), L36402, EF175743.

Coleaceae. *Colea sytsmae* M. Zjhra, M. Zjhra 917, EF105001, EF105058. *Ophiocolea floribunda* (Bojer ex Lindl.) H. Perrier, G.E. Schatz, et al 3448 (MO; Spangler and Olmstead 1999), AF102634, EF105075. *Phyllarthron articulatum* (Desf. ex Poir) K. Schum., M. Zjhra 752, EF105019, EF105079. *Rhodocolea racemosa* (Lam.) H. Perrier, Miller 6222, EF105021, EF105081.

Tecomeae. *Campsidium valdivianum* (Phil.) Skotts., Gardner and Knees 4050 (E), EF104999, EF105056. *Catophractes alexandri* D. Don, Huntington BG #50485, EF105000, EF105057.

Delostoma lobii Seem., P. Hutchison & J.K. Wright 5465 (UC), EF105005, EF105063. *Delostoma integrifolium* D. Don, MA Blanco 2155, EF105004, EF105062. *Deplanchea tetraphylla* (R. Br.) F. Muell. ex Steenis, R.A. Howard 19758, EF105006, EF105064. *Fernandoa madagascariensis* (Baker) A. H. Gentry, M.W. Chase 5570 (K), EF105009, EF105067. *Heterophragma adenophyllum* (Wall. ex G. Don) Seem. ex Benth. & Hook., Waimea, BG # 79s763, EF105011, EF105069. *Jacaranda mimosifolia* D. Don, L. Lohmann 369 (MO), EF105012, EF105070. *Jacaranda rufa* Manso, L. Lohmann 262 (MO), EF105013, EF105071. *Kigelia africana* (Lam.) Benth., R.C.A. Rica s.n., AF102632, EF105072. *Lamiodendron magnificum* Steenis, Takeuchi & Ama 16634, EF105014, EF105073. *Newbouldia laevis* (Beauv.) Seem. ex Bur., RBGE #19671901, EF105015, EF105074. *Pandorea pandorana* Andrews, Scot Kelchner LQ8, EF105016, EF105076. *Rhigozum obovatum* Burch., M.W. Chase 3892 (K), EF105020, EF105080. *Spathodea campanulata* Beauv., Waimea #78p1079, EF105023, EF105083. *Stereospermum nematocarpum* DC, M.W. Chase 3891 (K), EF105025, EF105085. *Tecomathe hillii* F. Muell., no voucher, EF105051, EF105113.