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

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Abstract—The tribe Crescentieae includes Amphitecna (21 species), Crescentia (six species), and Parmentiera (10 species), three genera of understory trees with a center of diversity in Central America and a small number of species in the Antilles and northern South America. Species in Crescentieae are united by their fleshy, indehiscent fruit and cauliflorous, bat-pollinated flowers. To lay a foundation for examining morphological, ecological, and biogeographic patterns within the tribe, we inferred the phylogeny for Crescentieae using both chloroplast (ubqF, trnL-F) and nuclear markers (PepC, ITS). The most recent circumscription of Crescentieae, containing Amphitecna, Crescentia, and Parmentiera is supported by our phylogenetic results. Likewise, the sister relationship between Crescentieae and the Antillean-endemic Spirotecnum is also corroborated by our findings. This relationship implies the evolution of fleshy and indehiscent fruits from dry and dehiscent ones, as well as the evolution of bat pollination from insect pollination.

Fruits and seeds from species in Crescentieae are consumed by humans, ungulates, birds, and fish.

Keywords—Amphitecna, Crescentia, fruit dispersal, mammal dispersal, molecular phylogeny, Parmentiera.

Bignoniaceae Juss. is a family of approximately 850 species and 82 genera with a pantropical distribution (Fischer et al. 2004; Olmstead et al. 2009; Lohmann and Ullioa Ulloa 2006). The family is best known as trees and woody vines, with trumpet shaped flowers that usually form showy inflorescences (Lohmann and Hopkins 1999). The fruits of most taxa are bilocular and dehiscent, releasing the winged seeds for wind (Gentry 1980). However, Coleeae Bojer ex Meisn., are bilocular and dehiscent, releasing the winged seeds for dispersal by wind (Gentry 1980). However, Coleeae Bojer ex Reveal, Crescentieae G.Don, and Kigelia DC. have fruits that are indehiscent and adapted to dispersal by mammals. Historically, all of these taxa were united in tribe Crescentieae (De Candolle 1838; Bentham and Hooker 1876; Gentry 1980), or in a family Crescentieae (Seemann 1854; Miers 1861).

Besides fruits adapted for mammal dispersal, species in Coleeae, Crescentieae, and Kigelia share a similar habit and cauliflory (Perrier de la Bathie 1938). A problem with the traditional concept of Crescentieae was that it had a disjunct distribution of its taxa, with most species restricted to either Central America or Madagascar, and a single species, Kigelia africana (Lam.) Benth., found on mainland Africa (Gentry 1976). To explain this distribution, one needed to invoke either widespread extinction or an unusual long-distance dispersal event. Gentry (1976) examined the morphology in depth and suggested that the African and Madagascar species formed one group, tribe Coleeae, while the Central American taxa comprised a second group, tribe Crescentieae. He further suggested that each evolved autochthonously. Under the circumscription of Crescentieae proposed by Gentry (1980), the tribe included 33 species distributed in three genera: Amphitecna Miers, Crescentia L., and Parmentiera DC. With the subsequent addition of three new species of Amphitecna and one new Parmentiera (Gentry 1982; Burger and Gentry 2000; Ortiz-Rodriguez et al. 2016; see Appendix S1 in Ragsac et al. 2021), although Parmentiera also includes large trees (Gentry 1980). Representatives of this clade are easily recognized by their cauliflorous flowers, often with white-green corollas, and fleshy and indehiscent fruits (Fig. 1), except for Parmentiera (10 spp.), which includes species with elongate indehiscent fruits with a fibrous-fleshy core that lack a hard shell (Gentry 1980). The large fruits of Parmentiera are often yellow or purple, persisting for a long time on the trunk and branches (S. Grose pers. obs.). Crescentia and Amphitecna share pepo or calabash fruits, spherical to ellipsoid in shape, with pulpy cores containing unwinged seeds (Gentry 1980). The hard exocarps of Crescentia cujete L. fruit have been used as containers throughout their native range, and to bail water out of native canoes, as reported by Columbus as early as 13 October 1492, the day he arrived to the New World. Smaller exocarps are used to make maracas in much of Mexico and Central America (Gentry 1992b).

Crescentia has sessile and simple leaves borne in fascicles along their branches, giving them a unique appearance. Crescentia flowers have white to yellow flowers with maroon
penciling and 3- or 6-colpate pollen (Gentry and Tomb 1979; Gentry 1980). The largest genus of the tribe, *Amphitecna*, contains 21 species characterized by polyporate and finely reticulate pollen (Gentry 1980; Ortiz-Rodriguez et al. 2016). Due to their simple, alternate leaves that are not arranged in fascicles, species in *Amphitecna* are difficult to identify when not in flower (Gentry 1980). When flowering, the fallen corollas are scattered on the ground under and around the tree (S. Grose pers. obs.). Species in *Amphitecna* are not collected often and most of what is known about their distributions can be attributed to occasional collecting, as in many other tropical species (Funk et al. 1999; Funk and Richardson 2002).

All species in *Amphitecna* are restricted to Central America except for *A. latifolia* (Mill.) A.H.Gentry, which has a much larger distribution spanning from the Greater Antilles to coastal Venezuela and Costa Rica to Ecuador (Gentry 1980). In *Crescentia*, *C. alata* Kunth and *C. cujete* are widespread throughout Central America and the West Indies, while *C. amazonica* Ducke, occurs in northern South America as far south as the Upper Orinoco and Amazon basins (Gentry 1980). The remaining three species in the genus, *C. linearifolia* Miers, *C. mirabilis* Ekman ex Urb, and *C. portoricensis* Britton are restricted to the West Indies (Gentry 1980). *Parmentiera* is restricted to Central America, except for *P. stenocarpa* Dugand & L.B.Sm. which is endemic to northwest Colombia (Gentry 1980). Species in Crescentieae that are not widely distributed are usually restricted to a particular mountain range, sometimes only known from a single mountain (Gentry 1980).

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

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**Fig. 1.** Floral and fruit morphology of Crescentieae. A–C. Flowers illustrating off-white to maroon corollas and cauliflorous habits. A. *Amphitecna tuxtensis* A.H.Gentry. B. *Crescentia cujete*. C. *Parmentiera valerii*. D. *Amphitecna bresloaei* A.H.Gentry, fruit. E. *Crescentia alata*, fruit. F. *Parmentiera aculeata*, fruit. G. *Amphitecna gentryi*, fruit section demonstrating fleshy pulp and unwinged seeds. Photos: SOG.
prehistoric gomphotheres have been suggested to be dispersers of C. cujete and other trees with large indehiscent fruits (Janzen and Martin 1982).

Grose and Olmstead (2007a) found an unexpected relationship between Crescentieae and Antillean-endemic Spirotecoma (Baill.) Dalla Torre & Harms. Spirotecoma includes 4 species, three of which are endemic to Cuba (Gentry 1980). Within the Tabebuia Alliance, Spirotecoma is the only genus that shares ramiflorous inflorescences reduced to one or two flowers with Crescentieae. The fruits of Spirotecoma are linear, terete, and spirally coiled, with small-winged seeds embedded in pits in the enlarged septum. Otherwise, the fruits of Spirotecoma are bilocular and dehiscent capsules, like other Bignoniaceae (Gentry 1992a). Understanding the relationship among Spirotecoma and members of the Crescentieae can lead to insights on the morphological evolution and biogeographic history of these important Neotropical clades.

The goal of this paper is to reconstruct the phylogeny of Crescentieae and use the inferred relationships to discuss morphological and biogeographical aspects within the tribe. Sequence data from two chloroplast loci, ndhF and trnL-F, and two nuclear loci, PepC and ITS, are used in the phylogenetic analyses.

**Materials and Methods**

**Taxonomic Sampling**—A total of 30 accessions representing 28 out of the 37 spp. of Crescentieae and 75% of the overall diversity of the clade were sampled. Our sampling scheme includes seven (out of 10) species of *Parmentiera*, all six species of *Crescentia*, and 15 (out of 21) species of *Amphitecna*. Two accessions were included for *C. cujete* and *A. brellavid*: Collections, including silica gel preserved samples of fresh tissue, were made in Puerto Rico, Cuba, Panamá, Costa Rica, Nicaragua, México, and Guatemala, as well as at the U.C. Berkeley Botanical Gardens and Fairchild Tropical Botanic Gardens. Additional tissue was provided by the Missouri Botanical Garden and Jardín Botánico Nacional de Cuba (Havana).

Thirteen outgroups were included from a previous phylogenetic study of the Tabebuia alliance (Grose and Olmstead 2007a, b). Vouchers were deposited in herbaria in their country of origin or WTU (Appendix 1).

DNA extraction was performed using a modified CTAB protocol (Doyle and Doyle 1987) and amplified product was further purified using the Qiagen kit (Qiagen Co., Valencia, California) as described in Beardsley and Olmstead (2002) or polyethylene glycol (PEG) precipitation.

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

For chloroplast loci and ITS, standard PCR methods were used to amplify the target regions. We used the ndhF primers described in Olmstead and Sweere (1994) and Olmstead and Reeves (1995). For the amplification of trnL-F, we used primers described in Taberlet et al. (1991) and Beardsley and Olmstead (2002). ITS sequences were amplified using the ITS4 (5′-TCCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGAAAGTGAAGCTCGTACAC-3′) primers modified for Eukaryotes from White et al. (1990). Products from these gene regions were then directly sequenced using DYEnamic ET chemistry (Amersham Biosciences, Piscataway, New Jersey), in the Center for Comparative Genomics at the University of Washington, or by Geneviz (Seattle, WA). Sequences were edited and aligned using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, Michigan), Se-Al Carbon (A. Rambaut, University of Oxford, UK), or Geneious 10.2.3 (Biomatters Ltd., Auckland, New Zealand).

Twenty-six taxa in Crescentieae were included in the nuclear analyses: six species of *Parmentiera*, six species of *Crescentia*, and 15 species of *Amphitecna*. Outgroups were Spirotecoma holguinensis, Tabebuia bullata, Rouxendron donnell-smithii, Cybistax antisypialithica, Sparattosperma leucanthum, and eight species in Handroanthus. Twenty-seven taxa in Crescentieae were included in the chloroplast analyses. The ingroup consisted of the same taxa as the nuclear dataset, with the addition of a second *C. cujete* specimen from Puerto Rico, A. kennedyae, and P. milispagnaiana, but excluding C. amazonica. All taxa used in the nuclear and chloroplast datasets were included in the combined dataset.

Appropriate substitution models were determined using PartitionFinder 2.1.1 (Lanfear et al. 2016). For ndhF, three models of evolution were assigned based on codon position: TVM+I+G for position 1, HKY+G for position 2, and GTR+G for position 3. For the remaining regions, trnLF was assigned TVM+I+G, ITS was assigned TIM+G, and PepC was assigned GTR+G. Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist et al. 2012) for 1 million MCMC generations on 2 runs with 2 heated chains each subsequently checked for stationarity in Tracer 1.6 (Rambaut et al. 2014) with the first 25% of trees discarded as burn-in. Maximum likelihood analyses were performed in RAxML 8.2.4 (Stamatakis 2014) with the autoMRE bootstrap setting to detect when the analysis had run to convergence. For the combined dataset, loci were concatenated as chloroplast, PepC, and ITS. Coalescent analyses were performed in BEAST 2.6.0 using the BEAST template (Bouckaert et al. 2014). *Amphitecna kennedyae*, *Amphitecna steyermarkii*, *Crescentia amazonica*, *Crescentia cujete* (Puerto Rico), and *Parmentiera milispagnaiana* were excluded from the *BEAST analysis due to a lack of chloroplast and PepC sequence data. Nuclear regions were treated as autosomal, whereas the concatenated chloroplast data was treated as haploid (Y or mitochondrial). Priors were set to GTR substitution models, uncorrelated relaxed clock, Yule model, birthrate, and population mean were set to a Gamma distribution with a shape of 2. *BEAST analysis was run twice for 1.0 × 10⁶ generations, sampling every 1.0 × 10⁶ trees. Trees were summarized using TreeAnnotator 2.5.0 (Rambaut and Drummond 2017), with the first 25% of trees discarded as burn-in.

**Results**

**Datasets**—Of the 28 taxa in Crescentieae used in this study, 17 were sampled here for the first time. We were unable to sample nine taxa with very restricted distributions, namely *A. costata* A.H.Gentry, *A. donnell-smithii* (Sprague) L.O.Williams, *A. isthmica* (A.H.Gentry) A.H.Gentry, *A. loreae* Ortiz-Rodr. & Burelo, *A. lundellii* A.H.Gentry, *A. parviflora* A.H.Gentry, *P. dressleri* A.H.Gentry, *P. morii* A.H.Gentry, and *P. stenocarpa* Dugand & L.B.Sm. The ndhF dataset consisted of 41 taxa (13 outgroup, 28 ingroup), with sequence lengths ranging from 1746–2101 bp, 75.1% identical sites, and 0.6% missing data. The trnL-F dataset consisted of 42 taxa (13 outgroup, 29 ingroup), with sequence lengths ranging from 671–908 bp, 75% identical sites, and 2.5% missing data. The PepC alignment consisted of 38 taxa (13 outgroups, 23 ingroup), and individual sequences of PepC ranged from 431–645 bp with 26.9% identical sites and 7.7% missing data. ITS was sequenced for 21 taxa (2 groups, 19 ingroup), with individual sequences ranging from 356–711 bp, 75.3% identical sites, and 1.9% missing data.

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

**Phylogenetic Analyses**—PartitionFinder indicated that a mix of models were appropriate for the combined dataset. In inference programs where it was allowed (MrBayes, RAXML), these appropriate models were assigned to each locus or codon position before analysis. In *BEAST*, each locus was run under the GTR+G model, which was also appropriate because the GTR+G model is inclusive of all other models (Abadi et al. 2019). Both Bayesian and maximum likelihood analyses of the nuclear dataset (Fig. 2) recovered a clade consisting of *Spirotecoma holguinensis* and Crescentieae [Posterior Probability (PP) = 1.0, Bootstrap (BS) = 100%]. A monophyletic Crescentieae received mixed support (0.57, 85%). *Amphitecna*, *Crescentia*, and *Parmentiera* were all supported as monophyletic (1.0, 97%; 0.98, 83%; 1.0, 99%, respectively). A clade comprising *Crescentia* and *Amphitecna* was strongly supported (1.0, 100%). Despite a range of support values, both methods produced matching topologies in *Crescentia* and *Parmentiera*, as well as provided support for two clades within *Amphitecna* (Figs. S1, S2). Supplementary figures and appendices are available on Dryad (Ragsac et al. 2021).

The topologies generated by the chloroplast dataset (Fig. 3) were nearly identical in Bayesian and maximum likelihood frameworks (Figs. S3, S4), with the exception of the inter-specific relationships in *Amphitecna*. A clade consisting of *S. holguinensis* and Crescentieae was well supported in both analyses (0.98, 82%), and a monophyletic Crescentieae received moderate support (0.91, 69%). Only the monophyly of *Parmentiera* (1.0, 97%) was strongly supported. *Crescentia* was weakly supported as monophyletic, and *Amphitecna* received mixed support between analyses (0.99, 53%). Two *Crescentia* clades emerged: one with the widespread species *C. alata* and the Mexican accession of *C. cujete* (0.99, 93%), and the second with all other *Crescentia* species, including the Antillean accession of *C. cujete* (0.95, 64%).

In the tree generated from the combined dataset (Fig. 4), a clade containing *S. holguinensis* and Crescentieae was strongly supported (1.0, 100%). Crescentieae was supported as monophyletic (0.98, 93%), and so was the monophyly of each genus [*Amphitecna* (1.0, 94%), *Crescentia* (0.97, 71%), and *Parmentiera* (1.0, 100%)]. A clade of *Crescentia* and *Amphitecna* was also well supported (1.0, 99%). Interspecific relationships within *Crescentia*, *Parmentiera*, and one clade in *Amphitecna* matched across analyses, while those within the larger clade in *Amphitecna* were weakly resolved (Figs. S5, S6).

The maximum clade credibility *BEAST* tree (Fig. 5) produced strong support for a clade containing *Spirotecoma* and Crescentieae (PP = 0.95), but weak support for a monophyletic Crescentieae (0.67). *Amphitecna*, *Crescentia*, and *Parmentiera* were each strongly supported as monophyletic (1.0, 0.99, 1.0).

**Discussion**

**Phylogeny of Crescentieae**—The most recent circumscription of Crescentieae, containing *Amphitecna*, *Crescentia*, and *Parmentiera* is supported by our phylogenetic results, as is its sister relationship with *Spirotecoma*. *Amphitecna* and *Parmentiera* were both strongly supported as monophyletic, while *Crescentia* received strong support only in the species tree derived from the analysis of the combined dataset. Crescentieae was monophyletic in all analyses, receiving especially strong support in the combined analysis (Fig. 4).

Species relationships in *Parmentiera* varied among trees. In trees derived from the analyses of the combined-data and chloroplast trees, *Parmentiera aculeata* (Kunth) Seem., *Parmentiera cereifera* Seem., *Parmentiera millspaghiana* L.O.Williams, *Parmentiera parviflora* Lundell, *Parmentiera valerii* Standl., and *Parmentiera macrophylla* Standl. form a clade (combined data: 1.0, 98%; chloroplast: 1.0, 98%). This clade was not recovered in the nuclear gene tree and in the species tree it formed a grade with *P. macrophylla* and *P. trunciflora* nested within it (*P. millspaghiana* only has chloroplast data). *Parmentiera aculeata* and *Parmentiera parviflora* formed a clade in all analyses (combined data: 1.0, 100%; nuclear: 1.0, 99%; chloroplast: 1.0, 100%; species tree: 0.87). *Parmentiera trunciflora* Standl. & L.O.Williams was either recovered as sister to the rest of the genus, but with weak support across analyses (combined data: 0.74, 63%; nuclear: 0.78, 48%), or in a clade with *Parmentiera macrophylla* (chloroplast: 0.85, 84%; species tree: 0.53). Species in *Parmentiera* not sampled in this study were *P. dressleri* A.H.Gentry, *P. morii* A.H.Gentry, and *P. stenocarpa* Dugand & L.B.Sm.

In *Crescentia*, the phylogenetic analyses suggested the existence of two groups. The widespread Central American *C. alata* and the Mexican accession of *C. cujete* formed a well-supported clade in the chloroplast tree (0.99, 93%) that was sister to a clade of Antillean species that was well supported in all analyses. However, in the nuclear gene tree, *C. alata* and the Mexican accession of *C. cujete* formed a grade along with South American *C. amazonica*, for which only nuclear data were available. In the combined data analyses, the concatenated analysis with all taxa found a clade of mainland species (*C. alata*, *C. amazonica*, and Mexican *C. cujete*) sister to the Antillean clade with modest support, while the *BEAST* species tree, with only those accessions for which both plastid and nuclear data were available, found weak support for that relationship. Additional sequence data will be needed to fully resolve this relationship.

There was little well-supported resolution among species in *Amphitecna*. However, there were two clades recovered in some analyses. The first, containing *A. gentry*, *A. megalophylla*, and *A. moline*, was well supported in trees from combined (0.91, 98%) and nuclear (1.0, 99%) datasets, but was not supported in chloroplast or species trees. A clade containing all other species in the genus was recovered in the combined dataset (0.82, 94%) and nuclear (1.0, 97%) trees, but the relationships between species within this large clade were unresolved. Five species in *Amphitecna* were not sampled for this study: *A. costata* A.H.Gentry, *A. donnell-smithii* (Sprague) L.O.Williams, *A. isthmica* (A.H.Gentry) A.H.Gentry, *A. loreae* Ortiz-Rodr. & Burelo, and *A. lundellii* A.H.Gentry. Because *Amphitecna* is the most species-rich clade in Crescentieae, these results demonstrate the need for improved sampling in terms of characters and taxa to resolve species relationships.
Biogeographic Implications—Species in *Parmentiera* are restricted to mainland Central America and northernmost South America. *Parmentiera aculeata* and *P. parviflora* formed a Mexican endemic clade nested within *Parmentiera* in all trees except the species tree. This clade was usually sister to a group containing species with a collective widespread distribution across Central America: *P. cereifera* in Panama, *P. macrophylla* in Panama and Nicaragua, *P. valerii* in Costa Rica, and *P. millspaughiana* in Mexico. *Parmentiera trunciflora*, most commonly sister to the rest of the genus in our analyses, is Nicaraguan in distribution, occasionally forming a clade with *P. macrophylla*, also distributed in Nicaragua. *Parmentiera stenocarpa* is the only species found in South America. While not sampled here, it is likely to...
represent a recent extension of the distribution of *Parmentiera* into South America.

In *Crescentia*, evidence from chloroplast and combined analyses suggested that the mainland and Antillean members form sister groups, although the nuclear data suggested an origin on the mainland from which the Antillean clade is derived. *Crescentia amazonica*, distributed in northern South America, appears to be part of the mainland radiation, and is the only species in the tribe restricted to South America.

Two accessions of *C. cujete* were included in our dataset that represent the mainland and Antillean distribution of this species. Each accession fell out with other accessions in the
same geographic region. It is possible that a broadly distributed ancestor may have given rise to the rest of the genus autochthonously, or that two separate lineages may have converged on the same morphology of *C. cujete*, or that chloroplast transfer may have occurred in one geographic region. Extensive population level sampling will be necessary to sort among these scenarios.

**Morphological Implications**—The monophyly of *Crescentia* received mixed support across analyses; it was strongly supported in the nuclear and species trees, but weakly supported in the chloroplast tree and this was reflected in the tree from the concatenated dataset. Species in *Crescentia* exhibit a unique combination of morphological traits, including 3- or 6-colpate pollen, fasciculate, often simple leaves, the smallest seeds of the tribe, and white-maroon corollas (Gentry and Tomb 1979; Gentry 1980), some of which are derived traits within Crescentieae, suggesting monophyly of *Crescentia*. Additional sequence data is likely to confirm its monophyly.

**Fig. 4.** Bayesian 50% majority rule consensus tree generated from analysis of the combined dataset. Branches leading to clades with both > 0.9 posterior probability and > 90% bootstrap support from maximum likelihood (ML) analysis are in bold. Other relationships in Crescentieae recovered in both Bayesian and ML analyses are indicated by posterior probability and bootstrap values in nodes. The scale bar represents the expected number of substitutions per site.
The sister relationship between Crescentieae and *Spirotecoma* presents insights into the directionality of morphological and biogeographic evolution in this group. *Spirotecoma* has opposite, unifoliolate to digitate leaves with three to seven leaflets, inflorescences in a few-flowered raceme or one to two individual flowers borne ramiflorously from leaf axils, tubular-campanulate flowers with reddish-purple to yellowish-brown corollas, cylindrical capsules and winged seeds (Gentry 1992a). It shares opposite and palmately compound leaves with *Parmentiera* and *Crescentia*. *Spirotecoma*’s ramiflorous inflorescences bear developmental similarity to the cauliflorous inflorescences of Crescentieae. Cauliflory follows ramiflory when the site of ramiflorous inflorescences does not become inactive after abscission of the spent inflorescence, the perennial inflorescences becoming situated on old branches as the plant ages (Endress 2010). Furthermore, while most species in *Spirotecoma* have yellow to red corollas, *S. spiralis* has greenish-purple to yellowish-brown corollas that are likely bat pollinated, a syndrome shared among species in Crescentieae (Gentry 1992a).

*Spirotecoma* has a thickened, fleshy septum with seeds embedded in it. *Parmentiera* fruits also have a fibrous central core (Gentry 1980), while *Amphitecna* and *Crescentia* have a more uniformly fleshy interior derived from placental tissue (S. Grose pers. obs.).

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

**Bat Pollination and Cauliflory**—Gentry described the syndrome of bat pollination in Bignoniaceae as “Crescentia-type,” observing that several species of bats visit *C. alata* and *C. cujete*, and citing a “characteristic musky odor” and “night flowering, extremely copious production of nectar, an open rachitic branching pattern, and cauliflorous flowers” as adaptations that facilitate visits by bats (Gentry 1974). Thompson (2014) documented 366 nectivorous bats, from three species, over 25 nights of mist netting next to *C. cujete* trees in Western Mexico. The number of nectivorous bats was significantly and positively associated with the number of open flowers on *C. cujete* trees.

Cauliflory may also be an adaptation for supporting large, heavy fruits, such as those in Crescentieae, *Theobroma cacao* (Malvaceae), or other large-fruited, cauliflorous species. Cauliflorous flowers have also been documented in *Kigelia*, Coleeae, and *Adenocalymma* Mart. ex Meisn. (Bignoniaceae) in Bignoniaceae (Zjhra et al. 2004; Fonseca and Lohmann 2017), all of which have large fruits, with *Kigelia* dispersed by baboons, Coleeae by lemurs, and *Adenocalymma* by wind. Some, but not all, of these groups have evidence of bat pollination; the exception is Coleeae, which are pollinated mainly by insects and birds (Gentry 1974; Machado and Vogel 2004; Zjhra 2008).

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

Animal dispersal of the large, heavy indehiscent fruit of species in Crescentieae have piqued the imaginations of many, considering a lack of large native mammals in the Americas following Pleistocene extinctions (Simpson 1980; Lessa and Farina 1996). Many organisms, ranging from monkeys to gomphotheres, tapirs to agoutis, have been suggested as dispersers (Gentry 1974, 1976; Janzen and Martin 1982; Haber et al. 2000), but little observational evidence exists to confirm their effectiveness as dispersers. Janzen (1982a, p. 1267), explored the consumption and dispersal of *C. alata* fruit, finding that “once the hard hull is broken by a large dispersal agent, the seeds are largely protected from grinding by the agent’s molar by being small and embedded in a sweet, slippery, and messy matrix that is easily and eagerly swallowed with little effort and chewing.” Furthermore, Janzen (1982b) presented a herd of 17 horses with 160 *C. alata* fruits, and “they broke and ate the contents of all of them in an afternoon.” We (SOG and RGO) observed cows eagerly eating fruits of *Parmentiera trunciflora* in Nicaragua. This behavior may serve as a proxy for the eating preferences of now extinct Pleistocene megafauna responsible for past seed dispersal in Crescentieae. Thompson (2014) found low levels of genetic structure in *C. cujete* populations in Western Mexico, the opposite of what one would expect with the extinction of a dispersal agent. Therefore, Thompson (2014) proposed high pollen-mediated gene flow facilitated by bat pollinators as a possible mechanism leading to the homogenization of genetic diversity in *C. cujete*. It is also possible that, because *C. cujete* trees are found along arroyos in Western Mexico, flooding during the rainy season promotes seed-mediated gene flow of fruit that can survive water dispersal. Seeds from *C. alata* fruits have been documented as feed supplements in rats and chickens (Janzen 1982a). The fruits and seeds of Crescentieae have also made it into the human diet. *Parmentiera aculeata* is a cultivated fruit tree in the Mayan region of Central America, and *P. stenocarpa* produces an edible fruit that has a flavor “apt for desserts or fruit juices” (Gentry 1992b). *Crescentia cujete* seeds are also used to make “semilla de jicaro” a locally popular drink in Nicaragua (Gentry 1992b).

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

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**Author Contributions**

ACR and SOG wrote the manuscript. ACR sampled herbarium collections, provided data, assembled sequence alignments, and performed phylogenetic analyses. SOG did field work and provided data and photographs. RGO provided guidance on the project and revised the manuscript.

**Literature Cited**

Abadi, S., D. Azouri, T. Pupko, and I. Mayrose. 2019. Model selection may not be a mandatory step for phylogeny reconstruction. *Nature Communications* 10: 1–11.

Beardsley, P. M. and R. G. Olmstead. 2002. Redefining Phrymaceae: The placement of *Mimusus*, 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: Lovell Reeve and Company.
Zhara, M. L. 2008. Facilitating sympatric species coexistence via pollinator partitioning in endemic tropical trees of Madagascar. *Plant Systematics and Evolution* 271: 157–176.

Zhara, M. L., K. J. Sytsma, and R. G. Ohmscheid. 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**. Specimen information for sampled taxa. These data are provided in the following order: taxon, locality, voucher, herbarium, GenBank accession numbers for ndhF, trnL-trnF, PepC, and ITS. Herbarium acronyms are according to Thiers (2020).

*Amphitecna apiculata* A.H.Gentry, México, SOG 176 (WTU), MT679616, MT679636, MT679578, MT623520. *Amphitecna brodleioid A.H.Gentry* (upland), México, Cult. Miranda BG, Santiago Las Tuxtlas, SOG 163 (WTU), MT679617, MT679637, – MT623521. *Amphitecna brodleioid A.H.Gentry* (lowland), Guatemala, SOG 174 (WTU), MT679618, MT679638, MT679597, MT623522. *Amphitecna gentryi* W.C.Burger, Costa Rica, MAB 2155 (FLU), EF104996.1, EF105054.1, MT623523. *Amphitecna kennedyae* (A.H.Gentry) A.H.Gentry, Costa Rica, SOG 133 (WTU), MT679577, MT679640, – *Amphitecna latifolia* (A.H.Gentry) A.H.Gentry, Cuba, RGO 96-101 (WTU), EF104997.1, EF175740.1, MT679582. – *Amphitecna macrophylla* (Seem.) Miers ex. Baill., México, SOG 182 (WTU), MT679619, MT679641, MT679593, – *Amphitecna megalophylla* (Donn.Sm.) A.H.Gentry, Guatemala, SOG 173 (WTU), MT679620, MT679642, MT679584, – *Amphitecna molinae* L.O.Williams, Nicaragua, SOG 152 (HULE), MT679621, MT679643, MT679585, MT623524. *Amphitecna montana* L.O.Williams, UC Berkeley, H. Forbes s.n. (UC), FJ887850, FJ870014, MT679586, MT623525. *Amphitecna regalis* (Linden) A.H.Gentry, México, SOG 181 (WTU), MT679622, MT679644, MT679587, MT623526. *Amphitecna sessilifolia* (Donn.Sm.) L.-O.Williams, Costa Rica, SOG 130 (WTU), MT679623, MT679639, MT679581, – *Amphitecna serratiloba* L.O.Williams, México, SOG 166 (WTU), MT679624, MT679645, MT679588, – *Amphitecna sphericulis A(H.Gentry)* A.H.Gentry, Panamá, SOG 125 (WTU), MT679625, MT679646, MT679589, – *Amphitecna steinmarkii* (A.H.Gentry) A.H.Gentry, México, SOG 168 (WTU), – MT679647, MT679590, MT623527. *Amphitecna tuzilensis* A.H.Gentry, México, SOG 160 (WTU), EF104998.1, EF105055.1, MT679591, MT623528. *Crescentia alata* Kunth, UC Berkeley, None, FJ887856, FJ870025, MT679592, MT623529. *Crescentia andonianica* Duke, Venezuela, Díaz 5096 (NY), –, –, MT623530. *Crescentia cuyite* L., Puerto Rico, SOG 060 (WTU), MT679626, MT679649, –, *Crescentia chrysanthus* L., *Crescentia guayacan* L, Miers, SOG 157 (WTU), MT679627, MT679650, MT679593, MT623531. *Crescentia linearifolia* Miers, Puerto Rico, SO 058 (WTU), EF105021.1, EF105059.1, MT679594, MT623532. *Crescentia mirabilis* Ekman ex Urb., Cuba, Cult. Jardín Botánico Nacional, Havana, None, MT679628, MT679651, MT679595, MT623533. *Crescentia portoricensis* Britton, Puerto Rico, Gentry 50488 (MO), EF105267, EF105061.1, MT679596, MT623534. *Cyphistia antisipholististra* (Mart.) Mart., Bolivia, None and Bohn 51868 (NY), EF105003.1, EF105061.1, MT679597, MT623535. *Handroanthus capitatus* (Bureau and K.Schum.) Mattos, Guyana, KM Redden 1657 (US), EF105045.1, EF105071.1, MT679607, – *Handroanthus chrysanthus* (Jacq.) S.O.Grose, México, SOG 164 (WTU), EF105030.1, EF105090.1, MT679608, MT623540. *Handroanthus chrysanthus* ssp. *chrysanthus* (Jacq.) S.O.Grose, Ecuador, MAB 2512 (FLU), EF105091.1, MT679609, – *Handroanthus chrysotrichus* (Mart. ex. DC) Mattos, Cult at UC Berkeley BG, L. Anderson s.n. (UC), EF105032.1, EF105092.1, MT679610, – *Handroanthus guajacum* (Seem.) S.O.Grose, Panamá, SOG 122 (WTU), EF105033.1, EF105094.1, MT679612, – *Handroanthus impetiginosus* (Mart. ex. DC) Mattos, Cult at UC Berkeley BG, None, EF105035.1, EF105097.1, MT679613, – *Handroanthus obscurus* (Bunou and K.Schum.) Mattos, Guyana, HD Clarke 10977 (US), EF105047.1, EF105091.1, MT679614, – *Handroanthus serratifolius* (Vahl) S.O.Grose, Cult at Waimae BG, None, EF105043.1, EF105051.1, MT679615, – *Parmentiera acutifolia* (Kunth) Seem., Cult Fairchild BG, Unvouched live material FBG #x4241A, MT679629, MT679652, MT679598, MT623536. *Parmentiera creniflora* Seem., Panamá, cult at Fairchild BG, Unvouched live material FBG #x4183, MT679630, MT679653, MT679599, – *Parmentiera macrophylla* Standl., Panamá, SOG 126 (WTU), EF105017.1, EF105077.1, MT679600, MT623537. *Parmentiera miliaepaughiana* L.O.Williams, Nicaragua, SOG 154 (HULE), MT679631, MT679654, – *Parmentiera parviflora* Lundell, Guatemala, SOG 170 (WTU), EF105018.1, EF105078.1, MT679601, – *Parmentiera trichilolia* Standl. and L.O.Williams, Nicaragua, SOG 141 (HULE), MT679632, MT679655, MT679602, MT623538. *Parmentiera turcifolia* Standl., Costa Rica, SOG 135 (WTU), MT679633, MT679656, MT679603, MT623539. *Rosedendron donnell-smithii* (Rose) Miranda, Cult. at Waimae BG, Unvouched live material Waimae #89P166, MT679634, EF105093.1, MT679611, – *Sparattosperma leucanthum* (Vell.) K.Schum., Cult at Waimae BG, Unvouched live material Waimae #875446, EF105022.1, EF105082.1, MT679604, – *Sparattosperma holguinensis* (Britton) Alain, Cuba, No Voucher, EF105024.1, EF105084.1, MT679605, – *Tabebuia bullata* A.H.Gentry, Dominican Republic, SOG 77 (WTU), MT679635, MT679657, MT679606, –.