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# TRIBAL AND INTERGENERIC RELATIONSHIPS OF MESECHITEAE (APOCYNOIDEAE, APOCYNACEAE): EVIDENCE FROM THREE NONCODING PLASTID DNA REGIONS AND MORPHOLOGY[1]

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The Neotropical tribe Mesechiteae (Apocynaceae) is currently considered to include nine genera: *Allomarkgrafia, Galactophora, Macrosiphonia, Mandevilla, Mesechites, Quiotania, Secondatia, Telosiphonia,* and *Tintinnabularia.* Tribal and intergeneric relationships, however, are in dispute. To test the monophyly of the tribe and evaluate intratribal relationships, a maximum parsimony analysis was conducted based on DNA sequences from the plastid *rpl16* intron, *rps16* intron, and *trnS-G* intergenic spacer region as well as morphological data for 23 taxa of Mesechiteae and 11 taxa from other tribes of Apocynoideae. Mesechiteae, as currently circumscribed, was found to be polyphyletic. Only removal of *Secondatia* and *Galactophora* and inclusion of *Forsteronia* rendered the tribe monophyletic. Thus defined, Mesechiteae forms a strongly supported clade including seven genera in three subclades: the *Mesechites* subclade (comprising *Tintinnabularia, Allomarkgrafia,* and *Mesechites*), the *Forsteronia* subclade (containing only *Forsteronia*) and the *Mandevilla* subclade (comprising *Macrosiphonia, Mandevilla,* and *Telosiphonia*). *Allomarkgrafia* is nested in *Mesechites*. *Macrosiphonia* and *Telosiphonia* form two distinct monophyletic clades. Both, however, are nested in *Mandevilla*. Results suggest upholding the following genera in Mesechiteae: *Allomarkgrafia, Forsteronia, Mandevilla, Mesechites,* and *Tintinnabularia*. The status of *Quiotania* could not be evaluated.

**Key words:** Apocynaceae; Apocynoideae; Mesechiteae; morphology; phylogenetic systematics; *rpl16*; *rps16*; *trnS-G*.

Mesechiteae is one of the five tribes comprising the subfamily Apocynoideae (Endress and Bruyns, 2000) and includes nine genera and about 150 species. It is restricted to the Neotropics, where it has a broad distribution, ranging from the southwestern United States throughout Mesoamerica and the Caribbean to southern South America in rainforests, montane forest, savanna, and desert habitats. The tribe is extremely variable in habit and includes vines, erect subshrubs, and small trees. Floral structure is also remarkably diverse, especially the corolla, which ranges from small, inconspicuous, whitish, and tubular to large, variously colored, and infundibuliform.

Although several recent phylogenetic studies have addressed the circumscription of the Apocynaceae and their relationships with the Asclepiadaceae (Judd et al., 1994; Sennblad and Bremer, 1996, 2002; Potgieter and Albert, 2001), resulting in the amalgamation of these two families into Apocynaceae sensu lato (Endress and Bruyns, 2000), many other aspects of classification within the family remain controversial. One main controversy has been the delimitation and composition of tribes (Table 1). This is exemplified by Mesechiteae, for which relationships with other tribes are unknown and the generic circumscription is confusing and shows little consistency (Zarucchi, 1991; Henrickson, 1996; Williams, 1999).

Pichon (1950) recognized four tribes in Apocynoideae: Parsonsieae, Nerieae, Ecdysanthereae, and Ichnocarpeae. His tribal delimitations were based mainly on the form of the ''retinacle,'' a term he coined for the specialized region of the anther that unites it with the style head (Pichon, 1948a). Except for *Galactophora* and *Secondatia*, most of the genera included in the ingroup in our study were placed in his tribe Ichnocarpeae, which was defined by the presence of a glabrous, concave retinacle. Within Ichnocarpeae, five subtribes were recognized, only two of which are pertinent to our study: Forsteroniinae and Mandevillinae.

A new classification for the Apocynaceae sensu stricto was published by Leeuwenberg (1994). Although his work was clearly influenced by Pichon's ideas, only three tribes were recognized in Apocynoideae: Echiteae, Wrightieae, and Apocyneae. This was the first classification to take into account the priority rule, necessitating changing the name of Pichon's (1950) tribe Parsonsieae to Echiteae and Nerieae to Wrightieae. Leeuwenberg's third tribe, Apocyneae, was more or less equivalent to Pichon's tribes Ecdysanthereae and Ichnocarpeae combined. Leeuwenberg's tribes, however, are difficult to distinguish, and Sennblad et al. (1998) showed that they are artificial. Leeuwenberg (1994) arranged the genera he included in his Echiteae into two subtribes, Echitinae and Parsonsiinae, without any explanation of the criteria he used to delimit them. In his classification, most of the genera that comprise the ingroup in this study were placed in Echitinae. The only excep-

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TABLE 1. Comparative tribal placement of genera that have been included in Mesechiteae.

| Genus | Pichon (1950) | Leeuwenberg (1994) | Endress and Bruyns (2000) | This project |
|---|---|---|---|---|
| *Allomarkgrafia* | —[a] | — | Mesechiteae | Mesechiteae |
| *Forsteronia* | Ichnocarpeae | Apocyneae | Apocyneae | Mesechiteae |
| *Galactophora* | Parsonsieae | Echiteae | Mesechiteae | ?[a] |
| *Macrosiphonia* | — | Echiteae | Mesechiteae | Mesechiteae |
| *Mandevilla* | Ichnocarpeae | Echiteae | Mesechiteae | Mesechiteae |
| *Mesechites* | Ichnocarpeae | Echiteae | Mesechiteae | Mesechiteae |
| *Quiotania* | — | — | Mesechiteae | ? |
| *Secondatia* | Ecdysanthereae | Echiteae | Mesechiteae | Apocyneae |
| *Telosiphonia* | — | — | Mesechiteae | Mesechiteae |
| *Tintinnabularia* | Ichnocarpeae | Wrightieae | Mesechiteae | Mesechiteae |

[a] —= genus not recognized,? = uncertain placement.

tion is *Tintinnabularia*, which was placed near *Beaumontia* in Wrightieae. Although Leeuwenberg (1994) gave no reasons for this placement of *Tintinnabularia*, presumably he was influenced by Woodson's (1936) comment that *Tintinnabularia* and *Beaumontia* share three character states: (1) domatia on the lower surface of the leaves, (2) long anther filaments, and (3) foliaceous sepals. The first two are relatively rare in the family.

The most recent classification of the family is that by Endress and Bruyns (2000). Theirs was a unified classification taking into account both Apocynaceae and Asclepiadaceae, and the first one to incorporate phylogenetic considerations. Two of the five subfamilies they recognized, Rauvolfioideae and Apocynoideae, correspond to the traditional Apocynaceae sensu stricto (s.s.), the other three to the Asclepiadaceae as traditionally circumscribed. Their classification differed most dramatically from those of previous authors with regard to the circumscription of tribes in Rauvolfioideae and Apocynoideae. Although the subfamilies and tribes proposed by Endress and Bruyns (2000) were based mainly on morphological characters, this was the first classification to incorporate evidence from molecular studies as well (e.g., Endress et al., 1996; Sennblad and Bremer, 1996; Sennblad et al., 1998; K. Potgieter and V. Albert, University of Illinois, personal communication).

Endress and Bruyns (2000) recognized five tribes in Apocynoideae: Wrightieae, Malouetieae, Echiteae, Mesechiteae, and Apocyneae. Two of the most important morphological characters they used to define these tribes are the structure of the style head and the retinacle, both of which are also important characters in Pichon's (1948a, 1950) classification, but were not considered reliable by A. Leeuwenberg (Wageningen Agricultural University, personal communication). Endress and Bruyns (2000) significantly changed tribal circumscription and composition from those in the classification systems of Pichon (1950) and Leeuwenberg (1994). Most taxa included in a more narrowly circumscribed Mesechiteae by Endress and Bruyns (2000) were part of the heterogeneous Ichnocarpeae of Pichon (1950) and the Echiteae of Leeuwenberg (1994).

Another controversy concerns whether or not smaller satellite genera should be recognized as distinct from their closely related, larger genus. Within Mesechiteae, one such case is whether *Allomarkgrafia* should be included in the synonymy of *Mesechites* (as proposed by Pichon, 1950 and followed by Leeuwenberg, 1994) or recognized as a distinct genus (as in the classification of Endress and Bruyns, 2000). Another problematic taxonomic question concerns the large genus *Mandevilla* and its smaller satellite genera *Macrosiphonia*, *Telosiphonia*, and, more recently, *Quiotania*. In this group of taxa, one to four genera have been recognized by specialists in the family (Zarucchi, 1991; Leeuwenberg, 1994; Henrickson, 1996; Williams, 1999).

The classification of Apocynaceae sensu lato (s.l.) proposed by Endress and Bruyns (2000) represents a considerable advance in the systematics of the family and is a logical starting point for studies that test the phylogenetic relationships of the groupings proposed therein. This type of analysis was done by Potgieter and Albert (2001), based on the *trnL-F* intergenic spacer and morphology in a broad phylogenetic study of the family. They found well-defined clades in Rauvolfioideae, several of which are reflected in the current classification. For Apocynoideae, however, no clearly defined groups were retrieved using that plastid region.

The aims of the present article are to test the monophyly of Mesechiteae sensu Endress and Bruyns (2000) and the relationships among its constituent genera using both morphology and molecular sequence data from the plastid *rpl16* intron, *rps16* intron, and *trnS-G* intergenic spacer. The resulting hypothesis of phylogenetic relationships within Mesechiteae is compared with the current classification, morphological features that characterize clades are discussed, and a modified circumscription of Mesechiteae is proposed.

## MATERIALS AND METHODS

***Taxon sampling***—Twenty-three taxa, representing eight of the nine genera currently recognized in Mesechiteae by Endress and Bruyns (2000), were defined as the ingroup and included in this study (*Allomarkgrafia*, *Galactophora*, *Macrosiphonia*, *Mandevilla*, *Mesechites*, *Secondatia*, *Telosiphonia*, and *Tintinnabularia*). *Mandevilla tenuifolia* exhibits a high degree of polymorphism in its habit and vegetative parts, one morphotype (*Mandevilla tenuifolia*2) of which is very similar to *M. myriophyllum*. Therefore, in order to determine the relationship of these two species, we included two samples of the most extreme forms of *M. tenuifolia* and one from *M. myriophyllum*. *Quiotania* could not be included, as none of the type material could be located, suggesting that it was never distributed. Outgroup taxa were chosen from all but the basalmost tribe of the subfamily (Wrightieae), based largely on previous studies, which suggest that the closest relative of Mesechiteae is either Apocyneae or Echiteae (Sennblad et al., 1998; Sennblad and Bremer, 2002). Two genera from Echiteae (*Prestonia* and *Rhodocalyx*) and five genera from Apocyneae (*Beaumontia*, *Chonemorpha*, *Forsteronia*, *Odontadenia*, and *Trachelospermum*) were included. Three representatives of Malouetieae (two species of *Pachypodium* and one species of *Mascarenhasia*) were used to root the cladograms. Taxon names, voucher information, and GenBank accession numbers are given in Appendix 1 (see Supplemental Data accompanying the online version of this article).

TABLE 2. Summary of sequence length, variability, and parsimony-tree parameters for individual and combined data sets. Tree length, consistency index (CI), and retention index (RI) were calculated based on parsimony-informative characters only.

| Parameter | *rpl16* intron | *rps16* intron | *trnS-G* intergenic spacer | Molecular combined | Morphology | Total evidence |
|---|---|---|---|---|---|---|
| Aligned length | 1436 | 902 | 1365 | 3708 | 29 | 3737 |
| Range of sequence length | 900–1092 | 790–815 | 445–801 | — | — | — |
| No. of coded gaps (no. of parsimony-informative gaps) | 179 (36) | 71 (19) | 164 (27) | 420 (82) | — | 420 (82) |
| No. of characters excluded (nucleotides + gaps) | 195 | 56 | 382 | 633 | — | 633 |
| Total no. of parsimony-informative characters[a] | 143 (10%) | 98 (10.7%) | 99 (8.6%) | 346 (9.9%) | 29 (100%) | 375 (10.7%) |
| Tree length | 241 | 141 | 131 | 525 | 117 | 659 |
| CI | 0.718 | 0.762 | 0.671 | 0.723 | 0.350 | 0.680 |
| RI | 0.818 | 0.854 | 0.811 | 0.826 | 0.550 | 0.801 |

[a] Percentage of total number of characters.

***DNA extraction, amplification, and sequencing***—Total genomic DNA was extracted from silica-dried leaf material or from herbarium specimens using DNeasy Plant mini kits (Qiagen, Valencia, California, USA) following the manufacturer's protocol. Three noncoding plastid regions, the *rpl16* intron, *rps16* intron, and *trnS-G* intergenic spacer, were amplified for all taxa. Double-stranded DNA was amplified by polymerase chain reaction (PCR) on a Biometra Tgradient machine (Biometra, Göttingen, Germany), applying a thermal cycling program consisting of 34 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 1 min, and extension at 72°C for 90 s. Reactions were terminated with a final extension of 4 min at 72°C. All PCR reactions were performed in a total volume of 25 μL, using 2.5 mmol/L MgCl, 10% PCR* Buffer (Amersham Biosciences, Otelfingen, Switzerland), 0.25 mmol/L dNTP, 0.5 units *Taq* DNA polymerase (Amersham Biosciences), 1–4 μL bovine serum albumin (BSA, Sigma, Steinheim, Germany) and 0.1 mmol/L of each primer. Primer information is presented in Appendix 2 (see Supplemental Data accompanying the online version of this article). For a few taxa, internal primers were also used to amplify the *rpl16* intron and *trnS-G* intergenic spacer, with the following changes in the thermal cycling program: 40 instead of 34 cycles and extension time shortened to 1 min. Successfully amplified PCR products were then purified using GFX PCR DNA and a gel band purification kit (Amersham Biosciences).

The same primers used for PCR amplification were also used for the cycle-sequencing reactions, carried out with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Extraction Kit (Perkin Elmer, Applied Biosystems, Applera Europe BV, Rotkreuz, Switzerland). Sequence products were purified on MicroSpin G-50 columns (Amersham Pharmacia Biotech Europe, Dübendorf, Switzerland) and loaded on an ABI Prism 377 DNA sequencer (Perkin Elmer). Complementary strands were edited and assembled with Sequencher 3.1.1 (Gene Codes, Ann Arbor, Michigan, USA).

***Data matrix composition and parsimony analysis***—Nucleotide sequences of the *rpl16* intron, *rps16* intron, and *trnS-G* intergenic spacer were aligned using Clustal W, version 1.8 (Thompson et al., 1994) and adjusted by eye. Regions of ambiguous alignment were excluded from the analysis. Individual gap positions were treated as missing data, unequivocally aligned gaps being coded as presence/absence of characters with the software Gapcoder (Young and Healy, 2003) and then added to the sequence matrix.

Twenty-nine morphological characters were scored using herbarium and fresh specimens, pickled flowers, and when available, flower sections provided by the second author. For some taxa, the literature was also consulted (e.g., Woodson, 1933; Pichon, 1950; Leeuwenberg, 1997; Morales, 1998, 2002). The morphological matrix, a list of the characters, character states, and explanatory notes on characters are given in Appendices 3 and 4 (see Supplemental Data). Exemplars were used as terminal taxa, because morphological variation is considerable in the two larger genera, *Mandevilla* and *Forsteronia*, which would lead to difficulties in coding character states.

The following data sets were subjected to phylogenetic analysis: (1) *rpl16* intron, (2) *rps16* intron, (3) *trnS-G* intergenic spacer, and (4) morphology. As the results of individual analyses did not show any major topological conflict, data partitions were combined in the following ways: all molecular data sets combined together (molecular combined) and all molecular and morphological data sets combined (total evidence).

Maximum parsimony analyses were performed using PAUP* 4.0b (Swofford, 2000). All characters were unordered and equally weighted. Polymorphisms in the data matrix were treated as such, rather than as uncertainties. A heuristic search for most parsimonious trees (MPT) included an initial round of tree searches with 1000 random addition sequence replicates (RASR), holding 10 trees at each step, tree bisection-reconnection (TBR) branch swapping with MULTREES and steepest descent in effect, saving a maximum of 100 trees at each replicate. All shortest trees retained in memory were then included in a second round of searches involving exhaustive TBR branch swapping. Relative support for each node was estimated using the bootstrap resampling procedure (Felsenstein, 1985) as implemented in PAUP employing a full heuristic search with 1000 replicates, 250 RASR, three trees held at each step, TBR branch swapping with steepest descent and MULTREES in effect, saving 10 trees at each RASR.

Morphological characters were optimized onto the strict consensus tree of the total evidence analysis using Winclada, version 1.00.08 (Nixon, 2002), in order to identify the synapomorphies that were congruent with each of the major clades of the ingroup retrieved in our analyses. The proportion of nodes in the individual molecular data partitions that were congruent with the topology of the total evidence tree was assessed in WinClada by optimizing the unambiguous character changes of each data set separately onto the topology of the strict consensus tree obtained from the total evidence matrix. Subsequently, the ratio of nodes supported by such character changes divided by the total number of nodes was calculated. This method is analogous to the Partition Bremer Support (Baker and DeSalle, 1997), although it has the disadvantage that, unlike Partition Bremer Support, it cannot identify whether or not a data partition contradicts a particular node.

## RESULTS

***Size and structure of individual and combined data sets***—Detailed information for both individual and combined data sets is given in Table 2. Multiple sequence alignment was straightforward for the *rps16* intron, but proved to be more difficult for the *rpl16* intron and the *trnS-G* intergenic spacer due to the large number of gaps and AT-rich regions. A total of 382 characters, including nucleotides and gaps, were excluded from this last data set because of ambiguous alignment.

***Parsimony analyses***—To test hypotheses of the tribal delimitation proposed by Endress and Bruyns (2000) (see Table 1), *Galactophora* was also included in the initial round of taxon sampling. Results from a heuristic search showed that

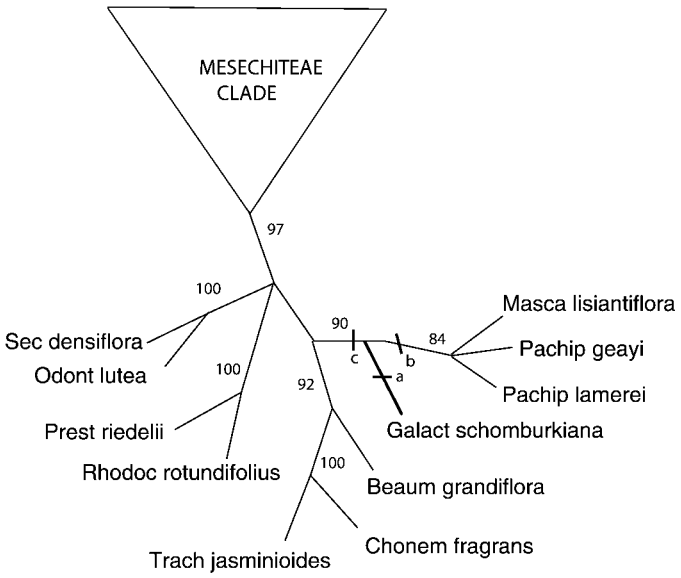



Fig. 1. Unrooted cladogram based on the total evidence data set, showing the relationship of *Galactophora* to the other taxa used in this analysis. Different root positions (a, b, c) show that *Galactophora* never groups with the Mesechiteae clade. Full taxon names are given in Appendix 3 (see Supplemental Data accompanying the online version of this article).

*Galactophora* did not group with the Mesechiteae, but rather came out as sister to the rest of the ingroup species, as illustrated in Fig. 1. Based on its position in the unrooted tree and its long branch compared to other taxa in our sampling, *Galactophora* might actually be less closely related to the ingroup than the taxa used for rooting (*Pachypodium geayi*, *P. lamerei*, and *Mascarenhasia lisianthiflora*). Due to this uncertainty, *Galactophora* was excluded from further analysis.

Tree length, consistency index (CI), and retention index (RI) for the cladograms that resulted from the analyses of the individual and combined data sets are summarized in Table 2. Individual analyses of the three plastid regions showed similar results, and visual inspection of the strict consensus trees of the individual molecular data sets showed no topological conflict. Assuming that simultaneous analysis of combined data is the best approach to phylogenetic inference (e.g., Brower, 1996; Nixon and Carpenter, 1996), and because none of the nodes involved in topological discrepancies in our analysis were supported by high bootstrap values (Fig. 2), the individual molecular data sets were combined. The best resolved cladograms were provided by the *rpl16* and *rps16* intron data sets, with most of the internal nodes receiving bootstrap support (BS) higher than 50%. Of the cladograms generated by the separate molecular data sets, only the *rps16* intron tree defined a clade, corresponding to what will later be defined as the Mesechiteae clade, with BS higher than 50%, identical to that of the molecular combined and total evidence trees. The least resolved cladogram was that based on the *trnS-G* intergenic spacer data set.

Analysis of the morphological data set resulted in a poorly resolved cladogram, with only a few groups supported by a bootstrap value higher than 50% (Fig. 3). Except for a weakly supported clade composed of the species of *Macrosiphonia* and *Telosiphonia* (BS = 54%), which is not found in any of the molecular trees, no incongruence was detected when comparing the morphological tree with either the strict consensus of the individual or combined molecular trees. Therefore, the morphological and combined molecular data sets were combined into a total evidence data set. All further discussion will be based on the total evidence tree (Fig. 4).

A clade including representatives of *Allomarkgrafia, Forsteronia, Mesechites, Mandevilla, Macrosiphonia, Telosiphonia,* and *Tintinnabularia* is strongly supported (BS = 97%) and will be referred to hereafter as the Mesechiteae clade. Within this clade, three other subclades are defined: (1) a strongly supported (BS = 100%) subclade comprised of *Allomarkgrafia, Mesechites,* and *Tintinnabularia* and hereafter referred to as the *Mesechites* subclade; (2) a strongly supported (BS = 99%) subclade comprising the two *Forsteronia* species (the *Forsteronia* subclade); and (3) a larger, moderately supported (BS = 77%) subclade composed of taxa of *Mandevilla, Macrosiphonia,* and *Telosiphonia,* hereafter referred to as the *Mandevilla* subclade. *Mandevilla tenuifolia* is paraphyletic to *M. myriophyllum*. One morphotype (*Mandevilla tenuifolia*1) forms a strongly supported clade (BS = 94%) with *M. myriophyllum* rather than with the other morphotype of *M. tenuifolia* (*Mandevilla tenuifolia*2). The tree topology resulting from MP analyses of the total evidence data set showed a clade formed by *Odontadenia* and *Secondatia* to be sister to the Mesechiteae clade, although this relationship received a BS of less than 50%.

The taxa belonging to tribe Apocyneae sensu Endress and Bruyns (2000) are polyphyletic, with the six representatives included in this study dispersed among three different parts of the cladogram. The Neotropical genus *Forsteronia* is nested in the Mesechiteae clade, whereas *Odontadenia,* a large genus widely distributed in the Neotropics, forms a strongly supported clade (BS = 98%) with *Secondatia,* a Neotropical genus included in tribe Mesechiteae by Endress and Bruyns (2000). *Beaumontia, Chonemorpha,* and *Trachelospermum,* all genera of tropical and subtropical regions of Asia, form a strongly supported clade (BS = 96%) somewhat closer to the base of the tree. A clade composed of *Prestonia* and *Rhodocalyx,* both Neotropical members of Echiteae sensu Endress and Bruyns (2000), is strongly supported (BS = 100%).

Analysis of the percentage of nodes supported by unambiguously optimized characters for each individual partition onto the strict consensus tree of the total evidence analysis showed that the individual data sets had varying degrees of resolving power (Table 3). The highest power of resolution consistent with the total evidence tree was found for nucleotides in the *rpl16* intron, with more than 80% of the nodes in the strict consensus tree of the combined analysis supported by at least one unambiguously optimized nucleotide substitution of the *rpl16* intron. Slightly lower percentages were found for the *rps16* intron and *trnS-G* intergenic spacer, with the lowest percentage provided by the gaps in the *rps16* intron. A relatively high percentage (45%) of the nodes in the strict consensus of the total evidence tree was supported by at least one unambiguously optimized morphological character.

## DISCUSSION

***Molecular characteristics of noncoding plastid DNA***—The three plastid regions used in our analysis showed a set of characteristics similar to that reported by Kelchner (2000) for noncoding plastid regions, such as the occurrence of strings of mononucleotide repeats and small tandem repeat units. Considerable length variation among the individual sequences re-

quired the insertion of a large number of gaps in the alignments (Table 2), especially for the *trnS-G* intergenic spacer, which is consistent with the results reported by Perret et al. (2003) for the same region. Gaps provided a considerable amount of information for our analysis, representing one-quarter of the total number of informative characters. The nucleotide substitutions were not uniform across sequences of the same DNA region, but rather alternated between more conserved and more variable regions. This heterogeneity was highest in the *trnS-G* intergenic spacer region. These more variable regions, which could provide potential sources of phylogenetic information at lower taxonomic levels, were excluded from our analysis because of ambiguous alignment.

***Phylogenetic utility of individual data partitions***—All three plastid regions were characterized by similar percentages of parsimony informative characters, ranging from 8.6% in the *trnS-G* intergenic spacer to 10.6% in the *rps16* intron, consistent with the results reported by Schönenberger and Conti (2003). In contrast to this uniformity in the percentage of parsimony informative characters provided by each data set, marked differences were observed in the resolving power of the separate data partitions. The highest resolving power was found in the *rpl16* intron, which yielded the highest percentage of nodes supported by at least one unambiguously optimized character onto the strict consensus of the total evidence tree (Table 3). All individual data partitions, however, including morphology, contributed partially to the resolution in the total evidence tree.

Although the *rpl16* intron, *rps16* intron, and *trnS-G* intergenic spacer have been used in several recent studies, either individually or combined with other plastid and/or nuclear regions (e.g., Oxelman et al., 1997; Downie et al., 2000; Asmussen and Chase, 2001; Perret et al., 2003), the combination of all three regions in the same data matrix has been applied only recently (Schönenberger and Conti, 2003). This study shows their phylogenetic utility to resolve relationships at the tribal and intergeneric levels, especially when combined with morphology.

***Comparison between phylogenetic hypothesis and current classification***—The total evidence tree does not support the monophyly of Mesechiteae as circumscribed by Endress and Bruyns (2000) with its nine constituent genera: *Allomarkgrafia, Galactophora, Quiotania, Macrosiphonia, Mandevilla, Mesechites, Secondatia, Telosiphonia,* and *Tintinnabularia*. Of the eight genera included in this study, six (*Allomarkgrafia, Macrosiphonia, Mandevilla, Mesechites, Telosiphonia,* and *Tintinnabularia*) form a strongly supported clade together with *Forsteronia,* a genus placed in Apocyneae by Endress and Bruyns (2000). *Secondatia,* on the other hand, groups with *Odontadenia,* the Neotropical representative of Apocyneae included among the outgroup taxa in our study. *Galactophora* clearly does not belong in Mesechiteae, but its relationships are uncertain at present (see Results and Fig. 1). The status of *Quiotania* could not be evaluated, because collection of leaf tissue was not possible (see Materials and Methods).

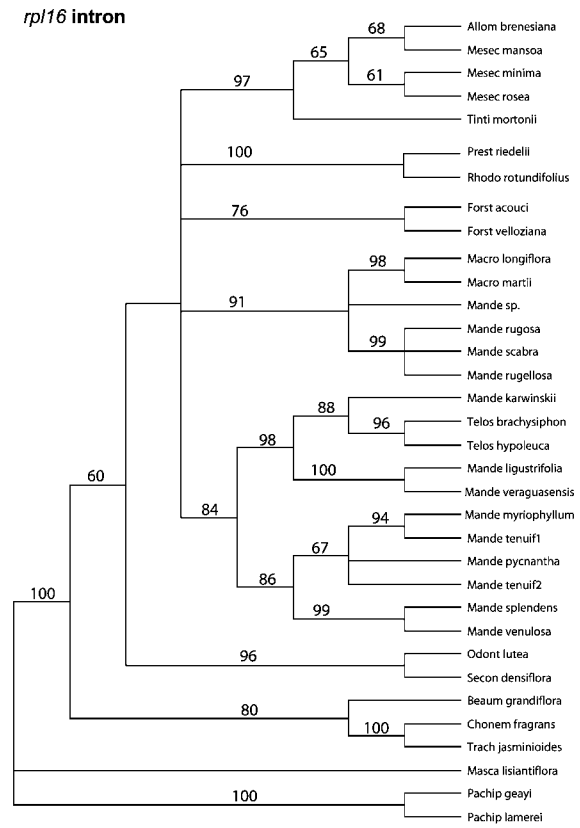
Addition of morphological characters to the combined molecular data set increased bootstrap support for the Mesechiteae clade from 81 to 97%. Four morphological synapomorphies were identified, which are congruent with the Mesechiteae clade, as defined in the total evidence tree: (1) leaf blade with one to many colleters on the adaxial surface, (2) anthers with a blunt-cordate to truncate base, (3) retinacle strongly united with the style head by cellular fusion, and (4) style head with five strongly protruding longitudinal ribs (Fig. 4). The structure of the style head and the manner in which it is united with the anthers (retinacle type) are both key characters in the specialized flowers of the Apocynaceae. The style head is a product of postgenital fusion of the two carpel apices, which develop into an enlarged, secretory structure. The retinacle is the region of the anther that becomes postgenitally united with the style head, thus forming a gynostegium (Fallen, 1986; Sennblad et al., 1998). All members of the Mesechiteae clade have a style head that is often referred to in the earlier literature as ''umbraculiform'' (umbrella shaped). This type of style head is characterized by having a star-like shape in cross section, from the five strongly projecting vertical ribs, which may extend along its entire length or be more or less restricted to the base. The anthers are postgenitally united with these ribs by the retinacle, which in Mesechiteae consists of a strip of specialized cells that are unusually short and strongly attached to the style head by cellular fusion. This type of retinacle sets the Mesechiteae clade apart from other Neotropical Apocynoideae, in which the retinacle is composed of longer hairs, characterized by a weaker attachment to the style head, without cellular fusion.

In contrast to these ''good'' characters, many of the morphological characters (e.g., habit, presence of domatia, absence vs. presence or arrangement of calycine colleters, corolla shape, length of staminal filaments, and nectary height), which have previously been used to define taxonomic groups (Woodson, 1933; Zarucchi, 1991; Morales, 1997, 1998; Williams, 1999, 2002), appear to be phylogenetically unreliable. For example, Woodson (1936) and Leeuwenberg (1994) considered *Tintinnabularia* to be related to *Beaumontia* (a genus of the outgroup tribe Apocyneae in this study), based on their shared possession of domatia and long staminal filaments.

***Relationships between genera***—Next we briefly discuss relationships among the three subclades of Mesechiteae, focusing on the morphological synapomorphies that are consistent within these clades.

*Mesechites subclade*—The *Mesechites* subclade is defined by one morphological synapomorphy: having the ribs of the style head restricted to the base (Fig. 4). This distinguishes the *Mesechites* subclade from the other two subclades of Mesechiteae, both of which are characterized by having ribs along the entire length of the style head. In the literature (e.g., Woodson, 1933; Williams, 1999), the inflorescences of *Allomarkgrafia, Mesechites,* and *Tintinnabularia* are often said to be cymose (to distinguish these genera from *Mandevilla,* which is considered to have racemose inflorescences). However, inflorescence type is a character that is difficult to interpret, especially as the inflorescence is often reduced (e.g., in *Tintinnabularia mortonii, Mesechites minima,* and *M. rosea*).

In the *Mesechites* subclade, *Tintinnabularia* is sister to a strongly supported clade composed of *Allomarkgrafia* and *Mesechites*. *Tintinnabularia,* described by Woodson (1936) and comprising three species, is restricted to Mexico, Guatemala, and Honduras. It is one of the most rarely collected and thus poorly known genera in the Apocynoideae. It has been considered to be allied to other members of the Mesechiteae by most Neotropical Apocynaceae specialists (e.g., Zarucchi, 1991; Henrickson, 1996; Williams, 1999), but except for Pi-


rpl16 intron
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Mesec rosea
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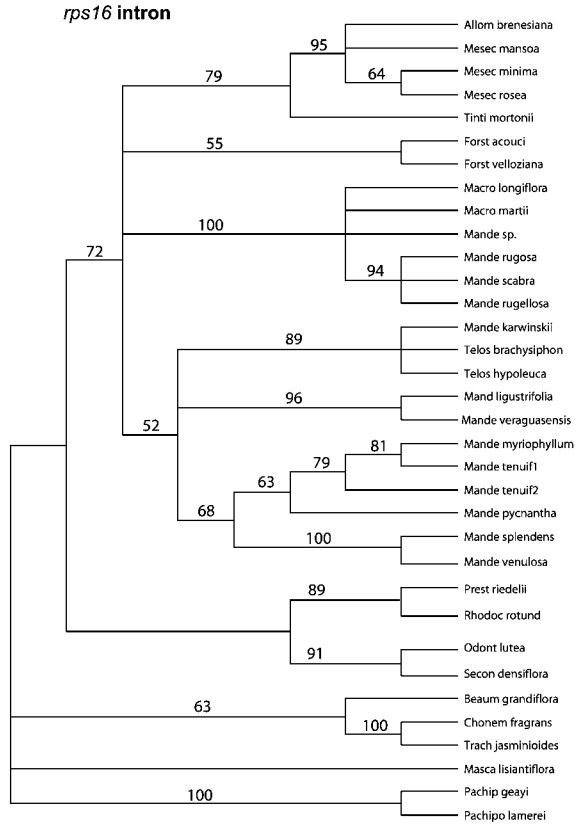
Forst acouci
Forst velloziana
Macro longiflora
Macro martii
Mande sp.
Mande rugosa
Mande scabra
Mande rugellosa
Mande karwinskii
Telos brachysiphon
Telos hypoleuca
Mande ligustrifolia
Mande veraguasensis
Mande myriophyllum
Mande tenuif1
Mande pycnantha
Mande tenuif2
Mande splendens
Mande venulosa
Odont lutea
Secon densiflora
Beaum grandiflora
Chonem fragrans
Trach jasminioides
Masca lisiantiflora
Pachip geayi
Pachip lamerei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Telos hypoleuca
Mand ligustrifolia
Mande veraguasensis
Mande myriophyllum
Mande tenuif1
Mande tenuif2
Mande pycnantha
Mande splendens
Mande venulosa
Prest riedelii
Rhodoc rotund
Odont lutea
Secon densiflora
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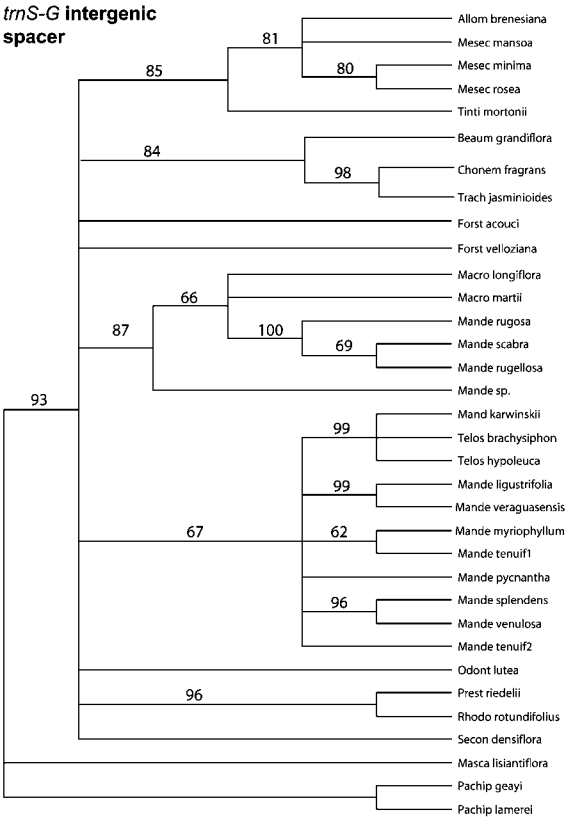
Pachipo lamerei


trnS-G intergenic spacer
Allom brenesiana
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Forst acouci
Forst velloziana
Macro longiflora
Macro martii
Mande rugosa
Mande scabra
Mande rugellosa
Mande sp.
Mand karwinskii
Telos brachysiphon
Telos hypoleuca
Mande ligustrifolia
Mande veraguasensis
Mande myriophyllum
Mande tenuif1
Mande pycnantha
Mande splendens
Mande venulosa
Mande tenuif2
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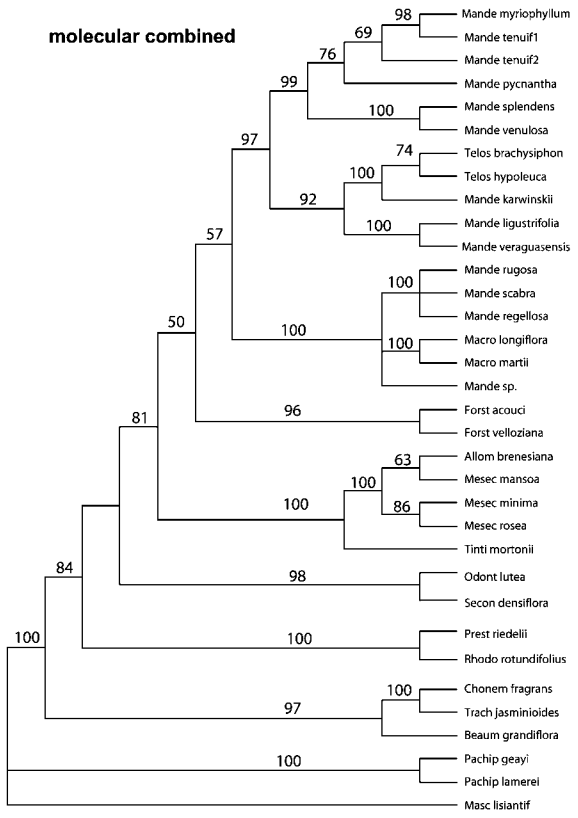
molecular combined
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Mande tenuif1
Mande tenuif2
Mande pycnantha
Mande splendens
Mande venulosa
Telos brachysiphon
Telos hypoleuca
Mande karwinskii
Mande ligustrifolia
Mande veraguasensis
Mande rugosa
Mande scabra
Mande regellosa
Macro longiflora
Macro martii
Mande sp.
Forst acouci
Forst velloziana
Allom brenesiana
Mesec mansoa
Mesec minima
Mesec rosea
Tinti mortonii
Odont lutea
Secon densiflora
Prest riedelii
Rhodo rotundifolius
Chonem fragrans
Trach jasminioides
Beaum grandiflora
Pachip geayi
Pachip lamerei
Masc lisiantif

chon (1950), who considered *Tintinnabularia* to be most closely related *Forsteronia,* no other specialist in the family has made an attempt to elucidate its closest relatives within the tribe.

*Mesechites* currently comprises 10 species, divided between the two subgenera, *Eumesechites* and *Didymadenia,* described by Woodson (1933). Of the three species of *Mesechites* included in our study, the two Cuban species, *M. minima* and *M. rosea,* representing subgenus *Didymadenia,* are strongly supported as sister taxa (BS = 94%). The South American representative from subgenus *Eumesechites, M. mansoana,* however, groups with *Allomarkgrafia* rather than with the other *Mesechites* species. This suggests that *Mesechites* may not be a monophyletic genus as currently circumscribed. The two genera have long been considered to be closely related. Pichon (1948b, 1950) considered *Allomarkgrafia* to be a synonym of *Mesechites,* arguing that the style head form was the same for the two genera and that no diagnostic character states supported the generic distinction of *Allomarkgrafia*. Our current results do not contradict this taxonomic interpretation; no morphological synapomorphy that could distinguish the two genera was found. More species need to be analyzed, however, before firm conclusions as to generic circumscription can be reached.

*Forsteronia*—The inclusion of *Forsteronia* in the Mesechiteae clade is somewhat unexpected; this relationship has never been proposed. *Forsteronia* is a relatively large Neotropical genus with some 46 recognized species, characterized by having small, subrotate flowers in thyrsiform, often dense inflorescences. Although easily distinguished from other Neotropical genera, the placement of *Forsteronia* within the Apocynoideae has proved difficult. Pichon (1950) placed *Forsteronia* in his tribe Ichnocarpeae based on its glabrous, concave retinacle and created the subtribe Forsteroniinae to accommodate this genus together with *Tintinnabularia*. This subtribe was mainly defined by the presence of domatia on the abaxial surface of the leaves. Leeuwenberg (1994) agreed for the most part with Pichon and placed *Forsteronia* in his tribe Apocyneae, together with many of the genera originally placed in Pichon's Ichnocarpeae. He did not recognize subtribe Forsteroniinae, however, and he placed *Tintinnabularia* in a different tribe, Wrightieae. Endress and Bruyns (2000) included *Forsteronia* in Apocyneae, assuming that it shares the diagnostic characters states of the tribe: style head fusiform and retinacle formed by a horseshoe-shaped rim and a narrow longitudinal strip of hairs.

One of the main difficulties concerning the placement of *Forsteronia* is due to the morphological variation within the genus. In order to estimate this variation and the consistency of our phylogenetic results, the flower structure of an additional five species of *Forsteronia, F. australis* Müll.-Arg., *F. portoricensis* Woodson, *F. rufa* Müll.-Arg., *F. spicata* (Jacq.) G. Mey., and *F. velloziana* (A. DC.) Woodson, was examined, based on herbarium vouchers, pickled flowers and, when available, sections of flowers provided by the second author. All species were found to share two of the key characters states

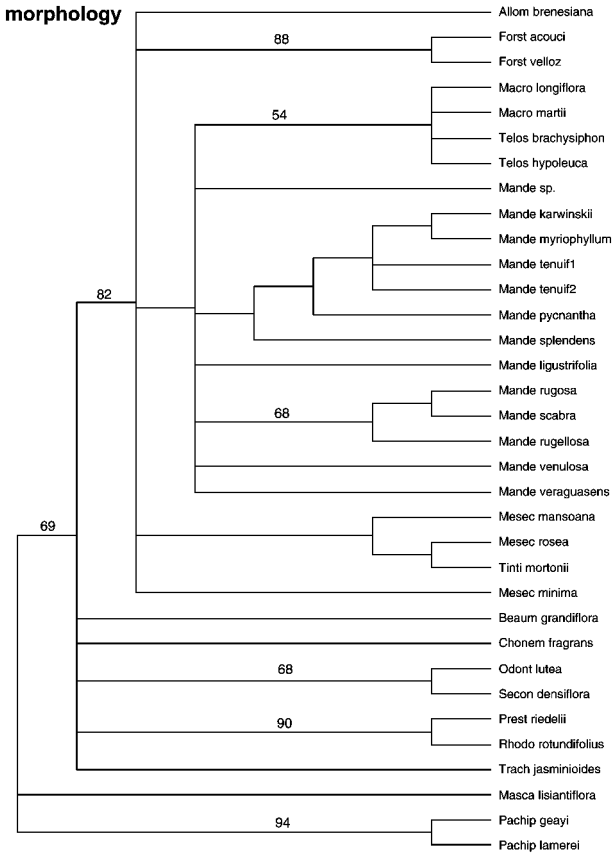


Fig. 3. Strict consensus of the most parsimonious trees generated by the morphological data set. Bootstrap values >50% are indicated above the branches. Full taxon names are given in Appendix 3 (see Supplemental Data accompanying the online version of this article).

that define the Mesechiteae clade: the presence of colleters at the leaf base and anthers with a bluntly cordate to truncate base. Other characteristics, however, are more variable and are more or less intermediate between Mesechiteae and Apocyneae. One such character is the structure of the retinacle. As in other Mesechiteae, the retinacle of *Forsteronia* has a glabrous, concave region; but it also has a small to well-developed row of hair-like cells beneath this. These hair-like cells form a weak union between the anthers and the base of the style head, generally only by agglutination but sometimes with accompanying cellular fusion (e.g., *F. spicata*), as in other Mesechiteae. Furthermore, the longitudinal ribs of the style head are not always well developed. In some species they are quite conspicuous, with the characteristic ''Mesechiteae'' star-like shape in cross section. In other species, however, the ribs are scarcely developed (e.g., *F. acouci*), so that the style head is more or less pentagonal in cross section, which is more characteristic of the Apocyneae. Despite this variation in some of its morphological features, the results of our analyses of morphological variation indicate that the inclusion of *Forsteronia* in the Mesechiteae clade is warranted. In order to test the

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Fig. 2. Strict consensus of the most parsimonious trees generated by the three individual molecular data sets and the combined molecular data set. Bootstrap values >50% are indicated above the branches. Full taxon names are given in Appendix 3 (see Supplemental Data accompanying the online version of this article).

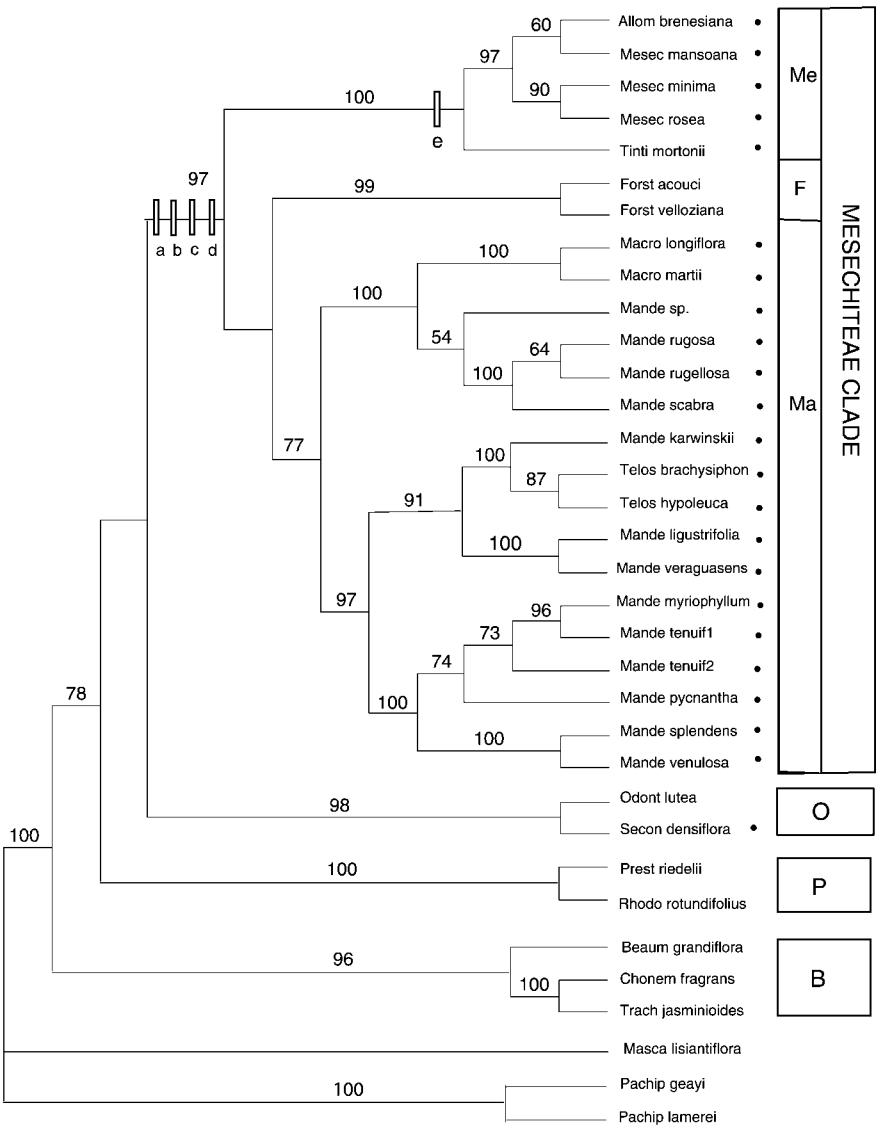


Fig. 4. Strict consensus of the most parsimonious trees generated by the total evidence data set. Bootstrap values >50% are indicated above the branches. Clade names are indicated as follows: Me = *Mesechites* clade; F = *Forsteronia* clade; Ma = *Mandevilla* clade; O = *Odontadenia* clade; P = *Prestonia* clade: B = *Beaumontia* clade. The letters a, b, c, d, e denote key morphological synapomorphies diagnostic for the Mesechiteae and Mesechites clades and are taken from the morphological data set (see Appendices 3 and 4 in Supplemental Data accompanying the online version of this article); a: leaf colleters on the adaxial surface of the leaf; b: anther base bluntly cordate to truncate; c: anthers attached to the style head by cellular fusion; d: style head with five strongly protruding ribs in cross section; e: ribs of the style head restricted to the base. Dots indicate species previously placed in Mesechiteae by Endress and Bruyns (2000). Full taxon names are given in Appendix 3 (see Supplemental Data accompanying the online version of this article).

monophyly of *Forsteronia*, however, a broader taxon sampling of species chosen to adequately represent the range of variation within the genus is required.

*The Mandevilla subclade*—The three genera of this subclade (*Macrosiphonia*, *Mandevilla*, and *Telosiphonia*) have always been considered to be closely related and sometimes even synonymous. They all have racemose inflorescences, sometimes reduced to a single flower, and strongly protruding ribs extending along the entire length of the style head.

*Mandevilla* is the largest Neotropical genus in the Apocynoideae. It is extremely variable, with about 120 species dis-

TABLE 3. Percentage of nodes supported by at least one unambiguously optimized character for each individual partition on the strict consensus tree of total evidence analysis.

| | *rpl16* intron | *rps16* intron | *trnS-G* intergenic spacer | Morphology |
|---|---|---|---|---|
| Nucleotides | 83.8% | 67.7% | 64.5% | — |
| Gaps | 51.6% | 25.8% | 35.4% | — |
| Morphological characters | — | — | — | 45.1% |

tributed throughout the Neotropics, from Mexico to Argentina, and includes vines, erect shrubs, and even epiphytes. The flower size and structure also spans a broad range, from inconspicuous whitish, tubular flowers less than 1 cm long to brightly colored, showy infundibuliform flowers up to 5 cm long. *Macrosiphonia* and *Telosiphonia,* in contrast, contain only five and six species, respectively, which share a number of morphological characteristics. Both are erect shrubs or subshrubs with leaves covered by a dense, wooly indument on the abaxial side and occur in savannas or arid habitats. The flowers are white with a long slender tube and are presumably adapted to hawkmoth pollination. *Telosiphonia* was originally described as a subgenus of *Macrosiphonia* by Woodson (1933). Henrickson (1996), however, elevated subgenus *Telosiphonia* to generic rank based on characters such as inflorescence type, style head structure and pollen size. He suggested that the many similarities between *Macrosiphonia* and *Telosiphonia* are the result of adaptation to a similar habitat and pollination syndrome. The distribution of the two genera roughly coincides with the extreme northern and southern distribution of *Mandevilla*. *Telosiphonia* is restricted to the arid zones of Mexico and the southwestern United States, whereas *Macrosiphonia* is found in the savannas of central Brazil to Argentina. The separation of *Macrosiphonia* and *Telosiphonia* into two distinct clades strongly supports Henrickson's (1996) ideas that the two taxa are not congeneric. Nevertheless, their recognition as distinct genera probably cannot be upheld in light of the present data because both clades are nested in *Mandevilla*. The morphological characteristics that have been used to distinguish *Mandevilla* from *Macrosiphonia* and *Telosiphonia* are rather minor, being based only on leaf indument and superficial aspects of flower structure. Woodson (1933, p. 778) maintained them as distinct genera, but stated that ''The existing distinctions between *Macrosiphonia* and *Mandevilla* are extremely tenuous.'' Pichon (1948b) proposed the inclusion of *Macrosiphonia* in the synonymy of *Mandevilla,* arguing that the distinguishing characters used by Woodson (1933) to differentiate between the two genera were inconsistent and arbitrary, making impossible an unambiguous distinction between them. Our morphological analyses identified no apomorphies exclusive to either genus, reinforcing the difficulty of upholding their current generic rank.

In his taxonomic revision of *Mandevilla,* Woodson (1933) recognized two subgenera: *Eumandevilla* and *Exothostemon*. Within subgenus *Eumandevilla*, he recognized five sections: Laxae, Montanae, Tenuifoliae, Torosae, and Tubiflorae. The monophyly of subgenus *Exothostemon,* represented in our study by *M. rugosa, M. rugellosa,* and *M. scabra,* is strongly supported (BS = 100%). However, these results must be interpreted as preliminary due to the small number of *Mandevilla* species sampled for a genus of this size. Similarly, no conclusions can be made at this time about relationships among the sections of *Mandevilla* sensu Woodson (1933), also due to the insufficient taxon sampling. Our finding of paraphylly in *M. tenuifolia* with regard to *M. myriophyllum* suggests that the latter could be merely an extreme morphotype of the former. However, a significantly broader taxon sampling in *M. tenuifolia* would need to be undertaken in order to determine this with more certainty. Further studies based on more intensive taxon sampling in *Mandevilla,* needed to address these questions, are underway.

***Galactophora and Secondatia***—The exclusion of *Secondatia* and *Galactophora* from the Mesechiteae, suggested by our phylogenetic analyses, is congruent with morphology. In both genera, leaf colleters are absent, the anther base is strongly sagittate, and the retinacle is protuberant with no detectable concave region—all character states that are at odds with the synapomorphies that support the Mesechiteae clade. In *Secondatia,* the style head is almost circular in cross section, with no longitudinal ribs, similar to that found in *Odontadenia*. The retinacle structure is also of the same type as that in *Odontadenia*. *Secondatia* and *Odontadenia* comprised Pichon's (1950) subtribe Secondatinae of Ecdysanthereae, and our results also support a close relationship between these two genera. In *Galactophora,* in contrast, the style head has five well-developed projecting ribs at the base. The presence of these ribs was the main reason for the inclusion of *Galactophora* in Mesechiteae by Endress and Bruyns (2000). Our results, however, suggest that the ribs on the style head are independently derived in *Galactophora* and the Mesechiteae clade. Preliminary analysis of the style-head structure of *G. calycina* reinforces this hypotheses. In this species, the ribs are not continuous with the main body of the style head, as in taxa of the Mesechiteae clade, but rather are formed by soft tissue that is distinct from that of the rest of the style head. The union between the style head and the anthers in *Galactophora* is also quite weak; the anthers are easily detached from the style head. This is in sharp contrast to the situation in taxa of the Mesechiteae clade in which the anthers are so strongly united with the style head that they can usually only be removed by ripping off an adjacent piece of the style head.

***Apocyneae***—Our results, especially in light of the position of *Forsteronia* in Mesechiteae, strongly suggest that Apocyneae sensu Endress and Bruyns (2000) is not monophyletic. This is not unexpected, confirming their prediction (Endress and Bruyns, 2000, p. 8) that ''The Apocyneae, especially, will probably need to be divided in some way, and some rearrangement of taxa will no doubt be necessary as more data accumulate.'' No further conclusions can be drawn about relationships within the Apocyneae, however, due to the small number of taxa from this tribe sampled in our analysis.

***Conclusions***—The phylogenetic analysis presented here provides the first broad study of the Mesechiteae including representatives of all but one of its constituent genera, using both morphological and molecular characters. This represents the first step towards resolving long-standing disputes over generic delimitation and intergeneric relationships within the tribe. The newly defined Mesechiteae comprise taxa previously ascribed to eight genera: *Allomarkgrafia, Forsteronia, Macrosiphonia, Mandevilla, Mesechites, Quiotania, Telosiphonia,* and *Tintinnabularia*.

Topics to be addressed in a future study include testing the monophyly and determining the systematic position of *Forsteronia* in the Mesechiteae; defining the generic circumscription of *Allomarkgrafia*, *Macrosiphonia,* and *Telosiphonia*; testing the monophyly of the currently recognized sections within *Mandevilla*; and elucidating character evolution and the biogeographic history of the group.

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