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IS *MANDEVILLA* (APOCYNACEAE, MESECHITEAE) MONOPHYLETIC? EVIDENCE FROM FIVE PLASTID DNA LOCI AND MORPHOLOGY

IS *MANDEVILLA* (APOCYNACEAE, MESECHITEAE) MONOPHYLETIC? EVIDENCE FROM FIVE PLASTID DNA LOCI AND MORPHOLOGY[1]

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ABSTRACT

In order to test the monophyly of *Mandevilla* Lindl., the largest genus in tribe Mesechiteae (Apocynaceae, Apocynoideae), and its affinities to other genera in the tribe, maximum parsimony analysis was conducted on a data set comprising DNA sequences from five plastid loci (*rpl*16, *rps*16, and *trn*K introns; *trn*S^{GCU}-*trn*G^{UUC} intergenic spacer; and *mat*K gene), as well as morphological data for 65 taxa of Mesechiteae (48, *Mandevilla*) and nine taxa from other tribes of the subfamily. *Mandevilla*, as circumscribed by Pichon, was found to be monophyletic, whereas Woodson's circumscription proved to be polyphyletic. Thus defined, *Mandevilla* forms a strongly supported clade that can be divided into six clades of species groups. Most of the infrageneric taxa of *Mandevilla* proposed by Woodson and Pichon are polyphyletic. Many of the diagnostic characters previously used to define taxonomic groups are shown to have arisen multiple times, rendering them unsuitable for classificatory purposes. The similar growth form and tubular flowers of *Macrosiphonia* Müll. Arg. and *Telosiphonia* (Woodson) Henr., two geographically disjunct segregates, represent the most extreme case of parallel evolution within *Mandevilla*, with their striking similarities most likely correlated to colonization of open, dry habitats and pollination by hawkmoths.

Key words: Apocynaceae, *Mandevilla*, *mat*K, Mesechiteae, morphology, phylogenetic systematics, *rpl*16, *rps*16, *trn*K, *trn*S^{GCU}-*trn*G^{UUC}.

Mandevilla Lindl., a member of tribe Mesechiteae, is the largest Neotropical genus in Apocynaceae and comprises about 150 species (Simões et al., 2004; Sales et al., 2006). It is distributed throughout the Neotropics, from Mexico to Argentina, in a wide variety of habitats such as deserts, savannas, tepuis, open grasslands, and forests. Morphological variation is remarkable in the genus in both vegetative and reproductive parts. Most species are vines, but erect shrubs are also common, while unbranched subshrubs and epiphytes occur less frequently. Flower size and structure are also very diverse, ranging from inconspicuous white, tubular flowers less than 1 cm long to brightly colored, showy infundibuliform flowers up to 9 cm long. The genus is traditionally characterized by the following set of traits: racemose inflorescence; leaf blade with one to many colleters on the adaxial surface, sometimes extending along the midrib; and style head with five strongly protruding, well-developed longitudinal ribs (Woodson, 1933; Pichon, 1948; Henrickson, 1996; Morales, 1998; Simões & Kinoshita, 2002; Simões et al., 2004).

A combination of high morphological diversity and broad geographic distribution makes *Mandevilla* one of the most challenging and complex genera of Neotropical Apocynaceae, a fact that is reflected in its taxonomic history. The currently accepted circumscription of *Mandevilla* was defined by Woodson in

1933. In a broad taxonomic study of the Neotropical species of subfamily Apocynoideae, he made significant changes in the circumscription of *Mandevilla*, including in its synonymy such genera as *Exothostemon* (G. Don) Woodson, *Dipladenia* A. DC., *Laseguea* A. DC., *Amblyanthera* Müll. Arg., *Heterothrix* Müll. Arg., and part of *Echites* P. Browne. *Macrosiphonia* Müll. Arg., a small group of shrubby species with long, white, tubular flowers and a disjunct distribution in the arid zones of the southwestern U.S.A. and Mexico and the savannas of southern South America, was maintained by Woodson as a separate genus. He admitted, however, that the distinctions between *Macrosiphonia* and *Mandevilla*, based on plant habit, flowering time, and style head structure, were extremely tenuous. He also cautiously recognized two subgenera in *Macrosiphonia* (Woodson, 1933: 778), *Telosiphonia* Woodson and *Eumacrosiphonia* Woodson (= *Macrosiphonia*), comprising the species that occur in the Northern and Southern Hemispheres, respectively.

In addition to broadening the limits of *Mandevilla*, Woodson (1933) also proposed a morphologically based infrageneric classification of the genus, with the subgenera *Exothostemon* and *Eumandevilla* Woodson (= *Mandevilla*). The two subgenera were differentiated based on the following suite of morphological characters: species of subgenus *Exothostemon* have leaf colleters distributed along the entire length of the midrib, calycine colleters with an opposite arrangement, and a curved corolla tube, whereas species of subgenus *Mandevilla* have leaf colleters restricted to the base of the midrib, calycine colleters with an alternate or continuous arrangement, and a straight corolla tube.

Within subgenus *Mandevilla*, Woodson (1933) proposed a further subdivision with five sections: *Laxae* Woodson, *Montanae* Woodson, *Tenuifoliae* Woodson, *Torosae* Woodson, and *Tubiflorae* Woodson, which were differentiated based mainly on corolla shape, anther base shape, and number and size of nectaries. The largest section, *Laxae*, included 46 species distributed throughout South America and was characterized by infundibuliform corollas. Section *Montanae* consisted of 16 species also distributed in South America and was characterized by flowers with salverform to tubular-salverform corollas, anthers with a truncate base, and nectaries varying in number from two to five or even absent in some species. The smallest section, *Tenuifoliae*, comprising only two South American species, *M. myriophyllum* (Taub.) Woodson and *M. tenuifolia* (J. C. Mikan) Woodson, was distinguished from section *Montanae* by having anthers with auriculate bases and two nectaries. The two remaining sections, *Torosae* and *Tubiflorae*, have

five and eight species, respectively, and are distributed in Mexico and Mesoamerica. Both of these sections were characterized by flowers with salverform to tubular-salverform corollas, anthers with auriculate bases, and five nectaries surrounding the ovary, but differed from one another in the size of the nectaries, which were said to be equal to or taller than the ovary in section *Tubiflorae* and shorter than the ovary in section *Torosae*.

A revised classification of *Mandevilla* was published by Pichon in 1948. He expanded Woodson's (1933) circumscription by including *Macrosiphonia*, which he justified by arguing that the characters used by Woodson to differentiate between the two genera were inconsistent and arbitrary. He did not consider Woodson's subgenera *Macrosiphonia* and *Telosiphonia* to be each other's closest relatives, however, and placed them in two distinct sections, based on the absence of a pedicel, longer staminal filaments, and larger pollen grains of the former. Within *Mandevilla*, Pichon recognized Woodson's subgenus *Mandevilla* and subgenus *Exothostemon* as valid groups but did not recognize his five sections within subgenus *Mandevilla*. According to Pichon, the characters supporting these two subgenera were reliable, whereas those supporting the sections were highly inconsistent, with no real diagnostic states to define them. Pichon (1948) proposed a new infrageneric classification within *Mandevilla*, recognizing four sections (*Orthocaulon* Pichon, *Exothostemon* Pichon, *Megasiphon* Pichon, and *Telosiphonia* Pichon). A summarized comparison between the infrageneric classification of Woodson (1933) and Pichon (1948) is provided in Table 1.

Since Pichon's work (1948, 1950), very few studies have investigated the taxonomy of *Mandevilla* and related genera. In 1991, Zarucchi described *Quiotania* Zarucchi as a monotypic genus morphologically very similar to *Mandevilla*, and Woodson's subgenus *Telosiphonia* was later elevated to generic status by Henrickson (1996). Another relevant work was a synopsis of the Mexican and Central American species of *Mandevilla* by Morales (1998), with new taxonomic combinations involving the species from Woodson's sections *Tubiflorae* and *Torosae*, many of these reduced to synonymy. In addition, a large number of new species of *Mandevilla* have been described in the past few decades, increasing the number of published species from the 108 recognized by Woodson in 1933 to about 150 at present. Although new information has been accumulating for the genus, no overall classification within *Mandevilla* as a whole has been proposed since Pichon (1948). Taxonomic difficulties involving both generic and infrageneric concepts have persisted for the past

Table 1. Comparison of Woodson's (1933) subgenera and sections of *Mandevilla* and their corresponding ranks in Pichon's (1948) classification.

Woodson (1933)	Pichon (1948)
Mandevilla subg. *Exothostemon*	*Mandevilla* sect. *Exothostemon*
Mandevilla subg. *Mandevilla*	*Mandevilla* sect. *Orthocaulon*
Section *Laxae*	
Section *Montanae*	
Section *Tenuifoliae*	
Section *Torosae*	
Section *Tubiflorae*	
Genus *Macrosiphonia*	
Subgenus *Macrosiphonia*	*Mandevilla* sect. *Megasiphon*
Subgenus *Telosiphonia*	*Mandevilla* sect. *Telosiphonia*

seven decades and still remain, as pointed out by Zarucchi (1991: 35): "The last word concerning generic limits of the *Mandevilla–Mesechites–Macrosiphonia* complex and near relatives has obviously not yet been written."

The use of phylogenetic methods has been successfully applied in Apocynaceae to solve controversial aspects of classification within the family. Previous studies have addressed the circumscription of Apocynaceae s. str. and their relationships with the former Asclepiadaceae (e.g., Judd et al., 1994; Sennblad & Bremer, 1996, 2002; Potgieter & Albert, 2001), but a growing number of works have focused on relationships within Apocynaceae s. str. Examples include overviews by Endress et al. (1996) and Sennblad et al. (1998) for tribe Wrightieae, a study by Endress et al. (2007) for Alyxieae, and a larger-scale study of subfamily Apocynoideae by Livshultz et al. (2007). Phylogenetic studies based on morphological characters were also published by Sidiyasa (1998) for *Alstonia* R. Br., van der Ham (2001) for Alyxieae, and Williams (2004) for *Echites*.

Simões et al. (2004) provided the first phylogenetic study of tribe Mesechiteae, with suggestions for taxonomic improvements in tribal and intergeneric delimitations. Preliminary results were obtained for *Mandevilla* and related genera, but due to the limited taxon sampling within *Mandevilla*, no firm conclusions could be drawn as to infrageneric relationships. Our present study represents the subsequent second step in interpreting the phylogeny of Mesechiteae by focusing on the intergeneric and infrageneric relationships of its largest genus, *Mandevilla*.

The aims of the present article are to test the monophyly of *Mandevilla* and determine its relationships to the putatively affined genera *Macrosiphonia*, *Telosiphonia*, and *Quiotania*, using both morphology

and DNA sequence data from five chloroplast DNA loci. The resulting phylogenetic hypotheses of monophyly and infrageneric relationships of *Mandevilla* are compared with the classifications of Woodson (1933) and Pichon (1948). Morphological features consistent with the retrieved clades and/or used to define taxonomic ranks are also discussed.

MATERIALS AND METHODS

TAXON SAMPLING

Sixty-five taxa of Mesechiteae, including representatives from all genera of the tribe recognized by Simões et al. (2004) (*Allomarkgrafia* Woodson, *Forsteronia* G. Mey., *Macrosiphonia*, *Mandevilla*, *Mesechites* Müll. Arg., *Telosiphonia*, and *Tintinnabularia* Woodson), were included in this study (Appendix 1). In order to test the infrageneric classifications of *Mandevilla* proposed by Woodson (1933) and Pichon (1948) (Table 1), 48 accessions (from 47 species) of *Mandevilla*, representing all subgenera and sections, were sampled (Table 2). Nine outgroup taxa representing all but the basalmost tribe (Wrightieae) of subfamily Apocynoideae were chosen, based largely on previous studies suggesting that the closest relatives of Mesechiteae are either Apocyneae or Echiteae (Sennblad et al., 1998; Sennblad & Bremer, 2002; Simões et al, 2004). Two genera from Echiteae (*Prestonia* R. Br. and *Rhodocalyx* Müll. Arg.) and five from Apocyneae (*Beaumontia* Wall., *Chonemorpha* G. Don, *Odontadenia* Benth., *Secondatia* A. DC., and *Trachelospermum* Lem.) were included. Two species of *Pachypodium* Lindl. (Malouetieae) were used to root the cladograms.

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, U.S.A.) following the manufacturer's protocol. Five plastid loci (*rpl*16, *rps*16, and *trn*K introns; *trn*S^GCU-*trn*G^UUC intergenic spacer; and *mat*K gene) were amplified. Double-stranded DNA was amplified by polymerase chain reaction (PCR) on a Biometra Tgradient machine (Biometra, Göttingen, Germany), applying a thermal cycling program of 34 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 1 minute, and extension at 72°C for 90 seconds. The *trn*K intron and *mat*K gene were co-amplified in a single PCR reaction, and the thermal cycling program was modified in the following steps: denaturation at 94°C for 30 seconds and annealing at 54°C for 1 minute. For some taxa, amplification of the entire *trn*K intron/*mat*K locus

Table 2. List of the sampled taxa of *Macrosiphonia*, *Mandevilla*, and *Telosiphonia* and their placement in the classification of Woodson (1933) and the clades observed in the present study.

Taxon name and current classification	This study
Genus *Macrosiphonia* Müll. Arg.	
Macrosiphonia longiflora (Desf.) Müll. Arg.	Clade I
Macrosiphonia martii Müll. Arg.	Clade I
Macrosiphonia velame (A. St.-Hil.) Müll. Arg.	Clade I
Genus *Mandevilla* Lindl.	
Subgenus *Mandevilla* Woodson, as "*Eumandevilla*"	
Section *Laxae* Woodson	
Mandevilla atroviolacea (Stadelm.) Woodson	Clade III
Mandevilla callista Woodson	Clade I
Mandevilla coccinea (Hook. & Arn.) Woodson	Clade III
Mandevilla convolvulacea (A. DC.) Hemsl.	Clade IV
Mandevilla duartei Markgr.	Clade III
Mandevilla fragrans (Stadelm.) Woodson	Clade III
Mandevilla funiformis (Vell.) K. Schum.	Clade I
Mandevilla glandulosa (Ruiz & Pav.) Woodson	Clade IV
Mandevilla harleyi M. F. Sales, Kin.-Gouv. & A. O. Simões	Clade I
Mandevilla illustris (Vell.) Woodson	Clade III
Mandevilla laxa (Ruiz & Pav.) Woodson	Clade IV
Mandevilla ligustriflora Woodson	Clade IV
Mandevilla martiana (Stadelm.) Woodson	Clade III
Mandevilla moricandiana (A. DC.) Woodson	Clade III
Mandevilla oaxacana (A. DC.) Hemsl.	Clade IV
Mandevilla pendula (Ule) Woodson	Clade III
Mandevilla pohliana (Stadelm.) A. H. Gentry	Clade III
Mandevilla sancta (Stadelm.) Woodson	Clade III
Mandevilla sellowii (Müll. Arg.) Woodson	Clade III
Mandevilla spigeliiflora (Stadelm.) Woodson	Clade III
Mandevilla splendens (Hook. f.) Woodson	Clade III
Mandevilla urophylla (Hook. f.) Woodson	Clade III
Mandevilla venulosa (Müll. Arg.) Woodson	Clade III
Mandevilla veraguasensis (Seem.) Hemsl.	Clade IV
Section *Montanae* Woodson	
Mandevilla cercophylla Woodson	Clade IV
Mandevilla emarginata (Vell.) C. Ezcurra	Clade IV
Mandevilla jamesonii Woodson	Clade IV
Mandevilla pentlandiana (A. DC.) Woodson	Clade IV
Mandevilla pycnantha (Steud. ex A. DC.) Woodson	Clade III
Mandevilla tricolor Woodson	Clade IV
Section *Tenuifoliae* Woodson	
Mandevilla myriophyllum (Taub.) Woodson	Clade III
Mandevilla tenuifolia (J. C. Mikan) Woodson	Clade III
Section *Torosae* Woodson	
Mandevilla foliosa (Müll. Arg.) Hemsl.	Clade IV
Mandevilla karwinskii (Müll. Arg.) Hemsl.	Clade IV
Section *Tubiflorae* Woodson	
Mandevilla syrinx Woodson	Clade IV
Mandevilla tubiflora (M. Martens & Galeotti) Woodson	Clade IV
Subgenus *Exothostemon* (G. Don) Woodson	
Mandevilla anceps Woodson	Clade I
Mandevilla dodsonii A. H. Gentry	Clade I
Mandevilla hirsuta (Rich.) K. Schum.	Clade I
Mandevilla krukovii Woodson	Clade I
Mandevilla lancifolia Woodson	Clade I
Mandevilla leptophylla (A. DC.) K. Schum.	Clade I
Mandevilla nerioides Woodson	Clade I

Table 2. Continued.

Taxon name and current classification	This study
Mandevilla rugellosa (Rich.) L. Allorge	Clade I
Mandevilla rugosa (Benth.) Woodson	Clade I
Mandevilla scabra (Hoffmanns. ex Roem. & Schult.) K. Schum.	Clade I
Mandevilla subsagittata (Ruiz & Pav.) Woodson	Clade I
Genus *Telosiphonia* (Woodson) Henr.	
Telosiphonia brachysiphon (Torr.) Henr.	Clade IV
Telosiphonia hypoleuca (Benth.) Henr.	Clade IV
Telosiphonia nacalpulensis Felger & Henr.	Clade IV

was only possible by fragmenting that region into two parts, using a combination of external and internal primers. Reactions were terminated with a final extension of 4 minutes at 72°C for the *rpl16* intron, *rps16* intron, and *trn*S^{GCU}-*trn*G^{UUC} spacer, and 7 minutes for the *trn*K intron and *mat*K gene. All PCR reactions were performed in a total volume of 25 μL, using 2.5 mM MgCl$_2$ 10× PCR* buffer (Amersham Biosciences, Piscataway, New Jersey, U.S.A.), 0.25 mM of dNTP, 0.5 units of Taq DNA polymerase (Amersham Biosciences, lot 17544), 1 to 4 μl of bovine serum albumin (BSA; Sigma, Steinheim, Germany), and 0.1 mM of each primer.

Primer information is presented in Table 3. For some taxa, internal primers were also used to amplify the *rpl16* intron and *trn*S^{GCU}-*trn*G^{UUC} intergenic spacer, with the following changes in the thermal cycling program: 40 instead of 34 cycles and extension time shortened to 1 minute. Successfully amplified PCR products were then purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences).

For some taxa with DNA extracted from herbarium vouchers, no amplified products were obtained with our initial PCR protocols and primer sets. In those cases, amplification was only successful using a two-step amplification procedure. A first round of PCR amplification was performed using the total genomic DNA as a template, followed by a second round using one external and one internal primer and a 10% dilution of the product of the first amplification as a template. The two-round amplification procedure was used to obtain products of the *trn*K intron and *mat*K gene for six species (*Mandevilla anceps* Woodson, *M. krukovii* Woodson, *M. lancifolia* Woodson, *M. leptophylla* (A. DC.) K. Schum., *M. nerioides* Woodson, *M. tricolor* Woodson, and *Tintinnabularia mortonii* Woodson).

Cycle-sequencing reactions were carried out using an 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, U.S.A.).

DATA MATRIX COMPOSITION AND PARSIMONY ANALYSES

Nucleotide sequences of the studied plastid loci were aligned using Clustal W version 1.8 (Thompson et al., 1994) and adjusted visually. Regions of mononucleotide repeats with variable length between taxa, as well as those composed of nested gaps resulting from ambiguous alignment, were excluded from the analysis. Aligned gaps were manually coded as presence/absence characters by applying the single indel coding method described by Simmons and Ochoterena (2000) for the *mat*K gene and by using the software Gapcoder (Young & Healy, 2003) for the other loci. All coded gaps were then added to the sequence matrix and used in further analyses.

Thirty-two morphological characters were coded using a combination of herbarium and fresh specimens, pickled flowers, and, when available, flower sections prepared by the second author. For some taxa, the literature was also consulted (e.g., Woodson, 1933; Pichon, 1950; Leeuwenberg, 1997; Morales, 1996, 1997, 1998). The morphological matrix and the characters and character states, including some explanatory notes on characters, are given in Appendices 2 and 3, respectively.

A total of six data sets were subjected to phylogenetic analysis, corresponding to the five loci sequenced plus morphology. Because simultaneous analysis of combined data sets has been proposed as the best approach to phylogenetic inference (Nixon & Carpenter, 1996), we tested the combinability of all partitions by searching for incongruence between individual data sets. For this, we compared the results on a node-to-node basis of all individual data sets with respect to levels of resolution and bootstrap support (BS), as applied by other authors (e.g., Wiens, 1998;

Table 3. DNA sequences of the primers used for amplification and sequencing of the five plastid loci used in this study. Primers designed for the two-step amplification are indicated by an asterisk (*).

cpDNA locus		Primers	Primer source
*rpl*16 intron	F71	5′-GCTATGCTTAGTGTGTGACTCGTTG-3′	Baum & Wendel, 1998
	R1516	5′-CCCTTCATTCTTCCTCTATGTTG-3′	Baum & Wendel, 1998
	513F	5′-GGGAACGATGGAAGCTGTGAATGC-3′	Simões et al., 2004
	542R	5′-CGCGGGCGAATATTTACTCTTC-3′	Simões et al., 2004
	*F73	5′-CYCATTACTTCGCATTATCTC-3′	This study
	*1R582	5′-CGACCAGTGAATCATTAAGAT-3′	This study
	*IF479	5′-ACAAATTTCATTATGAGCTCC-3′	This study
	*R1060	5′-GCGAATAAAAGAATTMAAA-3′	This study
rps 16 intron	*rps*F	5′-TGGTAGAAAGCAACGTGCGACTT-3′	Oxelman et al., 1997
	*rps*R2	5′-TCGGGATCGAACATCAATTGCAAG-3′	Oxelman et al., 1997
	387F	5′-CACCGAAGTAATGCCTAAACC-3′	Simões et al., 2004
	497R	5′-GGATTCTKAAGTCTGGCCCAG-3′	Simões et al., 2004
	*IF486	5′-WAACTGGGCCAGACTTMAGAA-3′	This study
	*R768	5′-CGAATAAATTACATAAAAGG-3′	This study
	*R782	5′-ATGGAATTCGAATAAATTACA-3′	This study
*trn*S^GCU-*trn*G^UUC spacer	*trn*S	5′-GCCGCTTTAGTCCACTCAGC-3′	Hamilton, 1999
	*trn*G	5′-GAACGAATCACACTTTTACCAC-3′	Hamilton, 1999
	309F	5′-GATGATTTTTCATTTATMTGA-3′	Simões et al., 2004
	527R	5′-GTGCTWAAATATTTCYYATTMAC-3′	Simões et al., 2004
*trn*K intron + *mat*K gene	*trn*K 3914F	5′-GGGGTTGCTAACTCAACGG-3′	Civeyrel & Rowe, 2001
	*mat*K 8F	5′-AATTTCAAATGGAAGAAATC-3′	Civeyrel & Rowe, 2001
	*mat*K 174R	5′-CGAKTAATTAAMCGTTTCAC-3′	Civeyrel & Rowe, 2001
	*mat*K 8F	5′-AATTTCAAATGGAAGAAATC-3′	Civeyrel & Rowe, 2001
	*mat*K 503R	5′-GCATCTTTTACCCAATAGCG-3′	Civeyrel & Rowe, 2001
	*mat*K 503F	5′-TCGCTATTGGGTAAAAGATGC-3′	Civeyrel & Rowe, 2001
	*mat*K 681F	5′-GTGAATACGAATCYATTTTC-3′	Civeyrel & Rowe, 2001
	*mat*K 900F	5′-TGGAAATTTTACCTTGTCAA-3′	Civeyrel & Rowe, 2001
	*mat*K 1628R	5′-CATGCTACATCAACATTTCAG-3′	Civeyrel & Rowe, 2001
	*mat*K 1309F	5′-GACTTTCTTGTGCTAGAACT-3′	Civeyrel & Rowe, 2001
	*trn*K 2R	5′-AACTAGTCGGATGGAGTA-3′	Civeyrel & Rowe, 2001

Sheahan & Chase, 2000; Whitten et al., 2000; Reeves et al., 2001). Because the trees generated from the individual data sets did not show any topological conflict when supported by bootstrap values greater than 75%, data partitions were then combined as follows: all molecular data sets combined (molecular combined) and all molecular data sets combined with morphology (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 (1) an initial round of tree searches with 1000 random addition sequence replicates (RASR), holding 10 trees at each step, and (2) tree bisection-reconnection (TBR) branch swapping with MULTREES and steepest descent in effect, saving a maximum of 50 trees at each replicate. All shortest trees retained in the 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 heuristic search with 500 replicates, 250 RASR with three trees held at each step, and TBR branch swapping with steepest descent and MULTREES in effect, saving 10 trees at each RASR.

Morphological characters were mapped onto the two most parsimonious trees resulting from the total evidence analysis using MacClade 4.0 (Maddison & Maddison, 2000) in order to identify the synapomorphies that are congruent with each of the major clades of *Mandevilla* retrieved in our analyses. Unambiguous changes were then reconstructed with maximum parsimony applying both accelerated (ACCTRAN) and delayed (DELTRAN) character state optimizations.

RESULTS

Amplification of the five selected loci was routine for most taxa. The two-round amplification of the *trn*K/ *mat*K locus was only partially successful for *Mande-*

Table 4. Summary of sequence characteristics and tree statistics for separate and combined data sets. Tree length, consistency index (CI), and retention index (RI) were calculated based on parsimony-informative characters only.

	*rpl*16 Intron	*rps*16 Intron	*trn*S^GCU-*trn*G^UUC Intergenic spacer	*trn*K Intron	*mat*K Gene	Molecular combined	Morphology	Total evidence
Aligned length	1554	931	1275	1110	1554	6344	32	6376
Range of sequence length	908–1092	789–830	437–801	903–1005	1497–1554	NA	NA	NA
No. of coded gaps	212	131	177	20	6	546	NA	546
No. of characters excluded (nucleotides + gaps)	62	21	63	19	NA	165	NA	165
Total no. of parsimony-informative characters (% of total no. of characters)	242 (13.7%)	127 (12%)	127 (8.8%)	149 (13.3%)	216 (13.9%)	858 (12.5%)	32 (100%)	893 (12.9%)
Tree length	460	283	305	273	430	1653	117	1796
CI	0.521	0.551	0.475	0.667	0.644	0.619	0.205	0.594
RI	0.777	0.825	0.756	0.862	0.865	0.848	0.412	0.836

villa anceps, *M. leptophylla*, and *Tintinnabularia mortonii* and failed completely for *M. krukovii*, *M. nerioides*, and *M. tricolor*.

Multiple sequence alignment for the *mat*K gene required only a few gaps that, without exception, occurred in multiples of three. Alignment was also unproblematic for the *trn*K and *rps*16 introns but proved to be somewhat more difficult for the *rpl*16 intron and *trn*S^GCU-*trn*G^UUC spacer due to the larger number of gaps and mononucleotide repeats. A total of 165 characters, including nucleotides and gaps, were thus excluded from further analyses of the noncoding loci, mainly from the *rpl*16 intron. Manual verification of the coded gap characters showed that Gapcoder performed well, even in cases of overlapping gaps with different start and/or ending positions, and no further adjustments in the matrices were necessary. More detailed information for the individual and combined data sets is given in Table 4.

PARSIMONY ANALYSES

Tree length, consistency index (CI), and retention index (RI) for the cladograms resulting from the analyses of the individual and combined data sets are summarized in Table 4. From the individual molecular data sets, the best-resolved cladogram was provided by the *mat*K gene, with most of the nodes receiving greater than 50% BS (Fig. 1). The highest proportion of parsimony informative characters, as well as the highest CI and RI values, were also provided by this data set. Of the other data sets, the *trn*K intron (Fig. 1) and *rpl*16 and *rps*16 introns (Fig. 2) had similar levels of resolution; the least resolved cladogram was provided by *trn*S^GCU-*trn*G^UUC intergenic spacer, with the lowest number of nodes supported by at least 50% BS (Fig. 3). Of these cladograms, only the *mat*K and *trn*S^GCU-*trn*G^UUC trees defined a clade containing all species of *Mandevilla*, *Macrosiphonia*, and *Telosiphonia* with BS higher than 50%. Because no strongly supported (> 75%) incongruent clades were found between individual partitions, all molecular data sets were combined. Their analyses yielded the tree shown in Figure 4.

Analysis of the morphological data set resulted in a poorly resolved cladogram with only a few groups supported by a BS value higher than 50% (Fig. 3). No incongruent clades with BS greater than 75% were 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 to form a total evidence data set.
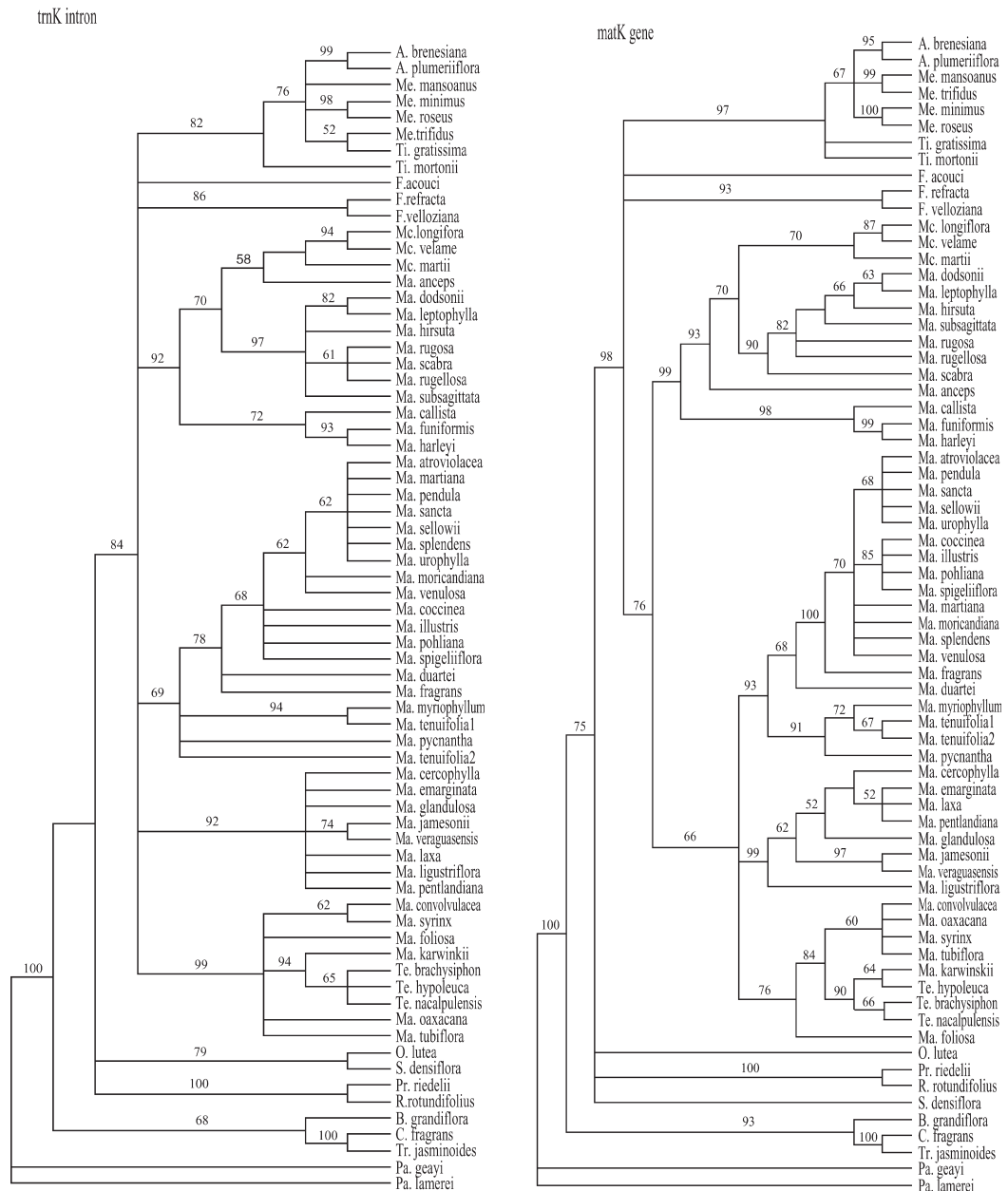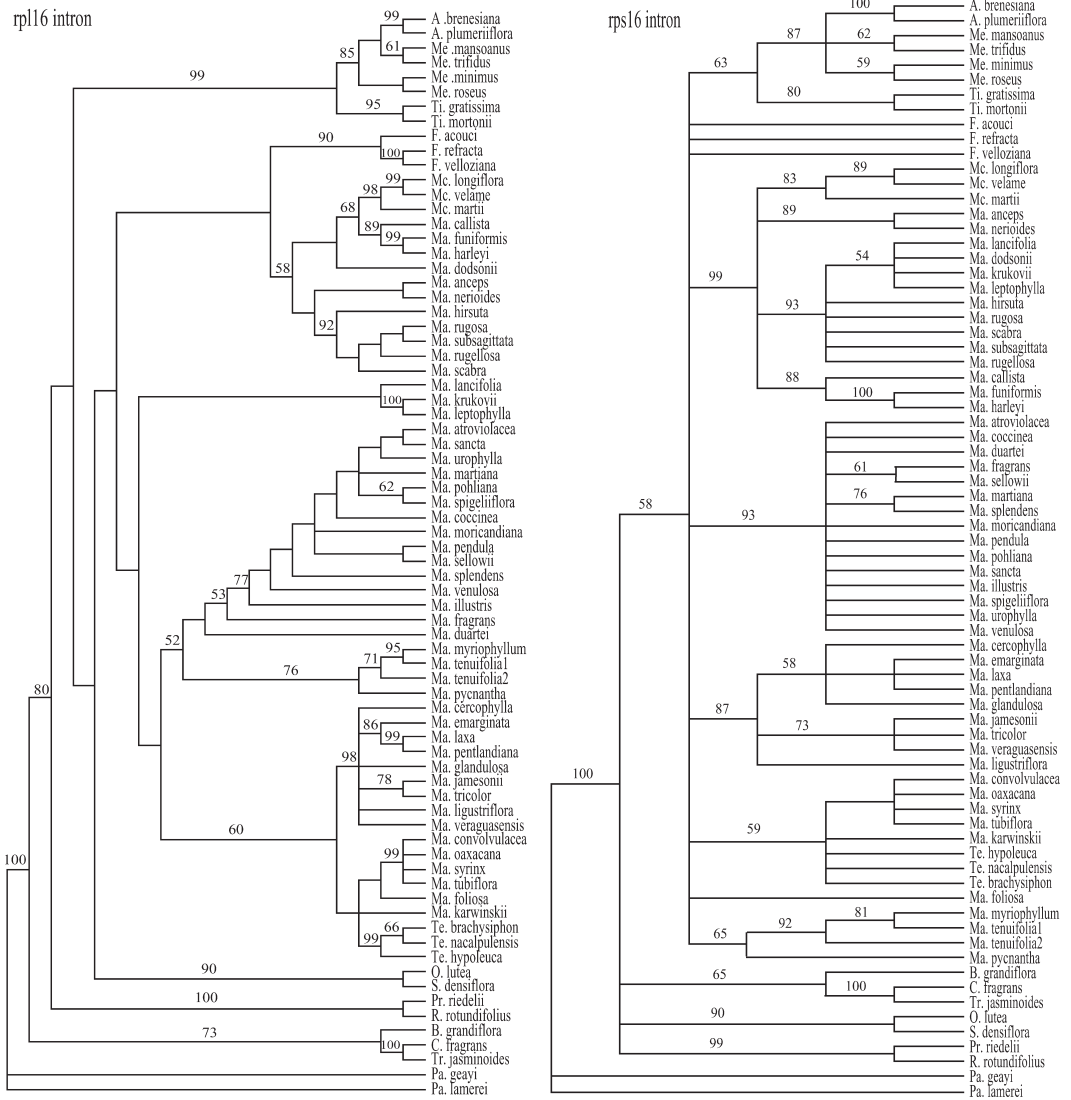
Figure 1. Strict consensus of the most parsimonious trees generated by the *trn*K intron and *mat*K gene data sets. Bootstrap values > 50% are indicated above the branches. Full taxon names are given in Appendix 1.

The total evidence tree (Fig. 5) contains a strongly supported clade (BS = 100%) including representatives of *Allomarkgrafia*, *Forsteronia*, *Macrosiphonia*, *Mandevilla*, *Mesechites*, and *Tintinnabularia* (the Mesechiteae clade). Within this clade, three strongly supported clades were recovered: (1) the *Mesechites* clade (BS = 100%), comprising *Allomarkgrafia*, *Mesechites*, and *Tintinnabularia*; (2) the *Forsteronia* clade (BS = 99%), formed by the three sampled

species of this genus; and (3) the *Mandevilla* clade (BS = 94%), comprising species of *Macrosiphonia*, *Mandevilla*, and *Telosiphonia*, a result that is consistent with our earlier findings (Simões et al., 2004).

Within the *Mandevilla* clade, two major, strongly supported clades (Fig. 5; BS = 100%) were recovered: (1) one formed by *Macrosiphonia*, 11 species of *Mandevilla* subg. *Exothostemon*, and three species

Figure 2. Strict consensus of the most parsimonious trees generated by the *rpl*16 intron and *rps*16 intron data sets. Bootstrap values > 50% are indicated above the branches. Full taxon names are given in Appendix 1.

of subgenus *Mandevilla* (*M. callista* Woodson, *M. funiformis* (Vell.) K. Schum., and *M. harleyi* M. F. Sales, Kin.-Gouv. & A. O. Simões), hereafter referred to as Clade I, and (2) another formed by *Telosiphonia* and all remaining species of *Mandevilla* sampled, hereafter referred to as Clade II.

Within Clade II, which is more morphologically diverse than Clade I and more extensively sampled in our study, two strongly supported clades were recovered: (1) a clade comprised of species of *Mandevilla* mostly from central to southern South America, hereafter referred to as Clade III, and (2) a clade consisting of *Telosiphonia* and species of *Mandevilla* with a wide range of distribution from

Mexico to southern South America, hereafter referred to as Clade IV. Clade IV can, in turn, be subdivided into two smaller clades: (1) Clade V, a heterogeneous assemblage composed of the South American species of *Mandevilla* with truncate anther bases, and (2) Clade VI, formed by *Telosiphonia* and all Mexican species of *Mandevilla*.

Mapping morphological characters onto the *Mandevilla* clade shows that Clades I, IV, and V are supported by unambiguous changes in character state (Fig. 6). In Clade I, opposite calycine colleters are derived from colleters with an alternate to continuous arrangement (character no. 15, Fig. 6), with a reversal to the ancestral state in the subclade formed by
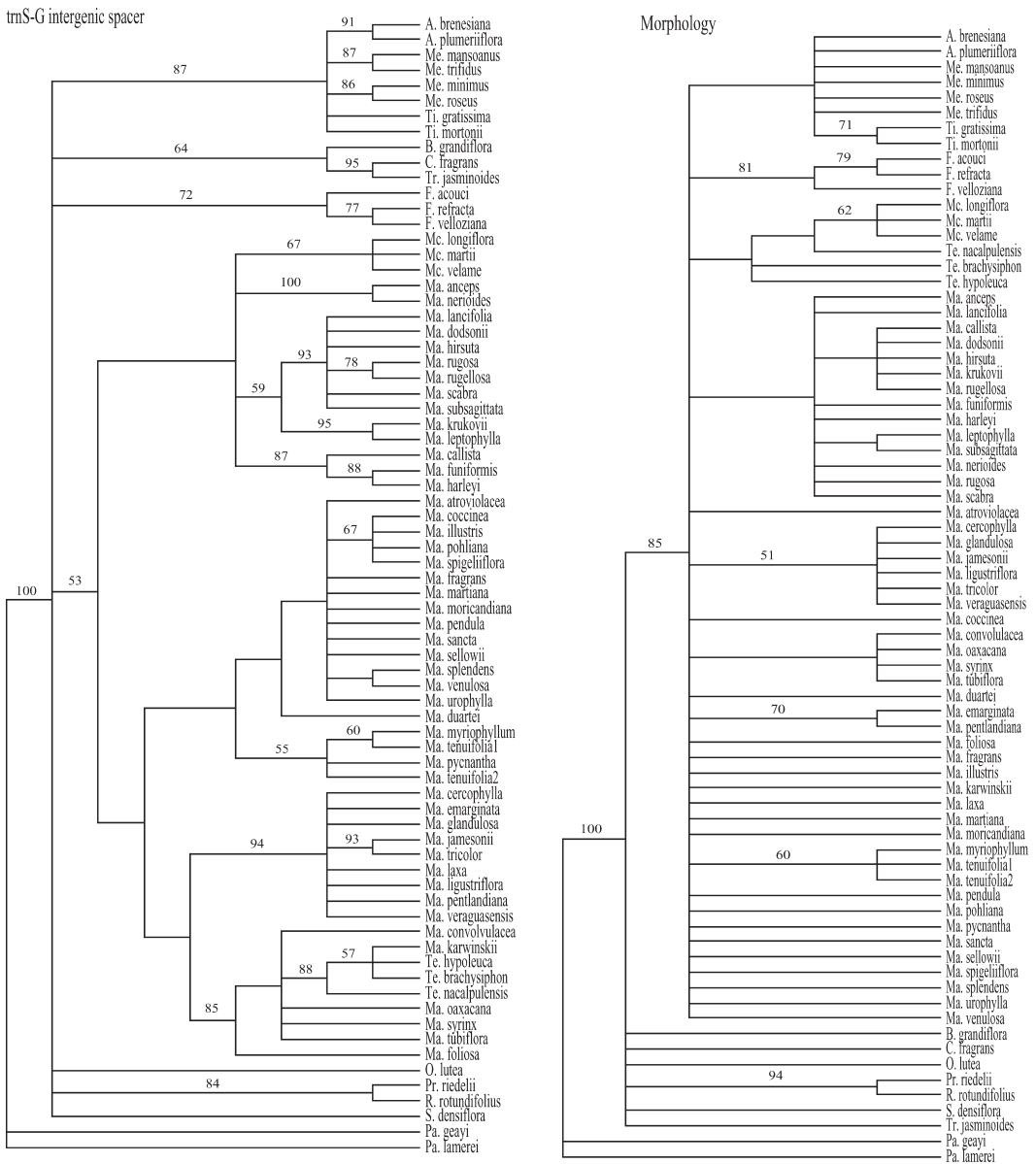
Figure 3.   Strict consensus of the most parsimonious trees generated by the *trn*S^GCU-*trn*G^UUC intergenic spacer and morphological data sets. Bootstrap values > 50% are indicated above the branches. Full taxon names are given in Appendix 1.

species of *Macrosiphonia*. In Clade IV, a shift from short to long style head appendages was noted on all terminal branches, except in the subclade formed by species of *Telosiphonia* (character no. 29, Fig. 6). In Clade V, anthers with a truncate base evolved unambiguously from the ancestral state of a cordate base (character no. 20, Fig. 6). No unequivocal morphological synapomorphies were found to support Clades II, III, and IV, as ACCTRAN and DELTRAN optimizations resulted in different reconstructions of character state

changes (Fig. 6). A more detailed explanation of morphological characters and changes of state is given in the discussion of each individual clade.

DISCUSSION

PHYLOGENETIC HYPOTHESIS AND CURRENT CLASSIFICATIONS

In our study, the clade formed by species of *Mandevilla*, *Macrosiphonia*, and *Telosiphonia* (*Mandevilla* clade) largely corresponds to the circumscrip-

Molecular combined
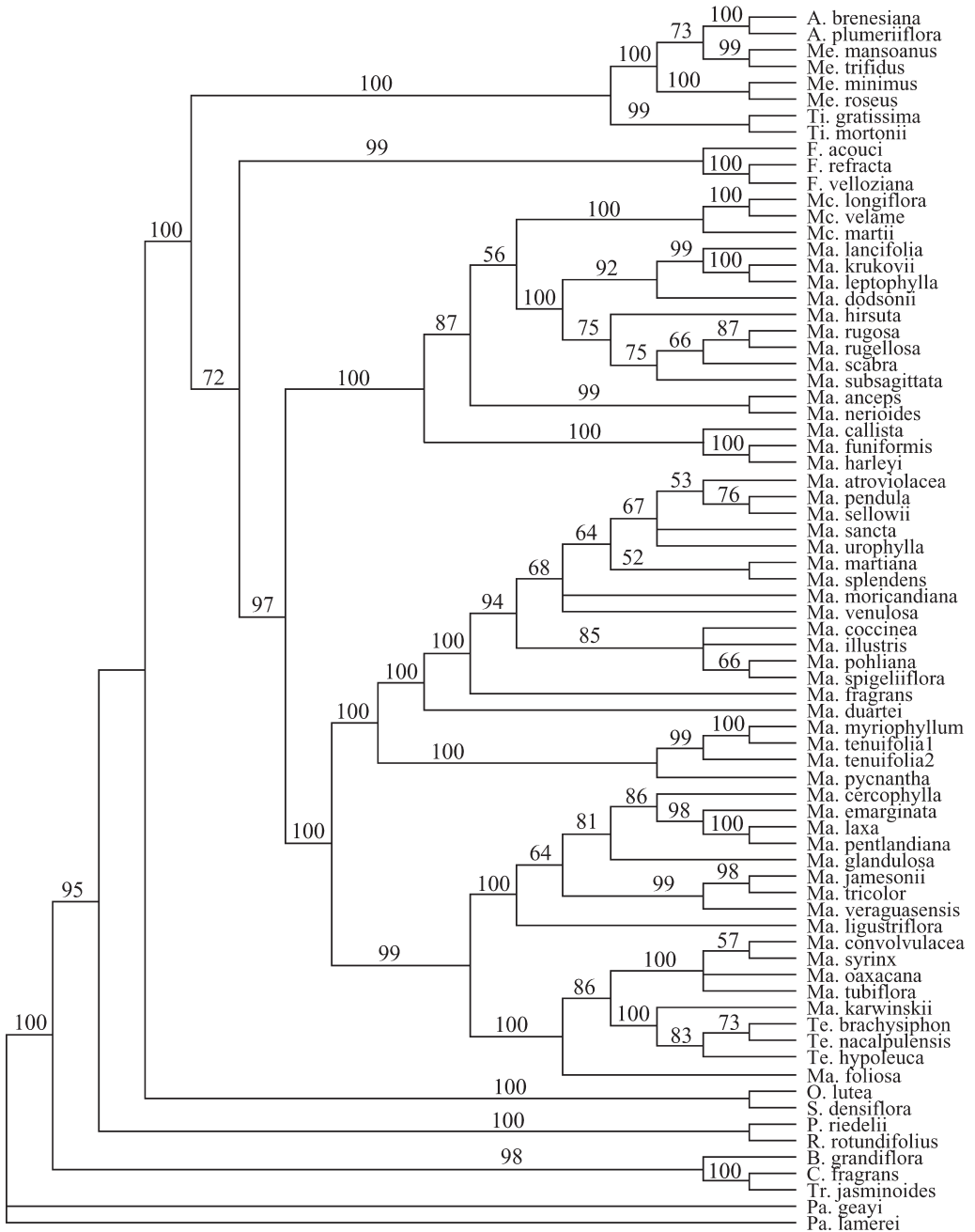
Figure 4.   Strict consensus of the most parsimonious trees generated by the molecular combined data set. Bootstrap values > 50% are indicated above the branches. Full taxon names are given in Appendix 1.

tion of *Mandevilla* proposed by Pichon (1948) but only partially corresponds to that of Woodson (1933). The main difference between the two classifications concerns the rank of *Macrosiphonia* and *Telosiphonia*. Woodson (1933) recognized *Macrosiphonia* as a dis-

tinct genus with two disjunct subgenera, subgenus *Macrosiphonia* in the Southern Hemisphere and subgenus *Telosiphonia* in the Northern Hemisphere. Pichon (1948), in contrast, included *Macrosiphonia* in the synonymy of *Mandevilla*. He cited a set of
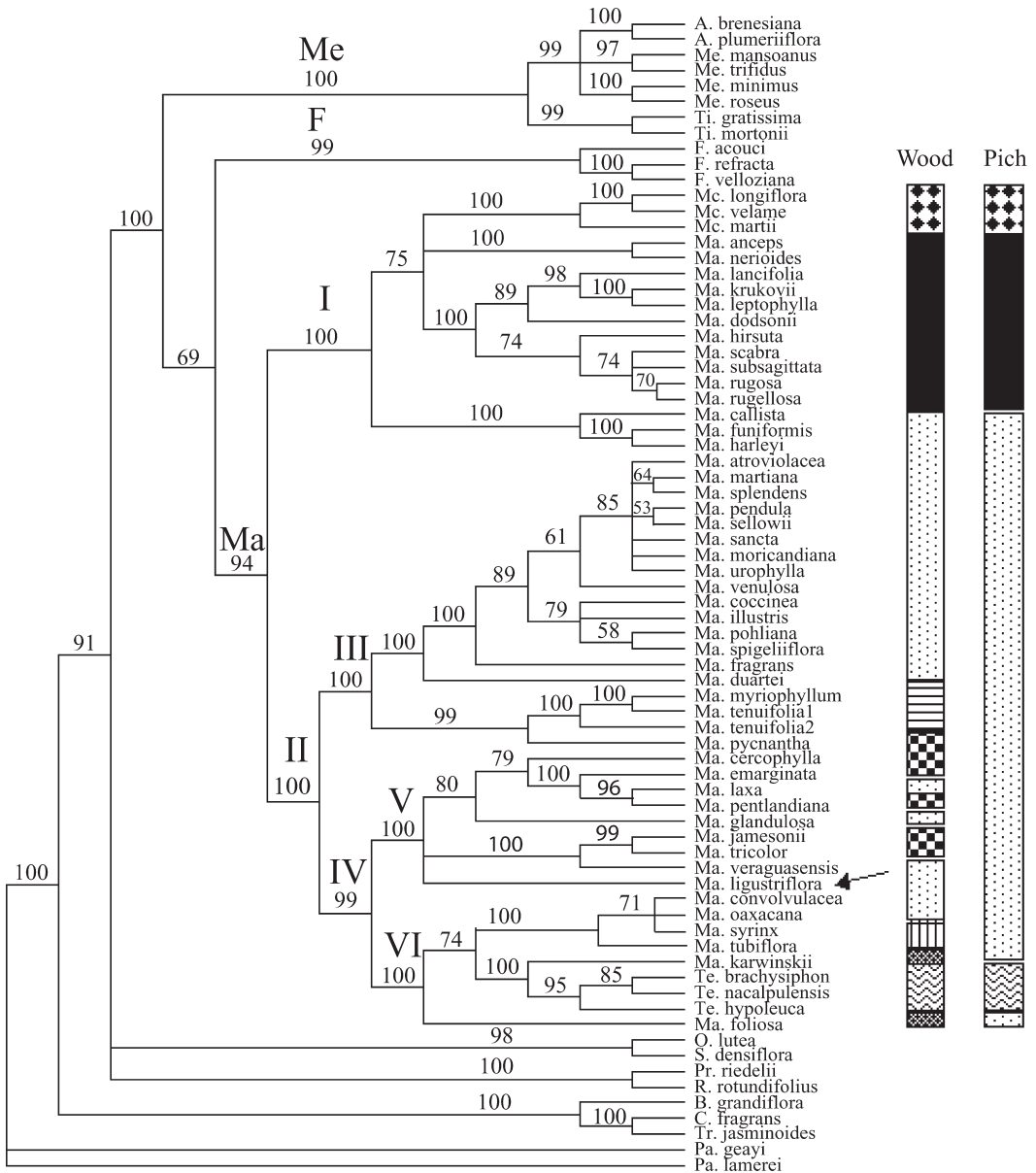
Figure 5.   Strict consensus of the most parsimonious trees generated by the total evidence data set. Bootstrap values > 50% are indicated above the branches. Ma = *Mandevilla* clade; Me = *Mesechites* clade; F = *Forsteronia* clade. The six

morphological characters to differentiate between Woodson's subgenus *Macrosiphonia* and subgenus *Telosiphonia* (presence vs. absence of a pedicel, structure of staminal filaments, and pollen size) and recognized them as two different sections of *Mandevilla*, section *Megasiphon* and section *Telosiphonia*, respectively. Our results suggest that *Telosiphonia* and *Macrosiphonia* are not closely related to each other, although both are clearly nested within *Mandevilla*, and confirm the preliminary results from our previous study (Simões et al., 2004).

Most of the infrageneric groups of *Mandevilla* proposed by Woodson (1933) are not monophyletic. The two subgenera he proposed, subgenus *Exothostemon* and subgenus *Mandevilla*, correspond for the most part to the two major clades within *Mandevilla* identified in our analyses, Clades I and II, respectively (Fig. 5). To render Woodson's subgenera monophyletic, the following new classifications must be made: (1) *Macrosiphonia* must be included in subgenus *Exothostemon*, (2) *Telosiphonia* must be included in subgenus *Mandevilla*, and (3) *Mandevilla callista*, *M. funiformis*, and *M. harleyi* must be transferred from subgenus *Mandevilla* to subgenus *Exothostemon*. Of the five sections of subgenus *Mandevilla* proposed by Woodson (1933), only the smallest, section *Tenuifoliae*, containing two species, constitutes a monophyletic group (BS = 100%) in our study. All of the other sections are polyphyletic, with their constituent taxa scattered throughout the *Mandevilla* clade (Fig. 5). The most extreme case of polyphyly is found in Woodson's section *Laxae*, the largest of subgenus *Mandevilla*, which he characterized by having infundibuliform corollas. In our study, the 24 species sampled from this section are scattered among all larger subclades of the *Mandevilla* clade (Fig. 5, Table 2).

With regard to the infrageneric ranks proposed by Pichon (1948), our results support the monophyly of two of his sections, namely, *Megasiphon* and *Telosiphonia*. His other sections, *Orthocaulon* and *Exothostemon*, correspond to Woodson's subgenus *Mandevilla* and subgenus *Exothostemon*, respectively, and do not constitute monophyletic groups as indicated above. Despite their strongly supported monophyly, recognition of sections *Megasiphon* and *Telosiphonia* is untenable both taxonomically and morphologically, due to the considerable number of additional sections

without morphological synapomorphies that would need to be recognized in *Mandevilla*. The same justification can be applied for not recognizing Woodson's section *Tenuifoliae*, despite its monophyly.

After a detailed examination of herbarium vouchers and phototypes, we have concluded that *Mandevilla ligustriflora* Woodson and *Quiotania colombiana* Zarucchi are conspecific. As *Q. colombiana* is the only described species of the genus and *M. ligustriflora* is nested within Clade IV with a strong bootstrap support (see Fig. 5), *Quiotania* cannot be recognized as a valid genus and should, therefore, be included in the synonymy of *Mandevilla*. The required nomenclatural changes have been undertaken in a separate paper (Simões et al, 2007).

CLADE I

Clade I contains representatives from three disparate taxonomic groups of Woodson's (1933) classification. Of the 17 species included in this clade, the majority (11) belong to Woodson's subgenus *Exothostemon*. All 11 sampled species of *Exothostemon* in our study are within this clade. Of the six remaining taxa, three (*Macrosiphonia longiflora* (Desf.) Müll. Arg., *M. martii* Müll. Arg., and *M. velame* (A. St.-Hil.) Müll. Arg.) belong to Woodson's genus *Macrosiphonia*, and the other three (*Mandevilla callista*, *M. funiformis*, and *M. harleyi*) fall under the circumscription of his subgenus *Mandevilla*. Clade I is characterized by one morphological synapomorphy: the calycine colleters have an opposite arrangement in relation to the calyx lobes (character no. 15, Fig. 6; see Appendix 3).

Subgenus *Exothostemon* forms a morphologically distinctive group within *Mandevilla*. Flower structure is quite homogeneous, with the presence of three character states considered by both Woodson (1933) and Pichon (1948) as diagnostic for the group: (1) leaf surface with many colleters distributed along the midrib on the adaxial surface; (2) opposite calycine colleters; and (3) corolla lower tube more or less gibbous or arcuate. Variation in vegetative characters and geographic distribution is, however, remarkable in the subgenus, and groups within Clade I can be discerned based on morphology. The first group, represented in our study by eight species (*M. dodsonii* A. H. Gentry, *M. hirsuta* (Rich.) K. Schum., *M. krukovii*, *M. leptophylla*, *M. rugellosa* (Rich.) L. Allorge, *M. rugosa* (Benth.) Woodson, *M. scabra*

←

subclades within the *Mandevilla* clade are indicated as I, II, III, IV, V, and VI. The arrow indicates the position of *Mandevilla ligustriflora*, which is conspecific with *Quiotania colombiana*. A comparison between the classifications of Woodson (1933) and Pichon (1948) is illustrated in the two columns on the right of the cladogram. Each pattern of the columns is associated to its corresponding taxonomic rank in Woodson's (W) and Pichon's (P) classification. Full taxon names are given in Appendix 1.
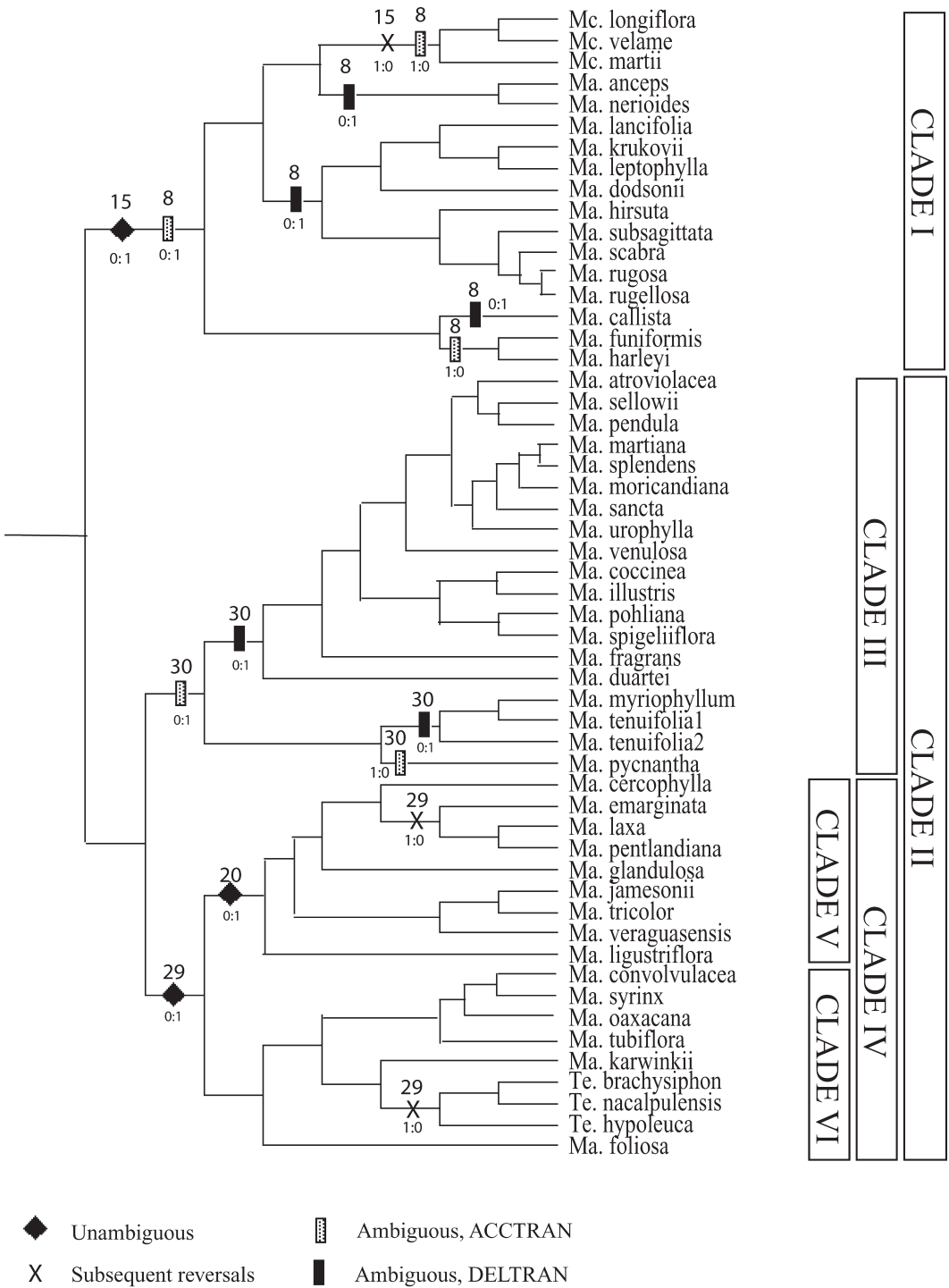
Figure 6.   One of the two most parsimonious total evidence trees showing optimized morphological character-state changes within the *Mandevilla* clade. Optimizations were identical in both trees. Diamonds show unambiguously optimized character-state changes diagnostic for clades IV and V, with subsequent reversals indicated by an X. Bars denote ambiguously optimized character-state changes. Character-state changes using DELTRAN optimization are indicated by solid bars; character-state changes using ACCTRAN optimization are indicated by dotted bars. All numbers above the symbols correspond to the character number as indicated in Appendix 3. The directions of character-state changes are reported below the symbols.

(Hoffmanns. ex Roem. & Schult.) K. Schum., and *M. subsagittata* (Ruiz & Pav.) Woodson), is composed of taxa that show the most common morphological pattern in the subgenus: vines with terete stems and yellow flowers, often with a red center (white flowers in *M. rugosa*), that occur mainly in forests and their bordering zones throughout the Neotropics. The second group, represented in our study by three species (*M. anceps*, *M. lancifolia*, and *M. nerioides*), is composed of taxa that have a unique set of characters within the subgenus: they are shrubs or woody lianas with strongly angled to winged stems (tetragonal in cross section) and flowers of various colors that are found mainly in the open habitats of white sand savannas and tepuis of northern South America. Neither of these two groups is monophyletic, however. All species from the first group form a strongly supported clade together with one species from the second group, *M. lancifolia*. The two remaining species of the second group, *M. anceps* and *M. nerioides*, form a strongly supported clade (BS = 100%), but this clade's relationship to the remaining species of Clade I is not resolved in our analysis. Therefore, no further conclusions on relationships and patterns of evolution within *Exothostemon* can be drawn at this time. Broader taxon sampling, especially including representatives from the poorly collected species of the winged-stem group, is needed to address these questions.

The inclusion of three species from *Mandevilla* subg. *Mandevilla* (*M. callista*, *M. funiformis*, and *M. harleyi*) in Clade I is central to understanding character evolution within the genus, because they possess characteristics of both of Woodson's (1933) subgenera. According to Woodson, *M. funiformis* has five calycine colleters in an opposite arrangement, as is characteristic for subgenus *Exothostemon*, but this species also has leaf colleters restricted to the base of the midrib, a key character of subgenus *Mandevilla*. Conversely, in *M. callista*, Woodson noted that leaf colleters are spread along the length of the midrib, as is characteristic for taxa in subgenus *Exothostemon*, while calycine colleters form a continuous ring. Woodson (1933) recognized this "intermediate" status of *M. callista* and *M. funiformis* but justified their inclusion in subgenus *Mandevilla* based on the presence of continuous calycine colleters in the former and leaf colleters restricted to the base of the

midrib in the latter. We examined vouchers of the three species in order to compare morphological variation with the taxonomic descriptions provided by Woodson (1933) and Sales et al. (2006). We found that specimens of *M. funiformis* have leaf colleters restricted to the base of the midrib and calycine colleters in an opposite arrangement, confirming Woodson's observations. The same set of characters was also seen in specimens of *M. harleyi*. Our observations for *M. callista*, however, do not agree completely with Woodson's original description. We found that leaf colleters are spread along the entire midrib, but, in the specimens we studied, calycine colleters had the opposite arrangement typical for subgenus *Exothostemon*.

The well-supported inclusion of representatives of *Macrosiphonia* in Clade I is surprising and somewhat unexpected from a morphological standpoint. In *Macrosiphonia*, the leaf blade is covered by white woolly trichomes abaxially, the leaf colleters are restricted to the adaxial base of the midrib, the flowers lack a pedicel, and the calycine colleters are arranged in a continuous ring (Woodson, 1933; Ezcurra et al., 1992; Henrickson, 1996). The other species in Clade I, in contrast, have glabrous to tomentose (but never woolly) leaves with colleters spread along the midrib (except in *Mandevilla funiformis* and *M. harleyi*, where the colleters are restricted to the base), pedicellate flowers, and calycine colleters with an opposite arrangement. Increased taxon sampling and additional studies focused on features that have scarcely been addressed previously in *Mandevilla* and *Macrosiphonia* (e.g., palynology, floral ontogeny, and anatomy) could provide useful information to support relationships within this clade.

The arrangement of leaf and calycine colleters are key characters to understanding phylogenetic relationships and morphological evolution in *Mandevilla*. Colleters distributed along the entire length of the midrib were observed in species of Clade I. This character state is unique and has never been reported in any other group within Apocynaceae. Calycine colleters with opposite arrangement were also observed only in species of Clade I within the *Mandevilla* clade, but the same state has been reported in other groups of Apocynaceae, as in the Neotropical genera of tribe Echiteae (e.g., *Thenardia* HBK, *Prestonia* R. Br., and *Temnadenia* Miers)

←

(Pichon, 1950; Ezcurra et al., 1992; Simões & Kinoshita, 2002). Opposite calycine colleters were unambiguously reconstructed as the ancestral state of Clade I (character no. 15, Fig. 6), but two equally parsimonious hypotheses could explain the evolution of leaf colleters in this clade (character no. 8, Fig. 6). Using ACCTRAN optimization, the presence of colleters distributed along the midrib represents a synapomorphy for Clade I, with two subsequent reversals to colleters clustered at the leaf base in both species of *Macrosiphonia* and in the *Mandevilla funiformis/M. harleyi* subclade. DELTRAN optimization, in contrast, suggests three parallel origins of leaf colleters distributed along the midrib: in *Mandevilla callista*, in the *M. anceps/M. nerioides* subclade, and in the largest subclade of Clade I. Given the unique status of this feature in Apocynaceae and its occurrence only in species of Clade I, a single origin of this state seems more likely than three parallel changes in character state, in which case it would represent another synapomorphy for Clade I. No further conclusions can be drawn from our results. Future studies focusing on the morphology and ontogeny of leaf colleters in *Mandevilla* could help to clarify the evolution of this character in the genus.

CLADE II

This clade, which comprises *Telosiphonia* and the majority of *Mandevilla* species, corresponds to Pichon's (1948) sections *Orthocaulon* and *Telosiphonia*, and for the main part to Woodson's (1933) subgenus *Mandevilla* and subgenus *Telosiphonia*. From a morphological standpoint, Clade II spans almost the entire spectrum of morphological variation found in *Mandevilla*, from subshrubs with large, showy, lilac to pink infundibuliform flowers, as in *M. sancta* (Stadelm.) Woodson, to vines with small, inconspicuous, white tubular flowers, as in *M. ligustriflora*. This clade is also represented throughout the entire geographic range of *Mandevilla*, from the southwestern United States and Mexico to subtropical Argentina.

All species from this clade share two morphological character states: leaf colleters restricted to the base of the midrib and calycine colleters with an arrangement that varies from alternate to continuous. These are, however, plesiomorphic states within the *Mandevilla* clade and therefore cannot be recognized as synapomorphies of Clade II. Simões et al. (2004) showed that colleters restricted to the leaf base is one of the four morphological synapomorphies that characterize the tribe Mesechiteae, and calycine colleters with alternate to continuous arrangement are found in the two

other clades of Mesechiteae (*Mesechites* and *Forsteronia* clades), as well as in some outgroup taxa.

CLADE III

This clade is primarily composed of species of *Mandevilla* occurring in forests, savannas, and campo rupestre formations of northeastern to southern Brazil, also reaching Paraguay and Argentina. Most of these species belong to Woodson's (1933) section *Laxae*, with the exception of *M. myriophyllum* and *M. tenuifolia*, both ascribed to his section *Tenuifoliae*, and *M. pycnantha* (Steud. ex A. DC.) Woodson, attributed to section *Montanae*. With the exception of *M. pycnantha*, all species in this clade share one morphological character state: the presence of only two nectaries alternate to the carpels.

Other morphological characters, however, are more variable within this clade, in both vegetative and reproductive parts. Some species, such as *Mandevilla pendula* (Ule) Woodson and *M. urophylla* (Hook. f.) Woodson, are vines from the Atlantic Rainforest in southwestern Brazil, but others, including *M. illustris* (Vell.) Woodson, *M. pohliana* (Stadelm.) A. H. Gentry, and *M. spigeliiflora* (Stadelm.) Woodson, are small, unbranched subshrubs of savannas and campo rupestre formations from central and southern South America. Branched, woody shrubs are also common, with some species, such as *M. duartei* Markgr. and *M. venulosa* (Müll. Arg.) Woodson, endemic to specific mountain formations of southwestern Brazil. Their flowers are showy and variously colored and, in most cases, have an infundibuliform corolla. Woodson (1933) used corolla shape as a diagnostic character and defined his entire section *Laxae* according to the shared occurrence of infundibuliform corollas among its members. Even though species with an infundibuliform corolla comprise a strongly supported subclade (BS = 100%) within Clade III, this character state clearly appears to have arisen independently multiple times within *Mandevilla*, undermining its taxonomic utility.

The number of floral nectaries is an easily defined character, with no intermediate states. Most members of Clade III, with the exception of *Mandevilla pycnantha*, are characterized by the presence of two nectaries in the flower (character no. 30, Fig. 6). This state could be considered as a synapomorphy for Clade III, with a later reversal to five nectaries in *M. pycnantha*. However, an equally parsimonious reconstruction would involve a switch from five to two nectaries occurring independently twice: once in the clade composed by *M. myriophyllum* and *M. tenuifolia*, and again in the clade composed by the remaining species of Clade III. Given that the occurrence of two
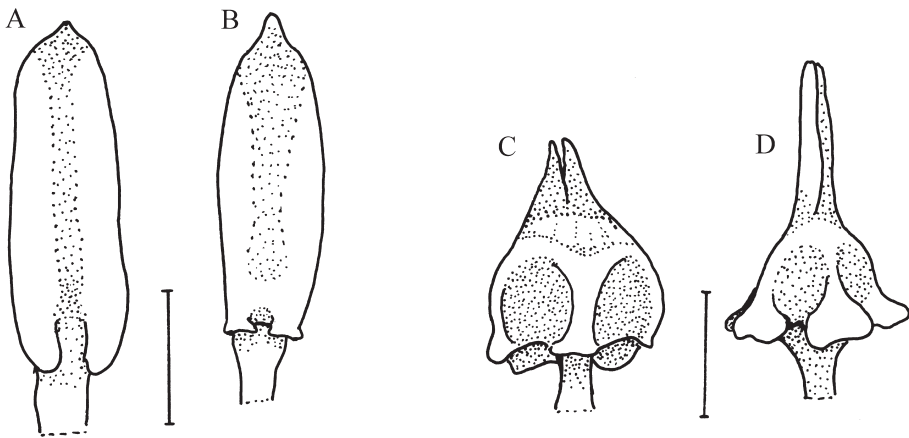
Figure 7. Diagrammatic drawings of the anther base form and style head apical appendages in *Mandevilla*. —A. *M. tenuifolia*, anther base cordate. —B. *M. veraguasensis*, anther base truncate. —C. *M. tenuifolia*, short style head appendages. —D. *M. syrinx*, long style head appendages. Scale bar = 1 mm.

nectaries is restricted to species of Clade III within *Mandevilla* and the relatively small number of taxa that exhibit this character state in Apocynaceae, the first hypothesis, of a single switch from five to two nectaries, seems more likely than two parallel changes within Clade III. Further studies focusing on the structure and development of floral nectaries in Apocynaceae, however, are needed to test these alternative hypotheses.

CLADE IV

This clade comprises a heterogeneous group of 15 species of *Mandevilla* from Woodson's (1933) sections *Laxae*, *Montaneae*, *Torosae*, and *Tubiflorae*, plus the three sampled species of *Telosiphonia*, with members distributed mainly from Mexico and the southwestern United States to northern South America, but also reaching southern Brazil and Argentina.

A morphological synapomorphy for Clade IV is the presence of elongate apical appendages of the style head that are the same size or longer than its main body (character no. 29, Fig. 6; Fig. 7). These are found in all species, with the exception of two subclades: the one formed by the three sampled species of *Telosiphonia* and the one comprising *Mandevilla emarginata* (Vell.) C. Ezcurra, *M. laxa* (Ruiz & Pav.) Woodson, and *M. pentlandiana* (A. DC.) Woodson. Two equally parsimonious reconstructions of ancestral states are possible for this character (Fig. 6). In one optimization, elongate apical appendages evolved in the ancestor of Clade IV and were independently lost in the two subclades mentioned above. In the alternative optimization, the evolution of elongate apical appendages in the ancestor of Clade V was followed by a single reversal to short appendages

in the clade comprising *M. emarginata*, *M. laxa*, and *M. pentlandiana*.

CLADE V

This clade is mainly composed of species from Woodson's (1933) *Mandevilla* section *Montanae*, but three species (*M. glandulosa* (Ruiz & Pav.) Woodson, *M. laxa*, and *M. veraguasensis* (Seem.) Hemsl.) were assigned to his section *Laxae*. Most are vines, with the exception of *M. emarginata*, an unbranched subshrub, and *M. pentlandiana*, which has both vine and shrub forms. Flowers in this clade are generally salverform or tubular, white to greenish, but *M. veraguasensis*, *M. glandulosa*, and *M. laxa* have showy, infundibuliform to campanulate corollas. One morphological feature, found nowhere else in the *Mandevilla* clade, was unambiguously reconstructed as a synapomorphy of this clade: the anther base is truncate, with no discernible auricles or protruding extensions (character no. 20, Fig. 6; Fig. 7). In *M. emarginata*, *M. laxa*, and *M. pentlandiana*, auriculate anther bases can occasionally be found in some individuals, but in most cases the base is truncate. The presence of truncate anthers was used by Woodson (1933) to distinguish his section *Montanae*, although *M. pycnantha* has conspicuously auriculate anthers. Interestingly, our parsimony analyses did not support the inclusion of *M. pycnantha* in Clade V, while they firmly placed *M. glandulosa*, *M. laxa*, and *M. veraguasensis* in it. These latter species, included by Woodson (1933) in section *Laxae*, are also characterized by truncate anthers.

A distinctive aspect of Clade V is its geographical distribution. In contrast to Clade VI, which is restricted to a single region, two geographically disjunct groups can be distinguished in Clade V.

The majority of its species are found in the forests of Central America and northwestern South America. Three species (*M. emarginata*, *M. laxa*, and *M. pentlandiana*), however, occur in the Atlantic Rainforest in southern and southeastern Brazil, as well as in more arid habitats from southern Bolivia and Peru to Paraguay, Uruguay, and Argentina, with *M. emarginata* also reaching the savannas of central Brazil. The fragmentary, circum-Amazonian distribution found in this clade has also been reported in other Neotropical plant groups (e.g., Plowman, 1979, in *Brunfelsia*, Solanaceae) and might be related to the climatic fluctuations of the Quaternary, as well as the geologic history of the Andes during the Pliocene/Pleistocene.

From the Paleocene to the Miocene, the continuous occurrence of everwet climates in South America is thought to have resulted in the spread of tropical rain forests across the continent, forming a continuous belt from the Atlantic to the Pacific coast (Morley, 2000). A general cooling and/or overall reduction of precipitation on the continent during the Late Miocene and Early Pliocene resulted in the retraction of forested areas and expansion of savannas (Prance, 1982; Morley, 2000). With the simultaneous climatic fluctuations and the major uplift of the northern Andes (van der Hammen, 1974; Flenley, 1979; Morley, 2000) during the Late Tertiary and Early Quaternary and the subsequent expansion of rain forest through the Amazon basin, the northwestern part of South America became isolated from the central and southern parts of the continent. Thus the geologic and climatic history of South America during the Cenozoic could explain the close phylogenetic relationships among greatly disjunct species observed in Clade V (see Fig. 5).

CLADE VI

This clade is composed of species from Woodson's *Mandevilla* sections *Laxae* (*M. convolvulacea* (A. DC.) Hemsl., *M. oaxacana* (A. DC.) Hemsl.), *Torosae* (*M. foliosa* (Müll. Arg.) Hemsl., *M. karwinskii* (Müll. Arg.) Hemsl.), and *Tubiflorae* (*M. syrinx* Woodson, *M. tubiflora* (M. Martens & Galeotti) Woodson), plus genus *Telosiphonia*, all of which occur in deserts and dry forests of Mexico and the southwestern United States. Morphological traits in this clade are extremely variable, especially those related to flower structure. *Mandevilla syrinx* and *M. tubiflora* have many-flowered inflorescences bearing small, tubular, white flowers, whereas *M. convolvulacea* and *M. oaxacana* have few-flowered inflorescences with showy, yellow, infundibuliform flowers. The most striking floral morphology of this clade is found in *Telosiphonia*,

characterized by long, narrowly tubular, white flowers forming 1- to few-flowered inflorescences.

Although species of Woodson's (1933) sections *Torosae* and *Tubiflorae* are restricted to this clade, they do not form monophyletic groups, and thus their continued recognition might be questionable. The distinction between these two sections is based on nectary height: in section *Torosae*, the nectaries are shorter than the ovary, whereas in section *Tubiflorae* they are the same size or taller than the ovary. We observed that species of section *Torosae* always have nectaries taller than the ovary, but the same condition occurs in three other species from this clade, all of which belong to different sections sensu Woodson (1933): *Mandevilla foliosa* (sect. *Torosae*), and *M. convolvulacea* and *M. oaxacana* (both in sect. *Laxae*). Nectaries shorter than the ovary are found in all *Telosiphonia* species and in *M. karwinskii*, which together form a strongly supported subclade (BS = 100%).

The sister relationship between *Mandevilla karwinskii* and species of *Telosiphonia*, which has never been proposed before, is congruent with their geographic distribution and habitat preferences. Both taxa are rhizomatous shrubs occurring sympatrically in the deserts of Mexico and the southwestern United States. Apart from their short nectaries, morphological traits are quite different between *M. karwinskii* and species of *Telosiphonia*, especially leaf indument, flower size, and style head structure.

The striking similarity in morphology between species of *Telosiphonia* and *Macrosiphonia* is the most extreme example of parallel evolution in the *Mandevilla* clade. The two genera, each of which comprises a well-supported subclade nested within the *Mandevilla* clade (*Macrosiphonia* in Clade I and *Telosiphonia* in Clade VI, see Fig. 5), occur in disjunct geographic areas that roughly coincide with the extreme northern and southern range of *Mandevilla*. *Macrosiphonia* is found in the savannas of southern South America in arid, usually sandy cerrado and campo rupestre vegetation from southern Bolivia and Peru to central Brazil, Paraguay, Uruguay, and Argentina, whereas *Telosiphonia* is restricted to the arid zones of Mexico and the southwestern United States. Despite their geographic disjunction, the two genera share a suite of morphological characteristics. Both are erect shrubs or subshrubs, sometimes rhizomatous, with a well-developed underground storage system and leaves covered by a dense, wooly indument on the abaxial surface. The most remarkable similarities, however, are related to flower structure. In both genera, flowers are white and tubular, with some of the longest corolla tubes in Apocynaceae, reaching up to 17 cm in *Macrosiphonia longiflora*,

and are only fully open at dusk, when they produce a distinctive scent, suggesting pollination by hawk-moths.

The apparent parallelism in vegetative characters observed in these two groups could be explained as an adaptation to similar environmental conditions. A shrubby, erect habit, the presence of a dense indu-ment covering both vegetative and reproductive organs, and well-developed underground storage organs such as tubers and xylopods are common traits of plants of open, seasonally dry habitats (Rizzini, 1997; Dallman, 1998). On the other hand, parallelism in floral structure is more likely driven by pollinator preferences. The distinctive features shared by *Macrosiphonia* and *Telosiphonia* are typical of the sphingophilous (hawkmoth) pollination syndrome reported by many authors (e.g., Vogel, 1954; Faegri & van der Pijl, 1966; Baker & Hurd, 1968; Endress, 1994; Galetto, 1997). Reports of hawkmoths visiting flowers of various *Telosiphonia* and *Macrosiphonia* species are congruent with the hypothesis of hawk-moth pollination. For example, two hawkmoth species, *Manduca sexta* L. and *Hyles lineata* Fabricius, have been observed visiting *Telosiphonia nacapulensis* Felger & Henr. and *T. brachysiphon* (Torr.) Henr. in the Sonoran Desert of southern Arizona (R. Raguso, pers. comm., 2004). In Cordoba Province and in the El Palmar National Park, Entre Ríos Province, Argen-tina, *Manduca sexta*, *M. rustica* Fabricius, and *Agrius cingulata* Fabricius have been found carrying pollen of *Macrosiphonia petraea* (A. St.-Hil.) K. Schum attached only to the very tips of their proboscis (A. Cocucci, pers. comm., 2004). Given the remarkable length of the floral tube of this plant (ca. 105 mm), it is reasonable to expect that only insects with a very long proboscis could reach the nectar (Marcela Moré, in prep.).

CONCLUSIONS

The phylogenetic results presented here show that *Mandevilla*, as circumscribed by Pichon (1948), is monophyletic, but Woodson's (1933) circumscription of the genus is paraphyletic. *Quiotania*, *Macrosipho-nia*, and *Telosiphonia* are nested within *Mandevilla* and therefore should be included in its synonymy. Representatives from *Macrosiphonia* and *Telosiphonia* form distinct clades embedded within *Mandevilla*, and their striking morphological similarities may have evolved in parallel, possibly as a result of similar selective pressures driven by colonization of open, dry habitats and hawkmoth pollination.

All infrageneric taxa within *Mandevilla* proposed by both Woodson (1933) and Pichon (1948) were found to be paraphyletic or polyphyletic, with the

exception of Woodson's section *Tenuifoliae* and Pichon's sections *Megasiphon* and *Telosiphonia*. Recognition of these three sections is, however, untenable for the moment, as this would require recognizing additional sections that lack clear mor-phological synapomorphies. Six major clades were recognized within *Mandevilla* in our study, although only three have unambiguous morphological synapo-morphies. It is hoped that more detailed morpholog-ical studies in *Mandevilla* could uncover additional characters that might prove useful for delimitation within this group. Until such evidence becomes available, we think it is most prudent to withhold from erecting a new intrageneric classification of *Mandevilla*.

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Appendix 1. Voucher information and Genbank accession numbers for the taxa used in this paper. Additional vouchers selected for morphological analyses are indicated by an asterisk (*).

Species	Voucher/citation	*rpl*16 Intron	*rps*16 Intron	*trn*S^{GCU}-*trn*G^{UUC} Intergenic spacer	*trn*K Intron	*mat*K Gene
Allomarkgrafia brenesiana Woodson	Costa Rica, *Endress 97-06* (Z)	AY597546	AY597580	AY597614	DQ522660	DQ522591
Allomarkgrafia plumeriiflora Woodson	Costa Rica, *Morales 9338* (INB)	DQ522730	DQ522812	DQ522771	DQ522661	DQ522592
Beaumontia grandiflora Wall.	Cultivated, Bot. Gard. Munich, *G. Gerlach 5/06* (M); *India, Feb. 1909, *coll. Native collector s.n.* (Z)	AY597547	AY597581	AY597615	DQ522662	Z98174
Chonemorpha fragrans (Moon) Alston	Cultivated, Queensland, Australia, *Forster 2009* (BRI); * India, *Ridsale 109* (Z)	AY597548	AY597582	AY597616	DQ522663	DQ522593
Forsteronia acouci (Aubl.) A. DC.	French Guiana, *Prévost 3720* (CAY); * Peru, *Revilla 291* (Z); * Venezuela, *Breteler 5029* (Z)	AY597549	AY597583	AY597617	DQ522664	DQ522594
Forsteronia refracta Müll. Arg.	Brazil, *Yamamoto 02/108* (UEC)	DQ522731	DQ522813	DQ522772	DQ522665	DQ522595
Forsteronia velloziana (A. DC.) Woodson	Brazil, *Simões 343* (UEC)	AY597550	AY597584	AY597618	DQ522666	DQ522596
Macrosiphonia longiflora (Desf.) Müll. Arg.	Brazil, *Schütz Rodrigues 1227* (UEC); * Brazil, *Simões 47, 859, 930* (UEC)	AY597551	AY597585	AY597619	DQ522667	DQ522597
Macrosiphonia martii Müll. Arg.	Brazil, *Simões 1245* (UEC); * Brazil, *Duarte 2445* (RB); * Brazil, *Simões 1205, 1206* (UEC)	AY597552	AY597586	AY597620	DQ522668	DQ522598
Macrosiphonia velame (A. St.-Hil.) Müll. Arg.	Brazil, *Simões 1199* (UEC); * Brazil, *Leitão-Filho 15307* (UEC); * Brazil, *Kinoshita 2000/67* (UEC)	DQ522732	DQ522814	DQ522773	DQ522669	DQ522599
Mandevilla anceps Woodson	Venezuela, *Huber & Medina 5793* (Z)	DQ522733	DQ522815	DQ522774	DQ522670	DQ522600
Mandevilla atroviolacea (Stadelm.) Woodson	Brazil, *Meireles 1290* (UEC)	DQ522734	DQ522816	DQ522775	DQ522671	DQ522601
Mandevilla callista Woodson	Ecuador, *Webster & Castro 31319* (Z); * Ecuador, *Céros 2874* (AAU); * Ecuador, *Gentry 12457, 30842* (Z)	DQ522735	DQ522817	DQ522776	DQ522672	DQ522602
Mandevilla cercophylla Woodson	Ecuador, *Matezki 420* (Z)	DQ522736	DQ522818	DQ522777	DQ522673	DQ522603
Mandevilla coccinea (Hook. & Arn.) Woodson	Brazil, *Flores 452* (UEC)	DQ522737	DQ522819	DQ522778	DQ522674	DQ522604
Mandevilla convolvulacea (A. DC.) Hemsl.	Mexico, *Alvarado 162* (MEXU)	DQ522738	DQ522820	DQ522779	DQ522675	DQ522605
Mandevilla dodsonii A. H. Gentry	Ecuador, *Fallen 875* (Z)	DQ522739	DQ522821	DQ522780	DQ522676	DQ522606
Mandevilla duartei Markgr.	Brazil, *Simões 1281* (UEC)	DQ522740	DQ522822	DQ522781	DQ522677	DQ522607

Appendix 1. Continued.

Species	Voucher/citation	$rpl16$ Intron	$rps16$ Intron	$trnS^{GCU}$-$trnG^{UUC}$ Intergenic spacer	$trnK$ Intron	$matK$ Gene
Mandevilla emarginata (Vell.) C. Ezcurra	Brazil, *Quast 1* (UEC); * Brazil, *Bicudo 1235* (UEC); * Brazil, *Oliveira 35* (SP)	DQ522741	DQ522823	DQ522782	DQ522678	DQ522608
Mandevilla foliosa (Müll. Arg.) Hemsl.	Mexico, *Reína 2000-447* (Z)	DQ522742	DQ522824	DQ522783	DQ522679	DQ522609
Mandevilla fragrans (Stadelm.) Woodson	Brazil, *Pansarin & Micheliunas 1022* (UEC)	DQ522743	DQ522825	DQ522784	DQ522680	DQ522610
Mandevilla funiformis (Vell.) K. Schum.	Brazil, *Simões 1105* (UEC); * Brazil, *Custódio-Filho 733* (SP); * Brazil, *Leitão-Filho 10764* (UEC); * Brazil, *Shepherd & Vidal 11221* (UEC)	DQ522744	DQ522826	DQ522785	DQ522681	DQ522611
Mandevilla glandulosa (Ruiz & Pav.) Woodson	Ecuador, *Matezki 427* (Z)	DQ522745	DQ522827	DQ522786	DQ522682	DQ522612
Mandevilla harleyi M. F. Sales, Kin-Gouv. & A. O. Simões	Brazil, *Simões 1303* (UEC); * Brazil, *Harley 25194* (SPF)	AY597559	AY597593	AY597627	DQ522683	DQ522613
Mandevilla hirsuta (Rich.) K. Schum.	Brazil, *Kinoshita 02/114* (UEC)	DQ522746	DQ522828	DQ522787	DQ522684	DQ522614
Mandevilla illustris (Vell.) Woodson	Brazil, *Kinoshita & Matsumoto 0000/562* (UEC)	DQ522747	DQ522829	DQ522788	DQ522685	DQ522615
Mandevilla jamesonii Woodson	Ecuador, *Jorgensen 1467* (Z)	DQ522748	DQ522830	DQ522789	DQ522686	DQ522616
Mandevilla karwinskii (Müll. Arg.) Hemsl.	Mexíco, *Fishbein 2966* (ARIZ)	AY597553	AY596587	AY597621	DQ522687	DQ522617
Mandevilla krukovii Woodson	Brazil, *Kirkbride & Lleras 2907* (Z)	DQ522749	DQ522831	DQ522790		
Mandevilla lancifolia Woodson	Venezuela, *Davidse & Huber 14887* (Z)	DQ522750	DQ522832	DQ522791		
Mandevilla laxa (Ruiz & Pav.) Woodson	Argentina, *Galetto 809* (CORD); * Argentina, *Hatschbach 40681* (Z); * Argentina, *Novara 8347, 8568* (Z)	DQ522751	DQ522833	DQ522792	DQ522688	DQ522618
Mandevilla leptophylla (A. DC.) K. Schum.	Venezuela, *Steyermark 119835* (Z)	DQ522752	DQ522834	DQ522793	DQ522689	DQ522619
Mandevilla ligustriflora Woodson	Ecuador, *Matezki 340* (Z); * Ecuador, *Espinosa 1547* (MO)	AY597554	AY596588	AY597622	DQ522690	DQ522620
Mandevilla martiana (Stadelm.) Woodson	Brazil, *Simões & Pansarin 1100* (UEC)	DQ522753	DQ522835	DQ522794	DQ522691	DQ522621
Mandevilla moricandiana (A. DC.) Woodson	Brazil, *Simões 1130* (UEC)	DQ522754	DQ522836	DQ522795	DQ522692	DQ522622
Mandevilla myriophyllum (Taub.) Woodson	Brazil, *Pansarin 878* (UEC)	AY597555	AY596589	AY597623	DQ522693	DQ522623
Mandevilla nerioides Woodson	Colombia, *Franco 618* (Z)	DQ522755	DQ522837	DQ522796		
Mandevilla oaxacana (A. DC.) Hemsl.	Mexico, *Alvarado 190* (MEXU)	DQ522756	DQ522838	DQ522797	DQ522694	DQ522624

Appendix 1.   Continued.

Species	Voucher/citation	*rpl*16 Intron	*rps*16 Intron	*trn*S^GCU-*trn*G^UUC Intergenic spacer	*trn*K Intron	*mat*K Gene
Mandevilla pendula (Ule) Woodson	Brazil, *Ribeiro 2520* (UEC)	DQ522757	DQ522839	DQ522798	DQ522695	DQ522625
Mandevilla pentlandiana (A. DC.) Woodson	Brazil, *Simões 1272* (UEC); * Brazil, *Silva 1081* (UEC); * Brazil, *Lewinsohn 15901* (UEC)	DQ522758	DQ522840	DQ522799	DQ522696	DQ522626
Mandevilla pohliana (Stadelm.) A. H. Gentry	Brazil, *Feres 98/49* (UEC)	DQ722759	DQ522841	DQ522800	DQ522697	DQ522627
Mandevilla pycnantha (Steud. ex A. DC.) Woodson	Brazil, *Yamamoto 02/107* (UEC)	AY597556	AY596580	AY597625	DQ522698	DQ522628
Mandevilla rugellosa (Rich.) L. Allorge	French Guiana, *Prévost 3720* (CAY); * Surinam, *Lindeman 1976* (Z)	AY597561	AY597595	AY597629	DQ522699	DQ522629
Mandevilla rugosa (Benth.) Woodson	Brazil, *Simões 1204* (UEC)	AY597557	AY597591	AY597625	DQ522700	DQ522630
Mandevilla sancta (Stadelm.) Woodson	Brazil, *Simões 1060* (UEC)	DQ522760	DQ522842	DQ522801	DQ522701	DQ522631
Mandevilla scabra (Hoffmanns. ex Roem. & Schult.) K. Schum.	Brazil, *Simões 1126* (UEC)	AY597558	AY597592	AY597626	DQ522702	DQ522632
Mandevilla sellowii (Müll. Arg.) Woodson	Brazil, *Ribeiro 2522* (UEC)	DQ522761	DQ522843	DQ522802	DQ522703	DQ522633
Mandevilla spigeliiflora (Stadelm.) Woodson	Brazil, *Gomes 513* (UEC)	DQ522762	DQ522844	DQ522803	DQ522704	DQ522634
Mandevilla splendens (Hook. f.) Woodson	Brazil, *Simões 1268* (UEC)	AY597560	AY597594	AY597628	DQ522705	DQ522635
Mandevilla subsagittata (Ruiz & Pav.) Woodson	Mexico, *Alvarado 288* (MEXU)	DQ522763	DQ522845	DQ522804	DQ522706	DQ522636
Mandevilla syrinx Woodson	Mexico, *Calzada 21305* (MEXU)	DQ522764	DQ522846	DQ522805	DQ522707	DQ522637
Mandevilla tenuifolia (J. C. Mikan) Woodson, acc. 1	Brazil, *Simões 1171* (UEC)	AY597562	AY597596	AY597630	DQ522708	DQ522638
Mandevilla tenuifolia (J. C. Mikan) Woodson, acc. 2	Brazil, *Kinoshita & Matsumoto 00/609* (UEC)	AY597563	AY597597	AY597631	DQ522709	DQ522639
Mandevilla tricolor Woodson	Ecuador, *Jorgensen 1484* (Z)	DQ522765	DQ522847	DQ522806		
Mandevilla tubiflora (M. Martens & Galeotti) Woodson	Mexico, *Alvarado 106* (MEXU)	DQ522766	DQ522848	DQ522807	DQ522710	DQ522640
Mandevilla urophylla (Hook. f.) Woodson	Brazil, *M. P. Quast 6* (UEC)	DQ522767	DQ522849	DQ522808	DQ522711	DQ522641
Mandevilla venulosa (Müll. Arg.) Woodson	Brazil, *Simões 1107* (UEC)	AY597564	AY597598	AY597632	DQ522712	DQ522642
Mandevilla veraguasensis (Seem.) Hemsl.	Costa Rica, *Endress 97-76* (Z)	AY597565	AY597599	AY597633	DQ522713	DQ522643
Mesechites mansoanus (A. DC.) Woodson	Brazil, *Simões 1087* (UEC)	AY597567	AY597601	AY597635	DQ522714	DQ522644
Mesechites minimus (Britton & P. Wilson) Woodson	Cuba, Feb. 2001, *Nilsson s.n.* (Z)	AY597568	AY597602	AY597636	DQ522715	DQ522645

Appendix 1. Continued.

Species	Voucher/citation	$rpl16$ Intron	$rps16$ Intron	$trnS^{GCU}$-$trnG^{UUC}$ Intergenic spacer	$trnK$ Intron	$matK$ Gene
Mesechites roseus (A. DC.) Miers	Cuba, Feb. 2001, *Nilsson s.n.* (Z)	AY597569	AY597603	AY597637	DQ522716	DQ522646
Mesechites trifidus (Jacq.) Müll. Arg.	Ecuador, *Liede & Meve 3471* (UBT)	DQ522768	DQ522850	DQ522809	DQ522717	DQ522647
Odontadenia lutea (Vell.) Markgr.	Brazil, *Kinoshita 2002/56* (UEC)	AY597570	AY597604	AY597638	DQ522718	DQ522648
Pachypodium geayi Costantin & Bois	Cultivated, Bot. Gard. Chèvreloup, *Lieberherr s.n.* (unvouchered)	AY597571	AY597605	AY597640	DQ522719	DQ522649
Pachypodium lamerei Drake	Cultivated, Zürich Bot. Gart., *Simões 1333* (Z)	AY597572	AY597606	AY597639	DQ522720	DQ522650
Prestonia riedelii (Müll. Arg.) Markgr.	Brazil, *Simões 1274* (UEC)	AY597573	AY597607	AY597641	DQ522721	DQ522651
Rhodocalyx rotundifolius Müll. Arg.	Brazil, *Kinoshita 2000/66* (UEC)	AY597574	AY597608	AY597642	DQ522722	DQ522652
Secondatia densiflora A. DC.	Brazil, *Simões 1218* (UEC)	AY597575	AY597609	AY597643	DQ522723	DQ522653
Telosiphonia brachysiphon (Torr.) Henr.	U.S.A., *Jenkins 00-185* (TUC); * U.S.A., *Worthington 25068* (TEX)	AY597576	AY597610	AY597644	DQ522724	DQ522654
Telosiphonia hypoleuca (Benth.) Henr.	Mexico, *Reina 2000-362* (Z); * Mexico, *Richardson 1526* (TEX)	AY597579	AY597611	AY597645	DQ522725	DQ522655
Telosiphonia nacalpulensis Felger & Henr.	U.S.A., Arizona, July 2000, *Van Devender s.n.* (Z)	DQ522769	DQ522851	DQ522810	DQ522726	DQ522656
Tintinnabularia gratissima J. F. Morales	Mexico, *Ventura 107* (ENCB)	DQ522770	DQ522852	DQ522811	DQ522727	DQ522657
Tintinnabularia mortonii Woodson	Mexico, *Breedlove 34900* (TEX)	AY597578	AY597612	AY597646	DQ522728	DQ522658
Trachelospermum jasminoides (Lindl.) Lem.	Cultivated, Zürich Bot. Gard., *Simões 1334* (Z)	AY597577	AY597613	AY597647	DQ522729	DQ522659

Appendix 2.   Morphological matrix. ? signifies missing data. Polymorphic states are indicated by numbers in brackets. Characters and coding for character states as in Appendix 3.

Taxon	Character states for characters 1–32
Allomarkgrafia brenesiana	10000111000001000001101032100010
Allomarkgrafia plumeriiflora	10000111000001000001101032100010
Beaumontia grandiflora	10010100000101000001102021000001
Chonemorpha fragrans	10020100000001000001101021000000
Forsteronia acouci	10000111010001030011101\{23\}2200001
Forsteronia refracta	10000111010001030011101\{23\}2200001
Forsteronia velloziana	10000111000001030011101\{23\}2200001
Macrosiphonia longiflora	20000111101011010001101032200000
Macrosiphonia martii	20000111101011010001101032200000
Macrosiphonia velame	20000111101011010001101032200000
Mandevilla anceps	20100112001001100001101132200000
Mandevilla atroviolacea	10000111001001000001101032200100
Mandevilla callista	10000112001101100101101132200000
Mandevilla cercophylla	10000111011001010002101032201000
Mandevilla coccinea	20000111001001010001101032200100
Mandevilla convolvulacea	10000111001001000001101032201011
Mandevilla dodsonii	10000112001101100101101132200000
Mandevilla duartei	20000111001001000001101032200100
Mandevilla emarginata	20000111001101020001\{12\}101032200000
Mandevilla foliosa	20000111001001010001101032201010
Mandevilla fragrans	10000111001001000001101032200100
Mandevilla funiformis	10000111001001100101101132200000
Mandevilla glandulosa	10000111001001000002101032201000
Mandevilla harleyi	20000111001001100101101132200000
Mandevilla hirsuta	10000111001101100101101132200000
Mandevilla illustris	20020111001001000001101032200100
Mandevilla jamesonii	10000111001001010002101032201000
Mandevilla karwinskii	20000111001001010001101032201000
Mandevilla krukovii	10000112001101100101101132200000
Mandevilla lancifolia	\{12\}0100\{12\}12001001100001101032200000
Mandevilla laxa	10021111001001000000\{12\}101032200000
Mandevilla leptophylla	10021112001001100101101132200000
Mandevilla ligustriflora	10000111011001000002101032201000
Mandevilla martiana	\{12\}00211110010010000001101032200100
Mandevilla moricandiana	10021111001001000001101032200100
Mandevilla myriophyllum	20020111001001010001101032200100
Mandevilla nerioides	20100112001001100001101?32200000
Mandevilla oaxacana	10000111001001000001101032201011
Mandevilla pendula	10000111001001000001101032200100
Mandevilla pentlandiana	\{12\}0000111001101102000\{12\}101032200000
Mandevilla pohliana	20020111001001000001101032200100
Mandevilla pycnantha	20000111001001010001101032200000
Mandevilla rugellosa	10000112001101110101101132200000
Mandevilla rugosa	10000112001001100101101132200000
Mandevilla sancia	\{12\}00211110010010000001101032200100
Mandevilla scabra	10000112001001100101101132200000
Mandevilla sellowii	10021111001001000001101032200100
Mandevilla spigeliiflora	20000111001001000001101032200100
Mandevilla splendens	10021111001001000001101132200101
Mandevilla subsagittata	10020112001001110101101?32200000
Mandevilla syrinx	10000111001001020001101032201011
Mandevilla tenuifolia 1	20020111001001010001101032200100
Mandevilla tenuifolia 2	20020111001001010001101032200100
Mandevilla tricolor	10000111001001010002101032201000
Mandevilla tubiflora	10000111001001020001101032201010

Appendix 2.   Continued.

Taxon	Character states for characters 1–32
Mandevilla urophylla	10021111001001000001101032200100
Mandevilla venulosa	20000111001001000001101032200100
Mandevilla veraguasensis	10000111001001000002102032201000
Mesechites mansoanus	10000111000001010001101032100010
Mesechites minimus	1000011100?001010001101032100010
Mesechites roseus	1000011100?001010001101032100010
Mesechites trifidus	10000111000001010001101032100010
Odontadenia lutea	10020100000001000001111021000000
Pachipodium geayi	000-0000000000-00010000-00000000
Pachipodium lamerei	000-0000000000-00000000-00000000
Prestonia riedelii	11010100001101111000111010010000
Rhodocalyx rotundifolius	21000100000101111000101010010010
Secondatia densiflora	10020100000001010000111021000000
Telosiphonia brachysiphon	20000111010010100001101032200000
Telosiphonia hypoleuca	20000111101001010001101032200000
Telosiphonia nacalpulensis	20000111101001010001101032200000
Tintinnabularia gratissima	1000011101?001000001101032100010
Tintinnabularia mortonii	1000011101?101000001102032100010
Trachelospermum jasminoides	10020100000001010000101021000000

Appendix 3. Characters and character states for the morphological matrix used in the cladistic analyses.

1. **Habit**: 0, trees; 1, lianas or vines; 2, erect shrubs or subshrubs, these often with a xylopod.
2. **Latex**: 0, white; 1, translucent.
3. **Stem in cross section**: 0, circular; 1, pentagonal.
4. **Nodal colleters**: 0, interpetiolar; 1, intrapetiolar; 2, continuous. Colleters are small glandular structures found on the margin or in axillary position to both vegetative and reproductive organs in the Apocynaceae (Thomas, 1991). Their number and organization have been traditionally used as taxonomic characters in the family. The arrangement of colleters on the branch nodes constitutes an easily coded character that has not received taxonomic scrutiny in *Mandevilla* and related genera.
5. **Spiny ring of nodal colleters**: 0, absent; 1, present. In some species of *Mandevilla*, the nodal colleters are greatly expanded and form a somewhat spiny crown around the nodes.
6. **Phyllotaxis**: 0, alternate; 1, opposite.
7. **Leaf colleters**: 0, absent; 1, present.
8. **Leaf colleter position**: 0, clustered at the base of the leaf blade adaxially; 1, spread along the midrib of the leaf blade adaxially.
9. **Abaxial leaf surface**: 0, thick indument of white wooly trichomes absent; 1, thick indument of white wooly trichomes present.
10. **Domatia**: 0, absent; 1, present.
11. **Inflorescence type**: 0, branched (cymose); 1, unbranched (racemose).
12. **Bracts**: 0, scarious; 1, petaloid.
13. **Pedicel**: 0, present; 1, absent.
14. **Calycine colleters**: 0, absent; 1, present.
15. **Calycine colleter arrangement**: 0, alternate to continuous; 1, opposite.
16. **Corolla shape**: 0, infundibuliform or campanulate to tubular-campanulate; 1, salverform; 2, tubular; 3, rotate.
17. **Annular corona**: 0, absent; 1, present.
18. **Form of the lower corolla tube**: 0, straight; 1, curved.
19. **Stamens**: 0, completely included; 1, tips of the anthers exserted, stamens ± completely exserted.
20. **Anther base**: 0, strongly sagittate; 1, cordate; 2, truncate.
21. **Anther guide-rails**: 0, composed mainly of endothecial thickenings; 1, composed mainly of sclerenchyma.
22. **Dorsal side of anthers**: 0, completely glabrous; 1, with trichomes.
23. **Filament length**: 0, anthers ± sessile; 1, < 1 cm long; 2, > 3 cm long.
24. **Junction of filament and anther connection**: 0, flat; 1, with a globose swelling.
25. **Anther/style head union**: 0, anthers attached by a circular patch of trichome-like cells; 1, anthers attached by a horseshoe-shaped rim of hairs; 2, anthers attached by a horseshoe-shaped rim of hairs and a narrow longitudinal strip; 3, anthers attached by cellular fusion.
26. **Style head shape in cross section**: 0, circular or subcircular; 1, pentagonal; 2, with five strongly projecting ribs.
27. **Style head ribs**: 0, absent; 1, restricted to the base; 2, along the entire length of the body of the style head.
28. **Collar or wreath at base of style head**: 0, absent; 1, present.
29. **Proportion between the apical appendages and main body of the style head**: 0, < 1:1; 1, 1:1 or appendages bigger than the main body. The style head is divided in two portions: two apical appendages and a massive main body. The appendages are variable in size within different species of *Mandevilla*, and their size in proportion to the main body constitutes a character that has never been used before in the genus.
30. **Nectaries number**: 0, five; 1, two.
31. **Nectaries height**: 0, smaller than the ovary; 1, equal or greater than the ovary.
32. **Ovary indument**: 0, absent; 1, present.