
PHYLOGENY AND SYSTEMATICS
OF THE RAUVOLFOIDEAE
(APOCYNACEAE) BASED ON
MOLECULAR AND
MORPHOLOGICAL EVIDENCE¹

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ABSTRACT

To elucidate deeper relationships within Rauvolfioideae (Apocynaceae), a phylogenetic analysis was conducted using sequences from five DNA regions of the chloroplast genome (*matK*, *rbcL*, *rpl16* intron, *rps16* intron, and 3' *trnK* intron), as well as morphology. Bayesian and parsimony analyses were performed on sequences from 50 taxa of Rauvolfioideae and 16 taxa from Apocynoideae. Neither subfamily is monophyletic, Rauvolfioideae because it is a grade and Apocynoideae because the subfamilies Periplocoideae, Secamonoideae, and Asclepiadoideae nest within it. In addition, three of the nine currently recognized tribes of Rauvolfioideae (Alstonieae, Melodineae, and Vinceae) are polyphyletic. We discuss morphological characters and identify pervasive homoplasy, particularly among fruit and seed characters previously used to delimit tribes in Rauvolfioideae, as the major source of incongruence between traditional classifications and our phylogenetic results. Based on our phylogeny, simple style-heads, syncarpous ovaries, indehiscent fruits, and winged seeds have evolved in parallel numerous times. A revised classification is offered for the subfamily, its tribes, and inclusive genera.

Key words: Apocynaceae, classification, homoplasy, molecular phylogenetics, morphology, Rauvolfioideae, systematics.

During the past decade, phylogenetic studies, especially those employing molecular data, have significantly improved our understanding of higher-level relationships within Apocynaceae s.l., leading to the recognition of this family as a strongly supported clade composed of the traditional Apocynaceae s. str. and Asclepiadaceae s.l. (Judd et al., 1994; Endress et al., 1996; Sennblad & Bremer, 1996, 2002; Potgieter & Albert, 2001). The greatest progress has been made within the speciose asclepiads, where we now have a fairly reliable backbone upon which to base a tribal and, in some cases even a subtribal, classification

(Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Liede et al., 2002a, b; Rapini et al., 2003; Meve & Liede, 2002, 2004; Verhoeven et al., 2003; Liede & Meve, 2004; Liede-Schumann et al., 2005).

Despite significant insights gained from studies over the past 10 years, especially from the broad-scale studies based on *rbcL* by Sennblad and Bremer (1996, 2002) and the *trnL-F* intergenic spacer by Potgieter and Albert (2001), a number of uncertainties remain within the Apocynaceae s. str. In particular, the resolution of the deeper nodes in both Rauvolfioideae as well as Apocynoideae has proven to be

¹The authors thank the following persons for providing plant material: Tony Abbott (PRU), Leonardo Alvarado-Cárdenas (MEXU), Paul Berry (MICH), Frieda Billiet (BR), Melany Chapin (NTBG), Paul I. Forster (BRI), Günter Gerlach (M), Anne Hutchings (ZULU), Luiza Kinoshita and Ingrid Koch (UEC), Stephanie Lieberherr (P), Sigrid Liede and Ulrich Meve (UBT), David Middleton (E), Marie Françoise Prévost (CAY), Ernest Schmidt (PRU), Jürg Schönenberger (SUNIV), Ian M. Turner (SING), and Bram Van Wyk (PRU); without their generosity, this study would not have been possible. We thank Gretchen Ionta (FLAS) for kindly sharing her modified CTAB protocol. We are also grateful to the Botanical Garden in Meise, Belgium, and the Agricultural University of Wageningen, The Netherlands, for their help and support during visits by the first author and allowing him to collect samples both from the living collection as well as the herbarium. The rich herbarium collections of these two botanical institutes were invaluable to this study, allowing us to include taxa that otherwise would not have been possible if we had had to collect all of them in the field. We also thank the Missouri Botanical Garden (MO) for permission to remove samples from their herbarium collection. The fourth author thankfully acknowledges the hospitality and support of the National Tropical Botanical Garden in Kauai and for permission to collect samples from the living collection. This study was supported by grants to A. Simões from the Nachwuchsförderungskredit/Stieffel-Zanger-Stiftung, University of Zürich, Switzerland. The first author is particularly grateful for the support from two Synthesys grants (BE-TAF-1031 for the National Botanical Garden of Belgium at Meise and NL-TAF-977 for the Agricultural University at Wageningen). The second author gratefully acknowledges the support of a Mercer Fellowship from the Arnold Arboretum of Harvard University.

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problematic. More narrowly focused studies have concentrated for the most part on improving understanding of relationships in the Apocynoideae (Sennblad et al., 1998; Simões et al., 2004; Livshultz et al., 2007, this issue). In contrast, studies in the subfamily Rauvolfioideae have lagged behind. This is probably due in part to a lack of charisma in several genera having similar-looking small, inconspicuous, white, salverform flowers. Due to the relative dearth of taxonomically useful traits in the flowers, traditional classifications of Rauvolfioideae were based almost exclusively on fruit and seed characters (Schumann, 1895; Pichon, 1948a, b, 1949; Leeuwenberg, 1994a). Although the diverse array of fruit and seed types in Rauvolfioideae provides easy characters to develop a classification, preliminary molecular and morphological phylogenetic studies suggest that fruit and seed characters are homoplasious and that most higher taxa in previous classifications are not monophyletic (Potgieter & Albert, 2001).

In this study, we aim to elucidate the deeper relationships in Rauvolfioideae that remained unresolved or poorly supported in recent phylogenetic studies in order to evaluate the congruence between the retrieved monophyletic groups and the genera, as well as supergeneric taxa recognized in the most recent classification (Endress & Bruyns, 2000). Because denser taxon and character sampling is known to improve phylogenetic resolution (Graybeal, 1998; Rannala et al., 1998; Soltis et al., 1998, 2001), we expanded both for Rauvolfioideae compared to previous phylogenetic studies (Potgieter & Albert, 2001; Sennblad & Bremer, 2002), including a total of 76 taxa and characters from five plastid loci (*matK*, *rbcL*, *rpl16* intron, *rps16* intron, and 3' *trnK* intron), as well as morphology. We compare the resulting phylogenetic hypotheses within Rauvolfioideae with the classifications of Leeuwenberg (1994a) and Endress and Bruyns (2000), discuss the morphological features traditionally used to define tribes that proved to be consistent with the retrieved clades, and propose conservative modifications to the subfamily's classification until better resolved and more strongly supported phylogenies become available. We then use this information as a basis to discuss character evolution in this subfamily and, finally, to provide improved circumscription at the tribal level.

MATERIALS AND METHODS

TAXON SAMPLING

Fifty taxa (41 genera) of Rauvolfioideae and 16 genera of Apocynoideae were employed in this study, including representatives from all 14 tribes recog-

nized in Apocynaceae s. str. by Endress and Bruyns (2000). In addition, two genera of Periplocoideae, one genus of Secamonoideae, and two genera of Asclepiadoideae were included. Outgroup taxa, *Gentiana* L. (Gentianaceae), *Gelsemium* Juss. (Gelsemiaceae), *Strychnos* L., and *Mitrasacme* Labill. (Loganiaceae), were chosen from other families of Gentianales to include potential close sister groups to Apocynaceae (Backlund et al., 2000). The taxa analyzed, voucher information, and GenBank accession numbers are listed in Appendix 1. Sequences for 12 genera and 37 species are reported here for the first time and are analyzed together with sequences generated in previous studies (Sennblad & Bremer, 1996, 2002; Endress et al., 1996; Sennblad et al., 1998; Potgieter & Albert, 2001; Simões et al., 2004; Livshultz et al., 2007).

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. For some problematic taxa, particularly those samples taken from herbarium material, a modified CTAB methodology based on the standard CTAB DNA extraction method (Doyle & Doyle, 1987) but using proteinase K instead of β -mercaptoethanol, proved to be a more efficient means of extraction. Five plastid loci, comprising three non-coding (*rpl16*, *rps16*, and 3' *trnK* introns) and two coding (*matK* and *rbcL*) regions, 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 consisting of 40 cycles of denaturation (95°C, 30 sec.), annealing (52°C, 1 min.), and chain extension (72°C, 90 sec.). The *trnK* intron and *matK* were co-amplified in a single PCR reaction, and the thermal cycling program was modified in the following steps: denaturation (94°C, 30 sec.) and annealing (58°C, 1 min.). Reactions were terminated with a final extension of 4 min. at 72°C for the *rpl16* and *rps16* introns, and 7 min. for the *trnK* intron, *matK*, and *rbcL* genes. All PCR reactions were performed in a total reaction volume of 25 μ l, using 2.5 mM MgCl₂, 10 \times PCR* Buffer (Amersham Biosciences), 0.25 mM of dNTP, 0.5 units of Taq DNA polymerase (Amersham Biosciences, lot 17544), 1 to 4 μ l of bovine serum albumin (Sigma, Steinheim, Germany), and 0.1 mM of each primer. Amplification as one piece was possible for most of the taxa. In those cases where no products were obtained by using primers external to the target regions, smaller fragments were amplified by using

a combination of external and internal primers. Most of the primers utilized in our analyses were obtained from previous studies, as follows: Zurawski et al. (1981) for the *rbcL* gene, Simões et al. (2004) for the *rpl16* and *rps16* introns, and Civeyrel and Rowe (2001) for the *trnK* intron and *matK* gene. As reported in Sennblad and Bremer (1996), primers *rbcLz*-234 and *rbcLz*-895 were modified for use in the *rbcL* gene, as follows: 5'-CGTTACAAAGGKCGATGCTAC-CAYATCGA-3' for the former, and 5'-GCAGTTATTGATAGACAGAARAATCATGG-3' for the latter. The primers *matK*438F (5'-TTTKGKGTAGATATAY-TAAT-3') and *matK*756R (5'-TAGAAATAGAT-TAGTTCAAGA-3') were designed by us for use in sequencing of the *matK* gene. Amplified PCR products were then purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences). No amplified products of the 3' *trnK* intron were obtained for *Gelsemium sempervirens* Ait. and *Thevetia peruviana* Merr.

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 4.2 (Gene Codes, Ann Arbor, Michigan, U.S.A.).

DATA MATRIX COMPOSITION AND ALIGNMENT

Nucleotide sequences of the studied plastid loci were manually assembled for each taxon. Aligned sequence matrices of the individual regions are available from the first author upon request. Aligned gaps were manually coded as presence/absence characters applying the single indel coding method described by Simmons and Ochoterena (2000) for *matK*, and by using the software GapCoder (Young & Healy, 2003) for the other loci. Manual confirmation of the coded gap characters showed that the GapCoder software performed well even in cases of overlapping gaps with different start and/or ending positions, and no further adjustments in the matrices were necessary. All coded gaps were then added to the sequence matrix and used in further analyses.

Forty-one parsimony-informative morphological characters were coded and analyzed with maximum parsimony. For character coding, herbarium and fresh specimens, pickled flowers, and when available, flower sections provided by the last author were used. Information for some characters was taken from the literature (e.g., Woodson, 1928; Pichon, 1948a, b, 1949;

Erdtman, 1952; Bisset, 1960, 1980, 1992; Hegnauer, 1970, 1989; Ornduff, 1970; Gadella, 1980; Leeuwenberg & Leenhouts, 1980; Barink, 1983; Coppen & Cobb, 1983; Kisakürek et al., 1983; Goldblatt, 1984, 1988; Nilsson, 1986; Chatterjee et al., 1987; Endress et al., 1990; Goldblatt & Johnson, 1990, 1991; Leeuwenberg, 1991; Middleton, 1993, 2003; Hartmann & Witte, 1995; Li et al. 1995a, b; Doll et al., 1996; Forster & Williams, 1996; Albers & Meve, 2001; van der Ham et al., 2001; Verhoeven & Venter, 2001; Humberto et al., 2004; Middleton et al., 2005). The morphological matrix, including explanatory notes on characters, is given in Appendices 2 and 3.

PARSIMONY ANALYSIS

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 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, as applied by other authors (e.g., Wiens, 1998; Sheahan & Chase, 2000; Whitten et al., 2000; Reeves et al., 2001).

Maximum parsimony (MP) 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 the most parsimonious trees 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 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, three trees held at each step, TBR branch swapping with steepest descent and MULTREES in effect, saving 10 trees at each RASR.

In order to identify the synapomorphies that are congruent with each of the major clades of Rauvolfioideae retrieved in our analyses, morphological characters were mapped onto one of the 28 most parsimonious trees resulting from the total evidence parsimony analysis, using MacClade 4.0 (Maddison &

Maddison, 2000). Unambiguous changes were then reconstructed with maximum parsimony, applying both accelerated (ACCTRAN) and delayed (DELTRAN) character state optimizations. Alternative optimizations were also reconstructed in five other most parsimonious trees to investigate the influence of alternative topologies on possible interpretations of character evolution.

BAYESIAN ANALYSIS

Bayesian inference was performed using Mr Bayes version 3.0 (Ronquist & Huelsenbeck, 2003). Data were partitioned into the categories: *rpl16* intron, *rps16* intron, 3' *trnK* intron, *matK*, *rbcL*, and gaps. The most optimal model of sequence evolution for each partition was selected using Modeltest version 3.6 (Posada & Crandall, 1998). A combined data set of all partitions, including gaps, was analyzed, applying separate models to each data partition and with parameters estimated separately for each individual partition. Starting model parameters were assigned uniform prior probabilities and further estimated during the analysis, by allowing them to vary independently among data partitions. One million generations were run, with parameters sampled every 1000 generations. Based on the inspection of the likelihood scores for each generation, the first 250 sampled generations were considered as burn-in and thus discarded from subsequent analyses.

RESULTS

ANALYSES OF THE CHLOROPLAST DNA DATA SETS

Multiple sequence alignment was straightforward for *rbcL*, with no observed gaps, and for *matK*, required only a few gaps that, without exception, occurred in multiples of three. Alignment was also straightforward for the 3' *trnK* intron and the *rps16* intron, but proved to be more difficult for the *rpl16* intron due to the large number of gaps and mononucleotide repeats. A total of 274 characters, including regions of mononucleotide repeats with variable length between taxa as well as composed by nested gaps resulting from ambiguous alignment, were excluded from the analysis.

Tree length, consistency index (CI), and retention index (RI) values for the cladograms that resulted from the maximum parsimony analyses of individual and combined data sets are summarized in Table 1. Among the individual molecular data sets, *matK* provided the best-resolved cladogram, with most of the nodes receiving bootstrap support (BS) higher than 50%. The highest proportion of parsimony-informative characters, as well as the highest CI and RI values,

Table 1. 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. Coded gaps, when present, were considered for calculations of the total number and percentage of parsimony-informative characters for both individual and combined data sets. NA = Data not applicable.

	<i>matK</i> gene	3' half of <i>trnK</i> intron	<i>rbcL</i> gene	<i>rps16</i> intron	<i>rpl16</i> intron	Molecular combined	Morphology
Aligned length	1683	366	1232	1097	1181	5563	41
Range of sequence length	1098–1164	190–247	NA	675–849	886–1081	NA	NA
No. of coded gaps	48	79	NA	169	194	490	NA
Total no. of parsimony-informative characters	528 (30.5%)	122 (27.3%)	188 (15.3%)	310 (24.5%)	378 (27.8%)	1581 (26.1%)	41 (100%)
Tree length	1781	533	787	966	1238	5225	371
CI	0.477	0.457	0.328	0.466	0.471	0.459	0.189
RI	0.675	0.601	0.557	0.635	0.623	0.645	0.441

were also provided by this data set. Of the other data sets, the *rpl16* and *rps16* introns performed equally well, with similar tree statistics and levels of resolution. The 3' *trnK* intron provided the least-resolved cladogram, and *rbcL* provided the lowest proportion of parsimony-informative characters and CI and RI values. No strongly supported (> 75%) incongruent clades were found between individual partitions; therefore, all molecular data sets were combined, and only the combined tree is shown (Fig. 1). Analysis of the morphological data set resulted in a poorly resolved cladogram (not shown, available upon request from the first author).

For the Bayesian analysis, the model GTR + γ was selected for *rbcL* and the *rpl16*, *rps16*, and 3' *trnK* introns, whereas the model TVM + γ was selected for *matK* by Modeltest. Parameter values of each molecular data set suggested by Modeltest are summarized in Table 2. Application of both parsimony and Bayesian methods to the molecular combined data set resulted in similar topologies, although posterior probability values (PP) for clades were higher in most cases than parsimony bootstrap values (Figs. 1, 2). All further discussion will be based on the strict consensus cladogram resulting from parsimony analysis of the molecular combined data set as well as the Bayesian tree obtained from the same data set.

Apocynaceae s.l. are strongly supported as monophyletic in both the MP and the Bayesian analyses (BS, PP = 100%), a result also supported by analysis of each individual molecular data set (except for the 3' *trnK* intron), as well as the morphological data set. Within Apocynaceae, 13 strongly supported (BS, PP = 100%) clades were recovered, of which one, the APSA clade, included all exemplars of Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae. The remaining taxa form the paraphyletic Rauvolfioideae, a grade with its representatives dispersed among 12 remaining clades. Our selection of these clades was based on their correspondence to the tribes in Rauvolfioideae recognized by Endress and Bruyns (2000): (1) the Aspidospermeae clade (ASP); (2) the Alstonieae clade (ALS); (3) the Vinceae clade (VIN); (4) the Willughbeieae clade (WIL); (5) the Tabernaemontaneae clade (TAB); (6) the Hunterieae clade (HUN); (7) *Diplorhynchus* (DI); (8) the Melodineae clade (MEL); (9) the *Amsonia* clade (AMS); (10) the Alyxieae clade (ALY); (11) the Plumerieae clade (PLU); and (12) the Carisseae clade (CAR).

CHARACTER MAPPING

Mapping morphological characters onto one of the most parsimonious trees showed that only four of the 11 major clades of Rauvolfioideae (Alyxieae, Aspidosper-

meae, Vinceae, and Tabernaemontaneae clades) are supported by unique, unambiguous changes. For most characters, reconstruction of character state changes resulted in ambiguous assumptions of ancestral state and numerous shifts in character state (Figs. 3, 4), even when different most parsimonious trees were selected for optimization. An example is given by character number 24, in which congenitally syncarpous ovaries were observed in all representatives of the Willughbeieae, Melodineae, and Carisseae clades, but also in one species of the Alyxieae clade (*Chilocarpus costatus* Miq.) and two from the Tabernaemontaneae clade (*Ambelania acida* Aubl. and *Molongum laxum* (Benth.) Pichon).

DISCUSSION

RELATIONSHIPS WITHIN RAUVOLFOIOEAE

The structure of the combined molecular trees in Figures 1 and 2 is in general agreement with previously published phylogenies of Apocynaceae based on chloroplast DNA (e.g., Civeyrel et al., 1998; Potgieter & Albert, 2001; Sennblad & Bremer, 2002). Neither of the two subfamilies of Apocynaceae s. str. (Rauvolfioideae and Apocynoideae) is monophyletic. Rauvolfioideae, as traditionally defined, comprises the taxa from *Aspidosperma* Mart. & Zucc. through *Carissa* L. (Figs. 1, 2), forming a grade that is paraphyletic to the rest of the family. Periplocoideae, Secamonoideae, and Asclepiadoideae are nested within Apocynoideae, thus rendering it paraphyletic as well. The results of this study are compared here with those of Potgieter and Albert (2001) and Sennblad and Bremer (2002), and to the classification of Endress and Bruyns (2000).

Within Rauvolfioideae, most of the terminal clades retrieved in our analyses correspond to clades based on the *trnL* intron and the *trnL-F* spacer (Potgieter & Albert, 2001) and *rbcL* (Sennblad & Bremer, 2002) and, in most cases, equate to tribes in the current classification (sensu Endress & Bruyns, 2000). The support for these clades is, in general, higher here than in these previous studies. Resolution for the relationships among these clades at the tips of the branches is considerably improved as well, compared to the two earlier studies, especially in the region of the tree comprising the Aspidospermeae through the Tabernaemontaneae/Willughbeieae clades. The region of the tree (cf. Figs. 1, 2) including the Hunterieae, Melodineae, *Amsonia* Walter, and Alyxieae clades, as well as *Diplorhynchus* Welw. ex Ficalho & Hiern continues to remain unresolved.

The Aspidospermeae clade, comprising *Aspidosperma* (represented by *A. australe* Müll. Arg., *A.*

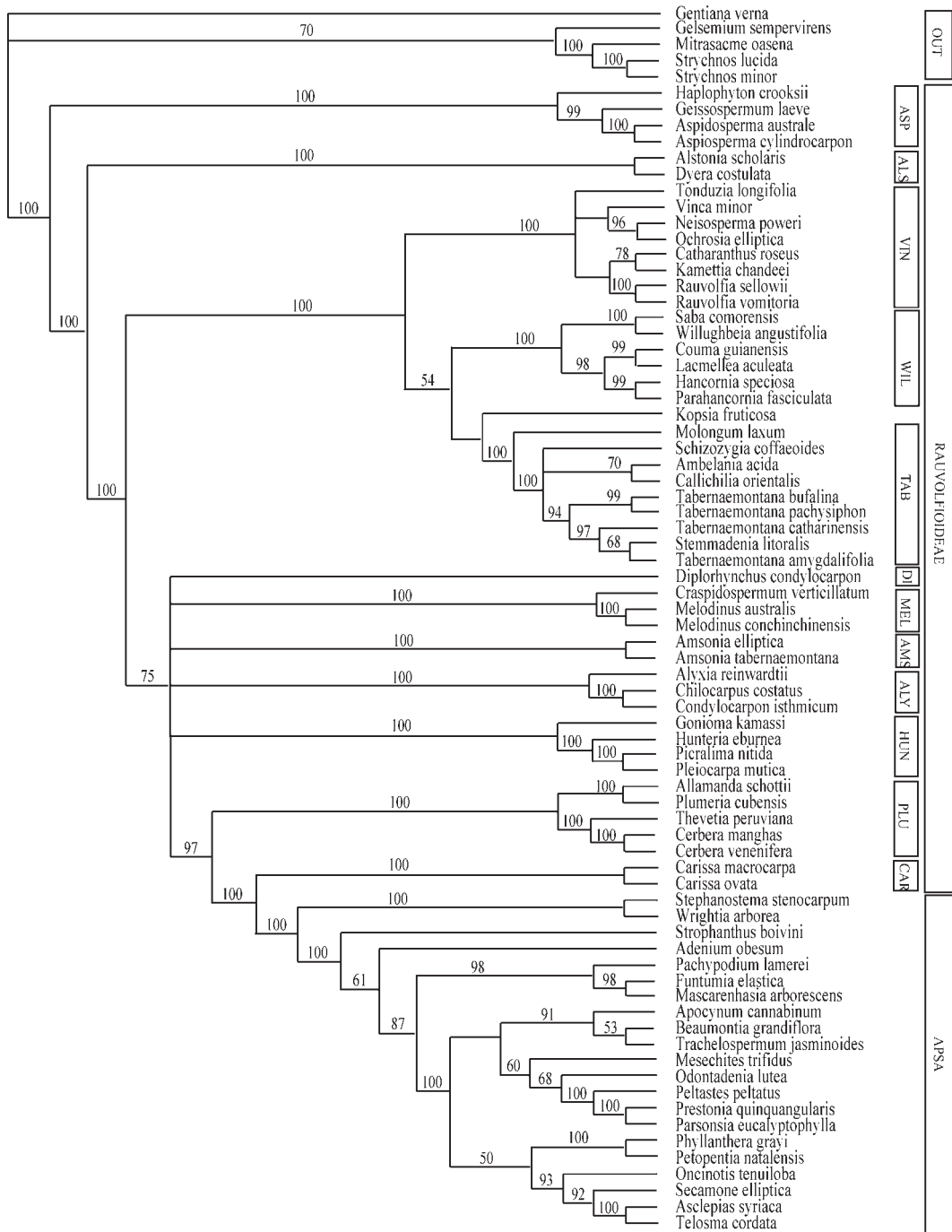


Figure 1. Strict consensus of the 28 most parsimonious trees generated by the molecular combined data set. Bootstrap values greater than 50% are indicated above the branches. Full taxon names are given in Appendix 1. The clades identified are abbreviated as follows: OUT = Outgroup; ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; WIL = Willughbeieae clade; TAB = Tabernaemontaneae clade; DI = *Dyplorhynchus*; MEL = Melodieae clade; AMS = *Amsonia* clade; ALY = Alyxieae clade; HUN = Hunterieae clade; PLU = Plumerieae clade; CAR = Carisseae clade; APSA = Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae.

Table 2. Summary of the model parameters suggested by Modeltest 3.06 for the molecular data sets.

Molecular data set	Substitution model	-ln L	Gamma shape parameter	Base frequency				Rate matrix					
				A	C	T	G	A-C	A-G	A-T	C-G	C-T	G-T
<i>matK</i>	TVM+I+G	14345.0674	1.0878	0.2897	0.1699	0.1705	0.3699	1.1761	1.7446	0.2101	0.8363	1.7446	1.0000
<i>rbcL</i>	GTR+I+G	7323.1772	0.7065	0.2435	0.2005	0.2646	0.2915	1.7303	2.2893	0.4607	1.1604	3.0710	1.0000
<i>Rpl16</i> intron	GTR+I+G	8850.9785	1.0623	0.4269	0.1330	0.1574	0.2828	0.8470	1.0078	0.3027	0.6600	1.3899	1.0000
<i>Rps16</i> intron	GTR+I+G	7388.5928	1.1501	0.3498	0.1521	0.1834	0.3417	1.0032	1.2574	0.3209	0.6133	1.7876	1.0000
<i>3'trnK</i> intron	GTR+G	2979.7278	3.0211	0.3759	0.1100	0.1923	0.3218	1.1913	1.1633	0.5878	1.1038	1.7375	1.0000

cylindrocarpon Müll. Arg.), *Geissospermum* Allemão (*G. laeve* Baill.), and *Haplophyton* A. DC. (*H. crooksii* L. D. Benson) sensu Alstonieae (Endress & Bruyns, 2000) and Aspidospermeae (Sennblad & Bremer, 2002), is strongly supported as sister to the rest of the family. The basal position of this clade of New World taxa, as well as the separation of the Old World genus *Alstonia* R. Br. (Alstonieae) as a distinct clade, concurs with the findings of Potgieter and Albert (2001). The three genera of the Aspidospermeae clade are characterized by having conspicuous slits in the corolla tube just above the level of stamen insertion (Appendix 3, char. 12: 1) that are usually visible under the dissecting scope or with the naked eye. These slits are formed when the flower reaches anthesis before the postgenital fusion of the upper part of the corolla tube is completed. Remnants of epidermis along the suture line or tiny gaps a few microns in length in this region, visible in serial sections and at high magnification, have been reported in various genera of both Rauvolfioideae and Apocynoideae (Endress et al., 1996; Sennblad et al., 1998). It is extremely rare, however, that the gaps are so long that they can be seen as slits under a normal dissecting scope. Visible slits in the corolla tube were considered to be a key character to distinguish *Aspidosperma* from related genera by Woodson (1951) and Markgraf (1974). However, visible slits in the corolla tube were also reported within the other two genera represented in the Aspidospermeae clade, *Geissospermum* and *Haplophyton*, by Fallen (1986).

The large and widespread Paleotropical genus *Alstonia*, also recognized as a clade in the analyses of Endress et al. (1996) and Potgieter and Albert (2001), here with *A. scholaris* R. Br., forms a strong sister relationship with *Dyera* Hook. f. (*D. costulata* Hook. f. included as exemplar), another genus of the Old World tropics containing only two species. Although currently included in the Melodineae, it has previously been suggested that *Dyera* is the closest relative of *Alstonia*, based on morphological similarities including the large tree habit, the presence of several leaves in a whorl, and an unusual type of outgrowth at the base of the petiole in both *Dyera* species and *A. scholaris* (Monachino, 1949; Sidiyasa, 1998; D. J. Middleton, pers. comm.).

The Vinceae clade (Figs. 1, 2) corresponds to previous Vinceae clades (Potgieter & Albert, 2001; Sennblad & Bremer, 2002). *Catharanthus* G. Don, *Rauwolfia* L., *Vinca* L., *Neisosperma* Raf., and *Ochrosia* Juss. are all members of Vinceae in Endress and Bruyns (2000). The current results also support inclusion of *Tonduzia* Pittier (*T. longifolia* (A. DC.) Woodson), previously classified in Alstonieae, and

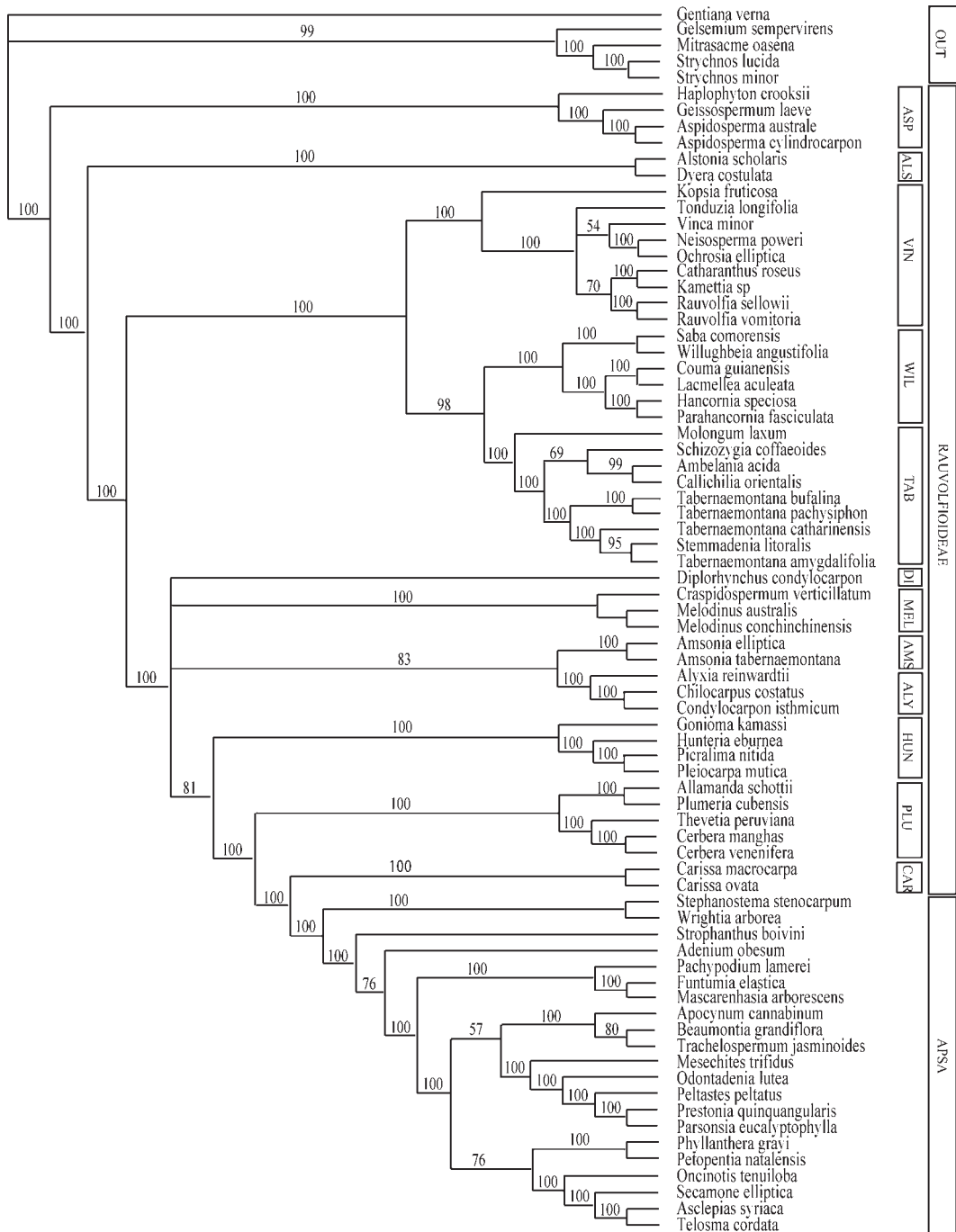


Figure 2. Majority rule consensus tree based on the Bayesian analysis of the combined molecular data set. Posterior probabilities are indicated above the branches. Full taxon names are given in Appendix 1. The clades identified are abbreviated as follows: OUT = Outgroup; ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; WIL = Willughbeieae clade; TAB = Tabernaemontaneae clade; DI = *Dyplorhynchus*; MEL = Melodineae clade; AMS = *Amsonia* clade; ALY = Alyxieae clade; HUN = Hunterieae clade; PLU = Plumerieae clade; CAR = Carisseae clade; APSA = Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae.

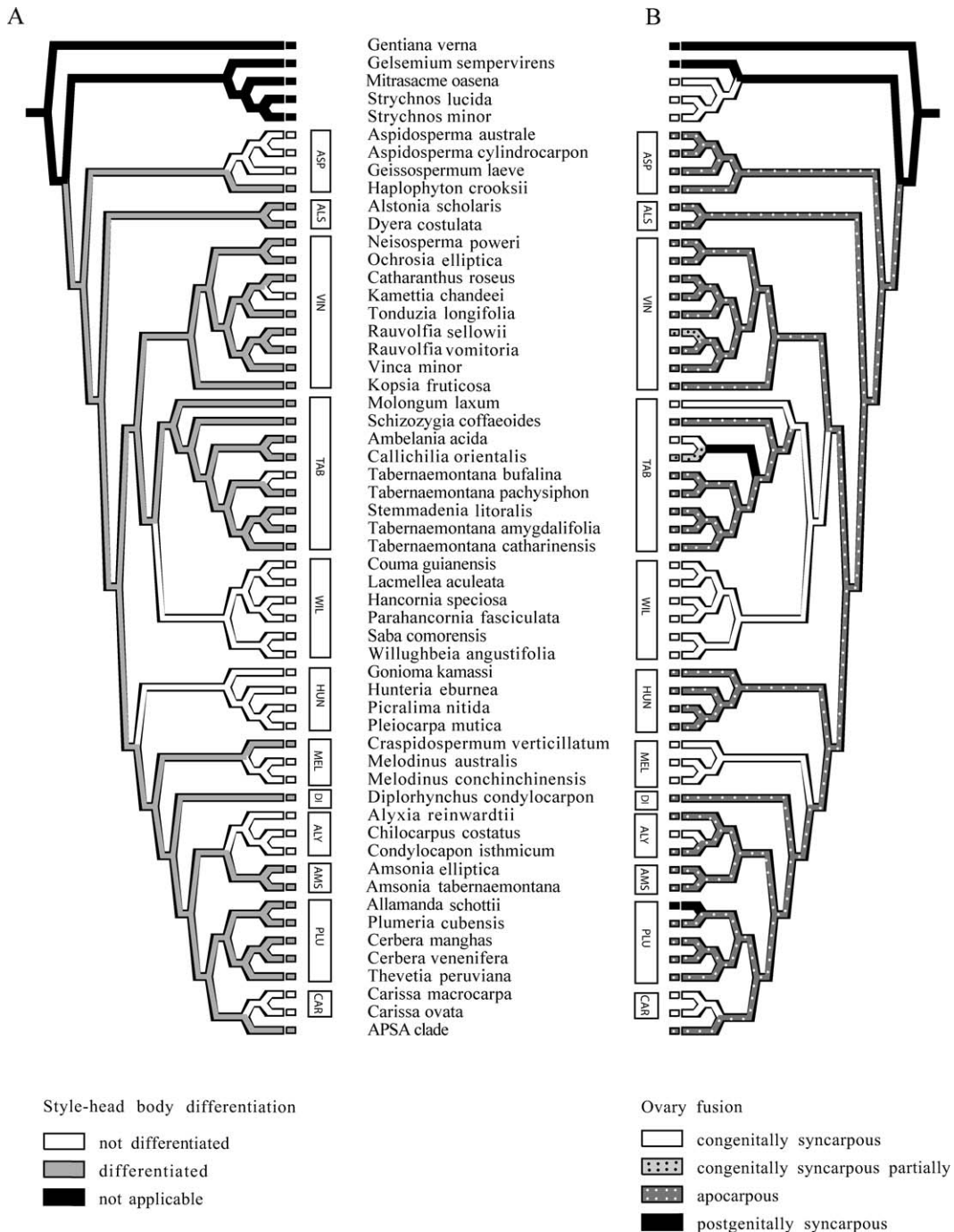


Figure 3. Evolution of two selected flower characters in Rauvolfioideae optimized onto one of the most parsimonious trees from the combined molecular data set. Only ACCTRAN optimization is shown. —A. Style-head body differentiation (char. 20). —B. Ovary fusion (char. 24). Clade names within Rauvolfioideae are abbreviated as follows: ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; TAB = Tabernaemontaneae clade; WIL = Willughbeieae clade; HUN = Hunterieae clade; MEL = Melodineae clade; DI = *Dyplorhynchus*; ALY = Alyxieae clade; AMS = *Amsonia* clade; PLU = Plumerieae clade; CAR = Carisseae clade.

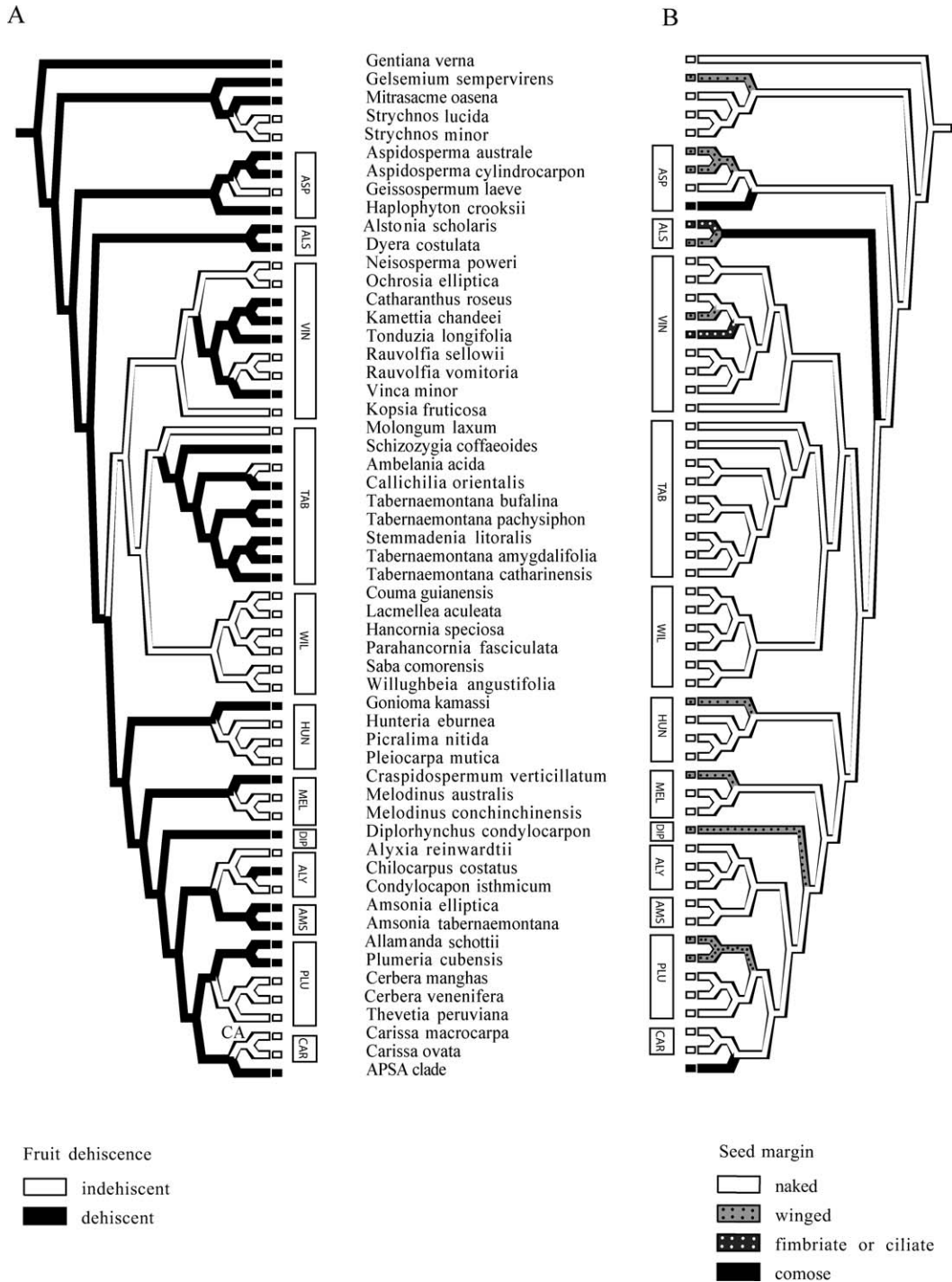


Figure 4. Evolution of two selected fruit and seed characters optimized onto one of the most parsimonious trees from the combined molecular data set. Only ACCTRAN optimization is shown. —A. Fruit dehiscence (char. 26). —B. Seed margin (char. 29). Clade names within Rauvolfioideae are abbreviated as follows: ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; TAB = Tabernaemontaneae clade; WIL = Willughbeieae clade; HUN = Hunterieae clade; MEL = Melodineae clade; DI = *Dyplorynchus*; ALY = Alyxieae clade; AMS = *Amsonia* clade; PLU = Plumerieae clade; CAR = Carisseae clade.

Kamettia Kostel., previously placed in Melodineae, in the Vinceae. *Tonduzia* was put into synonymy under *Alstonia* by Pichon (1947) and maintained there by subsequent authors (Gentry, 1983; Morales, 1995; Williams, 1996; Sidiyasa, 1998). In the Endress and Bruyns (2000) classification, *Tonduzia* is recognized as a distinct genus in Alstonieae. In the study by Potgieter and Albert (2001), *Tonduzia* as well as *Laxoplumeria* Markgr. were strongly supported as members of the Vinceae. *Kamettia*, a rare and poorly collected genus known previously from a single species in the Western Ghats of India, is represented in this study by *K. chandeei* D. J. Middleton, a new species discovered by fellow apocynologist David Middleton during fieldwork in Thailand (Middleton et al., 2005). *Kamettia* is currently included in Melodineae by Endress and Bruyns (2000) but is strongly supported by the one exemplar here as a member of Vinceae. The ongoing dispute over the status of *Neisosperma* as a genus distinct from *Ochrosia* based on morphological criteria (Fosberg & Sachet, 1977; Hendrian, 2004) could not be adequately tested here due to insufficient taxon sampling. The strongly supported sister relationship of the two included exemplars, however, attests to their close affinity from a molecular perspective and does not contradict their being as congeners, as they have been treated by several specialists of Paleotropical Apocynaceae in more recent years (Leeuwenberg, 1994a; Hendrian, 2004; Middleton, 2004b). Therefore, we have decided to follow their lead and treat *Neisosperma* as a synonym of *Ochrosia*.

The position of *Kopsia* Blume, an Asian genus of some 24 species (Middleton, 2004a) currently included in Vinceae, could not be resolved satisfactorily in the parsimony analysis, in which *K. fruticosa* (Roxb.) A. DC. was sister to Tabernaemontaneae (but with < 50% bootstrap support). Bayesian inference placed *Kopsia* as sister to the rest of Vinceae with 100% support, a position very weakly supported in the study by Sennblad and Bremer (2002). Morphologically, *Kopsia* resembles other members of Vinceae in a number of characters. The style-head has a short membranous basal collar (Middleton, 2004a, b) and two well-developed nectary lobes alternating with the carpels, as also found in *Vinca*, *Catharanthus*, and *Laxoplumeria*; two much reduced nectaries in the same position are also present in *Neisosperma* (Forster & Williams, 1996). These characteristics are typical for Vinceae, but otherwise relatively uncommon in Rauvolfioideae. In addition, the corolla lobes are dextrorsely convolute, as found in *Ochrosia* (including *Neisosperma*), whereas in the great majority of Rauvolfioideae, the corolla lobes are sinistrorsely convolute. Chemotaxonomically, the presence of

exclusively complex plumeran alkaloids with a rearranged secologanin backbone in *Kopsia* suggests a somewhat isolated position for the genus in Vinceae (Kisakürek et al., 1983; Homberger & Hesse, 1984). The co-occurrence of these characters in *Kopsia* is the basis for its inclusion in Vinceae in the classification of Endress and Bruyns (2000).

The Willughbeieae clade, comprising *Couma* Aubl. (*C. guianensis* Aubl.), *Hancornia* Gomez (*H. speciosa* Gomez), *Lacmellea* H. Karst. (*L. aculeata* (Ducke) Monach.), *Parahancornia* Ducke (*P. fasciculata* (Poir.) Benoist), *Saba* (Pichon) Pichon (*S. comorensis* (Bojer) Pichon), and *Willughbeia* Roxb. (*W. angustifolia* (Miq.) Markgr.), largely corresponds to clades found by Potgieter and Albert (2001) and Sennblad and Bremer (2002). All taxa included in the Willughbeieae clade in our study, as well as in the studies by Potgieter and Albert (2001) and Sennblad and Bremer (2002), are currently included in Willughbeieae sensu Endress and Bruyns (2000), strongly supporting the monophyly of the tribe. Whereas in Sennblad and Bremer (2002) the Willughbeieae clade contained only closely related African taxa and in Potgieter and Albert (2001) it remained unresolved, here it is divided into two strongly supported clades: a New World clade containing *Couma*, *Hancornia*, *Lacmellea*, and *Parahancornia*, all from South America, and an Old World clade containing *Saba* from Africa and *Willughbeia* from Asia. Willughbeieae have a pantropical distribution, and with 18 recognized genera (Endress & Bruyns, 2000), they are the second largest tribe in Rauvolfioideae. The tribe comprises mainly trees in the New World and mostly robust lianas in the Old World. In most Old World species as well as *Pacouria* Aubl. in the New World, the inflorescences are modified into grappling, curled tendrils (found nowhere else in the family), allowing them to climb high into the canopy (char. 6: 1). The tribe is quite homogeneous and characterized by the congenitally syncarpous, mostly unilocular, fruits with parietal placentation, opposite leaves, long, slender, pale-colored corollas, and undifferentiated style-heads. Unlike most Apocynaceae, the fruits of Willughbeieae are almost always edible (Monachino, 1943; Leeuwenberg & van Dilst, 1989; Vonk, 1989; Zarucchi, 1991; Persoon et al., 1992; Vonk et al., 1994).

The broader circumscription of Tabernaemontaneae as delimited in the classification by Endress and Bruyns (2000), including Ambelanieae of earlier classifications and corroborated by Potgieter and Albert (2001) and Sennblad and Bremer (2002), is also strongly supported here by the inclusion of *Molongum* Pichon (*M. laxum* (Benth.) Pichon) and *Ambelania* Aubl. (*A. acida* Aubl.) in the Tabernaemontaneae clade. Tabernaemontaneae is the largest

tribe in the Rauvolfioideae and is also the most phenotypically homogeneous. With a few rare exceptions, all genera are trees with opposite leaves and the fruit a pair of follicles. The two most distinctive character states typically found in Tabernaemontaneae are the highly modified anthers with large lignified guide rails (char. 16: 1; found nowhere else in Rauvolfioideae) and the arillate seeds (char. 28: 1). The only other genus in Apocynaceae known to have arillate seeds is *Chilocarpus* Blume of the Alyxieae. These arils are a fleshy, cushion-like outgrowth restricted to the funicle, whereas in Tabernaemontaneae, the pulpy aril surrounds the seed (Corner, 1976a, b). *Tabernaemontana* L., as circumscribed by Leeuwenberg (1994b), is a large pantropical genus of some 110 species, divided into seven sections. The species included in our study represent four of these sections, but they have also been treated earlier as separate genera: *T. bufalina* Lour. as *Ervatamia* (A. DC.) Stapf, *T. pachysiphon* Stapf as *Conopharyngia* G. Don, *T. catharinensis* A. DC. as *Peschiera* A. DC., and *T. amygdalifolia* Sieber ex A. DC. as *Tabernaemontana* s. str. (Stapf, 1902; Allorge, 1985). Our results suggest paraphyly of *Tabernaemontana*, as the four sampled species of the genus form a strongly supported clade with the sampled species of *Stemmadenia* Benth. (*S. litoralis* (Kunth) L. Allorge).

A sister relationship of the Willughbeieae and Tabernaemontaneae clades was supported in both the MP and Bayesian analyses, although this topology received a bootstrap support of only 54% in the parsimony tree.

Sister to the Tabernaemontaneae, Willughbeieae, and Vincae clades is a polytomy composed of six clades: *Diplorhynchus*, the Melodineae clade, the *Amsonia* clade, the Alyxieae clade, the Hunterieae clade, and a large clade composed of the Plumerieae, Carisseae, and APSA clades (Fig. 2).

Diplorhynchus, a monotypic genus restricted to southern Africa, has traditionally been considered to be closely related to *Pycnobotrya* Benth., *Aspidosperma*, *Geissospermum*, and *Microplumeria* Baill. based on similarities of their fruit and seeds (Pichon, 1950; Plazier, 1980; van der Ploeg, 1983). Endress and Bruyns (2000) placed the last three genera in Alstonieae, whereas *Diplorhynchus* and *Pycnobotrya* are included in Melodineae. Although *Diplorhynchus condylocarpon* (Müll. Arg.) Pichon is part of an unresolved polytomy in our analyses, its position would preclude a close relationship with *Aspidosperma*. We hypothesize the closest relative of *Diplorhynchus* may be *Pycnobotrya*, another monotypic and African genus. Both genera have latex that is often yellow, terminal, many-flowered inflorescences, anthers with sterile appendages at the base

and apex, apocarpous ovary with four seeds per carpel, and stout, widely divergent follicular fruits and compressed, winged seeds. Our attempts to obtain leaf samples of *Pycnobotrya* failed, and future molecular studies should make every effort to include this genus.

The Melodineae clade, representing Melodineae sensu Endress and Bruyns (2000), was also retrieved in previous analyses (Potgieter & Albert, 2001; Sennblad & Bremer, 2002). The strongly supported sister relationship of *Craspidospermum verticillatum* Bojer with two species of *Melodinus* is difficult to understand morphologically. Whereas the monotypic Madagascan endemic, *Craspidospermum* Bojer ex A. DC., is a tree with leaves in whorls of three to four, flowers without a petaloid corona, and dry capsular fruits, species of *Melodinus* J. R. Forst. & G. Forst., a genus of about 25 species with a wide distribution in Asia and Australasia (D. Middleton, pers. comm.), are climbers with opposite (rarely ternate) leaves, flowers with a conspicuous petaloid corona in the throat, and indehiscent berries with the seeds embedded in pulp. The porate pollen of *Craspidospermum*, shed as pantoporate tetrads (Lienau et al., 1986), is otherwise unknown in Rauvolfioideae and superficially resembles the pollen of some Periplocoideae (Nilsson et al., 1993). The Melodineae is the most poorly understood tribe in Rauvolfioideae, and although *Melodinus* was recently revised (Leeuwenberg, 2003), the treatment is unsatisfactory.

The position of the *Amsonia* clade here is at odds with its previous inclusion in Vincae (Endress & Bruyns, 2000). *Amsonia* was also distinct from Vincae in the analysis of Potgieter and Albert (2001). Its suggested relationship to *Thevetia* L. in that study may be incorrect, however, because both here and in other studies (Endress et al., 1996; Sennblad & Bremer, 1996, 2002; Civeyrel et al., 1998; Alvarado-Cárdenas & Ochoterena, 2007, this issue) *T. peruviana* is shown to be related to genera in the Plumerieae. Morphological support for the position of *Amsonia* as sister to the Alyxieae clade herein (Fig. 2) is lacking at present. The short collar at the base of the style-head and slender dehiscent follicles with naked seeds (e.g., Rosatti, 1989: 360) make *Amsonia* morphologically similar to some Vincae (e.g., *Catharanthus* and *Vinca*). The genus needs comprehensive study.

All members of the Alyxieae clade here (*Alyxia reinwardti* Blume, *Chilocarpus costatus*, and *Condylocarpon isthmicum* (Vell.) A. DC.) agree with previous results (Potgieter & Albert, 2001; Sennblad & Bremer, 2002) and belong to the Alyxieae sensu Endress and Bruyns (2000), which is composed of *Alyxia* Banks ex R. Br., a large genus (100+ species) widespread in Asia and Australasia (Middleton, 2000, 2002), and six

small genera, four of them island endemics (Lorence & Wagner, 1997; Endress et al., 1997, 2007). Morphologically, this tribe is best characterized by its unusual pollen. The pollen apertures are large pores, usually with a thickened margin, and are irregularly spaced on the grain (char. 36: 1). This is not homologous to the porate pollen found in the other subfamilies and is restricted to the Alyxieae (van der Ham et al., 2001). A sister relationship between the *Amsonia* and Alyxieae clades was retrieved in the Bayesian tree, but this topology received only moderate support (PP = 83%).

The Hunterieae clade corresponds to similar clades found by both Potgieter and Albert (2001) and Sennblad and Bremer (2002) and the Hunterieae sensu Endress and Bruyns (2000); it is comprised of the three genera: *Hunteria* Roxb. (*H. eburnea* Pichon), *Picalima* Pierre (*P. nitida* Th. & H. Dur.), and *Pleiocarpa* Benth. (*P. mutica* Benth.), all African. *Gonioma* E. Mey., a genus of two species restricted to South Africa and Madagascar, characterized by dehiscent fruits with small winged seeds and currently included in Melodineae, is strongly supported here by *G. kamassi* E. Mey. in the Hunterieae clade, in which all other genera have fleshy, indehiscent fruits. The Hunterieae clade received moderate support (PP = 81%) as sister to the next higher clade (composed of Carisseae, Plumerieae, and the APSA clades) in the Bayesian tree, but this was not retrieved in the strict consensus tree of the MP analysis.

Our phylogenetic analyses support the monophyly of Plumerieae, as circumscribed by Endress and Bruyns (2000). As noted, the position of *Thevetia peruviana* as sister to *Amsonia* in the study by Potgieter and Albert (2001) is most likely due to human error, because *T. peruviana* comes out together with the other members of Plumerieae, as expected, here, as well as in previous studies (Endress et al., 1996; Civeyrel et al., 1998; Sennblad & Bremer, 2002). In a detailed morphological cladistic study including all eight recognized species of *Thevetia* (including *Cascabela* Raf.), all taxa formed a clade (Alvarado-Cárdenas, 2005; Alvarado-Cárdenas & Ochoterena, 2007).

Molecular studies have greatly altered our understanding of the phylogenetic relationships of the genera traditionally included in the Carisseae. Historically considered to contain the most plesiomorphic taxa in the family (e.g., Leeuwenberg, 1994a), Carisseae in the conventional sense (e.g., Pichon, 1948a; Leeuwenberg, 1994a) is polyphyletic here, with its constituent genera scattered among four clades (see Table 3). The position of Carisseae sensu Endress and Bruyns (2000) comprising only *Carissa* and *Acokanthera* G. Don as sister to the Apocyno-

deae, Periplocoideae, Secamonoideae, and Asclepiadoideae concurs with results of Civeyrel et al. (1998) and Potgieter and Albert (2001). The highly nested position of *Carissa* (here *C. macrocarpa* A. DC., *C. ovata* R. Br.) and *Acokanthera* (Potgieter & Albert, 2001) indicates that characters that had been interpreted as plesiomorphic (e.g., the undifferentiated style-head and fleshy, indehiscent fruits) are derived in these two genera. Fleshy fruits are commonly associated with dispersal by animals, but observations on dispersal mode are lacking, not only for taxa of Carisseae, but for most other fleshy-fruited genera in the family. Comparative studies of the pollination biology and fruit dispersal are necessary to understand their functional and adaptive significance.

PHYLOGENY COMPARED TO PREVIOUS CLASSIFICATIONS

In Table 3, the relationships of the genera included in the present study are compared to their tribal placement in the two most recent classifications—Leeuwenberg (1994a) for the Apocynaceae s. str. and Endress and Bruyns (2000) for Apocynaceae s.l. Leeuwenberg's classification of Rauvolfioideae was based mainly on fruit and seed characters, and it recognized nine tribes: Carisseae, Chilocarpeae, Ambelanieae, Macoubeae, Tabernaemontaneae, Plumerieae, Alyxieae, Cerbereae, and Allamandaeae, three of which (Chilocarpeae, Macoubeae, and Allamandaeae) are monogeneric. Of the six remaining tribes, only one, Cerbereae (including *Cerbera* and *Thevetia*, nested in the Plumerieae clade), was found to be monophyletic, and five (Carisseae, Ambelanieae, Tabernaemontaneae, Plumerieae, and Alyxieae) were found to be nonmonophyletic. Alyxieae, Carisseae, and Plumerieae sensu Leeuwenberg (1994a) represented the most extreme cases of polyphyly here, with their constituent genera scattered across several clades in Rauvolfioideae, results that concur with those of Endress et al. (1996), Civeyrel et al. (1998), Sennblad et al. (1998), Potgieter and Albert (2001), and Sennblad and Bremer (2002).

The classification of Apocynaceae s.l. proposed by Endress and Bruyns (2000) also recognized nine tribes in Rauvolfioideae, but their circumscription differed significantly from that of Leeuwenberg. Tribal delimitation was based on floral, fruit and seed, and pollen characters, supplemented, when possible, by molecular phylogenetic results. Our results, corroborating the phylogenies obtained by Potgieter and Albert (2001) and Sennblad and Bremer (2002), show that the tribal classification of Rauvolfioideae by Endress and Bruyns (2000) contains more monophyletic groups than that of Leeuwenberg. Six of the nine tribes were found to be monophyletic (Will-

ughbeieae, Tabernaemontaneae, Hunterieae, Alyxieae, Plumerieae, and Carisseae), and three were found to be polyphyletic (Alstonieae, Melodineae, and Vinceae). Melodineae represented the most extreme case of polyphyly, with its genera scattered across five different clades.

MORPHOLOGICAL HOMOPLASY

The mapping of morphological character state changes onto one of the most parsimonious trees obtained from the combined molecular matrix indicates extensive morphological homoplasy among the characters that were traditionally used to circumscribe Rauvolfioideae. Fruit and seed characters, in particular, seem to be especially affected by homoplasy. The following characters are examined, because they were considered to be key features in traditional classifications: style-head body differentiation, ovary fusion, fruit dehiscence, and seed margin.

- Style-head body differentiation (char. 20). A style-head body with a simple ovoid or globular shape and a non-differentiated, uniformly secretory epithelium that functions both as stigma and for production of pollen transport adhesive (Appendix 3) is characteristic for certain genera of Rauvolfioideae. Optimization of character state changes on all selected most parsimonious trees produced similar unambiguous reconstructions. Non-differentiated style-heads have arisen independently eight times in taxa scattered throughout the Rauvolfioideae, from the basalmost Aspidospermeae clade (except *Haplophyton*) to the most derived Carisseae clade (Fig. 3A). Concerted switches to non-differentiated style-heads, syncarpy, and indehiscent fruits co-occurred along the same branches leading to Willughbeieae, *Melodinus*, and Carisseae.
- Ovary fusion (char. 24). In traditional classifications, syncarpy was thought to be the primitive condition in the family, and most taxa with syncarpous ovaries were grouped into Carisseae, which was placed at the base of the family (Bentham & Hooker, 1876; Schumann, 1895; Pichon, 1949; Li, 1982; Lý, 1986; Leeuwenberg, 1994a). The plesiomorphic condition in the family is apocarpy, as previously suggested by Endress et al. (1996) and Potgieter and Albert (2001). Shifts from apocarpous to syncarpous ovaries (Fig. 3B) have occurred in parallel five to six times in Rauvolfioideae. Evolution of this character within the Tabernaemontaneae/Willughbeieae clade is equivocal, and reconstruction of character state changes resulted in two equally parsimonious hypotheses. In the first, syncarpy is the apo-

carpous state, with frequent reversals to apocarpous ovaries. Alternatively, syncarpous ovaries could have evolved independently three times, once in the Willughbeieae clade and twice in the Tabernaemontaneae clade (demonstrated once by *Molongum laxum* and again in *Ambelania acida*). A broader sampling of Tabernaemontaneae (sensu Endress & Bruyns, 2000), especially from genera with syncarpous ovaries, such as *Rhigospira* and *Spongiosperma*, is necessary for a better understanding of this character in the Tabernaemontaneae/Willughbeieae clade.

- Fruit dehiscence (char. 26). Fruit dehiscence also shows considerable homoplasy, underscoring the evolutionary lability in this character (Fig. 4A). From an ancestral state of dehiscent fruits, shifts to indehiscent fruits have occurred six to 11 times within Rauvolfioideae. This remarkable discrepancy in the number of hypothesized changes may be due to the weakly resolved phylogenetic relationships between the Alyxieae, *Amsonia*, Hunterieae, and Melodineae clades, plus *Diplorhynchus*. In one of the most parsimonious trees, reconstruction of character state changes resulted in one single origin of indehiscent fruits with several subsequent reversals to dehiscent fruits in this group, whereas four independent origins of indehiscent fruits can be equally reconstructed when a different most parsimonious tree is used as a template. The switches to syncarpy and indehiscence often occurred along the same branches in our optimizations. Almost all of the genera included in the Carisseae in traditional classifications have congenitally syncarpous berries. The exceptions are *Hunteria*, *Picalima*, and *Pleiocarpa*. These three genera formed the subtribe Pleiocarpinae of the Carisseae in traditional classifications (Pichon, 1948a; Leeuwenberg, 1994a; Omino, 1996), which corresponds to the core Hunterieae clade here. The berries in these genera are superficially similar to those in Willughbeieae. However, because these taxa are apocarpous, each carpel in the gynoeceum matures into a separate berry. In the case of *Pleiocarpa*, one of the few genera of Apocynaceae in which some species have a gynoeceum with more than two carpels, the fruit can be composed of up to five berries per flower. The congenitally syncarpous genus *Carissa*, the epitome of the primitive Apocynaceae (Leeuwenberg, 1994a) in traditional classifications, is now found to be well embedded in the phylogeny of Apocynaceae, as sister to higher Apocynaceae (Figs. 1, 2).
- Seed margin (char. 29). The seeds of many genera in Rauvolfioideae have a wing or wings at the

Table 3. Comparison of the classifications of Leeuwenberg (1994a) and Endress and Bruyns (2000) for taxa considered within Apocynaceae with the current phylogeny. NA = not applicable.

GENERA	Leeuwenberg (1994a)	Endress & Bruyns (2000)	This paper
RAUVOLFIOIDEAE			
<i>Allamanda</i> L.	Allamandaceae	Plumeriaceae	Plumeriaceae clade
<i>Alstonia</i> R. Br.	Plumeriaceae	Alstonieae	Alstonieae clade
<i>Alyxia</i> Banks ex R. Br.	Alyxieae	Alyxieae	Alyxieae clade
<i>Ambelania</i> Aubl.	Ambelanieae	Tabernaemontaneae	Tabernaemontaneae clade
<i>Amsonia</i> Walter	Plumeriaceae	Vinceae	<i>Amsonia</i> clade
<i>Aspidosperma</i> Mart. & Zucc.	Plumeriaceae	Alstonieae	Aspidospermeae clade
<i>Callichilia</i> Stapf	Tabernaemontaneae	Tabernaemontaneae	Tabernaemontaneae clade
<i>Carissa</i> L.	Carisseae	Carisseae	Carisseae clade
<i>Catharanthus</i> G. Don	Plumeriaceae	Vinceae	Vinceae clade
<i>Cerbera</i> L.	Cerbereae	Plumeriaceae	Plumeriaceae clade
<i>Chilocarpus</i> Blume	Chilocarpeae	Alyxieae	Alyxieae clade
<i>Condylocarpon</i> Desf.	Alyxieae	Alyxieae	Alyxieae clade
<i>Couma</i> Aubl.	Carisseae	Willughbeieae	Willughbeieae clade
<i>Craspidospermum</i> Bojer ex A. DC.	Plumeriaceae	Melodineae	Melodineae clade
<i>Diplorhynchus</i> Welw. ex Ficalho & Hiern	Plumeriaceae	Melodineae	<i>Diplorhynchus</i> clade
<i>Dyera</i> Hook. f.	Plumeriaceae	Melodineae	Alstonieae clade
<i>Geissospermum</i> Allemão	Plumeriaceae	Alstonieae	Aspidospermeae clade
<i>Gonioma</i> E. Mey.	Plumeriaceae	Melodineae	Hunterieae clade
<i>Hancornia</i> Gomes	Carisseae	Willughbeieae	Willughbeieae clade
<i>Haplophyton</i> A. DC.	Plumeriaceae	Alstonieae	Aspidospermeae clade
<i>Hunteria</i> Roxb.	Carisseae	Hunterieae	Hunterieae clade
<i>Kamettia</i> Kostel.	Plumeriaceae	Melodineae	Vinceae clade
<i>Kopsia</i> Blume	Alyxieae	Vinceae	Vinceae clade
<i>Lacmellea</i> H. Karst.	Carisseae	Willughbeieae	Willughbeieae clade
<i>Melodinus</i> J. R. Forst. & G. Forst.	Carisseae	Melodineae	Melodineae clade
<i>Molongum</i> Pichon	Ambelanieae	Tabernaemontaneae	Tabernaemontaneae clade
<i>Neisosperma</i> Raf.	NA	Vinceae	Vinceae clade
<i>Ochrosia</i> Juss.	Alyxieae	Vinceae	Vinceae clade
<i>Parahancornia</i> Ducke	Carisseae	Willughbeieae	Willughbeieae clade
<i>Picalima</i> Pierre	Carisseae	Hunterieae	Hunterieae clade
<i>Pleiocarpa</i> Benth.	Carisseae	Hunterieae	Hunterieae clade
<i>Plumeria</i> L.	Plumeriaceae	Plumeriaceae	Plumeriaceae clade
<i>Rawolfia</i> L.	Alyxieae	Vinceae	Vinceae clade
<i>Saba</i> (Pichon) Pichon	Carisseae	Willughbeieae	Willughbeieae clade
<i>Schizogygia</i> Baill.	Tabernaemontaneae	Tabernaemontaneae	Tabernaemontaneae clade
<i>Stemmadenia</i> Benth.	Tabernaemontaneae	Tabernaemontaneae	Tabernaemontaneae clade
<i>Tabernaemontana</i> L.	Tabernaemontaneae	Tabernaemontaneae	Tabernaemontaneae clade
<i>Thevetia</i> L.	Cerbereae	Plumeriaceae	Plumeriaceae clade
<i>Tonduzia</i> Pittier	Plumeriaceae	Alstonieae	Vinceae clade
<i>Vinca</i> L.	Plumeriaceae	Vinceae	Vinceae clade
<i>Willughbeia</i> Roxb.	Carisseae	Willughbeieae	Willughbeieae clade
APOCYNIOIDEAE			
<i>Adenium</i> Roem. & Schult.	Wrightieae	Wrightieae	APSA clade
<i>Apocynum</i> L.	Apocynaeae	Apocynaeae	APSA clade
<i>Beaumontia</i> Wall.	Wrightieae	Apocynaeae	APSA clade
<i>Funtumia</i> Stapf	Wrightieae	Malouetieae	APSA clade
<i>Mascarenhasia</i> A. DC.	Wrightieae	Malouetieae	APSA clade
<i>Mesechites</i> Müll. Arg.	Echiteae	Mesechiteae	APSA clade
<i>Odontadenia</i> Benth.	Echiteae	Apocynaeae	APSA clade
<i>Oncinotis</i> Benth.	Apocynaeae	Apocynaeae	APSA clade
<i>Pachypodium</i> Lindl.	Echiteae	Malouetieae	APSA clade
<i>Parsonsia</i> R. Br.	Echiteae	Echiteae	APSA clade
<i>Peltastes</i> Woodson	Echiteae	Echiteae	APSA clade

Table 3. Continued.

GENERA	Leeuwenberg (1994a)	Endress & Bruyns (2000)	This paper
<i>Prestonia</i> R. Br.	Echiteae	Echiteae	APSA clade
<i>Stephanostema</i> K. Schum.	Wrightieae	Wrightieae	APSA clade
<i>Strophanthus</i> DC.	Wrightieae	Wrightieae	APSA clade
<i>Trachelospermum</i> Lem.	Echiteae	Apocyneae	APSA clade
<i>Wrightia</i> R. Br.	Wrightieae	Wrightieae	APSA clade
PERIPLOCOIDEAE			
<i>Petopentia</i> Bullock	NA	Periplocoideae	APSA clade
<i>Phyllanthera</i> Blume	NA	Periplocoideae	APSA clade
SECAMONOIDEAE			
<i>Secamone</i> R. Br.	NA	Secamonoideae	APSA clade
ASCLEPIADOIDEAE			
<i>Asclepias</i> L.	NA	Asclepiadoideae	APSA clade
<i>Telosma</i> Coville	NA	Asclepiadoideae	APSA clade

margin, and this is a key character for Plumerieae in Leeuwenberg's (1994a) classification. These wings are variable, and assessing their development requires detailed ontogenetic studies. Some taxa have a single, concentric, usually papery, wing (e.g., *Dyera*, *Pycnobotrya*, *Stephanostegia* Baill., many *Aspidosperma*). Other genera are characterized by seeds with a wing at only one end (*Plumeria* Tourn. ex L., *Diplorhynchus*, some *Aspidosperma*) or a wing at each end (e.g., *Gonioma*, *Kamettia*, *Plectaneia* Thou., *Strempeleopsis* Benth.). In *Craspidospermum*, the margin of the seed is completely surrounded by peculiar flattened appendages (see Leeuwenberg, 1997: fig. 1), here interpreted as a special type of wing (Appendix 3).

Mapping character state changes shows that wings have evolved several times within Rauvolfioideae, with six to seven independent origins of winged seeds observed in our reconstructions (Fig. 4B). This variation is due to the unstable position of *Diplorhynchus*, which had three different placements in our selected subset of most parsimonious trees. Switches to winged seeds and to dehiscent fruits occurred, not surprisingly, along the same branches. In *Cameraria* L. and *Cerberiopsis* Vieill. ex Pancher & Sebert (both Plumerieae), the fruit itself, an indehiscent samaroid, develops a wing.

Only three genera of Rauvolfioideae—*Alstonia*, *Tonduzia*, and *Laxoplumeria*—have flat seeds with a ciliate or fimbriate margin, which are not considered to be homologous to winged or comose seeds and are coded separately (Appendix 3). Because this type of seed is so rare in the family, taxonomists considered these three genera to be closely related. Pichon (1947) assigned *Tonduzia* to its own section within *Alstonia*,

and with *Laxoplumeria* (as *Bisquamaria* Pichon) as a sister genus, and this was followed by subsequent specialists (Morales, 1995; Williams, 1996; Sidiyasa, 1998). Not until 2001, in the paper by Potgieter and Albert, were *Tonduzia* and *Laxoplumeria* shown to be unrelated to *Alstonia*.

The existence of different types of syncarpous, fleshy fruits and of winged seeds within Rauvolfioideae and their repeated evolution, as inferred from the molecular phylogeny (Figs. 3, 4), most likely correlate with fruit and seed dispersal syndromes. This may explain why traditional tribal circumscriptions based on interpretation of superficial fruit and seed characters in Rauvolfioideae resulted in nonmonophyletic groups, because they likely subsumed different morphological, evolutionary, and developmental pathways. Similar recurrent fruit and seed types in higher-level classification have been reported in a number of other tropical woody plant families, e.g., Myrtaceae (Johnson & Briggs, 1984); Rubiaceae (Bremer & Eriksson, 1992); Gesneriaceae (Smith & Carroll, 1997); Malvales (Alverson et al., 1999); and Melastomataceae (Clausing et al., 2000).

TAXONOMIC CONSEQUENCES

The following taxonomic changes (Appendix 4) are proposed to deal with paraphyly at the tribal level within Rauvolfioideae: (1) recognition of the *Aspidospermeae* Miers as a tribe separate from *Alstonieae*; (2) recognition of *Dyera* within the *Alstonieae*, of *Gonioma* within the *Hunterieae*, and of *Laxoplumeria*, *Tonduzia*, and *Kamettia* within the *Vinceae*; (3) and finally, the recognition of *Amsonia* and *Diplorhynchus*, originally included in *Vinceae* and *Melodineae*, respectively, as *incertae sedis*.

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Appendix 1. Species sequenced for the present study, voucher information, and GenBank accession numbers. For previously published sequences, EMBL Nucleotide Sequence Database numbers are not given here; instead the publication is cited by a letter as follows: A = Olmstead et al. (1993); B = Semblad and Bremner (1996); C = Endress et al. (1996); D = Semblad et al. (1998); E = Semblad and Bremer (2002); F = Simões et al. (2004); G = Simões et al. (2006) (see Materials and Methods).

Species	Voucher	GenBank accession numbers			
		<i>rpl16</i> intron	<i>rps16</i> intron	3' <i>trnK</i> intron	<i>rbcL</i>
INGROUP – APOCYNACEAE					
<i>Adenium obesum</i> (Forssk.) Roem. & Schult.	cult., U.S.A., Cornell Univ., <i>T. Linschultz 03-21</i> (BH)	DQ660746	DQ660556	DQ660678	DQ660494 D
<i>Allamanda schottii</i> Pohl	cult., U.S.A., Cornell Univ., <i>T. Linschultz 03-26</i> (BH)	DQ660747	DQ660557	DQ660679	DQ660495 DQ660626
<i>Alstonia scholaris</i> (L.) R. Br.	Sabah, S. J. Davies et al. <i>SJD99150</i> (A)	DQ660748	DQ660558	DQ660680	C B
<i>Alyxia reinwardtii</i> Blume	Thailand, D. J. Middleton <i>172</i> (A)	DQ660749	DQ660559	DQ660681	DQ660496 DQ660627
<i>Ambelania acida</i> Aubl.	French Guiana, M. F. Prévost <i>3799</i> (CAY, P, Z)	DQ660750	DQ660560	DQ660682	DQ660497 DQ660628
<i>Amsonia elliptica</i> (Thunb.) Roem. & Schult.	cult., Netherlands, Bot. Gard. Wageningen, acc. nr. <i>1991BG19812</i>	DQ660751	DQ660561	DQ660683	DQ660498 DQ660629
<i>Amsonia tabernaemontana</i> Walter	cult., Switzerland, Bot. Gard. Zurich, A. O. Simões <i>1330</i> (Z)	DQ660752	DQ660562	DQ660684	DQ660499 DQ660630
<i>Apocynum cannabinum</i> L.	U.S.A., <i>T. Linschultz 03-28</i> (BH)	DQ660753	DQ660563	DQ660685	DQ660500 B
<i>Asclepias syriaca</i> L.	U.S.A., <i>T. Linschultz 03-33</i> (A)	DQ660754	DQ660564	DQ660686	DQ660501 DQ660631
<i>Aspidosperma australe</i> Müll. Arg.	Brazil, L. S. Kinoshita <i>02/110</i> (UEC)	DQ660755	DQ660565	DQ660687	DQ660502 DQ660632
<i>Aspidosperma cylindrocarpon</i> Müll. Arg.	cult., Brazil, University of Campinas, A. O. Simões <i>1328</i> (UEC)	DQ660756	DQ660566	DQ660688	DQ660503 DQ660633
<i>Beaumontia grandiflora</i> Wall.	cult., Germany, Bot. Gard. Munich, G. Gerlach/ <i>I. Babczinsky 5/06</i> (M).	F	F	G	G D
<i>Callihibitia orientalis</i> S. Moore	cult., South Africa, A. Hutchings s.n. (ZULU 4000)	DQ660757	DQ660567	DQ660689	DQ660504 DQ660634
<i>Carissa macrocarpa</i> (Eckl.) A. DC.	cult., Switzerland, Bot. Gard. Zürich, A. O. Simões <i>1331</i> (Z)	DQ660758	DQ660568	DQ660690	DQ660505 DQ660635
<i>Carissa ovata</i> R. Br.	Australia, P. J. Forster <i>28689</i> (BRI)	DQ660759	DQ660569	DQ660691	DQ660506 DQ660636
<i>Catharanthus roseus</i> (L.) G. Don	cult., Brazil, University of Campinas, A. O. Simões <i>1329</i> (UEC)	DQ660760	DQ660570	DQ660692	DQ660507 B
<i>Cerbera venenifera</i> (Poir.) Steud.	Thailand, D. J. Middleton et al. <i>2047</i> (A)	DQ660761	DQ660571	DQ660693	DQ660508 DQ660637
<i>Chilocarpus costatus</i> Miq.	cult., Belgium, Nat. Bot. Gard. Belgium, F. Billiet <i>S526</i> (BR)	DQ660762	DQ660572	DQ660694	DQ660509 E
<i>Condylocarpon isthmicum</i> (Vell.) A. DC.	Thailand, D. J. Middleton et al. <i>370</i> (A)	DQ660763	DQ660573	DQ660695	DQ660510 DQ660638
<i>Couma guianensis</i> Aubl.	Brazil, L. S. Kinoshita et al. <i>98-267</i> (UEC)	DQ660764	DQ660574	DQ660696	DQ660511 DQ660639
<i>Craspidospermum verticillatum</i> Bojer ex A. DC.	French Guiana, M. F. Prévost <i>3798</i> (CAY)	DQ660765	DQ660575	DQ660697	DQ660512 DQ660640
<i>Diplorhynchus condylocarpon</i> (Müll. Arg.) Pichon	Madagascar, Schönberger et al. <i>D36</i> (UPS)	DQ660766	DQ660576	DQ660698	DQ660513 DQ660641
<i>Dyera costulata</i> Hook. f.	South Africa, 21 Apr. 2000, E. Schmidt s.n. (PRU)	DQ660767	DQ660577	DQ660699	DQ660514 E
	cult., Singapore, Bot. Gard. Singapore, 23 May 2000, Ian Turner s.n. (SING)	DQ660768	DQ660578	DQ660700	DQ660515 DQ660642
<i>Funtumia elastica</i> (Preuss) Slapf	cult., Belgium, Nat. Bot. Gard. Belgium, F. Billiet <i>S3927</i> (BR)	DQ660769	DQ660579	DQ660701	DQ660516 D
<i>Geissospermum laeve</i> (Vell.) Miers	French Guiana, M. F. Prévost <i>3802</i> (CAY, P, Z)	DQ660770	DQ660580	DQ660702	DQ660517 DQ660643
<i>Gonioma tamassi</i> E. Mey.	South Africa, T. Abbott <i>7713</i> (PRU, Z)	DQ660773	DQ660583	DQ660704	DQ660518 DQ660645
<i>Hancornia speciosa</i> Gomes	Brazil, L. S. Kinoshita <i>02/101</i> (UEC)	DQ660774	DQ660584	DQ660705	DQ660519 DQ660646
<i>Haplophyton crookii</i> (L. D. Benson) L. D. Benson	U.S.A., Arizona, P. Jenkins <i>4901</i> (ARIZ)	DQ660775	DQ660585	DQ660706	DQ660520 DQ660647

Appendix 1. Continued.

Species	Voucher	GenBank accession numbers				
		<i>rpl16</i> intron	<i>rps16</i> intron	3' <i>trnK</i> intron	<i>matK</i>	<i>rbcL</i>
<i>Hunteria eburnea</i> Pichon	cult., Belgium, Nat. Bot. Gard. Belgium, <i>F. Billiet</i> S3029 (BR)	DQ660776	DQ660586	DQ660707	DQ660521	DQ660648
<i>Kometia chandeei</i> D. J. Middleton	Thailand, <i>D. J. Middleton</i> 3341 (E)	DQ660777	DQ660587	DQ660708	DQ660522	DQ660649
<i>Kopsia fruticosa</i> (Ker Gawl.) A. DC.	Thailand, <i>D. J. Middleton</i> et al. 452 (A)	DQ660778	DQ660588	DQ660709	C	B
<i>Laemellea aculeata</i> (Ducke.) Monach.	French Guiana, <i>M. F. Prévost</i> 3803 (CAY, P, Z)	DQ660779	DQ660589	DQ660710	DQ660523	DQ660650
<i>Mascarenhasia arborescens</i> A. DC.	cult., Belgium, Nat. Bot. Gard. Belgium, <i>F. Billiet</i> S3053 (R)	F	F	G	G	D
<i>Melodinus australis</i> (F. Muell.) Pierre	Australia, <i>P. I. Forster</i> 27503 (BR)	DQ660780	DQ660590	DQ660711	DQ660524	DQ660651
<i>Melodinus cochinchinensis</i> (Lour.) Merr.	cult., Belgium, Nat. Bot. Gard. Belgium, <i>F. Billiet</i> S3441 (BR)	DQ660781	DQ660591	DQ660712	DQ660525	DQ660652
<i>Mesechites trifidus</i> (Jacq.) Müll. Arg.	Ecuador, <i>Liede & Mene</i> 3471 (UBT)	G	G	G	G	E
<i>Molongun laxum</i> (Benth.) Pichon	Venezuela, <i>P. Berry & E. Melgueiro</i> 5400 (Z)	DQ660783	DQ660593	DQ660714	C	B
<i>Neisosperma pouerei</i> (F. M. Bailey) Fosberg & M.-H. Sachet	Australia, <i>P. I. Forster</i> 26594 (BR)	DQ660784	DQ660594	DQ660715	DQ660527	DQ660654
<i>Ochrosia elliptica</i> Labill.	Australia, <i>P. I. Forster</i> 27100 (BR)	DQ660785	DQ660595	DQ660716	DQ660528	DQ660655
<i>Odontadenia lutea</i> (Vell.) Markgr.	Brazil, <i>L. S. Kinoshita</i> 2005/56 (UEC)	F	F	G	G	DQ660656
<i>Oncinotis tenuiloba</i> Stapf	South Africa, <i>T. Abbott</i> 7707 (PRU, Z)	DQ660786	DQ660596	DQ660717	DQ660529	DQ660657
<i>Pachypodium lamerei</i> Drake	cult., Switzerland, Bot. Gard. Zurich, <i>A. O. Simões</i> 1333 (Z)	F	F	G	G	D
<i>Parahancornia fusciculata</i> (Poir.) Benoist	French Guiana, <i>M. F. Prévost</i> 3801 (CAY, P, Z)	DQ660787	DQ660597	DQ660718	DQ660530	DQ660658
<i>Parsonsia eucalyptophylla</i> F. Muell.	Australia, <i>P. I. Forster</i> 20118 (BR)	DQ660788	DQ660598	DQ660719	DQ660531	DQ660659
<i>Peltastes peltatus</i> (Vell.) Woodson	Brazil, <i>M. P. Quast</i> 9 (UEC); cult., Belgium, Nat. Bot. Gard. Belgium, <i>F. Billiet</i> S3526 (BR)	DQ660789	DQ660599	DQ660720	DQ660532	E
<i>Petropentia natalensis</i> (Schltr.) Bullock	cult., U.S.A., Cornell, <i>Linschultz</i> 03-2 (BH)	DQ660790	DQ660600	DQ660721	DQ660533	E
<i>Phyllanthera grayi</i> (P. I. Forst.) Venter	Australia, <i>P. I. Forster</i> s.n. (BR)	DQ660791	DQ660601	DQ660722	DQ660534	DQ660660
<i>Pterocalima nitida</i> (Stapf) Th. & H. Dur.	cult., Belgium, Nat. Bot. Gard. Belgium, <i>F. Billiet</i> S3440 (BR)	DQ660792	DQ660602	DQ660723	C	B
<i>Pleiocarpa mutica</i> Benth.	cult., Belgium, Nat. Bot. Gard. Belgium, <i>F. Billiet</i> S1766 (BR)	DQ660793	DQ660603	DQ660724	DQ660535	E
<i>Plumeria cubensis</i> Urb.	cult., Hawaii, Nat. Trop. Bot. Gard. Kauai, Aug. 2003, <i>M. Endress</i> s.n. (NTBG)	DQ660794	DQ660604	DQ660725	DQ660536	DQ660661
<i>Prestonia quinqueangularis</i> (Jacq.) Spreng.	cult., Belgium, Nat. Bot. Gard. Belgium, <i>F. Billiet</i> S205 (BR)	DQ660795	DQ660605	DQ660726	C	B
<i>Rauwolfia sellowii</i> Müll. Arg.	Brazil, <i>J. Koch</i> 868 (UEC)	DQ660796	DQ660606	DQ660727	DQ660537	DQ660662
<i>Rauwolfia vomitoria</i> A.Zel.	cult., University of Campinas, <i>J. Koch</i> 871 (UEC)	DQ660797	DQ660607	DQ660728	DQ660538	DQ660663
<i>Saba comorensis</i> (Bojer ex A. DC.) Pichon	cult., Belgium, Nat. Bot. Gard. Belgium, <i>F. Billiet</i> 3442 (BR)	DQ660798	DQ660608	DQ660729	DQ660539	DQ660664
<i>Schizogygia coffaeoides</i> Baill.	cult., France, Bot. Gard. Chèvreloup, acc. nr. 6331/61.3331 (unvouchered)	DQ660799	DQ660609	DQ660730	DQ660540	E
<i>Secamone elliptica</i> R. Br.	Thailand, <i>D. J. Middleton</i> et al. 1162 (A)	DQ660800	DQ660610	DQ660731	DQ660541	DQ660665
<i>Stemmadenia litoralis</i> (Kunth) L. Allorge	cult., Hawaii, Nat. Trop. Bot. Gard. Kauai, Aug. 2003, <i>M. Endress</i> s.n. (NTBG)	DQ660801	DQ660611	DQ660732	DQ660542	DQ660666

Appendix 1. Continued.

Species	Voucher	GenBank accession numbers			
		<i>rpl16</i> intron	<i>rps16</i> intron	3' <i>trnK</i> intron	<i>matK</i>
<i>Stephanostema stenocarpum</i> K. Schum.	Tanzania, Luke 3754 (MO)	DQ660802	DQ660612	DQ660733	DQ660543 B
<i>Strophanthus boivinii</i> Baill.	cult., Switzerland, Bot. Gard. Zurich, Dec. 2003, M. Endress s.n. (Z)	DQ660803	DQ660613	DQ660734	DQ660544 DQ660667
<i>Tabernaemontana amygdalifolia</i> Jacq.	Mexico, R. Ramirez & M. Garcia 527 (MEXU)	DQ660806	DQ660616	DQ660737	DQ660547 DQ660670
<i>Tabernaemontana bufalina</i> Lour.	Thailand, D. J. Middleton 187 (A)	DQ660807	DQ660617	DQ660738	DQ660548 DQ660671
<i>Tabernaemontana catharinensis</i> A. DC.	Brazil, A. O. Simões 1039 (UEC)	DQ660808	DQ660618	DQ660739	DQ660549 DQ660672
<i>Tabernaemontana pachysiphon</i> Stapf DQ6606703	cult., Brazil, Agronomic Institute of Campinas, A. O. Simões 1325 (UEC)	DQ660809	DQ660619	DQ660740	DQ660550
<i>Telosma cordata</i> (Burm. f.) Merr.	cult., U.S.A., Cornell, T. Lischultz 01-33 (BH)	DQ660810	DQ660620	DQ660741	DQ660551 DQ660674
<i>Theretia peruviana</i> (Pers.) K. Schum.	cult., U.S.A., Univ. Conn. campus, T. Lischultz 03-34 (A)	DQ660811	DQ660621		C B
<i>Tonduzia longifolia</i> (A. DC.) Markgr.	Mexico, Munn-Estrada 2165 (MEXU)	DQ660812	DQ660622	DQ660742	DQ660552 DQ660675
<i>Trachelospermum jasminoides</i> (Lindl.) Lem.	cult., U.S.A., Univ. Fl. campus, G. Ionta 420 (FLAS)	F	F	C	C DQ660676
<i>Vinca minor</i> L.	cult., Switzerland, Bot. Gard. Zurich, A. O. Simões 1332 (Z)	DQ660813	DQ660623	DQ660743	DQ660553 E
<i>Willughbeia angustifolia</i> (Miq.) Markgr.	Brunei, D. J. Middleton et al. 727 (A)	DQ660814	DQ660624	DQ660744	DQ660554 DQ660677
<i>Wrightia arborea</i> (Dennst.) Mabb.	Thailand, D. J. Middleton 240 (A)	DQ660815	DQ660625	DQ660745	DQ660555 D
OUTGROUP					
<i>Gelsemium sempervirens</i> (L.) J. St.-Hil. (Gelsemiaceae)	cult., Switzerland, Bot. Gard. Zurich (plant died)	DQ660771	DQ660581		C A
<i>Gentiana verna</i> L. (Gentianaceae)	Switzerland, M. P. Quast 14 (Z)	DQ660772	DQ660582	DQ660703	C DQ660644
<i>Mitrasacme oasena</i> Dunlop (Loganiaceae)	Australia, P. I. Forster 26711 (BRU)	DQ660782	DQ660592	DQ660713	DQ660526 DQ660653
<i>Strychnos lucida</i> R. Br. (Loganiaceae)	Australia, P. I. Forster 24078 (BRU)	DQ660804	DQ660614	DQ660735	DQ660545 DQ660668
<i>Strychnos minor</i> Dennst. (Loganiaceae)	Australia, P. I. Forster 26993 (BRU)	DQ660805	DQ660615	DQ660736	DQ660546 DQ660669

Appendix 2. Morphological matrix for Apocynaceae and outgroups. ? = missing data. Polymorphic states are indicated by numbers in brackets.

	1	6	11	16	21	26	31	36	41
<i>Adenium obesum</i>	00111	01003	00100	10111	00021	11231	11021	20001	0
<i>Allamanda schottii</i>	00102	01012	10000	00011	01030	11212	21021	02100	0
<i>Alstonia scholaris</i>	00102	01003	00000	00011	00021	11222	21021	0{12}200	0
<i>Alyxia reinwardtii</i>	10102	01003	00000	00010	00021	00302	20021	14000	0
<i>Ambelania acida</i>	00100	01003	00000	10011	00002	00002	21021	00???	?
<i>Amsonia elliptica</i>	20101	01003	00000	00011	00021	11202	21021	00300	0
<i>Amsonia tabernaemontana</i>	20101	01003	00000	00011	00021	11202	21021	00300	0
<i>Apocynum cannabinum</i>	20100	01003	00100	10111	11121	11230	11121	2{05}001	0
<i>Asclepias syriaca</i>	20100	01110	00121	10111	33021	10230	11310	30001	0
<i>Aspidosperma australe</i>	00101	00003	01000	00010	00021	11212	21021	00300	0
<i>Aspidosperma cylindrocarpon</i>	00101	00003	01000	00010	00021	11212	21021	00300	0
<i>Beaumontia grandiflora</i>	10100	01012	00100	10111	01032	11230	11021	24001	0
<i>Callichilia orientalis</i>	00100	01012	00000	10011	01011	10102	20021	00400	0
<i>Carissa macrocarpa</i>	01100	00012	00000	00010	00002	00302	21021	00000	0
<i>Carissa ovata</i>	0?100	00003	00100	00010	00002	00302	21021	00000	0
<i>Catharanthus roseus</i>	30100	01003	00000	00011	01021	11202	21021	05400	0
<i>Cerbera manghas</i>	00101	01003	10000	01011	00021	00302	21021	01001	0
<i>Cerbera venenifera</i>	00101	01003	10000	01011	00021	00302	21021	01001	0
<i>Chilocarpus costatus</i>	10100	01003	00000	00010	00000	11102	20021	1????	?
<i>Condylocarpon isthmicum</i>	10102	01003	00000	00010	00021	01202	20120	?????	?
<i>Couma guianensis</i>	00102	01003	00000	00010	00100	00002	21021	0????	?
<i>Craspidospermum verticillatum</i>	00102	01003	00000	00011	00000	11212	21121	2?300	0
<i>Diplorhynchus condylocarpon</i>	00100	01003	00000	00011	00121	11212	21021	00200	0
<i>Dyera costulata</i>	00102	01012	00000	00011	00121	11212	21021	0????	?
<i>Funtumia elastica</i>	00100	01010	00100	10111	01021	11231	01021	20010	0
<i>Geissospermum laeve</i>	00101	00003	01100	00010	00021	00002	21021	0?200	0
<i>Gonioma kamassi</i>	00102	01003	00000	00010	00021	11212	21021	01300	0
<i>Hancornia speciosa</i>	00100	01003	00000	00010	00000	00002	21021	00???	?
<i>Haplophyton crooksii</i>	00100	01003	01000	00011	00021	11231	11021	0?300	0
<i>Hunteria eburnea</i>	00100	00012	00000	00010	00021	00002	21021	00300	0
<i>Kametia chandeei</i>	10102	01003	00000	00010	00021	11212	21021	0????	?
<i>Kopsia fruticosa</i>	00100	01003	00100	00011	01021	00302	21021	02300	0
<i>Lacmellea aculeata</i>	00100	01003	00000	00010	00000	00002	21021	00???	?
<i>Mascarenhasia arborescens</i>	00100	01012	00100	10111	01021	11230	11021	200?0	0
<i>Melodinus australis</i>	10100	01003	00000	00010	00002	00002	21021	00300	0
<i>Melodinus conchinchinensis</i>	10100	01003	00000	00010	00002	00002	21021	00300	0
<i>Mesechites trifidus</i>	10100	01112	00100	10111	01021	11230	11021	2????	?
<i>Molongum laxum</i>	00100	01012	00000	10011	00000	00002	21021	00???	?
<i>Neisosperma poweri</i>	00102	01003	00100	00011	01021	00302	21021	01200	0
<i>Ochrosia elliptica</i>	00102	01003	00100	00011	01021	00302	21021	01200	0
<i>Odontadenia lutea</i>	10100	01010	00100	10111	01021	11230	11021	24????	?
<i>Oncinotis tenuiloba</i>	10100	00110	00100	10111	01121	11230	11021	20000	0
<i>Pachypodium lamerei</i>	00101	01003	00100	10111	01021	11230	11021	22001	0
<i>Parahancornia fasciculata</i>	00100	01003	00000	00010	00000	00002	21021	0????	?
<i>Parsonsia eucalyptophylla</i>	10110	01011	00100	10111	01032	11230	11021	22000	1
<i>Peltastes peltatus</i>	10110	01011	00100	10111	01021	11230	11021	2????	?
<i>Petopentia natalensis</i>	10100	01010	00110	00111	22121	11230	11221	20001	0
<i>Phyllanthera grayi</i>	10100	01010	00110	00111	22121	11230	11221	2????	?
<i>Picralima nitida</i>	00100	00003	00000	00010	00021	00002	21021	00200	0
<i>Pleiocarpa mutica</i>	00100	00003	00000	00010	00021	00002	21021	00300	0
<i>Plumeria cubensis</i>	00101	01003	00000	00011	00121	11212	21021	02100	0
<i>Prestonia quinqueangularis</i>	10110	01011	00100	10111	01021	11230	11021	25000	1
<i>Rauwolfia sellowii</i>	01102	01003	00000	00011	01012	00302	21021	00200	0
<i>Rauwolfia vomitoria</i>	01102	01003	00000	00011	01021	00302	21021	00200	0

Appendix 2. Continued.

	1	6	11	16	21	26	31	36	41
<i>Saba comorensis</i>	10100	11003	00000	00010	00000	00002	21021	00???	?
<i>Schizogygia coffaeoides</i>	00100	01012	00100	10011	01021	11102	20021	00300	0
<i>Secamone elliptica</i>	10100	01010	00120	10111	34021	11230	11300	30001	0
<i>Stemmadenia litoralis</i>	00100	01012	00000	10011	01021	10102	20021	00400	0
<i>Stephanostema stenocarpum</i>	00100	01010	00000	10111	00021	11231	01021	20???	?
<i>Strophanthus boivini</i>	00100	01010	00100	10111	00112	11231	11021	21001	0
<i>Tabernaemontana amygdalifolia</i>	00100	01012	00000	10011	00021	10102	21021	00400	0
<i>Tabernaemontana bufalina</i>	00100	01012	00000	00010	00021	10102	21021	00400	0
<i>Tabernaemontana catharinensis</i>	00100	01012	00000	10011	00021	10102	21021	00400	0
<i>Tabernaemontana pachysiphon</i>	00100	01012	00000	10011	00021	10102	21021	00400	0
<i>Telosma cordata</i>	10100	01010	00121	10111	33021	10230	11310	30001	0
<i>Thevetia peruviana</i>	00101	01012	10000	01011	01021	00302	21021	01001	0
<i>Tonduzia longifolia</i>	00102	01010	00000	00011	01021	11222	21021	0?200	0
<i>Trachelospermum jasminoides</i>	10100	01012	00100	10111	01021	11230	11021	21???	?
<i>Vinca minor</i>	10100	01003	00000	00011	01021	11202	21021	00300	0
<i>Willughbeia angustifolia</i>	10100	10003	00000	00010	00000	00002	21021	0?000	0
<i>Wrightia arborea</i>	00100	01012	00000	10111	00032	11231	01021	20010	0
<i>Gelsemium sempervirens</i>	10020	01003	00300	02002	40032	11212	21021	06200	0
<i>Gentiana verna</i>	20020	00003	00100	02002	41030	11202	21021	0?100	0
<i>Mitrasacme oasena</i>	30020	00003	00200	01002	40002	11202	21021	000??	?
<i>Strychnos lucida</i>	00020	00003	00200	00002	40002	00002	21021	00200	0
<i>Strychnos minor</i>	10020	10003	00200	00002	40002	00002	21021	00200	0
	1	6	11	16	21	26	31	36	41

APPENDIX 3. Characters and character states for the morphological matrix used in the parsimony analyses.

1. Habit

Trees and shrubs (0); lianas or vines (1); perennial herbs (2); annual herbs (3).

The species of *Strophanthus* used in this analysis is an erect shrub; however, many species of this genus are large lianas.

2. Sex expression

Plants with perfect flowers (0); plants functionally dioecious or gynodioecious (1).

Functional dioecy has been reported in *Rawolfia sellowii* and functional gynodioecy in *R. vomitoria* (Koch et al., 2002), coincidentally the two species of *Rawolfia* chosen for this study. The only other genus of Apocynaceae in which functionally male or female flowers have been reported is *Carissa*, which also includes both functionally dioecious (e.g., *C. macrocarpa*, Correll & Correll, 1982; included in this study) as well as functionally gynodioecious (e.g., *C. grandiflora*, Marloth, 1932: 61; Koch et al., 2002) species. In functionally dioecious and gynodioecious species of both *Rawolfia* and *Carissa*, the female plants have normal-appearing but sterile anthers, and the style is shorter than in the flowers of pollen-producing male plants.

3. Non-articulated laticifers

Absent (0); present (1).

Non-articulated laticifers are one of the key characters for the Apocynaceae s.l., distinguishing them from other families of the Gentianales.

4. Latex color

White, rarely yellow or orange (0); translucent (1).

Latex is typically white in Apocynaceae, but translucent latex is frequent within Echiteae, Wrightieae, and Malouetieae.

5. Phyllotaxis

Leaves opposite (0); leaves alternate (1); leaves mainly whorled, occasionally opposite at some nodes (2).

Opposite leaf arrangement is the usual condition in the family, with sporadic occurrence of other phyllotaxes. In the Alstonieae and Plumerieae, however, both alternate or whorled leaves are seen, and in Vinceae, whorled leaves are observed. Frequently in taxa with clearly whorled phyllotaxis, some nodes may have opposite leaves, especially in taxa with ternate phyllotaxis and in nodes closer to inflorescences. In such cases the taxon is coded as having a whorled phyllotaxis rather than being polymorphic.

6. Tendrils

Absent (0); present (1).

In Apocynaceae, hook-like tendrils are restricted to the Willughbeieae. The tendrils are modified inflorescences and may also bear flowers.

7. Colleters in petiole axils

Absent (0); present (1).

8. Colleters adaxially at the base of the leaf blade

Absent (0); present (1).

In Apocynaceae, colleters clustered or paired at the base of the leaf blade adaxially are common in the Asclepiadoideae and in some Secamonoideae and Periplocoideae. In Apocynaceae s. str., they are rare and characteristic for only two apocynoid clades: the Mesechiteae (Simões et al., 2004)

and the close-knit trio of genera in Apocynaceae subtribe Baisseinae sensu De Kruijff (1983).

9. Calycine colleters

Absent (0); present (1).

In the Apocynaceae s. str., the absence or presence of calycine colleters is mostly consistent within a genus and is thus often used to distinguish genera. In addition, the distribution pattern of colleters within the calyx can be a useful tool to identify specimens and predict relationships (Woodson, 1933; Omino, 1996; Middleton, 1999; Endress & Bruyns, 2000; Simões & Kinoshita, 2002).

10. Colleter arrangement within calyx

One–15, in the sepal sinuses at the base (0); five, centered at the base sepal (1); 15 or more, more or less in a continuous ring, sometimes in more than one row (2).

11. Infrastaminal appendages

Absent (0); present (1).

Infrastaminal appendages, first described by Pichon (1948b), are outgrowths of the lower (congenitally fused) part of the corolla tube in the staminal sectors and are found in Plumeriaceae (e.g., *Thevetia*, *Cerbera*). The outgrowths in the congenitally fused part of the corolla tube in *Apocynum* are in the alternistaminal sectors and are therefore not considered to be homologous to infrastaminal appendages (Nilsson et al., 1993).

12. Visible gaps in corolla tube

Absent (0); present (1).

In Apocynaceae, the lower corolla tube (composed of the united petal and stamen primordia) is congenitally fused; the upper portion fuses postgenitally and basipetally, so that the last region to fuse is just above the insertion of the stamens. In a few genera (e.g., *Pleiocarpa*, *Picalima*, *Nerium*), flowers reach anthesis before fusion of the upper corolla is complete, leaving epidermal remnants or even visible gaps (Nishino, 1982, 1983; Erbar, 1991). Large gaps, visible with the naked eye or under a stereoscope, are characteristic for some Rauvolfioideae, e.g., *Aspidosperma* (Woodson, 1951), *Geissospermum* and *Haplophyton* (Fallen, 1986), and *Stephanostegia* (Leeuwenberg, 1997).

13. Corolla lobe aestivation

Sinistrorsely convolute (0); dextrorsely convolute (1); valvate (2); quincuncial imbricate (3).

Corolla lobe aestivation is one of the key taxonomic characters in the Apocynaceae. With a few exceptions, petals are almost always sinistrorsely convolute in the Rauvolfioideae, whereas in the other four subfamilies they are normally dextrorsely convolute or, in certain assemblages (e.g., Ceropogonaceae), valvate. The genera *Alstonia* (Sidiyasa, 1998), *Geissospermum* (Fallen, 1986; Allorge & Poupat, 1991), *Haplophyton* (Williams, 1995), and *Carissa* (Leeuwenberg & van Dilst, 2001) are rare in the family in that they contain both dextrorsely and sinistrorsely convolute species.

14. Stamens

Adnate to the corolla tube (0); adnate to five thickened basal corona lobes (1); adnate to a completely fused basal tube (2).

In Rauvolfioideae and Apocynoideae, stamens are always adnate to the corolla tube. In the other subfamilies, the stamens are more commonly inserted on coronas. In the Periplocoideae and Secamonoideae, this is in the form of a basal corona of five free to nearly fused lobes (Nilsson et al., 1993; Endress & Bruyns, 2000).

15. Number of anther locules

4 (0); 2 (1).

The anthers have four locules in all subfamilies of Apocynaceae except the Asclepiadoideae, in which there has been a loss of the two dorsal locules (Kunze, 1996).

16. Lignified guide rails

Absent (0); present (1).

Lignified guide rails are a specialization of the lateral parts of the anther and have a function in the complex pollination mechanism in Apocynaceae (Schick, 1982; Fallen, 1986; Kunze, 1991, 1996; Moré et al., 2007, this issue); they are absent in most Rauvolfioideae, but characteristic for Apocynoideae. Lignified guide rails are also characteristic for the majority of the genera of the Tabernaemontaneae but are absent, however, in species of *Tabernaemontana* section *Ervatamia* (see Leeuwenberg, 1994b: xv).

17. Anther dehiscence

Introrse (0); latrorse (1); extrorse (2).

18. Gynostegium

Absent (0); present (1).

The presence of a gynostegium (anthers postgenitally united with the style-head) is a key character in the family and distinguishes the subfamilies of the APSA clade (Livshultz et al., 2007) from the Rauvolfioideae (Leeuwenberg, 1994a; Endress & Bruyns, 2000).

19. Style-head

Absent (0); present (1).

Diagnostic within Apocynaceae, the style-head is the product of the postgenital fusion of the apical parts of the carpels and is enlarged and specialized, with zones covered by a secretory epithelium (Schick, 1980; Endress et al., 1983; Fallen, 1986).

20. Style-head body differentiation

Body of style-head with epidermis not clearly differentiated into regions specialized for pollen transport adhesive production and for receptive function (0); body of style-head with epidermis clearly differentiated into distinct regions specialized for the production of pollen transport adhesive or for receptive function (1).

Typically in Apocynaceae, the uppermost parts of the carpels do not fuse, resulting in two free apices at the top of the style-head. Schick (1980, 1982) demonstrated that these free appendages are sterile and that the receptive region of the style-head is typically at the base, often beneath a membranous collar or wreath of longer hairs. In some Rauvolfioideae (e.g., *Picalima*, *Alyxia*), the style-head has a simple shape, usually ovoid or globular, without collar or wreath, and is not differentiated into distinct zones. In these genera, except for the sterile free apices, the entire surface of the style-head is covered with an epithelium that functions both as stigma and for the production of pollen transport adhesive (Schick, 1980; Endress et al., 1996).

21. Pollen transport adhesive

A foamy adhesive (0); five amorphous sticky translators via desiccation (1); five firm differentiated translators with spoon and viscidium (2); five firm differentiated translators with corpusculum and caudicles (3); absent (4).

Translators are composed of secretions of the style-head that harden through desiccation and form the apparatus for the transport of pollen. Complex, functionally differentiated translators are found in all Periplocoideae, Secamonoideae, and Asclepiadoideae (Kunze, 1993). Simple, functionally uniform translators have been reported in *Apocynum* and *Forsteronia* of Apocynoideae (Nilsson et al., 1993).

22. Nectar secreting tissue

Absent or adnate and indistinct on outer wall of ovary or stipe (0); on lobes around ovary, these sometimes fused into an annulus (1); in the alternistaminal sectors on the sides of between the basal corona lobes (2); in alternistaminal pockets on the basal tube (3).

Nectaries are absent in many Rauvolfioideae; they are present as lobes surrounding the base of the ovary in most

Apocynoideae. In Periplocoideae, Secamonoideae, and Asclepiadoideae, nectar is secreted in alternistaminal pockets (Endress & Bruyns, 2000).

23. Ovary position

Superior (0); half-inferior (1); inferior (2).

24. Ovary fusion

Congenitally syncarpous (0); congenitally syncarpous to about halfway, apocarpous above (1); apocarpous (2); postgenitally syncarpous (3).

Postgenitally syncarpous ovaries are apocarpous in origin but fuse early during development. Postgenital syncarpy in Apocynaceae results most commonly in so-called double follicles, as found in *Parsonsia*, *Thenardia* Kunth, or *Temnadenia* Miers; the capsular fruit of *Allamanda* is also postgenitally syncarpous (Fallen, 1985).

25. Placentation

Parietal (0); marginal (1); axile (2).

26. Fruit dehiscence

Indehiscent (0); dehiscent (1).

27. Pericarp consistency

Fleshy (0); dry or woody (1).

28. Seed presentation

Embedded in pulp (0); with aril (1); naked (2); surrounded by a lignified or sclerified stone (3).

In most cases in which the seed is enclosed within a lignified or sclerified envelope, this is formed by the endocarp. The genera of the Carisseae present a special situation, in which the seed is surrounded by the lignified placentas, and thus not embedded in pulp, although the fruits of this tribe are fleshy berries (Pichon, 1952).

29. Seed margin

Naked (0); winged (1); fimbriate or ciliate (2); comose (3).

In *Craspidospermum*, the seed margin is surrounded by unusual-shaped, flattened appendages (Leeuwenberg, 1997: 14), which are here interpreted as a special kind of dissected wing and are not homologous with the fimbria or cilia found around the margin of the seed in, for example, *Alstonia* or *Tonduzia* (Sidiyasa, 1998, fig. 2.11). The coma is a terminal tuft of hairs on a seed and is thus not considered to be homologous to hairs, cilia, or fimbria surrounding the seed margin.

30. Chalazal coma

Absent (0); present (1).

Imprecise terminology, such as, "coma apical," with reference to a coma present at either end of the seed, has led to considerable confusion (see *Funtumia* in Sennblad et al., 1998). In the Apocynaceae, chalazal comas are on the end of the seed directed toward the base of the fruit, in contrast to the micropylar comas, which are on the end of the seed directed toward the apex of the fruit. The orientation of the coma hairs is irrelevant in determining whether a coma is chalazal or micropylar. For example, in *Kibatalia* G. Don, the seeds have a long-rostrate coma on the end of the seed directed toward the base of the fruit; the long hairs of the coma itself, however, are bent backward toward the fruit apex. Despite the direction of the hairs, *Kibatalia* is considered to have a basal coma due to its position on the seed in relation to the fruit. Due to difficulties in determining homology, chalazal and micropylar comas are coded as two separate characters. Chalazal comas are found only in the two tribes at the base of Apocynoideae: Wrightieae and Malouetieae. In some genera, only a chalazal coma is present, whereas in others, the seeds may have a coma at both the chalazal and micropylar ends, often with the micropylar one being the more robust and the chalazal one early deciduous. In other Apocynoideae and in all Periplocoideae, Secamonoideae, and Asclepiadoideae, the coma is

exclusively micropylar, except for a few rare instances where it has been secondarily lost, usually in conjunction with a shift to water dispersal (e.g., several species of *Malouetia*, Apocynoideae (Woodson, 1933); *Finlaysonia obovata* Wall., Periplocoideae (Venter & Verhoeven, 2001); *Marsdenia rubrofusca* Benth. ex Fourn. and *Matelea amazonica* Morillo, Asclepiadoideae (Morillo, 1978, 1997).

31. Micropylar coma

Absent (0); present (1).

32. Endosperm

Ruminate (0); not ruminate (1).

Ruminate endosperm, though relatively rare in Apocynaceae, is characteristic for *Tabernaemontana* and is also found in most genera of the Alyxieae (Endress et al., 2007).

33. Pollen dispersal unit

Monads (0); tetrads without thinner inner walls (1); tetrads with thinner inner walls (2); pollinia (3).

In Rauvolfioideae and Apocynoideae, pollen is normally in monads. In Apocynoideae, only *Apocynum* has pollen in tetrads, whereas in Rauvolfioideae, tetrads occur sporadically in a few genera: *Callichilia*, *Condyllocarpon*, *Craspidospermum*, and some *Melodinus* (Erdtman, 1952; Lienau et al., 1986). Tetrads are characteristic for the Periplocoideae, but these differ from those in Apocynoideae and Rauvolfioideae by their thinner inner walls (Nilsson et al., 1993).

34. Sporopollenin layer covering pollinium

Absent (0); present (1).

In Secamonoideae and a few genera of Periplocoideae, all tetrads of a theca form a pollinium. The pollinia in these taxa differ from those in the Asclepiadoideae in that they lack an outer sporopollenin layer (Verhoeven & Venter, 2001).

35. Pollen apertures

Absent (0); present (1).

In Asclepiadoideae and Secamonoideae, pollen grains within the pollinia are inaperturate. Pollen is aperturate in all other subfamilies with the exception of *Condyllocarpon* (Rauvolfioideae, Alyxieae), which has inaperturate, nearly exineless pollen grains that remain in tetrads (van der Ham et al., 2001).

36. Aperture type

Colporate (0); porate with large (6–22 μm), irregularly disposed and \pm unequal-sized pores (1); porate with small (2–5 μm), regularly disposed and equal-sized pores (2).

Apocynoideae, Periplocoideae, Secamonoideae, and *Craspidospermum* (Melodineae) are characterized by having porate pollen grains. In Rauvolfioideae, in contrast, pollen grains are almost always colporate (Nilsson, 1986). Within Rauvolfioideae, several genera of the Alyxieae, however, have porate pollen; this pollen differs from the porate pollen in the other subfamilies in that the pores are of varying sizes, often very large (up to 86 μm), and are not arranged in a regular pattern on the grain (van der Ham et al., 2001).

37. Chromosome number

$x = 11$ (0); $x = 10$ (1); $x = 9$ (2); $x = 6$ (3); $x = 12$ (4); $x = 8$ (5); $x = 4$ (6).

38. Secoiridoids and complex indole alkaloids

Absent (0); secoiridoids present, indole alkaloids absent (1); characteristic indole alkaloids present, these with secologanin skeleton non re-arranged (2); characteristic indole alkaloids present, including those with re-arranged secologanin skeleton of the eburnan and/or plumeran type (3); characteristic indole alkaloids present, including those with re-arranged secologanin part of the ibogan type (4).

An estimated 1600 different structural representatives of alkaloids have been isolated in Apocynaceae, which are classified by their biogenetic relationship. Of these, complex indole alkaloids are restricted to Rauvolfioideae and are

categorized by their skeletal type (Kisakürek et al., 1983). Here we used a slightly simplified version of this system, adapted for our taxon sampling.

39. Steroidal alkaloids

Absent (0); present (1).

Steroidal alkaloids do not occur in Rauvolfioideae. They seem to be most common in the Malouetieae of the Apocynoideae (Endress et al., 1990; Bisset, 1992).

40. Cardenolides

Absent (0); present (1).

Cardenolides are found sporadically in various parts of the family. In Rauvolfioideae, they are found, for example, in *Acokanthera* (Carisseae), *Thevetia*, and *Cerbera* (Plumerieae) (Hegnauer, 1970, 1989).

41. Pyrrolizidine alkaloids

Absent (0); present (1).

Pyrrolizidine alkaloids have been reported from various genera of Apocynoideae, particularly *Parsonsia* and *Prestonia* of the tribe Echiteae (Brehm et al., 2007, this issue).

APPENDIX 4. Revised tribal classification of Rauvolfioideae. Genera transferred to tribes different from those in the classification of Endress and Bruyns (2000) are indicated in bold. A description of each tribe is provided.

RAUVOLFIOIDEAE Kostel., Allg. Med.-Pharm. Fl. 3: 1054. 1834. TYPE: *Rauwolfia* L., Sp. Pl. 1: 208. 1753.

ASPIDOSPERMEAE Miers, Apocyn. S. Amer. 7. 1878. TYPE: *Aspidosperma* Mart. & Zucc., Flora 7 (Beil. 4): 135. 1824, nom. cons.

Trees or shrubs. Latex usually milky, but sometimes red or yellow in *Aspidosperma*. Leaves alternate or opposite, rarely whorled. Calyx 5-, rarely 4-, 6-, or 7-parted, calycine colleters absent; corolla salverform or somewhat funnelliform in *Haplophyton*, with slits in the tube behind the stamens, corolla lobe aestivation sinistrorsely convolute (dextrorsely convolute in *Geissospermum* and one species of *Haplophyton*); corona absent; style-head body mostly undifferentiated, concomitantly receptive and secretory, with a short basal collar in *Haplophyton*; ovary apocarpous; disc absent. Fruit normally with dry pericarp and dehiscent, a pair of follicles, or sometimes one aborted, but fruit fleshy in *Geissospermum* and *Vallesia*. Seeds various: thin and compressed with papery wing in *Aspidosperma* and *Strepeliopsis*, or not compressed and with coma at both ends (*Haplophyton*), or naked (*Microplumeria*, *Vallesia*). Pollen 3-colporate. Secondary chemistry indole alkaloids.

Aspidosperma Mart. & Zucc., Flora 7 (Beil. 4): 135. 1824, nom. cons.

Geissospermum Allemão, Pl. Nov. Bras. 707. 1846.

Haplophyton A. DC., Prodr. 8: 412. 1844.

Microplumeria Baill., Bull. Mens. Soc. Linn. Paris 1: 749. 1889.

Strepeliopsis Benth. in Benth. & Hook. f., Gen. Pl. 2: 702. 1876.

Vallesia Ruiz & Pav., Fl. Peruv. Chil. Prodr. 28. 1794.

ALSTONIEAE G. Don, Gen. Hist. 4: 70, 86. 1838. TYPE: *Alstonia* R. Br., Asclepiadeae 64. 1810, nom. cons.

Trees or shrubs with milky latex. Leaves whorled or opposite. Flowers mostly small, salverform to tubular (rotate in *Dyera*); calycine colleters absent except in *Dyera*; corolla lobe aestivation either sinistrorse or dextrorse; corona absent; anthers normally ovate, unspecialized (connective with an apical extension in *Dyera*); style-head usually without a basal collar; ovary apocarpous, or partially syncarpous at the base and apocarpous above (completely syncarpous in *Alstonia*

rostrata) and more than half inferior in *Dyera*; disc absent, adnate, or inconspicuous, (some species of *Alstonia* with a distinct annular disc). Fruit with dry pericarp and dehiscent, a pair of follicles or these rarely fused; ovules numerous per carpel. Seeds thin, compressed, testa covered with long hairs (*Alstonia*) or surrounded by a papery wing (*Dyera*). Pollen 3-colporate. Secondary chemistry indole alkaloids.

Alstonia R. Br., Asclepiadeae 64. 1810, nom. cons.

Dyera Hook. f., J. Linn. Soc. Bot. 19: 293. 1882.

VINCEAE Duby, Bot. Gall. 1: 324. 1828. TYPE: *Vinca* L., Sp. Pl. 1: 209. 1753.

Trees or shrubs, more rarely lianas, vines or herbs, with milky latex. Leaves whorled or opposite, rarely alternate. Calycine colleters absent; corolla salverform (infundibuliform in *Vinca*); free corona lobes absent, but the orifice sometimes with a thickened ring; corolla lobe aestivation usually sinistrorse (dextrorse in *Kopsia*, *Neisosperma*, and *Ochrosia*); stamens inserted at the middle of the corolla tube or above, included; anthers ovate, without lignified basal appendages, unspecialized (except in *Vinca*), filaments almost always short (except in *Vinca*); style-head usually with the stigmatic region beneath a basal (sometimes membranous) collar; ovary apocarpous (in some *Rauwolfia* species hemi-syncarpous), usually glabrous, often only one carpel maturing; disc normally present. Fruit composed of 2 separate mericarps (sometimes only one developing), these either indehiscent, drupaceous, with a fleshy pericarp and stony endocarp, or with a pair of thin-walled follicles; seeds 1 to 4(to 6) per carpel, rarely more, ovoid or compressed, mostly not winged (the flat margins forming a narrow rim in *Kopsia*, *Ochrosia*, and *Neisosperma*); in *Tonduzia* and *Laxoplumeria* seeds numerous, elliptic-compressed with long cilia around the margin; endocarp mostly forming a stone. Pollen mostly 3-colporate. Secondary compounds indole alkaloids.

Catharanthus G. Don, Gen. Hist. 4(1): 95. 1837.

Kametia Kostel., Allg. Med.-Pharm. Fl. 3: 1062. 1834.

Kopsia Blume, Cat. Gew. Buitenzorg (Blume) 12. 1823, nom. cons.

Laxoplumeria Markgr., Notizbl. Bot. Gart. Berlin-Dahlem 9: 981. 1926.

Ochrosia Juss., Gen. Pl. 144. 1789.

Petchia Livera, Ann. Roy. Bot. Gard. (Peradeniya) 10: 140. 1926.

Rauwolfia L., Sp. Pl. 1: 208. 1753.

Tonduzia Pittier, Contr. U.S. Natl. Herb. 12: 103. 1908.

Vinca L., Sp. Pl. 1: 209. 1753.

WILLUGHBEIEAE A. DC., Prodr. 8: 318. 1844. TYPE: *Willughbeia* Roxb., Pl. Coromandel 3: 77, t. 280. 1819, nom. cons.

Trees, shrubs, or woody lianas, the last often with grappling tendrils, with milky latex. Leaves opposite (verticillate in *Couma*); calycine colleters present or absent; corolla salverform; corolline corona absent; corolla lobe aestivation almost always sinistrorse; stamens included; apical connective appendages of anthers often scarcely developed; style-head usually without basal collar, sometimes body uniformly receptive; ovary congenitally syncarpous; disc mostly absent, when present adnate or indistinct from ovary. Fruit indehiscent, a berry with fleshy, non-fibrous pericarp and placentas becoming pulpy in fruit; seeds ovoid, embedded in pulp, with or without a longitudinal furrow; endosperm smooth or wrinkled longitudinally. Pollen (1 to)3(to 4)-colporate. Secondary chemistry indole alkaloids.

- Ancylotryps* Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 91. 1898.
- Bousigonia* Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 35. 1898.
- Chamaecitandra* (Stapf) Pichon, Mém. Inst. Franç. Afrique Noire 35: 202. 1953.
- Clitandra* Benth. in Hook., Niger Fl. 445. 1849.
- Couma* Aubl., Hist. Pl. Guiane Suppl. 39, t. 392. 1775.
- Cyclocotyla* Stapf, Bull. Misc. Inform. 1908: 259. 1908.
- Cylindropsis* Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 38. 1898.
- Dictyophleba* Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 92. 1898.
- Hancornia* Gomes, Mem. Math. Phis. Acad. Real Sci. Lisboa 3: 51. 1812.
- Lacmellea* H. Karst., Linnaea 28: 449. 1857.
- Landolphia* P. Beauv., Fl. Oware Bénin 1: 54. 1804, nom. cons.
- Leuconotis* Jack, Trans. Linn. Soc. 14: 121. 1823.
- Orthopichonia* H. Huber, Kew Bull. 15: 437. 1962.
- Pacouria* Aubl., Hist. Pl. Guiane 268, t. 105. 1775.
- Parahancornia* Ducke, Arch. Jard. Bot. Rio de Janeiro 3: 242. 1922.
- Saba* (Pichon) Pichon, Mém. Inst. Franç. Afrique Noire 35: 302. 1953.
- Vahadenia* Stapf in Thiselton-Dyer, Fl. Trop. Africa 4(1): 29. 1902.
- Willughbeia* Roxb., Pl. Coromandel 3: 77, t. 280. 1819, nom. cons.
- TABERNAEMONTANEAE** G. Don, Gen. Hist. 4: 70, 87. 1838. TYPE: *Tabernaemontana* L., Sp. Pl. 1: 210. 1753.
- Trees or shrubs, rarely lianas, with milky latex. Leaves opposite, sometimes anisophyllous. Inflorescences axillary, terminal, or (usually paired) in the forks of branches; calycine colleters mostly present and centered on the lower part of the sepal, often multiseriate, rarely absent, very rarely (*Tabernanthe*) alternisepalous; corolla usually salverform, less often tubular-campanulate or infundibuliform; corona absent; corolla lobe aestivation almost always sinistrorse (dextrorse in *Schizogygia*, 1 species of *Callichilia*, and 2 species of *Ervatamia*); stamens mostly sessile or nearly so, anthers mostly with lignified guide-rails (these absent in *Ervatamia*); style-head with the stigmatic region beneath a basal flange and usually with an (often five-ribbed) upper crest or the style-head subglobose and without a basal collar and upper crest and body uniformly receptive; ovary syncarpous or apocarpous; disc surrounding the base of the ovary, adnate, indistinct, or free. Fruit with fleshy pericarp, usually an indehiscent berry with the seeds embedded in pulp or pair of dehiscent follicles with arillate seeds; seed testa often wrinkled, pitted, or with longitudinal ridges, often with a long hilar groove. Pollen 3- to 5-colporate, sometimes zono-colporate; in *Callichilia* in tetrads. Secondary chemistry highly evolved indole alkaloids of the heynean type.
- Ambelania* Aubl., Hist. Pl. Guiane 265, t. 104. 1775.
- Bonafousia* A. DC., Prodr. 8: 359. 1844.
- Callichilia* Stapf in Thiselton-Dyer, Fl. Trop. Afr. 4(1): 130. 1902.
- Calocrater* K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 175. 1895.
- Carvalhoa* K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 189. 1895.
- Crioceras* Pierre, Bull. Mens. Soc. Linn. Paris 1: 1311. 1897.
- Macoubea* Aubl., Hist. Pl. Guiane Suppl. 17, t. 378. 1775.
- Molongum* Pichon, Mém. Mus. Natl. Hist. Nat. 24: 167. 1948.
- Mucoa* Zarucchi, Agric. Univ. Wageningen Pap. 87(1): 40. 1988.
- Neocouma* Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 33. 1898.
- Rhigospira* Miers, Apocyn. S. Am. 67, t. 10A. 1878.
- Schizogygia* Baill., Bull. Mens. Soc. Linn. Paris 1: 752. 1888.
- Spongiosperma* Zarucchi, Agric. Univ. Wageningen Pap. 87(1): 48. 1988.
- Stemmadenia* Benth., Bot. Voyage Sulphur 124, t. 44. 1845.
- Stenosolen* (Müll. Arg.) Markgr. in Pulle, Fl. Suriname 4(1): 455. 1937.
- Tabernaemontana* L., Sp. Pl. 1: 210. 1753.
- Tabernanthe* Baill., Bull. Mens. Soc. Linn. Paris 1: 782. 1888.
- Voacanga* Thouars, Gen. Nov. Madag. 10. 1806.
- Woytkowskia* Woodson, Ann. Missouri Bot. Gard. 47: 74. 1960.
- MELODINEAE** G. Don, Gen. Hist. 4: 71, 101. 1838. TYPE: *Melodinus* J. R. Forst. & G. Forst., Char. Gen. Pl.: 37, t. 19. 1776.
- Trees or shrubs; latex milky. Leaves opposite or whorled. Calycine colleters usually absent; corolla salverform, sometimes unfused (with gaps) just above the insertion of the stamens; corolla lobe aestivation sinistrorse; small corolline corona often present in petal sinuses (well developed and usually annular and petaloid in *Melodinus*); stamens included, anther connective with both apical connective extension and small sterile basal lobes in *Pycnobotrya*; style-head mostly without basal collar or upper wreath; ovary apocarpous or congenitally syncarpous; disc absent or an adnate, indistinct annulus around the base of the ovary. Fruit mostly a pair of follicles with dry pericarp (fruit a capsule in *Craspidospermum* and a fleshy berry in *Melodinus*); seeds usually numerous, smooth, flat and winged, often peltately attached with a long funicle (somewhat verrucose, only slightly compressed and embedded in pulpy placenta in *Melodinus*). Pollen usually 3-colporate (in tetrads in some *Melodinus* species, and porate tetrads in *Craspidospermum*). Secondary compounds indole alkaloids.
- Craspidospermum* Bojer ex A. DC., Prodr. 8: 323. 1844.
- Melodinus* J. R. Forst. & G. Forst., Char. Gen. Pl.: 37, t. 19. 1776.
- Pycnobotrya* Benth. in Benth. & Hook. f., Gen. Pl. 2: 715. 1876.
- Stephanostegia* Baill., Bull. Mens. Soc. Linn. Paris 1: 748. 1888.
- ALYXIEAE** G. Don, Gen. Syst. 4: 70, 96. 1838. TYPE: *Alyxia* Banks ex R. Br., Prodr. 469. 1810, nom. cons.
- Trees, shrubs, or vines, with milky latex. Leaves often whorled, sometimes opposite, rarely alternate. Calycine colleters absent; corolla salverform (sometimes throat somewhat expanded in *Condylocarpon*); corolla lobes inflexed in bud or not; aestivation sinistrorse in bud; corona absent; stamens inserted in the upper half of the corolla tube, included; filaments short; anthers ovate, unspecialized; style-head without basal collar or upper wreath, body uniformly receptive; ovary apocarpous or syncarpous, normally bicarpellate with axile or marginal placentation (up to 5-carpellate in *Lepinia* and *Lepiniopsis*, and in *Chilocarpus* placentation parietal), often stipitate; disc absent, indistinct, or adnate. Fruit usually indehiscent (dehiscent in *Chilocarpus* and *Plectanea*), with fleshy pericarp and stony endocarp (pericarp dry in *Condylocarpon*, *Chilocarpus*, and *Plectanea*); seeds usually globular or ovoid, with deep hilar groove or depression (seeds arillate in *Chilocarpus* and compressed with wings and in *Plectanea*). Pollen 2- to 3-porate (mostly

barrel-shaped with two pores, but in tetrads in *Condylocarpon*). Neither alkaloids nor cardenolides are known to occur in the tribe.

- Alyxia* Banks ex R. Br., Prodr. 469. 1810, nom. cons.
Chilocarpus Blume, Cat. Gew. Buitenzorg (Blume) 22. 1823.
Condylocarpon Desf., Mém. Mus. Nat. 8: 119. 1822.
Lepinia Decne., Ann. Sci. Nat., Bot. sér. 3, 12: 194, t. 9. 1849.
Lepiniopsis Valetton, Ann. Jard. Bot. Buitenzorg 12: 251. 1895.
Plectaneaia Thouars, Gen. Nov. Madag. 11. 1806.
Pteralyxia K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 151. 1895.

HUNTERIEAE Miers, Apocyn. S. Amer. 6. 1878. TYPE: *Hunteria* Roxb., Fl. Indica 2: 531. 1824.

Trees or shrubs, rarely lianas, with milky latex. Leaves opposite, rarely in whorls of 3. Calycine colleters mostly present and centered at the base of the sepal, often multiseriate, rarely absent; corolla salverform; aestivation sinistorse; corona absent; anthers ovate, without lignified guide-rails, sometimes shortly sagittate at the base, included; style-head ovoid to narrowly clavate, without basal collar or upper crest, but often with slender, elongate non-receptive apices; ovary apocarpous, 2- to 5-carpellate, glabrous; disc an adnate or indistinct annulus around the base of the ovary. Fruit indehiscent, composed of 2 to 5 baccate mericarps with fleshy, fibrous pericarp; seeds ovoid, without longitudinal furrow, embedded in pulp. Pollen 3-colporate. Secondary chemistry indole alkaloids.

- Gonioma* E. Mey., Comment. Pl. Africae Austr. 188. 1838.
Hunteria Roxb., Fl. Indica 2: 531. 1824.
Picalima Pierre, Bull. Mens. Soc. Linn. Paris 2: 1278. 1896.
Pleiocarpa Benth. in Benth. & Hook. f., Gen. Pl. 2: 699. 1876.

PLUMERIEAE E. Mey., Comm. Pl. Africae Austr. 2: 188. 1838. TYPE: *Plumeria* L., Sp. Pl. 1: 209. 1753.

Trees or shrubs, the latter sometimes scrambling, with milky latex. Leaves usually alternate, sometimes opposite (whorled in *Allamanda*). Calycine colleters absent or present; corolla salverform, infundibuliform, or tubular-campanulate; corolline corona often present below the petal sinuses behind stamen (usually much dissected in *Allamanda*) and infrastaminal appendages generally present; aestivation sinistorse; anther connective often broadened, resulting in sublateral dehiscence and with an elongate apical extension, these often exerted, anthers otherwise included; style-head mostly with basal collar or lobes (no distinct basal collar in *Plumeria*, *Himatanthus*, and *Mortoniella*), mostly without upper wreath (well-developed upper wreath present in *Allamanda*), but often with free apices conspicuously enlarged; ovary apocarpous or syncarpous at the very base (postgenitally syncarpous in *Allamanda*), hemi-inferior in *Plumeria*, *Himatanthus*, and *Mortoniella*; disc absent or present. Fruit either indehiscent, a pair of drupes or samaroids with fleshy or dry pericarp or dehiscent and a pair of follicles (in *Allamanda* fruit a capsule, usually with

spines); seeds 1 to 4 (to many) per carpel, usually with compressed winglike margin or distinct papery wing. Pollen 3-colporate. Secondary compounds cardenolides or iridoid glycosides (in *Skytanthus*, *Skytanthus* alkaloids).

- Allamanda* L., Mant. 146: 214. 1771.
Anechites Griseb., Fl. Brit. W.I. (Grisebach) 410. 1861.
Cameraria L., Sp. Pl. 1: 210. 1753.
Cerbera L., Sp. Pl. 1: 208. 1753.
Cerberiopsis Vieill. ex Pancher & Sébert in Sébert, Not. Bois. Nov. Caledonie 187. 1874.
Himatanthus Willd. ex Schult. in Roem. & Schult., Syst. Veg. 5: 221. 1819.
Mortoniella Woodson, Ann. Missouri Bot. Gard. 26: 257. 1939.
Plumeria L., Sp. Pl. 1: 209. 1753.
Skytanthus Meyen, Reise 1: 376. 1834.
Thevetia L., Opera Varia 212. 1758, nom. cons.

CARISSEAE Dumort., Anal. Fam. Pl. 26. 1829. TYPE: *Carissa* L., Mant. 52. 1767, nom. cons.

Shrubs or small trees, rarely scandent, with or without (branched) spines in leaf axils, with milky latex. Leaves opposite, less frequently verticillate. Inflorescences usually axillary, cymose, mostly very contracted, \pm umbelliform; calycine colleters almost always absent; corolla salverform, usually white or cream, sometimes flushed with pink, the lobes not inflexed in bud; aestivation sinistorse or dextrorse; corona absent; stamens inserted at the middle of the corolla tube or above; anthers free from the style-head, ovate, unspecialized; style-head scarcely differentiated, without basal collar, body uniformly receptive; ovary congenitally syncarpous, bilocular, usually glabrous; placentation axile; disc absent or an indistinct annulus adnate to the base of the ovary. Fruit indehiscent, a berry with fleshy, non-fibrous, non-sclerotic pericarp; endocarp not stony, but placentas becoming indurated in fruit forming pseudo-stone; seeds 2 to 6 (to 12), rarely more, compressed, without longitudinal furrow. Pollen 3-colporate. Secondary chemistry cardenolides.

- Acokanthera* G. Don, Gen. Hist. 4(2): 485. 1838.
Carissa L., Mant. 52. 1767, nom. cons.

INCERTAE SEDIS WITHIN RAUVOLFOIDEAE

Amsonia Walter, Fl. Carol. 98. 1788.

Amsonia has long been considered related to taxa belonging to Vinceae sensu Endress and Bruyns (2000). Although in our analysis it is clearly unrelated to Vinceae, its affinities are uncertain. A sister relationship to Alyxieae is suggested, but this is only moderately supported and without substantiating morphological evidence.

Diplorhynchus Welw. ex Ficalho & Hiern, Trans. Linn. Soc. London, Bot. ser. 2, 2: 22, t. 5. 1881.

Diplorhynchus was included in the Melodineae sensu Endress and Bruyns (2000). Due to the unresolved placement of this genus in our analysis, we feel it is most prudent to place it as incertae sedis until more data become available.