
Volume 94
Number 1
2007

MISSOURI BOTANICAL

MAY 1 1 2007

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Annals
of the
Missouri
Botanical
Garden



A PHYLOGENETIC ANALYSIS OF
ALYXIEAE (APOCYNACEAE)
BASED ON *RBCL*, *MATK*, *TRNL*
INTRON, *TRNL-F* SPACER
SEQUENCES, AND
MORPHOLOGICAL CHARACTERS¹

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ABSTRACT

Within Rauvolfioideae (Apocynaceae), genera have long been assigned to tribes based mainly on only one or two superficial fruit and seed characters. Taxa with drupaceous fruits were included in Alyxieae. To elucidate relationships within Alyxieae, we analyzed phylogenetically a data set of sequences from four plastid DNA regions (*rbcL*, *matK*, *trnL* intron, and *trnL-F* intergenic spacer) and a morphological data set for 33 genera of Apocynaceae, including representatives of all genera previously included in Alyxieae and two non-Apocynaceae species. Results of parsimony analysis indicate that Alyxieae as previously delimited are polyphyletic, with most genera falling into two main clades. The *Alyxia* clade includes seven genera: *Alyxia* Banks ex R. Br., *Lepinia* Decne., *Lepiniopsis* Valetton, *Pteralyxia* K. Schum., and *Condylocarpon* Desf. together with *Plectaneia* Thouars. (earlier included in Plumerieae) and *Chilocarpus* Blume (earlier included in Chilocarpeae). The *Vinca* clade includes eight genera: *Cabucala* Pichon, *Petchia* Livera, *Rauwolfia* L., *Catharanthus* G. Don, *Vinca* L., *Neisosperma* Raf., *Ochrosia* Juss., and *Kopsia* Blume. *Vallesia* Ruiz & Pav. and *Anechites* Griseb. are not related to either clade and come out as sister to *Aspidosperma* Mart. & Zucc. (Aspidospermeae) and *Thevetia* L. (Plumerieae), respectively. The fruit and seed

¹We wish to thank the following persons who provided plant material or DNA samples: A. Assi, Paul Berry, F. Billiet, Birgitta Bremer, V. Ferreira, P. Garnock-Jones, P. Kessler, I. Koch, A. Leeuwenberg, S. Liede, D. Neill, R. Omlor, H. Petignat, M. Prévost, G. Romero, A. Specht, S. Tucker, and S. Zona. For technical assistance and photographic help with the pollen contribution sincere thanks are due to Elisabeth Grafström and Magnus Hellbom, Palynological Laboratory, Swedish Museum of Natural History, Stockholm, and Bertie Joan van Heuven and Wim Star, Nationaal Herbarium Nederland, Leiden. This study was supported by a grant from the Helge Ax:son Johnson Foundation and the Swedish Foundation for International Cooperation Research (STINT) to B. Sennblad.

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[†]Siwert Nilsson passed away unexpectedly before the manuscript was completed. We lost both an excellent collaborator and a dear friend. We dedicate this paper to him.

characters previously used to demarcate Alyxieae are homoplasious, as are other morphological characters such as style head structure and syncarpy versus apocarpy. Conversely, pollen morphology, which has not previously played much of a role in tribal delimitation, was shown to be the most useful morphological character for delimiting Alyxieae from other tribes of Rauvolfioideae.

Key words: Alyxieae, Apocynaceae, classification, *matK*, morphology, phylogeny, pollen, *rbcL*, systematics, *trnL*, *trnL-F*.

Tribal delimitation in Rauvolfioideae (usually referred to as Plumerioideae in the older literature) has previously been based on fruit and seed characters (Schumann, 1895; Pichon, 1949a, 1949c; L y, 1986; Leeuwenberg, 1994a). One reason that fruit and seed characters have been so frequently used in classifications and keys is that they are readily observed, permitting easy recognition of many genera. The other main reason for the fruit-based tribal classifications is that the flowers of many Rauvolfioideae tend to be superficially similar. Many are relatively small with a whitish, salverform corolla, and there are few obvious distinguishing floral characters useful for differentiating tribes in this subfamily. The most detailed studies of Rauvolfioideae were those of Pichon (1948a, 1948b, 1949a, 1950b), who published extensively on the family. His classification was a great improvement over its predecessors. Its main weakness was that tribal delimitation was based mainly on a single fruit character. He split the rauvolfioid tribes into two main groups, depending on whether the deeper layers of the mesocarp were fleshy or dry. The group with a fleshy inner mesocarp was divided into five tribes: Carisseae, Ambelanieae, and Macoubeeae comprised those taxa with indehiscent berries and Chilocarpeae and Tabernaemontaneae included those taxa with fruit consisting of dehiscent follicles with arillate seeds. In the group with a dry mesocarp, he recognized three tribes. Two of them are characterized by dehiscent fruits: Alstonieae (Plumerieae sensu Leeuwenberg, 1994a), in which the fruit consists of a pair of follicles, and the monotypic Allamandaeae, in which the fruit is a spiny unilocular capsule. The last tribe, Rauvolfieae (Alyxieae sensu Leeuwenberg, 1994a), contained all taxa in which the fruit is an indehiscent drupe with a stony endocarp. It is the relationships within this group that are the focus of this paper.

Pichon (1949a) recognized five subtribes within his Rauvolfieae: Rauvolfinae (including *Cabucala* Pichon, *Petchia* Livera, *Rauvolfia* L., and *Podochrosia* Baill.), Alyxiinae (including *Alyxia* Banks ex R. Br., *Lepinia* Decne., and *Lepiniopsis* Valetton), Ochrosiinae (including only *Ochrosia* Juss.), Vallesiinae (including *Vallesia* Ruiz & Pav. and *Kopsia* Blume), and Condylocarpaceae (including *Rhipidia* Markgr. and *Condylocarpon* Desf.). Pichon included two genera as incertae sedis: *Anechites* Griseb. and *Notonerium*

Benth. *Notonerium* has since been shown to belong to Boraginaceae (Crisp, 1983).

In his classification from 1994a, Leeuwenberg maintained Pichon's (1949a) circumscription of Rauvolfieae as well as the five subtribes included there but gave no insight into the delimitation of the subtribes, stating only that the relatively slight differences between its subtribes are not easily described in a concise way. The only differences between Leeuwenberg's (1994a) and Pichon's (1949a) tribal circumscriptions are that Leeuwenberg changed the name of the tribe to Alyxieae, put *Podochrosia* into synonymy under *Rauvolfia* and *Rhipidia* in *Condylocarpon* (following Fallen, 1983b), and included *Anechites* in Condylocarpaceae, although Fallen (1983a) had suggested earlier that a position closer to taxa previously included in Cerbereae (*Cameraria* L., *Cerbera* L., *Cerberiopsis* Vieill. ex Pancher & Sebert, *Thevetia* L., and *Skytanthus* Meyen) was more appropriate. More recently, Leeuwenberg (1997) put *Cabucala* into synonymy under *Petchia*.

In addition to the taxa mentioned above, there are four other genera characterized by drupaceous fruits: *Cerbera*, *Thevetia*, *Cerberiopsis*, and *Cameraria*. These, together with *Skytanthus*, with follicular fruits, were split out of Rauvolfioideae and treated by Pichon (1948b) as a separate subfamily, Cerberoideae. The characters he used for delimitation of this subfamily are ambiguous. Leeuwenberg (1994a) recognized the group as defined by Pichon, but at the tribal level as Cerbereae. Morphological studies by Fallen (1985) suggested a close relationship between Cerbereae and *Allamanda* L., the sole genus placed in Allamandaeae by Pichon (1949a) and Leeuwenberg (1994a). Studies based on molecular or combined morphological and molecular data (Endress et al., 1996; Sennblad & Bremer, 1996, 2000, 2002) indicated that the genera previously included in Cerbereae do form a natural group (see Potgieter & Albert, 2001, for a different opinion) and that they are only a part of a larger group that includes not only *Allamanda*, but also *Plumeria* L. (usually included in the Plumerieae: Rauvolfioideae). An analysis of Cerbereae is not the aim of this study, although some representatives from that tribe are included in our analyses.

Using fruit characters to delimit tribes in Rauvolfioideae is appealing because it allows taxa to be easily categorized and keys to be constructed.

However, other characters of these taxa do not indicate the same patterns of relationships. Phylogenetic analyses of mainly molecular data have shown that these fruit- and seed-based classifications are considerably more artificial than previously suspected. An *rbcL* analysis by Sennblad and Bremer (1996) indicated that *Catharanthus* G. Don (with dry dehiscent follicles and included in Plumerieae) was more closely related to taxa previously placed in Alyxieae sensu Leeuwenberg (1994a) than to other Plumerieae. In larger studies (Sennblad, 1997; Sennblad & Bremer, 2000, 2002), *Catharanthus* and *Vinca* L. formed a well-supported clade together with *Rauwolfia*, *Ochrosia*, and *Kopsia*, which have fleshy drupes. In the same study, *Chilocarpus* Blume, which has always been thought to be most closely related to Carisseae, formed a strongly supported clade with *Alyxia* and *Lepinia* (Alyxieae), confirming results of a strongly supported *Chilocarpus*–Alyxieae clade reported previously by Civeyrel (1996) and van der Ham et al. (2001). Pichon (1949a) already realized that *Geissospermum* Allemão (with indehiscent fruits and seeds embedded in pulp) is probably the nearest relative of *Aspidosperma* Mart. & Zucc. (with dry dehiscent follicles and wind-dispersed seeds with a diaphanous wing), a position supported by Potgieter and Albert (2001) and Simões et al. (2007). In addition, Potgieter and Albert (2001) found that *Vallesia* (with drupaceous fruits and seeds embedded in juicy pulp) is closely related to *Haplophyton* A. DC. (with dry dehiscent follicles and wind-dispersed comose seeds). Such results indicate that fruit characters in Apocynaceae are evolutionarily plastic in response to selective pressures for adaptations associated with wind or animal dispersal.

The most recent classifications of Apocynaceae s.l. (Endress & Bruyns, 2000; Sennblad & Bremer, 2002) attempted to rectify some of these anomalies. In the classification of Endress and Bruyns (2000), *Anechites* was moved to a newly defined Plumerieae (including Cerbereae sensu Leeuwenberg (1994a) as well as *Allamanda*), a position suggested by Fallen (1983a), and *Vallesia* was included with *Aspidosperma*, *Geissospermum*, and *Haplophyton* in a newly defined Alstonieae. The remainder of Alyxieae (sensu Leeuwenberg, 1994a) was split into two tribes, Alyxieae and Vinceae, based on molecular results as well as additional morphological characters. Alyxieae sensu Endress and Bruyns (2000) included seven genera: *Alyxia*, *Pteralyxia* K. Schum., *Lepinia*, *Lepiniopsis*, *Plectaneaia* Thouars, *Condylocarpon*, and *Chilocarpus*. Vinceae included *Amsonia* Walter, *Catharanthus*, *Vinca*, *Rauwolfia*, *Petchia*, *Kopsia*, *Neisosperma* Raf., and *Ochrosia*. *Rhazya* Decne. was considered to be synonymous with *Amsonia*, and *Cabucala* with *Petchia*

(following Leeuwenberg, 1997). Simões et al. (2007) treated *Amsonia* as a genus incertae sedis and transferred *Laxoplumeria* Markgr., *Tonduzia* Pittier, and *Kamettia* Kostel. to Vinceae, bringing the total number of genera in the tribe up to 10.

The classification of Sennblad and Bremer (2002) proposed a new system that is compatible with traditional Linnaean nomenclature but uses a variant of the definitions used in phylogenetic nomenclature to improve the stability of classifications. Although they do not provide lists of included genera, their definitions of the tribes containing traditional Alyxieae genera are completely congruent with those of Endress and Bruyns (2000). The aim of this study is to cladistically evaluate Alyxieae and Vinceae as circumscribed by Endress and Bruyns (2000) in comparison with previous classifications, to re-examine the usefulness of fruit and seed characters for tribal delimitation within Rauvolfioideae, and to discover new morphological characters that have hitherto received little attention in classification of this subfamily but show phylogenetic potential.

MATERIALS AND METHODS

TAXON SAMPLING

The ingroup taxa were chosen to include representatives of all genera of Alyxieae (sensu Leeuwenberg, 1994a), as well as other putatively related genera. The outgroup taxa are one genus each of Loganiaceae and Gelsemiaceae, which several studies (Bremer & Struwe, 1992; Chase et al., 1993; Savolainen et al., 2000; Soltis et al., 2000) have demonstrated to be closely related to Apocynaceae (Appendices 1, 2). Other more narrowly focused studies on Gentianales (Struwe et al., 1994; Endress et al., 1996; Backlund et al., 2000) have also indicated that Loganiaceae and Gelsemiaceae are the closest families to Apocynaceae. For the morphological analyses, we omitted the outgroups altogether because, in preliminary analyses, one or the other of these genera was embedded in different portions of the ingroup due to obvious parallelisms of certain characters; we arranged the morphological tree with the same group sister to the rest as in the molecular results.

FLORAL STRUCTURE

Fixed flowers at or near anthesis (only buds were available for *Lepiniopsis*) were dehydrated in an alcohol-xylene series, embedded in paraplast, cut with a rotary microtome at 10 μ m, and stained with safranin and astra blue. For SEM studies, material was critical-point dried and then sputter-coated with gold.

POLLEN MORPHOLOGY

Pollen material was sampled from the following herbaria: BISH, BR, COL, G, L, P, PTBG, QCA, S, UB, WAG, and Z. Pollen studies were carried out in Leiden and Stockholm. For light microscopy (LM), pollen material was acetolyzed (except for *Condylocarpon* and *Vinca*), mounted in glycerine jelly, and sealed with paraffin. Generally, 10 pollen grains were measured for polar axis (P) and equatorial diameter (E). For SEM, pollen was sputter-coated with gold and examined with a JSM 5300 or JSM 6300 scanning electron microscope (JEOL, Tokyo). Frozen sections were made using an Ames Tissue-TEK Cryostat. For transmission electron microscopy (TEM), unacetolyzed material (whole anthers) was embedded in Spurr resin or 3/7 Epon, sectioned with a LKB Ultratome III or V, poststained with uranylacetate and lead citrate, and examined with a Zeiss 10, a JEOL 100-S, or a JEM 1010. Terminology is according to Punt et al. (1994).

OTHER MORPHOLOGICAL AND CHEMICAL CHARACTERS

Information on fruit and seeds were taken from observations of herbarium specimens as available. Several fruit and seed characters were taken from the literature, as were data on the presence of laticifers and intraxylary phloem (Solereider, 1892; Schumann, 1895; Valetton, 1895; Degener, 1946; Pichon, 1947a, 1947b, 1948a, 1948b, 1948c, 1948d, 1949a, 1949b, 1949c, 1950a, 1950b, 1950c, 1952; Gensel, 1969; Markgraf, 1971, 1976, 1979; Markgraf & Huber, 1975; Corner, 1976; Conn, 1980; Leeuwenberg & Leenhouts, 1980; Rogers, 1986; Rudjiman, 1986; Pagen, 1987; Metcalf & Chalk, 1989; Rosatti, 1989; Wagner et al., 1990; Sévenet et al., 1994; Forster & Williams, 1996; Omino, 1996; Leeuwenberg, 1997; Sidiyasa, 1998; Lin & Bernardello, 1999). Chemical data were taken from the literature (Johns et al., 1968; Hegnauer, 1970, 1989; Coppen & Cobb, 1983; Kiskurek et al., 1983; Homberger & Hesse, 1984; Bisset, 1987; Endress et al., 1990; Wagner et al., 1990; Zhu et al., 1990; Attaurrahman et al., 1989, 1991; Arambewela & Ranatunge, 1991; Jensen, 1992; Sévenet et al., 1994; Zeches et al., 1995; Kam et al., 1997).

MOLECULAR METHODS

Nine new sequences of *rbcL*, 16 of *matK*, and 11 of the *trnL* intron and *trnL-F* intergenic spacer were produced for this study; the other sequences were published previously (Appendix 2). Total DNA was extracted from fresh leaves, silica gel-dried material (Chase & Hills, 1991), or herbarium material using the methods of Saghai-Marroof et al. (1984) or modified

Doyle and Doyle (1987). For the latter, DNA samples were purified by ultracentrifugation in CsCl-ethidium bromide gradients (1.55 g/ml). Additional purification using the QIAquick PCR purification kit (Qiagen, Valencia, California) was performed in cases with problematic polymerase chain reaction (PCR) amplification using the manufacturer's protocol. Double-stranded DNA was amplified with PCR primers for *rbcL* from Fay et al. (1998); the *trnL* intron and *trnL-F* intergenic spacer (hereafter, *trnL-F*) were amplified using the c and f primers of Taberlet et al. (1991); *matK* primers were those of Endress et al. (1996) and Johnson and Soltis (1994). Direct sequencing of PCR products was performed using the PCR primers plus internal sequencing primers. For *rbcL*, the internal primers were those of Fay et al. (1998); for *trnL-F*, we used the d and e primers of Taberlet et al. (1991); and for *matK*, we designed two new internal primers: 734F, 5'-ATGTATGTGACTACGAATCA-3' and 829R, 5'-ACTTCTATTTTCCATAGA-3'. In a number of cases, we also used the internal sequencing primers as PCR primers to amplify shorter products. For sequencing, we used either the Dye Deoxy Terminator Cycle Sequencing or Big Dye kits of Applied Biosystems (ABI; Warrington, Cheshire, United Kingdom). Sequencing reactions were carried out directly on the cleaned PCR products and run on an ABI 277a automated sequencer at Kew following the manufacturer's protocols.

CLADISTIC ANALYSES

The data matrix comprised four submatrices: each of the three DNA regions plus morphology. The morphological submatrix (Appendix 3) comprises 54 characters from floral, fruit, vegetative, and pollen morphology and phytochemistry (Appendix 4). All analyses were performed using PAUP* 4.0b10 (Swofford, 2002). Heuristic searches were performed with all characters given unit weight (Fitch parsimony; Fitch, 1971), and each submatrix was analyzed separately before their joint combined analysis. Each of the searches used the following settings: 1000 replicates of random taxon entry, the subtree pruning re-grafting (SPR) branch swapping algorithm, and MULTREES on (saving multiple equally parsimonious trees) but holding only 10 trees per replicate. All the shortest trees were then collected and used as starting trees for a search with a 25,000-tree limit. If the tree limit was reached, then swapping was allowed to continue until all 25,000 trees were swapped to completion.

Bootstrap percentages (BP) (Felsenstein, 1985) were calculated with 500 replicates on each of the submatrices as well as on the two combined matrices

(molecular combined and molecular/morphological combined). We used the following settings: SPR branch swapping and MULTREES on, holding only 10 trees per step. All other settings were those of the standard defaults of PAUP* 4.0. This strategy produces results statistically indistinguishable from other, more thorough bootstrap protocols (Salamin et al., 2003). We apply the following arbitrary scale in our discussion: 50%–74%, weakly supported; 75%–84%, moderately supported; 85%–100%, strongly supported. Alignment for *rbcL* and *matK* was a simple matter; the former had no length variation, and the latter had only a few easily characterized insertions/deletions (indels). For *trnL-F*, we started with the alignment of Potgieter and Albert (2001) and added the additional taxa needed for this analysis, which required adding a few more insertions; we did adjust their alignment in places following Kelchner (2000). We analyzed the *trnL* intron and *trnL-F* intergenic spacer in a single analysis (which can be considered “non-coding” because there is only about a 30 bp region of the *trnL* exon included); this region, termed *trnL-F*, is composed of two unrelated parts, but the number of variable sites is the lowest and, even when combined, these produced a highly unresolved strict consensus tree. We do report statistics for these two regions separately (Appendix 2) but consider results only for the two combined.

Incongruence of different regions of plastid DNA would be unexpected because recombination is unknown in the generally uniparentally inherited plastid genome. Several tests for combinability have been developed, but we have not used any of them in this paper. Such tests have proven to be unreliable indicators of incongruence (Reeves et al., 2001), so we attach no particular significance to results of these tests but instead prefer to look for cases of strongly supported and incongruent patterns of relationships. Differences in relationships are to be expected with different matrix components simply due to sampling effects where there are too few variable characters to obtain clear patterns. If, however, there are only a few characters in a matrix, such as is the case here with the morphological characters, differentiating between sampling error and incongruent patterns is extremely difficult. We note that the morphologically based estimates of relationships deviate from those based on DNA data, particularly for the Venceae, but the small number of morphological characters (only 54) does not permit us to say whether the differences between molecular and morphological patterns are evidence of true incongruence.

Character state distributions of selected morphological characters were individually mapped onto the total evidence tree (Figs. 10, 11) using MacClade 4.0

(Maddison & Maddison, 2000) to illustrate character evolution and compare the usefulness of characters that have previously been used in delimitation of Alyxieae.

RESULTS

MORPHOLOGY

Analysis of the morphological matrix produced 31 trees in three islands (18, 4, and 9 trees) of equally parsimonious trees, each of 246 steps with a consistency index (CI) of 0.37 and a retention index (RI) of 0.62. A strict consensus tree of all three islands (Fig. 1; numbers below the branches are BPs) shows that the position of several genera is unclear with these morphological data, and the three islands place them in different relative positions to the clades that are consistently resolved in all three islands. The positions of *Vallesia antillana* Woodson, *V. glabra* (Cav.) Link, and the *Plumeria* clade (*Allamanda cathartica* L., *Anechites nerium* (Aubl.) Urb., *Plumeria rubra* L., and *Thevetia peruviana* (Pers.) K. Schum.) are consistent in all three islands, as is a clade composed of all remaining taxa. Within the last, *Acokanthera oblongifolia* (Hochst.) Codd, *A. oppositifolia* (Lam.) Codd, *Molongum laxum* (Benth.) Pichon, *Picralima nitida* (Stapf) T. Durand & H. Durand, *Pleiocarpa mutica* Benth., *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult., and *T. pandacaqui* Lam. occupy different positions in each of the three islands with respect to the two consistently resolved clades: (1) *Alstonia scholaris* (L.) R. Br. to *Rhazya stricta* Decne. and (2) *Alyxia oblongata* Domin and *A. ruscifolia* R. Br. to *Nerium oleander* L. (Fig. 1). The other conspicuously differently placed group is that composed of *Amsonia ciliata* Walter, *A. tabernaemontana* Walter, *Rhazya stricta*, and *Catharanthus roseus* (L.) G. Don + *Vinca major* L. and *V. minor* L., which in two of the islands (18 and 9 tree islands) are a clade but in the other island form a grade. Clades that receive moderate to strong BPs are the following: *Neisosperma nakaiana* (Koidz.) Fosberg & Sachet + *Ochrosia coccinea* (Teijsm. & Binn.) Miq. (BP 83), *Alyxia oblongata* and *A. ruscifolia* + *Lepinia marquisensis* Lorence & W. L. Wagner, *L. solomonensis* Hemsl. and *L. taitensis* Decne. + *Lepiniopsis ternatensis* Valetton and *L. trilocularis* Markgr. + *Pteralyxia kauaiensis* Caum and *P. macrocarpa* (Hillebr.) K. Schum. (BP 97; the last two genera with BP 96; the *Alyxia* clade), *Kibatalia gitingensis* (Elmer) Woodson + *Mascarenhasia arborescens* A. DC. + *Nerium oleander* (BP 98; the first two genera with BP 94), *Picralima nitida* + *Pleiocarpa mutica* (BP 84), and *Allamanda cathartica*

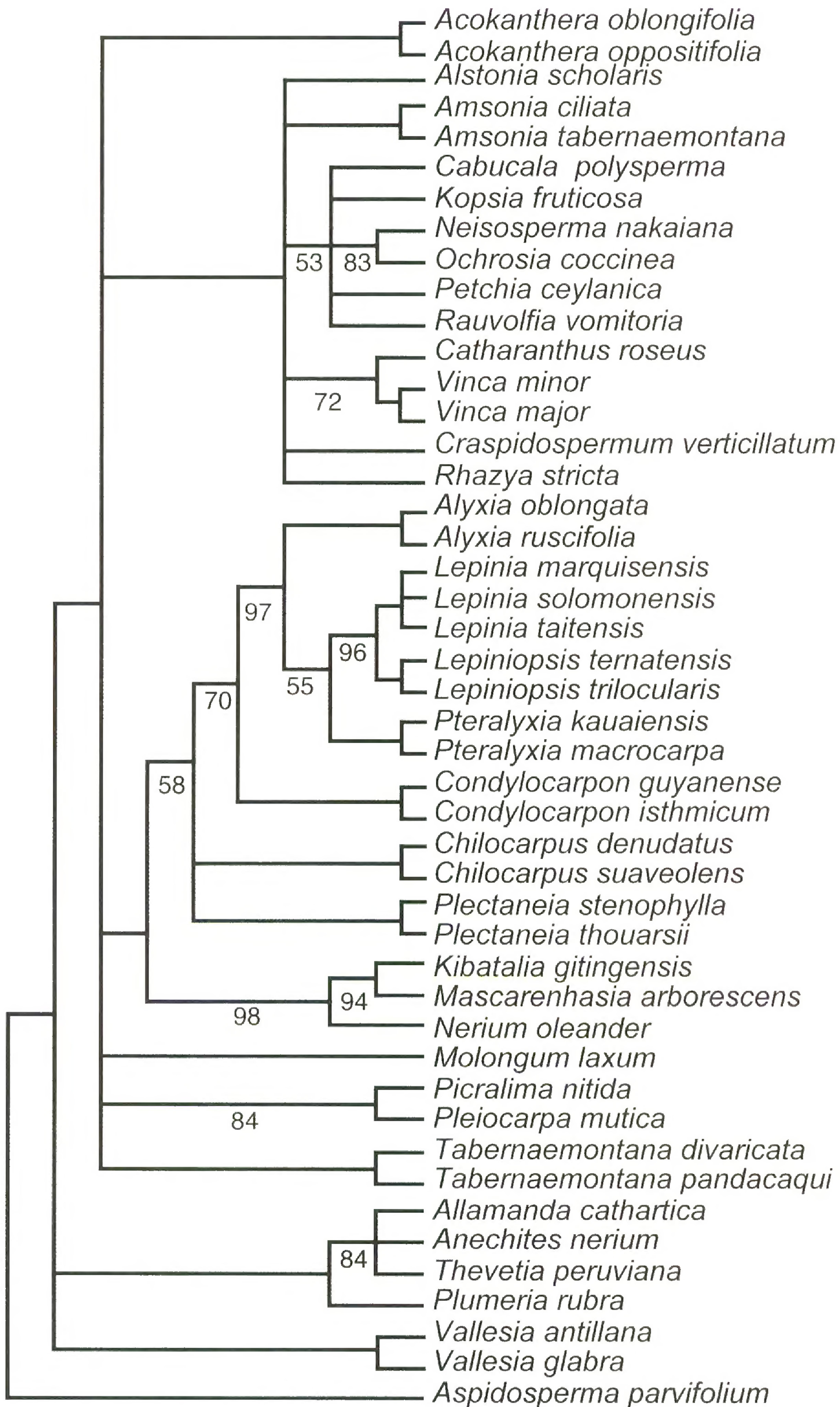


Figure 1. Strict consensus tree of the three islands found in the morphological analysis. Numbers below the branches indicate bootstrap percentages greater than 50%.

+ *Anechites nerium* + *Thevetia peruviana* (BP 84). *Catharanthus roseus* + *Vinca major* and *V. minor* nearly reached the moderate level (BP 72).

ANALYSIS OF *RBCL*

Of the 1398 included positions, 244 (18%) were variable and 146 (10%) were potentially parsimony informative. Analysis produced two islands of equally parsimonious trees, one of 39 trees and the other of 16 trees; they had 479 steps with CI (including uninformative positions) of 0.59 and RI of 0.63. The two islands differ in the relative positions of *Neisosperma oppositifolia* (Lam.) Fosberg & Sacht and *Rauwolfia mannii* Stapf and *Chilocarpus suaveolens* Blume + *Condylocarpon guyanense* Desf. In island one, the latter form a clade with the *Kibatalia gitingensis* + *Mascarenhasia arborescens* + *Nerium oleander* clade, and *Neisosperma oppositifolia* and *Rauwolfia mannii* are unresolved members of the clade including *Cabucala polysperma* (Scott-Elliott) Pichon + *Petchia ceylanica* (Wight) Livera + *Ochrosia coccinea*. In island two, *Neisosperma oppositifolia* + *Rauwolfia mannii* are sister to *Catharanthus roseus* + *Vinca minor*, and *Chilocarpus suaveolens* + *Condylocarpon guyanense* are unresolved. The strict consensus of both islands (Fig. 2) therefore shows these taxa to be unresolved. Supported clades that are also found in the morphological analysis include the following: *Allamanda cathartica* + *Plumeria inodora* Jacq. (BP 100) and *Anechites nerium* + *Thevetia peruviana* (BP 97; the whole *Plumeria* clade, BP 62); *Alyxia ruscifolia* + *Lepinia taitensis* + *Lepiniopsis trilocularis* + *Pteralyxia kauaiensis* (the *Alyxia* clade; BP 99); *Picralima nitida* sister to *Pleiocarpa mutica* (BP 100); *Catharanthus roseus* + *Vinca minor* (BP 68); and *Kibatalia gitingensis* + *Mascarenhasia arborescens* + *Nerium oleander* (the *Nerium* clade; BP 93). Well-supported clades that are not strongly in conflict with the morphological results include: *Aspidosperma triternatum* Rojas Acosta as sister to *Vallesia antillana* (BP 100); *Plectaneia stenophylla* Jum. as sister to the *Alyxia* clade (BP 98), within which the topology is the same as in the morphological results; *Amsonia tabernaemontana* as sister to *Rhazya stricta* (BP 100); *Cabucala polysperma* as sister to *Petchia ceylanica* (BP 100); and *Molongum laxum* as sister to *Tabernaemontana divaricata* (BP 95). *Chilocarpus suaveolens* as sister to *Condylocarpon guyanense* (BP 85) is contradicted in the morphological results by a weak BP 70 for the latter to be sister to *Alyxia* clade. Weakly supported results not contradicting relationships produced by morphology are the *Plumeria* clade (BP 62; *Allamanda cathartica*, *Anechites nerium*, *Plumeria inodora*, and *Thevetia peruviana*); *Picralima*

nitida + *Pleiocarpa mutica* as sister to the *Alyxia* clade, plus *Plectaneia stenophylla* (BP 70); and the *Vinca* clade (BP 65; *Cabucala polysperma*, *Petchia ceylanica*, *Ochrosia coccinea*, *Catharanthus roseus*, *Vinca minor*, *Neisosperma oppositifolia*, and *Rauwolfia mannii*).

ANALYSIS OF *MATK*

The aligned *matK* matrix contained 1647 bp, of which 561 (34%) were variable and 250 (15%) were potentially parsimony informative. We were unable to amplify the following taxa for *matK*: *Anechites*, *Lepinia*, *Lepiniopsis*, *Ochrosia*, and *Plectaneia*. Analysis produced a single, most parsimonious tree of 970 steps with a CI of 0.73 and an RI of 0.59. Patterns of relationships are nearly identical to those found with *rbcL*, but in general, BPs are higher than with *rbcL* or *trnL-F* (Fig. 3). Patterns that were not observed with *rbcL* include: *Chilocarpus suaveolens* + *Condylocarpon guyanense* (BP 100) as sister (BP 99) to the *Alyxia* clade (BP 100); *Alstonia scholaris* strongly supported in an isolated position as sister (BP 93) to all but *Aspidosperma triternatum* + *Vallesia antillana*; and *Aspidosperma triternatum* + *Vallesia antillana* (BP 82) moderately supported as sister to the rest of the ingroup (BP 97).

ANALYSIS OF *TRNL-F*

The aligned *trnL-F* matrix consisted of 1206 bp (761 bp from the *trnL* intron and 445 bp from the *trnL-F* spacer). We were unable to amplify the following taxa for *trnL-F*: *Kibatalia* G. Don and *Lepinia*. Analysis produced over 25,000 trees of 460 steps with a CI of 0.78 and an RI of 0.70 (*trnL* intron: 292 steps with a CI of 0.76 and an RI of 0.69; *trnL-F* intergenic spacer: 168 steps with a CI of 0.82 and an RI of 0.72) (Fig. 4). Relationships from these two, largely non-coding regions are similar to those estimated from *rbcL* and *matK* (Figs. 2, 3). The major noteworthy result (also observed in the *matK* results, but which received BP < 50) is a strongly supported clade (BP 97) composed of *Nerium oleander* and *Mascarenhasia arborescens* of the *Nerium* clade, observed with morphology, *rbcL*, and *matK*, with *Acokanthera oppositifolia* weakly supported as sister (BP 60) also observed but without support with *matK*, and *Allamanda indet.* + *Plumeria alba* Kunth (BP 95), *Anechites nerium*, and *Thevetia ahouai* (L.) A. DC. (the last two unresolved with respect to the *Nerium* clade).

COMBINED MOLECULAR ANALYSES

The combined data set produced 46 equally parsimonious trees of 1925 steps with a CI of 0.70

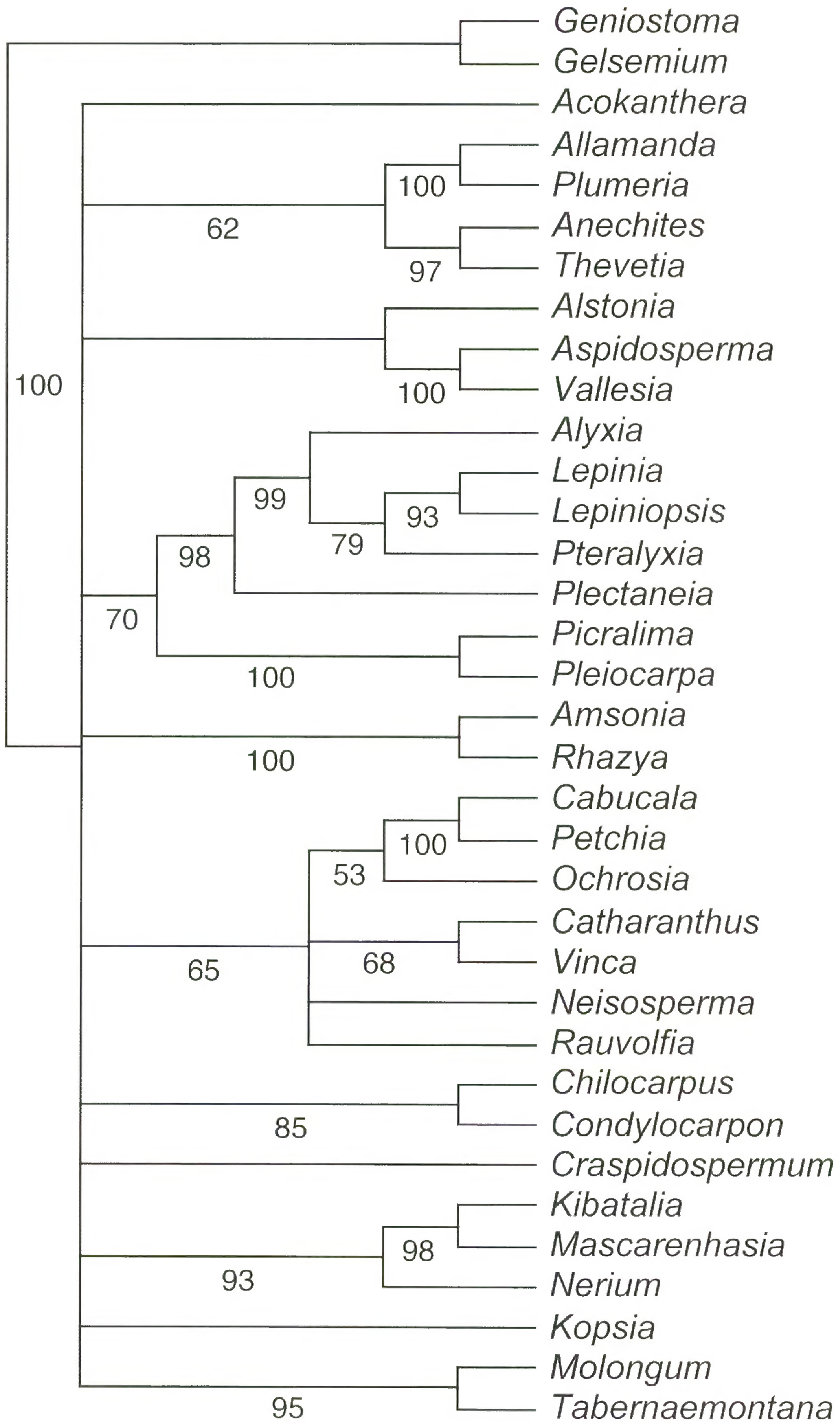


Figure 2. Strict consensus tree of the two islands found with the *rbcL* data. Numbers below the branches indicate bootstrap percentages greater than 50%.

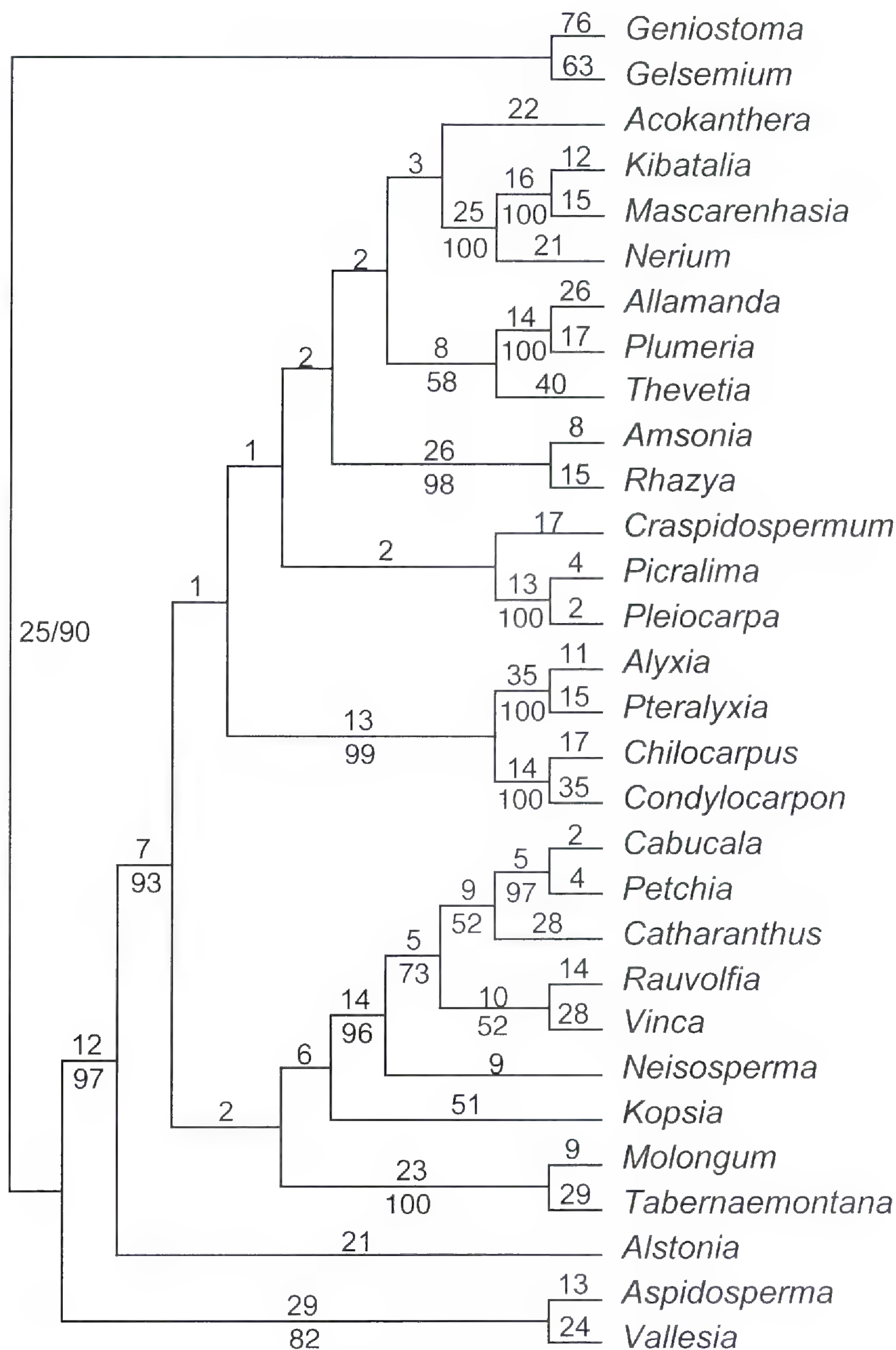


Figure 3. The single most parsimonious tree found with the *matK* data. Numbers above the branches indicate estimated substitutions, ACCTRAN optimization. Numbers below the branches indicate bootstrap percentages greater than 50%.

and an RI of 0.62. One of the shortest individual trees is shown in Figure 5, with estimated substitutions (ACCTRAN optimization) indicated above the branches and BPs below; groups not present in all shortest trees are marked with an arrowhead. We show a single tree to illustrate relative levels of genetic divergence. The contribution of each region to this tree was: *rbcL*, 488 steps (vs. 479 for the *rbcL* trees); *matK*, 974 steps (vs. 970 for the *matK* tree); *trnL* intron, 293 steps (vs. 292 on the *trnL-F* trees); and

trnL-F intergenic spacer, 170 steps (vs. 168 on the *trnL-F* trees). Patterns of relationships are much like those in the previous analyses, and BPs are generally higher than in any of the individual analyses.

ANALYSIS OF ALL DATA COMBINED

The combined data produced a single, most parsimonious tree of 2226 steps with a CI of 0.65 and an RI of 0.60 (Fig. 6). The DNA optimized onto

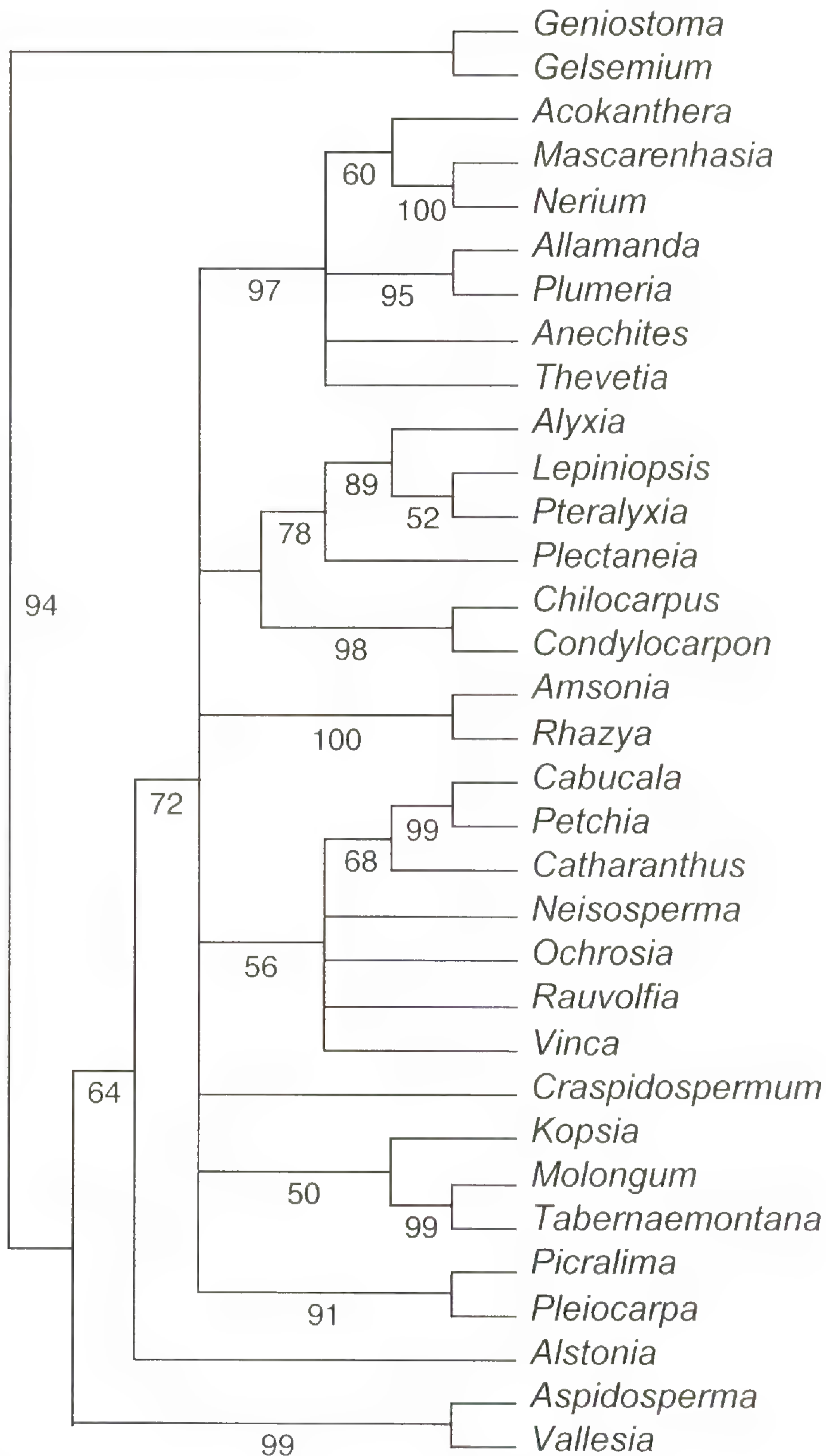


Figure 4. Strict consensus tree of the 25,000 equally most parsimonious trees found with the *trnL-F* data. Numbers below the branches indicate bootstrap percentages greater than 50%.

this tree is 1926 steps, one step longer than the combined DNA tree: this step is caused by shifting *Kopsia fruticosa* (Ker. Gawl.) A. DC. and *Molongum laxum* + *Tabernaemontana* (two spp., Appendix 2) from an unresolved position with respect to the major clades into positions as a grade with respect to the *Vinca* clade. Otherwise, relationships are exactly as with the combined molecular data. The morphological data optimized onto the combined trees (ACCTRAN optimization) was 276 steps with a CI of 0.33 and an RI of 0.55, versus 266 steps with a CI of 0.38 and an RI of 0.61 in the morphological analysis.

DISCUSSION

EVALUATION OF TRADITIONAL CIRCUMSCRIPTIONS OF ALYXIEAE

Both the morphological and the molecular analysis indicate that Alyxieae as previously circumscribed are polyphyletic. Of the individual data sets analyzed, the tree based on *matK* provided the best support, followed by that of *trnL-F*. BPs in the tree based on *rbcL* and the morphological data set were low, with much of the tree a polytomy, and the positions of several genera were equivocal. Even then, however,

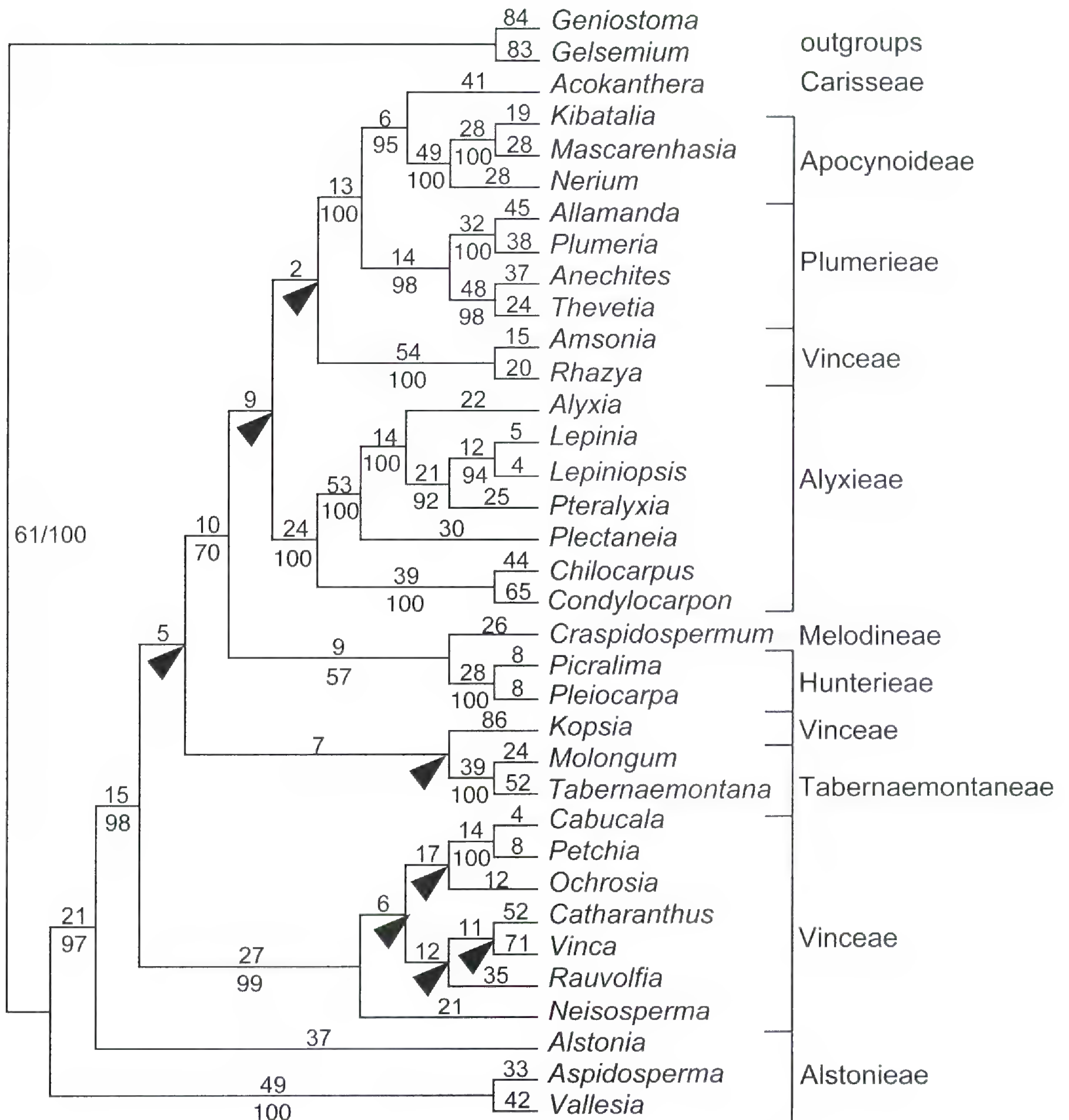


Figure 5. One of the most parsimonious trees found with the combined molecular data. Numbers above the branches indicate estimated substitutions, ACCTRAN optimization. Numbers below the branches indicate bootstrap percentages greater than 50%. Groups not present in all 46 shortest trees are noted with an arrowhead.

clusters of genera are present. The combined data tree is similar to that produced by the combined molecular data and provides much better support for patterns of relationship already seen in each of the individual trees. Therefore, the remainder of the discussion will be based on the total combined tree (Fig. 6). This tree is not intended to represent relationships within or among tribes other than Alyxieae and Vinceae.

All genera traditionally included in Alyxieae are preceded by a dot in Figure 6. *Vallesia* and *Anechites*, both included in Alyxieae by Pichon (1949a, 1950b,

as Rauvolfieae) and maintained there by Leeuwenberg (1994a), are not closely affiliated with any of the other members of the ingroup. *Vallesia* is sister to *Aspidosperma*, a position that supports results of previous phylogenetic studies (Sennblad & Bremer, 2000, 2002; Potgieter & Albert, 2001; Simões et al., 2007). It is unlikely that a close relationship between *Vallesia* and *Aspidosperma* would have been predicted based on their floral or fruit structures; they are too plesiomorphic to be of much help, and the small indehiscent drupaceous fruits of the former look very

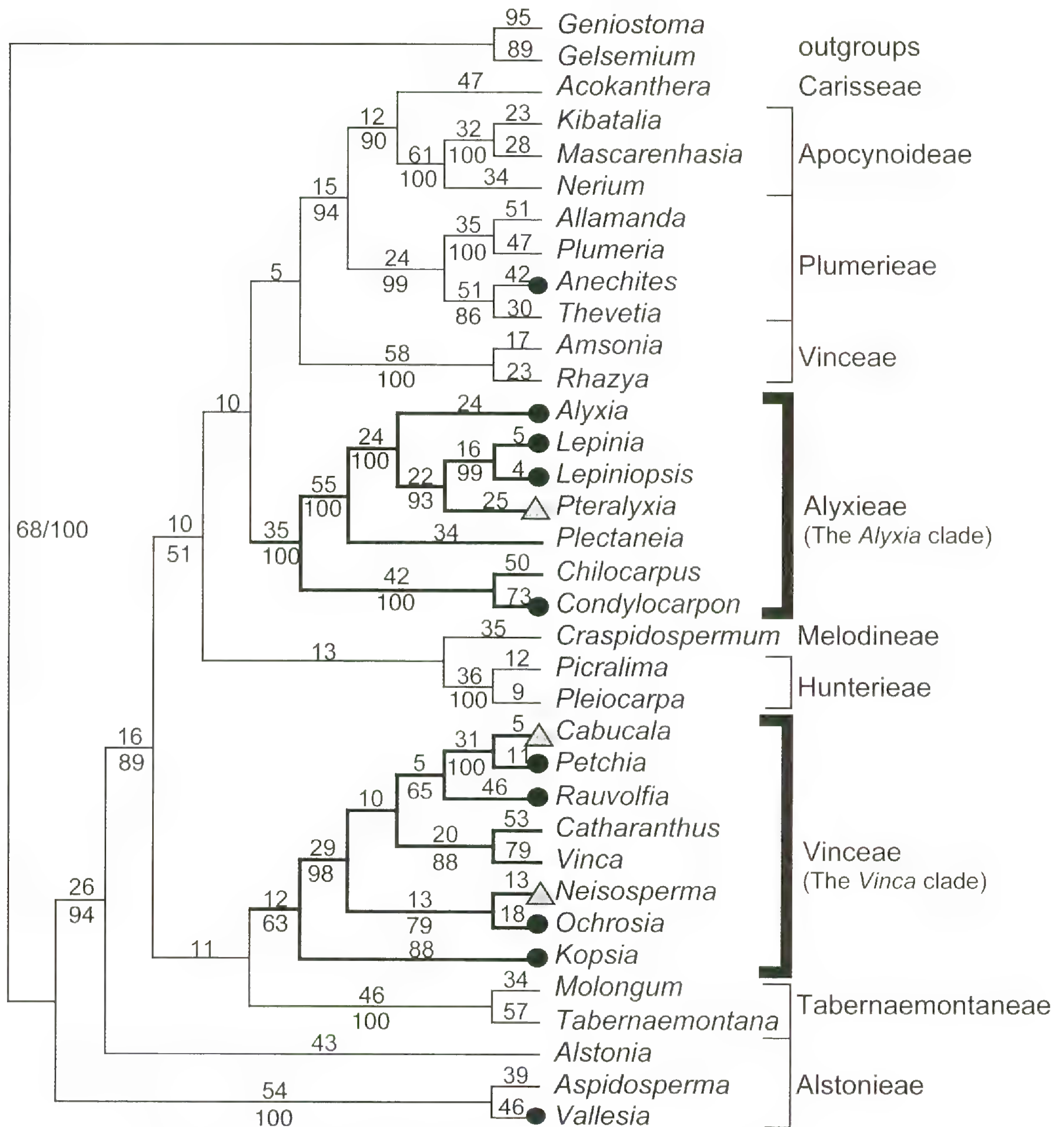


Figure 6. The single most parsimonious tree found in the total combined analysis of the morphological and molecular data. Numbers above the branches indicate estimated substitutions, ACCTRAN optimization. Numbers below the branches indicate bootstrap percentages greater than 50%. The *Alyxia* clade and the *Vinca* clade are indicated with thicker branches and brackets. • = Alyxieae (Rauvolfieae in earlier classifications). Δ = taxa not recognized at generic rank by Leeuwenberg (1994a). Tribal names on right follow the classification of Endress and Bruyns (2000).

different from the dehiscent follicular fruits of the latter. A close relationship between the two genera is, however, supported by pollen morphology. In both genera the pollen has five or six apertures (as opposed to the usual 3-aperturate condition), which are surrounded by distinctive prominent ridges. The inclusion of *Vallesia* in Alstonieae sensu Endress and Bruyns (2000) is supported by previous *rbcL* data (Sennblad & Bremer, 2000, 2002), but not by *trnL-F* data (Potgieter & Albert, 2001), which included more

genera near the base of Apocynaceae. In our analysis, *Anechites* is sister to *Thevetia*, grouping with members of Cerbereae of traditional classifications (Cerberoi-deae of Pichon, 1948b), which confirms results based on morphology (Fallen, 1983a; Alvarado-Cárdenas & Ochoterena, 2007) and earlier *rbcL* data (Sennblad & Bremer, 2000, 2002) and supports Endress and Bruyns' (2000) inclusion of *Anechites* in Plumerieae. The remainder of the genera of Alyxieae fall into two main clades.

RELATIONSHIPS WITHIN VINCEAE AND ALYXIEAE

The first main cluster of Alyxieae in the combined analysis (Fig. 6) is the *Alyxia* clade and includes *Alyxia*, *Lepinia*, *Lepiniopsis*, and *Condylocarpon*. In addition, the placement of *Chilocarpus*, *Plectaneia*, and *Pteralyxia* in this clade is well supported. This corroborates the positions of *Alyxia*, *Lepinia*, and *Chilocarpus* in the study by Sennblad and Bremer (2000, 2002) based on *rbcL* data. Except for *Condylocarpon*, the members of this group have irregular pollen grains with relatively large porate apertures. The aperture number is usually two; *Lepinia* and *Lepiniopsis* have three or four apertures (van der Ham et al., 2001). Within the *Alyxia* clade, *Alyxia*, *Lepinia*, *Lepiniopsis*, *Pteralyxia*, and *Plectaneia* form a clade (Fig. 6). The first three genera correspond to Pichon's (1949a) and Leeuwenberg's (1994a) Alyxiinae. *Pteralyxia* was considered to be a synonym of *Alyxia* by Pichon (1949a) and a synonym of either *Alyxia* or *Ochrosia* by Leeuwenberg (see Gunn et al., 1992 and van der Ham et al., 2001: 169, 187). *Plectaneia*, in contrast, has previously been included in Plumerieae and has usually been considered to be related to genera such as *Gonioma* E. Mey., *Stephanostegia* Baill., and *Craspidospermum* Bojer ex A. DC. (Alstoniinae of Schumann, 1895; Plectaneiinae of Pichon, 1949a; Craspidosperminae of Leeuwenberg, 1994a), all of which are included in Melodineae in Endress and Bruyns (2000). In terms of pollen morphology, *Alyxia*, *Lepinia*, *Lepiniopsis*, and *Pteralyxia* form a tight-knit monophyletic group (Fig. 7). The porate apertures can be large (maximum 21–33 μm), their margins are clearly thickened (less clearly also in *Chilocarpus*), the inner exine surface is granular (also in subfamily Apocynoideae), the inner exine layer (nexine) is completely endexinous, the infratectum is hardly recognizable (being reduced to \pm sparse gaps in the inner ectexine), and a relatively thick tectum is present (also in several other taxa). *Lepinia* and *Lepiniopsis* share the presence of an ornamentation consisting of anastomosed verrucae (van der Ham et al., 2001). In the large analysis of *trnL-F* by Potgieter and Albert (2001), *Alyxia*, *Lepiniopsis*, *Condylocarpon*, and *Plectaneia* were supported in Alyxieae, whereas *Pteralyxia* affined with Plumerieae. This is an unlikely position for *Pteralyxia* considering its distinctive pollen, which is a synapomorphy of Alyxieae and found nowhere else in Apocynaceae (van der Ham et al., 2001). As the same vouchered specimen was used as the source of DNA for both studies, the reason for this discrepancy between our *trnL-F* results and those of Potgieter and Albert (2001) is most likely due to a mix-up in the laboratory. It is noteworthy that of the seven genera in

the Alyxieae, four, *Lepinia*, *Lepiniopsis*, *Pteralyxia*, and *Plectaneia*, are island endemics (Leeuwenberg, 1997; Lorence & Wagner, 1997), and *Alyxia* has its greatest species diversity in the Pacific (Middleton, 2000, 2002).

Chilocarpus and *Condylocarpon* are sister genera that are the sister group to the remainder of Alyxieae (Fig. 6). Because of its syncarpous ovary, *Chilocarpus* was included in Carisseae (Willughbeieae sensu Endress & Bruyns, 2000) by Schumann (1895, as Arduineae) and the invalid Chilocarpaceae by Pichon (1948a). Leeuwenberg (1994a) provided a Latin diagnosis, raised Pichon's subtribe to tribal level, and in his recent revision of *Chilocarpus* (Leeuwenberg, 2002) considers Chilocarpaceae to fall somewhere between Carisseae and Ambelanieae. *Condylocarpon* is the only New World member of Alyxieae and exhibits a set of deviating pollen features (tetrads, inaperturate, and reduced exine; Fig. 7) that strongly indicate the pollen to be pedomorphic (i.e., underdeveloped regarding pollen wall features, yet viable). The basically decussate tetrad configuration indicates *Condylocarpon* pollen to be derived from a 2-aperturate rather than from a 3-aperturate ancestor, providing additional support for its present position in Alyxieae (van der Ham et al., 2001). Despite its aberrant, inaperturate, nearly exineless pollen, *Condylocarpon* resembles *Chilocarpus* in a number of other morphological aspects, especially the distinctive globose head of the flower buds. The fruits exhibit some superficial similarities; in most species of both genera, they are moniliform and woody, although the gynoeceum is syncarpous and dehiscent in *Chilocarpus* and apocarpous and indehiscent in *Condylocarpon* (Fallen, 1983b).

The second main cluster of Alyxieae in the combined analysis (Fig. 6) is the *Vinca* clade and includes *Cabucala*, *Petchia*, *Rauwolfia*, *Ochrosia*, *Neisosperma*, *Kopsia*, *Catharanthus*, and *Vinca*. All taxa in this clade are characterized by a differentiated style head with a distinct annulus at the base, but this is a plesiomorphic feature and is also found in other tribes (e.g., *Alstonia* R. Br., Alstonieae; *Allamanda*, Plumerieae). Similarly, the pollen morphology of this clade is unspecialized (Fig. 8). The occurrence of well-developed colpal and mesocolpal plates due to the presence of distinct supplementary endocolpi (absent in *Vinca*, weak in *Kopsia* and several taxa outside the Vincae: *Acokanthera* G. Don, *Allamanda*, *Aspidosperma*, and *Plumeria*) is their most discriminating feature. Of the taxa in the *Vinca* clade, *Rauwolfia*, *Ochrosia*, and *Kopsia* were treated in Schumann's (1895) classification. He included *Rauwolfia* in Rauwolfinae, together with nine other genera, none of which shows a close relationship with

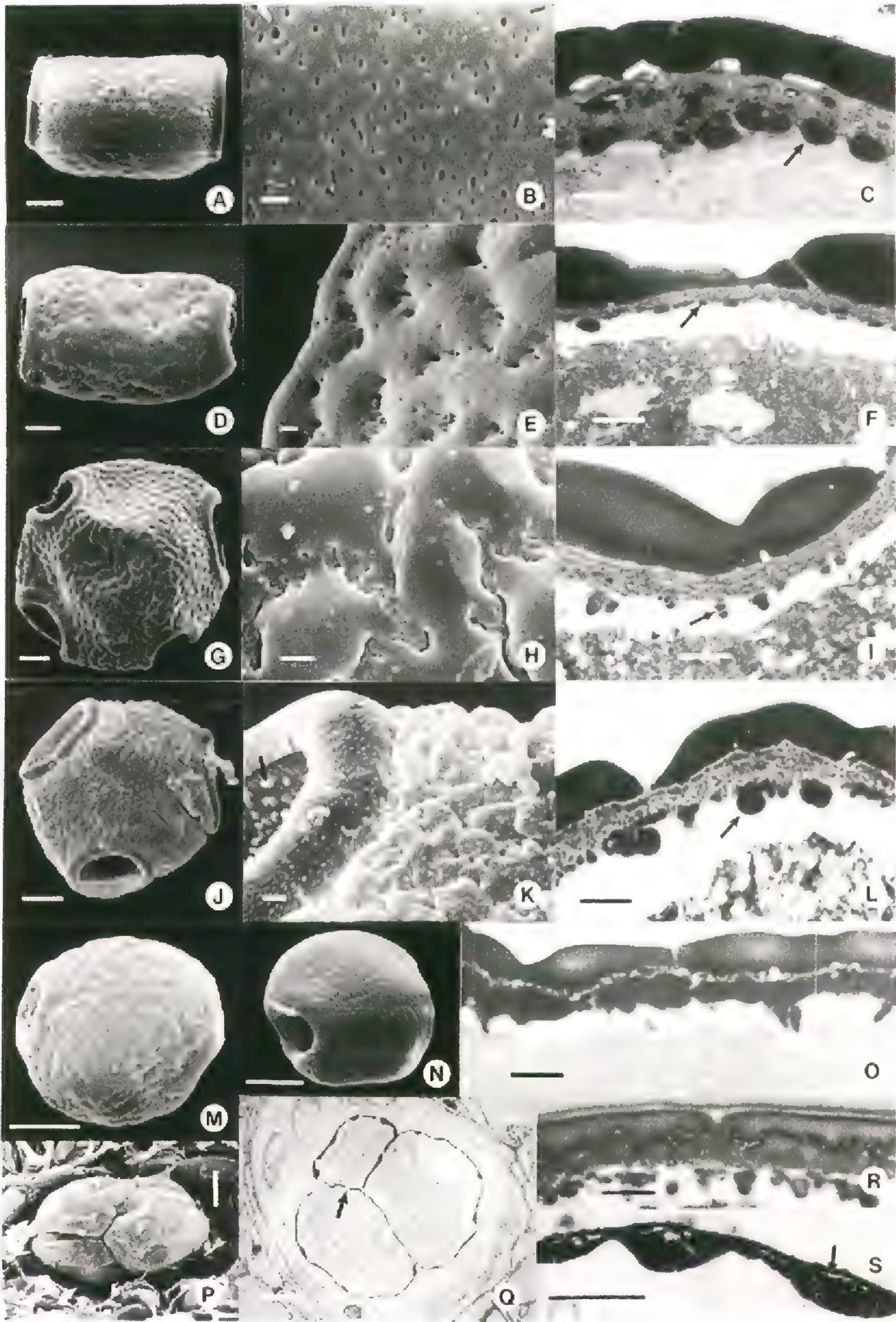


Figure 7. Apocynaceae, Alyxieae. SEM and TEM images of pollen grains. A–C. *Alyxia ruscifolia*. —A. Barrel-shaped 2-porate pollen grain (orientation unknown) with differently sized pores. —B. Detail of A, showing psilate, perforate ornamentation. —C. TEM section of exine and intine, showing tectum (above), infratectum (gaps), and endexinous inner layer with dark inclusions and dark granules on the inner surface (arrow). D, E. *Pteralyxia kauaiensis*. —D. Barrel-shaped 2-porate pollen grain (orientation unknown) with differently sized pores. —E. Detail of undulate ornamentation with perforations in the depressions. —F. *Pteralyxia macrocarpa*. TEM section of exine and intine, showing thick tectum (above), infratectum (commissural line and several small gaps), and endexinous inner layer with small dark inclusions and larger dark surface granules (arrow). G, H. *Lepinia solomonensis*. —G. Tetraporate pollen grain (orientation unknown) with unequal sides and differently sized pores. —H. Detail of \pm verrucate ornamentation. —I. *Lepinia taitensis*. TEM section of exine and intine, showing thick tectum (above), infratectum (commissural line and gap), and endexinous inner layer with small dark inclusions and larger (locally stacked) dark surface granules (arrow). J–L. *Lepiniopsis ternatensis*. —J. 3-porate pollen grain (orientation unknown) with unequal sides and differently sized pores. —K. Detail of J, showing \pm verrucate ornamentation, psilate annulus, and inner surface granules (arrow) inside pore. —L. TEM section of exine and intine, showing thick tectum (above), infratectum (commissural line and sparse gaps), and endexinous inner layer with small dark inclusions and large dark surface granules (arrow). M, O. *Chilocarpus denudatus*. —M. Psilate to finely fossulate 2-porate pollen grain (orientation unknown). —O. TEM section of exine and intine, showing tectum (above), granular infratectum, and ectexinous inner verrucate layer. N, R. *Plectaneia thouarsii*. —N. Psilate, perforate 2-porate pollen grain (orientation unknown). —R. TEM section of exine and

Rauwolfia in this study or other recent analyses (Sennblad & Bremer, 2000, 2002; Potgieter & Albert, 2001; Simões et al., 2007), whereas *Ochrosia* and *Kopsia* were included in a different subtribe, Cerberaeae (Plumerieae of Endress & Bruyns, 2000). Rauwolfieae as circumscribed by Pichon (1949a) included four subtribes. The Rauwolfinae comprised *Cabucala*, *Petchia*, and *Rauwolfia*, which are supported as monophyletic in the *Vinca* clade. Ochrosinae contained only *Ochrosia* (including *Neisosperma*), and Vallesinae included *Vallesia* and *Kopsia*, which are only distantly related here. His other two subtribes, Alyxinae and Condylocarpaceae, belong to elements of *Alyxia* clade in our study. Pichon (1949a) included *Anechites* as a genus incertae sedis, noting that he did not have sufficient material to place it, but he thought that it most probably belonged with *Condylocarpon* or in a tribe of its own.

The classification of Leeuwenberg (1994a) followed Pichon's, with the same tribal circumscription of Alyxieae and the same subtribal circumscriptions. The only differences were that *Anechites* was included as a member of Condylocarpaceae and the name of the tribe was changed to Alyxieae. More recently, Leeuwenberg (1997) placed *Cabucala* in synonymy under *Petchia*. Although there are some differences in pollen morphology (differing colpus length, tectum thickness and ornamentation, and the presence/absence of deviating mesocolpium centers) in the species studied, the floral structure of the two genera is nearly identical, and our results do not contradict the synonymy of *Cabucala*. *Neisosperma*, which was placed into synonymy under *Ochrosia* by Pichon (1949a) and maintained there by Leeuwenberg (1994a) and Hendrian (2004), is often considered to be a distinct genus, especially by specialists dealing with species of the Pacific Basin, where both genera have their greatest species density (Fosberg et al., 1977; Markgraf, 1979; Boiteau, 1981; Smith, 1988; Wagner et al., 1990; Forster & Williams, 1996). Macromorphologically, plants of *Neisosperma* and *Ochrosia* have a clear resemblance (e.g., trees with whorled leaves, corolla lobe aestivation dextrorsely contort, fruits large, fleshy colorful drupes). Their pollen, however, differs in several aspects (size, ectoaperture shape and margin, endoaperture margin,

and ornamentation). In our analyses, inclusion of *Neisosperma* in *Ochrosia* is only moderately supported. A detailed study including more species of both genera is needed in order to elucidate their relationship.

Also in the *Vinca* clade (Fig. 6) are two genera conventionally included in Plumerieae: *Catharanthus* and *Vinca* (Schumann, 1895 as Alstoniinae; Pichon, 1949a as Alstonieae; Leeuwenberg, 1994a). The pollen of *Vinca* is peculiar and unique by its indistinct ectoapertures, relatively large endoapertures, and thin exine, which makes it difficult to compare with the pollen of other taxa. *Vinca* also shows some derived floral characteristics, such as the enlarged spathulate, apical anther appendage, which most likely plays a role in inhibiting desiccation of the secondarily presented pollen (Church, 1908), possibly related to the temperate habitat of this genus.

The genera included here in the *Vinca* clade are the same as those included in Vinceae of Endress and Bruyns (2000), with the exception of *Amsonia* and *Rhazya*. These two genera have traditionally been considered to be closely related to *Catharanthus* and *Vinca*, and thus conventionally included in Plumerieae (Schumann, 1895 as Alstoniinae). *Amsonia*, *Rhazya*, *Catharanthus*, and *Vinca* constituted Lochnerinae of Pichon (1949a) and Catharanthinae of Leeuwenberg (1994a). Flowers, fruits, seeds, and pollen of *Rhazya* scarcely differ from those of *Amsonia* (Pichon, 1949a; Nilsson, 1986), which was therefore treated as a synonym of *Amsonia* by Endress and Bruyns (2000) and not contradicted by our study (Fig. 6). However, *Amsonia* and *Rhazya* together group here with neither Alyxieae nor Vinceae. Instead, they are placed as sister to the Plumerieae and Carisseae + Apocynoideae, although bootstrap support for this is less than 50% (Fig. 6). In the study by Potgieter and Albert (2001), *Amsonia* was in a clade together with *Thevetia peruviana*, which was included in a large polytomy. Floral structure of *Amsonia* and *Rhazya*, including details of the style head, is similar to that of *Catharanthus* and *Vinca* and does not agree with the more derived position for the former two genera indicated by the molecular analysis. Similarly, *Amsonia* and *Rhazya* occupy an unexpected position in the molecular tree considering their secondary

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intine, showing tectum (above), undulate granular infratectum, and ectexinous inner layer with surface verrucae (partly loose?). P, Q, S. *Condylocarpon isthmicum*. —P. Tetrad almost filling anther locule, showing the four constituent, psilate members in decussate configuration. —Q. TEM section through tetrad almost filling anther locule, showing three of the four constituent members, locally fused internal walls with sparse pores (arrow), and thin, locally thickened, external walls. —S. TEM section through external wall, showing the thin tectum, the poorly defined infratectum (arrow), and the locally thickened ectexinous inner layer. Scale bar = 10 μm in A, D, G, J, M, N, P, Q; scale bar = 1 μm in B, C, E, F, H, I, K, L, O, R, S.

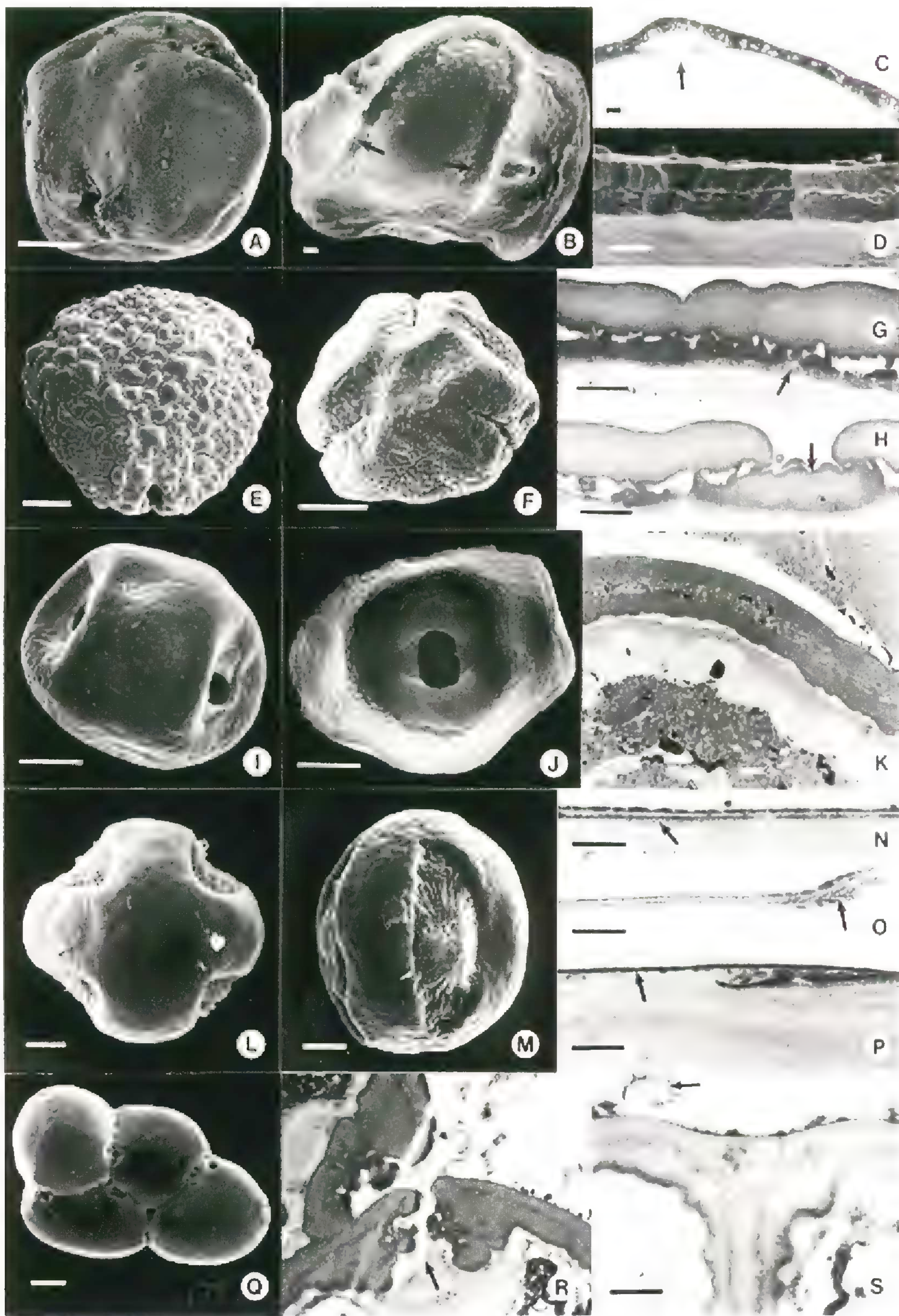


Figure 8. Apocynaceae, Vinceae (A–P), Melodineae (Q–S). SEM and TEM images of pollen grains. —A. *Petchia ceylanica*. Psilate 3-colporate pollen grain in oblique view; colpi short with relatively large endopore. B, C. *Cabucala caudata*. —B. Inside of tricolporate pollen grain showing two colpal plates, each enclosing an endopore surrounded by a distinct endoannulus; in the center a mesocolpal plate delimited from the colpal plates by supplementary endocolpi (arrows). —C. TEM section through supplementary endocolpus (arrow), colpal plate (left), and mesocolpal plate (right). —D. *Kopsia fruticosa*. SEM section, showing psilate to scabrate exine stratified into two equally thick strata (tectum above) separated by a thin infratectum. —E. *Ochrosia coccinea*. Verrucate 3-colporate pollen grain in polar view. F–H. *Neisosperma nakaiana*. —F. Psilate/finely fossulate tricolporate pollen grain in polar view. —G. TEM section through mesocolpal exine and intine, showing tectum (above), irregular infratectum and foot layer, and spongy endexine (arrow). —H. TEM section through apertural exine, showing tectum (above), irregular infratectum, foot layer, which is much thickened under the colpus (arrow), and spongy endexine. I–K. *Rhazya stricta*. —I. Psilate, perforate pollen grain in equatorial view; colpi short and wide with relatively large endopores. —J. Inside of pollen grain showing longitudinal endopore delimited polewards by horizontally oriented colpal plates; to the left and to the right, psilate mesocolpal plates delimited by wide verrucate zones (supplementary endocolpi). —K. TEM section through exine and intine, showing tectum (above), slightly thinner, granular-reticulate infratectum, and inner layer of the same thickness as tectum. L, M. *Vinca minor* (critical-point dried). —L. Psilate 4-aperturate pollen grain in polar view. —M. Psilate 4-aperturate pollen grain in equatorial view, showing the indistinct ectoaperture (porous area) in center of depressed oblong zone that is delimited by the endoapertural costae. N–P. *Vinca major*. —N. TEM section through thin mesocolpal exine and intine, showing tectum (above) with indistinct perforations, distinct

chemistry. Both genera contain numerous complex indole alkaloids of the plumerane type (Ganzinger & Hesse, 1976; Kısakürek et al., 1983), whereas in all genera above them in the uppermost clades of the tree and in Alyxieae, indole alkaloids have been lost. In the comparative study by Nilsson (1986), however, pollen of *Amsonia* and *Rhazya* was found to be nearly identical but showed no close relationships with pollen of *Catharanthus* or *Cabucala* (Vinceae). Because the focus of our study was Alyxieae, this was the only group densely sampled. To better assess the phylogenetic position of *Amsonia* and *Rhazya*, additional representatives of other tribes, especially previously unstudied genera of Melodineae sensu Endress and Bruyns (2000), should be included.

The unexpected positioning of *Amsonia* and *Rhazya* in the combined molecular tree (Fig. 5) away from the rest of Vinceae could be regarded as a case of incongruence with morphology. However, in the morphological analysis, *Amsonia* and *Rhazya* did not always appear together with *Catharanthus* + *Vinca*, and their position was relatively unclear (e.g., no BP > 50). Furthermore, combining the DNA and morphological data resulted in generally better resolution and higher support in the all-data combined tree relative to the combined DNA data tree, which would not be expected if there were highly incongruent basic patterns in each of them. The exact position of *Amsonia/Rhazya* is also not clear with the DNA data, except that their exclusion from the clade with the rest of Vinceae is not strongly supported (Figs. 5, 6), which concurs with the results of Simões et al. (2007).

SIGNIFICANCE OF MORPHOLOGICAL AND CHEMICAL CHARACTERS

As has been demonstrated here and elsewhere (Fallen, 1983a; Sennblad & Bremer, 2000, 2002; Potgieter & Albert, 2001), superficial resemblance of characters such as fruits and seeds of Apocynaceae that are correlated with dispersal mode are extremely labile, and there is a tendency for the repeated independent evolution of certain fruit and seed types. Similar findings for other angiosperm families have been reported by Bremer and Eriksson (1992),

Armbruster (1996), Endress (1996), Hufford (1997), and Clausen et al. (2000). Fruit and seed characters are thus particularly unreliable when used alone for determining relationships among genera in Apocynaceae. Examples of these characters include: fruit dehiscence, mesocarp consistency, and seed appendages. It was the use of such simple, single character-based categories that led to the artificial tribal classifications of Schumann (1895), Pichon (1949a), and Leeuwenberg (1994a). The most reliable characters are likely to be more subtle, and one must be willing to invest some effort to determine what they are. It is also unrealistic to believe that any single character is going to provide a non-homoplasious synapomorphy for any large genus or tribe, but rather it is more reasonable to expect that these groups can be circumscribed by a specific combination of characters.

Alyxieae pollen is characterized by large porate apertures (Fig. 7), which are distinct from the usual small aperturate porate grains found in Apocynoideae, completely different from the colporate grains found in Vinceae, and characteristic for all other Rauvolfioideae (Figs. 8, 9). Details of the pollen ectoapertures proved to be the most important morphological characters for defining Alyxieae because the unusual and distinct pollen type is synapomorphic for the tribe. Aperture type is less informative in the other tribes of Rauvolfioideae because, with a few exceptions (e.g., *Craspidospermum*; Fig. 8), all Rauvolfioideae have colporate pollen grains. Within Alyxieae, aberrant, inaperturate, nearly exineless pollen that remains in tetrads is an autapomorphy for *Condylocarpon*. Inaperturate pollen is otherwise known in the family only in Secamonoideae and Asclepiadoideae as well as some genera of Periplocoideae that have pollinia (e.g., *Finlaysonia* Wall., *Hemidesmus* R. Br.; Schill & Jäkel, 1978; Verhoeven & Venter, 2001). *Condylocarpon* is also of interest biogeographically, being the only Neotropical member of Alyxieae; all other genera are found in southeastern Asia and the Pacific. Aperture type thus provides a clear distinction between the Alyxieae and Vinceae.

Other morphological characteristics that distinguish Alyxieae from Vinceae (but not necessarily

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infratectum, and inner layer consisting of foot layer and spongy endexine (arrow). —O. TEM section through endoapertural costa (arrow) in ectoapertural area. —P. TEM section through endoapertural costa outside ectoapertural area, showing continuous tectum (arrow), discontinuous inner exine, and slightly bulging intine. Q–S. *Craspidospermum verticillatum*. —Q. Rhomboidal tetrad, showing several pores (in adjacent positions) near sutures. —R. TEM section through two adjacent pores of neighboring grains, showing small ectopores and heavily costate endopore (arrow). —S. TEM section through two adjacent pollen grains, showing exine stratigraphy with joint tecta, separate infratecta, and inner exine layers; note Ubisch bodies (arrow). Scale bar = 10 µm in A, E, F, L, M, Q; scale bar = 5 µm in I–K; scale bar = 1 µm in B–D, G, H, N–P, R, S.

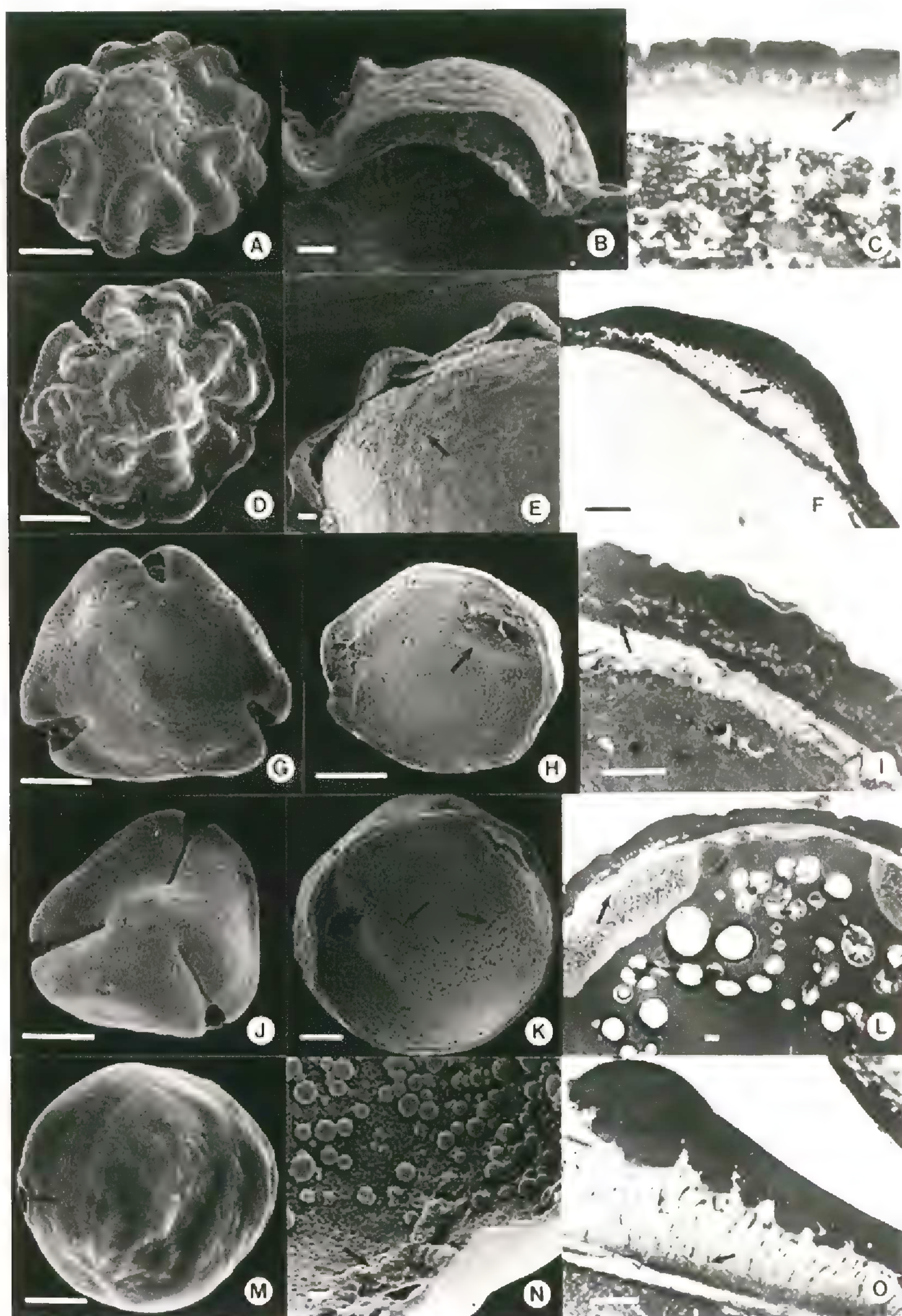


Figure 9. Apocynaceae. Aspidospermeae (A, B, D–F), Alstonieae (C), Hunterieae (G–I), Plumerieae (J), Carisseae (K, L), Malouetieae (Apocynoideae) (M–O). SEM and TEM images of pollen grains. A, B. *Aspidosperma parvifolium*. —A. Psilate/fossulate 6-colporate pollen grain in slightly oblique polar view, showing prominent arcus-like ridges surrounding the colpi. —B. SEM section of ridge, showing a thin, psilate, perforate tectum, thick granular infratectum, and thin inner exine layer. —C. *Alstonia scholaris*. TEM section through exine and intine, showing thick psilate, perforate tectum, granular infratectum, dimerous foot layer, and indistinct spongy endexine (arrow). D–E. *Vallesia glabra*. —D. Psilate pentacolporate pollen grain in polar view, showing prominent ridges surrounding colpi. —E. Part of fractured pollen grain showing three 'empty' ridges and psilate to scabrate inside with elongated, unevenly distributed granular marks (arrow). —F. *Vallesia antillana*. TEM section through ridge, showing tectum subtended by granular infratectum (arrow) and cavity, and a + dimerous inner layer. G, H. *Pleiocarpa mutica*. —G. Psilate, perforate tricolporate pollen grain in polar view. —H. Scabrate inside of colporate pollen grain showing two apertures (right and extreme left); endopores have polar costae (arrow). —I. *Picralima nitida*. TEM section through exine and intine; exine consists of relatively thick, undulated tectum, slightly thinner granular-reticulate infratectum subtended by a thin foot layer and thin indistinct endexine (arrow). —J. *Anechites nerium*. Psilate, finely perforate tricolporate pollen grain in polar view. K, L. *Acokanthera oppositifolia*. —K. Inside of tricolporate pollen grain showing colpal plates with lalongate endopores (left and right) and system of endocracks (supplementary endocolpi; arrows) delimiting psilate mesocolpal and polar plates. —L. TEM section through supplementary endocolpus (arrow) subtended by thickened intine (left) and mesocolpal exine (right). M–O. *Mascarenhasia arborescens*. —M. Psilate triporate pollen grain, showing pores with weak annulus (arrow). —N. Exine fragment showing part of psilate, sparsely perforate tectum, inner side beset with diversely

from other tribes of Rauvolfioideae) include the simple style head, which is uniformly secretory and receptive and lacks a pollen-trapping annulus at the base (vs. the style head differentiated into distinct morphological and functional zones, with the receptive region located beneath an annulus at the base). Except for *Plectaneia*, Alyxieae seeds have a tough and usually conspicuously ruminate endosperm (vs. a smooth and soft endosperm in Vinceae).

Because tribes within Rauvolfioideae were so artificial in earlier classifications (Schumann, 1895; Pichon, 1949a, 1950b; Leeuwenberg, 1994a), it was impossible to understand the evolution of secondary chemistry in this subfamily. As tribal circumscriptions become more natural, it is now possible to gain a better understanding of the phylogenetic pattern of indole alkaloids and cardenolides. Generally, indole alkaloids are considered to be characteristic for Rauvolfioideae. However, indole alkaloids characterize only five of the tribes included in this study (Alstonieae, Tabernaemontaneae, Vinceae, Hunterieae, and Melodineae), whereas indole alkaloids have not been reported in the other three tribes (Fig. 6). *Acokanthera* and *Carissa* L. contain cardenolides. Based on their syncarpous gynoecium, they were conventionally included with indole alkaloid-containing genera in a polyphyletic Carisseae (Leeuwenberg, 1994a). Here and in other phylogenetic studies (Endress et al., 1996; Potgieter & Albert, 2001; Simões et al., 2007), the Carisseae sensu Endress and Bruyns (2000) are placed with other taxa in which indole alkaloids have been lost or replaced by other secondary compounds (Johns et al., 1968; Hegnauer, 1970; Coppen & Cobb, 1983; Kiskurek et al., 1983; Jensen, 1992).

All genera of Vinceae contain various complex indole alkaloids (Hegnauer, 1970, 1989; Ganzinger & Hesse, 1976; Kiskurek et al., 1983). In contrast, all genera of Alyxieae studied for secondary chemistry lack indole alkaloids. *Alyxia* and *Lepiniopsis* contain coumarins (Johns et al., 1968; Hegnauer, 1970, 1989), and although *Lepinia* has never been analyzed for secondary compounds, the crushed leaves are known to emit a strong coumarin scent (D. Lorence, pers. comm., 1999). Coumarins are absent from *Plectaneia*, and no reliable data are available on the secondary chemistry of *Pteralyxia*, *Chilocarpus*, and *Condylocarpon*.

CHARACTER EVOLUTION

Gynoecium, fruit, and seed morphology are complex and homoplasious in Rauvolfioideae. In this analysis, the plesiomorphic state of the gynoecium is apocarpous, in concurrence with Potgieter and Albert (2001; but see Sennblad & Bremer, 2000, 2002, for an alternative view), and the majority of the genera are apocarpous. Even in the small sample here, almost every clade includes at least one syncarpous genus; similar findings are reported by Simões et al. (2007).

The style head is a useful character for distinguishing genera in Apocynaceae (see Fig. 10), but it is structurally complex, making it difficult to break down into meaningful character states for coding morphological characters. The two taxa sister to the rest in this analysis, *Vallesia* and *Aspidosperma*, have a simple style head that is vertically undifferentiated. A simple style head is also characteristic for all Alyxieae and for the Hunterieae and Carisseae (Fallen, 1986; Endress et al., 1996; Endress & Bruyns, 2000). A similar type of style head is found in some (but not all) genera of Alstonieae and Plumerieae. *Tabernaemontana* s.l., as currently circumscribed by Leeuwenberg (1991, 1994b), includes species with a simple, undifferentiated style head, such as the species included in this study, as well as ones with a complex, vertically differentiated style head with distinct functional regions and a broad pollen-trapping flange at the base (e.g., all the New World species). These results indicate that style head specialization has probably evolved in parallel in various clades of Rauvolfioideae, as was suggested by Potgieter and Albert (2001), and that this probably has proceeded in both directions (Fig. 10).

Within Alyxieae, *Lepinia* and *Lepiniopsis* have a 3- to 5-carpellate ovary, which is partially to completely syncarpous, respectively (Endress et al., 1997). A 3- to 5-carpellate ovary is otherwise known in Apocynaceae only in *Pleiocarpa* (Hunterieae). All other genera have two carpels.

Fruit type and seed margin are equivocally optimized: *Vallesia* has naked seeds in a small juicy drupe, whereas *Aspidosperma* has thick woody follicles and seeds with a diaphanous wing. This is in sharp contrast to the traditional view of the berries of Carisseae (a conglomeration of Willughbeieae, Melodineae, Hunterieae, and Carisseae, sensu Endress &

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sized granules, and endoannulus (arrow). —O. TEM section through exine and intine near pore; exine is thickened into an ecto- and endoannulus; innermost exine layer consists of granules or irregular elements that are separated from tectum by granular infratectum; the intine contains numerous radially oriented dark inclusions (arrow). Scale bar = 10 μm in A, D, G–J; scale bar = 5 μm in K, M; scale bar = 1 μm in B, C, E, F, L, N, O.

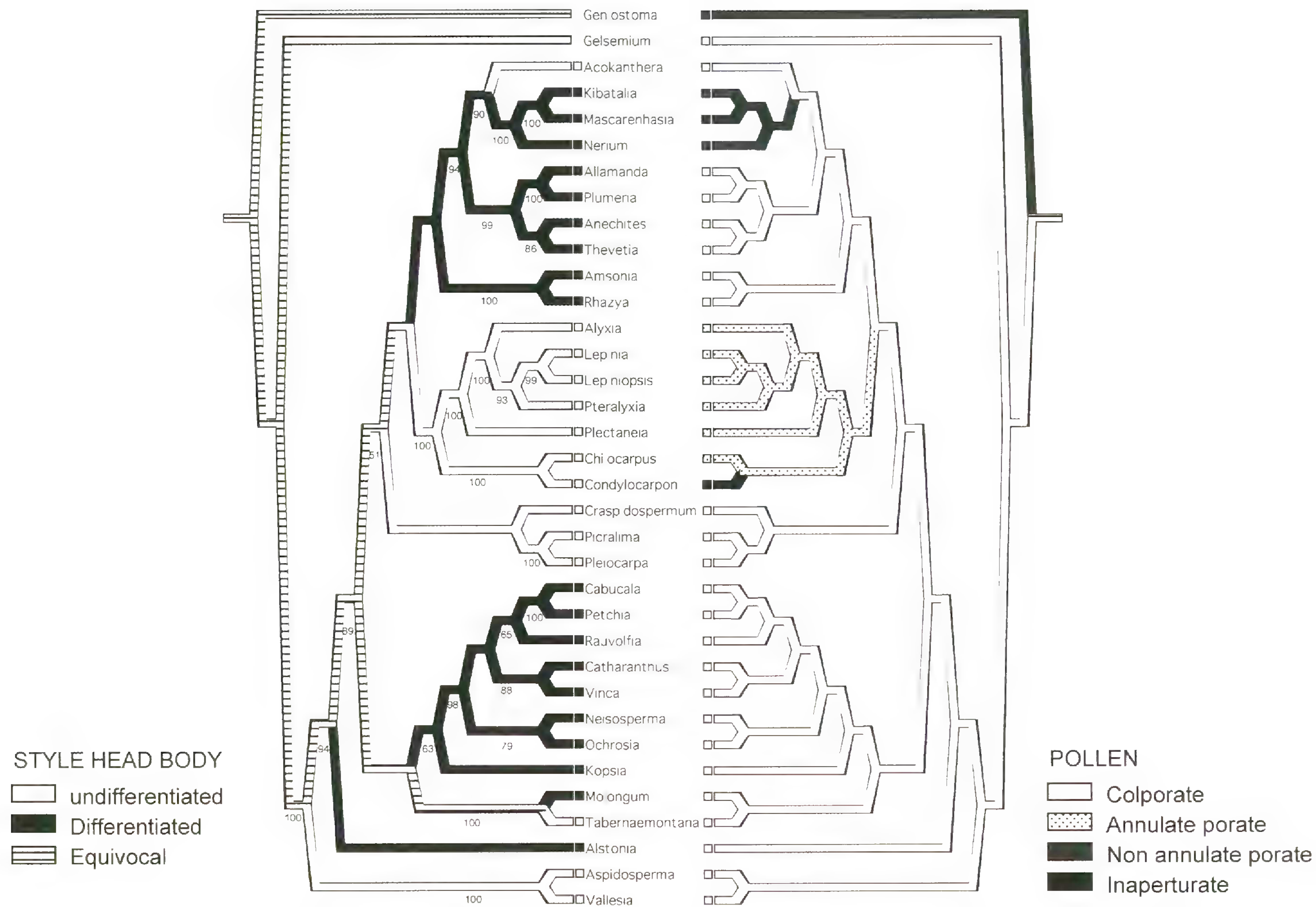


Figure 10. Evolution of style head body differentiation and pollen aperture type mapped onto the single most parsimonious tree from the combined analysis of morphological and molecular data using ACCTRAN optimization.

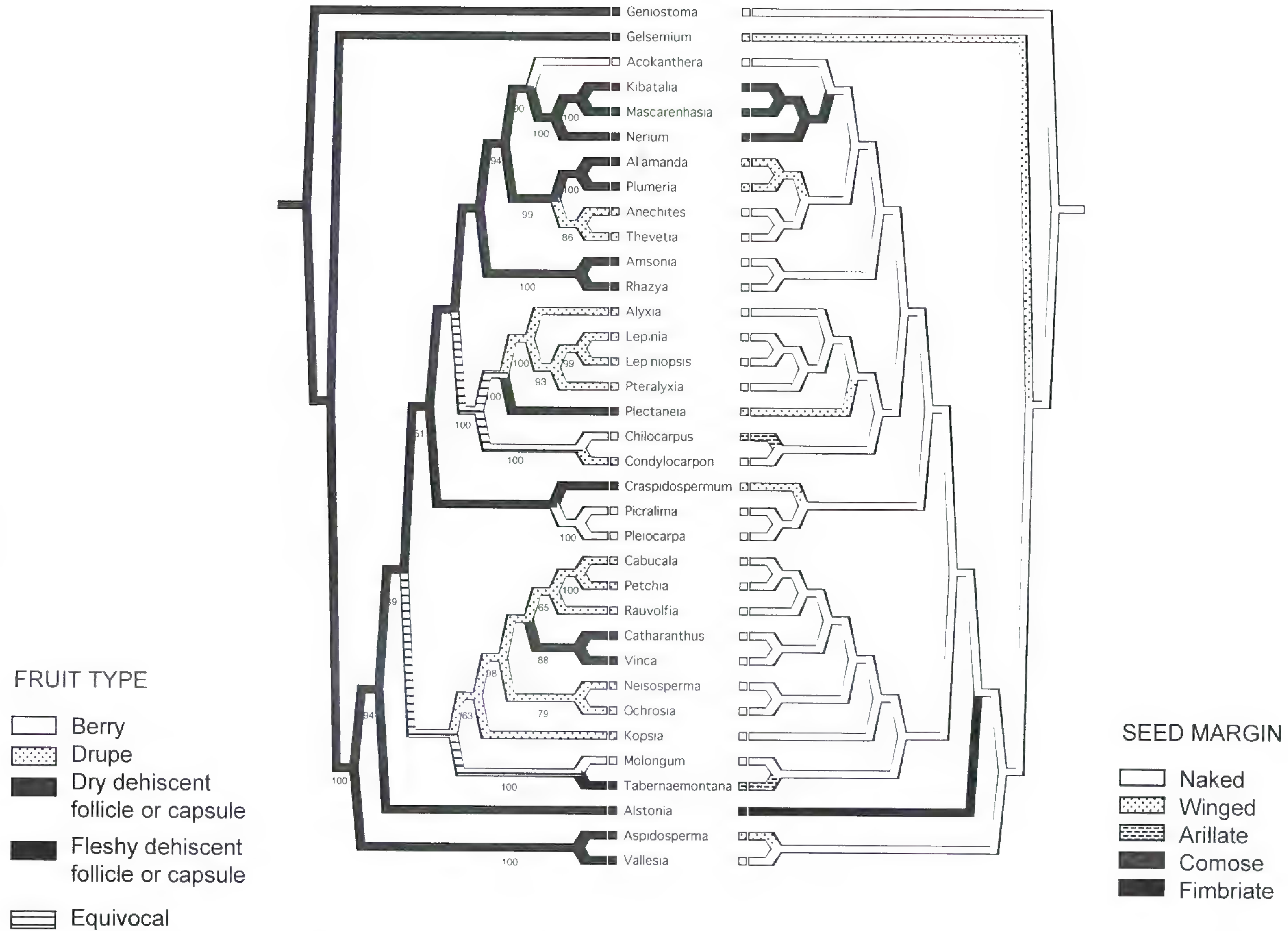


Figure 11. Evolution of fruit type and seed margin mapped onto the single most parsimonious tree from the combined analysis of morphological and molecular data using ACCTRAN optimization.

Bruyns, 2000) as the most unspecialized fruit type in the family. Vinceae are homogeneous with regard to seed margin, with the seeds being unwinged (see Simões et al., 2007, regarding *Kamettia* and *Tonduzia*, not included here). In the genera included in our analysis, the seeds are enclosed in an indehiscent drupe. Only *Catharanthus* and *Vinca* deviate, in having delicate, papery dehiscent follicles (Fig. 11). *Catharanthus* and *Vinca* also have a derived herbaceous habit, whereas the other genera in Vinceae are trees or shrubs.

The seed margin is evolutionarily plastic in Alyxieae (Fig. 11). The four morphologically most specialized genera—*Alyxia*, *Lepinia*, *Lepiniopsis*, and *Pteralyxia*, as well as *Condylocarpon*—all have distinctive cylindrical seeds that are longitudinally rolled with a deep hilar groove. *Plectaneia*, in contrast, has flat seeds with a wing at each end. In *Chilocarpus* the seeds have a small pink corky aril on the funiculus and are presented in an unusual type of leathery dehiscent fruit that splits apart along one or two valves (Leeuwenberg, 2002). Arils are rare in Apocynaceae, otherwise known only in *Tabernaemontanaeae*. Similar types of fruits are known as display fruits in Gesneriaceae (Wiehler, 1983; Smith, 2000) and Melastomataceae (Clausing et al., 2000) or as dehiscent berries in Oleaceae (Lawrence & Green, 1993; Li et al., 2002). Seed margins also vary considerably in Plumerieae (Pichon, 1949b, 1950b as *Cerberoideae*). In contrast to this diversity in Rauvolfioideae, in Apocynoideae and all other subfamilies, the fruit and seed type is uniform: the fruit is a pair of follicles (rarely postgenitally united) that dehisce to release small, comose seeds.

Palynologically, starting from the basic regular 3-colporate condition typical in the Rauvolfioideae (Figs. 8, 9; Nilsson, 1986), an entire suite of changes characterizes the derivation of Alyxieae (Fig. 10): pollen grain shape irregular, aperture number mostly two (sometimes three), and ectoapertures porate with thickened margins (Fig. 7). Within the clade, several other characters change: maximum pore diameter is 9 μm in *Chilocarpus*, 12 μm in *Plectaneia*, and 21–33 μm in the subclade including *Alyxia*, *Pteralyxia*, *Lepinia*, and *Lepiniopsis*. Possibly due to paedomorphosis, *Condylocarpon* pollen is inaperturate (van der Ham et al., 2001). Together with the larger maximum pore diameter, the *Alyxia* clade also shows much larger, barrel-shaped (two pores) or depressed (three or four pores) pollen grains, an endexinous inner exine layer with a granular surface, and an indistinct (reduced) infratectum. Within the *Alyxia* clade, there is a change toward a more heavily sculptured tectum in the subclade including *Alyxia*, *Pteralyxia*, *Lepinia*, and *Lepiniopsis*, from psilate in *Alyxia*, via undulate in

Pteralyxia, toward \pm verrucate in *Lepinia* and *Lepiniopsis*. Pollen of the last two genera mostly has more than two apertures: three (less often two) in *Lepiniopsis* and three or four in *Lepinia* (van der Ham et al., 2001). This is not simply a reversal to basic aperture conditions, as the apertures are still irregular or diverse within a single grain regarding size, configuration, and orientation.

The functional significance of the remarkable shift in pollen morphology toward and within Alyxieae is not understood. The oldest fossils of the *Alyxia* pollen type date from the Paleocene of northwestern Borneo (Muller, 1981), which demonstrates the considerable age of the syndrome.

CLASSIFICATION

The topologies of this study support recognition of Vinceae and Alyxieae sensu Endress and Bruyns (2000), as well as the exclusion of *Vallesia* and *Anechites* from either tribe and their placement in *Aspidospermeae* sensu Simões et al. (2007) and *Plumerieae*, respectively. Our results do not support inclusion of *Amsonia* and *Rhazya* in Vinceae; however, these two genera are not supported in any of the other groups included in this study. The position of *Amsonia* was also not resolved in the study by Simões et al. (2007). Because the position of *Amsonia* and *Rhazya* remains equivocal, these genera are withdrawn from Vinceae and left unplaced for the time being. Although it is unsatisfying to leave them in limbo, a classification should reflect phylogeny, so it seems best to keep them as unplaced genera until more data are available to place them more definitely.

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- APPENDIX 1.
- Voucher specimens used for morphological character assessment in the Alyxieae study. Herbarium acronyms are in parentheses. Specimens used to study floral structure are indicated with FS, those used for light microscopy, scanning electron microscopy, and transmission electron microscopy of pollen grains are indicated by LM, SEM, and TEM, respectively, following the herbarium acronym.
- APOCYNACEAE
- Acokanthera oblongifolia* (Hochst.) Codd
cult. Bot. Gard. Wageningen, *Kas et al. s.n.* (Z) FS
- Acokanthera oppositifolia* (Lam.) Codd
South Africa, *Bayliss BRI 544* (S) LM, SEM, TEM
- Allamanda cathartica* L.
cult. Royal Bot. Gard. Kew, 1983, *Fallen s.n.* (Z) FS; Gabon, *Leeuwenberg 12540* (WAG) LM, SEM, TEM
- Alstonia scholaris* (L.) R. Br.
cult. Fairchild Trop. Gard., *Gillis 6995* (Z) FS; New Guinea, *Schodde 2472* (L) LM, SEM, TEM
- Alyxia oblongata* Domin
Australia, *Dockrill 835* (L) LM
- Alyxia ruscifolia* R. Br.
cult. Montpellier, *Civeyrel 1055* (Z) FS; Australia, *Clark et al. 1753* (L) SEM, TEM
- Amsonia ciliata* Walter
U.S.A., *Sasseen s.n.* (WAG) LM, SEM, TEM
- Amsonia tabernaemontana* Walter
cult. Bot. Gard. Zürich, *Endress s.n.* (Z) FS
- Anechites nerium* (Aubl.) Urb.
Ecuador, *Asplund 16471* (Z) FS; Dominican Republic, *Ekman 15239* (S) LM, SEM
- Aspidosperma parvifolium* A. DC.
Brazil, *Ferreira s.n.* (Z) FS; Brazil, *Heringer 10672* (UB) LM, SEM
- Cabucala caudata* Markgr.
Madagascar, *Capuron 23701-SF* (P) LM, SEM, TEM
- Cabucala polysperma* (Scott-Elliott) Pichon
Madagascar, *Civeyrel 1281* (Z) FS
- Catharanthus roseus* (L.) G. Don
cult. Bot. Gard. Zurich, *Endress s.n.* (Z) FS; Liberia, *Van Harten 29* (WAG) LM, SEM, TEM
- Chilocarpus denudatus* Blume
cult. Bot. Gard. Bogor, *Burck s.n.* (Z) FS; India, *Ridsdale 757* (L) LM; Java, *Blume s.n.* (L) LM; Java, *anon. s.n.* (S) SEM; Sarawak, *Richards 1463* (L) TEM
- Chilocarpus suaveolens* Blume
Java, *Hochreutiner 2547* (L, Z) FS
- Condylocarpon guyanense* Desf.
French Guiana, *Sastre 5470* (P, Z) FS
- Condylocarpon isthmicum* (Vell.) A. DC.
Brazil, *Koch s.n.* (Z) FS; Brazil, *Hatschbach 13030* (Z) LM, SEM, TEM
- Craspidospermum verticillatum* Bojer ex A. DC.
Madagascar, *Civeyrel 1234* (Z) FS, LM, SEM, TEM
- Kibatalia gitingensis* (Elmer) Woodson
Philippines, *Liede 3268* (Z) FS; Philippines, *Wenzel 652* (G) LM, SEM, TEM
- Kopsia fruticosa* (Ker Gawl.) A. DC.
Java, *Prévost 167* (Z); cult. Victoria, Trinidad, *Broadway 5963* (S) LM, SEM, TEM
- Lepinia marquisensis* Lorence & W. L. Wagner
Fatu Hiva, Marquesas Islands, *Perlman 10271* (BISH, Z) FS
- Lepinia solomonensis* Hemsl.
Solomon Islands, *BSIP 13496* (L) LM, TEM
- Lepinia taitensis* Decne.
Society Islands, Moorea, *Perlman et al. 15071* (PTBG, Z) FS; Society Islands, Tahiti, *Whistler 4932* (BISH) SEM
- Lepiniopsis ternatensis* Valeton
Moluccas, *Mochtar 306* (L) LM, SEM; *PNH 17362* (L) TEM
- Lepiniopsis trilocularis* Markgr.
Palau Islands, *Lorence 8265* (PTBG, Z) FS
- Mascarenhasia arborescens* A. DC.
cult. Fairchild Bot. Gard., *Bird s.n.* (Z) FS; Madagascar, *Schlieben 8128* (Z) LM, SEM; Madagascar, *Capuron 22808-SF* (P) LM, SEM, TEM
- Molongum laxum* (Benth.) Pichon
Venezuela, *Berry 5400* (MO, Z) FS; Colombia, *Duntii 36267* (COL) LM, SEM, TEM
- Neisosperma nakaiana* (Koidz.) Fosberg & Sacht
cult. Waimea Arboretum, Hawaiian Is., *Neill 5291* (Z) FS, LM, SEM, TEM
- Nerium oleander* L.
cult. Bot. Gard. Zurich, *Fallen s.n.* (Z) FS; France, *Segal 232* (WAG) LM, TEM; cult. Perpignan, *Leeuwenberg 12206* (WAG) LM, SEM, TEM
- Ochrosia coccinea* (Teijsm. & Binn.) Miq.
cult. Bogor Bot. Gard., Java, *anon. s.n. 30/8/1982* (Z) FS, LM, SEM, TEM
- Petchia ceylanica* (Wight) Livera
cult. Bot. Gard. Kaiserslautern, *Omlor s.n.*, *Kessler s.n.* (Z) FS; Sri Lanka, *Wambeck 2510* (S) LM, SEM
- Picalima nitida* (Stapf) T. Durand & H. Durand
cult. Bot. Gard. Wageningen, *Leeuwenberg 10779* (Z) FS; Zaire, *Gille 100* (BR) LM, SEM, TEM
- Plectaneia stenophylla* Jum.
cult. Madagascar, *Petignat s.n.* (Z) FS
- Plectaneia thouarsii* Roem. & Schult.
Madagascar, *Bernardi 11820* (L) LM, SEM, TEM
- Pleiocarpa mutica* Benth.
cult. Bot. Gard. Wageningen, *van Setten 415* (WAG, Z) FS; Ivory Coast, *Leeuwenberg 12145* (WAG) LM, SEM
- Plumeria rubra* L.
cult. Bot. Gard. Zurich, *Fallen s.n.* (Z) FS; Ghana, *Leeuwenberg 11089* (WAG) LM, SEM, TEM
- Pteralyxia kauaiensis* Caum
Kauai, *Perlman 15456* (Z) FS; Hawaii, *Flynn 269* (PTBG) SEM
- Pteralyxia macrocarpa* (Hillebr.) K. Schum.
Hawaii, *Swezey s.n.* (L) LM, TEM
- Rauwolfia vomitoria* Afzel.
Ivory Coast, *Aké Assi s.n.* (Z) FS; Nigeria, *Leeuwenberg 11337* (WAG) LM, SEM, TEM; Ivory Coast, *Leeuwenberg 12122* (WAG) LM, SEM

Rhazya stricta Decne.

Yemen, *Brunner 31* (Z) FS; Saudi Arabia, *Schimper 812* (L) LM, SEM, TEM

Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult.
cult. Bot. Gard. Calcutta, *anon. s.n.* (Z) FS

Tabernaemontana pandacaqui Lam.
Australia, *Alkin s.n.* (Z) LM, SEM, TEM

Thevetia peruviana (Pers.) K. Schum.
cult. Bot. Gard. Zurich, *Fallen s.n.*, (Z) FS; cult.
Florida, *Gillis 9227* (S) LM, SEM, TEM

Vallesia antillana Woodson
cult. Fairchild Trop. Gard, *Zona s.n.* (Z) FS; Florida,
Killip 43415 (S) TEM

Vallesia glabra (Cav.) Link
Galapagos Is., *A. & H. Andersen 1009* (QCA) LM, SEM

Vinca minor L.

cult. Zurich, *Fallen s.n.* (Z) FS; cult. Schipluiden,
Netherlands, *De Kort s.n.* (L) SEM; cult. Sollentuna,
Sweden, *Nilsson s.n.* (S) SEM

Vinca major L.
cult. Pijnacker, Netherlands, *Van der Ham s.n.* (L) LM,
TEM

GEISEMIACEAE

Gelsemium sempervirens (L.) J. St.-Hil.
U.S.A., Louisiana, *Tucker 28771* (Z) FS; South
Carolina, *Wall. s.n.* (S) LM, SEM, TEM

LOGANIACEAE

Geniostoma rupestre (J. R. Forst. & G. Forst.) var.
ligustrifolium (A. Cunn.) B. J. Conn
New Zealand, *Garnock-Jones s.n.* (WELTU, Z)
FS; New Zealand, *Nilsson NZ 9* (S) LM, SEM,
TEM

Appendix 2. Voucher specimens used for molecular analyses and GenBank accession numbers.

Taxon		GenBank Accession No.			
APOCYNACEAE	Voucher/Literature Citation	<i>matK</i>	<i>rbcL</i>	<i>trnL</i>	<i>trnL-F</i>
<i>Acokanthera oblongifolia</i> (Hochst.) Codd	Endress et al., 1996 Sennblad & Bremer, 1996	Z70182	X91758		
<i>Acokanthera oppositifolia</i> (Lam.) Codd	Potgieter & Albert, 2001			AF214302	AF214148
<i>Allamanda cathartica</i> L.	Endress et al., 1996 Sennblad & Bremer, 1996	Z70190	X91759		
<i>Allamanda</i> indet.	Potgieter & Albert, 2001			AF214304	AF214150
<i>Alstonia boonei</i> De Wild.	Potgieter & Albert, 2001			AF102374	AF214151
<i>Alstonia scholaris</i> (L.) R. Br.	Endress et al., 1996 Sennblad & Bremer, 1996	Z70189	X91760		
<i>Alyxia buxifolia</i> R. Br.	Potgieter & Albert, 2001			AF214306	AF214152
<i>Alyxia ruscifolia</i> R. Br.	cult. Montpellier, <i>Civeyrel 1055</i> (TL) Sennblad & Bremer, 2002	DQ837536	AJ419731		
<i>Amsonia tabernaemontana</i> Walter	Potgieter & Albert, 2001 cult. Royal Bot. Gard. Kew, <i>Civeyrel 1057</i> (TL)	AM295066	AM295078	AF214307	AF214153
<i>Anechites nerium</i> (Aubl.) Urb.	Sennblad & Bremer, 2002		AJ419733	AM295087	AM295087
<i>Aspidosperma quebracho-</i> <i>blanco</i> Schltdl.	Potgieter & Albert, 2001			AF214319	AF214165
<i>Aspidosperma triternatum</i> Rojas Acosta	cult. Bot. Garden, Meise, <i>Bremer</i> <i>3029</i> (UPS) Sennblad & Bremer, 2002	AM295077	AJ419735		
<i>Cabucala polysperma</i> (Scott-Elliott) Pichon	Madagascar, <i>Civeyrel 1281</i> (TL)	AM295067	AM295079	AM295088	AM295088
<i>Catharanthus roseus</i> (L.) G. Don	Potgieter & Albert, 2001 cult. Stockholm Univ., <i>Bremer</i> <i>3128</i> (UPS) Sennblad & Bremer, 1996	AM295068	X91757	AF102392	AF214175
<i>Chilocarpus suaveolens</i> Blume	Endress et al., 1996 cult. Bot. Gard. Bogor, <i>Chase</i> <i>1208</i>	Z70184	X92445	AM295089	AM295089
<i>Condylocarpon</i> <i>amazonicum</i> (Markgr.) Ducke	Potgieter & Albert, 2001			AF214337	AF214183
<i>Condylocarpon guyanense</i> Desf.	French Guiana, <i>M. F. Prevost s.n.</i> (CAY)	DQ837537	AM295080		
<i>Craspidospermum</i> <i>verticillatum</i> Bojer ex A. DC.	Madagascar, <i>Civeyrel 1234</i> (TL) Sennblad & Bremer, 2002	DQ837538	AJ419743	AM295090	AM295090
<i>Kibatalia gitingensis</i> (Elmer) Woodson	Philippines, <i>Liede 3268</i> (Z) Sennblad & Bremer, 2002	AM295069	AJ419745		
<i>Kopsia fruticosa</i> (Ker Gawl.) A. DC.	cult. Bot. Gard. Meise, <i>Bremer</i> <i>3033</i> (UPS) Endress et al., 1996	Z70178	X91763	AM295091	AM295091
<i>Lepinia taitensis</i> Decne.	Sennblad & Bremer, 1996 Sennblad & Bremer, 2002		AJ419746		
<i>Lepiniopsis ternatensis</i> Valeton	Potgieter & Albert, 2001			AF214374	AF214220
<i>Lepiniopsis trilocularis</i> Markgr.	Palau Islands, <i>Lorence 8265</i> (PTBG)		AM295081		

Appendix 2. Continued.

Taxon		GenBank Accession No.			
APOCYNACEAE	Voucher/Literature Citation	<i>matK</i>	<i>rbcL</i>	<i>trnL</i>	<i>trnL-F</i>
<i>Mascarenhasia arborescens</i> A. DC.	Potgieter & Albert, 2001 cult. Wageningen Agric. Univ., nr. 80-16, <i>Setten 625</i> (WAG) Sennblad et al., 1998	AM295070		AF214378	AF214224
<i>Molongum laxum</i> (Benth.) Pichon	Potgieter & Albert, 2001 Endress et al., 1996	Z70185	X91765	AF214383	AF214229
<i>Neisosperma nakaiana</i> (Koidz.) Fosberg & Sachet	Potgieter & Albert, 2001			AF214385	AF214231
<i>Neisosperma oppositifolia</i> (Lam.) Fosberg & Sachet	cult. Nat. Trop. Bot. Gard., Kauai, <i>Lorence s.n.</i> , NTBG 970511 (PTBG)	AM295071	AM295082		
<i>Nerium oleander</i> L.	Potgieter & Albert, 2001 Civeyrel et al., 1998 Sennblad et al., 1998	Z98173		AF214386	AF214232
<i>Ochrosia coccinea</i> (Teijsm. & Binn.) Miq.	Sennblad & Bremer, 2002 cult. Wageningen Agric. Univ. nr. 78PTHB 354, <i>van der Laan</i> 389 (WAG)			AM295092	AM295092
<i>Petchia ceylanica</i> (Wight) Livera	cult. Univ. Kaiserslautern, Germany, <i>Omlor s.n.</i> (Z)	AM295072	AM295083	AM295093	AM295093
<i>Picralima nitida</i> (Stapf) T. Durand & H. Durand	Potgieter & Albert, 2001 Endress et al. 1996 Sennblad & Bremer, 1996	Z70179		AF214404	AF214250
<i>Plectaneia stenophylla</i> Jum.	Madagascar, <i>Petignat s.n.</i> (Z) Potgieter & Albert, 2001			AF214405	AF214251
<i>Pleiocarpa mutica</i> Benth.	Potgieter & Albert, 2001 cult. Royal Bot. Gard. Kew, <i>Civeyrel 1086</i> (TL) Sennblad & Bremer, 2002	DQ837539		AF214407	AF214253
<i>Plumeria alba</i> Kunth	Potgieter & Albert, 2001			AF214408	AF214254
<i>Plumeria inodora</i> Jacq.	Sennblad & Bremer, 1996		X91767		
<i>Plumeria rubra</i> L.	Endress et al., 1996	Z70191			
<i>Pteralyxia kauaiensis</i> Caum	Kauai, Hawaii, <i>Lorence 7768</i> (PTBG, Z)	AM295073	AM295085	AM295094	AM295094
<i>Rauwolfia mannii</i> Stapf	Endress et al., 1996 Sennblad & Bremer, 1996	Z70181		X91769	
<i>Rauwolfia serpentina</i> Benth. ex Kurz	Potgieter & Albert, 2001			AF214415	AF214261
<i>Rhazya stricta</i> Decne.	<i>Agosti 29</i> (Z)	AM295074	AM295086	AM295095	AM295095
<i>Tabernaemontana</i> <i>citrifolia</i> L.	Potgieter & Albert, 2001			AF214431	AF214277
<i>Tabernaemontana</i> <i>divaricata</i> (L.) R. Br. ex Roem. & Schult.	Endress et al., 1996 Sennblad & Bremer, 1996	Z70187		X91772	
<i>Thevetia ahouai</i> (L.) A. DC.	Potgieter & Albert, 2001			AF214435	AF214281
<i>Thevetia peruwiana</i> (Pers.) K. Schum.	Endress et al., 1996 Sennblad & Bremer, 1996	Z70188		X91773	
<i>Vallesia antillana</i> Woodson	cult. Fairchild Trop. Gard., <i>Meagher 966</i> (FTG) Sennblad & Bremer, 2002 Potgieter & Albert, 2001	AM295075		AF214447	AF214293

Appendix 2. Continued.

Taxon		GenBank Accession No.			
APOCYNACEAE	Voucher/Literature Citation	<i>matK</i>	<i>rbcL</i>	<i>trnL</i>	<i>trnL-F</i>
<i>Vinca minor</i> L.	cult. Bot. Gard. Uppsala, <i>Sennblad</i> 230 (UPS) Sennblad & Bremer, 2002 Potgieter & Albert, 2001	AM295076			
			AJ419768		
				AF214449	AF214295
GELSEMIACEAE					
<i>Gelsemium sempervirens</i> (L.) J. St.-Hil.	Endress et al., 1996 Olmstead et al., 1993 cult. Royal Bot. Gard. Kew, <i>Civeyrel 1069</i> (TL)	Z70195			
			L14397		
				AM295096	AM295096
LOGANIACEAE					
<i>Geniostoma rupestre</i> J. R. Forst. & G. Forst.	Endress et al., 1996 Wellington, New Zealand, <i>Garnock-Jones 2200</i> (WELTU)	Z70194	Z68828		
				AM295097	AM295097

APPENDIX 3. Matrix based on the morphological character coding. For some genera more than one species was used for character coding. a = species used to code characters 1–37 (the non-pollen characters); b = species used to code characters 38–54 (the pollen characters). See Appendices 1 and 4.

	1 2 3 4 5																																				
	123456789012345678901234567890123456789012345678901234																																				
<i>Acokanthera oblongifolia</i> ^a , <i>A. oppositifolia</i> ^b	010000000000000010000000000000201101000020100110011001																																				
<i>Allamanda cathartica</i>	112000011001100121111012010200200010001020000100101200																																				
<i>Alstonia scholaris</i>	012000100000000020110012000300202120000020002010001100																																				
<i>Alyxia oblongata</i> ^b , <i>A. ruscifolia</i> ^{a,b}	012010000000000010000010101002020100001112210010322000																																				
<i>Amsonia ciliata</i> ^b , <i>A. tabernaemontana</i> ^a	211000010000000021100012001000202130000020000210011200																																				
<i>Anechites nerium</i>	1100010110000011201110120100002021???0?0200000001?1240																																				
<i>Aspidosperma parvifolium</i>	0110001000000000100100120102002010300000303001000?1201																																				
<i>Cabucala caudata</i> ^b , <i>C. polysperma</i> ^a	012000000000000021100010100000001120000020001200011101																																				
<i>Catharanthus roseus</i>	210000000000000021102012001001202130001020002200001201																																				
<i>Chilocarpus denudatus</i> ^{a,b} , <i>C. suaveolens</i> ^a	1100100000100000100100120130011000???00112201010211100																																				
<i>Condylocarpon guyanense</i> ^a , <i>C. isthmicum</i> ^{a,b}	1120000000100000100000121010021201???1010???0?1011200																																				
<i>Craspidospermum verticillatum</i>	012000100000000020110002000100202130010133001000011100																																				
<i>Kibatalia gitingensis</i>	010002010101110230001012012013101100100023101000311200																																				
<i>Kopsia fruticosa</i>	0100000001000000211020111000001--130001020002100011101																																				
<i>Lepinia marquisensis</i> ^a , <i>L. solomonensis</i> ^b , <i>L. taitensis</i> ^{a,b}	011000000010000010000111101002020100001122210010322010																																				
<i>Lepiniopsis ternatensis</i> ^b , <i>L. trilocularis</i> ^a	011000000010000010000111101002020100001122210010322010																																				
<i>Mascarenhasia arborescens</i>	010001010100110230001012012013101100100023101010311000																																				
<i>Molongum laxum</i>	0100010000011101213100100030003011???00031201010211100																																				
<i>Veisosperma nakaiana</i>	012000000100000021200011100100101120000020002200001001																																				
<i>Verium oleander</i>	0120011101000102312100120120131011010001231010?0311000																																				
<i>Ochrosia coccinea</i>	012000000100000021200010100100101120001021200200001021																																				
<i>Petchia ceylanica</i>	0120000000000000211000101000000011???00021001200211000																																				
<i>Picralima nitida</i>	010001100000000010000011003000300120000021002000101040																																				
<i>Plectaneia stenophylla</i> ^a , <i>P. thouarsii</i> ^b	1100000000000000100000120102010001???00112010010311100																																				
<i>Pleiocarpa mutica</i>	0100001000000000100001110030003001300000210020001?1100																																				
<i>Plumeria rubra</i>	011000010000000010010002000200202010000020000110101201																																				
<i>Pteralyxia kauaiensis</i> ^{a,b} , <i>P. macrocarpa</i> ^b	0110000000000000100000111010020201???01112210010322000																																				
<i>Rauwolfia vomitoria</i>	012000000000000021101010100000001120001020003200011100																																				
<i>Rhazya stricta</i>	211000000000000021100012000100212120000020000210011200																																				
<i>Tabernaemontana divaricata</i> ^a , <i>T. pandacaqui</i> ^b	0100010000100000100100100030013211400000200020?0211200																																				
<i>Thevetia peruviana</i>	0110010110011011201110011132002020010010200000?0101230																																				
<i>Vallesia antillana</i> ^{a,b} , <i>V. glabra</i> ^b	0110000000000000110000111030002--030000030300000011200																																				
<i>Vinca major</i> ^b , <i>V. minor</i> ^{a,b}	2100000000000000121102012001001200120001024023001001200																																				
<i>Gelsemium sempervirens</i>	100100010200002-0---0012000200201120000020000000000250																																				
<i>Geniostoma rupestre</i>	0001020102000000000000020130012011???00023101010101100																																				

APPENDIX 4.

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

The characters and character states used in this study are based on the exemplar method; only the characteristics of the species used in the analysis are considered in assigning codes. In cases of large genera with a range of states, this is indicated here.

1. Habit: 0 = trees or shrubs; 1 = lianas or vines; 2 = perennial herbs. The species used to represent *Alyxia* here, *A. ruscifolia*, is a shrub; the great majority of *Alyxia* species, however, are lianas.

2. Non-articulated laticifers: 0 = absent; 1 = present. Non-articulated laticifers are one of the key characters that distinguish Apocynaceae s.l. from other Gentianales.

3. Phyllotaxis: 0 = leaves opposite; 1 = leaves alternate; 2 = leaves verticillate. Some taxa have leaves that are predominantly verticillate but may have some nodes with only two leaves. These taxa are coded as verticillate here.

4. Stipules: 0 = absent; 1 = present. Apocynaceae are here considered to be estipulate in the sense of Cronquist (1981) and Rosatti (1989). The colleters or interpetiolar ridges found in some taxa are not considered to be homologous with true stipules, nor are the adaxial outgrowths at the base of the petiole in *Alstonia scholaris* (Sidiyasa, 1998). Small bract- or scale-like organs that are found in some species of *Rauwolfia* have sometimes been called stipules. In a recent revision of the Neotropical species, however, Koch (2002) argued convincingly that these organs are better interpreted as cataphylls.

5. Supernumerary bracteoles: 0 = absent; 1 = present. Supernumerary bracteoles are clusters of bracteoles on the pedicel subtending the calyx. These bracteoles often resemble the sepals.

6. Calycine colleters: 0 = absent; 1 = several, across the inner face of the sepal (these sometimes coalesced at the base); 2 = few, mostly in the sepal sinuses. Calycine colleters are a common feature in Gentianales, and their lack or presence and arrangement is often used in Apocynaceae as an aid in distinguishing genera (e.g., Stapf, 1902; Woodson, 1933; Rosatti, 1989: 338–339; Ezcurra et al., 1992: 9–10; Omino, 1996: 87–88; Middleton, 1999, fig. 1). In *Alyxia ruscifolia*, although colleters are lacking at the base of the sepals themselves, they are well developed in the many supernumerary bracteoles clustered below the calyx. In Endress et al. (1996), *Plumeria* was coded as having a continuous row of calycine colleters. Detailed examination, however, has shown that they are not homologous to typical calycine colleters in that the entire inner surface of the upper part of the sepal is glandular. Because no other taxon shares this condition in this study, it is non-informative and thus not coded here.

7. Fusion of corolla tube: 0 = fused just above the level of stamen insertion; 1 = unfused (with gaps) just above the level of stamen insertion. In Apocynaceae, the lower corolla tube (composed of the united petal and stamen primordia) is congenitally fused; the upper part fuses postgenitally and basipetally, so that the last region to fuse is just above the insertion of the stamens. In some genera, flowers reach anthesis before fusion of the upper corolla is complete, resulting in gaps in the corolla tube (Boke, 1948; Nishino, 1982; Erbar, 1991). These gaps are especially long in *Aspidosperma*, *Geissospermum*, *Haplophyton*, and *Stephanostegia*, resulting in conspicuous slits that are easily visible with a dissecting microscope (Woodson, 1951; Fallen, 1980;

Leeuwenberg, 1997). Shorter gaps of some microns in length (visible in microtome serial sections) are also found in *Alstonia*, *Craspidospermum*, and *Hunteria*, whereas in other genera epidermal remnants are still visible, although there are no distinct gaps. In Endress et al. (1996), epidermal remnants and gaps were treated together as a single character state: corolla incompletely fused. Because, however, the tube may be fused yet still show epidermal remnants, here only the presence of distinct gaps, visible with dissecting microscope or in serial sections, is coded as unfused.

8. Corolla tube mouth: 0 = constricted; 1 = not constricted.

9. Infrastaminal appendages: 0 = absent; 1 = present. Infrastaminal appendages is a term used by Pichon (1948b) for outgrowths of the lower, congenitally fused part of the corolla tube in the staminal sectors (see Alvarado-Cárdenas & Ochoterena, 2007). They are found mainly in taxa previously included in *Cerberaeae* (e.g., *Cerbera*, *Thevetia*, *Cerberiopsis*). These genera have a long, thin style and a disproportionately large, broad style head.

10. Corolla lobe aestivation: 0 = sinistrorsely contort; 1 = dextrorsely contort; 2 = imbricate. Corolla lobe aestivation is one of the most important morphological characters in Apocynaceae. With a few exceptions, the direction is constant within a genus. In *Rauvolfioideae* corolla lobes are almost always sinistrorsely contort, whereas in *Apocynoideae*, corolla lobes are normally dextrorsely contort or, rarely, valvate. The corolla lobes in *Kopsia*, *Ochrosia*, and *Neisosperma* (all *Rauvolfioideae*) are consistently dextrorsely contort (Hendrian, 2004; Middleton, 2004) and thus an exception to the rule. *Alstonia* is one of the few genera in the family in which both sinistrorsely as well as dextrorsely contort species occur, and this feature is constant only at the species level.

11. Petals in bud: 0 = not inflexed; 1 = inflexed. In most Apocynaceae, contorted petals in bud are spiraled upward into a tip. Petals that are inflexed in bud are, instead, folded downward and spiral into the mouth of the corolla tube and only unfold at anthesis. Inflexed petals is a relatively uncommon condition in Apocynaceae.

12. Corolline corona below petal sinus, behind and/or just above anther: 0 = absent; 1 = a compact protruding lobe. All outgrowths in the staminal sector and above the insertion of the anther are interpreted here as a corona. These include the vertical ridges in *Molongum* Pichon, as well as the protuberances termed suprastaminal appendages by Pichon (1948b) in *Thevetia*; the fimbriate lobes of *Allamanda* are also interpreted as a corona (see Endress et al., 1996).

13. Anthers: 0 = atop filaments that arise from the corolla tube; 1 = sessile upon enlarged staminal ridges.

14. Lignified guide rails: 0 = absent; 1 = present. Lignified guide rails are a specialization of the lateral parts of the anther and have a function in the complex pollination mechanism in Apocynaceae; they are absent in most *Rauvolfioideae*, but are characteristic for *Apocynoideae*. It is important to note that lignified guide rails are also characteristic for the majority of *Tabernaemontana* species (including all of the Neotropical taxa), although absent in the two representative species used in this study and in all of section *Ervatamia* to which they belong (see Leeuwenberg, 1994b: xv). Thus, for this character, most species of *Tabernaemontana* would show more affinity morphologically to *Molongum* than is apparent from the representative species used here (compare with Endress et al., 1996, in which a Neotropical species was used in the morphological analysis).

15. Anther dehiscence: 0 = introrse; 1 = latrorse; 2 = extrorse.

16. Anther/style head synorganization: 0 = anthers situated above or below, but not closely synorganized with the style head; 1 = anthers at about the same level as, and connivent over and encircling the style head; 2 = anthers agglutinated to the style head via hair pads and adhesive. Synorganization of the anthers and the style head has always been a key character in Apocynaceae. It is the most important traditional character that separates Apocynoideae (in which the anthers are postgenitally united with the style head) from Rauvolfioideae (in which the anthers are free from the style head).

The lack of close synorganization of the anthers and style head in *Tabernaemontana divaricata* is not typical of the whole genus (as defined by Leeuwenberg, 1991). In all Neotropical species of *Tabernaemontana* and in some Paleotropical ones as well, the style head and anthers are more closely synorganized and would be coded as character state 1 in this study.

17. Style apex specialization: 0 = style apex without secretory epithelium; 1 = style apex transformed into an enlarged style head with epithelium of the body uniformly secretory and receptive; 2 = style apex transformed into an enlarged style head with epithelium of the body vertically differentiated with stigmatic region at base; 3 = style apex transformed into an enlarged style head, with epithelium of the body vertically differentiated, stigmatic zone at base, and radially mechanically interrupted by the adnate anthers. All Apocynaceae are characterized by having the carpel apices forming an enlarged style head with secretory epithelium. The degree and manner of histological differentiation of the style head and the epithelium is variable, with a specific type often characteristic of a particular tribe (Schick, 1980; Fallen, 1986). Although the gynoecium apex in *Geniostoma* J. R. Forst. & G. Forst. is post-genitally fused and enlarged, it is not covered with a secretory epithelium like that found in Apocynaceae. Instead, on male flowers, enlarged glue-filled irritable hairs with an abscissable tip are found scattered among the more numerous smaller, normal papillae (Endress et al., 1996). Specialized glue hairs like those found in *Geniostoma* are unknown in Apocynaceae.

18. Style head upper hair wreath: 0 = absent; 1 = present. Some style heads have a wreath of longer hairs just below the unfused carpel tips. The main function of the wreath is for pollen deposition and secondary presentation. The flowers are protandrous; shortly before anthesis, the anthers dehisce and shed their pollen toward the center of the flower. If the style head has an upper wreath, the pollen is shed onto this ring of hairs, which plays a role in the complex pollination mechanism of Apocynaceae (Church, 1908; Schick, 1980; Fallen, 1986).

19. Style head base: 0 = without collar or flange; 1 = with a distinct, thin collar; 2 = with a wreath of longer hairs; 3 = with thick flange. The base of the style head is often equipped with a means of scraping off donor pollen from the proboscis of an insect visitor. The presence (or absence) and type of scraper is often diagnostic of a particular tribe, and thus a useful character in the family. When a scraper is present at the base of the style head, the receptive zone is located beneath it (Schick, 1980; Fallen, 1986).

20. Style head unfused apices: 0 = small, inconspicuous, less than 1/3 the length of the total style head; 1 = enlarged, conical and tapering to blunt and clavate, at least 1/3 the length of the total style head.

21. Free disc nectary: 0 = absent; 1 = entire, annular; 2 = two separate lobes. In Apocynaceae, a free nectar disc is

often present. Sometimes the nectar disc is adnate to the base of the ovary. Some taxa (especially in Rauvolfioideae) are nectarless and apparently use deceit pollination (Haber, 1984; Lin & Bernardello, 1999). In some cases it is difficult to distinguish whether or not the base of the ovary is nectariferous. For this reason, only the presence versus absence of a distinct nectary disc is coded here. In the large genus *Alstonia*, this character varies from species to species. The species included in this study, *A. scholaris*, has a shallow nectar disc. In some other species of *Alstonia*, a slight thickening can be discerned at the base of the ovary, and in yet others there is no indication of a nectary at all.

22. Ovary: 0 = 2-carpellate; 1 = 3–5-carpellate. Throughout Apocynaceae s.l., the gynoecium is composed of two carpels. The only exceptions are found in *Lepinia* and *Lepiniopsis* in the Alyxieae and in *Pleiocarpa* in Hunterieae (Endress et al., 1997).

23. Placentas: 0 = lignified or indurated in fruit; 1 = not lignified or indurated in fruit.

24. Mesocarp consistency: 0 = fleshy, without fibers; 1 = fleshy, with fibers; 2 = dry or woody.

25. Endocarp: 0 = not forming a stone around the seed; 1 = lignified or sclerified and forming a stone around the seed.

26. Seeds: 0 = sessile; 1 = funiculate.

27. Seed shape: 0 = broad, compressed, not folded, mostly circular to ovoid; 1 = cylindrical, as if longitudinally rolled; 2 = narrowly fusiform, flattened, with a longitudinal fold; 3 = irregularly shaped, globular or angular, not flattened, or flattened on one side only, the other side convex.

28. Seed margin: 0 = with neither flattened edge nor wing; 1 = with a narrow flattened edge, this sometimes dissected; 2 = with a well-developed, usually membranous wing(s); 3 = fimbriate.

29. Seed coma: 0 = absent; 1 = present. A coma is a tuft of hairs all arising from a small restricted region at the end(s) of a seed. It is not considered to be homologous to the fimbria that are found around the margin of the seed in, for example, *Alstonia*.

30. Hilar depression: 0 = absent; 1 = an ovate depression, less than 50% the length of the seed; 2 = a deep, broad furrow, traversing the entire seed length; 3 = a deep, narrow fissure, traversing 75%–80% of the length of the seed.

31. Hilum shape: 0 = linear, traversing the length of the seed; 1 = linear, but shorter than the seed; 2 = small, circular (punctiform); 3 = ovate, covering a larger area.

32. Endosperm: 0 = not ruminant; 1 = with shallow, irregular tubercles or ruminations; 2 = with deep longitudinal ruminations. Ruminant endosperm, although relatively rare in Apocynaceae, is characteristic for *Tabernaemontana* and is also found in several genera of Alyxieae. *Chilocarpus* is unusual in this aspect in that the genus can be divided into two groups: those with smooth and those with ruminant endosperm (Pichon, 1949c; Markgraf, 1971). The representative species used in the analysis here belongs to the group with smooth endosperm; had a species from the other group been selected, *Chilocarpus* would fit better with other Alyxieae as to this character.

33. Endosperm: 0 = tough and corneous to subcartilaginous; 1 = firm, fleshy or starchy; 2 = delicate, soft or mealy. There is considerable variation in the thickness of the endosperm. For example, the endosperm of *Alyxia*, *Chilocarpus*, *Condylocarpon*, *Lepinia*, *Lepiniopsis*, and *Pteralyxia* is especially thick and tough (even difficult to cut with a razor blade). In *Allamanda*, *Picralima*, *Plectaneaia*, and *Pleiocarpa*, endosperm is also tough but much thinner, but because no

clear demarcation between "thick" and "thin" could be found, only the consistency of the endosperm is coded.

34. Cotyledon base: 0 = auriculate; 1 = not auriculate. Although cotyledons are typically auriculate at the base in *Tabernaemontaneae* (sensu Leeuwenberg, 1991), this was not the case in the species included here.

35. Secoiridoids and complex indole alkaloids: 0 = absent; 1 = secoiridoids present, indole alkaloids absent; 2 = dominant indole alkaloids present, but only those with non-rearranged secologanin skeleton; 3 = dominant indole alkaloids present, including those with rearranged secologanin skeleton of the eburnan and/or plumeran type; 4 = dominant indole alkaloids present, including those with rearranged secologanin part of the ibogan type.

36. Cardenolides: 0 = absent; 1 = present.

37. Steroidal alkaloids: 0 = absent; 1 = present.

38. Pollen unit: 0 = monad; 1 = tetrad. Tetrads are rare in *Rauvolfioideae*, and of the taxa sampled here they occur only in *Condylocarpon* and *Craspidospermum*.

39. Pollen grain: 0 = small ($\leq 51 \mu\text{m}$); 1 = large ($\geq 60 \mu\text{m}$). Average largest pollen grain size (either the length of the polar axis, P, or the diameter of the equatorial plane, E) varies between 25 and 90 μm . It appears that a relatively large gap exists between 51 and 60 μm and that only *Anechites* (56 μm) falls between. Coding pollen size either as small ($\leq 51 \mu\text{m}$) or as large ($\geq 60 \mu\text{m}$), with one ambiguous case, gives two rather well-separated size classes.

40. Pollen grain shape: 0 = regular; 1 = irregular. Pollen grains with a regular shape have a zonoaperturate aperture system with equally spaced apertures on the equator. The polar axis and the equatorial plane can be easily indicated. In pollen grains with an irregular shape, the position/orientation of the polar axis and the equatorial plane cannot be indicated because there are only one or two porate apertures that are unequally spaced and sized and have an oblique orientation. Irregular pollen grains with three porate apertures have unequally spaced and sized apertures with oblique orientations.

41. Aperture number: 0 = zero; 1 = one or two; 2 = three or four; 3 = five or more. In *Rauvolfioideae*, only *Condylocarpon* has inaperturate pollen (aperture number = zero). Two-aperturate pollen (sometimes mixed with 1-aperturate) occurs in *Alyxia*, *Chilocarpus*, *Plectaneia*, and *Pteralyxia*, while 3- and/or 4-aperturate pollen (often mixed within a single sample) is found in most other genera. A few genera have five or more apertures (up to 10 apertures are found in *Craspidospermum*). In most samples studied, minor percentages of pollen grains with deviating aperture numbers are found, which is a common phenomenon in dicots. The coding given is for the dominant aperture numbers.

42. Ectoapertures: 0 = long colpi; 1 = short colpi; 2 = large pori; 3 = small pori; 4 = indistinct. Colpate ectoapertures are either longer than ca. 2/3 (long colpi) or shorter than ca. 1/3 (short colpi) the length of the polar axis (P). Large pori are at least 6 μm ; if 6 μm (*Chilocarpus*, *Plectaneia*), then they are always accompanied by larger pori (up to 9 and 12 μm , respectively) in the same pollen grain. Small pores are 2–5 μm and do not vary much in size in a single grain. Due to its thin outer exine, *Vinca* pollen has indistinct ectoapertures.

43. Ectoaperture margin: 0 = not outwardly thickened; 1 = outwardly weakly thickened; 2 = outwardly distinctly thickened; 3 = with conspicuous arcus-like ridges. The ectoaperture margin is usually not thickened. In genera with large pores, but also in the brachycolpate *Molongum*, it is distinctly thickened into a well-delimited, protruding margin (aspidate pollen). In the genera with small pores, the

ectoaperture margin is not or only weakly thickened. *Aspidosperma* and *Vallesia* have conspicuous ridges (massive and partly hollow, respectively) along the colpi joining toward the poles.

44. Endoapertures: 0 = distinct from and smaller than ectoapertures; 1 = not distinct from ectoapertures; 2 = distinct from and larger than ectoapertures. In *Alyxia*, *Lepinia*, *Lepiniopsis*, *Plectaneia*, and *Pteralyxia* (all with porate pollen), the endoapertures are not delimited from the ectoapertures (endo- and ectoapertures congruent). In other porate genera, the endopore is distinct by being situated in a differentiated inner exine layer, and also in all other taxa the endo- and ectoapertures are incongruent. In colpate pollen grains, the endoapertures are always smaller than the ectoapertures, except in *Vinca*, in which the endoapertures (delimited by costae) are larger than the ectoapertures.

45. Endoaperture margin: 0 = not inwardly thickened; 1 = with endoannulus; 2 = with polar costae; 3 = with lateral costae. This character can only be assessed by using LM and/or SEM images of the inner pollen-wall surface. An endoannulus is an inward thickening encircling the endoaperture. Polar costae are thickenings at the polar sides of usually lalongate endoapertures. Lateral costae are thickenings at the lateral sides of circular to lalongate endoapertures.

46. Supplementary endocolpi: 0 = absent (no endoplates recognizable); 1 = weak (zones of endocracks; endoplates indistinct); 2 = distinct (endoplates well recognizable). Supplementary endocolpi are narrow (e.g., *Cabucula*) to wide (e.g., *Rhazya*) zones along the colpi (one at each side) where the inner exine layer is more or less missing. They may join interaperturally toward the poles. In some genera (e.g., *Catharanthus*), they seem to have taken over at least some of the function of the ectocolpi, bordering on distinctly thicker intine parts (see El-Ghazaly, 1990, fig. 17). Usually, supplementary endocolpi have a granular inner surface and delimit smooth endoplates in the mesocolpium centers (mesocolpial plates) and under the colpi at the polar sides of the endoapertures (colpal plates). Supplementary endocolpi occur only in colpate genera (not all) and are absent in all porate genera.

47. Intine protrusions: 0 = absent; 1 = present. Coding of this character is largely based on data provided by Pichon (1947c, 1948a, b, c, 1950a, b). Protruding intine bulges at the endoapertures occur in both genera with porate pollen and genera with colpate pollen. In some cases, it could be observed (TEM) that the outer zone of a bulge has an intricately channeled structure. In *Alyxia*, there seems even to be a kind of relatively rigid operculum topping the protrusion (Huang, 1986).

48. Exine: 0 = not reduced; 1 = reduced (thin). *Condylocarpon* and *Vinca* have a thin exine (0.1–0.6 μm and ca. 0.1 μm , respectively), whereas in the other genera, exine thickness is at least 0.9 μm , but is usually much thicker.

49. Inner exine surface: 0 = psilate; 1 = scabrate; 2 = verrucate; 3 = granular. This character codes for the inner ornamentation of the exine (nexine surface) and was taken from the inside of the mesocolpia (mesoporia) centers. A scabrate surface has elements smaller than 1 μm . Verrucate and granular elements are larger than 1 μm , the former with a broad base, the latter with a constricted base.

50. Inner exine layer (nexine): 0 = ectexinous/endexinous (foot layer/endexine); 1 = ectexinous (endexine absent); 2 = endexinous (foot layer absent). This character codes for the composition of the inner exine layer (nexine).

Endexinous parts are indicated by 'white lines,' a lamellate structure and/or a \pm spongy aspect. Ectexinous parts are \pm homogeneous. Usually endexinous and ectexinous parts differ in contrast.

51. Infratectum: 0 = columellate; 1 = granular, reticulate or irregular; 2 = not recognizable (commissural line). A columellate condition is found only in *Gelsemium*. In most other genera, the infratectum is granular, reticulate or irregular, and varying in thickness. In *Alyxia*, *Lepinia*, *Lepiniopsis*, and *Pteralyxia*, an infratectum is indistinct (sparse gaps in inner exine layer), the contact between ectexine and endexine being largely a commissural line.

52. Tectum: 0 = thicker than infratectum + inner exine layer; 1 = equal to infratectum + inner exine layer; 2 = thinner than infratectum + inner exine layer. This character codes for the thickness of the tectum compared with the rest of the exine (infratectum + inner exine layer). It is also an approximate measure for the relative position of the infratectum. Usually the tectum is well delimited. When

the boundary is irregular (e.g., in *Plectaneia*), the average tectum thickness was measured.

53. Outer exine surface: 0 = psilate (even to undulate); 1 = verrucate, with angular anastomosing verrucae; 2 = verrucate, with \pm circular isolated verrucae; 3 = microreticulate; 4 = scabrate; 5 = striate-reticulate. This character codes for the outer ornamentation of the exine (tectum surface). Most genera have psilate pollen (no protuberances), with an even to undulate, often perforate surface. *Lepinia* and *Lepiniopsis* have verrucate pollen with anastomosing verrucae. The other states, except for scabrate exine, occur in single genera.

54. Mesocolpium/mesopodium centers: 0 = outer surface hardly or not deviating from surrounding exine; 1 = outer surface clearly deviating from surrounding exine. In about 1/4 of the sampled genera with colporate pollen, the outer surfaces of the mesocolpium centers have a different ornamentation compared with the surrounding areas. Usually the mesocolpium centers have a rugulate, microfossulate to verrucate, or a less distinctly perforate ornamentation.