Morphology and molecular data in phylogenetic fraternity: the tribe Wrightieae (Apocynaceae) revisited[1]

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The monophyly and classification of the tribe Wrightieae of the subfamily Apocynoideae (Apocynaceae) are cladistically investigated. Nine taxa from the Wrightieae sensu Leeuwenberg, nine from other Apocynoideae sensu lato (s.l., including two from the traditional Asclepiadaceae), and two outgroup taxa from the Plumerioideae (Apocynaceae) were scored for *rbcL* sequence data and morphological data, mainly floral characters, and analyzed using successive weighting parsimony analysis. The Wrightieae sensu Leeuwenberg are shown to be largely paraphyletic, the constituent taxa being dispersed among four monophyletic clades. Previously not suggested relationships indicated by the study are the association of *Pachypodium* with *Funtumia, Holarrhena,* and *Mascarenhasia* and the position of *Beaumontia* close to *Trachelospermum.* A reclassification of the Wrightieae is discussed, in which three of the identified clades are recognized as tribes, the Wrightieae sensu stricto (s.s.), the Nerieae, and the Malouetieae. The support for the Wrightieae s.s. is very strong, as evaluated with Bremer support and bootstrap analysis. The Malouetieae are also strongly supported, but the Nerieae less so. Using potential morphological synapomorphies identified in the study, circumscription of the tribes is discussed. A potential pseudogene of *rbcL* is reported for *Beaumontia.*

Key words: Apocynaceae; chloroplast DNA; classification; floral morphology; Malouetieae; Nerieae; phylogeny; *rbcL*; Wrightieae.

Morphology has, without parallel, been the traditionally most important source of information in plant taxonomy. A majority of taxonomic groups recognized today are defined by cardinal characters mainly from floral morphology. The relatively recent addition of molecular data together with a phylogenetic concept in systematics challenges many of these groups, showing them to be paraphyletic assemblages. However, morphology should not simply be dismissed in favor of molecular data. Instead, combined analyses of morphological and molecular data sets provide a strong basis for phylogenetic hypotheses and thus also for classification. Morphological synapomorphies identified in such an analysis may also allow rapid prediction of the placement of further taxa. Here, this is exemplified in a phylogenetic study of the tribe Wrightieae from the subfamily Apocynoideae of the Apocynaceae s.l.

The Apocynaceae s.l. (de Jussieu, 1789) have traditionally been divided into the Apocynaceae s.s. and the Asclepiadaceae (e.g., Brown, 1810; Schumann, 1895a, b). Recent phylogenetic studies based on *rbcL* sequence data (Olmstead et al., 1993; Sennblad and Bremer, 1996) show that the Asclepiadaceae are nested within the subfamily Apocynoideae of the Apocynaceae, and thus support the wider circumscription of Apocynaceae s.l. of Jussieu. The group comprising the Apocynoideae and the traditional Asclepiadaceae is, henceforth, referred to as the Apocynoideae s.l.; the Apocynoideae as traditionally circumscribed (e.g., Schumann, 1895a; Pichon, 1950a; Leeuwenberg, 1994, non sensu Woodson, 1930) are referred to as the Apocynoideae s.s.

The subdivision of the subfamily Apocynoideae s.s. has always been considered problematic (e.g., Leeuwenberg, 1994); the number of tribes recognized has varied from two to four (e.g., Schumann, 1895a; Pichon, 1950a; Leeuwenberg, 1994; see Table 1). However, the tribe Wrightieae (also as Nerieae) has been recognized as distinct (Leeuwenberg, 1988, 1994), and its constituent genera have by some authors been interpreted as ''basal'' compared to the rest of the subfamily (MacFarlane, 1933; Pichon, 1950a). This interpretation has been based on the lower degree of specialization in the tribe, which thus shows a wide array of presumed plesiomorphic characters (see Appendix in Pichon, 1950a).

Plesiomorphic characters, however, may not identify a monophyletic group, and in the analysis by Sennblad and Bremer (1996) the tribe Wrightieae was also indicated to be paraphyletic, with the included representatives *Stephanostema* and *Strophanthus* being successive sister taxa to a clade comprising the rest of the Apocynoideae s.l.

History—The tribe Wrightieae was first described by Don (1838). It was based on presence of a chalazal hair tuft (coma) on the seed and included the two genera *Wrightia* and *Kibatalia* (Table 1). This treatment was fol-

TABLE 1.   Tribal and subtribal (if recognized) classification, and genera included in the cladistic analysis. For the Plumerioideae taxa only subfamily is given. (ASC) indicates that the taxon was placed in the Asclepiadaceae. All other taxa are classified in the Apocynoideae. A dash indicates that the taxon was not treated by the author heading the column.

Taxa	Don (1838)	Schumann (1895a, b)	Pichon (1948a, b; 1950a, b)	Leeuwenberg (1994)
Acokanthera	—	Plumerioideae[a]	Plumerioideae	Plumerioideae
Picralima	—	Plumerioideae	Plumerioideae	Plumerioideae
Thevetia	—	Plumerioideae	Cerberoideae	Plumerioideae
Adenium	Echiteae	Echiteae[b]	Wrightieae[c]: Adeniinae	Wrightieae: Neriinae
Nerium	Echiteae	Echiteae	Wrightieae: Neriinae[d]	Wrightieae: Neriinae
Beaumontia	Echiteae	Echiteae	Wrightieae: Beaumontiinae	Wrightieae: Wrightiinae
Stephanostema	—	—	Wrightieae: Wrightiinae	Wrightieae: Wrightiinae
Strophanthus	Echiteae	Echiteae	Wrightieae: Strophanthinae	Wrightieae: Wrightiinae
Wrightia	Wrightieae	Parsonsieae	Wrightieae: Wrightiinae	Wrightieae: Wrightiinae
Funtumia	—	Echiteae	Wrightieae: Kibataliinae	Wrightieae: Malouetiinae
Mascarenhasia	—	Echiteae	Wrightieae: Mascarenhasiinae	Wrightieae: Malouetiinae
Holarrhena	Echiteae	Plumerioideae	Plumerioideae	Wrightieae: Alafiinae
not sampled			Wrightieae: Alafiinae	
Apocynum	Echiteae	Echiteae	Ecdysanthereae: Apocyninae	Apocyneae: Apocyninae
Baissea	—	Echiteae	Ichnocarpeae: Baisseinae	Apocyninae: Ichnocarpinae
Mandevilla	—	Echiteae	Ichnocarpeae: Mandevillinae	Echiteae: Echitinae
Pachypodium	Echiteae	Echiteae	Parsonsieae: Pachypodiinae	Echiteae: Pachypodiinae
Parsonsia	Echiteae	Parsonsieae	Parsonsieae: Parsonsiinae	Echiteae: Parsonsiinae
Prestonia	Echiteae	Parsonsieae	Parsonsieae: Prestoniinae	Echiteae: Echitinae
Trachelospermum	—	Echiteae	Parsonsieae: Chonemorphinae	Echiteae: Echitinae
Periploca	Periploceae (ASC)	Periploceae (ASC)	—	—
Secamone	Secamoneae (ASC)	Secamoneae (ASC)	—	—

[a] as Plumieroideae.
[b] as Echitideae.
[c] as Nerieae.
[d] as Amphineuriinae.

lowed by contemporary authors (e.g., Endlicher, 1841; de Candolle, 1844) and recently also by Ly (1986). In the classification of Schumann (1895a) only two tribes of the Apocynoideae s.s. were recognized: the Parsonsieae and the Echiteae (Table 1). The former Wrightieae taxa were dispersed between these two tribes.

A much wider circumscription of the Wrightieae was presented by Pichon (1950a; see Table 1). His circumscription has been accepted by most subsequent authors (e.g., Wagenitz, 1964; Leeuwenberg, 1988, 1994), with minor additions and exclusions of genera. One such change is the inclusion of *Holarrhena, Carruthersia,* and *Spirolobium,* proposed by Endress et al. (1990).

Pichon subdivided the Wrightieae into nine rather narrowly delimited subtribes (Table 1), some of which were, or have now due to fusion of genera become, monogeneric. In the most recent classification of the Apocyna-

ceae s.s. by Leeuwenberg (1994), a wider subtribal delimitation was applied, and only four subtribes were recognized (Table 1).

Aim of the study—In the present study, we combine data from the chloroplast gene *rbcL* and floral morphology to investigate the monophyly of the Wrightieae and its position in the Apocynaceae and to identify morphological synapomorphies useful in classification of the group.

MATERIALS AND METHODS

Nine taxa were sampled so as to include representatives for all the described subtribes of Wrightieae according to the classification of Leeuwenberg (1994) and as many subtribes as possible from other classifications (Table 1). This corresponds to approximately half of the gen-

TABLE 2.   Sources and EMBL accession numbers for *rbcL* sequences used in the analysis. For the taxa sequenced in this study, the vouchers for the material are given. For material extracted from other sources, the publication is given.

Taxa	Source	EMBL number
Acokanthera oblongifolia	Sennblad and Bremer, 1996	X91758
Adenium obesum	Sennblad 238, UPS	AJ002880
Apocynum cannabinum	Sennblad and Bremer, 1996	X91761
Baissea leonensis	Sennblad 258, UPS	AJ002881
Beaumontia grandiflora	Sennblad 264, UPS	AJ002882, AJ002961[a]
Funtumia elastica	Sennblad 259, UPS	AJ002883
Holarrhena pubescens	Taylor, Gereau, and Lovett 8403, UPS	AJ002884
Mandevilla sanderi	Sennblad and Bremer, 1996	X91764
Mascarenhasia arborescens	Setten 625, WAG	AJ002885
Nerium oleander	Sennblad 265, UPS	AJ002886
Pachypodium lamerei	Sennblad 232, UPS	AJ002887
Parsonsia heterophylla	Garnock-Jones 2243, WELTU	AJ002888
Periploca graeca	Sennblad 255, UPS	AJ002889
Picralima nitida	Sennblad and Bremer, 1996	X91766
Prestonia quinquangularis	Sennblad and Bremer, 1996	X91768
Secamone afzelii	Sennblad and Bremer, 1996	X91779
Stephanostema stenocarpum	Sennblad and Bremer, 1996	X91770
Strophanthus eminii	Sennblad and Bremer, 1996	X91771
Thevetia peruviana	Sennblad and Bremer, 1996	X91773
Trachelospermum jasminoides	Gillis 9146, FTG	AJ002890
Wrightia arborea	Leeuwenberg 14225, WAG	AJ002891

[a] This is a potential pseudogene for *rbcL*.

era included in the Wrightieae sensu Leeuwenberg (1994). To investigate the monophyletic or paraphyletic status of the tribe (see Sennblad and Bremer, 1996), seven representatives from the other tribes of the Apocynoideae s.s. and one representative each from the Periploceae and Secamoneae of the traditional Asclepiadaceae were included (Table 1). The outgroup taxa were chosen from the subfamily Plumerioideae to include representatives of potential sister groups as indicated by recent studies (Endress et al., 1996; Sennblad and Bremer, 1996; see Table 1). In addition, taxa presently or formerly included in the Wrightieae were investigated for morphological synapomorphies identified in this study. These taxa are as follows: *Pleioceras, Spirolobium, Vallaris* (Wrightiinae sensu Leeuwenberg, 1994), *Kibatalia, Malouetia* (Malouetiinae sensu Leeuwenberg, 1994), *Alafia, Farquharia,* and *Carruthersia* (Alafiinae sensu Leeuwenberg, 1994), *Amphineurion* (= *Aganosma*), *Dewevrella, Isonema,* and *Pottsia* (Neriinae of Pichon, 1950a, excluded by Leeuwenberg, 1994).

rbcL—Nucleotide sequences of *rbcL* for *Adenium, Baissea, Beaumontia, Funtumia, Holarrhena, Mascarenhasia, Nerium, Pachypodium, Parsonsia, Periploca, Trachelospermum,* and *Wrightia* are published in this study, whereas sequences for the outgroup taxa and six ingroup taxa were accessed from other sources (Table 2). Total DNA was extracted from fresh leaves or herbarium material using the method of Saghai-Maroof et al. (1984) and Doyle and Doyle (1987). The extractions from fresh leaves were purified by ultracentrifugation in CsCl gradients, whereas the herbarium material extractions were purified through ethanol precipitation. Double-stranded DNA of the *rbcL* gene was amplified by PCR (polymerase chain reaction) using Taq-polymerase kit (Promega Corp., Madison, Wisconsin, WI) and two synthetic primers, the 5′-primer corresponding to the first 26 bases of the *rbcL* gene of tobacco, and the 3′-primer corresponding to a region ~100 nucleotides downstream from the coding region (Olmstead et al., 1992). In cases where PCR amplification proved difficult, a PCR reaction using Taq extender PCR additive (Stratagene Inc., La Jolla, California, CA) was performed, following the protocol provided by the supplier. A second round of PCR, with only one of the primers, respectively, was performed to achieve single-stranded DNA (Kaltenboeck et al., 1992). Single-stranded DNA was sequenced (Sanger, Nicklen, and Coulson, 1977) using internal primers designed by G. Zurawski (DNAX Research Institute, Palo Alto, California, CA).

Morphology—Floral and vegetative macromorphological characters were studied on herbarium material and material preserved in alcohol. Floral micromorphology was studied on 10-μm sections of fixed flowers at or near anthesis. Two stages of young buds of *Prestonia* were sectioned for study of corona development. Additional sections of anthers were made separately (20 μm) for studies of sclerenchyma. To compare ontogeny of colleters in leaf axils, vegetative buds of taxa representing different types of colleter distribution were sectioned (20 μm). All sectioned material was dehydrated in an alcohol-xylene series, embedded in paraplast, cut with a rotary microtome, and stained with safranin and astra blue. Floral venation was studied on corollas mounted in Hoyer's solution. Seed characters were collected from literature (see below).

For most included genera, more than one species was studied. To account for the variation within the genera, characters have also been checked, if possible, against information given in literature (Schumann, 1895a; Woodson, 1930, 1933, 1935, 1936a, b; Pichon, 1948a, b, 1949a, b, c, d, 1950a, b; Ngan, 1965; Browicz, 1966; Plaizier, 1980; de Kruif, 1981; Zwetsloot, 1981; Beentje, 1982; Kupicha, 1982; Rudjiman, 1982, 1986; Schick, 1982b; Fallen, 1983, 1986; Huber, 1983; van der Ploeg, 1983, 1985; Barink, 1984; Leeuwenberg et al., 1985; Leeuwenberg, 1987; Pagen, 1987; Anusansunthorn, 1989; Endress et al., 1990; Goyder, 1992; Klackenberg, 1992a, b; Nilsson, Endress, and Grafström, 1993; van Dilst, 1995; Zarucchi et al., 1995; Middleton, 1996; Morales, 1996; Omino, 1996; Williams, 1996).

Cladistics—The data matrix for the phylogenetic analysis consists of a molecular submatrix comprising characters corresponding to the nucleotide positions 27–1428 of the *rbcL* gene and a morphological submatrix comprising 25 characters. Database accession (EMBL) numbers for *rbcL* sequences are given in Table 2. The coding of the morphological characters is included in Appendix 1 and the morphological submatrix is presented in Table 3. Only phylogenetically informative characters (97 in total) were analyzed. Significance of incongruence between the two submatrices was evaluated using the incongruence length difference test, as implemented in the programme XArn (Farris et al., 1995), with 1000 random repartitions of the unit weighted matrix.

Cladistic analysis was performed using PAUP 3.1.1 (Swofford, 1993). An initial heuristic search with 100 replicates with random addition sequence, TBR branch-swapping, and all characters given unit weight, was followed by a successive weighting analysis (Farris, 1969,

TABLE 3. Morphological matrix. For coding of characters, refer to Appendix 1. An "A" denotes polymorphism for states 0 and 1, a "B" polymorphism for states 1 and 2, a "C" polymorphism for states 2 and 3, and a "D" denotes polymorphism for states 0, 1, and 3. A question mark indicates uncertainty, and a dash indicates that the character is inapplicable.

Taxon	5	10	15	20	25
Acokanthera	0 0 2 0 0	1 0 0 0 –	– 0 0 1 0	0 0 0 0 0	1 0 0 0
Adenium	1 0 2 1 1	0 1 0 0 0	0 1 1 1 0	1 0 0 0 0	0 1 1 0
Apocynum	0 0 2 1 0	1 0 1 0 0	0 0 2 1 0	3 1 1 1 1	1 1 0 0
Baissea	0 0 B 1 1	0 0 1 0 0	0 2 1 0 3	0 1 1 1 1	1 1 0 0
Beaumontia	0 0 A 1 ?	0 0 1 0 0	0 0 2 1 0	1 1 1 0 1	1 0 1 0 0
Funtumia	0 1 A 1 0	1 0 0 0 1	– 0 1 0 1	1 0 0 1 1	0 1 1 0 1
Holarrhena	0 1 1 1 1	1 0 0 0 1	– 0 0 0 1	1 0 0 0 1	0 1 1 0 0
Mandevilla	0 0 D 1 0	0 0 0 0 0	1 0 2 1 0	3 1 1 1 1	1 0 1 0 0
Mascarenhasia	0 1 0 1 0	1 0 0 0 1	– 0 1 0 1	1 0 0 1 1	0 1 1 0 0
Nerium	0 0 0 1 1	0 1 0 0 0	0 1 1 1 1	0 0 0 0 0	0 1 0 0
Pachypodium	1 ? 2 1 0	0 0 0 0 0	0 0 1 1 1	2 0 0 1 1	0 1 1 0 0
Parsonsia	0 0 C 1 0	1 0 0 0 0	0 0 2 1 0	2 0 1 1 1	0 0 1 0 0
Periploca	0 0 1 1 2	– 0 1 0 0	0 0 0 1 0	4 1 1 0 1	1 0 1 0 0
Picralima	0 0 0 0 1	0 0 0 0 –	– 0 0 0 0	0 0 0 0 0	0 1 0 0 0
Prestonia	0 0 3 1 1	0 0 0 1 0	1 0 2 1 0	2 0 1 1 1	0 0 1 0 A
Secamone	0 0 1 A 1	1 0 1 0 0	0 0 2 1 1	4 0 0 0 1	0 1 1 0 0
Stephanostema	0 0 1 0 1	1 1 0 0 0	0 0 2 0 1	0 0 0 0 0	0 0 1 0
Strophanthus	0 0 A 1 1	1 1 1 0 0	0 A 1 0 0	1 0 0 0 0	0 1 1 1
Thevetia	1 0 0 0 0	0 0 0 1 –	– 1 0 0 0	0 0 0 1 0	0 0 0 0
Trachelospermum	0 0 0 1 0	0 0 0 0 1	– 0 2 1 0	2 0 1 1 1	0 1 1 0 0
Wrightia	0 0 1 0 0	0 1 0 0 0	0 0 2 0 0	1 0 0 0 0	0 0 1 0

Figs. 1–2. Cross sections of leaf buds. **1.** *Wrightia,* showing presence of marginal and intrapetiolar colleters. **2.** *Holarrhena,* showing presence of intrapetiolar colleters only. Hatched = colleters, le = leaves. Bar = 0.1 mm in Fig. 1; this bar is also for Fig. 2.

1989), using heuristic searches with ten replicates with random addition sequence, TBR branch-swapping, and characters reweighted according to their rescaled consistency index. Successive weighting is here not used as a means of choosing between equally parsimonious trees from the unit-weighted analysis (Carpenter, 1988), but as a means to improve the original matrix, as originally suggested by Farris (1969). As such, it is a powerful tool to detect conflict in data and to enhance the signal present in data (Goloboff, 1993; Sennblad and Bremer, 1996). Bremer support, b (Bremer, 1988, 1994; Källersjö et al., 1992; sometimes termed branch support or less appropriately decay index), was calculated on the weighted data matrix using the methods outlined in Bremer (1994). Note, however, that the rescaling suggested by Bremer (1994) was not performed. Bootstrap analysis (Felsenstein, 1985), with 10 000 replicates, was performed on the weighted data matrix, using the following PAUP settings: retain groups with 50% bootstrap frequency, sample characters with equal probability but apply weights, random addition sequence − 1 replicate, MULPARS not in effect, and SPR branch-swapping. The base weight 1000, applied in computer calculation, is removed from tree lengths and Bremer support values; they are also rounded off to integers in the case of tree lengths and to two significant numbers in the case of Bremer supports. The reason for this is to relate both lengths and support values back to characters. In a perfect matrix without homoplasy the number of synapomorphies supporting a node would equal the branch length and also the Bremer support of that node. A similar, although not as straightforward, relationship exists for bootstrap support (Felsenstein, 1985; Harshman, 1994; Farris et al., 1996). A node supported by one (1) uncontradicted character would yield a bootstrap support of 63%, two characters would yield 86%, three characters 95%, etc. In real matrices, however, these relationships are obscured by homoplasy. The effect of homoplasy is complex; it involves the addition of homoplastic characters to branch lengths, but also the addition of support for numerous alternative (sub)topologies, and can give either higher or lower support values. Successive weighting offers a means to reduce the effects of homoplastic characters, and performing support analysis on the successive weighting matrix is likely to enhance the relation of support back to synapomorphies (Sennblad, 1997). One unambiguous synapomorphy
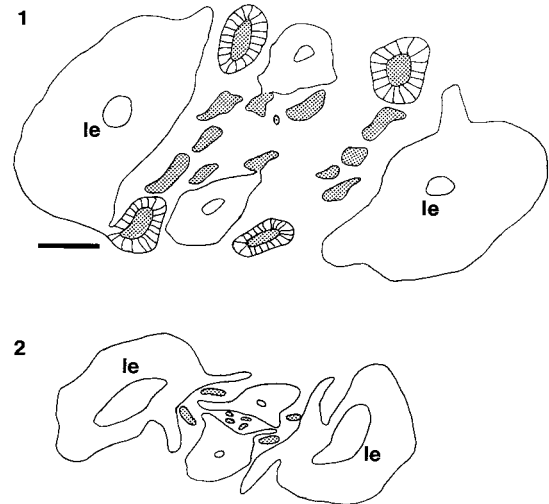
will thus equal one step in these values, while down-weighted homoplastic characters will contribute fractions of steps. Branch support may therefore take decimal values. To simplify the discussion of the results, a rough scale of the relative support of the clades in the successive weighting analysis was used: clades with a Bremer support higher than one step ($boot \approx 63\%$) are considered supported, whereas clades with a Bremer support higher than two steps ($boot \approx 85\%$) are considered strongly supported. This scale merely relates to the support distribution within the analysis.

Morphological characters were investigated by optimizing all most parsimonious state assignments onto the tree from the successive weighting analysis. In figures, ACCTRAN optimization is used and characters for which ambiguous optimizations exist are indicated.

COMMENTS ON CODING OF MORPHOLOGICAL CHARACTERS

Vegetative parts—The Apocynaceae s.l. are lactiferous trees, bushes, lianas, vines, or herbs; a few taxa are succulent. The leaves of the taxa studied usually lack, or have much reduced, true stipules and are regularly opposite, in a few taxa alternate or verticillate. In *Pachypodium,* which has alternate leaves, it seems that the leaves, at least in young plants, are initiated in an opposite or subopposite manner (Uhlarz, 1975). The later apparent alternate phyllotaxy in *Pachypodium* could be due to the succulent habit of the plant. The same could be the case for *Adenium,* which is also succulent. Succulence and phyllotaxy are thus potentially interdependent characters in the ingroup. To avoid intrinsic weighting, only one of the characters is therefore included in the matrix. Phyllotaxy (character 1) is chosen, as the coding is more straightforward (definition of succulence is avoided).

Colleters are finger-like, multicellular glands, commonly occurring in the axils of leaves and sepals in the families of the Gentianales (Wagenitz, 1992). In the Rubiaceae, colleters in the leaf axils mainly occur inside the stipules (Lersten, 1975). In the Apocynoideae s.l., leaf axil colleters may occur independently both in a marginal, sometimes interpetiolar, position or in an intrapetiolar position. Colleters in a marginal position (character 2) here indicates colleters initiated early in leaf ontogenesis and in a truly marginal position on the base of the petiole (cf. Figs. 1–2). These marginal colleters could be assumed to be associated with reduced stipules, in homology with the colleters inside the stipules of the Rubiaceae (see, e.g., Kunze, 1990). The coding of the character
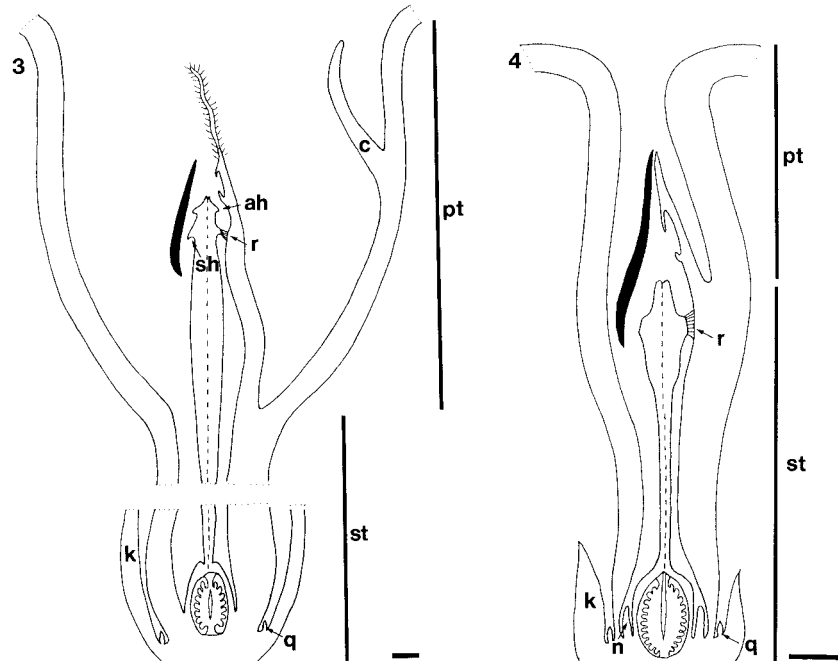
Figs. 3–4.   Longitudinal sections of flowers. **3.** *Nerium,* showing presence of corona, anthers with elongated apical appendage, and conical style head with apical hair wreath and stigmatic hollow. **4.** *Funtumia,* showing sessile anthers and presence of nectaries. ah = apical hair wreath, black = sclerenchymatic tissue, c = corona, k = calyx, n = nectary, pt = proper corolla tube, q = colleter, r = retinacle, sh = stigmatic hollow, st = stamen corolla tube. Horisontal bars = 1 mm in Figs. 3–4.

is based on the pattern and should not be affected even if the above assumption would be false. For intrapetiolar colleters, no consistent pattern was found, and variation within genera was considerable. Intrapetiolar colleters were therefore not coded.

Calyx—Calycine colleters (character 3) may show a similar separation into marginal and intrasepal position inside the five calyx lobes. This separation is, however, more obscure and would require ontogenetic investigation to be detected. Kunze (1990) suggested that colleters in a marginal position (stipules in his interpretation) are generally absent from the calyx in the Apocynaceae s.l.

Corolla—The aestivation of the five corolla lobes (character 4) is typically contorted, either to the left or to the right, as viewed from the outside.

The corolla tube of Apocynaceae s.l. consists of two parts (Figs. 3–4). The lower part of the corolla tube, i.e., below the insertion of the filaments, is here called stamen-corolla tube (Boke, 1948; Nishino, 1982). The upper part is the "proper corolla tube" and consists of the sympetalous petals. According to Erbar (1991), the Apocynaceae s.s. exhibit late sympetaly, whereas the traditional Asclepiadaceae cannot unambiguously be categorized as either early or late sympetalous. The resulting congenitally fused part of the proper corolla tube usually does not elongate; instead, the main part of the proper tube is, in the Apocynaceae s.s., postgenitally fused (Boke, 1948; Nishino, 1982). This postgenital fusion starts at an adnation point of two adjacent petals some distance from the base of the petals, and then proceeds basipetally. In some cases, the basal fusion is incomplete, resulting in unfused regions, slits, behind the stamens (character 5). According to Nishino (1983), postgenital fusion does not occur in the traditional Asclepiadaceae. However neither Erbar nor Nishino investigated any representatives of the subfamilies Periplocoideae and Secamonoideae or of the tribe Stapelieae of the Asclepioideae of the traditional Asclepiadaceae. In Stapelieae, postgenital fusion of petals has been reported in *Ceropegia*

(Vogel, 1961; Endress, 1994). We have here found that the petals of *Secamone* are postgenitally fused.

In the postgenitally fused region of adjacent petals, branches of the lateral veins of each petal may approach each other and anastomose or fuse into interpetal veins (character 6). Veins occurring over postgenitally fused regions are unusual according to von Gumppenberg (1924). At each sinus of the postgenital corolla tube, the interpetal vein divides into branches supplying each of the adjacent petals. Usually one of the initial branches constituting the interpetal vein is more weakly developed and may sometimes be completely reduced. The position of the fusion of the initial lateral branches may vary, presumably due to differential elongation of the corolla tube. Here only presence or absence of an interpetal vein is coded.

Small unvascularized appendages or pockets in the sinuses of the postgenitally fused corolla tube occur in all the included taxa but are developed in a few taxa into vascularized corona appendages (character 7; Fig. 3).

Another type of emanation from the corolla is situated lower down in the proper corolla tube, approximately at the level of stamen insertion. These emanations or coronas vary in structure and position among different taxa. We have distinguished two characters based on position. The first type (character 8) consists of more or less well-developed transversal ridges, flanking and confluent with the filament insertion. Adjacent ridges can be confluent epipetally, as in *Apocynum* and *Periploca*. The second type (character 9) is a corona lobe, sometimes with flanking ridges, above the stamens. This lobe is not associated with the filament insertion and is inserted in the postgenitally fused part of the corolla tube, i.e., above the slits in the corolla tube, if such are present.

Stamens—The five stamens of the Apocynoideae s.l. have massive anthers with lateral guide rails. The basal part of the anther connective is adnate to the style head (i.e., the enlarged apical part of the style). The filaments, here defined as the portion between the point of adnation to the style head and the insertion in the corolla tube, may be of varying

length. The elongation of the filaments (character 10) occurs late in ontogeny (Boke, 1949; Nishino, 1982) and in some taxa this elongation seems to be absent, i.e., they are sessile. When elongated, the filament can sometimes be geniculate towards the style, forming a "knee" below the adnation to the style head, as in *Mandevilla* and *Prestonia* (character 11). This "knee" may have a supportive function for the often slender style. Filaments that are less sharply geniculate occur in, e.g., *Parsonsia* and *Apocynum.* In these cases the "knee" is also situated lower down on the filament. We have here employed a narrow definition of the character, comprising only the sharply geniculate "knee" of *Mandevilla* and *Prestonia.*

In all the included taxa, the anthers have sterile apical appendages; in *Adenium, Nerium, Thevetia,* and in some species of *Strophanthus,* this appendage is filiform and much to extremely elongated (character 12; cf. Figs. 3–4).

Sclerenchymatic tissue, which constitutes the guide rails of the anther (Fallen, 1986), occurs apically as two strands or a continuous plate abaxial to the vascular strand. This expands laterally towards the base, where it is centrally interrupted and forms two anther tails. In some taxa the sclerenchyma also extends adaxial to the vascular strand in the apical and median region of the anther. Presence of adaxial sclerenchyma is coded into the matrix (character 13; cf. Figs. 7 and 10).

The vascular strand is usually surrounded by normal cellular, parenchymatous, or sclerenchymatous tissue. However, in some taxa, it is instead surrounded by a large air space (character 14), with a loose, scattered, spongy tissue (Fallen, 1986; cf. Figs. 7 and 10).

Calcium oxalate crystals present in packages in the stomium (i.e., the wall between the locules in the theca, character 15) occur in several plant families (D'Arcy, Keating, and Buchmann, 1996). In some Apocynaceae, they are present in extracellular spaces in the adaxial part of the stomium, where they can still be seen also in dehisced anthers (cf. Fig. 11).

Pichon (1948c) coined the term "retinacle" for the region of the anther connective that participates in the bond with the style head. The retinacle usually consists of a pad of hairs, situated at the basal part of the connective, by which the connective is adaxially adnate to the style head (Figs. 5 and 8). The outline of this pad of hair can be in the shape of a compact, often longitudinally elongated circle (Fig. 12). It can also be horseshoe shaped, with an apical beak and marginal rows of hairs (Fig. 13). The central hairs of the beak can be reduced, leaving only the marginal hairs, or all hairs may be reduced (Fig 14). In these two last types, the connective is directly adnate to the style head (Fig. 8).

In addition to the retinacle, the basal part of the thecae can be adnate to the style head (cf. Figs. 7 and 10). In some taxa, there is also a ridge present in the region between the thecae and the retinacle. This ridge is adnate to the style head as well (cf. Figs. 6 and 9). The retinacle, the adnate thecae, and the adnate ridge are here coded as separate characters in the matrix (characters 16–18).

Gynoecium—Nectaries, if present, are in most cases situated around the ovary (character 19). In *Periploca,* nectar-secreting tissue is situated on alternistaminal appendages just below the stamen insertion in the corolla tube. In *Secamone,* nectar-secreting tissue is difficult to identify (cf. Kunze, 1996) but is probably situated in alternistaminal troughs on the staminal tube. The nectaries of *Secamone* and *Periploca* are not considered homologous to the nectaries found around the ovary in the rest of the ingroup.

The gynoecium of most taxa of the Apocynaceae s.l. is usually referred to as apocarpous. However, the two carpels are usually congenitally fused for a short distance from the base, the remaining part of the ovary is apocarpous, and the style and the style head are postgenitally fused (Fallen, 1983). Of the taxa included in the present study, only *Acokanthera* is completely congenitally syncarpous, which, thus, is autapomorphic. Some individual species of the included genera have postgenitally fused ovaries (e.g., *Nerium* and *Prestonia*), and the character is effectively autapomorphic.

The apical part of the style is enlarged and is called the style head. On this the pollen is secondarily presented, and some taxa have specialized structures, apical hair wreaths, for this function (character 20, cf. Figs. 3–4). The main body of the style head may in some taxa be angular, with ridges opposite the anthers (character 21, cf. Figs. 6 and 9). The anthers are adnate to the style head, usually at its broadest part. The stigmatic area is located below the region of anther adnation and is sometimes situated in a "stigmatic hollow" (character 22, cf. Figs. 3–4), an annular invagination of the style head; the resulting collar, equipped with a lower wreath of hairs, functions as a "pollen scraper." See Schick (1980, 1982a) and Fallen (1986) for a more detailed description of the style head.

Seeds—The seeds of the Apocynoideae s.l. are wind dispersed by means of a brush of long hairs, a coma, which can be situated on the micropylar or the chalazal end of the seed (characters 23–24). The seeds of *Adenium* and *Strophanthus* have both a micropylar and chalazal coma, a fact that supports the treatment of micropylar and chalazal coma as separate characters. The coma, whether micropylar or chalazal, may be situated on a rostrum on the seed. Among the studied taxa, only a micropylar rostrum (character 25) is informative.

RESULTS

The unit weight analyses of the individual morphological and *rbcL* submatrices resulted in 16 trees, 77 steps long, with a consistency index (CI) = 0.416 and a retention index (RI) = 0.648, and 18 trees, 177 steps long, with CI = 0.486 and RI = 0.533, respectively. The results from the successive weighting analyses of the individual submatrices are presented in Figs. 15 and 16. The morphological analysis produced a single tree, 19 steps long, with CI = 0.607 and RI = 0.839; the length with unit-weighted characters is 79 steps. The *rbcL* analyses resulted in one tree, 42 steps long, with CI = 0.780 and RI = 0.854; the length with unit-weighted characters is 177 steps. The incongruence between the morphological and the *rbcL* submatrices is not significant as evaluated by the incongruence length difference test (α = 1000/ 1000; Farris et al., 1995).

The unit-weighted analysis of the combined data set resulted in six trees 257 steps long, with CI = 0.459 and RI = 0.570. The result from the successive weighting analysis, a single most parsimonious tree, 61 steps long, with CI = 0.727 and RI = 0.849, is presented in Fig. 17. The length of this tree with unit-weighted characters is 257 steps; this tree is thus identical to one of the trees from the unit-weighted analysis. Branches not present in the strict consensus tree from the unit-weighted analysis are indicated in Fig. 17. Unless otherwise stated, only the successive weighting analyses are considered.

Relationships—The positions of the outgroup taxa, *Acokanthera, Picralima,* and *Thevetia,* does not violate the initial assumption on ingroup monophyly.

The present analysis indicates that the Wrightieae sensu Leeuwenberg (1994) are paraphyletic, with these representatives dispersed among four clades: the *Stephanostema* and *Wrightia* clade; the *Adenium, Nerium,* and *Strophanthus* clade; the *Pachypodium, Mascarenhasia, Funtumia,* and *Holarrhena* clade; and the *Beaumontia* and *Trachelospermum* clade.

The relationship between *Stephanostema* and *Wrightia* is strongly supported (b = 8.1, *boot* = 100%). The sister
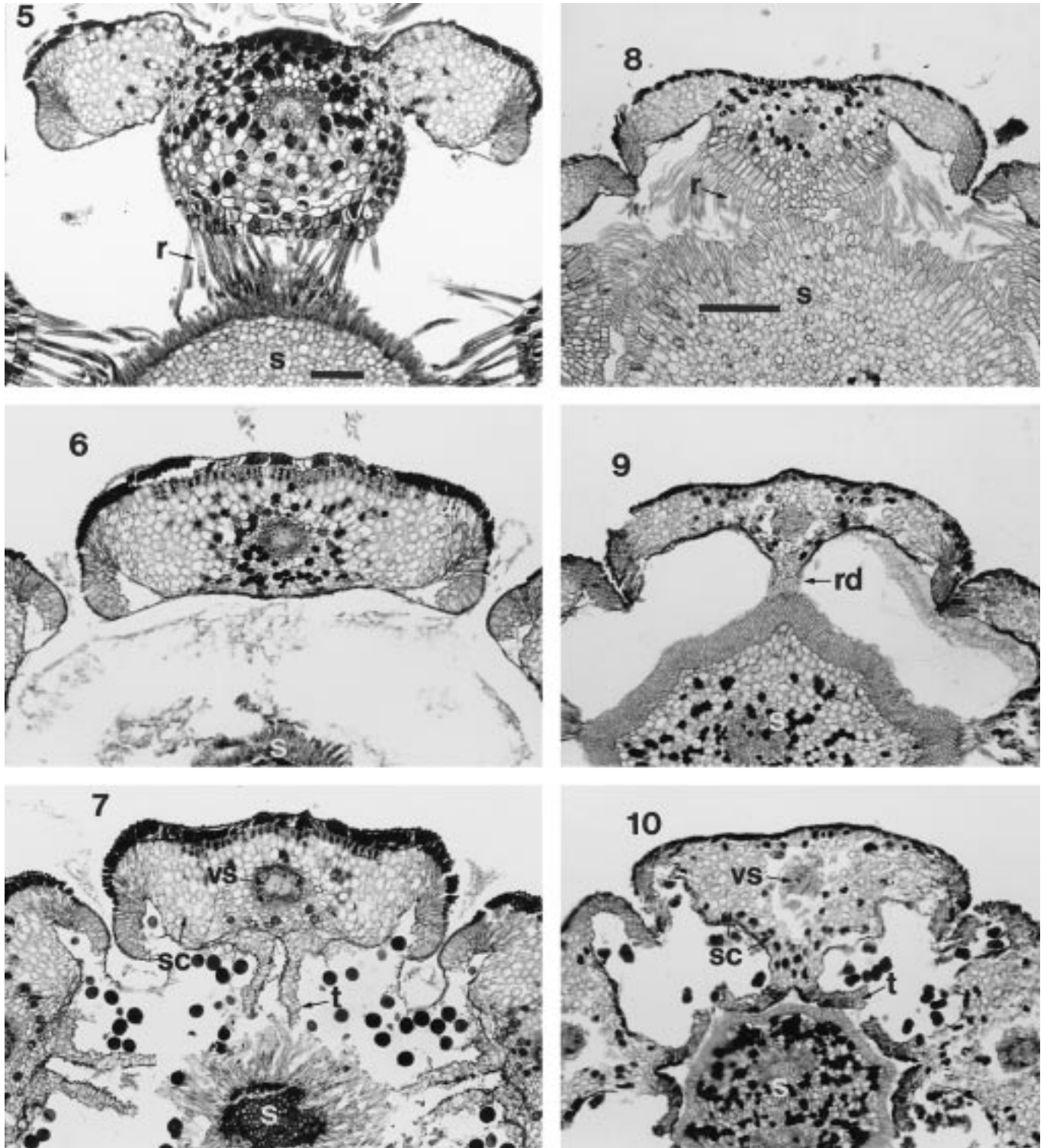
Figs. 5–10.   Cross sections of anthers. Figs. 5–7. *Mascarenhasia*. Figs. 8–10. *Apocynum*. **5.** At level of retinacle, showing compact circular retinacle. **6.** At level between thecae and retinacle, showing absence of a ridge adnate to style head. **7.** At level of thecae, showing thecae free from the style head and absence of sclerenchyma adaxial to vascular strand. **8.** At level of retinacle, showing retinacle with central hairs reduced. **9.** At level between thecae and retinacle, showing ridge adnate to style head. **10.** At level of thecae, showing thecae adnate to style head, air spaces around vascular strand, and sclerenchyma adaxial to vascular strand. r = retinacle, rd = ridge between thecae and retinacle, sc = sclerenchyma, s = style head, t = thecae, vs = vascular strand. Bars = 0.1 mm in Figs. 5 and 8. Bar in Fig. 5 is also for Figs. 6–7, and bar in Fig. 8 is also for Figs. 9–10.
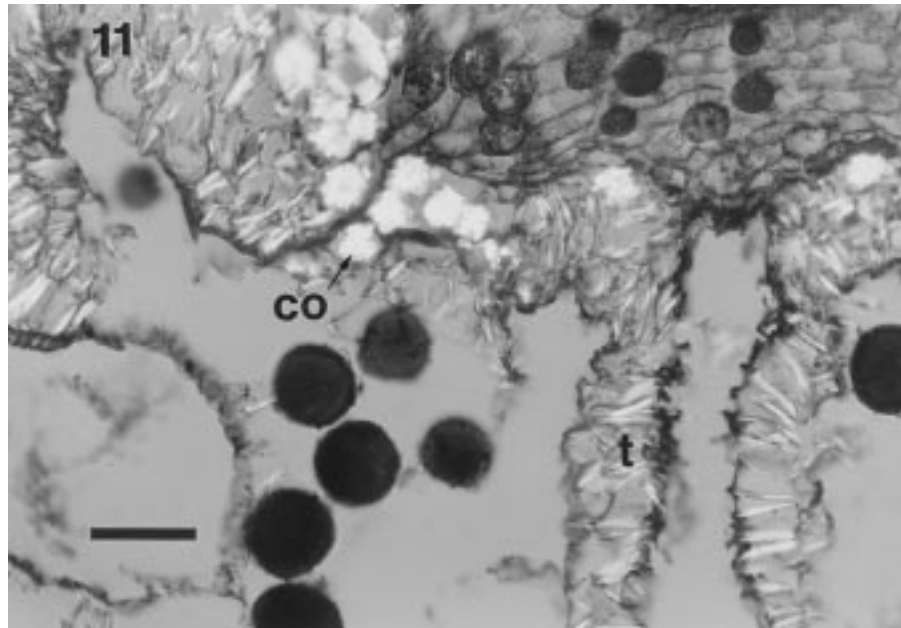
Fig. 11. Cross section of anther of *Mascarenhasia* at level of thecae. Dark-field photograph showing calcium oxalate packages in stomium. co = calcium oxalate crystals, t = thecae. Bar = 0.05 mm.
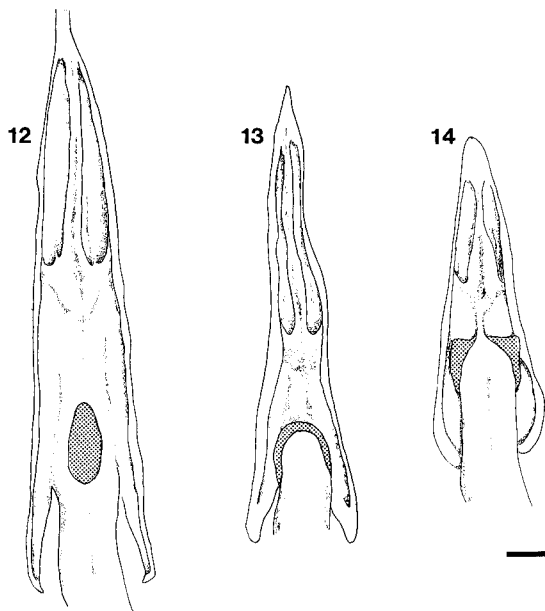
group of this clade is a supported clade consisting of the *Nerium, Adenium,* and *Strophanthus* clade and a clade comprising the rest of the ingroup ($b = 1.6$, *boot* = 84%). The *Adenium, Nerium,* and *Strophanthus* clade, as well as the subclade consisting of *Adenium* and *Strophanthus,* is weakly supported (both $b = 0.21$, *boot* < 50%).

The third of the main ''Wrightieae'' clades is the *Pachypodium, Mascarenhasia, Funtumia,* and *Holarrhena*

Figs. 12–14. Adaxial view of anthers, showing outline of retinacle. **12.** *Adenium,* compact circular retinacle. **13.** *Pachypodium,* horseshoe-shaped retinacle, with apical beak and marginal rows of hair. **14.** *Apocynum,* retinacle with the central hairs of the beak reduced. Hatched = retinacle. Bar = 0.5 mm in Fig. 14; this bar is also for Figs. 12–13.

clade, which is strongly supported ($b = 2.3$, *boot* = 93%). *Pachypodium* forms the sister group to the other constituent genera ($b = 2.1$, *boot* = 100%), and a sister relationship between *Holarrhena* and *Mascarenhasia* is moderately supported ($b = 1.0$, *boot* = 70%).

Beaumontia forms a group with *Trachelospermum* ($b = 0.61$, *boot* = 72%), a relationship not suggested before. This clade is the sister group to the remaining Apocynoideae s.l. ($b = 2.4$, *boot* = 84%). These comprise *Baissea* and *Secamone,* which form a moderately supported clade ($b = 1.2$, *boot* = 75%), and the remaining ingroup taxa, which constitute two sister clades. *Parsonsia* and *Prestonia* form a clade that is weakly supported with Bremer support ($b = 0.72$), but strongly supported by bootstrap analysis (*boot* = 94%). The last clade of the tree is the supported *Mandevilla, Apocynum,* and *Periploca* clade ($b = 1.4$, *boot* = 88%), the last two taxa forming a supported subclade ($b = 1.7$, *boot* = 93%).

As predicted, the two support measures for the successive data are approximately correlated. However, for some clades, bootstrap indicates a relatively higher support than does Bremer support, e.g., the *Parsonsia* and *Prestonia* clade ($b = 0.72$, *boot* = 94%).

Pseudogene—A potential pseudogene of *rbcL* was found in *Beaumontia*. This potential pseudogene differs from the functional *rbcL* in having two large deletions at the beginning of the gene (at positions 120–165 and 251–300, respectively), as well as several smaller deletions. The sequencing of the functional *rbcL* gene of *Beaumontia* was complicated due to the presence of the potential pseudogene, since the pseudogene amplified better with the PCR primers used. By designing new PCR primers (BS128F: ctcct caacc cggag ttcca cccga a; BS257R: cttct ccagg aacgg gctcg atgt) for the *rbcL* sequences corresponding to the deletions in the pseudogene, amplifica-
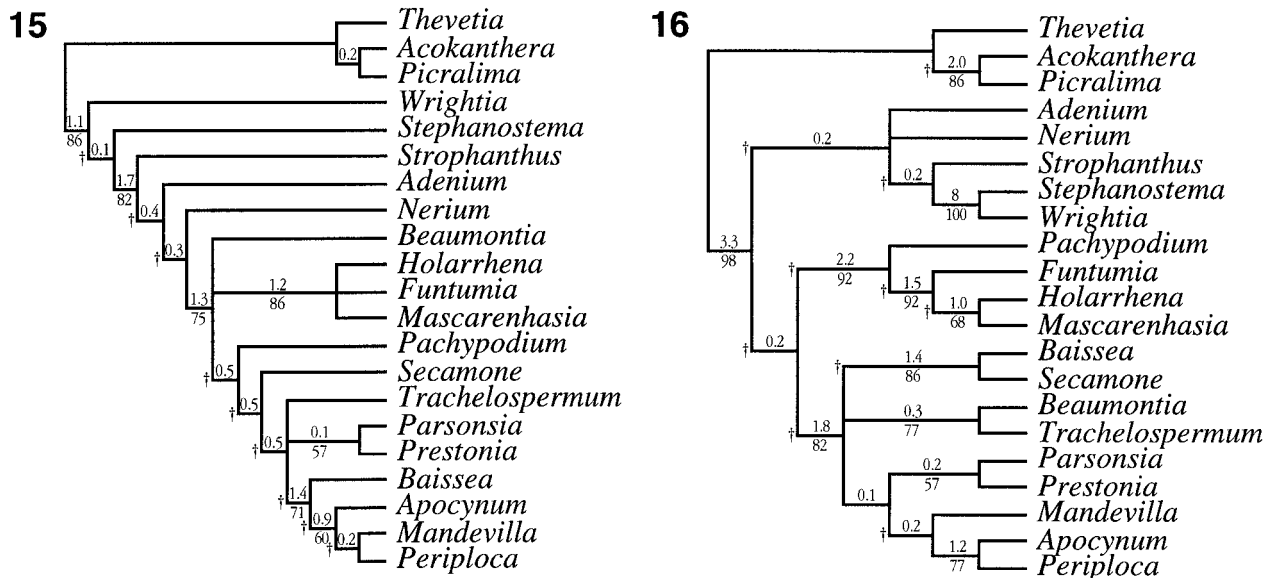
Figs. 15–16.   Results from the successive weighting analyses of the two submatrices. **15.** Single most parsimonious tree from the morphological submatrix. **16.** Single most parsimonious tree from the *rbcL* submatrix. Bremer support values are indicated above and bootstrap frequencies below branches. Bootstrap frequencies below 50% are not given. A cross (†) to the left of a branch indicates that the branch collapses in the strict consensus tree from the unit weight analysis.

tion of the functional *rbcL* alone was possible. The EMBL accession number for the potential pseudogene in *Beaumontia* is given in Table 2.

Reports of pseudogenes of *rbcL* are rare in the literature. A search for pseudogenes of *rbcL* in the GenBank sequence data base returned 27 accessions. Of these, 20 were from angiosperm genomes and the remaining were rhodophyte sequences. However, for most of these accessions, no reference was made to the pseudogene in the source publication. They furthermore showed no length
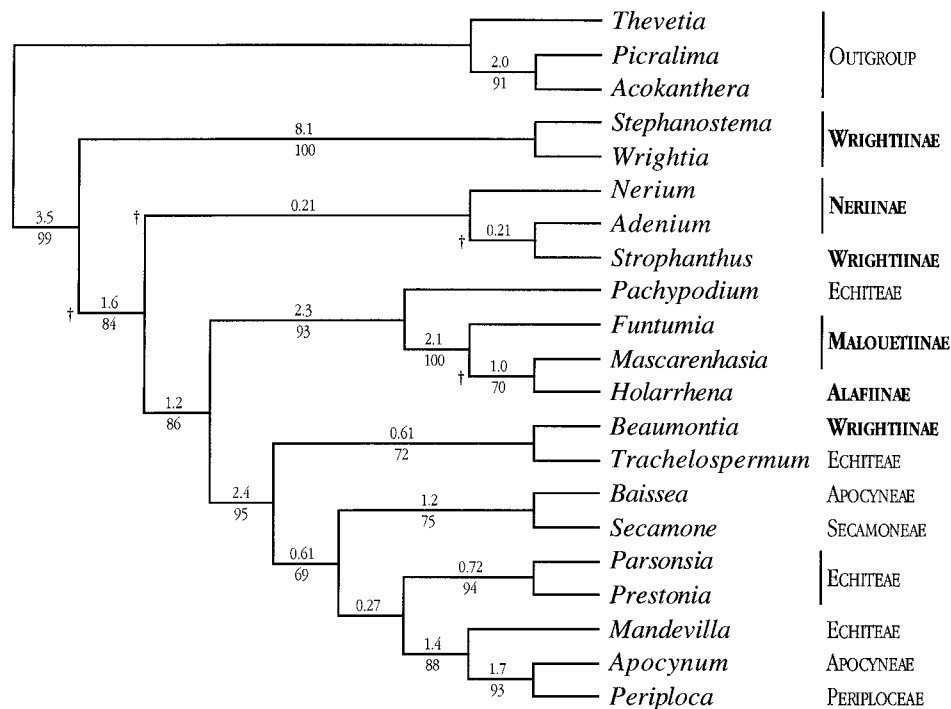
Fig. 17.   Single most parsimonious tree from the successive weighting analysis of the combined matrix. Subtribes of the Wrightieae (boldface) and tribes of other Apocynoideae s.l. (both sensu Leeuwenberg, 1994; sensu Liede and Albers, 1994, for traditional Asclepiadaceae) are indicated. Bremer support values are indicated above and bootstrap frequencies below branches. Bootstrap frequencies below 50% are not given. A cross (†) to the left of a branch indicates that the branch collapses in the strict consensus tree from the unit weight analysis.

variation, but contained an internal stop codon in the indicated reading frame. It is therefore possible that these sequences have been designated as pseudogenes, solely because of the internal stop codons. An exception is the study by Manhart (1994), in which the presence of internal stop codons was verified in *Megaceros* (Anthocerotaceae, Bryophyta). In *Epifagus* (Orobanchaceae), a nonphotosynthetic parasite, a pseudogene consisting of a truncated functional *rbcL* gene has been found (Wolfe, Morden, and Palmer, 1992). A truncated *rbcL* gene has also been found in a presumed heterogenous chloroplast population in *Oryza* (Moon, Kao, and Wu, 1987). Pseudogenes of *rbcL* situated in the mitochondrial genome have been reported in Brassicaceae (Nugent and Palmer, 1988), *Canella* (Canellaceae; Qui et al., 1993), *Ipomoea* (Convolvulaceae; Olmstead and Palmer, 1994), *Oryza* (Poaceae; Moon, Kao, and Wu, 1987), and in *Zea* (Poaceae; Lonsdale et al., 1983). In these cases, a relatively recent gene duplication and transfer of *rbcL* to mitochondria were indicated. A recent gene duplication may also be the case for the pseduogene of *Beaumontia*. Even if the pseudogene contains too many alterations to be unambiguously placed, it clearly groups within the Apocynoideae s.l.

DISCUSSION

Old classification—The present analysis (Fig. 17) shows that a portion of the Wrightieae sensu Pichon (1950a) or Leeuwenberg (1994) has a sister relationship to the rest of the Apocynoideae s.l., as suggested by Pichon (1950a) and Endress et al. (1996). However, the tribe is largely paraphyletic, as was also indicated in Sennblad and Bremer (1996) and is dispersed among four clades.

The first of these clades, the *Stephanostema* and *Wrightia* clade, corresponds to the subtribe Wrightiinae sensu Pichon (1950a). The close relationship between *Stephanostema* and *Wrightia* was also recognized by Barink (1984). The more widely circumscribed Wrightiinae sensu Leeuwenberg (1994; see Table 1), however, are paraphyletic, with *Strophanthus* grouping with *Adenium* and *Nerium* and *Beaumontia* with *Trachelospermum.*

Except for the inclusion of *Strophanthus,* the *Adenium, Nerium,* and *Strophanthus* clade corresponds to the Neriinae of Leeuwenberg (1994). An alliance between *Wrightia, Adenium, Nerium,* and *Strophanthus* (*Stephanostema* not discussed) was proposed by Beentje (1982) based on presence of a corona, absence of nectaries, and morphology of the style head.

Whether a vascularized sinal corona has evolved in parallel in the *Stephanostema* and *Wrightia* clade and the *Adenium, Nerium,* and *Strophanthus* clade or is plesiomorphic in the Apocynoideae cannot be unambiguously resolved by the present study. However, the coronas of *Adenium, Nerium,* and *Strophanthus* all consist of two adjacent lobes in the corolla sinuses, whereas the corona of *Stephanostema* is annular and that of *Wrightia* consists of several dissected lobes. It is thus possible that a paired sinal corona forms a potential synapomorphy for the *Adenium, Nerium,* and *Strophanthus* clade; to assume that it is homologous to the corona in *Stephanostema* and *Wrightia,* however, demands either a dissection or fusion

of the corona parts. The other two characters, absence of nectaries and a style head with an apical hair wreath and a basal stigmatic hollow are, according to the present study, probably best interpreted as plesiomorphies. Whether the stigmatic hollow of *Parsonsia, Prestonia, Mandevilla,* and *Periploca* is plesiomorphic as well cannot be unambiguously resolved.

The third main Wrightieae clade includes *Funtumia* and *Mascarenhasia* from the subtribe Malouetiinae, *Holarrhena* from the Alafiinae, and *Pachypodium* from the tribe Echiteae in the classification of Leeuwenberg (1994). The sister relationship between *Holarrhena* and *Mascarenhasia* makes the Malouetiinae sensu Leeuwenberg (1994) paraphyletic. *Holarrhena* was formerly placed in the subfamily Plumerioideae (Pichon, 1950b; de Kruif, 1981) based mainly on the presumed absence of a retinacle. Endress et al. (1990), however, showed that *Holarrhena* possesses a retinacle, albeit weakly developed. They therefore proposed a transfer to the Wrightieae, and in the classification of Leeuwenberg (1994) it was placed in the subtribe Alafiinae. Inclusion of *Pachypodium* in the Wrightieae has never been suggested. Due to the succulent habit of both *Pachypodium* and *Adenium,* an association of the two genera has been suggested (e.g., Plaizier, 1980) but usually dismissed. A close relationship to the Malouetiinae or Alafiinae is suggested for the first time in this study.

The present study indicates that the other tribes of the Apocynoideae s.l. are also paraphyletic. These "higher apocynoids" (i.e., Apocynoideae s.l. excluding the Wrightieae sensu Leeuwenberg, 1994) form a strongly supported clade ($b = 2.4$, $boot = 95\%$) and could be defined by having the thecae adnate to the style head. However, the putative groupings among the higher apocynoids are not the topic of this study and, thus, are not well sampled.

The Echiteae sensu Leeuwenberg (1994) are paraphyletic with the four included representatives dispersed among three different clades. *Trachelospermum* is sister ($b = 0.61$, $boot = 72\%$) to *Beaumontia* (Wrightiinae), a relationship not suggested before. This can possibly be explained by the fact that we found no morphological synapomorphies for this clade in our analysis.

Parsonsia and *Prestonia,* both Echiteae sensu Leeuwenberg, form a weakly supported clade as evaluated with Bremer support ($b = 0.72$), but strongly supported by bootstrap support ($boot = 94\%$). The *Parsonsia* and *Prestonia* clade could be defined by fused episepalous calycine colleters; these colleters are, however, absent in some species of *Parsonsia.*

The fourth member of Echiteae sensu Leeuwenberg, *Mandevilla,* forms a clade together with *Apocynum* and *Periploca.* This clade is supported ($b = 1.4$, $boot = 88\%$) and is defined by the anthers having a ridge between the retinacle and thecae that is adnate to the style head.

The Apocyneae sensu Leeuwenberg (1994) are paraphyletic since *Apocynum* (Apocyneae) and *Periploca* (Periploceae, traditional Asclepiadaceae) form a supported subclade ($b = 1.7$, $boot = 93\%$) in the *Mandevilla, Apocynum,* and *Periploca* clade, whereas the other representative of the Apocyneae, *Baissea,* forms a moderately supported clade ($b = 1.2$, $boot = 75\%$) with *Secamone* (Secamoneae, traditional Asclepiadaceae).

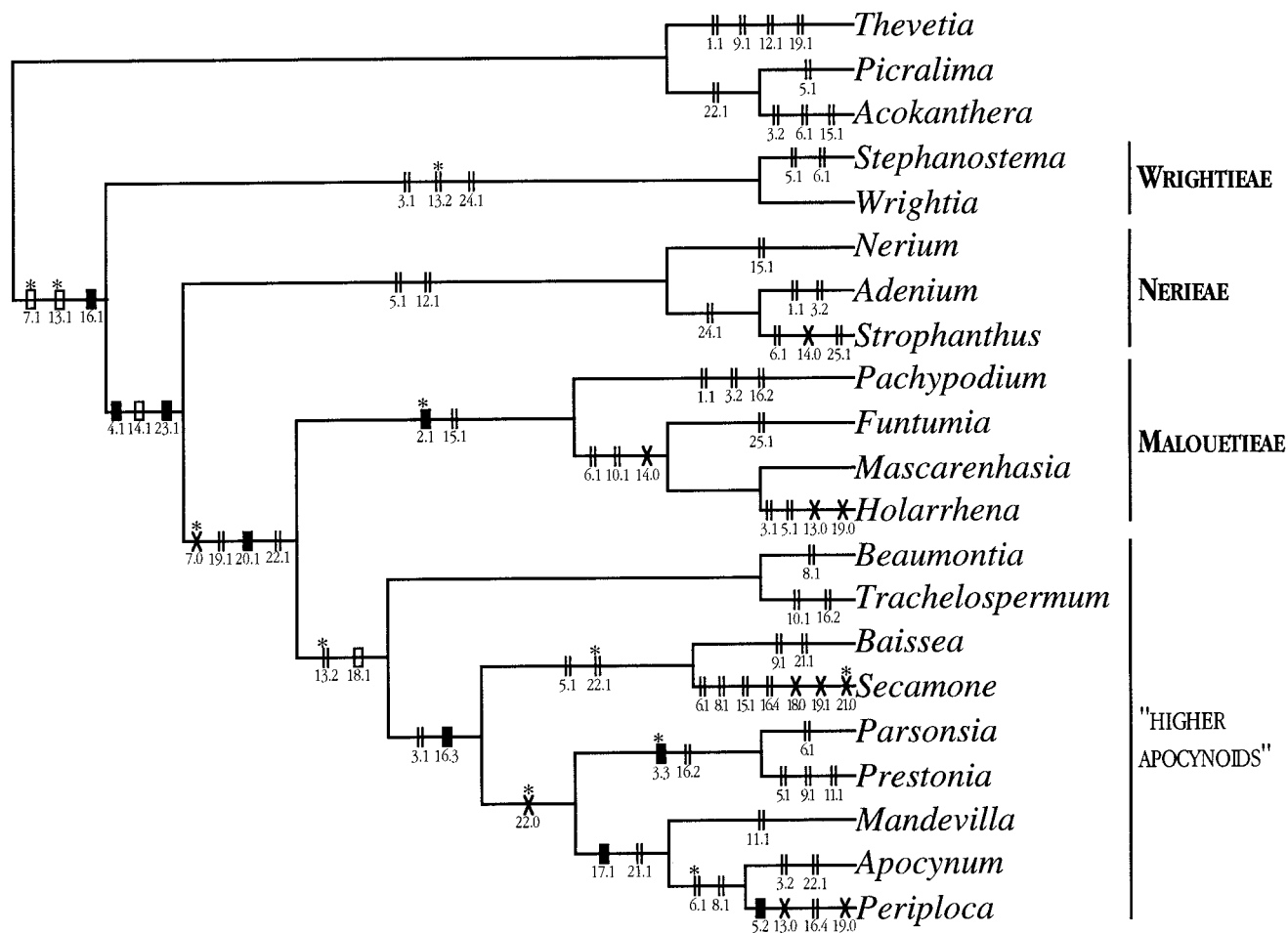Fig. 18. Reclassification of the Wrightieae based on the single most parsimonious tree from the successive weighting analysis of the combined matrix. Clades recognized as tribes by this study are indicated in boldface. Morphological characters are optimized on the tree using ACCTRAN optimization. An asterisk (*) above the character symbol indicates that optimization is ambiguous for that character. Characters are numbered as in Appendix 1. Filled bars indicate uncontradicted synapomorphies, open bars indicate synapomorphies for which reversals exist, parallel thin bars indicate parallelisms, and X's indicate reversals.

From this it also follows that the traditional Asclepiadaceae (sensu, e.g., Schumann, 1895b; Liede and Albers, 1994), represented by *Periploca* and *Secamone,* are biphyletic.

The *Mandevilla, Apocynum, Periploca, Baissea,* and *Secamone* clade is characterized by a reduction of the central hairs of the retinacle and possibly by a style head with ridges opposite the anthers.

The present study also shows that all the Apocynoideae tribes of the classification of Pichon (1950a) are paraphyletic, with the exception of the Ecdysanthereae (only one representative, *Apocynum,* included). The widely circumscribed tribes Echiteae of Don (1838), and Echiteae and Parsonsieae of Schumann (1895a) are also paraphyletic.

New classification—It is desirable that classification reflects phylogeny; this is best achieved by having monophyletic taxonomic units. The present circumscription of the Wrightieae is paraphyletic; the constituent genera being dispersed among four individual clades, of which two are strongly supported. In fact, enforcing monophyly of

the tribe would demand eight extra steps in the successive weighting analysis (12 steps with unit weights). Therefore, a reclassification is suggested to fulfill the criteria of monophyly at the tribal level of the Apocynaceae s.l. (Fig. 18).

The clade comprising *Stephanostema* and *Wrightia* constitutes the new Wrightieae s.s. This clade is the most strongly supported in the analysis ($b = 8.1$, *boot* = 100%); morphological synapomorphies for this clade are presence of calycine colleters in a marginal position only and presence of a chalazal coma. Both these characters, however, show parallelisms in other taxa in the tree. The clade also retains certain plesiomorphic traits, such as left-contorted aestivation, absence of air spaces in the anthers, and absence of a micropylar coma. These traits usually do not occur in other ingroup taxa; absence of air spaces in the anthers, however, shows reversals in the *Funtumia, Holarrhena,* and *Mascarenhasia* clade and in *Strophanthus.* The potential synapomorphy, presence of adaxial sclerenchyma, cannot be unambiguously optimized.

The *Adenium, Nerium,* and *Strophanthus* clade consti-

tutes the new Nerieae. This clade is weakly supported ($b = 0.21$, $boot < 50\%$). The main morphological apomorphies are presence of slits in the corolla tube (which shows parallelisms in several other taxa) and an elongated apical appendage of the anther connective (which is polymorphic in *Strophanthus* and paralleled in *Thevetia*). *Adenium* and *Strophanthus* also share the presence of a chalazal coma with *Stephanostema* and *Wrightia*. As noted above, the Nerieae and the Wrightieae share certain plesiomorphic states, such as a vascularized sinal corona (paired in the Nerieae, dissected or annular in the Wrightieae), a conical style head with a stigmatic hollow and an apical hair wreath, and absence of nectaries.

Also sharing a set of the abovementioned character states, but not analyzed in this study, are *Pleioceras* and *Isonema,* both formerly included in the Wrightieae (Pichon, 1950a; Leeuwenberg, 1988). *Pleioceras* and *Isonema* both have marginal calycine colleters (sometimes absent in *Pleioceras*), slits in the corolla tube, lack nectaries, have a conical style head with stigmatic hollow and apical hair wreath, and a chalazal coma. *Pleioceras* (Wrightiinae sensu Leeuwenberg, 1994) furthermore has left-contorted aestivation and a vascularized dissected sinal corona similar to the corona of *Wrightia*. This together with the marginal calycine colleters corresponds to the Wrightieae. The presence of slits in the corolla tube and air spaces in the anthers indicate a relationship to the Nerieae. However, presence of slits in the corolla tube also conforms to *Stephanostema*. *Pleioceras* is, therefore, best placed in the Wrightieae. A close relationship between *Wrightia* and *Pleioceras* has also been suggested by several authors (Pichon, 1950a; Ngan, 1965; Barink, 1984; Leeuwenberg, 1994). *Isonema* (Nereiinae sensu Pichon, 1950a, but excluded from the Wrightieae by Leeuwenberg, 1994), however, has right-contorted aestivation and a micropylar coma, which together with the presence of slits in the corolla tube support a position close to the Nerieae, rather than in the Wrightieae. Although *Isonema* lacks the vascularized corona and apical anther appendage of the Nerieae, several species of *Strophanthus* also lack an apical appendage. Furthermore, the presence of a chalazal as well as a micropylar coma on the seeds of *Isonema* corresponds well with the seeds of *Adenium* and *Strophanthus*. *Isonema* may therefore be included in the Nerieae. However, the possibility that *Isonema* may form a separate clade forming a sister group to the Nerieae and its sister clade cannot be excluded.

The *Pachypodium, Funtumia, Holarrhena,* and *Mascarenhasia* clade constitutes the new Malouetieae and is strongly supported ($b = 2.3$, $boot = 93\%$). The only unambiguous morphological apomorphy, however, is presence of calcium oxalate packages in the stomium of the anther (paralleled in other clades). The optimization of absence of leaf axil colleters in a marginal position as a synapomorphy for this clade is ambiguous. The spines of *Pachypodium* have been interpreted as transformed colleters. In sections of vegetative buds, young lateral spines show a characteristic secretory epidermis, thus being similar in appearance to colleters, or because of the size, perhaps to fused colleters. The specialization of these putative colleters and of the leaf base due to the succulence of the stem, however, makes it difficult to establish whether the spines or colleters are in a marginal position

(cf. also Uhlarz, 1975). Likewise, the absence of sclerenchyma adaxial to the vascular strand cannot unambiguously be resolved as a synapomorphy or as a symplesiomorphy shared with the Nerieae. There are more synapomorphies for the subclade consisting of *Funtumia, Holarrhena,* and *Mascarenhasia* ($b = 2.1$, $boot = 100\%$), however. Absence of an interpetal vein, filaments that are not elongated, and absence of air spaces around the vascular bundle of the anther (all paralleled in other clades) support this clade.

To extrapolate which other genera to include in the Malouetieae is difficult since many of the potentially synapomorphic characters as well as plesiomorphic characters are shared with the *Trachelospermum* and *Beaumontia* clade, e.g., sessile filaments and style head lacking an apical hair wreath and stigmatic hollow. Nevertheless, a synapomorphy for the ''higher apocynoids'' is having thecae basally adnate to the style head.

Using this synapomorphy, many taxa that have been suggested to be included in the Wrightieae s.l. may be better placed in the ''higher apocynoids.'' These are: *Vallaris* (Wrightiinae Leeuwenberg, 1994, and possibly also *Vallariopsis,* close to *Vallaris* according to Rudjiman, 1982, but excluded in Leeuwenberg, 1994), *Amphineurion* (= *Aganosma,* e.g., Middleton, 1996), *Dewevrella,* and *Pottsia* (all Neriinae, sensu Pichon, 1950a, excluded by Leeuwenberg, 1994), all of which have the thecae adnate to the style head. *Tintinnabularia* was included in the Wrightiinae by Leeuwenberg (1994). Few potential apomorphies could be scored for this genus. Supporting a position close to the Nerieae are the presence of an apical anther appendage and (according to Pichon, 1950a, fig. 10:20) thecae free from the style head. Contradicting this are the absence of a sinal corona and the presence of nectaries. The style head has a stigmatic hollow, but lacks an apical hair wreath. This, and the strongly five-angled style head, indicates a position close to the *Mandevilla, Apocynum,* and *Periploca* clade. A position close to *Mandevilla* or *Allomarkgrafia* has also been suggested by other authors (Woodson, 1936a; Pichon, 1950a; Zarucchi, 1991; Morales, 1996).

Kibatalia, Malouetia (both Malouetiinae sensu Leeuwenberg, 1994), *Alafia, Farquharia* (both Alafiinae sensu Leeuwenberg, 1994) all have calcium oxalate packages in the stomium (no data for *Farquharia*), lack interpetal veins and air spaces in the anthers, and do not have the thecae adnate to the style head (in Pichon, 1949a, fig. 2: 32, it is indicated that this is the case for *Alafia*; sectioned material, nevertheless, shows that actual adnation is not present). They, therefore, fit well into the Malouetieae. Whereas *Kibatalia* and *Farquharia* also match the *Funtumia, Mascarenhasia,* and *Holarrhena* subclade, *Malouetia* (and probably also the presumably closely related *Allowoodsonia* and *Malouetiella,* all Malouetiinae Leeuwenberg, 1994, *Malouetiella* not recognized by Leeuwenberg) and *Alafia* (Alafiinae Leeuwenberg, 1994) differ in having leaf axil colleters in a marginal position; *Alafia* also has elongated filaments and some species of *Malouetia* have a horseshoe-shaped retinacle. They may therefore be placed outside the *Funtumia, Mascarenhasia,* and *Holarrhena* subclade and possibly belong close to *Pachypodium.*

That *Carruthersia* and *Spirolobium* (earlier placed to-

gether with *Holarrhena* in the Plumerioideae Pichon, 1950b, but moved to the Alafiinae and Wrightiinae, respectively, by Leeuwenberg, 1994) also belong in the Malouetieae and higher apocynoid clade, is indicated by the absence of a sinal corona, a style head lacking an apical hair wreath and stigmatic hollow, and presence of nectaries. Furthermore, having thecae free from the style head places them outside the higher apocynoids. We lack data, however, for most of the potential synapomorphies of the Malouetieae for these two genera; only the absence of elongated filaments supports a position in the Malouetieae. The two genera seem to show a specialization of the retinacle. The weak retinacle, situated at the level of stamen insertion in the corolla tube, of *Spirolobium* is reminiscent of *Holarrhena,* whereas in *Carruthersia* the main adnation to the style head seems to have been shifted even farther down in the corolla tube. A reduced retinacle is possibly present in a similar position as in *Spirolobium* (Endress et al., 1990). *Carruthersia* and *Spirolobium* are here referred to the Malouetieae, but they may well form a separate clade apart from the Malouetieae.

Conclusions—The tribe Wrightieae (sensu, e.g., Pichon, 1950a; Leeuwenberg, 1988, 1994, Table 1) is shown to be paraphyletic. Its constituent genera are in this study dispersed among four monophyletic clades; one of these, the *Beaumontia* and *Trachelospermum* clade, is here referred to the "higher apocynoids" (i.e., Apocynoideae s.l. excluding the Wrightieae sensu Leeuwenberg, 1994). The three remaining clades are given tribal status, the Wrightieae s.s., the Nerieae, and the Malouetieae. All of these, except the Nerieae, are strongly supported.

Potential morphological synapomorphies for these three tribes are identified from the present data set. On the basis of these synapomorphies, additional taxa to be included are discussed. The Wrightieae may thus comprise *Pleioceras, Stephanostema,* and *Wrightia*; the Nerieae *Adenium, Isonema, Nerium,* and *Strophanthus*; and the Malouetieae *Alafia, Carruthersia, Farquharia, Funtumia, Holarrhena, Kibatalia, Malouetia, Mascarenhasia, Pachypodium,* and *Spirolobium.* The genera *Isonema, Carruthersia,* and *Spirolobium* are not unambiguously referred to tribes and may form independent clades from the tribe to which they are referred (the Nerieae for the first and the Malouetieae for the latter two). In such case, additional tribes must be described. *Aganosma, Dewevrella, Pottsia,* and *Vallaris* are referred to the higher apocynoids. For *Allowoodsonia, Malouetiella, Tintinnabularia,* and *Vallariopsis,* we had insufficient data; based on presumed relationships they are likely to belong in the Malouetieae and higher apocynoids clade.

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APPENDIX 1

Coding of morphological characters used in the cladistic analysis.

VEGETATIVE PARTS

1 Phyllotaxy 0, opposite; 1, alternate
Verticillate leaves occur only in *Nerium* and only as a polymorphism

with opposite leaves. It is therefore interpreted as a autapomorphic subset of opposite leaves and is not coded as a separate state.
2 Marginal colleters 0, present; 1, absent

CALYX

3 Calycine colleters 0, continuous; 1, marginal; 2, absent; 3, fused episepalous

COROLLA

4 Corolla aestivation contorted 0, left; 1, right
In the *Mascarenhasia* representatives studied, the aestivation is contorted to the right with induplicate margins. According to Pichon (1949c), some species of *Mascarenhasia* are induplicate without being contorted. Induplicate aestivation would in this study be a polymorphic autapomorphy, and is therefore not coded as a separate state.
5 Postgenital fusion of proper corolla tube 0, complete; 1, with slits above stamens; 2, absent
6 Interpetal vein supplying both petals 0, present; 1, absent
7 Vascularized sinal corona 0, absent; 1, present
Generally these corona appendages are vascularized by branches from the lateral and the midveins. An exception is *Adenium,* in which it is vascularized by branches from the lateral veins only, *Adenium* also has the smallest corona.
8 Staminal-corolline transverse ridges 0, absent; 1, present
9 Lower corona lobe 0, absent; 1, present
In *Baissea* the lobes are unvascularized, while the lobes of *Thevetia* and *Prestonia* are vascularized. The lobes of *Baissea* and *Thevetia* also have flanking ridges.

STAMENS

10 Elongation of free filament 0, elongated; 1, not elongated
The outgroup taxa lack a retinacle and are therefore coded as inapplicable (cf. definition of ''free filament'' in text).
11 Free filament 0, straight; 1, geniculate below retinacle
The outgroup taxa and taxa in which elongation of the filaments is absent (see character 10) are coded as inapplicable.
12 Elongated apical connective appendage 0, absent; 1, present
13 Sclerenchyma distribution 0, absent; 1, present, except adaxial to the vascular strand; 2, present also adaxial to the vascular strand
In some species of *Secamone* the sclerenchymatic cells may be strongly suberized, and do not stain with safranin.
14 Air-spaces around vascular strand 0, absent; 1, present
15 Calcium oxalate packages in stomium 0, absent; 1, present
16 Retinacle 0, absent 1, a circular, hairy patch; 2, horseshoe-shaped, with marginal hairs; 3, central hairs reduced; 4, all hairs reduced
17 Vertical ridge between thecae and retinacle that is adnate to style head 0, absent 1, present
18 Theca bases 0, free; 1, adnate to style head

GYNOECIUM

19 Nectaries around ovary 0, absent; 1, present
The number of nectaries is usually five; *Mandevilla* is polymorphic for two and five nectaries, thus two nectaries is a polymorphic autapomorphy.
20 Style head apical hair wreath 0, present; 1, absent
21 Style head vertical ridges opposite anthers 0, present; 1, absent
22 Style head basal stigmatic hollow 0, present; 1, absent

SEEDS

23 Micropylar coma 0, absent; 1, present
24 Chalazal coma 0, absent; 1, present
25 Micropylar rostrum 0, absent; 1, present

APPENDIX 2

Specimens investigated. Abbreviations: mm = macromorphology, co = corolla mounted in Hoyer's solution, as = anther sections, fs = flower sections, vs = vegetative bud sections.
Acokanthera oblongifolia (Hochst.) Codd, Sennblad 234, UPS (mm, co, as); Kas et al. s.n., permanent slides at Z (fs); *A. oppositifolia* (Lam.)

Codd, Fallen s.n., permanent slides at Z (fs); *A. schimperi* (A. DC.) Schweinf., Thulin 5605, UPS (mm)

Adenium multiflorum Klotzsch, Gilbert, Ensermu, and Vollesen 7553, UPS (mm, co, as); *A. obesum* (Forssk.) Roem. et Schult., Sennblad 238 (mm), UPS; *A. oleifolium* Stapf, Fallen s.n., permanent slides at Z (fs); *A. swazicum* Stapf, Fallen s.n. (mm)

Aganosma marginata (Roxb.) G. Don, without collector name, UPS (mm); Bird s.n., permanent slides at Z (fs)

Alafia barteri Oliv., Leeuwenberg 8105 (mm, co), UPS; Leeuwenberg 12156, WAG, permanent slides at Z (fs)

Apocynum androsaemifolium L., Fallen s.n., permanent slides at Z (fs, mm); *A. cannabinum* L., Bodin s.n., UPS (mm, co); McPherson and Lindsborg s.n., UPS (mm); Dorr 2385, TEX, permanent slides at Z (fs); *A. cannabinum* L. *x sibiricum* Jacq., Gereau 989, UPS (mm, co, fs, as)

Baissea bailloni Hua, Yallah 112, UPS (mm, vs); *B. leonensis* Benth., Yallah 48, UPS (mm, co, fs, as); *B. multiflora* A. DC., De Kruif 675, WAG, permanent slides at Z (fs); Louis 10689, UPS (mm, co)

Beaumontia grandiflora Wall., P. Endress 6004, permanent slides at Z (fs); Ludlow and Sherriff 3006, UPS (mm, co, as); Stainton, Sykes, and Williams 106, UPS (mm)

Dewevrella cochliostema de Wild., Louis 5586, UPS (mm)

Farquharia elliptica Stapf, Breteler 6123, UPS (mm, co)

Funtumia africana (Benth.) Stapf, Fay 7239, UPS (mm); Zwetsloot 6, UCI, WAG, permanent slides at Z (fs); *F. elastica* (Preuss) Stapf, Carvalho 2814, UPS (mm); Leeuwenberg 11072, WAG, permanent slides at Z (fs); Sennblad 259, UPS (mm, co, as)

Holarrhena floribunda, (G. Don) Durand et Schinz Leeuwenberg 9664, UPS, WAG, permanent slides at Z (mm, co, as, fs); *H. mitis* (Vahl.) R. Br., Leeuwenberg 10709, L, WAG, permanent slides at Z (fs); *H. pubescens* (Buch.-Ham.) G. Don, Leeuwenberg 10867, WAG, permanent slides at Z (fs); Richards and Arasululu 26350, UPS (mm, vs); Taylor, Gereau, and Lovett 8403, UPS (mm)

Isonema smeathmanni Roem. et Schult., Afzelius s.n., UPS (mm); Van Veldhuizen 24, WAG, permanent slides at Z (fs)

Kibatalia gitingensis (Elmer) Woodson, Liede 3268, Z, permanent slides at Z (mm, co, fs)

Malouetia bequaertiana Woodson, Bos 3690, UPS (mm, co); *M. glandulifera* Miers, Berry 5270, MO, permanent slides at Z (fs); *M. tamaquarina* (Aubl.) A. DC., Kubitzki et al. 84–247, HBG, permanent slides at Z (fs); *M. virescens* Müll.-Arg., Berry 5401, MO, permanent slides at Z (fs)

Mandevilla erecta (Vell.) Woodson, Ferreira s.n., permanent slides at Z (fs); *M. hirsuta* (Rich.) Malme, Bremer, Andreasen, Backlund, Kukka, and Sennblad 3384, UPS (mm); *M. laxa* (Ruiz et Pav.) Woodson, M. Jenny s.n., permanent slides at Z (fs); *M. sanderi* (Hemsl.) Woodson, Fallen s.n., permanent slides at Z (fs); Sennblad 253, UPS (mm, co, as); *M. tenuifolia* (J. C. Mikan) Woodson, Taylor et al. 1404, SSZ, permanent slides at Z (fs); *M. torosa* (Jacq.)Woodson, Meyer and Rogers 2604, UPS (mm, co)

Mascarenhasia arborescens A. DC., Bird s.n., permanent slides at Z (fs); Leeuwenberg 14 132, UPS (mm); Leeuwenberg 14187, UPS (mm); Malcomber et al 1667, UPS (mm, co, as)

Nerium oleander L., Fallen s.n., permanent slides at Z (mm, fs); Sennblad 265, UPS (mm, vs); Strid 2702, UPS (mm); Zohary and Andursky 456, UPS (mm, co, as)

Pachypodium baronii Costantin et Bois var. *windsori* Pichon, Fallen s.n., permanent slides at Z (fs)*; P. lamerei* Drake, Fallen s.n., permanent slides at Z (mm, fs); Sennblad, UPS 232 (mm, co, as, vs)

Parsonsia albiflora Raoul, Berggren s.n., UPS (mm); *P. capsularis* (G. Forst.) R. Br., P. Endress 6385, permanent slides at Z (fs); Tibell NZ41, UPS (mm, co, as); *P. eucalyptophylla* F. Muell., Forster 20118, BRI, permanent slides at Z (fs); *P. straminea* (R. Br.) F. Muell., P. Endress s.n., permanent slides at Z (mm, co, fs)

Periploca graeca L., Sennblad 255, UPS (mm, co, as, vs); Fallen s.n., permanent slides at Z (fs)

Picralima nitida (Stapf) T. et H. Durand, Deschamps 171, UPS (mm); Leeuwenberg 12025, WAG (mm, co, as); Leeuwenberg 10779, WAG, permanent slides at Z (fs)

Pleioceras barteri Baill., Aké Assi s.n., permanent slides at Z (fs)

Pottsia laxiflora (Blume) Kuntze, Chow and Wan 80100, UPS (mm, vegetative only); Ying 685, Z, permanent slides at Z (fs)

Prestonia acutifolia (Benth. ex Müll.-Arg.) K. Schum., Bremer, Andreasen, Backlund, Kukka, and Sennblad 3337, UPS (mm, co, as); *P. mollis* Humb., Bonpl. et Kunth, Bird s.n., permanent slides at Z (fs); *P. quinquangularis* (Jacq.) Spreng., Bremer 3028, UPS (mm); *P. riedelii* (Müll.-Arg.) Markgr., Ferreira s.n., permanent slides at Z (fs); *P. rotundifolia* Woodson, Bremer, Andreasen, Backlund, Kukka, and Sennblad 3379, UPS (mm, fs)

Secamone alpinii Schult., Bayliss 3168?, Z, permanent slides at Z (fs); *S. elliptica* R. Br., Forster 3309, BRI, permanent slides at Z (fs); *S. fryeri* Hemsl., Renvoize 927, UPS (mm, co); *S. punctulata* Decne., Sennblad 208, UPS (mm, co, as)

Stephanostema stenocarpum K. Schum., Fallen s.n., permanent slides at Z (mm, fs); Sennblad 200, UPS (mm, co, as); Sennblad 202, UPS (mm)

Strophanthus divaricatus (Lour.) Hook. et Arn., Ferreira s.n., permanent slides at Z (fs)*; S. eminii* Asch. et Pax, Sennblad 213, UPS (mm); *S. gratus* (Wall. et Hook.) Baill., Afzelius s.n., UPS (mm, co); *S. mirabilis* Gilg., Thulin 6386, UPS (mm, co, as)

Thevetia ahouai (L.) A. DC., Fallen s.n., permanent slides at Z (fs, mm); *T. peruviana* (Pers.) K. Schum., Fallen s.n., permanent slides at Z (fs); Nilsson s.n., UPS (mm, co, as); Sennblad 223, UPS (mm, co, as)

Trachelospermum difforme (Walter) A. Gray, Dorr 2 410, TEX, permanent slides at Z (fs); *T. jasminoides* (Lindl.) Lem., Block 54, MEISE (co, as); Fallen s.n., permanent slides at Z (mm, fs); Gills 9146, UPS (mm)

Vallaris solanacea (Roth) Kuntze, Thanikaimani 1760, permanent slides at Z (fs); Stainton, Sykes, and Williams 5442, UPS (mm)

Wrightia natalensis Stapf, Ward 1633, UPS (mm, co, as, vs); *W. religiosa* (Teijsm. et Binn.) Bentham, without collector name, permanent slides at Z (fs); *W. tinctoria* R. Br., Thomson s.n., UPS (mm, co); Swarupanandan s.n., permanent slides at Z (fs)