

## The familial and subfamilial relationships of *Apocynaceae* and *Asclepiadaceae* evaluated with *rbcL* data

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Received July 18, 1995; in revised version January 15, 1996

**Key words:** *Gentianales*, *Apocynaceae*, *Asclepiadaceae*. – Phylogeny, *rbcL*, parsimony, familial classification, subfamilial classification.

**Abstract:** Sequence data for the *rbcL* gene from twenty-four taxa of the families *Apocynaceae* and *Asclepiadaceae* were cladistically analysed in order to evaluate the existing familial and subfamilial classification. The taxa sampled represent all described subfamilies and a majority of the described tribes. The cladistic analysis shows that the *Asclepiadaceae* are nested within the *Apocynaceae*. An amalgamation of the two families is therefore recommended. The subfamilial classification is also in need of revision: the subfamilies *Plumerioideae* and *Apocynoideae* of the current classifications are paraphyletic, as are many of the tribes. Potential subfamily candidates and characters traditionally used in the classification are discussed.

The *Apocynaceae* and *Asclepiadaceae* are two relatively large families (in total approximately 5000 species) in the order *Gentianales* (*Asteridae*). The current circumscription of the *Gentianales* was made by WAGENITZ (1959, revised 1992), who included the families *Gentianaceae*, *Rubiaceae* (excluded by CRONQUIST 1981), *Apocynaceae*, *Asclepiadaceae*, and *Loganiaceae* (sometimes divided into several families, CRONQUIST 1981, DAHLGREN 1983, TAKHTAJAN 1987, STRUWE & al. 1994). Other families are sometimes also included (see CRONQUIST 1981, DAHLGREN 1983, TAKHTAJAN 1987, THORNE 1992, NICHOLAS & BAJNATH 1994). Recent molecular studies (DOWNIE & PALMER 1992, OLMSTEAD & al. 1993), however, support the circumscription proposed by WAGENITZ. WAGENITZ (1992) gave the following characters for the *Gentianales*: They are woody plants with opposite, entire leaves often with stipules. Colleters, a special type of multicellular glands on the stipules or the calyx lobes, are present. The flowers are sympetalous and actinomorphic with isomerous stamens. The endosperm formation is nuclear and intraxylary phloem is present (except in the *Rubiaceae*). Chemical characters include presence of indole alkaloids and cardenolides. Today, most systematists agree that the *Apocynaceae* and the *Asclepiadaceae* are closely related within the order (e.g., DAHLGREN 1980, CRONQUIST 1981, TAKHTAJAN 1987, THORNE 1992). Several recent studies have shown that they form a monophyletic group, and have indicated that the nearest

sister groups of the *Apocynaceae-Asclepiadaceae* complex are to be found in the paraphyletic family *Loganiaceae* (e.g., DOWNIE & PALMER 1992, OLMSTEAD & al. 1993, STRUWE & al. 1994).

The families *Apocynaceae* and *Asclepiadaceae* consist of mainly pantropical/subtropical plants with abundant latex and usually with opposite leaves. They have five-merous sympetalous flowers and are mainly insect-pollinated. The morphology of the pollination apparatus is heterogeneous in the *Apocynaceae*, ranging from a simple organisation with fully fertile anthers that are free from the style, to a complex compartmentalisation and synorganisation of the pollination apparatus, including basally sterile anthers that are connate to an apically enlarged style (frequently termed the style-head), and corolline corona appendages. The gynoecium usually consists of two carpels, and is syncarpous or secondarily apocarpous. Fruit types include berries, drupes, and follicles, and seed features to be found in the family include wings, comas, and arils. The *Asclepiadaceae* are more constant in morphological traits and the main difference relative to *Apocynaceae* is connected to pollination specialisation. The trend from *Apocynaceae*, concerning synorganisation of pollination structures, is continued and the anthers and the style-head are fused into a gynostegium, often with a staminal corona developing on the filaments. The pollen grains are shed as tetrads or agglutinated into pollinia. The main family-delimiting character is the translator, a special structure that is secreted by the style-head and enhances pollen removal and deposition. The fruits of the *Asclepiadaceae* are always secondarily apocarpous, bicarpellate and follicular, with comose seeds.

The *Apocynaceae* were first described by JUSSIEU (1789) as "*Apocinae*". It was a widely circumscribed family based on presence of laticifers, abundant endosperm (the term "Perispermo", used in JUSSIEU 1789, is here understood as endosperm), an often contorted corolla limb, and mainly bifollicular fruits. The family comprised genera later included in the *Asclepiadaceae* or in the *Loganiaceae*. A relatively short time later a part of the family was separated into a new family, the *Asclepiadaceae*, by BROWN (1810a) as "*Asclepiadeae*". The family was defined by "having pollen coalescing into masses, which are fixed or applied to the stigma [style-head], in a determinate manner". Ever since the *Asclepiadaceae* were separated from the *Apocynaceae* by BROWN (1810a), the families have been kept separate in most systems. Even if many systematics have acknowledged the close relationship between the two families and that they could be treated as one group (e.g., BAILLON 1891, WOODSON 1930, BULLOCK 1955, CRONQUIST 1981, TAKHTAJAN 1987, ROSATTI 1989, NICHOLAS & BAIJNATH 1994), only some of them have proposed to unite them (e.g., HALLIER 1912, DEMETER 1922, SAFWAT 1962, STEBBINS 1974, THORNE 1992, JUDD & al. 1994, STRUWE & al. 1994). Alternative treatments have included placing them as families in a separate order, the *Apocynales* (HUTCHINSON 1973, also including *Plocospermataceae*) or in a suborder of the *Gentianales*, the *Apocyninae* (ROSATTI 1989, NICHOLAS & BAIJNATH 1994).

**Infrafamilial classification of *Apocynaceae* s. str.** In the early systems, infrafamilial classification of *Apocynaceae* s. str. was based on the family's diversity in seed and fruit (ovary) characters (e.g., BROWN 1810b, DUMORTIER 1829, BARTLING 1830, CANDOLLE 1844). ENDLICHER (1838, revised 1841), who was the first to formally delimit subfamilies in the *Apocynaceae*, used two fruit characters, syn-/apo-

carpy and uni-/bilocularity, to divide the family into four subfamilies (“subordos”): the *Carissoideae* (as “*Carisseae*”) with syncarpous bilocular berries, *Allamandoideae* (as “*Allamandaeae*”) with syncarpous unilocular capsules, *Cerberoideae* (as “*Ophioxyleae*”) with apocarpous drupes, and *Apocynoideae* (as “*Euapocyneae*”) with apocarpous follicles. (For the convenience of comparison, here and in the following the subfamilies and tribes are given with the nomenclaturally appropriate name, the name actually used by the author is given in parentheses the first time the taxon is mentioned.) Presence and type of a coma on the seed were used to delimit four tribes in the *Apocynoideae* (a fifth tribe included genera later placed in *Loganiaceae*). A recent example of a subfamilial classification mainly based on fruit and seed characters, is the classification of LY (1986), who used these characters to delimit four subfamilies: the *Carissoideae* including all genera with syncarpous fruits, *Cerberoideae* with apocarpous berries or drupes, *Tabernaemontanoideae* with apocarpous fruits and arillate seeds, and *Apocynoideae* with apocarpous, follicular fruits. The subfamilies were further divided into a total of fifteen tribes (see Table 1 a).

BENTHAM (1876a) introduced the presence/absence of sterile anther appendages and the agglutination of the anthers to the style-head as the main characters for infrafamilial classification. The subfamilies that are the base for the current classifications of *Apocynaceae* were described by SCHUMANN (1895a). These were the *Plumerioideae* (as “*Plumieroideae*”), with anthers free from the style-head and mainly left-contorted aestivation, and the *Apocynoideae* (as “*Echitoideae*”), with anthers adherent to the style-head and right-contorted aestivation. The *Plumerioideae* were divided into four tribes based on fruit characters, and the *Apocynoideae* were divided into two tribes based on anther and corolla characters.

The main changes in subfamilial classification since then have been proposed additions of new subfamilies raised from within the *Plumerioideae*. The subfam. *Tabernaemontanoideae* was elevated by STAPF (1902) from the subtribe *Tabernaemontaninae* in the *Plumerioideae* sensu SCHUMANN primarily on the basis of the presence of an aril on the seeds. Additional genera have later been included (e.g., by BOITEAU & ALLORGE 1978, FALLEN 1986, see Table 1 a) and five tribes have been defined within the *Tabernaemontanoideae* (BOITEAU & SASTRE 1975). WOODSON (1930) redefined the subfam. *Apocynoideae* to include only three genera close to *Apocynum*, having pollen shed as tetrads. The other genera of the *Apocynoideae* sensu SCHUMANN were placed in the subfam. *Echitoideae* sensu WOODSON (see Table 1 a).

In the years between 1947 and 1954, PICHON (see, e.g., PICHON 1948a, b, c: the appendix, 1950) revised the family and recognised three subfamilies: the *Plumerioideae*, *Cerberoideae*, and *Apocynoideae* (as “*Echitoideae*”). He defined the subfam. *Cerberoideae*, consisting of genera formerly placed in the *Plumerioideae*, on the basis of mode of dehiscence of the anthers (see Table 1 a). The tribes of *Plumerioideae* and *Cerberoideae* were defined by fruit and anther characters. The anther-style-head connection was of prime importance to delimit the *Apocynoideae* and different modes of this connection were used to define the included tribes. The system of WAGENITZ (1964) essentially follows PICHON, but the *Cerberoideae* were reduced to a tribe in the *Plumerioideae*.

In the latest treatment of the *Apocynaceae* by LEEUWENBERG (1994), two subfam-

Table 1. Classification of *Apocynaceae*, *Asclepiadaceae*, and the outgroups. *a* Intrafamilial classification of the *Apocynaceae* according to LY (1986) and LEEUWENBERG (1994), with reference to the taxa included in the analysis. Miscellaneous subfamilies of other authors are given when these have a deviating circumscription. A single-letter code is used for references: E ENDLICHER (1841), F FALLEN (1986), P PICHON (1948C), W WOODSON (1930). *b* Intrafamilial classification of the *Asclepiadaceae* according to BENTHAM (1876b), SCHUMANN (1895b) and LIEDE & ALBERS (1994), with reference to the taxa included in the analysis. *c* Classification of the outgroup according to WAGENITZ (1964) and STRUWE & al. (1994), with reference to the taxa included in the analysis. A “=” denotes the same family/subfamily/tribe as the previous author, a dash denotes no corresponding tribe, and parentheses denote that the included taxon was not treated by the author

<i>a: Apocynaceae</i>	LY 1986	LEEUWENBERG 1994	Miscellaneous
Included taxon			
<i>Acokanthera</i>	<i>Carissoideae</i> : <i>Carisseae</i>	<i>Plumerioideae</i> : =	
<i>Dictyophleba</i>	<i>Carissoideae</i> : <i>Allamandeeae</i> <sup>1</sup>	<i>Plumerioideae</i> : <i>Carisseae</i>	
<i>Allamanda</i>	<i>Carissoideae</i> : <i>Allamandeeae</i> <sup>1</sup>	<i>Plumerioideae</i> : =	<i>Allamandoideae</i> sensu E
<i>Molongum</i>	<i>Carissoideae</i> : <i>Ambelanieae</i>	<i>Plumerioideae</i> : =	<i>Tabernaemontanoideae</i> sensu F
<i>Tabernaemontana</i>	<i>Tabernaemontanoideae</i> : <i>Tabernaemontaneae</i>	<i>Plumerioideae</i> : =	<i>Tabernaemontanoideae</i> sensu F
not sampled	—	<i>Plumerioideae</i> : <i>Macoubeae</i>	
not sampled	—	<i>Plumerioideae</i> : <i>Chilocarpeae</i>	
<i>Picralima</i>	<i>Cerberoideae</i> : <i>Alyxieae</i> <sup>2</sup>	<i>Plumerioideae</i> : <i>Carisseae</i>	
<i>Kopsia</i>	<i>Cerberoideae</i> : <i>Alyxieae</i> <sup>2</sup>	<i>Plumerioideae</i> : =	
<i>Rauvolfia</i>	<i>Cerberoideae</i> : <i>Alyxieae</i> <sup>2</sup>	<i>Plumerioideae</i> : =	
<i>Thevetia</i>	<i>Cerberoideae</i> : <i>Cerberae</i>	<i>Plumerioideae</i> : =	<i>Cerberoideae</i> sensu P, F
not sampled	<i>Cerberoideae</i> : <i>Pleiocarpeae</i>	—	
<i>Catharanthus</i>	<i>Apocynoideae</i> : <i>Plumerieae</i>	<i>Plumerioideae</i> : =	
<i>Plumeria</i>	<i>Apocynoideae</i> : <i>Plumerieae</i>	<i>Plumerioideae</i> : =	
<i>Alstonia</i>	<i>Apocynoideae</i> : <i>Alstonieae</i>	= : =	
<i>Prestonia</i>	<i>Apocynoideae</i> : <i>Parsonsieae</i>	= : <i>Echiteae</i>	<i>Echitoideae</i> sensu W
<i>Mandevilla</i>	<i>Apocynoideae</i> : <i>Echiteae</i> <sup>3</sup>	= : =	<i>Echitoideae</i> sensu W
<i>Stephanostema</i>	<i>Apocynoideae</i> : <i>Wrightieae</i>	= : =	<i>Echitoideae</i> sensu W
<i>Strophanthus</i>	<i>Apocynoideae</i> : <i>Apocyneae</i>	= : <i>Wrightieae</i>	<i>Echitoideae</i> sensu W
<i>Apocynum</i>	<i>Apocynoideae</i> : <i>Apocyneae</i>	= : =	<i>Apocynoideae</i> sensu W
not sampled	<i>Apocynoideae</i> : <i>Holarrheneae</i>	—	
not sampled	<i>Apocynoideae</i> : <i>Anodendreae</i>	—	

	BENTHAM 1876	SCHUMANN 1895	LIEDE & ALBERS 1994
<i>b: Asclepiadaceae</i>			
Included taxon			
<i>Parquetina</i>	( <i>Periplocoideae</i> : <i>Periploceae</i> )	<i>Periplocoideae</i> : <i>Periploceae</i>	= : =
<i>Secamone</i>	<i>Asclepioideae</i> : <i>Secamonaceae</i>	= : =	<i>Secamonoideae</i> : =
not sampled	—	—	<i>Asclepioideae</i> : <i>Fockeeae</i>
<i>Asclepias</i>	<i>Asclepioideae</i> : <i>Asclepiadeae</i>	= : =	= : =
<i>Hoya</i>	<i>Asclepioideae</i> : <i>Marsdeniaceae</i>	= : =	= : =
<i>Tylophora</i>	<i>Asclepioideae</i> : <i>Marsdeniaceae</i>	= : =	= : =
<i>Ceropegia</i>	<i>Asclepioideae</i> : <i>Ceropegieae</i>	= : <i>Marsdeniaceae</i>	= : <i>Stapelieae</i>
<i>Stapelia</i>	<i>Asclepioideae</i> : <i>Stapelieae</i>	= : <i>Marsdeniaceae</i>	= : <i>Stapelieae</i>
not sampled	<i>Asclepioideae</i> : <i>Gonolobeae</i>	= : =	= :
<i>c: Outgroup</i>			
Included taxon	WAGENITZ 1964	STRUWE & al. 1995	
<i>Luculia</i>	<i>Rubiaceae</i>	(=)	
<i>Coffea</i>	<i>Rubiaceae</i>	=	
<i>Gentiana</i>	<i>Gentianaceae</i>	=	
<i>Mostuea</i>	<i>Loganiaceae</i>		<i>Gelsemiaceae</i>
<i>Strychnos</i>	<i>Loganiaceae</i>		<i>Strychnaceae</i>

<sup>1</sup> As *Willughbeieae* in Ly 1986<sup>2</sup> As *Rauvolfieae* in Ly 1986<sup>3</sup> As *Ichnocarpae* in Ly 1986

ilies, *Plumerioideae* and *Apocynoideae*, were recognised. He more or less followed PICHON's (1948c) division of *Plumerioideae*, but reduced the *Cerberoideae* to a tribe in the *Plumerioideae*. He also followed PICHON's (1950) circumscription of *Apocynoideae*, but recognised only three tribes, none of them similar to the tribes circumscribed by PICHON (see Table 1 a).

**Intrafamilial classification of *Asclepiadaceae*.** Since its recognition, the infrafamilial classification of *Asclepiadaceae* has been based on characters of the translator and the pollinia. BROWN (1810a) recognised three groups within the *Asclepiadaceae*: the "*Asclepiadeae verae*" with ten pollinia, an unnamed group including only *Secamone* with 20 pollinia, and the "*Periploceae*" with granular pollen. In subfamilial treatments of the *Asclepiadaceae* either all three groups are recognised as subfamilies: the *Periplocoideae*, *Secamonoideae*, and *Asclepioideae* (ENDLICHER 1838, LIEDE & ALBERS 1994), or only two subfamilies are recognised with *Secamone* included in the *Asclepioideae* (BENTHAM 1876b, SCHUMANN 1895b). Based mainly on pollinium characters, three to six tribes are traditionally recognised in the *Asclepioideae* (see Table 1 b). BARTLING (1830), DECAISNE (1844), and BAILLON (1891) followed BROWN (1810a) in not recognising any subfamilies. SCHLECHTER (1927) described the "*Periploceae*" as a separate family, the *Periploceae*; this treatment has been followed by some other systematists (e.g., BULLOCK 1957, HUTCHINSON 1973, VERHOEVEN & VENTER 1988, DAVE & KURIACHEN 1991, KUNZE 1993, LIEDE & KUNZE 1993).

The aim of the present study is to investigate the relationships of *Apocynaceae* and *Asclepiadaceae* using *rbcL* sequence data. The *rbcL* gene is a single copy, chloroplast encoded gene, coding for the large subunit of ribulose 1,5-bisphosphate carboxylase (rubisco), an enzyme in the photosynthetic pathway. Sequences of *rbcL* have been used to investigate relationships at different taxonomic levels, and have proven particularly successful at the familial and ordinal level (see, e.g., OLMSTEAD & al. 1993, PRICE & PALMER 1993, BREMER & al. 1995, GUSTAFSSON & al. 1996). The present study is concentrated on reconstructing phylogeny and using this as a base to evaluate the familial and subfamilial classification of the *Apocynaceae-Asclepiadaceae* complex and to test different hypotheses of character evolution within the complex. Henceforth, unless otherwise stated, the subfamilies and tribes of *Apocynaceae* will be used sensu LEEUWENBERG (1994) and those of *Asclepiadaceae* sensu LIEDE & ALBERS (1994).

## Material and methods

The ingroup taxa were chosen to include representatives from all described subfamilies and as many tribes of the existing classifications as possible (Table 1 a, b). The outgroup taxa were chosen among the other families of *Gentianales* to include representatives of potential sister groups (Table 1 c). The choice of potential sister groups was based on previous classifications and are supported by studies of the *Gentianales* (BREMER & STRUWE 1992, OLMSTEAD & al. 1993, BREMER & al. 1994, Struwe & al. 1994).

All ingroup taxa have been sequenced by the first author, while the outgroup taxa were accessed from different sources (see Table 2). Total DNA was extracted from fresh leaves or herbarium material using the method of SAGHAI-MAROOF & al. (1984) and DOYLE & DOYLE (1987). The extractions from fresh leaves were purified by ultracentrifugation in

Table 2. Sources for the 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. In addition the EMBL-numbers for all taxa in the analysis are included

Included species	Source/voucher	EMBL-number
<b><i>Apocynaceae</i></b>		
<i>Acokanthera oblongifolia</i> (HOCHST.) CODD	SENNBLAD 234, UPS	X91758
<i>Allamanda cathartica</i> L.	SENNBLAD 226, UPS	X91759
<i>Alstonia scholaris</i> (L.) R. BR.	FANNING 212, FTG	X91760
<i>Apocynum cannabinum</i> L.	BREMER 3103, UPS	X91761
<i>Catharanthus roseus</i> (L.) G. DON	BREMER 3128, UPS	X91757
<i>Dictyophleba lucida</i> (K. SCHUM.) PIERRE	SENNBLAD 204, UPS	X91762
<i>Kopsia fruticosa</i> (KER) A. DC.	BREMER 3033, UPS <sup>1</sup>	X91763
<i>Mandevilla sanderi</i> (HEMSL.) WOODSON	SENNBLAD 253, UPS	X91764
<i>Molongum laxum</i> (BENTH.) PICHON	ROMERO & LLAMOZAS 3017, VEN	X91765
<i>Picralima nitida</i> (STAPF) T. & H. DUR.	LEEUEWENBERG 12025, WAG	X91766
<i>Plumeria inodora</i> JACQ.	SENNBLAD 225, UPS	X91767
<i>Prestonia quinquangularis</i> (JACQ.) SPRENG.	BREMER 3028, UPS	X91768
<i>Rauwolfia mannii</i> STAPF	SENNBLAD 218, UPS	X91769
<i>Stephanostema stenocarpum</i> K. SCHUM.	VAN DILST 1, WAG	X91770
<i>Strophanthus eminii</i> ASCH. ex PAX	SENNBLAD 213, UPS	X91771
<i>Tabernaemontana divaricata</i> (L.) R. BR. ex (ROEM. & SCHULT.	BREMER 3000, UPS	X91772
<i>Thevetia peruviana</i> (PERS.) K. SCHUM.	SENNBLAD 223, UPS	X91773
<b><i>Asclepiadaceae</i></b>		
<i>Asclepias curassavica</i> L.	BREMER 3100, UPS	X91774
<i>Ceropegia woodii</i> SCHLTR.	SENNBLAD 233, UPS	X91775
<i>Hoya bella</i> HOOK.	SENNBLAD 231, UPS	X91776
<i>Parquetina nigrescens</i> (AFZEL.) BULLOCK	SENNBLAD 220, UPS	X91777
<i>Secamone afzelii</i> (SCHULT.) K. SCHUM.	BREMER 3023, UPS	X91779
<i>Stapelia leendertziae</i> N. E. BR.	SENNBLAD 227, UPS	X91778
<i>Tylophora sylvatica</i> DECNE.	CARVALHO 3935, UPS	X91780
<b>Outgroups</b>		
<i>Coffea arabica</i> L.	BREMER & al. 1995	X83631
<i>Gentiana procera</i> HOLM	OLMSTEAD & al. 1993	L11684
<i>Luculia grandifolia</i> GHOSE	BREMER & al. 1995	X83648
<i>Mostuea brunonis</i> DIDR.	OLMSTEAD & al. 1993	L14404
<i>Strychnos nux-vomica</i> L.	OLMSTEAD & al. 1993	L14410

<sup>1</sup> This *Kopsia* sequence is a completion and correction to the *Kopsia* sequence published in OLMSTEAD & al. (1993)

CsCl-gradients, while the herbarium material extractions were purified through ethanol precipitation. Double-stranded DNA of the *rbcL* gene was amplified by PCR using Taq-polymerase kit (Promega Corp.) 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 ca. 100 nucleotides downstream from the coding region (OLMSTEAD & al. 1992). In cases where PCR-amplification proved difficult, a PCR reaction using Taq extender PCR additive (Stratagene Inc.) was performed, following the protocol provided by the suppli-

er. A second round of PCR, with only one of the primers, respectively, was performed to achieve single stranded DNA (KALTENBOECK & al. 1992). Single stranded DNA was sequenced (SANGER & al. 1977) using internal primers designed by G. ZURAWSKI (DNAX Research Institute). Both the 3'- and the 5'-strands of the *rbcL* gene were sequenced.

The data matrix for the phylogenetic analysis comprises characters corresponding to the nucleotide positions 27–1428 of the *rbcL* gene. Only phylogenetically informative characters (140) were analysed. Two types of cladistic analyses were performed using PAUP 3.1.1 (SWOFFORD 1993): a heuristic search with random starting trees, 10 000 replicates, with tree bisection and reconnection (TBR) branch swapping and all characters with unit weight, and a successive weighting analysis (FARRIS 1969), using heuristic searches with TBR branch swapping and characters reweighted according to their unit rescaled consistency index. Bremer support, *b* (K. BREMER 1988, 1995; KÄLLERSJÖ & al. 1992) – sometimes termed branch support or decay index – rescaled Bremer support for the successive weighting analysis, and a subsequent total support index, *ti* (see K. BREMER 1995), were calculated using the methods outlined in K. BREMER (1995). Jackknife frequencies, *j* (see FARRIS & al. 1995), 10 000 replicates, were calculated for the unit weight analysis using a prototype of the program Jac (FARRIS & al. 1995). This prototype cannot handle weighted data. To enhance 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 rescaled Bremer support higher than 9 steps are considered well supported, while clades with a rescaled support higher than 18 steps are considered strongly supported. This scale merely relates to the support distribution within the analysis.

## Results

The heuristic search with 10 000 replicates of random starting trees resulted in three trees 400 steps long, with a consistency index *ci* = 0.46, a retention index *ri* = 0.59, and a total support index *ti* = 0.17. The strict consensus tree from this analysis is given in Fig. 1. The single tree from the successive weighting analysis is shown in Fig. 2. The weighted length of this tree is 95 steps, the consistency index *ci* = 0.78, the retention index *ri* = 0.89, and the total support index *ti* = 0.52. The length with unit weight characters is 403 steps. The only incongruences between the trees from the two analyses are the positions of *Kopsia*, *Acokanthera*, and *Picralima*. Discussion of the clades will be made with reference to the result from the successive weighting analysis. Clades with high support values are, nevertheless, the same in both analyses.

Well supported ingroup clades (i.e., with a rescaled Bremer support *b* > 9 steps) are: the *Molongum* and *Tabernaemontana* clade, the *Rauvolfia* and *Catharanthus* clade, the *Thevetia*, *Allamanda*, and *Plumeria* clade, and the *Apocynoideae* and *Asclepiadaceae* clade (*Stephanostema*, *Strophanthus*, *Apocynum*, *Mandevilla*, *Prestonia*, *Parquetina*, *Secamone*, *Asclepias*, *Tylophora*, *Hoya*, *Ceropegia*, and *Stapelia*) which is nested within the *Plumerioideae*. Strongly supported clades (i.e., with a rescaled Bremer support *b* > 18 steps) are: the *Allamanda* and *Plumeria* clade and the *Ceropegia* and *Stapelia* clade.

## Discussion

**Assumptions.** Assumptions made in this study include the hypothesis that a hierarchical evolution is reflected by the data. This assumption, although not always explicitly expressed, is included in all studies tracing phylogeny (i.e., all studies



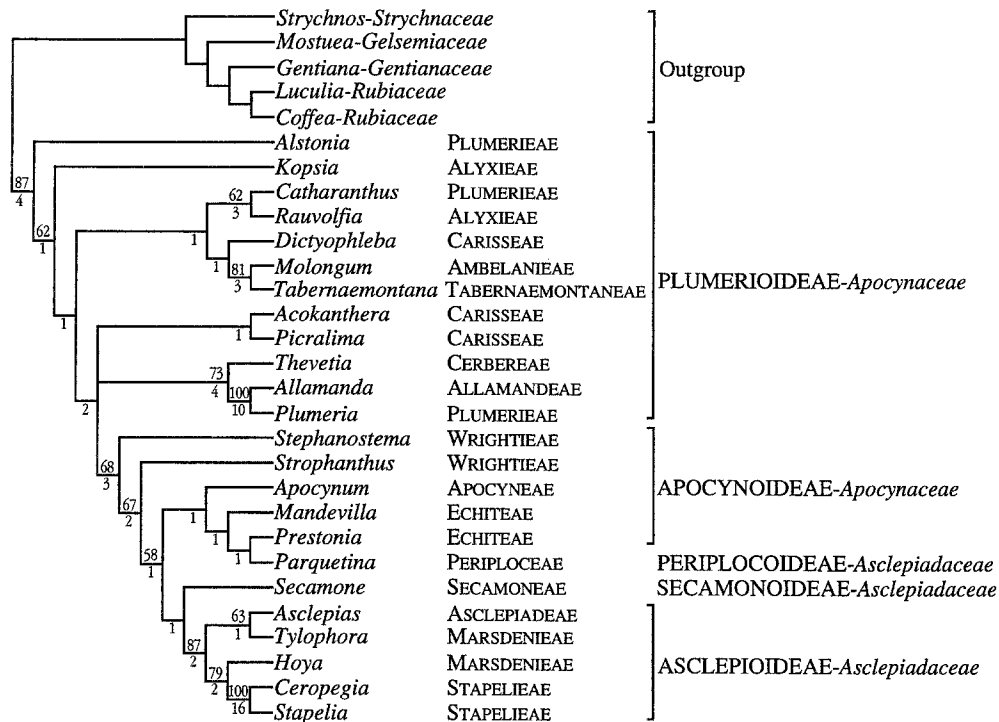


Fig. 1. Strict consensus tree of three most parsimonious trees from the unit weight analysis. The tribal and subfamilial positions (sensu LEEUWENBERG 1994 for the *Apocynaceae*, sensu LIEDE & ALBERS 1994 for the *Asclepiadaceae*, and sensu STRUWE & al. 1994 for the outgroups) are indicated, with subfamilies indicated by bars. Jackknife frequencies above 50% are indicated above branches. Numbers below branches are Bremer support values. Orientation of the tree does not reflect any hypothesis about outgroup relationships

ending up with an evolutionary tree) independently of the method used to trace hierarchy in data. The method chosen in this study is parsimony analysis (cladistics), as it makes least a priori assumptions on evolutionary events (see FARRIS 1973). The only assumptions adhering to the method itself are that the characters used are homologous and independent of each other. The homology assumption for the data used here, that each nucleotide position in one taxon is homologous to the same nucleotide position in the other taxa, is fairly safe, as structural mutations (e.g., insertions, deletions) are rare in the *rbcL* gene and there is no evidence for this in the taxa studied. The independence assumption raises an important issue, namely that all characters, i.e., the nucleotides, are part of the particular gene studied. All characters may therefore be biased by the same functional history. Using land plant phylogeny as a case study, ALBERT & al. (1994) showed that *rbcL* is under functional constraints. This makes it prone to “spurious branch attraction” or “long branch attraction” (FELSENSTEIN 1978) due to chance similarities between taxa. They also suggested that the probability of spurious branch attraction is reduced if the time sample is low, i.e., if the branching events between the taxa included are relatively recent. In this study, the probability of spurious branch attraction is assumed to be low, due to the relatively recent cladogenetic events in

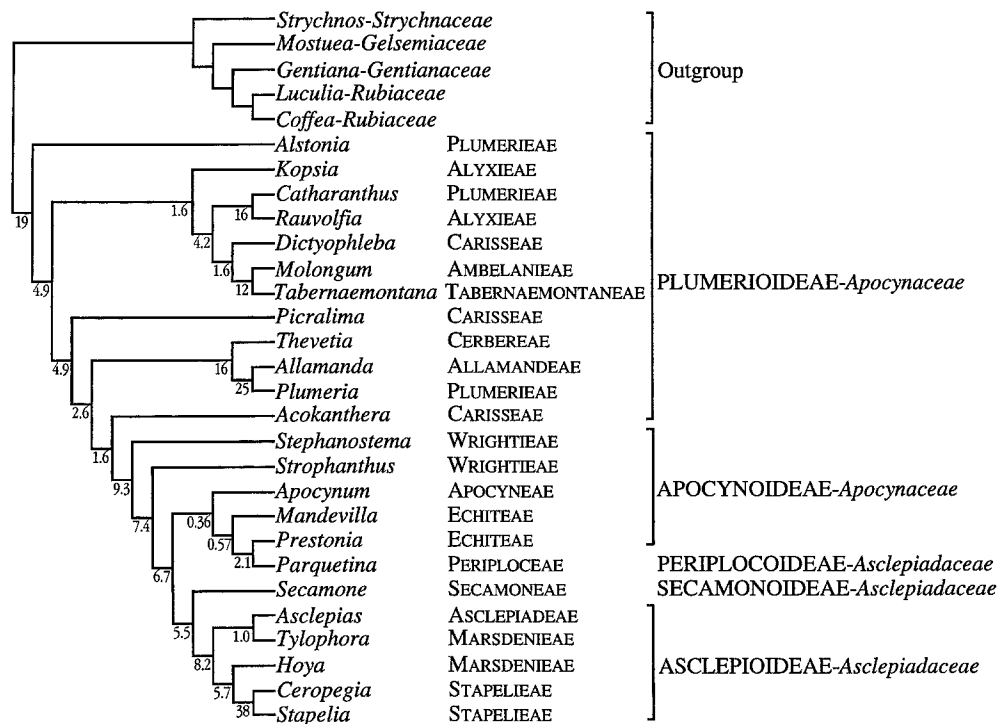


Fig. 2. Single most parsimonious tree from the successive weighting analysis. The tribal and subfamilial positions (sensu LEEUWENBERG 1994 for the *Apocynaceae*, sensu LIEDE & ALBERS 1994 for the *Asclepiadaceae*, and sensu STRUWE & al. 1994 for the outgroups) are indicated, with subfamilies indicated by bars. Numbers below branches are rescaled Bremer support values. Orientation of the tree does not reflect any hypothesis about outgroup relationships

the group investigated. Evidence for the correctness of this assumption can, however, only be achieved through congruence with additional data, as is also pointed out by ALBERT & al. (1994).

Another issue in connection with molecular data is the unequal probability for transitions and transversions in a nucleotide position, as well as for nucleotide exchange in different positions within a codon. The result from the study of ALBERT & al. (1993), in which the impact of this unequal probability is investigated, supports the use of unit weighting, given an even taxon sampling. Following their recommendation, an a priori unit weighting of all characters and character states is used in this study.

Orienting trees by the outgroup criterion involves choosing an outgroup from taxa outside the ingroup, e.g., as in this study, from potential sister groups. All taxa are then analysed together and the resulting network is "rooted" between the outgroup and the ingroup (NIXON & CARPENTER 1993). The outgroup criterion relies on the assumption that the ingroup is monophyletic. The relationships of the outgroup are not evaluated by the study. The outgroup may be paraphyletic towards the ingroup or may form a monophyletic sister group; the study cannot distinguish between these alternatives. Due to the usually weak sampling in the outgroup, an

interpretation of the outgroup relationships would also suffer from a great amount of uncertainty.

Traditionally the *Loganiaceae* have been interpreted as the sister group of the *Apocynaceae-Asclepiadaceae* complex. The family *Loganiaceae* has now been shown to be paraphyletic (BREMER & STRUWE 1992, OLMSTEAD & al. 1993, BREMER & al. 1994, STRUWE & al. 1994), and STRUWE & al. (1994) treated it as several separate families. The relationships within the *Gentianales* are not unambiguously resolved. The molecular investigation of OLMSTEAD & al. (1993) concluded that the sister group of the *Apocynaceae-Asclepiadaceae* complex is a clade with two loganiaceous genera *Gelsemium* and *Mostuea* (equalling the *Gelsemiaceae* in the treatment of STRUWE & al. 1994). In the morphological study of STRUWE & al. (1994), however, a clade comprising the loganiaceous genera *Geniostoma* and *Labordia* (*Geniostomaceae* sensu STRUWE & al. 1994) and the *Apocynaceae-Asclepiadaceae* group together.

The outgroup taxa in this study have been chosen from the *Gentianales* following the results of the above studies. The outgroup consists of two taxa from the *Rubiaceae*: *Luculia* and *Coffea*, representing the subfamilies *Cinchonoideae* and *Ixoroideae*, respectively, *Gentiana* from the *Gentianaceae*, and two taxa from the *Loganiaceae* s.l.: *Strychnos* and *Mostuea*, representing the *Strychnaceae* and the *Gelsemiaceae* in the classification of STRUWE & al. (1994). Sequences for the *Geniostomaceae* (sensu STRUWE & al. 1994) were not available.

The most parsimonious tree(s) achieved from the cladistic analysis is the hypothesis best supported by the data. Nevertheless, a number of methods to evaluate the relative support of the different clades, or branches, of the most parsimonious tree(s) have been developed in recent years. The Bremer support,  $b$  (K. BREMER 1988, 1995; KÄLLERSJÖ & al. 1992), for a branch is defined as the number of extra steps (i.e., in trees longer than the most parsimonious tree(s)) that is needed for the branch in study to be lost in the strict consensus tree. For trees achieved through successive weighting, rescaling is performed by dividing the number of extra steps by a factor  $s_w/s$ , where  $s_w$  = the length of the tree with successive weights applied and  $s$  = the length of the tree with unit weights applied (K. BREMER 1995). K. BREMER (1995) also suggests a total support index,  $ti$ , as a measure of supported resolution. The  $ti$  is the sum of the Bremer supports (or rescaled Bremer supports) for all branches on the tree (this is the total support, originally suggested by KÄLLERSJÖ & al. 1992) divided by the (unit weight) length of the tree  $s$ .

FARRIS & al. (1995) suggested jackknifing the data (i.e., random deletion of character(s)) with a high number of replicates and a subsequent evaluation of the percentage of trees in which a particular branch is present as a means for estimating branch support. Bootstrapping (FELSENSTEIN 1985) may also be used; however, jackknifing is preferred as it simplifies the relationships between support and group frequencies, and it is also much faster (FARRIS & al. 1995).

In this study the support for individual clades (branches) is evaluated by two methods: Bremer support (or the rescaled Bremer support of K. BREMER (1995) in the case of the successive weighting tree) and the jackknife procedure of FARRIS & al. (1995; see Figs. 1 and 2). A covariation between these two measures exists: both are a function of the number of uncontradicted characters that support a

branch on the most parsimonious tree(s) (FARRIS & al. 1995). In an indefinitely large matrix with no homoplastic characters, the number of characters setting off a clade would equal the Bremer support. Likewise, using the formula given in FARRIS & al., the jackknife frequencies can be calculated in relation to the number of characters setting off a clade, e.g., 1 character = 63%, 2 characters = 86%, 3 characters = 95%, and 4 characters = 98%. However, the effect of conflicting characters on this correlation has not been investigated (FARRIS, pers. comm.). In addition, both Bremer supports and jackknife frequencies are determined heuristically, and are therefore approximations of the actual support. An expected error would be overestimation of the support values. The results from the present study, where, for some clades, relatively high Bremer support values correspond to relatively low jackknife frequencies (Fig. 1: e.g., the *Catharanthus-Rauvolfia* clade:  $b = 3$ ,  $j = 62\%$ , the *Thevetia-Allamanda-Plumeria* clade:  $b = 4$ ,  $j = 73\%$ ), indicate that the Bremer support values may be more predisposed to these types of errors than the jackknife frequencies. If this is the case, the latter should provide a more conservative measure than the Bremer support, at least for large data sets. A similar disagreement between Bremer support values and bootstrap frequencies has also been reported (B. BREMER 1995). The support for the whole tree is evaluated using the total support index of K. BREMER (1995). The total support index for the successive weighting tree ( $ti = 0.33$ ) is higher than for the unit weight tree ( $ti = 0.17$ ) indicating an overall increase in the stability of the analysis using successive weighting.

Successive weighting was originally suggested by FARRIS (1969) as a method to make a cladistic analysis favouring the most congruent set of characters, i.e., those that show least homoplasy. The successive weighting procedure has mainly been used to choose between multiple equally parsimonious trees after an initial analysis, as suggested by CARPENTER (1988). A situation sometimes encountered in such an approach, is that the trees after successive weighting are incongruent with the trees from the initial analysis. This incongruence might be due to a conflict between a relatively high number of characters with high homoplasy supporting one resolution, and a lower number of characters supporting another resolution – incongruent with the first – and showing lower homoplasy on the initial tree. We believe that the successive weighting procedure is a powerful tool to detect such conflicts in data and that weighting against homoplasy probably provides the most objective solution to the problem (see GOLOBOFF 1993 for further discussion).

In the present study, there is little difference between the most parsimonious trees from the successive weighting analysis and the unit weight analysis. The only conflicts are in the positions of *Kopsia*, *Acokanthera*, and *Picralima*. In the unit weight analysis (Fig. 1) *Kopsia* is the sister group to the rest of the ingroup except *Alstonia* ( $b = 1$ ,  $j = 62\%$ ), whereas in the successive weighting analysis (Fig. 2), it is included in the *Catharanthus*, *Rauvolfia*, *Dictyophleba*, *Molongum*, and *Tabernaemontana* clade ( $b = 1.6$ ). *Acokanthera* and *Picralima* form a weakly supported clade ( $b = 1$ ,  $j < 50\%$ ) in the unit weight analysis, while in the successive weighting analysis *Acokanthera* is the sister group to the *Apocynoideae* and *Asclepiadaceae* clade ( $b = 1.6$ ), and *Picralima* is the sister group to a clade consisting of *Apocynoideae*, *Asclepiadaceae*, *Acokanthera*, *Plumeria*, *Allamanda*, and *Thevetia* ( $b = 4.9$ ). The supports for all of these positions are very low, how-

ever. The relative Bremer support values for corresponding clades in both analyses are fairly similar; clades with relatively high  $b$  in the unit weight analysis have a relatively high  $b$  in the successive weighting analysis. However, as might be expected, the amplitude of the support values is generally higher in the successive weighting analysis.

Because of the more highly resolved result provided by the successive weighting analysis, and because the successive weighting analysis probably provides the result based on the strongest evidence – something that is also indicated by the higher stability, as shown by  $ti$  – the discussion of the existing classifications will be made with reference to the result from the successive weighting analysis. Due to the small difference between the results from the two analyses, most arguments relating to the topology of the tree will apply also to the results from the unit weight analysis. The rescaled Bremer supports for the different clades in the successive weighting tree will be given in parentheses when the support of the clade is discussed.

**Systematic consequences.** The initial assumption on the monophyly of the ingroup is consistent with the successive weighting analysis, as all included outgroup taxa are grouped outside the ingroup ( $b = 19$ ). This is concordant with the results of DOWNIE & PALMER (1992), OLMSTEAD & al. (1993), and STRUWE & al. (1994). There is also good support for the *Asclepiadaceae* being nested within the *Apocynaceae* ( $b = 9.3$ , the *Apocynoideae-Asclepiadaceae* clade); this renders the *Apocynaceae* paraphyletic, as discussed by, e.g., SCHUMANN (1895b), WANNTORP (1988), DOWNIE & PALMER (1992), and OLMSTEAD & al. (1992). The main character separating the *Apocynaceae* from the *Asclepiadaceae* is the absence of a translator in the *Apocynaceae* while it is present in the *Asclepiadaceae*. The *Apocynaceae* are thus, as SCHUMANN (1895a) noted, defined by the absence of a character, often indicating a symplesiomorphy, which, as in this case, generally renders the group defined by such a character paraphyletic. Furthermore, homology has been suggested between the *Asclepiadaceae* translator and the stigma secretions aiding in pollen removal in the *Apocynaceae* (SCHICK 1980) and simple translators have also been found in genera of the *Apocynoideae* (DEMETER 1922, NILSSON & al. 1993).

The subfam. *Plumerioideae* of the *Apocynaceae* has often been interpreted as a paraphyletic group, from which the other subfamilies of the *Apocynaceae* have evolved (see, e.g., SCHUMANN 1895a, PICHON 1948c, FALLEN 1986). This is consistent with the result from this analysis, in which the subfam. *Plumerioideae* is strongly paraphyletic, with all other subfamilies nested within it. In the subfam. *Plumerioideae*, the *Carisseae* are usually interpreted as the most basal tribe (SCHUMANN 1895a, PICHON 1948c, FALLEN 1986). This is not supported by the present study; instead *Alstonia* (*Plumerieae*) is the sister group to the rest of the family, a relationship not previously suggested. The *Carisseae* are biphyletic with *Dictyophleba* associated with *Tabernaemontana* (*Tabernaemontaneae*) and *Molongum* (*Ambelanieae*), and *Acokanthera* and *Picralima* placed in different positions in a clade with *Thevetia* (*Cerberaeae*), *Allamanda* (*Allamandaeae*), *Plumeria* (*Plumerieae*), the *Apocynoideae*, and the *Asclepiadaceae*.

The position of *Dictyophleba* coincides well with the proposed ancestry of the *Tabernaemontaneae* of PICHON (1948c) and BOITEAU & SASTRE (1975), in which an

evolutionary series from the *Carisseae* via the *Ambelanieae* to the *Tabernaemontaneae* (*Tabernaemontanoideae* BOITEAU & SASTRE 1975) was proposed. The clade with *Tabernaemontana* and *Molongum* is well supported ( $b = 12$ ) and coincides with FALLEN'S (1986) circumscription of *Tabernaemontanoideae*. The sister group relationship of the *Dictyophleba*, *Molongum*, and *Tabernaemontana* clade and a clade consisting of *Rauvolfia* and *Catharanthus* has not been proposed before, nor is the well supported association of *Rauvolfia* (*Alyxieae*) and *Catharanthus* (*Plumerieae*;  $b = 16$ ) reflected in any infrafamilial classification. Instead the *Plumerieae*, as traditionally circumscribed, are paraphyletic, with none of the included genera, *Plumeria*, *Alstonia*, and *Catharanthus*, grouped together. *Kopsia*, which is a genus of uncertain systematic position, is in this study the sister group to the *Dictyophleba*, *Molongum*, *Tabernaemontana*, *Catharanthus*, and *Rauvolfia* clade mentioned above. The inclusion of *Kopsia* in the *Alyxieae* (PICHON 1948c, FALLEN 1986, LEEUWENBERG 1994) is not supported by the result of this analysis.

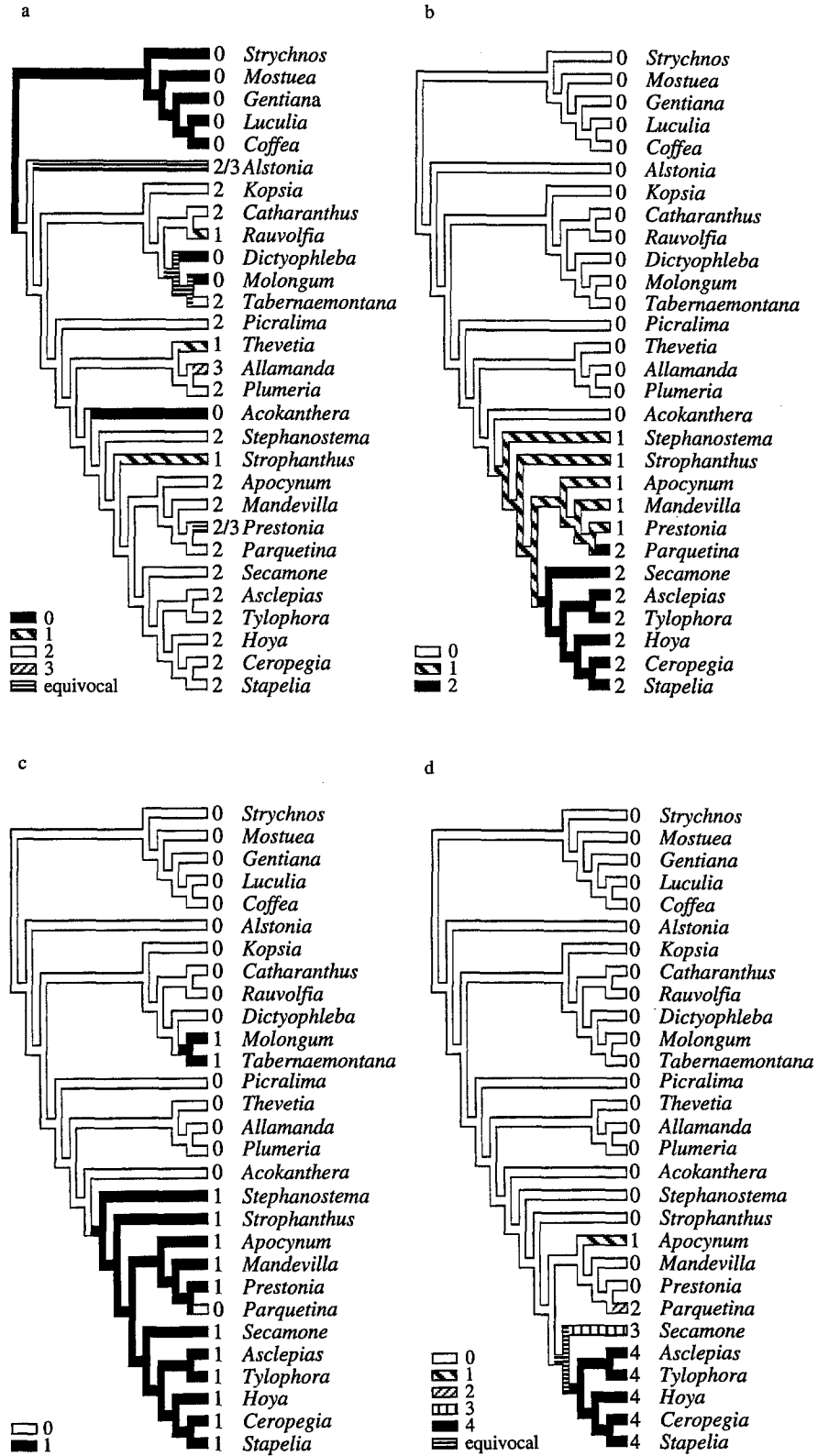
The monophyly of *Cerberoideae* (PICHON 1948b) cannot be evaluated, as only one representative of the subfamily, *Thevetia*, is included in the analysis. The *Cerberoideae* have been interpreted by FALLEN (1986) as descendants of the *Alyxieae*, with *Kopsia* as the closest link. A similar view was expressed by SCHUMANN (1895a) who placed *Thevetia* and *Kopsia* together in the subtr. *Cerberinae* of the *Plumerieae*, and also by ENDLICHER (1841) and LY (1986) who included the *Alyxieae* – including *Kopsia* – in the *Cerberoideae*. These views are not supported by the present study. Although not classifying them together, FALLEN (1985) noted an association between *Cerberoideae*, *Plumeria*, and *Allamanda*, an association strongly supported by the present study ( $b = 16$ ). In particular the branch connecting *Allamanda* and *Plumeria* has very strong support ( $b = 25$ ). *Allamanda* has been a genus with uncertain position, usually classified in a tribe of its own (PICHON 1948c, FALLEN 1986, LY 1986, LEEUWENBERG 1994) or even in a subfamily of its own (ENDLICHER 1841).

The delimiting character for *Apocynoideae*, i.e., the basally sterile anthers that are connate to the style-head, applies to the *Asclepiadaceae* as well. Therefore it is not surprising to find that the *Apocynoideae* are paraphyletic with the *Asclepiadaceae* nested within the group. The more narrowly circumscribed *Apocynoideae* of WOODSON (1930) cannot be evaluated by the present study, as only one genus of this subfamily, *Apocynum*, is included. However, several recent studies have reduced WOODSON'S *Apocynoideae* to a single genus, *Apocynum* (e.g., ROSATTI 1989, NILSSON & al. 1993). The *Echitoideae* (sensu WOODSON), in which WOODSON (1930) placed the remaining apocynoid genera, are in the present analysis strongly paraphyletic. The enlarged *Apocynoideae* of ENDLICHER (1841) and LY (1986), including the apocarpous genera of *Plumerioideae* (LY 1986 excepting *Tabernaemontaneae*), are of course also strongly paraphyletic relative to the *Asclepiadaceae*. The tribe *Wrightieae* is usually considered to be the more basal tribe in the *Apocynoideae* (e.g., PICHON 1950, as *Nerieae*). The *Wrightieae* taxa included in the present study, *Stephanostema* and *Strophanthus*, accordingly come out as the sister groups to the rest of the *Apocynoideae-Asclepiadaceae* clade. The tribe itself is, however, paraphyletic with the *Apocynoideae* and *Asclepiadaceae* nested within it. The other *Apocynoideae* taxa in the study, *Apocynum* (*Apocynaeae*), *Mandevilla*, and *Prestonia* (both in *Echiteae*), form a clade with *Parqueti-*

*na* (*Periplocoideae*). The *Echiteae* sensu LEEUWENBERG (1994) are paraphyletic in this analysis. LEEUWENBERG (1994) also indicated that the delimitation of tribes in the *Apocynoideae* was extremely difficult.

The sister group of the *Apocynum*, *Mandevilla*, *Prestonia*, and *Parquetina* clade consists of *Asclepioideae* and *Secamonoideae*. Due to the position of *Parquetina* (*Periplocoideae*) nested within the sister group to the rest of the *Asclepiadaceae*, the *Asclepiadaceae* are biphyletic in the present study. A biphyletic *Asclepiadaceae* have been suggested earlier by SCHUMANN (1895b), although he proposed the *Periplocoideae* as descendants of the *Plumerioideae* and the *Asclepioideae* (sensu SCHUMANN, i.e., including *Secamonoideae*) as descendants of the *Apocynoideae*. NILSSON & al. (1993) regarded the *Periplocoideae* as a sister group of the *Apocynoideae*. Neither of these views is supported by the present study as *Parquetina* (*Periplocoideae*) is nested within the *Apocynoideae*. The monophyly of the subfamilies *Periplocoideae* and *Secamonoideae* cannot be evaluated; the monophyly of *Asclepioideae*, however, is moderately well supported ( $b = 8.2$ ). The *Secamonoideae* are generally considered to be the sister group to the *Asclepioideae* (SAFWAT 1962, KUNZE 1993), and are sometimes included as a tribe in the *Asclepioideae* (BENTHAM 1876b, SCHUMANN 1895b). This view is consistent with the most parsimonious trees from the present study. Within the *Asclepioideae* the tribe *Marsdenieae* is paraphyletic, as *Tylophora* (*Marsdenieae*) forms a clade with *Asclepias* (*Asclepiadeae*), whereas *Hoya* (*Marsdenieae*) is grouped with *Ceropegia* and *Stapelia* (both *Stapelieae*) in the sister clade. The monophyly of the *Ceropegieae* and *Stapelieae* sensu BENTHAM (1876b) cannot be evaluated. However, the wider *Stapelieae* sensu LIEDE & ALBERS (1994), i.e., the *Ceropegia* and *Stapelia* clade, is the best supported clade in the present analysis ( $b = 38$ ).

**Characters used in classification.** These include, e.g., syn/apocarpy, which traditionally has been considered important in the *Apocynaceae*. It has been used for delimiting subfamilies (ENDLICHER 1841, LY 1986) or tribes (BENTHAM 1876b, SCHUMANN 1895a). Syncarpy has generally been interpreted as being the plesiomorphic state in the family, while apocarpy has been viewed as being derived secondarily (SCHUMANN 1895a, FALLEN 1986). Alternative views exist, however. Basing his arguments on the syncarpous fruit of *Allamanda*, which otherwise has advanced features, WOODSON (1930) proposed the following evolutionary series in the *Plumerioideae*: *Plumerieae* with syncarpous fruits are plesiomorphic in the subfamily, followed by *Carisseae* with syncarpous bilocular fruits, and finally *Allamanda* with syncarpous unilocular fruits. FALLEN (1985) dismissed the evolutionary series of WOODSON (1930), by showing that in the syncarpous fruit of *Allamanda*, the carpels are postgenitally fused, while in the fruits of *Carisseae*, they are congenitally fused. PICHON (1948a) pointed out that syn/apocarpy varies within several tribes, as well as within genera in the *Plumerioideae*. Consequently, he ignored syn/apocarpy and based his *Plumerioideae* tribes on other fruit characters (dry/fleshy pericarp, dehiscence/indehiscence) and anther characters. ROSATTI (1989) noted the same phenomenon and proposed that the pluricarpellate, apocarpyous fruits of SCHUMANN'S (1895a) tribe *Pleiocarpeae* in the *Plumerioideae* represent the plesiomorphic stage followed by the bicarpellate, apocarpyous *Plumerioideae*, *Apocynoideae*, and *Asclepioideae*; from the bicarpellate *Plumerioideae* he proposed that syncarpous fruits evolved at least twice.





The character states and the possible state assignments for the different branches on the tree from the successive weighting analysis are given in Fig. 3 a. The *Apocynaceae* taxa are coded as suggested by M. ENDRESS (FALLEN 1986, NILSSON & al. 1993, and pers. comm.); the *Asclepiadaceae* are all coded as being apocarpous, following SCHUMANN (1895b). Congenital syncarpy, occurring in *Acokanthera*, *Dictyophleba*, and *Molongum*, is coded as a state different from postgenital syncarpy, occurring in *Allamanda*. Partial congenital syncarpy (in *Thevetia*, *Rauvolfia*, and *Strophanthus*) is coded as a separate state. *Alstonia* and *Prestonia* contain both apocarpous and syncarpous species; the type of syncarpy is, however, not known. Considering the fruit type in *Alstonia* and *Prestonia* and the fact that the majority of the species in the two genera are apocarpous, it can be assumed probable, although not certain, that a fusion of carpels is postgenital (M. ENDRESS, pers. comm.). It is therefore assumed in this study that the syncarpy in *Alstonia* and *Prestonia* is of the postgenital type and they are consequently coded as polymorphic for apocarpy and postgenital syncarpy. Given this coding and the tree from the successive weighting analysis, apocarpy is interpreted as the plesiomorphic state in the family with syncarpy evolving several times (Fig. 3 a). The result from this analysis indicates that syncarpy is of limited value for the classification of *Apocynaceae* at this level as it does not form any potential synapomorphies. Further support for this view is provided by the fact that all taxa defined by syncarpy that could be evaluated by the present study (i.e., the *Carisseae* sensu LEEUWENBERG 1994, the *Allamandaeae* and the *Carissoideae* sensu LY 1986) have turned out to be paraphyletic.

Two important characters for current subfamilial classification of the *Apocynaceae* are the presence of sterile anther appendages and the agglutination of the anthers to the style-head, both used to define the subfam. *Apocynoideae*. These characters were first suggested by BENTHAM (1876a) but have been used also by several later authors (e.g., SCHUMANN 1895a, WAGENITZ 1964, LEEUWENBERG 1994). PICHON (1948d) used the term rétinacle for the part of the anther connective involved in the bond, and used the character to define not only the subfam. *Apocynoideae* but the tribes of this subfamily as well. In the treatment of FALLEN (1986), the structure of the bond is described in detail: a part of the connective just below the anther forms a pad of hairs; these hairs adjoin to the papillous style-head and become cemented to it by a viscin secreted by the style-head. NILSSON & al. (1993) described the similar anther-style-head connection in the *Periplocoideae*. Here, however, the anthers and the style-head are postgenitally fused by

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Fig. 3. Possible state assignments on the most parsimonious tree from the successive weighting analysis for some characters traditionally used in subfamilial classification. States for each taxon are given in front of the taxon. States separated by a slash indicate that the taxon is variable between these states. *a* Apo-/syncarpy: 0 = congenital syncarpy, 1 = partial congenital syncarpy, 2 = apocarpy, 3 = postgenital syncarpy. *b* Anther-style-head connection: 0 = anthers free from style-head, 1 = anthers agglutinated to style-head, 2 = anthers postgenitally fused to style-head. *c* Sclerenchymatic side wings or guide rails: 0 = absent, 1 = present. *d* Translator: 0 = absent, 1 = band-like translators, 2 = periplocoid translator, 3 = secamonoid translator, 4 = asclepioid translator

sclerenchymatic tissue. The same applies for investigated genera of the *Secamonoideae* and *Asclepioideae* (M. ENDRESS, pers. comm.). FALLEN 1986 also defined the “sterile anther appendages” more sharply as “presence of sclerenchymatic guide rails on the anthers”. Using this definition, the character is present in the *Apocynoideae*, the *Tabernaemontanoideae* sensu FALLEN (*Molongum* and *Tabernaemontana* in this study), the *Secamonoideae*, and the *Asclepioideae*. The codings and the possible state assignments for the two characters are shown in Fig. 3 b and c.

The codings follow the suggestions of M. ENDRESS (FALLEN 1986, NILSSON & al. 1993, and pers. comm.); *Parquetina* is assumed to have the same states as other taxa of *Periplocoideae* investigated. The rétinacle, i.e., adhesion by means of a pad of hair and viscin, evolved once and forms a potential synapomorphy for the *Apocynoideae-Asclepiadaceae* clade. The rétinacle is, however, replaced by the firmer adhesion in postgenital fusion, which evolved twice: once in *Parquetina* (*Periplocoideae*) and once in the *Secamonoideae-Asclepioideae* clade. Sclerenchymatic guide rails evolved twice: once in the *Molongum-Tabernaemontana* clade (the *Tabernaemontanoideae* of FALLEN 1986) and once in the *Apocynoideae-Asclepiadaceae* clade – with a reversal in *Parquetina* (*Periplocoideae*) – forming potential synapomorphies for these clades.

The translator, which provides the characters delimiting the family *Asclepiadaceae* and its subfamilies, is a hard structure secreted by the style-head aiding in pollen removal and deposition. Homology has been suggested with the viscous secretions of the style-head of *Apocynaceae* (SCHICK 1980). All taxa of the *Asclepiadaceae* have five translators located between the anthers in each flower, but they are of three main different types. The *Periplocoideae* have the pollen deposited as tetrads into a spoon-shaped translator lying on the style-head. At the base of this structure (the shaft of the spoon) a sticky plate is attached. This plate is directed outwards from the style-head and attaches to the pollinator (e.g., on the mouth parts). The *Secamonoideae* and the *Asclepioideae* have the pollen agglutinated into pollinia. The pollinia from anther halves of adjacent anthers are attached to a translator, which consists of a corpusculum – a structure that clasps on to the pollinator – and translator arms. The *Secamonoideae* have four pollinia attached to the corpusculum, while the *Asclepioideae* have two pollinia attached to the corpusculum. Furthermore, in the *Secamonoideae* the translator arms are poorly developed, while in the *Asclepioideae* well-developed translator arms usually are inserted between the corpusculum and the pollinia. Simple band-like translators have also been reported from the genera *Apocynum* and *Forsteronia* in the *Apocynoideae* (DEMETER 1922, NILSSON & al. 1993). Several attempts have been made to trace an evolutionary series between the different translator types. Although differing in the details of the suggested evolutionary process, most authors (DEMETER 1922, SAFWAT 1962, SCHICK 1980, KUNZE 1993) agree that, within the *Asclepiadaceae*, the *Periplocoideae* translator represents the most primitive translator type, which evolved into the translator type of *Secamonoideae* and then further into the *Asclepioideae* translator type. The position of the translator type of *Apocynum* and *Forsteronia* in this evolutionary series seems to be less certain and most authors avoid making any explicit hypothesis on this issue. The different translator types, described above, are in this study coded as states in a multistate character.

The codings and the possible state assignments are shown in Fig. 3 d. The result from this study indicates that the translator has evolved independently three times: once in *Apocynum*, once in *Parquetina* (*Periplocoideae*), and once in the *Secamonoideae-Asclepioideae* clade. The homology of the *Periplocoideae* translator type and the *Asclepioideae-Secamonoideae* translator types, suggested by DEMETER (1922), SAFWAT (1962), SCHICK (1980), and KUNZE (1993), is thus not supported, nor is the use of “presence of a translator” as a synapomorphy for the *Asclepiadaceae*. However, a close relationship of the translator types of *Secamonoideae* and *Asclepioideae* is supported, as is suggested also by the morphological similarity between the two translator types. These translator types with pollinia attached to a corpusculum form a potential synapomorphy for the *Secamonoideae-Asclepioideae* clade.

**Classification.** A classification should reflect a hypothesis of the phylogeny of the taxa. Groups that constitute taxonomic units should therefore be monophyletic. The position of the *Asclepiadaceae* nested within the *Apocynaceae* is well supported ( $b = 9.3$ ) by the present study, and is also suggested by several other authors (SCHUMANN 1895b, DEMETER 1922, SAFWAT 1962, DOWNIE & PALMER 1992, OLMSTEAD & al. 1993, STRUWE & al. 1994). In order to fulfil the criterion of monophyly, either an amalgamation of the two families into one, or a division into several smaller, monophyletic families is necessary. We believe that an amalgamation will serve systematic treatment as well as nomenclatural stability best. An enlarged family *Apocynaceae* including the *Asclepiadaceae* is therefore proposed, in agreement with earlier authors (JUSSIEU 1789, HALLIER 1912, DEMETER 1922, SAFWAT 1962, DOWNIE & PALMER 1992, THORNE 1992, JUDD & al. 1994, STRUWE & al. 1994).

Similar reasoning can be applied to the subfamilial delimitation. Many of the subfamilies of the *Apocynaceae* are paraphyletic and a redefinition of the subfamilies would be desirable. One potential subfamily could be the *Apocynoideae-Asclepiadaceae* clade, which is well supported ( $b = 9.3$ ) in this study and could be defined by characters traditionally used to delimit the *Apocynoideae*. This clade would constitute the new *Apocynoideae*, which consequently include the *Asclepiadaceae*. An alternative would be to recognise the *Asclepioideae* of SCHUMANN (1895b) including *Secamonoideae*. The sister group comprising the *Periplocoideae* and *Apocynoideae*, but excluding the *Wrightieae*, would then form the new *Apocynoideae*. However, in that circumscription the *Apocynoideae* would be weakly supported ( $b = 0.36$ ) and hard to define, and reclassification of the paraphyletic *Wrightieae* would demand at least two new subfamilies. A well supported group ( $b = 16$ ) is the one including *Thevetia* (*Cerberaeae*), *Allamanda* (*Allamandaeae*), and *Plumeria* (*Plumerieae*). This group would constitute the new *Plumerioideae*. The *Tabernaemontanoideae* sensu FALLEN (1986), i.e., *Molongum* and *Tabernaemontana*, are well supported ( $b = 12$ ); however, an enlarged subfam. *Tabernaemontanoideae*, including *Dictyophleba*, *Catharanthus*, and *Rauvolfia*, and also *Kopsia*, following the result from the successive weighting analysis, is less well supported ( $b = 1.6$ ). Weakly supported are also the positions of *Alstonia* ( $b = 4.9$ ), *Acokanthera* ( $b = 1.6$ ), and *Picralima* ( $b = 2.6$ ), which each would demand a separate subfamily. A possible solution, advocated by, e.g., BREMER (1994), is to leave taxa with uncertain or weakly supported position unclassified,

recognising only the well supported subfamilies. In addition to the problem with weakly supported groups, there is another difficulty. In most cases where more than one representative for a tribe of the current classifications were included in the present study, the tribe turned out to be paraphyletic (*Carisseae*, *Alyxieae*, *Plumerieae*, *Wrightieae*, *Echiteae*, and *Marsdenieae*). This indicates that to extrapolate the terminal units in this study to represent tribes and base the circumscription of the subfamilies on the tree could be unwise. Consequently, considering the rather weak branch support for the position of some of the taxa and the fact that several tribes have proved to be paraphyletic, it is believed that further sampling and possibly also further data are needed before any subfamilies can be safely delimited.

We wish to thank STEVE FARRIS for kindly providing a prototype of his program Jac and for valuable discussions, MARY ENDRESS for providing plant material and for comments on the coding of the morphological characters, ANTONY LEEUWENBERG and ELMAR ROBBRECHT for providing plant material, and ANDERS BACKLUND, KÅRE BREMER, MATS GUSTAFSSON, MARI KÄLLERSJÖ, ELISABETH LÖNN, NADINA LAURENT, RICHARD OLMSTEAD, LARS-GUNNAR REINHAMMAR, MATS THULIN, and an anonymous reviewer for comments on the manuscript. The Uppsala Botanical Garden, the Department of Plant Taxonomy at Wageningen Agricultural University, the National Botanical Garden of Belgium, the Bergius Botanical Garden, and the Fairchild Tropical Garden kindly provided plant material. This study was supported by the Swedish Natural Science Research Council and a travel grant from the P. F. Wahlberg memory fund.

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Accepted on January 17, 1996 by F. EHRENDORFER