

## Phylogeny and classification of the subfamily Rubioidae (Rubiaceae)

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**Abstract.** We performed phylogenetic analyses of the subfamily Rubioidae (Rubiaceae) based on three different pieces of chloroplast DNA, the protein coding *rbcL* gene, the spacer sequence between *atpB* and *rbcL* (*atpB-rbcL*), and the recently published (Andersson and Rova 1999) *rps16* intron data. New *rbcL* sequences have been produced for 41 taxa and there are 52 new *atpB-rbcL* spacer sequences. All analyses gave similar results concerning the phylogeny, but they differ slightly in resolution and support for the various branches. The minor tribes Ophiorrhizeae, Urophyllaeae, Lasiantheae, and Coussareeae form a grade to the rest of the subfamily, which consists of two well-supported branches, the Psychotriaceae alliance and the Spermaceae alliance, including a majority of all genera and species. Based on the resulting phylogenies we present a revised classification of the Rubioidae. We accept 16 tribes of which 12 more or less correspond to earlier tribal circumscriptions: Anthospermeae, Argostemmateae, Craterispermeae, Gaertnereae, Morindeae, Paederieae, Psychotriaceae, Schradereae, Spermaceae, Rubieae, Theligoneae, and Urophyllaeae; two tribes have received new and very different circumscriptions: Ophiorrhizeae and Coussareeae; and two are new to science: Lasiantheae and Danaideae.

**Key words:** Rubiaceae, Rubioidae, Danaideae, Lasiantheae, chloroplast DNA, *rbcL*, *atpB-rbcL* spacer, *rps16* intron, phylogeny, classification.

Progress in understanding of the subfamily Rubioidae of the Rubiaceae is relatively recent and includes many important contributions from many different scientists. Before the middle of the 20th century the “Rubioidae” taxa were dispersed in the two subfamilies Coffeoidae and Cinchonoideae, a classification of the Rubiaceae based on ovule number (Schumann 1891). Bremekamp (1952, 1954) and Verdcourt (1958) argued against this artificial division of the family and instead proposed that all Rubiaceae tribes with species containing raphides (calcium oxalate crystals) should be set aside as a new subfamily, Rubioidae. There are large similarities between the systems of these two authors. Verdcourt included 16 tribes (and the unplaced genus *Hillia*) in the subfamily Rubioidae, and Bremekamp (1966), who used a more narrow tribal circumscription, included 19 tribes and excluded some tribes to other subfamilies: Hillieae to Hillioideae, Ophiorrhizeae to Ophiorrhizoideae, and Urophyllaeae and Pauridiantheae to Urophyllloideae. Characters, except the raphides, that were used to distinguish Rubioidae were valvate aestivation (with few exceptions, *Hamelia*, *Deppea*, and *Hillia* – all these have later been shown to belong to Cinchonoideae; e.g. Bremer et al.

1995) and indumentum of septated (articulated) hairs. The next major change in the Rubioideae circumscription was presented by Robbrecht (1988). His Rubioideae with 16 tribes agreed largely with Bremekamp's but Robbrecht included Ophiorrhizeae and Theligoneae (shown to be related to Rubiaceae by Wunderlich 1971) and excluded Knoxiaceae and Craterispermeae to the subfamily Antirhoeidae. These three classification schemes of Bremekamp, Verdcourt, and Robbrecht have been important sources of inspiration for later more focused studies of taxonomy and morphology. Comprehensive taxonomic studies of different Rubioideae tribes that should be mentioned are those by Puff and Robbrecht with co-authors (Puff 1982, 1988; Puff and Robbrecht 1989; Puff and Buchner 1998; Robbrecht 1982; Robbrecht et al. 1991), and Johansson (1987a, b, 1988, 1989, 1994). Other examples are chromosome investigations by Kiehn (e.g. 1986, 1995), morphological studies by Igersheim and co-authors (Igersheim 1992, Igersheim and Rohrhofer 1993, Igersheim et al. 1994), and pollen investigations by e.g. Robbrecht and co-workers (Robbrecht 1982, Jansen et al. 1996).

The classification systems as well as the taxonomic/morphological investigations have in their turn inspired phylogenetic investigations based on molecular data. In many of these, Rubioideae taxa have been included even if the focus has been on other groups or at other taxonomic levels. Several studies have aimed at resolving the whole family phylogeny (Bremer and Jansen 1991, Bremer et al. 1995, Bremer 1996a), others at specific tribes, e.g. Rubiaceae (Manen et al. 1994, Manen and Natali 1995, Manen and Natali 1996, Natali et al. 1996), and Psychotriaceae (Nepokroeff et al. 1999). These studies are based on molecular markers designed to elucidate phylogenies at different taxonomic levels but the results are congruent to a great extent. The results in some parts agree with classification but in other parts they are totally different and there are several important conclusions concerning Rubioideae, e.g. that Hamelieae

(Bremer and Jansen 1991) and Hillieae (Bremer et al. 1995, Natali et al. 1995) do not belong in the subfamily, that Theligoneae belong in the Rubioideae close to Rubiaceae (Bremer 1996a, Natali et al. 1996), and that Knoxiaceae belong in the Rubioideae and are not part of Antirhoeidae (Bremer 1996a).

Molecular investigation has proved to be very informative for reconstruction of phylogeny but several tribes are yet to be represented in molecular investigations. Just before completion of this manuscript a detailed analysis of the Rubioideae based on the *rps16* intron was published (Andersson and Rova 1999). That study confirms, in most parts, earlier molecular studies (Bremer 1996a, Natali et al. 1996) but as more taxa are included it also contributes a lot of new information.

The present study aims at presenting new phylogenetic analyses of the Rubioideae based on two different sequences of chloroplast DNA, the protein coding *rbcL* gene and the spacer sequence between *atpB* and *rbcL* (*atpB-rbcL*), a combined analysis of these data, and also an analysis of our data in combination with the recently published *rps16* data, *rbcL/atpB-rbcL/rps16* (also referred to as the 3-data matrix or analysis). As a result of the phylogenetic analyses we present a new classification of the subfamily Rubioideae.

## Material and methods

The sampling strategy in the phylogenetic analyses was to cover as many of the described tribes of the subfamily Rubioideae as possible, and also to include several taxa from large and diverse tribes. We did not manage to find material from two of the twenty-eight tribes (Lathraeocarpeae and Perameae) and for economical and technical reasons we did not sequence all taxa for both *rbcL* and the spacer. All 151 genera included in the resulting classification are listed in the appendices 1 and 2.

The outgroups were chosen to represent one Gentianales member outside the Rubiaceae, *Gelsemium* (Gelsemiaceae; in the spacer and combined analyses), and members of other subfamilies of the Rubiaceae (cf. Bremer 1996a), *Gardenia* from the subfamily Ixoroideae, and *Cinchona* and

*Luculia* (in the spacer analysis also *Coptosapelta* and *Exostema*) from the subfamily Cinchonoideae. In the analysis of our data in combination with the *rps16* data we used *Gelsemium* and *Cinchona* as outgroups.

Silica gel-dried or herbarium leaves were used in the DNA investigations. DNA was extracted, amplified, and sequenced according to Bremer et al. (1995) and Backlund et al. (2000) for *rbcL*, and according to Manen et al. (1994) for the *atpB-rbcL* spacer. Table 1 provides a list of taxa included in the phylogenetic analyses of *rbcL* and *atpB-rbcL* data, with EMBL/GenBank accession number of the corresponding sequences, and with voucher information for new sequenced taxa. New *rbcL* sequences have been produced for 41 taxa and there are 52 new *atpB-rbcL* spacer sequences. All other sequences used in these analyses have been published earlier by the first or second author alone or in collaboration with co-authors (Olmstead et al. 1993; Bremer et al. 1995; Bremer 1996a, b; Andreassen and Bremer 1996; Manen et al. 1994; Manen and Natali 1995, 1996). For references to the *rps16* data see Andersson and Rova (1999).

The *rbcL* and *atpB-rbcL* spacer matrices comprise 106 and 63 taxa, respectively. The combined *rbcL/atpB-rbcL* matrix comprises 59 taxa. Most of the taxa used in the combined matrix are of the same species. However, *Coprosma*, *Spermacoce*, *Manettia*, *Faramea*, *Coussarea*, *Praravinia*, *Hamelia* and *Gardenia* are represented by two distinct species in the *rbcL* and in the *atpB-rbcL* matrix, respectively. In one analysis we included *rps16* data from Anderson and Rova (1999) and performed a combined analysis. In this 3-data matrix we included 42 genera from the two studies. In several cases we had to compare different species from a genus, but in genera suspected to be paraphyletic, e.g., *Psychotria* and *Hedyotis*, we selected species that are supposed to be close and belong to the same monophyletic group.

The alignments of the *atpB-rbcL* spacer and the *rps16* matrices were first done by the Clustal program (Thompson et al. 1994) followed by manual corrections. Some short regions could not be aligned and were excluded from the analyses. Homologous indel events were coded as presence/absence (1/0). When it was not possible to decide for homology, the event was coded with a question-mark. The aligned matrices are available on request or at <http://www.unige.cjb.ch>. The *rbcL* matrices

were without indels and the alignments were simple and without any alternatives.

The parsimony analyses were done using PAUP 3.1 (Swofford 1993). Only phylogenetically informative characters were included. The search method for the combined matrices was heuristic with 100 replications of RANDOM stepwise additions of sequences, the TBR branch swapping, and MULPARS options in effect. Support of the clades was calculated with bootstrap analyses with 1000 replicates and TBR branch swapping and MULPARS off.

## Results

The *atpB-rbcL* spacer matrix (63 taxa) comprises 970 aligned positions, 453 of which are variable and 255 are potentially informative, 35 of which are indel characters. The *rbcL* matrix (106 taxa) comprises 1 402 sites, 560 of which are variable and 404 are potentially informative. The combined *rbcL/atpB-rbcL* matrix (59 taxa) comprises 2 364 sites, 899 of which are variable and 533 are potentially informative. The combined *rbcL/atpB-rbcL/rps16* matrix (42 taxa) comprises 3 254 aligned positions, 1 269 of which are variable and 737 are potentially informative, of which 57 are indel characters.

**The *atpB-rbcL* spacer analysis (Fig. 1).** The bootstrap analysis of the 63 spacer sequences (52 from Rubioideae and eleven outgroup taxa) resulted in a tree illustrated in a simplified form showing mainly the relationships of the tribes (Fig. 1).

In the *atpB-rbcL* spacer tree, based on 255 phylogenetically informative characters, the mean bootstrap value for the nodes is 55%, and 35% of the nodes have bootstrap values  $\geq 75\%$ .

In agreement with earlier studies (Bremer et al. 1995, Natali et al. 1995, Andersson and Rova 1999) the tribes Hamelieae and Hillieae do not belong in the subfamily Rubioideae but instead in the Cinchonoideae. *Virectaria* (former Virectarieae) is close to *Gardenia* of the Ixoroideae (as in Bremer and Thulin 1998).

The subfamily Rubioideae is well-supported with a bootstrap value of 100%. The base of the

**Table 1.** List of taxa sequenced for *rbcL* and *atpB-rbcL*. Earlier unpublished sequences are indicated with a \* and voucher information is given. All other taxa have been published earlier by the first or second author alone or in collaboration with co-authors (see Material and Methods). Names of herbaria are abbreviated according to Holmgren et al., 1990. All *rps16* sequences analysed in this paper are from Andersson and Rova 1999

	Accession EMBL/ GenBank <i>rbcL</i>	Accession EMBL/ GenBank <i>atpB-rbcL</i>	Source/ voucher information
<i>Agathisanthemum bojeri</i> (Hiern.) Klotzsch	Z68787		
<i>Amaracarpus</i> Blume sp.	AJ002176		
<i>Amphiasma luzuloides</i> (K. Schum.) Bremek.	AJ288594*		Tanzania, Herb. material, Iversen et al. 87694 (UPS)
<i>Amphidasya ambigua</i> (Standley) Standley	Y11844		
<i>Anthospermum</i> <i>herbaceum</i> L.f.	X83623	AJ234028*	Tanzania, Bremer 3093 (UPS)
<i>Appunia guatemalensis</i> Donn. Smith	AJ288593*	AJ234009*	North America, Herb. material, Martinez 13581 (G)
<i>Arcytophyllum aristaum</i> Standley	AJ288595*		Ecuador, Bremer et al. 3371 (UPS)
<i>Argostemma hookeri</i> King	Z68788	AJ234032*	Malaysia, Wanntorp s.n. (S)
<i>Asperula laevigata</i> L.	X81092		
<i>Batopedina pulvinellata</i> E. Robbrecht	AJ288596*		Zaire, Herb. material, Malaisse 7695 (UPS)
<i>Bouvardia glaberrima</i> Engelm.	X83626		
<i>Bouvardia glaberrima</i> Engelm.		X76478	
<i>Carpacoce</i> Sond. sp.	AJ288597*		South Africa, Bremer 3708 (UPS)
<i>Carphalea glaucescens</i> (Hiern.) Verdc.	Z68789		
<i>Chasalia parviflora</i> Benth.	Z68790		
<i>Chazaliella abrupta</i> (Hiern) E. Petit & Verd.	Z68791		
<i>Cinchona pubescens</i> Vahl	X83630		
<i>Cinchona pubescens</i> Vahl		AJ233990*	Jamaica, McDowell. 4613 (DUKE)
<i>Coccocypselum hirsutum</i> (Bartling ex DC.) L.O. Williams	X87145		
<i>Coccocypselum</i> P. Br. sp.		X811678	
<i>Coelospermum balansanum</i> Baill.	AJ288598*	AJ234010*	New Caledonia, Herb. material, Veillon 3765 (G)
<i>Commitheca liebrechtsiana</i> (De Wild & Th. Dur.) Bremek.		AJ233999*	Gabon, Herb. material, Vilks 478 (ME)
<i>Conostomium quadrangulare</i> (Rendle) Cufod.	Z68792		

Table 1. (Continued)

	Accession EMBL/ GenBank <i>rbcL</i>	Accession EMBL/ GenBank <i>atpB-rbcL</i>	Source/ voucher information
<i>Coprosma ernodeoides</i> A. Gray		AJ234029*	Hawaii, Kiehn 910114 (WU)
<i>Coprosma pumila</i> Hook. f.	X87146		
<i>Coptosapelta diffusa</i> (Champ. ex Beneth.) van Steenis		AJ233987*	China, Herb. material, Bartholomew et al. 1188 (ME)
<i>Coussarea contracta</i> Beneth. & Hook. f.		AJ234007*	Paraguay, Zardini and Velasquez 9774 (G)
<i>Coussarea macrophylla</i> Muell. Arg.	Y11847		
<i>Craterispermum</i> <i>brachynematum</i> Hiern	AJ288629*	AJ234011*	Zaire, Herb. material, Lejoly 2707 (ME)
<i>Cruciata glabra</i> (L.) Ehrend.	X81097		
<i>Cruckshanksia hymenodon</i> Hook. & Arn.	AJ288599*		Chile, Herb. material, Rodriguez 10 (K)
<i>Cruckshanksia hymenodon</i> Hook. & Arn.		AJ234004*	Chile, Herb. material, Billiet and Jardin 5570 (ME)
<i>Damnacanthus indicus</i> Gaertn. f.	Z68793	AJ234015*	Cult., Missouri Bot. Gard. Bremer 3107 (UPS)
<i>Danais xanthorrhoea</i> (K. Schum.) Bremek.	Z68794	AJ234019*	Tanzania, Bremer 3079 (UPS)
<i>Declieuxia fruticosa</i> Kuntze	AJ002177		
<i>Didymaea alsinoides</i> (Cham. & Schlecht.) Standley	Z68795		
<i>Didymaea alsinoides</i> (Cham. & Schlecht.) Standley		AJ234036*	Costa Rica, Kiehn sn (WU)
<i>Diodia sarmentosa</i> SW.	AJ288600*		Puerto Rico, Taylor 11749 (MO)
<i>Ernodea litoralis</i> SW.	AJ288601*	AJ234025*	Cuba, Kiehn sn (WU)
<i>Exostema caribaeum</i> (Jacq.) Roem. & Schult.	X83636		
<i>Exostema caribaeum</i> (Jacq.) Roem. & Schult.		AJ233991*	Cuba, Kiehn 910526 (WU)
<i>Faramea multiflora</i> A. Rich.	Z68796		
<i>Faramea porophylla</i> Muell. Arg.		AJ234008*	Paraguay, Herb. material, Zardini 8630 (G)
<i>Gaertnera</i> Retz. sp.		AJ234012*	Madagascar, Herb. material, Malcomber 999 (BR, G, MO, TAN, WAG)
<i>Gaertnera</i> Retz. sp.	Z68797		
<i>Gaillonia yemenensis</i> Thulin	AJ288630*		Yemen, Thulin et al. 9365 (UPS)
<i>Galium album</i> Mill.	X81090		
<i>Galopina circaeoides</i> Thunb.	AJ288602*		South Africa, Bremer 3797 (UPS)

Table 1. (Continued)

	Accession EMBL/ GenBank <i>rbcL</i>	Accession EMBL/ GenBank <i>atpB-rbcL</i>	Source/ voucher information
<i>Gardenia taitensis</i> DC.		AJ233988*	Cult., Geneva Bot. Gard., Natali and Manen 007 (G)
<i>Gardenia thunbergia</i> Linn. f.	X83637		
<i>Gelsemium sempervirens</i> Ait.	L14397	AJ233985*	
<i>Geophila repens</i> (L.) I.M. Johnston	Z68798	AJ234017*	Cult., Uppsala Bot. Gard. Bremer 3130 (UPS)
<i>Gynochthodes coriacea</i> Blume	AJ288603*		Cult., Bogor Bot. Gard., Ridsdale XVII.C.103 (L)
<i>Hamelia cuprea</i> Griseb.	X83641		
<i>Hamelia papillosa</i> Urb.		AJ233992*	Jamaica, McDowell. 4600 (DUKE)
<i>Hedyotis caerulea</i> Wight & Arn.	AJ288604*		Cult., Uppsala. Bot. Gard. Bremer s.n. (UPS)
<i>Hedyotis fruticosa</i> Linn.	Z68799	AJ234026*	
<i>Hedyotis littoralis</i> (Hillebr.) Fosberg "Kadua"	AJ288605*	AJ234027*	Cult., Vienna Bot. Gard., Kiehn & Luegmayer 920823 (WU)
<i>Hedyotis nigricans</i> (Lam.) Fosberg	AJ288606*		USA, Miller et al., 8232 (MO)
<i>Hemidiodia ocimifolia</i> Schum.	AJ288607*		Ecuador, Bremer et al., 3340 (MO, QCA, QCNE, UPS)
<i>Hillia triflora</i> (Oerst.) C.M. Taylor	X83642	AJ233993*	Cult., Univ. of Colorado, Bremer 3101 (UPS)
<i>Hillia valeris</i> Standley	AJ288608*	X81683	Costa Rica, Kiehn 880331 (WU)
<i>Hoffmannia refulgens</i> Hemsl. x <i>ghiesbreghtii</i> Hemsl.	X83644		
<i>Hydnophytum formicarum</i> Jack	X83645		
<i>Hydnophytum formicarum</i> Jack		X76480	
<i>Hymenocoleus hirsutus</i> (Benth.) Robbrecht	AJ002178		
<i>Knoxia platycarpa</i> Arn.	AJ288631*		Sri Lanka, Herb. material, Lundqvist 11302 (UPS)
<i>Kohautia caespitosa</i> Schnizl.	Z68800		
<i>Lasianthus pedunculatus</i> E.A. Bruce	Z68802	AJ234003*	Tanzania, Andreasen 71 (UPS)
<i>Lelya prostrata</i> (Good) W.H. Lewis	AJ288609*		Malawi, Herb. material, Thomson & Rawlins 5482 (K)
<i>Lerchea bracteata</i> Valeton	AJ288610*	AJ233997*	Sumatra, Herb. material, Axelius 343 (S)
<i>Luculia grandifolia</i> Ghose	X83648	AJ233986*	Cult., Stockholm Univ., Bremer 2713 (S)
<i>Manettia bicolor</i> Paxt.	Z68803		

Table 1. (Continued)

	Accession EMBL/ GenBank <i>rbcL</i>	Accession EMBL/ GenBank <i>atpB-rbcL</i>	Source/ voucher information
<i>Manettia cordifolia</i> Mart.		AJ234022*	Argentina, Herb. material, Novara 9814 (G)
<i>Mapouria</i> cf. <i>umbrosa</i> Muell. Arg.	Z68804		
<i>Maschalocorymbus</i> <i>corymbosus</i> (Blume) Bremek.	AJ288611*		Sabah, Ridsdale 2471 (L)
<i>Mitchella repens</i> Linn. <i>Mitchella</i> Linn. sp.	Z68805	AJ234016*	Japan, Ehrendorfer 930905-1601 (WU)
<i>Mitracarpum villosum</i> Cham. & Schlecht.	AJ288632*		Sri Lanka, Herb. material, Fagerlind 810 (S)
<i>Morinda citrifolia</i> Linn.	X83651	AJ234013*	Cult., Uppsala Bot. Gard., Bremer 3106 (UPS)
<i>Mycetia malayana</i> Craib	Z68806	AJ234033*	Cult., Univ. of Aarhus, Larsen et al., 42486 (AAU, UPS)
<i>Myrmecodia platyrea</i> Becc.	X87147		
<i>Nertera granadensis</i> (L.F.) Druce	X83654		
<i>Neurocalyx zeylanicus</i> Hook.	Z68807	AJ233995*	Sri Lanka, Bremer 937 (PDA, S, US)
<i>Oldenlandia</i> cf. <i>corymbosa</i> Linn.	X83655		
<i>Oldenlandia goreensis</i> Summerhayes	Z68808		
<i>Opercularia vaginata</i> Labill.		AJ234030*	Australia, Herb. material, Weber 9157 (G)
<i>Opercularia vaginata</i> Labill.	Z68809		
<i>Ophiorrhiza mungos</i> Linn.	X83656		Cult., Meisse Bot. Gard. Robbrecht s.n. (UPS)
<i>Ophiorrhiza</i> Linn. sp.		X81677	Sumatra, Frimmel s.n. (WU)
<i>Oreopolus glacialis</i> (Poepp. & Endl.) Ricardi	AJ288612*		Argentina, Swenson 328 (UPS)
<i>Otiophora cupheoidea</i> N.E. Br.	AJ288613*		South Africa, Bremer 3805 (UPS)
<i>Otomeria oculata</i> S. Moore	AJ288614*		Ethiopia, Herb. material, Puff 821222-2/1 (K)
<i>Paederia foetida</i> Linn.		AJ234006*	Japan, Ehrendorfer 930830-0801 (WU)
<i>Palicourea</i> Aubl. sp.	Z68810		
<i>Parapentas silvatica</i> (K. Schum.) Bremek.	X83657	AJ234021*	Tanzania, Bremer 3091 (UPS)
<i>Paratriainia xerophila</i> Bremek.	AJ288633*		Madagascar, Herb. material, Croat 30548 (MO)
<i>Pauridiantha paucinervis</i> (Hiern) Bremek.	Z68811	AJ233998*	Tanzania, Bremer 3090 (UPS)

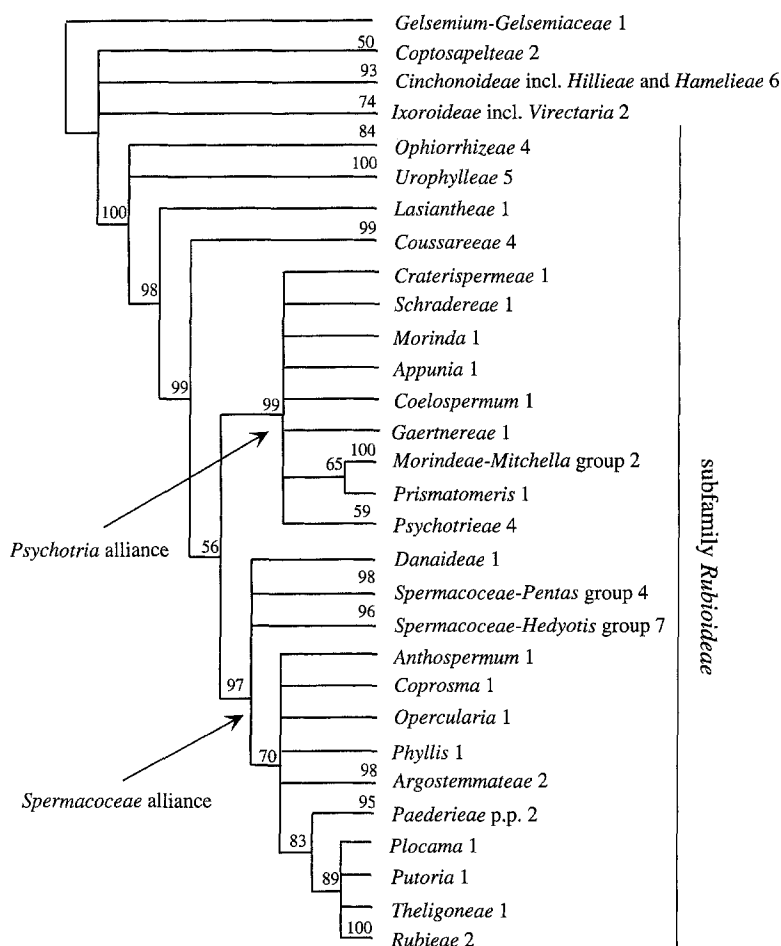
Table 1. (Continued)

	Accession EMBL/ GenBank <i>rbcL</i>	Accession EMBL/ GenBank <i>atpB-rbcL</i>	Source/ voucher information
<i>Pentania longituba</i> Oliver	Z68812		
<i>Pentania microphylla</i> Chior.		AJ234022*	South Africa, Herb. material, Moll 610 (G)
<i>Pentanopsis fragrans</i> Rendle	Z68813		
<i>Pentas lanceolata</i> (Forsk.) Deffl.	X83659		Cult., Univ. of Connecticut, Bremer 2702 (S)
<i>Pentas lanceolata</i> (Forsk.) Deffl.		X76479	Geneva Bot. Gard., Natali and Manen 004 (G)
<i>Pentodon pentandrus</i> Vatke	X83660	AJ234024*	Tanzania, Bremer 3082 (UPS)
<i>Phuopsis stylosa</i> Beneth. & Hook. f.	X81103		
<i>Phyllis nobla</i> Linn.	Z68814		
<i>Phyllis nobla</i> Linn.		AJ234031*	Canary Islands, Herb. material, Charpin and Rodriguez 18576 (G)
<i>Phylohydrax carnosa</i> (Hochst.) C. Puff	AJ288615*		South Africa, Bremer 3783 (UPS)
<i>Placopoda virgata</i> Bolf. f.	Z68815		
<i>Plocama pendula</i> Ait.	Z68816	AJ234035*	Canary Islands, Andreasen 1 (UPS)
<i>Praravinia densiflora</i> Korth.		AJ234000*	Borneo, Clemens 33777 (G)
<i>Praravinia suberosa</i> (Merill) Bremek.	AJ288616*		Sabah, no voucher, Ridsdale
<i>Pravinaria leucocarpa</i> Bremek.	AJ288617*	AJ234001*	Borneo, Herb. material, Collenette 21654 (G)
<i>Prismatomeris labordei</i> (Leveille) Merrill apud Rehder		AJ234005*	China, Herb. material, Bartholomew et al. 2118 (ME)
<i>Prismatomeris beccarianum</i> (Baillon) J.T. Johansson	AJ288618*		Sabah, Herb. material, Ridsdale 2461 (L)
<i>Psychotria kirkii</i> Hiern ( <i>bacteriophila</i> ) Yalet.	X83663		
<i>Psychotria kirkii</i> Hiern ( <i>bacteriophila</i> ) Yalet.		X76481	
<i>Psychotria peteri</i> Verdcourt	Z68817		
<i>Psychotria poeppigiana</i> Muell. Arg.	Z68818	AJ234018*	Ecuador, Bremer et al. 3030 (MO, QCA, QCNE, UPS)
<i>Psychotria</i> Linn. sp. " <i>cephaelis</i> "	AJ002188		
<i>Psyllocarpus</i> <i>laricoides</i> Mart.	AJ288619*		Brasil, Herb. material., Andersson et al. 355750 (UPS)



**Table 1.** (Continued)

	Accession EMBL/ GenBank <i>rbcL</i>	Accession EMBL/ GenBank <i>atpB-rbcL</i>	Source/ voucher information
<i>Putoria calabrica</i> (L.) DC.	AJ288620*		Herb. material, Jonsell 4216 (UPS)
<i>Putoria calabrica</i> (L.) DC.		X81672	Greece, Ehrendorfer 930409-2301 (WU)
<i>Relbunium hypocarpium</i> Hemsl.	AJ288621*		Cult., Meise Bot. Gard., Billiet 3788 (BR)
<i>Richardia pilosa</i> Ruiz. & Pav.	Z68820		
<i>Rubia tinctorum</i> Linn.	X83666		
<i>Rubia tinctorum</i> Linn.		X76465	
<i>Rudgea sessiliflora</i> Standley	AJ002186		
<i>Rutidea orientalis</i> D.M. Bridson	Z68862		
<i>Schenckia blumenaviensis</i> K. Schum.	AJ288622*		Cult., Copenhagen Bot. Gard., Ryding 2359 (C)
<i>Schenckia blumenaviensis</i> K. Schum.		AJ233994*	Brazil, Kiehn sn (WU)
<i>Schradera subandina</i> Krouse	Y11859	AJ234014*	Ecuador, Clark & Watt 783 (MO, QCNE, UPS)
<i>Serissa fetida</i> Lam.	Z68822	AJ234034*	Cult., Univ. of Connecticut, Bremer 2717 (UPS)
<i>Sherardia arvensis</i> Linn.	X81106		
<i>Spermacoce assurgens</i> Ruiz & Pav.		X81679	
<i>Spermacoce hispida</i> Linn.	AJ288623*		Sri Lanka, Herb. material, Wanntorp et al. 2667 (S)
<i>Spermacoce laevis</i> Roxb.	Z68823		
<i>Spermadictyon suaveolens</i> Roxb.	Z68824		
<i>Synaptantha tillaeacea</i> (F. Muell.) J.D. Hook.	AJ288624*		Australia, Herb. material, Lazarides & Palmer 272 (K)
<i>Theligonum cynocrambe</i> Huth		X81680	Greece, Ehrendorfer 930416-6201 (WU)
<i>Theligonum cynocrambe</i> Huth	X83668		
<i>Triainolepis hildebrandtii</i> Vatke	AJ288625*	AJ234020*	Kenya, Herb. material, Bally 13258 (G)
<i>Trichostachys</i> Hook f. in Beneth. & Hook. f. sp.	AJ288626*		Cameroun, Sonké 1725 (UPS)
<i>Urophyllum ellipticum</i> Thw.	AJ288627*	AJ234002*	Herb. material, Lundqvist 11085 (UPS)
<i>Valantia muralis</i> L.	X81107		
<i>Virectaria major</i> K. Schum.	Y11861	AJ233989*	Herb. material, Reekmans 10916 (UPS)
<i>Xanthophytum capitellatum</i> Ridley	AJ288628*	AJ233996*	Sabah, Ridsdale 2473 (L)



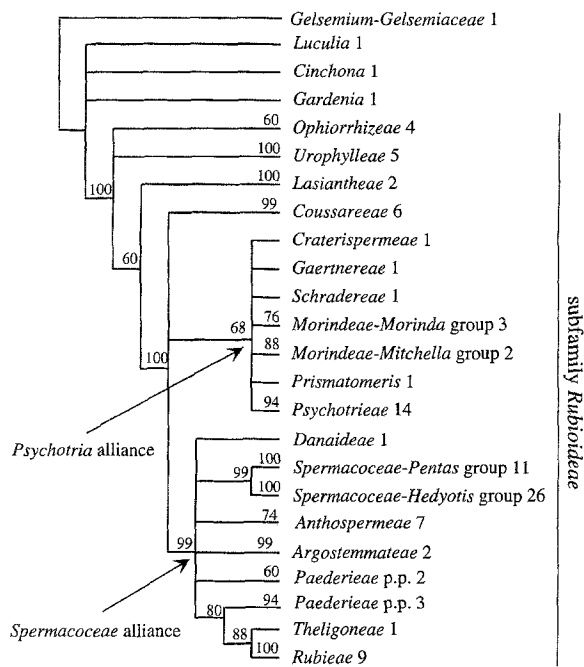
**Fig. 1.** Simplified phylogenetic tree illustrating the relationships of Rubioideae based on *atpB-rbcL* sequences (analysis includes 52 ingroup taxa). Numbers above nodes indicate bootstrap values ( $\geq 50\%$ ) from 1000 replicates with TBR branch swapping, all bootstrap values  $\geq 50\%$  are indicated. Numbers behind names represent number of investigated taxa on this branch

Rubioideae forms a trichotomy. One branch is the Ophiorrhizeae (bootstrap 84%), another is the Urophyllaeae (bootstrap 100%), and the third branch includes the remaining part of the subfamily (bootstrap 98%). In this large clade, *Lasianthus* (Lasiantheae) is the first taxon to branch off followed by the clade Coussareeae (bootstrap 99%, including *Coussarea*, *Faramea*, *Cruckshanksia*, former Hedyotideae/Cruckshanksiae and *Coccocypselum*). The sister group to Coussareeae is not well-supported (56%). However, within this clade there are two well-supported subclades. One of them here called the Psychotrieae alliance (bootstrap 99%) contains Psychotrieae, Craterispermeae, Gaertnereae, Schradereae, and Morindeae (not

supported as monophyletic). The other branch (bootstrap 97%) is here called the Spermaceae alliance and contains Spermaceae (not supported as monophyletic), Anthospermeae (not supported as monophyletic), Argostemmaeae, Danaideae, Paederieae (in this analysis paraphyletic), Rubieae, and Theligoneae.

**The *rbcL* analysis (Fig. 2).** The bootstrap analysis of the 106 *rbcL* sequences resulted in a tree illustrated in a simplified form showing mainly the relationships of the tribes (Fig. 2).

The topology of this *rbcL* tree (of 102 Rubioideae taxa and four outgroup taxa) is similar to the spacer tree (Fig. 1) but the support values are slightly higher. In the *rbcL* tree, based



**Fig. 2.** Simplified phylogenetic tree illustrating the relationships of Rubioideae based on *rbcL* sequences (analysis includes 102 ingroup taxa). Numbers above nodes indicate bootstrap values ( $\geq 50\%$ ) from 1000 replicates with TBR branch swapping, all bootstrap values  $\geq 50\%$  are indicated. Numbers behind names represent number of investigated taxa on this branch

on 404 phylogenetically informative characters, the mean bootstrap value for the nodes is 60%, and 38% of the nodes have bootstrap values  $\geq 75\%$ . All tribes that are supported in the spacer tree are also supported in the *rbcL* tree. Further, in the *rbcL* tree there is also support (74%) for a monophyletic Anthospermeae and a monophyletic Spermacoceae (99%), which are not supported in the spacer tree.

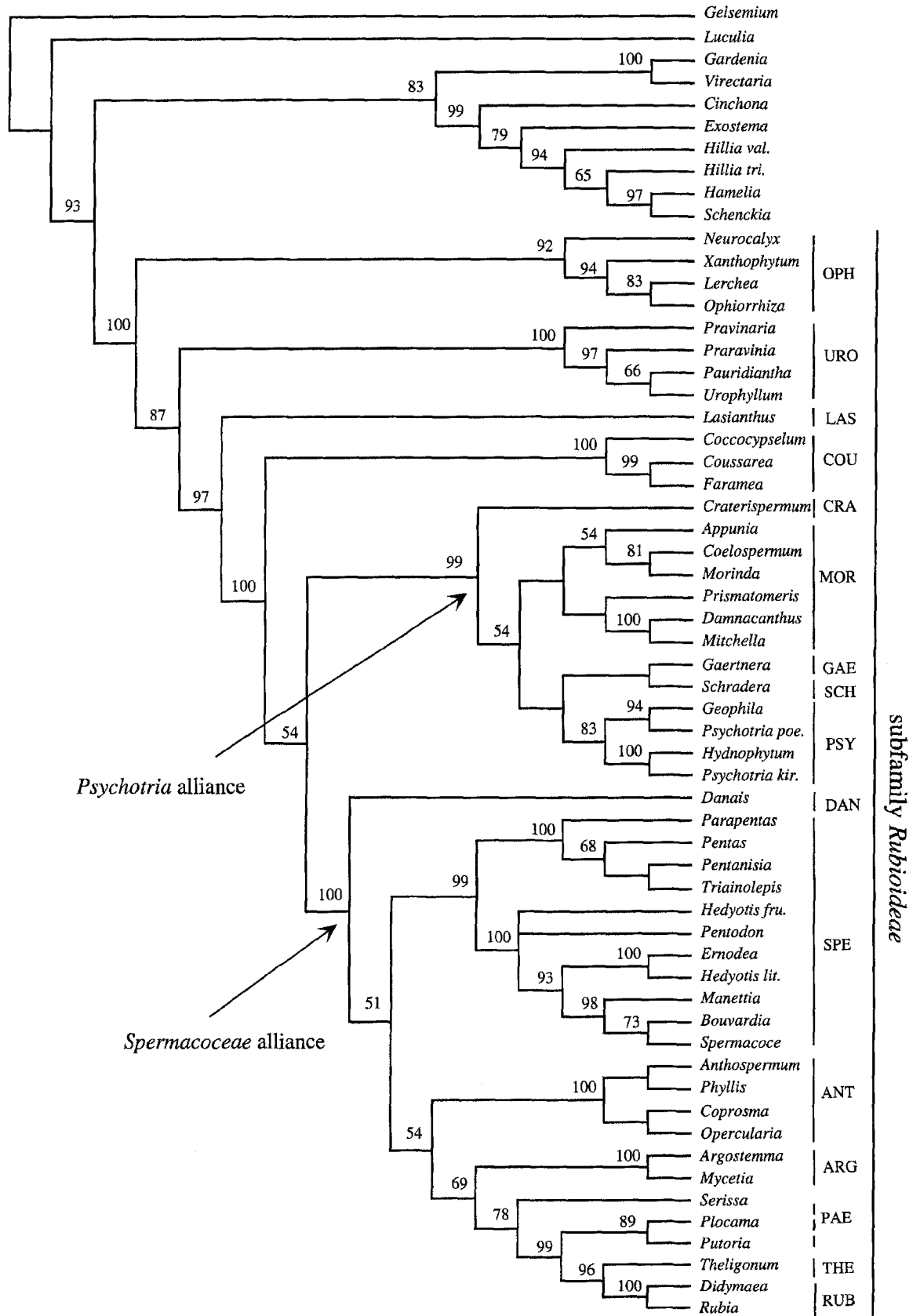
**The combined *rbcL/atpB-rbcL* analysis (Fig. 3).** The analysis of 59 taxa (49 Rubioideae taxa and ten outgroup taxa) from a combined matrix of *rbcL* and the spacer sequences resulted in two trees 1 787 steps long with a consistency index of 0.427 (excluding uninformative characters) and a retention index of 0.713. The strict consensus tree with bootstrap values indicated is presented in Fig. 3.

The analysis of the combined data produced trees very similar to the topology of the

spacer and *rbcL* trees. The main differences are the better resolution within the trees and much higher support values for many branches. In this combined tree, based on 533 phylogenetically informative characters, the mean bootstrap value for the nodes is 81%, and 68% of the nodes have bootstrap values  $\geq 75\%$ .

The Rubioideae clade is supported by 100%. The most basal clade to branch off within the Rubioideae is the Ophiorrhizeae (bootstrap 92%; including *Ophiorrhiza*, *Lerchea*, *Neurocalyx*, and *Xantophytum*), separated (87%) from the following Urophyllaeae (bootstrap 100%; including *Urophyllum*, *Pravinaria*, *Praravinia*, and *Pauridiantha*). High bootstrap values (97%) support the isolation of *Lasianthus* from the basal clades and the remaining taxa (100%). The sister group of *Lasianthus* comprises three main branches. The relationships between these are not supported ( $< 50\%$ ). The three branches are the Coussareae (bootstrap 100%, including here also *Coccocypselum*), the Psychotriaceae alliance (bootstrap 99%), and the Spermacoceae alliance (bootstrap 100%). Inside the Psychotriaceae alliance clade, the tribe Craterispermeae is sister to the remaining part (bootstrap 54%), which comprises Gaertnereae, Morindeae, Schradereae, and Psychotriaceae. The relationships between these tribes are not supported ( $< 50$ ), and of these tribes only two are represented by several taxa of which only tribe Psychotriaceae has high support (83%).

Inside the Spermacoceae alliance clade, the position of Danaideae has low support (bootstrap 51%). The remaining taxa are included in two clades, one is the Spermacoceae (bootstrap 99%) which are clearly divided in two: one subclade (bootstrap 100%) containing the *Pentas* group and former members of Knoxieae and Triainolepideae, and another subclade (bootstrap 100%) containing e.g. the *Spermacoce* and the *Oldenlandia* groups. The other main branch of the Spermacoceae alliance is not well-supported (54%) but includes the relatively well-supported clades of Anthospermeae (bootstrap 100%), Argostemmateae (bootstrap 100%), and Paederieae, Theligoneae



neae, and Rubieae (bootstrap 78%). Paederieae are paraphyletic.

**The combination of our *rbcL/atpB-rbcL* data with *rps16* data (Fig. 4).** The analysis of 42 taxa (38 Rubioideae taxa and four outgroup taxa) from a combined matrix of data from the *rbcL*, the *atpB-rbcL* spacer and the *rps16* intron (Anderson and Rova 1999) resulted in 24 trees 2 096 steps long with a consistency index of 0.503 (excluding uninformative characters) and a retention index of 0.682. The strict consensus tree with bootstrap values indicated is presented in Fig. 4.

The topology of the consensus tree is almost identical or congruent with the *rbcL/atpB-rbcL* tree but the support value for each branch is higher, as could be expected since more characters are included. For example, the separation between the Coussareae and Psychotriaceae and Spermaceae alliances are better supported (70% compared to <50%), and the Anthospermeae, Argostemmateae, Paederieae, Theligoneae, and Rubieae branch has higher support (91% compared to 54%). This 3-data tree, based on 737 phylogenetically informative characters, has a mean bootstrap value for the nodes of 81%, and 72% of the nodes have bootstrap values  $\geq 75\%$ .

Two minor differences between this tree and the combined *rbcL/atpB-rbcL* tree are that the trichotomy of Ophiorrhizeae, Urophyllaeae and the rest of the subfamily is unresolved, and that the relationships within the Psychotriaceae are less resolved, but these relationships are not supported in the *rbcL/atpB-rbcL* (<50%) tree.

**Classification.** As a result of the phylogenetic analyses of molecular data in combination with morphological characters and comprehensive information from literature we here present a revised classification of the subfamily Rubioideae (below and Table 2). We have only listed

those genera that we have sequenced and genera with a close relationship to sequenced genera as judged from morphology.

**Subfamily Rubioideae** Verdcourt, Bull. Jard. Bot. État Brux. 28: 280 (1958)

– Cephaelidoideae Rafinesque, Ann. Gén. Sci. Phys. Brux. 6: 86 (1820)

– Richardioideae Rafinesque, Ann. Gén. Sci. Phys. Brux. 6: 84 (1820)

– Spermacocoideae Chevall., Fl. Gén. Env. Paris 2: 605 (1827) as “Spermacocceae”

– Anthospermoideae Kostel., Allg. Med.-Pharm. Fl. 2: 537 (1833) as “Anthospermeae”

– Hedyotidoideae Kostel., Allg. Med.-Pharm. Fl. 2: 538, 569 (1833) as “Hedyotidae”

– Opercularioideae Kostel., Allg. Med.-Pharm. Fl. 2: 537 (1833) as “Opercularieae”

– Paederioideae Kostel., Allg. Med.-Pharm. Fl. 2: 537, 564 (1833) as “Paederieae”

– Gaertneroideae Arn., Pug. Pl. Ind. Or.: 351, 352 (1836) as “Gaertnerieae”

– Coccocypseloideae Burmeister, Handb. Naturgesch., 286 (1837) as “Coccocypseae”

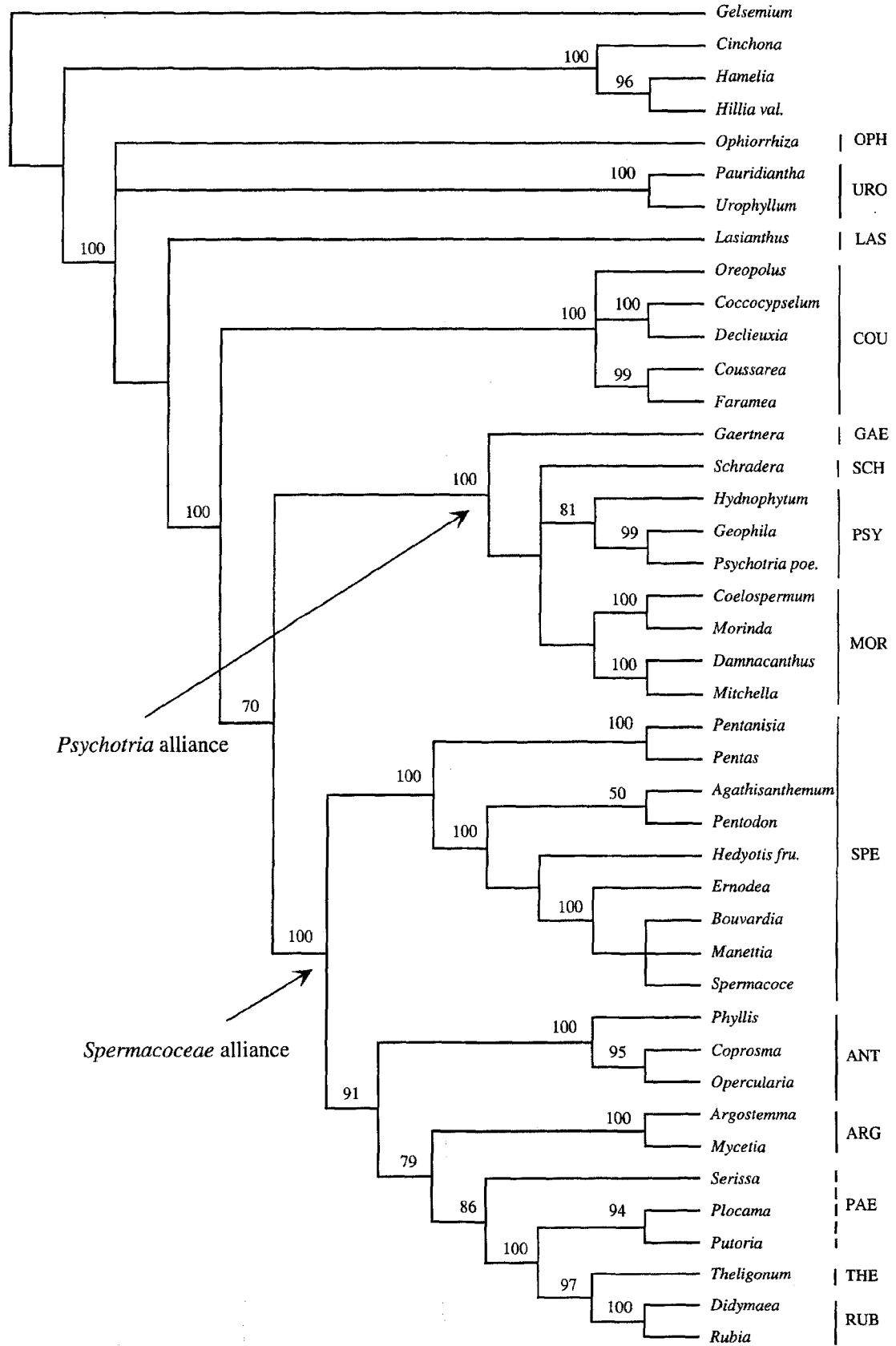
– Manettioideae Burmeister, Handb. Naturgesch., 286 (1837) as “Manettieae”

– Aparinoideae Pfeiff., Nomencl. Bot. 1(1): 320 (1872)

– Pomazotoideae Bremekamp ex S. Darwin, Taxon 25: 605 (1976)

Shrubs, herbs, or less common trees. Raphides present. Heterostylous flowers common. Stipules entire, bifid or often fimbriate. Corolla aestivation always valvate. Ovary 1-12-locular, most often 2-locular either with many or with single ovules in each locule, more rarely two or few in each locule. Fruits dry or fleshy, dehiscent or indehiscent.

**Fig. 3.** Strict consensus tree of two equally parsimonious trees of Rubioideae based on *rbcL* and *atpB-rbcL* sequences, after a heuristic search with 100 replicates and TBR branch swapping. Numbers above nodes indicate bootstrap values ( $\geq 50\%$ ) of 1000 replicates with TBR branch swapping, all bootstrap values  $\geq 50\%$  are indicated. Vertical bars and corresponding letters (first three letters of the tribes cf. Table 2) represent the tribal classification according to Bremer and Manen



Tribes included (here investigated): Anthospermeae, Argostemmateae, Coussareeae, Craterispermeae, Danaideae, Gaertnereae, Lasiantheae, Morindeae, Ophiorrhizeae, Paederieae, Psychotrieae, Rubieae, Schradereae, Spermacoceae, Theligoneae, Urophyllaeae.

Uncertain status (not investigated): Lathraeocarpeae, Perameae.

**Tribe Ophiorrhizeae** Bremekamp ex Verdcourt, Bull. Jard. Bot. État Brux. 28: 281 (1958)

– Pomazoteae Bremekamp ex S. Darwin, Taxon 25: 605 (1976)

Useful recent studies: Darwin (1976), Bremer (1979), Axelius (1987, 1990)

Herbs or subshrubs to small trees. Stipules entire, bifid to fimbriate. Flowers homo- or heterostylous, *Neurocalyx* with adnate anthers. Ovary 2-locular, with many ovules. Fruits dry, dehiscent (flattened capsules in *Ophiorrhiza*) or indehiscent with thin to thick pericarps, with many small (dust) seeds. Pollen 3-colporate. Chromosome basic number  $x = 11$  (12) with 2- or 4-ploidy level (Kiehn 1995).

Genera included (here investigated): *Ophiorrhiza*, *Neurocalyx*, *Lerchea*, *Xanthophytum*.

Inclusion based on morphology: *Coptophyllum* (incl. *Pomazota*), *Spiradiclis*, (and included in *Xanthophytum*: *Paedicalyx* and *Xanthophytopsis*).

**Tribe Urophyllaeae** Bremekamp ex Verdcourt, Bull. Jard. Bot. État Brux. 28: 281 (1958)

– Pauridiantheae Bremekamp ex S. Darwin, Taxon 25: 605 (1976)

Shrubs, subshrubs, or small trees. Stipules entire or fringed. Ovary 2-pluri-locular. Fruits fleshy with many seeds. Flowers unisexual or hermaphroditic, homo- or heterostylous. Pollen 3-colporate. Chromosome basic number  $x = 9$  with 2- or 6-ploidy level (Kiehn 1995).

Genera included (here investigated): *Urophyllum*, *Amphidasya*, *Commitheca*, *Maschalocorymus*, *Praravinia*, *Pravinaria*, *Pauridiantha*.

*Urophyllum* and *Pauridiantha* both have the rare basic number  $x = 9$  and they also have bimodal karyotypes (Kiehn: pers. comm.). In Andersson and Rova (1999) *Raritebe* belongs to the Urophyllaeae clade. *Raritebe* was earlier (Robbrecht 1993) placed in the Isertieae (subfamily Cinchonoideae) and reported not to contain raphides. We have not seen any material of the genus but if the position is correct it is the first report of a genus without raphides in Rubioideae.

**Tribe Lasiantheae** B. Bremer and Manen, trib. nov.

– Dressleriopsidae Dwyer, Ann. Missouri Bot. Gard. 67:11 (1980) - not validly published

Type genus: *Lasianthus*

Frutices, arbores parvae vel suffrutices. Stipulae integrae saepe triangulares. Flores interdum heterostyli. Ovaria 2- vel 4-12-locularia, ovulo in quoque loculo singulari. Fructus drupacei, nec compositi nec aggregati, saepe cyanei ad ateri pyrenis 2 vel 4-12. – Differt a tribus affinibus fructibus cyaneis vel atris, pyrenis 4-12 (*Trichostachys* 2) et inflorescentiis saepe axillaribus sessilibus.

Shrubs, small trees, or subshrubs. Stipules entire, often triangular. Flowers sometimes heteostylous. Ovary 2- (*Trichostachys*) or 4-12-locular, with a single ovule in each locule, erect from the base. Fruits drupaceous, not compound or aggregated, often blue to black, with 2 or 4-12 pyrenes. Chromosome basic number  $x = 11$  with 4- (Bir et al. 1984) or 22-ploidy level (Kiehn pers. comm.).

Genera included (here investigated): *Lasianthus*, *Trichostachys*.

←  
**Fig. 4.** Strict consensus tree of 24 equally parsimonious trees of Rubioideae based on *rbcL*, *atpB-rbcL*, and *rps16* sequences, after a heuristic search with 100 replicates and TBR branch swapping. Numbers above nodes indicate bootstrap values ( $\geq 50\%$ ) of 1 000 replicates with TBR branch swapping, all bootstrap values  $\geq 50\%$  are indicated. Vertical bars and corresponding letters (first three letters of the tribes cf. Table 2) represent the tribal classification according to Bremer and Manen

**Table 2.** Tribes included in or associated with the subfamily Rubioideae (Rubiaceae)

Tribes included in or associated with the Rubioideae by Bremekamp (1952, 1954, 1966), Verdcourt 1958, 1975), or Robbrecht (1988, 1993) and compared to the classification of Bremer and Manen. All investigated and accepted Rubioideae tribes are in boldface. Under the authors, tribal names are indicated with the first three letters of the tribal names; subfamilies are indicated with the first four letters of subfamilial names ANTIrheoideae, CINChonoideae, HILLioideae, POMAzotoideae, UROPhylloideae. "x": the tribe is accepted in the subfamily Rubioideae; "?": uncertain position according to the author; "in": the tribe is included in another tribe

	Bremekamp	Verdcourt	Robbrecht	Bremer and Manen
Tribes of Rubioideae				
<b>Anthospermeae</b>	x	x	x	x
<b>Argostemmateae</b>	x	x	x	x
<b>Coussareeae</b>	x	x	x	new circumscription
<b>Craterispermeae</b>	x	x	ANTI	x
<b>Danaideae</b>				new tribe
<b>Gaertnereae</b>	x	in PSY?	in PSY	x
<b>Lasiantheae</b>				new tribe
<b>Morindeae</b>	x	x	x	x
<b>Ophiorrhizeae</b>	UROP	x	x	new circumscription
<b>Paederieae</b>	x	x	x	paraphyletic?
<b>Psychotrieae</b>	x	x	x	x
<b>Rubicae</b>	x	x	x	x
<b>Schradereae</b>	x	x	x	x
<b>Spermacoceae</b>	x	x	x	x
<b>Theligoneae</b>			x	x
<b>Urophyllaeae</b>	UROP	x	CINC	x
Included in other tribes of Rubioideae				
Coccocypseleae	x	x	x	in COU
Cruckshanksieae	x	x	in HED	in COU
Hedyotideae	x	x	x	in SPE
Knoxieae	x	x	ANTI	in SPE
Manettieae	in HED	in HED	? in HED/CIN	in SPE
Opercularieae	?		in ANT	in ANT
Pauridiantheae	UROP	in URO	CINC	in URO
Pomazoteae	POMA	?	in HED	in OPH
Triainolepideae	x	in PSY	x	in SPE
Included in other Subfamilies				
Hamelieae	x	x	x	CINC
Hillieae	HILL	?x	CINC	CINC
Virectarieae	in OPH	CINC	in HED	IXOR
Incertae sedis				
Lathraeocarpeae	x		x	? no material
Perameae	x		x	? no material

Inclusion based on morphology (cf. Puff and Igersheim 1994); *Metabolus* (incl. *Alla-eophania*).

**Tribe Coussareeae** J. D. Hooker, in G. Bentham and J. D. Hooker, *Genera Plantarum* 2: 9, 24 (1873)



– *Coccocypseleae* Bremekamp, Rec. Trav. Bot. Neerl. 31: 253 (1934)

– *Cruckshanksiae* J. D. Hooker Genera Plantarum 2: 9, 20 (1873)

Useful recent studies: Dwyer (1966), Kirkbride (1976), Taylor (1996a)

Herbs (creeping in *Coccocypselum*), subshrubs, shrubs, or small trees. Stipules entire, cleft, as a ridge with appendages, or deeply bilobed. Flowers homo- or heterostylous, often 4-merous, white, blue, or bright yellow (*Cruckshanksia*, *Oreopolus*). Ovary 1-2-locular, with 1-2 or many (*Coccocypselum*) ovules per locule. Fruits often flattened (not *Coccocypselum*), fleshy, white (*Coussarea*) or often blue (*Faramea*, *Coccocypselum*) berries, schizocarps (*Declieuxia*), or thin-walled capsules (*Cruckshanksia*, *Oreopolus*), with 1–2 or many seeds, often flattened. Pollen 3-colporate or 2-4-porate. Chromosome basic number  $x = 10, 11?$  with 2- or 4-ploidy level (Kiehn 1995).

Genera included (here investigated): *Coussarea*, *Coccocypselum*, *Declieuxia*, *Cruckshanksia*, *Faramea*, *Oreopolus*.

In Andersson and Rova (1999) *Hindsia* and *Heterophyllaea* are nested within our *Coussareae*. We have not had the possibility to sequence these genera, but *Hindsia* shows at least macromorphological similarity to *Declieuxia*. If this position is correct the fruit morphology of the tribe is even more variable as the fruits of *Hindsia* are capsular with hard walls and winged seeds.

**Tribe Craterispermeae** Verdcourt, Bull. Jard. Bot. État Brux. 28: 281 (1958)

Useful recent study: Igersheim (1992)

Shrubs or trees. Stipules entire. Inflorescences axillary and condensed and borne on a stout short peduncle. Flowers heterostylous. Ovary 2-locular, with a single pendulous ovule in each cell (one aborted?). Fruits fleshy (thickness of endocarp varies) with one seed. Pollen 3-colporate. Chromosome basic number  $x = 11$  with 2-ploidy level (Kiehn 1995).

Genus included (here investigated): *Craterispermum*.

**Tribe Gaertnereae** Bremekamp ex S. Darwin, Taxon 25: 601 (1976)

Useful recent studies: Igersheim et al. (1994), Jansen et al. (1996)

Shrubs or trees. Stipules entire and united into a long cylindrical sheath, often with setae. Flowers often small and white. Ovary superior, 2-locular, with single erect ovule in each locule. Fruits fleshy, with one-seeded pyrenes. Pollen 2-3-colporate.

Genus included (here investigated): *Gaertnera*.

Inclusion based on morphology (cf. Igersheim et al. 1994, and Jansen et al. 1996): *Pagamea*.

**Tribe Morindeae** Miquel, Flora van Nederlandsch Indie 2: 239, 241 (1857)

Useful recent study: Igersheim and Robbrecht (1993)

– Subtribe Morindinae de Candolle, Prodr. Systematis Naturalis 4: 342, 446 (1830)

Useful recent studies: Johansson (1988, 1994)

Shrubs, small trees, or lianas. Stipules entire or dentate, usually connate to sheathing. Flowers usually not heterostylous. Ovary 2-locular, with 2 erect ovules in each locule, later subdivided by secondary septum. Fruits drupaceous, often aggregated. Pollen 3-4-colporate. Chromosome basic number  $x = 11$  with 2-, 4-, 8-, or 20-ploidy level (Kiehn 1995).

Genera included (here investigated): *Morinda*, *Appunia*, *Coelospermum*, *Gynochthodes*.

Inclusion based on morphology: *Pogonolobus*.

– Subtribe Prismatomerinae Ruang, Acta Phytotaxon. Sin. 26: 446 (1988)

Useful recent studies: Johansson (1987A, 1987B, 1989), Robbrecht et al. (1991)

Shrubs or trees. Stipules entire or often bilobed. Flower often heterostylous. Ovary 2-locular, with a single ovule in each locule. Fruits fleshy berries, often dark purple or blue, free or fused. Pollen 3-5-colporate.

Genus included (here investigated): *Prismatomeris*.

Inclusion based on morphology: *Renellia* (incl. *Didymoecium*), *Motleyia*.

– The *Mitchella* group

Useful recent study: Robbrecht et al. (1991)

Thorny shrubs or creeping herbs. Stipules entire or rarely bilobed. Flowers homo- (*Damnacanthus*) or hetero-stylous (*Mitchella*). Ovary 2-locular, with one pendulous ovule in each locule. Fruits fleshy, free or fused in pairs. Pollen 3-6-colporate. Chromosome basic number  $x=11$  with 2-ploidy level (Robbrecht, et al. 1991).

Genera included (here investigated): *Mitchella*, *Damnacanthus*.

**Tribe Psychotrieae** Cham. and Schldl., Linnaea 4: 4. 1829

– Psathureae A. Rich. ex Dumort., Anal. Fam. Pl.: 32. 1829

Useful recent treatment: Taylor (1996b), Nepokroeff et al. (1999)

Shrubs, trees or herbs, or epiphytes. Stipules divided or rarely entire. Flowers often small, white and heterostylous. Ovary often 2-locular, with single erect ovule in each locule. Fruits fleshy, with one-seeded pyrenes. Seeds often with horny endosperm. Pollen very variable, 0-5-aperturate. Chromosome basic number  $x=(10), 11$  with 2-12-ploidy level (Kiehn 1995).

Genera included (here investigated, an \* indicates a paraphyletic taxon): *Psychotria*\*, *Amaracarpus*, *Cephaelis*, *Chasallia*, *Chazaliella*, *Geophila*, *Hydnophytum*, *Hymenocoleus*, *Myrmecodia*, *Palicourea*\*, *Rudgea*, *Uragoga*.

Inclusion based on morphology: *Anthorrhiza*, *Myrmephytum*, *Squamellaria*.

This tribe usually includes many genera (e.g. Robbrecht 1988) and *Psychotria* has been shown to be highly paraphyletic and many of the described genera are nested within it. Major revision of the whole tribe with new circumscriptions of genera will probably follow from initiated phylogenetic analysis (e.g. Nepokroeff pers. comm). Andersson and Rova (1999) have shown that *Readea*, *Strebl-osa* and *Margaretopsis* belong to this group of taxa but we have not sequenced these genera and it is not obvious from the morphology that they belong here.

**Tribe Schradereae** Bremekamp, Rec. Trav. Bot. Neerl. 31: 253 (1934)

Useful recent studies: Puff et al. (1993), Puff and Buchner (1998), Puff et al. (1998a), Puff et al. (1998b)

Shrub or often epiphytic climbers with adhesive roots. Stipules entire. Ovary 2-(3-4)-locular. Flowers in congested spherical inflorescences, heterostyly has been reported. Fruits fleshy, baccate, with many seeds. Chromosome basic number  $x=11$  with 2-ploidy level (Kiehn 1995). Pollen 2-3-(4)-porate.

Genera included (here investigated): *Schrad-dera*.

Inclusion based on morphology (cf. Puff et al. 1993): *Lecananthus*, *Leucocodon*.

**Tribe Danaideae** B. Bremer and Manen, trib. nov.

Type genus: *Danais*

Useful recent studies: Buchner and Puff (1993), Puff and Buchner (1994)

Lianae lignosae, frutices interdum scandentes vel arbores parvae. Stipulae integrae, bifidae vel fimbriatae. Flores heterostyli. Corollae valvatae vel reduplicatae-valvatae. Ovaria 2-locularia in quoque loculo ovulis numerosis. Fructus capsulares loculicidi vel septicidales seminibus alatis. Pollen 3-4 (-5)-colporatum. – Differt a tribuluis ceteris habitibus plerumque scandentibus lignosis et seminibus alatis (itidem in *Bouvadria* et *Manettia*; Spermaceae).

Woody lianas, climbing shrubs, shrubs, or small trees. Flowers heterostylous. Stipules entire, bifid or fimbriate. Corolla valvate or valvate-reduplicate (*Danais* and *Schismatoclada*). Ovary 2-locular with numerous ovules in each locule. Fruits capsular loculicidal (*Danais* and *Payera*) or septicidal (*Schismatoclada*), with winged seeds. Pollen 3-4(-5)-colporate.

Genus included (here investigated): *Danais*.

Inclusion based on morphology: (cf. Buchner and Puff 1993) *Schismatoclada* (inclusion supported by unpublished *rbcL* data: Bremer), *Payera*.

**Tribe Spermaceae** Bercht. and J. Presl, Prir. Rostlin: 256 (1820) – the change of the authors from earlier A. Rich. ex Dumort., Analyse des familles des plantes: 33 (1829) was first noted by J. Reveal (pers. comm.)

– Hedyotideae Chamisso and Schlechtendal ex de Candolle, Prodromus Systematis Naturalis 4: 342, 401(1830)

– Knoxieae Hooker f. in Bentham and Hooker, Genera plantarum 2: 9, 21 (1873)

– Manettieae Bremekamp, Rec. Trav. Bot. Neerl. 31: 253 (1934)

– Triainolepidae Bremekamp, Proc. Kon. Akad. Wetensch. 59: 3 (1956)

Useful recent studies: Puff (1988), Puff and Robbrecht (1989), Mena (1990)

Herbs or subshrubs (a few species are small trees). Stipules fimbriate. Flowers quite often heterostylous. Ovary 1-5- or often 2-locular, with many to single ovules in each locule. Fruits dry, dehiscent or indehiscent, or rarely fleshy (e.g. *Triainolepis*) with many to single seeds. Pollen often 3-colporate-pluricolpate. Chromosome basic number very variable  $x=6-17$  with 2-20-ploidy levels (Kiehn 1995).

Genera included (here investigated, an \* indicates a paraphyletic taxon): *Spermaceae\** (incl. *Borreria*), *Agathisanthemum*, *Amphiasma*, *Arcytophyllum*, *Batopedina*, *Bouvardia*, *Carphealea* (incl. *Dirichletia*), *Conostomium*, *Diodia*, *Ernodea*, *Hedyotis\** (incl.

*Kadua*), *Hemidiodia*, *Knoxia*, *Kohautia*, *Lelya*, *Manettia*, *Mitracarpus*, *Oldenlandia\**, *Otiophora*, *Otomeria*, *Parapentas*, *Paratriaina*, *Pentanisia*, *Pentanopsis*, *Pentas*, *Pentodon*, *Phylohydrax*, *Placopoda*, *Psyllocarpus*, *Richardia*, *Triainolepis*.

A majority of the former Hedyotideae, Knoxieae, and Spermaceae s.s. genera should be included in this tribe. Inclusion based on morphology is supported for at least the following genera: *Chaemepentas*, *Crusea*, *Dentella*, *Dibrachionostylus*, *Dolichometra*, *Hedythyrus*, *Hydrophylax*, *Lucya*, *Manostachya*, *Mitrasacmopsis*, *Neohymenopogon*, *Nodocarpaea*, *Pseudohedyotis*, *Schwendera*, *Synaptantha*, *Staelia*, *Thecorchus*, *Thyridocalyx*.

**Tribe Anthospermeae** Chamisso and Schlechtendal ex de Candolle, Prodromus Systematis Naturalis 4: 343, 578 (1830)

– Opercularieae A. Rich. ex de Candolle, Prodromus Systematis Naturalis 4: 343, 614 (1830)

– Durringtonieae Henderson and Guymer, Kew Bull. 40: 97–107 (1985)

Useful recent studies: Puff (1982, 1986), Robbrecht (1982)

Herbs, shrubs, dwarf shrubs, or small trees. Stipules entire or divided. Flowers usually unisexual, anemophilous, not heterostylous. Stamens usually inserted low in the corolla. Stigma long and filiform. Ovary 1-2(-5)-locular, with a single erect ovule in each locule. Fruits dry and splitting into cocci or capsular, or fleshy. Chromosome basic number  $x=11$  (one exception of 10) with 2-20-ploidy levels (Kiehn 1995). Pollen 3-colporate.

Genera included (here investigated): *Anthospermum*, *Carpacoce*, *Coprosma*, *Galopina*, *Nenax*, *Nertera*, *Opercularia*, *Phyllis*.

Inclusion based on morphology: *Durringtonia*, *Eleutheranthus*, *Leptostigma* (incl. *Corynula*), *Normandia*, *Peratanthe*, *Pomax* (inclusion of *Pomax* supported by unpublished *rbcL* data Bremer).

**Tribe Argostemmateae** Bremekamp ex Verdcourt, Bull. Jard. Bot. État Brux. 28: 281 (1958)

Useful recent study: Bremer (1989)

Herbs with iso- or anisophyllous leaves. Stipules entire or slightly cleft. Flowers hermaphroditic. Stamens usually inserted at base of the corolla, adnate into an anther cone or free. Anthers open with vertical slits or rarely with pores. Ovary 2-6-locular. Fruit a succulent capsule opening by an apical operculum (*Argostemma*) or a berry (*Mycetia*), with many small seeds. Chromosome basic number  $x = 11$ , 14 with 2- or 4-ploidy levels (Kiehn 1995).

Genera included (here investigated): *Argostemma*, *Mycetia*.

**Tribe Paederieae** de Candolle, Prodr. Systematis Naturalis 4: 342, 470 (1830)

– Putorieae de Candolle ex Sweet, Sweet's Hortus Britannicus, ed 3, 325 (1839)

Useful recent studies: Puff (1982), Thulin (1997)

Shrubs, climbers, dwarf shrubs and herbs, many with a foetid smell. Stipules divided or entire. Flowers often heterostylous, Stamens inserted in upper part of corolla, Ovary 2-5-locular, with a single erect ovule in each locule. Fruits dry, dehiscent into mericarps or opening with operculum, or fleshy, indehiscent. Chromosome basic number  $x = 11$  (rarely 10, 12, 13) with 2-, 4-, 6- or 8-ploidy levels (Kiehn 1995). Pollen 3-colpate.

Genera included (here investigated): *Paederia*, *Gaillonia*, *Plocama*, *Putoria*, *Serissa*, *Spermadictyon*.

Inclusion based on morphology: *Chouletia*, *Jaubertia*, *Kelloggia*, *Leptodermis*, *Pseudogaillonia*, *Pseudopyxis*, *Pterogaillonia*.

**Tribe Theligoneae** Wunderlich ex S. Darwin, Taxon 25: 607 (1976)

Useful recent study: Wunderlich (1971)

Herbs with upper leaves alternate, anisophyllous. Stipules sheathing. Flowers unisexual, anemophilous. Male flowers usually with many, up to 30 stamens. Ovary 1-locular, with

one ovule. Fruits fleshy, with elaiosome and a single seed. Pollen (3-)4-8-porate. Chromosome basic number  $x = 10, 11$  with 2-ploidy level (Kiehn 1995).

Genus included (here investigated): *Theligonum*.

**Tribe Rubieae** Baill., Hist. Pl. 7: 365, 390 (1880)

– Galieae A. Rich. ex Dumort., Anal. Fam. Pl.: 33. (1829)

– Asperuleae A. Rich., Mém. Soc. Hist. Nat. Paris 5: 126 (1830)

Useful recent studies: Manen et al. (1994), Natali et al. (1996)

Herbs or rarely shrubs, with polygonal stems. Leaves (and or leaf-like stipules) verticillate. Flowers generally perfect and heterostylous, *Phuopsis* with secondary pollen presentation. Calyx rudimentary. Ovary 2-locular, with single ovule in each locule. Fruits dry or fleshy, didymous, seeds usually adhering to the pericarp. Pollen pluricolpate. Chromosome basic number  $x = 9-12$  with 2-12-ploidy levels.

Genera included (here investigated, an \* indicates a paraphyletic taxon): *Rubia*, *Asperula\**, *Galium\**, *Relbunium*, *Didymaea*, *Cruciata*, *Phuopsis*, *Sherardia*, *Valantia*.

Inclusion based on morphology: *Callipeltis*, *Crucianella*, *Mericarpacea* (inclusion supported by unpublished *atpB-rbcL* data: Manen), *Microphysa*, *Warburgia*.

## Discussion

The power of the present study is that the phylogeny and proposed classification is based on many characters from different molecular markers. One is protein-coding (*rbcL*), one is a non-coding spacer (the *atpB-rbcL* spacer), and the third is a non-coding intron marker (*rps16* from Andersson and Rova 1999).

Another strength of our study is the comprehensive tribal sampling, e.g., we include, for the first time, representatives of the tribe Craterispermeae and the former tribes Pomazoteae and Triainolepideae. Our analysed taxa represent all except two of the 28

Rubiaceae tribes that have been associated with the subfamily Rubioideae. We accept 16 in Rubioideae (Table 2), nine are included as synonyms, three are included in other subfamilies and two have been left "incertae sedis" as we did not manage to get any material. If compared to earlier classifications of the subfamily (Bremekamp 1954, 1966; Verdcourt 1958; Robbrecht 1988, 1993) it seems that Verdcourt's scheme is best supported by our data and our classification is in many parts similar to his. Of our 16 tribes 12 more or less correspond to earlier tribal circumscriptions, two tribes have received new very different circumscriptions, and two are new to science.

The topologies of the trees are almost identical or congruent in our four analyses. The resolution and support are highest in the combined analyses, which is expected as more characters are included (cf. Bremer et al. 1999). In all analyses (Figs. 1–4) the same seven tribes are monophyletic: Argostemmaeae, Coussareae, Ophiorrhizeae (3-data matrix includes only one taxon), Psychotriaceae, Rubiaceae, Spermaceae, and Urophyllaeae. Furthermore, Anthospermeae have good support in the *rbcL* (Fig. 2) and combined analyses (Fig. 3) but not in the *atpB-rbcL* data (Fig. 1). Lasiantheae are represented by two taxa in the *rbcL* analysis (but only one in the other analyses) and are highly supported (100%; Fig. 2). Five tribes are represented by single taxa or are monogeneric and thus the monophyly can not be tested: Craterispermeae, Danaideae, Gaertnereae, Schradereae, and Theligoneae. Morindeae are monophyletic in the combined analyses (but not supported <50%; Figs. 3, 4). Finally, the tribe Paederieae is not supported in our data, but instead paraphyletic. However, at this moment, we have taken a conservative approach and maintain Paederieae as a tribe while awaiting a more detailed study (under revision by M. Backlund pers. comm.).

Our results are also in many parts similar to those from the recent study by Andersson and Rova (1999) of *rps16* data, but, there are important differences. Notably, the results

from our combined analysis including their *rps16* data are different from their results. However, in our analysis the *rps16* data are a minor part of the matrix. Other explanations might be that we use different alignments, or different ways of analysis. They never completed their analysis as they ran out of computer memory when 14 600 trees were found. Had it been possible to save all equally parsimonious trees, their consensus could be much more collapsed, so it is difficult to judge to what extent the *rps16* data alone are phylogenetically informative. Clades not supported by bootstrap values well over 50% (preferably 75%) in the trees presented by Andersson and Rova should not be considered reliable. Two examples concern the circumscription and position of Spermaceae and Theligoneae, respectively.

Andersson and Rova (1999) found what we call tribe Spermaceae paraphyletic. The incongruence between our studies must be of the "soft incongruency" type (Seelanaen et al. 1997). Our tribe Spermaceae is monophyletic and supported in most of our analyses (in *atpB-rbcL* not supported but congruent, *rbcL* 99%, *rbcL/atpB-rbcL* 99%, *rbcL/atpB-rbcL/rps16* 100%). Andersson and Rova illustrate (in one out of 14 600 trees) the Knoxieae (in our data included in the Spermaceae) as the sister group to the rest of the Spermaceae alliance. However, this relationship is not supported by their data (<50%). Andersson and Rova also found Theligoneae sister to *Plocama*, a member of the Paederieae (56%). In our analyses the sister group relationship of Theligoneae is not *Plocama* but with the Rubiaceae, strongly supported by our data (*rbcL* 88%, *rbcL/atpB-rbcL* 96%, *rbcL/atpB-rbcL/rps16* 97%, not supported by the *atpB-rbcL*). This difference is of the "hard incongruency" type (Seelanaen et al. 1997).

The following discussion of phylogeny and classification is based on the tree from the combined analysis of the *rbcL/atpB-rbcL* data (Fig. 3), unless otherwise stated, since this analysis has the best taxon sampling relevant for the tribal phylogeny.

**The basal clades.** The first dichotomy of the ingroup (Fig. 3) in the tree is between a newly circumscribed Ophiorrhizeae and the rest of the subfamily. In agreement with Bremer (1996a), the genus *Neurocalyx* (formerly Argostemmatae cf. Bremer 1987) belongs to this clade and for the first time we here show that the genera *Lerchea* and *Xanthophytum* formerly in Pomazoteae (Bremekamp 1966) or Hedyotideae (Robbrecht 1993) also are closely related. The tribe is supported by a high bootstrap value (92%) and is distinctly separated (87%) from the rest of the subfamily. One uniting morphological character for the taxa of the Ophiorrhizeae is the occurrence of very small seeds (dust seeds). Andersson and Rova (1999) found *Ophiorrhiza* to be sister to Urophylleae and mention that the subfamily Urophylloideae (incl. *Ophiorrhiza*) "is defensible, being the sister group to the rest of the Rubioideae, but it may not be a useful entity". We certainly agree with the latter statement but disagree with their recognition of the subfamily Urophylloideae. Our data do not support inclusion of *Ophiorrhiza* in the same clade as the Urophylleae. The differences in results might be due to taxon sampling and analysis methods. We included more representatives of Ophiorrhizeae (4 genera compared to 1) and Urophylleae (5 genera compared to 3) and our analyses run to completion. Morphologically Ophiorrhizeae differ from Urophylleae by type of fruits and habit, Urophylleae are woody with fleshy berries while Ophiorrhizeae never have berries and are often herbaceous.

The next branch to split off (100% bootstrap value) is the tribe Urophylleae, with *Pauridiantha* nested within the tribe (as in Andersson and Rova 1999). Inclusion of *Pauridiantha* within Urophylleae was first proposed by Verdcourt (1958) in contrast to Bremekamp (1954, 1966) and Robbrecht (1988, 1993), who recognised Pauridiantheae as a separate tribe next to the Urophylleae. These two tribes were included in the separate subfamily Urophylloideae by Bremekamp. Robbrecht did not accept this subfamily and

he moved Urophylleae and Pauridiantheae to subfamily Cinchonoideae. Verdcourt was the first author to include the Urophylleae in the Rubioideae which is in concordance with the present study. The Urophylleae are characterised by berries with many seeds.

*Lasianthus* is the next branch to split off. It is distinctly separated from the Urophylleae part of the tree (97%) and the rest of the subfamily (100%). The genus was earlier included in Psychotrieae (by most earlier authors) or in Morindeae (Robbrecht 1988, 1993). A position within the Psychotrieae alliance has earlier been refuted by Bremer (1996a). As *Lasianthus* in our study is the sister to the rest of the subfamily (confirmed by an unpublished *rbcL* sequence of *Lasianthus strigosus*, Bremer) and unless all the remaining Rubioideae taxa should be lumped into one tribe, *Lasianthus* must form a new tribe. Further, the genus *Trichostachys* belongs to this distinct branch (*rbcL* analysis). The Lasiantheae are characterised by fleshy drupes with 2 to 12 pyrenes, often blue or black. The position of Lasiantheae is supported by the 3-data matrix and by the results of Andersson and Rova (1999). In their analysis the *Lasianthus* branch is sister to *Perama* of the tribe Perameae (supported by 100%). If this relationship is correct one could argue that *Lasianthus* should be included in the tribe Perameae instead of being elevated to a new tribe Lasiantheae. However, we agree with Andersson and Rova (1999) that such a taxon would be morphologically undefinable. *Perama* is a genus of tiny, hairy plants, with very small or reduced stipules, flowers in terminal heads, calyx of two lobes, and dry 3-locular capsular fruits with a single ovule in each locule. The single ovule is the only similarity to Lasiantheae, but solitary ovules occur and have evolved several times in the subfamily.

The next node includes the Coussareeae (100%) as sister to the Psychotrieae alliance (99%) and the Spermaceae alliance (100%), a relationship with low support (54%). For the Coussareeae a new, highly unexpected, relationship was shown between *Faramea* (Cous-

sareeae) and *Coccocypselum* (former Coccocypseleae) by Bremer (1996a, *Coussarea* was also added to this clade in Bremer and Thulin 1998). Further, Nepokroeff et al. (1999) and Andersson and Rova (1999) showed that the genus *Declieuxia*, formerly in Psychotriaceae also belongs to this group. Another interesting result from the present study (and Andersson and Rova 1999) is the position of *Cruckshanksia* (*rbcL* analysis) and *Oreopolus* (*rbcL* and *atpB-rbcL* analyses) also placed in this same clade. These last two genera have been accepted as a separate tribe Cruckshanksieae or they have been included in Hedyotideae (cf. Table 2). We have decided to include all these six genera in the tribe Coussareeae despite morphological variation (large parts of the variation is found within the "Coussareeae s.s." and the "Coccocypseleae" branches, respectively). There are morphological traits that support their relationship, e.g., often 4-merous flowers, flattened berry-like or thin-walled capsular fruits, and flattened seeds. Earlier Coussareeae were supposed to have drupaceous fruits which has been refuted (C. Taylor pers. comm.). Interestingly all Coussareeae genera are American and most of them tropical. The African genus *Schizocolea* was earlier included in Coussareeae but unpublished *rbcL* data (Bremer) contradict that position.

**The Psychotriaceae alliance** is well-supported with a high bootstrap value (99%; Fig. 3). This group comprises many species (probably ca 2000) which can be classified in five tribes: Psychotriaceae, Craterispermeae, Gaertnereae, Morindeae, and Schradereae. They are characterised by fleshy drupes with one ovule per carpel (one or two in Morindeae). Many are important as food sources for frugivorous birds in the tropics (cf. Snow 1981). The relationships and delimitations of the *Psychotria* alliance are still unclear and need further investigations. The largest tribe is the Psychotriaceae s.s. which is distinctly separated from the other taxa (83% bootstrap values). Molecular data (Bremer 1996a) have earlier shown that the type genus of the tribe, *Psychotria*, is

paraphyletic. It has recently been demonstrated by Nepokroeff et al. (1999) that *Psychotria* together with the other genera of the tribe nicely can be split into minor monophyletic groups, e.g., one includes *Psychotria* s.s. (subgenera *Psychotria* and *Tetramerae* and a Pacific group including e.g. the myrmecophilous genera *Anthorrhiza*, *Hydnophytum*, *Myrmecodia*, and *Myrmephytum*), another group includes the subgenus *Heteropsychotria* and the genus *Palicourea*. Also other genera such as *Chasallia*, *Chazaliella*, *Geophila*, *Rudgea*, and *Hymenocoleus* belong to this tribe.

Since the relationships and circumscriptions of the other tribes of the *Psychotria* alliance group are uncertain it is premature to propose a classification. However, from our results it is evident that the tribes Craterispermeae, Gaertnereae, Schradereae, and Morindeae all belong to this group and that they are distinctly separated from Psychotriaceae. Further, all these tribes have been described before, are accepted by several authors, and are morphologically distinct. We find it more informative to accept them as tribes than sink them in Psychotriaceae. Craterispermeae were placed close to Psychotriaceae by Verdcourt (1958) but Robbrecht removed them to subfamily Antirheoideae, a position not supported by our data. Further, from the present study as well as from Nepokroeff et al. (1999) and Andersson and Rova (1999) there are no indications that the genus *Gaertnera* should be included in the tribe Psychotriaceae as was suggested e.g. by Verdcourt (1958, although with some hesitation) and Robbrecht (1988, 1993). The Schradereae have recently been expanded to include also *Lecananthus*, *Leucocodon* (Puff and Buchner 1998, Puff et al. 1998, Puff et al. 1998) and it seems correct that they form a distinct monophyletic group. A monophyletic tribe Morindeae is not contradicted by our data but there is no support for the tribe either (<50%). The delimitation of and relationships within the Morindeae have been subject to recent discussions (e.g. Igersheim and Robbrecht 1993). In our study we find the Morindeae representatives in three

groups, corresponding to subtribe Morindinae, subtribe Prismatomerinae, and the *Mitchella* group (Robbrecht 1993). The relationship between *Mitchella* and *Damnacanthus* of the *Mitchella* group was first pointed out by Robbrecht et al. (1991) and was also highly supported in Bremer (1996a). Andersson and Rova (1999) included four of the tribes in the Psychotriaceae alliance but no representative of Craterispermeae. Their sample tree is fully resolved. Psychotriaceae and Gaertnereae are shown to be well-supported, but the relationships between the Gaertnereae, Schradereae, and Morindeae have only low support (59%, 55%). The monophyly of Morindeae has good support (80%), but Andersson and Rova did not include any representative of the subtribe Prismatomerinae. In our 3-data analysis the support for the Morindeae (including Prismatomeridae is very low (48%).

**The Spermaceae alliance** includes the rest of the subfamily Rubioideae. It is a strongly supported clade (100%; Fig. 3) and represents a majority of the dry-fruited taxa of the subfamily. In this group we accept seven tribes and of these six (Spermaceae, Anthospermeae, Argostemmateae, Danaideae, Rubieae, Theligoneae) are distinctly separated from each other with bootstrap values ranging from 99% to 100%. One tribe, the Paederieae, is found to be biphyletic. As the tribe is under revision (M. Backlund pers. comm.) we refrain from doing any taxonomic changes at the moment.

At the most basal dichotomy in this Spermaceae alliance we find the genus *Danais*. It is one of the genera with winged seeds that Bremekamp (1952) transferred from the Cinchoneae to the Hedyotideae (here synonym to Spermaceae), a position that was accepted by Verdcourt (1958). Robbrecht (1988, 1993) on the other hand considered it a link between Rubioideae and Cinchonoideae. In a detailed study of *Danais* Buchner and Puff (1993) showed it to be closely related to *Schismatoclada* and *Payera*. The close relationship of *Danais* and *Schismatoclada* has been confirmed by sequence data (Bremer unpublished). The present analysis supports the conclusion from

Bremer (1996a) that *Danais* belongs to the Rubioideae, and is nested within the subfamily as the sister group to the rest of the Spermaceae alliance separated from the other tribes and as there are certain morphological characters that are rather rare in this part of Rubiaceae (woody lianas, climbing shrubs or shrubs to small trees with capsular fruits containing many winged seeds), we do not hesitate to propose a new tribe.

The next node in the tree is a split between the tribe Spermaceae (fide Bremer 1996a) and the rest of the tribes. The Spermaceae includes all investigated taxa of the former tribes Hedyotideae (excluding *Danais* as noted above), Manettieae, Knoxieae, and Spermaceae and further also *Triainolepis* of the former tribe Triainolepideae. None of these tribes can be accepted as often circumscribed (Verdcourt 1958; Bremekamp 1966; Robbrecht 1998, 1993). Within this large group of taxa there is a basal split into two highly supported branches (100% bootstrap fractions for each), one representing the *Pentas/Triainolepis* group and the other including the rest of the clade, here called the *Hedyotis/Spermaceae* group. These two groups were initially identified by morphological characters (Bremer 1987) and supported by molecular data (Bremer et al. 1995, Bremer 1996a). The *Pentas/Triainolepis* group includes also the tribe Knoxieae (represented by *Pentansia*), a tribe Robbrecht (1988) transferred to the subfamily Antirhoeidae. Our placement of *Pentansia*, and the former Knoxieae, is corroborated by the *rbcL* analysis which also includes *Knoxia* in the same branch. One of the characters for Knoxieae was solitary pendulous ovules, but at least ovule reduction occurs several times in this part of the Rubioideae, also in the closely related *Placopoda* and *Carphalea*. In the *Hedyotis/Spermaceae* group many of the genera have numerous ovules and wing-less seeds, e.g., *Hedyotis*, *Oldenlandia*, *Kohautia*, and *Pentodon*, but there are also genera with many ovules and winged seeds, earlier included in *Manettieae* (*Bouvardia* and *Manettia*) as well as the genera of the tribe Spermaceae s.s.



with solitary, erect ovules. As shown before (Bremer 1996a), the large genera *Hedyotis* and *Oldenlandia* are paraphyletic. The position of Spermacoceae s.s. within the former Hedyotideae (pointed out by Bremer et al. 1995 and Natali et al. 1995, Bremer 1996a) made the Hedyotideae paraphyletic.

The next clade in the tree corresponds to the tribe Anthospermeae, supported in the combined analyses (100% in both analyses), the *rbcL* analysis (74%), and in Andersson and Rova (1999; 54%). However, in our *atpB-rbcL* tree there is no support for the tribe.

The next branch represents the new circumscription of Argostemmatae (fide Bremer 1996a), well-supported (100%), so also in Andersson and Rova (1999; 99%). The former circumscription of the tribe (including *Neurocalyx* and *Steenisia*) was mainly based on a supposedly unique character, adnate anthers. However, that character is homoplastic and has evolved at least three times in the family (in *Argostemma*, *Neurocalyx* of the Ophiorrhizaceae, and *Steenisia* in the subfamily Cinchoinoideae, Bremer 1984).

Sister to Argostemmatae is a clade containing the tribes Rubieae, Theligoneae, and representatives of the tribe Paederieae. The circumscription and taxonomic position of the tribe Paederieae has been much discussed, e.g., Puff (1982) was of the opinion that the tribe is close to Theligoneae and Anthospermeae and he transferred all insect-pollinated genera from Anthospermeae to Paederieae. Today the tribe includes ca. 15 genera (Robbrecht 1988, 1993) but it is difficult to identify characters that unite all taxa, as most characters are variable. We have investigated five genera molecularly (*Gaillonia*, *Plocama*, *Putoria*, *Serissa*, *Spermatidion*). The analyses indicate that the taxa

are related to the Rubieae and Theligoneae but not to Anthospermeae, nor do they form a natural group; instead they constitute a grade (same result in Andersson and Rova 1999).

The monogeneric tribe Theligoneae is the sister group to the Rubieae. This close relationship was earlier indicated by Bremer et al. (1995) and Natali et al. (1995), although contradicted in Andersson and Rova (1999), however, with low support. Finally, the Rubieae is supported by 100% in all our analyses. The strong support for this group is congruent with earlier results (Natali et al. 1995, Bremer 1996a, Andersson and Rova 1999).

Representatives of the Lathraeocarpeae remain to be investigated and the recently proposed position of the Perameae (Andersson and Rova 1999) needs to be confirmed. Material of these remaining taxa has so far been difficult to obtain or analyse. With this study we have nevertheless provided a comprehensive phylogeny of Rubioideae and most of the tribes earlier associated with the subfamily have been investigated. A classification including ca 150 genera in 16 well-supported monophyletic tribes summarises our results.

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**Appendix 1.** Genera included in the Rubioideae, fide Bremer and Manen. A list of genera sorted within tribes in alphabetical order. Tribal position is indicated with the first three letters of the tribal name. Morindeae (MOR) contain also: -M, -M1 or -P indicating subgroups. \*paraphyletic taxon

Genus	Tribe	Genus	Tribe	Genus	Tribe
<i>Anthospermum</i>	ANT	<i>Kelloggia</i>	PAE	<i>Dibrachionostylus</i>	SPE
<i>Carpacoce</i>	ANT	<i>Plocama</i>	PAE	<i>Diodia</i>	SPE
<i>Coprosma</i>	ANT	<i>Pseudogaillonia</i>	PAE	<i>Dirichletia</i>	SPE
<i>Durringtonia</i>	ANT	<i>Pterogaillonia</i>	PAE	<i>Dolichometra</i>	SPE
<i>Eleutheranthus</i>	ANT	<i>Putoria</i>	PAE	<i>Ernodea</i>	SPE
<i>Galopina</i>	ANT	<i>Leptodermis</i>	PAE	<i>Hedyotis*</i>	SPE
<i>Leptostigma</i>	ANT	<i>Pseudopyxis</i>	PAE	<i>Hedythyrus</i>	SPE
<i>Nenax</i>	ANT	<i>Serissa</i>	PAE	<i>Hemidiodia</i>	SPE
<i>Nertera</i>	ANT	<i>Spermadictyon</i>	PAE	<i>Houstonia</i>	SPE
<i>Normandia</i>	ANT	<i>Amaracarpus</i>	PSY	<i>Hydrophylax</i>	SPE
<i>Opercularia</i>	ANT	<i>Anthorrhiza</i>	PSY	<i>Kadua</i>	SPE
<i>Peratanthe</i>	ANT	<i>Cephaelis</i>	PSY	<i>Knoxia</i>	SPE
<i>Phyllis</i>	ANT	<i>Chasallia</i>	PSY	<i>Kohautia</i>	SPE
<i>Pomax</i>	ANT	<i>Chazaliella</i>	PSY	<i>Lelya</i>	SPE
<i>Argostemma</i>	ARG	<i>Geophila</i>	PSY	<i>Lucya</i>	SPE
<i>Mycetia</i>	ARG	<i>Hydnophytum</i>	PSY	<i>Manettia</i>	SPE
<i>Coccocypselum</i>	COU	<i>Hymenocoleus</i>	PSY	<i>Manostachya</i>	SPE
<i>Coussarea</i>	COU	<i>Myrmecodia</i>	PSY	<i>Mitracarpus</i>	SPE
<i>Cruckshanksa</i>	COU	<i>Myrmephytum</i>	PSY	<i>Mitrasacmopsis</i>	SPE
<i>Declieuxia</i>	COU	<i>Palicouria</i>	PSY	<i>Neohymenopogon</i>	SPE
<i>Faramea</i>	COU	<i>Psychotria*</i>	PSY	<i>Nodocapaea</i>	SPE
<i>Oreopolus</i>	COU	<i>Rudgea</i>	PSY	<i>Oldenlandia*</i>	SPE
<i>Craterispermum</i>	CRA	<i>Squamellaria</i>	PSY	<i>Otiophora</i>	SPE
<i>Danais</i>	DAN	<i>Uragoga</i>	PSY	<i>Otomeria</i>	SPE
<i>Payera</i>	DAN	<i>Asperula*</i>	RUB	<i>Parapentas</i>	SPE
<i>Schismatoclada</i>	DAN	<i>Callipeltis</i>	RUB	<i>Paratriaina</i>	SPE
<i>Gaertnera</i>	GAE	<i>Crucianella</i>	RUB	<i>Pentanisia</i>	SPE
<i>Pagamea</i>	GAE	<i>Cruciata</i>	RUB	<i>Pentanopsis</i>	SPE
<i>Lasianthus</i>	LAS	<i>Didymaea</i>	RUB	<i>Pentas</i>	SPE
<i>Trichostachys</i>	LAS	<i>Galium*</i>	RUB	<i>Pentodon</i>	SPE
<i>Metabolus</i>	LAS	<i>Mericalpaea</i>	RUB	<i>Phylohydrax</i>	SPE
<i>Appunia</i>	MOR-M	<i>Microphysa</i>	RUB	<i>Placopoda</i>	SPE
<i>Coelospermum</i>	MOR-M	<i>Phuopsis</i>	RUB	<i>Pseudohedyotis</i>	SPE
<i>Gynochthodes</i>	MOR-M	<i>Relbunium</i>	RUB	<i>Psyllocarpus</i>	SPE
<i>Morinda</i>	MOR-M	<i>Rubia</i>	RUB	<i>Richardia</i>	SPE
<i>Pogonolobus</i>	MOR-M	<i>Sherardia</i>	RUB	<i>Schwendera</i>	SPE
<i>Damnacanthus</i>	MOR-MI	<i>Valantia</i>	RUB	<i>Spermacoce*</i>	SPE
<i>Mitchella</i>	MOR-MI	<i>Warburgia</i>	RUB	<i>Staelia</i>	SPE
<i>Motleya</i>	MOR-P	<i>Lecananthus</i>	SCH	<i>Synaptantha</i>	SPE
<i>Prismatomeris</i>	MOR-P	<i>Leucocodon</i>	SCH	<i>Thecorchus</i>	SPE
<i>Renellia</i>	MOR-P	<i>Schradera</i>	SCH	<i>Thyridocalyx</i>	SPE
<i>Coptophyllum</i>	OPH	<i>Agathisanthemum</i>	SPE	<i>Triainolepis</i>	SPE
<i>Lerchea</i>	OPH	<i>Amphasma</i>	SPE	<i>Theligonum</i>	THE
<i>Neurocalyx</i>	OPH	<i>Arcytophyllum</i>	SPE	<i>Amphidasya</i>	URO
<i>Ophiorrhiza</i>	OPH	<i>Batopedina</i>	SPE	<i>Commitheca</i>	URO
<i>Spiradielis</i>	OPH	<i>Bouvardia</i>	SPE	<i>Maschalocorymus</i>	URO

**Appendix 1.** (Continued)

Genus	Tribe	Genus	Tribe	Genus	Tribe
<i>Xanthophytum</i>	OPH	<i>Carphalea</i>	SPE	<i>Pauridiantha</i>	URO
<i>Paederia</i>	PAE	<i>Chaemepentas</i>	SPE	<i>Praravinia</i>	URO
<i>Choulettia</i>	PAE	<i>Conostomium</i>	SPE	<i>Pravinaria</i>	URO
<i>Gaillonia</i>	PAE	<i>Crusea</i>	SPE	<i>Urophyllum</i>	URO
<i>Jaubertia</i>	PAE	<i>Dentella</i>	SPE		

**Appendix 2.** Genera included in the Rubioideae, fide Bremer and Manen. A list of genera in alphabetical order. Tribal position is indicated with the first three letters of the tribal name. Morindeae (MOR) contain also: -M, -MI or -P indicating subgroups. \*paraphyletic taxon

Genus	Tribe	Genus	Tribe	Genus	Tribe
<i>Agathisanthemum</i>	SPE	<i>Hedyotis*</i>	SPE	<i>Pauridiantha</i>	URO
<i>Amaracarpus</i>	PSY	<i>Hedythyrus</i>	SPE	<i>Payera</i>	DAN
<i>Amphiasma</i>	SPE	<i>Hemidiodia</i>	SPE	<i>Pentanisia</i>	SPE
<i>Amphidasya</i>	URO	<i>Houstonia</i>	SPE	<i>Pentanopsis</i>	SPE
<i>Anthorrhiza</i>	PSY	<i>Hydnophytum</i>	PSY	<i>Pentas</i>	SPE
<i>Anthospermum</i>	ANT	<i>Hydrophylax</i>	SPE	<i>Pentodon</i>	SPE
<i>Appunia</i>	MOR-M	<i>Hymenocoleus</i>	PSY	<i>Peratanthe</i>	ANT
<i>Arcytophyllum</i>	SPE	<i>Jaubertia</i>	PAE	<i>Phuopsis</i>	RUB
<i>Argostemma</i>	ARG	<i>Kadua</i>	SPE	<i>Phyllis</i>	ANT
<i>Asperula*</i>	RUB	<i>Kelloggia</i>	PAE	<i>Phylohydrax</i>	SPE
<i>Batopedina</i>	SPE	<i>Knoxia</i>	SPE	<i>Placopoda</i>	SPE
<i>Bouvardia</i>	SPE	<i>Kohautia</i>	SPE	<i>Plocama</i>	PAE
<i>Callipeltis</i>	RUB	<i>Lasianthus</i>	LAS	<i>Pogonolobus</i>	MOR-M
<i>Carhpalea</i>	SPE	<i>Lecananthus</i>	SCH	<i>Pomax</i>	ANT
<i>Carpacoe</i>	ANT	<i>Lelya</i>	SPE	<i>Praravinia</i>	URO
<i>Cephaelis</i>	PSY	<i>Leptodermis</i>	PAE	<i>Pravinaria</i>	URO
<i>Chaemepentas</i>	SPE	<i>Leptostigma</i>	ANT	<i>Prismatomeris</i>	MOR-P
<i>Chasallia</i>	PSY	<i>Lerchea</i>	OPH	<i>Pseudogaillonia</i>	PAE
<i>Chazaliella</i>	PSY	<i>Leucocodon</i>	SCH	<i>Pseudohedyotis</i>	SPE
<i>Choulettia</i>	PAE	<i>Lucya</i>	SPE	<i>Pseudopyxis</i>	PAE
<i>Coccocypselum</i>	COU	<i>Manettia</i>	SPE	<i>Psychotria*</i>	PSY
<i>Coelospermum</i>	MOR-M	<i>Manostachya</i>	SPE	<i>Psyllocarpus</i>	SPE
<i>Commitheca</i>	URO	<i>Maschalocorymus</i>	URO	<i>Pterogaillonia</i>	PAE
<i>Conostomium</i>	SPE	<i>Mericalpaea</i>	RUB	<i>Putoria</i>	PAE
<i>Coprosma</i>	ANT	<i>Metabolus</i>	LAS	<i>Relbunium</i>	RUB
<i>Coptophyllum</i>	OPH	<i>Microphysa</i>	RUB	<i>Renellia</i>	MOR-P
<i>Coussarea</i>	COU	<i>Mitchella</i>	MOR-MI	<i>Richardia</i>	SPE
<i>Craterispermum</i>	CRA	<i>Mitracarpus</i>	SPE	<i>Rubia</i>	RUB
<i>Cruckshanksiae</i>	COU	<i>Mitrasacmopsis</i>	SPE	<i>Rudgea</i>	PSY
<i>Crusea</i>	SPE	<i>Morinda</i>	MOR-M	<i>Schismatoclada</i>	DAN
<i>Crucianella</i>	RUB	<i>Motleyia</i>	MOR-P	<i>Schradera</i>	SCH
<i>Cruciata</i>	RUB	<i>Mycetia</i>	ARG	<i>Schwendera</i>	SPE
<i>Dammacanthus</i>	MOR-MI	<i>Myrmecodia</i>	PSY	<i>Serissa</i>	PAE
<i>Danais</i>	DAN	<i>Myrmephytum</i>	PSY	<i>Sherardia</i>	RUB
<i>Declieuxia</i>	COU	<i>Nenax</i>	ANT	<i>Spermacoe*</i>	SPE
<i>Dentella</i>	SPE	<i>Neohymenopogon</i>	SPE	<i>Spermadictyon</i>	PAE

## Appendix 2. (Continued)

Genus	Tribe	Genus	Tribe	Genus	Tribe
<i>Dibrachionostylus</i>	SPE	<i>Nertera</i>	ANT	<i>Spiradichlis</i>	OPH
<i>Didymaea</i>	RUB	<i>Neurocalyx</i>	OPH	<i>Squamellaria</i>	PSY
<i>Diodia</i>	SPE	<i>Nodocapaea</i>	SPE	<i>Staelia</i>	SPE
<i>Dirichletia</i>	SPE	<i>Normandia</i>	ANT	<i>Synaptantha</i>	SPE
<i>Dolichometra</i>	SPE	<i>Oldenlandia*</i>	SPE	<i>Thecorchus</i>	SPE
<i>Durringtonia</i>	ANT	<i>Opercularia</i>	ANT	<i>Theligonum</i>	THE
<i>Eleutheranthus</i>	ANT	<i>Ophiorrhiza</i>	OPH	<i>Thyridocalyx</i>	SPE
<i>Ernodea</i>	SPE	<i>Oreopolus</i>	COU	<i>Triainolepis</i>	SPE
<i>Faramea</i>	COU	<i>Otiophora</i>	SPE	<i>Trichostachys</i>	LAS
<i>Gaertnera</i>	GAE	<i>Otomeria</i>	SPE	<i>Uragoga</i>	PSY
<i>Gaillonia</i>	PAE	<i>Paederia</i>	PAE	<i>Urophyllum</i>	URO
<i>Galium*</i>	RUB	<i>Pagamea</i>	GAE	<i>Valantia</i>	RUB
<i>Galopina</i>	ANT	<i>Palicouria</i>	PSY	<i>Warburgia</i>	RUB
<i>Geophila</i>	PSY	<i>Parapentas</i>	SPE	<i>Xanthophytum</i>	OPH
<i>Gynochthodes</i>	MOR-M	<i>Paratriaina</i>	SPE		

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