Evolutionary relationships in the Spermacoceae alliance (Rubiaceae) using information from six molecular loci: insights into systematic affinities of *Neohymenopogon* and *Mouretia*

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Several recent phylogenetic studies of Rubiaceae have dealt with enigmatic taxa whose systematic positions have been previously unknown or controversial. We address evolutionary relationships in the Spermacoceae alliance (Rubioideae) with special emphasis on the Asian genera *Mouretia* and *Neohymenopogon*, here sequenced for the first time. Both genera belong in the tribe Argostemmateae and have persistent calyx lobes on the fruit in common with *Argostemma* and *Mycetia*. Other previous uncertainties are resolved with strong support; *Saprosma* is sister to Paederieae s.str. and *Carpacoce* is sister to remaining Anthospermeae. Our results further reveal some phylogenetic problems. Danaideae is sister to remaining taxa in the Spermacoceae alliance with high posterior probability, which contradicts results in a recent study. The uncertainty concerning evolutionary relationships of *Dunnia* and *Theligonum* is reinforced, despite a denser taxon sampling in the Spermacoceae alliance compared with earlier studies. We also demonstrate yet another example of the controversial correlation between molecular substitution rate and plant life history.

KEYWORDS: Anthospermeae, Argostemmateae, Danaideae, Paederieae, rate heterogeneity, *Saprosma*, *Theligonum*

INTRODUCTION

The large coffee family, Rubiaceae, comprises more than 13,000 species (Govaerts & al., 2006), a diversity which is estimated to have originated in the mid-Cretaceous (Bremer & Eriksson, 2009). Rubiaceae display a great diversity in growth habit ranging from arborescent, suffrutescent, lianescent, epiphytic to herbaceous habits (Robbrecht, 1988; Bremer & al., 1995). A woody habit is the predominant life form in the subfamilies Cinchonoideae and Ixoroideae, whereas herbaceous as well as woody habits occur in Rubioideae (Verdcourt, 1958). Climbers are also relatively common in the family and about 250 species are epiphytic (Robbrecht, 1988), but a parasitic habit is very unusual, probably unknown.

The epiphytic to terrestrial plant *Neohymenopogon parasiticus* (Wallich, 1824; Bennet, 1981), distributed in Bhutan, Vietnam, Tibet and China (Yunnan) (Govaerts & al., 2006), was originally described under the name *Hymenopogon parasiticus*, as a small branchy shrub, attached parasitically to trees by means of fibrous roots (Wallich, 1824). The interpretation of the plant as a parasite was likely a misconception (see, e.g., Puff & al., 2005), but the matter has to our knowledge never been thoroughly investigated. The leaves are membranous (but green), the flowers are pentamerous with the stamina inserted near

the apex of the corolla lobe and the capsule has numerous seeds. A thin but leafy bract, "elegantly nerved" underneath is attached to the node where the peduncles unite (Wallich, 1824: 157). *Hymenopogon* Wall. (Wallich, 1824), comprising three species, was an illegitimate homonym and Bennet (1981) suggested it should be replaced by *Neohymenopogon*. Andersson & Persson (1991) included the genus in a morphological study and found support for Bremekamp's (1952, 1966) transfer of *Neohymenopogon* from Cinchoneae to Hedyotideae. Apart from this work, short descriptions in floras and other surveys (Wallich, 1824; Hooker, 1880; Classen-Bockhoff, 1996) and Puff & al.'s more comprehensive flora (2005), we have not found any published information on *Neohymenopogon*. In order to get a first indication on the systematic affinity of this genus, we produced and analysed a nrITS sequence and the preliminary results indicated that *Neohymenopogon parasiticus* belongs in Rubioideae, Rubiaceae, probably in the Spermacoceae alliance (further described below).

Several recent phylogenetic studies of Rubiaceae have investigated enigmatic taxa whose systematic positions have been unknown or controversial, e.g., *Kelloggia* (Nie & al., 2005), *Rhopalobrachium* (Mouly & al., 2007), *Dunnia* (Rydin & al., 2008), *Schizocolea* (Razafimandimbison & al., 2008; Rydin & al., 2008), *Petitiocodon* (Tosh & al., 2008) and *Acranthera* (Rydin & al., 2009). Another

genus of uncertain systematic affinity is *Mouretia* (Pitard, 1922), which comprises five species distributed in China, Laos, Vietnam and Thailand (Govaerts & al., 2006). Tange (1997) made a revision of the genus and described *Mouretia* as herbs to small shrubs, often anisophyllous and with distylous flowers (Tange, 1997; see also Puff & al., 2005). *Mouretia* is currently classified in the tribe Hedyotideae (Robbrecht, 1988, 1993), and this was tentatively accepted by Tange (1997). Tange suggested a possible affinity between *Mouretia* and *Mycetia* but did not do any formal changes of the classifications. *Mycetia* was at that time placed in the tribe Isertieae, subfamily Cinchonoideae (Robbrecht, 1988, 1993), though Bremekamp (1952, 1966) had suggested that it belonged in Hedyotideae. Hypotheses on the systematic position of *Mouretia* have to our knowledge never been tested using phylogenetic analyses.

The Spermacoceae alliance and the aim of this study. — The Spermacoceae alliance (Bremer & Manen, 2000), also known as the Hedyotideae-Rubieae clade (Andersson & Rova, 1999) and supertribe Rubiidinae (Robbrecht & Manen, 2006), is a well-corroborated clade consisting of nearly 3,000 species. It comprises two major subclades (Bremer & Manen, 2000); subclade 1: the tribes Knoxieae and Spermacoceae (Bremer, 1996; Robbrecht & Manen, 2006; Kårehed & Bremer, 2007) (not discussed in the present paper), and subclade 2: Anthospermeae, Argostemmateae, Dunnieae, Paederieae, Putorieae, Rubieae and Theligoneae (Bremer & Manen, 2000; Backlund & al., 2007; Rydin & al., 2008, 2009). Further, the small tribe Danaideae belongs to the Spermacoceae alliance (Bremer & Manen, 2000) but its relationship to the remaining tribes has been inconsistent (results differ between studies).

Here, we investigate evolutionary relationships within the Spermacoceae alliance with special focus on *Neohymenopogon* and *Mouretia*. The study is based on information from six loci and utilises a denser taxon sampling in the Spermacoceae alliance compared with previous studies.

MATERIALS AND METHODS

Selection of species and laboratory procedures. — We selected 159 taxa from subfamily Rubioideae for this study (Appendix). We have included 91 terminals from the ingroup, the Spermacoceae alliance (Bremer & Manen, 2000), representing ten tribes. Due to limited access to material, we were only able to include one species each of *Mouretia* and *Neohymenopogon*. The material investigated during the revision of *Mouretia* (Tange, 1997) has unfortunately not been possible to trace but other material of *Mouretia larsenii* Tange has been studied and used to produce sequences in a first attempt to

elucidate the evolutionary origin of the genus. *Neohymenopogon parasiticus* is investigated using material from two specimens. Relationships within the Knoxieae-Spermacoceae clade are investigated elsewhere (i.e., Kårehed & Bremer, 2007; Kårehed & al., 2008; Groeninckx & al., 2009) and will not be discussed here, but to relevantly address the topics of the present paper, we have included 24 representatives from the Knoxieae-Spermacoceae clade. We further included a comprehensive set of terminals (68) from remaining Rubioideae, representing 14 tribes. All trees were rooted on *Colletoecema*, the sister group of all other Rubioideae species (Robbrecht & Manen, 2006; Rydin & al., 2008; Sonké & al., 2008).

We utilised information from six loci: five chloroplast regions (*rbcL*, the *rps16* intron, *ndhF*, *atpB-rbcL* spacer, the *trnT-L-F* region), and the internal transcribed spacer of the nuclear ribosomal DNA, nrITS (nrITS1, 5.8S, nrITS2). Primer references are given in Table 1. We used information from GenBank if available and we also produced 252 new sequences for this study (Table 2). GenBank accession numbers are given in Appendix. DNA was extracted, amplified and sequenced using standard procedures previously described (Kårehed & Bremer, 2007).

Table 1. Primers.

DNA region	Primer name	Reference
rbcL	5′F, 3′R and 427F	Bremer & al. (2002)
rbcL	Z895R	Zurawski, DNAX
rps16	F and 2R	Oxelman & al. (1997)
nrITS	P17 and 26S-82R	Popp & Oxelman (2001)
nrITS	P25	Oxelman (1996)
nrITS	ITSForwRub	Rydin & al. (2009)
nrITS	ITSRevRub	Rydin & al. (2009)
ndhF	2F	Rydin & al. (2008)
ndhF	1000R	Rydin & al. (2008)
ndhF	720F	Rydin & al. (2008)
ndhF	1700R	Rydin & al. (2008)
ndhF	1320F	Rydin & al. (2008)
ndhF	2280R	Rydin & al. (2008)
atpB-rbcL spacer	rbcL5′R	Rydin & al. (2008)
atpB-rbcL spacer	atpB5′R	Rydin & al. (2008)
trnT-L-F	A1	Bremer & al. (2002)
trnT-L-F	940R	Rydin & al. (2008)
trnT-L-F	820F	Rydin & al. (2008)
trnT-L-F	IR	Bremer & al. (2002)
trnT-L-F	1250F	Rydin & al. (2008)
trnT-L-F	D	Taberlet & al. (1991)
trnT-L-F	1880F	Rydin & al. (2008)
trnT-L-F	2670R	Rydin & al. (2008)

Sequence fragments were assembled using the Staden package (Staden, 1996).

Alignment and phylogenetic reconstruction. — Sequences were aligned visually, using computer programs Seaview 2.2 (Galtier & al., 1996) and Se-Al v.2.0 (Rambaut, 1996). Insertion/deletion events were visually inferred, following the alignment criteria outlined in Oxelman & al. (1997).

All matrices were analysed with two approaches: Bayesian inference and parsimony. We analysed each gene separately, and we also analysed all gene regions together in a combined dataset. Bayesian analyses were performed in MrBayes 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). For each single-gene dataset, the best-performing evolutionary model was identified under several different model selection criteria: Akaike information criterion (AIC) (Akaike, 1973), AICc, the Bayesian information criterion (BIC) (Schwartz, 1978) and hierarchical likelihood ratio tests (four different hierarchies described in Posada & Crandall, 2001). We performed these calculations in softwares MrAIC ver. 1.4.3 and PHYML (Guindon & Gascuel, 2003; Nylander, 2004a), and in MrModeltest ver. 2.3 and PAUP* ver. 4.0b10 for Unix (Swofford, 1998; Nylander, 2004b).

Prior probabilities were set following outputs from MrAIC: a flat Dirichlet prior probability (all values are 1.0) was selected for the substitution rates (revmatpr) and the nucleotide frequencies (statefreqpr). The prior probability for the shape parameter of the gamma distribution of rate variation (shapepr) was uniformly distributed in the interval (0.1, 50.0). For analyses using a gamma distribution with a proportion of invariable sites, we specified a prior probability for this proportion (pinvarpr), uniformly distributed on the interval (0.0, 1.0). When applicable, the prior for the transition/transversion rate ratio (tratio) was set so as to put equal emphasis on transition/transversion rate ratios above 1.0 and below 1.0 (beta 1.0, 1.0).

The combined dataset was partitioned in two ways: (1) six partitions (one for each locus) and (2) two partitions (chloroplast data and nuclear data). Partitions were unlinked so that each partition was allowed to have its own set of parameters. Five million generations were run with a sample frequency of 1,000, six parallel chains and the temperature set to 0.15. For single-gene analyses, 1.5 million generations were run with a sample frequency of 1,000, four parallel chains and the temperature set to 0.2. Convergence of the Markov chain was assumed to be reached when the likelihood values and individual parameters were stably fluctuating. At this point, the potential scale reduction factor was approaching 1.000 for all parameters and standard deviation of split frequencies for the two parallel runs was <0.007. Trees sampled from the first two million generations (200,000 generations for single-gene analyses) were discarded as burn-in.

Table 2. Data description.

Taxon	rbcL	rps16	ndhF	atpB-rbcL spacer	trnT/F	nrITS	Combined matrix, 6 partitions	Combined matrix, 2 partitions
Total number of taxa in matrix (number of new sequences)	155 (32)	153 (30)	127 (61)	139 (53)	149 (32)	134 (44)	159 (252)	159 (252)
Total number of characters in matrix	1,402	1,689	2,223	1,144	3,513	893	10,864	10,864
Number of variable characters	517	967	1,060	556	1,905	586	5,591	5,591
Number of informative characters	393	636	765	338	1,183	486	3,801	3,801
Best fitting evolutionary model; AICc weights[a]	GTR+I+Γ	HKY+Γ	GTR+Γ	GTR+Γ	GTR+I+Γ	GTR+I+Γ	—	—
Best fitting evolutionary model; AIC weights[a]	GTR+I+Γ	HKY+Γ	GTR+Γ	GTR+Γ	GTR+I+Γ	GTR+I+Γ	—	—
Best fitting evolutionary model; BIC weights[a]	GTR+I+Γ	HKY+Γ	GTR+Γ	GTR+Γ	GTR+I+Γ	GTR+I+Γ	—	—
Best fitting evolutionary model; hLRTs[b]	GTR+I+Γ	GTR+Γ	GTR+I+Γ	GTR+Γ	GTR+I+Γ	GTR+I+Γ	—	—
Conflicts between Bayesian and parsimony analyses	No	No	No	No	No	No	No	No
Bootstrap analysis (bootstrap replicates /random sequence additions in each replicate)	1,000/10	1,000/10	1,000/10	1,000/10	1,000/10	1,000/10	5,000/10	5,000/10
Bayesian analysis (number of generations run)	1.5 million	1.5 million	1.5 million	1.5 million	1.5 million	1.5 million	5 million	5 million
Employed evolutionary model (AICc weights)	GTR+I+Γ	HKY+Γ	GTR+Γ	GTR+Γ	GTR+I+Γ	GTR+I+Γ	←[c]	GTR+I+Γ

[a] AIC, Akaike information criterion; AICc, corrected AIC; BICC, Bayesian information criterion; all calculated in software MrAIC.pl (Nylander, 2004a).
[b] hLRTs, hierarchical likelihood ratio tests; calculated in software MrModeltest (Nylander, 2004b).
[c] Evolutionary model employed for each data partition are as specified to the left (for single genes).

Parsimony analyses were performed for single gene datasets, as well as for the combined dataset, in PAUP* ver. 4.0b10 for Unix (Swofford, 1998). Most parsimonious trees were calculated using the heuristic search option, 500 random sequence additions and tree bisection reconnection branch swapping and multrees off. Support values were obtained by using bootstrap in PAUP*, performing 5,000 bootstrap replicates, each with ten random sequence additions. For single-gene analyses, 1,000 bootstrap replicates were run. A majority rule consensus tree was produced from the resulting trees, in which nodes with a bootstrap support <50% were collapsed.

RESULTS

Data and model choice. — The combined matrix comprised 10,864 aligned characters. Of the 5,591 variable characters, 3,801 were phylogenetically informative (Table 2). The hierarchical likelihood ratio tests (hLRTs in MrModeltest) and calculations of AIC, AICc, and BIC weights (performed in MrAIC) for estimation of best performing nucleotide substitution models sometimes identified different optimal models for a given locus (Table 2). Bayesian and AIC approaches have been shown to have important advantages over the hLRTs for model selection (Posada & Buckley, 2004) and when forced to make a choice (*ndhF*, *rps16* intron), we used the model identified under the corrected Akaike information criterion because AICc is appropriate when the ratio between sample size and number of parameters is small (n / K < 40; Burnham & Anderson, 2003: 66), but also for higher ratios because AICc will then converge to AIC (Posada & Buckley, 2004).

For the *rbcL*, *trnT-L-F* and nrITS data, the general time reversible model (GTR; Tavare, 1986) with gamma distributed rates (Yang, 1993) and a proportion of invariable sites was selected (GTR+Γ+I). For *ndhF* and the *atpB-rbcL* spacer, GTR+Γ was selected. For the *rps16* intron the Hasegawa, Kishino and Yano model (Hasegawa & al., 1985) with gamma distributed rates (HKY+Γ) was selected (see also Table 2). For the combined analysis with two partitions (chloroplast data; nuclear data), GTR+Γ+I was selected for the chloroplast partition.

Phylogenetic relationships in the Spermacoceae alliance: the combined analyses. — The majority rule consensus tree from the Bayesian analysis with six data partitions is shown in Fig. 1 with posterior probabilities of clades above branches. Bootstrap indices for nodes in the ingroup (the Spermacoceae alliance) and major divergences in the Rubioideae are mapped below branches. Results from the two different Bayesian analyses of the combined dataset (six partitions; two partitions) did not differ, but a few more nodes were resolved and/or better supported

in the six-partition analysis. There were no supported conflicts between Bayesian and parsimony analyses.

All nodes presented below received 100% posterior probability and ≥85% bootstrap support unless otherwise is stated as follows (Bayesian posterior probability/bootstrap support).

The Spermacoceae alliance was monophyletic with Danaideae being sister clade to remaining species (Fig. 1A, B). The genera *Danais* and *Schismatoclada* were monophyletic. One Malagasy species, *Danais nigra*, was sister to remaining *Danais*, within which the African species *Danais xanthorrhoea* was sister to *Danais comorensis*.

Remaining species of the Spermacoceae alliance (95/–), consist of the sister clades Knoxieae-Spermacoceae and Anthospermeae-Argostemmateae-Dunnieae-Paederieae-Putorieae-Theligoneae-Rubieae. Within the latter, Anthospermeae are sister to the other tribes. Anthospermeae are well resolved; *Carpacoce* is sister to the remaining species: a (*Phyllis*(*Anthospermum-Nenax*)) clade and an ((*Opercuclaria-Pomax*) + ((*Normandia*(*Coprosma-Nertera*))) clade (100/64).

Within the next diverging clade (Argostemmateae), *Mouretia* is sister to the remaining species, followed by *Mycetia*. *Neohymenopogon* and *Argostemma* are sisters (97/70). *Mycetia javanica* and *M. malayana* are sister to a poorly supported (71/–) clade ((*M. gracilis* + (*M. cauliflora* + (two unknown species of *Mycetia*))). (*Argostemma bifolium* + (*A. gracile* + (*A. brachyantherum–A. psychtrioides*))) are sisters to a clade comprising ((*A. elatostemma–A. hookerii*) and (*A. geesinkii* + (*A. yappii* + (*A. rupestre* + (*A. parvifolium* var. *involucratum–A. parvifolium*))))). Support was less than 100% posterior probability and 85% bootstrap for the (*A. yappii* + (*A. rupestre* + (*A. parvifolium* var. *involucratum–A. parvifolium*) clade (85/–), and for the (*A. rupestre* + (*A. parvifolium* var. *involucratum–A. parvifolium*) clade (98/–).

Argostemmateae + (Dunnieae-Paederieae-Putorieae-Theligoneae-Rubieae) are well supported in Bayesian analysis (100%) but the node is collapsed in the bootstrap analysis. Within the latter clade (64/–), Dunnieae are sister to the other tribes.

Within Paederieae-Putorieae-Theligoneae-Rubieae (100/72), Paederieae s.str. (Backlund & al., 2007) is sister to the other tribes and *Saprosma* is well supported as sister to remaining Paederieae. The (Theligoneae + (Rubieae-Kelloggia)) clade is poorly supported (58/75).

Single-gene datasets. — Generally, single gene analyses (not shown) produced the same topologies as those described above, but partly collapsed. There are some deviations and "supported" differences (here arbitrarily defined as nodes receiving a Bayesian posterior probability higher than 85%, and/or a bootstrap support higher than 50%) are presented here. The differences mainly concern

groups, whose systematic positions are poorly supported in the combined tree (Dunnieae, Theligoneae).

rps16. In the single-gene *rps16* analyses, Theligoneae are sister to a clade containing three species of Putorieae (100/86). *Neohymenopogon* is sister to *Mycetia* (99/70).

ndhF. In the *ndhF* trees, Danaideae are sister to the Knoxieae-Spermacoceae clade (96/57). Dunnieae are sister to the Anthospermeae-Argostemmateae-Paederieae-Putorieae-Theligoneae-Rubieae clade (96/-).

atpB-rbcL **spacer.** Theligoneae are part of a paraphyletic Putorieae (98/72).

nrITS. Anthospermeae are sister (98/–) to (Theligoneae + (Rubieae-Putorieae)) (99/–). *Neohymenopogon* is sister to *Mycetia* (–/77).

■ DISCUSSION

New insights into evolutionary relationships. — The present study reveals several new findings on the phylogeny of the Spermacoceae alliance, for example within Argostemmateae, Anthospermeae, Danaideae and Paederieae. These results are discussed in detail below. We were particularly interested in the phylogenetic position of *Mouretia* and *Neohymenopogon*, here sequenced for the first time, and the results show that both genera belong to Argostemmateae. *Neohymenopogon parasiticus* is sister to *Argostemma* (Fig. 1B), thus, the type species of a small genus comprising only three species in Southeast Asia (*Neohymenopogon*) is sister to the large genus *Argostemma*, which comprises more than 160 species (Govaerts & al., 2006), with a probable centre of origin in tropical Asia. *Mouretia*, also sequenced here for the first time, is strongly supported as sister to remaining species of Argostemmateae (Fig. 1B). Tange's (1997) argumentation for strong similarity between *Mouretia* and *Mycetia* is thus supported, even if the clade also comprises *Argostemma* and *Neohymenopogon*.
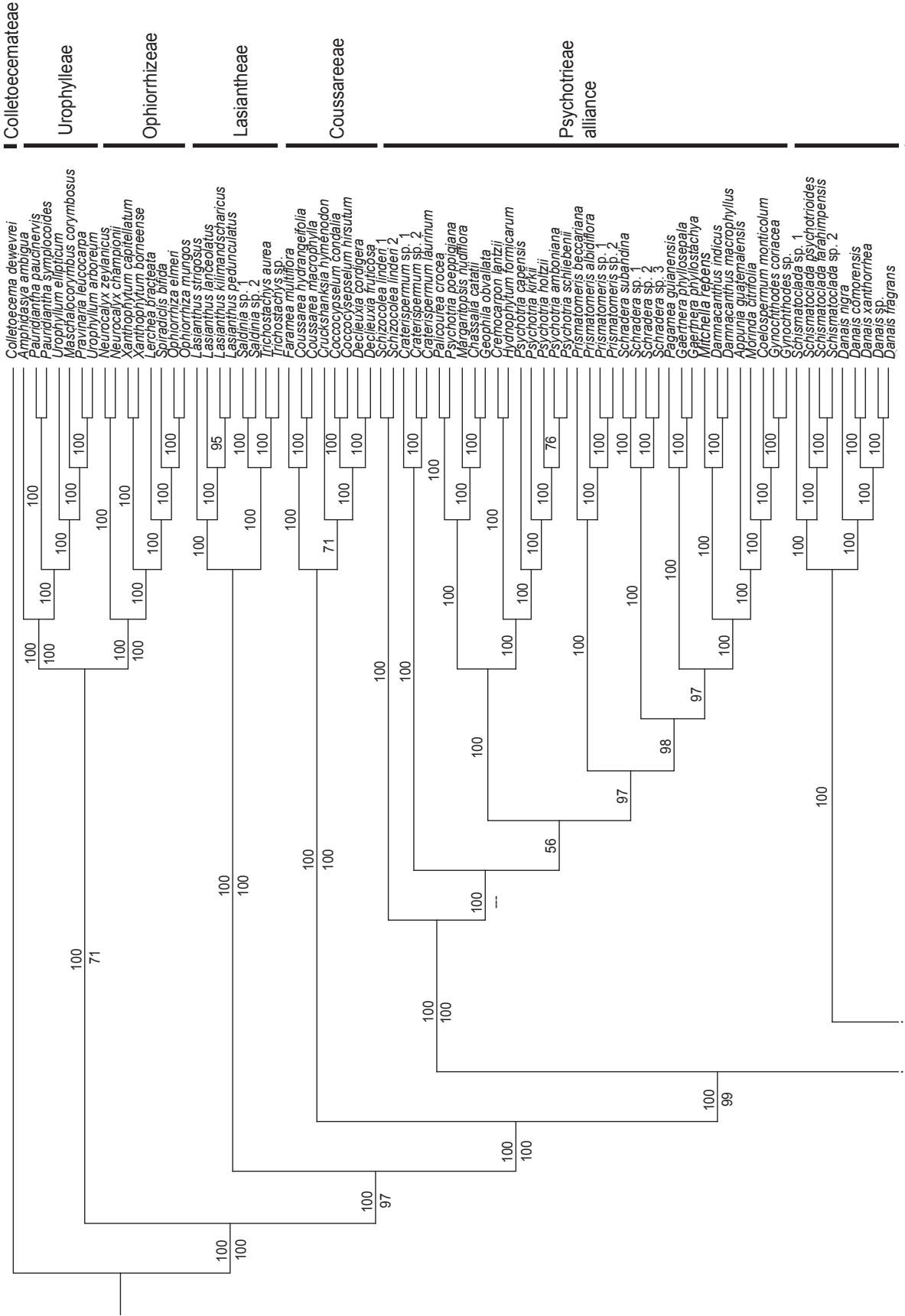
Conflicting results. — The results also reveal several conflicts and potentially difficult phylogenetic problems, i.e., regarding Danaideae, Dunnieae and Theligoneae. The position of Danaideae is perhaps the most striking example. Danaideae comprise the woody climber genus *Danais* and the arborescent genera *Schismatoclada* and *Payera*, all with heterostylous flowers and capsular fruits (Buchner & Puff, 1993; Bremer & Manen, 2000). In previous studies, Danaideae has been suggested to be sister to the remaining species of the Spermacoceae alliance (Bremer & Manen, 2000) or sister to the Anthospermeae-Argostemmateae-Paederieae-Rubieae-Theligoneae clade (Robbrecht & Manen, 2006) and in a recent study (Rydin & al., 2009), Danaideae were sister to the Spermacoceae-Knoxieae clade, a result which was very well supported. Here, Danaideae are sister to remaining

species in the Spermacoceae alliance. The relationship is well supported in Bayesian analysis, but collapses in the bootstrap analysis (Fig. 1B) and *ndhF* data resolve Danaideae as sister to Knoxieae-Spermacoceae. Thus, there are supported conflicts on the position of Danaideae and the result presented here should probably be considered uncertain despite a high posterior probability and a much denser species representation in Danaideae; we have included nine terminals, whereas previous studies used one or two terminals.

Dunnieae contain a single species, the highly endangered *Dunnia sinensis* (Tutcher, 1905), endemic to the southern Guangdong Province of China (Chen, 1999). It is a woody shrub with pentamerous flowers in clusters, subtended by a few showy, petaloid structures, often referred to as enlarged calyx lobes (e.g., Chen, 1999; Ge & al., 2002). New investigations show, however, that they are modified bracts (C. Taylor, pers. comm.; Chen & Taylor, in prep.) and this seems also to be in line with the original description, in which the showy structures are described as bract-like lobes near the inflorescence (Tutcher, 1905). Dunnieae were sister to the Anthospermeae-Argostemmateae-Paederieae-Rubieae-Theligoneae clade in Rydin & al. (2008) and to the Paederieae-Rubieae-Theligoneae clade in Rydin & al. (2009). The studies of Rydin & al. (2008, 2009) addressed major evolutionary events within the entire family (Rubiaceae) and many nodes within the Spermacoceae alliance were poorly supported, conceivably due to limited taxon sampling. However, despite the much denser taxon sampling within the Spermacoceae alliance employed here, the position of Dunnieae (sister to the Paederieae-Putorieae-Rubieae-Theligoneae clade in the present study; Fig. 1B) is again poorly supported due to conflicting information in investigated loci.

The species of *Theligonum* are herbs, anisophyllous and with unisexual, anemophilous flowers (Bremer & Manen, 2000). The genus has an interesting disjunct distribution, with one species in Macaronesia, the Mediterranean region and the Near East and three species in Taiwan, China and Japan (one in each region). In recent studies, Theligoneae are generally resolved as sister to Rubieae (Nie & al., 2005; Backlund & al., 2007; Rydin & al., 2009), but often with week support. Moreover, it has generally been the same sequences (i.e., those originally produced by Bremer & al., 1995; Andersson & Rova, 1999) that have been used over again. Based on new data from two additional specimens of *Theligonum cynocrambe*, we confirm its sister relationship to Rubieae. The support is very low, however, and some loci support a sister relationship between Theligoneae and Putorieae (*rps16, atpB-rbcL* spacer) or between between Theligoneae and Putorieae–Rubieae (nrITS).

The morphology of *Theligonum* is in many respects different from that of other Rubiaceae (see, e.g.,

Fig. 1A. Phylogeny of Rubioideae (overview), estimated using Bayesian inference of phylogeny (six-data partitions) based on molecular data from chloroplast regions *rbcL*, *rps16* intron, *ndhF*, *atpB-rbcL* spacer, *trnT-L-F* and from the nuclear ribosomal ITS. Posterior probabilities are given above branches. Selected bootstrap values (under parsimony) are mapped on this Bayesian tree (below branches).

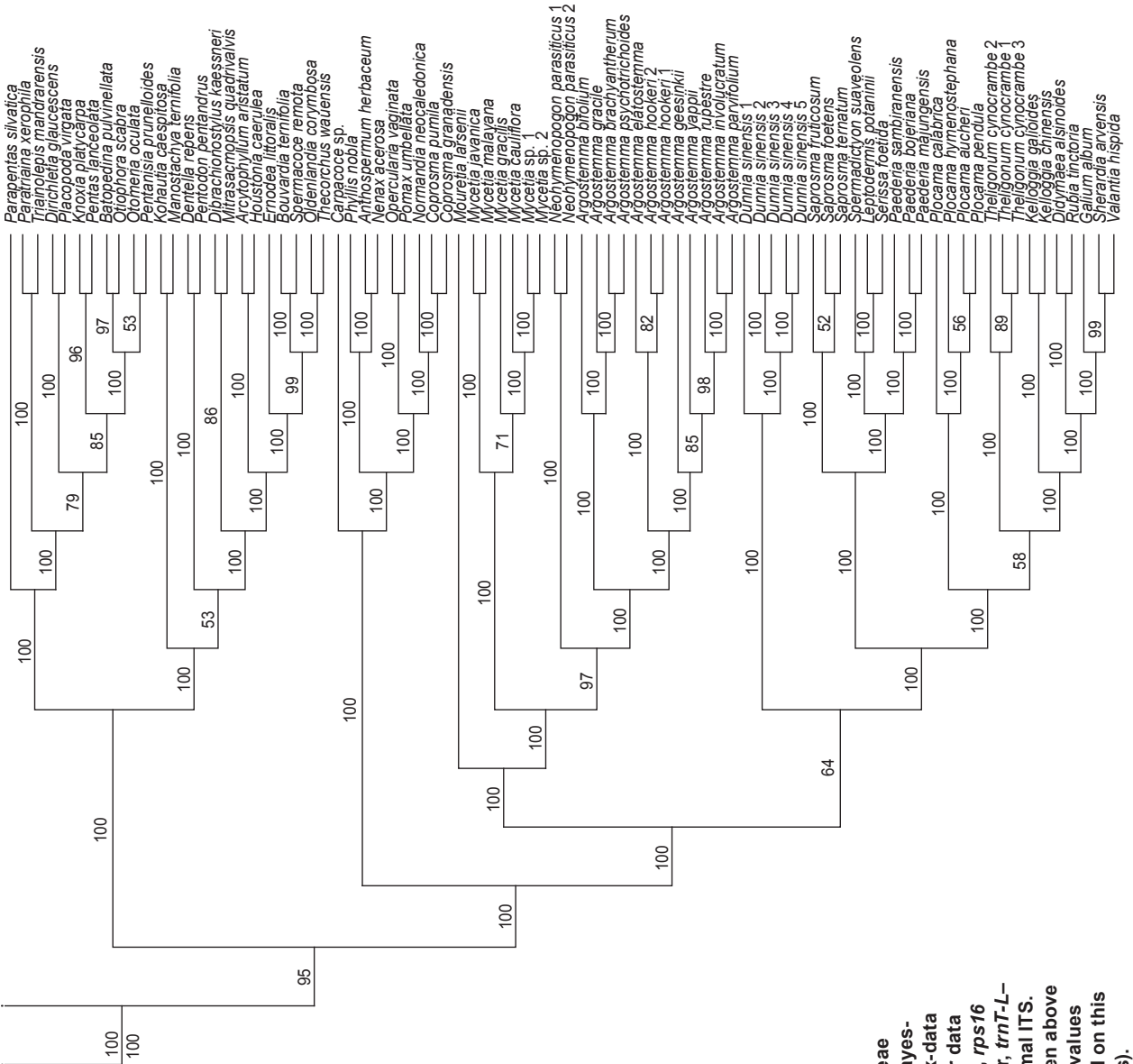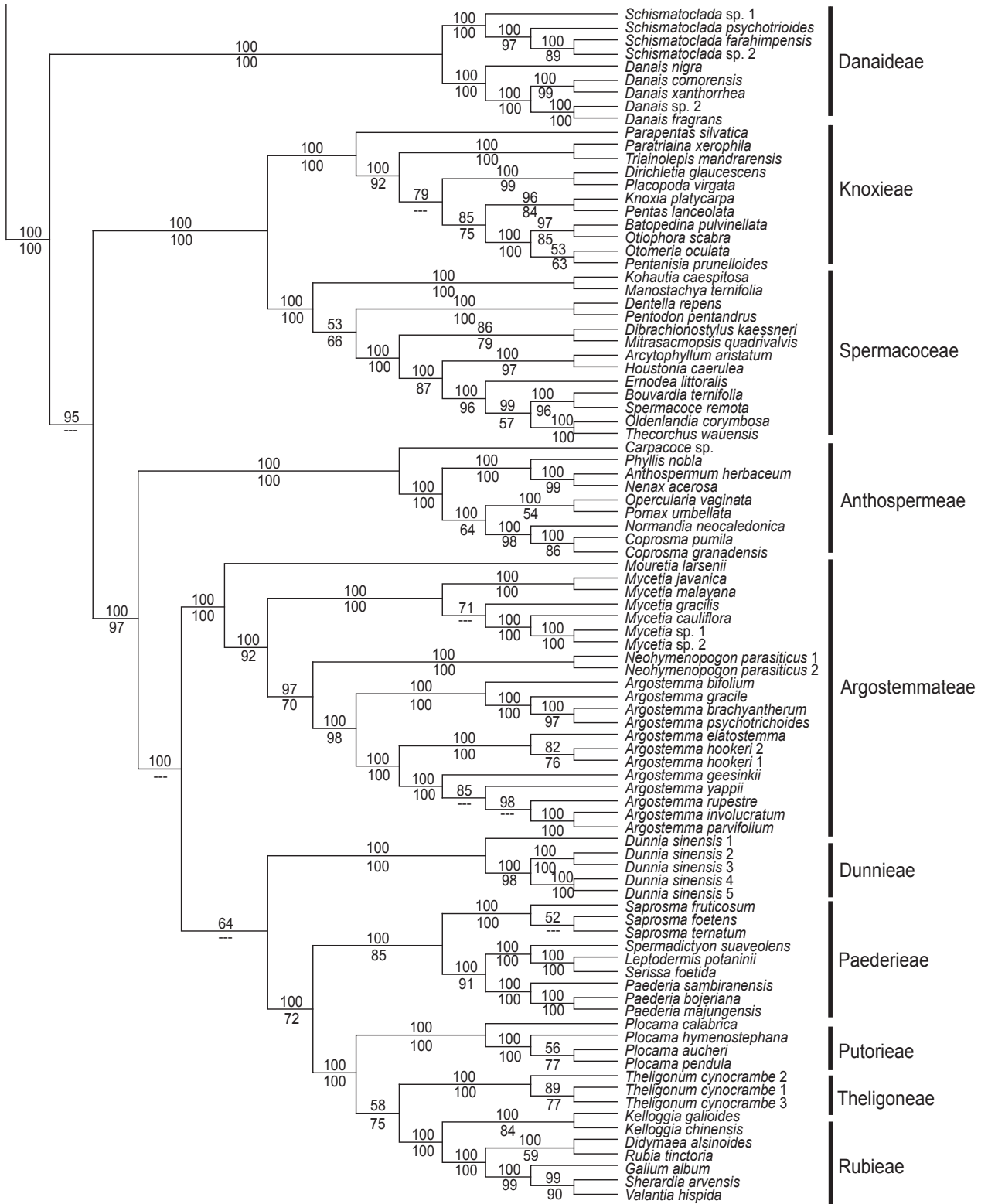**Fig. 1B. Phylogeny of the Spermacoceae alliance, estimated using Bayesian inference of phylogeny (six-data partitions) based on molecular data from chloroplast regions *rbcL, rps16* intron*, ndhF, atpB-rbcL* spacer, *trnT-L-F* and from the nuclear ribosomal ITS. Posterior probabilities are given above branches, bootstrap values (under parsimony) are mapped on this Bayesian tree (below branches).**

Rutishauser & al., 1998, for a discussion of this) and the systematic affinity of the genus has been much debated, even its inclusion in Rubiaceae. Robbrecht & Manen (2006) argue, based on investigations in Dessein & al. (2005), that pollen morphology supports the relationship between Theligoneae and Rubieae. They maintain that Paederieae and Putorieae (and *Kelloggia*) have retained ancestral 3-colpate pollen, whereas evolutionary changes have occurred, to 4–8-porate grains in *Theligonum* and to pluricolpate pollen in Rubieae (Robbrecht & Manen, 2006). However, because *Kelloggia* is sister to Rubieae, an inferred change to several apertures in the common ancestor of Theligoneae and Rubiaceae requires a reversal back to 3-colpate pollen in *Kelloggia*. Additional data, not least from remaining species of *Theligonum*, are needed to further assess relationships and character evolution in the Putorieae-Theligoneae-Rubieae clade.

Rate heterogeneity. — We find indications that the mostly herbaceous clade, the Spermacoceae alliance, overall has higher substitution rates (longer branches) than its mostly woody sister clade the Psychotrieae alliance (Fig. 2). Danaideae, here resolved as the earliest diverging clade within the Spermacoceae alliance, has shorter branch lengths than remaining Spermacoceae alliance, but this clade comprises woody species. The statistical significance of these results remains to be tested, but several previous studies have indicated significant rate heterogeneity between annual and perennial lineages (Bousquet & al., 1992; Andreasen & Baldwin, 2001), or a (negative) correlation between generation time and rates of molecular evolution (Gaut & al., 1992). These results may be analogous and coupled to generation time rather than to a herbaceous versus woody plant body. Bousquet & al. (1992) found for example no differences between woody and herbaceous perennials. The link between generation time and molecular evolutionary rate variation, including its theoretical basis in plants, has been questioned (Whittle & Johnston, 2003), but accumulating evidence suggests that rates of evolution is consistently lower in angiosperms with longer generation times, than in angiosperms with shorter generation times (Smith & Donoghue, 2008).

More details on evolutionary relationships in Danaideae. — The close relationship between *Danais* and *Schismatoclada* suggested by Buchner & Puff (1993) was supported by an unpublished *rbcL* sequence, according to Bremer & Manen (2000: 61) and this has later been confirmed by additional molecular data (Rydin & al., 2008). However, previous studies investigated only one specimen of each genus: the single African species *Danais xanthorrhoea* and an unknown species of *Schismatoclada*. We have added new information from three additional species of *Schismatoclada* and we include new Malagasy and Comoro Island species of *Danais*. Both genera are monophyletic as investigated here. *Payera* (suggested to

be related to *Danais* and *Schismatoclada* based on morphology; Buchner & Puff, 1993) is not investigated in the present study.

The 31 recognised species of *Danais* have an interesting distribution; the majority of species are from Madagascar, but a few are from Mauritius and there is one species each from the Comoro Islands (*D. comorensis*) and the African mainland (*D. xanthorrhea*). Because a Malagasy species (*D. nigra*) is sister to remaining *Danais* and the African *Danais xanthorrhea* is nested within the genus (sister to *D. comorensis* from the Comoro Islands), the topology tentatively indicates dispersal from the islands in the Indian Ocean into the African mainland, but this hypothesis needs to be further tested including more species and biogeographical analysis.

Morphology-based conclusions on Anthospermeae supported. — Anthospermeae are herbs, shrubs or small trees with unisexual, wind-pollinated flowers; the fruit can be fleshy or dry and Puff (1982) argued that, e.g., fruit characters and pollination biology (dichogamy) are useful for subdivisions of the ca. 200 species of the group.

We included representatives from eight of the twelve genera of Anthospermeae (Puff, 1982; Bremer & Manen, 2000) and our results confirm many of the hypotheses established by Puff (1982). Based on morphology, Puff (1982) subdivided Anthospermeae into three biogeographically supported subtribes. Our (*Phyllis* + (*Anthospermum-Nenax*)) clade largely corresponds to the afrotropical subtribe Anthospermineae sensu Puff (1982), characterised by unisexual and/or protandrous hermaphroditic flowers and dry, often dehiscent fruits (Puff, 1982). We further find a clade consisting of (*Normandia* + (*Coprosma-Nertera*)), which corresponds to Puff's circumpacific subtribe Coprosminae, and is characterised by unisexual and/or protogynous flowers and more or less fleshy fruits (Puff, 1982). In contrast to Anderson & al. (2001), we further find strong support for Puff's Australian subtribe Operculariinae, here represented by *Opercularia* and *Pomax*, and characterised by umbel- or head-like inflorescences, unisexual to protogynous hermaphroditic flowers and fruits that open by means of an operculum (Puff, 1982).

The only difference between our results and those of Puff (1982) is that the latter study included *Carpacoce* in subtribe Anthospermineae. In Anderson & al. (2001) *Carpacoce* was sister to Knoxieae and they questioned the inclusion of *Carpacoce* in Anthospermeae. However, in Bremer & Eriksson (in press), *Carpacoce* is included in Anthospermeae (higher-level relationships unresolved) and here, the South African (Cape) endemic *Carpacoce* is strongly supported as sister to remaining species in Anthospermeae, a finding that may be in accordance with Puff's (1982) view that the genus is distantly related to the other genera in Anthospermineae.

Mostly woody species

Woody species

Mostly herbaceous species

Colletoecema dewevrei
Amphidasya ambigua
Pauridiantha paucinervis
Pauridiantha symplocoides
Urophyllum ellipticum
Maschalocorymbus corymbosus
Pravinaria leucocarpa
Urophyllum arboreum
Neurocalyx zeylanicus
Neurocalyx championii
Xanthophytum capitellatum
Xanthophytum borneense
Lerchea bracteata
Spiradiclis bifida
Ophiorrhiza elmeri
Ophiorrhiza mungos
Lasianthus strigosus
Lasianthus lanceolatus
Lasianthus kilimandscharicus
Lasianthus pedunculatus
Saldinia sp. 1
Saldinia sp. 2
Trichostachys aurea
Trichostachys sp.
Faramea multiflora
Coussarea hydrangeifolia
Coussarea macrophylla
Cruckshanksia hymenodon
Coccocypselum condalia
Coccocypselum hirsutum
Declieuxia cordigera
Declieuxia fruticosa
Schizocolea linderi 1
Schizocolea linderi 2
Craterispermum sp. 1
Craterispermum sp. 2
Craterispermum laurinum
Palicourea crocea
Psychotria poeppigiana
Margaritopsis acutifolia
Chassalia catatii
Geophila obvallata
Cremocarpon lantzii
Hydnophytum sp.
Psychotria capensis
Psychotria kirkii
Psychotria holtzii
Psychotria amboniana
Psychotria schliebenii
Prismatomeris beccariana
Prismatomeris albidiflora
Prismatomeris sp. 1
Prismatomeris sp. 2
Schradera subandina
Schradera sp. 1
Schradera sp. 2
Schradera sp. 3
Pagamea guianensis
Gaertnera phyllosepala
Gaertnera phyllostachya
Mitchella repens
Damnacanthus indicus
Damnacanthus macrophyllus
Appunia guatemalensis
Morinda citrifolia
Coelospermum monticolum
Gynochthodes coriacea
Gynochthodes sp.
Schismatoclada sp. 1
Schismatoclada psychotrioides
Schismatoclada farahimpensis
Schismatoclada sp. 2
Danais nigra
Danais comorensis
Danais xanthorrhea
Danais sp. 2
Danais fragrans
Parapentas silvatica
Paratriaina xerophila
Triainolepis mandrarensis
Carphalea glaucescens
Placopoda virgata
Knoxia platycarpa
Pentas lanceolata
Batopedina pulvinellata
Otiophora scabra
Otomeria oculata
Pentanisia prunelloides
Kohautia caespitosa
Manostachya ternifolia
Dentella repens
Pentodon pentandrus
Dibrachionostylus kaessneri
Mitrasacmopsis quadrivalvis
Arcytophyllum aristatum
Houstonia caerulea
Ernodea littoralis
Bouvardia ternifolia
Spermacoce remota
Oldenlandia corymbosa
Thecorchus wauensis
Carpacoce sp.
Phyllis nobla
Anthospermum herbaceum
Nenax acerosa
Opercularia vaginata
Pomax umbellata
Normandia neocaledonica
Coprosma pumila
Coprosma granadensis
Mouretia larsenii
Mycetia javanica
Mycetia malayana
Mycetia gracilis
Mycetia cauliflora
Mycetia sp. 1
Mycetia sp. 2
Neohymenopogon parasiticus 1
Neohymenopogon parasiticus 2
Argostemma bifolium
Argostemma gracile
Argostemma brachyantherum
Argostemma psychotrichoides
Argostemma elatostemma
Argostemma hookeri 2
Argostemma hookeri 1
Argostemma geesinkii
Argostemma yappii
Argostemma rupestre
Argostemma involucratum
Argostemma parvifolium
Dunnia sinensis 1
Dunnia sinensis 2
Dunnia sinensis 3
Dunnia sinensis 4
Dunnia sinensis 5
Saprosma fruticosum
Saprosma foetens
Saprosma ternatum
Spermadictyon suaveolens
Leptodermis potaninii
Serissa foetida
Paederia sambiranensis
Paederia bojeriana
Paederia malungensis
Plocama calabrica
Plocama hymenostephana
Plocama aucheri
Plocama pendula
Theligonum cynocrambe 2
Theligonum cynocrambe 1
Theligonum cynocrambe 3
Kelloggia galioides
Kelloggia chinensis
Didymaea alsinoides
Rubia tinctoria
Galium album
Sherardia arvensis
Valantia hispida

0.04

***Saprosma* is sister to Paederieae s.str.** — Relationships among species assigned to Paederieae have long been debated. Puff (1982) considered Paederieae and Anthospermeae closely related and he transferred insect-pollinated genera from Anthospermeae to Paederieae. However, Paederieae was later shown to be paraphyletic (Bremer, 1996; Andersson & Rova, 1999; Bremer & Manen, 2000; Backlund & al., 2007), and Backlund & al. (2007) divided the tribe into a monophyletic Paederieae s.str. and a re-recognised Putorieae comprising the members of the former Paederieae with fruits splitting into two indehiscent, one-seeded mericarps (Backlund & al., 2007). *Saprosma* (included in Paederieae by Puff, 1992; Robbrecht, 1993) was weakly supported as sister to a Putorieae-Rubieae-Theligoneae clade (Backlund & al., 2007).

Our results support relationships among and within Paederieae and Putorieae found in Backlund & al. (2007), however, in contrast to Backlund & al. (2007), we find that the Southeast Asian genus *Saprosma* is strongly supported as sister to Paederieae s.str. *Saprosma* is monophyletic as represented here (*S. foetens, S. fruticosum, S. ternatum*), however, the genus comprises more than 40 species and Xiao & Zhu (2007) suggested that one of them (*Saprosma crassipes*) is nested within *Lasianthus*.

Within remaining Paederieae, *Paederia* is sister to a clade comprising *Spermadictyon, Leptodermis, Serissa*, all woody species with their main distribution in Southeast Asia (*Paederia* has a pantropical distribution). The close relationship between *Spermadictyon, Leptodermis* and *Serissa* was recognised by Puff (1982), based on morphology.

Phylogeny and morphology of Argostemmateae. — Argostemmateae are here shown to comprise *Mouretia, Mycetia, Argostemma* and *Neohymenopogon* (Figs. 1B, 3). A literature survey, based on Wallich (1824), Hooker (1880), Bremer (1989), Tange (1997), Bremer & Manen (2000) and Puff & al. (2005), reveals that *Argostemma, Mouretia, Mycetia* and *Neohymenopogon* all have hermaphroditic flowers, bilocular ovaries (sometimes 3–5-celled in *Mycetia*), fruits crowned by a persistent calyx lobe, and many small seeds (dust seeds). Terminal cymes and stamens inserted near the base of the corolla tube are also ancestral in the group, with a change in *Neohymenopogon* to stamens inserted in the upper part of the corolla. The showy bract, which subtends the inflorescence is unique to *Neohymenopogon* within Argostemmateae, but found in some distantly related rubiaceous tribes (Psychotrieae, Robbrecht, 1988; Hymenodictyeae, Razafimandimbison & Bremer, 2006; Dunnieae, Rydin & al., 2008; see also Classen-Bockhoff,

1996). *Mouretia, Mycetia* and *Neohymenopogon* are all restricted to Southeast Asia. *Argostemma* has a disjunct, palaeotropical distribution; most species occur in tropical Asia, but two are from the African mainland. In the sister clade of Argostemmateae (i.e., Dunnieae-Paederieae-Putorieae-Theligoneae-Rubieae), several genera have a broad palaeotropical or pantropical distribution (e.g., *Paederia, Plocama*), sometimes even extending into temperate regions (e.g., *Galium, Sherardia*).

Mycetia is sister to the *Argostemma-Neohymenopogon* clade (Figs. 1B, 3). The relationship between *Mycetia* and *Argostemma* (*Neohymenopogon* not included) was very well supported in previous studies (e.g., Bremer, 1996; Bremer & Manen, 2000), but only a single specimen from each genus was investigated. Here we have included six species of *Mycetia* and twelve species of *Argostemma*, and both genera are monophyletic as represented here. There is little morphological support for the subclade consisting of *Mycetia + Argostemma-Neohymenopogon* (Fig. 3). A possible synapomorphy is the shape of the stipules, with a change from bilobed in *Mouretia* to entire in remaining species but with variable states in *Argostemma* (entire, bilobed–cleft-fringed or leaf-like; Bremer, 1989; Puff & al., 2005).

The relationship between *Argostemma* and *Neohymenopogon* is morphologically manifested in a change
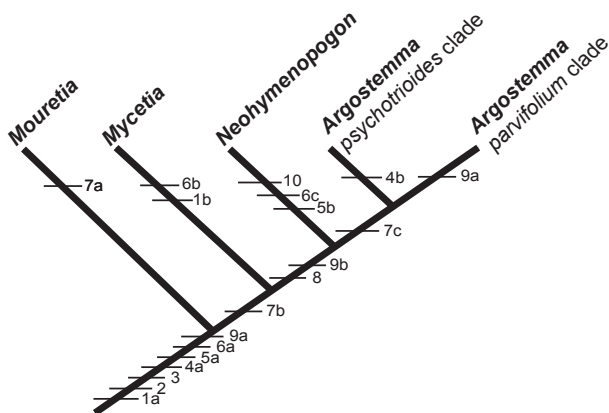

Fig. 3. An illustration to the discussion of the evolution of selected characters in Argostemmateae. *Argostemma* is a large genus and many characters are variable within the genus. We do not cover the full variation here. 1, ovaries, a) bilocular, b) 2–5-celled; 2, fruits crowned by persistent calyx lobes; 3, dust seeds; 4, inflorescence, a) terminal cyme, b) umbelliform; 5, stamens, a) inserted near corolla base, b) higher up; 6, fruit, a) fleshy capsule, b) berry, c) dry capsule; 7, stipules, a) bi-lobed, b) entire, c) variable shape; 8, isostylous flowers; 9, shoots, a) often anisophyllous, b) isophyllous; 10, showy bract subtending the inflorescence.

◄ **Fig. 2.** Rate heterogeneity in Rubioideae as indicated by a phylogram based on the Bayesian analysis of the combined dataset. The mostly woody lineages, the Psychotrieae alliance and Danaideae are marked with bold branches. The mostly herbaceous Spermacoceae alliance (except Danaideae) is marked with thin branches. Gray indicates outgroups.

to isostylous flowers (Fig. 3). Many plants in Rubioideae are heterostylous (e.g., Verdcourt, 1958: 227) and so are most species of *Mouretia* (Tange, 1997) and *Mycetia* (Puff & al., 2005). The *Argostemma-Neohymenopogon* clade might be further supported by a change to isophyllous shoots from mostly anisophyllous, which appear ancestral in Argostemmateae, and by an epiphytic habit. The latter characters are variable within the genera though, not least in *Mycetia* and *Argostemma*.

Fruit characters are variable in Argostemmateae, but the fruit is generally crowned by the persistent calyx lobes in all genera (occurs also in other rubiaceous plants). *Mouretia* fruits are fleshy capsules, which open by an operculum (Tange, 1997) and the fruits become aggregated. The fruits of *Mycetia* are baccate and indehiscent, but crowned by small calyx lobes. *Argostemma* has retained the ancestral state with a fleshy capsule, which opens by an operculum, but *Neohymenopogon* has a capsule that splits into valves. There are no reports of fleshiness in the fruits of *Neohymenopogon* (mature fruits were not available to us).

Our results provide a first attempt to test the monophyly of *Argostemma*, as well as hypotheses on intra-generic relationships based on morphology, using molecular data. Bremer (1989) studied *Argostemma* in Borneo and argued that the species could be divided into at least three major subclades, of which we have included specimens from two: the parvifolium group and the psychotrioides group.

The parvifolium group (*Argostemma parvifolium* and *A. elatostemma* with a broader distribution in Southeast Asia, and the Borneo endemics *A. rupestre* and *A. geesinkii* plus eight more species not included in the present study) is defined, e.g., by anisophyllous shoots, rotate corollas and a glabrous style (Bremer, 1989). It is, as represented here, very well supported by molecular data (Fig. 1B) and further includes three species not investigated in Bremer (1989): *A. parvifolium* var. *involucratum*, *A. yappii* and *A. hookeri*, all from Malaysia and Sumatra. The clade appears to ancestrally have had a broad distribution in Southeast Asia.

The psychotrioides group (*Argostemma psychotrioides*, *A. gracile*, *A. brachyantherum* and eleven additional species not investigated here) is also very well supported by molecular data (Fig. 1B). Morphologically, it is defined, e.g., by umbelliform inflorescences and the style is often much longer than the stamens (Bremer, 1989). All species of the psychotrioides group are restricted to Borneo, but its sister-taxon (*Argostemma bifolium*) is from Peninsular Malaysia.

Conclusions. — This study presents new insights into the phylogeny of the Spermacoceae alliance (Rubiaceae). Some genera (*Neohymenopogon* and *Mouretia*) are sequenced and analysed in a modern cladistic framework for the first time and some previous uncertainties (e.g., the position of *Saprosma* and relationships within Anthospermeae) are also resolved with strong support;

Neohymenopogon and *Mouretia* belong to Argostemmateae, *Saprosma* is sister to Paederieae s.str., *Carpacoce* is sister to the remaining species in Anthospermeae. The results further indicate a possible correlation between molecular substitution rate and generation time, something which has been questioned and considered controversial for plants.

However, our results also reveal conflicts and perhaps difficult phylogenetic problems. The small tribe Danaideae is here sister to the remaining Spermacoceae alliance with high posterior probability, but the topology conflicts with well-supported results in a recent study (Rydin & al., 2009). Theligoneae and Dunnieae are additional examples of small isolated clades, which represent unique lineages within the Spermacoceae alliance, also in terms of morphology (see, e.g., Rutishauser & al., 1998; Rydin & al., 2008). The uncertainty of their relationships to other tribes in the group is emphasised in the present study, mainly due to conflicting information.

Because of the apparent conflicts between results from different loci in the chloroplast genome, it might conceivably be difficult to confidently resolve these phylogenetic problems only by analysing larger sets of molecular data. Detailed morphological and histological information from reproductive structures (i.e., from serial sectioning and SEM investigations) may allow to discriminate between alternative hypotheses indicated by molecular data. Gross morphological characters are, however, less likely to be useful because of the great diversity of life forms, morphology and ecology among the numerous species of Rubiaceae.

■ TAXONOMIC IMPLICATIONS

Based on the new results we re-define the tribe Argostemmateae to comprise four genera: *Argostemma*, *Mouretia*, *Mycetia* and *Neohymenopogon*. Robbrecht (1993) transferred *Saprosma* to Paederieae based on morphological observations by Puff (1992) but Paederieae sensu Robbrecht (1993) comprised genera from the Putorieae-Rubieae-Theligoneae clade as well as from Paederieae s.str. We re-define Paederieae s.str. to comprise *Leptodermis*, *Paederia*, *Saprosma*, *Serissa* and *Spermadictyon*.

Family – Rubiaceae Juss.
Subfamily – Rubioideae Verdc.

Tribe Argostemmateae Bremek. ex Verdc. in Bull. Jard. Bot. État Bruxelles 28: 281. 1958 – Type: *Argostemma* Wall. in W. Roxburgh (1824).

Description: Herbs (mainly), or shrubs to small treelets, iso- or anisophyllous. Stipules entire or slightly cleft, rarely leaf-like. Flowers hermaphroditic. Stamens usually inserted at base of the corolla or higher up (in

Neohymenopogon), adnate into an anther cone or free. Anthers open with vertical slits or rarely with pores. Ovary 2(–6)-locular. Fruit a capsule (often fleshy) or a berry (*Mycetia*), with many small seeds.

Genera included: *Argostemma, Mouretia, Mycetia, Neohymenopogon*.

Useful studies: Wallich (1824), Hooker (1880), Bremer (1989), Puff & al., (1995), Tange (1997), Bremer & Manen (2000), Puff & al., (2005), the present study.

Tribe Paederieae DC., Prodr. 4: 342, 470. 1830 – Type: *Paederia* L. (1767).

Description: Small shrubs or climbers (*Paederia*), often foetid. Stipules divided (*Paederia*) or entire. Flowers 4- (*Saprosma*) to 5-merous, stamens inserted in the corolla tube, ovary 2–5-locular, with a single erect ovule in each locule. Fruits dry, dehiscent into valves (*Leptodermis, Paederia, Spermadictyon*), or fleshy, indehiscent (*Saprosma, Serissa*).

Genera included: *Leptodermis, Paederia, Saprosma, Serissa, Spermadictyon*.

Useful studies: Puff (1982), Robbrecht (1982), Bremer & Manen (2000), Robbrecht & Manen (2006), Backlund & al., (2007), the present study.

ACKNOWLEDGMENTS

LITERATURE CITED

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. Pp. 267–281 in: Petrov, B.N. & Csaki, F. (eds), *Second International Symposium of Information Theory.* Akademiai Kiado, Budapest.

Alejandro, G.D., Razafimandimbison, S.G. & Liede-Schumann, S. 2005. Polyphyly of *Mussaenda* inferred from ITS and *trnT-F* data and its implication for generic limits in Mussaendeae (Rubiaceae). *Amer. J. Bot.* 92: 544–557.

Anderson, C.L., Rova, J.H.E. & Andersson, L. 2001. Molecular phylogeny of the tribe Anthospermeae (Rubiaceae): systematic and biogeographic implications. *Austral. Syst. Bot.* 14: 231–244.

Andersson, L. 2001. *Margaritopsis* (Rubiaceae, Psychotrieae) is a pantropical genus. *Syst. Geogr. Pl.* 71: 73–85.

Andersson, L. 2002. Relationships and generic circumscription in the *Psychotria* complex (Rubiaceae, Psychotrieae). *Syst. Geogr. Pl.* 72: 167–202.

Andersson, L. & Antonelli, A. 2005. Phylogeny of the tribe Cinchoneae (Rubiaceae), its position in Cinchonoideae, and description of a new genus, *Ciliosemina. Taxon* 54: 17–28.

Andersson, L. & Persson, C. 1991. Circumscription of the tribe Cinchoneae (Rubiaceae)—a cladistic approach. *Pl. Syst. Evol.* 178: 65–94.

Andersson, L. & Rova, J.H.E. 1999. The *rps16* intron and the phylogeny of Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 214: 161–186.

Andreasen, K. & Baldwin, B.G. 2001. Unequal evolutionary rates between annual and perennial lineages of checker mallows (*Sidalcea*, Malvaceae): evidence from 18S-26S rDNA internal and external transcribed spacers. *Molec. Biol. Evol.* 18: 936–944.

Backlund, M., Bremer, B. & Thulin, M. 2007. Paraphyly of Paederieae, recognition of Putorieae and expansion of *Plocama* (Rubiaceae-Rubioideae). *Taxon* 56: 315–328.

Backlund, M., Oxelman, B. & Bremer, B. 2000. Phylogenetic relationships within the Gentianales based on *ndhF* and *rbcL* sequences, with particular reference to the Loganiaceae. *Amer. J. Bot.* 87: 1029–1043.

Bennet, S.S.R. 1981. Rubiaceae. *Indian Forester* 107: 436–437.

Bousquet, J., Strauss, S.H., Doerksen, A.H. & Price, R.A. 1992. Extensive variation in evolutionary rate of *rbcL* gene-sequences among seed plants. *Proc. Natl. Acad. Sci. U.S.A.* 89: 7844–7848.

Bremekamp, C.E.B. 1952. The African species of *Oldenlandia* L. sensu Hiern et K. Schumann. *Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2* 48: 1–297.

Bremekamp, C.E.B. 1966. Remarks on the position, the delimitation and the subdivision of the Rubiaceae. *Acta Bot. Neerl.* 15: 1–33.

Bremer, B. 1989. The genus *Argostemma* (Rubiaceae-Argostemmateae) in Borneo. *Ann. Missouri Bot. Gard.* 76: 7–49.

Bremer, B. 1996. Phylogenetic studies within Rubiaceae and relationships to other families based on molecular data. *Opera Bot. Belg.* 7: 33–50.

Bremer, B., Andreasen, K. & Olsson, D. 1995. Subfamilial and tribal relationships in the Rubiaceae based on *rbcL* sequence data. *Ann. Missouri Bot. Gard.* 82: 383–397.

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Källersjö, M. & Barkhordarian, E. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molec. Phylog. Evol.* 24: 274–301.

Bremer, B. & Eriksson, T. 2009. Timetree of Rubiaceae—phylogeny and dating the family, subfamilies and tribes. *Int. J. Pl. Sci.* 170: 766–793.

Bremer, B., Jansen, R.K., Oxelman, B., Backlund, M., Lantz, H. & Kim, K. 1999. More characters or more taxa for a robust phylogeny–case study from the coffee family (Rubiaceae). *Syst. Biol.* 48: 413–435.

Bremer, B. & Manen, J.F. 2000. Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 225: 43–72.

Bremer, B. & Thulin, M. 1998. Collapse of Isertieae, re-establishment of Mussaendeae, and a new genus of Sabiceeae (Rubiaceae); phylogenetic relationships based on *rbcL* data. *Pl. Syst. Evol.* 211: 71–92.

Buchner, R. & Puff, C. 1993. The genus complex *Danais-Schismatoclada-Payera* (Rubiaceae). Characters states, generic delimitation and taxonomic position. *Adansonia* 1–4: 23–74.

Burnham, K.P. & Anderson, D.R. 2003. *Model Selection and Multimodel Inference: A Practical Information-theoretic Approach*, 2nd ed. Springer, New York.

Chen, W.C. 1999. *Dunnia* Tutch. Pp. 233–235, plate 258 in: Lo, H.S., (ed.), *Flora Reipublicae Popularis Sinicae*, vol. 71/2. Science Press, Beijing. [In Chinese.]

Church, S.A. 2003. Molecular phylogenetics of *Houstonia* (Rubiaceae): descending aneuploidy and breeding system evolution in the radiation of the lineage across North America. *Molec. Phylog. Evol.* 27: 223–238.

Church, S.A. & Taylor, D.R. 2005. Speciation and hybridization among *Houstonia* (Rubiaceae) species: the influence of polyploidy on reticulate evolution. *Amer. J. Bot.* 92: 1372–1380.

Classen-Bockhoff, R. 1996. A survey of flower-like inflorescences in the Rubiaceae. *Opera Bot. Belg.* 7: 329–367.

Dessein, S., Ochoterena, H., De Block, P., Lens, F., Robbrecht, E., Schols, P., Smets, E., Vinckier, S. & Huysmans, S. 2005. Palynological characters and their phylogenetic signal in Rubiaceae. *Bot. Rev.* 71: 354–414.

Galtier, N., Gouy, M. & Gautier, C. 1996. SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput. Appl. Biosci.* 12: 543–548.

Gaut, B.S., Muse, S.V., Clark, W.D. & Clegg, M.T. 1992. Relative rates of nucleotide substitution at the *rbcL* locus of monocotyledonous plants. *J. Molec. Evol.* 35: 292–303.

Ge, X.J., Chiang, Y.C., Chou, C.H. & Chiang, T.Y. 2002. Nested clade analysis of *Dunnia sinensis* (Rubiaceae), a monotypic genus from China based on organelle DNA sequences. *Cons. Genet.* 3: 351–362.

Govaerts, R., Andersson, L., Robbrecht, E., Bridson, D., Davis, A., Schanzer, I. & Sonke, B. 2006. *World Checklist of Rubiaceae*. The Board of Trustees of the Royal Botanic Gardens, Kew.

Groeninckx, I., Dessein, S., Ochoterena, H., Persson, C., Motley, T., Kårehed, J., Bremer, B., Huysmans, S. & Smets, E. 2009 Phylogeny of the herbaceous tribe Spermacoceae (Rubiaceae) based on plastid DNA data. *Ann. Missouri Bot. Gard.* 96: 109–132.

Guindon, S. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52: 696–704.

Hasegawa, M., Kishino, H. & Yano, T. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Molec. Evol.* 22: 160–174.

Hooker, J.D. 1880. *Flora of British India*. Reeve, London.

Huelsenbeck, J.P. & Ronquist, F.R. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Kårehed, J. & Bremer, B. 2007. The systematics of Knoxieae (Rubiaceae)—molecular data and their taxonomic consequences. *Taxon* 56: 1051–1076.

Kårehed, J., Groeninckx, I., Dessein, S., Motley, T. & Bremer, B. 2008. The phylogenetic utility of chloroplast and nuclear DNA markers and the phylogeny of the Rubiaceae tribe Spermacoceae. *Molec. Phylog. Evol.* 49: 843–866.

Manen, J.F. & Natali, A. 1995. Comparison of the evolution of ribulose-1,5-biphosphate carboxylase (*rbcL*) and *atpB-rbcL* noncoding spacer sequences in a recent plant group, the tribe Rubieae (Rubiaceae). *J. Molec. Evol.* 41: 920–927.

Manen, J.F., Natali, A. & Ehrendorfer, F. 1994. Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergene region. *Pl. Syst. Evol.* 190: 195–211.

Mouly, A., Razafimandimbison, S.G., Achille, F., Haevermans, T. & Bremer, B. 2007. Phylogenetic placement of *Rhopalobrachium fragrans* (Rubiaceae): evidence from molecular (*rps16* and *trnT-F*) and morphological data. *Syst. Bot.* 32: 872–882.

Nakamura, K., Chung, S.W., Kokubugata, G., Denda, T. & Yokota, M. 2006. Phylogenetic systematics of the monotypic genus *Hayataella* (Rubiaceae) endemic to Taiwan. *J. Pl. Res.* 119: 657–661.

Natali, A., Manen, J.F. & Ehrendorfer, F. 1995. Phylogeny of the Rubiaceae Rubioideae, in particular the tribe Rubieae—evidence from a noncoding chloroplast DNA-sequence. *Ann. Missouri Bot. Gard.* 82: 428–439.

Nepokroeff, M., Bremer, B. & Sytsma, K.J. 1999. Reorganization of the genus *Psychotria* and tribe Psychotrieae (Rubiaceae) inferred from ITS and *rbcL* sequence data. *Syst. Bot.* 24: 5–27.

Nie, Z.L., Wen, J., Sun, H. & Bartholomew, B. 2005. Monophyly of *Kelloggia* Torrey ex Benth. (Rubiaceae) and evolution of its intercontinental disjunction between western North America and eastern Asia. *Amer. J. Bot.* 92: 642–652.

Novotny, V., Basset, Y., Miller, S.E., Weiblen, G.D., Bremer, B., Cizek, L. & Drozd, P. 2002. Low host specificity of herbivorous insects in a tropical forest. *Nature* 416: 841–844.

Nylander, J.A.A. 2004a. MrAIC.pl. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, Uppsala. http://www.abc.se/~nylander/.

Nylander, J.A.A. 2004b. MrModeltest v2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, Uppsala. http://www.abc.se/~nylander/.

Oxelman, B. 1996. RAPD patterns, nrDNA ITS sequences and morphological patterns in *Silene* section Sedoineae (Caryophyllaceae). *Pl. Syst. Evol.* 201: 93–116.

Oxelman, B., Liden, M. & Berglund, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* 206: 393–410.

Piesschaert, F., Andersson, L., Jansen, S., Dessein, S., Robbrecht, E. & Smets, E. 2000. Searching for the taxonomic position of the African genus *Colletoecema* (Rubiaceae): morphology and anatomy compared to an *rps16*-intron analysis of the Rubioideae. *Canad. J. Bot.* 78: 288–304.

Pitard, J. 1922. Rubiaceae. Pp. 71 in: Lecomte, P.H. (ed.), *Flore Génerale de L'Indo-Chine*. Masson et Cie, Paris.

Popp, M. & Oxelman, B. 2001. Inferring the history of the polyploid *Silene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. *Molec. Phylog. Evol.* 20: 474–481.

Posada, D. & Buckley, T.R. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Syst. Biol.* 53: 793–808.

Posada, D. & Crandall, K.A. 2001. Selecting the best-fit model of nucleotide substitution. *Syst. Biol.* 50: 580–601.

Puff, C. 1982. The delimitation of the tribe Antospermeae and its affinities to the Paederieae (Rubiaceae). *Bot. J. Linn. Soc.* 84: 355–377.

Puff, C. 1992. On the correct tribal position of *Saprosma* Bl. (Rubiaceae). Pp. 34 in: *Second Flora Malesiana Symposium, Yogyakarta 7–12 September 1992. Programme & Summaries of Papers and Posters.* Herbarium Bogoriense, Bogor. [Abstract.]

Puff, C., Chayamarit, K. & Chamchumroon, V. 2005. *Rubiaceae of Thailand: A Pictorial Guide to Indigenous and Cultivated Genera.* The Forest Herbarium, National Park, Wildlife and Plant Conservation Department, Bangkok.

Puff, C., Igersheim, A., Buchner, R. & Rohrhofer, U. 1995. United stamens of Rubiaceae. Morphology, anatomy; their role in pollination ecology. *Ann. Missouri Bot. Gard.* 82: 357–382.

Rambaut, A. 1996. Se-Al: Sequence Alignment Editor. Available at http://evolve.zoo.ox.ac.uk.

Razafimandimbison, S.G. & Bremer, B. 2006. Taxonomic revision of the tribe Hymenodictyeae (Rubiaceae, Cinchonoideae). *Bot. J. Linn. Soc.* 152: 331–386.

Razafimandimbison, S.G., Rydin, C. & Bremer, B. 2008. Evolution and trends in the Psychotrieae alliance (Rubiaceae)—a rarely reported evolutionary change of many-seeded carpels from one-seeded carpels. *Molec. Phylog. Evol.* 48: 207–223.

Robbrecht, E. 1982. Pollen morphology of the tribes Anthospermeae and Paederieae (Rubiaceae) in relation to taxonomy. *Bull. Jard. Bot. Natl. Belg.* 52: 349–366.

Robbrecht, E. 1988. Tropical woody Rubiaceae. *Opera Bot. Belg.* 1: 1–271.

Robbrecht, E. 1993. Supplement to the 1988 outline of the classification of the Rubiaceae. Index to genera. Pp. 173–196 in: Robbrecht, E. (ed.), *Advances in Rubiaceae Macrosystematics.* Opera Botanica Belgica 6. National Botanic Garden of Belgium, Meise.

Robbrecht, E. & Manen, J.-F. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-trnF* and *atpB-rbcL* data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. *Syst. Geogr. Pl.* 76: 85–146.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Rova, J.H.E., Delprete, P.G., Andersson, L. & Albert, V.A. 2002. A *trnL-F* cpDNA sequence study of the Condamineeae-Rondeletieae-Sipaneeae complex with implications on the phylogeny of the Rubiaceae. *Amer. J. Bot.* 89: 145–159.

Rutishauser, R., Ronse Decraene, L.P., Smets, E. & Mendoza-Heuer, I. 1998. *Theligonum cynocrambe*: developmental morphology of a peculiar rubiaceous herb. *Pl. Syst. Evol.* 210: 1–24.

Rydin, C., Kainulainen, K., Razafimandimbison, S.G., Smedmark, J.E.E. & Bremer, B. 2009. Deep divergences in the coffee family and the systematic position of *Acranthera*. *Pl. Syst. Evol.* 278: 101–123.

Rydin, C., Razafimandimbison, S.G. & Bremer, B. 2008.

Rare and enigmatic genera (*Dunnia*, *Schizocolea*, *Colletoecema*), sisters to species-rich clades: phylogeny and aspects of conservation biology in the coffee family. *Molec. Phylog. Evol.* 48: 74–83.

Schwartz, G. 1978. Estimating the dimensions of a model. *Ann. Statist.* 6: 461–464.

Smedmark, J.E.E., Rydin, C., Razafimandimbison, S.G., Khan, S.A., Liede-Schumann, S. & Bremer, B. 2008. A phylogeny of Urophylleae (Rubiaceae) based on *rps16* intron data. *Taxon* 57: 24–32.

Smith, S.A. & Donoghue, M.J. 2008. Rates of molecular evolution are linked to life history in flowering plants. *Science* 322: 86–89.

Sonké, B., Dessein, S., Taedoumg, H., Groeninckx, I. & Robbrecht, E. 2008. A new speices of *Colletoecema* (Rubiaceae) from southern Cameroon with a discussion of relationships among basal Rubioideae. *Blumea* 53: 533–547.

Staden, R. 1996. The Staden sequence analysis package. *Molec. Biotechnol.* 5: 233–241.

Struwe, L., Thiv, M., Kadereit, J.W., Pepper, A.S.-R., Motley, T.J., White, P.J., Rova, J.H.E., Potgieter, K. & Albert, V.A. 1998. *Saccifolium* (Saccifoliaceae), an endemic of Sierra de la Neblina on the Brazilian-Venezuelan border, is related to temperate-alpine lineages of Gentianaceae. *Harvard Pap. Bot.* 3: 199–214.

Swofford, D.L. 1998. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Tange, C. 1997. A revision of the genus *Mouretia* (Rubiaceae). *Nord. J. Bot.* 17: 123–132.

Tavare, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. Pp. 57–86 in: Miura, R.M. (ed.), *Some Mathematical Questions in Biology—DNA Sequence Analysis.* American Mathematical Society, Providence.

Thulin, M. & Bremer, B. 2004. Studies in the tribe Spermacoceae (Rubiaceae-Rubioideae): the circumscriptions of *Amphiasma* and *Pentanopsis* and the affinities of *Phylohydrax*. *Pl. Syst. Evol.* 247: 233–239.

Tosh, J., De Block, P., Davis, A.P., Dessein, S., Robbrecht, E. & Smets, E.F. 2008. The tribal placement of the monospecific tropical African genus *Petitiocodon* (Rubiaceae) based on molecular data and morphology. *Blumea* 53: 549–565.

Tutcher, W.J. 1905. Description of some new species, and notes on other Chinese plants. *J. Linn. Soc. Bot.* 37: 58–70.

Verdcourt, B. 1958. Remarks on the classification of the Rubiaceae. *Bull. Jard. Bot. État Bruxelles* 28: 209–281.

Wallich, N. 1824. *Hymenopogon* Wall. Pp. 156–158 in: Roxburgh, W. (ed.), *Flora Indica*. Mission press, Serampore.

Whittle, C.A. & Johnston, M.O. 2003. Broad-scale analysis contradicts the theory that generation time affects molecular evolutionary rates in plants. *J. Molec. Evol.* 56: 223–233.

Xiao, L.Q. & Zhu, H. 2007. Paraphyly and phylogenetic relationships in *Lasianthus* (Rubiaceae) inferred from chloroplast *rps16* data. *Bot. Stud.* 48: 227–232.

Yang, Z. 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molec. Biol. Evol.* 10: 1396–1401.

Appendix. Information on included species and GenBank accessions.

Taxon, **classification, voucher (if applicable),** *rbcL*, *rps16*, *ndhF*, *atpB-rbcL* **spacer,** *trnT/F*, **nrITS (– indicates missing data)**

Amphidasya ambigua (Standl.) Standl., Rub-Urophylleae, –, Y11844[07], AF129271[13], –, EU145337[36], EU145576[36], EU145383[36]; *Anthospermum herbaceum* L. f., Rub-Anthospermeae, –, X83623[01], EU145496[36], AJ236284[09], AJ234028[02], EU145544[36], EU145355[36]; *Arcytophyllum aristatum* Standl., Rub-Spermacoceae, *Bremer 3371* (UPS), AJ288595[02], AF333348[10], FJ695282*, FJ695343*, AF333349[10], AM182061[32]; *Argostemma bifolium* Ridl., Rub-Argostemmateae, *B & K Bremer 1797* (S), FJ695220*, –, FJ695283*, FJ695344*, FJ695396*, FJ695428*; *Argostemma brachyantherum* Stapf, Rub-Argostemmateae, *Beaman 8931* (S), FJ695221*, FJ695252*, FJ695284*, FJ695345*, FJ695397*, FJ695429*; *Argostemma elatostemma* Hook. f., Rub-Argostemmateae, *B & K Bremer 1722* (S), FJ695222*, –, –, FJ695346*, –, –; *Argostemma geesinkii* B. Bremer, Rub-Argostemmateae, *Beaman 8935* (S), FJ695223*, FJ695253*, FJ695285*, FJ695347*, FJ695398*, FJ695430*; *Argostemma gracile* Stapf, Rub-Argostemmateae, *Beaman 8885* (S), FJ695224*, FJ695254*, FJ695286*, FJ695348*, FJ695399*, FJ695431*; *Argostemma hookeri* King Spec. 1, Rub-Argostemmateae, –, Z68788[11], EU145497[36], EU145419[36], AJ234032[02], EU145545[36], EU145356[36]; *Argostemma hookeri* King Spec. 2, Rub-Argostemmateae, *Wanntorp 88-27* (S), FJ695225*, FJ695255*, FJ695287*, FJ695349*, FJ695400*, FJ695432*; *Argostemma involucratum* Hemsl., Rub-Argostemmateae, *Wanntorp 3047* (S), FJ695226*, FJ695256*, FJ695288*, FJ695350*, FJ695401*, FJ695433*; *Argostemma parvifolium* Benn., Rub-Argostemmateae, *B & K Bremer 1811* (S), FJ695227*, FJ695257*, FJ695289*, FJ695351*, FJ695402*, FJ695434*; *Argostemma psychotrichoides* Ridl., Rub-Argostemmateae, *B & K Bremer 1744* (S), FJ695228*, FJ695258*, FJ695290*, FJ695352*, –, FJ695435*; *Argostemma rupestre* Ridl., Rub-Argostemmateae, *B & K Bremer 1675* (S), FJ695229*, FJ695259*, –, FJ695353*, FJ695403*, FJ695437*; *Argostemma yappii* King, Rub-Argostemmateae, *B & K Bremer 1609* (S), FJ695230*, FJ695260*, –, FJ695354*, FJ695403*, FJ695437*; *Batopedina pulvinellata* Robbr., Rub-Knoxieae, *Dessein 264* (BR), AJ288596[02], AM117285[22], FJ695291*, FJ695355*, AM117349[22], AM266989[23]; *Bouvardia ternifolia* (Cav.) Schltdl. (synonym: *Bouvardia glaberrima* Engelm.), Rub-Spermacoceae, *Forbes s.n.* (S), X83626[01], AF002758[05], FJ695292*, X76478[40], DQ359165[03], DQ358884[03]; *Carpacoce* sp. Sond., Rub-Anthospermeae, *B. Bremer & al. 4365, 4385* (UPS), FJ695231*, FJ695261*, FJ695293*, FJ695356*, FJ695404*, FJ695438*; *Chassalia catatii* Drake ex Bremek., Rub-Psychotrieae, –, AM945305[37], AM945331[37], AM945283[37], AM945251[37], AM945363[37], AM945218[37]; *Coccocypselum condalia* Pers., Rub-Coussareeae, –, AM117217[22], EU145499[36], EU145420[36], EU145324[36], EU145547[36], EU145358[36]; *Coccocypselum hirsutum* Bartl. ex DC., Rub-Coussareeae, –, X87145[22], EU145500[36], EU145421[36], EU145325[36], EU145548[36], EU145359[36]; *Coelospermum fragrans* (Montrouz.) Baill. ex Guillaumin (synonym: *Coelospermum monticolum* Baill. ex Guillaumin.), Rub-Morindeae, –, AF331644[10], AF001438[05], AM945255[37], AM945221[37], AM945334[37], AM945194[37]; *Colletoecema dewevrei* (De Wild.) E.M.A. Petit, Rub-Colletoecemeae, –, EU145457[36], AF129272[13], EU145409[36], DQ13171[36], EU145532[36], EU145353[36]; *Coprosma granadensis* Mutis ex L. f. (synonym: *Nertera granadensis* (Mutis ex L. f.) Druce), Rub-Anthospermeae, –, X83654[01], AF002741[05], –, –, AF152623[06], –; *Coprosma pumila* Hook. f., Rub-Anthospermeae, *Forbes s.n.* (S), X87146[01], FJ695262*, FJ695294*, –, FJ695405*, FJ695439*; *Coussarea hydrangeifolia* (Benth.) Benth. & Hook. f. ex Müll.Arg., Rub-Coussareeae, –, EU145460[36], EU145501[36], EU145422[36], EU145326[36], EU145549[36], EU145360[36]; *Coussarea macrophylla* (Mart.) Müll. Arg., Rub-Coussareeae, –, Y11847[07], AF004040[05], –, –, AF152612[06], –; *Craterispermum laurinum* (Poir.) Benth., Rub-Craterispermeae, –, AM945300[37], AM945325[37], AM945276[37], AM945243[37], AM945356[37], AM945212[37]; *Craterispermum* sp. Benth. Spec. 1, Rub-Craterispermeae, –, AM945297[37], AM945273[37], AM945241[37], AM945353[37], AM945209[37]; *Craterispermum* sp. Benth. Spec. 2, Rub-Craterispermeae, –, AM945298[37], AM945313[37], AM945274[37], AM945242[37], AM945354[37], AM945210[37]; *Cremocarpon lantzii* Bremek., Rub-Psychotrieae, –, AM117222[22], AM117296[22], –, –, AM117356[22], –; *Cruckshanksia hymenodon* Hook. & Arn., Rub-Coussareeae, –, AJ288599[02], EU145502[36], –, AJ234004[02], EU145550[36], –; *Damnacanthus indicus* C.F. Gaertn., Rub-Mitchelleae, –, Z68793[11], AF331647[10], AM945256[37], AJ234015[02], AM945335[37], AY514061[29]; *Damnacanthus macrophyllus* Siebold ex Miq., Rub-Mitchelleae, –, AM945285[37], AM945308[37], AM945257[37], AM945222[37], AM945336[37], AM945195[37]; *Danais comorensis* Drake, Rub-Danaideae, *Mouly 687* (P), FJ695232*, FJ695263*, –, FJ695358*, FJ695406*, –; *Danais fragrans* (Lam.) Pers., Rub-Danaideae, *Eriksson & al. 966* (S), FJ695233*, FJ695264*, FJ695295*, FJ695359*, FJ695407*, –; *Danais* sp. Comm. ex Vent., Rub-Danaideae, *Eriksson & al. 1032* (S), FJ695234*, FJ695265*, FJ695296*, FJ695360*, FJ695408*, FJ695441*; *Danais nigra* Homolle, Rub-Danaideae, *Kårehed & al. 254* (UPS), FJ695235*, FJ695266*, FJ695297*, FJ695361*, FJ695409*, FJ695441*; *Danais xanthorrhoea* (K. Schum.) Bremek., Rub-Danaideae, –, Z68794[11], AM117297[22], AJ236293[09], AJ234019[02], DQ662138[19], EU145364[36]; *Declieuxia cordigera* Mart. & Zucc. ex Schult. & Schult. f., Rub-Coussareeae, –, AM117224[22], AM117298[22], EU145423[36], EU145327[36], EU145551[36], EU145361[36]; *Declieuxia fruticosa* (Willd. ex Roem. & Schult.) Kuntze, Rub-Coussareeae, –, AJ002177[12], EU145503[36], –, DQ13172[16], EU145552[36], EU145362[36]; *Dentella repens* (L.) J.R. Forst. & G. Forst., Rub-Spermacoceae, –, –, AF333370[10], –, –, AF381540[27], –; *Dibrachionostylus kaessneri* (S. Moore) Bremek., Rub-Spermacoceae, –, AJ616211[17], AF002761[05], –, –, EU145574[36], –; *Didymaea alsinoides* (Cham. & Schltdl.) Standl., Rub-Rubieae, *Keller 1901* (CAS), Z68795[11], –, FJ695298*, AJ234036[02], EU145570[36], FJ695442*; *Dirichletia glaucescens* Hiern (synonym *Carphalea glaucescens* (Hiern) Verdc.), Rub-Knoxieae, *SMP 215* (UPS), Z68789[11], AM117288[22], AJ236287[09], AM117351[22], AM26699323; *Dunnia sinensis* Tutcher Spec. 1, Rub-Dunnieae, –, EU145467[36], EU145515[36], EU145442[36], EU145339[36], EU145583[36], EU145390[36]; *Dunnia sinensis* Tutcher Spec. 2, Rub-Dunnieae, –, EU145468[36], EU145516[36], EU145443[36], EU145340[36], EU145584[36], EU145391[36]; *Dunnia sinensis* Tutcher Spec. 3, Rub-Dunnieae, –, EU145469[36], EU145517[36], EU145444[36], EU145341[36], EU145585[36], EU145392[36]; *Dunnia sinensis* Tutcher Spec. 4, Rub-Dunnieae, –, EU145470[36], EU145518[36], EU145445[36], EU145342[36], EU145586[36], EU145393[36]; *Dunnia sinensis* Tutcher Spec. 5, Rub-Dunnieae, –, EU145471[36], EU145519[36], EU145446[36], EU145343[36], EU145587[36], EU145394[36]; *Ernodea littoralis* Sw., Rub-Spermacoceae, –, AJ288601[02], AF002763[05], –, AJ234025[02], –, –; *Faramea multiflora* A. Rich., Rub-Coussareeae, –, Z68796[11], AF004048[05], EU145424[36], EU145328[36], AF102422[26], EU145363[36]; *Gaertnera phyllosepala* Baker, Rub-Gaertnereae, –, AM945288[37], AM945311[37], AM945261[37], AM945227[37], AM945340[37], AM945200[37]; *Gaertnera phyllostachya* Baker, Rub-Gaertnereae, –, AM945289[37], AM945312[37], AM945262[37], AM945228[37], AM945341[37], AM945201[37]; *Galium album* Mill., Rub-Rubieae, *Bremer 3321* (UPS), X81090[16], AF004050[05], FJ695299*, X76459[40], –, –; *Geophila obvallata* Didr., Rub-Psychotrieae, –, AM117228[22], AF369845[15], AM945259[37], –, EU145569[36], AM945196[37]; *Gynochthodes* sp. Blume, Rub-Morindeae, –, AM945284[37], AM945307[37], AM945254[37], AM945220[37], AM945333[37], AM945193[37]; *Gynochthodes coriacea* Blume, Rub-Morindeae, –, AJ288603[02], AM117311[22], AM945253[37], AM945219[37], AJ847407[25], AM945192[37]; *Houstonia caerulea* L., Rub-Spermacoceae, *Bremer s.n.* (UPS), AJ288604[02], AF333379[10], FJ695300*, FJ695362*, AF381524[27], DQ012706[33], DQ012774[33]; *Hydnophytum formicarum* Jack, Rub-Psychotrieae, –, X83645[01], AF001339[05], –, X76480[40], –, AF034912[12]; *Kelloggia chinensis* Franch., Rub-, –, AY570776[38], AY570771[38], –, AY570765[38], –, –; *Kelloggia galioides* Torr., Rub-, *Garyfield & al. 2437* (UPS), DQ662179[04], DQ662203[04], FJ695301*, AY570768[38], DQ662146[04], FJ695443*; *Knoxia platycarpa* Arn., Rub-Knoxieae, *Lundqvist 11302* (UPS), AJ288631[07], AM266826[23], FJ695302*, FJ695363*, AM117367[22], AM267002[23]; *Kohautia caespitosa* Schnizl., Rub-Spermacoceae, *Bremer & al. 42566B* (UPS), Z68800[11], AM117324[22], FJ695303*, FJ695364*, EU145573[36], FJ695444*; *Lasianthus kilimandscharicus* K. Schum., Rub-Lasiantheae, –, AM117237[22], AM117327[22], EU145426[36], EU145330[36], DQ662147[19], –; *Lasianthus lanceolatus* (Griseb.) Urb., Rub-Lasiantheae, –, AM117238[22], AF004062[05], –, EU145331[36], EU145554[36], EU145367[36]; *Lasianthus pedunculatus* E.A. Bruce, Rub-Lasiantheae, –, Z68802[11], EU145504[36], EU145427[36], AJ234003[02], EU145555[36], EU145368[36]; *Lasianthus strigosus* Wight, Rub-Lasiantheae, –, AM117239[22], EU145505[36], EU145428[36], –, EU145556[36], EU145369[36]; *Leptodermis potaninii* Batalin, Rub-Paederieae, *KA 230 Berkeley* (UCBG), AM117241[22], DQ662204[04], FJ695304*, FJ695365*, DQ662148[04], FJ695445*; *Lerchea bracteata* Valeton, Rub-Ophiorrizeae, –, AJ288610[02], EU145508[36], EU145433[36], AJ233997[02], EU145561[36], EU145374[36]; *Manostachya ternifolia* E.S. Martins, Rub-Spermacoceae, *Bamps & Martins 4410* (UPS), AJ616213[17], AM117328[22], FJ695305*, FJ695366*, EU145572[36], FJ695446*; *Margaritopsis nudiflora* (Griseb.) K. Schum. (synonym: *Margaritopsis acuifolia* C. Wright, Rub-Psychotrieae, –, AM117247[22], AF001340[05], –, AM9452[25], EU145568[36], AM945198[37]; *Maschalocorymbus corymbosus* (Blume) Bremek., Rub-Urophylleae, –, AJ288611[02], AM900611[35], –, –, EU145577[36], EU145384[36]; *Mitchella repens* L., Rub-Mitchelleae, –, Z68805[11], AF001441[05], AM945258[37], AM945223[37], AM945337[37], AB103535[30], AB103536[30]; *Mitrasacmopsis quadrivalvis* Jovet, Rub-Spermacoceae, –, AJ616214[17], AM117329[22], EU145439[36], EU145336[36], EU145575[36],

Appendix. Continued.

EU145382[36]; *Morinda citrifolia* L., Rub-Morindeae, –, AJ318448[14], AJ320078[14], AJ236300[09], AJ234013[02], AF152616[06], AY762843[31]; *Morinda guatemalensis* (Donn. Sm.) Steyerm. (synonym: *Appunia guatemalensis* Donn. Sm.), Rub-Morindeae, –, AJ288593[02], AM945306[37], AM945252[37], AJ234009[02], AM945332[37], AM945191[37]; *Mouretia larsenii* Tange, Rub-Argostemmateae, *Beusekom & al. 4743* (P), FJ695236*, FJ695267*, FJ695306*, FJ695367*, FJ695410*, FJ695447*; *Mycetia cauliflora* Reinw., Rub-Argostemmateae, *Larsen & al. 46287* (AAU), FJ695237*, FJ695268*, FJ695307*, FJ695368*, FJ695411*, FJ695448*; *Mycetia gracilis* Craib, Rub-Argostemmateae, *Larsen & al. 46250* (AAU), FJ695238*, FJ695269*, FJ695308*, FJ695369*, FJ695412*, FJ695449*; *Mycetia javanica* (Blume) Reinw. ex Korth., Rub-Argostemmateae, *Larsen & al. 43970* (AAU), FJ695239*, FJ695270*, FJ695309*, FJ695370*, FJ695413*, FJ695450*; *Mycetia malayana* (G. Don) Craib, Rub-Argostemmateae, *Larsen 42486* (UPS), Z68806[11], AF002771[05], FJ695310*, AJ234033[02], AF152622[06], –; *Mycetia* sp. Reinw. Spec. 1, Rub-Argostemmateae, *Larsen & al. 44635* (AAU), FJ695240*, FJ695271*, FJ695311*, FJ695371*, FJ695414*, FJ695451*; *Mycetia* sp. Reinw. Spec. 2, Rub-Argostemmateae, *Larsen & al. 43706* (AAU), FJ695241*, FJ695272*, FJ695312*, FJ695372*, FJ695415*, FJ695452*; *Nenax acerosa* Gaertn., Rub-Anthospermeae, –, –, AF003606[05], –, –, –, –; *Neohymenopogon parasiticus* (Wall.) Bennet Spec. 1, Rub-Argostemmateae, *Vidal 5729* (P), FJ695242*, FJ695273*, FJ695313*, FJ695373*, FJ695416*, FJ695453*; *Neohymenopogon parasiticus* (Wall.) Bennet Spec. 2, Rub-Argostemmateae, *B. Bremer 2743* (UPS), FJ695243*, FJ695274*, FJ695314*, FJ695374*, FJ695417*, –; *Neurocalyx championii* Benth. ex Thwaites, Rub-Ophiorrizeae, –, EU145463[36], EU145509[36], EU145435[36], –, EU145563[36], EU145376[36]; *Neurocalyx zeylanicus* Hook., Rub-Ophiorrizeae, –, Z68807[11], AM900594[35], EU145434[36], AJ233995[02], EU145562[36], EU145375[36]; *Normandia neocaledonica* Hook. f., Rub-Anthospermeae, *Munzinger 532* (MO), AM117250[22], AF257931[18], FJ695315*, FJ695375*, EU145543[36], AF257930[18]; *Oldenlandia corymbosa* L., Rub-Spermacoceae, –, X83655[01], AF333381[10], AJ130837[09], –, AF381537[27], AY854053[34]; *Opercularia vaginata* Juss., Rub-Anthospermeae, *K. Bremer & Gustafsson 25* (UPS), Z68809[11], AF257936[18], FJ695316*, FJ695376*, FJ695418*, AF257935[18]; *Ophiorrhiza elmeri* Merr., Rub-Ophiorrizeae, –, EU145464[36], EU145510[36], EU145436[36], –, EU145564[36], EU145378[36]; *Ophiorrhiza mungos* L., Rub-Ophiorrizeae, –, X83656[01], AF004064[05], AJ130838[09], –, DQ66215[16], EU145377[36]; *Otiophora scabra* Zucc., Rub-Knoxieae, *Iversen & Martinson 89078* (UPS), FJ695244*, AM266839[23], –, DQ131756[03], AM266928[23], AM267015[23]; *Otomeria oculata* S. Moore, Rub-Knoxieae, *Puff & al. 82/222-21* (K), AJ288614[02], AM266844[23], FJ695317*, FJ695377*, AM117374[22], AM267019[23]; *Paederia bojeriana* (A. Rich. ex DC.) Drake, Rub-Paederieae, *Razafimandimbison & H. Bremer 483* (UPS), DQ662181[04], DQ662206[04], FJ695318*, DQ131757[03], DQ662152[04], FJ695454*; *Paederia majungensis* Homolle ex Puff, Rub-Paederieae, *Nilsson & al. D152* (UPS), DQ662184[04], DQ662209[04], FJ695319*, FJ695378*, DQ662155[04], –; *Paederia sambiranensis* Homolle ex Puff, Rub-Paederieae, *Kårehed & al. 238* (UPS), DQ662188[04], DQ662213[04], –, FJ695379*, DQ662159[04], –; *Pagamea guianensis* Aubl., Rub-Gaertnereae, –, AM945290[37], AF002744[05], AM945263[37], AM945229[37], AM945342[37], AF333846[62]; *Palicourea crocea* (Sw.) Schult, Rub-Psychotrieae, –, AM117253[22], AF147510[20], AM945280[37], AM945247[37], AM945259[37], AF149322[20]; *Parapentas silvatica* (K. Schum.) Bremek., Rub-Knoxieae, *Bremer 3091* (UPS), X83657[01], AM117332[22], FJ695320*, AJ234021[02], AM117376[22], AM267023[23]; *Paratriaina xerophila* Bremek., Rub-Knoxieae, *Razafimandimbison & Bremer 489* (UPS), AJ28863[02], AM266850[23], FJ695321*, FJ695380*, AM266938[23], AM267024[23]; *Pauridiantha paucinervis* (Hiern) Bremek., Rub-Urophylleae, –, Z68811[11], AM900600[35], AJ236302[09], AJ233998[02], EU145578[36], EU145385[36]; *Pauridiantha symplocoides* (S. Moore) Bremek., Rub-Urophylleae, –, AY538502[08], AF004068[05], EU145440[36], EU145338[36], AF102467[26], EU145386[36]; *Pentanisia prunelloides* (Klotzsch) Walp., Rub-Knoxieae, *B. Bremer & al. 4316* (UPS), AM117255[22], AM266860[23], FJ695322*, FJ695381*, AM266948[23], AM267033[23]; *Pentas lanceolata* (Forssk.) Deflers, Rub-Knoxieae, –, X83659[01], AM117334[22], AJ236304[09], X76479[40], AM117379[22], AB247275[28]; *Pentodon pentandrus* (Schumach. & Thonn.) Vatke, Oesterr., Rub-Spermacoceae, *Bremer 3082* (UPS), X83660[01], AF003612[05], FJ695323*, AJ234024[02], FJ695419*, FJ695455*; *Phyllis nobla* L., Rub-Anthospermeae, *K. Bremer 3008* (UPS), Z68814[11], AF003613[05], FJ695324*, AJ234031[02], AY538468[08], AF257939[18]; *Placopoda virgata* Balf. f., Rub-Knoxieae, *Thulin & Gifri 8528* (UPS), Z68815[11], AM117335[22], FJ695325*, FJ695382*, AM117382[22], AM267064[23]; *Plocama aucheri* (Guill.) M. Backlund & Thulin, Rub-Putorieae, *Thulin 9963* (UPS), DQ662178[04], DQ662202[04], FJ695326*, FJ695383*, DQ662145[04], FJ695456*; *Plocama calabrica* (L. f.) M. Backlund & Thulin, Rub-Putorieae, *Jonsell 4216* (UPS), AJ288620[02], FJ695275*, FJ695327*, FJ695384*, DQ662166[04], FJ695457*; *Plocama hymenostephana* (Jaub. & Spach) M. Backlund & Thulin, Rub-Putorieae, *Thulin 9993* (UPS), DQ662190[04], DQ662215[04], FJ695328*, FJ695385*, DQ662163[04], FJ695458*; *Plocama pendula* Aiton, Rub-Putorieae, *Andreasen 1* (UPS), Z68816[01], FJ695276*, FJ695329*, AJ234035[02], DQ662162[04], FJ695459*; *Pomax umbellata* (Gaertn.) Sol. ex A. Rich., Rub-Anthospermeae, *B. & K. Bremer 3918* (UPS), AM117260[22], AF257941[18], –, DQ131767[03], FJ695420*, AF257940[18]; *Pravinaria leucocarpa* Bremek., Rub-Urophylleae, –, AJ288617[02], AM900613[35], EU145441[36], AJ234001[02], EU145580[36], EU145388[36]; *Prismatomeris albidiflora* Thwaites, Rub-Psychotrieae, –, AM945296[37], AM945320[37], AM945270[37], AM945237[37], AM945351[37], AM945205[37]; *Prismatomeris beccariana* (Baill. ex K. Schum.) J.T. Johanss., Rub-Psychotrieae, –, AJ288618[02], AF331652[10], AM945271[37], AM945238[37], AM945206[37]; *Prismatomeris* sp. Thwaites Spec. 1, Rub-Psychotrieae, –, AM945292[37], AM945316[37], AM945266[37], AM945233[37], AM945347[37], AM945202[37]; *Prismatomeris* sp. Thwaites Spec. 2, Rub-Psychotrieae, –, AM945293[37], AM945317[37], AM945267[37], AM945234[37], AM945348[37], –; *Psychotria amboniana* K. Schum., Rub-Psychotrieae, –, AM945302[37], AM945328[37], AM945281[37], AM945248[37], AM945360[37], AM945215[37]; *Psychotria capensis* (Eckl.) Vatke, Rub-Psychotrieae, –, AM945301[37], AM945326[37], AM945277[37], AM945245[37], AM945357[37], AM945213[37]; *Psychotria holtzii* (K. Schum.) E.M.A. Petit, Rub-Psychotrieae, –, AM945304[37], AM945330[37], –, AM945250[37], AM945362[37], AM945217[37]; *Psychotria kirkii* Hiern, Rub-Psychotrieae, –, X83663[01], AF410728[21], AJ236307[09], X76481[40], AY538469[08], AF072038[12]; *Psychotria poeppigiana* Müll.Arg., Rub-Psychotrieae, –, Z68818[11], AF002748[05], AM945279[37], AJ234018[02], –, AF14940020; *Psychotria schliebenii* E.M.A. Petit, Rub-Psychotrieae, –, AM945303[37], AM945329[37], AM945282[37], AM945249[37], AM945361[37], AM945216[37]; *Rubia tinctorum* L., Rub-Rubieae, *Bremer 3300* (UPS), X83666[01], –, DQ359167[03], X76465[40], FJ695421*, DQ358885[03]; *Saldinia* sp. A. Rich. ex DC. Spec. 1, Rub-Lasiantheae, –, AM117269[22], AF129275[13], EU145429[36], EU145332[36], EU145557[36], EU145370[36]; *Saldinia* sp. A. Rich. ex DC. Spec. 2, Rub-Lasiantheae, –, EU145461[36], EU145506[36], EU145430[36], EU145333[36], EU145558[36], EU145371[36]; *Saprosma foetens* (Wight) K. Schum., Rub-Paederieae, *Klackenberg 325* (S), DQ662193[04], DQ662218[04], –, FJ695386*, DQ662168[04], FJ695460*; *Saprosma fruticosum* Blume, Rub-Paederieae, *Ridsdale IV.E.157* (L), DQ662194[04], FJ695277*, FJ695330*, FJ695387*, DQ662169[04], FJ695461*; *Saprosma ternatum* (Wall.) Hook. f., Rub-Paederieae, –, –, DQ282646[39], –, –, –, –; *Schismatoclada farahimpensis* Homolle, Rub-Danaideae, *Kårehed & al. 267* (UPS), FJ695245*, FJ695278*, FJ695331*, FJ695388*, FJ695422*, FJ695462*; *Schismatoclada* sp. Baker Spec. 1, Rub-Danaideae, *Razafimandimbison 375* (S), AM117271[22], AM117341[22], EU145425[36], EU145329[36], EU145553[36], EU145365[36]; *Schismatoclada* sp. Baker Spec. 2, Rub-Danaideae, *Razafimandimbison & Ravelonarivo 625* (S), FJ695246*, FJ695279*, FJ695332*, FJ695389*, FJ695423*, FJ695463*; *Schismatoclada* aff. *psychotrioides* Baker, Rub-Danaideae, *Eriksson & al. 988* (S), FJ695247*, –, FJ695333*, FJ695390*, FJ695424*, FJ695464*; *Schizocolea linderi* (Hutch. & Dalziel) Bremek. Spec. 1, Rub-Schizocoleeae, *Adam 20116* (UPS), AM117272[22], EU145498[36], FJ695334*, EU145323[36], EU145546[36], EU145357[36]; *Schizocolea linderi* (Hutch. & Dalziel) Bremek. Spec. 2, Rub-Schizocoleeae, *Adam 789* (P), AM945286[37], AM945309[37], FJ695335*, AM945224[37], AM945338[37], AM945197[37]; *Schradera subandina* K. Krause, Rub-Schradereae, –, Y11859[07], AM945313[37], AM945264[37], AJ234014[02], AM945343[37], –; *Schradera* sp. Vahl Spec. 1, Rub-Schradereae, —, –, AF003617[05], –, –, AF152613[06], –; *Schradera* sp. Vahl Spec. 2, Rub-Schradereae, –, —, AM945265[37], AM945230[37], AM945244[37], –, AM945231[37], AM945345[37], –; *Schradera* sp. Vahl Spec. 3, Rub-Schradereae, –, —, AM945303[37], AM945329[37], AM945282[37], AM945249[37], AM945361[37], –; *Serissa japonica* Thunb. (synonym: *Serissa foetida* (L. f.) Lam.), Rub-Paederieae, *Bremer 2717* (UPS), Z68822[11], AF004081[05], FJ695336*, AJ234034[02], AF152618[06], FJ695465*; *Sherardia arvensis* L., Rub-Rubieae, *K. Andreasen 345* (SBT), X81106[16], AF004082[05], FJ695337*, X76458[40], EU145571[36], FJ695466*; *Spermacoce remota* Lam., Rub-Spermacoceae, *Bremer 3062* (UPS), Z68823[11], –, AJ236309[09], –, FJ695425*, FJ695467*; *Spermadictyon suaveolens* Roxb., Rub-Paederieae, *Bremer 3133* (UPS), Z68824[11], DQ662219[04], FJ695338*, FJ695391*, DQ662171[04], FJ695468*; *Spiradiclis bifida* Kurz, Rub-Ophiorrizeae, , EU145465[36], EU145511[36], EU145437[36], –, EU145565[36], EU145379[36]; *Thecorchus wauensis* (Schweinf. ex Hiern) Bremek., Rub-Spermacoceae, *Friis & al. 2560* (C), AM117282[22], AM266901[23], –, FJ695392*, AM266987[23], AM267070[23]; *Theligonum cynocrambe* L. Spec. 1,

Appendix. Continued.

Rub-Theligoneae, –, X83668[01], AF004087[05], –, X81680[24], AF152621[06], –; *Theligonum cynocrambe* L. Spec. 2, Rub-Theligoneae, *Thor 654* (S), FJ695248*, FJ695280*, FJ695339*, FJ695393*, FJ695426*, FJ695469*; *Theligonum cynocrambe* L. Spec. 3, Rub-Theligoneae, Reuterswärd & Forsslund 2 (S), FJ695249*, FJ695281*, FJ695340*, –, FJ695427*, FJ695470*; *Triainolepis mandrarensis* Homolle ex Bremek., Rub-Knoxieae, *Razafimandimbison 521* (UPS), FJ695250*, AM266899[23], FJ695341*, FJ695394*, AM266985[23], AM267068[23]; *Trichostachys aurea* Hiern, Rub-Lasiantheae, –, EU145462[36], EU145507[36], EU145431[36], EU145334[36], EU145559[36], EU145372[36]; *Trichostachys* sp. Hook. f., Rub-Lasiantheae, –, AJ288626[02], AM900595[35], EU145432[36], DQ13179[26], EU145560[36], EU145373[36]; *Urophyllum arboreum* (Reinw. ex Blume) Korth., Rub-Urophylleae, –, –, AM900617[35], –, DQ131793[03], EU145582[36], –; *Urophyllum ellipticum* (Wight) Thwaites, Rub-Urophylleae, –, AJ288627[02], AM900619[35], –, AJ234002[02], EU145581[36], EU145389[36]; *Valantia hispida* L., Rub-Rubieae, *Bremer 3131* (UPS), FJ695251*, AF004090[05], FJ695342*, FJ695395*, AM117385[22], FJ695471*; *Xanthophytum borneense* (Valeton) Axelius, Rub-Ophiorrizeae, –, EU145466[36], EU145513[36], EU145438[36], EU145335[36], EU145567[36], EU145381[36]; *Xanthophytum capitellatum* Ridl., Rub-Ophiorrizeae, –, AJ288628[02], EU145512[36], –, AJ233996[02], EU145566[36], EU145380[29].

*Previously unpublished sequence. Published sequences: 01: Bremer & al. (1995). 02: Bremer & Manen (2000). 03: Manen, J.-F. (GenBank unpublished). 04: Backlund & al. (2000). 05: Andersson & Rova (1999). 06: Rova & al. (2002). 07: Bremer & Thulin (1998). 08: Andersson & Antonelli (2005). 09: Bremer & al. (1999). 10: Andersson, L. (GenBank unpublished). 11: Bremer (1996). 12: Nepokroeff & al. (1999). 13: Piesschaert & al. (2000). 14: Novotny & al. (2002). 15: Andersson (2001). 16: Manen & Natali (1995). 17: Thulin & Bremer (2004). 18: Anderson, C.L. & al. (GenBank unpublished). 19: Backlund, M. (GenBank unpublished). 20: Andersson, L. & Taylor, C. (GenBank unpublished). 21: Andersson (2002). 22: Bremer & Eriksson (2009). 23: Kårehed & Bremer (2007). 24: Natali & al. (1995). 25: Alejandro & al. (2005). 26: Struwe & al. (1998). 27: Church (2003). 28: Nakamura & al. (2006). 29: Ding, P. & al. (GenBank unpublished). 30: Yokoyama, J. & al. (GenBank unpublished). 31: Proujansky, A.D. & Stern, D.L. (GenBank unpublished). 32: Wolff, D. & Liede-Schumann, S. (GenBank unpublished). 33: Church & Taylor (2005). 34: Yuan, C.I. (GenBank unpublished). 35: Smedmark & al. (2008). 36: Rydin & al. (2008, 2009). 37: Razafimandimbison & al. (2008). 38: Nie & al. (2005). 39: Xiao L.-Q. & Zhu H. (GenBank unpublished). 40. Manen & al. (1994).