Evolution and trends in the Psychotrieae alliance (Rubiaceae)—A rarely reported evolutionary change of many-seeded carpels from one-seeded carpels

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ABSTRACT

Bayesian and parsimony analyses of five plastid gene and nrITS regions from 58 Rubioideae (Rubiaceae) taxa further support the sister-group relationship between the African monotypic genus *Schizocolea* and the Psychotrieae alliance sensu Bremer & Manen. Our analyses show that the Psychotrieae alliance can be subdivided into in four well-supported clades: *Schizocolea*, (Schradereae(Gaertnereae(Mitchelleae–Morindeae s.s.))), Palicoureeae–Psychotrieae s.s., and Craterispermeae–Prismatomerideae. The relationships between the latter three clades remain unsettled. Our study further reveals much higher numbers of molecular autapomorphies of the tribes compared with those of molecular synapomorphies of two sister tribes or groups of tribes. Within the newly delimited Psychotrieae alliance a one-seeded carpel was inferred as ancestral and many- and two-seeded carpels evolved once each. We describe Mitchelleae to accommodate *Damnacanthus* and *Mitchella* and restrict Morindeae to include only *Appunia, Coelospermum, Gynochthodes, Morinda, Pogonolobus*, and *Syphonandrium*. Mitchelleae is characterized e.g., by placentae inserted near the top of the septum and a single campylotropous ovule per carpel, while Morindeae s.s. has massive and T-shaped placentae inserted in the middle of the septum and two anatropous ovules per carpel.

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1. Introduction

Rubioideae Verdc. (Verdcourt, 1958), the largest subfamily in the coffee family (Rubiaceae) with ca. 7475 species (Govaerts et al., 2006), is a well-defined monophyletic group that can easily be diagnosed by the presence of raphide idioblasts, valvate corolla aestivation and often heterostylous flowers. Our knowledge of the subfamily has recently been improved as a result of a series of independent molecular phylogenetic studies of Rubiaceae (Bremer, 1996a; Andersson and Rova, 1999; Bremer and Manen, 2000; Robbrecht and Manen, 2006). For examples, the sister-group relationship between the African monotypic genus *Colletoecema* Petit (1963) and the remaining Rubioideae was shown for the first time by Robbrecht and Manen (2006). The tribes Lasiantheae Bremer and Manen (2000) and Coussareeae Hooker (1873) were demonstrated to be successive sister groups to the large Psychotrieae and Spermacoceae alliances (both sensu Bremer and Manen, 2000), which are in turn sisters (e.g., Andersson and Rova, 1999; Bremer and Manen, 2000). Despite the largely congruent results

from these independent studies, there were some noticeable discrepancies such as, the phylogenetic positions of the tribes Urophylleae Bremek. ex Verdc. and Ophiorrhizeae Bremek. ex Verdc. (Verdcourt, 1958) and the tribal delimitations and relationships within the Psychotrieae alliance (see Fig. 1A–D). This latter group is the main focus of the present study.

The Psychotrieae alliance sensu Bremer and Manen (2000) [also known as Psychotrieae s.l., Bremer (1996a); the Gaertnereae-Morindeae–Psychotrieae complex, Andersson and Rova (1999); supertribe Psychotriidinae, Robbrecht and Manen (2006)] is mostly tropical, with the exception of the East Asian *Damnacanthus* C.F.Gaertn. and the North American and East Asian *Mitchella* L., which are restricted to the temperate regions. The alliance is characterized by its fleshy (drupes or berries) fruits, which are considered important food sources for many tropical frugivorous birds (Snow, 1981). It is a predominantly woody group consisting of ca. 3000 species (ca. 1/4 of Rubiaceae) (Govaerts et al., 2006) belonging to ca. 54 genera, which have previously been classified in four to six tribes (see Table 1). These tribes are morphologically distinct and apparently share no obvious morphological synapomorphies. As a result, the tribal relationships within the Psychotrieae alliance have always been unsettled (see Fig. 1A–D) and are the major issues in the recent discussions about the classification

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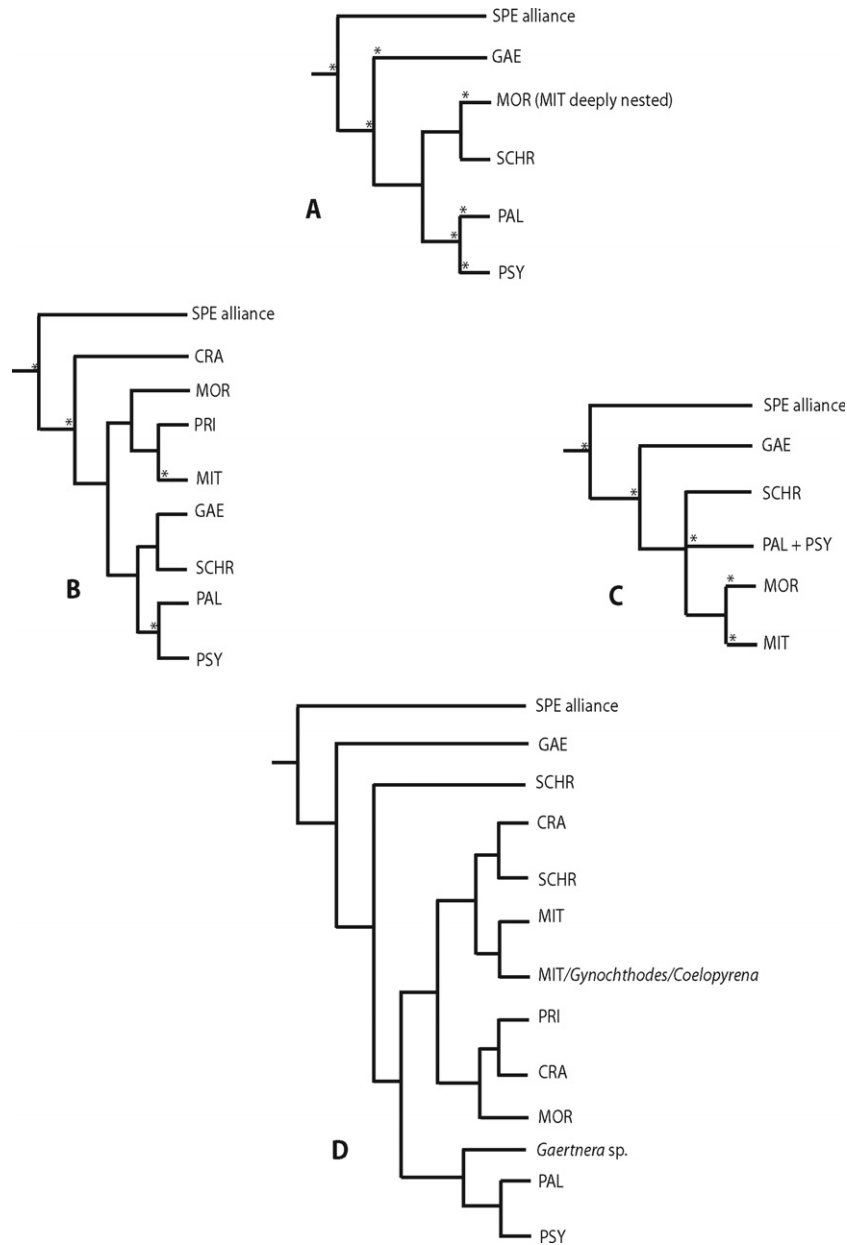
Fig. 1. Intertribal phylogenetic relationships in the Psychotrieae alliance, as inferred in three different studies. CRA, Craterispermeae; GAE, Gaertnereae; MIT, *Mitchella* group; MOR, Morindeae s.s.; PRI, Prismatomerideae; and SCHR, Schradereae. Branches marked by an asterisk correspond to clades supported by bootstrap or jackknife values ⩾80%. (A) *rps*16 tree from Andersson and Rova (1999: Fig. 5); (B) combined *atp*B-*rbc*L/*rbc*L tree from Bremer and Manen (2000: Fig. 3); (C) combined *atp*B-*rbc*L/*rbc*L/*rps*16 tree from Bremer and Manen (2000: Fig. 4); (D) and supertree based *atp*B-*rbc*L/*rbc*L/*rps*16/*trn*L-F data from Robbrecht and Manen (2006: Fig. 4C).

Table 1
Tribal circumscriptions of the Psychotrieae alliance based the recent molecular phylogenetic studies

Andersson and Rova (1999)	Bremer and Manen (2000)	Robbrecht and Manen (2006)	Razafimandimbison et al. (this study)
	Craterispermeae[5]	Craterispermeae[5]	Craterispermeae[5]
Gaertnereae[1]	Gaertnereae[1]	Gaertnereae[1]	Gaertnereae[1]
			Mitchelleae trib. nov.
Morindeae s.l.[2]	Morindeae s.l.[2]	Morindeae s.l.[2]	Morindeae s.s.
		Palicoureeae[6]	Palicoureeae[6]
			Prismatomerideae[7]
Psychotrieae s.l.[3]	Psychotrieae s.l.[3]	Psychotrieae s.s.[6]	Psychotrieae s.s.[6]
Schradereae[4]	Schradereae[4]	Schradereae[4]	Schradereae[4]
			Schizocolea

[1]Darwin (1976); [2]including the *Mitchella* group and Prismatomerideae sensu Robbrecht et al. (1991); [3]including Palicoureeae sensu Robbrecht and Manen (2006); [4]Puff and Buchner (1998); Puff et al. (1998a,b); [5]Verdcourt (1958); [6]Robbrecht and Manen (2006); and [7]Robbrecht et al. (1991).

of Rubioideae. Almost all morphological (including anatomical and palynological) studies focusing on one or more tribes of the Psy-

chotrieae alliance (e.g., Robbrecht et al., 1991; Igersheim, 1992; Puff et al., 1993; Igersheim et al., 1994; Jansen et al., 1996) seemed

in favor of separating the small tribes Craterispermeae Verdc. (Verdcourt, 1958), Gaertnereae Bremek. ex Darwin (Darwin, 1976), Prismatomerideae Ruan (1988) (sensu Robbrecht et al., 1991), and Schradereae Bremekamp (1934) from the large tribes Morindeae Miquel (1857) and Psychotrieae Chamisso and von Schlechtendal (1828). On the other hand, previous molecular phylogenetic studies (Fig. 1A–D) have shown largely inconsistent but mostly poorly supported results regarding the phylogenetic positions of the tribes in the alliance.

It has been widely accepted that uni-seeded carpels and a syndrome of adaptations in fruits and/or seeds go hand in hand. This has been thought so advanced that it excludes a reversal to many ovules (e.g., Stebbins, 1950, 1974). In the Psychotrieae alliance, most of its members have uni-seeded carpels, with the exception of Morindeae s.s. (Igersheim and Robbrecht, 1993) and Schradereae (Puff et al., 1993), respectively, with two- and many-seeded carpels. In Robbrecht and Manen (2006: Fig. 4C), the two sequenced *Schradera* species did not form a clade. *Schradera* sp. was the next lineage to branch off after Gaertnereae and sister to a large clade containing the rest of the Psychotrieae alliance. The other species (*S. subandina*) was nested within the Craterispermeae-Morindeae clade. Based on these results Robbrecht and Manen (2006: 124) argued that the basal position of *Schradera* is more consistent with "morphological facts", because the alternative position would require a reversal from pluri-seeded condition to uni-seeded state, which has been considered to be very unlikely.

Sequence data from *rbc*L and *rps*16 intron have been used alone (Bremer, 1996a; Andersson and Rova, 1999, respectively) or in combination with either morphological data (Piesschaert et al., 2000) or sequence data from other chloroplast gene regions (e.g., *atp*B-*rbc*L, Bremer and Manen, 2000; *atp*B-*rbc*L and *trn*L-F, Robbrecht and Manen, 2006) for reconstructing phylogenies at high taxonomic levels (subfamilies or tribes) in Rubiaceae (see Fig. 1A–D) Phylogenetic analyses of Rubiaceae (Rydin et al., 2008) based on six chloroplast gene regions (four abovementioned markers plus *trn*T-L and *ndh*F) and the internal transcribed spacer region of the nuclear ribosomal DNA (nrITS) demonstrated that combined data from these markers seemed to have enough phylogenetic signals for resolving tribal circumscriptions and relationships within the Psychotrieae alliance. The present study attempted to reconstruct a robust phylogeny for the Psychotrieae alliance based on a much larger sampling and the same five chloroplast and nrITS regions. The resulting phylogenies were used to rigorously assess the phylogenetic relationships and evolutionary trends within the alliance.

2. Materials and methods

2.1. Taxon sampling

We sampled two to six taxa from each of the following groups: Coussareeae, Lasiantheae, Ophiorrhizeae, Urophylleae, and the Spermacoceae alliance. As the present study focused mainly on the Psychotrieae alliance, we made efforts to sample as many representatives as possible of all tribes that we recognize in the Psychotrieae alliance (see Table 1). The monotypic genus *Schizocolea* Bremekamp (1950) was included in our study, as it has recently shown by Rydin et al. (2008) to be sister to the Psychotrieae sensu Bremer and Manen (2000). We failed to obtain reliable sequences from the Southeast Asian genera *Gentingia* J.T.Johanss. & K.M.Wong and *Motleya* J.T.Johanss. (both Prismatomerideae), the Australian genus *Pogonolobus* F. & Muell., the Samoan genus *Sarcopygme* Setchell. & Christophersen, and the New Guinean genus *Syphonandrium* K.Schum. (all Morindeae sensu Darwin, 1979). The monogeneric

African genus *Colletoecema* was chosen as outgroup taxon to root trees, which were generated from single gene and combined analyses based on 58 Rubioideae taxa. Origins and voucher specimens are listed in Table 2.

2.2. DNA extraction, amplification, and sequencing

Total DNA, extracted from leaves dried in silica gel (Chase and Hills, 1991) and/or herbarium material, was isolated following the mini-prep procedure of Saghai-Maroof et al. (1984), as modified by Doyle and Doyle (1987). Isolated DNA was amplified and sequenced according to the protocols outlined in the following articles: Bremer et al. (1995) for *rbc*L, Oxelman et al. (1997) for *rps*16, Rydin et al. (2008) for *atp*B-*rbc*L spacer, Olmstead and Reeves (1995) for *ndh*F, Razafimandimbison and Bremer (2002) for *trn*T-F, and Razafimandimbison and Bremer (2001) and Razafimandimbison et al. (2004) for nrITS. In all PCRs, one reaction was run with water instead of DNA as a negative control to check for contamination. The primers from these previous studies (see Table 3) were used for the nrITS, *rbc*L, *rps*16, and *trn*T-F regions, while two and six primers, all designed by C. Rydin (Table 3), were utilized for *atp*B-*rbc*L and *ndh*F, respectively. All sequencing reactions were performed using the Big Dye® Terminator v3.1 Cycle Sequencing kit and Bid Dye® Terminator v1.1 Cycle Sequencing kit (Applied Biosystems) and subsequently analyzed with the 3100 Genetic Analyzer (Applied Biosystems).

2.3. Data analyses

Sequence fragments were assembled using the Staden package (Staden, 1996). For each marker all new sequences and the published ones taken from the international sequence database were aligned together using the computer program CLUSTAL-X (Thompson et al., 1997) to produce an initial alignment and manually adjusted using software Se-Al v.2.0 (Rambaut, 1996). Insertion/deletion events were inferred by eye and gaps were treated as missing data in the alignments. All new sequences have been submitted to EMBL (Table 2). We performed separate and combined analyses without and with coded indels. Furthermore, the aligned matrices were analyzed with Bayesian inference and maximum parsimony.

Separate and combined Bayesian analyses of sequence data from the five chloroplast gene and nrITS regions of the 58 Rubioideae taxa were performed in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For each single gene data set, the best performing nucleotide substitution model was selected using the computer programs MrModeltest 2.0 (Nylander, 2001) and MrAIC ver. 1.4.3 (Nylander, 2004). In the latter, the best performing evolutionary models was estimated under three different model selection criteria: Akaike information criterion (AIC) (Akaike, 1974), AICc (a second order AIC, necessary for small samples) and the Bayesian information criterion (BIC) (Schwartz, 1978). All combined Bayesian analyses were conducted with four independent Markov chains run for 5×10^6 Metropolis-coupled MCMC generations, with tree sampling every 1×10^3 generations, and burn-in after 2×10^5 trees (as detected by plotting the log likelihood scores against generation number). We partitioned the combined data sets into two partitions: partition # 1 with GTR+G+I applied to the nrITS, *ndh*F, and *rbc*L data; and partition # 2 with GTR+G applied to the *atp*B-*rbc*L, *rps*16, and *trn*T-F data. In all analyses, partitions were unlinked so that each partition was allowed to have its own sets of parameters. Flat prior probabilities were specified according to suggestions produced by the software MrAiC (Nylander, 2004).

We further explored the information in the data sets by running a set of additional combined Bayesian analyses based on data sets

Table 2
List of taxa investigated in this study, voucher information, tribal classifications (Bremer and Manen, 2000; Razafimandimbison et al., this study), and accession numbers

Taxa	Voucher (of previously unpublished sequences)	Tribal classifications	*rbc*L	*rps*16	*ndh*F	*atp*B-*rbc*L spacer	*trn*T-F	ITS
Anthospermum herbaceum L.f.		Anthospermeae	X83623[1]	EU145496[2]	AJ236284[7]	AJ234028[2]	EU145544[26]	EU145355[26]
Appunia guatemalensis Donn.Sm.	Sine loc., Lundell 6675 (S)	Morindeae s.s.	AJ288593[2]	AM945306	AM945252	AJ234009[2]	AM945332	AM945191
Argostemma hookeri King		Argostemmateae	Z68788[9]	EU145497[26]	EU145419[26]	AJ234032[2]	EU145545[26]	EU145356[26]
Chassalia catati Drake ex Bremek.	Madagascar, Razafimandimbison 480 (UPS)	Palicoureeae	AM945305	AM945331	AM945283	AM945251	AM945363	AM945218
Coccocypselum hirsutum Bartl. ex DC.		Coussareeae	X87145[10]	EU145500[26]	EU145421[26]	EU145325[26]	EU145548[26]	—
Coelospermum monticola Baill. ex Guillaumin	New Caledonia, Johansson 87 (S)	Morindeae s.s.	AF331644[8]	AF001438[4]	AM945255	AM945221	AM945334	AM945194
Colletoecema dewevrei (De Wild.) E.M.A.Petit		Colletoecemeae	EU145457	AF129272[12]	EU145409[26]	DQ131713[3]	EU145532[26]	—
Coussarea hydrangeifolia (Benth.) Benth. &Hook.f. ex Müll.Arg.		Coussareeae	EU145460[26]	EU145501[26]	EU145422[26]	EU145326[26]	EU145549[26]	—
Craterispermum laurinum	Madagascar, Kårehed et al. 303 (UPS)	Craterispermeae	AM945300	AM945325	AM945276	AM945243	AM945356	AM945212
Craterispermum sp. 1 Eriksson et al. 999	Madagascar, Eriksson et al. 999 (S)	Craterispermeae	AM945297	—	AM945273	AM945241	AM945353	AM945209
Craterispermum sp. 2 Razafimandimbison and Ravelonarivo 656	Madagascar, Razafimandimbison and Ravelonarivo 656 (S)	Craterispermeae	AM945298	AM945313	AM945274	AM945242	AM945354	AM945210
Craterispermum sp. 3 24169-SF	Madagascar, 24169-SF (P)	Craterispermeae	AM945299	AM945324	AM945275	AM945244	AM945355	AM945211
Damnacanthus indicus C.F.Gaertn.	Sine loc., Bremer 3107 (S)	Mitchelleae	Z68793[9]	AF331647[8]	AM945256	AJ234015[2]	AM945335	AY514061[23]
Damnacanthus macrophyllus Siebold ex Miq.	Japan, Fukuoka 8792 (P)	Mitchelleae	AM945285	AM945308	AM945257	AM945222	AM945336	AM945195
Danais xanthorrhoea (K.Schum.) Bremek.		Danaideae	Z68794[9]	AM117297[17]	AJ236293[7]	AJ234019[2]	DQ662138[15]	—
Declieuxia cordigera Mart. & Zucc. ex Schult. & Schult.f.		Coussareeae	AM117224[17]	AM117298[17]	EU145423[26]	EU145327[26]	EU145551[26]	—
Gaertnera phyllosepala Baker	Madagascar, Kårehed et al. 274 (UPS)	Gaertnereae	AM945288	AM945311	AM945261	AM945227	AM945340	AM945200
Gaertnera phyllostachya Baker	Madagascar, Kårehed et al. 272 (UPS)	Gaertnereae	AM945289	AM945312	AM945262	AM945228	AM945341	AM945201
Gaertnera sp. Bremer et al. 4008	Madagascar, Bremer et al. 4008 (UPS)	Gaertnereae	AM945287	AM945310	AM945260	AM945226	AM945339	AM945199
Geophila obvallata Didr.		Psychotrieae s.s.	AM117228[17]	AF369845[14]	AM945259	—	EU145569[26]	AM945196
Gynochthodes coriacea Blume	Alejandro et al. (2005)	Morindeae s.s.	AJ288603[2]	AM117311[17]	AM945253	AM945219	AJ847407[19]	AM945192
Gynochthodes sp. Davis 4062	Vietnam, Davis 4062 (K)	Morindeae s.s.	AM945284	AM945307	AM945254	AM945220	AM945333	AM945193
Hydnophytum formicarum Jack		Psychotrieae s.s.	X83645[1]	AF001339[4]	—	X76480[18]	—	AF034912[11]
Lasianthus kilimandscharicus K.Schum.		Lasiantheae	AM117237[17]	AM117327[17]	EU145426[26]	EU145330[26]	DQ662147[15]	—
Lasianthus lanceolatus (Griseb.) Urb.		Lasiantheae	AM117238[17]	AF004062[4]	—	EU145331[26]	EU145554[26]	—
Lucinaea sp. 1 Ridsdale 2152	Ridsdale 2152 (L.)	Schradereae	—	AM945314	AM945265	AM945230	AM945344	—
Lucinaea sp. 2 Ridsdale 2152a	Ridsdale 2152a (L.)	Schradereae	—	AM945315	—	AM945231	AM945345	—
Margaritopsis acuifolia C.Wright (accepted name *Margaritopsis nudiflora* (Griseb.) K. Schum.)		Psychotrieae s.s.	AM117247[17]	AF001340[4]	—	AM945225	EU145568[26]	AM945198
Mitchella repens L.		Mitchelleae	Z68805[21]	AF001441[4]	AM945258	AM945223	AM945337	AB103535[20] AB103536
Morinda citrifolia L.		Morindeae s.s.	AJ318448[13]	AJ320078[13]	AJ236300[7]	AJ234013[2]	AF152616[5]	AY762843[21]
Ophiorrhiza mungos L.	Bremer 3301 (UPS)	Ophiorrhizeae	X83656[1]	AF004064[4]	AJ130838[7]	—	DQ662151[3]	—
Pagamea guianensis Aubl.	McDowell 5738 (ETSU)	Gaertnereae	AM945290	AF002744[4]	AM945263	AM945229	AM945342	AF333846[22]
Palicourea crocea (Sw.) Schult	Cordiero 2736 (SP)	Palicoureeae	AM117253[17]	AF147510[16]	AM945280	AM945247	AM945259	AF149322[16]
Pauridiantha paucinervis (Hiern) Bremek.		Urophylleae	Z68811[9]	AM900600[24]	AJ236302[7]	AJ233998[2]	EU145578[26]	—
Pentas lanceolata (Forssk.) Deflers		Knoxieae	X83659[1]	AM117334[17]	AJ236304[7]	X76479[18]	AM117379[17]	—
Praravinia suberosa (Merr.) Bremek.	Rydin et al. (in press)	Urophylleae	AJ288616[2]	EU145514[26]	—	—	—	—
Prismatomeris albidiflora Thw.	Cambodge, Marie 89 (P)	Prismatomerideae	AM945296	AM945320	AM945270	AM945237	AM945351	AM945205
Prismatomeris beccariana (Baill. ex K.Schum.) J.T.Johanss.	Ridsdale 2461 (L.)	Prismatomerideae	AJ288618[2]	AF331652[8]	AM945271	AM945238	AM945352	AM945206
Prismatomeris filamentosa Craib.	Thailand, Geesink & Hiepko 7830 (P)	Prismatomerideae	—	AM945321	AM945272	AM945239	—	AM945207
Prismatomeris griffithii Ridl.	Thailand, Geesink & Santisuck 5466 (P)	Prismatomerideae	—	AM945322	—	AM945240	—	AM945208

Table 2 (continued)

Taxa	Voucher (of previously unpublished sequences)	Tribal classifications	*rbc*L	*rps*16	*ndh*F	*atpB-rbc*L spacer	*trn*T-F	ITS
Prismatomeris sp. 1 Davis 4057	Vietnam, Davis 4057 (K)	Prismatomerideae	AM945292	AM945316	AM945266	AM945233	AM945347	AM945202
Prismatomeris sp. 2 Davis 4037	Vietnam, Davis 4037 (K)	Prismatomerideae	AM945293	AM945317	AM945267	AM945234	AM945348	—
Prismatomeris sp. 3 Davis 4026	Vietnam, Davis 4026 (K)	Prismatomerideae	AM945294	AM945318	AM945268	AM945235	AM945349	AM945203
Prismatomeris sp. 4 Davis 4068	Vietnam, Davis 4068 (K)	Prismatomerideae	AM945295	AM945319	AM945269	AM945236	AM945350	AM945204
Psychotra capensis Vatke	South Africa, Bremer et al. 4284 (UPS)	Psychotrieae s.s.	AM945301	AM945326	AM945277	AM945245	AM945357	AM945213
Psychotria amboniana K.Schum.	Kenya, Luke 8344 (UPS)	Psychotrieae s.s.	AM945302	AM945328	AM945281	AM945248	AM945360	AM945215
Psychotria holtzii (K.Schum.) E.M.A.Petit	Kenya, Luke 8342 (UPS)	Psychotrieae s.s.	AM945304	AM945330	—	AM945250	AM945362	AM945217
Psychotria kirkii Hiern	Cult. Uppsala Bot. Gard., Bremer 3102 (UPS)	Psychotrieae s.s.	AY538469[25]	AM945327	AM945278	AM945246	AM945358	AM945214
Psychotria poeppigiana Müll.Arg.	Ecuador, Bremer 3330 (UPS)	Palicoureeae	Z68818[9]	AF002748[4]	AM945279	AJ234018[2]	—	AF149400[16]
Psychotria schliebenii E.M.A.Petit	Kenya, Luke 8348 (UPS)	Psychotrieae s.str.	AM945303	AM945329	AM945282	AM945249	AM945361	AM945216
Rennellia elliptica Korth.	Malaysia, Deveru 60 (P)	Prismatomerideae	AM945291	—	—	AM945232	AM945346	—
Rubia tinctorum L.	Cult. Uppsala Bot. Gard., Bremer 3300 (UPS)	Rubieae	X83666[1]	—	DQ359167[3]	X76465[18]	—	—
Rubiaceae indet.	Taishan 10		EU145468	EU145516	EU145443	EU145443	EU145584	EU145391
Schizocolea linderi 1(Hutch. & Dalziel) Bremek.			AM117272[17]	EU145498[26]	—	EU145323[26]	EU145546[26]	EU145357[26]
Schizocolea linderi 2 (Hutch. & Dalziel) Bremek.	Liberia, Adam 789 (P)		AM945286	AM945309	—	AM945224	AM945338	AM945197
Schradera sp. Andersson 2107	Andersson and Rova (1999)	Schradereae	—	AF003617[4]	—	—	AF152613[5]	—
Schradera subandina K.Krause	Sine loc., Clark & Watt 878 (QCNE)	Schradereae	Y11859[6]	AM945313	AM945264	AJ234014[2]	AM945343	—
Xanthophytum borneense (Valeton) Axelius		Ophiorrhizeae	EU145466[26]	EU145513[26]	—	—	—	—

Accession numbers underlined are new sequences. Published sequences: [1](Bremer et al., 1995); [2](Bremer and Manen, 2000); [3]Manen, J.-F. (Genbank unpublished); [4](Andersson and Rova, 1999); [5]Rova et al. (2002); [6](Bremer and Thulin, 1998); [7](Bremer et al., 1999); [8]Andersson, L. (Genbank unpublished); [9](Bremer, 1996a); [10](Bremer, 1996b); [11](Nepokroeff et al., 1999); [12](Piesschaert et al., 2000); [13](Novotny et al., 2002); [14](Andersson, 2001); [15]Backlund, M. (Genbank unpublished); [16]Andersson, L. & Taylor; C. (GenBank unpublished); [17]B. Bremer (in prep.); [18](Manen et al., 1994); [19](Alejandro et al., 2005); [20]J. Yokoyama et al. (Genbank unpublished); [21]A.D. Proujansky and Stern (Genbank unpublished); [22]Malcomber (2002); [23]P. Ding et al. (GenBank unpublished); [24](Smedmark et al., 2008); [25](Andersson and Antonelli, 2005); [26]Rydin et al. (2008).

Table 3
List of the primers used in this study

DNA region	Primer names	Sequence 5′–3′/reference
*rbc*L	5′F, 3′R and 427F	Bremer et al. (1995)
*rbc*L	Z895R	Zurawski, DNAX Research institute
*rps*16	F and 2R	(Oxelman et al., 1997)
ITS	P17 and 26S-82R	(Bolmgren and Oxelman in Popp and Oxelman, 2001)
ITS	P25	(Oxelman, 1996)
*ndh*F	2F	Rydin et al. (2008)
*ndh*F	1000R	Rydin et al. (2008)
*ndh*F	720F	Rydin et al. (2008)
*ndh*F	1700R	Rydin et al. (2008)
*ndh*F	1320F	Rydin et al. (2008)
*ndh*F	2280R	Rydin et al. (2008)
*atpB-rbc*L spacer	rbcL5′R	Rydin et al. (2008)
*atpB-rbc*L spacer	atpB5′R	Rydin et al. (2008)
*trn*T-F	A1	Razafimandimbison and Bremer (2002)
*trn*T-F	IR	Razafimandimbison and Bremer (2002)
*trn*T-F	C	Taberlet et al. (1991)
*trn*T-F	F	Taberlet et al. (1991)

from: (1) the five chloroplast gene regions of the 58 Rubioideae taxa; and (2) the five chloroplast gene and nrITS regions of the 48 taxa of the Psychotrieae and Spermacoceae alliances. The sampled taxa of Spermacoceae alliance were used as outgroup taxa for the latter the analysis.

All separate and combined Bayesian analyses were repeated two times using different random starting trees to evaluate the convergence of the likelihood values and posterior probabilities. All saved trees (after excluding burn-ins) from the two independent runs were pooled for a consensus tree. Groups characterized by posterior probabilities over 95% were regarded as strongly supported.

We also performed separate and combined parsimony analyses, respectively, with the 58 Rubioideae and 48 Psychotrieae and Spermacoceae taxa using PAUP* version 4.0b10 for Unix (Swofford, 1998). Most equally parsimonious trees were generated using the following settings: heuristic search option, 5000 random sequence additions, tree bisection reconnection (TBR), branch swapping, and MULTREES option on. The consistency index (CI; Kluge and Farris, 1969) and retention index (RI; Farris, 1989) were calculated (all uninformative characters excluded) to estimate homoplasy. A phylogram of the single most parsimonious tree yielded from a parsimony analysis of the combined *atpB-rbc*L/*ndh*F/*rbc*L/*rps*16/*trn*T-F/ nrITS (called combined chloroplast-nuclear hereafter) data was depicted to show branch lengths, which are proportional to inferred amount of character changes. Jackknife (JK, Farris et al., 1995) values were computed using heuristic searches, with MULTREES on, TBR branch swapping, five random additions, and 500 replicates

Table 4
Testing the monophyly of earlier proposed clades in the Psychotrieae alliance tested with the data used in this study

Hypotheses tested	References for the tested phylogenetic hypotheses	Posterior probabilities for the monophyly of the constraint groups
H1. Forcing (MIT + MOR) sister to SCHR	Andersson and Rova (1999)	0.0004
H2. Forcing GAE sister to SCHR	Bremer and Manen (2000: Fig. 3)	0.00125
H3. Forcing CRA sister to SCHR	Robbrecht and Manen (2006)	0
H4. Forcing GAE sister to (PAL + PSY)	Baillon (1879), Solereder. (1890), Jansen et al. (1996), Robbrecht and Manen (2006)	0
H5. Forcing GAE sister to the rest of PA sensu Bremer and Manen (2000)	Andersson and Rova. (1999); Bremer and Manen (2000: Fig. 4), Robbrecht and Manen (2006)	0
H6. Forcing (PAL + PSY) sister to (GAE + SCHR)	Bremer and Manen (2000: Fig. 3)	0
H7. Forcing (MIT + MOR + PRI) monophyletic	Bremer and Manen (2000)	0
H8. Forcing (PAL + PSY) sister to (MIT + MOR s.s. + SCHR)	Andersson and Rova (1999)	0
H9. Forcing CRA sister to the rest of PA sensu Bremer and Manen (2000)	Bremer and Manen (2000)	0

CRA, Craterispermeae; GAE, Gaertnereae; MIT, *Mitchella* group (Mitchelleae); MOR, Morindeae s.s.; PRI, Prismatomerideae; PA, Psychotrieae alliance; PAL, Palicoureeae; PSY, Psychotrieae s.s.; and SCHR, Schradereae.

to assess relative support of retained clades. Strict consensus trees were produced from the resulting most parsimonious trees saved from each data set.

2.4. Testing hypotheses on intertribal relationships in the Psychotrieae alliance

As mentioned earlier, previous molecular phylogenetic studies (Fig. 1A–D) were inconsistent regarding the intertribal relationships within the Psychotrieae alliance. We used the "Filter trees" command, implemented in PAUP*, to search among the pooled Bayesian trees from the chloroplast-nuclear data of the 58 Rubioideae taxa saved after stationary (i.e., burn-ins excluded) those consistent with the constraint tree (i.e., the alternative intertribal relationship tested). The number of trees retained by filter divided by the total number of the post-stationarity trees is the posterior probability of the hypothesis represented by the constraint tree. In total, we computed Bayesian posterior probabilities for the monophyly of nine clades (Hypotheses # 1–9, see Table 4) suggested in previous molecular studies.

2.5. Assessment of the evolution of seed numbers in the Psychotrieae alliance

We used MacClade 4.06 (Maddison and Maddison, 2003) to trace the character states (one, two, and numerous) of the seed numbers by overlaying them onto the one randomly selected most parsimonious tree from the combined chloroplast-nuclear analysis of the 48 taxa of the Psychotrieae and Spermacoceae alliances. We scored the selected trait for the taxa in the phylogeny using information either from the literature or from personal observation and both ACCTRAN and DELTRAN optimizations.

3. Results

3.1. Sequence characteristics

Information about all sequence data from the six markers is summarized in Table 5. The non-aligned sequences of *ndh*F were the longest and those of the nrITS region were the shortest regions. In contrast, the aligned matrix of the *trn*T-F data (3344 bp) became the longest and that of the *rps*16 data was the third longest data mainly due to introduction of many gaps in the alignment (Table 5). For the ITS data the alignment of the sequences of the 48 taxa of the Psychotrieae and Spermacoceae alliances were straightforward. However, we had difficulty in aligning these sequences with those from taxa investigated from the rest of Rubioideae (Coussareeae, Lasiantheae, Ophiorrhizeae, Urophylleae, and *Colletoecema*); therefore, the ITS sequences of the latter groups were not included in our analyses.

According to Fig. 2, the nrITS matrix contained the highest number of parsimony informative characters (31.97%), followed by the *ndh*F (19.22%) and *rbc*L (17.89%) matrices. The *trn*T-F (15.10%) and *atp*B-*rbc*L (14.49%) matrices were the least variable. The combined data from the *atp*B-*rbc*L (8.46%), *rbc*L (13.11%), and *rps*16 (16.5%) partitions, the same markers used in Bremer and Manen (2000: Fig. 4), together yielded ca. 38% of the total number of parsimony informative characters (PIC); the three other data sets (nrITS with 13.89%, *ndh*F with 21.6%, and *trn*T-F with 26.38%) together produced a total of ca. 62% of the PIC. Finally, The *rbc*L (0.422) and nrITS (0.433) data had the lowest CI (i.e., with highest levels of homoplasy), whereas the *trn*T-F (0.669) and *rps*16 (0.661) data had the highest CI (i.e., lowest levels of homoplasy) (see Table 5).

Table 5
Number of investigated sequences and some useful information for the separate analyses

| Markers	*atp*B-*rbc*L	*ndh*F	*rbc*L	*rps*16	*trn*T-F	ITS
Number of sequences investigated	53	48	55	53	49	42
Number of new sequences	33	33	24	26	32	31
Ranges of sequence lengths (bp)	283–769	1212–2095	674–1402	676–931	508–1886	225–717
Lengths of aligned matrices (bp)	1118	2154	1403	1903	3344	832
Number of parsimony informative characters (PIC)	162	414	251	316	505	266
Consistency index	0.651	0.551	0.422	0.661	0.669	0.433
Retention index	0.801	0.709	0.679	0.806	0.793	0.649
Best tree lengths	341	1099	767	644	1087	1183
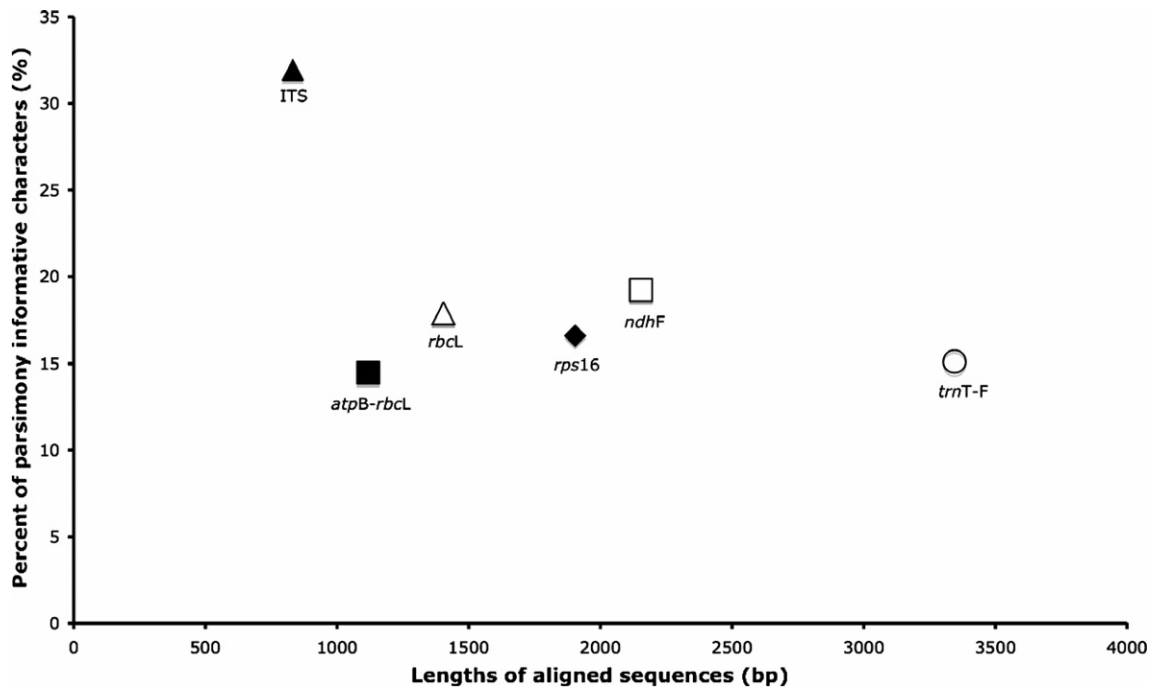
Fig. 2. Correlation between lengths of aligned matrices and percent of parsimony informative characters.

3.2. Separate analyses

The same best-fit models of nucleotide substitution were estimated by the three model selection criteria utilized in this study: GTR+G+I (Yang, 1993) for the nrITS, *rbc*L, and *trn*T-F data and GTR+G (Yang, 1993) for the *atp*B-*rbc*L, *ndh*F, and *rps*16 data. For each separate analysis we summarized in Table 5 the number of the investigated sequences and some useful information about the parsimony analyses. The present study used 300 sequences, of which 28 nrITS, 33 *atp*B-*rbc*L, 32 *ndh*F, 22 *rbc*L, 26 *rps*16, and 32 *trn*T-F, a total of 173 (ca. 58%), new sequences are published here. Each data set was analyzed separately without and with gap coding characters to see the presence of any well-supported topological conflicts. All separate Bayesian and parsimony analyses retained the same overall tree topologies, with only some differences in support values of retained clades. Accordingly, we merged data sets from the six markers in a single matrix for conducting combined Bayesian and parsimony analyses.

3.3. Combined analyses

The merged data sets of the six (without coded indels) of 58 Ruboideae taxa yielded 10757 positions, of which 1908 positions were parsimony informative characters. A parsimony analysis based on the combined chloroplast-nuclear data of the 58 Rubioideae taxa yielded one single most parsimonious and fully resolved tree (L = 5227; CI = 0.536; and RI = 0.710), which had identical tree topologies with the Bayesian majority rule consensus tree from a combined Bayesian analysis (see Fig. 3) of the same data sets. Within the Psychotrieae alliance a phylogram of the single most parsimonious tree (Fig. 4) revealed higher numbers of molecular autapomorphic characters of the tribes (with the exception of Palicoureeae) compared with those of molecular synapomorphies of two sister tribes or groups of tribes. Both the Bayesian and parsimony analyses of the combined chloroplast-nuclear data identified the same lineages (Fig. 3), which received higher support values than any of the trees from the separates analyses or those from the combined chloroplast data (results not presented). Urophylleae was resolved as sister to the rest of Rubioideae. Ophiorrhizeae and

Lasiantheae were the successive next diverging clades to branch off, followed by Coussareeae, which was sister-group to a very large clade forming *Schizocolea* and all sampled members of the Psychotrieae and Spermacoceae alliances sensu Bremer and Manen (2000). The Spermacoceae alliance was resolved with high support (PP = 1.00; JK = 94) as sister to a large clade (= Psychotrieae alliance sensu Razafimandimbison et al., this study) consisting of *Schizocolea* (Fig. 3A) and the Psychotrieae alliance sensu Bremer and Manen (2000) (Fig. 3B). All sequenced members of the Spermacoceae alliance and an unknown Rubiaceae constituted a well-supported clade.

Within the Psychotrieae alliance (as circumscribed here) *Schizocolea* (Fig. 3A), here represented by two accessions of the type species *S. linderi* (Hutch. & Dalziel) Bremek., was highly supported (PP = 1.00) as sister to a strongly supported clade (Fig. 3B) containing all sampled taxa of the Psychotrieae alliance sensu Bremer and Manen (2000) (PP = 1.00). In the latter clade the sequenced taxa were resolved in two large subclades: a strongly subclade (PP = 1.00) forming Schradereae, Gaertnereae, the *Mitchella* group, and Morindeae s.s. (Fig. 3C); and a poorly supported (PP = 0.66) subclade (Fig. 3D) consisting of Palicoureeae, Psychotrieae s.s., Craterispermeae, and Prismatomerideae. Within the former subclade Schradereae was the first basal lineage to branch off, followed by Gaertnereae, which was resolved as sister to the Mitchelleae–Morindeae group. In the latter subclade, Palicoureeae and Psychotrieae s.s. (Fig. 3E) and Craterispermeae and Primatomerideae (Fig. 3F), respectively, formed were sister groups, both with PP = 1.00. When the combined chloroplast-nuclear data of the 58 Rubioideae taxa were analyzed with 33 coded indels from the *atp*B-*rbc*L, *rbc*L, and *rps*16 data, we obtained Bayesian majority consensus and strict consensus parsimony trees identical to those generated from the same data without coded indels (see Fig. 3). The support value for the sister-group relationship between the Craterispermeae–Prismatomerideae and Palicoureeae–Psychotrieae clades decreased from 0.66 to 0.53 (PP) (results not shown).

The overall tree topologies from the Bayesian and parsimony analyses of the combined chloroplast data of the 58 Rubioideae taxa were similar to those from the Bayesian and parsimony analyses of the combined chloroplast-nuclear data (Fig. 3). The only difference
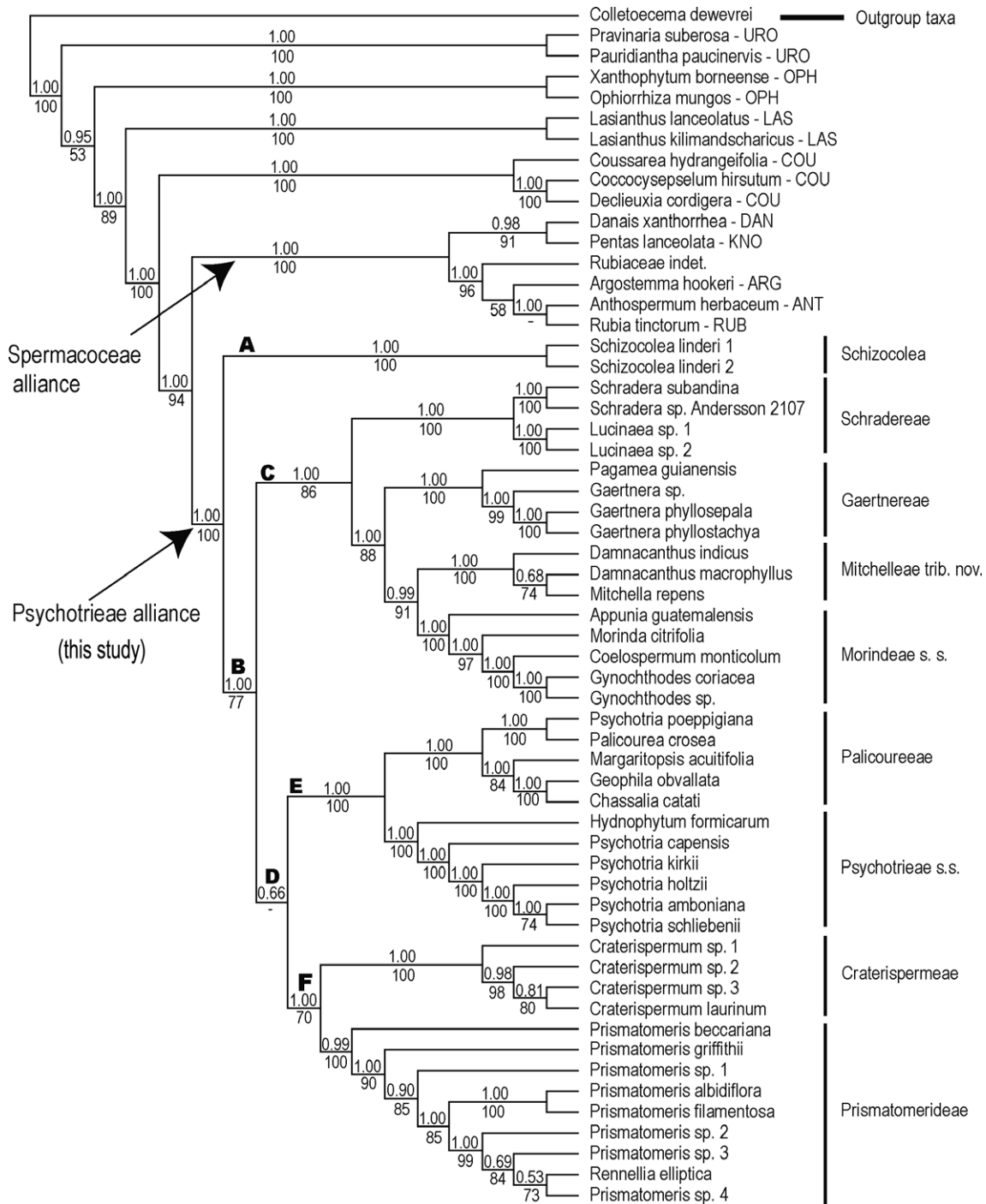
Fig. 3. Bayesian majority rule consensus tree of 58 Rubioideae taxa based on combined sequence data (without coded indels) from the *atpB-rbcL* spacer, *ndhF*, *rbcL*, *rps16* intron, *trnT-L-F*, and nrITS regions, estimated using Bayesian inference. The data sets were partitioned into two partitions (GTR+G+I applied to the nrITS, *ndhF*, and *rbcL* data; and GTR+G applied to *atpB-rbcL*, *rps16*, and *trnT-F* data) and 5 million generations were run. Values above nodes are posterior probabilities and those below nodes are jackkniffe support ⩾50%. Horizontal bar delimits outgroup taxon; vertical bars denote tribal limits of the Psychotreae alliance. ANT, Anthospermeae; ARG, Argostemmateae; COU, Coussareeae; DAN, Danaideae; KNO, Knoxieae; LAS, Lasiantheae; OPH, Ophiorrhizeae; RUB, Rubieae; and URO, Urophylleae.

was that the poorly supported sister-group relationship (Fig. 3D) between the Palicoureeae–Psychotrieae (Fig. 3E) and Craterispermeae–Prismatomerideae (Fig. 3F) clades collapsed in the former (results not shown). Instead, the Palicoureeae–Psychotrieae clade (Fig. 3E) formed a weakly supported (PP = 0.76) monophyletic group with the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade (Fig. 3C) in a combined Bayesian analysis of the chloroplast-nuclear data. This latter relationship collapsed in a combined parsimony analysis of the chloroplast data (results not presented).

A parsimony analysis of the combined chloroplast-nuclear data of 48 taxa of the Psychotrieae and Spermacoceae alliances resulted in identical tree topologies in the Psychotrieae alliance with minor differences in support values. For examples, the Palicoureeae–Psychotrieae (Fig. 3E) and Craterispermeae–Prismatomerideae (Fig. 3F) clades as sister groups (Fig. 3D) were poorly resolved as sister groups (JK = 56%). This relationship collapsed in a Bayesian analysis of the same data (results not shown). In all above combined Bayesian and parsimony analyses, the support values for
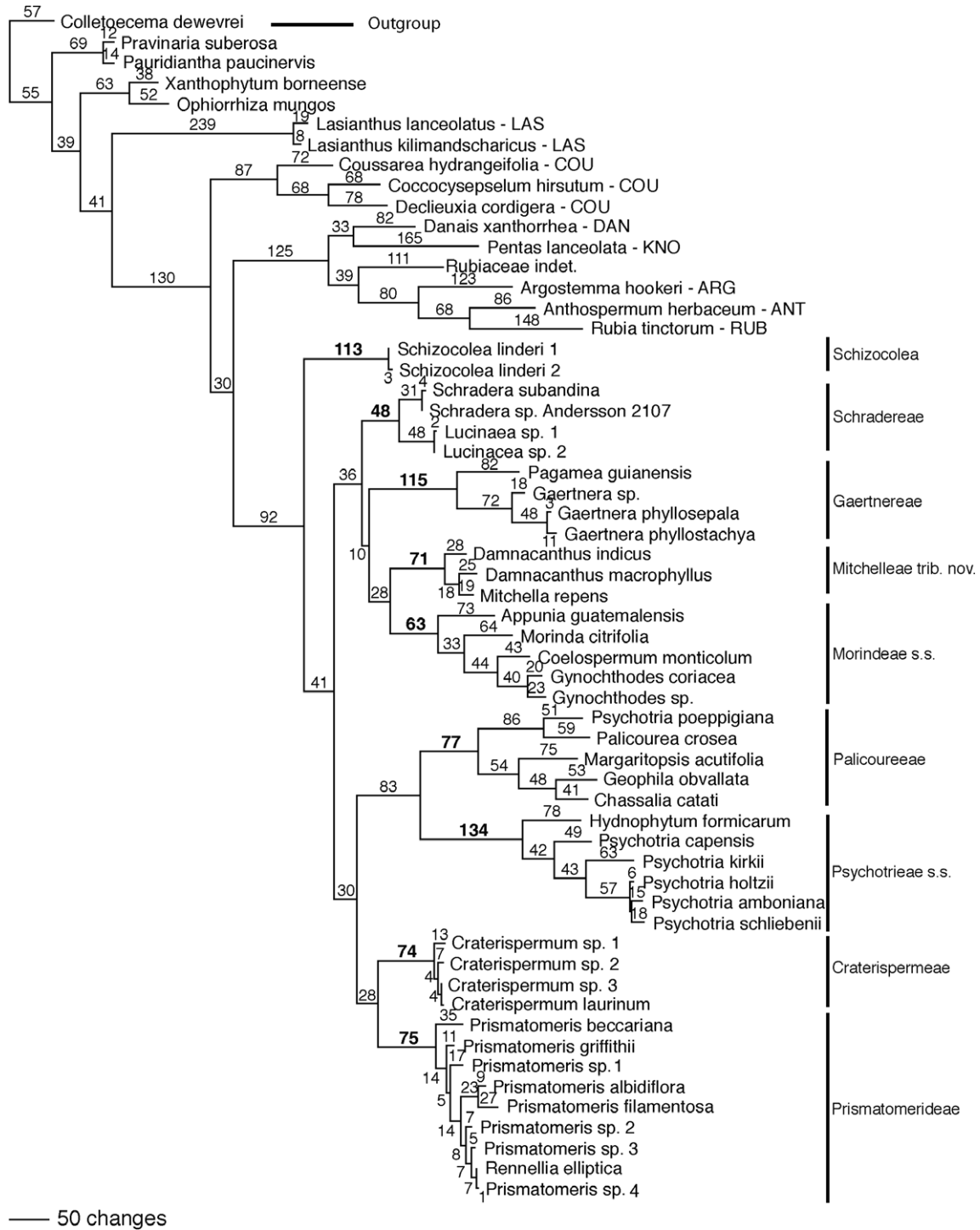
Fig. 4. A phylogram of the single most parsimonious tree generated from the combined chloroplast-nuclear analysis of 58 Rubioideae taxa. Numbers above nodes are numbers of evolutionary changes.

the *Schizocolea* (Fig. 3A), Schradereae–Gaertnereae–Mitchelleae–Morindeae (Fig. 3C), Palicoureeae–Psychotrieae (Fig. 3E), and Craterispermeae–Prismatomerideae (Fig. 3F) clades and those for the eight tribes recognized in the Psychotrieae alliance sensu Bremer and Manen (2000) were always very high (Fig. 3).

3.4. Testing the monophyly of alternative intertribal relationships in the Psychotrieae alliance

We summarized in Table 4 the Bayesian posterior probabilities of the monophyly of nine earlier suggested relationships of taxa

(mainly tribes) in the Psychotrieae alliance tested with our data. The Bayesian posterior probabilities of these nine clades (Hypotheses # 1–9) ranged from 0 to 0.00125. Constraining the Craterispermeae–Prismatomerideae clade (Fig. 3F) as sister to the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade (Fig. 3C) had a posterior probability of 0.5, while constraining the Palicoureeae–Psychotrieae clade (Fig. 3E) as sister to the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade (Fig. 3C) received a posterior probability of 0.29. These values were much lower than those for the sister-group relationship between the Craterispermeae–Prismatomerideae and Palicoureeae–Psychot-

rieae clades in the combined chloroplast-nuclear tree (PP = 0.66, Fig. 3D).

3.5. Character optimization

When the character states of seed numbers were optimized on the single most parsimonious tree from the combined chloroplast-nuclear data of the 48 taxa of the Psychotrieae and Spermacoceae alliances (see Fig. 5), a one-seeded carpel was inferred as ancestral in the alliance. Within the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade many- and two-seeded carpels each evolved once (Fig. 5). The outcomes of the optimization did not change when ACCTRAN or DELTRAN optimization was used.

4. Discussion

The present study is the first molecular phylogenetic study, which is specifically designed to rigorously assess the tribal limits and relationships within the Psychotrieae alliance sensu Bremer and Manen (2000). Our analyses include the representatives of all tribes of the Psychotrieae alliance recognized in our study. We base our conclusions regarding the tribal limits and relationships in the Psychotrieae alliance on the fully resolved tree shown in Fig. 3, as it is the best supported hypothesis, maximizing congruence among all of the characters sampled. Below, we first characterize the major lineages of the Psychotrieae alliance and discuss their phylogenetic relationships. We then discuss the tribal limits and relationships in the alliance in the light of the results of this study. We further discuss some evolutionary trends in the alliance based on the single parsimonious tree from the combined chloroplast-nuclear data of the 48 taxa of the Psychotrieae and Spermac-oceae alliances (Fig. 5). Finally, we formally describe a new tribe Mitchelleae Razafim. & B.Bremer.

4.1. Characterizations of and relationships between the major lineages in the Psychotrieae alliance

The sister-group relationship between Schizocolea (Fig. 3A) and the Psychotrieae alliance sensu Bremer and Manen (2000) (Fig. 3B) is further confirmed by our study. In other words, the present analyses support the inclusion of Schizocolea in the Psychotrieae alliance and reveals for the first time that the alliance can be subdivided into four well-supported major lineages: the Schizocolea clade (Fig. 3A, PP = 1.00), the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade (Fig. 3C, PP = 1.00), the Palicoureeae–Psychotrieae clade (Fig. 3E, PP = 1.00), and the Craterispermeae–Primatomerideae clade (Fig. 3F, PP = 1.00). The present analyses are inconclusive regarding the relationships within the latter group. The relationship between the sister groups (Fig. 3D) Palicoureeae–Psychotrieae (Fig. 3E) and Craterispermeae–Prismatomerideae (Fig. 3F) clades is poorly supported (PP = 0.66) in the combined chloroplast-nuclear tree (Fig. 3). The support value for this relationship decreases (PP = 0.53) when 33 coded indels are added (results not shown). On the other hand, a sister-group relationship between the Palicoureeae-Psychotrieae clade Fig. 3E) and the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade (Fig. 3C) receives a posterior probability of 0.5 only and that of between the Craterispermeae–Prismatomerideae clade (Fig. 3F) and the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade (Fig. 3C) has a support value of 0.29 in the combined chloroplast-nuclear analyses (Fig. 3). Additional sequence data from slowly evolving nuclear markers (e.g., 18S or 26S) could perhaps help
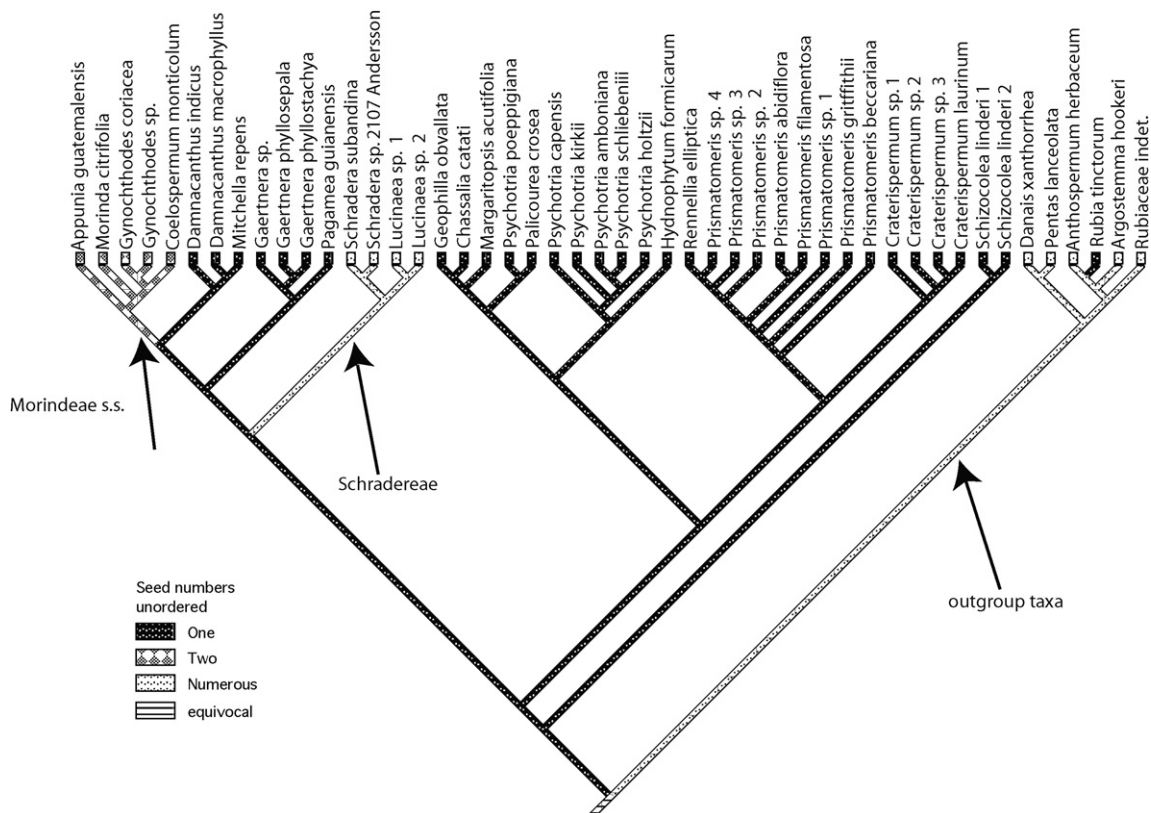

Fig. 5. Overlay of seed number on the single most parsimonious tree generated from the combined chloroplast-nuclear analysis of 48 taxa of the Psychotrieae and Spermacoceae alliances.

for a further re-assessment of the relationships between the three major lineages of the alliance.

There seem to be no obvious morphological characters, which can be used to diagnose the four major lineages of the alliance. We compile in Table 6 some morphological characters and important information, which can be used in combination for characterizing these groups. According to Table 6, the Palicoureeae-Psychotrieae clade is the most species-rich group with ca. 2800 species (Govaerts et al., 2006), while the Schizocolea clade is the smallest group with only two species. The Schradereae–Gaertnereae–Mitchelleae–Morindeae clade is the most heterogeneous because of some odd characters in Schradereae and Gaertnereae (see Table 6). Finally, detailed morphological (including anatomical and palynological) investigations are presently lacking and therefore are needed for Schizocolea.

4.2. Tribal delimitations and relationships in Psychotrieae alliance

4.2.1. Schizocolea Bremek.

The position of Schizocolea linderi, the type species of Schizocolea, as sister to the Psychotrieae alliance Bremer and Manen (2000) implies that Schizocolea will have to be placed in its own tribe (Rydin et al., submitted for publication). On the other hand, the monophyly of Schizocolea is yet to be tested, as the Congolian species Schizocolea ochreata E.M.A. Petit, has not been investigated.

4.2.2. Schradereae Bremek.

The tribe Schradereae was originally established by Bremekamp (1934) to accommodate the Neotropical genus Schradera Aubl. and the Southeast Asian genus Lucinaea DC. These genera were initially placed in the tribe Isertieae s.l. (including Mussaendeae) of Cinchonoideae. That circumscription of Schradereae was later adopted by Verdcourt (1958), Robbrecht (1988), and Puff et al. (1993). Puff et al. (1993) merged Lucinaea in Schradera and recently, Puff and Buchner (1998) transferred the monotypic Sri Lankan genus Leucocodon Gardn. and the Southeast Asian genus Lecananthus Jack from Isertieae s.l. to Schradereae. Schradereae as circumscribed by Puff and Buchner (1998) and Puff et al. (1998a,b) are diagnosed by its scandent epiphytic habits with adhesive adventitious rootlets borne along the internodes and/or nodes, head-like inflorescences bearing free congested flowers,

ovaries containing numerous, campylotropous ovules in each carpel, and succulent, berry-like fruits (see Table 6). The tribe and its type genus Schradera sensu Puff et al. (1993) have disjunct distributions occurring in the Neotropics, Sri Lanka, and Southeast Asia (from Peninsular Malaysia to New Guinea). In Andersson and Rova (1999), the single sampled Schradera species is poorly resolved as sister to a clade forming Morindeae s.s. and the Mitchella group (H1, Table 4). In Bremer and Manen (2000: Fig. 3), Schradera, represented by S. subandina, is poorly resolved as sister to Gaertnera sp. (H2, Table 4) in the combined atpB-rbcL/rbcL tree (see also Fig. 1B); however, the genus is left unresolved in a polytomy also containing a subclade forming Palicoureeae and Psychotrieae s.s., a subclade consisting of Morindeae s.s. and the Mitchella group in their combined atpB-rbcL-rbcL-rps16 tree (Bremer and Manen, 2000: Fig. 4; see also Fig. 1C). In Robbrecht and Manen (2006: Fig. 4C), the two sampled Schradera species, S. subandina and Schradera sp., do not form a clade; the latter species is the next diverging taxon to branch off after Gaertnereae, while the former species forms a monophyletic group with Craterispermum caudatum Hutch. (H3, Table 4). In our analyses, we find no support for all above alternative sister-group relationships (H1–3 with PP = 0–0.00125, Table 4). Schradereae, here represented by two Neotropical Schradera species and two Southeast Asian Lucinaea species, is consistently resolved as monophyletic (PP = 1.00), inconsistent with Robbrecht and Manen (2006), and is sister to a highly supported (Gaertnereae(Mitchelleae–Morindeae s.s.)) (PP = 1.00). This sister-group relationship, (Schradereae–(Gaertnereae(Mitchelleae–Morindeae s.s.))), has never been proposed before. We maintain the current tribal status of Schradereae, because it is morphologically distinct from the rest of the Psychotrieae alliance (see also Table 6). Based on the present analyses, a resurrection of Southeast Asian Lucinaea could be argued for if the genus is shown to have enough morphological characters to distinguish it from the Neotropical Schradera. Leucocodon and Lecananthus are not included in our analyses, so the monophyly of Schradereae sensu Puff and Buchner (1998) and Puff et al. (1998a,b) and Schradera sensu Puff et al. (1993) are yet to be tested.

4.2.3. Gaertnereae Bremek. ex S.P.Darwin

Gaertnereae is a small pantropical tribe with a wide and disjunct geographic distribution. Representatives of the tribe are dis-

Table 6
Morphological characteristics and other important information of the major lineages of the Psychotrieae alliance

	Schizocolea	Schradereae–Gaertnereae–Mitchelleae–Morindeae clade	Palicoureeae–Psychotrieae clade	Craterispermeae–Prismatomerideae clade
Number of species/genera	2/1	310/13	ca. 2800/36	ca. 40/5
Geographic distributions	West and Central Africa	Pantropical and temperate	Pantropical	Tropical Africa, Madagascar, Seychelles, Southeast Asia
Habits	Shrubs	Shrubs, small trees, herbaceous (Mitchella), epiphytes (Schradereae)	Small shrubs, rarely herbaceous	Shrubs, small trees
Inflorescence positions	Axillaries	Axillaries or terminals	Terminals	Axillaries or terminals
Ovary positions	Inferior	Inferior, rarely superior (Gaertnereae)	Inferior	Inferior
Types flowers	Unknown	Mostly heterostylous, rarely monomorphic, but not homostylous (in Damnacanthus)	Heterostylous	Heterostylous
Ovule numbers per locule	One	Mostly one, two (Morindeae s.s.) or numerous (Schradereae)	One	One
Ovule orientation	Unknown	Anatropous, campylotropous (Mitchelleae and Schradereae)	Anatropous	Anatropous, hemianatropous (Primatomerideae)
Fruit types	Berries	Mostly drupes, berries (Schradereae)	Berries	Berries
Basic Chromosome numbers	Unknown	11	11	11
Ploidy levels	Unknown	2, 4, 8, 20–22	2–12	2
Pollen types	Unknown	2-3-4-6-colporate, 2-3-4-porate (Gaertnereae, Schradereae)	3-4-5-colporate, 3-4-5-copate, rarely 2-, 4-, 5-porate	3-4-5-colporate

tributed throughout the tropical rainforests of South America, Southeast Asia (from central Thailand through Peninsular Malaysia to Sulawesi), Sri Lanka, Western and Central Africa, Madagascar, and the Mascarenes (Mauritius and Réunion Islands) (Malcomber, 2002). Gaertnereae was originally established by Bremekamp (1966) to accommodate the only two rubiaceous genera with superior ovaries; however, it was Darwin (1976) who validly published the name. The members of the tribe were originally associated with the family Loganiaceae because of their superior ovaries. They were later transferred to Rubiaceae based mainly on the presence of intraxylary phloem, colletors on the stipules, and raphides (Baillon, 1879; Solereder, 1890), a position highly supported by molecular data (e.g., Bremer, 1996a). Gaertnereae contains ca. 100 species of mainly shrubs, which are currently classified in two genera, the paleotropical *Gaertnera* Lam. with ca. 70 species (Malcomber, in prep.) and the Neotropical genus *Pagamea* Aubl. with 29 species (Vicentini, 2007). Both Baillon. (1879) and Solereder. (1890) postulated close affinities of Gaertnereae with Psychotrieae (H4, Table 4), a position also supported by Jansen et al.'s (1996) anatomical and palynological studies. Accordingly, Jansen et al. (1996) recognized Gaertnereae as a subtribe of Psychotrieae, Gaertnerinae. We perceive no molecular support for that classification, as the *Gaertnera–Pagamea* group (= Gaertnereae) never forms a clade with Psychotrieae s.l. (including Palicoureeae) in our analyses (PP = 0, Table 4). The combined chloroplast-nuclear tree (Fig. 3) supports the monophyly of *Gaertnera* and Gaertnereae, congruent with Malcomber (2002) but incongruent with Robbrecht and Manen (2006), in which *Gaertnera* is resolved as paraphyletic. In addition, we find no support (PP = 0, Table 4) for the earlier proposition but poorly supported sister-group relationship between Gaertnereae and the remaining Psychotrieae alliance (H5, Table 4). Furthermore, the sister-group relationships between Gaertnereae and Schradereae (H2, Table 4) and the Schradereae–Gaertnereae and the Palicoureeae–Psychotrieae s.s. clades (H6, Table 4) are also inconsistent with our results (PP = 0.00125 and 0, respectively, Table 4). Gaertnereae is consistently resolved as sister to a clade consisting of the *Mitchella* group and Morindeae s.s. in our analyses (PP = 1.00, Fig. 3). This relationship has never been postulated before and appears to be supported only by molecular data. We maintain Gaertnereae at tribal level because it is morphologically distinct from the rest of the Psychotrieae alliance (Table 6).

4.2.4. Morindeae Miq. and Mitchelleae Razafim. & B.Bremer

We discuss the *Mitchella* group, formally recognized here as a new tribe Mitchelleae Razafim. & B.Bremer (see below), and Morindeae s.s. together, as the former has been associated with the latter. Morindeae is a pantropical tribe, which was originally described by Miquel (1857) to accommodate three genera *Morinda* L., *Tribrachya* Korth. (now *Morinda*), and *Rennellia* Korth. Since then, different authors (Hooker, 1873; Baillon, 1880; Robbrecht, 1988) added more genera, which have caused confusions for the circumscriptions of Morindeae, Psychotrieae, and Coussareeae. However, some of these genera have recently been transferred in other distantly or closely related tribes based on molecular evidence [e.g., *Carphalea* Juss. in knoxieae (Bremer, 1996a), *Cruckshanksia* Hook.f. in Coussareeae (Bremer and Manen, 2000), *Lasianthus* Jack. in Lasiantheae (e.g., Andersson and Rova, 1999), *Prismatomeris* Thw. and its satellite genera (*Gentingia, Motleyia,* and *Rennellia*) in Prismatomerideae (Robbrecht et al., 1991), all Rubioideae].

The East Asian genus *Damnacanthus* has always been associated with Morindeae (e.g., Hooker, 1873; Robbrecht, 1988), whereas the North American and East Asian genus *Mitchella* was initially associated with the tribe Anthospermeae (Hooker, 1873) before Robbrecht (1988) considered it to be of uncertain position.

Baillon (1879, 1880) was the first to report close affinities between *Damnacanthus* and *Mitchella* and even considered to reduce the former to a section of the latter. The close relationships between the two genera were recently corroborated by Robbrecht et al.'s (1991) morphological investigations and more recently by molecular data (e.g., Bremer, 1996a; this study). Robbrecht et al. (1991) additionally pointed out that the genus pair had "certain agreements with the core of the Morindeae" (=Morindeae s.s., including *Appunia* Hook.f., *Coelospermum* Blume, *Gynochthodes* Blume, *Morinda, Pogonolobus*, and probably the New Guinean genus *Siphonandrium*). They stressed that "their definite tribal placement should be withheld until a recircumscription of the Morindeae becomes available (Robbrecht et al., 1991: 307)." In addition, the same authors stated that the genus pair "seems to stand quite isolated and is to be excluded both from the Anthospermeae... (Robbrecht et al., 1991: 343)." Accordingly, Igersheim and Robbrecht (1993) provisionally placed *Damnacanthus* and *Mitchella* in an informal group, the *Mitchella* group. Andersson and Rova (1999) do not support the separation of a *Mitchella* group, as the sampled *Damnacanthus* and *Mitchella* are deeply nested within Morindeae s.s. Bremer and Manen (2000: Fig. 3), on the other hand, find very low (BS < 50%) support for a monophyletic Morindeae s.l. (H7, Table 4), which includes the *Mitchella* group and Prismatomerideae sensu Robbrecht et al. (1991); in other words, they find very low support for keeping the two latter groups separate from Morindeae s.s. Accordingly, they propose a broad circumscription of Morindeae, which includes the *Mitchella* group and two subtribes Morindinae DC. (= Morindeae s.s.) and Prismatomerinae Ruan (= Prismatomerideae sensu Robbrecht et al., 1991). This circumscription of Morindeae has recently been adopted by Robbrecht and Manen (2006). None of the above sister-group relationships are supported by our analyses (PP = 0, Table 4). In contrast with Andersson and Rova (1999) the present study strongly supports the monophyly of both Mitchelleae and Morindeae s.s. and their sister-group relationship, all congruent with the combined *atp*B-*rbc*L/*rbc*L/*rps*16 tree (Fig. 4) in Bremer and Manen (2000).

The present analyses indicate that Morindeae needs to be recircumscribed. Two alternative solutions are possible. One is to recognize a broader circumscription of Morindeae (including Mitchelleae), which would require no nomenclature changes. On the other hand, this would render the tribe heterogeneous morphologically. The other is to restrict Morindeae to include six genera (*Appunia, Coelospermum, Gynochtodes, Morinda, Pogonolobus*, and *Siphonandrium*) with massive and T-shaped (in cross-section) placentae, which are inserted in the middle of the septa and lay between two anatropous ovules (=Morindeae sensu Igersheim and Robbrecht, 1993) and describe a new tribe Mitchelleae to accommodate *Damnacanthus* and *Mitchella*. We favor the second alternative because it renders Morindeae s.s. morphologically homogeneous, which can additionally be characterized by its whitish to yellowish drupaceous fruits and pyrenes with single lateral preformed germination slits (see also Table 6). Igersheim and Robbrecht (1993) have questioned the inclusion of *Sarcopygme* in Morindeae s.s., because the genus has solitary and erect ovules. In contrast, Mitchelleae can be diagnosed by the presence of massive obturator inserted in the indistinct transition zone between placentae and funicule of the ovule; the tribe can additionally be characterized by a combination of the following characters: medium-sized placentae inserted in the upper part of the septum (near the top), four carpels each containing single campylotropous ovule, red drupaceous fruits, and pyrenes without preformed germination slits (Robbrecht et al., 1991; see also Table 6). In addition, Mitchelleae has a distinct and disjunct (North American and East Asian) geographic distribution (Robbrecht et al., 1991).

4.2.5. Palicoureeae Robbr. & Manen and Psychotrieae Cham. & Schtdl. s.s.

The Palicoureeae–Psychotrieae s.s. clade (Fig. 3E) is pantropical and the most species-rich group in Rubiaceae with ca. 36 genera and ca. 2800 species (Govaerts et al., 2006), which can be subdivided into two major subclades: Psychotrieae s.s. (= *Psychotria* complex sensu Andersson, 2001) and Palicoureeae sensu Robbrecht and Manen (2006) (= *Palicourea* complex sensu Andersson, 2001). While the Palicoureeae-Psychotrieae s.s. clade has been identified in previous molecular studies (e.g., Andersson and Rova, 1999; Bremer and Manen, 2000), its position in the Psychotrieae alliance remains elusive. In Andersson and Rova (1999) the Palicoureeae–Psychotrieae s.s. is poorly supported as sister to the Mitchelleae–Morindeae s.s.–Schradereae clade (H8, Table 4). No members of Craterispermeae and Prismatomerideae sensu Robbrecht et al. (1991) are included in this study. In contrast, the Palicoureeae–Psychotrieae s.s. clade is poorly supported as sister to the Gaertnereae–Schradereae clade (H6, Table 4) in Bremer and Manen (2000: Fig. 3). In Robbrecht and Manen (2006), the Palicoureeae–Psychotrieae s.s. clade and *Gaertnera* sp. are sisters (H4, Table 4), which are in turn resolved as sister to a large clade containing Craterispermeae, Morindeae s.s., Mitchelleae, *Coelopyrena salicifolia* Valeton (currently placed in Psychotrieae sensu Robbrecht, 1988), and *Schradera subandina*. Neither of the above relationships is supported by our results (PP = 0, Table 4). Our analyses are inconclusive regarding the position of the Psychotrieae s.l.; the combined chloroplast-nuclear tree (Fig. 4) resolves with poor support the clade as sister to the Craterispermeae–Prismatomerideae clade (PP = 0.66). We accept the recognition of the *Palicourea* complex sensu Andersson (2001) at tribal level (Palicoureeae) and the narrow circumscription of Psychotrieae, both proposed by Robbrecht and Manen (2006). The latter can be distinguished by having stipules that are shed from an abscission layer, while those in the former have non-caducous (but often marcescent) stipules (Andersson, 2002).

4.2.6. Craterispermeae Verdc.

The monogeneric tribe Craterispermeae was originally described by Verdcourt (1958) to accommodate the Afro-Malagasy-Seychellean genus *Craterispermum* Benth. with 16 species (Govaerts et al., 2006) plus at least five undescribed new species from Madagascar. *Craterispemum* can easily be diagnosed by its yellow–green foliage and axillary, condensed inflorescence borne on a stout and flattened peduncle (see also Table 6). Verdcourt (1958) classified his new tribe in Rubioideae based on the presence of raphides, a position highly supported by molecular data (e.g., Bremer and Manen, 2000; Robbrecht and Manen, 2006). The monophyly of Craterispermeae is strongly supported in our analyses; this is inconsistent with Robbrecht and Manen (2006), in which the three sampled *Craterispermum* species (*C. brachynematum* Hiern, *C. caudatum*, and *C. laurinum* Benth.) do not form a clade. In Bremer and Manen (2000), Craterispermeae, represented by the African species *C. bachynematum*, is poorly resolved as sister to the remaining Psychotrieae alliance (H9, Table 4), a relationship not supported by our results (PP = 0, Table 4). In Robbrecht and Manen (2006: Fig. 4C), *Craterispermum caudatum* forms a clade with *Schradera subandina* (H3, Table 4), while *Craterispermum brachynematum* and *C. laurinum* constitute a clade that is sister to *Prismatomeris beccariana*. The former relationship is not supported by our results (PP = 0, Table 4), whereas, this latter one, never discussed in Robbrecht and Manen (2006), is strongly supported by our results (Fig. 3F). The present analyses highly indicate that Craterispermeae and Prismatomerideae are sister groups. On the other hand, we find no obvious morphological synapomorphies to support this relationship. Accordingly, we maintain the tribal status of Craterispermeae.

4.2.7. Prismatomerideae Ruan

The tribe Prismatomerideae is a small Southeast Asian tribe, which was originally established by Ruan (1988) to accommodate the Southeast Asian genus *Prismatomeris*. Later, Igersheim and Robbrecht (1993) added three Southeast Asian genera (*Gentingia, Motleyia*, and *Rennellia*), considered by Johansson (1987a), Johansson and Wong, 1988) to be closely related to the type genus *Prismatomeris*. The members of Prismatomerideae were initially classified in Morindeae apparently because of the occurrence of ovary fusions, a feature commonly found in Morindeae s.s., Mitchelleae, and in some tribes in the Psychotrieae alliance (see Table 6) and some distantly related rubiaceous tribes [e.g., Naucleeae s.l. sensu Razafimandimbison and Bremer (2002) of Cinchonoideae]. As mentioned earlier, we find no support for merging Prismatomerideae sensu Robbrecht et al. (1991) in Morindeae s.l. sensu Bremer and Manen (2000) (H7 with PP = 0, Table 4). Plus, merging Prismatomerideae in Craterispermeae does not seem to be an attractive solution due to their morphological differences, terminal inflorescences, placentae attached to middle of the septum, sessile ovules, large seeds with frequently blue endosperm, and excavation filled with parenchyma-like possibly placental tissue. Based on the molecular evidence presented above and its morphological distinctness (Igersheim and Robbrecht, 1993) we maintain the current tribal status of Prismatomerideae (Table 6).

4.3. Evolutionary trends in the newly delimited Psychotrieae alliance

4.3.1. Unbalanced numbers between the molecular autapomorphies of the tribes and the molecular synapomorphies of groups of tribes: indication of rapid early radiations?

As mentioned earlier, solving the tribal limits and relationships within the Psychotrieae alliance has been difficult despite considerable efforts made by different groups of Rubiaceae specialists (e.g., Robbrecht et al., 1991; Puff et al., 1993; Igersheim et al., 1994; Bremer, 1996a; Jansen et al., 1996). A phylogram of the single most parsimonious tree from the combined chloroplast-nuclear data shown in Fig. 4 reveals unbalanced numbers between molecular autapomorphies of the tribes and that of the molecular synapomorphies of two sister tribes and groups of tribes in the alliance, particularly in the Schradereae–Gaertnereae–Mitchelleae–Morindeae and Craterispermeae–Prismatomerideae clades. Similar patterns are also observed at morphological level, as the tribes in the alliance are morphologically distinct (i.e., they have many morphological autapomorphies) and apparently share no obvious morphological synapomorphies. The same patterns observed at both morphological and molecular levels in the Psychotrieae alliance may well be an indication of a rapid early radiation, such that only few mutations became fixed in the common ancestors of many (tribes) subclades in the Psychotrieae alliance. Table 7 summarizes some important information for *Schizocolea* and all recognized tribes in this study.

Combined chloroplast data from *atp*B-*rbc*L, *rbc*L, and *rps*16 used in Bremer and Manen (2000: Fig. 4) do not yield enough phylogenetic signals for assessing intertribal affinities in the alliance. Only ca. 38% of the PIC in the combined chloroplast-nuclear analyses (Fig. 3) come from the markers used in Bremer and Manen (2000), with the remaining 62% from the nrITS, *ndh*F, and *trn*T-F data. Robbrecht and Manen (2006) produce a fully resolved supertree using various results from independent phylogenetic rubiaceous studies based on sequence data from *atp*B-*rbc*L, *rbc*L, *rps*16, and *trn*L-F. However, no support values of the resolved clades are provided and their results are largely incongruent with ours regarding the monophyly of the tribes in Psychotrieae alliance and their affinities.

In the present study, we have investigated much larger data sets with more taxa and more molecular characters. With the exception of Palicoureeae and Psychotrieae s.s., our samplings for the remain-

Table 7
Compilation of some important information for *Schizocolea* and all currently recognized tribes in the Psychotrieae alliance

Tribes	*Schizocolea*	Schradereae	Gaertnereae	Mitchelleae	Morindeae s.s.	Psychotrieae s.l.[*]	Craterispermeae	Prismatomerideae
Number of species	2	ca. 54	ca. 95	ca. 12	ca. 160	ca. 2800	at least 16	ca. 23
Geographic distributions	West and Central Africa	Neotropics and Southeast Asia	Tropical Asia, tropical Africa, Madagascar, and Mascarenes	North America, East Asia	Pantropical	Pantropical	Tropical Africa, Madagascar, Seychelles	Tropical Asia
Habits	Small Shrubs	Scandent Epiphytes	Shrubs, Small trees	Herbs, shrubs	Shrubs, woody climbers	Shrubs, epiphytes, rarely herbs	Shrubs, small trees	Shrubs
Inflorescence positions	Axillaries	Terminals	Terminals	Terminals	Terminals or axillaries	Terminals	Axillaries	Terminals
Heterostyly	Unknown	Distylous	Homostylous or distylous	Monomorphic but not homostylous or distylous	Homostylous or heterostylous	Distylous	Distylous	Distylous
Ovary positions	Inferior	Inferior	Superior	Inferior	Inferior	Inferior	Inferior	Inferior
Ovule numbers per locule	One	Numerous	One	One	Two	One	One	One
Ovule orientations	Unknown	Campylotropous	Unknown	Campylotropous	Anatropous, amphitropous	Unknown	Anatropous	Hemianatropous with slight tendency towards campylotropous
Fruit types	Berries	Berries	Drupes	Drupes	Drupes	Berries	Berries	Berries
Presence of multiple fruits	Absent	Absent	Present	Present	Present	Absent	Absent	Present
Basic chromosome	Unknown	11	Unknown	11	11	11	11	Unknown
Ploidy level	Unknown	2	Unknown	2	2–4, 8, 20–22	2–12	2	Unknown
Pollen types	Unknown	2-4-porate	2-3-coloporate, 2-3-4-colpoïdorate, 3-porate	3-6-colporate	3-4-colporate	3-5-colporate, 3-5-colpate, rarely 3-, 4-5-porate	3-colporate	3-5-colporate

[*] = Palicoureeae + Psychotrieae s.s.

ing tribes of the Psychotrieae alliance are much larger than those used in previous molecular studies (see Fig. 1A–D).

4.3.2. Assessment of the evolution of seed numbers in the newly circumscribed Psychotrieae alliance

Resolving the tribal limits and relationships within the Psychotrieae alliance is a crucial step for understanding the evolution of seed numbers in the group. We trace the states of seed numbers on the single most parsimonious tree from the combined chloroplast-nuclear analyses of 48 sampled taxa of the Psychotrieae alliance (Fig. 5). The result of the optimization unequivocally indicates a one-seeded carpel as ancestral in the alliance. The present analysis reveals that many- and two-seeded carpels appear to have occurred once each within the Psychotrieae alliance. This evolutionary change of two-seeded carpels from one-seeded carpels has already been reported by Bremer (1996a) within the Psychotrieae alliance. However, this is, to our current knowledge, the first report of a change from a one- to pluri-seeded carpel in Rubiaceae; a similar evolutionary change has recently been reported by Zhang et al. (2006) from the order Curcurbitales. Schradereae is the only tribe of the Psychotrieae alliance with numerous ovules per carpel (Puff et al., 1993), while Morindeae s.s. is the sole tribe with two ovules per carpels. It is, however, worth noting that the Samoan genus *Sarcopygme*, currently placed in the latter tribe by Darwin, 1979), has solitary and erect ovules. So, if its present position is correct, we have a case of reversal from bi- to uniseeded condition within Morindeae s.s.

5. Taxonomic implications

5.1. Mitchelleae Razafim. & B.Bremer, trib. nov

Type genus: Mitchella L., Diss. Chen. 24. 1751; Amoen. Acad. iii. 16. 1756.

Diagnosis: Frutices spinosus vel perennis, herbae repentes. Stipulae integrae vel bifidae. Inflorescentiis (1-)2-(-3-4-) floris. Ovaria 2-locularia, ovulo in quoque loculo singularis atque campylotropous. Fructus drupacei, liber vel coalitus binatin, vivide rubber coloratus.

Description: Thorny shrubs or perennial, creeping herbs with slightly woody stems at the base. Stipules entire or rarely bilobed. Inflorescences terminal, (1-)2-(3-4-) flowered. Flowers monomorphic, but not homostylous (*Damnacanthus*) or heterodi-stylous (four *Damnacanthus* species and *Mitchella*). Ovaries with 4 carpels, each containing a single campylotropous ovule; medium-sized placentae inserted in the upper part of the septum (near the top). Fruits drupaceous, free or fused in pairs, brightly red colored; Seeds with pyrenes without preformed germination slits. Pollen 3-6-colporate. Chromosome basic number $x = 11$ with 2- or 4-ploidy.

Genera included (here investigated): Damnacanthus and Mitchella.
Useful reference: Robbrecht et al. (1991), Naiki and Nagamasu (2003, 2004).

5.2. Morindeae Miq., Flora van Nederlandsch Indie 2: 239, 241. 1857

Morindinae DC., Prodromus Systematis Naturalis 4: 342, 446. 1830.

Type genus: Morinda L.

Description: Shrubs, small trees, or lianas. Stipules entire or dentate, usually connate to sheathing. Inflorescences predominantly terminal, sometimes leaf-opposed, paired or verticillate in the axils or single axillary, usually pedunculate, mostly head-like, sometimes paniculate or umbel-like or short to elongate cymes. Flowers usually homostylous, sometimes heterostylous. Ovary 2-locular, massive and T-shaped (in cross-section) placentae inserted in the

middle of the septa and lay between two anatropous ovules. Fruits drupaceous, often connate and forming multiple or compound fruits, sometimes free, whitish to yellowish. Seeds with pyrenes with single lateral preformed germination slits. Pollen 3-4-colporate. Chromosome basic number $x = 11$ with 2-, 4-, 8-, or 20-ploidy level (Kiehn, 1995).

Genera included (here investigated): Appunia, Coelopermum, Gynochthodes, and Morinda.
Inclusion based on morphology: Pogonolobus and Syphonandrium.
Useful references: Igersheim and Robbrecht (1993), Johansson (1987b, 1994).

6. Conclusions

The present study further support the sister-group relationship between *Schizocolea* and the Psychotrieae sensu Bremer and Manen (2000) and is the first to reveal with high support that the Psychotrieae alliance can be subdivided into four monophyletic groups: the *Schizocolea* clade, the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade, the Palicoureeae–Psychotrieae clade, and the Craterispermeae–Prismatomerideae clade. The relationships between the last three clades are unresolved. Within the clade with four tribes, the Schradereae–Gaertnereae–Mitchelleae–Morindeae clade, the relationships are: (Schradereae(Gaertnereae(Mitchelleae–Morindeae s.s.))). For now, we recognize a total of eight morphologically distinct and well-defined tribes in the alliance: Craterispermeae, Gaertnereae, Mitchelleae Razafim. & B.Bremer trib. nov., Morindeae s.s., Palicoureeae, Prismatomerideae, Psychotrieae s.s., and Schradereae, all with high supported. A new monogeneric tribe will be described elsewhere to accommodate *Schizocolea* (Rydin et al., submitted for publication). Furthermore, we propose a narrow circumscription of Morindeae, which includes only six genera (*Appunia, Coelospermum, Gynochthodes, Morinda, Pogonolobus*, and *Syphonandrium*). The new tribe Mitchelleae is described here to accommodate the members of the *Mitchella* group (sensu Robbrecht et al., 1991). Furthermore, our study reveals much higher numbers of molecular autapomorphies of the tribes compared with those of molecular synapomorphies of two tribes or groups of tribes. A oneseeded carpel is unequivocally shown to be the pleisiomorphic condition in the Psychotrieae alliance. Finally, we report for the first time in Rubiaceae one case of evolutionary change from one- to many-seeded condition.

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