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Phylogenetic affinities of *Myrioneuron* and *Cyanoneuron*, generic limits of the tribe Argostemmateae and description of a new Asian tribe, Cyanoneuroneae (Rubiaceae)

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Abstract Argostemmateae (Rubiaceae, subfam. Rubioideae) are a mostly tropical Asian group of about 200 species currently classified in four morphologically distinct genera (*Argostemma*, *Mouretia*, *Mycetia*, *Neohymenopogon*). The monophyly of the tribe and *Mycetia* is strongly supported by molecular data, however, the tropical Asian genus *Myrioneuron*, traditionally associated with *Mycetia* based on its berry fruits, has not previously been investigated. The Bornean and Sulawesian genus *Cyanoneuron*, described based on the species of *Myrioneuron* with drupaceous fruits, had not been sequenced. Therefore, the phylogenetic positions of *Cyanoneuron* and *Myrioneuron* within Rubiaceae and their generic status have yet to be assessed with molecular data. These genera have tentatively been placed in tribe Spermacoceae (Rubioideae). We reconstructed a robust phylogeny of Rubioideae with sequence data from five plastid regions of 176 accessions and using the Bayesian Markov chain Monte Carlo and parsimony methods. Once the positions of *Cyanoneuron* and *Myrioneuron* were revealed, a robust phylogeny of the Spermacoceae alliance was reconstructed with the combined plastid and nuclear data (nrETS, nrITS) from 61 accessions to reassess its tribal limits. *Mycetia* and *Myrioneuron* are non-monophyletic and intermixed, and formed a well-supported clade diagnosed by berry fruits. We formally transfer *Myrioneuron* to *Mycetia* (older name), and present nine new combinations in the latter genus. *Cyanoneuron* was resolved with high support as monophyletic, and appears to be closely related to the Chinese monogeneric tribes Foonchewieae and Dunnieae. A new tribe Cyanoneuroneae is described to accommodate *Cyanoneuron*. This tribe is morphologically distinct from related tribes by its stipules apically divided into multiple linear segments, condensed-cymose inflorescences and drupe-like fruits with numerous small seeds. A new key to the genera of Argostemmateae is provided.

Keywords Argostemmateae; Cyanoneuroneae; generic circumscription; molecular systematics; *Mycetia*; taxonomy; tribal circumscription; tropical Asia

Supplementary Material Alignment files are available in the Supplementary Data section of the online version of this article at http://www.ingentaconnect.com/iapt/tax

■ INTRODUCTION

Bremekamp (1952) described tribe Argostemmateae Bremek. ex Verdc. (Rubiaceae, subfam. Rubioideae) to accommodate the paleotropical genus *Argostemma* Wall., which was traditionally associated with the large genus *Oldenlandia* L. (tribe Spermacoceae, Rubioideae). However, it was Verdcourt (1958) who validly published this tribal name by providing a Latin diagnosis. Since then the generic delimitations of Argostemmateae have changed greatly (Bremekamp, 1966; Bremer, 1987, 1996a; Rydin & al., 2009b). Earlier circumscriptions of the tribe included *Argostemma*, *Clarkella* Hook.f., *Neurocalyx* Hook. and *Steenisia* Bakh.f. (Verdcourt, 1958; Bremekamp, 1966). This group of plants was characterized by having adnate anthers opening with a short slit or a pore and stamens attached at the base of the corolla lobes (Bremekamp, 1966). *Steenisia* was later excluded from Argostemmateae by Bremer (1984) based on the absence of raphides, its thick-walled hairs, left-contorted corollas, hard, bony endocarp, and seed coat structure similar to that of subfamily Cinchonoideae. The genus was transferred to tribe Rondeletieae of subfamily Cinchonoideae (Bremer, 1987), but was recently shown by Kainulainen & al. (2009, 2013) to be a member of subfamily Ixoroideae. It is currently placed in its own tribe Steenisieae (Kainulainen & al., 2013). Bremer (1987) restricted Argostemmateae to include only *Argostemma* and *Neurocalyx*; however, the *rbcL*-based phylogenetic study by Bremer (1996a) revealed that *Argostemma* and *Mycetia* Reinw.

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are closely related, while *Neurocalyx* belongs to tribe Ophiorrhizeae (Rubioideae). Andersson & Rova (1999) and Bremer & Manen (2000) confirmed the close relationship between *Argostemma* and *Mycetia*. Bremer (1987) excluded *Clarkella* from the tribe based on its funnel-shaped flowers, anthers not fixed to the base of corolla, stigmas with two linear lobes and very large pollen. This genus is currently classified in its own tribe Clarkelleae (Deb, 2001). Recently, Argostemmateae were re-circumscribed by Rydin & al. (2009b) as a result of their molecular phylogenetic study based on combined data from five plastid and one nuclear regions; the tribe includes four genera: *Argostemma*, *Mouretia* Pit., *Mycetia* and *Neohymenopogon* Bennet. The present study pinpoints for the first time the phylogenetic placements of the two enigmatic Asian genera *Myrioneuron* R.Br. ex Benth. & Hook.f. and *Cyanoneuron* Tange within the subfamily Rubioideae. The results of our analyses also allow us to re-assess the generic limits of tribe Argostemmateae and establish new tribal limits of the Spermacoceae alliance (Bremer & Manen, 2000).

Tribe Argostemmateae as delimited by Rydin & al. (2009b) is a member of the species-rich Spermacoceae alliance (Bremer & Manen, 2000) in subfamily Rubioideae, and sister to a large clade formed by tribes Dunnieae, Foonchewieae, Paederieae, Putorieae, Theligoneae and Rubieae (Rydin & al., 2009b; Wen & Wang, 2012). This group of plants exhibits a mostly tropical Asian distribution, with only two species of *Argostemma* endemic to tropical Africa. It can broadly be characterized by: herbs or small shrubs with entire or slightly cleft stipules; isostylous or heterostylous hermaphroditic flowers with anthers typically inserted at the base of the corolla tubes and opening with vertical slits or pores; and mostly capsules bearing numerous small seeds (Rydin & al., 2009b). The genera of Argostemmateae are morphogically distinct. *Argostemma* typically has adnate anthers (Bremer, 1989), while the other three genera have free anthers. *Mycetia* is the only genus with berries; *Neohymenopogon* has dry capsules, while *Argostemma* and *Mouretia* produce fleshy capsules. *Argostemma* and *Neohymenopogon* have isostylous flowers, whereas *Mouretia* and *Mycetia* have heterodistylous flowers. Moreover, *Neohymenopogon* are mostly epiphytic and have stamens inserted in the upper part of the corolla tubes; in contrast, the other genera are non-epiphytic and have stamens inserted at the base of the corolla tubes. Finally, the leafy bracts subtending the inflorescences of *Neohymenopogon* are unique within Argostemmateae. However this feature has evolved independently numerous times in Rubiaceae (e.g., Hymenodictyeae, Razafimandimbison & Bremer, 2006; Dunnieae, Rydin & al., 2008). Argostemmateae as defined by Rydin & al. (2009b) do not seem to have any obvious morphological synapomorphy but are highly supported as monophyletic by molecular data. The tropical Asian genus *Myrioneuron*, which has been traditionally associated with *Mycetia* and appeared to fall within *Mycetia*, has not previously been investigated in molecular phylogenetic studies of Rubiaceae. Therefore, the monophyly of *Mycetia* and Argostemmateae as defined by Rydin & al. (2009b) needs to be re-tested.

Myrioneuron was initially placed in tribe Mussaendeae based on its fleshy fruits (Hooker, 1882). Bremekamp (1952) moved the genus to tribe Hedyotideae (synonym of tribe Spermacoceae) based on the presence of raphides and the absence of large pits in the basal wall of testa cells. Currently, *Myrioneuron* consists of eight species that are distributed in Bhutan, China, Vietnam, India and Nepal. Its members can be characterized by their arborescent habit with spongy bark, large leaves and stipules, mostly terminal cymose inflorescences with white, 5-lobed tubular flowers, villous inside and valvate in bud, and berries with many small seeds (Deb, 1996; Chen &Taylor, 2011). *Myrioneuron* has sometimes been treated as a synonym of *Mycetia* (Bakhuizen, 1975) or *Keenania* Hook.f. (Van Steenis, 1987; Robbrecht, 1988). Deb (1996) hypothesized that these three genera are closely related (with *Myrioneuron* more closely related to *Mycetia* than to *Keenania*), but are morphologically distinct. He distinguished *Myrioneuron* from *Mycetia* by coriaceous (vs. membraneous) leaves, isostylous flowers (vs. heterostylous), and lack of stalked marginal glands on leaves, stipules, bracts and flower parts (vs. presence of this type of gland in *Mycetia*). However, it is erroneous to describe the flowers of *Myrioneuron* as isostylous, as they are clearly heterodistylous; furthermore, the stalked marginal glands are not always found in *Mycetia*, because some *Mycetia* species only have sessile marginal glands (Chen & Taylor, 2011). The monophyly of *Mycetia* and *Myrioneurion* has not been tested with a molecular-based phylogenetic analysis.

The Bornean and Sulawesian genus *Cyanoneuron* (Tange, 1998) was described based on three species of *Myrioneuron* with drupaceous fruits. Tange (1998: 148) described the fruits of *Cyanoneuron* as "drupe fruits with one stone separated into two loculi by a thin and soft septum, each loculus with numerous small seeds." The genus as delimited by Tange (1998) consists of five species of herbs or small shrubs with stipules divided into long linear segments, bluish nerves on the abaxial side of the leaves, "drupe-like" fruits and seeds with thickened exotesta cells. Based on its general morphology, *Cyanoneuron* was postulated to be related to *Myrioneuron* and *Hedyotis* L. (Spermacoceae). It has a two-layered upper epidermis, which has not been observed in other Rubiaceae, and multiseriate hairs, absent in *Myrioneuron* and *Hedyotis* (Tange, 1998). *Cyanoneuron* was tentatively placed by Tange (1998) in Spermacoceae, but so far no molecular phylogenetic studies of Rubiaceae have investigated any of its members.

The main objectives of the present study are to: (1) assess the phylogenetic positions of *Cyanoneuron* and *Myrioneuron* within Rubioideae; (2) re-test the monophyly of Argostemmateae sensu Rydin & al. (2009b) and *Mycetia*; and (3) re-assess the generic limits of Argostemmateae and the tribal limits of the Spermacoceae alliance.

■ MATERIALS AND METHODS

Taxon sampling and laboratory procedures. — A sampling of 180 Rubioideae accessions, including the 11 currently recognized tribes of the Spermacoceae alliance (Anthospermeae, Argostemmateae, Danaideae, Dunnieae, Foonchewieae, Knoxieae, Paederieae, Putorieae, Rubieae, Spermacoceae and

Theligoneae, Rydin & al., 2009b; Wen & Wang, 2012), was used for this study (Appendix 1). We focused our sampling on this subfamily, as *Myrioneuron* and *Cyanoneuron* exhibit the typical characteristics of this group, including the presence of raphides and heterostyly. In addition, the top 100 hits from BLAST searches of various *Myrioneuron* and *Cyanoneuron* sequences against published Rubiaceae sequences available in the international databases all belong to Rubioideae. The types of *Cyanoneuron* (*C. cyaneum* (Hallier f.) Tange), *Mycetia* (*M. caulifora* Reinw.) and *Myrioneuron* (*M. nutans* R.Br. ex Kurz) were included in our analyses. We were unable to identify at species level four specimens of *Mycetia* and one specimen of *Myrioneuron* from Vietnam using *Flora of China* (Chen & Taylor, 2011). We suspect that these specimens represent new undescribed species. Unfortunately, due to the lack of material, *Keenania* could not be studied. *Colletoecema*, sister to the rest of Rubioideae (Robbrecht & Manen, 2006; Rydin & al., 2008), was used as outgroup to root the trees generated from the large chloroplast dataset.

Total DNA was extracted from 30 newly available plant samples, following the standard CTAB protocol (Doyle & Doyle, 1987). Extracted DNA samples were then purified using the QIAquick PCR Purification Kit following the manufacturer's protocol (QIAGEN, Hilden, Germany). In total, purified DNAs of 47 accessions were amplified through the polymerase chain reaction (PCR) and subsequently sequenced for five plastid (*atpB-rbcL*, *ndhF*, *rbcL*, *trnTF*, *rps16*) and two nuclear (nrITS, nrETS) regions.

Amplifications were performed with the GeneAmp PCR System 9700 (Applied Biosystems, Singapore). Table 1 summarizes the amplification programs used. Each 50-µl PCR reaction contained: 5 µl reaction buffer, 5 µl 0.1M TMACl (Tetramethylammonium chloride), 4 µl dNTP, 0.5 µl Paq DNA polymerase, 0.5 µl 1% BSA (bovine serum albumin), 0.5 µl 20µM forward primer and 0.5 µl 20µM reverse primer (Table 2), 1 µl of template DNA (water for negative control) and water up to 50 µl. For nuclear regions (ETS, ITS), 0.7 µl of both forward and reverse primers (Table 2) were used. Products of successful amplifications were then purified using the Multiscreen$_{\mathrm{HTS}}$ PCR plates (Millipore, Billerica, Massachusetts, U.S.A.). Sequencing reactions of 10 µl were prepared as follows: 5 µl of PCR product and 5 µl of 10 µM primer were mixed in each well of the sequencing plate. The same primers (Table 2) were used for amplifications and sequencing. Finally, the prepared plates were sent to Macrogen (Amsterdam, Netherlands) for sequencing.

Sequence editing and alignment. — Sequence fragments were assembled and edited using the Staden package v.2.0.0b9 (Staden, 2013). Previously published sequences were obtained from GenBank. The alignment of matrices was done manually following similarity criterion (Simmons, 2004) using BioEdit v.7.2.5 (Hall, 2013).

Phylogenetic analyses. — The single-marker matrices were subjected to parsimony bootstrap analyses (Felsenstein, 1985) using PAUP* v.4.0b10 (Swofford, 2002) under the following settings: heuristic search, random sequence addition with 5 replicates, character resampling with 100 replicates, TBR branch swapping and MULTREES off and excluding parsimony-uninformative characters. Gaps were treated as missing data in all alignments and inferred indels/deletions were not coded as separate characters. The ensemble consistency index (CI) and ensemble retention index (RI) were calculated. The resulting bootstrap trees (not presented) were visually compared to detect any supported topological incongruence.

We performed combined Bayesian Markov chain Monte Carlo (MCMC) analyses (Yang & Rannala, 1997) of the datasets from five plastid regions (*atpB-rbcL*, *ndhF*, *rbcL*, *rps16*, *trnTF*) using the computer program MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The best-fitting nucleotide substitution models under the Akaike information criterion (AIC; Akaike, 1973) were determined for the protein-coding (combined *rbcL* and *ndhF*) and non-coding (combined *rps16*, *atpB-rbcL* and *trnTF*) matrices, using the computer program MrModeltest v.2.2 (Nylander, 2004). The best-fit model for the coding dataset was GTR+I+G, while for the non-coding data the best-fit model was GTR+G. The combined plastid matrix was subdivided into two partitions. The analysis consisted of two independent parallel runs of four Markov chains each, run for 9×10^6 generations, with trees sampled every 2000 generations. The convergence of the two runs was confirmed (standard deviation of split frequencies below 0.01). The first third of trees were excluded (burn-in) and a 50% Bayesian majority-rule consensus tree was calculated based on the remaining trees. The resulting cladogram was set to show branch lengths in order to illustrate inferred amount of character changes for each branch. In addition, a parsimony bootstrap analysis of the combined plastid matrix was performed under the following settings: heuristic search, random

Table 1. Amplification settings used for different DNA regions studied.

	rbcL, *rps16*, *trnTF*, *atpB-rbcL*	*ndhF*	nrETS and nrITS
Initialization	95°C – 2 min	94°C – 2 min	97°C – 1 min
Denaturation	97°C – 30 s	94°C – 45 s	97°C – 10 s
Annealing	50°C – 1 min	49°C – 1 min	97°C – 10 s
Elongation	72°C – 2 min	72°C – 1 min 20 s	55°C – 30 s, 72°C – 20 s (ramp 3°/cycle)
Final elongation	72°C – 7 min	72°C – 6 min	72°C – 7 min
Number of cycles	37	35	40

sequence addition with 10 replicates, character resampling with 1000 replicates, TBR branch swapping and MULTREES off.

After assessing the phylogenetic positions of *Myrioneuron* and *Cyanoneuron* within Rubioideae, the sampling was reduced to only include 61 accessions of the Spermacoceae alliance: 9 accessions of *Cyanoneuron* (representing 2 species), 41 accessions of Argostemmateae (including 13 accessions of *Myrioneuron*, representing 6 species) and 11 accessions (representing 11 species) from the 10 tribes of the Spermacoceae alliance (Anthospermeae, Danaideae, Dunnieae, Foonchewieae, Knoxieae, Paederieae, Putorieae, Rubieae, Spermacoceae, Theligoneae). *Danais xanthorrhea* (Danaideae, shown to be sister to the remaining Spermacoceae alliance, e.g., Rydin & al., 2009b) was utilized as outgroup. Sequence data of the five plastid and two nuclear regions from the 61 accessions were analyzed with the Bayesian MCMC method. The best-fitting model for the coding, non-coding and nuclear matrices was GTR+I+G. The Bayesian MCMC analysis of the combined plastid-nuclear matrix was performed using the same settings as before, except the matrix was subdivided into three partitions (combined coding plastid data: *ndhF*, *rbcL*; combined non-coding plastid data: *atpB-rbcL*, *rps16*, *trnT-F*; combined nuclear data), and was run

Table 2. Summary of primers used for DNA amplification and sequencing.

	Primer name	Sequence	Reference
atpB-rbcL	rbcL5R	CTCTTTAACACCAKCYTTGAATCC	Rydin & al., 2008
atpB-rbcL	atpB5R	CCGATGATTTGGACAATACG	Rydin & al., 2008
ndhF	720F	GCACAATTTCCCCTTCATGTATGG	Rydin & al., 2008
ndhF	17R	AGTATTATCCGATTCATAAGGAT	Rydin & al., 2008
ndhF	1320F	GGGATTAAC(CT)GCATTTTATATGTTTCG	Rydin & al., 2008
ndhF	2280R	AAGAAAAGATAAGAAGAGATGCG	Rydin & al., 2008
ndhF	1F	AGGTAAGATCCGGTGAATCGGAAAC	Bremer & al., 2002
ndhF	735	CAGCACACAAAGTAACAAAT	Unpublished
rbcL	5F	ATGTCACCACAAACAGAAACTAAAGC	Bremer & al., 2002
rbcL	1020R	ATCATCGCGCAATAAATCAACAAAACCTAAAGT	Bremer & al., 2002
rbcL	427bs	GCTTATATTAAAACCTTCCAAGGCCCGCC	Bremer & al., 2002
rbcL	3R	CTT TTA GTA AAA GAT TGG GCC GAG	Bremer & al., 2002
rps16	F	GTGGTAGAAAGCAACGTGCGACTT	Oxelman & al., 1997
rps16	2R	TCGGGATCGAACATCAATTGCAAC	Oxelman & al., 1997
trnTF	A1	ACAAATGCGATGCTCTAACC	Bremer & al., 2002
trnTF	I	CCAACTCCATTTGTTAGAAC	Bremer & al., 2002
trnTF	1250F	ATGGCGAAATTGGTAGACGC	Rydin & al., 2008
trnTF	D	GGGGATAGAGGGACTTGAAC	Taberlet & al., 1991
nrETS	EritF	CTTGTATGGGTTGGTTGGA	Negrón-Ortiz &Watson, 2002
nrETS	18S-E	GCAGGATCAACCAGGTAGCA	Baldwin & Markos, 1998
nrITS	P17	CTACCGATTGAATGGTCCGGTGAA	Popp & Oxelman, 2001
nrITS	P25	GGGTAGTCCCGCCTGACCTG	Oxelman, 1996

Table 3. Characteristics of the plastid markers used for the separate and combined parsimony analyses of the plastid datasets.

	atpB-rbcL	*ndhF*	*rbcL*	*rps16*	*trnTF*	Combined data
Number of accessions in each matrix	176	176	176	176	176	176
Number of sequences in each matrix	162	173	175	168	167	845 (96.02%)
Number of missing sequences in each matrix	14	3	1	8	9	35 (3.98%)
Number of new sequences in each matrix	26	28	29	26	29	138
Total characters (bp)	1148	2222	1403	1672	3522	9967
Parsimony-informative characters (PIC)	335	772	373	627	1113	3220
Tree length	926	3043	1718	2087	3541	11,554
Consistency index (CI)	0.562	0.428	0.317	0.494	0.532	0.457
Retention index (RI)	0.850	0.809	0.793	0.790	0.817	0.801

for 20 × 10^6 generations. A parsimony bootstrap analysis of the combined plastid-nuclear matrix was conducted under the settings described above.

■ RESULTS

Information about all sequence data from the five plastid markers is summarized in Table 3. This study used a total of 904 sequences, of which 825 sequences were from the five selected chloroplast regions (*atpB-rbcL*, *ndhF*, *rbcL*, *rps16*, *trnT-F*; Table 3) and 79 sequences from the nrETS and nrITS regions (28 and 51 respectively). One hundred eighty-six of the 904 sequences (ca. 20.57%) are newly published in this study.

Separate analyses. — Comparison of trees resulting from the single plastid analyses of the large datasets (not presented) showed no strongly supported topological conflicts. All sampled species of *Myrioneuron* were nested in Argostemmateae sensu Rydin & al. (2009b) in all trees, but resolution inside the tribe varied between trees. *Myrioneuron* and *Mycetia* were non-monophyletic and intermixed. *Cyanoneuron* was resolved as monophyletic. Its phylogenetic position varied from being well-supported sister clade to Argostemmateae (in the *ndhF* tree) to being nested within a largely unresolved Spermacoceae alliance (all other trees). Table 3 summarizes statistics for the separate and combined parsimony analyses. As a consequence, datasets from the five single plastid matrices were all combined in one large matrix in order to increase the number of informative characters.

Combined analyses. — The combined plastid data of the 176 Rubioideae accessions yielded a total of 9967 base pairs (bp), of which 3220 bp were parsimony-informative (Table 3). There were no strongly supported topological conflicts observed between the 50% Bayesian majority-rule consensus tree (Fig. 1) and the bootstrap parsimony tree (not shown). Therefore, the bootstrap values from the parsimony bootstrap analysis were

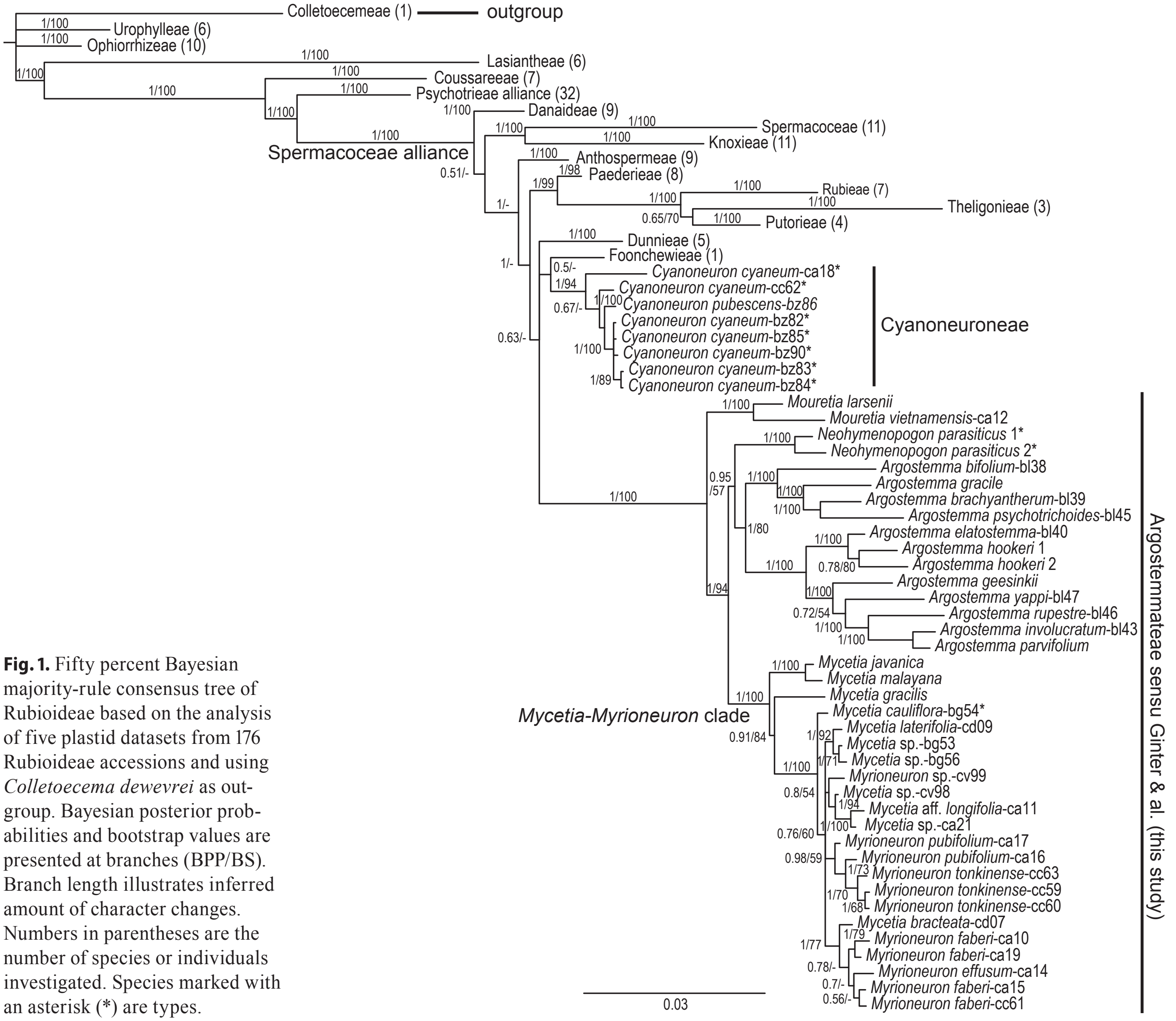

Fig. 1. Fifty percent Bayesian majority-rule consensus tree of Rubioideae based on the analysis of five plastid datasets from 176 Rubioideae accessions and using *Colletoecema dewevrei* as outgroup. Bayesian posterior probabilities and bootstrap values are presented at branches (BPP/BS). Branch length illustrates inferred amount of character changes. Numbers in parentheses are the number of species or individuals investigated. Species marked with an asterisk (*) are types.

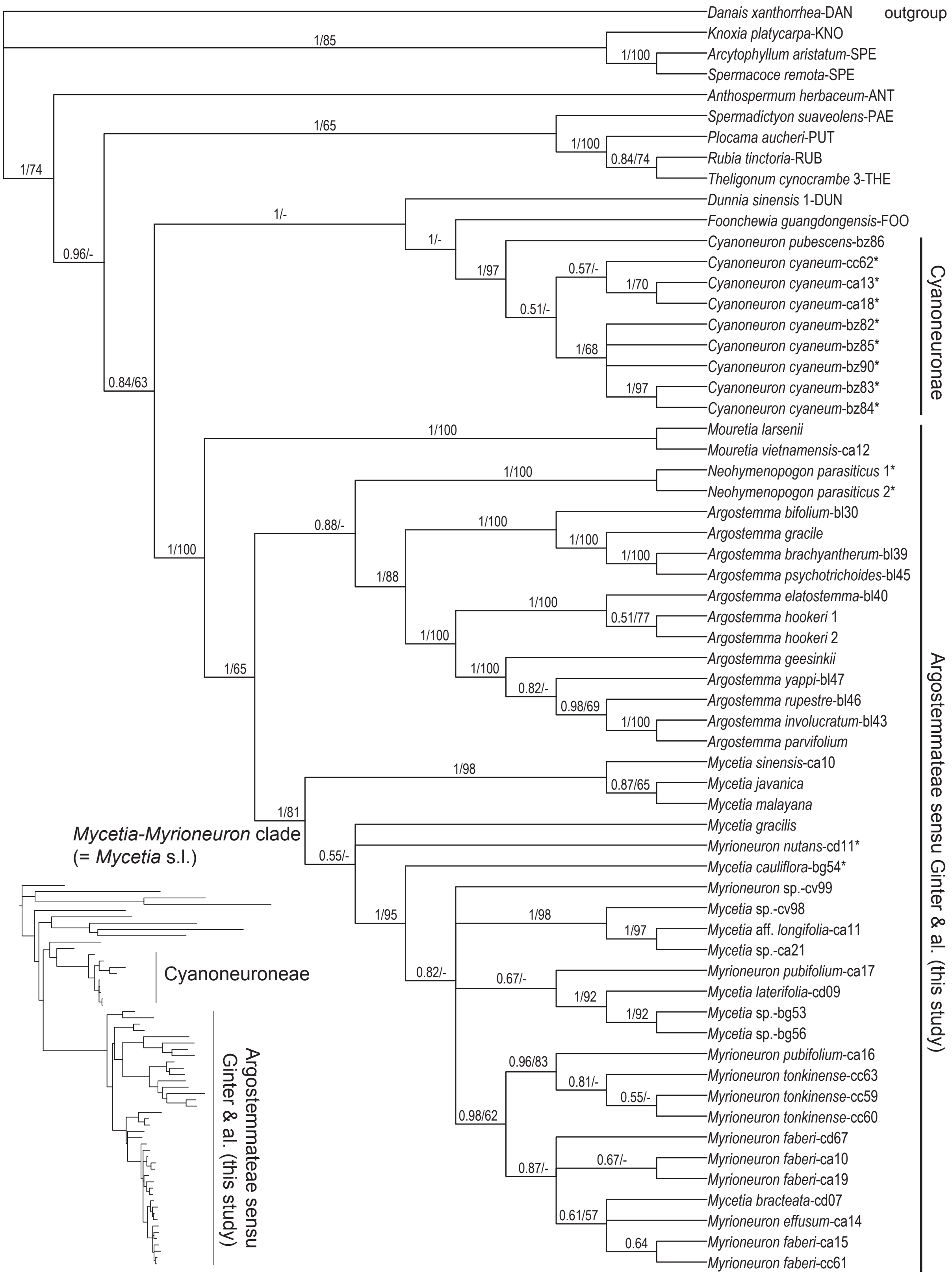

Fig. 2. Fifty percent Bayesian majority-rule consensus tree of the Spermacoceae alliance based on the analysis of the combined plastid-nuclear data from 61 accessions and using *Danais xanthorrhea* as outgroup. Posterior probabilities and bootstrap values are presented above branches (BPP/BS). Branch length illustrates inferred amount of character changes. Species marked with an asterisk (*) are types. ANT, Anthospermeae; DAN, Danaideae; DUN, Dunnieae; FOO, Foonchewieae; KNO, Knoxieae; PAE, Paederieae; PUT, Putorieae; RUB, Rubieae; SPE, Spermacoceae; THE, Theligoneae.

added onto the 50% Bayesian majority-rule consensus tree. *Myrioneuron* was nested within Argostemmateae sensu Rydin & al. (2009b), and formed a well-supported clade (Bayesian posterior probability, BPP = 1 and bootstrap, BS = 100) with *Mycetia* (hereafter called the *Mycetia-Myrioneuron* clade). The two genera were shown to be non-monophyletic and intermixed. Within the *Mycetia-Myrioneuron* clade phylogenetic relationships between the sampled species of *Mycetia* and *Myrioneuron* were only partly resolved. *Cyanoneuron* (represented by *C. cyaneum* and *C. pubescens* (Valeton) Tange) was nested within the Spermacoceae alliance, and was poorly supported (BPP = 0.5, BS < 50) as sister to the Chinese monogeneric tribe Foonchewieae. The genus was resolved as monophyletic with high support (BPP = 1, BS = 94).

There was no strongly supported topological conflict between the trees produced from the Bayesian and parsimony bootstrap analyses of the combined plastid and combined nuclear matrices of the 61 accessions of the Spermacoceae alliance (not presented). As a result, the datasets from the five plastid and two nuclear matrices were combined. In the combined plastid-nuclear tree generated from the Bayesian MCMC analysis (with bootstrap values added; Fig. 2), the support for the monophyly of *Cyanoneuron* was high (BPP = 1, BS = 97) and this lineage was resolved sister to the Chinese tribe Foonchewieae (BPP = 1, BS < 50). The Chinese monogeneric tribe Dunnieae was resolved as sister to the Foonchewieae-*Cyanoneuron* clade (BPP = 1, BS < 50). The Dunnieae-Foonchewieae-*Cyanoneuron* clade was resolved with weak support (BPP = 0.84, BS = 63) as sister to Argostemmateae (including *Myrioneuron*). The *Mycetia-Myrioneuron* clade received high support (BPP = 1, BS = 81) and *Myrioneuron* and *Mycetia* remained non-monophyletic and intermixed (Fig. 2).

■ DISCUSSION

Non-monophyly of *Myrioneuron* and *Mycetia* and phylogenetic position of *Myrioneuron*. — *Mycetia* and *Myrioneuron* have been considered to be closely related (e.g., Deb, 1996). This hypothesis is highly supported by the present molecular phylogenetic analyses. Deb (1996) recognized *Myrioneuron* and *Mycetia* as distinct genera; this is not supported by our results, because these genera are non-monophyletic and intermixed. Therefore, this study supports the decision of Bakhuizen (1975) who treated *Myrioneuron* as a synonym of *Mycetia*. The *Mycetia-Myrioneuron* clade is highly supported (BPP = 1, BS = 100 or 81; Figs. 1–2), and characterized by whitish soft bark, flowers with stamens inserted in the corolla tube at different positions and berries with many small seeds. As the general morphology of *Myrioneuron* falls within the range of *Mycetia*, its inclusion in the latter genus will not require any change in the current tribal description of Argostemmateae (Rydin & al., 2009b).

Monophyly and phylogenetic position of *Cyanoneuron*. — As described by Tange (1998), *Cyanoneuron* contains five species, and was tentatively placed in tribe Spermacoceae due to its overall morphological similarity with *Hedyotis*. The present study is the first to include *Cyanoneuron* in a molecular phylogenetic study of Rubiaceae, and supports its phylogenetic placement within the Spermacoceae alliance (Figs. 1–2). However, the genus does not belong to Spermacoceae and is not closely related to *Myrioneuron* as postulated by Tange (1998). *Cyanoneuron* is resolved as monophyletic with high support (BPP = 1, BS = 94 or 97; Figs. 1–2), and is strongly supported as sister to the Chinese tribe Foonchewieae in the Bayesian tree from the combined plastid-nuclear data. On the other hand, this sister-group relationship receives poor support in the corresponding parsimony bootstrap analysis (Fig. 2) and in both the Bayesian MCMC and parsimony analyses of the combined plastid data (Fig. 1). The Chinese tribes Dunnieae and Foonchewieae and *Cyanoneuron* form a highly supported clade in the Bayesian analysis of the combined plastid-nuclear data; however, this relationship collapses in the parsimony analysis (Fig. 2). More data are needed to test the close relationships of Dunnieae, Foonchewieae and *Cyanoneuron* as suggested by the Bayesian analysis of the plastid-nuclear datasets.

Non-monophyly of Argostemmateae sensu Rydin & al. and reassessment of its generic limits. — As described earlier, tribe Argostemmateae sensu Rydin & al. (2009b) comprises of four genera: *Argostemma*, *Mycetia*, *Neohymenopogon* and *Mouretia*. The monophyly of this tribe has been strongly supported by molecular data (e.g., Rydin & al., 2009b). However, this study shows that Argostemmateae sensu Rydin & al. (2009b) is paraphyletic unless *Myrioneuron* is also included (BPP = 1, BS = 100 or 81; Figs. 1–2).

The overall topology of Argostemmateae in this study is consistent with the results of Rydin & al. (2009b). In both studies, *Mouretia* is the first-diverging lineage, and sister to the rest of Argostemmateae. This genus is resolved as sister to a large clade formed by the *Mycetia-Myrioneuron* clade (BPP = 1, BS = 100 or 81) and the *Neohymenopogon-Argostemma* clade (BPP = 0.95 or 0.88, BS = 57 or <50). These two latter clades are sisters. In Rydin & al. (2009b), *Mycetia* is resolved with high support as monophyletic; however, *Myrioneuron* was not included in that study. In the present study, *Mycetia* and *Myrioneuron* are non-monophyletic and intermixed. In addition, the *Neohymenopogon-Argostemma* clade was strongly supported in Rydin & al. (2009b), which is not the case in this study. Based on the evidence presented above, we maintain the generic status of *Argostemma*, *Mouretia* and *Neohymenopogon*. The current circumscriptions of *Mycetia* and *Myrioneuron* are untenable.

New combinations in *Mycetia*. — Based on the evidence presented in this study, *Myrioneuron* and *Mycetia* have to be combined to represent a monophyletic genus. *Mycetia* Reinwardt 1825 has priority over *Myrioneuron* R.Br. ex Benth. & Hook.f. 1873 (McNeill & al., 2012). In consequence, nine species of *Myrioneuron* are formally transferred to *Mycetia* here.

Mycetia Reinw. in Hornschuch, Syll. Pl. Nov. 2: 9. 1825 – Type: *Mycetia cauliflora* Reinw.
= *Adenosacme* Wall. ex Endl., Gen. Pl.: 552. 1838 – Type: *Adenosacme longifolia* (Wall.) Hook.f. ≡ *Mycetia longifolia* (Wall.) Kuntze.
= *Lawia* Wight in Calcutta J. Nat. Hist. 7: 14. 1846 – Type:

Lawia acuminata Wight ≡ *Mycetia acuminata* (Wight) Kuntze.

= *Myrioneuron* R.Br. ex Benth. & Hook.f., Gen. Pl. 2: 69. 1873, **syn. nov.** – Type: *Myrioneuron nutans* R.Br. ex Kurz.

1. ***Mycetia angustifolia*** (Hook.f.) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron angustifolium* Hook.f., Fl. Brit. India 3: 97. 1880 – Holotype: Birma (= Burma), *W. Griffith 3088* (K barcode K000031972!).

2. ***Mycetia clarkei*** (Hook.f.) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron clarkei* Hook.f., Fl. Brit. India 3: 96. 1880 – Holotype: Chittagong (Bangladesh), 1 Feb 1857 (fl.), *J.D. Hooker & T. Thompson s.n.* (K barcode K000031970!; isotype: K barcode K000031971!).

3. ***Mycetia effusa*** (Pit.) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron nutans* var. *effusa* Pit., Fl. Indo-Chine 3: 192. 1923 ≡ *Myrioneuron effusum* (Pit.) Merr. in J. Arnold Arbor. 23: 195. 1942 – Holotype: Tonkin (Vietnam), Vallée de Lankok (Mont Bavi), 18 Oct 1887 (fr.), *B. Balansa 2725* (P barcode P03953370!).

4. ***Mycetia faberi*** (Hemsl.) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron faberi* Hemsl. in J. Linn. Soc. Bot. 23: 380. 1888 – Holotype: China, Szechwan (Sichuan), Mont Omei, 3500 ft., Dec 1887 (fl.), *E. Faber 260* (K barcode K000740563!).

= *Myrioneuron oligoneuron* Hand.-Mazz. in Sinensia 5: 21. 1934 – Holotype: China, Guizhou Province (Kweichou), Na-kan, Chengfeng, in fossa, 17 Oct 1930, *Y. Tsiang 4627* (CVH!).

5. ***Mycetia hirsuta*** (Kurz) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron hirsutum* Kurz, Forest Fl. Burma 2: 55. 1877 – Holotype: Ava, hills east of Bhamo, *J. Anderson s.n.* (not located).

Notes. – In his publication of *Myrioneuron hirsutum*, Kurz provided a description and mentioned Ava hills as the locality. The type specimen of *M. nutans* was not mentioned in the protologue. However, in his article entitled "Contributions towards a knowledge of the Burmese flora" published the same year (in J. Asiat. Soc. Bengal 46: 161. 1877), Kurz included *M. hirsutum* in his generic conspectus for *Myrioneuron*. He provided the following information: "*M. hirsutum*, Kurz For. Fl. II. 55. Hab. Ava, hills east of Bhamo (J. Anderson). - Fl. May." Based on the above information we consider the specimen *J. Anderson s.n.* to be the holotype of *M. hirsutum*.

6. ***Mycetia nutans*** (R.Br. ex Kurz) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron nutans* R.Br. ex Kurz, Forest Fl. Burma 2: 55. 1877 – Holotype: Chittagong-hills, Kassalong (Bangladesh), *C.B. Clarke s.n.* (not located).

Notes. – In his publication of *Myrioneuron nutans*, Kurz provided a description and mentioned Chittagong as the locality. The type specimen of *M. nutans* was not mentioned in the protologue. However, in his article entitled "Contributions towards a knowledge of the Burmese flora" published the same year (in J. Asiat. Soc. Bengal 46: 161. 1877), Kurz included *M. nutans* in his generic conspectus for *Myrioneuron*. He provided the following information: "*M. nutans*, R. Br. in Wall. Cat.; Kurz For. Fl. II. 55. Hab. Chittagong-hills, Kassalong (C. B. Clarke). Fr. CS." Based on the above information we consider the specimen *C.B. Clarke s.n.* to be the holotype of *M. nutans*.

7. ***Mycetia parviflora*** (Hook.f.) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron nutans* var. *parviflora* Hook.f., Fl. Brit. India 3: 96. 1880 – Holotype: Birma (= Burma), (fr.), *W. Griffith 3020* (K barcode K000031973!).

Note. – We recognize *Myrioneuron nutans* var. *parviflora* at species level, as it is clearly morphologically distinct from *Myrioneuron nutans* var. *nutans*.

8. ***Mycetia pubifolia*** (Pit.) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron pubifolium* Pit., Fl. Indo-Chine 3: 193. 1923 – **Lectotype (designated here):** Vietnam, Province Vienh Yen, Tam Dao, *P.A. Eberhardt 4983* (P barcode P02273470!; isolectotypes: P barcodes P02273471–3!).

9. ***Mycetia tonkinensis*** (Pit.) Razafim. & B.Bremer, **comb. nov.** ≡ *Myrioneuron tonkinense* Pit., Fl. Indo-Chine 3: 193. 1923 – **Lectotype (designated here):** Vietnam, Kien Khe, sub umbrosis rupibus, *Dong Ban, H.F. Bon 3022* (P barcode P02273474!; isolectotypes: P barcodes P02273475–6!).

Key to the genera of Argostemmateae

1. Herbaceous plants; flowers with adnate anthers .... ***Argostemma***
1. Epiphytic or non-epiphytic shrubs or small trees; flowers with free anthers 2
2. Epiphytic shrubs; leaf-like bracts subtending inflorescences; flowers isostylous .... ***Neohymenopogon***
2. Non-epiphytic shrubs or small trees; leaf-like bracts subtending inflorescences absent; flowers heterodistylous . 3
3. Inflorescences scorpioid cymes; fruits fleshy capsules .. .. ***Mouretia***
3. Inflorescences corymbose or capitate cymes; fruits berries .... ***Mycetia*** s.l. (including *Myrioneuron*)

Re-assessments of the tribal limits in the Spermacoceae alliance: description of the new tribe Cyanoneuroneae. — The results of this study have taxonomic implications for the tribal limits of the Spermacoceae alliance (Bremer & Manen, 2000). As already stated before, *Cyanoneuron* is resolved as a strongly supported monophyletic group, and is distinct by its stipules divided into long linear segments and drupe-like fruits (Tange, 1998; Table 4). In addition, the genus has a two-layered upper epidermis, which has not been observed in other Rubiaceae (Tange, 1998). Furthermore, *Cyanoneuron* is not nested within any of the other tribes of the Spermacoceae alliance. Its close relationship with the Chinese tribes Foonchewieae and Dunnieae as indicated by the Bayesian analysis of the combined plastid-nuclear data is not supported by the parsimony analysis of the same data. Unlike *Cyanoneuron*, these two tribes,

Table 4. Comparison between tribes Dunnieae, Foonchewieae and Cyanoneuroneae.

	Dunnieae (Rydin & al., 2009a, b)	Foonchewieae (Wen & Wang, 2012)	Cyanoneuroneae (Tange, 1998; this study)
Geographic distribution	China	China	Borneo and Sulawesi
Habit	subshrubs	subshrubs	herbs to subshrubs
Stipules	bifid	entire	divided into long linear segments
Presence of white, persistent, petaloid bracts	yes	no	no
Inflorescence type	panicles	panicles	condensed cymes
Flower	heterodistylous, 4–5-merous	heterodistylous, 5-merous	heterodistylous, 5-merous
Fruit type	capsules with lignified wall, dehiscent septicidally	capsules with lignified wall, dehiscent with apical pores	drupe-like
Seed	non-angular and winged	angular and unwinged	angular and unwinged
Pollen type	3-colporate	4-colporate	3-colporate

respectively, have bifid and entire stipules and capsular fruits dehiscing septicidally and through apical pores (see Table 4 for additional morphological characteristics of tribes Dunnieae, Foonchewieae and *Cyanoneuron*). Therefore, placing *Cyanoneuron* in its own tribe, Cyanoneuroneae, is the best solution (see below). Finally, the sister-group relationship between Dunnieae and Foonchewieae as revealed by Wen & Wang (2012) is not supported by our analyses.

Cyanoneuroneae Razafim. & B.Bremer, **tr. nov.** – Type: *Cyanoneuron* Tange

Description (slightly modified from Tange, 1998). – Herbs to small shrubs. Leaves with intramarginal nerves and upper epidermis two-layered. External hairs multicellular, thin-walled, and with constriction between the cells. Idioblasts with raphides. Stipules divided into multiple linear segments. Inflorescences terminal, condensed cymes. Flowers 5-merous, heterodistylous; corollas hypocrateriform, valvate in bud; stamens inserted in corolla tube; ovary 2-locular crowned by calyx and an annular disc, with peltate placentae attached to middle of septum, ovules numerous. Fruits drupes each with one stone separated into two loculi by a thin and soft septum, with numerous small seeds in each locule. Seeds angular. Pollen spherical, tricolporate, colpus narrow, tectum reticulate to foveolate.

Diagnostic characters. – Tribe Cyanoneuroneae is distinct from the closely related tribes Foonchewieae, Dunnieae and Argostemmateae by its stipules apically divided into multiple linear segments, condensed-cymose inflorescences and drupe-like fruits with numerous small seeds.

Genus included. – Cyanoneuron.

Geographic distribution. – Borneo and Sulawesi.

■ FUTURE PERSPECTIVES

Future studies of Argostemmateae should focus on in-depth morphological and molecular investigations of the *Mycetia-Myrioneuron* clade, as the current study provides poor resolution at species level. More species of the largest genus *Argostemma* should be investigated in order to assess the phylogenetic relationships within the genus. Finally, efforts should be made to obtain sequenceable material of *Keenania*, which has been traditionally associated with *Mycetia*, and *Clarkella*, previously included in Argostemmateae.

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Appendix 1. Information on the investigated taxa and markers.

Taxon, tribal classification, voucher information (for the new sequences only), accession numbers: *rbcL*, *rps16*, *ndhF*, *atpB-rbcL* spacer, *trnT-F*, nrITS and nrETS, respectively, a dash (–) denotes missing data.

Amphidasya ambigua (Standl.) Standl., Urophylleae, Y11844[1], AF129271[2], –, EU145337[3], EU145576[3], –, –; ***Anthospermum herbaceum*** L.f., Anthospermeae, X83623[4], EU145496[3], AJ236284[5], AJ234028[6], EU145544[3], EU145355[3], –; ***Appunia guatemalensis*** Donn.Sm., Morindeae, *Lundell 6675* (S), AJ288593[6], AM945306[34], AM945252[34], AJ234009[6], KP212892, –, –; ***Arcytophyllum aristatum*** Standl., Spermacoceae, AJ288595[6], AF333348[7], FJ695282[33], FJ695343[33], AF333349[7], AM182061[8], –; ***Argostemma bifolium***-bl38 Ridl., Argostemmateae, *Bremer 1797* (S), FJ695220[33], KP212839, FJ695283[33], FJ695344[33], FJ695396[33], FJ695428[33], KP212732; ***Argostemma brachyantherum***-bl39 Stapf, Argostemmateae, *Beaman 8931* (S), FJ695221[33], FJ695252[33], FJ695284[33], FJ695345[33],

Appendix 1. Continued.

FJ695397[33], FJ695429[33], KP212733; ***Argostemma elatostemma***-bl40 Hook.f., Argostemmateae, *B. Bremer & K. Bremer 1722* (S), FJ695222[33], –, –, FJ695346[33], –, –, KP212734; ***Argostemma geesinkii*** B.Bremer, Argostemmateae, FJ695223[33], FJ695253[33], FJ695285[33], FJ695347[33], FJ695398[33], FJ695430[33], –; ***Argostemma gracile***-bl42 Stapf, Argostemmateae, *Beaman 8885* (S), FJ695224[33], FJ695254[33], FJ695286[33], FJ695348[33], FJ695399[33], FJ695431[33], KP212735; ***Argostemma hookeri*** 1 King, Argostemmateae, Z68788[16], EU145497[15], EU145419[15], AJ234032[6], EU145545[15], EU145356[15], –; ***Argostemma hookeri*** 2 King, Argostemmateae, FJ695225[33], FJ695255[33], FJ695287[33], FJ695349[33], FJ695400[33], FJ695432[33], –; ***Argostemma involucratum***-bl43 Hemsl., Argostemmateae, *Wanntorp 3047* (S), FJ695226[33], FJ695256[33], FJ695288[33], FJ695350[33], FJ695401[33], FJ695433[33], KP212736; ***Argostemma parvifolium*** Benn., Argostemmateae, FJ695227[33], FJ695257[33], FJ695289[33], FJ695351[33], FJ695402[33], FJ695434[33], –; ***Argostemma psychotrichoides***-bl45 Ridl., Argostemmateae, *B. Bremer & K. Bremer 1744* (S), FJ695228[33], FJ695258[33], FJ695290[33], FJ695352[33], –, FJ695435[33], KP212737; ***Argostemma rupestre***-bl46 Ridl., Argostemmateae, *B. Bremer & K. Bremer 1609* (S), FJ695229[33], FJ695259[33], KP212782, FJ695353[33], KP212865, FJ695436[33], KP212738; ***Argostemma yappii***-bl47 King, Argostemmateae, *B. Bremer & K. Bremer 1675* (S), FJ695239[33], FJ695260[33], KP212783, FJ695354[33], FJ695403[33], FJ695437[33], KP212739; ***Batopedina pulvinellata*** Robbr., Spermacoceae, AJ288596[6], AM266813[9], FJ695291[33], FJ695355[33], AM266902[9], –, –; ***Bouvardia ternifolia*** (Cav.) Schltdl., Spermacoceae, X83626[4], AF002758[11], FJ695292[33], –, X76478[10], DQ359165[12], –; ***Carpacoce* sp.,** Knoxieae, FJ695231[33], FJ695261[33], FJ695293[33], FJ695356[33], FJ695404[33], –, –; ***Carphalea glaucescens*** (Hiern) Verdc. (accepted name ***Dirichletia glaucescens*** Hiern.), Knoxieae, Z68789[16], AM266817[9], AJ236287[5], FJ695357[33], AM266906[9], –, –; ***Chassalia catatii*** Drake ex Bremek., Palicoureeae, AM945305[34], AM945331[34], AM945283[34], AM945251[34], AM945363[34], –, –; ***Coccocypselum condalia*** Pers., Coussareeae, AM117217[13], EU145499[3], EU145420[3], EU145324[3], EU145547[3], –, –; ***Coccocysepselum hirsutum*** Bartl. ex DC., Coussareeae, X87145[14], EU145500[3], EU145421[3], EU145325[3], EU145548[3], –, –; ***Coelospermum monticolum*** Baill. ex Guillaumin (accepted name ***Coelospermum fragrans*** (Montrouz.) Baill. ex Guillaumin), Morindeae, AF331644[7], AF001438[11], AM945255[34], AM945221[34], AM945334[34], –, –; ***Colletoecema dewevrei*** (De Wild.) E.M.A.Petit, Colletoecemeae, EU145457[15], AF129272[2], EU145409[15], DQ131713[12], EU145532[15], –, –; ***Coprosma pumila*** Hook.f., Anthospermeae, X87146[4], FJ695262[33], FJ695294[33], –, FJ695405[33], –, –; ***Coussarea hydrangeifolia*** (Benth.) Müll.Arg., Coussareeae, EU145460[3], EU145501[3], EU145422[3], EU145326[3], EU145549[3], –, –; ***Craterispermum laurinum*** (Poir.) Benth., Craterispermeae, AM945300[34], AM945325[34], AM945276[34], AM945243[34], AM945356[34], –, –; ***Craterispermum* sp.** 1, Craterispermeae, *Eriksson & al. 999* (S), AM945297[34], KP212864, AM945273[34], AM945241[34], AM945353[34], –, –; ***Craterispermum* sp.** 2, Craterispermeae, AM945298[34], AM945323[34], AM945274[34], AM945242[34], AM945354[34], –, –; ***Cremocarpon lantzii*** Bremek. (accepted name ***Psychotria lantzii*** (Bremek.) Razafim. & B.Bremer), Psychotrieae, AM117222[13], AM117296[13], –, –, AM117356[13], –, –; ***Cruckshanksia hymenodon*** Hook. & Arn., Coussareeae, AJ288599[6], EU145502[3], –, AJ234004[6], EU145550[3], –, –; ***Cyanoneuron cyaneum***-bz82 (Hallier f.) Tange, Argostemmateae, *Coode & al. 6639* (L), KP212810, –, KP212784, KP212706, KP212866, –, –; ***Cyanoneuron cyaneum***-bz83 (Hallier f.) Tange, Argostemmateae, *Purseglove 5197* (S), KP212811, –, KP212785, KP212707, KP212867, –, KP212740; ***Cyanoneuron cyaneum***-bz84 (Hallier f.) Tange, Argostemmateae, *Bogner 1457* (L), KP212812, KP212840, KP212786, KP212708, KP212868, –, KP212741; ***Cyanoneuron cyaneum***-bz85 (Hallier f.) Tange, Argostemmateae, *Beaman 10186* (L), KP212813, KP212841, KP212787, KP212709, KP212869, –, –; ***Cyanoneuron cyaneum***-bz90 (Hallier f.) Tange, Argostemmateae, *Sundaling 135234* (L), KP212814, –, KP212788, KP212710, KP212870, –, –; ***Cyanoneuron cyaneum***-ca13, Argostemmateae, *Sundaling 129726* (L), –, –, –, –, –, KP212760, –; ***Cyanoneuron cyaneum***-ca18, Argostemmateae, *Sands 5797* (L), KP212815, KP212842, KP212789, KP212711, KP212761, KP212871, –; ***Cyanoneuron cyaneum***-cc62, Argostemmateae, *Axel D. Poulsen 292* (AAU), KP212816, KP212843, KP212791, –, KP212872, KP212762, –; ***Cyanoneuron pubescens***-bz86 (Valeton) Tange, Argostemmateae, *Ambriansyah 605* (L), KP212817, KP212844, KP212790, KP212712, KP212873, KP212763, –; ***Damnacanthus indicus*** C.F.Gaertn., Mitchelleae, Z68793[16], AF331647[7], AM945256[34], AJ234015[6], AM945335[34], –, –; ***Damnacanthus macrophyllus*** Siebold ex Miq., Mitchelleae, AM945285[34], AM945308[34], AM945257[34], AM945222[34], AM945336[34], –, –; ***Danais comorensis*** Drake, Danaideae, FJ695232[33], FJ695263[33], –, FJ695358[33], FJ695406[33], –, –; ***Danais fragrans*** (Lam.) Pers., Danaideae, FJ695233[33], FJ695264[33], FJ695295[33], FJ695359[33], FJ695407[33], –, –; ***Danais* sp.** 1-bg49, Danaideae, *Kårehed & al. 254* (UPS), KP212837, JQ729698[32], KP212893, FJ695297[33], FJ695361[33], –, –; ***Danais* sp.** 2, Danaideae, FJ695234[33], FJ695265[33], FJ695296[33], FJ695360[33], FJ695408[33], –, –; ***Danais xanthorrhea*** (K.Schum.) Bremek., Danaideae, Z68794[16], AM117297[13], AJ236293[5], AJ234019[6], DQ662138[17], EU145364[3], –; ***Declieuxia cordigera*** Mart. ex Zucc., Urophylleae, AM117224[13], AM117298[13], EU145423[3], EU145327[3], EU145551[3], –, –; ***Declieuxia fruticosa*** (Willd. ex Roem.) Kuntze, Urophylleae, AJ002177[18], EU145503[3], –, DQ131721[12], EU145552[3], –, –; ***Didymaea alsinoides*** (Cham. & Schltdl.) Standl., Rubieae, Z68795[16], –, FJ695298[33], AJ234036[6], EU145570[3], –, –; ***Dunnia sinensis*** 1 Tutcher, Dunnieae, EU145467[15], EU145515[15], EU145442[15], EU145339[15], EU145583[15], EU145390[3], –; ***Dunnia sinensis*** 2 Tutcher, Dunnieae, EU145468[15], EU145516[15], EU145443[15], EU145340[15], EU145584[15], –, –; ***Dunnia sinensis*** 3 Tutcher, Dunnieae, EU145469[15], EU145517[15], EU145444[15], EU145341[15], EU145585[15], –, –; ***Dunnia sinensis*** 4 Tutcher, Dunnieae, EU145470[15], EU145518[15], EU145445[15], EU145342[15], EU145586[15], –, –; ***Dunnia sinensis*** 5 Tutcher, Dunnieae, EU145471[15], EU145519[15], EU145446[15], EU145343[15], EU145587[15], –, –; ***Ernodea littoralis*** Sw., Spermacoceae, AJ288601[6], AF002763[11], –, AJ234025[6], –, –, –; ***Faramea multiflora*** A.Rich., Coussareeae, Z68796[16], AF004048[11], EU145424[3], EU145328[3], AF102422[19], –, –; ***Foonchewia guangdongensis*** R.J.Wang, Foonchewieae, JQ002641[37], JQ002637[37], JQ002645[37], JQ002649[37], –, –, –; ***Gaertnera phyllosepala*** Baker, Gaertnereae, AM945288[34], AM945311[34], AM945261[34], AM945227[34], AM945340[34], –, –; ***Gaertnera phyllostachya*** Baker, Gaertnereae, AM945289[34], AM945312[34], AM945262[34], AM945228[34], AM945341[34], –, –; ***Galium album*** Mill., Rubieae, *B. Bremer 3321* (UPS), X81090[20], AF004050[11], FJ695299[33], X76459[10], –, –, –; ***Geophila obvallata*** Didr., Palicoureeae, AM117228[13], AF369845[21], AM945259[34], –, EU145569[3], –, –; ***Gynochthodes coriacea*** Blume, Morindeae, AJ288603[6], AM117311[13], AM945253[34], AM945219[34], AJ847407[22], –, –; ***Gynochthodes* sp.,** Morindeae, AM945284[34], AM945307[34], AM945254[34], AM945220[34], AM945333[34], –, –; ***Houstonia caerulea*** L., Spermacoceae, AJ288604[6], AF333379[7], FJ695300[33], FJ695362[33], AF381524[23], –, –; ***Kelloggia chinensis*** Franch., Rubieae, AY570776[35], AY570771[35], –, AY570765[35], –, –, –; ***Kelloggia galioides*** Torr., Rubieae, DQ662179[17], DQ662203[17], FJ695301[33], AY570768[35], DQ662146[17], –, –; ***Knoxia platycarpa*** Arn., Knoxieae, AJ288631[6], AM266826[9], FJ695302[33], FJ695363[33], AM117367[13], AM267002[9], –; ***Kohautia caespitosa*** Schnizl., Spermacoceae, Z68800[16], AM117324[13], FJ695303[33], FJ695364[33], EU145573[3], –, –; ***Lasianthus kilimandscharicus*** K.Schum., Lasiantheae, AM117237[13], AM117327[13], EU145426[3], EU145330[3], DQ662147[17], –, –; ***Lasianthus pedunculatus*** E.A.Bruce, Lasiantheae, Z68802[16], EU145504[3], EU145427[3], AJ234003[6], EU145555[3], –, –; ***Leptodermis potaninii*** Batalin, Paederieae, AM117241[13], DQ662204[17], FJ695304[33], FJ695365[33], DQ662148[17], –, –; ***Lerchea bracteata*** Valeton, Ophiorrhizeae, AJ288610[6], EU145508[3], EU145433[3], AJ233997[6], EU145561[3], –, –; ***Manostachya ternifolia*** E.S.Martins, Spermacoceae, AJ616213[24], AM117328[13], FJ695305[33], FJ695366[33], EU145572[3], –, –; ***Margaritopsis acuifolia*** C.Wright, Palicoureeae, AM117247[13], AF001340[11], –, AM945225[34], EU145568[15], –, –; ***Maschalocorymbus corymbosus*** (Blume) Bremek., Urophylleae, AJ288611[6], AM900611[26], –, –, EU145577[3], –, –; ***Mitchella repens*** L., Mitchelleae, Z68805[16], AF001441[11], AM945258[34], AM945223[34], AM945337[34], –, –; ***Mitrasacmopsis quadrivalvis*** Jovet, Spermacoceae, AJ616214[24], AM117329[13], EU145439[3], EU145336[3], EU145575[3], –, –; ***Morinda citrifolia*** L., Morindeae, AJ318448[25], AJ320078[25], AJ236300[5], AJ234013[6], AF152616[27], –, –; ***Mouretia larsenii*** Tange, Argostemmateae, FJ695236[33], FJ695267[33], FJ695306[33], FJ695367[33], FJ695410[33], FJ695447[33], –; ***Mouretia vietnamensis*** Tange, Argostemmateae, *Razafimandimbison & al. 748* (S), KP212818, KP212845, KP212792, KP212713, KP212874, –, KP212742; ***Mycetia*** aff. ***longifolia***-ca11, Argostemmateae, *Krüger & al. 33* (S), KP212830, KP212857, KP212804, KP212725, KP212886, KP212777, KP212755; ***Mycetia bracteata***-cd07 Hutch., Argostemmateae, *Steward & Cheo 1105* (S), KP212819, KP212846, KP212793, KP212714, KP212875, KP212764, KP212743; ***Mycetia cauliflora***-bg54 Reinw., Argostemmateae, *Larsen & al. 46287* (AAU), FJ695237[33], FJ695268[33], FJ695307[33], FJ695368[33], FJ695411[33], FJ695448[33], KP212744; ***Mycetia gracilis*** Craib, Argostemmateae, FJ695238[33], FJ695269[33], FJ695308[33], FJ695369[33], FJ695412[33], FJ695449[33], –; ***Mycetia javanica*** (Blume) Reinw. ex Korth., Argostemmateae, FJ695239[33], FJ695270[33], FJ695309[33], FJ695370[33], FJ695413[33], FJ695450[33], –; ***Mycetia lateriflora***-cd09 (Blume) Reinw. ex Korth, Argostemmateae, *Axelius 367* (S), KP212821, KP212848, KP212795, KP212716, KP212877, KP212765, KP212746; ***Mycetia malayana*** (G.Don) Craib, Argostemmateae, Z68806[16], AF002771[11], FJ695310[33], AJ234033[6], AF152622[27], –, –; ***Mycetia sinensis***-cd10 (Hemsl.) Craib, Argostemmateae, *F.C. How 73075* (S), –, –, –, –, –, KP212766, KP212747; ***Mycetia* sp.**-bg53, Argostemmateae, *Larsen & al. 44635* (AAU), FJ695240[33], FJ695271[33], FJ695311[33], FJ695371[33], FJ695414[33], FJ695451[33], KP212748; ***Mycetia* sp.**-bg56, Argostemmateae, *Larsen & al. 43706* (AAU), FJ695241[33], FJ695272[33], FJ695312[33], FJ695372[33], FJ695415[33], FJ695452[33], KP212749; ***Mycetia* sp.**-ca21, Argostemmateae, *Krüger & al. 28* (S), KP212832, KP212859, KP212806, KP212727, KP212888, KP212779, KP212757; ***Mycetia* sp.**-cv98, Argostemmateae, *Swenson & al. 1285* (S), KP212820, KP212847, KP212794, KP212715, KP212876, –, KP212745; ***Myrioneuron effusum***-ca14 (Pit.) Merr., Argostemmateae, *Teng 90857* (L), KP212823, KP212850, KP212797, KP212718, KP212879,

Appendix 1. Continued.

KP212768, –; ***Myrioneuron faberi***-ca10 Hemsl., Argostemmateae, *Krüger & al. 23* (S), KP212829, KP212856, KP212803, KP212724, KP212885, KP212776, KP212754; ***Myrioneuron faberi***-ca15 Hemsl., Argostemmateae, *D.E. Boufford & Bartholomew 24308* (L), KP212824, KP212851, KP212798, KP212719, KP212880, KP212769, –; ***Myrioneuron faberi***-ca19 Hemsl., Argostemmateae, *Krüger & al. 22* (S), KP212831, KP212858, KP212805, KP212726, KP212887, KP212778, KP212756; ***Myrioneuron faberi***-cc61 Hemsl., Argostemmateae, *D.E. Boufford & B. Bartholomew 24308* (AAU), KP212825, KP212852, KP212799, KP212720, KP212881, KP212770, KP212751; ***Myrioneuron faberi***-cd67 Hemsl., Argostemmateae, *Wang & al. 870420* (UPS), –, –, –, –, –, KP212771, –; ***Myrioneuron nutans***-cd11 R.Br. ex Kurz, Argostemmateae, *Herbarium of the late East India company 3019* (S), –, –, –, –, –, KP212772, –; ***Myrioneuron pubifolium***-ca16 Pit., Argostemmateae, *Cuong 428* (L), KP212826, KP212853, KP212800, KP212721, KP212882, KP212773, –; ***Myrioneuron pubifolium***-ca17 Pit., Argostemmateae, *Kainulainen & al. 25* (S), KP212827, KP212854, KP212801, KP212722, KP212883, KP212774, KP212752; ***Myrioneuron*** **sp.**-cv99, Argostemmateae, *Swenson & al. 1246* (S), KP212828, KP212855, KP212802, KP212723, KP212884, KP212775, KP212753; ***Myrioneuron tonkinense***-cc60 Pit., Argostemmateae, *Nguyen Nghia Thin & al. 3390* (AAU), KP212834, KP212861, KP212808, KP212729, KP212890, KP212781, KP212759; ***Myrioneuron tonkinense***-cc59 Pit., Argostemmateae, *Nguyen Nghia Thin & al. 3285* (AAU), KP212833, KP212860, KP212807, KP212728, KP212889, KP212780, KP212758; ***Myrioneuron tonkinense***-cc63 Pit., Argostemmateae, *Nguyen Nghia Thin & al. 2353* (AAU), KP212822, KP212849, KP212796, KP212717, KP212878, KP212767, KP212750; ***Nenax acerosa*** Gaerth., Spermacoceae, –, AF003606[11], –, –, –, –, –; ***Neohymenopogon parasiticus*** 1 (Wall.) Bennet, Argostemmateae, FJ695242[33], FJ695273[33], FJ695313[33], FJ695373[33], FJ695416[33], FJ695453[33], –; ***Neohymenopogon parasiticus*** 2 (Wall.) Bennet, Argostemmateae, FJ695243[33], FJ695274[33], FJ695314[33], FJ695374[33], FJ695417[33], –, –; ***Nertera granadensis*** Druce, Spermacoceae, X83654[4], AF002741[11], –, –, AF152623[27], –, –; ***Neurocalyx championii*** Benth. ex Thwaites, Ophiorrhizeae, EU145463[3], EU145509[3], EU145435[3], –, EU145563[3], –, –; ***Neurocalyx zeylanicus*** Hook., Ophiorrhizeae, Z68807[16], AM900594[26], EU145434[3], AJ233995[6], EU145562[3], –, –; ***Normandia neocaledonica*** Hook.f., Anthospermeae, AM117250[13], AF257931[31], FJ695315[33], FJ695375[33], EU145543[15], –, –; ***Oldenlandia corymbosa*** L., Spermacoceae, X83655[4], AF333381[7], AJ130837[5], –, AF381537[23], –, –; ***Opercularia vaginata*** Juss., Anthospermeae, Z68809[16], AF257936[31], FJ695316[33], FJ695376[33], FJ695418[33], –, –; ***Ophiorrhiza elmeri*** Merr., Ophiorrhizeae, EU145464[3], EU145510[3], EU145436[3], –, EU145509[3], –, –; ***Ophiorrhiza mungos*** L., Ophiorrhizeae, X83655[4], AF004064[11], AJ130838[5], –, DQ662151[12], –, –; ***Ophiorrhiza*** **sp.**-ca20 L., Ophiorrhizeae, *Krüger & al. 35* (S), KP212835, KP212862, KP212809, KP212731, KP212891, –, –; ***Ophiorrhiza*** **sp.**-cv97 L., Ophiorrhizeae, *Swenson & al. 1411* (S), KP212836, KP212863, –, KP212730, –, –, –; ***Otiophora scabra*** Zucc., Knoxieae, FJ695244[33], AM266839[9], –, DQ131756[12], AM266928[9], –, –; ***Otomeria oculata*** S.Moore, Knoxieae, AJ288614[6], AM266844[9], FJ695317[33], FJ695377[33], AM117374[13], –, –; ***Paederia bojeriana*** (A.Rich. ex DC.) Drake, Paederieae, DQ662181[17], DQ662206[17], FJ695318[33], DQ662151[12], DQ662152[17], –, –; ***Paederia majungensis*** Homolle ex Puff, Paederieae, DQ662184[17], DQ662209[17], FJ695319[33], FJ695378[33], DQ662155[17], –, –; ***Paederia sambiranensis*** Homolle ex Puff, Paederieae, DQ662188[17], DQ662213[17], –, FJ695379[33], DQ662159[17], –, –; ***Pagamea guianensis*** Aubl., Gaertnereae, AM945290[34], AF002744[11], AM945263[34], AM945229[34], AM945342[34], –, –; ***Palicourea crocea*** (Sw.) Schult, Palicoureeae, AM117253[13], AF147510[28], AM945280[34], AM945247[34], AM945259[34], –, –; ***Parapentas silvatica*** (K.Schum.) Bremek., Knoxieae, X83657[4], AM266849[9], FJ695320[33], AJ234021[6], AM266937[9], –, –; ***Paratriaina xerophila*** Bremek. (accepted name ***Triainolepis xerophila*** (Bremek.) Kårehed & B.Bremer), Knoxieae, AJ288633[6], AM266850[9], FJ695321[33], FJ695380[33], AM266938[9], –, –; ***Pauridiantha paucinervis*** (Hiern), Urophylleae, Z68811[16], AM900600[26], AJ236302[5], AJ233998[6], EU145578[3], –, –; ***Pauridiantha symplocoides*** (S.Moore) Bremek., Urophylleae, AY538502[29], AF004068[11], EU145440[3], EU145338[3], AF102467[19], –, –; ***Pentanisia prunelloides*** (Klotzsch) Walp., Knoxieae, AM117255[13], AM266860[9], FJ695322[33], FJ695381[33], AM266948[9], –, –; ***Pentas lanceolata*** K.Schum., Knoxieae, X83659[4], AM266875[9], AJ236304[5], X76479[10], AM266963[9], –, –; ***Pentodon pentandrus*** Vatke, Spermacoceae, X83660[4], AF003612[11], FJ695323[33], AJ234024[6], FJ695419[33], –, –; ***Phyllis nobla*** L., Anthospermeae, Z68814[16], AF003613[11], FJ695324[33], AJ234031[6], AY538468[29], –, –; ***Placopoda virgata*** Balf.f., Knoxieae, Z68815[16], AM117335[13], FJ695325[33], FJ695382[33], AM117382[13], –, –; ***Plocama aucheri*** (Guill.) M.Backlund & Thulin, Putorieae, DQ662178[17], DQ662202[17], FJ695326[33], FJ695383[33], DQ662145[17], FJ695456[33], –; ***Plocama calabrica*** (L.f.) M.Backlund & Thulin, Putorieae, AJ288620[6], FJ695275[33], FJ695327[33], FJ695384[33], DQ662166[17], –, –; ***Plocama hymenostephana*** (Jaub. & Spach) M.Backlund & Thulin, Putorieae, DQ662190[17], DQ662215[17], FJ695328[33], FJ695385[33], DQ662163[17], –, –; ***Plocama pendula*** Aiton, Putorieae, Z68816[4], FJ695276[33], FJ695329[33], AJ234035[6], DQ662162[17], –, –; ***Pomax umbellata*** (Gaertn.) Sol. ex A.Rich., Anthospermeae, AM117260[13], AF257941[7], –, DQ131767[12], FJ695420[33], –, –; ***Pravinaria leucocarpa*** Bremek., Urophylleae, AJ288617[6], AM900613[26], EU145441[3], AJ234001[6], EU145580[3], –, –; ***Prismatomeris albidiflora*** Thwaites, Prismatomerideae, AM945296[34], AM945320[34], AM945270[34], AM945237[34], AM945351[34], –, –; ***Prismatomeris beccariana*** (Baill. ex K.Schum.) J.T.Johanss., Prismatomerideae, AJ288618[6], AF331652[7], AM945271[34], AM945238[34], AM945352[34], –, –; ***Prismatomeris*** **sp.** 1, Prismatomerideae, AM945292[34], AM945316[34], AM945266[34], AM945233[34], AM945347[34], –, –; ***Prismatomeris*** **sp.** 2, Prismatomerideae, AM945293[34], AM945317[34], AM945267[34], AM945234[34], AM945348[34], –, –; ***Psychotria amboniana*** K.Schum., Psychotrieae, AM945302[34], AM945328[34], AM945281[34], AM945248[34], AM945360[34], –, –; ***Psychotria capensis*** (Eckl.) Vatke, Psychotrieae, AM945301[34], AM945326[34], AM945277[34], AM945245[34], AM945357[34], –, –; ***Psychotria holtzii*** (K.Schum.) E.M.A.Petit, Psychotrieae, AM945304[34], AM945330[34], –, AM945250[34], AM945362[34], –, –; ***Psychotria kirkii*** Hiern., Psychotrieae, X83663[4], AF410728[30], AJ236307[5], X76481[10], AY538469[29], –, –; ***Psychotria poeppigiana*** Müll.Arg., Palicoureeae, Z68818[16], AF002748[11], AM945279[34], AJ234018[6], –, –, –; ***Psychotria schliebenii*** E.M.A.Petit, Psychotrieae, AM945303[34], AM945329[34], AM945282[34], AM945249[34], AM945361[34], –, –; ***Rubia tinctoria*** L., Rubieae, X83666[4], –, DQ359167[12], X76465[10], FJ695421[33], DQ358885[12], –; ***Saldinia*** **sp.** 1, Lasiantheae, AM117269[13], AF129275[2], EU145429[3], EU145332[3], EU145557[3], –, –; ***Saldinia*** **sp.** 2, Lasiantheae, EU145461[3], EU145506[3], EU145430[3], EU145333[3], EU145558[3], –, –; ***Saprosma foetens*** (Wight) K.Schum., Paederieae, DQ662193[17], DQ662218[17], –, FJ695386[33], DQ662168[17], –, –; ***Saprosma fruticosum*** Blume, Paederieae, DQ662194[17], FJ695277[33], FJ695330[33], FJ695387[33], DQ662169[17], –, –; ***Schismatoclada farahimpensis*** Homolle, Danaideae, FJ695245[33], FJ695278[33], FJ695331[33], FJ695388[33], FJ695422[33], –, –; ***Schismatoclada*** **sp.** 1, Danaideae, AM117271[13], AM117341[13], EU145425[15], EU145329[15], EU145553[15], –, –; ***Schismatoclada*** **sp.** 2, Danaideae, FJ695246[33], FJ695279[33], FJ695332[33], FJ695389[33], FJ695423[33], –, –; ***Schismatoclada*** **sp.** 3, Danaideae, FJ695247[33], –, FJ695333[33], FJ695390[33], FJ695424[33], –, –; ***Schizocolea linderi*** 1 (Hutch., Dalziel) Bremek., Schizocoleeae, AM117272[13], EU145498[15], FJ695334[33], EU145323[15], EU145546[15], –, –; ***Schradera*** **sp.** 2, *Ridsdale 2152* (L), KP212838, AM945314[34], AM945265[34], AM945230[34], AM945344[34], –, –; ***Schradera stellata*** Benth., Schradereae, Y11859[1], AM945313[34], AM945264[34], AJ234014[6], AM945343[34], –, –; ***Serissa foetida*** (L.f.) Lam., Paederieae, Z68822[16], AF004081[11], FJ695336[33], AJ234034[6], AF152618[27], –, –; ***Sherardia arvensis*** L., Rubieae, X81106[20], AF004982[11], FJ695337[33], X76458[10], EU145571[3], –, –; ***Spermacoce remota*** Lam., Spermacoceae, Z68823[16], –, AJ236309[5], –, FJ695425[33], –, –; ***Spermadictyon suaveolens*** Roxb., Paederieae, Z68824[16], DQ662219[17], FJ695338[33], FJ695391[33], DQ662171[17], FJ695468[33], –; ***Spiradiclis bifida*** Kurz, Urophylleae, EU145465[3], EU145511[3], EU145437[3], –, EU145565[3], –, –; ***Thecorchus wauensis*** (Schweinf. ex Hiern) Bremek., Spermacoceae, AM117282[13], AM266901[9], –, FJ695392[33], AM266987[9], –, –; ***Theligonum cynocrambe*** 1 L., Theligoneae, X83668[4], AF004087[11], –, X81860[36], AF152621[27], –, –; ***Theligonum cynocrambe*** 2 L., Theligoneae, FJ695248[33], FJ695280[33], FJ695339[33], FJ695393[33], FJ695426[33], –, –; ***Theligonum cynocrambe*** 3 L., Theligoneae, FJ695249[33], FJ695281[33], FJ695340[33], –, FJ695427[33], –, –; ***Triainolepis mandrarensis*** Homolle ex Bremek., Knoxieae, FJ695250[33], AM266899[9], FJ695341[33], FJ695394[33], AM266985[9], –, –; ***Trichostachys aurea*** Hiern., Lasiantheae, EU145462[3], EU145507[3], EU145431[3], EU145334[3], EU145559[3], –, –; ***Trichostachys*** **sp.**, Lasiantheae, AJ288626[6], AM900595[26], EU145432[3], DQ131792[12], EU145560[3], –, –; ***Urophyllum ellipticum*** (Wight) Thwaites, Urophylleae, AJ288627[6], AM900619[26], –, AJ234002[6], EU145581[3], –, –; ***Valantia hispida*** L., Rubieae, FJ695251[33], AF004090[11], FJ695342[33], FJ695395[33], AM117385[13], –, –; ***Xanthophytum borneense*** (Valeton) Axelius, Ophiorrhizeae, EU145466[3], EU145513[3], EU145438[3], EU145335[3], EU145567[3], –, –; ***Xanthophytum capitellatum*** Ridl., Ophiorrhizeae, AJ288628[6], EU145512[3], –, AJ233996[6], EU145566[3], –, –.

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[1] APG (1998); [2] Piesschaert & al. (2000); [3] Rydin & al. (2009a); [4] Bremer & al. (1995); [5] Bremer & al. (1999); [6] Bremer & Manen (2000); [7] Andersson & al. (2002); [8] Wolff & Liede-Schumann (2007); [9] Kårehed & Bremer (2007); [10] Manen & al. (1994); [11] Andersson & Rova (1999); [12] J.-F. Manen (GenBank unpublished); [13] Bremer & Eriksson (2009); [14] Bremer (1996b); [15] Rydin & al. (2008); [16] Bremer (1996a); [17] Backlund & al. (2007); [18] Nepokroeff & al. (1999); [19] Struwe & al. (1998); [20] Manen & Natali (1995); [21] Andersson (2001); [22] Alejandro & al. (2005); [23] Church (2003); [24] Thulin & Bremer (2004); [25] Novotny & al. (2002); [26] Smedmark & al. (2008); [27] Rova & al. (2002); [28] L. Andersson & C.M. Taylor (GenBank unpublished); [29] Andersson & Antonelli (2005); [30] Andersson (2002); [31] Anderson & al. (2001); [32] Krüger & al. (2012); [33] Rydin & al. (2009b); [34] Razafimandimbison & al. (2008); [35] Nie & al. (2005); [36] Natali & al. (1995); [37] Wen & Wang (2012).