

MOLECULAR PHYLOGENETICS AND BIOGEOGRAPHY

Molecular support for a basal grade of morphologically distinct, monotypic genera in the species-rich Vanguerieae alliance (Rubiaceae, Ixoroideae): Its systematic and conservation implications

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Abstract Many monotypic genera with unique apomorphic characters have been difficult to place in the morphology-based classifications of the coffee family (Rubiaceae). We rigorously assessed the subfamilial phylogenetic position and generic status of three enigmatic genera, the Seychellois *Glionnetia*, the Southeast Asian *Jackiopsis*, and the Chinese *Trailliaedoxa* within Rubiaceae, using sequence data of four plastid markers (*ndhF*, *rbcl*, *rps16*, *trnT-F*). The present study provides molecular phylogenetic support for positions of these genera in the subfamily Ixoroideae, and reveals the presence of a basal grade of morphologically distinct, monotypic genera (*Crossopteryx*, *Jackiopsis*, *Scyphiphora*, *Trailliaedoxa*, and *Glionnetia*, respectively) in the species-rich Vanguerieae alliance. These five genera may represent sole representatives of their respective lineages and therefore may carry unique genetic information. Their conservation status was assessed, applying the criteria set in IUCN Red List Categories. We consider *Glionnetia* and *Jackiopsis* Endangered. *Scyphiphora* is recognized as Near Threatened despite its extensive range and *Crossopteryx* as Least Concern. *Trailliaedoxa* is poorly known (Data Deficient). Finally, the generic status of *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* and the monogeneric tribe Jackieae as defined by Ridsdale are supported.

Keywords conservation; *Crossopteryx*; *Glionnetia*; *Jackiopsis*; molecular systematics; *Scyphiphora*; *Trailliaedoxa*

■ INTRODUCTION

Rubiaceae (or the coffee family) are one of the most species-rich flowering plant families with a particularly high number of monotypic genera, which represent 34.5% of the genera (Davis & al., 2009). The majority of these genera have been studied in previous molecular phylogenetic investigations of the family, and their generic status, systematic positions, and phylogenetic relationships have been assessed. Some monotypic genera have been synonymized, as they were shown to be part of much larger and well-defined genera (e.g., *Aphaenandra* Miq. now included in *Mussaenda* L., Alejandro & al., 2005; *Hymenocnemis* Hook. f. now included in *Gaertnera* Lam., Malcomber & Davis, 2005; *Litosanthes* Blume now included in *Lasianthus* Jack, Xiao & Xhu, 2008; *Rhopalobrachium* Schltr. & K. Krause now included in *Cyclophyllum* Hook. f., Mouly & al., 2007; *Schizostigma* Arn. ex Meisn. now included in *Sabicea* Aubl., Khan & al., 2008; *Neoleroya* Cavaco and *Scyphochlamys* Balf. f. now included in *Pyrostria* Comm. ex A. Juss., Razafimandimbison & al., 2009). In contrast, the generic status of some monotypic genera (e.g., *Strumpfia* Jacq., Bremer & al., 1995; *Crossopteryx* (Afzel. ex G. Don) Benth., Razafimandimbison & Bremer, 2001; *Burttdavya* Hoyle, Razafimandimbison & Bremer, 2002; *Landiopsis* Capuron ex Bosser, Alejandro & al., 2005; *Dunnia* Tutcher, Rydin & al., 2008) has been retained, because they are not nested within well-defined genera. Furthermore,

they have at least one autapomorphic character allowing them to be easily identified (Backlund & Bremer, 1998). The systematic placement of a few rubiaceous monotypic genera with particularly unique morphology remains either unknown or controversial due to the lack of sequenceable material. The present study focuses on the enigmatic genera *Trailliaedoxa* W.W. Sm. & Forrest, *Jackiopsis* Ridsdale, and *Glionnetia* Tirvengadam (Fig. 1A–J).

Jackiopsis was originally described by Wallich (in Roxburgh, 1824) as *Jackia* Wall., but this latter name was already used for another genus *Jackia* Blume in the family Polygalaceae. As a result, Ridsdale (1979) proposed the name *Jackiopsis* for Wallich's intended genus. When establishing *Jackia ornata* Wall. (= *Jackiopsis ornata* (Wall.) Ridsdale) Wallich (in Roxburgh, 1824) wrote a very detailed description based on individual plants in flower and immature fruit that he had discovered "on several of the small islands in the immediate vicinity of Singapore". *Jackiopsis* is a large tree up to 35 m tall with spreading buttresses. The stipules are large, persistent, and connate into a sheath with 8 to 15 stiff, filiform lobes (Fig. 1D). Its inflorescences are axillary, large, pendulous, and paniculate with scorpioid lateral branches. The peduncles are typically flattened, articulated, and are composed of a primary peduncle unit, a secondary node, a secondary peduncle unit, a tertiary node, and a tertiary peduncle unit. Each secondary node bears a sheathed bract, and a pair of reduced leaves; each tertiary node bears a sheathed

bract and is typically terminated by up to eight scorpioid inflorescences in umbel-like arrangement. Corolla lobe aestivation is valvate. Ovaries are bilocular with 2–5 ovules in each locule; the ovules are basally attached on ascending, stalked placentae. Fruits are dry, dehiscent, winged nutlets with persistent, reticulate, and accrescent calyx lobes (pterophylls) (Fig. 1E), commonly with only one seed per locule, as the remaining ovules are aborted (Wallich in Roxburgh, 1824; Ridsdale, 1979).

Korthals (1851) was the first to classify *Jackiopsis* (as *Jackia*) at tribal level. He placed the genus in his new tribe

Jackieae (as *Jackiae*) along with four other genera, *Tricalysia* A. Rich. and *Diplospora* DC. (now in tribe Coffeeae), *Hypobathrum* Blume (now in Octotropideae), and *Scyphiphora* Gaertn., all presently placed in the subfamily Ixoroideae. Hooker (1873) erroneously considered the corolla aestivation of *Jackiopsis* to be contorted and its fruits to be drupes with 5 to 7 pyrenes, and accordingly placed the genus in Retiniphylleae, now in subfamily Ixoroideae. Baillon (1878) postulated a close affinity between *Jackiopsis*, *Cruckshanksia* Hook. & Arn., and *Carphalea* Juss. based on similarities in ovary structure, placentation, and fruits with reticulate pterophylls.

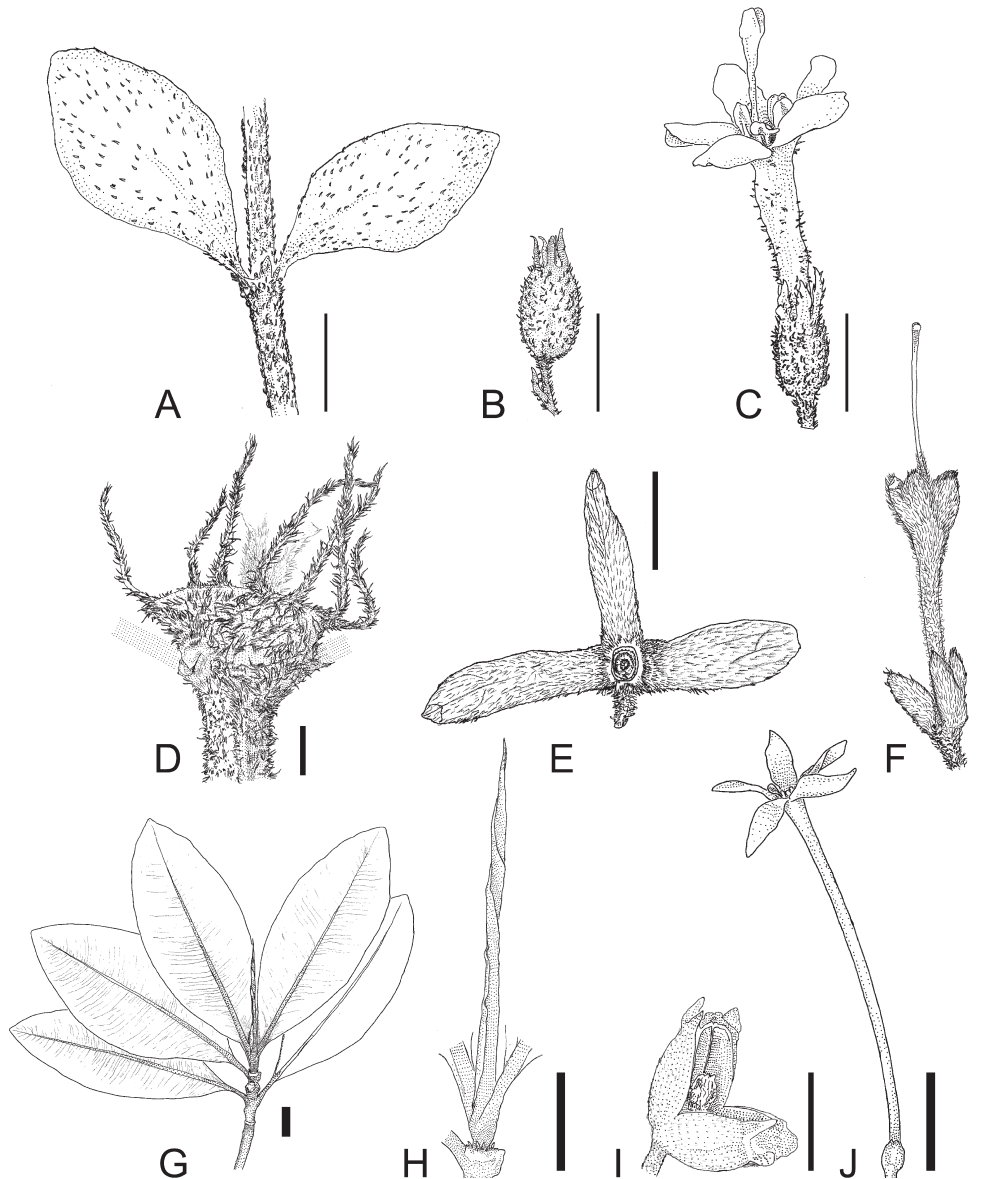


Fig. 1. Illustrations showing the morphological synapomorphies or characteristics of *Trailliaedoxa*, *Jackiopsis*, and *Glionnetia*. **A–C**, *Trailliaedoxa gracilis*: **A**, leafy twig; from Boufford & al. 35041 (MO); **B**, immature fruit; from Yü 1348 (HUH); **C**, flower showing partly exerted anthers and a fully exerted stigma; from Forrest 12638 (S). **D–F**, *Jackiopsis ornata*: **D**, large, densely pubescent stipule with filiform lobes; from Kalat & al. s.n. 1993-05-11 (L); **E**, dry, indehiscent fruit with three unequal lobes; from Van Balooy 6109 (MO); **F**, densely pubescent flower with three calyx lobes and a very long style; from Rahmat Si Toroes 3424 (S). **G–J**, *Glionnetia sericea*: **G**, habit showing a very long, convolute terminal vegetative bud (young stipule); from Beaver 03 (S); **H**, long, convolute stipule; from Beaver 03 (S); **I**, mature capsular fruit; from Beaver 17 (S); **J**, flower showing partly exerted anthers; from Beaver 17 (S). Scale bars: thin, 2 mm; medium, 5 mm; thick, 20 mm.

The latter two genera are currently placed in the tribes Cousareeae and Knoxieae, respectively, in the subfamily Rubioideae. Schumann (1891) associated *Jackiopsis* with Rubioideae, viz. Oldenlandieae. More recently, Ridsdale (1979) narrowly circumscribed Jackieae to include only the genus *Jackiopsis* and tentatively placed the tribe in the subfamily Cinchonoideae. Finally, the recent discovery of intra-ovarian trichomes in *Jackiopsis* (Puff & Igersheim, 1994) and in some Malagasy species of *Tricalysia* (Ranarivelo-Randriamboavonjy & al., 2007) could be taken as an indication of a close relationship between the two genera and would imply placing the former in the tribe Coffeae of Ixoroideae.

Trailliaedoxa is an ericoid, erect subshrub (up to 60 cm tall), with a broom-like branching and small, tough leaves (Robbrecht, 1988). According to the protologue (Smith, 1917), the genus grows in the rocky habitats and thickets on dry mountain slopes of Sichuan, China, between 1450 and 3000 m elevation. *Trailliaedoxa* is very distinct from other Rubiaceae in its ericoid habit, pubescent styles, and schizocarpous fruits. The genus is presently unclassified at tribal level in Rubiaceae (Robbrecht, 1988). Smith (1917) thought that the floral structure of the genus is similar to that of Alberteae, now in Ixoroideae. On the other hand, its ericoid habit is also comparable to that of *Spermadictyon* Roxb. of the tribe Paederieae in the subfamily Rubioideae (Smith, 1917). Robbrecht (1988) tentatively placed *Trailliaedoxa* in the subfamily Antirheoideae, which was shown to be highly polyphyletic (e.g., Bremer & al., 1995).

Finally, the genus *Glionnetia* was originally described by Tirvengadam (1984) to accommodate the Seychellois *Randia sericea* (Baker) Hemsl. (Hemsley, 1916: 18) that he excluded from the Neotropical genus *Randia* L. This monotypic genus is restricted to Mahé and Silhouette Islands of the Seychelles Archipelago; it is distinct by its large, long, intrapetiolar, and convolute stipules and can additionally be characterized by a combination of the following features: glabrous, coriaceous leaves clustered at the apex of the branches, corollas 6–7 cm long, left-contorted aestivation, and capsular fruits with loculicidal dehiscence (Fig. 1G–J). Tirvengadam (1984) originally classified *Glionnetia* in Rondeletieae of the subfamily Cinchonoideae. Robbrecht (1988), and more recently Rova & al. (2009) and Manns & Bremer (2010), tentatively followed this classification. None of the discussed hypotheses regarding systematic positions within Rubiaceae have been tested with molecular data.

We rigorously investigated the phylogenetic positions of *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* in Rubiaceae and assessed their generic status, using sequence data from four plastid markers (*ndhF*, *rbcl*, *rps16* intron, *trnT-F*). We found that these genera belong in the subfamily Ixoroideae, and that *Crossopteryx*, *Jackiopsis*, *Scyphiphora*, *Trailliaedoxa*, and *Glionnetia*, respectively, form a paraphyletic assemblage at the base of the species-rich Vanguerieae alliance. These results allowed us to hypothesize that these monotypic genera may represent sole representatives of their respective lineages. We discuss the systematic and conservation implications of these findings.

■ MATERIALS AND METHODS

Taxon sampling and laboratory work. — We extracted silica-gel leaf samples from three individuals of *Jackiopsis ornata* (from the Malay Peninsula), three individuals of *Glionnetia sericea* (from the Island of Mahé in the Seychelles Archipelago), and two individuals of *Trailliaedoxa gracilis* (from Yunnan, China), following the protocol outlined in Razafimandimbison & al. (2008). The extracted DNA was amplified and sequenced for four chloroplast regions (*ndhF*, *rbcl*, *rps16* intron, *trnT-F*), using the primers of Razafimandimbison & al. (2008) and Rydin & al. (2008).

Phylogenetic analyses. — Sequences were preliminary aligned using the Clustal W (default settings; Thompson & al., 1994), as implemented in BioEdit v.7.0.9 (Hall, 1999), and subsequently edited manually. We initially performed maximum parsimony (MP) and Bayesian analyses of 120 *rbcl* sequences of Rubiaceae (including all major lineages in the three subfamilies of Rubiaceae and the tribes Luculieae and Coptosapelteae, which do not belong in any of the three subfamilies) in order to pinpoint the subfamilial position(s) of the three genera within the family. Eight taxa from the other families of the order Gentianales (Apocynaceae, Gentianaceae, Gelsemiaceae, Loganiaceae) were utilized as outgroups following Bremer & Eriksson (2009). Bayesian analysis of the *rbcl* dataset was performed using the GTR+G+I model. We refer to these MP and Bayesian *rbcl* analyses hereafter as the “Family-Wide-*rbcl*” analyses. The results of the Family-Wide-*rbcl* analyses allowed us to narrow down our sampling to include only the three targeted genera and their more closely related groups and select the outgroup taxa from within Rubiaceae (*Cinchona* L. and *Cubanola Aiello* [subfamily Cinchonoideae], *Morinda* L. [subfamily Rubioideae], and *Luculia* Sweet [Luculieae]). MP and Bayesian analyses of 71 taxa based on combined sequence data from the chloroplast markers *ndhF*, *rbcl*, *rps16* intron, and *trnT-F* were subsequently performed, of which 67 species represented all tribes currently recognized in Ixoroideae (with the exception of Henriquezieae) (see Appendix). We refer to these analyses hereafter as the “Ixoroideae-Four-Locus” analyses. We hoped that we could better resolve the position of these genera within subfamily Ixoroideae, by combining the two more rapidly evolving non-coding regions (*rps16* intron, *trnT-F*) and the two coding regions (*ndhF*, *rbcl*) in a single data matrix.

Separate and combined MP analyses of the *ndhF*, *rbcl*, *rps16*, and *trnT-F* datasets of the 71 taxa were conducted using the program PAUP* v.4.0B10 (Swofford, 2002). MP analyses consisted of a heuristic search with the TBR branch swapping algorithm, Multrees on, 1000 random sequence addition replicates and a maximum of 10 trees saved per replicate. Clade bootstrap support (BS) was estimated using the same settings and three random sequence additions per replicate.

The combined dataset was also analyzed using Bayesian inference (BI), using the program MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The combined data were partitioned in a protein-coding (*rbcl*, *ndhF*) partition and a non-coding (*rps16* intron, *trnT-F*) partition; these were unlinked and analyzed under the GTR+G+I

and GTR+G models, respectively, which were suggested as best-fit to the data under the corrected Akaike information criterion (AICc). The Bayesian *rbcL* analysis of the 120 taxa and combined Bayesian analyses of the 71 taxa each comprised two runs of four chains, which were monitored for 15×10^6 generations, with every 1000th generation being sampled, and the temperature coefficient of the chain-heating scheme set to 0.15. Stationarity and convergence of runs, as well as the correlation of split frequencies between the runs were checked using the

program AWTY (Nylander & al., 2008). Trees sampled before the posterior probability (PP) of splits stabilized, were excluded from consensus as a burn-in phase. The effective sample size (ESS) of parameters was checked using the program Tracer v.1.4.1 (Rambaut & Drummond, 2007).

Finally, we assessed the conservation status of *Crossopteryx*, *Glionnetia*, *Jackiopsis*, *Scyphiphora*, and *Trailliaedoxa*, applying the criteria set in World Conservation Union (IUCN) Red List Categories (Version 3.1; IUCN, 2001).

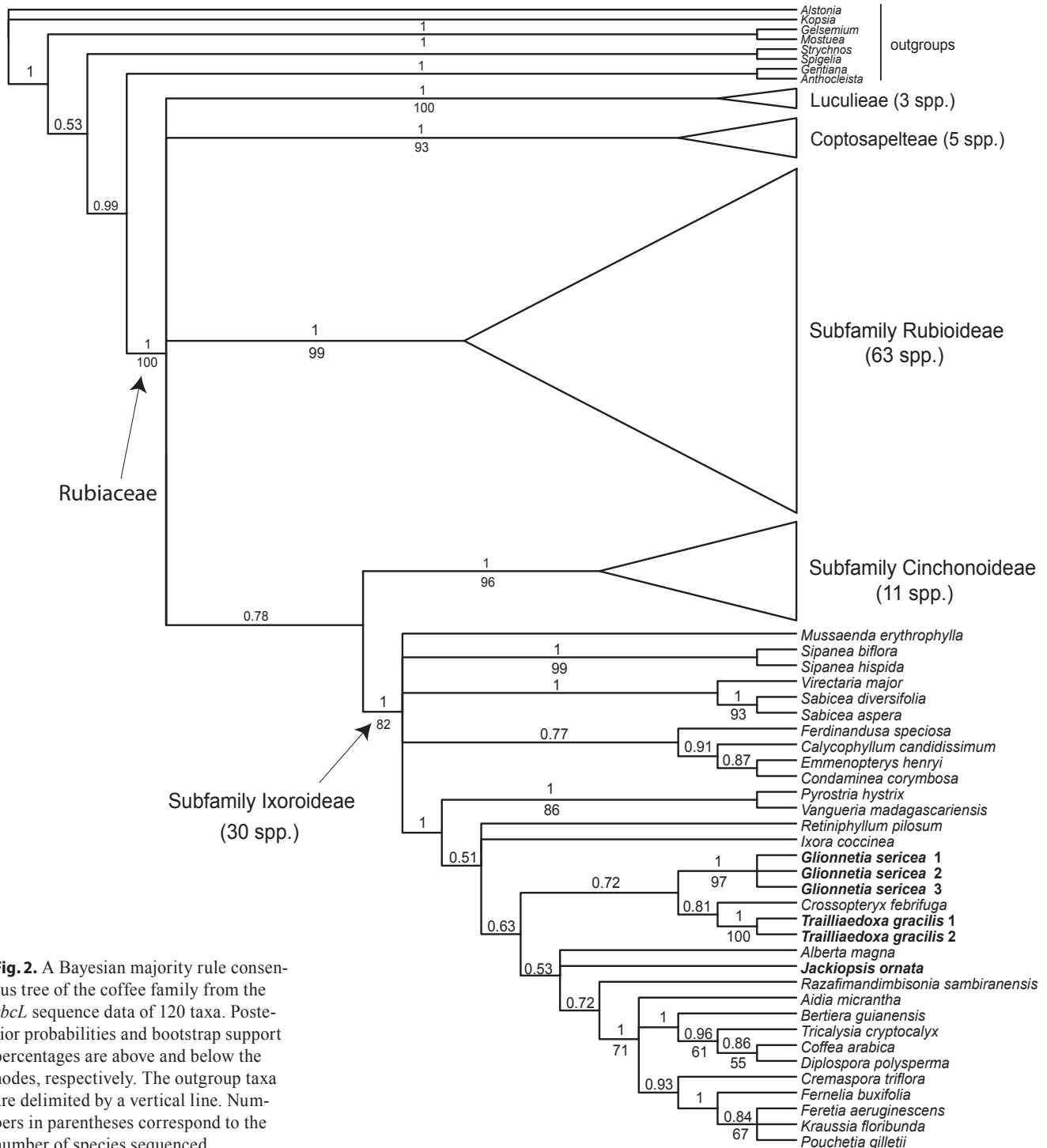


Fig. 2. A Bayesian majority rule consensus tree of the coffee family from the *rbcL* sequence data of 120 taxa. Posterior probabilities and bootstrap support percentages are above and below the nodes, respectively. The outgroup taxa are delimited by a vertical line. Numbers in parentheses correspond to the number of species sequenced.

■ RESULTS

Separate analyses. — The Family-Wide-*rbcL* analyses based on the 120 *rbcL* sequence data placed all sampled *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* in the subfamily Ixoroideae (PP = 1.00; BS = 82%). The monophyly of Ixoroideae and its sister-group relationship with the subfamily Cinchonoideae is consistent with other published Rubiaceae studies (e.g., Robbrecht & Manen, 2006; Bremer & Eriksson, 2009; Rydin & al., 2009). *Glionnetia* and *Trailliaedoxa* formed a poorly supported clade with *Crossopteryx*, while *Jackiopsis* was left unresolved in the poorly supported Coffeae alliance (PP = 0.53) (Fig. 2). A summary of the tree data and statistics from the analyses is given in Table 1. In the separate Ixoroideae analyses of the 71-taxa datasets, the incongruent nodes between the separate cpDNA markers generally received weak support (trees not shown), with the exception of a node inferred from the *trnT-F* data: a clade consisting of Condamineae, Sipaneeae and Posoquerieae, as sister to the rest of the Ixoroideae (BS = 81%; PP = 0.96) excluding Mussaendeae and Sabiceae (inferred as sister groups in a basal clade).

Combined analyses. — The phylogenetic hypotheses inferred from the MP and BI analyses of the Ixoroideae-Four-Locus datasets were in general agreement, with the exception of the position of *Tocoyena pittieri*, resolved as sister to *Gardenia hansemanii* in the former (BS = 53%), and as sister to *Aulacocalyx jasminiflora* in the latter (PP = 0.89).

The uncorrected potential scale reduction factor (PSRF; Gelman & Rubin, 1992) of the summarized parameters approached 1.0, and the split frequencies between the runs were highly correlated, indicating that the runs had converged. The ESS of all model parameters were >200, as recommended by Drummond & al. (2007) for adequate representation of the posterior probability. The 50% majority-rule consensus tree inferred from the BI analysis of the Ixoroideae-Four-Locus data is shown in Fig. 3.

The sampled specimens of *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* formed, respectively, highly supported clades (PP = 1.00; BS = 100) and were all nested in the highly supported Vanguerieae alliance (Fig. 3). Within this alliance the monotypic African genus *Crossopteryx* was the first to branch off, followed by *Jackiopsis*, *Scyphiphora*, *Trailliaedoxa*, and *Glionnetia*, respectively. *Glionnetia* was in turn resolved with poor support as

sister to a poorly supported large clade containing Vanguerieae, Greeneae, Aleisanthiae, and Ixoreae. Within this latter clade Aleisanthiae and Ixoreae formed a sister group, which in turn was sister to Greeneae. The (Greeneae(Aleisanthiae-Ixoreae)) clade was sister to Vanguerieae.

■ DISCUSSION

Subfamilial position of *Glionnetia*, *Jackiopsis* and *Trailliaedoxa* within Rubiaceae: New circumscription of the Vanguerieae alliance. — The present study is the first molecular phylogenetic investigation of the Seychellois genus *Glionnetia*, the Southeast Asian *Jackiopsis*, and the Chinese genus *Trailliaedoxa*. The results of the Family-Wide-*rbcL* analysis (Fig. 2) support the placement of these monotypic genera in the subfamily Ixoroideae sensu Bremer & Eriksson (2009). The phylogenetic hypothesis from the Ixoroideae-Four-Locus analyses (Fig. 3) reveals that the Vanguerieae alliance of the subfamily Ixoroideae contains the following major lineages: the African *Crossopteryx* (Crossopterygeae), *Jackiopsis* (Jackieae sensu Ridsdale), the mangrove genus *Scyphiphora*, *Trailliaedoxa*, *Glionnetia*, all currently unplaced at tribal level, and the tribes Vanguerieae, Aleisanthiae, Greeneae, and Ixoreae. This alliance is species-rich and morphologically diverse, and contains over 1100 species of shrubs and small trees; it does not seem to have any obvious synapomorphic character, however the majority of its members (i.e., those of the species-rich tribes Ixoreae and Vanguerieae) have flowers showing secondary pollen presentation, drupaceous fruits, and one-seeded carpels. Ixoreae is pantropical, while the remaining lineages are paleotropical. An overview of the morphological and ecological characteristics of these major lineages of the Vanguerieae alliance is shown in Table 2.

Tribal positions and generic status of *Glionnetia*, *Jackiopsis* and *Trailliaedoxa*. — The morphologically unique genera *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* represent a paraphyletic basal assemblage within the Vanguerieae alliance (Fig. 3). Assessment and recognition of these monotypic genera and their tribal positions are discussed on the basis of a combination of the following criteria (Backlund & Bremer, 1998; Razafimandimbison & Bremer, 2002): (1) if they are not nested within other well-defined genera; and (2) if they have at least one

Table 1. Characteristics of the markers/datasets used, including statistics of alignments, analyses, as well as parameter estimates and evolutionary models suggested by MrModeltest 2.2 (Nylander, 2004).

	<i>ndhF</i>	<i>rbcL</i>	<i>rps16</i>	<i>trnT-F</i>	Combined	Coding	Noncoding
Number of taxa	40	53	65	66	66	56	66
Included characters	2159	1403	1073	2441	7076	3562	3514
Variable characters	677	291	382	886	2236	968	1268
Parsimony-informative characters	353	158	227	465	1203	511	692
% informative characters	16.35	11.26	21.16	19.05	17.00	14.35	19.69
Consistency index (CI)	0.70	0.54	0.68	0.75	0.68	0.64	0.72
Retention index (RI)	0.73	0.64	0.82	0.82	0.77	0.69	0.82

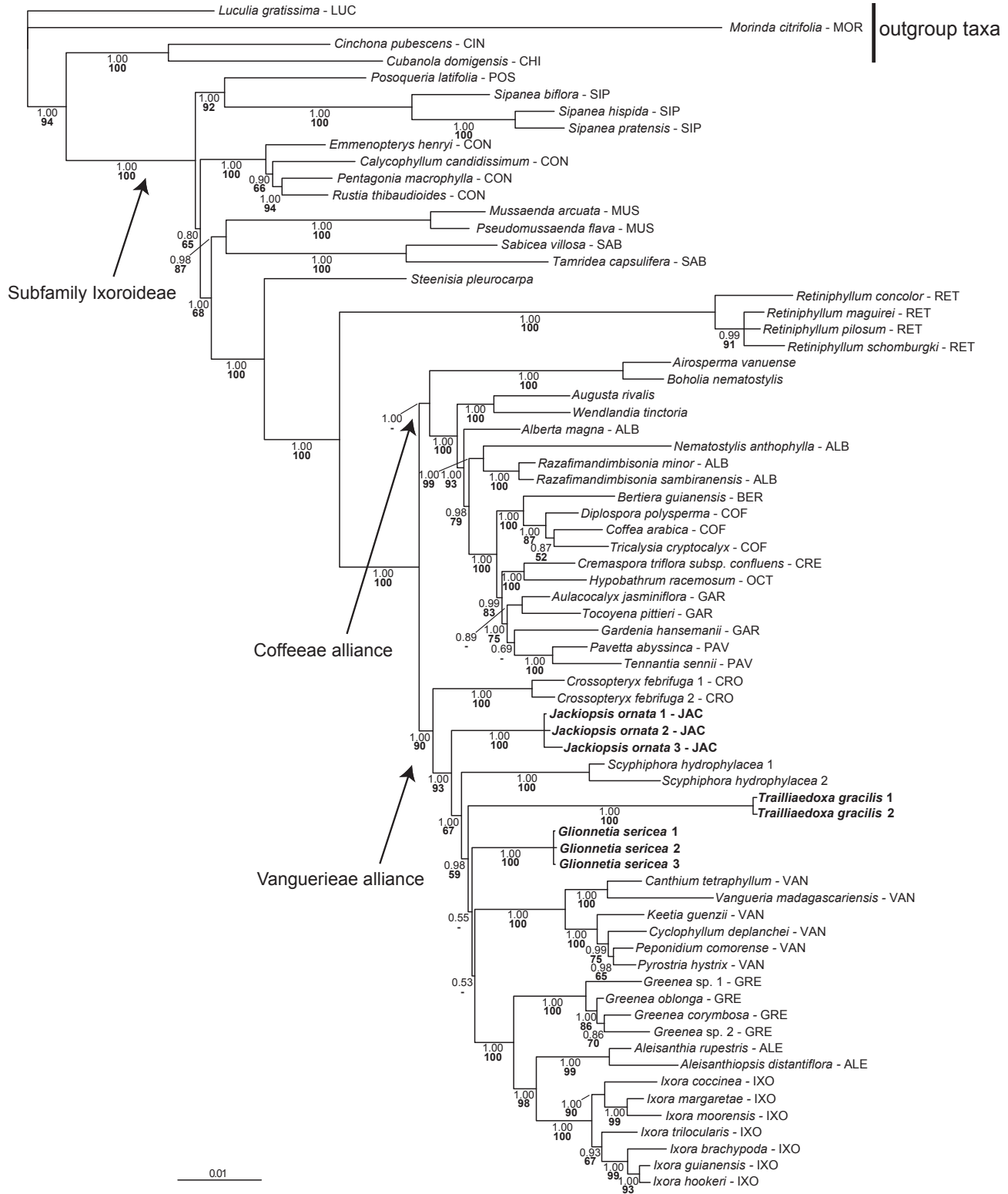


Fig. 3. Bayesian majority-rule consensus tree of the subfamily Ixoroideae from the combined *ndhF/rbcL/rps16/trnL-F* data. Below branches are posterior probabilities and bootstrap support percentages (bold). Bootstrap support <50% is indicated by a dash. The outgroup taxa are delimited by a vertical line. ALB, Alberteae; ALE, Aleisanthieae; CHI, Chiococceae; CIN, Cinchoneae; COF, Coffeae; CON, Condamineae; CRE, Cremasporae; CRO, Crossopterygeae; GAR, Gardenieae; GRE, Greeneae; IXO, Ixoreae; JAC, Jackieae; LUC, Luculieae; MUS, Mussaendeae; OCT, Octotropideae; PAV, Pavetteae; POS, Posoquerieae; RET, Retiniphyllae; SAB, Sabiceae; SIP, Sipaneeae; VAN, Vanguerieae. The positions of the subfamily Ixoroideae and the Coffeae and Vanguerieae alliances are indicated. The sampled specimens of *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* are in boldface.

autapomorphic character or a combination of plesiomorphic characters, allowing them to be recognized easily.

Glionnetia, *Jackiopsis* or *Trailliaedoxa* are not nested within the five currently recognized tribes (Aleisanthieae, Crossopterygeae, Greeneae, Ixoreae, Vanguerieae) of the Vanguerieae alliance. Like the monogeneric tribe Crossopterygeae, each of these three genera can also be placed in their own tribes, as they have distinct morphological features that warrant tribal recognition.

The large, persistent, and sheathed stipules with 8–15 filiform lobes and the large (Fig. 1D), paniculate inflorescences with scorpioid lateral branches are unique for *Jackiopsis* in the Vanguerieae alliance and may represent potential apomorphies for the genus. A combination of valvate aestivation and dry, indehiscent fruits with pterophylls (Fig. 1E) can additionally be used to characterize *Jackiopsis*. Within the Vanguerieae alliance the members of Vanguerieae also have valvate aestivation but their fruits are drupes (Table 2). Dry fruits with pterophylls are also found in some other rubiaceaceous genera (e.g., *Steenisia* Bahk. f., Bremer, 1984; *Cosmocalyx* Standl., Delprete, 1998; *Alberta* E. Mey, Kainulainen & al., 2009). Furthermore, the Ixoroideae-Four-Locus analyses (Fig. 3) support the monogeneric tribe Jackieae as circumscribed by Ridsdale (1979). Consequently, an older view of Jackieae (Korthals, 1851), as containing *Diplospora*, *Hypobathrum*, *Jackiopsis*, *Scyphiphora*, and

Tricalysia, is highly polyphyletic. The phylogenetic positions of *Diplospora* and *Tricalysia* in Coffeae and *Hypobathrum* in Octotropideae have been shown by many independent molecular phylogenetic studies (e.g., Andreasen & Bremer, 2000; Robbrecht & Manen, 2006). The fourth genus *Scyphiphora* has been shown to be closely related to Aleisanthieae, Greeneae, Ixoreae, and Vanguerieae of the Vanguerieae alliance (e.g., Robbrecht & Manen, 2006; Bremer & Eriksson, 2009; Cortés-B. & al., 2009; Mouly & al., 2009). A close relationship of *Jackiopsis* with the former Hedyotideae (now Knoxiaceae in subfamily Rubioideae) as put forward by Baillon (1878) and Schumann (1891) is not supported by this study (Fig. 2).

The Ixoroideae-Four-Locus analyses (Fig. 3) strongly support the position of *Glionnetia* in the Vanguerieae alliance, although its relationship to other taxa within the alliance is poorly resolved. This finding is inconsistent with Tirvengadam (1984), who tentatively placed the genus in Rondeletieae of the subfamily Cinchonoideae. Within Rondeletieae, Tirvengadam (1984) postulated a close relationship to the genus *Lindenia* Benth. (now *Augusta* Pohl), a genus that has recently been demonstrated to have a close affinity with the genus *Wendlandia* Bartl. ex DC. (Rova & al., 2002) of the Coffeae alliance of Ixoroideae (Fig. 3). The long, intrapetiolar, and convolute stipules are unique for *Glionnetia* within the Vanguerieae alliance, and may represent a potential apomorphy for the

Table 2. Morphological characteristics and other important information of the major lineages of the Vanguerieae alliance.

Character	Cross-opterygeae	Jackieae	<i>Scyphiphora</i>	<i>Trailliaedoxa</i>	<i>Glionnetia</i>	Vanguerieae	Aleisanthieae	Greeneae	Ixoreae
Distribution	Tropical Africa	SE Asia	SE Asia and Madagascar	Yunnan, Sichuan, China	Seychelles	Paleotropics	SE Asia	SE Asia	Pantropical
Habit	Shrubs to small trees	Tall to emergent trees	Shrubs	Subshrubs (up to 60 cm tall)	Small trees	Small trees, shrubs or herbs	Shrubs	Shrubs or small trees	Shrubs
Inflorescence position	Terminal	Axillary (rarely axillary/terminal)	Axillary	Terminal/axillary	Terminal	Axillary	Terminal/axillary	Terminal	Terminal/cauliflorous
Inflorescence type	Paniculate	Paniculate with scorpioid lateral branches	Cymose	Cymose to umbelliform or fasciculate	Paniculate	Cymose	Scorpioid	Scorpioid	Cymose/paniculate
Corolla aestivation	Left-contorted	Valvate	Left-contorted	Left-contorted	Left-contorted	Valvate	Left-contorted	Left-contorted	Left-contorted
Ovules per carpel	Few	2–5	2	1	Many	1	Many	Many	1
Fruit type	Capsular	Samara-like	Non-fleshy, drupaceous	Schizocarpous	Capsular	Drupaceous	Capsular	Capsular	Drupaceous
Dispersal mode	Anemochorous	Anemochorous	Sea currents	Anemochorous	Anemochorous	Zoochorous	Anemochorous	Anemochorous	Zoochorous
Pollen type	Colporate	Colporate	Colporate	Colporate	Colporate	Porate	Pororate/colporate	Colporate	Colporate

genus. The genus can be confused with some members of Ixoreae because of its arborescent growth habit, terminal and paniculate inflorescences, and large, 5-merous flowers with left-contorted aestivation. However, *Glionnetia* bears capsular fruits with many-seeded carpels, while the members of Ixoreae have drupaceous fruits with one-seeded carpels (Table 2). Furthermore, *Glionnetia* and *Crossopteryx* have arborescent growth habit, terminal, paniculate inflorescences, left-contorted aestivation, and capsular fruits. However, these genera have very distinct seeds: the former with very small (2–3 mm long), unwinged seeds (Tirvengadam, 1984) and the latter with large, flattened seeds with deeply fimbriate wings (Stoffelen & al., 1996). Additionally, *Crossopteryx* is restricted to tropical Africa, while *Glionnetia* is endemic to the Seychelles. For now, we leave *Glionnetia* unclassified at tribal level pending further analyses.

Trailliaedoxa was considered to be a genus with uncertain phylogenetic position within the family Rubiaceae (Smith, 1917; Robbrecht, 1988). Smith (1917) thought that the floral structure of the genus is similar to that of *Albeteae* sensu Hooker (1873), which is characterized by contorted aestivation and solitary pendulous ovules. On the other hand, its ericoid habit has been compared to that of *Spermadictyon* of the tribe Paederieae in the subfamily Rubioideae (Smith, 1917). Our Ixoroideae-Four-Locus analyses (Fig. 3) place *Trailliaedoxa* in the Vanguerieae alliance. This position is consistent with the frequent occurrence of contorted aestivation in Ixoroideae, particularly in the Coffeae and Vanguerieae alliances. *Trailliaedoxa* is very distinct from other genera in the alliance because of its ericoid habit, densely pubescent styles, and schizocarpous fruits.

Based on the results of the analyses presented (see also Table 2), we maintain the generic status of *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* and the tribal status of Jackieae as delimited by Ridsdale (1979). We suggest that *Trailliaedoxa* and *Scyphiphora* be recognized at tribal level. However, the formal recognition of the two respective new tribes will be published in another paper focusing on the tribal assessment and relationships in the subfamily Ixoroideae (Kainulainen & al., subm.).

A basal grade of monotypic genera in the species-rich Vanguerieae alliance and its conservation implications. — The Ixoroideae-Four-Locus analyses (Fig. 3) reveal a basal grade of morphologically distinct, monotypic genera (*Crossopteryx*, *Jackiopsis*, *Scyphiphora*, *Trailliaedoxa*, and *Glionnetia*, respectively) in the otherwise species-rich Vanguerieae alliance. In other words, these monotypic genera form a paraphyletic assemblage at the base of the alliance. These results here presented are interesting from both phylogenetic and conservation standpoints, as these genera may be sole representatives of their respective lineages and therefore may carry unique genetic information. Their extinction would mean loss of their entire lineages (see also Rydin & al., 2008; Rana & Ranade, 2009). These monotypic genera could be potential candidates for conservation priorities due to their genetic isolation, however this latter should not automatically be reason for conservation concern. Below we present conservation assessments of these five genera, applying the criteria set in World Conservation Union (IUCN) Red List Categories (IUCN, 2001).

■ *Crossopteryx febrifuga* is a shrub or small tree species restricted to the savanna woodlands of tropical Africa. The species is common and widespread throughout tropical Africa and is fire resistant due to its thick bark (Gignoux & al., 1997); it is widely used as a medicinal plant across much of tropical Africa but is not considered endangered. At present, it may represent less of a conservation priority, and therefore, we consider it to be Least Concern (IUCN, 2001).

■ *Jackiopsis ornata* is restricted to Southeast Asia and distributed in the Malay Peninsula, Sumatra, Borneo, and the Riau Archipelago (Ridsdale, 1979). According to Sosef & al. (1998), the species is “reported to be locally frequent but never common in lowland swamp forests and riverine habitats”; the wood of *J. ornata* is “rarely and only locally used”. It was considered by UNEP-WCMC (2008) to be a timber tree species, which is not in the international trade or of conservation concern. However, the occurrence of this species appears patchy and is not registered in most peat swamp forest inventories (e.g., Kishokumar, 2008). In Southeast Asia, there has been much large-scale conversion of peatlands into commercial agriculture, such as for oil palms (Aiken & Leigh, 1992; Jomo & al., 2004; Kathirithamby-Wells, 2005). In addition, the frequent fires degrading peat swamp forests on Borneo (Sosef & al., 1998) and Sumatra (Anderson & Bowen, 2000) already had a tremendous impact on the natural habitats of *J. ornata*. Based on the information presented above, we consider *J. ornata* Endangered (IUCN, 2001). Therefore, an ex situ conservation plan for this unique taxon is strongly encouraged. The gregarious flowering and stately habit of mature *J. ornata* trees, with spectacular displays of dense, brightly colored inflorescences, should provide an added incentive for future conservation and planting.

■ *Scyphiphora hydrophylacea* is a mangrove plant. It is distributed from East India throughout the Indo-Malesian region to the West Pacific and northern Australia, Sri Lanka, and Madagascar (Puff & al., 2007). The wide coastal distribution range can be attributed to the trans-oceanic dispersal of its buoyant fruits. Mangrove stands are highly threatened ecosystems and exploited for fuelwood and timber, local constructions, development of salt beds, and conversion to aquaculture ponds (e.g., Tomlinson, 1986; Primavera, 2000). For example, mangrove areas in the Philippines decreased from 428,382 ha (Villaluz, 1953) to 200,500 ha (DENR, 1996) between 1951 and 1995. About 50% of the Philippine mangroves that disappeared from 1950 to 1988 had been converted into culture ponds, making aquaculture the main cause of the mangrove loss in the Philippines (Primavera, 2000). *Scyphiphora hydrophylacea* is reported to be rare and highly threatened in Sri Lanka and Peninsular India, where seedlings and young plants are scarce. This situation has been attributed to a very low percentage of fertile fruits in *S. hydrophylacea* and its inability to produce healthy seedlings, which is possibly due to inbreeding depression in small, isolated populations (Hettiarachchi & Jayaratne, 2006). According to Puff & al. (2007), *S. hydrophylacea* is usually not present in large populations in Thailand. Within its center of distribution in Southeast Asia, there is much alteration and destruction of its habitat. Largely ignored by

forestry inventories or conservation projects, the occurrence of *S. hydrophylacea* is rare or patchy along the more frequently inundated mangrove fringes and adjacent shores. Occurrence is largely insignificant to absent within the main formation that is dominated by taller stands of Rhizophoraceae and other tree species. Moreover, conversion to other land use or subsequent degradation due to adjacent coastal development can drastically reduce mangrove extents (Chan & al., 1993; Sasekumar & Then, 2005). Based on the evidence for concern presented above, we consider *S. hydrophylacea* Near Threatened (IUCN, 2001) despite its extensive range.

■ *Trailliaedoxa gracilis* appears to have a narrow distribution range. Endemic to southern China, it grows in rocky habitats and thickets on the mountain slopes of Sichuan and Yunnan at an altitude of 1540 to 3000 m. The conservation status of the genus is currently unknown, as it is only known from a few specimens. *Trailliaedoxa gracilis* may be a potential candidate for a high conservation priority, however it is poorly known (= Data Deficient; IUCN, 2001).

■ Finally, *Glionnetia sericea* is confined to a very restricted area less than 1000 ha on Mahé and Silhouette Islands in the Seychelles Archipelago. It is one of two genera of Rubiaceae endemic to the Seychelles and has been considered Vulnerable D2 (IUCN, 2010). However, a recent assessment concluded that the species is Endangered B1ab(iii)+2ab(iii) (Huber, pers. comm.). *Glionnetia sericea* thrives mainly on high ridges in the mountains (Tirvengadam, 1984); it does not seem to grow well at lower altitudes (K. Beaver, pers. comm.). Conservation in situ is strongly encouraged.

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■ LITERATURE CITED

- Aiken, S.R. & Leigh, C.L. 1992. *Vanishing rain forests: The ecological transition in Malaysia*. Oxford: Clarendon Press.
- Alejandro, G.D., Razafimandimbison, S.G. & Liede-Schumann, S. 2005. Polyphyly of *Mussaenda* inferred from ITS and *trnT-F* data and its implication for generic limits in Mussaendeae (Rubiaceae). *Amer. J. Bot.* 92: 544–557.
- Anderson, I.P. & Bowen, M.R. 2000. *Fire zones and the threat to the wetlands of Sumatra, Indonesia*. Palembang: Forest Fire Prevention and Control Project.
- Anderson, L. & Antonelli, A. 2005. Phylogeny of the tribe Cinchoneae (Rubiaceae), its position in Cinchonoideae, and description of a new genus, *Ciliosemina*. *Taxon* 54: 17–28.
- Andersson, L. & Rova, J.H.E. 1999. The *rps16* intron and the phylogeny of the Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 214: 161–186.
- Andreasen, K., Baldwin, B.G. & Bremer, B. 1999. Phylogenetic utility of the nuclear rDNA ITS region in subfamily Ixoroideae (Rubiaceae): Comparisons with cpDNA *rbcl* sequence data. *Pl. Syst. Evol.* 217: 119–135.
- Andreasen, K. & Bremer, B. 1996. Phylogeny of the subfamily Ixoroideae (Rubiaceae). *Opera Bot. Belg.* 7: 119–138.
- Andreasen, K. & Bremer, B. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: Morphology, nuclear and chloroplast DNA data. *Amer. J. Bot.* 87: 1731–1748.
- Backlund, A. & Bremer, K. 1998. To be or not to be: Principles of classification and monotypic plant families. *Taxon* 47: 391–400.
- Backlund, M., Oxelman, B. & Bremer, B. 2000. Phylogenetic relationships within the Gentianales based on *ndhF* and *rbcl* sequences, with particular reference to the Loganiaceae. *Amer. J. Bot.* 87: 1029–1043.
- Baillon, H. 1878. Sur l'organisation et les affinités du *Jackia*. *Bull. Mens. Soc. Linn. Paris* 1: 185–188.
- Bremer, B. 1984. The genus *Steenisia* (Rubiaceae) and its taxonomic position. *Nord. J. Bot.* 4: 333–345.
- Bremer, B., Andreasen, K. & Olsson, D. 1995. Subfamilial and tribal relationships in the Rubiaceae based on *rbcl* sequence data. *Ann. Missouri Bot. Gard.* 82: 383–397.
- Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A., Källersjö, M. & Barkhordarian, E. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molec. Phylog. Evol.* 24: 274–301.
- Bremer, B. & Eriksson, T. 2009. Time tree of Rubiaceae: Phylogeny and dating of the family, subfamilies, and tribes. *Int. J. Pl. Sci.* 170: 766–793.
- Bremer, B., Jansen, R.K., Oxelman, B., Backlund, M., Lantz, H. & Kim, K.J. 1999. More characters or more taxa for a robust phylogeny — case study from the coffee family (Rubiaceae). *Syst. Biol.* 48: 413–435.
- Bremer, B. & Thulin, M. 1998. Collapse of Isertieae, re-establishment of Mussaendeae, and a new genus of Sabiceae (Rubiaceae); phylogenetic relationships based on *rbcl* data. *Pl. Syst. Evol.* 211: 71–92.
- Chan, H.T., Ong, J.E., Gong, W.K. & Sasekumar, A. 1993. The socio-economic, ecological and environmental values of mangrove ecosystems in Malaysia and their present state of conservation. Pp. 41–81 in: Clough, B.F. (ed.), *The economic and environmental values of mangrove forests and their present state of conservation in South-East Asia/Pacific region*. Mangrove Ecosystems Technical Reports, No. 1, ITTO/ISME/JIAM Project PD71/89, Rev. 1 (F). Nishihara, Japan: International Society for Mangrove Ecosystems (ISME), International Tropical Timber Organization (ITTO), Japan International Association for Mangroves (JIAM).
- Cortés-B., R., Delprete, R.P.G. & Motley, T. 2009. Phylogenetic placement of the tribe Retiniphyllae among the subfamily Ixoroideae (Rubiaceae). *Ann. Missouri Bot. Gard.* 96: 61–67.
- Davis, A.P., Govaerts, R., Bridson, D.M., Rushsam, M. Moat, J. & Brummitt, N.A. 2009. A global assessment of distribution, diversity, endemism, and taxonomic effort in the Rubiaceae. *Ann. Missouri Bot. Gard.* 96: 68–78.
- Delprete, R.P.G. 1998. Notes on calycophyllous Rubiaceae. Part III. Systematic position of the monotypic Mexican genus *Cosmocalyx* and notes on the calycophyll development. *Brittonia* 50: 309–317.
- Delprete, R.P.G. & Cortés-B., R. 2004. A phylogenetic study of the tribe Sipaneeae (Rubiaceae, Ixoroideae), using *trnL-F* and ITS sequence data. *Taxon* 53: 347–356.
- DENR. 1996. *The Philippine Environmental Quality Report, 1990–1995*. Quezon: Environmental Management Bureau, Department of Environment and Natural Resources.
- Drummond, A.J., Ho, S.Y., Rawlence, W.N. & Rambaut, A. 2007. *A rough guide to BEAST 1.4*. http://molecularevolution.org/molevol/files/beast/BEAST14_MANUAL-7-6-07.pdf.

- Gelman, A. & Rubin, D.** 1992. Inference from iterative simulation using multiple sequences. *Statist. Sci.* 7: 457–511.
- Gignoux, J., Clobert, J. & Menaut, J.C.** 1997. Alternative fire resistance strategies in savanna trees. *Oecologia* 110: 576–583.
- Hall, T.A.** 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Sér.* 41: 95–98.
- Hemsley, W.B.** 1916. Flora of Seychelles and Aldabra. *J. Bot.* 54[Suppl. 2]: 1–24.
- Hettiarachchi, P.L. & Jayaratne, R.K.** 2006. Factors limiting seed germination in *Scyphiphora hydrophyllacea* Gaertn f. and *Pemphis acidula* J R & Forst [sic]. <http://forestry2002.blogspot.com/2006/09/factors-limiting-seed-germination-in.html>.
- Hooker, J.D.** 1873. Ordo LXXXIV. Rubiaceae. Pp. 7–151 in: Bentham, G. & Hooker, J.D. (eds.), *Genera plantarum*, vol. 2, part 1. London: Reeve; Williams & Norgate.
- Huelsenbeck, J.P. & Ronquist, F.** 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.
- IUCN.** 2001. *IUCN Red List Categories and Criteria*, version 3.1. IUCN Cambridge: Species Survival Commission.
- IUCN.** 2010. *IUCN Red List of Threatened Species*, version 2010.4. www.iucnredlist.org (accessed 4 Feb. 2011).
- Jomo, K.S., Chang, Y.T. & Khoo, K.J.** 2004. *Deforesting Malaysia: The political economy and social ecology of agricultural expansion and commercial logging*. London: Zed Books.
- Khan, S., Razafimandimbison, S.G., Bremer, B. & Liede-Schumann, S.** 2008. Sabiceae and Virectariae (Rubiaceae, Ixoroideae): One or two tribes? – New tribal and generic circumscriptions of Sabiceae and biogeography of *Sabicea* s.l. *Taxon* 57: 7–23.
- Kainulainen, K., Mouly, A., Khodabandeh, A. & Bremer, B.** 2009. Molecular phylogenetic analysis of the tribe Alberteae (Rubiaceae), with description of a new genus *Razafimandimbisonia*. *Taxon* 58: 757–768.
- Kathirithamby-Wells, J.** 2005. *Nature and nation: Forests and development in Peninsular Malaysia*. Singapore: NIAS Press.
- Kishokumar, J.** 2008. *Integrated management plan of the South-East Pahang Peat Swamp Forest*. PSF Technical Series, No. 9. UNDP/GEF Peat Swamp Forest Project, Pahang Forestry Dept., Malaysia.
- Korthals, P.W.** 1851. Overzicht der Rubiaceën van de Nederlandsch-Oostindische Kolonien. *Ned. Kruidk. Arch.* 2: 196–198.
- Lantz, H. & Bremer, B.** 2004. Phylogeny inferred from morphology and DNA data: Characterizing well-supported groups in Vanguerieae (Rubiaceae). *Bot. J. Linn. Soc.* 146: 257–283.
- Malcomber, S.T. & Davis, A.P.** 2005. Six new species of *Gaertnera* (Rubiaceae) from Madagascar and phylogenetic analyses that support *Hymenocnemis* as a synonym of *Gaertnera*. *Syst. Bot. Missouri Bot. Gard.* 104: 371–398.
- Manns, U. & Bremer, B.** 2010. Towards a better understanding of intertribal relationships and stable tribal delimitations within Cinchonoideae s.s. (Rubiaceae). *Molec. Phylog. Evol.* 56: 21–39.
- Mouly, A., Razafimandimbison, S.G., Achille, F., Haevermans, T. & Bremer, B.** 2007. Phylogenetic placement of *Rhopalobrachium fragrans* (Rubiaceae): Evidence from molecular (*rps16* and *trnT-F*) and morphological data. *Syst. Bot.* 32: 872–882.
- Mouly, A., Razafimandimbison, S.G., Florence, J., Jérémie, J. & Bremer, B.** 2009. Paraphyly of *Ixora* and new tribal delimitation of Ixoreae (Rubiaceae): Inference from combined chloroplast (*rps16*, *rbcL*, and *trnT-F*) sequence data. *Ann. Missouri Bot. Gard.* 96: 146–160.
- Novotny, V., Basset, Y., Miller, S.E., Weiblen, G.D., Bremer, B., Cizek, L. & Drozd, P.** 2002. Low host specificity of herbivorous insects in a tropical forest. *Nature* 416: 841–844.
- Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L.** 2008. AWTY (Are We There Yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24: 581–583.
- Oxelman, B., Backlund, M. & Bremer, B.** 1999. Relationships of the Buddlejaceae s.l. investigated using parsimony jackknife and branch support analysis of chloroplast *ndhF* and *rbcL* sequence data. *Syst. Bot.* 24: 164–182.
- Primavera, J.** 2000. Development and conservation of Philippine mangroves: Institutional issues. *Ecol. Econ.* 35: 91–106.
- Puff, C., Chayamarit, C. & Chamchumroon, V.** 2007. *Rubiaceae of Thailand: A pictorial guide to indigenous and cultivated genera*. Bangkok: The Forest Herbarium, Department of National Parks, Wildlife and Conservation.
- Puff, C. & Igersheim, A.** 1994. Intra-ovarian trichomes in *Jackiopsis ornata* (Wallich) Risdale (Rubiaceae-Jackieae). *Bot. J. Linn. Soc.* 115: 29–33.
- Rambaut, A. & Drummond, A.J.** 2007. Tracer, version 1.4. <http://beast.bio.ed.ac.uk/Tracer>.
- Rana, T.S. & Ranade, S.A.** 2009. The enigma of monotypic taxa and their taxonomic implications. *Curr. Sci.* 96: 219–229.
- Ranarivelo-Randriambovonjy, T., Robbrecht, E., Rabakonandrianina, E. & De Block, P.** 2007. Revision of the Malagasy species of the genus *Tricalysia* (Rubiaceae). *Bot. J. Linn. Soc.* 155: 83–126.
- Razafimandimbison, S.G. & Bremer, B.** 2001 [publ. 2002]. Tribal delimitation of Naucleaeae (Cinchonoideae, Rubiaceae) inference from molecular and morphological data. *Syst. Geogr. Pl.* 71: 515–538.
- Razafimandimbison, S.G. & Bremer, B.** 2002. Phylogeny and classification of Naucleaeae s. l. (Rubiaceae) inferred from molecular (ITS, *rbcL*, and *trnT-F*) and morphological data. *Amer. J. Bot.* 89: 1027–1041.
- Razafimandimbison, S.G., Lantz, H., Mouly, A. & Bremer, B.** 2009. Evolutionary trends, major lineages, new generic limits in the dioecious group of the tribe Vanguerieae (Rubiaceae) insights into the evolution of functional dioecy. *Ann. Missouri Bot. Gard.* 96: 161–181.
- Razafimandimbison, S.G., Rydin, C. & Bremer, B.** 2008. Evolution and trends in the Psychotriaceae alliance (Rubiaceae): A rarely reported evolutionary change of many-seeded carpels from one-seeded carpels. *Molec. Phylog. Evol.* 48: 207–223.
- Ridsdale, C.** 1979. *Jackiopsis*, a new name for *Jackia* Wall. (Rubiaceae – Jackieae). *Blumea* 25: 295–296.
- Robbrecht, E.** 1988. Tropical woody Rubiaceae. *Opera Bot. Belg.* 1: 1–271.
- Robbrecht, E. & Manen, J-F.** 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-F* and *atpB-rbcL* data. *Syst. Geogr. Pl.* 76: 85–146.
- Ronquist, F. & Huelsenbeck, J.P.** 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Rova, J.H.E., Delprete, R.P.G., Andersson, L. & Albert, V.** 2002. A *trnL-F* cpDNA sequence study of the Condamineae-Rondeletiae-Sipaneeae complex with implications on the phylogeny of the Rubiaceae. *Amer. J. Bot.* 89: 145–159.
- Rova, J.H.E., Delprete, R.P.G. & Bremer, B.** 2009. The *Rondeletia* complex: An attempt to use ITS, *rps16*, and *trnL-F* sequence data to delimit Guettardeae, Rondeletiae, and sections within *Rondeletia*. *Ann. Missouri Bot. Gard.* 96: 182–193.
- Roxburgh, W.** 1824. *Flora Indica*. Calcutta: Mission Press.
- Rydin, C., Kainulainen, K., Razafimandimbison, S.G., Smedmark, J.E.E. & Bremer, B.** 2009. Deep divergences in the coffee family and systematic position of *Acranthera*. *Pl. Syst. Evol.* 278: 101–123.
- Rydin, C., Razafimandimbison, S.G. & Bremer, B.** 2008. Rare and enigmatic genera (*Dunnia*, *Schizocolea*, and *Colletocema*), sisters to species-rich clades: Phylogeny and aspects of conservation biology in the coffee family. *Molec. Phylog. Evol.* 48: 74–83.
- Samson, N., Bauscher, M.G., Lee, S.-B., Jensen, R.K. & Daniell, H.** 2007. The complete nucleotide sequence of the coffee (*Coffea arabica* L.) chloroplast genome: Organization and implications for biotechnology and phylogenetic relationships among angiosperms. *Pl. Biotechnol. J.* 5: 339–353.

- Sasekumar, A. & Then, A.Y.H.** 2005. *Vegetation, forestry and conservation of Selangor mangrove forests*. Pp. 57–65 in: Sasekumar, A. & Chong, V.C. (eds.), *Ecology of Klang Strait*. Kuala Lumpur: University of Malaysia.
- Schumann, K.** 1891. Rubiaceae. Pp. 1–156 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, vol. 4, 4: 1. Leipzig: Engelmann.
- Smith, W.W.** 1917. Diagnoses specierum novarum in herbario Horti Regii Botanici Edinburgensis cognitarum (species asiaticae). CCLI–CCCL. *Notes Roy. Bot. Gard. Edinburgh* 10: 1–78.
- Sosef, M.S., Prawirohatmoko, M.S. & Hong, L.T.** 1998. *Plant resources of South-East Asia*, vol. 5 no. 3, *Timber trees: Lesser-known timbers*. Leiden: Backhuys.
- Stoffelen, P., Robbrecht, E. & Smets, E.** 1996. A revision of *Corynanthe* and *Pausinystalia* (African Rubiaceae–Coptosapeltea). *Bot. J. Linn. Soc.* 120: 287–326.
- Swofford, D.L.** 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Sunderland, Massachusetts: Sinauer.
- Thompson, J.D., Higgins, D.G. & Gibson, T.J.** 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22: 4673–4680.
- Tirvengadam, D.D.** 1984. *Glionnetia*, un nouveau genre de Rubiacées (Rondéletiales) des Seychelles. *Bull. Mus. Natl. Hist. Nat., B, Adansonia* 6: 197–205.
- Tomlinson, P.H.** 1986. *The botany of mangroves*. Cambridge: Cambridge University Press.
- Tosh, J., Davis, A.P., Dessen, S., De Block, P., Huysmans, S., Fay, M.F., Smets, E. & Robbrecht, E.** 2009. Phylogeny of *Tricalysia* (Rubiaceae) and its relationships with allied genera based on based plastid DNA data: Resurrection of the genus *Empogona*. *Ann. Missouri Bot. Gard.* 96: 194–213.
- UNEP World Conservation Monitoring Centre.** 2008. *Strategies for the sustainable use and management of timber species subject to international trade: South East Asia*. Cambridge, U.K.: UNEP-WCMC.
- Villaluz, D.K.** 1953. *Fish farming in the Philippines*. Manila: Bookman.
- Xiao, L.-Q. & Zhu, H.** 2007. Paraphyly and phylogenetic relationships in *Lasianthus* (Rubiaceae) inferred from chloroplast *rps16* data. *Bot. Stud.* 48: 225–232.

Appendix. Species sampled for the study.

Species, accession nos. [*ndhF*, *rbcL*, *rps16*, *trnT-F*; an n-dash (–) denotes missing data; superscripts 1–27 refer to literature or GenBank sources, superscripts 28–37 refer to voucher specimens and herbarium acronyms, both given at the end of the Appendix].

Acranthera frutescens Valetton, –, AM117198⁹, –, –, *Acranthera grandiflora* Bedd., –, AM117199⁹, –, –, *Alstonia scholaris* (L.) R. Br., –, X91760²⁸, –, –, *Amphidasya ambigua* Standl. Standl., –, Y11844²⁰, –, –, *Arctophyllum aristatum* Standl., –, AJ288595²⁹, –, –, *Anthocleista Afzel.* ex R. Br., –, L14389³⁰, –, –, *Anthospermum herbageum* L. f., –, X83623¹⁰, –, –, *Airosperma vanuense* S.P. Darwin, AM949845¹, –, FM204700¹, FM207108¹; Alberta magna E. Mey., AJ236282³ EU817411⁷ FM204701¹ AJ620118⁴; *Aleisanthia rupestris* (Ridl.) Ridl., –, AF242902⁶, AF152660⁵; *Aleisanthiopsis distantiflora* (Merr.) Tange, HM164350²⁸, EU817411⁷, EU817434⁷, EU817453⁷; *Aidia micrantha* (K. Schum.) Bullock ex F. White, –, Z68844¹⁵, –, –, *Argostemma hookeri* King, –, Z68788³¹, –, –, *Augusta rivalis* (Benth.) J.H. Kirkbr., AM949846¹, AM949842¹, –, FM207118¹; *Aulacocalyx jasminiflora* Hook.f., –, EU817413⁷, EF205639⁸, EU817455⁷; *Bertiera guianensis* Aubl.; AM949847¹, AJ224845², FM204710¹, FM207119¹ & AF152670⁵; *Boholia nematosylis* Merr., AM949848¹, FM204711¹, AM117350⁹; *Calyculophyllum candidissimum* (Vahl) DC., AJ236285³, X83627¹⁰, FM204712¹, AJ847398¹¹; *Canthium tetraphyllum* (Schweinf. ex Hiern) Robyns, AM949849¹, X83649¹⁰, FM204713¹, FM207120¹; *Carphalea glaucescens* (Hiern) Verdc., –, Z68789³¹, –, –, *Catesbaea spinosa* L., –, X83628¹⁰, –, –, *Chiococca alba* (L.) Hitch., –, L14394³⁰, –, –, *Cephalanthus occidentalis* L., –, X83629¹⁰, –, –, *Cinchona pubescens* Vahl., AJ235843¹², X83630¹⁰, FM204714¹, AJ346963¹³; *Coccyzselum hirsutum* Bartl. ex DC., –, X87145¹⁰, –, –, *Coffea arabica* L., EF044213¹⁴, EF044213¹⁴, EF044213¹⁴, EF044213¹⁴; *Coltoecema dewevrei* (De Wild.) E.M.A. Petit, –, EU145457²³, –, –, *Condaminea corymbosa* (Ruiz & Pav.) DC., –, Y18713³, –, –, *Coptosapelta diffusa* Steenis, –, EU145452²³, –, –, *Coptosapelta flavescens* Korth., –, Y18714³, –, –, *Coptosapelta montana* Korth. ex Valetton, –, EU145451²³, –, –, *Coussarea macrophylla* (Mart.) Müll. Arg., –, Y11847³, –, –, *Cremaspora triflora* ssp. *confluens* (K. Schum.) Verdc., AM949850¹, Z68856¹⁵, FM204715¹, FM207121¹; *Cremocarpon lantzii* Bremek., –, AM117222⁹, –, –, *Crossopteryx febrifuga* (Afzel. ex G. Don) Benth. 1., –, AJ347009¹³, FM204716¹, FM207122¹; *Crossopteryx febrifuga* (Afzel. ex G. Don) Benth. 2., AM949851¹, AM117223⁹, FM204717¹, FM207123¹; *Cruckshanksia hymenodon* Hook. & Arn., –, AJ288599²⁹, –, –, *Cubanola domingensis* (Britton) Aiello, AM117345⁹, X83632¹⁰, FM204718¹, FM207124¹; *Cyclophyllum deplanchei* Hook.f., –, EU817416⁷, EF205640⁸, EF205631⁸; *Damnacanthus indicus* C.F. Gaertn., –, Z68793³¹, –, –, *Danais xanthorrhoea* (K. Schum.) Bremek., –, Z68794³¹, –, –, *Declieuxia cordigera* Mart. & Zucc. ex Scult. & Schult.f., –, AM117224⁹, –, –, *Dibrachionostylus kaessneri* (S. Moore) Bremek., –, AJ616211³², –, –, *Diplospora polysperma* Valetton, –, AJ286703¹⁷, AM117301⁹, EU145538²³; *Dunnia sinensis* Tutcher 1., –, EU145467²³, –, –, *Dydimaea alsinoides* (Cham. & Schtdl.) Standl., –, Z68795³¹, –, –, *Emmenopterys henryi* Oliv., AJ236294³, Y18715³, FM204719¹, FM207125¹; *Ernodea littoralis* Sw., –, AJ288601²⁹, –, –, *Faramaea multiflora* A. Rich., –, Z68796³¹, –, –, *Ferdinandusa speciosa* Pohl., –, AM117226⁹, –, –, *Feretia aeruginescens* Stapf, –, Z68857³¹, –, –, *Fernelia buxifolia* Lam., –, AJ286704¹⁷, –, –, *Galium album* Mill., –, X81090¹⁰, –, –, *Gardenia hansemannii* K. Schum., AM949852¹, AJ318446¹⁶, FM204720¹, FM207126¹; *Gelsemium* Juss., –, L14397³⁰, –, –, *Gentiana* L., –, L14398³⁰, –, –, *Geophila obvallata* Didr., –, AM117228⁹, –, –, *Glionnetia sericea* (Baker) Tirveng. 1., HM536216²⁹, HM536222²⁹, HM536228²⁹, HM536234²⁹; *Glionnetia sericea* (Baker) Tirveng. 2., HM536217³⁰, HM536223³⁰, HM536229³⁰, HM536235³⁰; *Glionnetia sericea* (Baker) Tirveng. 3., HM536218³¹, HM536224³¹, HM536230³¹, HM536236³¹; *Greenea corymbosa* (Jack) Volgt., –, AF242961⁶, AF152657⁵; *Greenea oblonga* Craib, HM164371³², –, EU817459⁷; EU817439⁷; *Greenea* sp. 1., –, EU817438⁷, EU817458⁷; *Greenea* sp. 2., –, EU817418⁷, EU817440⁷, EU817460⁷; *Guettarda uruguensis* Cham. & Schtdl., –, X83638¹⁰, –, –, *Gynochthodes coriacea* Blume, –, AJ288603²⁹, –, –, *Hedyotis fruticosa* L., –, Z68799³¹, –, –, *Hillia triflora* (Oerst.) C.M. Taylor, –, X8364210³, –, –, *Houstonia caerulea* L., –, AJ288604²⁹, –, –, *Hydnophytum formicarum* Jack., –, X83645¹⁰, –, –, *Hypobathrum racemosum* (Roxb.) Kurz, AM949853¹, AJ286705¹⁷, AM117318⁹, FM207127¹; *Ixora brachypoda* DC., –, EU817421⁷, EU817442⁷, EU817463⁷; *Ixora coccinea* L., AJ236299³, X83646¹⁰, EF205641⁸, EU817464⁷; *Ixora guineensis* Benth., –, EU817424⁷, EU817443⁷, EU817467⁷; *Ixora hookeri* (Oudem.) Bremek., –, EU817425⁷, EU817443⁷, EU817467⁷; *Ixora margaretae* N. Hallé, –, EU817425⁷, EU817444⁷, EU817468⁷; *Ixora moorensis* (Nadeau) Fosberg, –, EU817420⁷, EU817441⁷, EU817462⁷; *Ixora trilocularis* (Balf.f.) Mouly & B. Bremer, –, EU817417⁷, EU817437⁷, EU817457⁷; *Jackiopsis ornata* Ridsdale 1., HM536213³³, HM536219³³, HM536225³³, HM536231³³; *Jackiopsis ornata* Ridsdale 2., HM536214³⁴, HM536220³⁴, HM536226³⁴, HM536232³⁴; *Jackiopsis ornata* Ridsdale 3., HM536215³⁵, HM536221³⁵, HM536227³⁵, HM536233³⁵; *Keetia gueinzii* (Sond.) Bridson, –, AM117323⁹, AJ620143⁹; *Kohautia caespitosa* Schnizl., –, Z68800³¹, –, –, *Kraussia floribunda* Harv., –, Z68858³¹, –, –, *Lasianthus pedunculatus* (Griseb.) Urb., –, Z68802³¹, –, –, *Lerchea bracteata* Valetton, AJ288610²⁹; *Luculia grandifolia* Ghose, –, X83648¹⁰, –, –, *Luculia grattissima* (Wall.) Sweet, AJ011987¹⁸, AM117243⁹, AJ431036¹⁹, AJ430911¹⁹; *Luculia pincea* Hook., –, EU145447²³, –, –, *Manostachya ternifolia* (A. Martins, AJ616213³⁰; *Margaritopsis acutifolia* C. Wright, –, AM117247⁹, –, –, *Maschalocorymbus corymbus* (Blume) Bremek., –, AJ288611²⁹, –, –, *Mitchella repens* L., –, Z68805³¹, –, –, *Mitrasacmopsis quadravalvis* Jovet, –, AJ616214²⁰, –, –, *Morinda citrifolia* L., AJ236300³, X83651¹⁰, AJ320078¹⁶, FM207589¹ & AF152616⁵; *Mostuea* Didr., –, L14404², –, –, *Mussaenda arcuata* Poir., AJ236301³, Y11854²⁰, FM204721¹, FM207128¹; *Mussaenda erythrophylla* Schumach. & Thonn., –, X83652¹; *Mycetia malayana* (G. Don) Craib, –, Z68806³¹, –, –, *Nauclea orientalis* (L.) L., –, X83653¹⁰, –, –, *Nematostylis anthophylla* (A. Rich. ex DC.) Baill., FM207650¹, FM207648¹, FM204723¹, FM207130¹; *Nertera granadensis* (Mutis ex L.f.) Druce, –, X83654¹⁰, –, –, *Neurocalyx zeylanicus* Hook., –, Z68807³, –, –, *Normandia neocaledonica* Hook.f., –, AM117250⁹, –, –, *Oldenlandia corymbosa* L., –, X83655¹⁰, –, –, *Ophiorrhiza mungos* L., –, X83656¹⁰, –, –, *Oreopilos glacialis* (poepp.) Ricardi, –, AJ288612²⁹, –, –, *Paederia foetida* L., –, AF332373³³, –, –, *Palicourea crocea* (Sw.) Schult., –, AM117253⁹, –, –, *Parapentas silvatica* (K. Schum.) Bremek., –, X83657¹⁰, –, –, *Pauridiantha paucinervis* (Hiern) Bremek., –, Z68811³¹, –, –, *Pavetta abyssinica* Fresen., AM949854¹, Z68863¹⁵, FM204726¹, FM207133¹; *Pentagonia macrophylla* Benth., AJ236303³,

Appendix. Continued.

X83658¹⁰, FM204727¹, FM207134¹; *Pentas lanceolata* (Forssk.) Deflers., –, Z83659³¹, –, –, *Pentodon pentandrus* (Schumach. & Thonn.) Vatke. Oesterr., –, X83660¹⁰, –, –, *Peponidium comorense* Arènes, –, –, EF205646⁸ EF205632⁸; *Posoqueria latifolia* (Rudge) Roem. & Schult., AM949855¹, Z68850¹⁵, FM204728¹, FM207135¹; *Pouchetia gillettii* De Wild., –, Z68859¹⁵, –, –, *Praravinia suberosa* (Merr.) Bremek., –, AJ288616²⁹, –, –, *Pseudomussaenda flava* Verdc., AM949856¹, Y11855²⁰, FM204729¹, FM207136¹; *Psychotria kirkii* Hiern, –, X83663¹⁰, –, –, *Psychotria poeppigiana* Müll.Arg., –, Z68818³¹, –, –, *Pyrostria hystrix* (Bremk.) Bridson, EU145418²³, AM117262⁹, AM117338⁹, AJ620168³; *Razafimandimbisonia minor* (Baill.) Kainul. & B. Bremer, AM949844¹, EU817410⁷, EF205637⁸, EU817452⁷; *Razafimandimbisonia sambiranensis* (Homolle ex Cavaco) Kainul. & B. Bremer, –, EU817431⁷, EF205645⁸, EU817474⁷; *Retiniphyllum concolor* (Spruce ex Benth.) Müll.Arg., –, –, EU821629²⁴ EU821641²⁴; *Retiniphyllum maguirei* Standl., –, –, EU821632²⁴ EU821646²⁴; *Retiniphyllum pilosum* (Spruce ex Benth.) Müll.Arg., –, AF331654²¹, FM204730¹, FM207137¹; *Retiniphyllum schomburgki* (Benth.) Müll.Arg., –, –, EU821631²⁴, EU821645²⁴; *Rhachicallis americana* (Jacq.) Hitchc., –, X83664¹⁰, –, –, *Rondeletia odorata* Jacq., –, Y11857²⁰, –, –, *Rubia tinctorum* L., –, X83666¹⁰, –, –, *Rustia thibaudioides* (H. Karst.) Delprete, AJ236310³, Y18716³, FM204731¹, FM207138¹; *Sabicea aspera* Aubl., –, AY538508²², –, –, *Sabicea diversifolia* Pers., –, EU145459²³, –, –, *Sabicea villosa* Roem. & Schult., AM949857¹, Y11858²⁰, FM204732¹, FM207139¹; *Saldinia* sp. 1, –, AM117269⁹, –, –, *Schradera* K. Krausse sp., –, Y11859³, –, –, *Schismatoclada* sp., –, AM117271⁹, –, –, *Schizocolea linderi* (Hutch. & Dalziel) Bremek., –, AM117272⁹, –, –, *Scyphiphora hydrophylacea* C.F. Gaertn. 1, AJ236311³, Y18717³, FM204733¹, FM207140¹; *Scyphiphora hydrophylacea* C.F. Gaertn. 2, –, –, EU821634²⁴ EU821648²⁴; *Sherardia arvensis* L., –, X81106¹⁰, –, –, *Spermacoe laevis* Lam., –, Z68823³¹, –, –, *Spigelia* L., –, Y11863²⁰, –, –, *Sipanea biflora* (L. f.) Cham. & Schldt., EU145413²³, AY538509²², FM204734¹, FM207141¹; *Sipanea hispida* Benth. ex Wernham, EU145414²³, EU145458²³, EU145492²³, AY555107²⁷; *Sipanea pratensis* Aubl., –, –, AF243022⁶, AF152677⁵; *Spiradiclis bifida* Kutz., –, EU145465, –, –, *Steenisia pleurocarpa* (Airy Shaw) Bakh.f., –, AM117279⁹, FM204735¹, FM207142¹; *Strychnos* L., –, L14410²⁸, –, –, *Tamridaea capsulifera* (Balf.f.) Thulin & B. Bremer, –, Y11860²⁰, FM204736¹, FM207143¹; *Tennantia sennii* (Chiov.) Verdc. & Bridson, AM949858¹, AM949843¹, FM204737¹, FM207144¹; *Thecorchus wauensis* (Schweinf. ex Hiern) Bremek., –, AM117282⁹, –, –, *Theligonum cynocrambe* L., –, X83668¹⁰, –, –, *Tocoyena pittieri* (Standl.) Standl., AM949859¹, –, FM204738¹, FM207145¹; *Trailliaedoxa gracilis* W.W. Sm. & Forrest 1, HM164399³⁶, HM164182³⁶, HM164226³⁶, HM164338³⁶; *Trailliaedoxa gracilis* W.W. Sm. & Forrest 2, HM164400³⁷, HM164183³⁷, HM164227³⁷, HM164339³⁷; *Tricalysia cryptocalyx* Baker, –, Z68854¹⁵, AF004088²⁶, AM999487²⁵; *Trichostachys aurea* Hiern, –, EU145462²³, –, –, *Trichostachys* sp., –, AJ288626²⁹, –, –, *Urophyllum ellipticum* (Wight) Thwaites, –, X83670¹⁰, –, –, *Vangueria madagascariensis* J.F. Gmel., AJ130840³, X83670¹⁰, EU821636²⁴, FM207146¹; *Virectaria major* (K. Schum.) Verdc., –, Y11861²⁰, –, –, *Xanthophyllum borneense* (Valeton) Axelius, –, EU145466²³, –, –, *Wendlandia tinctoria* (Roxb.) DC., AM949860¹, FM207649¹, FM204739¹, FM207147¹.

Published sequences: 1, Kainulainen & al. (2009); 2, Andreasen & al. (1999); 3, Bremer & al. (1999); 4, Lantz & Bremer (2004); 5, Rova & al. (2002); 6, Rova, J.H.E. (Genbank unpub.); 7, Mouly & al. (2009); 8, Mouly & al. (2007); 9, Bremer & Eriksson (2009); 10, Bremer & al. (1995); 11, Alejandro & al. (2005); 12, Backlund & al. (2000); 13, Razafimandimbison & Bremer (2002); 14, Samson & al. (2007); 15, Andreasen & Bremer (1996); 16, Novotny & al. (2002); 17, Andreasen & Bremer (2000); 18, Oxelman & al. (1999); 19, Bremer & al. (2002); 20, Bremer & Thulin (1998); 21, Andersson, L. (Genbank unpub.); 22, Andersson & Antonelli (2005); 23, Rydin & al. (2008); 24, Cortes-B. & al. (2009); 25, Tosh & al. (2009); 26, Andersson & Rova (1999); 27, Delprete & Cortes-B. (2004).

Voucher information: 28, *Tange 46977* (AAU); 29, *Beaver 3* (S); 30, *Beaver 17* (S); 31, *Beaver 27* (S); 32, *Larsen K. & Larsen S.S. 33451* (P); 33, *Tan s.n.* (Malaysia), cA047; 34, *Tan s.n.* (Malaysia), cA048; 35, *Tan s.n.* (Malaysia), cA049; 36, *Yü 1348* (HUH); 37, *Boufford & al. 35041* (MO).