

Molecular phylogenetic analysis of the tribe Alberteae (Rubiaceae), with description of a new genus, *Razafimandimbisonia*

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The tribe Alberteae, presently classified in the subfamily Ixoroideae (Rubiaceae), has historically been an artificial grouping of genera. In the present study, phylogenetic analyses of the chloroplast DNA markers *rbcL*, *ndhF*, *trnS-G*, *trnT-F* and *trnH-psbA* as well as the ITS region of the nuclear ribosomal DNA, are done to assess the delimitation of Alberteae. The resulting phylogenetic hypothesis is highly resolved, with most clades strongly supported. The genus *Alberta* is found to be paraphyletic as presently circumscribed. As a consequence, we propose the new genus *Razafimandimbisonia* Kainul. & B. Bremer to accommodate the Malagasy species. The newly delimited *Alberta* is distinguished by having two calycophylls that expand after anthesis as well as awl-shaped stigma lobes. *Razafimandimbisonia* is distinguished from the remaining Alberteae by having dehiscent fruits and anthers without basal appendages. We demonstrate that the genera *Airosperma*, *Boholia* and *Crossopteryx* are not associated with Alberteae, as has previously been suggested. Alberteae is considered restricted to the genus *Alberta* endemic to Southeast Africa, and the two Malagasy endemic genera *Nematostylis* and *Razafimandimbisonia*.

KEYWORDS: *Airosperma*, *Alberta*, *Boholia*, cpDNA, *Crossopteryx*, Madagascar, *Nematostylis*, Rubiaceae

INTRODUCTION

Current understanding of the species-rich plant family Rubiaceae has been greatly improved by recent molecular phylogenetic studies. The former classification systems have been shown to contain many unnatural groups (for a review, see Bremer, 2009). The subfamily Ixoroideae, as defined by Bremekamp (1966), comprised genera with secondary pollen presentation, entire stipules and stamens inserted at the corolla throat. Lately, the merging of Ixoroideae with Cinchonoideae has been advocated (Bridson & Verdcourt, 2003; Robbrecht & Manen, 2006). In this paper we follow the classification as outlined by Bremer (2009), with Ixoroideae being comprised of the tribes Alberteae, Bertiereae, Coffeae, Condamineae, Cremasporeae, Ixoreae, Gardenieae, Mussaendeae, Octotropideae, Pavetteae, Posoquerieae, Retiniphyllae, Sabiceae, Sipaneeae and Vanguerieae.

The tribe Alberteae has a complex taxonomic history; for a more thorough review the reader is referred to Puff & al. (1984). Alberteae was originally described by Hooker (1873), comprising genera with solitary pendulous ovules and contorted aestivation of corolla lobes. He included the following genera: *Alberta* E. Mey., *Aulacocalyx* Hook. f., *Belonophora* Hook. f., *Cremaspora* Benth., *Nematostylis* Hook. f., *Polysphaeria* Hook. f., and *Rhabdostigma* Hook. f. This classification was followed by Schumann (1891), who also added *Lamprothamnus*

Hiern. and *Octotropis* Bedd. Bremekamp (1952), did not consider ovule number a morphological character of much value, but stressed as previously mentioned the importance of secondary pollen presentation. Based on what he perceived as an absence of secondary pollen presentation in the genera *Alberta*, *Belonophora* and *Nematostylis*, he split Alberteae, and consequently accommodated the remaining genera in a newly erected tribe Cremasporeae. No new tribal placement was given for *Belonophora*. Later Keay (1958a) found that the previously reported number of ovules in *Belonophora* was incorrect and suggested an exclusion of the genus from Alberteae. Secondary pollen presentation has since then been reported in both *Alberta* and *Nematostylis* (Puff & al., 1984; cf. Fig. 1A), as well as in *Belonophora* (Cheek & Dawson, 2000). In the classification of Verdcourt (1958), Alberteae consist of only *Alberta* and *Nematostylis*.

To Alberteae sensu Verdcourt (1958), Robbrecht (1988) in his classification tentatively (re-)included *Octotropis* as well as the genera *Airosperma* K. Schum. & Lauterb. and *Boholia* Merr., the latter two Pacific genera whose affinity to Alberteae had been suggested by their respective authors. Later, Robbrecht & al. (1994) transferred *Octotropis* to a resurrected Octotropideae. *Lamprothamnus* and *Polysphaeria* of Alberteae sensu Schumann (1891), have also been moved to Octotropideae (Robbrecht, 1980; as Hypobathreae), as well as *Rhabdostigma* syn. *Kraussia* Harv. (Andreasen & Bremer,

1996). A monogeneric Cremasporaeae has been shown to form a sister group to Octotropideae (Andreasen & Bremer, 1996, 2000), and *Belonophora* has been shown to be part of Coffeaeae (Robbrecht & Manen, 2006; Davis & al., 2007). Additionally, *Rhopalobrachium* Schltr. & K. Krause, was considered part of Alberteae by its authors, however this genus has recently been shown to be nested within Vanguerieae, with a close relationship with *Cyclophyllum* Hook. f. (Mouly & al., 2007).

In a thorough morphological study of *Alberta* and *Nematostylis*, Puff & al. (1984) concluded that the tribe occupies an isolated position in the subfamily Ixoroideae. Robbrecht (1988), however, placed Alberteae in Antirheoideae, a subfamily that was later shown to be polyphyletic by Bremer & Jansen (1991). In a molecular study by Bremer & al. (1999), *Alberta magna* E. Mey. formed a well-supported sister group to a clade consisting of *Coffea* L. and *Gardenia* Ellis, and in a study by Mouly & al. (2007) the Malagasy species *A. minor* Baill. and *A. sambiranensis* Homolle ex Cavaco (the latter as *Nematostylis anthophylla* (A. Rich. ex DC.) Baill.) formed a clade in a corresponding albeit weakly supported position. Recently, Robbrecht & Manen (2006) placed Alberteae in a “Vanguerieae alliance”, and included in this group also the genera *Aulacocalyx* and *Crossopteryx* Fenzl. In Razafimandimbison & Bremer (2001), *Crossopteryx* was shown to be nested within Ixoroideae, and the genus was later accommodated in the monogeneric Crossopterygeae by Bridson & Verdcourt (2003). *Aulacocalyx*, as previously mentioned, was initially placed in Alberteae by Hooker (1873) based on what he perceived as solitary pendulous ovules. As in the case with *Belonophora* this character was shown to be erroneous by Keay (1958b). *Aulacocalyx* as a member of Alberteae has also been rejected by Puff & al. (1984), and was subsequently accommodated in a tribe Aulacocalyceae together with five other genera by Robbrecht & Puff (1986). However, Aulacocalyceae was found nested within Gardenieae, and merged into this tribe in a molecular study by Andreasen & Bremer (2000), although this study did not include the type *Aulacocalyx*. In the study by Mouly & al. (2007), *Aulacocalyx* is indeed nested within Gardenieae.

The aims of the present study are to determine the delimitation and phylogeny of Alberteae using molecular DNA data, and to test if *Airosperma*, *Boholia* or *Crossopteryx* are part of, or associated with the tribe, as has previously been suggested.

MATERIALS AND METHODS

Taxon sampling. — Taxa from all tribes of Ixoroideae as defined by Bremer (2009) were included (*Berti*ereae, *Berti*era *guianensis* Aubl.; Coffeaeae, *Coffea*

arabica L.; Condamineaeae s.l., *Calycophyllum candidissimum* (Vahl) DC., *Emmenopterys henryi* Oliv., *Pentagonia macrophylla* Benth., *Rustia thibaudioides* (H. Karst.) Delprete; Cremasporaeae, *Cremaspora triflora* subsp. *confluens* (K. Schum.) Verdc.; Gardenieae, *Gardenia hansemannii* K. Schum., *Tocoyena pittieri* (Standl.) Standl.; Ixoreae, *Ixora coccinea* L.; Mussaendeae, *Mussaenda arcuata* Poir., *Pseudomussaenda flava* Verdc.; Octotropideae, *Hypobathrum racemosum* (Roxb.) Kurz; Pavetteae, *Pavetta abyssinica* Fresen., *Tennantia sennii* (Chiov.) Verdc. & Bridson; Posoquerieae, *Posoqueria latifolia* (Rudge) Roem. & Schult.; Retiniphyllaeae, *Retiniphyllum pilosum* (Spruce ex Benth.) Müll. Arg.; Sabiceaeae, *Sabicea villosa* Roem. & Schult., *Tamridaea capsulifera* (Balf. f.) Thulin & B. Bremer; Sipaneeae, *Sipanea biflora* (L. f.) Cham. & Schldt.; Vanguerieae, *Canthium tetraphyllum* (Schweinf. ex Hiern) Robyns, *Vangueria madagascariensis* J.F. Gmel.), as well as a number of taxa whose phylogenetic positions are poorly known (see Appendix).

We were able to obtain sequences from several specimens of four of the six currently recognized species of *Alberta*, including the type, *A. magna*, from Southeast Africa, and the Malagasy species *A. minor* (Fig. 1A, B), *A. humblotii* Drake and *A. sambiranensis* (Fig. 1F, G), as well as sequences from several specimens of *Nematostylis* (Fig. 1I–K), including one as of yet undescribed species. We were however unable to obtain material or sequences of the rare *A. orientalis* Homolle ex Cavaco and *A. regalis* Puff & Robbr.

Molecular markers previously shown to be informative in inferring molecular phylogenies in the Rubiaceae were chosen. These included the chloroplast DNA (cpDNA) markers *rbcl* (Bremer & al., 1995), *ndhF* (Bremer & al., 1999), *rps16* (Andersson & Rova, 1999), *trnT-F* (Razafimandimbison & Bremer, 2002), *trnL-F* (Rova & al., 2002) and the ITS region (Persson, 2000) of the nuclear-ribosomal DNA (nrDNA). Two additional cpDNA markers were also included; the highly variable *trnH-psbA* spacer, as well as the *trnS-trnG-trnG* region (from here on called *trnS-G*), shown to have a high number of parsimony informative characters in a survey of phylogenetic utility of non-coding cpDNA markers in the seed plants by Shaw & al. (2005). The sampling of sequences of the relatively slowly evolving markers *rbcl* and *ndhF* at low taxonomic level was incomplete (see Appendix), as these markers were not expected to provide much phylogenetic information within the crown group Alberteae. Contrary, the sampling of the fast evolving ITS and *trnH-psbA* markers was restricted due to alignment difficulties omitting early divergent clades of the subfamily (see Appendix).

DNA isolation and amplification. — DNA was extracted from silica-gel dried leaf tissue or herbarium material, in accordance with the protocol of Doyle & Dickson (1987), using a Mini-Beadbeater 3110BX (BioSpec). The



Fig. 1. Characteristics and morphological variation of Alberteae. A, B, *Razafimandimbisonia minor* (*Alberta minor*); C, *Razafimandimbisonia* cf. *humblotii* (*Alberta* cf. *humblotii*); D, E, *Razafimandimbisonia* sp. (*Alberta* sp.); F, G, *Razafimandimbisonia sambiranensis* (*Alberta sambiranensis*); H, *Alberta magna*; I, J, *Nematostylis anthophylla*; K, *Nematostylis* sp. nov. (Photos: A, F, Conservatoire et Jardin Botanique de la Ville de Genève; B, K by KK; C by Agneta Julia Borg; D, E, J by Torsten Eriksson; G by Sylvain Razafimandimbison; H, I by Birgitta Bremer).

DNA was purified using the QIAquick® PCR purification kit following the instructions of the manufacturer (Qiagen). The markers ITS, *rps16* and *trnT-F* were amplified using standard PCR settings. The following primers were used for both amplification and sequencing reactions: *rps16*, *rps16_2F* (Bremer & al., 2002) and *rpsR2* (Oxelman & al., 1997); *trnT-L*, *trnT-F_a1F* and *trnT-F_iR* (Razafimandimbison & Bremer, 2002); *trnL-F*, *c*, *d*, *e* and *f* (Taberlet & al., 1991); and ITS, P17 (Lidén & al., 1995), P16, P25 and 26S-82R (Popp & Oxelman, 2001). The *ndhF* sequences as well as a number of the *trnT-F* sequences were obtained using the primers of Rydin & al. (2008). The *trnH-psbA* and *trnS-G* regions were amplified using primers and protocols according to Shaw & al. (2005). PCR products were purified using Montage® PCR96 plates (Millipore). Sequence reactions were performed using Big Dye® terminator v3.1 cycle sequencing kit following the instructions of the manufacturer (Applied Biosystems), and analysed using a 3100 Genetic Analyzer (Applied Biosystems). Sequence fragments were assembled using the Staden package v1.5.3 (Staden, 1996). Additional sequences were obtained from GenBank (for references, see Appendix). Sequences new to this study were submitted to EMBL (see Appendix).

Data analyses. — A 10-bp inversion was found in the *trnS-G* marker (corresponding to positions 9714–9723 in the *Coffea arabica* chloroplast genome; GenBank accession number, EF044213; Samson & al., 2007), that was highly homoplastic and consequently excluded from analyses. In the *trnH-psbA* marker two inversions were found, one 55 bp long occurring in *Augusta rivalis* (Benth.) J.H.Kirkbr. (corresponding to positions 100–154 in the *Coffea* chloroplast genome), and a 4-bp inversion (corresponding to positions 305–308 in the *Coffea* chloroplast genome) occurring in *Crossopteryx* and the *Vanguerieae*

taxa. The former was in our analyses inverted and re-inserted, whereas the latter was excluded from the analyses. In addition an AT-rich region in the *trnS-G* marker (corresponding to positions 9183–9203 in the *Coffea* chloroplast genome) was also excluded from analyses due to difficulties in finding an unambiguous alignment. The sequences were preliminarily aligned using Clustal W (default settings; Thompson & al., 1994), as implemented in BioEdit (Hall, 1999) and then edited manually. Aligned matrices were analysed using maximum parsimony (MP) and Bayesian inference (BI). In addition to analyses of the separate markers in order to assess incongruences of individual gene trees, analyses of all chloroplast regions combined in a “cpDNA” dataset, as well as all chloroplast and the ITS data in a “combined” dataset, were done in order to increase resolution and clade support. Parsimony analyses were done using the program PAUP* v4.0B10 (Swofford, 2002), a heuristic search with TBR branch swapping algorithm, Multrees on, 1,000 random sequence addition replicates, and a maximum of ten trees saved per replicate. Clade bootstrap support (BS) was estimated using the same settings and three random addition replicates per replicate.

Bayesian inference analyses were done using MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Substitution models suggested as best-fit to the data (Table 1) under the Akaike Information Criterion (AIC), as implemented in MrModeltest 2.2 (Nylander, 2004), were used in the analyses of each separate marker as well as in the analyses of the cpDNA and combined datasets. The two latter datasets were partitioned corresponding to the individual markers, with unlinked parameter estimates. The analyses consisted of two independent runs of four chains each, with a chain temperature parameter of 0.1, and were monitored for

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>rbcL</i>	<i>ndhF</i>	<i>trnS-G</i>	<i>trnT-F</i>	<i>rps16</i>	<i>trnH-psbA</i>	ITS	cpDNA	cpDNA +ITS
Number of taxa	37	33	47	51	48	34	29	51	51
Excluded characters	0	0	173	0	0	4	0	177	177
Included characters	1,402	2,158	1,513	2,320	1,011	418	864	8,822	9,686
Variable characters	272	639	531	813	349	122	230	2,726	2,956
Parsimony informat. characters	130	322	261	419	185	63	168	1,380	1,548
% informative characters	9.27	14.92	17.25	18.06	18.30	15.07	19.44	15.64	15.98
Score of best trees	550	1,152	920	1,326	622	188	532	4,796	5,357
Number of trees retained	818	72	727	4,878	7,371	140	12	18	21
Consistency index (CI)	0.59	0.70	0.74	0.77	0.71	0.76	0.64	0.71	0.70
Retention index (RI)	0.60	0.72	0.78	0.81	0.79	0.81	0.78	0.75	0.75
Model suggested	GTR+I+G	GTR+I+G	GTR+I+G	GTR+I+G	GTR+G	F81+G	GTR+I+G	–	–

2×10^6 generations (5×10^6 generations in the analysis of the combined dataset). Trees sampled before stable posterior probability values (PP) had been reached, or $\geq 25\%$ of the first of the saved trees, were excluded from consensus as a burn-in phase.

RESULTS

Separate analyses. — A summary of the tree data and statistics from the analyses is given in Table 1. The cpDNA markers *trnH-psbA* and *trnS-G* were both phylogenetically informative, with percent informative characters intermediate to those of the fast evolving ITS, *trnT-F* and *rps16* and those of the more slow evolving *rbcL* and *ndhF* markers. The MP and BI analyses of the individual markers resulted in largely congruent phylogenetic trees (trees not shown). Supported incongruences of individual marker trees relative the resulting phylogenetic hypothesis from the combined data (Fig. 2) included; (1) *Morinda* L. as sister group to Ixoroideae (*ndhF*, BI, PP 0.94), (2) Musaeaceae + Sabiceae as sister to the rest of Ixoroideae (*trnT-F*, MP, BS 80%; BI, PP 0.59). (3) *Posoqueria* was in the *rps16* analyses not sister group to Sipaneeae, but grouped unresolved with the remainder of Ixoroideae in the MP analysis (BS 73%), whereas its position was further resolved in the BI tree as sister to the crown group (PP for nodes, from the basal node towards the crown group: 0.96, 0.92 and 0.87, respectively).

Alberta magna did not group with the Malagasy *Alberta* nor *Nematostylis* in any of the analysed cpDNA markers, nor in analyses of these markers combined. The species was either (1) unresolved relative to these taxa and the Gardenieae alliance (*ndhF*, MP; *trnT-F*, MP; *trnS-G*, MP; *rps16*, MP, BI; *trnH-psbA*, MP, BI), (2) a poorly supported sister group to the Gardenieae alliance (*ndhF*, BI, PP 0.62), (3) nested within the Gardenieae alliance but with an unresolved position (*trnS-G*, BI, PP 0.85), or (4) sister to Pavetteae (*trnT-F*, BI, PP 0.96). In the analyses of the cpDNA dataset, *Nematostylis* + the Malagasy *Alberta* were resolved as a poorly supported sister to the Gardenieae alliance (MP, BS 58%; BI, PP 0.57). In the same cpDNA analyses the bootstrap support for the position of *Crossopteryx* as sister to a clade including Ixoreae, Vanguerieae and *Scyphiphora* C.F. Gaertn., was considerably stronger (92%), compared to when ITS was included (55%; Fig. 2), whereas the posterior probability was unchanged (1.00). Above mentioned incongruences were not consistently strongly supported, and were considered as “minor points of topological disparity” (Wendel & Doyle, 1998).

Nematostylis was resolved as sister group to the Malagasy *Alberta* in several of the analysed chloroplast markers (*trnS-G*, BS 61%, PP 0.99; *trnT-F*, BS 97%, PP 1.00; *rbcL*, PP 0.85, *rps16*, PP 0.82), but was left unresolved or

not supported in the *ndhF* and *trnH-psbA* trees, as well as in the *rbcL* and *rps16* MP trees. In the ITS trees, Alberteae was recovered as a strongly supported (BS 98%, PP 1.00) trichotomy of *A. magna*, the Malagasy *Alberta*, and *Nematostylis*.

In all the trees resulting from analyses of cpDNA markers with a more extensive sampling within Alberteae (i.e., *rps16*, *trnH-psbA*, *trnS-G*, *trnT-F*) a moderately to strongly supported clade of three specimens of *Alberta minor* was resolved, either as sister to the remaining Malagasy *Alberta* (*rps16*, *trnS-G*) or unresolved relative to these. This was incongruent with the ITS phylogeny, where *A. sambiranensis* formed a strongly supported (BS 97%, PP 1.00) sister group to a clade of the remainder of the included Malagasy species (BS 81%, PP 0.70).

Combined data analyses. — In the analyses of the combined datasets, the consensus trees of the MP and BI analyses were similar (the former differing in the position of *Aulacocalyx* as sister to the Pavetteae clade; BS 51%), resulting in a well-resolved phylogenetic hypothesis with in most cases strongly supported clades (Fig. 2). In this phylogenetic tree the tribe Alberteae consisted of *Alberta* and *Nematostylis*. *Alberta* appeared paraphyletic, with the Malagasy species grouping with *Nematostylis*. The tribe formed a well supported sister group to a clade consisting of representatives of the tribes Bertiereae, Coffeae, Cremasporae, Gardenieae, Octotropideae and Pavetteae (hereafter called the Gardenieae alliance). Nested within this group was also *Aulacocalyx*.

Resolved as a sister group to the Alberteae-Gardenieae alliance clade was a clade formed by *Augusta* Pohl and *Wendlandia* Bartl. ex DC. A strongly supported clade consisting of *Airosperma* and *Boholia*, in turn formed a sister group to the common ancestor of previously mentioned groups. Outside these clades, representatives of the tribes Ixoreae and Vanguerieae grouped together, with *Scyphiphora* forming a sister group to these tribes. Resolved as a well-supported sister to the *Scyphiphora*-Ixoreae-Vanguerieae clade was the genus *Crossopteryx*. Relative to the mentioned clades in the phylogenetic tree, followed then a grade of decreasing relatedness consisting of representatives of, in turn, *Retiniphyllum* Humb. & Bonpl., *Steenisia* Bakh. f., and Musaeaceae grouped with Sabiceae. The tribe Condamineae s.l. and a clade formed by the tribes Sipaneeae and Posoquerieae, formed successive sister groups to the rest of the Ixoroideae.

DISCUSSION

Tribal delimitation of Alberteae. — This study confirms the position of Alberteae within the subfamily Ixoroideae. The tribe is positioned as sister group to a clade of the Gardenieae alliance consisting of the tribes

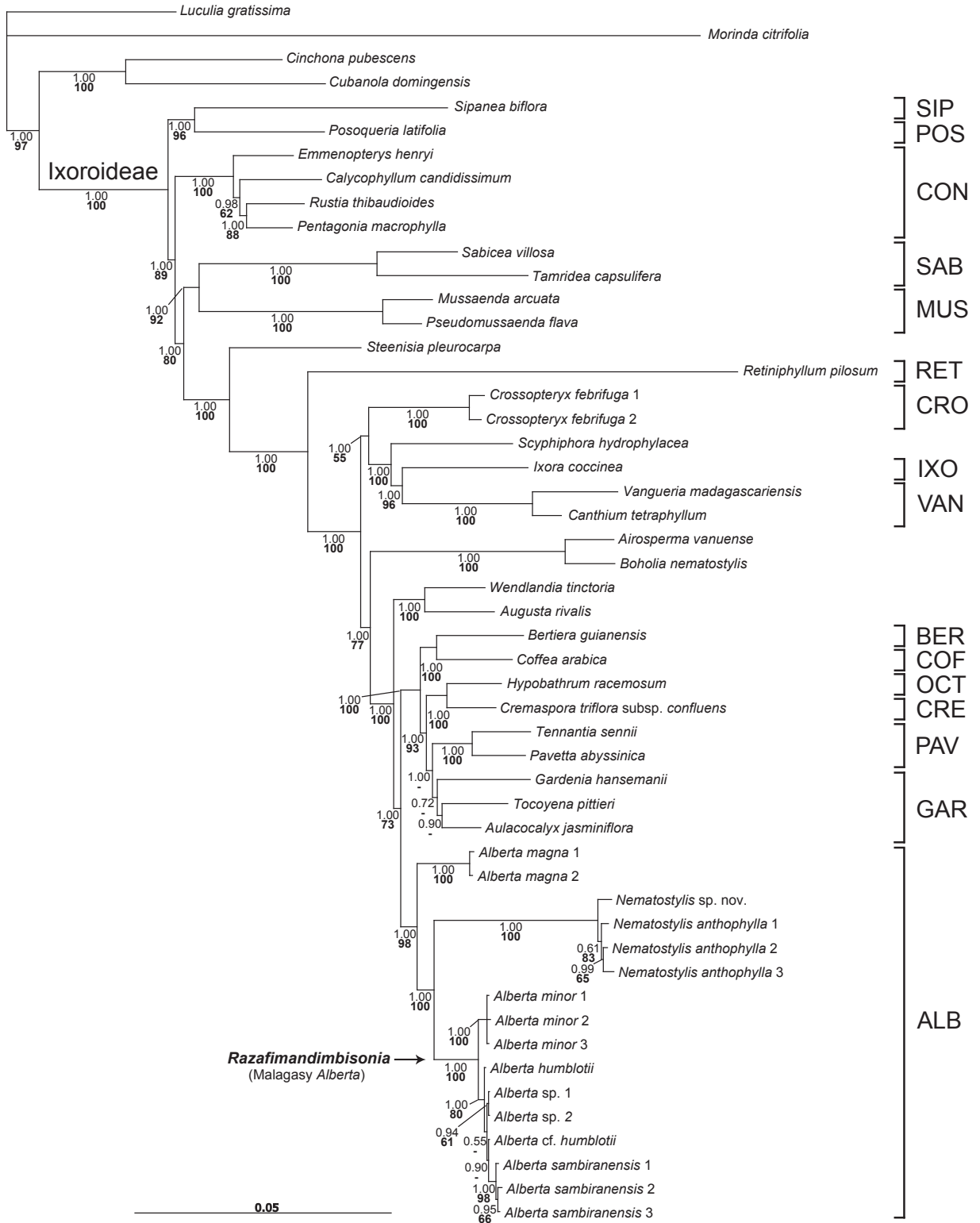


Fig. 2. 50% majority rule consensus tree of the combined data, from the Bayesian analyses. Below branches are posterior probabilities and bootstrap support percentages (bold). Bootstrap support <50% is indicated by a dash. Tribes of Ixoroideae indicated as, ALB = Alberteae, BER = Bertiereae, COF = Coffeae, CON = Condamineae s.l., CRE = Cremasporae, CRO = Crossopterygeae, IXO = Ixoreae, GAR = Gardenieae, MUS = Mussaendeae, OCT = Octotropideae, PAV = Pavetteae, POS = Posoquerieae, RET = Retiniphyllae, SAB = Sabiceae, SIP = Sipaneeae, VAN = Vanguerieae. Note that in the MP phylogeny *Gardenia* groups with *Tocoyena*, and *Aulacocalyx* groups with *Pavetteae* with a bootstrap support of 51%.

Bertierteae, Coffeae, Cremasporeae, Gardenieae, Octotropideae and Pavetteae, as in accordance with Bremer & al. (1999) and Mouly & al. (2007). This study also confirms the tribal delimitation of Alberteae sensu Verdcourt (1958) as to consist of the genera *Alberta* and *Nematostylis* only. *Aulacocalyx*, *Airosperma*, *Boholia* and *Crossopteryx* are not associated with the tribe, as has previously been suggested. The tribe Alberteae is consequently characterized by \pm zygomorphic flowers and 1–5 expanded and brightly coloured calyx lobes (calycophylls), on all flowers of the inflorescence (cf. Puff & al., 1984).

The monotypic *Crossopteryx* is resolved as a sister to the *Scyphiphora*-Ixoreae-Vanguerieae clade, as opposed to being allied to the Alberteae as indicated by Robbrecht & Manen (2006). The association of *Crossopteryx* with *A. magna* in their study goes back to *rbcL* data, and this conflicting signal relative the other cpDNA markers is seen in our analyses as well (in the MP strict consensus tree *A. magna* is sister group to a *Crossopteryx*-*Augusta* clade), but this relationship receives no support. The isolated position of *Crossopteryx* in the phylogenetic tree of the combined data supports the classification of Bridson & Verdcourt (2003), where the genus is accommodated in a tribe of its own, Crossopterygeae.

Airosperma and *Boholia* have previously been tentatively included in Alberteae (Robbrecht, 1988), but are in this study resolved as a sister group to the *Augusta*-*Wendlandia*-Alberteae-Gardenieae alliance clade. The two genera form a strongly supported Southeast Asian–Pacific clade. This relationship has previously been suggested by Darwin (1979). Both genera share a number of characters such as solitary pendulous ovules, membranaceous leaves and open, terminal inflorescences (Darwin, 1979). In addition, on the label of a specimen of *Boholia* sp.—*Sulit & Conklin 16910* (HUH), the fruits are described as “light blue to violet”. This is similar to the fruit colour of some of the *Airosperma* species (see Darwin, 1980).

Alberteae was not resolved in analyses of any of the separate cpDNA markers, nor in analyses of the combined cpDNA data, probably due to lack of informative characters. Even though resolved relationships were variable, no clear conflicting positions were supported. In the resulting ITS trees however, Alberteae was resolved with strong support (BS 98%, PS 1.00), and adding the cpDNA data did not reduce support (BS 98%, PP 1.00; Fig. 2).

Generic delimitation within Alberteae. — In the present study, three clades of Alberteae are resolved and in most analyses strongly supported, the Southeast African *Alberta magna*, the Malagasy *Alberta*, and *Nematostylis*. No molecular marker investigated shows support for a monophyletic *Alberta*. At least two ways of re-classification should be considered. Cavaco (1965), in describing two new species of *Alberta*, found the distinguishing characters of the two genera as defined by Hooker (1873)

to be obsolete (i.e., the number of stigma lobes and calycophylls, and the hairiness of the anthers), and merged *Nematostylis* with *Alberta*. However, in the study by Puff & al. (1984), *Nematostylis* was found to be significantly distinct from *Alberta* in a number of morphological characters (summarized in Table 2) and best maintained as a separate genus. The authors concluded that even though *Nematostylis* shares a number of characters with the Malagasy species of *Alberta*, and although the type, *A. magna*, was found to have significant differences to the Malagasy species, *Nematostylis* was still considered the more distantly related. In relation to the phylogenetic hypothesis presented in this paper (i.e., *A. magna* as sister group to the remaining tribe), this suggests that *Nematostylis* is morphologically derived. It should be noted that in the resulting phylograms of most analysed cpDNA markers, of the ITS region, and consequently in the phylogram of the combined data (Fig. 2), the branch length of the *Nematostylis* clade is exceptionally long, indicating a high rate of molecular substitutions.

Morphological characters distinguishing *Nematostylis* from *Alberta* include amphistomatic, semisucculent and deciduous leaves with a single vascular bundle in the petiole (as opposed to hypostomatic, leathery, evergreen leaves with three vascular bundles in the petiole), a flat-topped inflorescence of compound scorpioid cymes (as opposed to a panicle-like cyme, or a terminated inflorescence of a few pairs of cymose partial inflorescences, as in *A. magna* and the Malagasy *Alberta*, respectively), a tubular, glabrous and bicoloured corolla (as opposed to a funnel-shaped, pubescent and uniformly coloured corolla), and glabrous anthers (Puff & al., 1984). We consider *Nematostylis* a distinct genus; to sink it into *Alberta* would make the latter a morphologically heterogeneous genus. We consequently propose a new genus, *Razafimandimbisonia* K. Kainul. & B. Bremer, to accommodate the Malagasy species of *Alberta* (see synopsis).

Synapomorphies for the Malagasy *Alberta* clade include: inflorescence type (as mentioned), dehiscent fruits, anthers without basal appendages, and the number of calycophylls, being five in all species except *A. sambiranensis*, which like *Nematostylis* only has one. The two species *A. orientalis* and *A. regalis* that are missing in this study, conform to these characters, and can therefore be considered part of the Malagasy *Alberta* clade. Synapomorphies distinguishing *Nematostylis* and the Malagasy *Alberta* clade from *A. magna* include, number of calycophylls (one or all five, as opposed to two in *A. magna*), onset of calyx lobe expansion (before anthesis, as opposed to after as in *A. magna*) and stigma lobe shape (broad and rounded, as opposed to awl-shaped as in *A. magna*; Puff & al., 1984). Further, Puff & al. (1984) argued that the (robust) flowers of *A. magna* are bird pollinated, whereas those of *Nematostylis* and the Malagasy *Alberta* are presumably pollinated by butterflies

(cf. Fig. 11). It should be noted however, that on a label of a specimen of *A. sambiranensis*—*Schatz 2723* (MO), the flowers are described as “visited by sunbird”. The monophyletic Malagasy clade of *Nematostylis* and the Malagasy *Alberta*, indicate a single introduction of an Alberteae ancestor to Madagascar, followed by a subsequent diversification, which can be considered moderate in comparison to the radiation in other groups of Rubiaceae that have colonized Madagascar (see Malcomber, 2002; Alejandro & al., 2005). Alternatively, Madagascar is the ancestral area, from which dispersal to Southeast Africa has occurred.

The incongruence between the cpDNA and ITS data within the Malagasy *Alberta* is somewhat surprising (i.e., *A. minor* as sister to a clade consisting of *A. sambiranensis*, *A. humblotii* and *Alberta* sp. in the cpDNA marker trees, as opposed to the morphologically more aberrant *A. sambiranensis* being sister to the rest of the group, as is the case in the ITS trees). This discrepancy could be due to the short internal branches, or as a result of hybridization/introgression (Wendel & Doyle, 1998). Further, the specimens termed *Alberta* sp. (Fig. 1D, E) represent two collections from the Andringitra National Park that key out to *A. minor* as defined by Puff & al. (1984), but are nested within the *A. humblotii*–*A. sambiranensis* clade in the cpDNA dataset trees, as well as in the combined data tree (Fig. 2). Clearly the species delimitation within the Malagasy *Alberta* needs further study.

Tribal relationships in Ixoroideae — The clade of Alberteae and the Gardenieae alliance is sister group to a clade consisting of the genera *Augusta* and *Wendlandia*, two genera previously included in Rondeletieae (Cinchoideae) by Robbrecht (1988). This clade and its position in Ixoroideae, were first shown by Rova & al. (2002). The two genera have a disjunct distribution, with *Augusta* occurring in South and Central America and in the Pacific, while *Wendlandia* is distributed from northern Africa, across the subtropical and tropical Asia to northern Australia. Morphologically the two genera are also seemingly far apart; synapomorphies for the clade need further study.

Scyphiphora has been a taxonomically problematic genus (Puff & Rohrhofer, 1993). The genus is monotypic and the only genus of the Rubiaceae restricted to mangroves, and has a number of unusual characters such as biovulate ovaries and fruits adapted to sea dispersal (Puff & Rohrhofer, 1993). In this study *Scyphiphora* is strongly supported as sister to a Ixoreae-Vanguerieae clade, further confirming the results of Bremer & al. (1999) and the conclusion of Mouly & al. (2009) to exclude *Scyphiphora* from Ixoreae, where it was tentatively placed by Andreasen & Bremer (2000).

The genus *Steenisia*, previously classified in Rondeletieae (Bremekamp, 1952; Bremer, 1984; Robbrecht, 1988), is in our analyses nested within the Ixoroideae (Fig. 2), forming a strongly supported sister group to a clade

Table 2. Overview of distinguishing morphological characters within Alberteae (see Puff & al., 1984).

	<i>Alberta</i>	<i>Nematostylis</i>	<i>Razafimandimbsonia</i>
Habit	Monopodial shrubs or trees	Sympodial-dichasial semisucculent shrub	Monopodial shrubs or trees
Leaves	Leathery	Semisucculent	Leathery
	Hypostomatic	Amphistomatic	Hypostomatic
	Persistent	Deciduous	Persistent
	Venation not very distinct	Venation indistinct	Venation ±prominent
Petioles	3 vascular bundles	1 vascular bundle	3 vascular bundles
Inflorescences	Panicle-like cyme	Flat-topped, compound (scorpioid) cymes	Few pairs of cymose partial inflorescences
	With terminal flower	Occasional terminal flower	No terminal flower
	With transitional leaves	No transitional leaves	No transitional leaves
Calycophylls	2	1	1 or 5
	Expanding after anthesis	Expanding before anthesis	Expanding before anthesis
Corollas	Infundibular	Tubular	Infundibular
	Externally pubescent	Externally glabrous	Externally pubescent
	Colour uniform	Bicoloured	Colour uniform
Anthers	Pubescent	Glabrous	Pubescent
	With basal appendages	With basal appendages	No basal appendages
Stigma lobes	Awl-shaped	Broad, rounded	Broad, rounded
Fruits	Indehiscent	Indehiscent	Dehiscent

consisting of the Gardenieae alliance and the tribes *Albertainae*, *Ixoreae*, *Vanguerieae* and *Retiniphyllaeae*. *Steenisia* does have contorted corolla lobe aestivation, one of the characters used by Robbrecht (1988) in his classification of *Ixoroideae*.

The basal nodes within the *Ixoroideae* s.l. (i.e., *Condamineae* s.l., *Mussaendeae*, *Posoquerieae*, *Sabiceae*, *Sipaneae*) are in this study fully resolved and strongly supported (Fig. 2). *Sipaneae* and *Posoquerieae* form a sister group to the remaining subfamily, followed by a grade of, in turn, *Condamineae* s.l. and *Mussaendeae* + *Sabiceae*.

Conclusion. — The tribe *Albertainae* is nested within the subfamily *Ixoroideae* as sister to a clade consisting of the tribes *Bertiareae*, *Coffeae*, *Cremasporae*, *Gardenieae*, *Octotropideae* and *Pavetteae*, making the ‘*Vanguerieae* alliance’ of Robbrecht & Manen (2006) an unnatural group. *Albertainae* as defined by Verdcourt (1958) and Puff & al. (1984), consisting solely of *Alberta* and *Nematostylis*, is supported by our data. However, *Nematostylis* is nested within *Alberta* as presently circumscribed, and as a consequence we describe a new genus *Razafimandimbisonia*, to accommodate the Malagasy species of *Alberta*. Neither *Airosperma*, *Aulacocalyx*, *Boholia* nor *Crossopteryx* is associated with *Albertainae*.

SYNOPSIS

Alberta as presently circumscribed does not appear to be monophyletic. To accommodate the Malagasy species of *Alberta* we propose the new genus *Razafimandimbisonia*, in honour of the Malagasy botanist Sylvain Razafimandimbison for his many contributions to the knowledge of Rubiaceae. An overview of the distinguishing characters within the tribe *Albertainae* is given in Table 2.

Key to the genera of *Albertainae*

1. Leaves semisucculent, deciduous. Corolla tubular, bicoloured *Nematostylis*
1. Leaves leathery, persistent. Corolla funnel-shaped, monochromatic 2
2. Two calyx lobes expanded (after anthesis), fruits indehiscent, stigma lobes awl-shaped *Alberta*
2. One or all five calyx lobes expanded (before anthesis), fruits dehiscing into two mericarps, stigma lobes rounded *Razafimandimbisonia*

Alberta E. Mey. in *Linnaea* 12: 258. 1838 – Type: *Alberta magna* E. Mey. ≡ *Ernestimeyera* Kuntze in Post & Kuntze, *Lex. Gen. Phan.*: 205. 1903, nom. illeg. superfl.

Shrubs to small trees. Leaves leathery, elliptic-obovate. Lateral veins not very prominent. Inflorescence a panicle-like cyme with stout axes and peduncles. Flowers 5-merous, zygomorphic. Calyx lobes persistent, unequal,

with two lobes being enlarged after anthesis. Corolla red, curved and funnel-shaped, with contorted corolla lobes. Style long-exserted, stigma lobes short and awl shaped. Fruits indehiscent.

Number of species: One species in Southeast Africa.

Nematostylis Hook. f. in Bentham & Hooker, *Gen. Pl.* 2: 110. 1873 – Type: *Nematostylis anthophylla* (A. Rich. ex DC.) Baill.

Shrubs up to 3 m, with stems that are semisucculent and often dichasially branched. Leaves semisucculent, amphistomatic, with indistinct venation and a single vascular bundle in the petiole. Inflorescence flat-topped, of compound scorpioid cymes, rarely with a terminal flower. Flowers 5-merous, slightly zygomorphic. Calyx lobes persistent, unequal, with one lobe being enlarged before anthesis. Corolla tubular, ± curved, bicoloured, lobes contorted in bud. Style (long) exserted. Stigma lobes short and rounded. Anthers glabrous. Fruits indehiscent.

Number of species: One species in Madagascar is currently recognized, but the genus is in need of a revision.

Razafimandimbisonia Kainul. & B. Bremer, **gen. nov.** – Type: *Razafimandimbisonia minor* (Baill.) Kainul. & B. Bremer, **comb. nov.** ≡ *Alberta minor* Baill. in *Adansonia* 12: 247. 1879 – Type: interior of Madagascar, 1834, *W. Bojer s.n.* (HT: P!; IT: BM, K!, image seen).

Frutices vel arbores. Folia petiolata coriacea, venis plus minusve prominentibus. Inflorescentia determinata absque flore terminali, inflorescentiis partialibus cymis decussatis paucis paribus. Flores 5-meri plus minusve zygomorphi. Calyx lobis plus minusve aequalibus aut inaequalibus, ante anthesin totis expansis aut lobo uno petiolato expanso (*R. sambiranensis*). Corolla infundibularis plus minusve curva, lobis in alabastrio contortis. Stylus longi-exsertus. Stigma lobis brevibus rotundatis. Fructus in duo mericarpiis dehiscens.

Shrubs or trees. Leaves petiolate, leathery, veins ± prominent. Inflorescence determinate, lacking terminal flower; consisting of a few pairs of partial inflorescences of decussate cymes. Flowers 5-merous, ± zygomorphic. Calyx lobes ± equal or unequal, all expanded, or one lobe petiolate and expanded (*R. sambiranensis*), before anthesis. Corolla funnel-shaped, ± curved, lobes contorted in bud. Style long-exserted. Stigma lobes short and rounded. Fruits dehiscing into two mericarps.

Number of species: Five species in Madagascar. For distributions and a key to the species, see Puff & al. (1984).

Diagnostic characters: *Razafimandimbisonia* differs from *Alberta* in having rounded stigma lobes, dehiscent fruits, reduced inflorescences, anthers lacking basal appendages, as well as in the number of enlarged calyx lobes and in the onset of lobe-expansion.

Further new combinations

Razafimandimbisonia humblotii (Drake) Kainul. & B. Bremer, **comb. nov.** \equiv *Alberta humblotii* Drake in Grandidier, Hist. Phys. Madagascar 36: 439. 1897 – Type: Madagascar, *L. Humblot 554* (HT: P!).

Razafimandimbisonia orientalis (Homolle ex Cavaco) Kainul. & B. Bremer, **comb. nov.** \equiv *Alberta orientalis* Homolle ex Cavaco in Adansonia, n.s., 5: 517. 1965 – Type: Madagascar, Mt. Vatovavy, near Mananjary river, 1911, *H. Perrier de la Bâthie 3910* (HT: P!).

Razafimandimbisonia regalis (Puff & Robbr.) Kainul. & B. Bremer, **comb. nov.** \equiv *Alberta regalis* Puff & Robbr. in Bull. Jard. Bot. Natl. Belg. 54: 344. 1984 – Type: Madagascar, Manampanihy river basin, Mt. Vohimavo, 1947, *H. Humbert 20707* (HT: P!).

Razafimandimbisonia sambiranensis (Homolle ex Cavaco) Kainul. & B. Bremer, **comb. nov.** \equiv *Alberta sambiranensis* Homolle ex Cavaco in Adansonia, n.s., 2: 518. 1965 – Type: Madagascar, 1909, *H. Perrier de la Bâthie 3834* (HT: P!).

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Appendix. Sequences used, with EMBL/GenBank accession numbers. Voucher information is given for previously unpublished sequences (indicated with *). A dash indicates missing data.

Species, voucher specimen (herbarium), accession no.: *rbcL*, *ndhF*, *trnS-G*, *trnT-F*, *rps16*, *trnH-psbA*, ITS

Airosperma vanuense S.P. Darwin, *A.C. Smith 8214* (P), –, AM949845*, FM204740*, FM207108*, FM204700*, AM939386*, –, *Alberta* cf. *humblotii*, *Eriksson & al. 1027* (S), –, FM204751*, FM207117*, FM204709*, AM939398*, FM204686*, *Alberta humblotii* Drake, *Rakoto 306* (UPS), –, –, FM207109*, –, AM939387*, FM204676*, *Alberta magna* E. Mey. (1), *Tonkin 200* (UPS), Y18708¹, AJ236282², FM204741*, AJ620118³, FM204701*, AM939388*, AJ224842⁴; *Alberta magna* E. Mey. (2), *Bremer & Bremer 3773* (UPS), –, FM204742*, FM207110*, FM204702*, AM939389*, FM204677*, *Alberta minor* Baill. (1), *Razafimandimbison 558* (UPS), EU817410⁴, AM949844*, FM204743*, EU817452⁴, EF205637⁵, AM939390*, FM204678*, *Alberta minor* Baill. (2), *Razafimandimbison & al. 634* (S), –, FM204746*, FM207113*, FM204705*, AM939393*, FM204681*, *Alberta minor* Baill. (3), *Eriksson & al. 989* (S), –, FM204745*, FM207112*, FM204704*, AM939392*, FM204680*, *Alberta sambiranensis* Homolle ex Cavaco (1), *Ranaivojoana 214* (MO, TAN), –, FM204748*, FM207114*, FM204706*, AM939395*, FM204684*, *Alberta sambiranensis* Homolle ex Cavaco (2), *Razafimandimbison & Ravelonarivo 360* (MO, TAN), EU817431⁴, –, FM204747*, EU817474⁴, EF205645⁵, AM939394*, FM204683*, *Alberta sambiranensis* Homolle ex Cavaco (3), *Razafimandimbison & Ravelonarivo 618* (S), –, FM204750*, FM207116*, FM204708*, AM939397*, FM204685*, *Alberta* sp. (1), *Eriksson & al. 928* (S), –, FM204749*, FM207115*, FM204707*, AM939396*, FM204682*, *Alberta* sp. (2), *Eriksson & al. 904* (S), –, FM204744*, FM207111*, FM204703*, AM939391*, FM204679*, *Augusta rivalis* (Benth.) J.H. Kirkbr., *McDowell 5017* (ETSU), AM949842*, AM949846*, FM204752*, FM207118*, –, AM939399*, FM204687*, *Aulacocalyx jasminiflora* Hook. f., *Schmidt & al. 1672* (MO), EU817413⁴, –, FM204753*, EU817455⁴, EF205639⁵, AM939400*, FM204688*, *Bertiera guianensis* Aubl., *Andersson & al. 2029* (GB), AJ224845¹, AM949847*, FM204754*, FM207119* & AF152670⁶, FM204710*, AM939401*, AJ224841¹, *Boholia nematostylis* Merr., *Bicknell 1561A* (SUNIV), AM117210⁷, AM949848*, FM204755*, AM117350⁷, FM204711*, AM939402*, –, *Calyculophyllum candidissimum* (Vahl) DC., *Sanders 1805* (FTG), X83627⁸, AJ236285², FM204756*, AJ847398⁹, FM204712*, –, *Canthium tetraphyllum* (Schweinf. ex Hiern) Robyns, *Bremer 3074* (UPS), X83649⁸, AM949849*, FM204757*, FM207120*, FM204713*, AM939403*, AJ315083¹⁰, *Cinchona pubescens* Vahl., *Bremer 2733* (UPS), X83630⁸, AJ235843¹¹, FM204758*, AJ346963¹², FM204714*, –, *Coffea arabica* L., EF044213¹³, EF044213¹³, EF044213¹³, EF044213¹³, EF044213¹³, DQ153609¹⁴, *Cremastra triflora* subsp. *confluens* (K. Schum.) Verdc., *Andreasen 51* (UPS), Z68856¹⁵, AM949850*, FM204759*, FM207121*, FM204715*, AM939404*, AJ224824¹, *Crossopteryx febrifuga* (Afzel. ex G. Don) Benth. (1), *Bremer 3097* (UPS), AM117223⁷, AM949851*, FM204760*, FM207123*, FM204717*, AM939406*, FM204690*, *Crossopteryx febrifuga* (Afzel. ex G. Don) Benth. (2), *Razafimandimbison 298* (LBV, MO, P, TAN), AJ347009¹², –, FM207122*, FM204716*, AM939405*, FM204689*, *Cubanola domingensis* (Britton) Aiello, *McDowell 4427* (DUKE), X83632⁸, AM117345⁷, FM204761*, FM207124*, FM204718*, –, *Emmenopteryx henryi* Oliv., *Robbrecht s.n.* (UPS), Y18715², AJ236294², FM204762*, FM207125*, FM204719*, –, *Gardenia hansemanii* K. Schum., *Droz & Molem s.n.* 1998–11–13, AJ318446¹⁶, AM949852*, FM204763*, FM204763*, FM207126*, FM204720*, AM939407*, FM204691*, *Hypobathrum racemosum* (Roxb.) Kurz, *Ridsdale 81*, AJ286705¹⁷, AM949853*, –, FM207127*, AM117318⁷, AM939408*, –, *Ixora coccinea* L., *Bremer 2719* (UPS), X83646⁸, AJ236299², FM204764*, EU817464⁴, EF205641⁵, AM939409*, AJ224826¹, *Luculia gratissima* (Wall.) Sweet, cult. in Univ. Conn. Storrs 870064 (no voucher), AM117243⁷, AJ011987¹⁸, FM204765*, AJ430911¹⁹, AJ431036¹⁹, –, *Morinda citrifolia* L., *Droz & Molem s.n.* 1998–11–13, X83651⁸, AJ236300², FM204766*, FM207589*, AF152616⁶, AJ320078¹⁶, –, *Mussaenda arcuata* Poir., *McPherson 16213* (MO), Y11854²⁰, AJ236301², FM204767*, FM207128*, FM204721*, –, *Nematostylis anthophylla* (A. Rich. ex DC.) Baill. (1), *Lantz 160* (UPS), FM207648*, FM207650*, FM204769*, FM207130*, FM204722*, AM939411*, FM204693*, *Nematostylis anthophylla* (A. Rich. ex DC.) Baill. (2), *Eriksson & al. 977* (S), –, FM204768*, FM207129*, FM204722*, AM939410*, FM204692*, *Nematostylis anthophylla* (A. Rich. ex DC.) Baill. (3), *Anderberg & al. 75* (S), –, FM204770*, FM207131*, FM204724*, AM939412*, FM204694*, *Nematostylis* sp. nov., *Razafimandimbison & Ravelonarivo 616* (S), –, FM204771*, FM207132*, FM204725*, AM939413*, FM204695*, *Pavetta abyssinica* Fresen., *De Block 6* (BR), Z68863¹⁵, AM949854*, FM204772*, FM207133*, FM204726*, AM939414*, FM204696*, *Pentagonia macrophylla* Benth., *McDade 595A* (DUKE), X83658⁸, AJ236303², FM204773*, FM207134*, FM204727*, –, *Posoqueria latifolia* (Rudge) Roem. & Schult., *SU-C-88.10* Bergius Bot. Garden, Z68850¹⁵, AM949855*, FM204774*, FM207135*, FM204728*, –, *Pseudomussaenda flava* Verdc., *Nissen s.n.* (UPS), Y11855²⁰, AM949856*, FM204775*, FM207136*, FM204729*, –, *Retiniphyllum pilosum* (Spruce ex Benth.) Müll. Arg., *Wurdack & Adderley 43270* (S), AF331654²¹, –, FM204776*, FM207137*, FM204730*, –, *Rustia thibaudioides* (H. Karst.) Delprete, *Delprete 6378* (UPS), Y18716², AJ236310², FM204777*, FM207138*, FM204731*, –, *Sabicea villosa* Roem. & Schult., *Delprete 6396* (LL, TEX, UPS), Y11858²⁰, AM949857*, FM204778*, FM207139*, FM204732*, –, *Scyphiphora hydrophyllacea* C.F. Gaertn., *K. Bremer & al. 99* (S), Y18717², AJ236311², FM204779*, FM207140*, FM204733*, –, *Sipanea biflora* (L.f.) Cham. & Schltdl., *Rova & al. 2005* (GB, S), AY538509²², EU145413²³, FM204780*, FM207141*, FM204734*, –, *Steenisia pleurocarpa* (Airy Shaw) Bakh. f., *Puff BF 990619-1/4* (WU), AM117279⁷, –, FM204781*, FM207142*, FM204735*, –, FM204697*, *Tamridaea capsulifera* (Balf. f.) Thulin & B. Bremer, *Thulin & Gifri 8663* (Aden Univ., E, K, UPS), Y11860²⁰, –, FM204781*, FM207143*, FM204736*, –, *Tenantia semii* (Chiov.) Verdc. & Bridson, *Abdalla & al. 96/166* (P), AM949843*, AM949858*, FM204783*, FM207144*, FM204737*, AM939415*, –, *Tocoyena pittieri* (Standl.) Standl., *Rova & al. 2369* (GB), –, AM949859*, FM204784*, FM207145*, FM204738*, AM939416*, FM204698*, *Vangueria madagascariensis* J.F. Gmel., *Sanders 1798* (FTG), X83670⁸, AJ130840², FM204785*, FM207146*, –, AM939417*, –, *Wendlandia tinctoria* (Roxb.) DC., *Parker 3227* (S), FM207649*, AM949860*, FM204786*, FM207147*, FM204739*, AM939418*, FM204699*.

Published sequences: 1, Andreasen & al. (1999); 2, Bremer & al. (1999); 3, Lantz & Bremer (2004); 4, Mouly & al. (2009); 5, Mouly & al. (2007); 6, Rova & al. (2002); 7, Bremer & Eriksson (2009); 8, Bremer & al. (1995); 9, Alejandro & al. (2005); 10, Lantz & al. (2002); 11, Backlund & al. (2000); 12, Razafimandimbison & Bremer (2002); 13, Samson & al. (2007); 14, Maurin & 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).