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, Coffeaeae, Condamineae, Cremasporeae, Ixoreae, Gardenieae, Mussaendaeae, Octotropideae, Paveteae, Posoquerieae, Retiniphylleae, Sabiceaeae, Sipaneaeae 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 (1981), have also been moved to Octotropideae (Robbrecht, 1980; as Hypobathreae), as well as Rhabdostigma syn. Kraussia Harv. (Andreasen & Bremer,
A monogeneric Cremasporeae 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 Vangueriaeae, with a close relationship with Cyclophyllym 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 Ixoroi-deae. Robbrecht (1988), however, placed Alberteae in Antirheiroidea, 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 antithaphylla (A. Rich. ex DC.) Baill.) formed a clade in a corresponding albeit weakly supported position. Recently, Robbrecht & Manen (2006) placed Alberteae in a “Vangueriae alliance”, and included in this group also the genera Aulacocalyx and Crossopteryx Fenzl. In Razafimandibison & Bremer (2001), Crossopteryx was shown to be nested within Ixoroi-deae, 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, Boholía 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 Ixoroi-deae as defined by Bremer (2009) were included (Bertiereae, Bertiera guianensis Aubl.; Coffeacea, Coffea arabica L.; Condamineae s.l., Calycophyllum candidissimum (Vahl) DC., Enmenopterys henryi Oliv., Pentagonia macrophylla Benth., Rustia thibaudioides (H. Karst.) Delprete; Cremasporeae, Cremaspora triflora subsp. confluenus (K. Schum.) Verdc.; Gardenieaeae, Gardenia hansemannii K. Schum., Tocoyna pittieri (Standl.) Standli.; Ixoreaeae, Ixora cocinea L.; Mussaendaeeae, Mussaenda arcuata Poir., Pseudomussaenda flav Verdc.; Octotropi-deaeae, Hypobathrum racemosum (Roxb.) Kurz; Pavetteaeae, Pavetta abyssinica Fresen., Tennantia sennii (Giov.) Verdc. & Bridson; Podoqueriaeeae, Podoqueria latifolia (Rudge) Roem. & Schult.; Retiniphylleaeeae, Retiniphyllyea pilosum (Spruce ex Benth.) Müll.Arg.; Sabiceaeaeae, Sabicea villosa Roem. & Schult., Tamridaea capsules (Balf. f.) Thulin & B. Bremer; Sipaneeaeaeae, Sipanea biflora (L. f.) Cham. & Schldl.; Vangueriaeaeaeae, Canthium tetrathyllum (Schweinf. ex Hiern) Robyns, Vangueria madagascariensis J.F. Gmel.), as well as a number of taxa whose phyloge-netic 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. humblotti 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), trnF-F (Razafimandibison & 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).
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 et al., 2002) and rpsR2 (Oxelman et al., 1997); trnT-L, trnT-F, alF and trnT-F-iR (Razafimandimbison & Bremer, 2002); trnL-F, c, d, e and f (Taberlet et al., 1991); and ITS, P17 (Liden et al., 1995), P16, P25 and 26S-82R (Popp & Oxelman, 2001). The trnS-G 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 reinserted, 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 preliminary 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>
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</table>
$2 \times 10^6$ generations ($5 \times 10^6$ 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) *Musanga* + *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 Sipaneae, but grouped unresolved with the remainder of Ixoridaceae 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, Coffeeeae, Cremasporeae, Gardenieae, Octotropideae and Pavetteae (hereafter called the Gardenieae alliance). Nested within this group was also *Aulacocalyx*.

Resolved as a sister group to the Alberteae-Garde- nieae alliance clade was a clade formed by *Augusta* Pohl and *Wendlandia* Bartl. ex DC. A strongly supported clade consisting of *Atrosperma* 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 *Musanga* grouped with Sabiceae. The tribe Condaminaceae s.l. and a clade formed by the tribes Sipaneae 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 Ixoroi-deae indicated as: ALB = Alberteae, BER = Bertiereae, COF = Coffeeae, CON = Condamineae s.l., CRE = Cremasporeae, CRO = Crossopterygeae, I XO = Ixoreae, GAR = Gardenieae, MUS = Mussaendeae, OCT = Octotropideae, PAV = Pavetteae, POS = Posoquerieae, RET = Retiniphylleae, 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%.
Bertiereae, Coffeaceae, Cremasporaceae, Gardenieae, Octotropieae and Pavetteae, as in accordance with Bremer & al. (1999) and Moulty & 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 zygomorphic flowers and 1–5 expanded and brightly coloured calyx lobes (calycophyls), 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 (Robbrech, 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 scorpionid 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. sambrarenensis, 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.
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(cf. Fig. 1I). 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/introggression (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 (Cincho- noideae) 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).

<table>
<thead>
<tr>
<th>Alberta</th>
<th>Nematostylis</th>
<th>Razafimandimbisonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habit</td>
<td>Monopodial shrubs or trees</td>
<td>Sympodial-dichasial semisucent shrub</td>
</tr>
<tr>
<td>Leaves</td>
<td>Leathery</td>
<td>Semisucent</td>
</tr>
<tr>
<td>Hypostomatic</td>
<td>Persistent</td>
<td>Amphistomatic</td>
</tr>
<tr>
<td>Persistent</td>
<td>Venation not very distinct</td>
<td>Venation indistinct</td>
</tr>
<tr>
<td>Petioles</td>
<td>3 vascular bundles</td>
<td>1 vascular bundle</td>
</tr>
<tr>
<td>Inflorescences</td>
<td>Panicle-like cyme</td>
<td>Flat-topped, compound (scorpoid) cymes</td>
</tr>
<tr>
<td>With terminal flower</td>
<td>Occasional terminal flower</td>
<td>No terminal flower</td>
</tr>
<tr>
<td>With transitional leaves</td>
<td>No transitional leaves</td>
<td>No transitional leaves</td>
</tr>
<tr>
<td>Calycophylls</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Expanding after anthesis</td>
<td>Expanding before anthesis</td>
<td>Expanding before anthesis</td>
</tr>
<tr>
<td>Corollas</td>
<td>Infundibular</td>
<td>Tubular</td>
</tr>
<tr>
<td>Externally pubescent</td>
<td>Externally glabrous</td>
<td>Externally pubescent</td>
</tr>
<tr>
<td>Colour uniform</td>
<td>Bicoloured</td>
<td>Colour uniform</td>
</tr>
<tr>
<td>Anthers</td>
<td>Pubescent</td>
<td>Glabrous</td>
</tr>
<tr>
<td>With basal appendages</td>
<td>With basal appendages</td>
<td>No basal appendages</td>
</tr>
<tr>
<td>Stigma lobes</td>
<td>Awl-shaped</td>
<td>Broad, rounded</td>
</tr>
<tr>
<td>Fruits</td>
<td>Indehiscent</td>
<td>Indehiscent</td>
</tr>
</tbody>
</table>
consisting of the Gardenieae alliance and the tribes Alberteae, Ixoreae, Vanguerieae and Retiniphylleae. 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., Condamineeae s.l., Mussaendeae, Posoquerieae, Sabiceeae, Sipaneeae) are in this study fully resolved and strongly supported (Fig. 2). Sipaneeae and Posoquerieae form a sister group to the remaining subfamily, followed by a grade of, in turn, Condamineeae s.l. and Mussaendeae + Sabiceeae.

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

### SYNOPSIS

Alberto as presently circumscribed does not appear to be monophyletic. To accommodate the Malagasy species of Alberto 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 Alberteae is given in Table 2.

#### Key to the genera of Alberteae

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


Number of species: One species in Madagascar.


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


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LITERATURE CITED


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, trnF-psbA, ITS
