Sabiceeae and Virectarieae (Rubiaceae, Ixoroideae): one or two tribes? New tribal and generic circumscriptions of Sabiceeae and biogeography of *Sabicea* s.l.

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The results of two recent phylogenetic studies led to the reinstatement of the tribe Sabiceeae, currently classified in the subfamily Ixoroideae s.l. (Rubiaceae) but with conflicting circumscriptions. In the present study, phylogenetic analyses based on nrITS and *trnT-F* sequence data of 78 taxa are performed to evaluate the different circumscriptions of Sabiceeae, the generic limits within Sabiceeae, and the biogeography of *Sabicea*. The polyphyly of Sabiceeae sensu Andersson is confirmed, and *Pentaloncha* and *Temnopteryx* are shown not to belong to Ixoroideae s.l. but to the subfamily Rubioideae. Our results favour a broad circumscription of Sabiceeae that includes *Ecpoma*, *Hekistocarpa*, *Pseudosabicea*, *Sabicea*, *Schizostigma*, *Stipularia*, *Tamridaea* and *Virectaria*. *Sabicea* sensu Wernham is not monophyletic unless *Ecpoma*, *Pseudosabicea*, *Schizostigma*, and *Stipularia* are included. We find no support for the monophyly of *Stipularia*, *Sabicea* and *Pseudosabicea*. Therefore, our newly circumscribed Sabiceeae contains only *Hekistocarpa*, *Sabicea* s.l. (*Ecpoma*, *Pseudosabicea*, *Schizostigma*, *Stipularia*), *Tamridaea*, and *Virectaria*. Finally, our analyses indicate several dispersal events of *Sabicea* species between African phytogeographical regions and continental African origins of the Malagasy, São Tomean, Asian, and Neotropical species of *Sabicea* via perhaps four independent dispersal events.

KEYWORDS: biogeography, nrITS, Rubiaceae, *Sabicea*, Sabiceeae, *trnT-F*, Virectarieae

INTRODUCTION

Grisebach (1861) originally described the pantropical subtribe Sabiceinae (as "Sabicieae") of the tribe Cinchoneae in the subfamily Cinchonoideae (Rubiaceae) to accommodate two genera, *Sabicea* Aubl. and *Coccocypselum* P. Br., both with valvate corolla aestivation. Bremekamp (1934) established a monogeneric tribe Sabiceeae Bremek. (as "Sabiceae"), but no other rubiaceous taxonomists (except Bremekamp, 1966) accepted its tribal status between 1934 and 1996 (see Table 1). The type genus *Sabicea* was classified in the tribes Mussaendeae Benth. & Hook. f. (Verdcourt, 1958; Hallé, 1961; Hallé, 1966; Steyermark, 1962, 1972, 1974) or Isertieae A. Rich. ex DC. (Kirkbride, 1979, 1982; Robbrecht, 1988, 1993). For tropical Africa Hallé (1961) classified *Sabicea* and its four traditionally associated genera—*Ecpoma* K. Schum. (Schumann, 1896), *Pentaloncha* Hook. f. (Hooker, 1873a), *Stipularia* P. Beauv. (Palisot-Beauvois, 1807), and *Temnopteryx* Hook. f. (Hooker, 1873a)—in Mussaendeae. Hallé (1966) placed *Ecpoma* and *Pseudosabicea* and *Pentaloncha*, respectively, in his new subtribes Mussandenae and Urophyllinae of Mussaendeae. Steyermark (1962) classified the Neotropical *Pittierothamnus* Steyerm. in Mussaendeae s.l. but later merged it with *Amphidasya* Standl., also endorsed by Kirkbride (1979, 1982) and Robbrecht (1988). Bremekamp (1966) made the last attempt to re-establish Sabiceeae based on simple stipules, axillary inflorescences, and very narrow testa cells rather than bifid stipules, terminal inflorescences, and large testa cells of Mussaendeae. It is notable that some authors, mentioned above, used the tribal name Mussaendeae, although Isertiae had priority over Mussaendeae, because the tribe Mussaendeae contained the type genus (*Isertia* Schreb.) of Isertiae (Darwin, 1976). Robbrecht (1988) transferred to Isertieae the Indo-Malesian genus *Acranthera* Arn. ex Meisn. (Meisner, 1838), previously placed by Bremekamp (1966) in its own tribe, and all above genera traditionally associated with Mussaendeae plus *Schizostigma* Arn. ex Meisn., with the exception of *Pentaloncha*, which was left unclassified in Rubiaceae (see Table 1).

Sabiceeae was resurrected as a result of the morphological-based phylogeny of Isertieae sensu Robbrecht (1988) conducted by Andersson (1996). *Stipularia* was deeply nested within Sabiceeae (Andersson 1996: Fig. 5) but was not among the nine genera that he included in his Sabiceeae (Table 1). Based on a *rbcL* phylogeny Bremer & Thulin (1998) showed that Sabiceeae sensu Andersson (1996) was highly polyphyletic and additionally postulated that *Acranthera* might perhaps belong to the subfamily Rubioideae, consistent with Alejandro & al.'s (2005: Fig. 1) *trnT-F*-based phylogeny. Bremer & Thulin (1998) demonstrated for the first time that the broadly delimited Mussaendeae (sensu Hallé, 1961; Hallé, 1966) or Isertieeae (sensu Robbrecht, 1988) was also highly polyphyletic. As a result, they re-established Mussaendeae to accommodate *Mussaenda* and its satellite genera (*Aphaenandra* Miq., *Heinsia* DC., *Neomussaenda* C. Tange, *Pseudomussaenda* Wernham, *Schizomussaenda* H.L. Li) and restricted Isertieae to include the type genus *Isertia*. They further showed that the African genus *Virectaria* Bremek., previously placed by Verdcourt (1958) in its own tribe Virectarieae Verdc., is closely related to *Pseudosabicea* and *Sabicea*. Accordingly, they tentatively proposed a new circumscription of Sabiceeae, which included *Sabicea*, *Pseudosabicea*, the monotypic genus *Tamridaea* Thulin & B. Bremer, and *Virectaria*. They considered *Stipularia* to be closely related to *Pseudosabicea* and *Sabicea* based on morphological grounds. On the other hand, Dessein & al. (2001b: 22) considered *Virectaria* to be an isolated genus within Sabiceeae sensu Bremer & Thulin (1998) based on a few morphological characters (e.g., internal indument and seed anatomy). The *rbcL* jackknife tree of Dessein & al. (2001a) further confirmed the close relationships between *Tamridaea* and *Virectaria* and showed for the first time that the African monotypic genus *Hekistocarpa* Hook. f. (Hooker, 1873b) is closely related to these two genera. Dessein & al. (2001a: 75) additionally stressed that they "fail to find any morphological characteristics that are common to *Hekistocarpa*, *Pseudosabicea*, *Sabicea*, *Tamridaea*, and *Virectaria* of Sabiceeae in a broad sense." As a result, they resurrected the tribe Virectarieae to accommodate *Hekistocarpa*, *Tamridaea*, and *Virectaria* and restricted Sabiceeae to include only *Sabicea* and four of its traditionally allied genera (*Ecpoma*, *Pentaloncha*, *Pseudosabicea*, *Stipularia*). They admitted that their emended Virectarieae was difficult to diagnose morphologically. More recently, Robbrecht & Manen (2006) adopted another broader circumscription of Sabiceeae including eight genera and recognized two subtribes (Table 1): Sabiceinae (Bremek.) Robbr. & Manen and Virectariinae (Verdc.) Robbr. & Manen (= Virectarieae sensu Dessein & al., 2001a). The above conflicting circumscriptions of Sabiceeae drew our attention to further investigations.

Sabicea is the most species-rich genus of Sabiceeae with ca. 146 species of scandent shrubs, woody climbers, and scramblers or twiners. With two main centres of diversity, mainland Africa (ca. 82 species) and the Neotropics (ca. 54 species), *Sabicea* shows a trans-Atlantic

Table 1. Previous and new tribal positions of *Sabicea* and its traditionally and presently allied genera.

Genera	Bremekamp (1934)	Verdcourt (1958)	Hallé (1961)	Bremekamp (1966)	Hallé (1966)	Steyermark (1962)	Steyermark (1972)	Robbrecht (1988)	Andersson (1996)	Bremer & Thulin (1998)	Dessein & al. (2001a)	Robbrecht & Manen (2006)	This study
Acranthera Arn. ex Meisn.	–	–	–	Acr	–	–	–	Ise	Sab	Rubi	–	–	Rubi
Amphidasya Standl.	–	–	–	–	–	–	Mus	Ise	Sab	Rubi	–	Uro	Rubi
Ecpoma K. Schum.	–	–	Mus	–	Mus	–	–	Ise	Sab	–	Sab	SabS	Sab
Hekistocarpa Hook. f.	–	–	–	–	–	–	–	Hed	–	–	Vir	SabV	Sab
Pentaloncha Hook. f.	–	–	Mus	Pau	Mus	–	–	Ins	Sab	–	Sab	–	Rubi
Pittierothamnus Steyerm.	–	–	–	–	–	Mus	–	–	Sab	–	–	–	–
Pseudosabicea N. Hallé	–	–	–	–	Mus	–	–	Ise	Sab	Sab	Sab	SabS	Sab
Sabicea Aubl.	Sab	Mus	Mus	Sab	Mus	Mus	Mus	Ise	Sab	Sab	Sab	SabS	Sab
Schizostigma Arn. ex Meisn.	–	–	–	–	–	–	–	Ise	Sab	–	–	SabS	Sab
Stipularia P. Beauv.	–	–	Mus	–	Mus	–	–	Ise	–	Sab	Sab	SabS	Sab
Tamridaea Thulin & B. Bremer	–	–	–	–	–	–	–	–	–	Sab	Vir	SabV	Sab
Temnopteryx Hook. f.	–	–	Mus	Pau	Mus	–	–	Ise	Sab	–	–	–	Rubi
Virectaria Bremek.	–	Vir	–	Oph	Hed	–	–	Hed	–	Sab	Vir	SabV	Sab

Acr, tribe Acranthereae; Hed, Hedyotideae; Ins, Incertae sedis; Ise, Isertieae; Mus, Mussaendeae; Oph, Ophiorrhizeae; Pau, Pauridiantheae; Rubi, Rubioideae; Sab, Sabiceeae; SabS, Sabiceeae subtribe Sabiceinae; SabV, Sabiceeae subtribe Virectariinae; Vir, Virectarieae; Uro, Urophylleae s.l. (including Pauridiantheae); –, not mentioned.

distribution shared with few other Rubiaceae genera. Six species are endemic to Madagascar (Razafimandimbison & Miller, 1999), three to São Tomé and Príncipe (Joffroy, 2001), and one, *S. ceylanica* Puff. (Puff & al., 1998), originally described as *Schizostigma hirsutum* Arn. ex Meisn. (Meisner, 1838), to Sri Lanka. Aublet (1775) originally described *Sabicea* from South America including two species, *S. aspera* Aubl. and *S. cinerea* Aubl., with twining habits and 3–5-locular ovaries. Wernham (1914) proposed a broad circumscription of *Sabicea* including 105 species from Africa and South America with usually shrubby, climbing or prostrate to scrambling habits, isophylly or anisophylly, entire to fimbriate or laciniate stipules, axillary inflorescences, (sub-) free bracts, valvate corolla lobes, and (2)4–5-locular ovaries. Additionally, he recognized two subgenera in *Sabicea* based on the combination of habit and leaf and stipule sizes: *Sabicea* subgen. *Stipulariopsis* Wernham with 9 species and *Sabicea* subgen. *Eusabicea* Wernham with 96 species. Wernham (1914), endorsed by Hiern (1877), Hallé (1961), Hallé (1963, 1966), Andersson (1996), Bremer & Thulin (1998), and Dessein & al. (2001a), recognized the African *Stipularia* as a distinct genus because of its large stipules and well-developed campanulate involucral bracts completely surrounding the entire inflorescence (Palisot-Beauvois, 1807). On the other hand, Hepper's (1958) herbarium studies revealed that involucral bracts also occurred in few African *Sabicea* species (e.g., *S. capitellata* Benth, *S. dewevrei* De Wild. & T. Durand, *S. cordata* Hutch. & Dalziel, and *S. urceolata* Hepper) with variation in the degree of fusion. As a result, he merged the five described species of *Stipularia* (*S. africana* P. Beauv., *S. efulenensis* Hutch., *S. elliptica* Schweinf. ex Hiern, *S. gabonica* Hiern, and *S. mollis* Wernham) with *Sabicea*. Both Hallé (1961) and Hallé (1963, 1966) rejected Hepper's (1958) circumscription of *Sabicea* and reinstated *Stipularia* as a distinct genus. Plus, Hallé (1963) viewed *Sabicea* sensu Wernham (1914) as morphologically heterogeneous and accordingly restricted the genus to include only species with usually lianescent, slender and twining habits, long corollas, (4–)5-locular ovaries, accrescent fleshy axis of ovary, narrow, thin and sessile placentas, and fleshy juicy fruits with often-red carmine pulp. He then described the genus *Pseudosabicea* to accommodate all the African *Sabicea* species with creeping or climbing but non-twining habit, short corollas, 2(–3)-locular ovaries, non-fleshy axis of ovary, oblong, peltate and fleshy placentas, and scantly fleshy fruits with colourless pulp. In addition, Hallé (1963) transferred five African *Sabicea* species (*S. bicarpellata* K. Schum., *S. cauliflora* Hiern, *S. geantha* Hiern, *S. gigantostipula* K. Schum., *S. hierniana* Wernham) to the African genus *Ecpoma*.

Arnott (1839) viewed *Schizostigma* as closely related to *Sabicea* and more recently, Puff & al. (1998) merged *Schizostigma* in *Sabicea*, which they considered to be closely related to *Ecpoma*, *Pseudosabicea*, *Stipularia*, and *Temnopteryx*. Both Hallé (1961, 1966) and Puff & al. (1998) totally rejected Hiern's (1877) attempt to merge *Pentaloncha* and *Temnopteryx* with *Schizostigma*.

Although most Rubiaceae systematists seem to accept *Sabicea* sensu Hallé (1963, 1966), the monophyly of the above conflicting circumscriptions of *Sabicea* or its close allies have never been assessed before. Previous phylogenetic studies in some Rubiaceae groups based on the nrITS region of rDNA (e.g., Andreasen & al., 1999; Razafimandimbison & al., 2004; Motley & al., 2005) and the *trnT-F* region of chloroplast DNA (e.g., Razafimandimbison & Bremer, 2002; Alejandro & al., 2005) have demonstrated that both markers are useful for inferring phylogenetic relationships at tribal and generic levels in the family. The main objective of this study is to reconstruct phylogenies of *Sabicea* and its closely related genera using the sequence data from both the nuclear ribosomal internal transcribed spacer (nrITS1-5.8S-nrITS2 region) and the *trnT-F* regions of chloroplast DNA ($trnT_{\mathrm{UGU}}$-$trnL_{\mathrm{UAA}\,5'}$ exon, $trnL_{\mathrm{UAA}\,5'}$ exon-$trnL_{\mathrm{UAA}}$ intron, $trnL_{\mathrm{UAA}}$ intron-$trnL_{\mathrm{UAA}\,3'}$ exon, $trnL_{\mathrm{UAA}\,3'}$ exon-$trnF_{\mathrm{GAA}}$). The resulting phylogenies have been used to assess: *(1)* the conflicting circumscriptions of Sabiceeae, *(2)* the generic limits within Sabiceeae, and *(3)* the biogeography of *Sabicea*.

MATERIALS AND METHODS

Taxon selection. — A total of 36 species (38 individuals) belonging to *Sabicea* and 9 genera currently or traditionally associated with Sabiceeae and 19 genera presently placed in Cinchonoideae s.str., Ixoroideae s.l., and Rubioideae (Appendix) were included in the *trnT-F* analyses to assess the competing circumscriptions of Sabiceeae. Neither *Acranthera* nor *Amphidasya* were included in our analyses, as they have recently been shown to be related to Rubioideae (Bremer & Thulin 1998; Alejandro & al. 2005). No material was available for *Pittierothamnus*. The genus *Luculia* Sweet (*L. grandifolia* Ghose) was used as the outgroup taxon, in agreement with its basal position in Rubiaceae (Bremer & al., 1999; Rova & al., 2002). A total of 39 *Sabicea* species (40 individuals), 8 *Pseudosabicea* species (9 individuals), 2 species each of *Stipularia* and *Virectaria*, 1 *Ecpoma* species, and 1 individual each of the monotypic *Hekistocarpa*, *Schizostigma*, and *Tamridaea* were included in the the nrITS analyses and all of these accessions excluding *Tamridaea* were included in the combined nrITS + *trnT-F* analyses to assess the generic limits within Sabiceeae. One species each of *Heinsia* DC. (Mussaendeae sensu Bremer & Thulin, 1998), *Canthium* Lam. (Vanguerieae A. Rich. ex Dumort.), *Ixora* L. (Ixoreae sensu Andreasen & Bremer, 2000), and *Warszewiczia* Klotzsch (Condamineeae sensu Rova & al., 2002), all currently classified in Ixoroideae s.l., were selected to root the nrITS and combined analyses (see Appendix).

DNA isolation, amplification, and sequencing. — DNA isolation, amplification, and sequencing of the nrITS region were accomplished following the protocols described in Alejandro & al. (2005). The amplification and sequencing of the *trnT-F* region were performed following the protocols outlined in Razafimandimbison & Bremer (2002). For each 25 µL PCR reaction we added 15.8 µL dH_2O, 2 µL $MgCl_2$ (25 mM), 1.5 µL dNTP (2 mM), 1.0 µL each of forward (P17F, 5′-CTA CCG ATT GAA TGG TCC GGT GAA-3′) and reverse (26S-82R, 5′-TCC CGG TTC GCT CGC CGT TAC TA-3′) primers (10 pmol/µL), 2.5 µL PCR buffer (10×), 0.2 µL TAQ DNA polymerase, and 1.0 µL DNA sample.

Sequence alignment and coding of indels. — Forward and reverse sequences generated for both the nrITS and *trnT-F* regions were assembled using the Perkin Elmer Sequence Navigator, version 1.0.1 and Sequencher 3.1.1 and aligned with the CLUSTAL-W (Thompson & al., 1994) to obtain preliminary alignments, which were subsequently edited manually. We coded all informative indels using the simple gap coding method (Simmons & Ochoterena, 2000) and assessed their effects on the results.

Phylogenetic analyses. — Maximum parsimony analyses (hereafter MPA) of both the nrITS and nrITS + *trnT-F* data were performed with PAUP*, version 4.0b (Swofford, 2000) using the heuristic search settings: MulTrees option on, tree-bisection-reconnection (TBR) branch swapping, swap on best only in effect, 5,000 random addition sequences. We performed MPA of the *trnT-F* matrix using the same settings, but the searches were frequently terminated prematurely due to the limitation of computer memory. As a result, we analysed the *trnT-F* data using MulTrees option off, TBR branch swapping, swap on best only in effect, and 10,000 random addition sequences. To estimate homoplasy the consistency index (CI) and retention index (RI) were calculated. To assess the support of the retained clades the bootstrap values were computed using 1,000 replicates, MulTrees option on, TBR branch swapping, and five random addition sequences. We performed parsimony and bootstrap analyses of each of the *trnT-F*, nrITS and combined nrITS + *trnT-F* datasets with and without the coded indels to assess the effects of indel coding. No notable conflicts were found in the topologies of the *trnT-F*, nrITS, and combined nrITS + *trnT-F* trees or supports to the recognized clades for using the matrices with or without coded indels; therefore, finally we used the *trnT-F*, nrITS and combined nrITS + *trnT-F* matrices without indel coding. In all analyses, characters were of equal weight, gaps were treated as missing data, and only parsimony-informative characters were included. Visual comparisons between the *trnT-F* and nrITS trees from the preliminary parsimony analyses revealed topological conflicts regarding the position of *Tamridaea* (Figs. 1–2). The agreement on when the datasets should be combined is not generalized (Queiroz & al., 1995) and combinability tests have come under considerable criticism (Bremer 1996; Bayer & al. 2002). Therefore, we combined the *trnT-F* and nrITS data partitions examining the conflicting position of *Tamridaea* in the *trnT-F* and nrITS bootstrap trees. The supports for the conflicting positions of *Tamridaea* in the *trnT-F* and ITS trees (BS = 79–87 and BS = 65–78, respectively, depending on the alignment) were high, due to which finally we performed the combined nrITS + *trnT-F* analyses excluding *Tamridaea*.

We performed Bayesian analyses (hereafter BA) in MrBayes, version 3.1.2 (Huelsenbeck & Ronquist, 2001) using the substitution model parameters: Prset statefreqpr = dirichlet (1,1,1,1); Lset nst = 6 rates = equal; selected as best fit under Akaike Information Criterion (AIC) by MrModeltest, version 2.2 (Nylander, 2004) for the uncoded *trnT-F*, nrITS and combined nrITS + *trnT-F* datasets. In all searches, we used the default settings (MrBayes, version 3.1.2) for all active parameters for the corresponding substitution models, as well as, for the heating scheme. Eight chains under two simultaneous runs, with 100 sample frequencies were executed and monitored up to 3.4–4.0 × 10^6 Markov chain Monte Carlo (mcmc) generations for arriving at the stationary phase (with average standard deviation of split frequencies < 0.01 and PSRF = about 1.0). After discarding 25% of the samples as burn-in, the graphical presentations of summarized resulting trees were generated in PAUP* and Tree View (Page, 1996.) program. Internodes with posterior probabilities of more than 0.95 were considered as reliable support. In this study we infer the biogeography of *Sabicea* s.l. based on our results of MPA and BA of combined nrITS + *trnT-F* datasets.

RESULTS

Sequence and alignment characteristics. — The characteristics of the non-aligned *trnT-F* and nrITS sequences of Sabiceeae s.l. and the aligned matrices of the *trnT-F* and nrITS datasets and the nrITS and *trnT-F* partitions of the combined datasets are summarized in Table 2. The characteristics of the nrITS sequences and alignment were nearly the same in the nrITS and combined nrITS + *trnT-F* matrices. The 5.8S subunit was constant in length (165 bp) for all sequenced taxa.

***trnT-F* analyses (Fig. 1). —** The *trnT-F* analyses included 58 sequences, of which 39 are newly published here. The MPA of the *trnT-F* sequences data resulted in 8,067 equally parsimonious trees (each 977 steps long [L], CI = 0.679, RI = 0.879). All ingroup taxa were resolved in three strongly supported (BS = 100, PP = 1.00) major clades, corresponding to the subfamilies Rubioideae, Cinchonoideae s.str., and Ixoroideae s.l. (Bremer & al., 1999). The investigated members of Sabiceeae sensu Andersson (1996) were resolved in three separate highly supported subclades (Fig. 1): the *Pentaloncha* clade (BS = 100, PP = 1.00) and the *Temnopteryx* clade (BS = 100, PP = 1.00) both nested in Rubioideae, and the *Ecpoma*-*Pseudosabicea*-*Sabicea*-*Schizostigma*-*Stipularia* clade (BS = 83, PP = 1.00; called Sabiceeae s.str. hereafter) nested in Ixoroideae s.l. Within Ixoroideae s.l. Virectarieae sensu Dessein & al. (2001b), represented by *Hekistocarpa minutiflora* Hook. f., *Virectaria multiflora* (Sm.) Bremek. and *V. procumbens* (Sm.) Bremek., and *Tamridaea capsulifera* (Balf. f.) Thulin & B. Bremer, was not resolved as monophyletic. *Virectaria multiflora* and *V. procumbens* formed a strongly supported (BS = 100, PP = 1.00) monophyletic group, whereas *H. minutiflora* was left unresolved. *Tamridaea capsulifera* was resolved with moderate (BS = 80) and high (PP = 1.00) support, respectively, in the MPA and BA as sister to Sabiceeae s.str. The non-monophyletic Virectarieae sensu Dessein & al. (2001b) and Sabiceeae s.str. together (hereafter called Sabiceeae s.l.) formed a highly supported (BS = 100, PP = 1.00) monophyletic group. All studied Neotropical *Sabicea* species, with the exception of *S. mexicana* Wernham, formed a weakly (BS = 62) or highly (PP = 0.96) supported clade, respectively, in the MPA and BA.

nrITS analyses (Fig. 2). — A total of 61 nrITS sequences were included in the analyses and 56 are newly published here. The MPA of the nrITS data resulted in 210 equally parsimonious trees (L = 542, CI = 0.601, RI = 0.758). In the strict consensus tree (Fig. 2), *Hekistocarpa minutiflora* was resolved, with high support (BS = 100, PP = 1.00), as sister to a very large, moderately (BS = 83) and highly (PP = 0.98) supported clade, respectively, in the MPA and BA analyses. That clade contained all investigated members of *Tamridaea*, *Virectaria*, *Stipularia*, *Pseudosabicea*, *Sabicea*, *Schizostigma*, and *Ecpoma*. *Tamridaea capsulifera* and the two *Virectaria* species formed a moderately (BS = 70) and strongly (PP = 1.00) supported clade, respectively, in the MPA and BA anslyses. This *Tamridaea*-*Virectaria* clade was in turn resolved as sister to the strongly supported (BS = 100, PP = 1.00) Sabiceeae s.str. clade. Within the latter clade, *Stipularia elliptica* was resolved as sister to a moderately supported (BS = 75) clade containing *Stipularia efulenensis* and all sequenced species of *Ecpoma*, *Pseudosabicea*, *Sabicea*, and *Schizostigma* (hereafter called *Pseudosabicea*-*Sabicea*-*Stipularia*-*Schizostigma*-*Ecpoma* clade). Within this large clade all *Pseudosabicea* species were resolved in two highly supported clades: one formed by five *Pseudosabicea* species (BS = 99, PP = 1.00) and the other by three *Pseudosabicea* species, including the type species (Good, 1923; Hallé, 1970) *Pseudosabicea nobilis* (R. Good) N. Hallé (BS = 98, PP = 1.00). The former *Pseudosabicea* clade was resolved

Table 2. Characteristics of Sabiceeae sequences and the alignments used in the phylogenetic analyses.

Markers	Matrix	Range of non-aligned sequence lengths in Sabiceeae s.l. (bp)	Range of GC contents in Sabiceeae s.l. sequences (%)	Number of characters	Informative characters	Informative characters in Sabiceeae s.l.
trnT-F	*trnT-F*	1,574–1,688	28.9–32.5	2,348	495 (21.08%)	273 (11.63%)
trnT-L spacer	*trnT-F*	684–788	21.2–27.6	1,165	291 (12.39%)	165 (7.03%)
trnL intron	*trnT-F*	544–616	36.7–38.5	761	108 (4.60%)	62 (2.64%)
trnL-F spacer	*trnT-F*	268–324	32.1–36.2	422	96 (4.09%)	46 (1.96%)
ITS	ITS	566–599	53.7–65.5	670	202 (30.15%)	157 (23.43%)
ITS1	ITS	186–221	52.7–68.7	268	109 (16.27%)	78 (11.64%)
S5.8	ITS	165	54.5–53.3	165	6 (0.90%)	6 (0.90%)
ITS2	ITS	207–216	54.2–70.9	237	87 (12.98%)	73 (10.90%)
ITS	nrITS + *trnT-F*	589–599	53.7–65.5	670	201 (30.00%)	148 (22.09%)
ITS1	nrITS + *trnT-F*	216–221	52.7–68.7	268	109 (16.27%)	76 (11.34%)
S5.8	nrITS + *trnT-F*	165	54.5–53.3	165	6 (0.90%)	6 (0.90%)
ITS2	nrITS + *trnT-F*	207–216	54.2–70.9	237	86 (12.83%)	66 (9.85%)
trnT-F	nrITS + *trnT-F*	1,295–1,673	28.2–38.7	1,927	143 (7.63%)	64 (3.42%)
trnT-L spacer	nrITS + *trnT-F*	699–773	24.3–26.5	948	80 (4.15%)	47 (2.44%)
trnL intron	nrITS + *trnT-F*	523–616	36.5–44.4	642	29 (1.50%)	17 (0.88%)
trnL-F spacer	nrITS + *trnT-F*	185–331	35.4–37.3	337	34 (1.76%)	10 (0.56%)

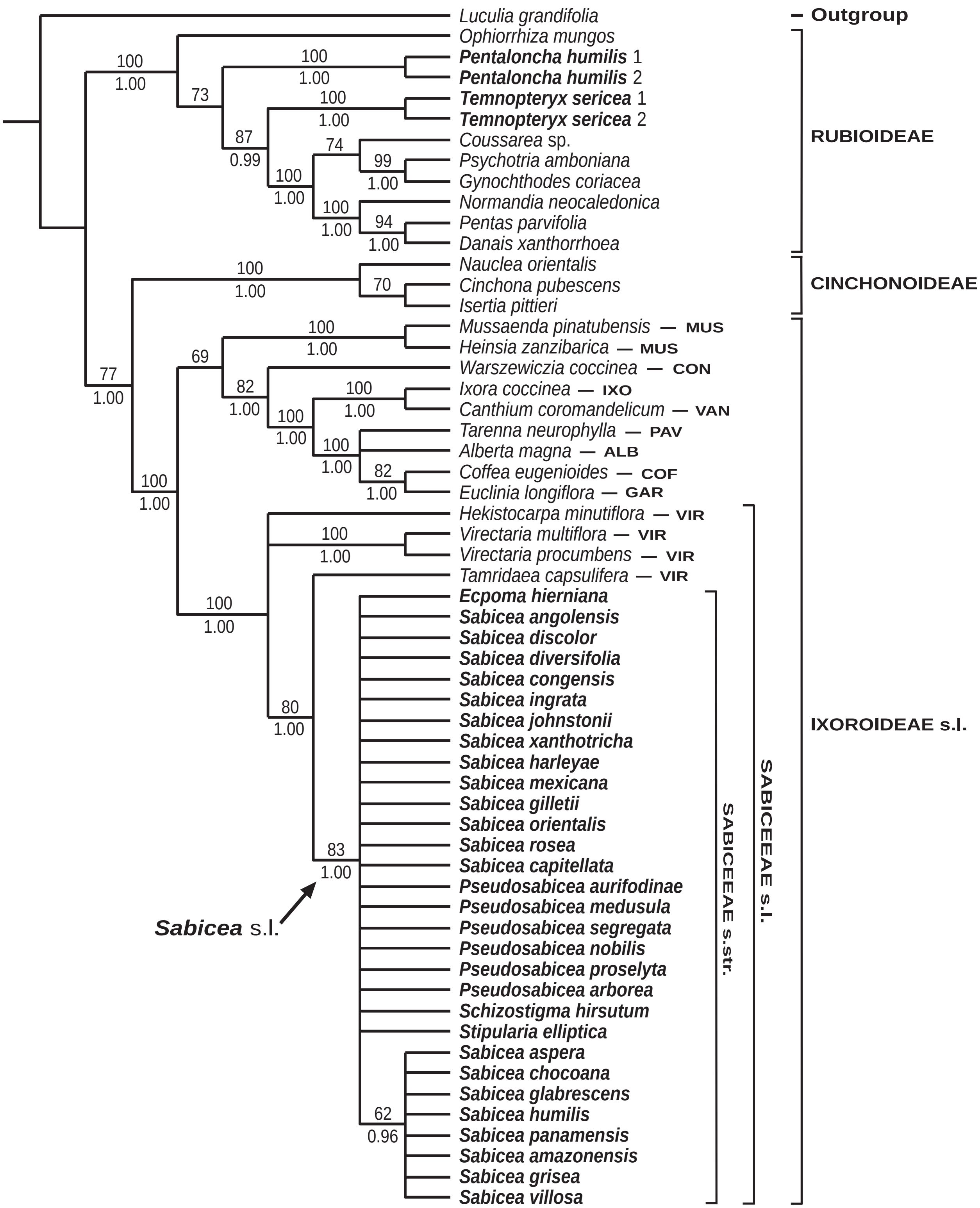

Fig. 1. Strict consensus tree generated from 8,067 equally parsimonious trees based on the phylogenetic analysis of the *trnT-F* data. The numbers above the branches represent bootstrap support values (> 50%) and those below the branches Bayesian posterior probabilities (> 0.95). ALB, Alberteae; COF, Coffeeae; CON, Condamineeae; GAR, Gardenieae; IXO, Ixoreae; MUS, Mussaendeae; PAV, Pavetteae; VAN, Vanguerieae; VIR, Virectarieae. Brackets delimit the three subfamilies (sensu Bremer & al., 1999), Sabiceeae s.l., and Sabiceeae s.str. The genera shown in boldface belong to Sabiceeae sensu Andersson (1996).

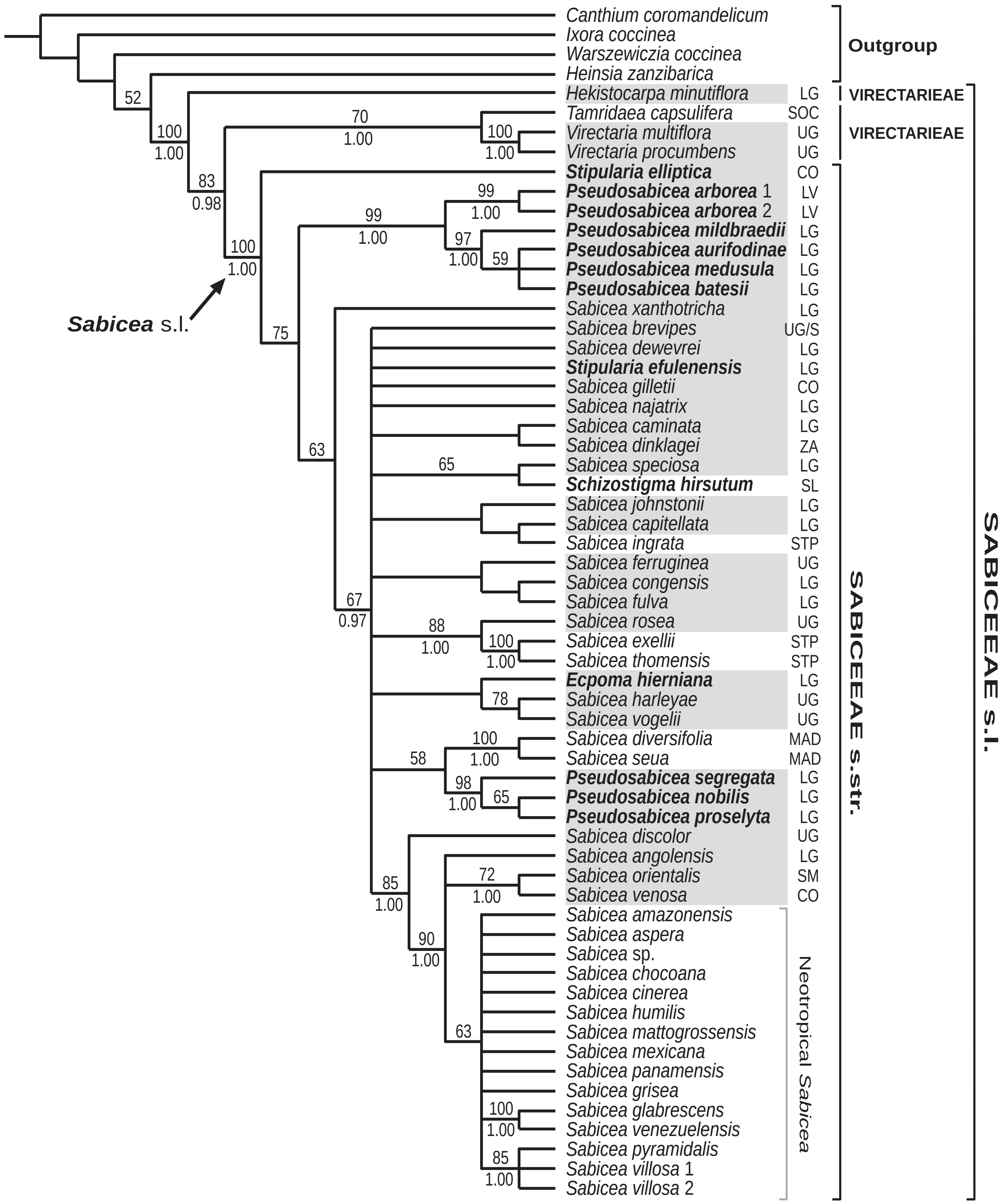

Fig. 2. Strict consensus tree generated from 210 equally parsimonious trees based on the phylogenetic analysis of the ITS data. The numbers above the branches represent bootstrap support values (> 50%) and those below the branches Bayesian posterior probabilities (> 0.95). Brackets delimit the outgroup taxa, Sabiceeae s.l., Sabiceeae s.str., and Neotropical *Sabicea*. Vertical bars delimit the genera of Virectarieae sensu Dessein & al. (2001a). CO, Congolian; LG, Lower-Guinean; LV, Lake Victorian; MAD, Madagascan; SL, Sri Lankan (Indian); SM, Somali-Masai; SOC, Socotran (Yemen); STP, São Tomean; UG, Upper-Guinean; UG/S, Upper-Guinean/Sudanian; ZA, Zambezian (African phytochoria; White, 1979, 1993). The phytogeographic data are mentioned only for the sampled African specimens. Sequenced species of *Ecpoma*, *Pseudosabicea*, *Schizostigma*, and *Stipularia* are shown in boldface. All shadowed taxa are from mainland Africa.

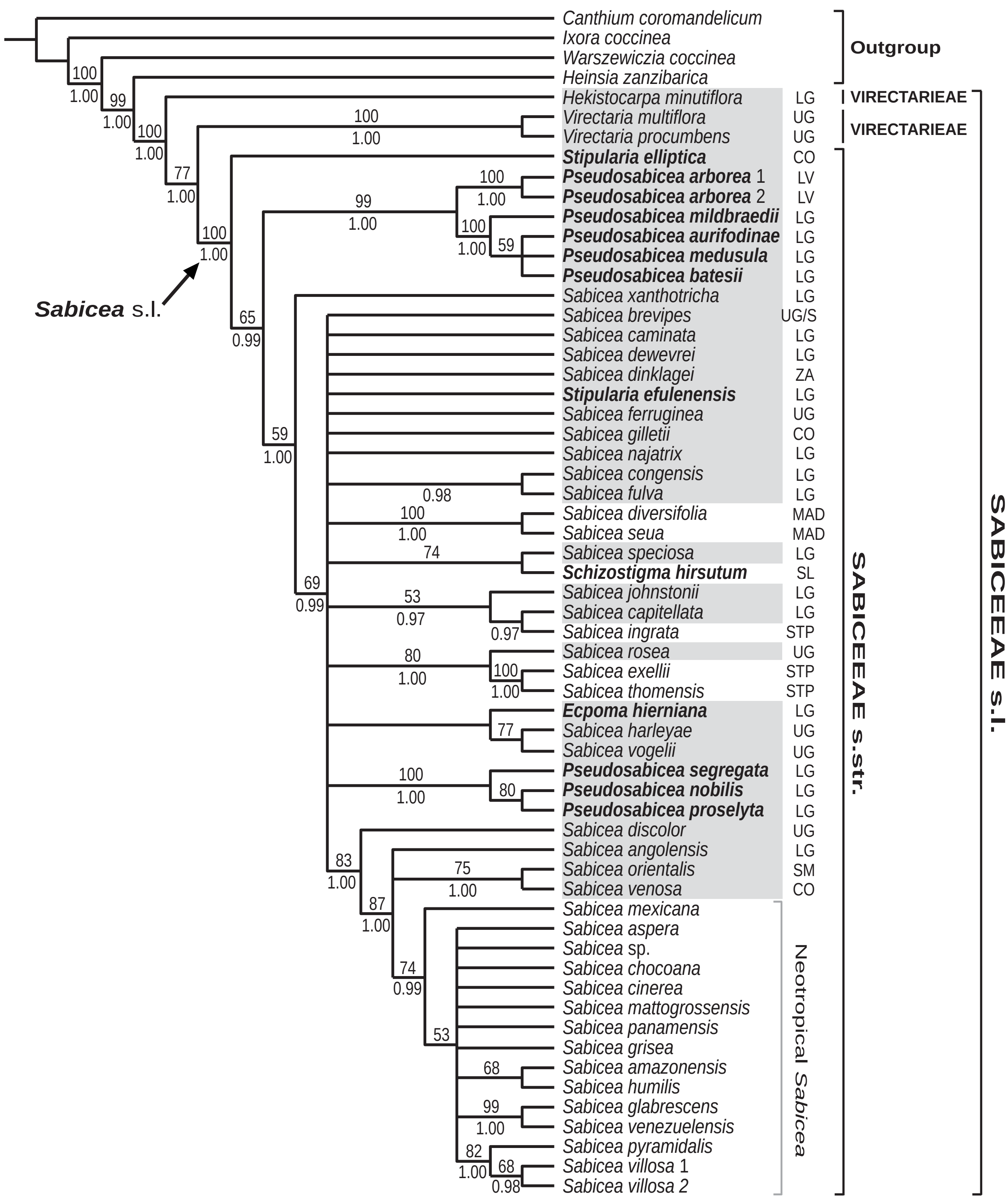

Fig. 3. **Strict consensus tree generated from 104,428 equally parsimonious trees based on the phylogenetic analysis of the ITS-*trnT-F* data. The numbers above the branches represent bootstrap support values (> 50%) and those below the branches Bayesian posterior probabilities (> 0.95). Brackets delimit the outgroup taxa, Sabiceeae s.l., Sabiceeae s.str., and Neotropical *Sabicea*. Vertical bars indicate the position of the genera of Virectarieae sensu Dessein & al. (2001a). CO, Congolian; LG, Lower-Guinean; LV, Lake Victorian; MAD, Madagascan; SL, Sri Lankan (Indian); SM, Somali-Masai; STP, São Tomean; UG, Upper-Guinean; UG/S, Upper-Guinean/Sudanian; ZA, Zambezian (African phytochoria; White, 1979, 1993). The phytogeographic data are mentioned only for the sampled African specimens. Sequenced species of *Ecpoma*, *Pseudosabicea*, *Schizostigma*, *Stipularia*, and *Tamridaea* are shown in boldface. All shadowed taxa are from mainland Africa.**

as sister to a weakly supported (BS = 63) and *Sabicea* dominated clade formed by *Stipularia efulenensis*, all studied species of *Ecpoma*, *Sabicea* and *Schizostigma* and the other *Pseudosabicea* clade (containing *P. segregata* (Hiern) N. Hallé, *P. nobilis*, and *P. proselyta* N. Hallé). Furthermore, two investigated Malagasy (*S. diversifolia* Pers. and *S. seua* Wernham) and two São Tomean *Sabicea* species (*S. exellii* G. Taylor and *S. thomensis* Joffroy) formed strongly supported (BS = 100, PP = 1.00) groups, respectively. Another São Tomean *Sabicea* (*S. ingrata* K. Schum.) formed an unsupported group with two continental African *Sabicea* (*S. johnstonii* K. Schum. and *S. capitellata*). Similarly, all sequenced Neotropical *Sabicea* formed a weakly supported (BS = 63) clade in the MPA. These three clades were nested within the largely African *Pseudosabicea*-*Sabicea*-*Stipularia*-*Schizostigma*-*Ecpoma* clade.

Combined nrITS-*trnT-F* analyses (Fig. 3). — Each of the nrITS and *trnT-F* partitions used in the combined analyses contained a total of 60 sequences including 55 new sequences. All of the 55 new sequences of nrITS partition were used in the nrITS analyses and 32 new sequences of *trnT-F* partition were used in the *trnT-F* analyses. The MPA of the combined nrITS + *trnT-F* matrix, composed of a total of 2,597 positions and 344 (13.24%) parsimony-informative characters (Table 2), generated 104,428 equally parsimonious trees (L = 714, Cl = 0.674, RI = 0.796). The overall tree topologies and support values of the resolved nodes in the strict consensus combined tree (Fig. 3) were largely similar to those of the strict consensus nrITS tree (Fig. 2). The support values in the combined tree were higher for some nodes (e.g., the Neotropical *Sabicea* clade and the *Sabicea speciosa*-*Schizostigma hirsutum* clade). The poorly supported (BS = 58) sister-group relationships between the Malagasy *Sabicea* clade (*S. diversifolia*, *S. seua*) and the *Pseudosabicea* clade formed by *P. segregata*, *P. nobilis* and *P. proselyta* (Fig. 2) collapsed in the combined tree (Fig. 3). Plus, the monophyletic group of one São Tomean *Sabicea* (*S. ingrata*) and two continental African *Sabicea* (*S. johnstonii* and *S. capitellata*), unsupported in the nrITS tree, was weakly (BS = 53) or strongly (PP = 0.97) supported in the combined tree.

DISCUSSION

Firstly, we compare the sequence characteristics between the nrITS and *trnT-F* sequences of Sabiceeae and those of the some other rubiaceous tribes (e.g., Naucleeae s.l., Mussaendeae, and Vanguerieae). Secondly, we discuss the new tribal circumscription of Sabiceeae, proposed in the light of our results. Accordingly, we propose the new circumscriptions for the genera of the tribe. Thirdly, we assess the biogeography of our newly delimited *Sabicea* s.l. and finally provide the updated description for *Sabicea* s.l. and make six new combinations.

Sequence characteristics. — Both the ranges of lengths (Table 2) and the average lengths of nrITS1 and nrITS2 of Sabiceeae taxa fall within the records for other angiosperms (Baldwin & al., 1995; Noyes, 2006). The constant length of 5.8S subunit is consistent with the reports for other Rubiaceae (e.g., Alejandro & al., 2005) and close to those for other angiosperms (Baldwin & al., 1995). The records of GC contents in nrITS1 and nrITS2 of Sabiceeae taxa coincide with the reports for Rubiaceae (Razafimandimbison & Bremer, 2001; Alejandro & al., 2005) and other angiosperms (Tate & al., 2005). The total lengths of the nrITS region of Sabiceeae (566–599 bp) are nearly similar to those of Mussaendeae sensu Bremer & Thulin (1998) (570–596 bp; Alejandro & al., 2005), shorter than those reported for the tribe Vanguerieae (611–671 bp; Lantz & Bremer, 2004), and fall within the known range for other Ixoroideae (565–654 bp; Andreasen & al., 1999). The parsimony informative characters (PIC) for the nrITS region of Sabiceeae (157) are higher than those reported for Mussaendeae (103; Alejandro & al., 2005) and other Ixoroideae tribe Gardenieae A. Rich. ex DC. (e.g., 121 for the *Alibertia* group; Persson, 2000). On the other hand, they are lower than the PIC recorded for Vanguerieae (188; Lantz & Bremer, 2004) and the Cinchonoidae tribe Naucleeae s.l. (210; Razafimandimbison & Bremer, 2002). So, there is a great variation of the lengths of nrITS regions and eventually the number of parsimony informative characters between the different rubiaceous tribes.

The range of the lengths of the *trnT-F* region of Sabiceeae (1574–1688 bp) coincides with the records for Mussaendeae sensu Bremer & Thulin (1998) (1662–1793 bp; Alejandro & al. 2005) and Vanguerieae (1559–1785 bp; Lantz & Bremer, 2004) but is shorter than that of Naucleeae s.l. (1707–1785 bp; Razafimandimbison & Bremer, 2002). The lengths of the *trnT-F* region of the studied Sabiceeae are 2.8 times longer than those of their nrITS region. In contrast, the *trnT-F* region of the sequenced Sabiceeae is less informative (11.63%) than their nrITS region (23.43%), concurring with Liede & Kunze (2002), Razafimandimbison & Bremer (2002), and Alejandro & al. (2005). In the *trnT-F* matrix, the *trnT-L* spacer (684–788 bp) is more variable than *trnL-F* spacer (268–324 bp), also consistent with Razafimandimbison & Bremer (2002), but our record of the *trnL* intron as more variable than the *trnL-F* spacer (Table 2) is inconsistent with their reports. The variations shown by the nrITS, *trnT-L*, *trnL* and *trnL-F* regions further indicate their usefulness for assessing the phylogenetic relationships in Rubiaceae and other families in the order Gentianales (e.g., Meve & Liede, 2002).

Tribal circumscriptions of Sabiceeae. — The polyphyly of Sabiceeae sensu Andersson (1996), which includes *Amphidasya*, currently classified by Bremer & Manen (2000) in the tribe Urophylleae Bremek. ex Verdc. (Rubioideae), and *Acranthera*, recently shown by Alejandro & al. (2005) to be associated with Rubioideae, is further corroborated by the *trnT-F* tree (Fig. 1), as both *Pentaloncha* and *Temnopteryx* are also resolved with high support (BS = 100, PP = 1.00) in Rubioideae. This is the first molecular phylogenetic study to include these African rubiaceous monotypic genera. We find no support either for the close relationships of *Temnopteryx* and *Pentaloncha* with *Ecpoma*, *Pseudosabicea*, *Sabicea*, and *Stipularia* postulated, respectively, by Puff & al. (1998) and Dessein & al. (2001a) or Hiern's (1877) attempt to merge both *Pentaloncha* and *Temnopteryx* with *Schizostigma* (= *Sabicea*; Puff & al., 1998). The combined nrITS + *trnT-F* tree (Fig. 3) shows that Sabiceeae sensu Bremer & Thulin (1998) is not monophyletic, unless *Ecpoma*, *Hekistocarpa*, and *Schizostigma* are also included. Dessein & al. (2001a) tentatively included *Pentaloncha* in Sabiceeae s.str. based on morphological grounds. But our results strongly support the exclusion of *Pentaloncha* from Sabiceeae.

Our results clearly favour a broad circumscription of Sabiceeae, which should include the following eight genera: *Ecpoma*, *Hekistocarpa*, *Pseudosabicea*, *Sabicea*, *Schizostigma*, *Stipularia*, *Tamridaea*, and *Virectaria* (Figs. 1–2), consistent with Robbrecht & Manen (2006). In all our parsimony and Bayesian analyses, we perceive no support for the monophyly of Virectarieae sensu Dessein & al. (2001a), as *Hekistocarpa*, *Virectaria*, and *Tamridaea* (Figs. 1–2) or *Hekistocarpa* and *Virectaria* (Fig. 3) never form a clade, and therefore, its tribal status is untenable. For the same reason our results do not support the new subtribal classification of Sabiceeae (Sabiceinae and Virectariinae) by Robbrecht & Manen (2006). The discrepancies between our results and the *rbcL* or *rps16* trees of Dessein & al. (2001a) are probably due to taxon sampling. In the *rbcL* tree of Dessein & al. (2001a), Sabiceeae, represented by one *Sabicea* species, is weakly resolved as sister to the strongly supported (BS = 87) Virectarieae sensu Dessein & al. (2001a). In their *rps16* tree, the support for Virectarieae, represented by *Hekistokarpa* and *Virectaria*, is weak (BS = 59), while that of Sabiceeae s.str., represented by four *Sabicea* and two *Pseudosabicea* species, is high (BS = 87). In other words, the support for the monophyly of Virectarieae sensu Dessein & al. (2001a) seems to decrease when more species from Sabiceeae are included in the *rps16* analysis. This is further confirmed by our ITS and combined nrITS + *trnT-F* analyses (Figs. 2–3), which contain a much larger sampling of Sabiceeae s.str. (51 species of *Ecpoma*, *Pseudosabicea*, *Sabicea*, *Schizostigma*, and *Stipularia*), in which Virectarieae sensu Dessein & al. (2001a) totally collapse. The Sabiceeae s.l. clade of our *trnT-F* tree (Fig. 1) is largely congruent with that of the nrITS (Fig. 2) and the combined nrITS + *trnT-F* trees (Fig. 3), with the exception of the position of *Tamridaea*.

We were unable to include the Neotropical genus *Pittierothamnus* (Steyermark, 1962) due to lack of material. Therefore, its phylogenetic position in Sabiceeae postulated by Andersson (1996) has yet to be tested with molecular-based phylogenies. We have not been able to find any potential morphological synapomorphy to diagnose our newly delimited Sabiceeae s.l. Therefore, the monophyly of the tribe is entirely based on molecular data.

Generic circumscriptions in Sabiceeae s.l. — Our newly circumscribed Sabiceeae contains the following four genera: *Hekistocarpa*, *Sabicea* s.l. (including *Ecpoma*, *Pseudosabicea*, *Schizostigma*, and *Stipularia*), *Tamridaea*, and *Virectaria*, and a total of ca. 180 species.

The monotypic genus *Hekistocarpa* is restricted to Cameroon and Nigeria (Dessein & al 2001a: Fig. 37). This genus can be characterized by the following characters: herbaceous growth habit, lateral scorpioid cymes, laterally compressed fruits, exotesta cells with strongly thickened walls, tuberculate surface and one perforation, and tricolpate pollens (Dessein & al. 2001a). *Hekistocarpa* was classified for a long time in the tribe Hedyotideae Cham. & Schltdl. ex DC. (Rubioideae) because of its herbaceous habit, scorpioid inflorescences and many-seeded fruits (Hooker, 1873b). The study of Dessein & al. (2001a) is the first to place *Hekistocarpa* in Virectarieae. All the *trnT-F*, nrITS and combined nrITS + *trnT-F* trees (Figs. 1–3) strongly (BS = 100, PP = 1.00) favour its placement in Sabiceeae s.l. Furthermore, *Hekistocarpa* is resolved as sister to the rest of Sabiceeae s.l. (e.g., Fig. 2) and therefore, its current generic status should be maintained.

All nrITS and combined analyses (Figs. 2–3) indicate that *Sabicea* sensu Wernham (1914) including *S. hierniana* Wernham (= *Ecpoma hierniana* (Wernham) N. Hallé & F. Hallé), *S. segregata* Wernham (= *Pseudosabicea segregata*), and *S. nobilis* R. Good (= *Pseudosabicea nobilis*) is only monophyletic if *Pseudosabicea proselyta*, *Schizostigma*, and *Stipularia efulenensis* are also included. The African genus *Stipularia* appears polyphyletic, as the two sequenced species, *S. elliptica* and *S. efulenensis*, are resolved in two separate clades (Figs. 2–3). The type species *S. africana* is not included in the present study, so the generic status of *Stipularia* could still be maintained if it turns out that *S. africana* forms a clade with *S. elliptica*. On the other hand, our results indicate that the generic concept of *Stipularia* based mainly on the presence of the large campanulate involucral bracts subtending the entire inflorescence is untenable, as the two sequenced *Stipularia* species bearing the same type of the involucral bracts (Hepper, 1958) do not form a clade. Plus, Hepper (1958: 289–291) convincingly explained that the involucral bracts of some African *Sabicea* species show a great range of the degree of fusion (from inconspicuous to distinct and totally free to partly or completely fused bracts). Also, Hallé (1966) showed that some African *Sabicea* species (e.g., *Sabicea duparquetiana* H. Baillon ex Wernham and *S. najatrix* N. Hallé) have large and partly fused campanulate involucral bracts. Based on the above evidence presented we concur with Hepper's (1958) decision to merge *Stipularia* with *Sabicea*.

Our analyses further reveal the polyphyly of *Sabicea* sensu Hallé (1963), as *Ecpoma*, represented by *E. hierniana*, *Pseudosabicea*, represented by *P. segregata*, *P. nobilis*, and *P. proselyta*, and *Schizostigma* are all resolved in the largely *Sabicea* clade with weak and high support (e.g., BS = 59, PP = 1.00; Fig. 3), respectively, in the MPA and BA. Similarly, *Pseudosabicea* sensu Hallé (1963) is also shown to be para- or polyphyletic, as the sequenced *Pseudosabicea* species group in two separate clades (Figs. 2–3). Accordingly, we merge *Pseudosabicea* with *Sabicea*. The range of variation in the characters of *Sabicea* includes the diagnostic characters of *Pseudosabicea* sensu Hallé (1963). One could recognize the strongly supported clade of five *Pseudosabicea* species at generic level; however, we find no distinctive morphological character for diagnosing this clade, once *P. nobilis*, *P. proselyta*, and *P. segregata* were included in *Sabicea*.

The African genus *Ecpoma* (Schumann, 1896) is comprised of six species and characterized by its shrubby habit, isophylly, colourless pulp of small fruits, bilocular ovaries, non-accrescent septa, rounded or twisted to peltate placentae (Hallé, 1963). *Ecpoma* was traditionally classified in Mussaendeae (Hallé, 1961; Hallé, 1963, 1966) or in Sabiceeae (Andersson, 1996; Robbrecht & Manen, 2006). In Andersson's (1996) study, *Ecpoma* did not form a monophyletic group with *Pseudosabicea*, *Sabicea*, and *Schizostigma*. In our nrITS and nrITS + *trnT-F* trees (Figs. 2–3), *Ecpoma*, represented by *E. hierniana*, however, is consistently and deeply nested within the *Pseudosabicea*-*Sabicea*-*Stipularia*-*Schizostigma*-*Ecpoma* clade, inconsistent with Hallé (1961), Hallé (1963) and Andersson (1996). Accordingly, we merge *Ecpoma* with *Sabicea* even if the type species is not included in our analyses because its character states clearly fall within the range of variation in *Sabicea* s.l.

Adopting the broadened circumscription of *Sabicea* including *Ecpoma*, *Pseudosabicea*, *Schizostigma*, and *Stipularia* requires only a maximum of six new combinations, as five of the six *Ecpoma* species (Hallé, 1963) and 8 of the 13 *Pseudosabicea* species (Hallé, 1963, 1966) were originally described as *Sabicea* (Wernham, 1914; Good, 1923). Plus, all five *Stipularia* species and

Table 3. Morphological distinctive characters of *Hekistocarpa*, *Sabicea* s.l., *Tamridaea*, and *Virectaria*.

Characters	*Hekistocarpa*	*Sabicea* s.l.	*Tamridaea*	*Virectaria*
Habit	Herbs	Lianas, vines, straggling to scrambling herbs, scandent or erect shrubs (up to 4 m tall), rarely small trees	Shrubs (ca. 1 m tall)	Herbs
Inflorescence position and types	Axillary, scorpioid cymes	Axillary, fasciculate or densely capitulate to paniculate or thyrsoid, simple to compound dichasial cymes or solitary flowers	Terminal, usually dichasial corymbose cymes	Terminal, dichasial thyrsoid to monochasial or simple cymes
Flower types	Homostylous	Hetero- and homostylous	Heterostylous	Homostylous
Corolla aestivation	Reduplicate valvate	True valvate	Reduplicate valvate	True valvate
Corolla lobes	Ovate to deltoid with (sub-) acute apices	Ovate with (sub-) acute apices	Obcordate corolla lobes with emarginate-mucronate apices	Lanceolate to deltoid with (sub-) acute apices
Anther fixation and position	Dorsimedifixed, included	Dorsimedifixed, included (short-styled flowers) and slightly exserted (long-styled flowers)	Dorsifixed, included (short-styled flowers) and slightly exserted (long-styled flowers)	Dorsimedifixed, exserted
Stigma branches	2, filiform	2–5(6), filiform to oblong or very narrowly elliptic or oblanceolate to widely spathulate or dilated	2, filiform-oblong	Initially 2, eventually truncated, spherical
No. of locules per ovary	ca. 10	2–5(7)	2	2
Fruit types	Dry, indehiscent or tardily dehiscent	Indehiscent berries	Dry, dehiscent capsules	Dry, dehiscent capsules with one caduceus valve
Pollen type	3-colporate	3–4-colporate	4-colporate	3-colporate

Schizostigma have already been merged, respectively, by Hepper (1958) and Puff & al. (1998) in *Sabicea*. *Sabicea* s.l. is very distinct from the other three genera (*Hekistocarpa*, *Tamridaea*, *Virectaria*) of Sabiceeae s.l. in some aspects (see Table 3).

All our nrITS and combined nrITS + *trnT-F* analyses contradict the monophyly of Wernham's (1914) two subgenera of *Sabicea* based on habit and leaf and stipule sizes. The two sequenced species of *Sabicea* subgen. *Stipulariopsis* (*Sabicea xanthotricha* Wernham and *S. hierniana* Wernham [= *Ecpoma hierniana*]) do not form a clade. The sequenced species of *Sabicea* subgen. *Eusabicea* (e.g., *S. batesii* Wernham [= *P. batesii*], S. *mildbraedii* [= *P. mildbraedii*], *S. segregata* [= *P. segregata*], *S. seua*, *S. speciosa* K. Schum., *S. vogelii* Benth., *S. angolensis* Wernham, *S. discolor* Stapf, *S. venosa* Benth., and *S. hirsuta* H.B. & K. [= *S. villosa* Willd. ex Roem. & Schult.], etc.) do not form a clade unless *Pseudosabicea* (*P. arborea* (K. Schum.) N. Hallé and *P. proselyta*), *Sabicea* subgen. *Stipulariopsis*, *Stipularia efulenensis*, and *Schizostigma* are also included.

New molecular phylogenetic investigations using multiple markers and a much broader sampling of *Ecpoma*, *Pseudosabicea*, *Stipularia*, and *Sabicea* will be performed in attempt to establish, if possible, new infrageneric classifications for our newly delimited *Sabicea* with ca. 170 species and also address some evolutionary questions.

The monotypic genus *Tamridaea*, endemic to Socotra (Yemen), is characterized by its shrubby habit, reduplicate-valvate aestivation, terminal cymes, flat, ± obcordate corolla lobes with emarginate-mucronate apices, bilobed stigma, bilocular ovaries (Bremer & Thulin, 1998), exotesta cells with verrucose thickenings, and 4-colporate pollens (Dessein & al., 2001a). Bremer & Thulin (1998) originally described *Tamridaea* to accommodate *Pseudomussaenda capsulifera* (Balf. f.) Wernham, previously classified in Isertieae sensu Robbrecht (1988), and placed it in their Sabiceeae s.l. Dessein & al. (2001b) accept the generic status of *Tamridaea* and its placement in Sabiceeae sensu Bremer & Thulin (1998), though Dessein & al. (2001a) placed the genus in their emended Virectarieae. *Tamridaea* has conflicting positions in our results. In our *trnT-F* tree (Fig. 1), it is moderately (BS = 80) and highly (PP = 1.00) resolved, respectively, as sister to Sabiceeae s.str. in the MPA and BA analyses. In the nrITS tree, the genus and *Virectaria* form a moderately to highly (BS = 70, PP = 1.00; Fig. 2) supported clade, consistent with Bremer & Thulin (1998) and Dessein & al. (2001a). When included in a combined nrITS + *trnT-F* analysis *Tamridaea* is weakly resolved (BS = 58) as sister to *Virectaria*.

The tropical African genus *Virectaria* comprises eight species, of which three species (*V. major* K. Schum., *V. multiflora*, *V. procumbens*) are Guineo-Congolian wide (Dessein & al., 2001b), while four species (*V. herbacoursi* N. Hallé, *V. belingana* N. Hallé, *V. salicoides* (C.H. Wright) Bremek., *V. angustifolia* (Hiern) Bremek.) are endemic to one of the domains of the Guineo-Congolian region (White, 1979), Lower Guinea and *V. tenella* J.B. Hall to Upper Guinea (Dessein & al. 2001a: Figs. 69–70). The genus can be characterized by its herbaceous to semi-woody habits, terminal inflorescences, truncated stigmas, flat trichomes of the corolla orifice or inside the corolla tubes, elongated floral disc, one persistent and one deciduous valve during fruit dehiscence, and exotesta cells of seeds with many small perforations (Dessein & al., 2001a). Our results support the placement of *Virectaria* in Ixoroideae s.l., also consistent with Bremer & Thulin (1998) and Dessein & al. (2001a) but inconsistent with Bremekamp (1952, 1966) who classified the genus in the tribe Ophiorrhizeae of his Cinchonoideae, and Verdcourt (1975) who placed it in Cinchonoideae as a monogeneric tribe Virectarieae. In both nrITS and combined nrITS + *trnT-F* trees (Figs. 2–3), *Virectaria* is strongly (BS = 100, PP = 1.00) resolved as a monophyletic group, which is moderately supported as sister to *Tamridaea* (Fig. 2), consistent with Dessein & al. (2001a) and Robbrecht & Manen (2006). However, our results are inconsistent with the placement of the genus pair and *Hekistokarpa* in a separate tribe Virectarieae (Dessein & al. 2001a) or subtribe Virectariinae (Robbrecht & Manen, 2006). *Tamridaea* and *Virectaria* are morphologically distinct (see Table 3) and therefore, their generic status can be maintained.

Biogeography of *Sabicea* s.l. — We are unable to perform a proper biogeographic analysis, because the clade of Sabiceeae s.str. is largely unresolved in all trees (Fig. 1–3). However, some biogeographical facts can be discussed for *Sabicea* s.l. The combined tree (Fig. 3) shows that neither the Upper-Guinean, nor the Lower-Guinean, nor the Congolian (White, 1976; Robbrecht, 1996) *Sabicea* species form a monophyletic group, and in contrast, the species of different phytogeographical regions (e.g., Lake Victoria and Lower-Guinea or Somali-Masai and Congolia; White, 1976, 1993) form highly supported clades. These results indicate that *Sabicea* species of these phytogeographical domains and regions are not closely related and there seem to be several dispersal events of *Sabicea* species between them.

The volcanic Island of São Tomé (Deruelle & al., 1991; Munhá & al., 2002) has three endemic *Sabicea* species (*S. exellii*, *S. ingrata*, *S. thomensis*; Joffroy, 2001), which are consistently nested in the almost continental African *Sabicea* clade (Figs. 2–3). One São Tomean *Sabicea* species (*S. ingrata*) groups together with the Lower-Guinean *S. capitellata* and *S. johnstonii*, and the other two São Tomean species (*S. ingrata*, *S. thomensis*) group with the Upper-Guinean *S. rosea* Hoyle (Fig. 3). These results indicate that the São Tomean species must have had two African ancestors, which appear to have reached the island via two independent dispersal events. Similarly, the two sequenced Malagasy species of *Sabicea*, *S. diversifolia* and *S. seua* (Razafimandimbison & Miller, 1999), form a highly supported (BS = 100, PP = 1.00) clade, which is nested in the large *Sabicea* clade. No record of *Sabicea* s.l. is known from the neighbouring Islands of Madagascar. Madagascar is about 400 km off the southwestern coast of Mozambique, whereas São Tomé & Príncipe are only within 225 to 250 km off of the northwestern coast of Gabon. All sequenced *Sabicea* species of the Neotropics form a moderately supported (BS = 74) clade in the MPA and a highly supported (PP = 0.99) clade in the BA (Fig. 3), indicating a single origin of all Neotropical *Sabicea*. The Neotropical *Sabicea* additionally appear to have originated from an African common ancestor.

Furthermore, our data (Fig. 3) indicate that the African common ancestors of the Malagasy, São Tomean, and Neotropical *Sabicea*, respectively, most likely reached Madagascar, São Tomé, and the Neotropics through four independent dispersal events either via wind and/or ocean currents or dispersal of seeds across the Mozambique Channel, the Gulf of Guinea, and the South Atlantic Ocean by birds. *Sabicea* s.l. produce fleshy and (sub-) globose or obovoid berries bearing many small seeds, which would presumably provide an important source of food for tropical frugivorous birds. This seems to favour a zoochorous mode of dispersal (but see Renner, 2004). The fact that the Neotropics and São Tomé do not share in common any *Sabicea* species seems to exclude stepping-stone long-distance dispersal (i.e., dispersal from the mainland Africa to the Neotropics via São Tomé) as the mode of dispersal responsible for the present trans-Atlantic distribution of *Sabicea* s.l. Our results (Fig. 3) further indicate that four African *Sabicea* species (*S. angolensis*, *S. discolor*, *S. orientalis* Wernham, *S. venosa*) are more closely related to each other than they are to the remaining *Sabicea* s.l. Plus, they appear to be most closely related to the Neotropical *Sabicea*, also consistent with morphological grounds. It is, however, important to note that these four African *Sabicea* species are presently either restricted to a domain of Guineo-Congolian region (e.g., *S. angolensis* and *S. discolor* occur in Lower- and Upper Guinea, repectively) or dispersed to two to three phytogeographical regions (e.g., *S. orientalis* occurs in Guineo-Congolian, Zambezian and Somalia-Masai region, and *S. venosa* in Guineo-Congolian and Lake Victoria regions; White, 1993).

Finally, *Sabicea* s.l. seems to have started to diversify in mainland Africa, where at least 106 species are presently known. A second major radiation of *Sabicea* appears to have occurred after the group began to colonize the Neotropics. The occurrence of the single Asian species *Sabicea ceylanica* (restricted to Sri Lanka) indicates that the genus seems to have failed to disperse to the rest of Asia.

CONCLUSIONS

The present phylogenetic analyses favour a broad circumscription of Sabiceeae, which includes the following four genera: *Hekistocarpa*, *Sabicea* s.l. (including *Ecpoma*, *Pseudosabicea*, *Schizostigma*, and *Stipularia*), *Tamridaea*, and *Virectaria*. *Pentaloncha* and *Temnopteryx* belong to subfamily Rubioideae. Sabiceeae sensu Bremer & Thulin (1998) is not monophyletic, unless *Ecpoma*, *Hekistocarpa*, and *Schizostigma* are also included. Virectarieae sensu Dessein & al. (2001a) appears to be para- or polyphyletic. Dessein & al.'s (2001a) Sabiceeae and Robbrecht & Manen's (2006) subtribal classification of Sabiceeae are not supported by our results. *Sabicea* sensu Wernham (1914) is monophyletic only if *Pseudosabicea proselyta*, *Stipularia efulenensis* and *Schizostigma* are included. Finally, our analyses support the monophyly of Malagasy and Neotropical *Sabicea*, but not of *Sabicea* and *Pseudosabicea* both sensu Hallé (1963, 1966) and *Stipularia*. Our results indicate several dispersal events of *Sabicea* species between few African phytogeographical domains and regions. The São Tomean, Malagasy, Asian and Neotropical species of *Sabicea* all appear to have had African origins and perhaps dispersed via four independent dispersal events.

TAXONOMIC IMPLICATIONS

Sabicea Aubl. Hist. Pl. Guiane Françoise 1: 192, t. 75. Jun-Dec 1775 – Lectotype: *S. cinerea* Aubl. designated by P.C. Standley, N. Amer. Fl. 32: 148. 10 May 1921. PHAN.-RUBIACEAE (75/104).

= *Cephaëlis* Sw., Prodr. (Swartz) 3, 45 ('*Cephaelis*'). 20 Jun-29 Jul 1788 (nom. cons.) – Type: *C. muscosa* (Jacq.) Sw. ≡ *Morinda muscosa* Jacq. (typ. cons.).

= *Paiva* Vell., Fl. Flum.: 104. 7 Sep-28 Nov 1829 ('1825') – Type: *P. verticillata* Vell.

= *Stipularia* P. Beauv., Fl. Owar. 2: 26. 1807 – Type: *S. africana* P. Beauv. – Holotype: South Nigeria, *Palisot de Beauvois s.n.* (G!), isotype (P, not seen) ≡ *Sabicea africana* (P. Beauv.) Hepper.

= *Ecpoma* K. Schum., Bot. Jahrb. 23: 430. 1896, **syn. nov.** – Type: *E. apocynaceum* K. Schum. – Holotype: Cameroon, near Lolodorf, *Staudt 204* (B, presumably destroyed; K, photo!).

= *Pseudosabicea* N. Hallé, Adansonia ser 2, 3: 170. 1963, **syn. nov.** – Type: *P. nobilis* (R. Good) N. Hallé ≡ *Sabicea nobilis* R. Good – Syntypes: Angola, Belize, Maiombe, *Gossweiler 7550*, *7043* (BM, P).

= *Schizostigma* Arn. ex Meisn., Pl. Vasc. Gen. 1: 164; 2: 115. 1838 – Type: *S. hirsutum* Arn. ex Meisn. (holotype or syntypes not designated) ≡ *Sabicea ceylanica* Puff.

Schwenkfeldia Wild. (Sp. Pl. 4 [post Reichardianum quinta]: 982. 1797) was described based on *Schwenkfelda* Schreb. (Gen. Pl. 1: 123. 1789), but the latter was described based on *Sabicea* Aubl. Therefore, *Schwenkfelda* and *Schwenkfeldia* are illegitimate names.

Lianas or woody vines, climbing or scrambling to erect herbs, scandent to erect shrubs, rarely small trees, stems rounded to shallowly quadrangular. Stipules interpetiolar, free, persistent, minute to vigorous, usually entire, sometimes fimbriate to deeply laciniate, usually with few to many colleters inside the base. Nodes isophyllous or anisophyllous. Leaves membranaceous to subcoriaceous. Inflorescences axillary, sessile to pedunculate, solitary to compactly capitate to lax thyrsoid and few to many flowered cymes, subtended by inconspicuous to distinct and free to completely united and variously lobed bracts with usually 2 to many colleters inside the base, with or without forming spreaded to deeply campanulate involucre, rarely followed by prophylls. Calyces shallowly to deeply campanulate to funnel-shaped, 3–5-lobed, lobes filiform to elliptic or obovate, antrorse to abruptly reflex, usually with 1–2 colleters in or below each sinus. Corollas hypocrateriform or broadly infundibuliform, usually white, occasionally pinkish, usually 5-lobed, lobes valvate, narrowly to widely ovate, margins entire, glabrous or papillate inside, (sub-) acute at apex. Stamens included to slightly exserted just beyond the corolla tubes, anthers linear to narrowly oblong, basally and apically acute to rounded, dehiscent by longitudinal slits, dorsifixed near the middle by the very short free part of filiform filaments, attached to the upper part of corolla tubes. Pollens colporate to pororate, apertures 3 or 4, exine surface minutely reticulate, released as monads. Styles filiform, usually glabrous and included to slightly exserted just beyond the corolla tubes, stigmatic lobes 2–5, filiform to oblong or very narrowly elliptic or oblanceolate to widely spathulate or dilated. Ovaries usually (sub-) globose, 2–7-locular with axile placentation and numerous ovules per locule. Fruits (sub-) globose, indehiscent berries. Seeds minute, usually numerous, variously angular, exotesta cells narrow and elongated, with few to many rounded pits, radial wall with verrucose thickenings. Indument of stem, branches, leaves, stipules, inflorescences, bracts, hypanthia and corolla tubes isolatedly to densely puberulous to hirsute or pilose, strigose or sericeous to villous, velutinous or arachnose and indument of corolla orifice or inside the corolla tubes usually moniliform. The karyologically reported taxa are tetraploid with basic chromosome numbers $x = 9$ or 11 (Kiehn, 1995). Number of species: ca. 170 species (106 confined to the African mainland, 54 restricted to the Neotropics, 6 endemic to Madagascar, 3 to São Tomé and Príncipe, and 1 to Sri lanka).

Sabicea s.l. can easily be distinguished from the other three genera of Sabiceeae s.l. by the combination of the following characters: axillary inflorescences usually composed of few to many flowered fascicles or densely capitulate to laxly paniculate cymes or solitary flowers, hypocrateriform or broadly infundibuliform corollas with ovate, (sub-) acute lobes, anthers and 2–6-lobed stigmata usually included in the corolla tubes, moniliform trichomes of corolla orifice or inside the corolla tubes, and narrow to elongated exotesta cells of seeds, with few to many rounded pits and verrucose thickenings on the radial wall (see also Table 3).

New combinations

Sabicea apocynaceum (K. Schum.) Razafim., B. Bremer, Liede & Khan, **comb. nov.** ≡ *Ecpoma apocynaceum* K. Schum. in Bot. Jahrb. 23: 430. 1897 – Type: Cameroon, Lolodorf, Feb (fl.), *Staudt 208* (holotype, B, presumably destroyed; K, photo).

Sabicea aurifodinae (N. Hallé) Razafim., B. Bremer, Liede & Khan, **comb. nov.** ≡ *Pseudosabicea aurifodinae* N. Hallé in Fl. Gabon 12: 201. 1966 – Type: Gabon, Moubigou-2, au bout de la route de Massima vers Moumba, région d'Etéké, *N. Hallé & G. Cours 6137* (holotype, P).

Sabicea becquetii (N. Hallé) Razafim., B. Bremer, Liede & Khan, **comb. nov.** ≡ *Pseudosabicea becquetii* N. Hallé in Bull. Jard. Bot. État Bruxelles 34: 400. 1964 – Type: Burundi, Bururi chefferi Arawe-territoire, alt. 1,600 m, *Becquet 115* (holotype, P; isotype, K).

Sabicea proselyta (N. Hallé) Razafim., B. Bremer, Liede & Khan, **comb. nov.** ≡ *Pseudosabicea proselyta* N. Hallé in Adansonia ser. 2, 3: 172. 1963 – Type: Gabon, la Nkoulounga, 11 Jul 1959, *N. Hallé 748* (holotype, P).

Sabicea sanguinosa (N. Hallé) Razafim., B. Bremer, Liede & Khan, **comb. nov.** ≡ *Pseudosabicea sanguinosa* N. Hallé in Adansonia ser. 2, 11: 313. 1971 – Type: Gabon, environs de la Station forestière du Petit Bam-Bam, 50 km SW de la base rivière Ramboué, au sud de l'Estuaire, pays de savanes, 21 Aug 1966 (fl.), *N. Hallé & A. Le Thomas 573* (holotype, P).

Sabicea sthenula (N. Hallé) Razafim., B. Bremer, Liede & Khan, **comb. nov.** ≡ *Pseudosabicea sthenula* N. Hallé in Fl. Gabon 12: 208. 1966 – Type: Gabon, Makokou, 27 Feb 1961, *N. Hallé 1339* (holotype, P).

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LITERATURE CITED

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.

Andersson, L. 1996. Circumscription of the tribe Isertieae (Rubiaceae). *Opera Bot. Belg.* 7: 139–164.

Andreasen, K., Baldwin, B.G. & Bremer, B. 1999. Phylogenetic utility of the nuclear rDNA ITS region in the subfamily Ixoroideae (Rubiaceae): comparisons with cpDNA *rbcL* sequence data. *Pl. Syst. Evol.* 217: 119–135.

Andreasen, K. & Bremer, B. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology, nuclear and chloroplast DNA data. *Amer. J. Bot.* 87: 1731–1748.

Arnott, G.A.W. 1839. Descriptions of some new or rare Indian plants. *Ann. Nat. Hist.* 3: 20–23.

Aublet, J.B.C.F. 1775. *Sabicea*, Pentandria, Monogynia. Pp. 192–196 in: *Histoire des plantes de la Guiane Françoise*, vol. 1. P.-F. Didot jeune, London & Paris.

Baldwin, B.G., Anderson, M.J.S., Porter, J.M., Wojciechowski, M.F., Campbell, C.S. & Donoghue, M.J. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann. Missouri Bot. Gard.* 82: 247–277.

Bayer, R.J., Greber, D.G. & Bagnall, N.H. 2002. Phylogeny of Australian Gnaphalieae (Asteraceae) based on chloroplast and nuclear sequences, the *trnL* intron, *trnL/trnF* intergenic spacer, *matK*, and ETS. *Syst. Bot.* 27: 801–814.

Bremekamp, C.E.B. 1934. Notes on Rubiaceae of Surinam. *Recueil Trav. Bot. Néerl.* 31: 248–308.

Bremekamp, C.E.B. 1952. The African species of *Oldenlandia* L sensu Hiern et K. Schumann. *Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2.*, 48: 1–297.

Bremekamp, C.E.B. 1966. Remarks on the position, the delimitation and the subdivision of the Rubiaceae. *Acta Bot. Neerl.* 15: 1–33.

Bremer, B. 1996. Combined and separate analyses of morphological and molecular data in the plant family Rubiaceae. *Cladistics* 12: 21–40.

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. & Manen, J.F. 2000. Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 225: 43–72.

Bremer, B. & Thulin, M. 1998. Collapse of Isertieae, re-establishment of Mussaendeae, and a new genus of Sabiceeae (Rubiaceae): phylogenetic relationships based on *rbcL* data. *Pl. Syst. Evol.* 211: 71–92.

Darwin, S.P. 1976. The subfamilial, tribal, and subtribal nomenclature of the Rubiaceae. *Taxon* 25: 595–610.

Dessein, S., Andersson, L., Robbrecht, E. & Smets, E. 2001a. *Hekistocarpa* (Rubiaceae): a member of an emended tribe Virectarieae. *Pl. Syst. Evol.* 229: 59–78.

Dessein, S., Jansen, S., Huysmans, S., Robbrecht, E. & Smets, E. 2001b. A morphological and anatomical survey of *Virectaria* (African Rubiaceae), with a discussion of its taxonomic position. *Bot. J. Linn. Soc.* 137: 1–29.

Deruelle, B., Moreau, C., Nkoumbou, C., Kambou, R., Lissom, J., Njongfang, E., Ghogomu, R.T. & Nono, A. 1991. The Cameroon Line: a review. Pp. 274–327 in: Kampunzu, A.B. & Lubala, R.T. (eds.), *Magmatism in Extensional Structural Settings*. Springer Verlag, Berlin.

Good, R. 1923. New Tropical African Rubiaceae. *J. Bot.*, 61: 86.

Grisebach, A.H.R. 1861. XCIV Rubiaceae. Pp. 316–351 in: *Flora of the British West Indian Islands*. L. Reeve & Co., London.

Hallé, F. 1961. Contribution à l'étude biologique et taxonomique des Mussaendeae (Rubiaceae) d'Afrique tropicale. *Adansonia* 1: 266–298.

Hallé, N. 1963. Délimitation des genres *Sabicea* Aubl. et *Ecpoma* K. Schum. en regard d'un genre nouveau: *Pseudosabicea* (Mussaendeae-Rubiaceae). *Adansonia* ser. 2, 3: 168–177.

Hallé, N. 1966. Famille des Rubiacées (1re partie). Vol. 12 of: Aubréville, A. (ed.), *Flore du Gabon*. Muséum National d'Histoire Naturelle, Laboratoire de Phanérogamie, Paris.

Hallé, N. 1970. Famille des Rubiacées (2e partie). Vol. 17 of: Aubréville, A. & Leroy, J.F. (eds.), *Flore du Gabon*. Muséum National d'Histoire Naturelle, Laboratoire de Phanérogamie, Paris.

Hepper, F.N. 1958. *Sabicea* Aubl. and *Stipularia* Beauv. (Rubiaceae-Mussaendeae) in Tropical Africa. *Kew Bull.* 289–294.

Hiern, W.P. 1877. Ordo LXX. Rubiaceae. Pp. 33–82 in: Oliver, D., Dyer, W.T.T., Prain, D. & Hill, A.W. (eds.), *Flora of Tropical Africa*, vol. 3. L. Reeve & Co., London.

Hooker, J.D. 1873a. Ordo LXXXIV, Rubiaceae. Pp. 7–151 in: Bentham, G. & Hooker, J.D. (eds.), *Genera Plantarum ad exemplaria imprimis in herbariis Kewensibus servanta defirmata*, vol. 2. L. Reeve & Co., London.

Hooker, J.D. 1873b. *Hekistocarpa minutiflora*. *Icones Plantarum* 12: 46. William Pamplin, London.

Huelsenbeck, J.P. & Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

Joffroy, G. 2001. Le genre *Sabicea* (Rubiaceae) à São Tomé (São Tomé et Príncipe). *Syst. Geogr. Pl.* 71: 383–390.

Kiehn, M. 1995. Chromosome survey of the Rubiaceae. *Ann. Missouri Bot. Gard.* 82: 398–408.

Kirkbride, M.C.G. 1979. Review of the Neotropical Isertieae (Rubiaceae). *Brittonia* 31: 313–332.

Kirkbride, M.C.G. 1982. A preliminary phylogeny for Neotropical Rubiaceae. *Pl. Syst. Evol.* 141: 115–121.

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.

Liede, S. & Kunze, H. 2002. *Cynanchum* and the Cynanchinae (Apocynaceae–Asclepiadoideae)—a molecular, anatomical and latex triterpenoid study. *Organisms Divers. Evol.* 2: 239–269.

Meisner, C.F. 1838. Plantarum Vascularium Genera, Secundum Ordines Naturales Digesta, 1: 162. Libraria Weidmannia, Lipsiae (Leipzig).

Meve, U. & Liede, S. 2002. A molecular phylogeny and generic rearrangement of the stapelioid Ceropegieae (Apocynaceae-Asclepiadoideae). *Pl. Syst. Evol.* 234: 171–209.

Motley, T.J., Wurdack, K.J. & Delprete, P.G. 2005. Molecular systematics of the Catesbaeeae-Chiococceae complex (Rubiaceae): flower and fruit evolution and biogeographic implications. *Amer. J. Bot.* 92: 316–329.

Munhá, J., Afonso, R., Caldeira, R. & Mata, J. 2002. Estudo geológico preliminar da região nordeste da Ilha de S Tomé (Folha n° 2, Ana Chaves). *Garcia de Horta, ser. Geol.* 18: 1–8.

Noyes, R.D. 2006. Intraspecific nuclear ribosomal DNA divergence and reticulation in sexual diploid *Erigeron strigosus* (Asteraceae). *Amer. J. Bot.* 93: 470–479.

Nylander, J.A.A. 2004. MrModeltest, v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Page, R.D.M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput. Applic. Biosci.* 12: 357–358.

Palisot-Beauvois, A.M.F.J. 1810 [1807]. Stipulaire. *Stipularia*, Fam. des Rubiacées. P. 26 in: *Flore d'Oware et de Bénin, en Afrique*. Imprimerie de Fain et compagnie, Paris.

Persson, C. 2000. Phylogeny of the Neotropical *Alibertia* group (Rubiaceae), with emphasis on the genus *Alibertia*, inferred from ITS and 5S Ribosomal DNA sequences. *Amer. J. Bot.* 87: 1018–1028.

Puff, C., Igersheim, A. & Buchner, R. 1998. Character states and taxonomic position of the monotypic Sri Lankan *Schizostigma* (Rubiaceae–Isertieae). Pp. 187–203 in: Dransfield, J., Coode, M.J.E. & Simpson, D.A. (eds.), *Plant Diversity in Malesia III*. Royal Botanic Gardens, Kew, London.

Queiroz, A. de, Donoghue, M.J. & Im, J.K. 1995. Separate versus combined analysis of phylogenetic evidence. *Annu. Rev. Ecol. Syst.* 26: 657–681.

Razafimandimbison, S.G. & Bremer, B. 2001 [2002]. Tribal delimitation of Naucleeae (Cinchonoideae, Rubiaceae): inference from molecular and morphological data. *Syst. Geogr. Pl.* 71: 515–538.

Razafimandimbison, S.G. & Bremer, B. 2002. Phylogeny and classification of Naucleeae s.l. (Rubiaceae) inferred from molecular (ITS, *rbcl*, and *trnT-F*) and morphological data. *Amer. J. Bot.* 89: 1027–1041.

Razafimandimbison, S.G., Kellogg, E.A. & Bremer, B. 2004. Recent origin and phylogenetic utility of divergent ITS putative pseudogenes: a case study from Naucleeae (Rubiaceae). *Syst. Biol.* 53: 177–192.

Razafimandimbison, S.G. & Miller, J.S. 1999. New taxa and nomenclatural notes on the flora of the Marojejy Massif, Madagascar. III. Rubiaceae. A new species of *Sabicea*. *Adansonia* ser. 3, 21: 41–45.

Renner, S. 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. *Int. J. Pl. Sci.* 165 (Supplement): S23–S33.

Robbrecht, E. 1988. *Tropical woody Rubiaceae*. Opera Botanica Belgica 1. National Botanic Garden of Belgium, Meise.

Robbrecht, E. 1993. Supplement to the 1988 outline of the classification of the Rubiaceae, index to genera. Pp. 173–196 in: Robbrecht, E. (ed.), *Advances in Rubiaceae Macrosystematics*. Opera Botanica Belgica 6. National Botanic Garden of Belgium, Meise.

Robbrecht, E. 1996. Generic distribution patterns in subsaharan African Rubiaceae (Angiospermae). *J. Biogeogr.* 23: 311–328.

Robbrecht, E. & Manen, J.F. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nrDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-trnF* and *atpB-rbcL* data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. *Syst. Geogr. Pl.* 76: 85–146.

Rova, J.H.E., Delprete, P.G., Andersson, L. & Albert, V.A. 2002. A *trnL-F* cpDNA sequence study of the Condamineeae-Rondeletieae-Sipaneeae complex with implications on the phylogeny of the Rubiaceae. *Amer. J. Bot.* 89: 145–159.

Schumann, K. 1896 [1897]. Beiträge zur Flora von Afrika, XIII, Rubiaceae africanae. *Bot. Jahrb. Syst.* 23: 412–470.

Simmons, M.P. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol.* 49: 369–381.

Steyermark, J.A. 1962. *Pittierothamnus*, new genus of Rubiaceae. *Bol. Soc. Venez. Ci. Nat.* 23: 92–95.

Steyermark, J.A. 1972. Rubiaceae. Pp. 227–832 in: Maguire, B. & Collaborators, The Botany of the Guayana Highland, Part IX. *Mem. New York Bot. Gard.* 23: 1–832.

Steyermark, J.A. 1974. Rubiaceae. Pp. 7–539 in: Lasser, T., *Flora de Venezuela*, vol. IX, primera parte. Ministerio de Agricultura y Cría, Caracas.

Swofford, D.L. 2000. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, version 4.0b. Sinauer Associates, Sunderland, Massachusetts.

Tate, J.A., Aguilar, J.F., Wagstaff, S.J., LaDuke, J.C., Bodo Slotta, T.A. & Simpson, B.B. 2005. Phylogenetic relationships within the tribe Malveae (Malvaceae, subfamily Malvoideae) as inferred from its sequence data. *Amer. J. Bot.* 92: 584–602.

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. *Nucleic Acids Res.* 22: 4673–4680.

Verdcourt, B. 1958. Remarks on the classification of the Rubiaceae. *Bull. Jard. Bot. État Bruxelles* 28: 209–314.

Verdcourt, B. 1975. New sectional names and a new tribe Virectarieae (Rubiaceae). *Kew Bull.* 30: 366.

Wernham, H.F. 1914. *A Monograph of the Genus Sabicea*. British Museum (Natural History), London.

White, F. 1979. The Guineo-Congolian region and its relationships to other phytochoria. *Bull. Jard. Bot. Natl. Belg.* 49: 11–55.

White F. 1993. The AETFAT chorological classification of Africa: history, methods and applications. *Bull. Jard. Bot. Natl. Belg.* 62: 225–281.

Appendix. Voucher information and accession numbers for all species included in this study.

Species, country origins, voucher, *trnT-F* acc. no., ITS acc. no.

Alberta magna E. Mey., GenBank, AJ620118, –; *A. magna*, GenBank, -, AJ224842; *Canthium coromandelicum* (Burm. f.) Alston, GenBank, AJ847401, –; *C. coromandelicum*, GenBank, -, AJ315081; *Cinchona pubescens* Vahl, GenBank, AJ346963, –; *Coffea eugenioides* S. Moore, GenBank, AJ847402, –; *Coussarea* sp., GenBank, AF152612, –; *Danais xanthorrhoea* (K. Schum.) Bremek., GenBank, AM409329, –; *Ecpoma hierniana* (Wernham) N. Hallé & F. Hallé, *Thompson 1803* (K), AM409140, AM409055; *Euclinia longiflora* Salisb., GenBank, AJ847399, –; *Gynochthodes coriacea* Blume, GenBank, AJ847407, –; *Heinsia zanzibarica* (Boj.) Verdc., GenBank, AJ847377, AJ846880; *Hekistocarpa minutiflora* Hook. f., Cameroon, *Sonké & al. 2708* (BR), AM409141, AM409056; *Isertia pittieri* (Standl.) Standl., GenBank, AJ847404, –; *Ixora coccinea* L., GenBank, AJ620117, –; *I. coccinea*, GenBank, -, AJ224826; *Luculia grandifolia* Ghose, GenBank, AJ346929, –; *Mussaenda pinatubensis* Elmer, GenBank, AJ847365, –; *Nauclea orientalis* (L.) L., GenBank, AJ346958, –; *Normandia neocaledonica* Hook. f., New Caledonia, *Munzinger 532* (MO), AM409177, –; *Ophiorrhiza mungos* L., GenBank, AF152610, –; *Pentaloncha humilis* Hook. f. (2), Gabon, *Wilde & al. 10235* (WAG), AM409173, –; *P. humilis* (1), Gabon, *Breteler & al. 10985* (WAG), AM409174, –; *Pentas parvifolia* Hiern, GenBank, AJ847406, –; *Pseudosabicea arborea* (K. Schum.) N. Hallé (1), Burundi, *Reekmans 11116* (K), AM409167, AM409049; *P. arborea* (2), Burundi, *Reekmans 11116* (WAG), AM409138, AM409050; *P. aurifodinae* N. Hallé, Gabon, *Wieringa & al. 5026* (WAG), AM409162, AM409046; *P. batesii* (Wernham) N. Hallé, Gabon, *Valkenburg & al. 2569* (WAG), AM409139, AM409048; *P. medusula* (K. Schum. ex Wernham) N. Hallé, Cameroon, *Andel & al. 3555* (WAG), AM409163, AM409047; *P. mildbraedii* (Wernham) N. Hallé, Gabon, *Wieringa & al. 5032* (WAG), AM409137, AM409051; *P. nobilis* (R. Good) N. Hallé, Gabon, *Valkenburg & al. 2604* (WAG), AM409165, AM409052; *P. proselyta* N. Hallé, Gabon, *Valkenburg & al. 2646* (WAG), AM409166, AM409053; *P. segregata* (Hiern) N. Hallé, Gabon, *Wieringa & al. 5025* (WAG), AM409164, AM409054; *Psychotria amboniana* K. Schum., GenBank, AJ847409, –; *Sabicea amazonensis* Wernham, Brazil, *Campbell & al. P22037* (MO), AM409157, AM409007; *S. angolensis* Wernham, Republic of the Congo, *Lisowski B-7136* (BR), AM409142, AM409006; *S. aspera* Aubl., French Guiana, *Andersson & al. 2003* (NY), AM409143, AM409008; *S. brevipes* Wernham, Ghana, *Jongkind & Nieuwenhuis 2793* (WAG), AM409178, AM409009; *S. caminata* N. Hallé, Gabon, *Wilde & Sosef 10311* (WAG), AM409118, AM409010; *S. capitellata* Benth., Equatorial Guinea, *Sonké & Esono 2533* (BR), AM409161, AM409012; *S. chocoana* C.M. Taylor, Colombia, *Delprete 6342* (NY), AM409144, AM409013; *S. cinerea* Aubl., French Guiana, *Andersson & al. 1903* (NY), AM409120, AM409014; *S. congensis* Wernham, Gabon, *Breteler 12428* (WAG), AM409146, AM409015; *S. dewevrei* De Wild. & T. Durand, Republic of the Congo, *Lemaire 1393* (BR), AM409121, AM409016; *S. dinklagei* K. Schum., Malawi, *Pawek 6510* (UPS), AM409122, AM409017; *S. discolor* Stapf, Ivory Coast, *Jongkind & al. 4880* (WAG), AM409145, AM409018; *S. diversifolia* Pers., GenBank, AJ847396, AJ846883; *S. exellii* G. Taylor, São Tomé and Príncipe, *Joffroy 188* (BRLU), AM409124, AM409020; *S. ferruginea* Benth., Liberia, *Jongkind & al. 5683* (WAG), AM409125, AM409021; *S. fulva* Wernham, Gabon, *Wieringa & al. 4094* (WAG), AM409126, AM409022; *S. gilletii* De Wild., Dem. Rep. of the Congo (Zaire), *Lejoly 82/903* (BR), AM409154, AM409023; *S. glabrescens* Benth., Guyana, *Gillespie & Tiwari 825* (NY), AM409147, AM409024; *S. grisea* Cham. & Schltdl., Brazil, *Arbo & al. 7191* (NY), AM409159, AM409040; *S. harleyae* Hepper, Ivory Coast, *Jongkind & al. 4867* (WAG), AM409152, AM409025; *S. humilis* S. Moore, Brazil, *Malme 2684* (S), AM409148, AM409026; *S. ingrata* K. Schum., São Tomé and Príncipe, *Ogonnovsky 10* (BRLU), AM409149, AM409027; *S. johnstonii* K. Schum. ex Wernham, Gabon, *Wieringa & al. 4652* (WAG), AM409150, AM409028; *S. mattogrossensis* Wernham, Bolivia, *Beck & Haase 9986* (NY), AM409127, AM409029; *S. mexicana* Wernham, Mexico, *Hahn 639* (NY), AM409153, AM409030; *S. najatrix* N. Hallé, Gabon, *Wieringa & al. 4653* (WAG), AM409128, AM409031; *S. orientalis* Wernham, Tanzania, *Mhoro 443* (UPS), AM409155, AM409032; *S. panamensis* Wernham, Ecuador, *Harling & Ståhl 26896* (S), AM409156, AM409033; *S. pyramidalis* L. Andersson, Ecuador, *Burnham 1455* (F), AM409129, AM409034; *S. rosea* Hoyle, Ivory Coast, *Jongkind 4550* (WAG), AM409158, AM409035; *S. seua* Wernham, Madagascar, *Malcomber & al. 1085* (WAG), AM409130, AM409036; *S. speciosa* K. Schum., Nigeria, *Meer 1623* (WAG), AM409131, AM409037; *S. thomensis* Joffroy, São Tomé and Príncipe, *Ogonnovsky 18* (BRLU), AM409132, AM409038; *S. venezuelensis* Steyerm., Venezuela, *Huber 4201* (NY), AM409133, AM409039; *S. venosa* Benth., Central Africa Republic, *Sonké & Beina 2797* (WAG), AM409134, AM409041; *S. villosa* Willd. ex Roem. & Schult. (1), Costa Rica, *Delprete 5102* (NY), AM409160, AM409042; *S. villosa* (2), Ecuador, *Delprete & Verduga 6396* (NY), AM409135, AM409043; *S. vogelii* Benth., Ivory Coast, *Jongkind & al. 4859* (WAG), AM409136, AM409044; *S. xanthotricha* Wernham, Cameroon, *Sonké 1082* (BR), AM409151, AM409045; *Sabicea* sp., Bolivia, *Nee 46014* (NY), AM409119, AM409011; *Schizostigma hirsutum* Arn. (= *S. ceylanica* Puff.), Sri Lanka, *Iwarsson 576* (UPS), AM409168, AM409057; *Stipularia efulenensis* Hutch., Cameroon, *Andel 3417* (WAG), AM409123, AM409019; *S. elliptica* Schweinf. ex Hiern, Dem. Rep. of the Congo (Zaire), *Lisowski 56663* (BR), AM409169, AM409058; *Tamridaea capsulifera* (Balf. f.) Thulin & B. Bremer, Yemen, *Miller & al. 10087* (UPS), AM409170, AM409059; *Tarenna neurophylla* (S. Moore) Bremek., GenBank, AJ847403, –; *Temnopteryx sericea* Hook. f. (1), Equatorial Guinea, *Wieringa & Haegens 2266* (WAG), AM409175, –; *T. sericea* (2), Gabon, *Tabak 99* (WAG), AM409176, –; *Virectaria multiflora* (Sm.) Bremek., Ivory Coast, *Leeuwenberg 2295* (UPS), AM409171, AM409060; *V. procumbens* (Sm.) Bremek., Liberia, *Adams 453* (UPS), AM409172, AM409061; *Warszewiczia coccinea* Klotzsch, GenBank, AJ847397, AJ846884.