The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2010, **162**, 453–476. With 4 figures

Phylogenetic relationships of Combretaceae inferred from nuclear and plastid DNA sequence data: implications for generic classification

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Received 27 August 2009; accepted for publication 8 January 2010

The putative complexity of Combretaceae and lack of information on phylogenetic relationships within the family led us to explore relationships between genera of Combretaceae by means of combined analyses of plastid and nuclear sequences. We collected DNA sequence data from the nuclear ribosomal internal transcribed spacer region and plastid *rbcL, psaA-ycf3* spacer and *psbA-trnH* spacer for 14 of the 17 genera of Combretaceae. The current classification of the family into two subfamilies, Strephonematoideae and Combretoideae, is corroborated. Within Combretoideae, division into two tribes, Laguncularieae and Combreteae, is strongly supported. Within Combreteae subtribe Terminaliinae, relationships between genera are largely unresolved. *Terminalia* is not supported as monophyletic and two groups were identified, one containing mainly African species and another of mostly Asian species. *Pteleopsis*, *Buchenavia* and *Anogeissus* are embedded within *Terminalia*, and we suggest that all genera of Terminaliinae, with the exception of *Conocarpus*, should be included in an expanded circumscrition of *Terminalia*. Within subtribe Combretinae, a clade formed by the two monotypic genera *Guiera* and *Calycopteris* is sister to the rest of the subtribe. Groupings in Combretinae are consistent with recent results based on morphological data. *Combretum* is currently divided into three subgenera: *Apethalanthum*, *Cacoucia* and *Combretum.* The last two were included in this study and supported as monophyletic if *Quisqualis* is included within subgenus *Cacoucia*. *Meiostemon* is sister to subgenus *Combretum*. We recommend that subgenus *Combretum* should be expanded to include *Meiostemon* and subgenus *Cacoucia* to include *Quisqualis.* The sectional classification within *Combretum* proposed in earlier morphological studies is confirmed except for the exclusion of *C. imberbe* from section *Hypocrateropsis* in a separate and monotypic section and the inclusion of *C. zeyheri* (section *Spathulipetala*) in section *Macrostigmatea*. In order to accommodate *C. imberbe*, a new section is suggested. The reinstatement of previously recognized sections *Grandiflora* and *Trichopetala*, both of which had been sunk into subgenus *Cacoucia* section *Poivrea*, is proposed.

ADDITIONAL KEYWORDS: *Combretum* – internal transcribed spacer (ITS) – Myrtales – phylogeny – *psaA-ycf3* – *rbcL* – *Terminalia* – *trnH-psbA*.

INTRODUCTION

Combretaceae R.Br. comprise 17 genera and approximately 525 species of trees, shrubs, lianas and mangroves distributed mainly in tropical and subtropical Africa, but also in Central and South America, southern Asia and northern Australia (Mabberley, 2008). The presence of combretaceous hairs, unicellular with slender thick walls and a distinctive basal compartment, on the epidermis of the leaves is

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one of the defining features of Combretaceae (Stace, 1965; Tilney, 2002). These can vary in form and shape and are useful for species identification. Combretaceae are also characterized by scales or stalked glands, which are multicellular structures that are generally more abundant on the lower surfaces of the leaf blade and can also occur on inflorescences and fruits. Scales generally occur in *Combretum* Loefl. subgenus *Combretum* Exell & Stace, *Guiera* Adans. ex Juss. and *Calycopteris* Lam. (Stace, 1965, 2007), whereas stalked glands are found in *Combretum* subgenus *Cacoucia* (Aublet) Exell and Stace, including *Quisqualis* L. and *Calopyxis* Tul. (Stace, 1965).

The family is currently divided into two subfamilies, Combretoideae Engl. & Diels and Strephonematoideae Engl. & Diels, the latter comprised of a single genus, *Strephonema* Hook.f., with three species of trees restricted to western tropical Africa. Strephonematoideae differ from Combretoideae in possessing a semi-inferior ovary, whereas it is inferior for all taxa of Combretoideae. Combretoideae are divided into two tribes, Laguncularieae Engl. & Diels and Combreteae DC., based on adnate prophylls on the hypanthium of Langunculareae (Stace, 2007), whereas these are unfused in members of Combreteae. Laguncularieae comprise four genera of trees, shrubs and mangroves: *Laguncularia* C.F.Gaertn., a monotypic mangrove genus of tropical America and western tropical Africa; *Lumnitzera* Willd. with two species of mangrove shrubs and trees distributed from eastern tropical Africa to Australia, including India and some islands in the Indian and Pacific Oceans; and *Macropteranthes* F.Muell. (five species) and *Dansiea* Byrnes (two species), which are trees and shrubs endemic to Australia.

Combreteae are divided into two subtribes, Terminaliinae (DC.) Exell & Stace and Combretinae Exell & Stace. Terminaliinae include pantropical *Terminalia* L., the largest genus of the subtribe comprising *c*. 150 species of trees (often used as timber) and shrubs. The name *Bucida* L. (1759) predates *Terminalia* (1767) and, according to Stace (2002), its adoption to cover species of *Terminalia* would require *c*. 200 new combinations and is therefore highly undesirable. *Bucida s.s.*, with three species all from Central America, differs from all other American species of *Terminalia* and from nearly all other *Terminalia* in possessing small fruits that retain the upper portion of the hypanthium. However, according to Stace (2002) *Terminalia tetrandra* (Danguy) Capuron, *T. mantaly* H.Perrier and *T. mantaliopsis* Capuron from Madagascar also have fruits like those of *Bucida*. Stace (2002) concluded that the retained upper hypanthium is not unique to *Bucida* and it should therefore be amalgamated with *Terminalia*. Other genera in Terminaliinae are: *Pteleopsis* Engl., an African tree genus of *c*. 10 species; *Buchenavia* Eichler, 20 species of trees and shrubs from tropical America; *Anogeissus* (DC.) Wall., seven species of trees and shrubs distributed from western tropical Africa to Southeast Asia, including India; monotypic *Finetia* Gagnep., a tree endemic to Thailand and Laos; and *Conocarpus* L., with two species of trees and shrubs growing in mangroves from tropical America to tropical Africa, including islands in the Atlantic Ocean to Yemen in the Arabian Peninsula.

Combretinae contain the largest genus of the family, *Combretum*, with approximately 250 species of trees, shrubs, scandent shrubs and lianas occurring in tropical and subtropical regions except in the Pacific and most of Australia. The most recent infrageneric classification (Stace, 1980b) divided *Combretum* into three subgenera: *Combretum*, *Cacoucia* and *Apetalanthum* Exell & Stace. The first two subgenera include most of the species and are distinguished by scales located mainly on the lower surface of the leaf in subgenus *Combretum* and by microscopic stalked glands in subgenus *Cacoucia* (Engler & Diels, 1899; Exell, 1953, 1968, 1970, 1978; Stace, 1969, 1980a, b, Wickens, 1973; Tilney, 2002). Subgenus *Apetalanthum* comprises a single species (*Combretum apetalum* Wall.) from Southeast Asia, which possesses both scales and glandular hairs. Africa is the centre of diversity for the genus with 163 species (Klopper *et al.*, 2006) compared with the Americas with 33, Asia with 27 and Australia with one (Stace, 1980b; Pedley, 1990). According to Stace (2007), *Combretum* currently includes *Quisqualis*, *Calopyxis*, *Thiloa* Eichler and *Meiostemon* Exell & Stace, but not all these taxonomic changes have been formally published. Jongkind (1990) questioned the delimitation of *Combretum* vs. *Quisqualis*. *Quisqualis* comprises 16 species of lianas restricted to the Old World tropics and shares the morphological characters of *Combretum* subgenus *Cacoucia* (i.e. absence of scales, presence of stalked glands). Jongkind (1995) also transferred *Calopyxis*, including *c*. 23 species endemic to Madagascar, to *Combretum*. *Thiloa*, with three species from the Neotropics, and *Meiostemon*, with two species from southern Africa and Madagascar, have often been seen as close relatives of *Combretum* subgenus *Combretum*. The two monotypic genera *Calycopteris* and *Guiera* are restricted to Southeast Asia and western tropical Africa, respectively.

As no comprehensive phylogenetic framework currently exists for Combretaceae, except for the study of Tan *et al.* (2002) with limited sampling, it has been impossible to evaluate any of the above-mentioned taxonomic hypotheses. Thus, in this study, we conducted a phylogenetic analysis of Combretaceae using DNA sequence data from three plastid regions (*rbcL, trnH-psbA* spacer and *psaA-ycf3* spacer) and the internal transcribed spacers (ITS) of nuclear ribosomal DNA. The main objectives were, firstly, to compare results of this study with the current infrageneric taxonomy to determine if classification changes are needed and, secondly, to evaluate the sectional classification within *Combretum*.

MATERIAL AND METHODS

Taxon sampling

Representatives of 14 of the 17 genera accepted in Combretaceae (including 101 species and subspecies) were analysed in this study. Samples were chosen to represent the full range of floral diversity and habits within the family (Table 1). Samples were collected during fieldwork in Limpopo, Mpumalanga, Gauteng, KwaZulu-Natal (all provinces of South Africa) and Botswana, Mozambique, Namibia and Zimbabwe. Samples from cultivated species from the Lowveld National Botanical Garden (Nelspruit, South Africa), Pretoria National Botanical Garden (Pretoria, South Africa), Kirstenbosch National Botanical Garden (Cape Town, South Africa), National Botanic Garden, Harare (Zimbabwe) and the Honolulu Botanical Gardens (USA) were also included, as well as material grown from seed collected in the wild, DNA banks and herbarium specimens from the collections of the Missouri Botanical Garden (MO) and the Royal Botanic Gardens, Kew (K). Four undescribed species from South Africa were also included and will be described jointly with the South African National Biodiversity Institute (SANBI, South Africa). Voucher specimen information and GenBank accession numbers for the taxa used in the study are listed in the Appendix. We were unable to obtain fresh material for *Dansiea, Macropterantes* and *Combretum apetalum*, and PCR was unsuccessful from DNA extracted from herbarium material, which prevents us from assessing relationships of the two Australian genera and between the *Combretum* subgenus *Apetalanthum* with subgenera *Combretum* and *Cacoucia*.

Outgroup and locus selection

The *psaA* and *ycf3* spacer, *rbcL* and ITS regions were chosen because a recent study used these genes (Tan *et al.*, 2002), which enabled us to add to this preexisting dataset. The *trnH-psbA* spacer was also sequenced as it has been shown to be useful at the species level in several families of angiosperms (Sang, Crawford & Steussy, 1997; Lahaye *et al.*, 2008). To select an appropriate outgroup for our analyses, a broadly sampled *rbcL* dataset was analysed; it included 20 representatives of Myrtales Lindl. and a representative of each of the following families: Annonaceae Juss., Escalloniaceae R.Br. ex Dumort., Geraniaceae Juss., Lamiaceae Martynov, Lauraceae Juss., Malvaceae Juss. and Zygophyllaceae R.Br. (Fig. 1). Previous studies did not reach a well-supported topology for relationships among the various families of Myrtales, so we wished to reconfirm outgroup relationships in this study using our own data. Our results confirmed that the pair *Strephonema mannii* Hook.f. and *Strephonema pseudocola* A.Chev. were sister to Combretoideae and thus we used the genus as outgroup in the single region analyses. For the combined molecular analysis of Combretaceae, we added three members of Myrtales (*Galpinia transvaalica* N.E.Br., Lythraceae J.St.-Hil.; *Ludwigia peploides* (Kunth) P.H.Raven, Onagraceae Juss.; *Eugenia uniflora* L., Myrtaceae Juss.) as outgroups because our *rbcL* study and previous studies indicated that Lythraceae, Onagraceae and Myrtaceae are the closest relatives of Combretaceae (Conti *et al.*, 1997; Sytsma *et al.*, 2004).

DNA extraction, PCR and sequencing

DNA was extracted from 0.3 g of silica-gel-dried leaf material (Chase & Hills, 1991) or herbarium material using the 2′ cetyl trimethylammonium bromide (CTAB) method described by Doyle & Doyle (1987), with the addition of 2% polyvinyl pyrrolidone (PVP) to help reduce the effects of high polysaccharide concentration in the samples. To avoid problems of PCR inhibition, all samples were purified using QIAquick purification columns (Qiagen Inc., Hilden, Germany) according to the manufacturer's protocol.

Amplification of *rbcL* was carried out in two overlapping fragments using the following primer combinations: 1F-724R and 636F-1426R (Olmstead *et al.*, 1992; Fay, Swensen & Chase, 1997). The *trnH-psbA* spacer was sequenced using primers 1F and 2R (Sang *et al.*, 1997). The *psaA-ycf3* spacer was amplified using the PG1f and PG2r primers (Huang & Shi, 2002). Additional *Combretum*-specific internal primers were designed to overcome regions of microsatellites located within the *psaA-ycf3* spacer: *psaA-ycf3* IR 5′-CTAGGAACTTCTAATTGAGA′-3 was used to sequence past a poly-T region located *c.* 350 bp from the beginning in several taxa; *psaA-ycf3* IF 5′-CATGTATTTCGAGTCTGTTT-3′ was used to sequence past a similar region located at the 3′ end of the fragment. ITS was amplified in two non-overlapping pieces using two internal primers with a pair of external primers: 17SE-ITS2 and ITS3-26SE (White *et al.*, 1990; Sun *et al.*, 1994).

All reactions were performed using Ready Master mix (Advanced Biotechnologies, Epsom, UK), with addition of 4.5% of dimethylsulphoxide (DMSO) to ITS amplification reactions to reduce secondary struc-

Table 1. Genera studied, including subfamily, tribal and sectional classification. Sections, classification and number of species included within each genus *Combretum* are from Stace (1980a, b), Van Wyk (1984) and Mabberley (2008). Number of species included in this study is indicated in parentheses. Sectional classification of *Terminalia* from Griffiths (1959)

Subfamily	Tribe	Subtribe	Genus	Subgenus	Section	No. of species	Distribution
Strephonematoideae Engl. & Diels			*Strephonema* Hook.f.			3–4 (2)	West tropical Africa
Combretoideae Engl. & Diels	Laguncularieae Engl. & Diels		*Laguncularia* C.F.Gaertn			1 (1)	Tropical America, western tropical Africa
			Lumnitzera Willd.			2 (2)	Eastern tropical Africa to Australia, including India and some islands in the Indian and Pacific Oceans
	Combreteae DC.	Terminaliinae (DC) Exell & Stace	*Anogeissus* (DC.) Wall.			8 (2)	Old World tropics
			Buchenavia Eichler			24 (2)	Tropical America
			Conocarpus L.			2 (2)	Tropical America and Africa, north-east Africa to southern Yemen
			Bucida L. (= *Terminalia* L.)			4 (1)	Florida, Central America, West Indies
			Pteleopsis Engl.			9 (2)	Tropical and southern Africa
			Terminalia L.		*Abreviatae*	(2)	–
					Catappa	(1)	–
					Mantaly	(1)	–
					Myrobalanus	(1)	Africa
					Pentaptera	(1)	–
					Platycarpae	(2)	Africa
					Psidioides	(1)	–
					Stenocarpae	(1)	Africa
					Unknown	(11)	–
		Combretinae Exell & Stace	*Calycopteris* Lam.			1 (1)	South-east Asia
			Calopyxis Tul.			23 (1)	Madagascar
			Combretum Loefl.	*Cacoucia* (Aubl.) Exell & Stace	*Conniventia*	20 (3)	Africa
					Megalantherum	1 (1)	Namibia, Angola
					Oxystachia	1 (1)	Namibia
					Poivrea	31 (6)	Africa, Madagascar and south-east Asia
				Combretum Loefl.	*Angustimarginata*	6 (6)	Southern Africa
					Breviramea	5 (1)	Africa
					Campestria	4 (1)	Africa
					Ciliatipetala	10 (9 + 3)	Africa and Arabian Peninsula
					Glabripetala	8 (3)	Africa
					Hypocrateropsis	6 (5)	Africa
					Macrostigmatea	4 (3 + 1)	Africa
					Mettalicum	4 (1)	Africa
					Micrantha	2 (1)	West Africa
					Spathulipetala	1 (1)	Africa
			Guiera Adans			1 (1)	North tropical Africa
			Meiostemon Exell & Stace			2 (2)	Zambia, Zimbabwe and Madagascar
			Quisqualis L.			16 (3)	Old World tropics
			Thiloa Eichler			3 (1)	Tropical South America

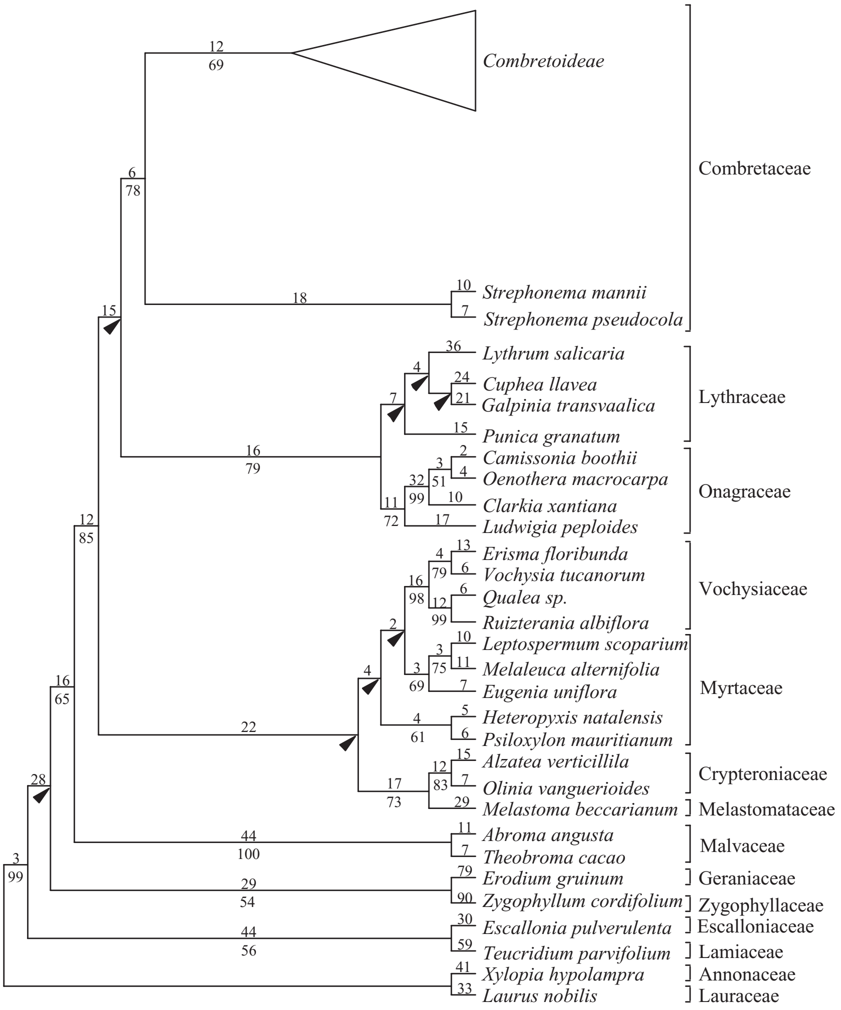

Figure 1. A single randomly selected (of 6340) equally most parsimonious tree based on *rbcL* for Myrtales plus outgroups (TL is 1148 steps, CI 0.48 and RI 0.76). Numbers above the branches are Fitch branch lengths (DELTRAN optimization) and those below the branches are bootstrap percentages above 50. Arrows indicate groups not present in the strict consensus tree. TL, tree length; CI, consistency index; RI, retention index.

ture problems common in ribosomal DNA (Álvarez & Wendel, 2003). PCR amplification was performed using the following programmes: for *rbcL* and *trnH-psbA* spacer 3 min at 94 °C followed by 28 cycles of 1 min 94 °C, 1 min at 48 °C and 1 min at 72 °C, with a final extension at 72 °C for 7 min; for *psaA-ycf3* spacer and ITS 1 min at 94 °C followed by 26 cycles of 1 min at 94 °C, 1 min at 48 °C and 3 min at 72 °C followed by a final 7 min extension (72 °C). Amplified products were purified using QIAquick columns (Qiagen) following the manufacturer's protocol. Cycle sequencing reactions were carried out using BigDye© V3.1 Terminator Mix [Applied Biosystems Inc. (ABI), Warrington, UK] and cleaned using the ethanol–sodium chloride (NaCl) method provided by ABI; they were then sequenced on an ABI 3130*xl* genetic analyser.

Sequencing and alignment

Complementary strands were assembled and edited using Sequencher ver. 4.6 (Gene Codes Corp., Ann Arbor, MI, USA), and sequences were aligned manually in PAUP* (ver. 4.0b1; Swofford, 2002) without difficulty because of low levels of insertions/deletions (indels), except for the *trnH-psbA* spacer for which large regions were excluded from analyses because of alignment difficulties. Nineteen indels were included for the plastid DNA analyses as presence/absence characters. The aligned matrices are available from OM and MWC (olive.maurin@gmail.com; m.chase@kew.org).

Phylogenetic analyses of molecular data

Maximum parsimony (MP) using PAUP* ver. 4.0b1 (Swofford, 2002) was implemented to analyse (1) the *rbcL* data including representative of Myrtales and several phylogenetically distantly related families to identify the appropriate outgroup for Combretaceae, (2) the combined plastid data for Combretaceae, (3) the ITS data for Combretaceae and (4) the combined sequence data for Combretaceae. Tree searches were conducted using 1000 replicates of random taxon addition, retaining 10 trees at each step, with tree–bisection–reconnection (TBR) branch swapping and MulTrees in effect (saving multiple equally parsimonious trees). The trees collected in the 1000 replicates were then used as starting trees for another search without a tree limit. Support for clades in all analyses was estimated using bootstrap analysis (Felsenstein, 1985) with 1000 replicates, simple sequence addition, TBR swapping, with MulTrees in effect but saving a maximum of 10 trees per replicate. Bootstrap support (BP) was classified as high (85–100%), moderate (75–84%) or low (50–74%). All data sets were analysed separately and the individual bootstrap consensus trees examined by eye to identify topological conflicts, i.e. moderate to high support for different placement of taxa. 'Congruence tests' such as the incongruence length test (ILD) can be unreliable (Reeves *et al.*, 2001; Yoder, Irwin & Payseur, 2001) and were not therefore used in this study. Delayed transformation character optimization (DELTRAN) was used to calculate branch lengths, as a result of reported errors (http://paup.csit.fsu.edu/problems.html) with accelerated transformation optimization (ACCTRAN) in PAUP ver. 4.0b1.

We could not amplify *rbcL*, *psaA-ycf3* and ITS for all taxa and thus the three matrices do not contain identical sets of taxa (Appendix). We investigated the effects of these missing sequences on patterns of relationships and support in the combined analysis by performing two combined analyses: (1) all taxa with all data; and (2) all taxa for which at least some data were present. We found that missing data did not affect the second analysis and thus illustrate the combined results with all taxa (analysis 4).

Bayesian analysis (BI; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) was performed using MRBAYES ver. 3.1.2. For each matrix (ITS, *rbcL*, *psaA-ycf3*, *trnH-psbA*) the most appropriate model was selected using MODELTEST ver. 3.06 (Posada & Crandall, 1998). For ITS, *rbcL and psaA-ycf3*, the model GTR+I+G was selected and, for *trnH-psbA*, TIM+G, in which the two resulting models share the same number of substitutions (six) and rates (gamma), base frequency (empirical), clock (unconstrained), and we used 2 000 000 generations with trees sampled every 200. Three analyses were performed: (1) all plastid loci combined; (2) ITS nrDNA; (3) combined plastid and nuclear regions. Partitioned analyses were run for (1) and (3) as different models were selected. The resulting trees were plotted against their likelihoods to determine the point where likelihoods converged on a maximum value and all the trees before the convergence were discarded as 'burn-in' (1000 trees). All remaining trees were imported into PAUP ver. 4.0b10 and a majority-rule consensus tree was produced showing frequencies (i.e. posterior probabilities or PP) of all observed bi-partitions. PP values are shown on the MP trees because the topologies are identical. The following scale was used to evaluate the PPs: < 0.95, weakly supported; 0.95–1.0, well supported.

RESULTS

MOLECULAR EVOLUTION

Statistics for MP analyses for the three plastid partitions and the combined three-region data set are shown in Table 2. Of the plastid regions used, *trnH-psbA* (excluding unalignable regions) had a significantly higher number of variable sites (31.0%; Table 2) than *psaA-ycf3* (24.2%) or *rbcL* (15.2%). The number of potentially parsimony informative characters for *rbcL* within Combretaceae (8.7%) is much lower than for the two other plastid regions, *trnH-psbA* and *psaA-ycf3* (17.4 and 16.0%, respectively). The variable sites evolve at a similar rate for *trnH-psbA* (1.65 changes per variable site) and *rbcL* (1.70 changes per variable site) and performed equally well (as measured by retention index; RI). One insertion of *c*. 300 bp was identified in the *trnH-psbA* data matrix that was cladistically informative, with *Meiostemon* and all species of *Combretum* subgenus *Combretum* sharing this character. The ITS region had more than twice the number of variable sites (45.3%) and potentially parsimony informative characters (54.3%) of any of the plastid regions (Table 2). It evolves at a much faster rate than the plastid genes (4.35 changes per variable site) with a lower consistency index (CI) and RI.

Results from four analyses are presented: the large *rbcL* dataset with a broad outgroup sampling (Fig. 1), combined plastid regions (*rbcL* + *psaA-ycf3* + *trnH-psbA*; Fig. 2), ITS (Fig. 3) and combined plastid and ITS (*rbcL* + *psaA-ycf3* + *trnH-psbA* + ITS; Fig. 4).

ANALYSIS OF *RBCL* WITH A BROAD OUTGROUP SAMPLING (ANALYSIS 1)

Parsimony analysis yielded 6340 most parsimonious trees of 1148 steps, CI 0.48, RI 0.76 (Table 2). One of the most parsimonious trees with branches collapsing in strict consensus tree marked is illustrated in Figure 1. Monophyly of Combretaceae and the two subfamilies, Strephonematoideae and Combretoideae, is moderately to weakly supported (82 and 68 BP,

Table 2. Maximum parsimony statistics from analyses of the separate and combined data sets

	rbcL Myrtales	*rbcL* Combretaceae	*trnH-psbA*	*psaA-ycf3*	Combined plastid	Internal transcribed spacer (ITS)	Combined plastid + ITS
No. of taxa	126	100	85	96	103	93	103
No. of included characters (= aligned length)	1349	1349	667	929	2945	738	3683
No. of constant characters	924	1144	460	704	2308	337	2645
No. of variable sites	425 (31.5%)	205 (15.19%)	207 (31.03%)	225 (24.22%)	637 (21.63%)	401 (54.34%)	1038 (28.18%)
No. of potentially parsimony informative sites	282 (20.9%)	117 (8.67%)	116 (17.39%)	149 (16.04%)	382 (12.97%)	334 (45.26%)	716 (19.44%)
No. of most parsimonious trees	6340	463	4	1426	3910	2500	184
Tree length (TL)	1148	347	341	314	1037	1744	3163
Consistency index (CI)	0.48	0.67	0.75	0.82	0.72	0.41	0.46
Retention index (RI)	0.76	0.87	0.91	0.92	0.88	0.78	0.76
Average number of changes per variable site (number of steps/number of variable sites)	2.70	1.70	1.65	1.39	1.63	4.35	3.05

respectively). Relationships within Combretoideae were unresolved resulting in a large polytomy in the strict consensus tree with only a few supported clades.

We initially included two sequences obtained from GenBank in our analysis namely, AF281477 (*Conocarpus erectus* L.) and AF281478 (*Calycopteris floribunda* Lam.) from the study of Tan *et al.* (2002). Our analysis of *rbcL* sequences placed *C. erectus* sister to *Abroma* Jacq. and *Theobroma* L. (Malvaceae) and *C. floribunda* grouped with *Teucridium* (Lamiaceae). This is in contrast with the results of Tan *et al.* (2002), in which *C. erectus* was placed as sister to Terminaliinae and *C. floribunda* sister to Combretinae. We thus exclude these two accessions from our combined analysis (analysis 4) as this discrepancy may represent a case of misidentification or deposition of the wrong *rbcL* sequences in GenBank.

COMBINED PLASTID ANALYSIS (ANALYSIS 2)

Individual plastid sequence analyses (results not shown) were topologically consistent (negligible to zero incongruence) and, for the purpose of the results and discussion, were combined and treated as a single analysis. The parsimony analysis yielded 3910 most parsimonious trees of 1037 steps, CI 0.72, RI 0.88 (Table 2). One of the most parsimonious trees is illustrated in Figure 2. Results support monophyly of Combretaceae (98 BP/1.0 PP). Subfamilies Strephonematoideae and Combretoideae are strongly supported (96 BP/1.0 PP and 87 BP/1.0 PP). Within Combretoideae, there is weak support for monophyly of tribes Laguncularieae, Terminaliinae and Combretinae excluding *Calycopteris* (52 BP/0.9 PP, 66 BP/1.0 PP and 57 BP/1.0 PP, respectively). Within Terminaliinae, *Terminalia* comprises at least four major clades.

Within the weakly supported Combretinae, the positions of *Calycopteris* and *Guiera* are unresolved. With the exception of *Calycopteris* and *Guiera,* two main clades can be distinguished: clade 1 is weakly (54 BP/1.0 PP) supported and includes all species of *Combretum* subgenus *Combretum* and *Meiostemon* and clade 2, although supported in the strict consensus tree, received support less than 50 BP in the bootstrap analysis. This last clade includes all representatives of *Combretum* subgenus *Cacoucia* and *Quisqualis* and *Calopyxis*.

ITS ANALYSIS (ANALYSIS 3)

Analysis resulted in 2500 equally most parsimonious trees of 1744 steps, CI 0.41, RI 0.78 (Table 2). One of the most parsimonious trees is illustrated in Figure 3. Tribe *Laguncularieae* are strongly supported (100 BP/1.0 PP) as sister to the rest of Combretaceae. Within

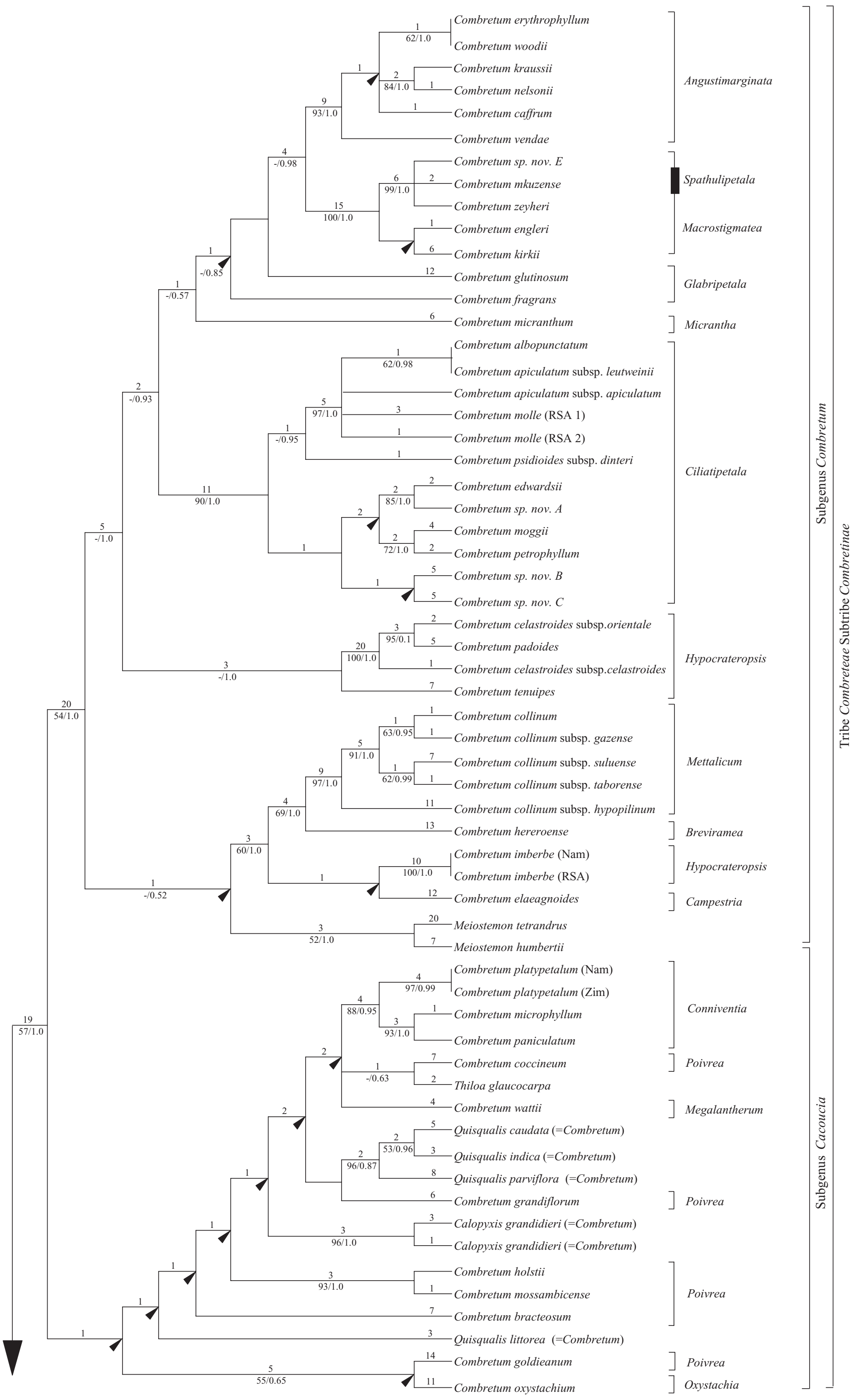

Figure 2. One of the 3910 equally parsimonious trees based on the combined plastid data (TL 1037 steps, CI 0.72 and RI 0.88). Numbers above the branches are Fitch lengths (DELTRAN optimization) and those below the branches are bootstrap percentages above 50/Bayesian PPs > 0.5. Arrows indicate clades not present in the strict consensus tree. Current subtribal (Vollessen, 1981) and sectional (Stace, 1980a) classification is indicated on the right. TL, tree length; CI, consistency index; RI, retention index.

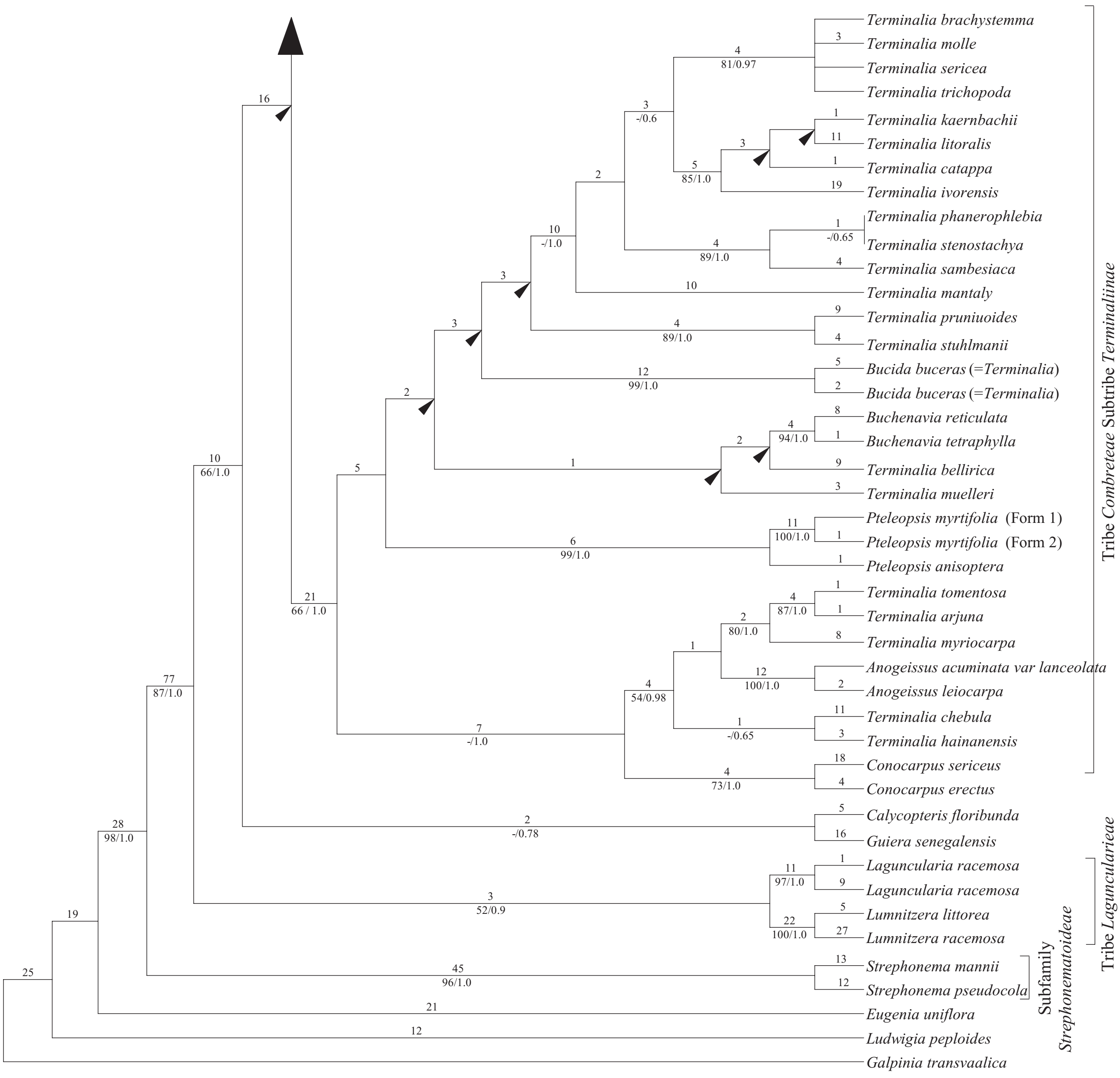

Figure 2. *Continued*

Combretoideae, two subclades are identified, namely Teminaliinae excluding *Conocarpus* (74 BP/1.0 PP) and Combretinae excluding *Calycopteris* and *Guiera* (91 BP/0.94 PP). The positions of *Conocarpus* (Terminaliinae), *Calycopteris* (Combretinae) and *Guiera* (Combretinae) are unresolved within tribe Combretaceae. Within the Terminaliinae clade, groupings comparable with the combined plastid analysis can be observed: *Terminalia* is polyphyletic with at least four clades. Although weakly supported, two main groups in Combretinae are observed; these correspond to *Combretum* subgenus *Cacoucia* (64 BP/0.99 PP) and *Combretum* subgenus *Combretum* (53 BP/0.98 PP). Species of section *Hypocrateropsis* Engl. & Diels form a well-supported clade (99 BP/1.0 PP) as sister to the rest of *Combretum* subgenus *Combretum* with support > 50 BP. As in the combined plastid analysis *Meiostemon*, *Combretum inberbe* Wawra and *Thiloa* are included in Combretinae, but their positions are unresolved.

COMBINED MOLECULAR ANALYSIS (ANALYSIS 4)

Results of the combined plastid analysis are largely congruent with those of the ITS analysis and all data

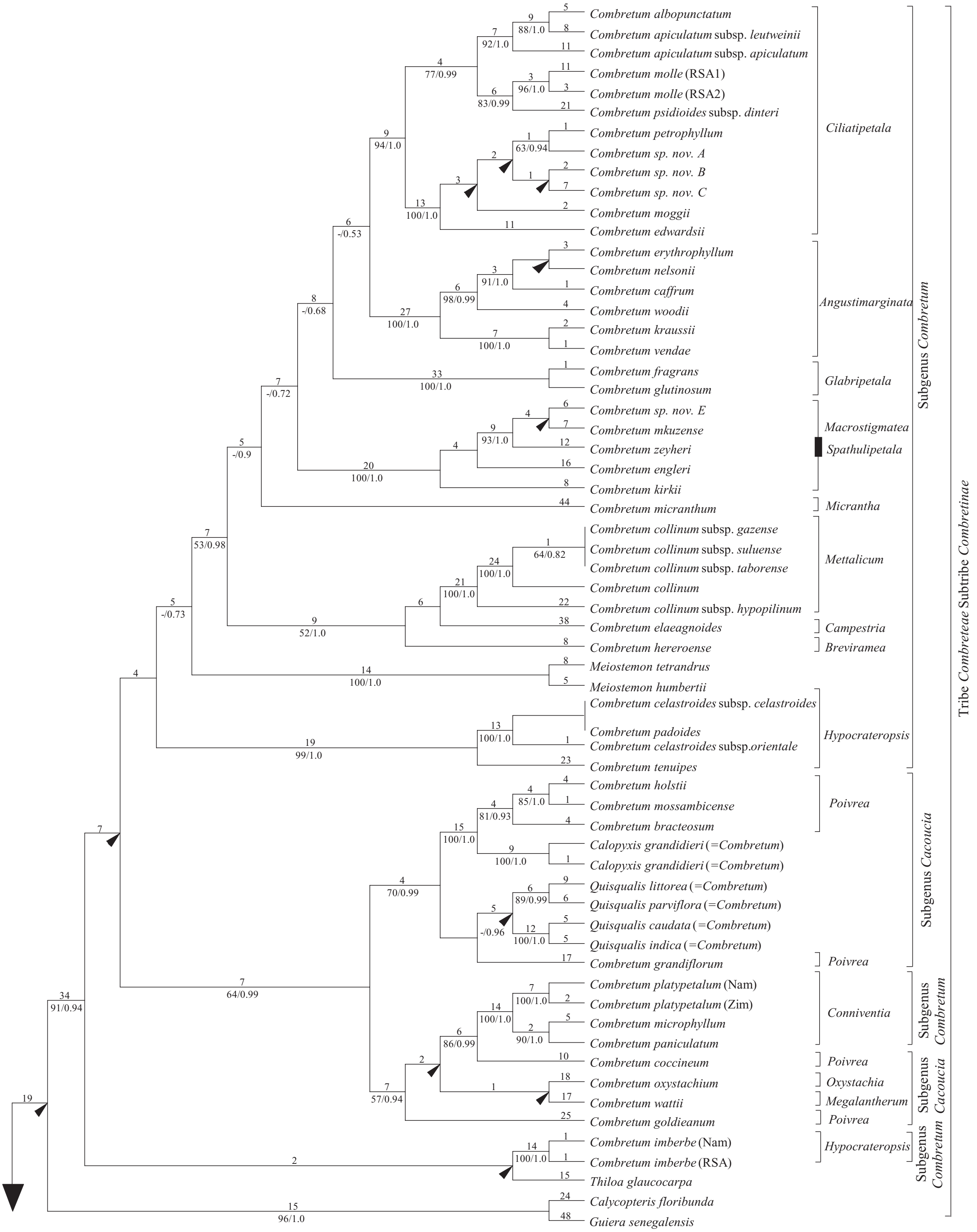

Figure 3. One of the 2500 Fitch trees obtained from the analysis of internal transcribed spacer (ITS). Numbers above the branches are Fitch lengths (DELTRAN optimization) and those below the branches are bootstrap percentages above 50/Bayesian PPs > 0.5. Arrows indicate clades not present in the strict consensus tree. (TL is 1744 steps, CI 0.41 and RI 0.78). Current subtribal (Vollessen, 1981) and sectional (Stace, 1980a) classification is indicated on the right. TL, tree length; CI, consistency index; RI, retention index.

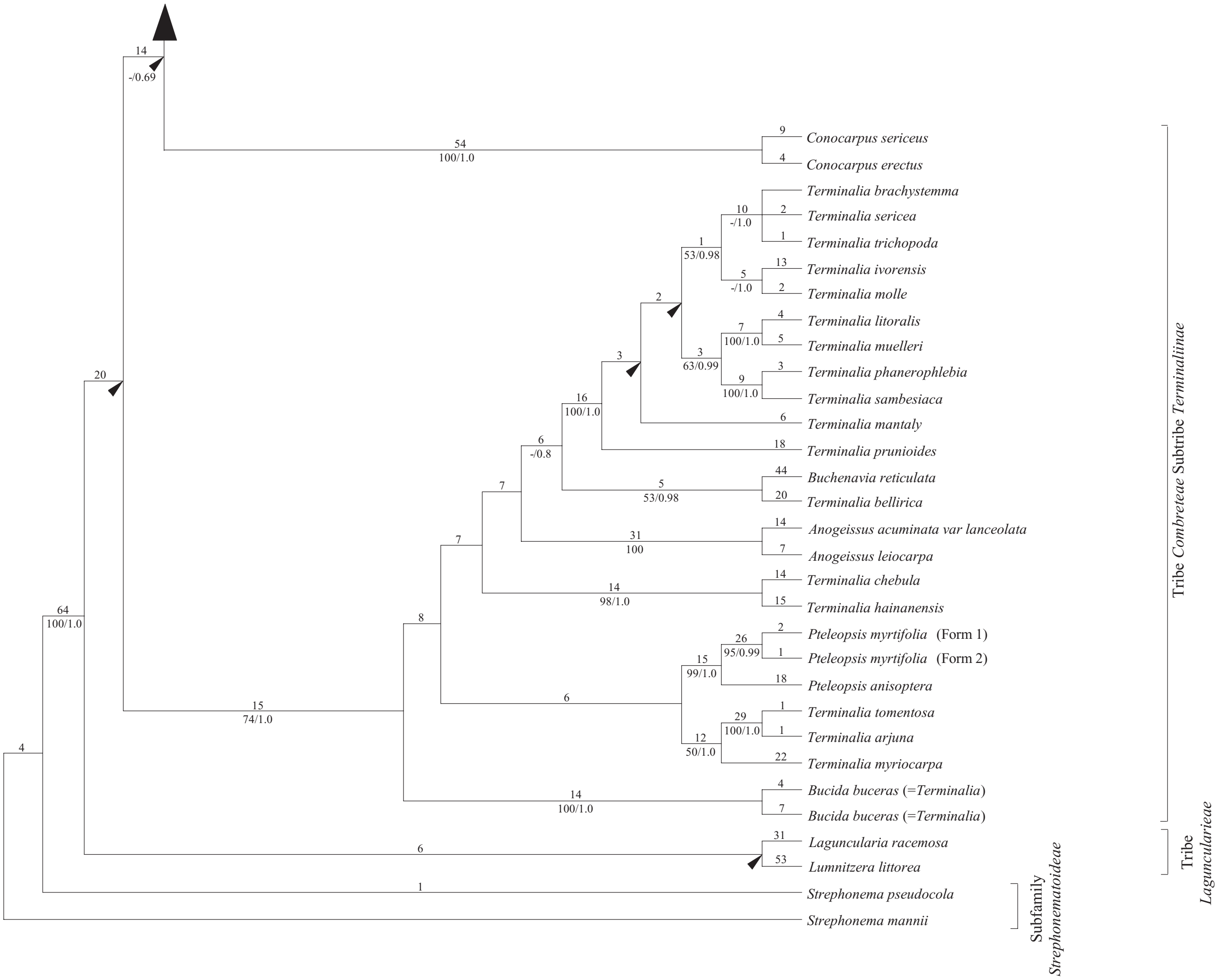

Figure 3. *Continued*

were therefore combined (3683 characters; Table 2). Parsimony analysis produced 184 trees of 3163 steps, CI 0.46, RI 0.76. One of the most parsimonious trees is shown in Figure 4. Combretaceae are strongly supported as monophyletic (96 BP/1.0 PP) with Strephonematoideae (*Strephonema* alone) sister to Combretoideae. Although there is no MP bootstrap support for monophyly of Laguncularieae, BI supports their monophyly (0.95 PP). BI also supports the sister relationship of Languncularieae to Combreteae (77 BP/1.0 PP). Monophyly of Combreteae is weakly supported in the MP analysis (68 BP) and strongly supported in the BP analysis (0.96 PP). Within Combreteae, both subtribes are well supported, Terminaliinae with 72 BP/1.0 PP and Combretinae with 64 BP/1.0 PP. Relationships within Terminaliinae are unresolved in the MP analysis and well resolved in the BI analysis. Two main clades are observed, with *Conocarpus* sister (79 BP/0.98 PP) to the remaining genera of this subtribe. Clade 1 (1.0 PP) contains representatives of *Terminalia* from Africa, Asia, Australia and the Pacific islands. *Buchenavia*, *Bucida* and *Pteleopsis* are embedded within this clade. The second clade (1.0 PP) consists of Asian *Terminalia* taxa. *Anogeissus* is embedded within this second *Terminalia* clade.

In the clade representing Combretinae, *Calycopteris* and *Guiera* are together sister to the remaining taxa (96 BP/1.0 PP). Bayesian analysis produced a more resolved topology than the MP analysis with two well-supported clades. Clade 1 includes all representatives of *Combretum* subgenus *Combretum* (0.99 PP) and *Meiostemon*, whereas clade 2 contains all taxa belonging to *Combretum* subgenus *Cacoucia*, *Quisqualis* and *Calopyxis* (81 BP/1.0 PP). The position of *Thiloa* is unresolved in both the MP and BI analyses. Within subgenus *Combretum*, two main groups are found. The first group (59 BP/1.0 PP) includes sections *Hypocrateropsis* (excluding *Combre-*

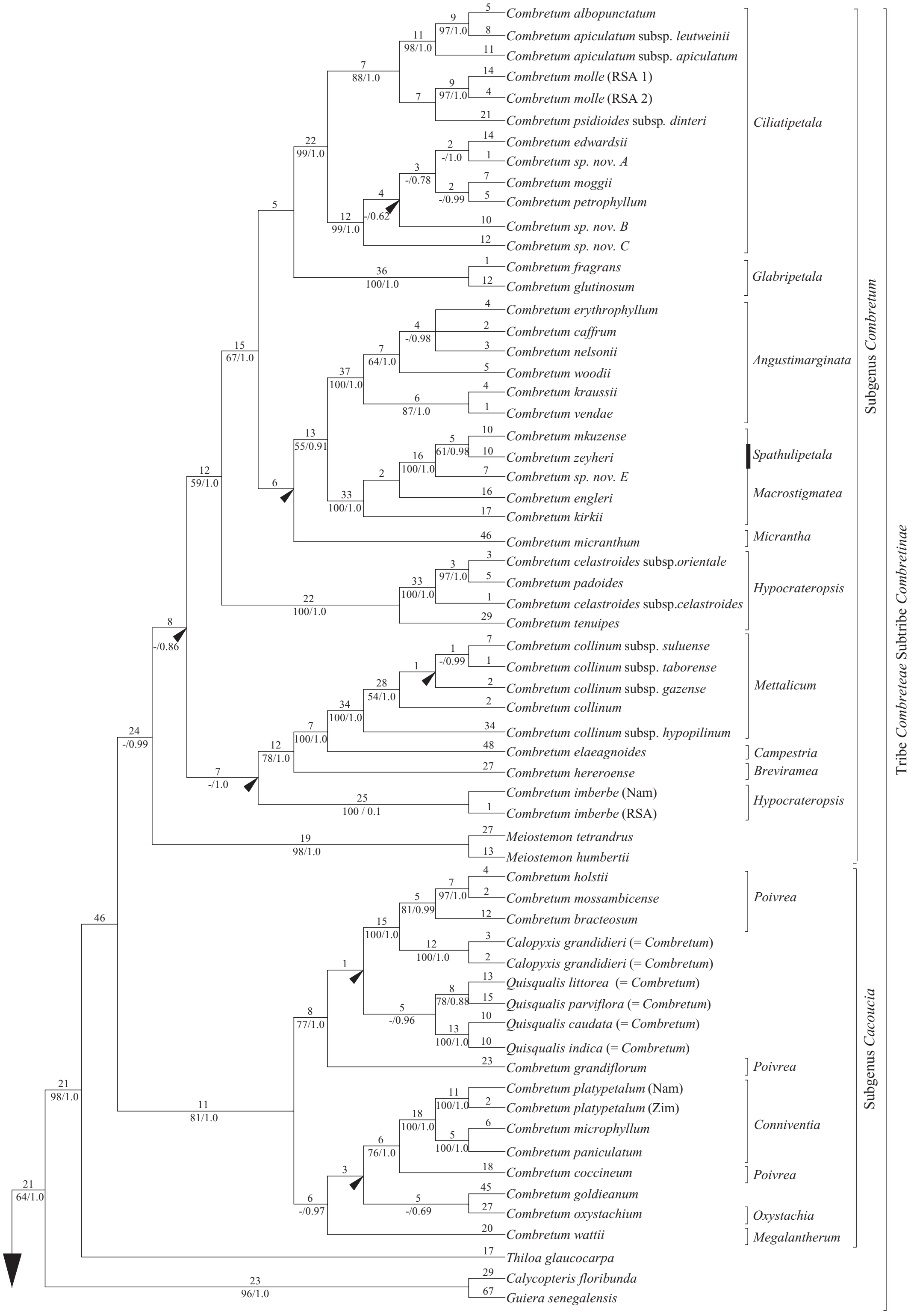

Figure 4. One of the 184 most parsimonious trees (3163 steps, CI 0.46, RI 0.76) from the combined plastid and nuclear internal transcribed spacer (ITS) data set. Numbers above the branches are Fitch lengths (DELTRAN optimization) and those below the branches are bootstrap percentages above 50/Bayesian PPs > 0.5. Arrows indicate clades that collapse in the strict consensus tree. Current subtribal (Vollessen, 1981) and sectional (Stace, 1980a) classification is indicated on the right. CI, consistency index; RI, retention index.

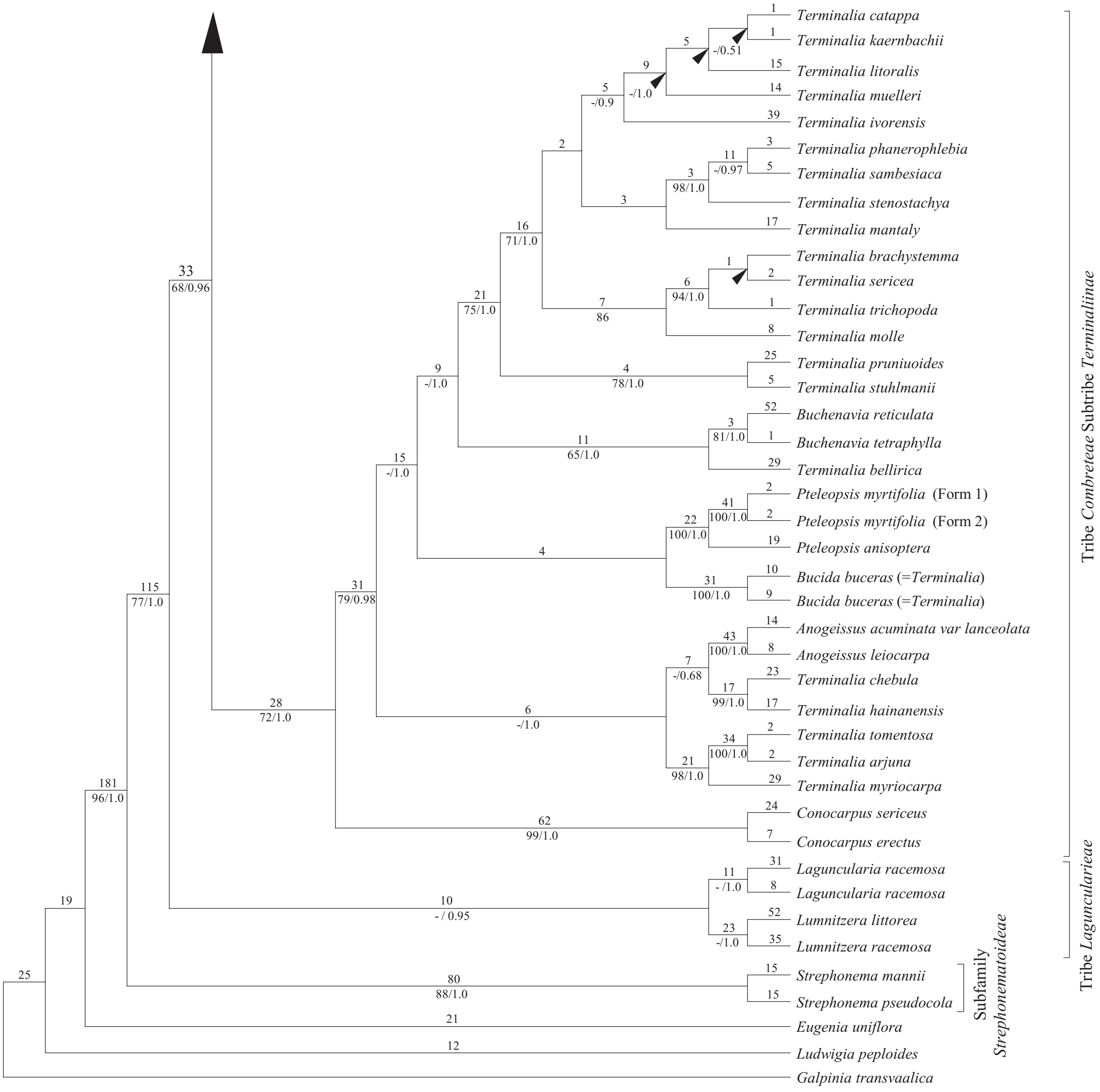

Figure 4. *Continued*

tum imberbe; 100 BP/1.0 PP), *Ciliatipetala* Engl. & Diels (99 BP/1.0 PP), *Macrostigmatea* Engl. & Diels/ *Spathulipetala* Engl. & Diels (100 BP/1.0 PP) and *Angustimaginata* Engl. & Diels (100 BP/1.0 PP). *Meiostemon* is supported as sister to subgenus *Combretum* in the BI analysis (1.0 PP), but its position is unresolved in the MP analysis. Group 2 (1.0 PP) includes sections *Glabripetala* Engl. & Diels, *Mettalicum* Engl. & Diels, *Breviramea* Engl. & Diels, *Campestria* Engl. & Diels and *C. imberbe* from section *Hypocrateropsis*.

DISCUSSION

Because the results of the plastid and ITS matrices are highly congruent, we will restrict our discussion to results obtained from the combined data set (Fig. 4). Our results confirm monophyly of Combretaceae with Strephonematoideae distinct from the remaining genera of the family (96 BP/1.0 PP). *Strephonema* (Strephonematoideae) was initially placed in Lythraceae (Bentham & Hooker, 1867), but Engler & Diels (1900) placed Strephonematoideae in Combretaceae because the genus also possesses combretaceous segmented hairs. *Strephonema* is easily distinguished from the other genera in the family by its revolute domatia, characteristic pattern of epidermal cells, paracytic subsidiary cells and two-armed hairs (Stace, 1965). Furthermore, according to Jongkind (1995), *Strephonema* exhibits more putatively plesiomorphic characters than any other genus in the family. It possesses semi-inferior ovaries and fruits without wings or other obvious adaptations for dispersal (Jongkind, 1995), whereas the remainder of the family possess inferior ovaries and fruits with wings.

RELATIONSHIPS WITHIN TRIBE LAGUNCULARIEAE

Engler & Diels (1899) first considered Languncularieae as one of four tribes within Combretoideae. It was distinguished from the other three tribes by a pair of bracteoles adnate to the lower receptacle and lack of obvious venation on the adaxial epidermis in the leaf surface (Stace, 1965). The three remaining tribes were lumped into a single tribe: Combreteae (Stace, 1965) that included *Laguncularia, Lumnitzera* and *Macropteranthes*. *Dansiea* was described in 1981 and included in Laguncularieae (Stace, 2007). In the MP analysis, monophyly of tribe Laguncularieae did not receive $BP > 50$, whereas it was strongly supported in the BI analysis (0.95 PP).

SUBTRIBAL RELATIONSHIPS WITHIN TRIBE COMBRETEAE

In our sampling of Combreteae we were able to include all genera with the exception of *Finetia*. Subdivision of Combreteae by Vollessen (1981) into two subtribes, Terminaliinae (72 BP/1.0 PP) and Combretinae (64 BP/1.0 PP), is well supported in the MP and BI analyses. The two monotypic genera *Guiera* and *Calycopteris* are together sister to the rest of Combretinae. Although only moderately supported in the MP analysis, *Conocarpus* is strongly supported as sister to the remaining genera and included in Terminaliinae in the BI analysis (79 BP/0.98 PP).

RELATIONSHIPS WITHIN SUBTRIBE TERMINALIINAE

Six genera are currently recognized within Terminaliinae: (1) *Anogeissus* (tropical West Africa to Southeast Asia); (2) *Buchenavia* (tropical America); (3) *Conocarpus* (tropical America, north-eastern Africa and southern Yemen); (4) *Finetia* (Thailand and Laos); (5) *Pteleopsis* (Africa); and (6) *Terminalia* (America, Africa, Madagascar and Asia to Australia; Stace, 2007).

Our results confirm that *Terminalia* is not monophyletic and contains two distinct groups, a mainly African group with a few taxa from the New World and the Pacific islands and a second mostly Asian group including some New World taxa. Although our sampling of *Terminalia* species is relatively small compared with the number of species in the genus, we are suggesting several taxonomical changes. These are based on (1) our phylogenetic results, (2) a recent proposal to transfer *Bucida* to *Terminalia* (Stace, 2002) and (3) the existing doubt regarding the relationship between *Pteleopsis* and *Terminalia* (Vollessen, 1981). We therefore propose that, with the exception of *Conocarpus*, which can easily be distinguished from all other genera of Terminaliinae by its stalked glands (Stace, 1965) and glandular trichomes (Stace, 1980b), all other genera of Terminaliinae should be transferred to *Terminalia*. We are following this option of merging these genera of Terminaliinae within *Terminalia* as the genus predates the others. Much greater sampling will be necessary to assess relationships and develop a modern classification for the tribe.

Pteleopsis spp. are shrubs to small trees without scales or stalked glands, but they often have conspicuous small, pink, conical buds in the axis of the leaf or leaf scars (Coates Palgrave, 2002). The genus has 12 species in tropical Africa (Klopper *et al.*, 2006), of which two species occur south of the Zambezi River. On the one hand, *Pteleopsis* was considered distinct from *Terminalia* because of its male flowers being found only at the base of the inflorescence and not at the apex, as is the case for *Terminalia* (Stace, 2007). This character, however, proved to be unreliable for the separation of these genera as there are species of *Terminalia* with only basal male flowers (section *Ramatuellea* Kunth.; Stace, 2007). On the other hand, according to Exell & Stace (1966) and Wickens (1973), *Pteleopsis* represents an intermediate between *Combretum* and *Terminalia* based on several characters. It shares some character states with *Combretum*: it has petals (petals are completely absent in *Terminalia*) and opposite leaves. However, it seems to be closely related to *Terminalia* based on characters such as spirally convolute cotyledons, flattened fruits andromonoecious flowers and lack of scales and stalked glands. Tilney & Van Wyk (2004) reported the occurrence of extrafloral nectaries on leaves of *Pteleopsis* and *Terminalia*, but these are absent in *Combretum*. *Pteleopsis* fruits have two to five wings, whereas those of *Terminalia* generally possess two wings and *Combretum* four to five wings. In the current study, we retrieved high support for an embedded position of *Pteleopsis* in *Terminalia*. We thus propose that *Pteleopsis* should be united with *Terminalia*, the latter having priority.

Buchenavia comprises 24 species (Mabberley, 2008) from tropical and subtropical Central and South

America (Stace, 1965). Our analysis suggests *Terminalia bellirica* (Gaertn.) Roxb. is sister to *Buchenavia* and that the latter is included in the clade containing mainly African species of *Terminalia*. According to Stace (1965), *Terminalia* and *Buchenavia* have only one or two minor floral characters distinguishing them. Molecular data here strongly support *Buchenavia* as embedded within *Terminalia*, and we therefore propose that *Buchenavia* should be transferred to *Terminalia*.

Anogeissus is a genus of eight species distributed from the Old World tropics including the Arabian Peninsula (Mabberley, 2008). It is difficult to distinguish from *Terminalia* based on epidermal and leaf morphological features and shares some similarities with *Conocarpus* in possessing numerous stomata on the upper epidermis as the primary difference from *Terminalia* (Stace, 1965). *Anogeissus* was once included in *Conocarpus* as section *Anogeissus* DC (1828), but Guillemin, Perrotet & Richard (1833) raised it to genus. Fruit characters are diagnostic for the two genera, with *Anogeissus* having cone-like heads with wings or ribs and an apical beak representing the persistent calyx stalk (lower receptacle above the ovary) and *Conocarpus* with a 4-ribbed, slightly flattened and achene-like fruit (Stace, 2007) with the calyx stalk deciduous (Scott, 1979). Molecular data from our study do not indicate a close relationship between *Conocarpus* and *Anogeissus*, but rather that *Anogeissus* is embedded in the mainly Asian clade of *Terminalia*. We propose to unite *Anogeissus* and *Terminalia*.

Bucida, when accepted as a separate genus, comprises three species (Mabberley, 1997) from Central America, the Caribbean and North America (Florida). In 2002, Stace transferred *Bucida* to *Terminalia*. *Bucida* differs consistently from other American *Terminalia* and nearly all other Terminaliinae in having a small, nut-like fruit that retains the withered crown-like upper portion of the hypanthium (Stace, 2002), whereas *Terminalia* fruits are generally hard and woody with two wings (Stace, 1965).

African species of *Terminalia* are relatively well studied and a sectional classification has been suggested (Griffiths, 1959). For the remainder of the genus, there is an insufficient or only localized sectional classification (Clarke, 1878; Exell, 1954; Griffiths, 1959; Capuron, 1967; Pedley, 1990). In our study, the genus is polyphyletic and has the closely related genera of Terminaliinae imbedded in it, with the exception of *Conocarpus*. The BI analysis revealed two main clades within *Terminalia*, as discussed above. We thus formally propose the transfer of *Pteleopsis*, *Buchenavia* and *Anogeissus* to *Terminalia*, *Bucida* having been already transferred (Stace, 2002).

RELATIONSHIPS WITHIN SUBTRIBE *COMBRETINAE*

In the MP analysis, the subfamily is weakly supported (64 BP), whereas in the BI analysis it receives strong support (1.0 PP). Currently, five genera are recognized within Combretinae (Stace, 2007): (1) *Combretum* (pantropical); (2) *Calycopteris* (Southeast Asia); (3) *Meiostemon* (tropical southern Africa, Madagascar); (4) *Thiloa* (Central America); and (5) *Guiera* (northern tropical Africa). Stace (2007) suggested transferring *Meiostemon* to *Combretum*, but this was never formally published. Delimitation of *Quisqualis* and *Combretum* has been discussed (Jongkind, 1990) and our molecular results support the transfer of *Quisqualis* to *Combretum*. We were unable to sequence *C. apetalum* in this study because of the difficulty of obtaining fresh material and PCR was unsuccessful from herbarium DNA. This species is important because it is the only representative of one of the three subgenera within *Combretum*, *C.* subgenus *Apetalanthum.* It shares morphological characters with the other two subgenera: the stalked glands of subgenus *Cacoucia* and scales of subgenus *Combretum* (Stace, 1980a, 2007).

The two monotypic genera, *Calycopteris* and *Guiera*, are together sister to the rest of Combretinae. According to Stace (1980b), *Calycopteris* and *Guiera* are morphologically distinct, although the upper and lower epidermis of these two genera is similar (Stace, 1965). The relationship of *Calycopteris* with other genera within the subtribe has never been fully investigated, even although it resembles *Guiera, Thiloa* and *Combretum* in possessesing scales (Stace, 1965). *Guiera* is easily distinguishable by its epidermal cells and numerous shortly segmented hairs (Stace, 1965). The position of *Thiloa* within Combretinae is unresolved. Eichler (1866) separated *Thiloa* from *Combretum* on the basis of its floral structure (lack of petals, a single row of stamens), whereas Stace (2007) included the genus in *Combretum* subgenus *Combretum*. These floral characters are not unique to *Thiloa*, being also present in other taxa within *Combretum* (absence of petals in *C. apetalum* and a single row of stamens in *Meiostemon*). *Thiloa* needs to be sampled more densely to assess its relationship with subgenus *Combretum.*

In the BI analysis, two main clades are found in Combretinae, one (0.99 PP) including all representatives with scales and the other in which they are absent (81 BP/1.0 PP). The first represents *Combretum* subgenus *Combretum* with *Meiostemon*, a small genus with two species, as their sister. Exell & Stace (1966) erected *Meiostemon* as a new genus based on the suppression of one whorl of stamens, which is different from *Combretum*. These authors also concluded that *Meiostemon* shows no affinity to any other section within *Combretum*, although both species possess scales like all species currently in subgenus *Combretum*. According to Exell (1978), the distribution of *Meiostemon* in Mozambique, Zambia, Zimbabwe and Madagascar could indicate a relatively ancient origin and could also have resulted from recent, long-distance dispersal. We propose its reinstatement as *Combretum* section *Haplostemon*, following Exell (1939).

The second clade includes representatives of subgenus *Cacoucia* and consists of two main subgroups, each moderately supported. There is strong support (86 BP/1.0 PP) for *Quisqualis*, *Combretum coccineum* (Sonn.) Lam. (syn: *Poivrea* Comm. ex DC.; now a section within *Combretum* subgenus *Cacoucia*) and *Calopyxis* as imbedded within subgenus *Cacoucia*. Jongkind (1990, 1995) argued for the inclusion of *Quisqualis* and *Calopyxis* in *Combretum*, just as Engler & Diels (1899, 1900) had for *Poivrea*. *Calopyxis*, with 18 out of the 19 species endemic to Madagascar, has flowers in which petals are absent as in the case of *Terminalia*, whereas *Quisqualis* has flowers with five petals. According to Stace (2007), these differences are not consistent. *Quisqualis* is a small genus comprising 16 species of lianas distributed in the Old World tropics (Mabberley, 1997). Morphologically they share important similarities with the climbing species of *Combretum* subgenus *Cacoucia* (Exell & Stace, 1966). *Quisqualis* spp. have microscopic stalked glands similar to those found in subgenus *Cacoucia*, whereas the scales characteristic of subgenus *Combretum* are absent (Exell & Stace, 1966; Wickens, 1973). Fruits of subgenus *Cacoucia* are 4- to 5-winged, whereas in *Quisqualis* they are generally 5-winged. In addition to this, *Quisqualis* spp. have long, tubular upper receptacles that are absent in *Combretum* (Hooker, 1867; Lawson, 1871) and stamens not exserted beyond the petals; in the case of *Combretum*, the species have long, protruding stamens. These characters have proved to be unreliable for the separation of *Quisqualis* and *Combretum*. Exell (1931) proposed a new generic classification system in which *Quisqualis* was separated from *Combretum* on account of the style being adnate to the upper receptacle for approximately half the length. In 1964, Exell & Stace reorganized the delimitation of these two genera (Exell & Stace, 1964), but this separation was found to be unreliable by Jongkind (1990). Jongkind (1990) stated that, as no character has been identified that would allow for the reliable and unambiguous separation of *Quisqualis* and *Combretum*, they must be united, with the name *Combretum* having priority. An additional character of *Quisqualis* spp. and most representatives of subgenus *Cacoucia* is the presence of attractive pinkish–red flowers, which are not found in the rest of the family. In the case of *Quisqualis*, the elongated calyx might represent a modification for moth pollination (Stace, 2007).

A revision of the generic classification within subtribe Combretinae appears necessary to conform to the criteria of monophyly. Apart from the primary principle of monophyly, Backlund & Bremer (1998) also proposed as secondary principles that a classification should maximize stability, phylogenetic information, support for monophyly and ease of classification. Based on this, we propose that *Combretum* subgenus *Combretum* should be expanded to include *Meiostemon* and that *Combretum* subgenus *Cacoucia* should include *Quisqualis*. Our study confirms the recent treatment of *Calopyxis* in *Combretum* subgenus *Cacoucia* (Jongkind, 1995).

RELATIONSHIPS WITHIN *COMBRETUM* SUBGENUS *COMBRETUM* INCLUDING *MEIOSTEMON*

Section Angustimarginata

This section was described by Engler & Diels (1899) and includes the following South African species: *C. erythrophyllum* (Burch.) Sond., *C. caffrum* (Eckl. & Zeyh.) Kuntze, *C. kraussii* Hochst., *C. nelsonii* Dümmer, *C. vendae* A.E. van Wyk and *C. woodii* Dümmer. It is restricted to southern Africa and has been well studied and amended with the work of Van Wyk (1984), who reinstated four species, *C. caffrum* (Eckl. & Zeyh.), *C. nelsonii*, *C. kraussii* and *C. woodii*, and described a new species, *C. vendae* A.E.van Wyk. Section *Angustimarginata* is a natural group, which is easily distinguished by inconspicuous scales, often obscured by the indumentum and/or glutinous secretions (Exell, 1970, 1978). Other characters for this section are: bark smooth or flaking in small papery pieces, young leaves that are white or creamy without chlorophyll (some turning red in autumn), reddish calyx lobes and mature fruit usually partially or completely tinted pink to dark red (Van Wyk, 1984). In this study, section *Angustimarginata* is strongly supported (100 BP/1.0 PP) with *C. kraussii* and *C. vendae* grouping together with strong support (87 BP/1.0 PP). According to Van Wyk (1984), *C. kraussii* is morphologically the most primitive species of the group, a hypothesis that a molecular study cannot refute. The current molecular study corroborates the hypothesis that *C. woodii* and *C. nelsonii* are distinct species from *C. kraussii* as Exell (1970, 1978) suggested.

Section Ciliatipetala

According to the latest revision of the sectional classification (Stace, 1980b), section *Ciliatipetala* comprises 10 species from Africa and Yemen in the Arabian Peninsula. In this study, six described and three undescribed species from Africa were included, and these members of section *Ciliatipetala* form a strongly supported clade (99 BP/1.0 PP) that is characterized by flowers with small ciliate petals generally produced in large numbers. Fruits are generally small and scales variable in size from 40 to 120 μm; they have 7–12 radial walls often with additional tangential walls (Exell, 1978). Although this group seems natural in our results and is one of the largest sections in *Combretum* subgenus *Combretum*, Stace (1969) considered it as one of the most problematic, with most of the species falling into two major groups centred around *C. molle* R.Br. and *C. apiculatum* Sond. Stace (1969) also hypothesized the existence of species complexes or aggregates around *C. psidioides* Welw., *C. moggii* Exell and *C. albopunctatum* Suesseng. Stace (1969) proposed that further characters should be studied and suggested that some of these taxa might even need to be separated into different groups. Although our sampling does not include many representatives of each aggregate as recognized by Stace (1969), our results indicate that section *Ciliatipetala* comprises two main clades. The first includes *C. albopunctatum*, *C. apiculatum* and *C. molle*, and the second *C. moggii*, *C. petrophilum* Retief and *C. edwardsii* Exell (88 BP/1.0 PP and 99 BP/1.0 PP, respectively). It will be necessary to add representatives of the aggregates around *C. nigricans* Lepr. mentioned by Stace (1969) and more representatives of each aggregate (for example, the different forms or ecotypes of *C. molle* and subspecies of *C. psidioides*) to gain a better understanding of this section and its subsectional groupings.

Sections Macrostigmatea and Spathulipetala

Our results support inclusion of section *Spathulipetala* in section *Macrostigmatea* (100 BP/1.0 PP). According to Stace (1980b), section *Macrostigmatea* comprises four species from Africa and section *Spathulipetala* only one, *C. zeyheri* Sond., that is widespread throughout southern Africa. The division of the group into two sections is questionable. Exell (1978) reported on relationships between the two sections and stated that they share similarities such as scale size, scale fragmentation into walls and fruit size. Our results strongly support lumping *C. zeyheri* in section *Macrostigmatea*. *Combretum zeyheri* shows a close relationship to *C. mkuzense* J.D.Carr & Retief from the sand forests in KwaZulu-Natal (70 BP/1.0 PP). This result is not unexpected as both species have large fruits, up to 50 × 50 mm and even larger in some specimens. A second specimen supposed to be *C. mkuzenze* (voucher *RBN 154*) was included in the analysis following suggestions that the localized species from the sand forest of KwaZulu-Natal might extend to similar vegetation in the north-west of the country. Our molecular data do not suggest that these two accessions are members of the same species, and this second taxa is now treated as a new species that will be described jointly with authors from South Africa National Biodiversity Institute (SANBI) in a forthcoming publication. Exell (1978) suggested that *C. kirkii* M.A.Lawson and *C. gilletianum* Liben could be segregated from *C. schumannii* Engl. into a separate section based on the floral disk being glabrous or not. Our study supports *C. kirkii* as sister to the rest of section *Macrostigmatea*, but before any conclusions can be made *C. gillettianum* should be included in the analysis.

Section Hypocrateropsis (excluding Combretum imberbe)

Section *Hypocrateropsis* is characterized mainly by petals that are linear–elliptic and glabrous with large leaf scales (50–150 μm in diameter); it comprises six species from Africa (Stace, 1980b). Until now, *C. imberbe* was accepted as a member of this group, a placement that our study, however, does not support. To confirm this result we included two specimens of *C. imberbe* from different localities (Namibia and Republic of South Africa) in the analysis. As mentioned previously, scale size and shape are consistent characters in this section (scales are generally <100 μm with primary and secondary radial and tangential walls); however, scale density is a character that distinguishes *C. imberbe* from the rest of the section. In *C. imberbe*, scales are mostly contiguous to overlapping, differing from those of *C. celastroides* Welw. ex M.A.Lawson and *C. padoides* Engl. & Diels, which are rarely contiguous (Exell, 1978). In this case, scale density in addition to shape and construction might be an important taxonomic character to be investigated at both species and sectional level. Stace (1969) also mentioned the epidermis with a well-developed venule reticulum, sparse to frequent hairs and usually undulate-walled areolar cells without papillae in the six species he studied; these are absent in *C. imberbe*. This is in contrast with the other species in which there are only obvious major laterals veins and midribs, no hairs and straight-walled cells, those beneath the scales with conspicuous, rounded papillae.

Sections Glabripetala and Mettalicum

Engler & Diels (1899) grouped *C. adenogonium* Steud. ex A.Rich (= *C. fragrans* F.Hoffm.) and the subspecies of *C. collinum* Fresen under section *Glabripetala*, but this section was later dismembered, with a new section for *C. collinum* (section *Mettalicum* Exell & Stace) based on differences in scale structure (Stace, 1969). Our results support the distinction of these taxa and their separation in sections *Glabripetala* and *Mettalicum*. Our phylogenetic results support *Glabripetala* as sister to the southern African sections *Angustimargina, Macrostigmatea* and *Spathulipetala*, and *Mettalicum* as more closely related to sections *Campestria*, *Breviramea* and *C. imberbe* (the last previously included in section *Hypocrateropsis*).

Sections Campestria and Breviramea

Stace (1980b) considered section *Elaegnoidea* Engl. & Diels a synonym of section *Campestria*. Sections *Campestria* and *Breviramea* are each represented by four species in Africa and only one representative of each is included in our study: *C. elaeagnoides* Klotzsch (*Campestria*) and *C. hereroense* Schinz (*Breviramea*). To have a more comprehensive overview of these two sections and their relationships with the apparently closely related sections *Mettalicum* and *Glabripetala*, more species of each section should be included in future analyses.

RELATIONSHIPS WITHIN *COMBRETUM* SUBGENUS *CACOUCIA* INCLUDING *QUISQUALIS* AND *CALOPYXIS*

As previously discussed, subgenus *Cacoucia* is unique in its glandular, compartmented hairs and absence of scales (Stace, 1980a). Flowers of this subgenus exhibit better developed petals than in subgenus *Combretum*, and they are often colourful with nectariferous disks, probably linked to adaptation for pollination by birds and moths. This becomes even more obvious in *Quisqualis*, which according to our results is closely related to subgenus *Cacoucia.* In our analysis, subgenus *Cacoucia* is well supported (81 BP/1.0 PP) and divided into two low to moderately supported clades in the MP analysis, but both of these are strongly supported in the BI analysis (1.0 PP, 0.97 PP, respectively). The first comprises two subclades, one with species belonging to *Quisqualis* (0.95 PP) and the second with several species of *Combretum* subgenus *Cacoucia* section *Poivrea* (Commerson ex DC.) G.Don and members of former *Calopyxis* now considered to be in *Combretum* (100 BP/1.0 PP). However, the three taxa, *C. holstii* Engl., *C. mossambicense* (Klotzsch) Engl. and *C. bracteosum* (Hochst.) Brandis (previously section *Trichopetaleae* Engl. & Diels) did not group with other representatives of section *Poivrea*. *Combretum grandiflorum* G.Don is unresolved and was previously included in section *Grandiflora* Engl. & Diels. We therefore suggest that the two sections *Trichopetaleae* and *Grandiflora* be reinstated to accommodate the paraphyletic section *Poivrea*.

Following the suggestion of Capuron (1967), Stace (1980a) included *Poivrea grandidieri* (Drake) H.Perrier in *Calopyxis* as *C. grandidieri* (Drake) Capuron ex Stace; however, according to Jongkind (1995), if this species is placed in *Calopyxis* the most important character that previously separated this genus from the related *Combretum* subgenus *Cacoucia* was no longer present in all species (i.e. the absence of petals). In 1995, Jongkind transferred *Calopyxis* back into *Combretum* as subgenus *Cacoucia* section *Calopyxis* Jongkind, which our results support.

The second clade is supported by 76 BP (MP) and 1.0 PP (BI). With BI, the clade is enlarged to include *C. oxystachyum* Welw. ex M.A.Lawson, *C. watti* Exell and *C. goldieanum* F.Muell (0.97 PP). This clade also includes section *Conniventia* Engl. & Diels, two taxa of section *Poivrea* as well as two monotypic sections from southern Africa: *Megalantherum* Exell (*C. wattii*) and *Oxystachia* Exell (*C. oxystachyum*).

Subgenus *Cacoucia* contains 13 sections in total with around 78 species (Stace, 1980b), most of them occurring in Africa and Madagascar with distributions mainly north of the Zambezi River. Our study included only ten species representing four sections, and therefore it is necessary to have more sampling of this subgenus to create a better understanding of relationships within and between sections.

Section Poivrea

Engler & Diels (1899, 1900) included *Poivrea* in subgenus *Cacoucia* and, according to Stace (1980b), the section comprises 31 species from Africa, Madagascar and Asia. Our study includes the widespread species *C. mossambicense* and an atypical species with round wingless fruit, *C. bracteosum*, from the eastern coastal forests of KwaZulu-Natal and the Eastern Cape (RSA). *Combretum holstii* is morphologically similar to *C. mossambicense*, a relationship confirmed by our analysis (100 BP/1.0 PP). These three species do not group with *C. coccineum* (also section *Poivrea*). They represent a group of morphologically similar species that were previously included in section *Trichopetala.* Reinstatement of the section sunk by Stace (1980a) in section *Poivrea* would be appropriate according to our results. However, these three species do not group with *C. goldieanum* F.Muell., an Asian species also previously considered a member of section *Trichopetala.* *Combretum goldieanum* appears more closely related to *C. oxystachyum* of section *Oxystachia*, although they form a clade with weak support (0.69 PP).

Section Conniventia

Section *Conniventia* Engl. & Diels occurs in Africa and is represented in our sampling by four out of the 20 species (Stace, 1980b). *Combretum platypetalum* Welw. ex M.A.Lawson includes four subspecies based on hairy receptacles and leaves (Exell, 1968). It is a perplexing group that warrants further study with more individuals from each subspecies. In our analysis, we included two specimens of *C. platypetalum*, one from the Caprivi Strip (Namibia) and the other collected close to Harare (Zimbabwe). The relationship between *C. platypetalum*, *C. paniculatum* Vent. and *C. microphyllum* Klotzsch (Exell, 1978) is equally unclear, which makes this group even more confusing. An investigation of *C. platypetalum* and its relationship to *C. paniculatum* and *C. microphyllum* is required.

Wickens (1973) considered *C. microphyllum* and *C. paniculatum* to be one species and *C. microphyllum* to be a subspecies of *C. paniculatum*; however, Exell (1978) preferred to treat these as two separate species. Our study supports the opinion of Exell (1978) and this is also confirmed by the distribution and differences in drought tolerance of *C. microphyllum* and *C. paniculatum.* The latter is a mesic forest species from tropical Africa with a southern distribution in the Soutpansberg (RSA). In contrast to this, *C. microphyllum* is more resistant to drought and found along riverbanks in savannah vegetation.

CONCLUSIONS

The well-resolved and supported results presented here allow a discussion of the classification and relationships between the genera of Combretaceae, with some suggestions for improving their classification. Our analyses generally support the current classification, particularly the transfer of genera that were solely based on equivocal morphological data, namely: (1) *Terminalia* (nom. cons.) for *Bucida*; and (2) *Combretum* (nom. cons.) for *Calopyxis* as well as the sectional classification proposed progressively by Engler & Diels (1899), Exell (1939), Exell (1953), Exell (1968) and Stace (1980b), except for inclusion of *C. imberbe* within section *Hypocrateropsis.* From our results, we propose the following: (1) combination of *Terminalia, Buchenavia, Anogeissus* and *Pteleopsis*, with the name *Terminalia* having priority; (2) transfer of the two species of *Meiostemon* (Exell, 1939) to *Combretum*; (3) reinstatement of subgenus *Combretum* section *Haplostemon* for which both species were previously placed in subgenus *Combretum*; (4) transfer of *Quisqualis* to *Combretum*, as suggested by Jongkind (1995) and Stace (2007); (5) reinstatement of sections *Grandiflora* and *Trichopetala*, which had previously been sunk in section *Poivrea*; and (6) establishment of a new section for *C. imberbe*.

Taxonomic decisions to adjust the generic limits will be better evaluated by inclusion of more species. Adding the two Australian genera, *Dansiea* and *Macropteranthes*, would be necessary to assess relationships with tribe Laguncularieae. In order to have a better understanding of the generic and subgeneric relationships with tribe Combreteae, addition of species from *Buchenavia, Pteleopsis*, *Thiloa* and *Combretum* subgenus *Combretum* section *Calopyxis* would be necessary. It will also be necessary to add the third and monotypic subgenus *Apetalanthum* and representatives of all sections of *Combretum* and *Terminalia*.

TAXONOMY

Combretum Loefl., Iter Hispanicum App.: 308 (1758) [Combret.], nom. cons. emend. prop. Type: Combretum fruticosum (Loefl.) Stuntz

(=) Quisqualis L., Species Plantarum ed. 2, 1 (1762) [Combret.], nom. rej. prop. Type: Quisqualis indica L.

(=) *Meiostemon* Exell & Stace, *Bol. Soc. Brot*. sér. 2, 40: 18, in adnot. (1966), *nom. rej. prop.* Type: *Meiostemon tetrandrum* Exell

Combretum Loefl., subgenus ***Combretum***, section ***Haplostemon*** Exell

Combretum Loefl. subgenus ***Combretum*** section ***Plumbea*** O.Maurin, Jordaan & A.E.van Wyk, sect. nov.

Sectioni *Hypocrateropsidi*, valde affinis sed habitu arboris unicaulis discretae, juventute ramis decussatis, ramulis brevibus lateralibus apice spinescente; foliis perdense lepidotis, squamis plerumque contiguis vel superpositis, 120–300 μm diametro, cellulis permultis parvis, solum in costa nervisque primariis lateralibus conspicuis; margine disci dense tomentoso; stylo squamis stipitatis velato; cotyledonibus infra planum humi orientibus, differt.

Type: *C. imberbe* Wawra in Sitzungsber. Acad. Wien, Math. -Nat., 38: 556 (1860).

This section is named *Plumbea*, derived from the Latin word *plumbum* which means lead, referring to the heartwood of *C. imberbe* which is extremely hard, heavy and durable, hence the common name leadwood.

Combretum Loefl. subgenus ***Cacoucia*** (Aublet) Exell & Stace section ***Grandiflora*** Engl. & Diels

Type: *C. grandiflorum* G.Don in Edinb. Phil. Journ. (1824) 346.

Combretum Loefl. subgenus ***Cacoucia*** (Aublet) Exell & Stace section ***Trichopetaleae*** Engl. & Diels

Type: *C. trichopetalum* Engl. = *C. mossambicense* (Klotsch) Engl. in Pflanzenw. Ost-Afrikas C (1895) 292.

Terminalia L., Syst. Nat., ed. 12, 2: 674. (15–31 Oct 1767) [*Combret.*], *nom. cons. emend. prop.*

Type: Terminalia catappa L.

(=) *Pteleopsis* Engl., Abh. Königl. Akad. Wiss. Berlin, 25. 1894, *nom. rej. prop.*

Type: *Pteleopsis variifolia* Engl.

(=) *Anogeissus* (DC) Wall., Florae Senegambiae Tentamen 1:279. 1832, *nom. rej. prop.*

Type: *Anogeissus acuminata*.

(=) *Buchenavia* Eichler, Flora 49(11): 1866, *nom. rej. prop.*

Type: *Buchenavia capitata* (Vahl) Eichler.

ACKNOWLEDGEMENTS

The authors thank the South African National Biodiversity Institute (SANBI), National Botanical Research Institute, Namibia (NBRI), Ministry of Tourism Namibia, Christine Bredenkamp, Johan Hurter, Ernst van Jaarsveld, Hugh Glen, Robert Archer, Pieter Winter (SANBI), Desiree Marimuthoo (KZN National Botanical Garden), Joshlyn Sand (Honolulu Botanical Garden), Clyde Imada (Bishop Museum Honolulu), Missouri Botanical Garden and Meg Coates-Palgrave for samples. Edith Kapinos and Laszlo Csiba, DNA Bank Managers at the Royal Botanic Gardens, Kew, are thanked for extracting some DNA samples. Clive Stace and Braam van Wyk are thanked for their helpful comments and Stephen Boatwright and Martyn Powell for proofreading the manuscript. Hugh Glen (SANBI) wrote the Latin for the diagnostic characters of the new section. The National Research Foundation (NRF; South Africa) and the University of Johannesburg financed the research.

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APPENDIX

Voucher information and GenBank accession numbers for taxa used in this study. A dash (—) indicates DNA regions not sampled and DNA sequences obtained from GenBank are underlined. Voucher specimens are deposited in the following herbaria: BISH, Bishop Museum, Honolulu, USA; JRAU, University of Johannesburg (UJ), Johannesburg, South Africa; K, Royal Botanic Gardens, Kew, Richmond, UK; MO, Missouri Botanical Garden, St Louis, USA; PRE, South African National Botanical Institute, Pretoria, South Africa.

Family. Taxon – Voucher (Herbarium), Country where collected, GenBank accession no.: *ITS, rbcL, PsaA-Ycf3, psbA-trnH*.

Annonaceae. *Xylopia hypolampra* Mildbr., —; AY337731; —; —.

Alzateaceae. *Alzatea verticillata* Ruiz & Pav.,—; AVU26316; —; —.

Combretaceae. *Anogeissus acuminata* Wall., AF334765; AF425708; AF425692; —; *Anogeissus leiocarpa* Guill. & Perr., AF334766; AF425709; AF425693; —; *Buchenavia reticulata* Eichler, *van der Werff H. & R. Vasquez 13866* (MO), Peru, FJ381770; FJ381804; FJ381841; FJ381877; *Buchenavia tetraphylla* (Aubl.) R.A. Howard, *Taylor, C.M. 11671* (MO), Puerto Rico, —; FJ381805; FJ381842; —; *Bucida buceras* L. *Harder, D.K. & M. Merello 1184* (MO),US Florida, FJ381771; FJ381806; FJ38184; FJ572675; *Bucida buceras* L. *Maurin 1670* (JRAU), Cult., FJ381772; FJ381807; FJ381844; FJ381878; *Calopyxis grandidieri* (Drake) Capuron ex Stace, *Phillipson and Rabesihanaka 3147* (K), Madagascar, FJ381762; FJ381796; —; FJ381870; *Calopyxis grandidieri* (Drake) Capuron ex Stace*, Willing s.n.* (K), Madagascar, FJ381761; FJ381795; FJ381834; FJ381869; *Calycopteris floribunda* (Roxb.) Lam. ex Poir., AF334770; —; AF425691; —; *Combretum albopunctatum* Suess.*, Maurin 1038* (JRAU), Namibia, EU338031; EU338141; EU338086; EU338196; *Combretum apiculatum* Sond. subsp. *apiculatum, Lahaye 1355* (JRAU), South Africa, EU338032; EU338142; EU338087; EU213796; *Combretum apiculatum* Sond. subsp. *leutweinii* (Schinz) Exell*, Maurin 1015* (JRAU), Namibia, EU338033; EU338143; EU338088; EU338197; *Combretum bracteosum* (Hochst.) Brandis *Maurin & van der Bank 22* (JRAU), South Africa, EU338018; EU338128; EU338073; EU338183; *Combretum caffrum* (Eckl. & Zeyh.) Kuntze, *Maurin & van der Bank 11* (JRAU), South Africa, EU338057; EU338167; EU338112; EU338221; *Combretum celastroides* Welw. ex M.A.Lawson subsp. *celastroides, Maurin & van der Bank 28* (JRAU), South Africa, EU338042; EU338152; EU338097; EU338206; *Combretum celastroides* Welw. ex M.A.Lawson subsp. *orientale* Exell*, Maurin & van der Bank 27* (JRAU), South Africa, EU338043; EU338153; EU338098; EU338207; *Combretum coccineum* Engl. & Diels, *Archer 2972* (PRE), Madagascar, FJ381766; FJ381800; FJ381838; FJ381874; *Combretum collinum* Fresen.*, Maurin 1524* (JRAU), South Africa, EU338041; EU338151; EU338096; EU338205; *Combretum collinum* Fresen. subsp. *gazense* (Swynn. & Baker f.), *Maurin 1024* (JRAU), South Africa, EU338048; EU338158; EU338103; EU338212; *Combretum collinum* Fresen. subsp. *suluense* (Engl. & Diels) Okafor, *Maurin & van der Bank 34* (JRAU), South Africa, EU338049; EU338159; EU338104; EU338213; *Combretum collinum* Fresen. subsp. *taborense* (Engl.) Okafor*, Bryden 170* (JRAU), South Africa, EU338050; EU338160; EU338105; EU338214; *Combretum collinum* Fresen. subsp. *hypopilinum* (Diels) Okafor, *Sanou 004* (K), Burkina Faso, FJ381756; FJ381790; FJ381829; FJ381865; *Combretum edwardsii* Exell*, Maurin 1584* (JRAU), South Africa, EU338034; EU338144; EU338089; EU338198; *Combretum elaeagnoides* Klotzsch*, Maurin 1021* (JRAU), Namibia, EU338040; EU338150; EU338095; EU338204; *Combretum engleri* Schinz*, Maurin 1025* (JRAU), Namibia, EU338051; EU338161; EU338106; EU338215; *Combretum erythrophyllum* (Burch.) Sond.*, Maurin 201* (JRAU), South Africa, EU338023; EU338133; EU338078; EU338188; *Combretum fragrans* F.Hoffm.*, Slageren and Sanou 866* (K), Burkina Faso, FJ381754; FJ381788; —; —; *Combretum glutinosum* Perr. ex DC.*, Slageren and Sanou 854* (K), Burkina Faso, FJ381755; FJ381789; FJ381828; —; *Combretum goldieanum* F.Muell.*, P.Hayers FL-1125* (BISH), Cult., FJ381767; FJ381801; FJ381839; FJ381875; *Combretum grandiflorum* G.Don, *P.C. Hutchinson 2849* (BISH), Cult., FJ381763; FJ381797; FJ381835; FJ381871; *Combretum hereroense* Schinz, *Maurin 238* (JRAU), South Africa, EU338028; EU338138; EU338083; EU338193; *Combretum holstii* Engl.*, Palgrave 504* (JRAU), Mozambique, EU338019; EU338129; EU338074; EU338184; *Combretum imberbe* Wawra*, Maurin 1012* (JRAU), Namibia, EU338044; EU338154; EU338099; EU338208; *Combretum imberbe* Wawra, *Lahaye 1380* (JRAU), South Africa, EU338045; EU338155; EU338100; EU338209; *Combretum kirkii* M.A.Lawson*, Palgrave 512* (JRAU), Mozambique, EU338052; EU338162; EU338107; EU338216; *Combretum kraussii* Hochst., *Maurin & van der Bank 36* (JRAU), South Africa, EU338024; EU338134; EU338079; EU338189; *Combretum micranthum* G.Don, *Slageren and Diallo 673* (K), Burkina Faso, FJ381759; FJ381793; FJ381832; FJ381868; *Combretum microphyllum* Klotzsch, *Maurin 205* (JRAU), South Africa, EU338020; EU338130; EU338075; EU338185; *Combretum mkuzense* J.D.Carr & Retief, *Maurin 1574* (JRAU), South Africa, EU338054; EU338164; EU338109; EU338218; *Combretum moggii* Exell, *Maurin 1585* (JRAU), South Africa, EU338035; EU338145; EU338090; EU338199; *Combretum molle* R.Br. ex G.Don*, Maurin 1571* (JRAU), South Africa, EU338036; EU338146; EU338091; EU338200; *Combretum molle* R.Br. ex G.Don, *Maurin 558* (JRAU), South Africa, EU338037; EU338147; EU338092; EU338201; *Combretum mossambicense* (Klotzsch) Engl., *Maurin 1011* (JRAU), Namibia, EU338021; EU338131; EU338076; EU338186; *Combretum nelsonii* Dummer, *van der Bank 26* (JRAU), South Africa, EU338025; EU338135; EU338080; EU338190; *Combretum oxystachyum* Welw. ex M.A.Lawson*, Maurin 1052* (JRAU), Namibia, EU338017; EU338127; EU338072; EU338182; *Combretum padoides* Engl. & Diels*, Maurin 1285* (JRAU), South Africa, EU338046; EU338156; EU338101; EU338210; *Combretum paniculatum* Vent.*, Maurin & van der Bank 16* (JRAU), South Africa, EU338022; EU338132; EU338077;

EU338187; *Combretum petrophilum* Retief, *Maurin & van der Bank 31* (JRAU), South Africa, EU338038; EU338148; EU338093; EU338202; *Combretum platypetalum* Welw. ex M.A.Lawson, *Maurin 1020* (JRAU), Namibia, EU338014; EU338124; EU338069; EU338179; *Combretum platypetalum* Welw. ex M.A.Lawson, *Maurin 1658* (JRAU), Zimbabwe, EU338015; EU338125; EU338070; EU338180; *Combretum psidioides* Welw. subsp. *dinteri* (Schinz) Exell, *Maurin 1039* (JRAU), Namibia, EU338039; EU338149; EU338094; EU338203. *Combretum sp. nov. A, Winter 7225* (PRE), South Africa, FJ381757; FJ381791; FJ381830; FJ381866; *Combretum sp. nov. B, Maurin 997* (JRAU), South Africa, EU338059; EU338169; EU338114; EU338222; *Combretum sp. C, Boon 3174* (PRE), South Africa, FJ381758; FJ381792; FJ381831; FJ381867; *Combretum sp. nov. E, Bryden 154* (JRAU), South Africa, EU338053; EU338163; EU338108; EU338217; *Combretum tenuipes* Engl. & Diels, *Maurin 1089* (JRAU), South Africa, EU338047; EU338157; EU338102; EU338211; *Combretum vendae* A.E.van Wyk, *Maurin & van der Bank 9* (JRAU), South Africa, EU338026; EU338136; EU338081; EU338191; *Combretum wattii* Exell, *Maurin 995* (JRAU), Namibia, EU338016; EU338126; EU338071; EU338181; *Combretum woodii* Dummer, *Maurin 1421* (JRAU), South Africa, EU338027; EU338137; EU338082; EU338192; *Combretum zeyheri* Sond., *Maurin 1041* (JRAU), Namibia, EU338056; EU338166; EU338111; EU338220; *Conocarpus erectus* L., AY050562; —; AF425700; —; *Conocarpus sericeus* (Griseb.) Jimenez, *Maurin 1668* (JRAU), Cult., FJ381784; FJ381822; FJ381860; FJ381894; *Guiera senegalensis* J.F.Gmel., *Daramola 233* (K), West tropical Africa, FJ381769; FJ381803; FJ381840; FJ381876; *Laguncularia racemosa* (L.) C.F. Gaertn., *PrinzieTh 132* (MO), US Florida, —; FJ381826; FJ381863; —; *Laguncularia racemosa* (L.) C.F. Gaertn., *Taylor, C.M.* 11787 (MO), Puerto Rico, FJ381787; FJ381825; —; —; *Lumnitzera littorea* Voigt, AF160468; AF425718; AF425704; —; *Lumnitzera racemosa* Willd., *Maurin 1675* (JRAU), South Africa, —; FJ381827; FJ381864; FJ381897; *Meiostemon humbertii* (H.Perrier) Exell & Stace, *Phillipson 2870* (K), Madagascar, FJ381760; FJ381794; FJ381833; —; *Meiostemon tetrandrus* (Exell) Exell & Stace, *Maurin 1653* (JRAU), Zimbabwe, EU338012; EU338122; EU338067; EU338177; *Pteleopsis anisoptera* (Welw. ex. M.A.Lawson) Engl. & Diels, *Maurin 1656* (JRAU), Zimbabwe, EU338005; EU338115; EU338060; EU338170; *Pteleopsis myrtifolia* (M.A.Lawson) Engl. & Diels, *Maurin & van der Bank 17* (JRAU), South Africa, EU338006; EU338116; EU338061; EU338171; *Pteleopsis myrtifolia* (M.A.Lawson) Engl. & Diels, *Maurin & van der Bank 19* (JRAU), South Africa, EU338007; EU338117; EU338062; EU338172; *Quisqualis caudata* Craib, AF160469; AF425706; AF425689; —. *Quisqualis indica* L., *Maurin 1669* (JRAU), Cult., FJ381764; FJ381798; FJ381836; FJ381872; *Quisqualis littorea* (Engl.) Exell, *Maurin & van der Bank 30* (JRAU), Cult., EU338013; EU338123; EU338068; EU338178; *Quisqualis parviflora* Gerr. ex Harv. & Sond., *Abbott 8891* (JRAU), South Africa, FJ381765; FJ381799; FJ381837; FJ381873; *Strephonema mannii* Hook f., *Sainge, M. & P. Mambo 807* (MO), Cameroon, FJ381785; FJ381823; FJ381861; FJ381895; *Strephonema pseudocola* A. Chev., *Sainge, M. & P. Mambo 823* (MO), Cameroon, FJ381786; FJ381824; FJ381862; FJ381896; *Terminalia arjuna* Wight & Arn., *Maurin 1671* (JRAU), Cult., FJ381783; FJ381821; FJ381859; FJ381893; *Terminalia bellirica* (Gaertn.) Roxb., *Maurin 1673* (JRAU), Cult., FJ381773; FJ381808; FJ381845; FJ381879; *Terminalia brachystemma* Welw. ex Hiern subsp. *brachystemma, Maurin & van der Bank 18* (JRAU), South Africa, FJ381774; FJ381810; FJ381847; FJ381881; *Terminalia catappa* L., *Archer 2941* (PRE), Madagascar, —; FJ381811; FJ381848; FJ381882; *Terminalia chebula* Willd. ex Flem., *Annable 3580* (BISH), Philippines, FJ381775; FJ381812; FJ381849; FJ381883; *Terminalia hainanensis* Exell., AF160466; AY050563; AF425694; —; *Terminalia ivorensis* A.Chev., *Annable & Canham 3718* (BISH). Nigeria, FJ381776; FJ381813; FJ381850; FJ381884; *Terminalia kaernbachii* Warb., *Kampong 3179* (BISH), Cult., —; —; FJ381851; FJ381885; *Terminalia litoralis* Seem *Miller & Merelo 7911* (BISH). Tonga, FJ381777; FJ381814; FJ381852; FJ381886; *Terminalia mantaly* H.Perrier, *Maurin 1088* (JRAU), Cult., FJ381778; FJ381815; FJ381853; FJ381887; *Terminalia mollis* M.Lawson, *Maurin & van der Bank 15* (JRAU), South Africa, EU338008; EU338118; EU338063; EU338173; *Terminalia muelleri* Benth., AF160472; AF425712; AF425697; —; *Terminalia myriocarpa* Van Heurck & Müll.Arg., *Lyon s.n.*, Cult., FJ381779; FJ381816; FJ381854; FJ381888; *Terminalia phanerophlebia* Engl. & Diels, *Maurin 1179* (JRAU), South Africa, EU338009; EU338119; EU338064; EU338174; *Terminalia prunioides* M.Lawson, *Maurin 327* (JRAU), South Africa, EU338010; EU338120; EU338065; EU338175; *Terminalia sambesiaca* Engl. & Diels, *Maurin & van der Bank 20* (JRAU), South Africa, FJ381780; FJ381817; FJ381855; FJ381889; *Terminalia sericea* Burch. ex DC., *Maurin 478* (JRAU), South Africa, EU338011; EU338121; EU338066; EU338176; *Terminalia stenostachya* Engl. & Diels, *Maurin 1665* (JRAU), Cult., —; FJ381818; FJ381856; FJ381890; *Terminalia stuhlmannii* Engl., *Zimba, N.B. et al. 899* (MO), Zambia, —; FJ381809; FJ381846; FJ381880; *Terminalia tomentosa* (Roxb.) Wight & Arn., *Maurin 1667* (JRAU), Cult., FJ381781; FJ381819; FJ381857;

FJ381891; *Terminalia trichopoda* Diels, *Maurin 1657* (JRAU), Cult., FJ381782; FJ381820; FJ381858; FJ381892; *Thiloa glaucocarpa* Eichler, *Giulietti et al. H51200* (K), Brasil, FJ381768; FJ381802; —; —.

Escalloniaceae. *Escallonia pulverulenta* Pers., —; AJ419696; —; —.

Geraniaceae. *Erodium gruinum* (L.) L'Hér., —; DQ452874; —; —.

Heteropyxidaceae. *Heteropyxis natalensis* Harv., —; AM235662; —; —.

Lauraceae. *Laurus nobilis* L., —; AY841668; —; —.

Lamiaceae. *Teucridium parvifolium* Hook.f., —; TPU78715; —; —.

Lythraceae. *Cuphea llavea* Lindl., —; AF495773; —; —; *Galpinia transvaalica* N.E.Br., —; AY905409; —; —. *Lythrum salicaria* L., —; AF495760; —; —; *Punica granatum* L., —; PUGRBCLX; —; —.

Malvaceae. *Abroma augustum* L.f., —; AJ012208; —; —; *Theobroma cacao* L., —; AF022125; —; —.

Melastomataceae. *Melastoma beccarianum* Cogn., —; AM235646; —; —.

Myrtaceae. *Eugenia uniflora* L., —; AM235654; —; —; *Leptospermum scoparium* J.R.Forst. & G.Forst., —; AM235656; —; —; *Melaleuca alternifolia* Cheel, —; AM235658; —; —.

Oliniaceae. *Olinia vanguerioides* Baker f., — AM235626; —; —.

Onagraceae. *Camissonia boothii (Douglas) P.H.Raven,* —; AF495766; —; —; *Clarkia xantiana* A.Gray, —; CLRRBCLX; —; —; *Ludwigia peploides* (Kunth) P.H.Raven, —; LUDRBCLY; —; —; *Oenothera macrocarpa* Pursh, —; AM235671; —; —.

Psiloxylaceae. *Psiloxylon mauritianum* Thou. ex Benth., —; AM235663; —; —.

Vochysiaceae. *Erisma floribundum* Rudge, —; EFU26324; —; —; *Qualea* Aubl., —; U02730; —; —; *Ruizterania albiflora* (Warm.) Marc.-Berti, —; AM235664; —; —; *Vochysia tucanorum* Mart.,—; AM235665; —; —.

Zygophyllaceae. *Zygophyllum cordifolium* L.f.,—; EF655993; —; —.