GENERIC DELIMITATION AND RELATIONSHIPS IN EBENACEAE SENSU LATO: EVIDENCE FROM SIX PLASTID DNA REGIONS[1]

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Phylogenetic relationships of the pantropical family Ebenaceae s.l. were investigated using plastid DNA sequence data from six regions: *atpB*, *matK*, *ndhF*, *trnK* intron, *trnL* intron, and *trnL-trnF* spacer. Sampling included representatives of all currently recognized genera of Ebenaceae, *Diospyros*, *Euclea*, and *Lissocarpa*, and nearly all taxa that were previously recognized at the generic level, e.g., *Cargillia*, *Gunisanthus*, *Maba*, *Macreightia*, *Royena*, and *Tetraclis*. Our results strongly support monophyly of Ebenaceae s.l. and demonstrate that the previous infrafamilar classifications of the family do not circumscribe monophyletic groups. A new infrafamilial classification based on a phylogenetic approach is proposed here and consists of two subfamilies, Lissocarpoideae and Ebenoideae, and four genera, *Lissocarpa*, *Euclea*, *Royena*, and *Diospyros*. Relationships and potential synapomorphic characters are discussed and summarized. This study supports a western Gondwanan origin of family and indicates that both vicariant and long-distance dispersal events played an important role in attaining current distributions.

Key words: *atpB*; Ebenaceae; infrafamilial classification; *matK*; *ndhF*; phylogenetic relationships; *trnK* intron; *trnL-trnF* region.

Ebenaceae sensu lato (s.l.) consist of Ebenaceae sensu stricto (s.s.) and Lissocarpaceae; they are a medium-sized pantropical family with the greatest number of species in Asia and the Indo-Pacific region, although the greatest morphological diversity is in Africa and Madagascar (White, 1983; Wallnöfer, 2001, 2004). The family is the source of several economically important products; the most valuable are timber (ebony) and fruits (persimmons). They also are a conspicuous forest component of Africa and Asia (Heywood, 1978; Judd et al., 2002).

Ebenaceae s.s. is a well-known family (Hiern, 1873; Bakhuizen, 1936–1955; White, 1980, 1983, 1993; Singh, 2005), but molecular studies (Berry et al., 2001; Anderberg et al., 2002; Bremer et al., 2002) have recently shown the family could also include *Lissocarpa* (formerly assigned to the monogeneric family Lissocarpaceae). Based on this molecular result, *Lissocarpa* has been transferred to Ebenaceae s.l. as

subfamily Lissocarpoideae (Wallnöfer, 2004). Unless noted otherwise, we will use the broader circumscription throughout this paper (i.e., including *Lissocarpa*). Previous placements of both Ebenaceae s.s. and Lissocarpaceae were in order Ebenales (sensu Cronquist, 1981, 1988; Dahlgren, 1989) or Styracales (sensu Thorne, 1992; Takhtajan, 1997). According to the APG system, Ebenaceae and other families of Ebenales and Styracales have been placed within an expanded order Ericales (Chase et al., 1993; Soltis et al., 2000; Berry et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; APG II, 2003; Schönenberger et al., 2005).

Infrafamilial classifications of Ebenaceae s.s. have been proposed by de Candolle (1844), Hiern (1873), Bakhuizen (1936–1955), White (1980, 1983, 1993), and Singh (2005). The previous classifications based on morphological and anatomical characters have been considered to be problematic; generic and infrageneric boundaries of each system have been different and much debated. The number of genera recognized in Ebenaceae s.s. has varied from two to eight (Table 1), and some of these circumscriptions are contradictory, so at least some of them do not circumscribe monophyletic groups. The earliest infrafamilial classification for Ebenaceae s.s. on a worldwide scale was that of de Candolle (1844), who recognized eight genera: *Cargillia*, *Diospyros*, *Euclea*, *Gunisanthus*, *Maba*, *Macreightia*, *Rospidios*, and *Royena*. Hiern (1873) placed the 249 then-recognized species in five genera, *Diospyros*, *Euclea*, *Maba*, *Royena*, and *Tetraclis*. In contrast to de Candolle's system, he proposed a new Madagscan endemic, *Tetraclis*, and lumped *Cargillia*, *Gunisanthus*, and *Rospidios* with *Diospyros* and *Macreightia* with *Maba*. Bakhuizen (1936–1955) in his regional revision united *Maba* with *Diospyros*, thus recognizing only four genera, *Diospyros*, *Euclea*, *Royena*, and *Tetraclis* (the last three genera only according to the literature; they were not in the area he covered). Bakhuizen also divided *Diospyros* into five subgenera (Table 1). White (1980, 1983) had a much broader generic concept, reducing *Royena* and *Tetraclis* to synonymy with

TABLE 1. Infrafamilial classification of Ebenaceae s.s. Genera, subgenera, and sections are ordered alphabetically; the number of species accepted by each author is given in parentheses.

de Candolle (1844)	Hiern (1873)	Bakhuizen (1936–1955)	White (1980, 1983, 1993)	Singh (2005)
Area treated: Worldwide (158)	Worldwide (249)	SE Asia and Pacific region (192)	Africa (103)	India (66)
Genus *Cargillia* (2)	Included in *Diospyros*	Included in *Diospyros*	Included in *Diospyros*	Included in *Diospyros*
Genus *Diospyros* (ca. 96)	Genus *Diospyros* (ca. 160)	Genus *Diospyros* (ca. 192)	Genus *Diospyros* (ca. 91)	Genus *Diospyros* (ca. 66)
Sect. *Amuxis*	Sect. *Amuxis*	Subgen. *Cargillia* (2)	Sect. *Asteropetala*	Sect. *Acanthebenus*
Sect. *Diospyros* (*Eudiospyros*)	Sect. *Cargillia*	Subgen. *Diospyros* (*Eudiospyros*; ca. 172)	Sect. *Brevistyla*	Sect. *Assimilis*
Sect. *Otogyne*	Sect. *Cavanillea*	Sect. *Acanthebenus*	Sect. *Brevituba*	Sect. *Atrata*
Sect. *Tetradiospyros*	Sect. *Cunalonia*	Sect. *Asterocalix*	Sect. *Calvitiella*	Sect. *Barberi*
	Sect. *Danzleria*	Sect. *Basithrix*	Sect. *Dodonium*	Sect. *Basithrix*
	Sect. *Ebenus*	Sect. *Brachycylix*	Sect. *Entia*	Sect. *Brachycylix*
	Sect. *Ermellinus*	Sect. *Campanulata*	Sect. *Erikesi*	Sect. *Candolleana*
	Sect. *Guaiacana*	Sect. *Cavanilleastrum*	Sect. *Forbesia*	Sect. *Campanulata*
	Sect. *Gunisanthus*	Sect. *Caudifera*	Sect. *Katula*	Sect. *Confertiflora*
	Sect. *Leucoxylum*	Sect. *Cladantha*	Sect. *Lagenaria*	Sect. *Crumenata*
	Sect. *Melonia*	Sect. *Confertiflora*	Sect. *Latibulum*	Sect. *Ebenaster*
	Sect. *Noltia*	Sect. *Didymanthera*	Sect. *Maba*	Sect. *Ebenopsis*
	Sect. *Paralea*	Sect. *Ebenaster*	Sect. *Marsupium*	Sect. *Ebenus*
	Sect. *Patonia*	Sect. *Ebenopsis*	Sect. *Myrmecophila*	Sect. *Eucarpon*
	Sect. *Rospidios*	Sect. *Ebenus*	Sect. *Noltia*	Sect. *Hirsuta*
		Sect. *Eriantha*	Sect. *Rhaphidanthe*	Sect. *Insignis*
		Sect. *Eucarpon*	Sect. *Royena*	Sect. *Kaki*
		Sect. *Glutinosa*	Sect. *Tabonaca*	Sect. *Kurzella*
		Sect. *Kurzella*		Sect. *Liophylla*
		Sect. *Liophylla*		Sect. *Lotus*
		Sect. *Lotus*		Sect. *Maba*
		Sect. *Nesindica*		Sect. *Nesindica*
		Sect. *Pachycylix*		Sect. *Ptychocylix*
		Sect. *Phyllosepala*		Sect. *Pyrrhocarpoides*
		Sect. *Podophora*		Sect. *Stelechantha*
		Sect. *Ptychocylix*		Sect. *Sylvatica*
		Sect. *Reflexocalix*		Sect. *Trichophylla*
		Sect. *Rigidophylla*		
		Sect. *Saccocalix*		
		Sect. *Sapotanigra*		
		Sect. *Stelechantha*		
		Sect. *Trisantha*		
		Sect. *Truncicalix*		
		Sect. *Verruculosa*		
		Subgen. *Hierniodendron* (3)		
		Subgen. *Maba* (ca. 14)		
		Sect. *Cupulifera*		
		Sect. *Ferreola*		
		Sect. *Rhipidostigma*		
		Sect. *Miquelia*		
		Subgen. *Mabacea* (1)		
Genus *Euclea* (16)	Genus *Euclea* (19)	Genus *Euclea* (–)	Genus *Euclea* (12)	Genus *Euclea* (–)
Sect. *Rymia*				
Sect. *Ortheuclea*				
Genus *Gunisanthus* (1)	Included in *Diospyros*	Included in *Diospyros*	Included in *Diospyros*	Included in *Diospyros*
Genus *Maba* (17)	Genus *Maba* (56)	Included in *Diospyros*	Included in *Diospyros*	Included in *Diospyros*
	Sect. *Barberia*			
	Sect. *Ferreola*			
	Sect. *Holochilus*			
	Sect. *Macreightia*			
	Sect. *Rhipidostigma*			
	Sect. *Trichanthera*			
Genus *Macreightia* (7)	Included in *Maba*	Included in *Diospyros*	Included in *Diospyros*	Included in *Diospyros*
Genus *Rospidios* (1)	Included in *Diospyros*	Included in *Diospyros*	Included in *Diospyros*	Included in *Diospyros*
Genus *Royena* (18)	Genus *Royena* (13)	Genus *Royena* (–)	Included in *Diospyros*	Included in *Diospyros*
—	Genus *Tetraclis* (1)	Genus *Tetraclis* (–)	Included in *Diospyros*	Included in *Diospyros*

Diospyros; he thus recognized only two genera, *Diospyros* and *Euclea*, in Ebenaceae s.s. Singh (2005), while dealing with Indian Ebenaceae, followed the generic concepts of White (1980, 1983). He divided Indian *Diospyros* species into 27 sections. Recently, the monogeneric family, Lissocarpaceae has been formally included in Ebenaceae (Wallnöfer, 2004); *Lissocarpa* was divided into two sections, *Lissocarpa* and *Enho*. Molecular studies previously suggested that *Diospyros* sensu White (1980) is paraphyletic because species of *Diospyros* section *Royena* form a clade with *Euclea* (Morton et al., 1996; Berry et al., 2001), and this relationship is also supported by morphological and anatomical features (club-shaped trichomes and ingrowth of testa wall) that are shared between *Diospyros* section *Royena* and *Euclea* (Morton et al., 1996; Wallnöfer, 2001). In fact, it is still unclear how many genera of Ebenaceae reflect monophyletic groups. A molecular phylogenetic approach should help to resolve infrafamilial relationships of Ebenaceae and aid in assessments of generic boundaries within the family as it had done in the other families, e.g., Rhamnaceae (Richardson et al., 2000a, b), Zingiberaceae (Kress et al., 2002) and Phyllanthaceae (Kathriarachchi et al., 2005).

No studies with a broad sampling of species and a large amount of data have focused upon phylogenetics of Ebenaceae (cf., de Franceschi, 1993; Berry et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; Geeraerts, 2003). Many phylogenetic questions about the family raised by Berry et al. (2001) still need to be investigated, such as (1) the position of *Lissocarpa* when more species are included in the analysis, (2) the position of *Diospyros* section *Royena* as sister to *Euclea*, and (3) the relationships of this sister pair with respect to the remaining species of *Diospyros*. To answer these questions, we have to develop clearer phylogenetic hypotheses, for which we need more taxon sampling, especially for the problematic genera, and additional DNA markers.

In this study, we assess phylogenetic relationships in Ebenaceae s.l. using plastid nucleotide sequences from three coding regions (*atpB*, *ndhF*, and *matK*) and three noncoding regions (*trnK* intron, *trnL* intron, and *trnL-trnF* intergenic spacer). Plastid coding regions, such as *atpB*, *ndhF*, and *matK*, have proven useful in addressing phylogenetic relationships at higher taxonomic categories, whereas noncoding regions (introns and intergenic spacers) are viewed as being more useful at lower taxonomic categories (see Soltis and Soltis, 1998), but there are exceptions to this general pattern (e.g., Chase et al., 2000; Pires et al., 2001). Many studies (Reeves et al., 2001; Kress et al., 2002; Wang et al., 2004; Barfuss et al., 2005; Kathriarachchi et al., 2005; Wurdack et al., 2005; Soejima and Wen, 2006) have used combined plastid DNA data from several regions, coding and noncoding, to provide improved resolution compared to single-region analyses. Our specific objectives were to (1) investigate monophyly of Ebenaceae s.l. when more taxa and more data are included in the analysis, (2) assess generic boundaries by evaluating relationships, and (3) identify characteristic features that may be putative synapomorphies useful for classification within Ebenaceae.

MATERIALS AND METHODS

Taxon sampling and plant material—Accession name, voucher citations, and GenBank numbers for all sequences are provided in Appendix 1. Ingroup sampling comprised 99 species, including all currently recognized genera of Ebenaceae, *Diospyros*, *Euclea*, and *Lissocarpa* (Wallnöfer, 2001, 2004) and representatives of taxa that were previously recognized at the generic level, e.g., *Cargillia*, *Gunisanthus*, *Maba*, *Macreightia*, *Royena*, and *Tetraclis* (de Candolle, 1844; Hiern, 1873). In the case of the largest genus, *Diospyros*, our sampling represents all parts of its distribution (South, Central and North America, Africa, Madagascar, Asia, Australia and New Caledonia). Outgroup sampling included Lecythidaceae, Maesaceae, Marcgraviaceae, Pentaphylacaceae, Sapotaceae, Styracaceae, and Theaceae, as found in previous studies (Anderberg et al., 2002; Bremer et al., 2002; Schönenberger et al., 2005).

Silica-gel-dried collections (Chase and Hills, 1991) were obtained during fieldtrips in Brunei, Madagascar, New Caledonia, South America, and Thailand, as well as from the DNA bank of the Missouri Botanical Garden (http://mobot.mobot.org/W3T/Search/dna/projsdna.html) and the Royal Botanic Gardens, Kew (http://www.rbgkew.org.uk/data/dnaBank/homepage.html). The remaining DNA extractions were obtained from herbarium material at the Royal Botanic Gardens, Kew (K) and the Natural History Museum Wien (W).

DNA extraction, amplification, and sequencing—Total DNA was extracted following the 2× cetyltrimethyl ammonium bromide (CTAB) procedure of Doyle and Doyle (1987) with minor modifications (see Muellner et al., 2005); we washed ground plant material with sorbitol buffer (Tel-zur et al., 1999) followed by centrifugation before incubating with CTAB buffer. Samples from Royal Botanic Gardens, Kew, and the DNA bank of Missouri Botanical Garden were extracted at Jodrell Laboratory, Royal Botanic Gardens, Kew, using the same procedure but with cleaning on a cesium chloride/ethidium bromide gradient (1.55 g·ml^{-1}).

Amplifications of selected regions used 50-μL reactions containing 45 μL 1.1× ReddyMix PCR Master Mix (Advanced Biotechnologies, ABgene House, UK), 2 μL 1.0% bovine serum albumin (BSA), 1 μL each primer (20 mmol/L) and 1 μL template DNA. The PCR profile consisted of an initial 3-min premelt at 94°C and 36 cycles of 1-min denaturation at 94°C, 1-min annealing at 48°C, and a 1-min (or 2-min, depending on length of the amplified fragment) extension at 72°C, followed by final extension of 10 min at 72°C.

Primers used are those described in Hoot et al. (1995) for *atpB*, Samuel et al. (2005) for *matK* and the rest of the *trnK* intron, Olmstead and Sweere (1994) and Oxelman et al. (1999) for *ndhF* (3′ portion), and Taberlet et al. (1991) for *trnL* intron and *trnL-trnF* intergenic spacer. Products were purified with the Invisorb Spin DNA Extraction kit (Invitek GmbH, Berlin, Germany) or ExoSAP-IT kit (USB Corp., Cleveland, Ohio, USA). Cycle sequencing was performed using the ABI PRISM BigDye Terminator Cycle Sequence kit, version 3.1 (Applied Biosystems, ABI, Vienna, Austria) using the same primers that we used for amplification and the manufacturer's protocols. Sequences were initially edited using Sequence Navigator (ABI), and complementary sequences were assembled using AutoAssembler version 1.4.0 (ABI).

A total of 107 *atpB*, 107 *ndhF*, 110 *matK-trnK* intron, and 109 *trnL* intron and *trnL-trnF* intergenic spacer sequences were newly generated for this study for the ingroup and outgroup taxa. The remaining 25 sequences of seven outgroup taxa were obtained from GenBank.

Sequencing alignment and phylogenetic analysis—Sequences were initially aligned with ClustalX (Thompson et al., 1997), and the alignment was adjusted visually following the guidelines of Kelchner (2000). No indels were found in alignment of *atpB*, whereas length variation (in multiples of three) was observed in *matK* and *ndhF*. Most of the data matrices contained 99 ingroup taxa except the *atpB* and *ndhF* matrices, which contained 97 ingroup taxa. In the *matK* and *trnK* matrices, there are 15 outgroup taxa, but the rest of the data matrices contained 16 outgroup taxa (Table 2). For some of the outgroup taxa, data from different species within the same genus were combined into a single terminal, i.e., (1) for *Marcgravia*, *M. rectiflora* was available for *atpB*, *ndhF*, *trnL* intron, and *trnL-trnF* spacer and *Marcgravia* sp. for *matK* and *trnK* intron; (2) for *Norantea*, *N. peduncularis* was available for *atpB* and *ndhF* and *N. guianensis* for *trnL* intron and *trnL-trnF* spacer; and (3) for *Maesa*, *M. myrsinoides* was available for *atpB* and *M. tenera* for *ndhF*, *matK*, *trnK* intron, *trnL* intron, and *trnL-trnF* spacer.

Phylogenetic analyses of Ebenaceae and closely related families were performed using both maximum parsimony (MP) and Bayesian inference. Fitch parsimony analyses (Fitch, 1971; equal weight for all changes) were performed as implemented in PAUP* version 4.0b10 (Swofford, 2003) with a two-stage search strategy. Nine data matrices were analyzed (see Table 2): (1) partial *atpB*, (2) partial *ndhF*, (3) *matK*, (4) *trnK* intron, (5) *trnL* intron (including *trnL* 3′ exon), (6) *trnL-trnF* intergenic spacer, (7) combined coding regions, (8)

Table 2.   Summary characteristics and maximum parsimony statistics for the nine matrices for phylogenetic analyses of Ebenaceae s.l.

Phylogenetic information	atpB	ndhF	matK	trnK Intron	trnL Intron	trnL-trnF Intergenic spacer	Combined coding region	Combined noncoding region	Combined six regions
No. ingroup taxa	97	97	99	99	99	99	99	99	99
No. outgroup taxa	16	16	15	15	16	16	16	16	16
Sequence length	1396	882–915	1497–1527	837–933	529–580	163–405			
(only ingroup)	(1396)	(882–903)	(1497–1527)	(837–919)	(529–568)	(163–387)			
Aligned length used in analysis	1396	942	1545	1,100	617	480			
No. variable characters (%)	288	427	635	346	177	201			
	(20.63)	(45.33)	(41.10)	(31.45)	(28.69)	(41.88)			
No. potentially parsimony-informative characters (%)	192	308	427	230	130	149			
	(13.75)	(32.70)	(27.64)	(20.91)	(21.07)	(31.04)			
No. trees retained	>15 000	>15 000	>15 000	>15 000	>15 000	47	>15 000	>15 000	>15 000
Tree length (step)	490	927	1144	620	293	353	2589	1295	3902
Consistency index (CI)	0.67	0.64	0.69	0.68	0.73	0.69	0.66	0.68	0.66
Retention index (RI)	0.84	0.80	0.81	0.81	0.82	0.83	0.80	0.81	0.80
Tree length/no. variable characters	1.70	2.17	1.80	1.79	1.66	1.76	2.79	2.54	2.72
No. clades present in combined all regions tree (ingroup only)	34	40	49	34	21	14	63	52	72
No. clades with low BP support (ingroup only)	10	16	18	8	13	6	11	14	15
No. clades with moderate BP support (ingroup only)	3	3	2	5	2	3	4	8	2
No. clades with high BP support (ingroup only)	17	16	30	17	3	5	46	26	54

combined noncoding regions, and (9) all six regions combined. Gaps were treated as missing data and not scored for inclusion because they did not provide any additional support for clades, i.e., the clades they characterized already had strong support from the sequence data; however, some indels are mapped onto Fig. 3 to demonstrate their congruence with the results found without their inclusion. Only the combined coding, noncoding, and all six regions results are shown. For each data set, heuristic searches were conducted using 1000 replicates random taxon addition, tree-bisection-reconnection (TBR) branch swapping, and "keeping multiple trees" (MulTrees) in effect, but saving only 10 trees per replicate. All trees found in the 1000 replicates were then used as starting trees for another search with a restriction of 15 000 trees, which were allowed to be swapped to completion. To assess support for each clade, bootstrap analyses (Felsenstein, 1985) were performed with 1000 bootstrap replicates, TBR branch swapping, and simple sequence addition. Bootstrap percentages are described as high (85–100%), moderate (75–84%), or low (50–74%).

Although all regions are located in plastid genome, which is inherited as a unit without recombination, we assessed incongruence of each data matrix using the partition homogeneity test (ILD test; Farris et al., 1994) as implemented in PAUP* version 4.0b10. We used 1000 replicates on parsimony-informative characters using TBR branch swapping with simple sequence addition and the MulTrees option in effect.

Bayesian inference (Huelsenbeck and Ronquist, 2001; Lewis, 2001) was performed with MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001) for a matrix of the six regions combined. Modeltest 3.7 (Posada and Crandall, 1998) was used to find the best-fitting substitution model; the GTR + I + G model was selected for the combined data matrix. Parameters set were nst = 6 and rate = invgamma; the others used were the default settings. The Markov chains, three heated and one cold, ran simultaneously starting from a random tree for one million generations, and trees were sampled every 100 generations. Trees that preceded stabilization of the likelihood value were discarded as the burn-in (1300 trees). The majority-rule consensus tree containing posterior probabilities (PP; Larget and Simon, 1999) was built from the remaining trees sampled. Bayesian analyses were repeated four times to confirm results.

RESULTS

The data characteristics and statistics from the maximum parsimony analyses of the nine matrices are given in Table 2. The results of the six individual region analyses support for monophyly of Ebenaceae s.l. but only partially resolved relationships within the family (trees not shown). These relationships are much more resolved for matrices of the combined noncoding, combined coding, and the six regions combined (Figs. 1–4). Because of poor congruence of our molecular results with previous classifications of Ebenaceae s.l. and a need for clade names, we named the major clades (Figs. 1–4) recovered from our DNA analyses as A–Q (Table 3). There is no significant conflict (p value of ILD test = 0.31) for tree topologies among the six (atpB, ndhF, matK, trnK intron, trnL intron, and trnL-trnF intergeneric spacer) individual data sets.

Individual analyses of the six plastid regions—Comparative characteristics of each plastid region included in this study are shown in Table 2. There are 192, 308, 427, 230, 130, and 149 potentially parsimony-informative characters in atpB, ndhF, matK, trnK intron, trnL intron, and trnL-trnF spacer, respectively, and thus relative variation (number of parsimony-informative characters per number of total characters in each data set analysis × 100%) is 13.75, 32.70, 27.64, 20.91, 21.06, and 31.40, respectively. The matK matrix contributed a greater number of steps (1144) than ndhF (927), trnK intron (620), atpB (490), trnL-trnF spacer (353), and trnL intron (293). The variable characters of ndhF change more frequently than those in matK, trnK intron, trnL-trnF spacer, atpB, and trnL intron; average change per variable character is 2.17, 1.80, 1.79, 1.76, 1.70, and 1.66, respectively. All six regions give nearly identical consistency (CI, 0.64–0.73) and retention indices (RI, 0.80–0.84) and have congruent tree topologies (p value of ILD test = 0.31), but they provide different levels of resolution within Ebenaceae s.l. The number of clades that are present in the combined analysis and strict consensus trees of atpB, ndhF, matK, trnK intron, trnL intron, and trnL-trnF spacer is 34, 40, 49, 34, 21, and 14, respectively.

Analysis of the six regions combined—Phylogenetic trees generated from maximum parsimony analysis of the combined matrix are shown as strict consensus tree in Fig. 2 and an individual tree (randomly selected) with branch lengths
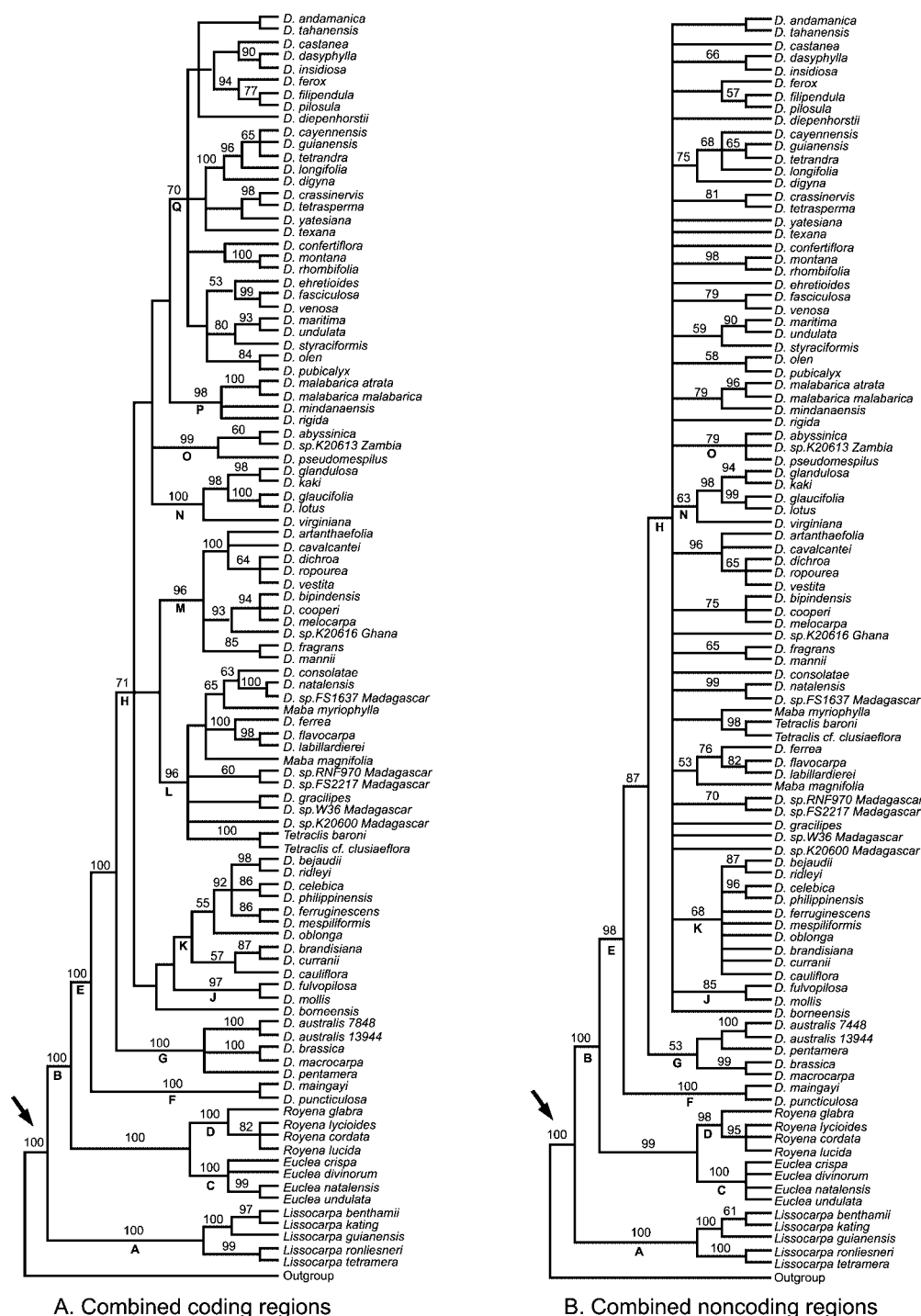
Fig. 1. Strict consensus tree of 15 000 equally parsimonious trees resulting from parsimony analysis of combined coding and noncoding regions (a tree limit of 15 000 trees was enforced). Arrows indicate Ebenaceae s.l. For relationships among the outgroup taxa, see Fig. 2; bootstrap percentages (BP) are given above branches; clades with less than 50 BP have nothing indicated. Letters below branches indicate clades discussed in the text. (A) Combined coding regions, (B) combined noncoding regions.

(DELTRAN optimization) in Fig. 3. Results show strong support for monophyly of all seven outgroup families, but interfamilial relationships between Ebenaceae s.l. and outgroups are not well resolved. Monophyly of Ebenaceae s.l. is strongly supported (BP 100) as well as that of the two major clades (labeled A and B, BP 100). The first major clade (A)

contains only members of *Lissocarpa* (subfamily Lissocarpoideae sensu Wallnöfer) and is divided into two subclades that are congruent with the infrageneric classification of Wallnöfer (2004). The second major clade (B) contains all members of Ebenaceae s.s. and has two well-supported subclades: C/D (BP 100) and E (BP 100). Subclade C/D consists of two well-
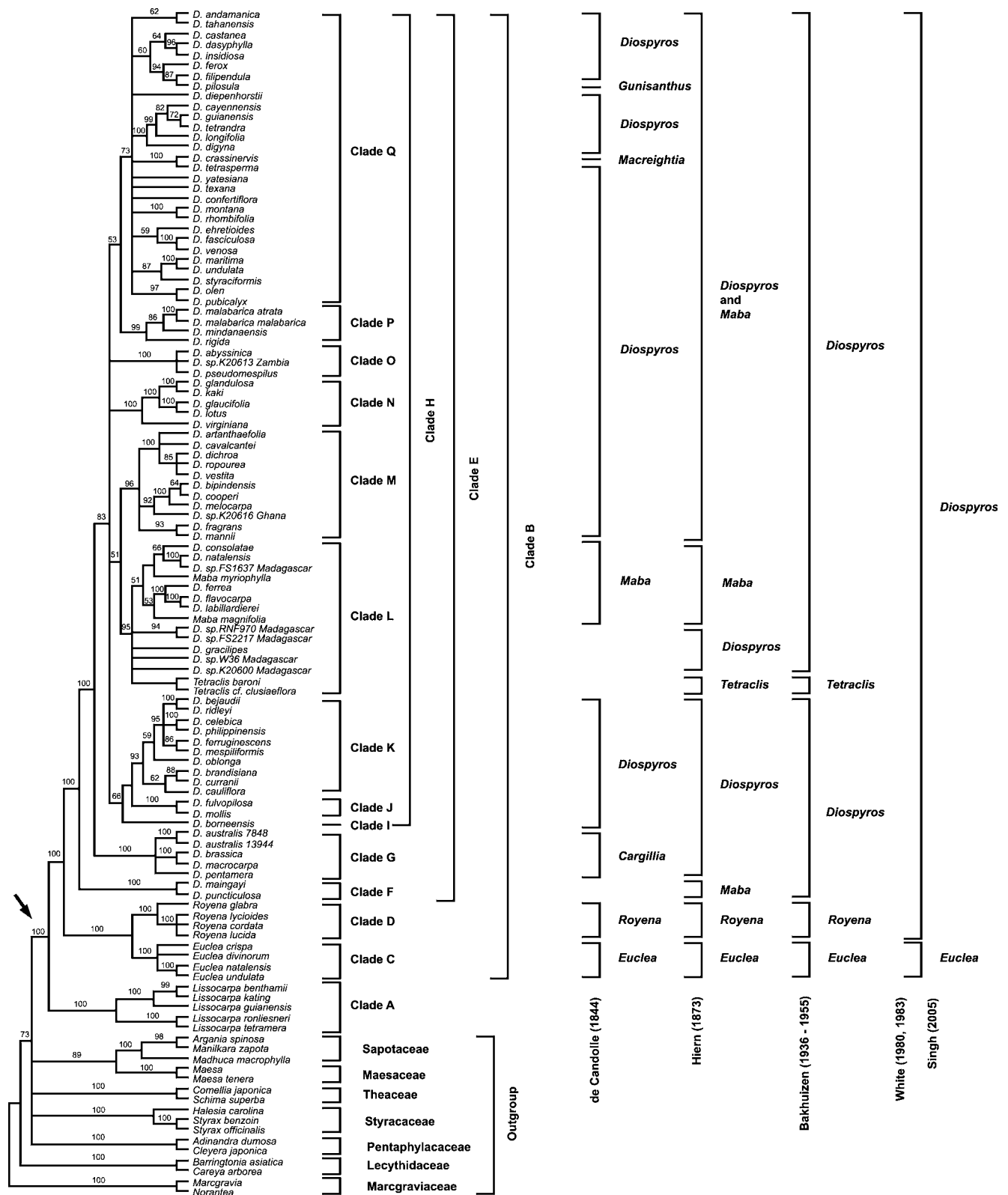
Fig. 2.   Strict consensus tree of 15 000 equally parsimonious trees resulting from analysis of the combined plastid regions (a tree limit of 15 000 trees was enforced). The arrow indicates Ebenaceae s.l. Bootstrap percentages (BP) are indicated above branches; clades with less than 50 BP have nothing indicated. The clades discussed in the text and circumscriptions from previous infrafamilial classifications (de Candolle, 1844; Hiern, 1873; Bakhuizen, 1936–1955; White, 1980, 1983; Singh, 2005) are labeled on the right side of the tree. Clades with no taxon indicated in de Candolle's classification (1844) were unknown at that time, so these are left unlabelled.
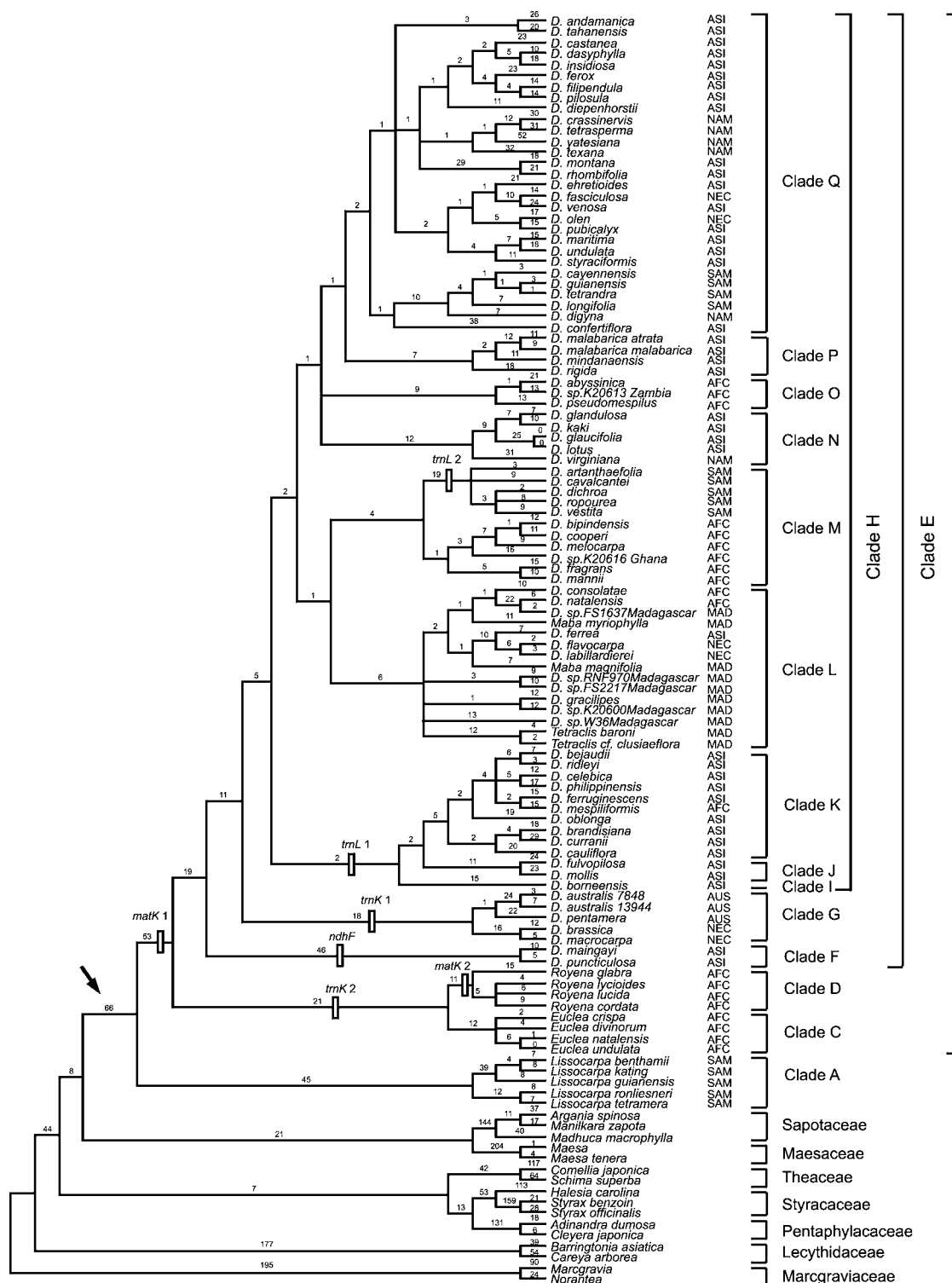
Fig. 3. One of the most parsimonious trees resulting from parsimony analysis of combined regions. The arrow indicates Ebenaceae s.l. Branch lengths (DELTRAN optimization) are indicated above branches. Potentially informative indels are indicated by hollow bars. Geographic distributions (AFC, Africa; ASI, Asia; AUS, Australia; MAD, Madagascar; NAM, North and Central America; NEC, New Caledonia; SAM, South America) are indicated to the right of the tree as well as clades that are referred to in the text.
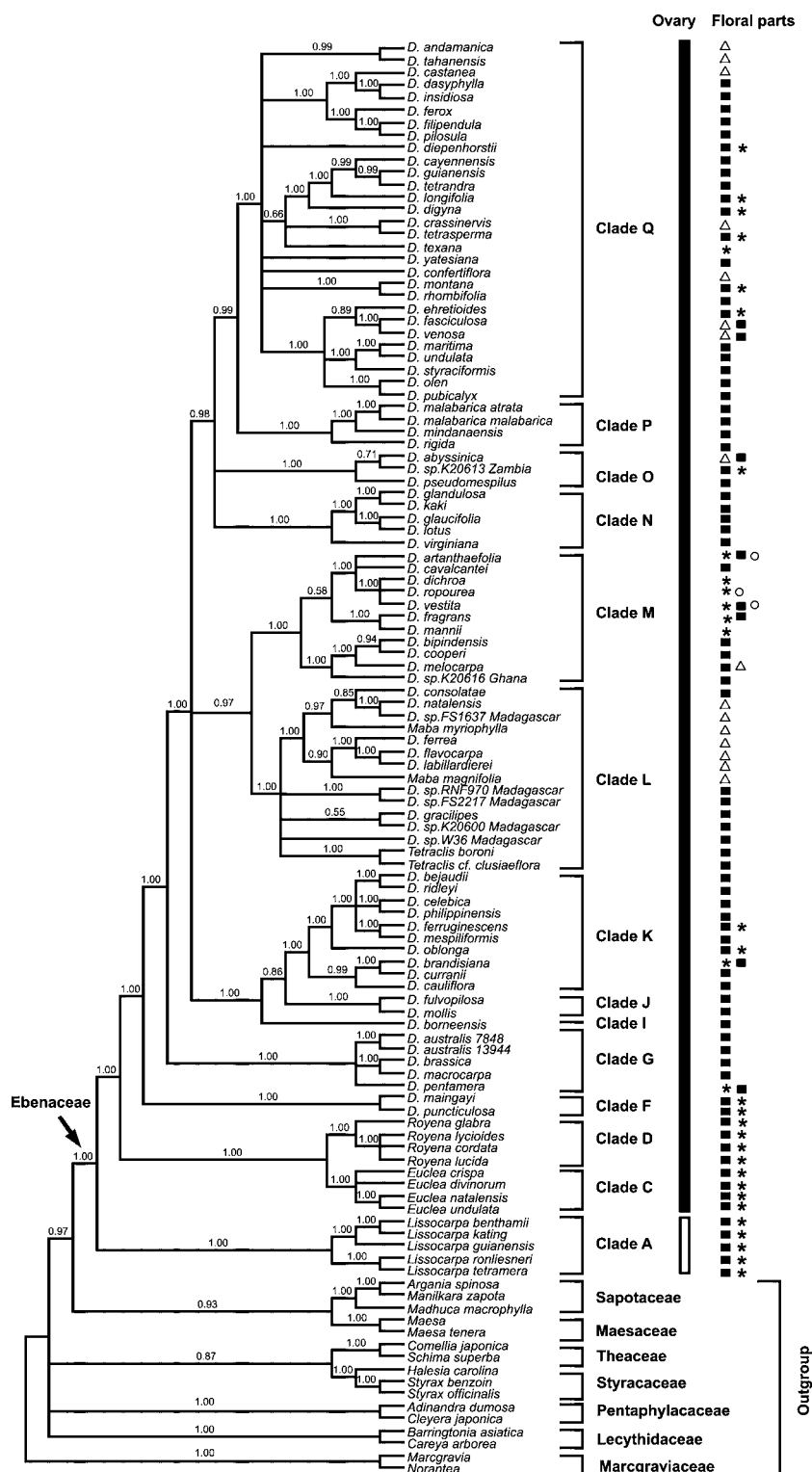
Fig. 4. Bayesian majority-rule probability tree from analysis of the combined plastid regions. Posterior probabilities are given above branches. Presence of ovary type and number of floral parts, which have been important characters used in Ebenaceae taxonomy in the past, are indicated with symbols to right of the tree. Ovary position can be either superior (black bar) or inferior (white bar). Number of floral parts can be divided into four classes: trimerous flowers (△), tetramerous flowers (■), pentamerous flowers (*), and hexamerous flowers (○).

TABLE 3. Clades indicated in figures. General geographical information; numbers of taxa are also provided.

Clade	Taxa (distribution; number of taxa within clade)
A	Genus *Lissocarpa* (South America; 5)
B	Ebenaceae s.s. (Pantropical; 94)
(including clades C to Q)	
C	Genus *Euclea* (Africa, Arabia, Socotra, and the Comoro Islands; 4)
D	Genus *Royena* (= *Diospyros* section *Royena* sensu White; Africa; 4)
E	Remaining *Diospyros* taxa (excluding *D*. section *Royena*) plus genus *Tetraclis* and two *Maba* species from Madagascar; (Pantropical; 86)
(including clades F to Q)	
F	*D*. subgenus *Hierniodendron* sensu Bakhuizen (Asia; 2)
G	*D*. subgenus *Cargillia* sensu Bakhuizen plus two *Diospyros* species from New Caledonia (Australia and New Caledonia; 4)
H	Remaining *Diospyros* taxa plus genus *Tetraclis* (Pantropical; 80)
(including clades I to Q)	
I	*D. borneensis* (Asia; 1)
J	*D. mollis* group (Asia; 2)
K	*D. celebica* and *D. mespiliformis* group (Asia and Africa; 10)
L	*D. ferrea* complex, including *Tetraclis*, all *Diospyros* and two *Maba* species from Madagascar (Africa, Asia, Madagascar, and New Caledonia; 15)
M	*D. bipindensis* and *D. artanthaefolia* group (Africa and South America; 11)
N	*D. kaki* group (temperate Asia and North America; 5)
O	*D. abyssinica* group (Africa; 3)
P	*D. malabarica* group (Asia; 4)
Q	Group of remaining *Diospyros* species from Asia, New Caledonia, North America, and South America (29)

supported sister taxa, *Euclea* and *Royena* (BP 100; the latter previously recognized as *Diospyros* section *Royena* sensu White, 1980, 1983). Within the diverse clade E, which comprises all other taxa of *Diospyros*, Madagascan *Maba* and *Tetraclis*, there are two groups (F, BP 100; and G, BP 100, respectively) that are successive sisters to a large internally unresolved clade (H BP 83). Clade F contains two species of *Diospyros* subgenus *Hierniodendron* sensu Bakhuizen and clade G consists of *Diospyros* subgenus *Cargillia* sensu Bakhuizen and other two *Diospyros* species from New Caledonia. Although relationships within the large clade (H) that contains the remaining species of *Diospyros* and genus *Tetraclis* are unresolved, there are within it nine well-supported clades: I, J (BP 100), K (BP 93), L (BP 95), M (BP 96), N (BP 100), O (BP 100), P (BP 99), and Q (BP 73).

Diospyros borneensis (I) is sister to clade J/K, but support is low. Clade J contains two species of Asian *Diospyros*, *D. fulvopilosa* and *D. mollis*. Clade K consists of nine species of Asian and one African *Diospyros*, *D. mespiliformis*. Clade L includes *Tetraclis* sensu Hiern, the *Diospyros ferrea* complex (including two Madagascan *Maba* species), and all *Diospyros* species from Madagascar. Clade M has several African and South American *Diospyros* and is further split into three subgroups. The sixth lineage is the *Diospyros kaki* group (N), which consists of five subtemperate to temperate species from Asia and North America. Clade O is a group of three African *Diospyros* species. Clade P contains four Asian species of *Diospyros*, and clade Q, which has high sequence divergence (Fig. 3), has 29 American, Asian, and New Caledonian *Diospyros* species.

In the Bayesian analyses of the combined matrix, there is no major conflict of tree topologies among the four independent analyses. The majority-rule consensus tree is shown in Fig. 4. The Bayesian tree is generally congruent with that inferred from maximum parsimony (Fig. 2), except for the sister-group relationship of clade N and clade O to clade P/Q; these three clades appear as a polytomy in the parsimony results.

DISCUSSION

Utility of the plastid regions used—The combined analysis of all regions provides clearer phylogenetic relationships within the Ebenaceae s.l. than any of the individual analyses; this was observed in other studies as well and is now an expected phenomenon (Reeves et al., 2001; Kress et al., 2002; Wang et al., 2004; Barfuss et al., 2005; Kathriarachchi et al., 2005; Wurdack et al., 2005; Soejima and Wen, 2006). Each of the six individual regions possessed different amounts of phylogenetic data (Table 2). Overall, *matK* and 3′ *ndhF* are more useful than the other four regions (*atpB*, *trnK* intron, *trnL* intron, and *trnL-trnF* spacer) because these two regions have more parsimony informative characters; furthermore, variable sites within 3′ *ndhF* change more often, which if sampled extensively provides more phylogenetic structure (Table 2; Asmussen and Chase, 2001). The strict consensus trees resulting from the individual analyses of *matK* and *ndhF* displayed more consistently resolved clades (49 and 40, respectively) than those of the other four regions (34, 34, 21, and 14 for *atpB*, *trnK* intron, *trnL* intron and *trnL-trnF* spacer, respectively), and topologies of the *matK* and *ndhF* trees are most similar to that from the combined matrix (Fig. 2). Overall, *ndhF* resolved the deeper nodes better than the more terminal ones, which is in contrast with the results from analysis of *matK*. As expected from previous studies, *atpB* has low numbers of phylogenetically informative sites within Ebenaceae s.l.; it has only 192 parsimony-informative sites (13.75% of the total number of characters). The three noncoding regions (*trnK* intron, *trnL* intron, *trnL-trnF* spacer) and even the combined analysis of these three regions (Fig. 1B) did not clearly resolve phylogenetic relationships within Ebenaceae s.l., especially within the large and diverse genus *Diospyros* (clade E). Nonetheless, the addition of data from these three regions resulted in improved resolution, increasing the number of supported clades from 61 (Fig. 1A) to 71 (Fig. 2) and also increasing the bootstrap percentages.

This study is one of only a few examples illustrating the

phylogenetic utility of *ndhF* at the infrageneric level (e.g., Clarkson et al. [2004] used it within *Nicotiana*, Solanaceae). Generally, this marker has been used for phylogenetic analyses at the generic level and above (Olmstead and Sweere, 1994; Olmstead and Reeves, 1995; Davis et al., 2001; Kim et al., 2001; Levin et al., 2003; Datwyler and Weiblen, 2004; Lohmann, 2006). In our study, *ndhF* helps resolve relationships within *Diospyros* (clade E, tree not shown). As mentioned, 3′ *ndhF* provided more parsimony-informative characters (32.70%) than any of the other regions (13.75–31.04%), and also each individual site within *ndhF* changed more often than those in the other regions. Not surprisingly, *matK* was also found to be useful at infrageneric levels; many previous studies have confirmed its utility at this level (e.g., Miller and Bayer, 2001; Wang et al., 2004; Rønsted et al., 2005; Kathriarachchi et al., 2006).

Comparison between molecular evidence and previous classifications—This extensive study, including approximately 20% of the species of Ebenaceae, supports monophyly of Ebenaceae s.l. and is consistent with the recent inclusion of *Lissocarpa* in Ebenaceae (Berry et al., 2001; APG II, 2003) as the monogeneric subfamily Lissocarpoideae (Wallnöfer, 2004). Putative synapomorphic characters of Ebenaceae s.l. are the presence of naphthoquinones, lack of stipules, extrafloral nectaries on abaxial leaf surfaces, a persistent calyx, usually unisexual flowers, and pendulous ovules (Wallnöfer, 2001, 2004). The similarity of wood anatomy of *Lissocarpa* and *Diospyros* was already pointed out in previous studies (Ng, 1971, 1991; Berry et al., 2001; Lens, 2005). Our results also provide a clear estimate of phylogenetic relationships within Ebenaceae s.l. and clarify generic boundaries that have been considered to be problematic in previous classifications. The infrafamilial classifications of de Candolle (1844), Hiern (1873), Bakhuizen (1936–1955), White (1980, 1983, 1993), and Singh (2005) (see Table 1) are only partially congruent with our molecular results (Fig. 2); at least some of the generic or infrageneric taxa in each system are not monophyletic.

De Candolle (1844) recognized the following eight genera within Ebenaceae s.s.: *Cargillia*, *Diospyros*, *Euclea*, *Gunisanthus*, *Maba*, *Macreightia*, *Rospidios*, and *Royena* (Table 1). Compared with our molecular results, only two genera, *Euclea* and *Royena*, are monophyletic and independent from *Diospyros*, and the other four genera, *Cargillia*, *Gunisanthus*, *Maba*, and *Macreightia*, are embedded within *Diospyros*. According to our results, two species of *Cargillia* (represented here by *D. australis* and *D. pentamera*) are grouped together with two species of *Diospyros* from New Caledonia (clade G) and are the sister group of *Diospyros* clade H (Fig. 2). The monotypic genus *Gunisanthus* with only *D. pilosula* does not warrant generic status on morphological grounds and is closely related to two Asian *Diospyros* species in clade Q (BP 94). All of them share long, slender pedicels and tetralocular ovaries. *Maba* sensu de Candolle forms a monophyletic group together with some Madagascan *Diospyros* and *Tetraclis* species. Only one species of the genus *Macreightia*, represented here by *D. crassinervis* (occurring in the Carribean islands), was included in our study. It is strongly supported as a sister to *D. tetrasperma* (occurring in Central America and the Carribean islands) in clade Q. In addition the infrageneric classification of *Diospyros* sensu de Candolle is artificial because the four sections of *Diospyros* that he recognized are not monophyletic.

In the last worldwide revision of Ebenaceae s.s., five genera

were recognized by Hiern (1873): *Diospyros*, *Euclea*, *Maba*, *Royena*, and *Tetraclis* (Table 1). He recognized *Tetraclis* as an independent genus because of its valvate corolla, and Perrier de la Bâthie (1952) used the same feature to distinguish three species of *Tetraclis*. As shown by our results (Figs. 2–4), *Tetraclis* (*T. baroni* and *T.* cf. *clusiaeflora*) does not deserve generic status because it groups with Madagascan species of *Diospyros* and other members of the *D. ferrea* complex (*Maba* sensu de Candolle) in clade L. The genus *Maba* sensu Hiern, which is broader than *Maba* sensu de Candolle, is a group of species with usually trimerous flowers. It consists of the following six sections, *Ferreola*, *Macreightia*, *Holochilus*, *Rhipidostigma*, *Barberia*, and *Trichanthera*. *Maba* as circumscribed by Hiern is polyphyletic in our results. Species of this concept appear in many different positions within *Diospyros*, such as in clade F (*Maba* section *Barberia*, represented here by *D. maingayi* and *D. puncticulosa*), clade L (*Maba* sections *Ferreola* and *Holochilus*, represented here by the *D. ferrea* complex and *D. natalensis*, respectively), clade O (*Maba* section *Holochilus*, with *D. abyssinica*), and clade Q (*Maba* sections *Macreightia* and *Rhipidostigma*, with *D. crassinervis*, *D. confertiflora*, *D. fasciculosa*, and *D. venosa*). *Tetraclis* and *Maba* are thus embedded within *Diospyros* sensu Hiern. As is the case in de Candolle's system, only two genera, *Euclea* and *Royena*, are strongly supported as monophyletic. Fifteen sections were recognized in the infrageneric classification of *Diospyros* sensu Hiern (Table 1). However, members of section *Melonia* turned up in three clades, J (*D. fulvopilosa* = *D. kurzii*), K (*D. mespiliformis*), and Q (*D. venosa* = *D. rotundiflora*). The species of section *Gunisanthus* are separated into two clades, L and Q (*D. gracilipes* and *D. pilosula*, respectively). The members of section *Patonia* are also placed in two clades: K (*D. philippinensis*) and Q (*D. maritima* and *D. undulata*). Section *Danzleria* is polyphyletic; the first group of this section occurs in clade N (*D. lotus*, *D. kaki*, and *D. virginiana*) and the second in clade Q (*D. montana*). Section *Paralea* is polyphyletic and embedded in three clades, G (*D. macrocarpa* and *D. pentamera*), K (*D. cauliflora* and *D. oblonga*), and Q (*D. diepenhorstii*, *D. olen*, and *D. texana*); even in those clades the representatives of section *Paralea* do not fall together.

Bakhuizen (1936–1955) studied Ebenaceae s.s. extensively in Southeast Asia; only *Diospyros* occurs there, and he basically adopted the system of Hiern (1873) for species occurring outside his area of study (Table 1). Bakhuizen pointed out that the distinction of *Maba* from *Diospyros* was unclear and often arbitrary, and so he recognized *Maba* as a subgenus of *Diospyros*. Although he included *Maba* within *Diospyros*, his concept of *Diospyros* is still not monophyletic because *Tetraclis* is embedded within it. Bakhuizen divided *Diospyros* into five subgenera: *Cargillia*, *Diospyros* (his "*Eudiospyros*"), *Hierniodendron*, *Maba*, and *Mabacea* (Table 1). The first four genera are included in our study. According to our results, only two subgenera, *Hierniodendron* (F) and *Cargillia* (G), are confirmed as monophyletic and both are small. Neither subgenus *Maba* nor *Diospyros* forms an independent clade (Fig. 2). Three sections of subgenus *Maba* are embedded within *Diospyros* and fall in two different clades. Section *Ferreola*, represented here by *D. ferrea*, *D. flavocarpa*, and *D. labillardierei*, is placed in clade L. Both sections *Miquelia* (represented here by *D. andamanica* and *D. tahanensis*) and *Rhipidiostigma* (with *D. confertiflora*, *D. fasciculosa*, and *D. venosa*) are placed in clade Q. Our study,

TABLE 4.   A key to subfamilies and genera of Ebenaceae s.l.

1. Ovary inferior, tetralocular and biovulate; indumentum absent; bracteoles subopposite; corona present in some species (section *Lissocarpa*); restricted to South America.
                    subfamily Lissocarpoideae, genus *Lissocarpa*
1. Ovary superior, (2) 3–10 (16)-locular, uniovulate or less frequently biovulate; indumentum present; bracteoles alternate; corona absent; pantropical.   subfamily Ebenoideae
  2. Radicle of the embryo surrounded by a deep invagination of the testa (for half or more its length).
    3. Flower unisexual; calyx not accrescent on fruits; fruit usually single seeded; seed usually subglobose; radicle of the embryo completely surrounded by invagination of the testa; restricted to Africa, Arabia, Socotra and the Comoro Islands.   genus *Euclea*
    3. Flowers always structurally hermaphroditic (pistillodes and antherodes well developed); calyx usually accrescent on fruits; fruit usually 2–8 (16)-seeded; seeds usually elongated and flattened laterally; only half of the radicle surrounded by invagination of testa; restricted to Africa.   genus *Royena*
  2. Radicle of the embryo not surrounded by an ingrowth of the testa; widely distributed in the tropics and subtropics.   genus *Diospyros*

which includes 16 of 32 sections of subgenus *Diospyros* sensu Bakhuizen finds the same problem at this level. Sections *Podophora* (*D. dasyphylla*, *D. diepenhorstii*, and *D. insidiosa*), *Kurzella* (*D. fulvopilosa* and *D. pubicalix*), and *Nesindica* (*D. macrocarpa*, *D. maritima*, *D. styraciformis*, and *D. undulata*) are polyphyletic. Although the remaining sections are not split, most of them do not form clades of their own. Thus, members of section *Brachycylix* (*D. philippinensis*) group with sections *Campanulata* (*D. bejaudii* and *D. ridleyi*), *Confertiflora* (*D. curranii*), *Ebenaster* (*D. celebica*), *Ptychocylix* (*D. ferruginescens* and *D. oblonga*), and *Stelechantha* (*D. cauliflora*); together, they make up most of clade K. Section *Rigidophylla* (*D. rigida*) forms a clade (P) with sections *Glutinosa* (*D. malabarica* var. *atrata* and *D. malabarica* var. *malabarica*) and *Saccocalix* (*D. mindanaensis*). Section *Acanthebenus* (*D. montana*) is placed in clade Q together with three other sections, *Basithrix* (*D. ferox*), *Ebenus* (*D. olen*), and *Podophora* (*D. dasyphylla*, *D. diepenhorstii*, and *D. insidiosa*), in which they are polyphyletic.

Only two genera, *Diospyros* and *Euclea*, were recognized in Ebenaceae s.s. by White (1980, 1983). He lumped *Royena* and *Tetraclis* within an enlarged *Diospyros* due to the lack of distinguishing characters (White and Barnes, 1958). *Diospyros* in its broader circumscription is paraphyletic. In our study, which included four species each of both *Euclea* and *Royena* (= White's *Diospyros* section *Royena*), clearly showed a close relationship of these two groups (clades C and D). Our results also do not support White's (1980, 1993) infrageneric classification of *Diospyros*. The African *Diospyros* taxa are not monophyletic as White (1980) suggested. Most of them do not form a clade of their own, but instead group with species from other areas, e.g., *D. mespiliformis* with taxa from Asia (clade K), *D. natalensis* and *D. consolatae* with Asian–Madagascan–New Caledonian taxa (L), whereas six more African species group with South American taxa in clade M. Only clade N consists entirely of African species (Fig. 3). Except for the problematic section *Royena* (as mentioned), there are eight other sections in his system, *Brevistyla*, *Brevituba*, *Calvitiella*, *Dodonium*, *Forbesia*, *Latibulum*, *Maba*,

and *Tabonaca* that are included in our study, and most of them are not monophyletic. *Diospyros consolatae* of section *Forbesia* grouped with members of section *Maba* (*D. ferrea* and *D. natalensis*) in clade L. Two species from section *Tabonaca* are embedded in different places: *D. fragrans* groups with *D. mannii* (section *Dodonium*) in clade M, but *D. pseudomespilus* falls with *D. abyssinica* (section *Brevituba*) in clade O. *Diospyros bipindensis* (section *Latibulum*) is embedded within section *Calvitella* (represented here by *D. cooperi* and *D. melocarpa*; clade M), which makes the latter paraphyletic.

The recent monograph of Singh (2005) covering the Indian region follows the generic concepts of White (1980, 1983). For an infrageneric classification, he divided the Indian *Diospyros* into 27 sections, which were mainly adopted from the system of Bakhuizen (1936–1955). The 12 sections that he adopted from Bakhuizen are not monophyletic. He also established 10 new sections to accommodate other Indian taxa. Our results do not support his sectional concepts. The members of his section *Lotus* occur in clade N (*Diospyros lotus*) and Q (*D. montana*) (Figs. 2–4). Additionally, *D. lotus* is grouped in clade N together with members of three of his other sections: *Kaki* (*D. kaki*), *Sylvatica* (*D. glandulosa*), and *Basithrix* (*D. virginiana*).

Circumscription and a new classification of Ebenaceae s.l.—The differing circumscriptions of the five previous systems just described suggest that there are problems of generic delimitation in Ebenaceae s.s.; furthermore, *Lissocarpa* has newly been included in Ebenaceae. Here, we present a new classification for Ebenaceae s.l. based on a phylogenetic approach. Our results clearly resolve relationships at the subfamily and generic levels. A classification of Ebenaceae compatible with our results (Figs. 2–4) is the following: two subfamilies, Lissocarpoideae and Ebenoideae, with four genera, *Lissocarpa*, *Euclea*, *Royena*, and *Diospyros*. Synapomorphic characters of this family were described in the first paragraph of section comparison between molecular evidence and previous classification. A key to the subfamilies and genera is presented in Table 4, and relationships among the subfamilies and genera are shown in a schematic diagram (Fig. 5). All clades, with the exception of *Diospyros* clade Q (BP 73) received high bootstrap support. The characters of the four genera of Ebenaceae are presented in Table 5.

Subfamily Lissocarpoideae and genus Lissocarpa—The systematic placement of genus *Lissocarpa* has been controversial since it was described. It was first assumed to be a member of Ebenaceae, but then it was moved to Styracaceae and finally to its own family, Lissocarpaceae (for details, see Wallnöfer, 2004). Based on previous molecular results with limited sampling (Berry et al., 2001; Anderberg et al., 2002; Bremer et al., 2002; APG II, 2003), *Lissocarpa* was formally transferred to Ebenaceae as the monogeneric subfamily Lissocarpoideae (Wallnöfer, 2004). Our much more extensive study is consistent with inclusion of *Lissocarpa* in Ebenaceae and placement in a distinct subfamily. *Lissocarpa* shares various characters with other members of Ebenaceae, e.g., the black color of roots and bark, extrafloral nectaries (*flachnektarien*) on abaxial leaf surfaces, a persistent calyx, unisexual flowers, biovulate carpels with pendulous ovules, and a similar wood anatomy (Ng, 1991; Berry et al., 2001; Wallnöfer, 2001, 2004; Lens, 2005). However, *Lissocarpa* is distinguished by several morphological characters from genera in Ebenoideae,

TABLE 5.   Characters of the genera of Ebenaceae s.l.

Character	*Lissocarpa*	*Euclea*	*Royena*	*Diospyros*
No. species	8	ca. 20	ca. 20	ca. 500
Geographical distribution	South America	Africa, Arabia, Socotra and the Comoro Islands	Africa	Pantropical, with few species in the temperate regions
Phellogen (cork cambium)	No data available	Usually of pericyclic origin	Usually of pericyclic origin	Subepidermal, rarely of pericyclic origin
Vessel perforation	Simple, sporadically scalariform	Simple	Simple	Simple
Indumentum	Absent	Usually present	Usually present	Usually present
Inflorescence position	Axillary	Axillary	Axillary	Axillary or cauline
Inflorescence type	Raceme (with terminal, aborting bud) or flowers solitary	Simple or branched pseudo-raceme, sometimes solitary	Usually solitary on long pedicels or few–flowered cymes	Usually cymes or fascicles, the female flowers usually solitary
No. floral parts	4 (5)-merous	4–5 (7)-merous	(4) 5 (8)-merous	3–5 (8)-merous
Bracteole arrangement	Subopposite	Alternate	Alternate	Alternate
Sex of flowers	Apparently always unisexual (but many flowers sterile); dioecious?	Unisexual; usually dioecious (rarely polygamous)	Structurally hermaphroditic and some functionally hermaphrodite; dioecious or polygamous	Usually unisexual, rarely structurally hermaphroditic; usually dioecious, less frequently polygamous or more rarely monoecious
Calyx	Persistent but not accrescent on fruits	Persistent but not accrescent on fruits	Persistent and usually accrescent on fruits	Persistent and usually accrescent on fruits
Corolla aestivation	Contorted	Contorted	Contorted	Cortorted or valvate
Corona	Only present in section *Lissocarpa*	Absent	Absent	Absent
Stamens	8, inserted in two (?) rows	(10) 12–20 (30), inserted in one or two rows	(5) 8–10 (14), inserted in one row	2–~100, inserted in two or more rows, rarely fused together forming a tube
Staminodes	Present in functionally female or sterile flowers	Usually absent, sometimes 2–4, some rudimentary	Usually present in female flower	Usually present, often 4–8, some rudimentary or absent at all
Pollen grains	Triporate	Tricolporate	Tricolporate	Tricolporate
Ovary	Inferior; 4-locular; biovulate	Superior; (2) 4 (6)-locular; uniovulate or less frequently biovulate	Superior; (2) 4–10-locular, uniovulate	Superior; 3–10 (16)-locular; uniovulate or less frequently biovulate
Fruits	Usually 1–2-seeded	1 (2)-seeded	(1) 2–8-seeded	1–10 (16)-seeded
Seed shape	Usually ellipsoidal	Subglobose	Elongated and usually flattened laterally	Elongated and usually flattened laterally
Testa	Radicle not surrounded by an ingrowth of the testa	Ingrowth of testa forming a cylinder around the whole radicle of the embryo	Ingrowth of testa forming a cylinder around a half of radicle of the embryo	Radicle not surrounded by an ingrowth of the testa
Endosperm	Smooth	Smooth or ruminate	Smooth or ruminate	Smooth or ruminate

e.g., absence of an indumentum, vessels with both scalariform and simple perforation plates, subopposite bracteoles, triporate pollen grains, and an inferior ovary (Table 5). Although *Lissocarpa* displays some advanced (derived) characters (inferior ovary and corona), it lacks a 12-bp deletion in *matK* (Fig. 4) that is present in all members of Ebenoideae. This molecular feature is plesiomorphic and distinguishes it from the rest of Ebenaceae.

Arrangement of the eight known species of *Lissocarpa* into two sections, *Lissocarpa* and *Enho* (Wallnöfer, 2004), is also compatible with the results of our study. Section *Lissocarpa* (represented here by *L. guianensis*, *L. benthamii*, and *L. kating*) is characterized by a corona that originated from staminodia and a strongly elevated midvein on the adaxial leaf surface. On the other hand, flowers of species belonging to section *Enho* (represented here by *L. ronliesneri* and *L. tetramera*) do not possess a corona, and the midvein on the adaxial leaf surface is strongly sunken.

Subfamily Ebenoideae—This subfamily consists of three genera, *Euclea*, *Royena*, and *Diospyros*. The shared features of this subfamily are the presence of indumentum, vessels with only simple perforation plates, alternate bracteoles, tricolporate pollen grains, superior ovary, and absence of a corona. This subfamily can be further subdivided into two major lineages. The first consists of two genera, *Euclea* and *Royena*, which are mainly restricted to southern Africa (except for a few species of *Euclea* that occur northward to the Arabian Peninsula, Socotra, and the Comoro Islands). This pattern of relationships was also observed in previous molecular studies (Morton et al., 1997; Berry et al., 2001). A relationship between *Euclea* and *Royena* is well supported by various characters such as seed anatomy and a deletion of 69 bp in the 3′ *trnK* intron (Fig. 3). On a morphological basis, species belonging to the first clade can be distinguished from the second by the presence of an ingrowth of the testa surrounding the radicle of the embryo. *Royena* and *Euclea* also usually possess a pericyclic phellogen, in contrast to *Diospyros*, which has a subepidermal phellogen (Hiern, 1906). The second lineage contains only the genus *Diospyros*, which is pantropical. It is more diverse in its morphology (Table 5) and DNA divergence (Fig. 3) than the other three genera. Our results allow us to lump all other previously
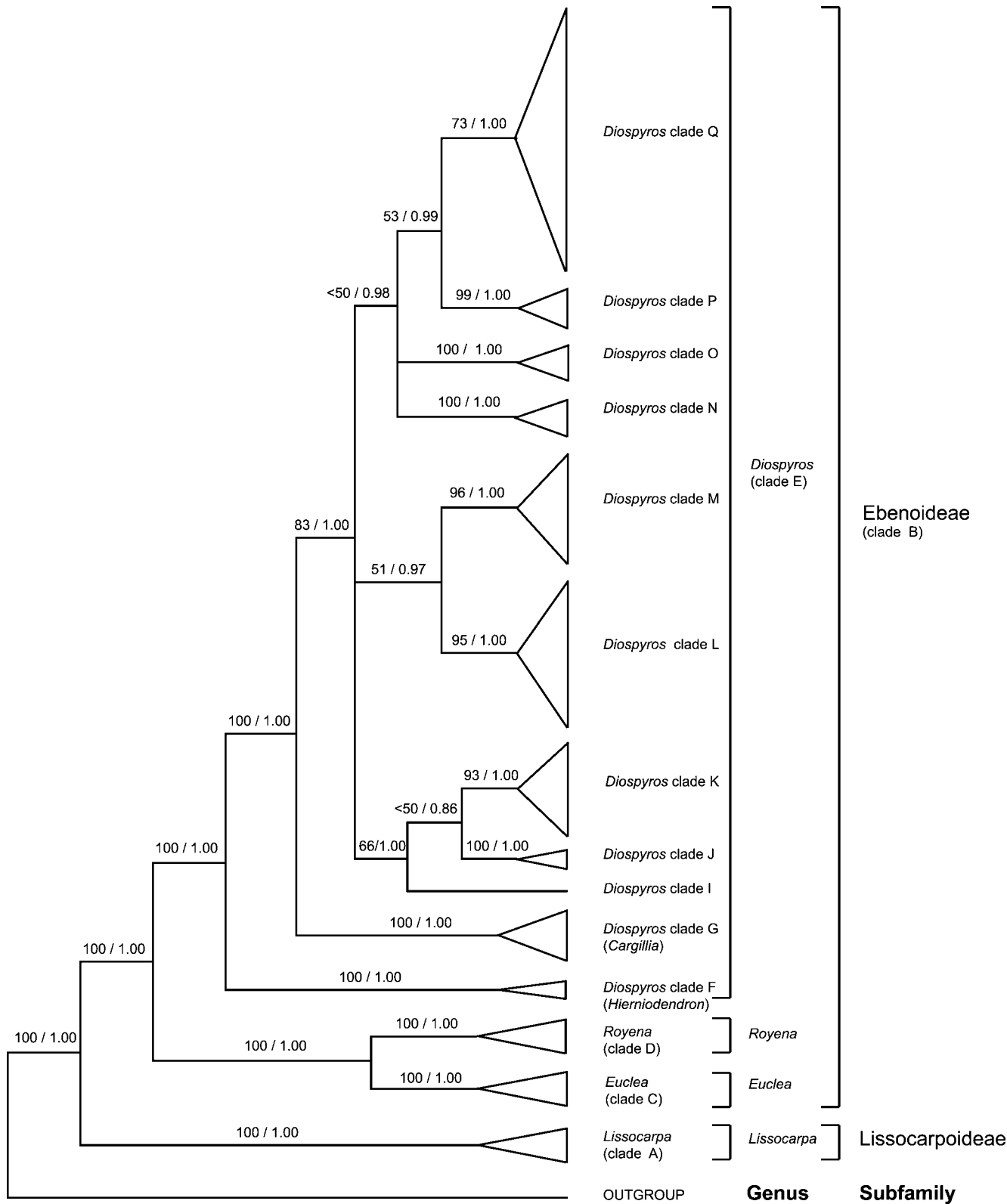
Fig. 5.   Schematic diagram summarizing relationships among well-supported clades within Ebenaceae as well as the revised infrafamilial classification of Ebenaceae s.l. based on phylogenetic analysis of six plastid regions. Bootstrap percentages and Bayesian posterior probabilities are indicated above branches.

recognized genera (*Cargillia*, *Gunisanthus*, *Maba*, *Macreightia*, and *Tetraclis*) within an expanded *Diospyros*. This genus is distinguished from the other two genera in Ebenoideae by the lack of an invagination surrounding the radicle.

Euclea—The ~20 species of *Euclea* are confined to Africa, Arabia, Socotra, and the Comoro Islands. This genus can be separated from *Royena* and *Diospyros* (including *Maba*) by the following characters: calyx not accrescent in fruit, staminodes usually absent from female flowers, inflorescence usually pseudoracemose, seed usually subglobose, radicle completely surrounded by invagination of testa, and an embryo with cotyledons flexed at right angles to the radicle (Hiern, 1873; Ng, 1971; White, 1983). *Euclea* female flowers are distinctly smaller than male flowers, but in *Diospyros* female flowers are often larger (White, 1983). With our limited sampling of species, we cannot evaluate de Candolle's (1844) infrageneic classification of *Euclea*.

Royena—This genus has been recognized for a long time as distinct (de Candolle, 1844; Hiern, 1873; Bakhuizen, 1936–1955). According to Hiern (1873, 1906), it can be distinguished from *Diospyros* and *Euclea* in having structurally hermaphroditic flowers (pistillodes and antherodes well developed so that the flowers appear bisexual) and stamens in one row (vs. dioecious or rarely polygamous; stamens usually in two or more rows, often in pairs). However, this genus was lumped in *Diospyros* only a few decades ago (de Winter and White, 1961; de Winter, 1963; White, 1980). The main reason for this action was the discovery of Salter (1953) that the flowers of *Royena glabra* are only structurally but not functionally hermaphroditic, and thus it is dioecious, as is (with some exceptions) usually the case in *Diospyros*. According to our results, which are basically congruent with those of previous studies (Morton et al., 1997; Berry et al., 2001; APG II, 2003), it is preferable to resurrect *Royena*. All species of this genus examined in our study share a 6-bp deletion in *matK*. *Royena* is distinguished from *Euclea* by its invariably structurally hermaphroditic flowers, usually accrescent calyx (vs. not accrescent), and many-seeded (vs. one-seeded) fruits (see Table 5). *Royena* has a center of distribution in southern Africa (White, 1988).

Diospyros—*Diospyros* contains more than 500 species distributed in the tropics and subtropics. As circumscribed here, it includes the other previously recognized genera, *Cargillia*, *Gunisanthus*, *Maba*, *Macreightia*, and *Tetraclis* (de Candolle, 1844; Hiern, 1873) and excludes members of section *Royena* sensu White (1980). Nomenclatural changes will be necessary for the taxa from Madagascar previously placed in *Maba* and *Tetraclis*. Our results support two successive sister clades (F and G) and a large internally unresolved clade within this genus. Although relationships within the last remain unclear, nine well-supported clades (I–Q) were found in our study (Fig. 2–4). As mentioned, earlier infrageneric classifications of *Diospyros* (i.e., de Candolle, 1844; Hiern, 1873; Bakhuizen, 1936–1955; White, 1980, 1993; Singh, 2005) are not compatible with our results. However, due to the lack of a clear estimate of relationships, we could not construct a new infrageneric classification. Further work is necessary and will not only require more data, both molecular and morphological, but also more extensive taxon sampling.

Diospyros clade F—This clade corresponds to Bakhuizen's subgenus *Hierniodendron* and is placed as sister to the rest of *Diospyros*. Both *D. maingayi* and *D. puncticulosa* are restricted to Southeast Asia where they grow in peat swamps and true tropical rain forest. Their filaments are fused together to form a hollow tube, which has not been observed in any other *Diospyros* species examined in our study. This group is also supported by a 6-bp indel in *ndhF* (Fig. 3).

Diospyros clade G—This group contains *D.* subgenus *Cargillia* sensu Bakhuizen (*D. australis* and *D. pentamera*) plus two New Caledonian species, *D. brassica* and *D. macrocarpa*. They share a 49-bp deletion in the *trnK* intron (Fig. 3). Their close relationship is supported by a combination of biogeographic and morphological evidence. All of them are confined to Australia and New Caledonia (eastern Gondwana) and have subsessile, axillary inflorescences, a deeply lobed corolla, and exserted anthers.

Diospyros clades I, J, and K—All species of these three clades share a 24-bp deletion in the *trnL* intron (Fig. 3). *Diospyros borneensis* (I) may be sister to the rest. It is distinguished from the other two clades by a cylindrical or funnel-shaped calyx and woody pericarp. Clade J has two Asian species, *D. fulvopilosa* and *D. mollis*, both of them restricted to Southeast Asia. Clade K consists of nine Asian species, *D. bejaudii*, *D. cauliflora*, *D. celebica*, *D. curranii*, *D. ferruginescens*, *D. oblonga*, *D. brandisiana*, *D. philippinensis*, and *D. ridleyi* and also the widespread African species, *D. mespiliformis* (White, 1988). Up to now, we have identified no unique morphological features for this clade as a whole. However, a ruminate endosperm is a unifying character for *D. mespiliformis* and five closely related species (*D. bejaudii*, *D. celebica*, *D. ferruginescens*, *D. philippinensis*, and *D. ridleyi*). Long-distance dispersal perhaps played a role for this Africa–Asia connection. The fruits of both African (White, 1983) and Asian (Ng, 1978b; S. Duangjai, personal observation) species are edible. In Thailand their fruits are eaten by mammals (S. Duangjai, personal observation), which may be dispersing seeds of this group.

Diospyros clade L—This clade contains the *D. ferrea* complex (including Madagascan *Maba*) and all Madagascan *Diospyros* species (including *Tetraclis*). The *D. ferrea* complex has been referred to genus *Maba* sensu de Candolle or *D.* sections *Ferreola* plus *Cupulifera* sensu Bakhuizen, and it is represented here by *D. ferrea*, *D. flavocarpa*, *D. labillardierei*, *D. natalensis*, *Maba magnifolia*, and *M. myriophylla*. This group has trimerous flowers with a trilocular ovary that is biovulate. It occurs throughout the Old World tropics: Africa, Madagascar, Asia, Polynesia, Australia, the Pacific islands, and Hawaii. Most have small fruits, which may be relatively easily dispersed across water barriers by migratory birds (Pannell and White, 1988; White, 1993). Our results strongly support a close relationship between the *D. ferrea* complex and the Madagascan *Diospyros* species, but within the clade, resolution is poor. The question of the origin of Madagascan *Diospyros* from the African mainland is still open. More intensive study will provide robust estimate of phylogenetic relationships, and this should enable us to evaluate better this hypothesis.

Diospyros clade M—This clade consists of African and South American species, and it is further divided into three

subclades. The first two subclades comprise only African species (*D. bipindensis*, *D. cooperi*, *D. fragrans*, *D. melocarpa*, *D. mannii*, and an unidentified species), all of them are distributed in the western side of Africa, mainly in the Guineo-Congolian region (White, 1978). The third subclade contains the South American species, all of which have a 42-bp deletion in the *trnL* intron (Fig. 3).

Diospyros* clade *N—This group contains five temperate or subtemperate species, *D. glandulosa*, *D. glaucifolia*, *D. kaki*, *D. lotus*, and *D. virginiana*. A relationship of these species is supported by a suite of morphological characteristics and was reported in a previous study (Morton et al., 1996). Fruits of this group are edible, and three species have been brought into cultivation: persimmon (*D. kaki*), date plum (*D. lotus*), and common persimmon (*D. virginiana*). Polyploidy is reported in *D. kaki* ($2n = 60$, 90, and 135) and *D. virginiana* ($2n = 60$ and 90). The other three species, *D. glandulosa*, *D. glaucifolia*, and *D. lotus*, are diploids ($2n = 30$). Based on morphological characters, *D. glandulosa* was proposed as the progenitor of *D. kaki* (Ng, 1978a). This hypothesis is consistent with our results: *D. glandulosa* is sister to *D. kaki*.

Diospyros* clade *O—This clade contains three African species, *D. abyssinica*, *D. pseudomespilus*, and an unidentified species. Although this clade is well supported by molecular data, shared morphological characters are still unclear. Further investigation with more extensive sampling is necessary to clarify synapomorphic characters for this African group.

Diospyros* clades *P* and *Q—The close relationship of these two clades has low support. Clade P consists of *D. malabarica*, *D. mindanaensis*, and *D. rigida*, all of which are distributed in southern and Southeast Asia. Species of this clade are characterized by a combination of the reddish inner bark and ruminate endosperm. Clade Q is formed by American, Asian, and New Caledonian species, and we could not find any unifying morphological features for this clade. According to the combined coding region tree (Fig. 1A), there are four subclades, which seem to be correlated with biogeography. The first subclade is confined to Asia and New Caledonia, the second contains American species, and the other two are restricted to Asia.

Character evolution—Our results (Fig. 4) indicate that a superior ovary is plesiomorphic in Ebenaceae s.l., with a shift to an inferior ovary only one time in *Lissocarpa*, because a superior ovary also appears in the outgroup families. Tetramerous and pentamerous flowers are plesiomorphic in Ebenaceae s.l., and they are more common than trimerous and hexamerous flowers (Fig. 4). Tetramerous and pentamerous flowers are present in all four genera, *Lissocarpa*, *Euclea*, *Diospyros*, and *Royena*, but trimerous and hexamerous flowers appear only in *Diospyros*. Trimerous and hexamerous flowers might not have evolved from the same ancestral condition; our results (Fig. 4) indicate that the trimerous flowers have evolved from the tetramerous flowers, whereas the hexamerous flowers are derived from the pentamerous flowers.

Mapping of the number of floral parts on the phylogenetic tree (Fig. 4) provides evidence that the trimery has evolved from tetramery more than one time. This is important to note because previous taxonomists (Hiern, 1873; Bakhuizen, 1936–1955) always used this character to separate the *Maba* (Table 1) from the other taxa. The first lineage of trimerous flowers is the *D. ferrea* complex in clade L. The others occur in clade M (*D. melocarpa*), O (*D. abyssinica*), and Q (*D. andamanica*, *D. tahanensis*, *D. castanea*, *D. confertiflora*, *D. crassinervis*, *D. fasciculosa*, and *D. venosa*). These clades differ in character of ovary: the first clade has three biovulate locules, but the others have an ovary with six uniovulate locules (Bakhuizen, 1936–1955). In addition, *D. venosa* has both trimerous and tetramerous flowers on the same tree (S. Duangjai, personal observation).

Hexamerous flowers are sometime found in *D. artanthaefolia*, *D. ropourea*, and *D. vestita* in clade M, whereas the members of this clade usually have tetramerous or pentamerous flowers (Fig. 4). Thus, hexamerous flowers seem to be derived from pentamerous flowers.

Biogeographical implications—The hypothesis of a western Gondwanan origin of Ebenceae (Raven and Axelrod, 1974) is supported by our molecular results (Fig. 3). The basal nodes can be assigned to only this region: *Lissocarpa* is found in the northern half of South America, while *Euclea* and *Royena* are centered in southern Africa. The distribution of the family perhaps is thus a result of both vicariant and long-distance dispersal events. As suggested earlier in our discussion, there are several intercontinentally disjunct clades: Asian–African (clade K), African–South American (clade M), Asian–North American (clade N), and American–Asian–Australian–New Caledonian–Pacific Island (clade Q). Furthermore, some members of *D. ferrea* complex (clade L) also occur on volcanic islands, e.g., Hawaii (Pannell and White, 1988) and Mauritius (Richardson, 1981). However, due to unresolved relationships within *Diospyros*, we are unable to identify the direction of migration. The biogeography of this family needs to be further explored with a phylogenetic hypothesis based on more extensive sampling in *Diospyros*.

Conclusions—The monophyly of Ebenaceae s.l. is strongly supported by analyses of data from six plastid DNA regions. Four genera, *Lissocarpa*, *Euclea*, *Royena*, and *Diospyros*, should be recognized within the family. Our results support inclusion in *Diospyros* of the other previously recognized genera, e.g., *Cargillia*, *Gunisanthus*, *Maba*, *Macreightia*, and *Tetraclis*. The family is split into two major clades corresponding to subfamilies Lissocarpoideae (only *Lissocarpa*) and Ebenoideae (the other three). Molecular evidence confirms the two sections (*Lissocarpa* and *Enho*) proposed by Wallnöfer (2004) within *Lissocarpa*. *Royena* is more closely related to *Euclea* than *Diospyros*. It also is clear that the previous infrageneric classifications of *Diospyros* are artificial and need to be revised. Further study will require more molecular and morphological data as well as more extensive sampling of species. Additionally, the plastid markers provide a powerful framework for identification of characters that represent synapomorphies useful in classification of this family, and by and large morphology confirms the molecular-based groups identified here.

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APPENDIX 1.   Voucher information and GenBank accession numbers for taxa used in this study. Abbreviations: BRUN = Brunei Herbarium; CICY = Centro de Investigación Científica de Yucatán, Mexico; K = Royal Botanic Gardens, Kew, Herbarium; KUFF = Faculty of Forestry, Kasetsart University Herbarium; MO = The Missouri Botanical Garden Herbarium; NCU = University of North Carolina Herbarium; NOU = Institut de Recherche pour le Développement Herbarium; PORT = BioCentro-UNELLEZ; U = Nationaal Herbarium Nederland, Utrecht University Branch; W = Natural History Museum, Vienna, Herbarium; WU = University of Vienna Herbarium.

Species	Locality; voucher	GenBank accession number			
		atpB	*ndhF*	*matK* and *trnK*	*trnL-F*
Ingroup					
Diospyros abyssinica (Hiern) F.White.	Africa; *Gilbert & Sebseke 8803* (K)	DQ923883	DQ924100	DQ923990	DQ924207
Diospyros andamanica (Kurz) Bakh.	Thailand; *Duangjai 068* (KUFF, W)	DQ923884	DQ924101	DQ923991	DQ924208
Diospyros artanthaefolia Mart. ex Miq.	Peru; *Pirie 62* (W)	DQ923885	DQ924102	DQ923992	DQ924209
Diospyros australis (R.Br.) Hiern	Australia; *Forster 7848* (K)	DQ923886	DQ924103	DQ923993	DQ924210
Diospyros australis (R.Br.) Hiern	Cult. Austria, Botanical Garden of University of Vienna living coll.; *Wallnöfer & Duangjai 13944* (W)	DQ923887	DQ924104	DQ923994	DQ924211
Diospyros bejaudii Lecomte	Thailand; *Duangjai 075* (KUFF, W)	DQ923888	DQ924105	DQ923995	DQ924212
Diospyros bipindensis Gürke	Gabon; *Stone & Niangadouma 3554* (MO)	DQ923889	DQ924106	DQ923996	DQ924213
Diospyros borneensis Hiern	Thailand; *Duangjai 079* (KUFF, W)	DQ923890	DQ924107	DQ923997	DQ924214
Diospyros brandisiana Kurz	Thailand; *Duangjai & Sinbumrung 007* (KUFF, W)	DQ923891	DQ924108	DQ923998	DQ924215
Diospyros brassica F.White	New Caledonia; *Munzinger 2898* (NOU)	DQ923892	DQ924109	DQ923999	DQ924216
Diospyros castanea (Craib) Fletcher	Thailand; *Duangjai 083* (KUFF, W)	DQ923893	DQ924110	DQ924000	DQ924217
Diospyros cauliflora Blume	Thailand; *Duangjai 087* (KUFF, W)	DQ923894	DQ924111	DQ924001	DQ924218
Diospyros cavalcantei Sothers	French Guiana; *Prévost et al. 4671* (W)	DQ923895	DQ924112	DQ924002	DQ924219
Diospyros cayennensis A.DC.	French Guiana; *Prévost 3430* (W)	DQ923896	DQ924113	DQ924003	DQ924220
Diospyros celebica Bakh.	Indonesia, Bogor Botanical Garden; *Chase 1242* (K)	DQ923897	DQ924114	DQ924004	DQ924221
Diospyros confertiflora (Hiern) Bakh.	Thailand; *Duangjai 091* (KUFF, W)	DQ923898	DQ924115	DQ924005	DQ924222
Diospyros consolatae Chiov.	Africa; *Beentje 2168* (K)	DQ923899	DQ924116	DQ924006	DQ924223
Diospyros cooperi (Hutchinson & Dalziel) F.White	Ghana; *Morello et al. 1353* (MO)	DQ923900	DQ924117	DQ924007	DQ924224
Diospyros crassinervis (Krug & Urb.) Standl.	Cuba; *Rainer s.n.* (W)	DQ923901	DQ924118	DQ924008	DQ924225
Diospyros curranii Merr.	Thailand; *Duangjai 094* (KUFF, W, WU)	DQ923902	DQ924119	DQ924009	DQ924226
Diospyros dasyphylla Kurz	Thailand; *Duangjai 096* (KUFF,W)	DQ923903	DQ924120	DQ924010	DQ924227
Diospyros dichroa Sandwith	French Guiana; *Sabatier et al. 4457* (W)	DQ923904	DQ924121	DQ924011	DQ924228
Diospyros diepenhorstii Miq.	Thailand; *Duangjai 103* (KUFF, W)	DQ923905	DQ924122	DQ924012	DQ924229
Diospyros digyna Jacq.	Cult. M. Selby Botanical Gardens; *Chase 212* (K)	DQ923906	DQ924123	DQ924013	DQ924230
Diospyros ehretioides Wall. ex G.Don	Thailand; *Duangjai 104* (KUFF, W)	DQ923907	DQ924124	DQ924014	DQ924231
Dispyros fasciculosa (F.Muell.) F.Muell.	New Caledonia; *Munzinger 2127* (NOU)	DQ923908	DQ924125	DQ924015	DQ924232
Diospyros ferox Bakh.	Brunei; *Duangjai et al. 012* (BRUN, W, WU)	DQ923909	DQ924126	DQ924016	DQ924233
Diospyros ferrea (Willd.) Bakh.	Thailand; *Duangjai 106* (KUFF, W)	DQ923910	DQ924127	DQ924017	DQ924234
Diospyros ferruginescens Bakh. var. *ferruginescens*	Brunei; *Duangjai et al. 007* (BRUN, W, WU)	DQ923911	DQ924128	DQ924018	DQ924235
Diospyros filipendula Pierre ex Lecomte	Thailand; *Duangjai 109* (KUFF)	DQ923912	DQ924129	DQ924019	DQ924236
Diospyros flavocarpa (Vieill. ex P.Parm.) F.White	New Caledonia; *McPherson & Lowry II 18563* (MO)	DQ923913	DQ924130	DQ924020	DQ924237
Diospyros fragrans Gürke	Gabon; *SIMAB 010610* (MO)	DQ923914	DQ924131	DQ924021	DQ924238
Diospyros fulvopilosa Fletcher	Thailand; *Duangjai 113* (KUFF, W)	DQ923915	DQ924132	DQ924022	DQ924239
Diospyros glandulosa Lace	Thailand; *Duangjai 114* (KUFF, W)	DQ923916	DQ924133	DQ924023	DQ924240
Diospyros glaucifolia Metcalf	Cult. UK, Kew living coll.; *Chase 14256* (K)	DQ923917	DQ924134	DQ924024	DQ924241
Diospyros gracilipes Hiern	Madagascar; *RNF 978* (W)	DQ923918	DQ924135	DQ924025	DQ924242
Diospyros guianensis (Aubl.) Gürke	French Guiana; *Prévost & Sabatier 4029* (W)	DQ923919	DQ924136	DQ924026	DQ924243
Diospyros insidiosa Bakh.	Thailand; *Duangjai 120* (KUFF, W)	DQ923920	DQ924137	DQ924027	DQ924244
Diospyros kaki L.f.	Cult. UK, Kew living coll.; *Chase 920* (K)	DQ923921	DQ924138	DQ924028	DQ924245
Diospyros labillardierei F.White	New Caledonia; *McPherson & Munzinger 18038* (MO)	DQ923922	DQ924139	DQ924029	DQ924246
Diospyros longifolia (Spruce ex Engl.) Sleumer & F.White	Brazil; *Maas et al. 9186* (W)	DQ923923	DQ924140	DQ924030	DQ924247
Diospyros lotus L.	Cult. UK, Kew living coll.; *Chase 965* (K)	DQ923924	DQ924141	DQ924031	DQ924248
Diospyros macrocarpa (Vieill.) Hiern	New Caledonia; *Munzinger 2829* (NOU)	DQ923925	DQ924142	DQ924032	DQ924249
Diospyros maingayi (Hiern) Bakh.	Thailand; *Duangjai 131* (KUFF, W)	DQ923926	DQ924143	DQ924033	DQ924250
Diospyros malabarica (Desr.) Kostel. var. *atrata* (Thwaites) Bakh.	Indonesia, Bogor Botanical Garden; *Chase 1247* (K)	DQ923927	DQ924144	DQ924034	DQ924251
Diospyros malabarica (Desr.) Kostel. var. *malabarica*	Thailand; *Duangjai 006* (KUFF, W)	DQ923928	DQ924145	DQ924035	DQ924252
Diospyros mannii Hiern	Ghana; *Merello et al.1348* (MO)	DQ923929	DQ924146	DQ924036	DQ924253

APPENDIX 1. Continued.

Species	Locality; voucher	GenBank accession number			
		atpB	*ndhF*	*matK* and *trnK*	*trnL-F*
Diospyros maritima Blume	Cult. Austria, Botanical Garden of University of Vienna living coll.; *Wallnöfer 13948* (W)	DQ923930	DQ924147	DQ924037	DQ924254
Diospyros melocarpa F.White	Gabon; *SIMAB 012319* (MO)	DQ923931	DQ924148	DQ924038	DQ924255
Diospyros mespiliformis Hochst. ex A.DC.	Cult. Austria, Botanical Garden of University of Vienna living coll.; *Wallnöfer & Duangjai 13945* (W)	DQ923932	DQ924149	DQ924039	DQ924256
Diospyros mindanaensis Merr.	Brunei; *Duangjai et al. 026* (BRUN, W, WU)	DQ923933	DQ924150	DQ924040	DQ924257
Diospyros mollis Griff.	Thailand; *Duangjai 132* (KUFF, W)	DQ923934	DQ924151	DQ924041	DQ924258
Diospyros montana Roxb.	Thailand; *Duangjai 136* (KUFF, W)	DQ923935	DQ924152	DQ924042	DQ924259
Diospyros natalensis (Harv.) Brenan	Zambia; *Bingham 10635* (K)	DQ923936	DQ924153	DQ924043	DQ924260
Diospyros oblonga Wall. ex G.Don.	Thailand; *Duangjai 141* (KUFF, W)	DQ923937	DQ924154	DQ924044	DQ924261
Diospyros olen Hiern	New Caledonia; *Lowry II et al. 5628* (MO)	DQ923938	DQ924155	DQ924045	DQ924262
Diospyros pentamera (Woolls & F.Muell.) F.Muell.	Australia; *Forster & Booth 25525* (K)	DQ923939	DQ924156	DQ924046	DQ924263
Diospyros philippinensis A.DC.	Indonesia, Bogor Botanical Garden; *Chase 1248* (K)	DQ923940	DQ924157	DQ924047	DQ924264
Diospyros pilosula (A.DC.) Hiern	Thailand; *Duangjai 150* (KUFF, W)	DQ923941	DQ924158	DQ924048	DQ924265
Diospyros pseudomespilus Mildbr.	Gabon; *Walters et al. 956* (MO)	DQ923942	DQ924159	DQ924049	DQ924266
Diospyros pubicalyx Bakh.	Thailand; *Duangjai & Sinbumrung 017* (KUFF, W)	DQ923943	DQ924160	DQ924050	DQ924267
Diospyros puncticulosa Bakh.	Brunei; *Duangjai et al. 018* (BRUN,W, WU)	DQ923944	DQ924161	DQ924051	DQ924268
Diospyros rhombifolia Hemsl.	Thailand; *Duangjai & Sinbumrung 016* (KUFF, W)	DQ923945	DQ924162	DQ924052	DQ924269
Diospyros ridleyi Bakh.	Brunei; *Duangjai et al. 002* (BRUN,W,WU)	DQ923946	DQ924163	DQ924053	DQ924270
Diospyros rigida Hiern	Brunei; *Duangjai et al. 004* (BRUN, W, WU)	DQ923947	DQ924164	DQ924054	DQ924271
Diospyros ropourea B.Walln.	French Guiana; *Wallnöfer et al. 13459* (W)	DQ923948	DQ924165	DQ924055	DQ924272
Diospyros styraciformis King & Gamble	Brunei; *Duangjai et al. 017* (BRUN, W, WU)	DQ923949	DQ924166	DQ924056	DQ924273
Diospyros tahanensis Bakh.	Thailand; *Duangjai 162* (KUFF)	DQ923950	DQ924167	DQ924057	DQ924274
Diospyros tetrandra Hiern	French Guiana; *Prévost et al. 4713* (W)	DQ923951	DQ924168	DQ924058	DQ924275
Diospyros tetrasperma Sw.	Mexico, Yucatan, Balancanche; *Chase 14254* (K, W) [orig. coll. = *Escalante 727* (CICY)]	DQ923952	DQ924169	DQ924059	DQ924276
Diospyros texana Scheele	Cult. Austria, Botanical Garden of University of Vienna living coll.; *Wallnöfer & Duangjai 13946* (W)	DQ923953	DQ924170	DQ924060	DQ924277
Diospyros undulata Wall. ex G.Don	Thailand; *Duangjai 170* (KUFF, W)	DQ923954	DQ924171	DQ924061	DQ924278
Diospyros venosa Wall ex A.DC.	Thailand; *Duangjai 177* (KUFF, W)	DQ923955	DQ924172	DQ924062	DQ924279
Diospyros vestita Benoist	French Guiana; *Molino 1849* (W)	DQ923956	DQ924173	DQ924063	DQ924280
Diospyros virginiana L.	Cult. UK, Kew living coll.; *Chase 14255* (K)	DQ923957	DQ924174	DQ924064	DQ924281
Diospyros yatesiana Lundell	Guatemala; *Frisch s.n.* (W)	DQ923958	DQ924175	DQ924065	DQ924282
Diospyros sp. FS1637	Madagascar; *FS 1637* (W)	DQ923959	DQ924176	DQ924066	DQ924283
Diospyros sp. FS2217	Madagascar; *FS 2217* (W)	DQ923960	DQ924177	DQ924067	DQ924284
Diospyros sp. K20600	Madagascar; *Rabenantoandro et al. 1246* (MO)	DQ923961	DQ924178	DQ924068	DQ924285
Diospyros sp. K20613	Zambia; *Zimba et al. 893* (MO)	DQ923962	DQ924179	DQ924069	DQ924286
Diospyros sp. K20616	Ghana; *Schmidt et al. 2207* (MO)	DQ923963	DQ924180	DQ924070	DQ924287
Diospyros sp. RNF 970	Madagascar; *RNF 970* (W)	DQ923964	DQ924181	DQ924071	DQ924288
Diospyros sp. W36	Madagascar; *Fischer et al. 258* (W)	DQ923965	DQ924182	DQ924072	DQ924289
Euclea crispa (Thunb.) Gürke	Cult. Austria, Botanical Garden of University of Vienna living coll.; *Wallnöfer 13949* (W)	DQ923966	DQ924183	DQ924073	DQ924290
Euclea divinorum Hiern	Cult. Austria, Botanical Garden of University of Vienna living coll.; *Wallnöfer & Duangjai 13947* (W)	DQ923967	DQ924184	DQ924074	DQ924291
Euclea natalensis A.DC.	South Africa; *Kurzweil E514* (W)	DQ923968	DQ924185	DQ924075	DQ924292
Euclea undulata Thunb.	Cult. Austria, Botanical Garden of University of Vienna living coll.; *Wallnöfer 13897* (W)		DQ924186	DQ924076	DQ924293
Lissocarpa benthamii Gürke	Venezuela; *Berry et al. 7217* (PORT)	DQ923969	DQ924187	DQ924077	DQ924294
Lissocarpa guianensis Gleason	Guyana; *Aret s.n.* (U)	DQ923970	DQ924188	DQ924078	DQ924295
Lissocarpa kating B.Walln.	Peru; *Vasquez & Ortiz-Gentry 25233* (MO)	DQ923971	DQ924189	DQ924079	DQ924296
Lissocarpa ronliesneri B.Walln.	Ecuador; *Miranda et al. 160* (W)	DQ923972		DQ924080	DQ924297
Lissocarpa tetramera (Rusby) P.E.Berry	Peru; *Solomon 14872* (W)			DQ924081	DQ924298
Maba magnifolia H.Perrier	Madagascar; *Rabevohitra et al.3660* (MO)	DQ923973	DQ924190	DQ924082	DQ924299
Maba myriophylla H.Perrier	Madagascar; *Fischer et al. 209*(W)	DQ923974	DQ924191	DQ924083	DQ924300
Royena cordata E.Mey. ex A.DC. [= *Diospyros scabrida* (Harv. ex Hiern) De Winter var. *cordata* (E.Mey. ex A.DC.) De Winter]	Cult. UK, Kew living coll.; *Chase 1144* (K)	DQ923975	DQ924192	DQ924084	DQ924301

APPENDIX 1.   Continued.

Species	Locality; voucher	GenBank accession number			
		atpB	*ndhF*	*matK* and *trnK*	*trnL-F*
Royena glabra L. [= *Diospyros grabra* (L.) De Winter]	South Africa; *Kurzweil 2097* (W)	DQ923976	DQ924193	DQ924085	DQ924302
Royena lucida L. [= *Diospyros whyteana* (Hiern) F.White]	Cult. Austria, Botanical Garden of University of Vienna living coll.; *Wallnöfer & Duangjai 13943* (W)	DQ923977	DQ924194	DQ924086	DQ924303
Royena lycioides (Desf.) A.DC. [= *Diospyros lycioides* Desf.]	Cult. UK, Kew living coll.; *Chase 977* (K)	DQ923978	DQ924195	DQ924087	DQ924304
Tetraclis baroni H.Perrier	Madagascar; *RNF 938* (W)	DQ923979	DQ924196	DQ924088	DQ924305
Tetraclis cf. *clusiaeflora* Hiern	Madagascar; *RNF 959* (W)	DQ923980	DQ924197	DQ924089	DQ924306
Outgroup					
Argania spinosa Skeels	Cult. UK, Kew living coll.; *Chase 978* (K)	DQ923981	DQ924198	DQ924090	DQ924307
Madhuca macrophylla (Hassk.) H.J.Lam	Indonesia, Bogor Botanical Garden; *Chase 1363* (K)	DQ923982	DQ924199	DQ924091	DQ924308
Manilkara zapota (L.) P.Royen	Cult. UMBG.; *Chase 129* (NCU)	DQ923983	DQ924200	DQ924092	DQ924309
Adinandra dumosa Jack var. *oblonga*	Indonesia, Bogor Botanical Garden; *Chase 1379* (K)	DQ923984	DQ924201	DQ924093	DQ924310
Cleyera japonica Siebold & Zucc.	Cult. UK, Kew living coll.; *Chase 1690* (K)	DQ923985	DQ924202	DQ924094	DQ924311
Barringtonia asiatica (L.) Kurz	Cult. UK, Kew living coll.; *Chase 328* (K)	DQ923986	DQ924203	DQ924095	DQ924312
Careya arborea Roxb.	Asia; *Chase 2256* (K)	DQ923987	DQ924204	DQ924096	DQ924313
Halesia carolina L.	Cult. UK, Kew living coll.; *Chase 910* (K)	DQ923988	DQ924205	DQ924097	DQ924314
Styrax benzoin Dryand.	Indonesia, Bogor Botanical Garden; *Chase 1371* (K)	DQ923989	DQ924206	DQ924098	DQ924315
Styrax officinalis L.	Anderberg et al. 2002	AF420984	AF421084		
Styrax officinalis L.	Cult. UK, Kew living coll.; *Chase 872* (K)			DQ924099	
Styrax officinalis L.	Fritsch et al. 2001				AF396197, AF396198
Camellia japonica L.	Soltis et al. 1999	AF209553			
Camellia japonica L.	Olmstead et al. 2000		AF130216		
Camellia japonica L.	Prince and Parks, 2001			AF380074	
Camellia japonica L.	Fritsch et al. 2001				AF396225, AF396226
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