

SYSTEMATIC AFFINITIES OF RHIZOPHORACEAE AND ANISOPHYLLEACEAE, AND INTERGENERIC RELATIONSHIPS WITHIN RHIZOPHORACEAE, BASED ON CHLOROPLAST DNA, NUCLEAR RIBOSOMAL DNA, AND MORPHOLOGY¹

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A cladistic analysis of sequences from the chloroplast gene *rbcL* was used to determine the systematic affinities of Rhizophoraceae and Anisophylleaceae. This analysis rejects close relationships of Rhizophoraceae with Celastraceae or Elaeocarpaceae, suggested previously, and identifies Erythroxylaceae as sister group within the Malpighiales, supported by several morphological and anatomical characters. Our molecular results also indicate that Anisophylleaceae are nested within Cucurbitales. Although this placement is novel, this affinity is also well supported by shared morphological characters. Tribal and generic relationships within Rhizophoraceae are evaluated with a combination of six molecular data sets (*rbcL*, *atpB-rbcL* intergenic spacer, *trnL-trnF* intergenic spacer, ITS1, ITS2, and 5.8S) and a morphological data set. These relationships are compared with results from previous morphological cladistic analyses. Against the background of the molecular results, we briefly discuss the evolution of morphological characters traditionally used for tribal subdivision as well as characters presumably significant for adaptation to mangrove habitats, namely, aerial stilt roots and vivipary.

Key words: Anisophylleaceae; *atpB-rbcL* spacer; cpDNA; Erythroxylaceae; ITS; mangroves; nrDNA; *rbcL*; Rhizophoraceae; *trnL-trnF* spacer; vivipary.

The Rhizophoraceae comprise 15 genera and ~140 species (Table 1). Although often described as a mangrove family, only four genera, including 16 species, live exclusively in mangrove habitats (Tobe and Raven, 1988a). The family is pantropical, and all members are either trees or shrubs. Rhizophoraceae mangroves are widely distributed along tropical coastlines and the terrestrial species grow in both primary and successional moist forests (Juncosa and Tomlinson, 1988a). A few

species, mainly in the genera *Cassipourea* and *Dactylopetalum*, inhabit drier environments.

The Anisophylleaceae consist of four woody genera and ~45 species growing in both New and Old World tropics. They occur in wet lowland primary forest, except for the monotypic genus *Combretocarpus*, which is restricted to peat swamp forests on Borneo (Juncosa and Tomlinson, 1988a).

The relationships of the two families and their delimitation have long been subjects of controversy (Table 1). Several authors have regarded the pantropical Anisophylleaceae as either closely related to Rhizophoraceae (Melchior, 1964) or as a separate tribe (Bentham and Hooker, 1865; Baillon, 1876; Ridley, 1922) or subfamily (Schimper, 1898) within the Rhizophoraceae. Takhtajan (1997) recently established a superorder Rhizophoranae containing the two single-family orders Anisophylleales and Rhizophorales, suggesting that although immediate relatives, the two families are not very close to each other. A series of thorough morphological and anatomical studies (Behnke, 1988; Dahlgren, 1988; Juncosa and Tobe, 1988; Juncosa and Tomlinson, 1988a, b; Keating and Randrianasolo, 1988; Tobe and Raven, 1988b; Raven and Tomlinson, 1988; Vezey et al., 1988) supported the exclusion of Anisophylleaceae from the Rhizophoraceae. In spite of these studies, the systematic positions of these families still have not been resolved. Several authors (Cronquist, 1981; Tobe and Raven, 1988a; Thorne, 1992) proposed that Anisophylleaceae are related to Rosales based mainly on floral and embryological characters. However, studies of wood anatomy (Van Vliet, 1976; Van Vliet and Baas, 1984) and leaf architecture supported the

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TABLE 1. Circumscription of Rhizophoraceae and Anisophylleaceae used by different authors. Numbers in parentheses mean (number of genera/species) or [species], respectively.

Juncosa and Tomlinson, 1988b, and this paper	Tobe and Raven, 1988a; Takhtajan, 1997 ^a	Melchior, 1964	Schimper, 1898
Rhizophoraceae (15/~143)	Rhizophoraceae	Rhizophoraceae	Rhizophoraceae
Macarisieae (7/87)	Macarisieae	Macarisieae	Rhizophoroideae
<i>Anopyxis</i> Pierre ex Engl. [3]	<i>Anopyxis</i>	<i>Macarisia</i>	Macarisieae
<i>Macarisia</i> Thou. [7]	<i>Macarisia</i>	<i>Blepharistemma</i>	<i>Macarisia</i>
<i>Blepharistemma</i> Wall. ex Benth. [2]	<i>Blepharistemma</i>	<i>Dactylopetalum</i>	
<i>Comiphyton</i> Floret [1]	<i>Comiphyton</i>	<i>Cassipourea</i>	Gynotrocheae
<i>Dactylopetalum</i> Benth. [14] ^b	<i>Dactylopetalum</i>	<i>Weihea</i>	Gynotrochineae
<i>Cassipourea</i> Aubl. [51] ^c	<i>Cassipourea</i>		<i>Crossostylis</i>
<i>Sterigmatapetalum</i> Kuhlm. [9]	<i>Sterigmatapetalum</i>	Gynotrocheae	<i>Ceriops</i>
		<i>Carallia</i>	<i>Gynotroches</i>
Gynotrocheae (4/~40)	Crossostylideae	<i>Crossostylis</i>	<i>Kandelia</i>
<i>Carallia</i> Roxb. [ca. 15]	<i>Crossostylis</i>	<i>Gynotroches</i>	<i>Rhizophora</i>
<i>Crossostylis</i> J. R. Forst. & G. Forst. [13]		<i>Pellacalyx</i>	
<i>Gynotroches</i> Blume [2-4]	Gynotrocheae		Carallinae
<i>Pellacalyx</i> Korth. [8]	<i>Carallia</i>	Rhizophoreae	<i>Carallia</i>
	<i>Gynotroches</i>	<i>Rhizophora</i>	
Rhizophoreae (4/16)	<i>Pellacalyx</i>	<i>Ceriops</i>	Anisophylloideae
<i>Rhizophora</i> L. [6]		<i>Kandelia</i>	<i>Anisophyllea</i>
<i>Ceriops</i> Arn. [3]	Rhizophoreae	<i>Bruguiera</i>	<i>Combretocarpus</i>
<i>Kandelia</i> (DC.) Wight & Arn. [1]	<i>Rhizophora</i>		
<i>Bruguiera</i> Sav. [6]	<i>Ceriops</i>	Anisophylleaceae	
	<i>Kandelia</i>	<i>Anisophyllea</i>	
Anisophylleaceae (4/~45)	<i>Bruguiera</i>	<i>Combretocarpus</i>	
<i>Anisophyllea</i> R. Br. ex Sabine [ca. 40]		<i>Poga</i>	
<i>Combretocarpus</i> Hook.f. [1-2]	Anisophylleaceae		
<i>Poga</i> Pierre [1]	<i>Anisophyllea</i>		
<i>Polygonanthus</i> Ducke [2]	<i>Combretocarpus</i>		
	<i>Poga</i>		
	<i>Polygonanthus</i>		

^a Takhtajan (1997) separated the two families in two orders Anisophylleales and Rhizophorales, both kept in the superorder Rhizophoranae.

^b Floret (1988) treated *Dactylopetalum* as a distinct subgenus in *Cassipourea*.

^c Including subgenera *Weihea* (32 species), *Lasiopetalum* (3), *Cassipourea* (13), *Pumiloweihea* (1), *Zenkeroweihea* (1), and *Dinklgeoweihea* (1) sensu Floret (1988), but not subgenus *Dactylopetalum*.

close relationship of the Anisophylleaceae and Rhizophoraceae to each other. One goal of the present analysis was to determine the relationships of these families within angiosperms.

With Anisophylleae removed, the Rhizophoraceae show less variation, but their position within the angiosperms still remains unclear. Several authors have placed the family in the Myrtales (Emberger, 1960; Melchior, 1964; Soó, 1975; Takhtajan, 1980), perhaps close to the Combretaceae with which they share several floral, vegetative, pollen (tricolporate), and embryological characters. The occurrence of other mangrove genera in the Myrtales, e.g., *Laguncularia* (Combretaceae) and *Sonneratia* (Lythraceae or Sonneratiaceae), also supported this grouping.

The Rhizophoraceae have also been placed in the Cornales (Cronquist, 1968; Thorne, 1968), and Airy Shaw (1966) postulated relationships with Combretaceae, Elaeocarpaceae, and Tiliaceae, and later with Combretaceae, Rubiaceae, and Elaeocarpaceae (Airy Shaw, 1973). More recent taxonomic treatments have excluded the Rhizophoraceae from the Myrtales (Johnson and Briggs, 1984) and treated them as a separate order Rhizophorales, close to Linales, Malpighiales, and Geraniales (Cronquist, 1981; Thorne, 1992). Other suggestions include relationships with Erythroxylaceae (Behnke, 1982, 1988) and with Hugoniaceae, Linaceae, Oxalidaceae, Celastraceae, and Lepidobotryaceae (Dahlgren, 1988).

Dahlgren (1988) conducted a systematic search for the relatives of Rhizophoraceae. He was guided by a comparison of characters that occur in most or all Rhizophoraceae, or at least in some taxa he regarded as having plesiomorphic character states. Additionally, he chose characters that are unusual in angiosperms at large, or have at least a limited distribution. Altogether six characters were chosen and their individual and combined occurrences within the angiosperms were evaluated: presence of endothelium in combination with crassinucellate ovules, presence of an aril formed by the exostome, fibrous exotegmic seed coat, chlorophyllous embryo, type of sieve-tube plastids, and presence of certain types of alkaloids. Some families that exhibit character similarities with the Rhizophoraceae are listed in Table 2. Dahlgren (1988) summarized his findings in a list of most likely sister taxa to Rhizophoraceae: 1, Elaeocarpaceae; 2, Celastraceae; 3, Erythroxylaceae; 4, Lepidobotryaceae and Ctenolophonaceae; 5, other Geraniales; 6, Theales, Ebenales, Sapindales, and Rutales; and 7, Myrtales.

Recently, Conti, Litt, and Sytsma (1996) suggested in a molecular cladistic analysis that the Rhizophoraceae do not belong in the Myrtales. Their *rbcL* tree shows a sister-group relationship to *Drypetes* Vahl. (Euphorbiaceae), but they sampled few groups outside the Myrtales and thus could not clarify phylogenetic affinities of Rhizophoraceae. In addition to the limitation of sparse sampling, *Drypetes* and Rhizophoraceae were united by few syna-

TABLE 2. Presence of characters according to Dahlgren (1988) for different angiosperm families. Families that share only one of the listed characters with Rhizophoraceae are not shown.

Character	Rhizophoraceae	Erythroxylaceae	Elaeocarpaceae	Celastraceae	Euphorbiaceae	Linaceae	Oxalidaceae	Clusiaceae	Zygophyllaceae	Violaceae
1. Presence of endothelium in combination with crassinucellate ovules	x	x	x	x		x	x		x	
2. Presence of aril formed from the exostome	x	x	x	x				x		
3. Seed coat with fibrous exotegmen	x	x	x	x	x	x	x			x
4. Chlorophyllous embryo	x	x	x	x		x	x	x	x	x
5. Sieve-tube plastids with numerous, variably large, square or polygonal protein bodies	x	x								
6. Presence of certain types of alkaloids:										
Hygroline	x	x								
Tropane alkaloids	x	x	x		x					
Pyrrolizidine alkaloids	x		x	x	x					
Σ of shared characters	8	7	6	5	3	3	3	2	2	2

pomorphies (4), particularly compared to the large number of autapomorphies in both (64 and 30, respectively). Such a pattern often results from long-branch attraction (Huelsenbeck, 1997; Lyons-Weiler and Hoelzer, 1997).

Despite considerable effort, the relationships of Rhizophoraceae and Anisophylleaceae were still unclear. Previous morphological studies were ambiguous, and sampling problems have limited the molecular studies. To resolve the relationships of the family Rhizophoraceae and Anisophylleaceae, we have sequenced the chloroplast gene *rbcL* for members of all tribes of the Rhizophoraceae and two (*Anisophyllea* and *Combretocarpus*) of the four genera of Anisophylleaceae. A large number of putative sister taxa were included to find the closest relatives of the families.

Intrafamilial relationships—After excluding Anisophylleae from Rhizophoraceae, the remainder of the family has traditionally been subdivided into three tribes, the

Gynotrocheae, Macarisieae, and Rhizophoreae (Table 1). However, a cladistic analysis of morphological characters (Juncosa and Tomlinson, 1988a) suggested that the Macarisieae and Gynotrocheae are paraphyletic (Fig. 1). Another cladistic analysis of seed and fruit characters (Tobe and Raven, 1988b) suggested that the Gynotrocheae are paraphyletic, but that the Macarisieae and Rhizophoreae are monophyletic (Fig. 2). Neither analysis included an explicit outgroup, but character polarity was determined by using the Elaeocarpaceae and Celastraceae as putative close relatives. Based on their study of seed and fruit characters, Tobe and Raven (1988b) proposed a revised classification, which was adopted by Takhtajan (1997), that split the Gynotrocheae into two separate tribes: Gynotrocheae containing *Gynotroches*, *Pellacalyx*, and *Carallia*, and the monogeneric Crossostylideae (Fig. 2).

Steyermark and Liesner (1983) recognized a fifth tribe.

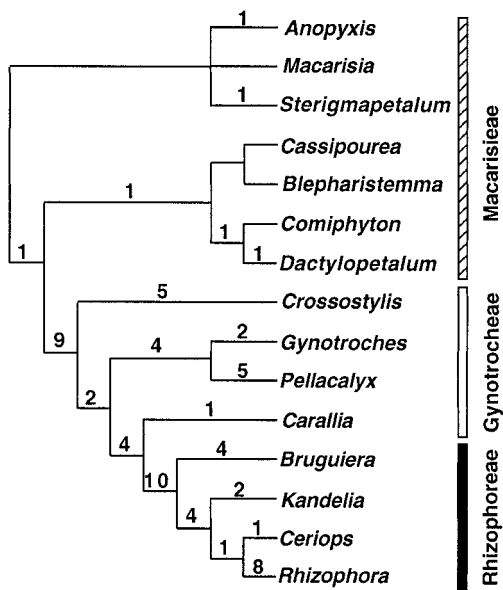


Fig. 1. Phylogenetic relationships of Rhizophoraceae based on a cladistic analysis of morphological characters by Juncosa and Tomlinson (1988a). Figures above branches indicate number of characters.

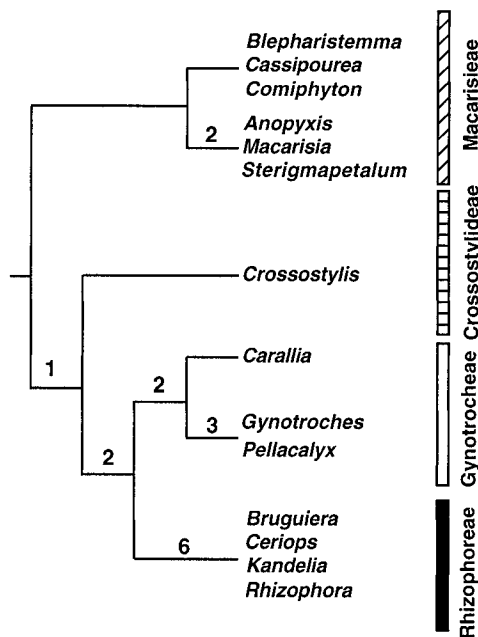


Fig. 2. Phylogenetic relationships of Rhizophoraceae based on seed morphology and anatomy by Tobe and Raven (1988b). Figures above branches indicate number of characters.

Based on floral characters, the genera *Sterigmapetalum* and *Cassipourea* (including *Dactylopetalum*) were separated as an additional tribe from the rest of the Macariseae (they used the illegitimate name Hypogyneae). This separation is also supported by mostly brochidodromous leaf venation in "Hypogyneae" instead of mostly eucamptodromous (or intermediate) venation in the Macariseae (Keating and Randrianasolo, 1988).

To examine intrafamilial relationships as well as monophyly of proposed tribes, we combined information from three chloroplast markers (*rbcL*, *atpB-rbcL* intergenic spacer, and *trnL-trnF* intergenic spacer), three nuclear ribosomal DNA regions (ITS1, ITS2, and 5.8S), and morphological data to construct a phylogenetic hypothesis for the Rhizophoraceae. In addition, we assess the evolution of morphological characters, especially those traditionally used for delimitation of systematic units in the Rhizophoraceae (e.g., fruit and seed characters), and those regarded as adaptations to mangrove habitats (e.g., aerial stilt roots and vivipary). The historical biogeography of the family will be discussed elsewhere (Schwarzbach and Ricklefs, unpublished data).

In summary, the main goals of the present study were (1) to find the closest relatives of Rhizophoraceae and Anisophylleaceae, (2) to reconstruct the intergeneric relationships within Rhizophoraceae and compare them with previously proposed classifications, and (3) to discuss the evolution of morphological attributes in Rhizophoraceae associated with exploitation of mangrove habitats.

MATERIALS AND METHODS

Taxon sampling—For the family-level analysis, *rbcL* was sequenced for 11 of the 15 genera and 17 of ~143 species of Rhizophoraceae representing all three tribes. Two species of *Anisophyllea* and the monotypic *Combretocarpus* represented the Anisophylleaceae. *RbcL* sequences of several putative sister families and representatives of groups within which these families may be nested were downloaded from GenBank. The following search strategies were employed to sample taxa. (1) Guided by large phylogenetic analyses of angiosperms based on different molecular markers (Chase et al., 1993; Soltis et al., 1997a, b), we sampled *rbcL* sequences from representatives of all major angiosperm clades and combined these data with our Rhizophoraceae/Anisophylleaceae sequences. This analysis narrowed the possible relationships of Rhizophoraceae/Anisophylleaceae to the rosid clades. (2) Accordingly, we next sampled representatives of each family in the rosid clade for which *rbcL* sequences were available, guided by phylogenetic analyses of groups within this clade (Albert, Williams, and Chase, 1992; Price and Palmer, 1993; Swensen, Mullin, and Chase, 1994; Conti, Litt, and Sytsma, 1996; Swensen, 1996; Fay, Swensen, and Chase, 1997; Savolainen, Spichinger, and Manen, 1997; Alverson et al., 1998; Fay et al., 1998). When sequences for several species of one family were available, we chose one or two representative species. We preferred species of unquestionable position in the above-mentioned analyses and for which a complete sequence was available. Due to the large number of taxa, this search could be conducted only with limited search strategies (especially reduced branch swapping procedures). (3) After this initial search, we added all available sequences for groups identified as close to either Rhizophoraceae or Anisophylleaceae. At the same time we removed several families that did not appear as close relatives to either one of our families in order to reduce the total number of taxa and to allow faster computing. The results of steps 1 and 2 are not shown in this paper, but can be requested from AES. The 81 taxa used for the

final search are listed in Appendix 1 (sequences obtained from GenBank) and Appendix 2 (sequences produced for this study).

For determining intrafamilial relationships in Rhizophoraceae we sampled 13 genera (material for the monotypic genera *Comiphyton* and *Blepharistemma* was not available) and 28 species of this family as well as all four genera of the Erythroxylaceae and *Drypetes* (Euphorbiaceae) as outgroups. Representatives of *Rhizophora mangle* from the Atlantic coast (AO; Ricklefs 186) and from the Pacific coast (PO; Ricklefs 171) of Panama were sequenced. For the combined analysis it was necessary to use sequences from different species in two instances: (1) *Erythroxylum confusum* (*rbcL*) combined with *Erythroxylum argentinum* (*trnL-trnF*, *atpB-rbcL*, and nrDNA) and (2) *Drypetes roxburghii* (*rbcL*) combined with *Drypetes deplanchei* (*trnL-trnF*).

Protocols for the molecular study—Total DNA from frozen, silica gel-dried, and herbarium material was extracted using the DNA easy extraction kit (QIAGEN Inc., Chatsworth, California, USA) mainly following the protocol provided by QIAGEN. The plant material was ground in Eppendorf tubes using sterilized sea-sand instead of liquid nitrogen. Herbarium and silica gel-dried material was ground in a dry state before adding extraction buffer, while the frozen material was ground after adding extraction buffer. DNA amplifications were performed in 50 μ L reactions containing 1.25U *Taq* polymerase (Promega, Madison, Wisconsin, USA), reaction buffer A supplied by Promega, 1.5 mmol/L MgCl₂, 25 pmol of each primer and 0.2 mmol/L of each dNTP. The flanking primer sequences for *rbcL* (1F and 1460R) were used as in Fay et al. (1998); additional internal primers were designed for maximum fit in the Rhizophoraceae (674F: 5'-TTTATAAAGCACAGCGGAA-3'; 795R: 5'-CTGTAAAGTAGTCATGCATT-3'). The *trnL-trnF* spacer region was amplified using primers E and F from Taberlet et al. (1991). The *atpB-rbcL* intergenic spacer region was amplified using the forward primer "oligo 2" described by Manen, Natali, and Ehrendorfer (1994) and a reverse primer complement to the forward primer (1F) that was used for amplification of *rbcL*. For amplification of the nuclear ribosomal DNA, primers designed by White et al. (1990) were used and 10% DMSO was added to the PCR mix. The PCR profile included 32 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, and a final cycle of 30 s at 95°C, 1 min at 55°C, and 8 min at 72°C. The extension time per cycle was increased to 2 min for *rbcL*. The product was separated from residual primers, dNTPs, and *Taq* polymerase by running the entire product on a low melting agarose gel. Amplified DNA was recovered by using the QIAquick Gel Extraction kit (QIAGEN Inc.). Part of the purified DNA (60–120 ng) was used for the cycle sequencing reaction following the protocol of the DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems Inc., Foster City, California). The samples were run on an acrylamide gel using an ABI 373 or ABI 377 DNA Sequencing System (Applied Biosystems Inc.). In most cases, both strands were sequenced.

Morphological data—We expanded the genus-level morphological data sets of Juncosa and Tomlinson (1988a), Keating and Randrianasolo (1988), and Tobe and Raven (1988b) to all species in the molecular study (Appendix 3) and added the outgroups Erythroxylaceae (Schulz, 1907; Badré, 1973a, b; Boesewinkel and Geenen, 1980; Verdcourt, 1980; Weberling, Lörcher, and Böhnke, 1980; Rury, 1981; Behnke, 1988) and *Drypetes* (Levin, 1986; Kapil and Bhatnagar, 1994; Dunlop, Leach, and Cowie, 1995; Tokuoka and Tobe, 1999). Additionally, some characters from published studies were recoded. For example, Juncosa and Tomlinson (1988a) coded fruit type (dehiscent or baccate) and seed appendages (no appendages, wings, or arils) as two separate characters. We treated these as nonindependent characters because certain combinations are not possible (e.g., berries with winged seeds or arils). Therefore, "fruit type" and "seed appendages" were united into a single character (see character 23 for coding, Appendix 3).

Data analysis—The DNA sequences were assembled and used to

construct consensus sequences. All sequences were aligned manually. For the ITS regions a Clustal W alignment (Thompson, Higgins, and Gibson, 1994) was performed in addition to the manual alignment, but this resulted in only minor differences that had no effect on the tree topologies. Gaps were coded as missing. Missing sequences were coded as missing data in the combined data set. All sequences have been deposited in GenBank (Appendix 2).

The aligned sequences were exported to PAUP* versions 4.0d64 (kindly provided by D. Swofford). The heuristic search algorithm, with the MULPARS and TBR branch swapping options, and 100 RANDOM additions to search for multiple islands of trees (Maddison, 1991), was used for all final tree searches. The morphological data were analyzed with the same PAUP settings. Larger preliminary analyses for the family relationships were performed with NNI branch swapping and CLOSEST taxon addition. For the intergeneric relationships the different data sets were analyzed both separately and combined. Strict consensus trees were constructed from all most parsimonious trees obtained from the individual and combined searches. Consistency (CI) and retention indices (RI) were calculated. Bootstrap analyses (Felsenstein, 1985) were used to assess support for monophyletic groups. The same PAUP settings were used for the bootstrap analysis (100 replicates) as for the original search, but SIMPLE addition was used instead of RANDOM. For the *trnL-trnF* data set a "fast bootstrap" with 10 000 replicates was performed, because a regular bootstrap could not be finished due to computational constraints.

MacClade 3.05 (Maddison and Maddison, 1992) was used for mapping characters and computation of tree lengths for alternative topologies. Both ACCTRAN and DELTRAN optimization were used and compared. The Templeton test (Templeton, 1983; Mason-Gamer and Kellogg, 1996) was used to test whether the alternative topologies obtained by moving branches in the family analysis were significantly longer than the most parsimonious trees.

The same test was used to test for significant differences between the morphological and combined molecular trees. The molecular trees resulting from the different molecular data sets do have slightly different taxon composition and were therefore only compared visually by comparing topology and branch supports, respectively.

RESULTS

Family-level search—During the third step of the family-level search 81 taxa were included (Appendices 1 and 2). The aligned sequences included 1402 bp, of which 411 characters were phylogenetically informative. The search based on *rbcL* sequences revealed three islands of trees, each containing 16 equally most parsimonious trees, 2365 steps long (excluding autapomorphies). One of the trees, which is identical to the majority rule consensus tree containing all compatible groupings, is shown in Fig. 3, but branches collapsing in the strict consensus tree are also indicated. The consistency index is 0.282 (excluding autapomorphies); the retention index is 0.558. Our results show that the family Rhizophoraceae is sister group to Erythroxylaceae, supported by a bootstrap value of 90%. Both families are part of the order Malpighiales within eurosids I (sensu APG, 1998). Additionally, our tree suggests a close relationship of Anisophylleaceae and Cucurbitaceae within the order Cucurbitales. Although this relationship is not well supported, Anisophylleaceae and Rhizophoraceae clearly are not immediate relatives. Forcing the Rhizophoraceae into a sister group relationship with Anisophylleaceae requires 37 extra steps on the tree and this new topology is significantly different ($P < 0.0001$). Conversely, moving Anisophylleaceae to the base of Rhizophoraceae creates a tree 32 steps longer

than the most parsimonious trees. Similarly, placing Rhizophoraceae in close relationship to families that were previously suggested as close relatives results in trees between 19 and 42 steps longer than the shortest trees (Fig. 3). All these alternative topologies require significantly more steps ($P < 0.0001$).

Intrafamilial search—*rbcL*—Altogether, 27 *rbcL* sequences, each 1402 bp long, were analyzed. The aligned data set contained 235 variable characters, of which 91 were phylogenetically informative. The tree search resulted in 144 equally most parsimonious trees (Table 3). All most parsimonious trees show the monophyly of the three tribes Macarisieae, Gynotrocheae, and Rhizophoreae (Fig. 4). The *rbcL* sequence data resulted in mainly well-resolved and well-supported deeper branches, but they fail to resolve more recent relationships.

AtpB-rbcL—Twenty-eight taxa were sequenced for this cpDNA spacer, which is adjacent to the *rbcL* gene. The sequences were between 647 and 736 bp long, and the aligned sequences contained 969 positions, of which 137 were variable and 73 phylogenetically informative (Table 3). The tree search resulted in 20 equally most parsimonious trees. All deep branches are well resolved (Fig. 4), but often less well supported than in the *rbcL* tree. This might be the result of fewer informative characters overall or, what seems to be more plausible to us, increasing difficulty in recovering multiple substitutions further back in time and therefore underestimation of deeper branch lengths.

TrnL-trnF—This cpDNA spacer region was sequenced for all 34 taxa included in this study. The sequences were between 331 and 437 bp long in the Rhizophoraceae and up to 467 bp in the outgroups. The aligned sequences were 567 bp long, of which 158 positions were variable and 87 phylogenetically informative (Table 3). The tree search could not be completed due to computational limitations and was stopped after obtaining 73 346 trees of 156 steps length. The strict consensus tree is shown in Fig. 4. This spacer region was the most variable, but also the shortest cpDNA piece sequenced in this study (Table 3).

NrDNA—ITS1 (206–251 bp), 5.8S (162 bp), ITS2 (196–222 bp), and a small part of the 26S nrDNA (50 bp) were sequenced for 20 taxa. We were not able to obtain a complete data set for two reasons. (1) Fungal and algal contaminations of the DNA that was extracted from some of the herbarium material leaves did not allow us in a few cases to obtain the Rhizophoraceae sequences. Even the use of primers that were designed to better fit angiosperm sequences (F. R. Blattner, personal communication, IPK Gatersleben, Germany) could not discriminate between fungal and Rhizophoraceae targets. All sequences obtained were tested with a GenBank BLAST search for possible contaminations, a caution suggested by Liston et al. (1996). (2) For some of the taxa we obtained two different copies of this repeat region, and for other taxa only "copy one" or "copy two." For this analysis we chose the copy that we obtained for a larger number of

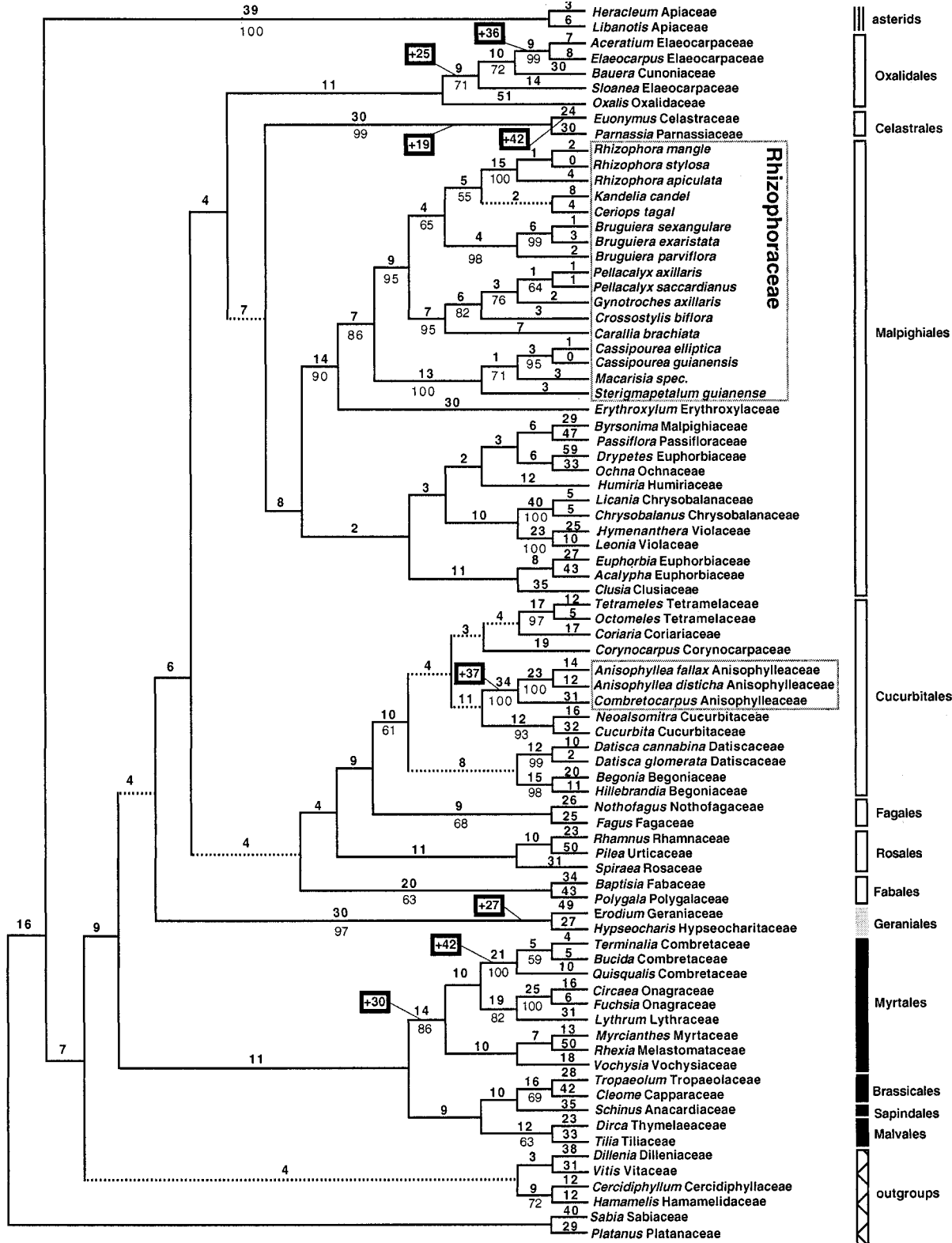


Fig. 3. One of 48 equally most parsimonious trees identical to the majority-rule consensus tree showing all compatible groupings based on the *rbcL*-based family search. Branches collapsing in the strict consensus tree are indicated by dotted lines. Figures above branches are number of nucleotide changes; figures below branches represent bootstrap values expressed as percentage of 100 bootstrap replications. Bootstrap values <50 % are not shown. The numbers shown in the boxes indicate the number of extra steps required forcing Rhizophoraceae into the indicated position. Tree length is 2365 steps (excluding autapomorphies), consistency index is 0.282 (excluding autapomorphies), retention index is 0.558. Boxed taxa show the delimitation of Rhizophoraceae and Anisophylleaceae, respectively. Eurosids I are indicated by black bars, eurosids II by white bars. Geraniales have not yet been assigned to either group (APG, 1998).

TABLE 3. Tree statistics for the different data sets and their combinations.

Data set	Number of included taxa ^a	Number of characters ^b	Number of informative characters	Number of trees	Tree length ^c	CI ^c	RI
Family relationships							
<i>rbcL</i>	81	1402	411 (29.2%)	48	2365	0.282	0.558
Intrafamilial relationships							
<i>rbcL</i>	27	1402	91 (6.5%)	144	153	0.686	0.887
<i>trnL-trnL</i>	34	567	87 (15.2%)	>73,346 ^d	156	0.673	0.877
<i>atpB-rbcL</i>	28	969	73 (7.6%)	20	113	0.761	0.902
ITS 1, ITS 2 and 5.8S, partial 26S	20	711	236 (33.2%)	2	614	0.658	0.734
Morphology	34	30	30 (100%)	55	77	0.649	0.899
Molecular combined	34	3649	487 (13.4%)	7	1047	0.669	0.818
All combined	34	3679	517 (14.1%)	1	1132	0.664	0.825

^a List of taxa in appendices 1 and 2, respectively.

^b Length of aligned sequences.

^c Excluding autapomorphies.

^d Result of an incomplete tree search (see text for details).

taxa, simply to avoid a large number of missing data in the combined analyses.

The aligned data set was 711 bp long and contained 410 variable characters, of which 236 were phylogenetically informative (Table 3). The tree search resulted in two equally most parsimonious trees and the strict consensus tree was calculated. The nrDNA phylogeny (Fig. 4) is largely congruent with the cpDNA trees. The main difference is the sister-group relationship of *Gynotroches* and *Crossostylis* (bootstrap value 79%) instead of *Gynotroches* being sister to *Pellacalyx* as found for cpDNA. This may well be a long-branch attraction problem because a small number of synapomorphies are opposed by a large number of autapomorphies (Fig. 4).

Morphology—The tree reconstruction based on 30 morphological characters (Appendix 3) resulted in 55 equally most parsimonious trees. The strict consensus tree is shown in Fig. 4. The phylogeny is not well resolved, most likely due to the small number of characters. ACCTRAN and DELTRAN optimizations did not result in any differences that would lead to an important difference in character interpretation.

Trees resulting from separate searches were highly congruent, although the separate analyses were sometimes less resolved, which would tend to obscure any incongruencies. Tree searches based on the *trnL-trnF* spacer sequences could not be completed, even though this spacer produced more informative characters than *atpB-rbcL* and the morphological data. *TrnL-trnF* may be too variable (Table 3) to unambiguously reconstruct deeper branches of the phylogeny. Multiple nucleotide substitutions over time seem to be the cause of the blurred deep relationships. The nrDNA was even more variable (Table 3), but the variability appeared to be distributed differently. Parts of ITS1 and ITS2 and especially the 5.8S DNA are relatively conservative (see also Hershkovitz and Lewis, 1996; Hershkovitz and Zimmer, 1996), allowing resolution of deep branches.

Combined analyses—We were not able to sequence the different regions for all taxa, mainly due to difficulties with amplification of DNA from herbarium material. For this reason the amount of data available for the different

taxa differs (Appendix 2). Because some sequences were incomplete, branch lengths generally do not reflect the actual number of transformations in the combined analysis. Missing data may also reduce bootstrap values in some cases. The combined molecular analysis based on an aligned data set composed of 3649 positions and 487 phylogenetically informative positions resulted in seven equally most parsimonious trees of 1047 steps length. The strict consensus tree is shown in Fig. 4. This tree is well resolved except within the Macarisieae, which might be due to the fact that most missing data are within this tribe.

When comparing molecular and morphological trees the Templeton test (Templeton, 1983) revealed some interesting insights. By plotting the combined molecular data set onto the 55 most parsimonious morphological tree topologies, as well as onto the 50% majority rule and strict consensus trees of these 55 trees, we found that only two of the 55 single-tree topologies did not differ significantly. Interestingly, even the consensus trees of the morphological analysis (and only morphological data were included in this case) differed significantly from the individual trees of this same analysis. This might indicate considerable internal conflict within the morphological trees, in our opinion most likely the result of the small number of informative characters. Conversely, plotting the morphological data onto the seven combined molecular trees resulted in no significant differences ($P = 0.15-0.32$). In summary, this means that the morphological data are compatible with the molecular tree topologies, but the molecular data are not compatible with the generally badly resolved morphological topologies. We think that the incongruence in only one direction is the result of an internal problem of the morphological data set and we therefore decided to combine the data sets.

Finally, the combination of all data sets, morphological and molecular, resulted in a single most parsimonious tree 1132 steps long (Fig. 5). Altogether, 517 informative characters were available. We do not believe that the larger molecular data set swamps the smaller morphological one resulting in a tree that more closely resembles the molecular tree than the morphological. A small number of added characters can alter tree topologies dramatically, and Chippindale and Wiens (1994) have shown that com-

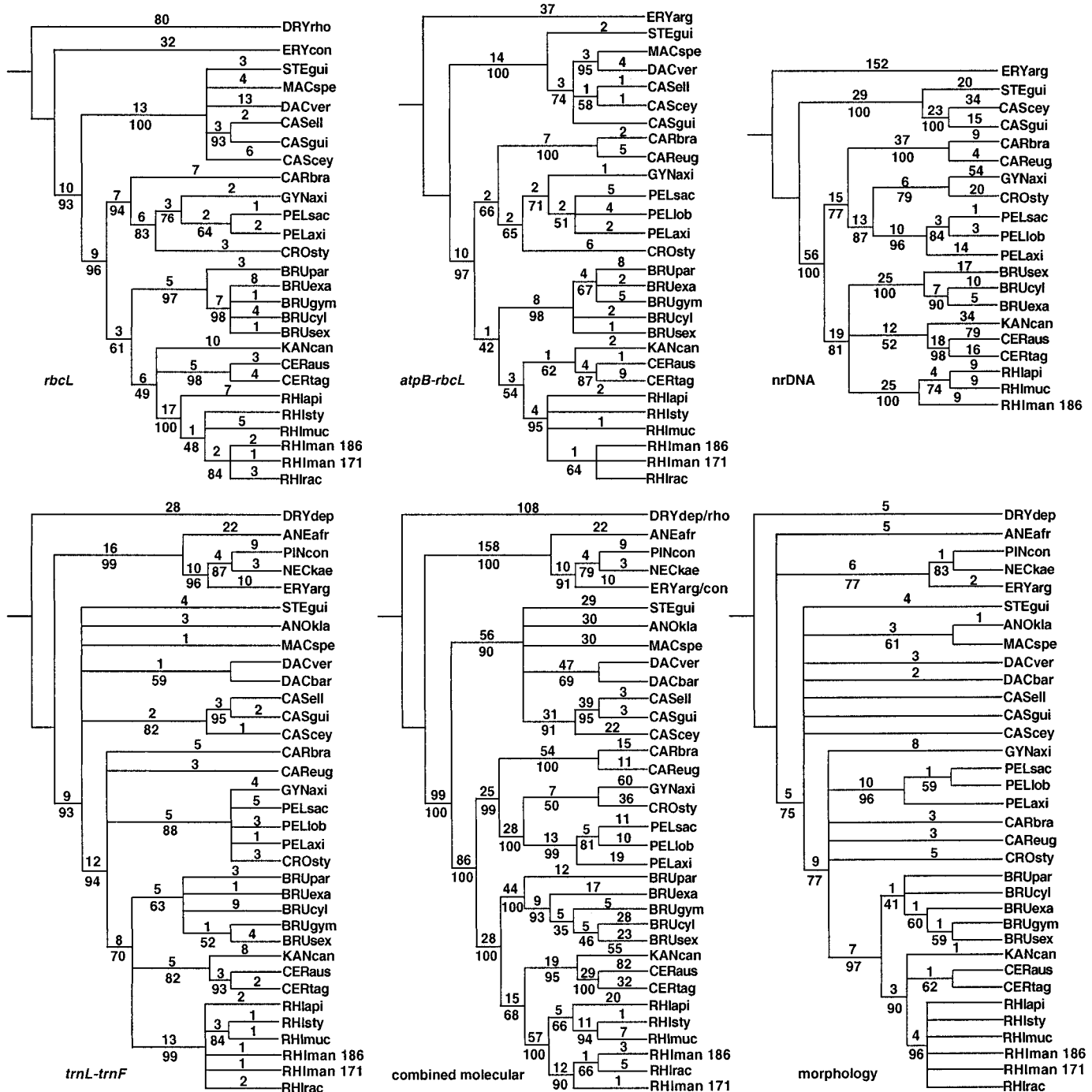


Fig. 4. Phylogenetic analyses of Rhizophoraceae based on *rbcL*, *atpB-rbcL* spacer, *trnL-trnF* spacer, and nuclear ribosomal DNA sequences (ITS1, ITS2, 5.8S, and partial 26S), all molecular data combined, and morphology. Strict consensus trees are shown; tree statistics are listed in Table 3. Due to computational constraints, the *trnL-trnF* tree represents the result of an uncompleted tree search (see text for details). Figures above branches are number of character changes (ACCTRAN optimization); figures below branches represent bootstrap values. Differences among trees are entirely based on badly supported or unresolved branches.

binations of smaller and larger data sets do not necessarily produce a tree that is identical to the tree obtained for the larger data set alone. In our study the single tree obtained with the total evidence approach is identical to one of the seven trees that resulted from the combined molecular analysis, indicating that the relatively few mor-

phological characters helped to discriminate between the alternative trees that resulted from molecular analyses.

DISCUSSION

Relationships of Anisophylleaceae—Our results indicate that the Anisophylleaceae belong in the Cucurbitales

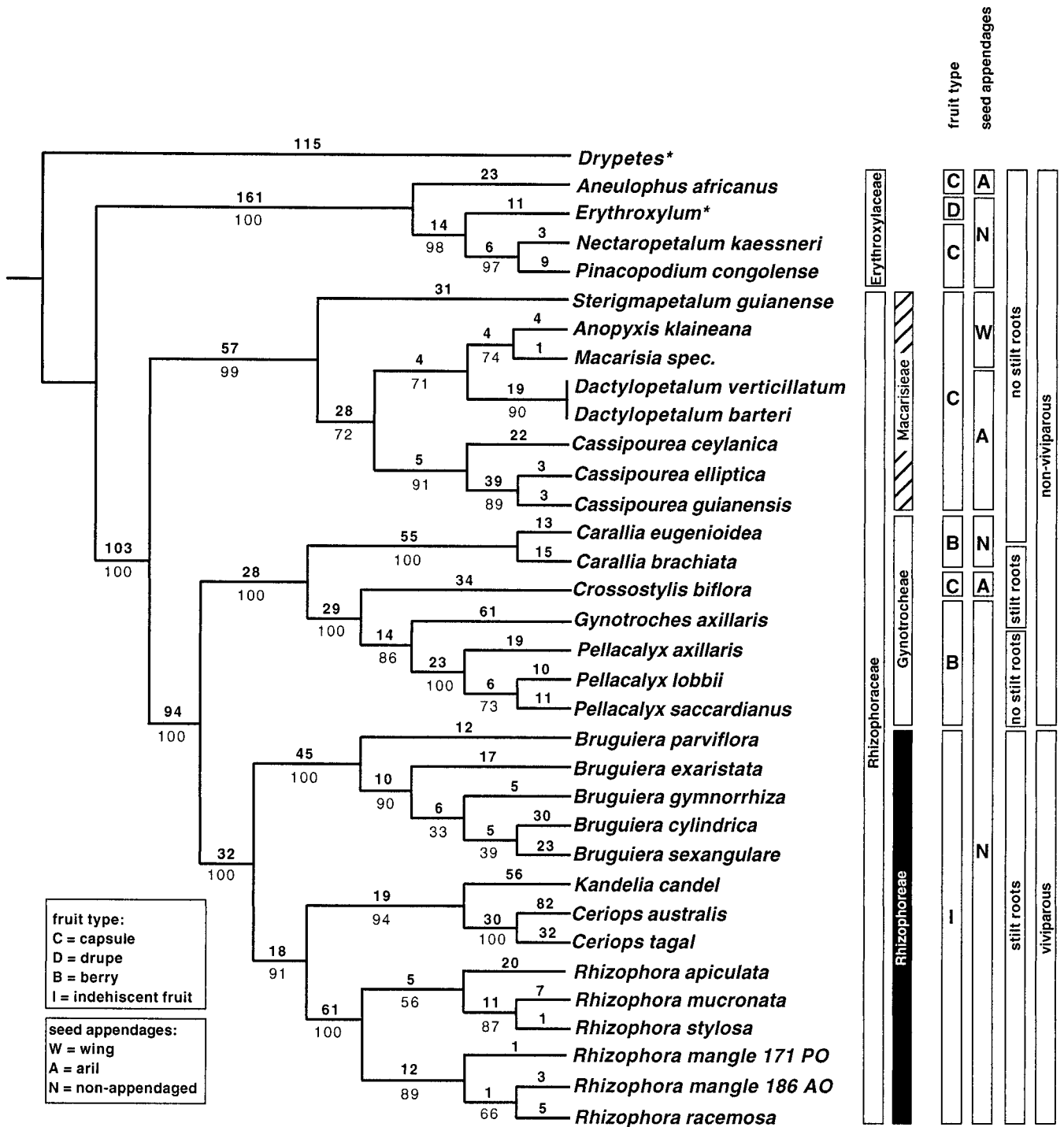


Fig. 5. Single most parsimonious tree based on the combined analysis of *rbcL*, *trnL-trnF* spacer, *atpB-rbcL* spacer, ITS1, ITS2, 5.8S, partial 26S sequences, and morphological characters. Figures above branches are number of character changes; figures below branches represent bootstrap values expressed as percentage of 100 bootstrap replications. Asterisks mark taxa where sequences originated from different species of the same genus (see text for details). Tree length is 1132 steps (excluding autapomorphies), consistency index is 0.664 (excluding autapomorphies), retention index is 0.825. Boxes to the right indicate distribution of morphological characters.

(sensu Bremer, Bremer, and Thulin, 1997; APG, 1998), which include the Cucurbitaceae, Begoniaceae, Datisca-ceae, Tetramelaceae, Coriariaceae, and Corynocarpaceae (see also Swensen, Mullin, and Chase, 1994; Swensen, Luthi, and Rieseberg, 1988), but are not closely related

to Rhizophoraceae. Our analysis results in a sister group relationship of the Anisophylleaceae and Cucurbitaceae, although this relationship is not well supported. The An-isophylleaceae and, to a large extent, the rest of Cucur-bitales share separate styles, an inferior ovary, and uni-

sexual flowers (apart from *Combretocarpus*). In contrast to most other Cucurbitales (but see *Octomeles*, a tree up to 60 m height), the Anisophylleaceae are often large tropical trees. Similarities between the Rhizophoraceae and the Anisophylleaceae, such as incised petals and similar vascular anatomy (Tobe and Raven, 1988a), probably should be regarded as the results of parallelism or convergent evolution. Tobe and Raven (1988a) argued that the Anisophylleaceae should be treated as distinct from Rhizophoraceae, but they postulated an intermediate position between the Rhizophoraceae and Myrtales. However, because several families are more closely related to one or another of these three groups (Fig. 3), character similarities among the Anisophylleaceae, Rhizophoraceae, and Myrtales should be regarded as plesiomorphies or parallelisms rather than synapomorphies. Cronquist (1981) and Thorne (1992) treated the Anisophylleaceae as a separate family in the order Rosales, but the close relationship to Rosaceae or other Rosales (Cronquist, 1981; Dahlgren, 1988) suggested by pollen and flower characters can also be rejected on the basis of our molecular results.

A better understanding of the relationships of the Anisophylleaceae will require a broader study including all four genera of the family as well as more members of the Cucurbitales, and perhaps a more variable marker. The genus *Polygonanthus* differs especially in many characters from the rest of the Anisophylleaceae, suggesting that it might be not part of this family, but unfortunately we were not able to obtain any material.

Relationships of the Rhizophoraceae—The hypotheses proposed by several authors of a close relationship of Rhizophoraceae with either Celastraceae or Elaeocarpaceae (Dahlgren, 1988; Juncosa and Tomlinson, 1988a; Tobe and Raven, 1988b) are not supported by our molecular analysis. Character similarities with the Rhizophoraceae, namely stipules, similar leaf venation, unicellular hairs, stomatal type, and presence of oxalate crystals in Elaeocarpaceae, and laticifers, oxalate crystals, stipules, inflorescence structure, and identical embryological features in Celastraceae (Dahlgren, 1988), should be regarded as plesiomorphies or parallelisms. Our analyses show, instead, that the sister family to the Rhizophoraceae is Erythroxylaceae, which was the third of the list of putative sister families suggested by Dahlgren (1988). The relationship between Rhizophoraceae and Erythroxylaceae is well supported by a high bootstrap value (90%) in the molecular analysis (*rbcL*) and by both families sharing morphological characters with other taxa in the order Malpighiales (sensu Bremer, Bremer, and Thulin, 1997; APG, 1998). When the Rhizophoraceae are forced to be the sister group to the alternative families suggested by Dahlgren (1988), at least 19 more steps (Celastrales) are required than in the most parsimonious tree (Fig. 3). Moreover, several nonmolecular characters listed by Dahlgren (1988) also support the sister group relationship of Erythroxylaceae and Rhizophoraceae, which share a woody habit, many embryological characters, the presence of the alkaloid hygroline, and, especially, a unique sieve-tube plastid type (Behnke, 1988). Indeed, the Erythroxylaceae shared with the Rhizophoraceae the highest number of the characters listed by Dahlgren (1988),

namely seven (Table 2). Compelling as this may be, Dahlgren (1988) preferred the Celastraceae and Elaeocarpaceae over the Erythroxylaceae as potential sister groups to the Rhizophoraceae. In particular, he found no reason to remove Erythroxylaceae from the Geraniales (or from Linales, if this order is separated from Geraniales, as by Cronquist, 1981) and to move them to the vicinity of Rhizophoraceae. Dahlgren (1988) thought that the similarities between Erythroxylaceae and Rhizophoraceae were “not as far-reaching” as those between Rhizophoraceae and Elaeocarpaceae. Consequently, according to his view, “the very particular kind of sieve-tube plastids that the two families [Erythroxylaceae and Rhizophoraceae] have in common has evolved by convergent evolution in the ancestors of each of the two families.”

Characters that vary in the Erythroxylaceae and Rhizophoraceae but also support a close relationship of the two families are discussed below with the results of the generic analysis.

Relationships within the Rhizophoraceae—Systematic inferences from the combined analysis (Fig. 5) agree with some traditional subdivisions of the family into the three tribes Macarisieae, Gynotrocheae and Rhizophoreae (Table 1). The monophyly of all three clades is well supported by bootstrap values of at least 99% (Fig. 5). The Gynotrocheae are the sister group to the mangrove tribe Rhizophoreae (bootstrap 100%) and both together are sister group to Macarisieae (bootstrap 100%; Fig. 5). Almost all separate analyses of molecular data show identical relationships of the tribes (except for *trnL-trnF*, for which this part of the tree is unresolved), and these contradict in several respects the arrangement indicated by the morphological analyses of Juncosa and Tomlinson (1988a; see Fig. 1) and Tobe and Raven (1988b; Fig. 2). Our morphological analysis did not resolve the tree on a tribal level. The three analyses differ in taxon and character sampling as well as in character coding, which may have been responsible for the different topologies. The main discrepancy can be found in the relationships of the Gynotrocheae and their position as a separate tribe. Based on our unambiguous molecular results and our interpretation of morphological character changes that will be presented below, we prefer a subdivision of the family into three tribes (Table 1).

With one exception, the three tribes can be circumscribed by fruit characters. Macarisieae share capsular fruits, Gynotrocheae (except *Crossostylis*) have berries, and Rhizophoreae have indehiscent fruits with seeds germinating on the mother plant (viviparous). In the analyses of Juncosa and Tomlinson (1988a) and Tobe and Raven (1988b), the capsular fruits with arillate seeds of *Crossostylis* resulted in a sister group relationship of *Crossostylis* to the rest of the Gynotrocheae and Rhizophoreae, leaving the Gynotrocheae paraphyletic. According to Juncosa and Tomlinson (1988a), the most parsimonious interpretation is that the capsular fruit constitutes the plesiomorphic character state in the family; a common ancestor of *Carallia*, *Gynotroches*, *Pellacalyx*, and the mangrove clade evolved berry-like fruits, which was followed by a shift to viviparous, indehiscent fruits in the mangrove group. Our most parsimonious interpretation

based on the combined analysis also suggests a plesiomorphic capsular fruit.

Juncosa and Tomlinson's (1988a) tree topology suggests that *Crossostylis* occupies a more basal position and retains the plesiomorphic fruit character state of the Rhizophoraceae. In contrast, our hypothesis suggests a more derived position for *Crossostylis* combined with a derived fruit type different from the plesiomorphic character state in the family. Several other apomorphic features also support a more derived position of *Crossostylis*. Species of this genus share a polyandrous androecium, which is clearly derived and elsewhere in the family is found only in *Kandelia*, a member of the Rhizophoreae, the most derived mangrove clade. The absence of terminal flowers in *Crossostylis* is a clearly derived feature that evolved in parallel in *Rhizophora* and *Kandelia*. Species with capsular fruits in the Macariseae share superior ovaries, which can be regarded as plesiomorphic, but *Crossostylis* has a half-inferior or inferior ovary (Tobe and Raven, 1988b; Setoguchi, Ohba, and Tobe, 1998) and the capsule opens with small slits. As far as we know, the capsular fruits of Macariseae open with longitudinal slits or fall from the tree unopened, dehiscing on the ground upon drying out (*Juncosa* and Tomlinson, 1988a). The hypothesis of a derived position of *Crossostylis* also agrees with the biogeography of this genus, which is endemic to South Pacific Islands. Based on morphological and cpDNA RFLP (restriction fragment length polymorphism) data, Setoguchi et al. (1998) suggested a successive distribution from more western islands (Solomon Islands, Vanuatu, and New Caledonia) to more eastern islands (Fiji, Samoa, Society Islands, and Marquesas Islands). Therefore, the arillate seeds in *Crossostylis* are most likely distributed by birds from island to island in contrast to the ant-dispersed arillate seeds of the Macariseae.

Not only fruit characters but also seed characters are important in the delimitation of clades within Rhizophoraceae. We have already suggested that the capsular fruit can be regarded as a plesiomorphic character state within the family and that the arillate seeds of *Crossostylis* are derived. For the entire family it is still questionable what seed characters are apomorphic or plesiomorphic. Regarding the evolution of seed appendages there have been two hypotheses. Based on their morphological cladistic analysis (Fig. 1), *Juncosa* and Tomlinson (1988a) considered that arillate-seeded genera were derived from winged-seeded ancestors. In contrast, other authors (Dahlgren, 1988; Tobe and Raven, 1988b) have suggested that the presence of an aril is the plesiomorphic character state. Their argument was based on the fact that all arillate seeds occur in taxa with superior ovaries, which they regarded as the plesiomorphic state. However, this is contradicted by *Crossostylis*, which has a half-inferior or inferior ovary, but arillate seeds (*Juncosa* and Tomlinson, 1988a), and by the fact that, without exception in the Rhizophoraceae, winged seeds also co-occur with superior ovaries (*Anopyxis*, *Macarisia*, and *Sterigmapetalum*).

Within the Erythroxylaceae, only *Aneulophus* has arillate seeds and a capsular fruit; the rest of the family has nonappendaged seeds, in either drupes or capsules. Concerning the entire family, it is equally parsimonious to assume nonappendaged or appendaged seeds as the ple-

siomorphic state. If we look closer at Macariseae, the common ancestor of this tribe very likely had appendaged seeds but it is not clear whether wings or arils were present. We have to infer parallel or reversal evolution for either one of the seed appendages, but unfortunately all of the most parsimonious scenarios require the same number of transformations and to date we can only provide ecological data that might favor the hypothesis of arillate seeds being apomorphic. Regarding arillate seeds as apomorphic within this tribe is supported by the fact that some species of the widespread and species-rich arillate-seeded genera *Cassipourea* and *Dactylopetalum* typically occur in drier habitats. This is clearly a derived feature within Rhizophoraceae, and the arillate seeds of these genera may be adapted to the characteristic seed dispersers of drier environments. More detailed anatomical studies might be able to tease apart homoplasious and homologous character states and solve this problem in the future.

Evolution of presumably adaptive morphological characters related to mangrove habitats—Tomlinson (1986) listed several apparently adaptive characters, including aerial (stilt) roots and vivipary, that are associated with living in mangrove habitats. We have examined the distribution of these characters and their order of occurrence within Rhizophoraceae (Fig. 5).

Vivipary—True vivipary is defined by the development of seedlings that remain attached to the parent plant. Thus, seedlings, rather than seeds or fruits, disperse (Elmqvist and Cox, 1996). Members of the tribe Rhizophoreae and a seagrass (*Amphibolis* Schott & Kotschy) are probably the only truly viviparous seed plants (*Juncosa*, 1982). Pseudovivipary sometimes occurs in the form of adventitious plantlets in inflorescences or facultative vivipary is found as premature germination in many cultivated fruits, e.g., *Citrus* L. In the case of cryptovivipary, the embryo emerges from the seed coat but not from the fruit before the entire entity is distributed (Carey, 1934; *Juncosa*, 1982). Cryptovivipary and vivipary have apparently evolved in parallel in many unrelated mangrove genera, such as *Pelliciera* Planch. & Triana (Pellicieraceae), *Avicennia* L. (Avicenniaceae), *Aegiceras* Gaertn. (Myrsinaceae), *Nypa* Steck (Arecaceae), and *Aegialitis* Trin. (Plumbaginaceae). As Tomlinson (1986) pointed out, embryological development of seeds is a continuous process, which for a longer or shorter time period is interrupted by seed dormancy. In viviparous plants this seed dormancy is either absent or very brief. Thus, vivipary represents the extreme of a continuum of seed dormancy relative to fruit abscission or dehiscence.

All members of the tribe Rhizophoreae are viviparous (Fig. 5). In *Bruguiera*, the seedling disperses initially with the fruit (Tomlinson, 1986), whereas in the more derived genera *Rhizophora*, *Kandelia*, and *Ceriops*, only the seedling disperses (*Juncosa*, 1982, 1984). The isolated position of *Bruguiera* from other Rhizophoreae is also evident in the phylogenetic tree (Fig. 5).

Aerial stilt roots—According to Tomlinson (1986), stilt roots are branched, looping aerial roots that arise from

the trunk and lower branches and often stabilize the tree trunk in older individuals. Aerial stilt roots can be found in many tropical plants growing in poorly aerated soils, but especially in swampy and mangrove habitats. All members of the mangrove tribe Rhizophoreae have aerial roots, although *Kandelia* develops them only in some limiting environments (Tomlinson, 1986). *Rhizophora* develops the largest and most elaborate stilt roots whereas *Ceriops* and *Bruguiera* form smaller stilt roots at the base of the trunk. In older plants of *Ceriops* and *Bruguiera* these separate roots coalesce and form a conical trunk base (Juncosa and Tomlinson, 1988a). Interestingly, some nonmangrove members of the family also have aerial stilt roots, suggesting that this character preceded the entry of the Rhizophoreae into coastal habitats. Among the inland genera, at least *Carallia brachiata* occurring in peat swamps (DingHou, 1958) as well as *Crossostylis* and *Gynotroches* of the Gynotrocheae develop aerial stilt roots (Juncosa and Tomlinson, 1988a). Based on the topologies found here (Fig. 5), the common ancestor of the Gynotrocheae and Rhizophoreae evolved the capacity to develop aerial stilt roots and stilt roots were lost in parallel in *Pellacalix* and some populations of *Kandelia*.

Conclusions—Our molecular phylogenetic analyses show how DNA sequence data can resolve systematic relationships that are difficult to infer from analysis of morphological data alone due to extensive homoplasy. Despite several excellent morphological and anatomical studies (Dahlgren, 1988; Juncosa and Tobe, 1988; Juncosa and Tomlinson, 1988a, b; Keating and Randrianasolo, 1988; Tobe and Raven, 1988a, b; Raven and Tomlinson, 1988; Vezev et al., 1988), it has not been possible to clearly identify the sister groups of Rhizophoraceae and Anisophylleaceae. Our molecular data allow us to reject previously suggested close relationships of the Rhizophoraceae with Celastraceae or Elaeocarpaceae and identify the Erythroxylaceae as sister group to the Rhizophoraceae and possibly the Cucurbitaceae as sister to the Anisophylleaceae. Although we were not able to obtain good support for the immediate relationships of the Anisophylleaceae, their position within Cucurbitales (sensu Bremer, Bremer, and Thulin, 1997; APG, 1998) can be regarded as both stable and surprising.

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APPENDIX 1. List of species ordered by family for *rbcL* sequences obtained from GenBank.

Species	Family	Authors of sequences or literature citation	GenBank acc. no ^a
<i>Schinus molle</i> L.	Anacardiaceae	Gadek et al., 1996	GBAN-U39270
<i>Heracleum dulce</i> Fisch.	Apiaceae	Kondo et al., 1996	GBAN-D44569
<i>Libanotis coreana</i> (Wolff.) Kitag.	Apiaceae	Kondo et al., 1996	GBAN-D44573
<i>Hillebrandia sandwicensis</i> Oliver	Begoniaceae	Swensen, 1996	GBAN-U59822
<i>Begonia herbacea</i> Vell	Begoniaceae	Swensen, 1996	GBAN-U59816
<i>Cleome hassleriana</i> Chod.	Capparaceae	Rodman et al., 1993	GBAN-M95755
<i>Euonymus alatus</i> (Thunberg) Siebold	Celastraceae	Chase et al., 1993	GBAN-L13184
<i>Cercidiphyllum japonicum</i> Siebold & Zucc.	Cercidiphyllaceae	Olmstead et al., 1992	GBAN-L11673
<i>Chrysobalanus icaco</i> L.	Chrysobalanaceae	Morgan and Soltis, 1993	GBAN-L11178
<i>Licania tomentosa</i> Fritsch.	Chrysobalanaceae	Morgan and Soltis, 1993	GBAN-L11193
<i>Clusia gundlachi</i> Stahl	Clusiaceae	Fay, Swensen, and Chase, 1997	GBAN-Z75673
<i>Bucida macrostachia</i> Standl.	Combretaceae	Conti, Litt, and Sytsma, 1996	GBAN-U26321
<i>Quisqualis indica</i> L.	Combretaceae	Albert, Williams, and Chase, 1992	GBAN-L01948
<i>Terminalia catappa</i> L.	Combretaceae	Conti, Litt, and Sytsma, 1996	GBAN-U26338
<i>Coriaria myrtifolia</i> L.	Coriariaceae	Albert, Williams, and Chase, 1992	GBAN-L01897
<i>Corynocarpus laevigata</i> Forst.	Corynocarpaceae	Martin and Dowd, 1993	GBAN-L28949
<i>Cucurbita pepo</i> L.	Cucurbitaceae	Swensen, Mullin, and Chase, 1994	GBAN-L21938
<i>Neolomandra sarcophylla</i> (Wall.) Hutchinson	Cucurbitaceae	Swensen, 1996	GBAN-U59823
<i>Bauera rubioides</i> Andr.	Cononiaceae	Morgan and Soltis, 1993	GBAN-L11174
<i>Datisca cannabina</i> L.	Datisceae	Swensen, Mullin, and Chase, 1994	GBAN-L21939
<i>Datisca glomerata</i> Baill.	Datisceae	Swensen, Mullin, and Chase, 1994	GBAN-L21940
<i>Dillenia indica</i> L.	Dilleniaceae	Albert, Williams, and Chase, 1992	GBAN-L01903
<i>Aceratium ferrugineum</i> C. T. White	Elaeocarpaceae	Martin and Dowd, 1993	GBAN-U06838
<i>Elaeocarpus grandis</i> F. Muell.	Elaeocarpaceae	Martin and Dowd, 1993	GBAN-U06842
<i>Sloanea latifolia</i> Schum.	Elaeocarpaceae	Alverson et al., 1998	GBAN-AFO22131
<i>Erythroxylum confusum</i> Britton	Erythroxylaceae	Chase et al., 1993	GBAN-L13183
<i>Acalypha rhomboidea</i> Rafin.	Euphorbiaceae	Gunter, Kochert, and Giannasi, 1994	GBAN-U00435
<i>Drypetes roxburghii</i> (Wall.) Hurusawa	Euphorbiaceae	Rodman et al., 1993	GBAN-M95757
<i>Euphorbia polychroma</i> A. Kerner	Euphorbiaceae	Chase et al., 1993	GBAN-L13183
<i>Baptisia tinctoria</i> R. Br.	Fabaceae	Käss and Wink, 1995	GBAN-Z70120
<i>Fagus sylvatica</i> L.	Fagaceae	Martin and Dowd, 1993	GBAN-L13340
<i>Erodium texanum</i> A. Gray	Geraniaceae	Price and Palmer, 1993	GBAN-U06493
<i>Hamamelis mollis</i> Oliv.	Hamamelidaceae	Albert, Williams, and Chase, 1992	GBAN-L01922
<i>Humiria balsamifera</i> Aubl.	Humiriaceae	Albert, Williams, and Chase, 1992	GBAN-L01926
<i>Hypseocharis spec.</i> Remy	Hypseocharitaceae	Price and Palmer, 1993	GBAN-L14699
<i>Lythrum hyssopifolium</i> L.	Lythraceae	Conti, Fischbach, and Sytsma, 1993	GBAN-M10218
<i>Brysonima crassifolia</i> (L.) Kunth.	Malpighiaceae	Albert, Williams, and Chase, 1992	GBAN-L01892
<i>Rhexia virginica</i> L.	Melastomataceae	Conti, Litt, and Sytsma, 1996	GBAN-U26334
<i>Myricianthes fragrans</i> (Sw.) McVaugh	Myrtaceae	Conti, Litt, and Sytsma, 1996	GBAN-U26328
<i>Nothofagus alpina</i> (Poepp. & Endl.) Oerst	Nothofagaceae	Martin and Dowd, 1993	GBAN-L13342
<i>Ochna multiflora</i> DC.	Ochnaceae	Fay, Swensen, and Chase, 1997	GBAN-Z75273
<i>Circaea alpina</i> L.	Onagraceae	Conti, Fischbach, and Sytsma, 1993	GBAN-L10216
<i>Fuchsia cyrtandroides</i> J. W. Moore	Onagraceae	Conti, Fischbach, and Sytsma, 1993	GBAN-L10220
<i>Oxalis dillenii</i> Jaqu.	Oxalidaceae	Albert, Williams, and Chase, 1992	GBAN-L01938
<i>Parnassia fimbriata</i> Banks	Parnassiaceae	Albert, Williams, and Chase, 1992	GBAN-L01939
<i>Passiflora quadrangularis</i> L.	Passifloraceae	Albert, Williams, and Chase, 1992	GBAN-L01940
<i>Platanus occidentalis</i> L.	Platanaceae	Albert, Williams, and Chase, 1992	GBAN-L01943
<i>Polygala cruciata</i> L.	Polygalaceae	Albert, Williams, and Chase, 1992	GBAN-L01945
<i>Rhamnus cathartica</i> L.	Rhamnaceae	Chase et al., 1993	GBAN-L13189
<i>Spiraea vanhouttei</i> (Briot) Zabel	Rosaceae	Morgan and Soltis, 1993	GBAN-L11206
<i>Sabia spec.</i> Colebr.	Sabiaceae	Qiu et al., 1993	GBAN-L12662
<i>Octomeles sumatrana</i> Miq.	Tetramelaceae	Swensen, Mullin, and Chase, 1994	GBAN-L21942
<i>Tetrameles nudiflora</i> R. Br.	Tetramelaceae	Swensen, Mullin, and Chase, 1994	GBAN-L21943
<i>Tilia americana</i> L.	Tiliaceae	Chase et al., 1993	GBAN-AFO22127
<i>Dirca palustris</i> L.	Thymelaeaceae	Conti, Litt, and Sytsma, 1996	GBAN-U26322
<i>Tropaeolum majus</i> L.	Tropaeolaceae	Price and Palmer, 1993	GBAN-L14706
<i>Pilea pumila</i> (L.) A. Gray	Urticaceae	Gunter, Kochert, and Giannasi, 1994	GBAN-U00438
<i>Hymenanthera alpina</i> (T. Kirk) W. R. B. Oliv.	Violaceae	Fay, Swensen, and Chase, 1997	GBAN-Z75692
<i>Leonia glycyarpa</i> Ruiz & Par.	Violaceae	Fay, Swensen, and Chase, 1997	GBAN-Z75693
<i>Vitis aestivalis</i> Torr.	Vitaceae	Albert, Williams, and Chase, 1992	GBAN-L01960
<i>Vochysia hondurensis</i> Sprague	Vochysiaceae	Conti, Litt, and Sytsma, 1996	GBAN-U26340

^a The prefix GBAN- has been added to link the online version of the *American Journal of Botany* to GenBank but is not part of the actual accession number.

APPENDIX 2. List of taxa and GenBank accession numbers^a for sequences generated for this study.

Taxon	Voucher	<i>rbcL</i>	<i>trnL-trnT</i>	<i>atpB-rbcL</i>	ITS1+2, 5.8S, partial 26S
Euphorbiaceae					
<i>Drypetes deplanchei</i> (Brongn. & Gris) Merrill	Schwarzbach AUS18-97 (MO)		GBAN-AF127727		
Erythroxylaceae					
<i>Aneulophus africanus</i> Benth.	McPherson 15849 (MO)		GBAN-AF127728		
<i>Nectaropetalum kaessneri</i> Engler	Robertson 4933 (MO)		GBAN-AF127729		
<i>Pinacopodium congolense</i> (S. Moore) Exell & Mendocá	McPherson 15533 (MO)		GBAN-AF127730		
<i>Erythroxylum argentinum</i> O. E. Schulz	Schwarzbach AUS2-97 (MO)		GBAN-AF127731	GBAN-AF127699	GBAN-AF130316
Rhizophoraceae					
Macarisiaceae					
<i>Sterigmopetalum guianense</i> subsp. <i>ichunense</i> Steyermark & Liesner	Aymard, Berry, Melguiero, Gomez 11287 (MO)	GBAN-AF127671	GBAN-AF127732	GBAN-AF127700	GBAN-AF130317
<i>Anopyxis klaineana</i> (Pierre) Engl.	Hart 962 (MO)		GBAN-AF127733		
<i>Cassipourea elliptica</i> Poir	Wright s.n., no voucher	GBAN-AF127672	GBAN-AF127734	GBAN-AF127701	GBAN-AF130318
<i>Cassipourea guianensis</i> Aubl.	Renner & Rosa 2159 (MO)	GBAN-AF127673	GBAN-AF127735	GBAN-AF127702	GBAN-AF130319
<i>Cassipourea ceylanica</i> (Gardn.) Alston	Cooray 6810212R (MO)	GBAN-AF127674	GBAN-AF127736	GBAN-AF127703	
<i>Macarisia</i> spec. Thou.	Clausing s.n. (MO)	GBAN-AF127675	GBAN-AF127737	GBAN-AF127704	
<i>Dacylopetalum verticillatum</i> Schiuz	Schwarzbach AUS16-97 (MO)	GBAN-AF127676	GBAN-AF127738	GBAN-AF127705	
<i>Dacylopetalum barteri</i> Hook f. ex Oliver	J. & A. Raynal 13572 (LE)		GBAN-AF127739		
Gynotrocheae					
<i>Carallia brachiata</i> (Lour.) Merr.	Schwarzbach AUS3-97 (MO)	GBAN-AF127677	GBAN-AF127740	GBAN-AF127706	GBAN-AF130320
<i>Carallia eugenioides</i> King	Meijer 5888 (LE)		GBAN-AF127741	GBAN-AF127707	GBAN-AF130321
<i>Gynotroches axillaris</i> Blume	Yong 49 (MO)	GBAN-AF127678	GBAN-AF127742	GBAN-AF127708	GBAN-AF130322
<i>Crossosylius biflora</i> J. R. et G. Forster	Gillett 2218 (LE)	GBAN-AF127679	GBAN-AF127743	GBAN-AF127709	GBAN-AF130323
<i>Pellacalyx saccardianus</i> Scortech.	Clausing s.n., no voucher	GBAN-AF127680	GBAN-AF127744	GBAN-AF127710	GBAN-AF130324
<i>Pellacalyx axillaris</i> Korth.	Yong 48 (MO)	GBAN-AF127681	GBAN-AF127745	GBAN-AF127711	GBAN-AF130325
<i>Pellacalyx lobbii</i> (Hook. f.) Schimp.	Ambri & Arigin W780 (LE)		GBAN-AF127746	GBAN-AF127712	GBAN-AF130326
Rhizophoreae					
<i>Kandelia candel</i> (DC.) Wight. & Arn.	Huang 5434 (TAD)	GBAN-AF127682	GBAN-AF127747	GBAN-AF127713	GBAN-AF130327
<i>Cerriops australis</i> (C. T. White) E. R. Ballment, T. J. Smith III & J. A. Stoddart					
<i>Cerriops tagal</i> (Perr.) C. B. Robinson	Schwarzbach AUS73-97 (MO)	GBAN-AF127683	GBAN-AF127748	GBAN-AF127714	GBAN-AF130328
<i>Rhizophora apiculata</i> Blume	Razifmandimbison 2 (MO)	GBAN-AF127684	GBAN-AF127749	GBAN-AF127715	GBAN-AF130329
<i>Rhizophora stylosa</i> Griff.	Meyer s.n. (MJG)	GBAN-AF127685	GBAN-AF127750	GBAN-AF127716	GBAN-AF130330
<i>Rhizophora mucronata</i> Lamk.	Schwarzbach AUS47-97 (MO)	GBAN-AF127686	GBAN-AF127751	GBAN-AF127717	
<i>Rhizophora mangle</i> L.	Yong 19 (MO)	GBAN-AF127687	GBAN-AF127752	GBAN-AF127718	GBAN-AF130331
<i>Rhizophora mangie</i> L.	Ricklefs 186, AO (MO)	GBAN-AF127688	GBAN-AF127753	GBAN-AF127719	GBAN-AF130332
<i>Rhizophora racemosa</i> G. F. W. Mey.	Ricklefs 171, PO (MO)	GBAN-AF127689	GBAN-AF127754	GBAN-AF127720	
<i>Bruguiera sexangula</i> (Lour.) Poir.	Yong 45 (MO)	GBAN-AF127690	GBAN-AF127755	GBAN-AF127721	GBAN-AF130333
<i>Bruguiera parviflora</i> (Roxb.) Arn.	Guillen s.n. (MO)	GBAN-AF127692	GBAN-AF127757	GBAN-AF127723	
<i>Bruguiera gymnorrhiza</i> (L.) Lamarck	Razifmandimbison 13 (MO)	GBAN-AF127693	GBAN-AF127758	GBAN-AF127724	
<i>Bruguiera cylindrica</i> (L.) Blume	Yong 8 (MO)	GBAN-AF127694	GBAN-AF127759	GBAN-AF127725	GBAN-AF130334
<i>Bruguiera exaristata</i> Ding Hou	Schwarzbach AUS61-97 (MO)	GBAN-AF127695	GBAN-AF127760	GBAN-AF127726	GBAN-AF130335
Anisophylleaceae					
<i>Anisophyllea disticha</i> (Jack) Baillon	Clausing s.n., no voucher	GBAN-AF127697			
<i>Anisophyllea fallax</i> S. Elliot	Clausing s.n. (MO)	GBAN-AF127696			
<i>Combretocarpus rotundatus</i> (Mig.) Danser	Beaman 8735 (LE)	GBAN-AF127698			

^aThe prefix GBAN- has been added to link the online version of the *American Journal of Botany* to GenBank but is not part of the actual accession number.

APPENDIX 3. Morphological data matrix.

Taxon ^a	Characters														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DRYdep	0	0	1	0	?	0	0	?	0	1	1	3	3	2	0
ANEafr	0	0	0	0	?	1	1	?	0	1	2	3	2	0	0
NECKae	0	0	1	0	?	0	1	?	0	1	2	3	2	0	0
PINcon	0	0	1	0	?	0	1	?	0	1	2	3	2	0	0
ERYarg	0	0	1	0	?	0	1	?	0	1	2	3	2	0	0
STEgui	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0
ANOkla	0	0	0	2	0	1	0	0	0	0	2	0	1	0	0
CASell	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0
CASgui	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0
CAScey	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0
MACspe	0	0	0	1 & 2	0	1	0 & 1	0	0	0	2	0	0	0	0
DACver	0	0	0	0	0	1	0	0	0	1	2	0	0	0	0
DACbar	0	0	0	0	0	1	0	0	0	1	2	0	0	0	0
CARbra	1	1	0	0	1	1	0	1	0	0	2	1	0	1	0
CAREug	0	1	0	0	1	1	1	1	0	0	2	1	0	1	0
GYNaxi	1	1	0	1	1	1	1	1	0	1	0 & 2	2	0	0	0
CRObif	1	1	0	0	1	1	1	1	0	0	2	1	1	0	1
PELsac	0	1	0	2	0	1	0 & 1	1	0	1	2	2	0	0	0
PELaxi	0	1	0	2	0	1	0 & 1	1	0	1	2	2	0	0	0
PELlob	0	1	0	2	0	1	0 & 1	1	0	1	2	2	0	0	0
KANcan	1	1	0	0	1	1	1	1	1	0	2	0	0	1	1
CERaus	1	1	0	0	1	1	1	1	1	1	2	0	0	1	0
CERtag	1	1	0	0	1	1	1	1	1	1	2	0	0	1	0
RHlapi	1	1	0	0	1	1	1	1	1	0	2	0	2	0	0
RHIsty	1	1	0	0	1	1	1	1	1	0	2	0	2	0	0
RHImuc	1	1	0	0	1	1	1	1	1	0	2	0	2	0	0
RHIman186	1	1	0	0	1	1	1	1	1	0	2	0	2	0	0
RHIman171	1	1	0	0	1	1	1	1	1	0	2	0	2	0	0
RHIRac	1	1	0	0	1	1	1	1	1	0	2	0	2	0	0
BRUsex	1	1	0	0	1	1	1	1	1	2	2	0	0	1	0
BRUpar	1	1	0	0	1	1	1	1	1	0	2	0	0	1	0
BRUgym	1	1	0	0	1	1	1	1	1	2	2	0	0	1	0
BRUcyl	1	1	0	0	1	1	1	1	1	0	2	0	0	1	0
BRUexa	1	1	0	0	1	1	1	1	1	2	2	0	0	1	0

^a Names abbreviated, first three capital letters stand for genus, last three small letters for species. Character legend: (1) stilt roots: 0 absent, 1 present; (2) root hairs: 0 present, 1 absent; (3) phyllotaxy: 0 opposite, 1 alternate; (4) leaf venation: 0 brachididromous, 1 eucamptodromous, 2 intermediate; (5) stipule vernation: 0 valvate, 1 imbricate; (6) stipule morphology: 0 intrapetiolar, 1 interpetiolar; (7) leaf margin: 0 serrate, 1 entire; (8) hypodermis: 0 absent, 1 present; (9) salt tolerant: 0 no, 1 yes; (10) inflorescence: 0 open-branched, 1 fasciculate, 2 solitary; (11) breeding system: 0 monoecious, 1 dioecious, 2 hermaphroditic; (12) floral laticifers: 0 one layer, 1 radially expanded, 2 idioblastic, 3 none; (13) lateral fringed petal appendages: 0 present, 1 abort, 2 absent, 3 no petals; (14) petal orientation: 0 reflexed, 1 erect, 2 no petals; (15) androecium: 0 diplostemonous, 1 polyandrous; (16) hypanthium: 0 absent, 1 present; (17) ovary position: 0 superior, 1 half-inferior, 2 inferior; (18) carpels: 0 five carpels, 1 three-many carpels, 2 two carpels, 3 three carpels; (19) ovules per locule: 0 two ovules, 1 five-eight, 2 one ovule; (20) nucellus: 0 crassinucellate, 1 tenuinucellate; (21) integument: 0 not vascularized, 1 vascularized; (22) seeds per fruit: 0 several to many, 1 one seed, 2 two seeds, 3 three seeds; (23) fruit-seed appendage type: 0 capsule-aril, 1 baccate-none, 2 capsule-wing, 3 capsule-none, 4 drupe-none, 5 indehiscent hard-walled-none; (24) cotyledons: 0 separate, 1 connate; (25) viviparous: 0 no, 1 yes; (26) germination process: 0 by hypocotyl, 1 by endosperm expansion; (27) seedling establishment: 0 by radicle, 1 by lateral roots; (28) sepals: 0 ten, 1 eleven, 2 twelve, 3 thirteen, 4 four, 5 five, 6 six, 7 seven, 8 eight, 9 fourteen; (29) trichomes: 0 stellate, 1 others; (30) stamen tube: 0 absent, 1 present.

APPENDIX 3. Extended.

Characters														
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
0	0	1	0	0	?	1	4	0	0	0	0	4 & 5	1	0
0	0	3	0	0	?	3	0	0	0	0	0	5	1	1
0	0	2	2	0	?	2	3	0	0	0	0	5	1	1
0	0	2	2	0	?	2	3	0	0	0	0	5	1	1
0	0	3	2	0	?	1	4	0	0	0	0	5	1	1
0	0	0	0	0	0	0	2	0	0	0	0	5	1	0
0	0	0	0	0	0	0	2	0	0	0	0	5	1	0
0	0	1	0	0	0	0	0	0	0	0	0	4 & 5	1	0
0	0	1	0	0	0	0	0	0	0	0	0	4 & 5	1	0
0	0	1	0	0	0	0	0	0	0	0	0	5	1	0
0	0	0	0	0	0	0	2	0	0	0	0	5	1	0
0	0	2	0	0	0	0	0	0	0	0	0	6 & 7	1	0
0	0	2	0	0	0	0	0	0	0	0	0	5 & 6	1	0
1	2	0	0	0	0	0	1	0	0	0	0	4 & 5 & 6 & 7 & 8	1	0
1	2	0	0	0	0	0	1	0	0	0	0	5	1	0
1	0	0	1	1	0	0	1	0	0	0	0	4 & 5	1	0
1	1	1	0	0	0	0	0	0	0	0	0	4	1	0
1	1	1	1	1	0	0	1	0	0	0	0	4	0	0
1	1	1	1	1	0	0	1	0	0	0	0	5	0	0
1	1	1	1	1	0	0	1	0	0	0	0	4	0	0
1	2	1	0	0	1	1	5	1	1	1	1	5 & 6	1	0
1	2	1	0	0	1	1	5	1	1	1	1	5	1	0
1	2	1	0	0	1	1	5	1	1	1	1	5	1	0
1	2	2	0	0	1	1	5	1	1	1	1	4	1	0
1	2	2	0	0	1	1	5	1	1	1	1	4	1	0
1	2	2	0	0	1	1	5	1	1	1	1	4	1	0
1	2	2	0	0	1	1	5	1	1	1	1	4	1	0
1	2	2	0	0	1	1	5	1	1	1	1	4	1	0
1	2	2	0	0	1	1	5	1	1	1	1	4	1	0
1	2	2	0	0	1	1	5	1	1	1	1	4	1	0
1	2	1	0	0	1	1	5	0	1	0	0	0 & 1 & 2	1	0
1	2	1	0	0	1	1	5	0	1	0	0	8	1	0
1	2	1	0	0	1	1	5	0	1	0	0	0 & 1 & 2 & 3 & 9	1	0
1	2	1	0	0	1	1	5	0	1	0	0	8	1	0
1	2	1	0	0	1	1	5	0	1	0	0	8	1	0