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CIRCUMSCRIPTION OF MYRTALES AND THEIR RELATIONSHIPS TO OTHER ROSIDS: EVIDENCE FROM *RBC*L SEQUENCE DATA[1]

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Despite intensive morphological and chemical studies on the Myrtales, the circumscription of the order remains poorly defined. To test the monophyly of Myrtales sensu Dahlgren and Thorne (*Annals of the Missouri Botanical Garden* 71: 633–694, 1984), determine the relationships of some controversial families, and identify the most likely sister group of Myrtales, we conducted parsimony analyses on 80 *rbc*L sequences representing 36 taxa from families traditionally included in Myrtales and 44 taxa from other Rosidae. The consensus tree resulting from these analyses supports the monophyly of Myrtales and is substantially congruent with the circumscription of the order proposed by Dahlgren and Thorne (*Annals of the Missouri Botanical Garden* 71: 633–694, 1984), with one notable exception: in the *rbc*L tree Vochysiaceae are placed in Myrtales. A reanalysis of morphological attributes of Vochysiaceae revealed that the inclusion of the family in Myrtales is also supported by the combined occurrence of two typical myrtalean features of the wood: vestured pits and bicollateral vascular bundles. Furthermore, our analyses excluded Thymelaeaceae, Lecythidaceae, Haloragaceae, and Gunneraceae from Myrtales, suggesting that the association of these families with Myrtales, as previously proposed by other authors, may not reflect common ancestry. Finally, our analyses support a sister group relationship between the order Myrtales and a clade formed by an expanded Malvales, Sapindales, and an expanded Capparales.

Key words: Myrtales; *rbc*L sequence data; Rosidae.

Myrtales are one of the most intensively studied orders of plants, as exemplified by the proceedings of a symposium held in 1984 at the XIII International Botanical Congress, Sydney, Australia (Raven, 1984). Despite the intensive morphological and chemical studies carried out in the order, a number of systematic issues remains unsolved. Foremost among these are (1) the circumscription of Myrtales and (2) their relationships to other clades in the Rosidae.

Myrtales are characterized primarily by two distinctive wood anatomical features that are not commonly found together in other groups of flowering plants: bicollateral vascular bundles in the primary stem and vestures in the bordered pits of the secondary xylem. The combined occurrence of these two anatomical characters is very rare and, outside of Myrtales, is known only in Apocynaceae, Asclepiadaceae, and Loganiaceae pro parte (Gentianiflorae of Dahlgren), Thymelaeaceae (Malviflorae of Dahlgren), Vochysiaceae (Polygalales), sporadically in Euphorbiaceae, and in the single genus *Centropodium* of the Polygonaceae (Polygonales; van Vliet and Baas, 1984).

The order Myrtales consists of woody and herbaceous plants characterized by production of tannins (Cronquist, 1981). Leaves are typically opposite, simple, and with entire margins. The flowers of Myrtales are bisexual, mostly four- or five-merous, actinomorphic or weakly zygomorphic, and often are distinguished by the presence of numerous stamens. The 2–5 carpels are fused to form a syncarpous pistil with several locules; the position of the ovary ranges from perigynous to epigynous, often with a short to long hypanthium. Pollen grains are tricolporate, often with pseudocolpi; pollination is usually performed by insects or birds (see also Dahlgren and Thorne, 1984). Other notable features that are typical, although not exclusive, of the Myrtales include unilacunar nodes, starch-accumulating plastids in the sieve tube elements, and chromosome numbers in multiples of 11 or especially 12 (Raven, 1975).

Disagreement on the placement and taxonomic rank of some families of Myrtales is common (reviewed in Dahlgren and Thorne, 1984). Table 1 summarizes the families included in Myrtales by seven different studies based on morphological, anatomical, embryological, palynological, and chemical characters. Some general conclusions can be drawn from comparisons among these treatments. All authors concur in including the following 14 families in the order: Myrtaceae, Heteropyxidaceae, Psiloxylaceae, Lythraceae, Punicaceae, Sonneratiaceae, Combretaceae, Melastomataceae, Memecylaceae, Onagraceae, Trapaceae, Crypteroniaceae, Alzateaceae, and Rhynchocalycaceae. Most systematic treatments concur in including Oliniaceae and Penaeaceae in Myrtales, with some exceptions. Emberger (1960), placed both families in the order Thymelaeales (together with Geissolomataceae,

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TABLE 1. Circumscription of Myrtales according to different authors: X = placed in Myrtales as a separate family; [X] = placed in Myrtales but within another family; O = not placed in Myrtales; ? = taxon not included in the study.

	Emberger, 1960	Melchior, 1964	Soo', 1975	Cronquist 1981	Takhtajan 1980	Dahlgren and Thorne, 1984	Johnson and Briggs, 1984
Myrtaceae	X	X	X	X	X	X	X
Heteropyxidaceae	X	[X]	[X]	[X]	[X]	X/[X]	X
Psiloxylaceae	[X]	[X]	[X]	[X]	[X]	X/[X]	X
Penaeaceae	O	O	O	X	X	X	X
Oliniaceae	O	X	X	X	X	X	X
Alzateaceae	?	?	?	?	?	X	X
Rhynchocalycaceae	?	?	?	?	?	X	X
Lythraceae	X	X	X	X	X	X	X
Punicaceae	X	X	X	X	X	[X]	[X]
Sonneratiaceae	X	X	X	X	X	[X]	[X]
Combretaceae	X	XC	X	X	X	X	X
Melastomataceae	X	X	X	X	X	X	X
Memecylaceae	[X]	[X]	[X]	[X]	[X]	X/[X]	X
Crypteroniaceae	[X]	X	?	X	X	X	X
Onagraceae	X	X	X	X	X	X	X
Trapaceae	X	X	X	X	X	X	X
Rhizophoraceae	X	X	X	O	X	O	O
Lecythidaceae	X	X	X	O	X	O	O
haloragaceae	X	X	X	O	X	O	O
Gunneraceae	X	[X]	X	O	O	O	O
Thymelaeaceae	O	O	O	X	O	O	O

Thymelaeaceae, and Elaeagnaceae); Melchior (1964) and Soo' (1975) followed Emberger's treatment, but placed Oliniaceae in Myrtales.

A number of other families have occasionally been associated with Myrtales. This paper discusses the relationships of five families (Thymelaeaceae, Lecythidaceae, Rhizophoraceae, Haloragaceae, and Gunneraceae) that have been included in the order by at least one of seven more recent taxonomic treatments (Table 1), plus one family (Vochysiaceae) that has never been included in Myrtales. Takhtajan's treatment (1980) conformed to those of Emberger (1960), Melchior (1964), and Soo' (1975) in keeping Rhizophoraceae, Lecythidaceae, Haloragaceae, and Gunneraceae in Myrtales. However, the most recent revisions of the order (Cronquist, 1981; Dahlgren and Thorne, 1984; Johnson and Briggs, 1984) excluded these four families from Myrtales. Thymelaeaceae have been retained in the order only by Cronquist (1981). The rationales that different authors invoked for excluding or including families in the order will be discussed later, in the light of the relationships suggested by parsimony analyses of *rbc*L sequence data.

To date, only two cladistic analyses of Myrtales have been published: one was based on morphological characters (Johnson and Briggs, 1984), the other on amino acid sequence data from the nuclear-encoded small subunit of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*S; Martin and Dowd, 1986). This last study, however, used only 40 amino acids at the N-terminus of *rbc*S and included only Myrtaceae, Melastomataceae, Combretaceae, Lythraceae, Onagraceae, Thymelaeaceae, and a few other rosid taxa representing Euphorbiales and Malvales. Johnson and Briggs (1984), on the other hand, provided a detailed morphological analysis of interfamilial relationships within Myrtales, but did not explore relationships outside the order. The most comprehensive review of morphological and chemical characters of Myrtales and associated families was provided by Dahlgren and Thorne (1984), although they did not furnish an explicit phylogenetic analysis of relationships in the group.

Therefore, a cladistic analysis of the circumscription and relationships of Myrtales based on molecular data is timely, as it will expand upon these previous studies both in terms of taxon sampling and methods of analysis. These studies have provided a wealth of background information for comparisons with the phylogenetic inferences generated by a cladistic molecular analysis of Myrtales. Presented here is a phylogenetic analysis of nucleotide sequence data from the chloroplast gene encoding the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) of Myrtales and putatively related families.

The main goals of the present study are to: (1) establish whether Myrtales sensu Dahlgren and Thorne (1984) constitute a monophyletic order; (2) determine whether some controversial families, such as Thymelaeaceae, Rhizophoraceae, Lecythidaceae, Haloragaceae, and Gunneraceae should be included or excluded from the order; and (3) identify the sister group of Myrtales. We conducted a parsimony analysis of DNA sequence data from the chloroplast gene *rbc*L, the first comprehensive molecular phylogenetic analysis on the relationships of Myrtales. The implications of *rbc*L sequence data at the intraordinal level are addressed elsewhere (Conti, 1994).

MATERIALS AND METHODS

Taxon sampling—The total number of *rbc*L sequences used for phylogenetic analyses of Myrtales was 80, 36 of which were obtained from Myrtales sensu Dahlgren and Thorne (1984). The taxa included in the analysis and the authors of the sequences are listed in Appendix 1. Myrtaceae and Melastomataceae, the two largest families of the order, were represented by five and three genera, respectively, and the three mid-size families (Lythraceae, Combretaceae, and Onagraceae) by five, three, and ten taxa, respectively. Onagraceae were the focus of a more detailed analysis of intrafamilial relationships (Conti, Fischbach, and Sytsma, 1993), thus explaining the more intensive taxon sampling available for this family. Sequences of Lythraceae were obtained in collaboration with S. Graham as part of a separate study on this family (Graham, Conti, and Sytsma, 1993). All of the small and monotypic families of Myrtales were sampled, with the exception of Crypteroniaceae, which includes ten species in three genera. The GenBank accession numbers for the sequences obtained specifically for this study are listed in Appendix 2.

To investigate the circumscription of Myrtales and their relationships within Rosidae, a total of 44 taxa were sampled from various rosid groups. Twelve new rosid *rbc*L sequences were obtained specifically for this study. These represent families occasionally associated with Myrtales (Rhizophoraceae, Thymelaeaceae, Lecythidaceae, Haloragaceae), and two rosid families (Vochysiaceae and Sarcolaenaceae) never included in the order by previous morphological analyses. One sequence of Vochysiaceae, *Qualea,* was kindly made available by M. Chase, and the three sequences of Lecythidaceae were contributed by K. Karol and W. Alverson in K. Sytsma's laboratory. The remaining rosid *rbc*L sequences were graciously provided by other workers (M. Chase, D. Soltis, and J. Rodman). The rationale that guided the choice of rosid taxa outside Myrtales is discussed in more detail under *Phylogenetic analyses.*

DNA extraction, rbcL amplification, and sequencing—The experimental approach followed the general procedure described in Conti, Fischbach, and Sytsma (1993). Total DNA was obtained from silica gel dried leaves of four taxa: *Penaea* (Penaeaceae), *Olinia* (Oliniaceae), *Erisma* (Vochysiaceae), and *Sarcolaena* (Sarcolaenacae); the other DNA extractions were made from fresh leaves maintained at −80°C. The procedure for total DNA extraction followed the method described in Protocol D of Smith et al. (1991), which employs a 6% hexadecyltrimethylammonium bromide (CTAB) extraction/lysis buffer. In vitro amplifications of the *rbc*L gene were obtained using a 5′ primer that annealed at the first 26 positions of the gene and a 3′ primer that annealed ≈100 nucleotides downstream from the stop codon of the gene in *Nicotiana* (Olmstead et al., 1992). However, for two of the taxa for which DNA was obtained from silica gel dried leaves (*Penaea* and *Erisma*), the use of the above-mentioned primers for *rbc*L amplifications failed repeatedly. Therefore, *rbc*L sequences of *Penaea* and *Erisma* were derived from two fragments. One fragment was obtained using an internal primer with the 5′ end starting at position 463 of the *rbc*L gene and an external primer that annealed in the *acc*D gene downstream from the 3′ end of the *rbc*L gene. The other fragment was obtained by using a 5′ primer that annealed in positions 1-26 of the *rbc*L gene and a 3′ primer that annealed in positions 1023–1057 of the gene.

Double-stranded *rbc*L products from in vitro amplifications were sequenced directly using the Sanger, Nicklen, and Coulson (1977) dideoxy method. Overlapping sequence fragments were obtained from both strands of the gene using a total of ten primers. The strand with the 5′ end at the ATG start codon ("forward" strand) was used as reference to indicate the positions of the sequencing primers. Eight primers ("forward" primers) annealed with their 5′ end at positions 1, 234, 437, 523, 674, 895, 1020, and 1204 on the reference strand. Two primers annealed with their 3′ end at positions 346 and 1020 on the reference strand. The sequences of most primers, of length varying between 26 and 34 nucleotides, were kindly provided by G. Zurawski (Zurawski et al., 1981; Zurawski, Whitfield, and Bottomley, 1986), except for primer 437, developed for the study of Lythraceae by S. Graham, and primer 523, developed for Myrtales by E. Conti. A total of 41 *rbc*L sequences were generated for a comprehensive phylogenetic study of Myrtales (see Appendix 1). Sequences were easily aligned by using the *rbc*L sequence of *Ludwigia peploides* as a template (Conti, Fischbach, and Sytsma, 1993). The accuracy of the sequences was checked by translating the nucleotide sequences to the corresponding amino acid sequences with the TRANSLATE program of the Genetics Computer Group (GCG) package; no internal stop codons were found.

Phylogenetic analyses—The search for the most parsimonious trees used 80 taxa representative of Myrtales and of all the major clades of Rosidae identified in the global analyses of *rbc*L sequence data from seed plants conducted by Chase et al. (1993). A total of 1401 nucleotides, from positions 27 to 1428 of the *rbc*L gene, were employed for reconstruction of phylogenetic relationships of Myrtales using the program PAUP 3.1.1 (Swofford, 1993). All known stop codons of the newly obtained sequences ended in position 1428, hence sequences were readily aligned by eye. Stop codons were unknown for the *rbc*L sequences of five species: *Dissotis rotundifolia, Acmena smithii, Myrcianthes fragrans, Dirca palustris,* and *Phaleria chermsideana.* Nucleotide positions were treated as multistate, unordered characters and unknown nucleotides were considered as uncertainties in PAUP analyses. To find the most likely sister group of Myrtales, our data set included representatives from all the major lineages within the clades defined as sisters to Myrtales in both searches of Chase et al. (1993), as well as four taxa (*Spinacia, Rheum, Saxifraga,* and *Hamamelis*) from two clades never associated with Myrtales in either of those two searches. These last four taxa were employed for global outgroup comparisons (Maddison, Donoghue, and Maddison, 1984).

In order to detect the presence of multiple "islands" of most parsimonious trees (Maddison, 1991), the tree-searching strategy suggested by Swofford (1993, p. 103), as implemented by Olmstead et al. (1992, 1993), was used. The search for the most parsimonious trees was conducted on a MacIntosh LC475 with 5,000 K allotted to PAUP 3.1.1 and involved the four steps described below, with Steepest Descent and Collapse option activated during all four steps. (1) An initial round of 1,000 random addition sequences was performed by using the Nearest Neighbor Interchange (NNI) algorithm, with MULPARS off. Random additions of taxa to the developing trees increased the chances that the heuristic algorithm would find multiple tree islands, if they existed (Swofford, 1993). (2) The tree or trees found at the end of step 1 were used independently as starting trees for searches performed with MULPARS off and Tree Bisection Reconnection (TBR); this algorithm produces a more global type of branch swapping than NNI (Swofford and Olsen, 1990). (3) The trees found at the end of step 2 were used to start a heuristic search with NNI and MULPARS on. (4) Tree swapping was performed on trees from step three using TBR with MULPARS on. The use of MULPARS option should allow the recovery of all the trees in a single island.

The results of this four-step search were compared with the results of a search performed with TBR on 100 random addition sequences, with Steepest Descent and MULPARS activated from the beginning of the search. All analyses were conducted only on the potentially informative characters by invoking accelerated transformation series (ACCTRAN). Considering the large size of the data set and the associated time constraints, analyses that differentially weight character-states (Albert, Chase, and Mishler, 1993) were not performed, and Fitch (1971), or equal-weighted, parsimony was employed.

To explore the support of the relationships specifically discussed in this paper, the number of extra steps necessary to move selected taxa outside (Vochysiaceae, Myrtaceae, Myrtaceae + Vochysiaceae, and Heteropyxidaceae + Psiloxylaceae) or inside Myrtales (*Haloragis, Haloragis* + *Myriophyllum,* Thymelaeaceae, Rhizophoraceae, and Lecythidaceae) was estimated by defining nine constraint trees and by invoking the "ENFORCE CONSTRAINT" option in PAUP (Price and Palmer, 1993; Baum, Sytsma, and Hoch, 1994). Heuristic searches with TBR, 100 random replications, and MULPARS off were conducted for each topological constraint on a MacIntosh Quadra 950. Each search required >3 d. Additionally, support for various lineages was tested by tree manipulations in MacClade ver. 3.0 (Maddison and Maddison, 1992). Because a thorough decay analysis of this large data set would be very time consuming (each branch must be tested using the same lengthy procedure outlined above to get reliable results) and because support for the specific relationships discussed in this paper was assessed by analyzing the above-mentioned constraint trees, decay values for each branch were not estimated.

Despite the limitations and criticisms of the bootstrap procedure (see Sanderson, 1989; Wendel and Albert, 1992), we proceeded to calculate bootstrap values to provide a further estimate of branch support. Due to the considerable size of the data matrix, the bootstrap analysis was performed with MULPARS off, and by using the TBR heuristic algorithm with ten random additions for each of 100 bootstrap replications. The phylogenetic signal present in the *rbc*L data set of Myrtales was estimated by analyzing the distribution of 1,000,000 random trees (RANDOM TREES option in PAUP). The skewness of this distribution (−g1) provides an estimate of the phylogenetic signal: the higher the absolute value of this statistic, the higher the amount of phylogenetic signal contained in a given data set (Hillis, 1991; Hillis and Hulsenbeck, 1992).

RESULTS

The results of the four-step phylogenetic analysis can be summarized as follows. At the end of step 1, with NNI and MULPARS off, only one tree 2,489 steps long (L = 2489) was found; this tree was recovered in replication 91, while the remaining 909 random additions did

not produce any shorter trees. TBR swapping in step 2 did not retrieve any shorter tree. The single tree of L = 2,489 was used as starting tree for NNI swapping with MULPARS activated in step 3; this search found 60 trees on the island at L = 2,489. In step 4, TBR swapping on these trees recovered 40 trees one step shorter (L = 2,488). To summarize, this search found a single island of 40 most parsimonious trees, all 2,488 steps long.

The other search for the most parsimonious trees, conducted in a single step, with MULPARS on, TBR, and 100 random additions, was stopped after 12 d, when 45 replications had been completed. This search identified two most parsimonious islands (L = 2,488) from different replications: one with 40 trees and one with 60 trees. It should be noted that this search strategy was more effective at recovering different tree islands in the *rbc*L data set for Myrtales than the four-step search described above (Olmstead et al., 1992, 1993).

The strict consensus trees for each of these two islands (not shown) exhibited identical topologies for Myrtales and their immediate sister group, but differed in the positions of a few taxa representing rosid groups more distantly related to Myrtales (see Chase et al., 1993). Because these taxa were placeholders for much larger clades, the differences between the two consensus trees most likely resulted from the phenomenon of branch attraction (Swofford and Olsen, 1990); therefore the strict consensus of all 100 trees on both islands was calculated (see Fig. 1). The following statistics describe these trees: Consistency Index (CI) = 0.278 (excluding autapomorphies), Retention Index (RI) = 0.537; Rescaled Consistency index (RC) = 0.149. The RI = 0.537 for the *rbc*L data matrix of Myrtales corroborates the finding of Maddison (1991), who reported that eight data matrices with RI < 0.67 produced two or more islands of most parsimonious trees. Trees were rooted with *Saxifraga, Hamamelis, Spinacia,* and *Rheum* designated as outgroups, which were allowed to remain paraphyletic with respect to the ingroup. Branch lengths (under ACCTRAN optimization) of one of these 100 most parsimonious Fitch trees are presented in Fig. 2. The number of potentially informative characters was 435.

Bootstrap values >50% are reported above the branches of the tree in Fig. 1. The results of the searches employing constraint trees are also included in Fig. 1 and will be presented under "Discussion". The g1 statistic of −0.306 was more negative than the critical value furnished by Hillis and Hulsenbeck (1992) for a much smaller data set (15 taxa, 500 characters), thus indicating the presence of considerable phylogenetic information in the *rbc*L data matrix of Myrtales and other rosids.

DISCUSSION

The main goals of this study were to test the monophyly of Myrtales sensu Dahlgren and Thorne (1984), to determine the relationships of some controversial families, and to identify the most likely sister group of the order.

Monophyly of Myrtales—The consensus of the 100 trees of L = 2,488, resulting from parsimony analyses of *rbc*L sequences from 80 taxa, supports the monophyly of a myrtalean clade consisting of the following families: Alzateaceae, Rhynchocalycaceae, Penaeaceae, Oliniaceae, Memecylaceae, Melastomataceae, Heteropyxidaceae, Myrtaceae, Vochysiaceae, Onagraceae, Lythraceae, Trapaceae, and Combretaceae (Fig. 1). Crypteroniaceae were not available for this study. Using constraint topologies and tree manipulation in MacClade, the monophyly of Myrtales breaks down maximally at +16 steps (see Fig. 1). The 18 steps in the lineage leading to the Myrtales (see Fig. 2), the high decay value of 16 for this branch, and a bootstrap value of 100% suggest strong support for a monophyletic order Myrtales.

The circumscription of Myrtales defined by the *rbc*L tree largely corresponds to that proposed by Dahlgren and Thorne (1984) on the basis of morphological characters, most notably the combined occurrence of bicollateral vascular bundles and vestured pits in the vessel elements. However, there is one remarkable exception to this overall pattern of agreement between the results of molecular and morphological analyses: the placement of the family Vochysiaceae. The inclusion of Vochysiaceae in Myrtales, as suggested by the *rbc*L tree, has never been proposed before. Vochysiaceae, represented in our study by *Qualea, Vochysia,* and *Erisma* (Fig. 2), constitute a subclade of the clade formed also by Myrtaceae and sister to *Heteropyxis+Psiloxylon*; 16 extra steps are required to force Vochysiaceae outside of Myrtales (Fig. 1). This value is slightly smaller than the number of extra steps necessary to exclude the following taxa from Myrtales: (1) Myrtaceae and Vochysiaceae (+17); (2) Myrtaceae only (+19); and (3) Heteropyxidaceae and Psiloxylaceae (+21). These results provide strong support for the inclusion of Vochysiaceae in Myrtales.

Traditionally, the neotropical family Vochysiaceae has been associated with Polygalales (represented in the *rbc*L tree by *Polygala*) on the basis of several morphological characters, such as the tree or shrub habit, the simple leaves with opposite or whorled arrangement, the zygomorphic flowers, the one- or three-locular ovary and the nonendospermic seeds (Heywood, 1993). However, the unique floral morphology of Vochysiaceae, with strongly asymmetric flowers, only one fertile stamen, and the number of petals often reduced to three, one, or even none (Cronquist, 1981), makes it difficult to find derived character states that this family shares with other families, and it is thus problematic to establish its relationships on morphological grounds. For example, Baillon (1878) referred to five families in four different orders as likely

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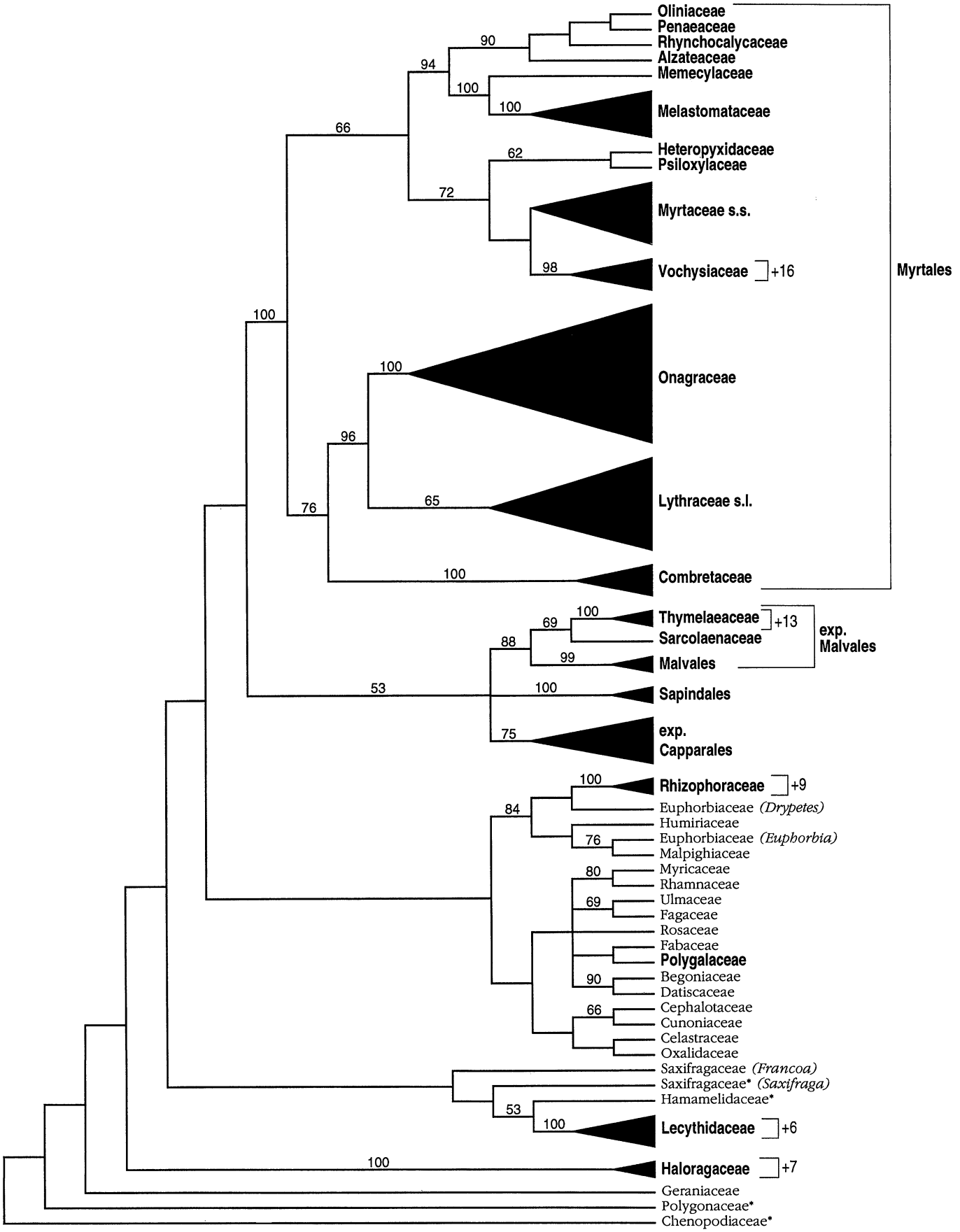
Fig. 1. Strict consensus of 100 most parsimonious trees of L = 2,488 representing two islands of trees. The numbers reported to the right of the tree indicate the number of extra steps necessary to change the placement of selected clades, either out of the Myrtales or to the base of the Myrtales. The numbers above the branches represent bootstrap values expressed as percentages of 100 bootstrap replications. Bootstrap values smaller than 50% are not reported. Outgroup taxa are marked by an asterisk. Terminal taxa are represented by families (except for Malvales, Sapindales, and expanded Capparales): see Appendix 1 and text for details on the 80 species included in the study.


Oliniaceae
Penaeaceae
Rhynchocalycaceae
Alzateaceae
Memecylaceae
Melastomataceae
Heteropyxidaceae
Psiloxylaceae
Myrtaceae s.s.
Vochysiaceae +16
Myrtales
Onagraceae
Lythraceae s.l.
Combretaceae
Thymelaeaceae +13
Sarcolaenaceae
Malvales
exp. Malvales
Sapindales
exp. Capparales
Rhizophoraceae +9
Euphorbiaceae (Drypetes)
Humiriaceae
Euphorbiaceae (Euphorbia)
Malpighiaceae
Myricaceae
Rhamnaceae
Ulmaceae
Fagaceae
Rosaceae
Fabaceae
Polygalaceae
Begoniaceae
Datiscaceae
Cephalotaceae
Cunoniaceae
Celastraceae
Oxalidaceae
Saxifragaceae (Francoa)
Saxifragaceae* (Saxifraga)
Hamamelidaceae*
Lecythidaceae +6
Haloragaceae +7
Geraniaceae
Polygonaceae*
Chenopodiaceae*
90
94
100
100
66
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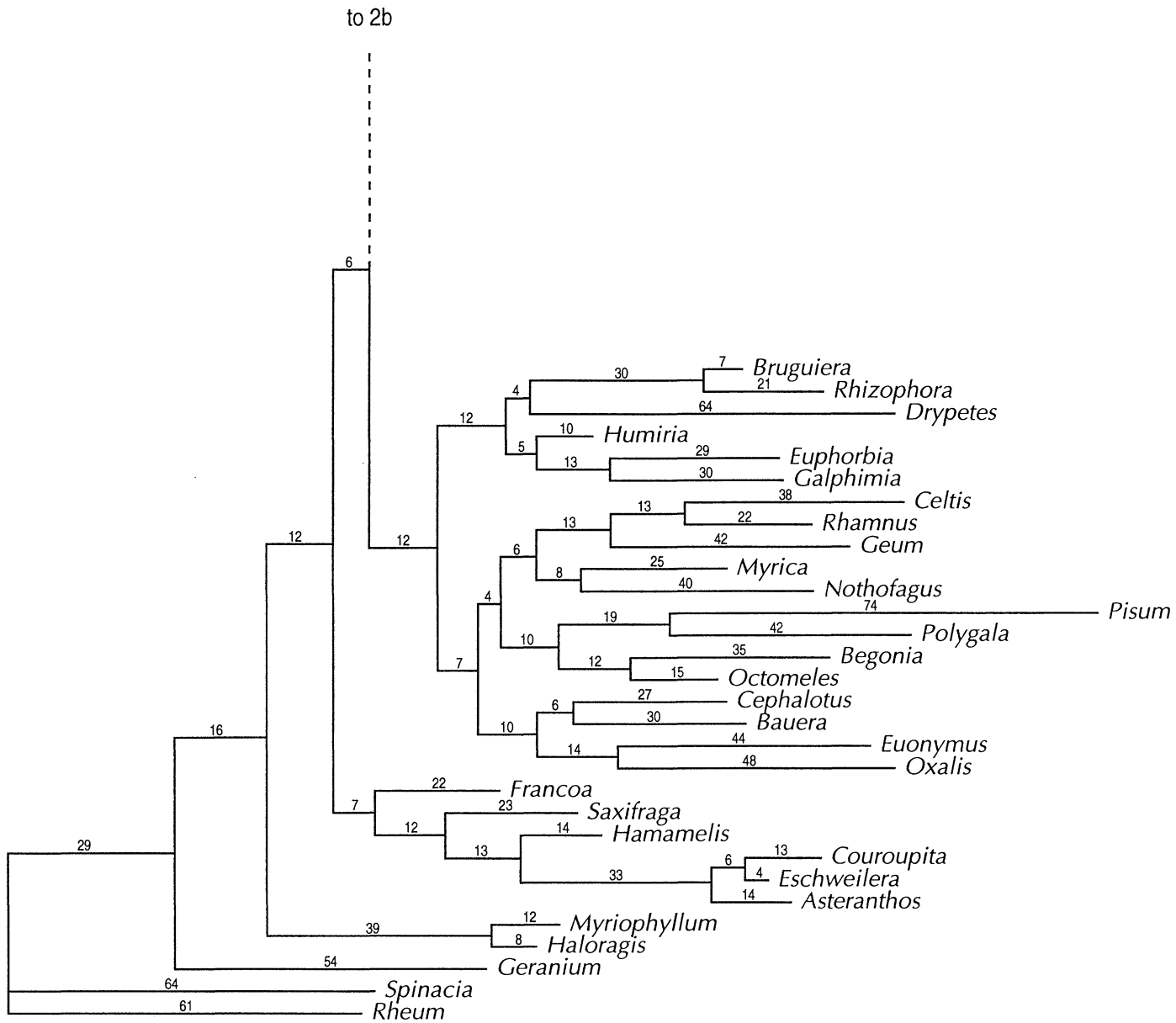

Fig. 2. Phylogram representing one of the 100 most parsimonious trees of length L = 2,488. The numbers reported above the branches indicate the branch length.

close relatives of Vochysiaceae. He reported that some authors suggested a possible affinity with Geraniaceae (Geraniales), due to similarities with the spurred flowers of *Pelargonium,* and with Dichapetalaceae (Celastrales) and Euphorbiaceae (Euphorbiales), because the irregular flowers of these two families are reminiscent of the flowers of some genera of Vochysiaceae. However, Baillon (1878) also mentioned Combretaceae and Onagraceae (Myrtales) as plausible relatives, owing to the presence of perigynous flowers in both families. In more recent times, Cronquist (1981) remarked that Vochysiaceae have not been adequately studied; for example, no detailed information is available on production of tannins in the family.

The results of molecular phylogenetic analyses presented here provide an alternative hypothesis suggesting that the relationships of Vochysiaceae should be re-examined in the context of Myrtales, rather than Polygala-

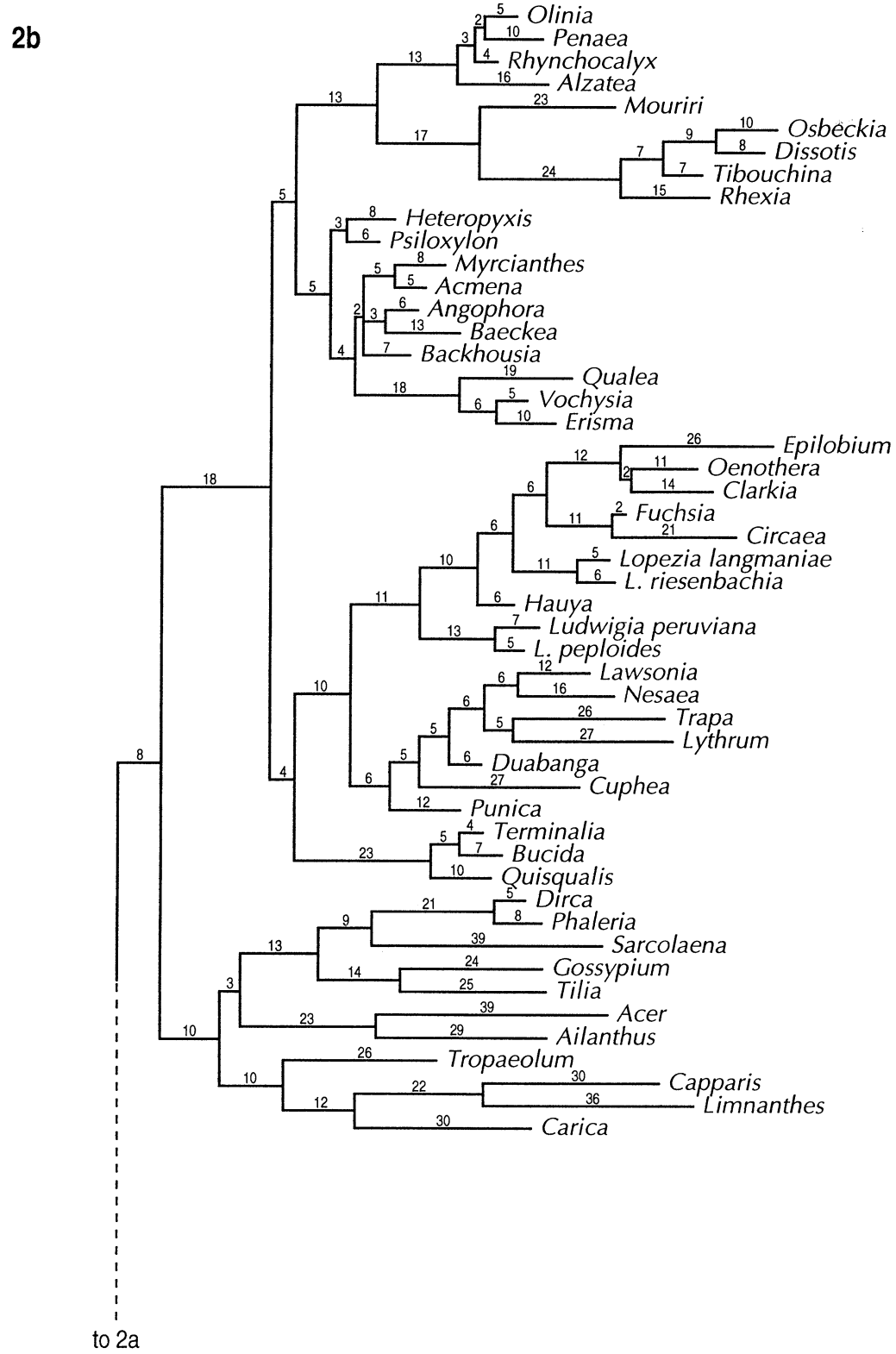

Fig. 2. Continued.

les. As noted by van Vliet and Baas (1984), Vochysiaceae is the only family traditionally included in Polygalales that is characterized by the combined occurrence of vestured pits in the secondary xylem and bicollateral vascular bundles in the primary stem, two wood anatomical traits used to define the order Myrtales. However, the highly modified floral morphology of Vochysiaceae has obscured the interpretation of relationships for this family and the phylogenetic significance of these two wood anatomical traits has been overlooked until now.

Controversial families—Parsimony analyses of DNA sequence data from *rbc*L contributed to the resolution of competing hypotheses about the relationships of the controversial families Thymelaeaceae, Lecythidaceae, Haloragaceae, and Gunneraceae (Table 1).

The cosmopolitan family Thymelaeaceae, with ≈50 genera and 500 species (Cronquist, 1981), consists mainly of shrubs and is especially well represented in Africa (Heywood, 1993). Two hypotheses of relationships have been proposed for Thymelaeaceae. One hypothesis, favored by Cronquist (1981), supported the inclusion of this family in Myrtales based on the co-occurrence of internal phloem, vestured pits, a prominent hypanthium, and elongated crystals in the wood, all considered typical myrtalean characters (van Vliet and Baas, 1984). Cladistic analyses of the first 40 amino acids from *rbc*S (Martin and Dowd, 1986) supported Cronquist's view, although it should be noted that these analyses were based on sequence data from only nine families and did not provide estimates of branch support. The other hypothesis, backed by the rest of the authors listed in Table 1, favored the exclusion of Thymelaeaceae from the order, owing to the absence of tannins, which characterize Myrtales, and the presence of the pseudomonomerous gynoecium, a condition not seen in Myrtales. Embryological and palynological evidence also argues against a close relationship of Thymelaeaceae to Myrtales. Tobe and Raven (1983) listed seven embryological characters that define Myrtales: (1) anther tapetum glandular; (2) ovule crassinucellate; (3) inner integument of the ovule with two layers (except in *Syzygium*); (4) micropyle formed by both integuments (except in *Syzygium* and *Trapa*); (5) antipodal cells ephemeral or absent; (6) nuclear-type endosperm formation; and (7) seed exalbuminous. These authors excluded from Myrtales the families that lack three or more of these characters. Thus, Tobe and Raven (1983) concluded that Thymelaeaceae should be omitted from the order because the family is characterized by ovules with an inner integument formed by three or four layers (instead of two), a micropyle formed by the inner integument alone (instead of both), persistent (instead of ephemeral) antipodal cells in the megagametophyte, and albuminous (instead of exalbuminous) seeds.

The *rbc*L tree (Fig. 1) strongly supports the exclusion of Thymelaeaceae (represented by the North American genus *Dirca* and the Indomalaysian genus *Phaleria*) from Myrtales, and instead suggests a close relationship with Malvales (included in Cronquist's Dilleniidae), represented in the *rbc*L tree by *Sarcolaena* (Sarcolaenaceae), *Gossypium* (Malvaceae), and *Tilia* (Tiliaceae); the expanded Malvales clade is supported by a bootstrap value of 88%. These results are corroborated by a detailed *rbc*L analysis of Malvales and relatives (Alverson et al., 1994). An extra 13 steps are required for Thymelaeaceae to fall in the same clade with Myrtales, suggesting that this relationship is unlikely. Analyses including a 70% completed sequence of *rbc*L from a third genus of Thymelaeaceae (the Mediterranean species *Daphne laureola*; not shown in Fig. 1) support the monophyly of the family and confirm its exclusion from Myrtales. The distinctive pollen morphology and seed wall structure (Dahlgren and Thorne, 1984) provide further evidence for the affinity of Thymelaeaceae with Malvales suggested by the *rbc*L tree.

It is relevant to mention that Gonystyloideae, considered as a primitive subfamily of Thymelaeaceae, lack bicollateral vascular bundles. According to Dahlgren and Thorne (1984) this would suggest that internal phloem was derived within the family, as a result of convergent evolution. As Thymelaeaceae are included in the sister group of Myrtales (discussed below), an alternative interpretation is that internal phloem evolved in parallel in Myrtales and in the more derived subfamilies of Thymelaeaceae. A wider sampling of the family, including representatives of Gonystyloideae, should be examined before more definitive claims on its detailed relationships are made.

The Lecythidaceae consist of ≈20 genera and 400 species distributed in tropical rain forests, especially of South America (Cronquist, 1981). Lecythidaceae have traditionally been associated with Myrtales owing to the presence of separate petals, numerous stamens, and syncarpous, inferior ovary with axile placentation (Emberger, 1960; Melchior, 1964; Soó, 1975; Takhtajan, 1980). However, several differences strongly argue against a myrtalean affinity of Lecythidaceae: a number of embryological features (summarized in Tobe and Raven, 1983); presence of alternate leaves; bitegmic tenuinucellar ovules; and absence of internal phloem and vestured pits (Cronquist, 1981; Dahlgren and Thorne, 1984; Johnson and Briggs, 1984). On these morphological grounds, Cronquist (1981) recognized a separate order Lecythidales to accomodate the family. An alternative treatment included the family in Theales on the basis of the centrifugally developing androecium and tenuinucellate ovules, in addition to the characters mentioned previously (Dahlgren, 1980; Thorne, 1981). Tannins, which characterize the Myrtales, are also present in both Lecythidaceae and Theales (Bate-Smith, 1962), and thus do not provide sufficient evidence to establish the affiliation of the family.

The placement of Lecythidaceae in the *rbc*L tree is far removed from the monophyletic myrtalean clade (Fig. 1), thus supporting the hypothesis that the family does not have a close relationship with the order. Lecythidaceae are placed at the base of the *rbc*L tree and an extra six steps are necessary to force Lecythidaceae at the base of the Myrtales clade (this seemingly low value is undoubtedly an underestimate due to the lower sampling of other rosid clades; inclusion of more rosids substantially increases the number of extra steps required to place Lecythidaceae with the Myrtales [data not shown]). Preliminary analyses suggest that Lecythidaceae are best placed at the base of Asteridae near Sapotaceae and Ebenaceae, among others (Morton, Chase, and Prance, 1995).

The relationships of the tropical family Rhizophoraceae, which includes four mangrove genera, have been at the center of much controversy. The questionable monophyly of this family, with the debatable placement of *Anisophyllea,* further complicates the issue of establishing its relationships to other families. Four of the treatments summarized in Table 1 (Emberger, 1960; Melchior, 1964; Soó, 1975; Takhtajan, 1980) retained the family in Myrtales, but the most recent taxonomic schemes (Cronquist, 1981; Dahlgren and Thorne, 1984; Johnson and Briggs, 1984) excluded it from the order. Characters suggesting the inclusion of Rhizophoraceae in Myrtales include the well-developed hypanthium in the genera with epigynous flowers, tricolporate pollen grains similar to the basic myrtalean type, and abundance of tannins (Dahlgren and Thorne, 1984). However, the lack of vestured pits and internal phloem argues against a close affinity of this family with Myrtales. The unique characteristics of Rhizophoraceae seem to isolate them from any other rosid group. Therefore, Cronquist (1981) assigned the family to its own separate order Rhizophorales. Thorne placed Rhizophoraceae in Cornales, with Haloragaceae, whereas Dahlgren argued that the lack of iridoid compounds and abundance of tannins do not support this placement (Dahlgren and Thorne, 1984). In a later revised classification of flowering plants, Thorne (1992), like Cronquist (1981), accomodated the family in the order Rhizophorales, that was included in the superorder Geranianae together with Linales, Geraniales, and Malpighiales.

The two mangrove species (*Rhizophora mangle* and *Bruguiera gymnorhiza*; see Fig. 2) sampled for this phylogenetic study are placed as sister species in the *rbc*L tree, and fall outside of Myrtales in a rosid clade that includes representatives of Euphorbiaceae, Humiriaceae, and Malpighiaceae (Fig. 1). An extra nine steps are required to force Rhizophoraceae into the Myrtales and argue against the inclusion of the family in Myrtales. However, the insufficient taxon sampling, most notably the absence of nonmangrove genera and especially *Anisophyllea,* does not allow any conclusions as to the monophyly of the family or to its detailed relationships within Rosidae.

The issue of the relationships of Haloragaceae is strictly intertwined with the placement of Gunneraceae, therefore these two families are discussed together. Contrasting hypotheses regarding the placement of Haloragaceae follow the same lines of division described for Rhizophoraceae and Lecythidaceae (see Table 1). Several vegetative and floral features suggest an affinity of Haloragaceae with Myrtales: often opposite leaves with small, vestigial stipules; four-merous diplostemonous flowers; exendospermous seeds; and similar embryological features (Orchard, 1975). However, the lack of internal phloem and vestured pits argues against the inclusion of the family in Myrtales (Dahlgren and Thorne, 1984). The spectrum of hypotheses regarding the placement of the monogeneric Gunneraceae resembles that described for Haloragaceae, except that Melchior (1964) considered Gunneraceae as a subfamily of Haloragaceae, and Takhtajan (1980) excluded Gunneraceae from Myrtales. The herbaceous habit of species in the single genus *Gunnera* strikingly differs from the mainly woody habit of Myrtales. The reduced flowers, cellular endosperm formation, seeds with abundant endosperm and a small embryo, as well as, most notably, the absence of internal phloem and vestured pits, differentiate the family from Myrtales (Dahlgren and Thorne, 1984). Thorne (1992) placed Gunneraceae in Cornales, together with Haloragaceae and other families, although the former family does not appear to have a close relationship to the latter (Dahlgren and Thorne, 1984). The most recent treatment of Takhtajan (1980) also suggested that *Gunnera* is not closely related to Haloragaceae, and thus the genus was removed from the family. Takhtajan included Gunneraceae in the order Laurales, remarking that their relationships are obscure and could possibly be placed in Saxifragales. Cronquist (1981) excluded both Haloragaceae and Gunneraceae from Myrtales, but placed them together in the separate order Haloragales.

Parsimony analyses of *rbc*L sequences shed some light on the confusing issue of the relationships of these two families (see Figs. 1, 2). *Haloragis* is sister to another genus of the family Haloragaceae (*Myriophyllum*) and the family is well removed from Myrtales. Seven extra steps are required to force *Haloragis* and *Myriophyllum* at the base of Myrtales. As discussed with the Lecythidaceae, the +7 value is an underestimate of the dissimilarity of the Haloragaceae to the Myrtales due to poor sampling (note the length of the branch leading to this family in Fig. 2). The *rbc*L trees of Chase et al. (1993) did not support the inclusion of *Gunnera* in Myrtales. The relationships of *Gunnera* in that study shifted between the heterogeneous Asterid V clade (Chase et al., 1993, fig. 12A) and an isolated position between Hamamelidae and Caryophyllidae (Chase et al., 1993, fig. 7B). In this latter case *Myriophyllum* was placed in two different rosid clades (Rosid IV in Chase et al., 1993, fig. 8A; Rosid III in Chase et al., 1993, fig. 8B), but never in the same clade with *Gunnera.* Because the placement of *Gunnera* in the *rbc*L tree of Chase et al. (1993) was far removed from Myrtales, this taxon was not included in our analysis of Myrtales, in order to reduce the effects of long branch attraction that are caused by the introduction of an isolated taxon with a high number of subsitutions along its branch (Felsenstein, 1978). However, the combined evidence from the present *rbc*L study and that of Chase et al. (1993) supports the conclusion that *Haloragis* and *Gunnera* do not belong to Myrtales, are not closely related to each other, and should not be included in the same family.

Sister group of Myrtales—The only point of agreement among different authors in regard to the relationships of Myrtales in the larger context of the dicotyledons seems to be the inclusion of the order in the subclass Rosidae (Takhtajan, 1980; Cronquist, 1981). Within this subclass, Myrtales have been associated with almost every other order, including Rosales, Cunoniales, Saxifragales, Rutales, Sapindales, Geraniales, and Theales (reviewed in Dahlgren and Thorne, 1984). The *rbc*L tree suggests that Myrtales are sister to a clade formed by an expanded Malvales (including Thymelaeaceae and Sarcolaenaceae), Sapindales (*Acer, Ailanthus*) and expanded Capparales (*Tropaeolum, Capparis, Limnanthes, Carica*; see Rodman et al., 1993). Support for this sister-group relationship is already lost in trees that are six steps lon-

ger than the most parsimonious trees and the bootstrap value is smaller than 50% (see Fig. 1). Therefore the issue of identifying the sister group of Myrtales cannot be considered definitely settled.

In summary, this study represents the first comprehensive (except for Crypteroniaceae) cladistic analysis of the circumscription of Myrtales and their relationships to other rosid families. Based on parsimony analyses of *rbc*L sequence data, the order Myrtales comprises the following families: Oliniaceae, Penaeaceae, Rhynchocalycaceae, Alzateaceae, Melastomataceae, Memecylaceae, Heteropyxidaceae, Psiloxylaceae, Myrtaceae, Vochysiaceae, Onagraceae, Lythraceae, Trapaceae, Punicaceae, and Combretaceae. However, other families that have been placed in Myrtales by different authors (Thymelaeaceae, Rhizophoraceae, Lecythidaceae, Haloragaceae, Gunneraceae), should not be included in the order.

A general conclusion emerging from these phylogenetic analyses of *rbc*L sequence data is that no single cluster of morphological or chemical characters can be used to unequivocally determine the circumscription of Myrtales. For example, the combined presence of internal phloem and vestured pits, as well as the abundance of tannins, can be considered as conditions necessary but not sufficient to determine the inclusion of a family in Myrtales as defined in the *rbc*L tree. Thus Thymelaeaceae, even though they are characterized by internal phloem and vestured pits, are excluded from the order in the *rbc*L tree. Conversely, no family lacking these features is included in the order, as demonstrated for Lecythidaceae, Rhizophoraceae, Haloragaceae, and Gunneraceae. Another major result of this *rbc*L study is the placement of Vochysiaceae in Myrtales, a conclusion never suggested before, but supported on morphological grounds by the presence of internal phloem and vestured pits in the wood of this family. Finally, this study of *rbc*L sequence data provides the first comprehensive phylogenetic framework for the reanalysis of the morphological characters that might link Myrtales to its suggested sister clade formed by an expanded Malvales, Sapindales, and an expanded Capparales.

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APPENDIX 1. The 80 species included in the study. The order of the families in this list follows their position in the tree of Fig. 1.

Species	Family	Author of sequence or literature citation	Source or voucher
Olinia cymosa Thunb.	Oliniaceae	E. Conti	N. Barker, U of Cape Town
Penaea mucronata L.	Penaeaceae	E. Conti	P. Goldblatt, MO
Rhynchocalyx lawsonioides Oliv.	Rhynchocalycaceae	E. Conti	Conti 102, WIS
Alzatea verticillata Ruiz & Pavon	Alzateaceae	E. Conti	Conti 107, WIS
Mouriri ciphocarpa Standl.	Memecylaceae	E. Conti	Nepokroeff & Hammel 724, WIS
Osbeckia stellata Wall.	Melastomataceae	E. Conti	Conti 1004, WIS
Dissotis rotundifolia (Sm.) Triana	Melastomataceae	E. Conti	Conti s.n., WIS
Tibouchina urvilleana (DC) Cogn.	Melastomataceae	E. Conti	P. Goldblatt, Golden Gate Park
Rhexia virginica L.	Melastomataceae	E. Conti	T. W. Post, Jasper Co., IN
Heteropyxis natalensis Harv.	Heteropyxidaceae	E. Conti	Conti 1002, WIS
Psiloxylon mauritianum Baill.	Psiloxylaceae	E. Conti	Conti, 101, WIS
Myrcianthes fragrans (Sw.) McVaugh	Myrtaceae	E. Conti	Conti 108, WIS
Acmena smithii (Poir.) Merrill & Perry	Myrtaceae	E. Conti	Conti 104, WIS
Angophora hispida (Sm.) Blaxell	Myrtaceae	E. Conti	Conti 109, WIS
Baeckea ramosissima A. Cunn.	Myrtaceae	E. Conti	Conti 103, WIS
Backhousia citriodora F. Muell.	Myrtaceae	E. Conti	Conti 110, WIS
Oualea sp.	Vochysiaceae	Chase et al., 1993	W. R. Anderson 13660, MICH
Vochysia hondurensis Sprague	Vochysiaceae	E. Conti	H. Iltis s.n., WIS
Erisma floribunda Rudge	Vochysiaceae	E. Conti	Mori 22847, NYBG
Epilobium angustifolium L.	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Sytsma 5012, WIS
Oenothera elata Kunth	Onagraceae	Winter and Herrmann, 1988	not available
Clarkia xantiana A. Gray	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Gottlieb 7436, DAV
Fuchsia cyrtandroides J. W. Moore	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Berry et al. 4618, MO
Circaea alpina L.	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Smith 1052, WIS
Lopezia langmaniae	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Sytsma s.n., WIS
Lopezia riesenbachia Plitmann, Raven & Breedlove	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Anderson & Anderson, 5626, MICH

APPENDIX 1. Continued.

Species	Family	Author of sequence or literature citation	Source or voucher
Hauya elegans DC	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Moreno 11352, MO
Ludwigia peruviana (L.) Hara	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Zardini & Gentry 2206, MO
Ludwigia peploides (Kunth) Raven	Onagraceae	Conti, Fischbach, and Sytsma, 1993	Sytsma 5010, WIS
Lawsonia inermis L.	Lythraceae s.s.	E. Conti/S. Graham	Phillipson 1724, MO
Nesaea aspera (Guill. & Perr.) Koehne	Lythraceae s.s.	E. Conti/S. Graham	R. B. Drummond 11446, KE
Lythrum hyssopifolia L.	Lythraceae s.s.	Conti, Fischbach, and Sytsma, 1993	Baldwin 500, DAV
Cuphea llavea Lex. in Llave & Lex.	Lythraceae s.s.	E. Conti/S. Graham	S. Graham 1024, KE
Duabanga grandiflora (Roxb. ex DC) Walp.	Lythraceae s.l. (Duabangoideae)	E. Conti/S. Graham	J. Maxwell s.n., in 1991, MO
Punica granatum L.	Lythraceae s.l. (Punicoideae)	E. Conti	Conti 1001, WIS
Trapa natans L.	Trapaceae	Conti, Fischbach, and Sytsma, 1993	unknown
Terminalia catappa L.	Combretaceae	E. Conti	Conti 1003, WIS
Bucida macrostachya Standl.	Combretaceae	E. Conti	Conti 111, WIS
Quisqualis indica L.	Combretaceae	Chase et al., 1993	W. R. Anderson s.n., MICH
Dirca palustris L.	Thymelaeaceae	E. Conti	M. Kuchenreuter s.n., WIS
Phaleria chermsideana (Bailey) C. White	Thymelaeaceae	E. Conti	Conti 106, WIS
Sarcolaena oblongifolia	Sarcolaenaceae	E. Conti	K. Sytsma s.n., WIS
Gossypium robinsonii F. Muell.	Malvaceae	Chase et al., 1993	Wendel s.n., ISC
Tilia americana L.	Tiliaceae	K. Karol	Alverson s.n., WIS
Acer saccharum L.	Aceraceae	Chase et al., 1993	M. W. Chase 106, NCU
Ailanthus altissima L.	Simaroubaceae	Chase et al., 1993	M. W. Chase 126, NCU
Tropaeolum majus L.	Tropaeolaceae	Price and Palmer, 1993	M. W. Chase 113, NCU
Capparis hastata Jacq.	Capparaceae	Rodman et al., 1993	Iltis 30315, WIS
Limnanthes douglasii R. Br	Limnanthaceae	Rodman et al., 1993	Price s.n., IND
Carica papaya L.	Caricaceae	Rodman et al., 1993	Wisconsin BG
Bruguiera gymnorhiza (L.) Savigny	Rhizophoraceae	E. Conti	Conti s.n., WIS
Rhizophora mangle L.	Rhizophoraceae	E. Conti	Conti s.n., WIS
Drypetes roxburghii (Wall.) Hurus.	Euphorbiaceae	Rodman et al., 1993	Lyon Arb., Hawaii
Humiria balsamifera Aubl.	Humiriaceae	Chase et al., 1993	W. R. Anderson 13701, MICH
Euphorbia polychroma A. Kerner	Euphorbiaceae	Chase et al., 1993	M. W. Chase 102, NCU
Galphimia gracilis Bartl.	Malpighiaceae	Chase et al., 1993	Adelson s.n. MICH
Myrica cerifera L.	Myricaceae	Chase et al., 1993	Qiu 91036, NCU
Rhamnus cathartica L.	Rhamnaceae	Chase et al., 1993	M. W. Chase 100, NCU
Celtis yunnanensis C. K. Schneid.	Ulmaceae	Chase et al., 1993	Qui P90002, NCU
Nothofagus balansae (Baill.) Steenis	Fagaceae	Chase et al., 1993	J. Read s.n., Monash U., Victoria
Geum chiloense Balb. ex Ser.	Rosaceae	Chase et al., 1993	Morgan 2137, WS
Pisum sativum L.	Fabaceae	Zurawski, Whitfield, and Bottomley, 1986	unknown
Polygala cruciata L.	Polygalaceae	Chase et al., 1993	M. W. Chase 155, NCU
Begonia metallica × *sanguinea*	Begoniaceae	Chase et al., 1993	M. W. Chase 225, NCU
Octomeles sumatrana Miq.	Datiscaceae	Chase et al., 1993	Philbrick 2273, RSA
Cephalotus folicularis Labill.	Cephalotaceae	Chase et al., 1993	M. W. Chase 147, NCU
Bauera rubioides Andrews	Cunoniaceae	Morgan and Soltis, 1993	Strybing Arb. 820237
Euonymus alatus (Thunb.) Siebold	Celastraceae	Chase et al., 1993	M. W. Chase 137, NCU
Oxalis dillenii Jacq.	Oxalidaceae	Chase et al., 1993	Price s.n., IND
Francoa sonchifolia Cav.	Saxifragaceae	Soltis et al., 1990	Soltis and Soltis 2479, WS
Saxifraga integrifolia Hook.	Saxifragaceae	Morgan and Soltis, 1993	Soltis and Soltis 2253, WS
Hamamelis mollis Oliv.	Hamamelidaceae	Chase et al., 1993	Qiu 91035, NCU
Couroupita guianensis	Lecythidaceae	Karol/Alverson	Alverson s.n., WIS
Eschweilera odorata	Lecythidaceae	Karol/Alverson	Alverson s.n., WIS
Asteranthos sp.	Lecythidaceae	Karol/Alverson	Alverson s.n., WIS
Myriophyllum exalbescens Fernald	Haloragaceae	Morgan and Soltis, 1993	Broch. 30 Aug 1991, WS
Haloragis serra Brongn.	Haloragaceae	E. Conti	Conti 105, WIS
Geranium cinereum Cav.	Geraniaceae	Price and Palmer, 1993	Price s.n., IND
Rheum × *cultorum*	Polygonaceae	Giannasi et al., 1992	unknown
Spinacia oleracea L.	Chenopodiaceae	Zurawski et al., 1981	unknown

APPENDIX 2. GenBank accession numbers for the *rbc*L sequences generated specifically for this study.

Acmena smithii (Poir.) Merrill & Perry: U26315; *Alzatea verticillata* Ruiz & Pavon: U26316; *Angophora hispida* (Sm.) Blaxell: U26317; *Backhousia citriodora* F. Muell.: U26318; *Baeckea ramosissima* A. Cunn.: U26319; *Brugueiera gymnorhiza* (L.) Savigny: U26320; *Bucida macrostachya* Standl.: U26321; *Dirca palustris* L.: U26322; *Dissotis rotundifolia* (Sm.) Triana: U26323; *Erisma floribunda* Rudge: U26324; *Haloragis serra* Brongn.: U26325; *Heteropyxis natalensis* Harv.: U26326; *Mouriri ciphocarpa* Standl.: U26327; *Myrcianthes fragrans* (Sw.) McVaugh: U26328; *Olinia cymosa* Thunb.: U26329; *Osbeckia stellata* Wall.: U26330; *Penaea mucronata* L.: U26331; *Phaleria chermsideana* (Bailey) C. White: U26332; *Psiloxylon mauritianum* Baill.: U26333; *Rhexia virginica* L.: U26334; *Rhizophora mangle* L.: U26335; *Rhynchocalyx lawsonioides* Oliv.: U26336; *Sarcolaena oblongifolia*: U26337; *Terminalia catappa* L.: U26338; *Tibouchina urvilleana* (DC) Cogn.: U26339; *Vochysia hondurensis* Sprague: U26340.