

PHYLOGENETIC RELATIONSHIPS IN THE ORDER ERICALES S.L.: ANALYSES OF MOLECULAR DATA FROM FIVE GENES FROM THE PLASTID AND MITOCHONDRIAL GENOMES¹

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Phylogenetic interrelationships in the enlarged order Ericales were investigated by jackknife analysis of a combination of DNA sequences from the plastid genes *rbcL*, *ndhF*, *atpB*, and the mitochondrial genes *atp1* and *matR*. Several well-supported groups were identified, but neither a combination of all gene sequences nor any one alone fully resolved the relationships between all major clades in Ericales. All investigated families except Theaceae were found to be monophyletic. Four families, Marcgraviaceae, Balsaminaceae, Pellicieraceae, and Tetrameristaceae form a monophyletic group that is the sister of the remaining families. On the next higher level, Fouquieriaceae and Polemoniaceae form a clade that is sister to the majority of families that form a group with eight supported clades between which the interrelationships are unresolved: Theaceae-Ternstroemiaceae with *Ficalhoa*, *Sladenia*, and *Pentaphylacaceae*; Theaceae-Theoideae; Ebenaceae and Lissocarpaceae; Symplocaceae; Maesaceae, Theophrastaceae, Primulaceae, and Myrsinaceae; Styrracaceae and Diapensiaceae; Lecythidaceae and Sapotaceae; Actinidiaceae, Roridulaceae, Sarraceniaceae, Clethraceae, Cyrillaceae, and Ericaceae.

Key words: *atpB*; *atp1*; cladistics; DNA; Ericales; jackknife; *matR*; *ndhF*; phylogeny; *rbcL*.

Understanding of phylogenetic relationships among angiosperms has greatly increased in the last few years, particularly as a result of analyses of molecular data accumulated from multiple genes. Among new discoveries is strong support for a large monophyletic group formed by the traditional sympetalous families of Asteridae, plus some from the Rosidae and Dilleniidae. The enlarged circumscription of the Asteridae includes three major clades, the euasterid clade, the cornealean clade, and the ericalean clade, each with a partly new circumscription (APG, 1998). In spite of a robust support for each of these three major clades, their interrelationships and the relationships between families within the ericalean clade are still unresolved.

The ericalean clade was referred to as order Ericales in the classification of the Angiosperm Phylogeny Group (APG, 1998), and in the following we will use their circumscription unless otherwise stated. Ericales comprise about 25 families, most of which have been placed in Dilleniidae, a few in Rosidae (Balsaminaceae, Roridulaceae), and one in Asteridae sensu stricto (s.s.) (Polemoniaceae) (cf. Cronquist, 1981; Thorne, 1983; Dahlgren, 1989; Takhtajan, 1997). Three families previously associated with families of the Ericales were left unclassified by APG (1998) because no molecular information

was available for them at the time, viz. Lissocarpaceae, Pentaphylacaceae, and Sladeniaceae. The circumscription of Ericales has been widened to include a number of families not traditionally considered close relatives of the Ericaceae or the Ericales sensu Cronquist (1981) or Takhtajan (1997), but corresponds well with the major part of superorder Theiflorae of Thorne (1968). The new circumscription of the Ericales includes a number of morphologically derived groups, but as a broad and nonexclusive generalization one could say that Ericales include families of sympetalous angiosperms with polystemonous or diplostemonous flowers and unitegmic, tenuinucellate ovules, or with haplostemonous flowers and bitegmic, tenuinucellate ovules. This means that the Ericales do not have the combination of haplostemonous flowers and unitegmic tenuinucellate ovules typical of the euasterid clade.

There are no obvious synapomorphies diagnosing the Ericales as a monophyletic group. In embryological characters they differ among themselves in the number of integuments, type of endosperm formation, presence of endosperm haustoria, presence of integumentary tapetum, etc. The only ubiquitous embryological character state is the presence of tenuinucellate ovules (e.g., Johri, Ambegaokar, and Srivastava, 1992), possibly with the exception of *Pentaphylax* of the theoid family Pentaphylacaceae. The presence of tenuinucellate ovules is a character state prevailing in Asteridae as a whole, and the combination of bitegmic-tenuinucellate ovules is found also outside the Ericales and is therefore not diagnostic of Ericales in particular. The distribution of wood anatomical features also gives the impression that Ericales are heterogeneous. However, there are some patterns of variation, e.g., most families have scalariform vessel perforations and exclusively simple perforations are being restricted to a few families (Baas, Wheeler, and Chase, 2000).

There is strong support for the monophyly of the Ericales in molecular data (Källersjö et al., 1998; Soltis et al., 2000),

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TABLE 1. Primers used for polymerase chain reaction and for sequencing the mitochondrial genes *atp1* and *matR*. Primers used for the plastid genes *atpB*, *rbcL*, and *ndhF* are those listed by Källersjö, Bergqvist, and Anderberg (2000).

| | |
|-----------------------------------|-----------------------------------|
| <i>atp1</i> ^a | |
| 1. TATGAGATCGGTCGAGTGGTCTCAGTTG | <i>atp1</i> 5'F = pos 1–28 |
| 2. ATTTCTTTTCAATTGGAAGTGGTG | <i>atp1</i> 3'R = pos 1253–1229 |
| 3. GAATATTCATTTCTTGTAGCAGCCACCGC | <i>atp1</i> 607F = pos 607–635 |
| 4. ATCATCATAGATTATTAATGCGTGCATTCC | <i>atp1</i> 738R = pos 709–738 |
| <i>matR</i> ^b | |
| 5. CGCGGCACCTGTAGTAGGACAGAGGA | <i>matR</i> 3'R = pos 1671–1696 |
| 6. GTTTTCACACCATCGACCGACATCG | <i>matR</i> 5'F = pos 134–158 |
| 7. CCGACATCGACTCATCCAATCTTTAAGG | <i>matR</i> 178F = pos 150–178 |
| 8. TCCTTTGCGCCGCTTCTCATAGAAG | <i>matR</i> 670F = pos 644–670 |
| 9. AGTATCGACCTCCCGCCAGTCTATCAGC | <i>matR</i> 800R = pos 771–800 |
| 10. AAGCTCTACGGCACCCACGATTTCC | <i>matR</i> 927R = pos 902–927 |
| 11. GACAGAGGACTTATCGCGATGCCCC | <i>matR</i> 1679R = pos 1655–1679 |

^a Position numbers refer to *Buxus sempervirens* AF197636. Primers 1 and 2 amplify a region of about 1250 bases.

^b Position numbers refer to *Buxus sempervirens* AF197786. Primers 5 and 6 amplify a region of about 1560 bases.

but as in many other groups of angiosperms the basal relationships in the Ericales are poorly supported. This has been pointed out by several authors who have presented tentative relationships between families in the Ericales (e.g., Bayer, Hufford, and Soltis, 1996; Morton et al., 1996, 1997; Soltis et al., 1997; Nandi, Chase, and Endress, 1998). To improve the picture on interfamilial relationships in the Ericales we have increased the number of taxa and analyzed DNA sequences from three genes of the plastid genome (*atpB*, *ndhF*, *rbcL*) in combination with sequences from two genes of the mitochondrial genome (*atp1*, *matR*). Following Eernisse and Kluge (1993), we consider the best hypothesis to be the one based on as much data as possible and on a combination of genes from different genomes and that a combined data set gives a more robust result than analyses of each genome separately.

MATERIALS AND METHODS

Taxa—For our analyses, we have used taxa representing all the families recognized in the order by APG (1998), and in most cases several representatives of each. In addition, we investigated the three unplaced (APG, 1998) families Sladeniaceae, Pentaphragmaceae, and Lissocarpaceae prior to our analyses of Ericales, by including their *rbcL* sequences in the large Angiosperm data set analyzed by Källersjö et al. (1998), where Ericales were found to be monophyletic. All three have been associated with families now included in Ericales (Theaceae, Ebenaceae) and therefore it was relevant to find out if they belonged in Ericales or not. A number of other taxa that also have been placed near Theaceae have already been shown to belong elsewhere. The genus *Asteropeia* Thouars from Madagascar, the only genus of Asteropeiaceae but often included in Theaceae (Melchior, 1925; Baretta-Kuipers, 1976; Cronquist, 1981), belongs to the Caryophyllales (Swensen and Chase, 1995; Morton et al., 1996). Furthermore, Caryocaraceae, Oncothecaceae, and Stachyuraceae, which were all included in the Theales by Takhtajan (1997), belong in the rosoid clade (Caryocaraceae, Stachyuraceae) or in Garryales of the eusterid clade (APG, 1998).

In our study, the sample of taxa is somewhat different in the five individual data sets because it was not always possible to obtain polymerase chain reaction (PCR) products for all genes. In a few cases sequences have been assembled from two species of the same genus. The combined five-gene study included sequences from 57 taxa.

Molecular methods—DNA was extracted from leaves taken from herbarium specimens, material dried in silica gel, or from living plants. Leaves were ground in liquid nitrogen with mortar and pestle and subsequently treated with the DNEasy plant DNA extraction kit from Qiagen (Qiagen, Valencia, California, USA), following the manufacturer's protocol. The PCR was performed with 10 μmol/L primers in 25-μL reactions using "Ready-to-go" PCR

beads from Pharmacia Biotech (Amersham Pharmacia Biotech, Uppsala, Sweden) following the manufacturer's standard protocol and suggested thermal cycling profile, generally 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 50°–60°C for 30 sec, 72°C for 2 min, and finally 72°C for 8 min. For sequencing reactions the "Big Dye Terminator Sequencing" kit (Applied Biosystems, Warrington, Cheshire, UK) was used, and fragments were separated on an ABI377 from Applied Biosystems. Primers used for PCR and for sequencing *atpB*, *ndhF*, and *rbcL* were those used by Källersjö, Bergqvist, and Anderberg (2000); primers used for *atp1* and *matR* are listed in Table 1. Sequences were assembled and carefully checked with the Staden software (Staden, Beal, and Bonfield, 1998) and aligned manually with the AssemblyLign software (Oxford Molecular Group, Campbell, California, USA). The 202 new sequences have been submitted to GenBank (accession numbers AF419239–AF419243 and AF420915–AF421111). All voucher information for material used in the present study is presented on the Botanical Society of America webpage (<http://ajbsupp.botany.org/v89/Anderberg.doc>).

Phylogenetic analyses—All data sets, combined and each individually, were analyzed with parsimony jackknifing (Farris et al., 1996) using the computer software "Xac" (Farris, 1997; Källersjö et al., 1998) with the following settings: 1000 replications, each with branch swapping and ten random addition sequences. For all analyses, *Cornus* L. was used as the outgroup (Farris, 1972), because it belongs to one of the three major clades of the Asteridae that is one of the potential sister groups of Ericales. Gaps were found in the *atp1*, *matR*, and *ndhF* sequences and treated as missing information in the analyses. Analyses were performed with all codon positions included as this has been demonstrated to give the best resolution and a higher number of supported groups than analyses of first and second codon positions only or of transversions only (Källersjö, Bergqvist, and Anderberg, 2000). When it was not possible to obtain PCR products, e.g., from *ndhF* in genera of Sarracenaceae, the data was coded as missing in the matrix.

A parsimony analysis of the five-gene data set was also performed using NONA version 1.6 (Goloboff, 1993). Searches for most-parsimonious trees were performed with 50 random additions and branch swapping on all trees (mult*50).

RESULTS

Combined five-gene data set—This data set included 57 taxa and 7682 characters, of which 1735 were informative. There is jackknife support for a number of clades, but not all relationships between families are resolved (Fig. 1). One small clade (100% jackknife support), here called clade I, with Balsaminaceae, Marcgraviaceae, Tetrameristaceae, and Pellicieraceae, is sister to a large group (89%) containing all other families (clades II–X). On the next higher level, Fouquieriaceae and Polemoniaceae (clade II) is sister to the rest, which

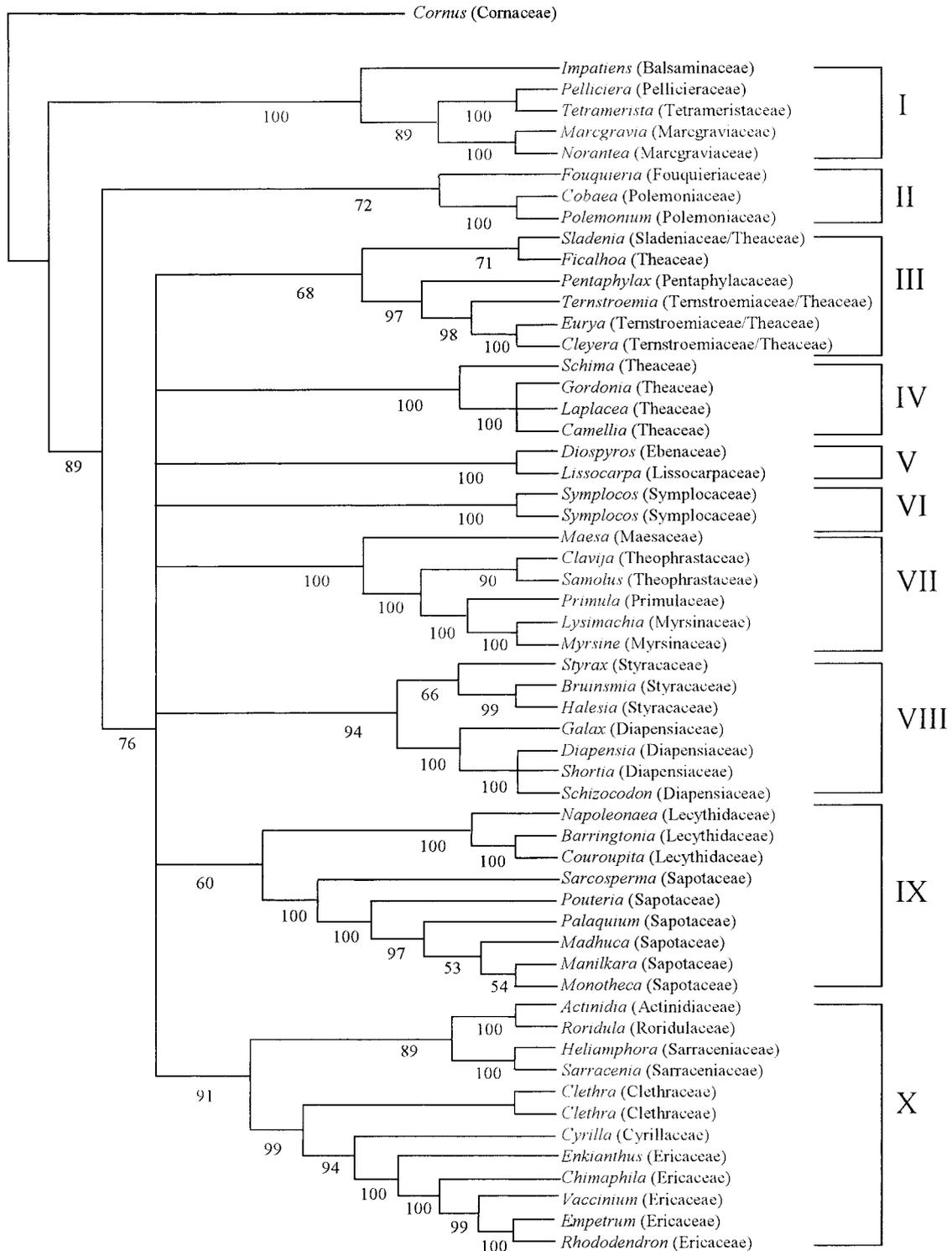


Fig. 1. Parsimony jackknife tree based on analysis of a combination of sequences from the five genes *atpB*, *ndhF*, *rbcl*, *atp1*, and *matR*. Jackknife support values are given for each node. The strict consensus tree from the parsimony analysis using NONA has exactly the same topology (28 trees, 7887 steps, consistency index = 0.43, retention index = 0.53). Roman numerals correspond to numbering of clades in the text and in Figs. 2 and 3.

is a monophyletic group (76%) comprising clades III–X. Clade I comprises Balsaminaceae, Marcgraviaceae, Tetrameristaceae, and Pellicieraceae with 100% jackknife support. Balsaminaceae are sister to a group (89%) in which Marcgraviaceae are sister to Tetrameristaceae and Pellicieraceae (100%). This

clade is the sister of all the remaining families. Clade II, a small clade of Polemoniaceae and Fouquieriaceae (72%), constitutes the sister group of the rest of the Ericales, except clade I. The members of clade III, Theaceae-Ternstroemiaceae (Ternstroemiaceae) including the theoid genera *Ficalhoa* and

Sladenia and *Pentaphylax* of Pentaphylacaceae, have 68% support. In one subclade *Ficalhoa* is sister to *Sladenia* (71%), and in the other subclade (97%) *Pentaphylax* is sister to *Ternstroemia* (98%) followed by *Cleyera* and *Eurya* (100%). In clade IV, Theaceae-Theoideae (Theaceae s.s.) have 100% support, with *Schima* as sister to a trichotomy formed by *Gordonia*, *Laplacea*, and *Camellia* (100%). In clade V, Ebenaceae and Lissocarpaceae form a clade with 100% support. Clade VI is composed of Symplocaceae with two species of *Symplocos* (100%). Clade VII is the primuloid clade with Maesaceae, Theophrastaceae, Primulaceae, and Myrsinaceae (100%). Maesaceae is sister to a clade (100%) with Theophrastaceae and *Samolus* (90%) as sister to Primulaceae and Myrsinaceae (100%). In clade VIII, Styracaceae and Diapensiaceae are sister groups (94%). Styracaceae has 66% jackknife support, with *Styrax* as sister to *Bruinsmia* and *Halesia* (99%). Diapensiaceae has 100% jackknife support with *Galax* as sister to a trichotomy with *Diapensia*, *Shortia*, and *Schizocodon* (100%). Lecythidaceae and Sapotaceae form clade IX with 60% support. In Lecythidaceae (100%), *Napoleonaea* is sister to *Barringtonia* and *Couroupita* (100%). In Sapotaceae (100%), *Sarcosperma* is sister to the remaining genera (100%), followed by *Pouteria* as sister to the rest (97%), and then *Palaquium* as sister (53%) to *Madhuca*, *Monothea* and *Manilkara*. Clade X is the ericoid clade with Ericaceae, Cyrillaceae, Clethraceae, Actinidiaceae, Sarraceniaceae, and Roridulaceae (91%) and has two groups. One group (89%) has Sarraceniaceae (100%) as sister to a group with Actinidiaceae and Roridulaceae (100%). The second group (99%) has Clethraceae as sister to Ericaceae and Cyrillaceae (94%), with Cyrillaceae as sister to Ericaceae (100%), with *Enkianthus* as sister to the rest of Ericaceae (100%), with *Chimaphila* as sister to the rest (99%) on the next higher node, and *Vaccinium* as sister to *Empetrum* and *Rhododendron* (100%).

The tree search in NONA resulted in 28 most-parsimonious trees, 7887 steps long, with a consistency index of 0.43 and a retention index of 0.53. The strict consensus tree (not shown) recognizes the same groups as the parsimony jackknife analysis and has exactly the same topology as the jackknife tree in Fig. 1.

Plastid genome, *rbcL*, *ndhF*, and *atpB*—The combined data set with the three plastid genes included 57 taxa and 4921 characters, of which 1329 were informative (Fig. 2). Jackknife support is found for 11 clades, which in the following are numbered in accordance with the clades identified in the five-gene tree (Fig. 1), and also in cases where the corresponding clade is not supported by the plastid data. As in the five-gene tree, there is a basal split between the small clade I, formed by the four families Balsaminaceae, Marcgraviaceae, Pellicieraceae, and Tetrameristaceae, and all other families of Ericales (clades II–X). Clade I (100%) has a trichotomy with *Impatiens*, with *Marcgravia* as sister to *Norantea* (100%), and *Tetramerista* as sister to *Pelliciera* (100%). The large clade (69%) including all other families is composed of nine supported clades (clades II–X). Clade II from the five-gene analysis is not supported by the plastid data only, because *Fouquieria* does not group with Polemoniaceae. Polemoniaceae are monophyletic with *Cobaea* as sister to *Polemonium* (100%), but the position of the genus *Fouquieria* is unresolved in relation to clades III–X. Theaceae-Ternstroemiaceae (Ternstroemiaceae) plus Pentaphylacaceae comprise clade III (51%), which has *Ficalhoa* as sister to *Sladenia* in one group

(56%) and a second group (96%) in which *Pentaphylax* is sister to *Ternstroemia*, *Cleyera*, and *Eurya* (97%). Theaceae-Theoideae (Theaceae s.s.) is clade IV (100%) in which *Schima* is sister to *Gordonia*, *Laplacea*, and *Camellia* (100%). In clade V, Ebenaceae are sister to Lissocarpaceae (100%). The Symplocaceae with two species of *Symplocos* group together in Clade VI (100%). Clade VII is formed by the primuloid families (100%), in which *Maesa* is sister to the rest (100%). On the next higher node, *Clavija* and *Samolus* form one clade with 88% support, and in another clade (100%) *Primula* is sister to *Lysimachia* and *Myrsine* (100%). In clade VIII (65%), Styracaceae is sister to Diapensiaceae. Styracaceae are monophyletic (65%) with *Styrax* as sister to *Bruinsmia* and *Halesia* (98%), and the support for Diapensiaceae is 100%, with *Galax* as sister to *Diapensia*, *Shortia*, and *Schizocodon* (100%). Clade IX (52%) is formed by genera from Sapotaceae (100%) as sister to Lecythidaceae (100%). In the former, *Sarcosperma* is sister to the rest of Sapotaceae (100%), followed by *Pouteria* as sister to *Palaquium*, *Madhuca*, *Manilkara*, *Monothea* (98%). In the latter, *Napoleonaea* is sister to *Barringtonia* and *Couroupita* (100%). Ericaceae, Cyrillaceae, Clethraceae, Actinidiaceae, Sarraceniaceae, and Roridulaceae form clade X with 78% jackknife support. It has two subclades: one clade (73%) is formed by Sarraceniaceae (100%) as sister to a group with Actinidiaceae and Roridulaceae (78%) and the other subclade (90%) has Clethraceae as sister to Cyrillaceae and Ericaceae (94%). Within Ericaceae (100%), *Enkianthus* is sister to the rest (100%), with *Chimaphila* as sister to the remaining genera at the next higher node (99%), and *Vaccinium* is sister to *Empetrum* and *Rhododendron* (100%).

Mitochondrial genome, *atp1* and *matR*—The combined data set with the two genes from the mitochondrial genome included 55 taxa and 2761 characters of which 403 were informative. The resulting tree (Fig. 3) is more unresolved than that from either the five-gene analysis (Fig. 1) or from the plastid gene analysis (Fig. 2). The mitochondrial genes support ten clades and leave a number of genera in a basal polychotomy. For comparison, clades are numbered in accordance with the clades of the five-gene tree in Fig. 1, although some are not supported by the mitochondrial data alone. Clade I (100%) has *Pelliciera* as sister to *Tetramerista* (100%) and *Marcgravia* as sister (85%) to *Norantea* and *Impatiens* (65%). There is no support for a position of clade I as sister to all other families. In clade II, *Cobaea* is sister to *Polemonium* (100%), but the Polemoniaceae do not group with *Fouquieria*, which has an unresolved position in relation to the ten clades. Clade III, the Theaceae-Ternstroemiaceae clade from the five-gene tree, is not supported by the mitochondrial data. All taxa belonging to clade III in the five-gene tree have unresolved positions in the basal polychotomy. Clade IV, the Theaceae-Theoideae clade (66%), has *Schima* as sister to *Gordonia* and *Camellia* (85%); *Laplacea* is missing. Clade V, the Ebenaceae-Lissocarpaceae clade from the five-gene tree, is not supported by the mitochondrial data. The taxa all have unresolved positions in the basal polychotomy. Clade VI, the Symplocaceae clade from the five-gene tree, is not supported by the mitochondrial data. The taxa all have unresolved positions in the basal polychotomy. Clade VII, the primuloid clade, has 100% support and has *Maesa* as sister to a group (98%) in which *Clavija*, *Samolus*, and a group (95%) with *Primula* as sister to *Lysimachia* and *Myrsine* (100%) form a trichotomy. Clade VIII, the clade with Styracaceae and Diapensiaceae, has 63%

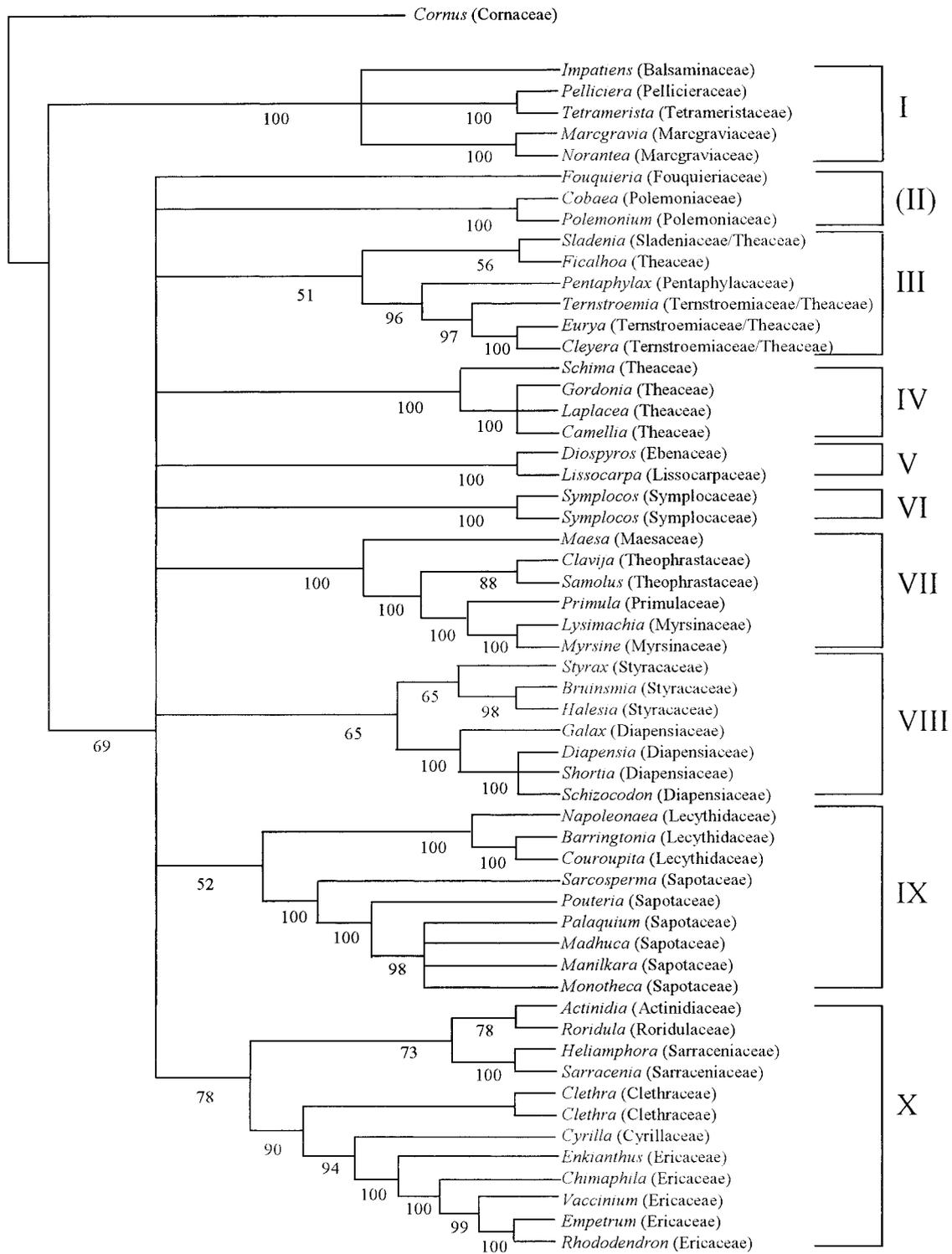


Fig. 2. Parsimony jackknife tree based on analysis of a combination of the plastid genes *atpB*, *ndhF*, and *rbcl*. Jackknife support values are given for each node. Roman numerals correspond to numbering of clades in the text and in Figs. 1 and 3. Roman numerals in parentheses indicate a clade from the analysis of five genes (Fig. 1) that is not supported by the plastid data only.

support, including a trichotomy with *Styrax*, a group with *Bruinsmia* as sister to *Halesia* (83%), and a group (100%) with *Galax* as sister to *Diapensia*, *Shortia* and *Schizocodon* (100%). Clade IX from the five-gene tree, with Lecythidaceae and Sapotaceae, is not supported by the mitochondrial data. The two

genera of Lecythidaceae as well as the genus *Sarcosperma* of Sapotaceae have unresolved positions in the basal polychotomy. The remaining Sapotaceae form a tetratomy (99%) with *Madhuca*, *Manilkara*, *Monotheca*, and a group with *Pouteria* as sister to *Palaquium* (60%). Clade X, the ericoid clade from

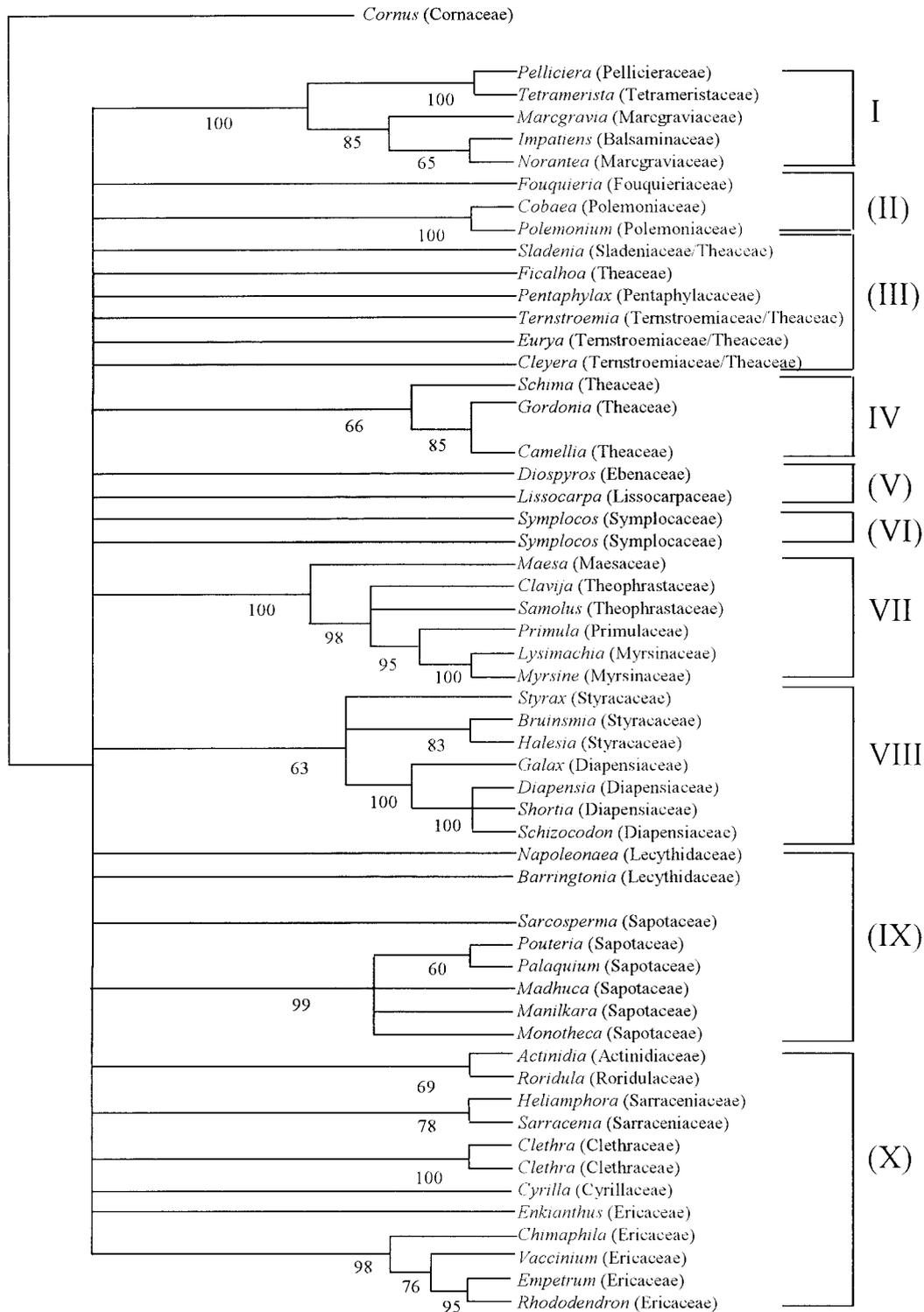


Fig. 3. Parsimony jackknife tree based on analysis of a combination of the mitochondrial genes *atp1* and *matR*. Jackknife support values are given for each node. Roman numerals correspond to numbering of clades in the text and in Figs. 1 and 2. Two genera are missing from the mitochondrial data set as compared to Figs. 1 and 2, viz. *Laplacea* (Theaceae) and *Couroupita* (Lecythidaceae); and this is indicated by blank spaces. Roman numerals in parentheses indicate a clade from the analysis of five genes (Fig. 1) that is not supported by the mitochondrial data only.

the five-gene tree, is not supported by the mitochondrial data. The two species of *Clethra* (100%), *Heliamphora* and *Sarracenia* (78%), and *Actinidia-Roridula* (69%) form three separate clades that are part of the basal polychotomy. Within the Ericaceae clade (98%), *Chimaphila* is sister to a group (76%) with *Vaccinium* as sister to *Empetrum* and *Rhododendron* (95%). The positions of *Cyrilla* of Cyrillaceae and *Enkianthus* of Ericaceae are also unresolved, being part of the basal polychotomy.

DISCUSSION

The results of the analyses give support for recognition of a number of clades in the Ericales. Each of the separate data sets gives a less resolved tree than that obtained from the analysis of all genes in combination, and the discussion will focus on the results obtained from the five-gene tree, which we consider the best estimate of the phylogeny. We will comment on the results of the other analyses only in cases where they provide additional information. The clades are numbered in accordance with the clades of the five-gene tree (Fig. 1).

Clade I: Marcgraviaceae, Balsaminaceae, Tetrameristaceae, and Pellicieraceae—The clade with these four families is the sister group of all other Ericales. In the combined analysis of all five genes, Balsaminaceae are sister to the three other families and Marcgraviaceae are sister to Tetrameristaceae and Pellicieraceae. The combined three genes from the plastid genome also support the monophyly of the group with Balsaminaceae, Marcgraviaceae, Tetrameristaceae, and Pellicieraceae and also its position as sister group to the other families. The data set with the two mitochondrial genes supports the monophyly of the clade, but only as part of a large polychotomy with unresolved position in relation to other families. Although *Impatiens* is sister to *Norantea* in the mitochondrial analysis, implying that Marcgraviaceae is paraphyletic, this is in conflict with morphology as well as molecular data from the plastid. Taxa of the four families all have raphides in the parenchymatic tissue, a rare feature in the Ericales and occurring elsewhere only in the Actinidiaceae. Both *Pelliciera* and *Tetramerista* have previously been included in Marcgraviaceae (Hallier, 1923) or more often in Theaceae (Melchior, 1925), from which they differ by having simple vessel perforations, multiple pores, and raphide-bearing ray cells (Barretta-Kuijpers, 1976). A morphological synapomorphy of the two families Tetrameristaceae and Pellicieraceae is the presence of glandular pits in the central part of the petals, a character that is not found in any other families. Both the Balsaminaceae and the Marcgraviaceae have micropylar endosperm haustoria, and as the embryology of Tetrameristaceae and Pellicieraceae is insufficiently investigated, it is possible that this could be another synapomorphy for this clade. Bitegmic ovules with an integumentary tapetum and cellular endosperm formation may be symplesiomorphies in this clade, as the opposite condition seems to be diagnostic of several other clades. *Pelliciera* (Pellicieraceae) has stamens forming a central column, and a staminal column with more or less connate filaments is characteristic of *Pentamerista* (Tetrameristaceae) and Balsaminaceae.

Clade II: Polemoniaceae and Fouquieriaceae—The five-gene analysis places these two families as sister to all Ericalean families except those of clade I, discussed in the previous paragraph. The support for Polemoniaceae-Fouquieriaceae is

72%, and the support for the large group with the remaining families is 76%. The Fouquieriaceae-Polemoniaceae clade is supported only in the five-gene tree, but not by the combined plastid genes alone, nor by the combined mitochondrial genes alone. The relatively low level of support for this clade in the molecular data is also reflected by the considerable difference in morphology between the two families. They have generally been placed in different groups of flowering plants, but Nash (1903) and others (e.g., Henrickson, 1967; Thorne, 1968; Downie and Palmer, 1992; Olmstead et al., 1992) have suggested a close relationship between them, something that our results support. More often their kinship has been rejected due to the numerous morphological and embryological differences, and the Fouquieriaceae have been notoriously difficult to place. Compared to the previous clade (clade I), unitegmic ovules and nuclear endosperm formation seem to have evolved in the Polemoniaceae, whereas the bitegmic condition of Fouquieriaceae, one of the major arguments against a relationship between them, could be a plesiomorphy.

Clades III and IV: Ternstroemiaceae and Theaceae—In our analyses, the Theaceae s.s., hereafter called Theaceae, and the Theaceae-Ternstroemiaceae, hereafter called Ternstroemiaceae, do not form a monophyletic group. There are no morphological synapomorphies for the Theaceae and Ternstroemiaceae and the delimitation of the Theaceae sensu lato (s.l.) has always been difficult. Some authors have recognized a thealean ancestry for many families in the Ericales (e.g., Cronquist, 1981; Takhtajan, 1997), and symplesiomorphic similarity that Theaceae share with taxa of other clades could explain this. One such feature is the presence of versatile anthers, which is typical of many genera in the Theaceae, in contrast to those of the Ternstroemiaceae. Polystemonous flowers are present also in the Symplocaceae clade (clade VI) and the ericoid clade (clade X), e.g., in Actinidiaceae. At least in some genera of Theaceae the stamens originate in five petal-opposed regions (Tsou, 1998), and this is also true for some Actinidiaceae. The Theaceae and Ternstroemiaceae both have nuclear endosperm formation, but this is also the case in families such as Lecythidaceae, Sapotaceae (clade IX), and the primuloid families (clade VII).

Tsou (1995, 1997) found that all genera of the Theaceae produce pseudopollen together with ordinary pollen from the connective, but no genus of the Ternstroemiaceae has this character, and therefore she considered this a synapomorphy for the genera of the former. Deng and Baas (1990, 1991) investigated the wood anatomy of genera in Theaceae s.l. and found it to be homogeneous, but also found that there were no wood anatomical synapomorphies for the family. *Ternstroemia*, but no investigated genus of the Theaceae, has silica grains in the wood (ter Welle, 1976; Morton et al., 1996). Silica grains also occur in the wood of most Sapotaceae, some Theophrastaceae and Ebenaceae, but seemingly not in most other representatives of Ericales. This character has so far not been sufficiently studied, and its usefulness is difficult to evaluate. The differences between the Theaceae and the Ternstroemiaceae are worth noting in this context.

In our five-gene analysis, the genera of Theaceae are supported by all data sets, and their circumscription is not controversial (clade IV). Ternstroemiaceae form a clade that also includes *Ficalhoa* from Theaceae proper and *Sladenia* of Theaceae-Sladenioideae (Sladeniaceae), as well as *Pentaphyllax* of Pentaphyllaceae (clade III). The plastid genes support

this clade with 51%, but it is not resolved by the mitochondrial data. In combination, all the five genes make the jackknife support for the clade increase to 68%, which means that there is information in the mitochondrial data adding to the support.

Pentaphylax is hitherto treated as the only genus of Pentaphylacaceae, a family that is sometimes considered to be close to Theaceae s.l. (e.g., Takhtajan, 1997). Pentaphylacaceae differ from Theaceae s.l. in having anthers with apical pores provided with small lids and allegedly crassinucellate ovules, but it has horseshoe-shaped embryos, and curved embryos are characteristic of Ternstroemiaceae. Mauritzon (1936) found the ovules to be bitegmic with only the inner integument forming the micropyle. He thought they were possibly crassinucellate, although the nucellus was "reduced." *Pentaphylax* is currently the only crassinucellate taxon known from Ericales. However, Mauritzon's investigation was based on herbarium material, and the embryological characteristics of *Pentaphylax* are still insufficiently studied. In an analysis of *rbcL*, Savolainen et al. (2000) found *Pentaphylax* to belong together with *Cardiopteris* and *Gonocaryum* of the Cardiopteridaceae in the eusterid clade, and this differs from our results. However, when we included a third recently available *rbcL* GenBank sequence from *Pentaphylax* (GBAN-AF320785) in a jackknife analysis, it was placed together with our own in the Ericales.

In our analysis, *Ficalhoa* and *Sladenia* appear as the sister group of *Pentaphylax*, *Ternstroemia*, *Cleyera*, and *Eurya*. *Pentaphylax* is firmly placed in the Ternstroemiaceae clade (clade III) where it is placed as sister group of *Ternstroemia*, *Cleyera*, and *Eurya*. We believe that *Ficalhoa*, *Sladenia*, and *Pentaphylax* should be included in Ternstroemiaceae and that the family names Sladeniaceae and Pentaphylacaceae should be treated as synonyms.

Sladenia, the only genus of Theaceae-Sladenioideae and sometimes treated as family Sladeniaceae, and the aberrant genus *Ficalhoa* from Theaceae-Theoideae form a monophyletic group in our analysis, which is placed as sister to the group with *Pentaphylax* and the genera of Ternstroemiaceae. The same position for *Sladenia* was found by Savolainen et al. (2000). *Sladenia* has previously been included in Actinidiaceae (Gilg and Werdermann, 1925) but differs in vegetative anatomy by lacking raphides in the parenchyma. *Ficalhoa* is usually included in Theaceae (Verdcourt, 1962; Airy Shaw, 1973), but has also been placed in Ericaceae, Actinidiaceae, or Sapotaceae (cf. Tsou, 1997).

There are several differences between the two genera *Sladenia* and *Ficalhoa*, e.g., presence of laticifers in *Ficalhoa* but not in *Sladenia* nor other Theaceae or Ternstroemiaceae, radially oriented vessel groups in *Sladenia* but scattered vessels in *Ficalhoa*, and three-carpellate fruits with few ovules per locule in *Sladenia* vs. five-carpellate fruits with many ovules per locule in *Ficalhoa*. Both genera have many apomorphic character states, and this has made them difficult to place. It seems that several of the diagnostic features are autapomorphies, but when the morphological aspects of the two species are compared, it becomes apparent that they also have several features in common. Among diagnostic similarities the most notable is the presence of flowers in axillary dichasial cymes (with prophylls in *Ficalhoa*), not found in any other genus of Ternstroemiaceae or Theaceae. The stamens are arranged in a single series alternating with the petals but adnate to the corolla; the anthers open with apical pores, which is also the case in *Pentaphylax*. The seeds are more or less winged with

straight embryo and with endosperm sparse (*Ficalhoa*) or absent (*Sladenia*). The close relationship between the two genera may seem odd from a biogeographical point of view. The fact that *Sladenia celsa* grows in Yunnan, Thailand, and Burma, and *Ficalhoa laurifolia* in east tropical Africa raises many questions. The genera of the Ternstroemiaceae clade are almost exclusively Asian and American, and only a few genera occur in Africa, viz. *Balthasaria* with two or three species in tropical Africa, the monotypic *Visnea* in Macaronesia, a few species of *Ternstroemia*, and *Ficalhoa*. The two genera *Sladenia* and *Ficalhoa* are today far separated but if their present distribution is the result of vicariance, the distribution of their common ancestor must have been much wider. A plant from Sudan dated back to the Albian-Cenomanian period of the Cretaceous with a wood anatomy similar to that of *Sladenia* could support this notion. The fossil was described by Giraud, Bussert, and Schrank (1992) and named *Sladenioxylon*, and, just like *Sladenia*, has radially arranged vessel, which is unique in Ternstroemiaceae (and Theaceae).

Clade V: Ebenaceae and Lissocarpaceae—The Ebenaceae and Lissocarpaceae comprise only three genera, two of which were included in the combined five-gene study. Lissocarpaceae were left unplaced by APG (1998) due to lack of molecular data, but it has previously been placed near Ebenaceae and differ from that family by having a corolla with a corona, inferior ovaries, and porate pollen. Our study shows that the systematic position of *Lissocarpa* is near *Diospyros*, and a recent study by Berry et al. (2001) indicates that *Lissocarpa* should be included in Ebenaceae. It is notable that the Ebenaceae-Lissocarpaceae clade does not group with other families of the former Ebenales, i.e., Styracaceae, Symplocaceae, or Sapotaceae.

Clade VI: Symplocaceae—Symplocaceae have unitegmic ovules, but unitegmic ovules have evidently evolved independently in many clades of Ericales and, in each case, may constitute a synapomorphy for particular monophyletic groups. Apart from Symplocaceae, unitegmic ovules are also found in Sapotaceae, Polemoniaceae, Diapensiaceae, some Styracaceae, and all families of the ericoid clade (clade X). The predominantly inferior ovaries of Symplocaceae are a synapomorphy diagnosing the family. Symplocaceae have previously been placed in Ebenales and considered related to Styracaceae (Takhtajan, 1997), but in our study the monophyly of the Ebenales was not supported, and Styracaceae were found to be closer to Diapensiaceae. The position of Symplocaceae is still not clear.

Clade VII: Maesaceae, Theophrastaceae, Primulaceae, Myrsinaceae—One of the major clades is formed by the primuloid families. The monophyletic group with Maesaceae as sister to the Theophrastaceae, Primulaceae, and Myrsinaceae is supported in the five-gene tree, as well as in the plastid and mitochondrial trees, respectively. There are also several morphological and embryological character states supporting this clade, such as haplostemonous flowers with stamens opposite the corolla lobes, free central placentation, bitegmic ovules where both integuments form the micropyle, and nuclear endosperm formation. In most other families of Ericales, haplostemonous flowers have stamens alternating with the corolla lobes and bitegmic ovules in which only the inner integument forms the micropyle, and all families except Polemoniaceae,

Theaceae, Lecythidaceae, and Sapotaceae have cellular endosperm formation. Sapotaceae also have petal-opposed stamens. The results support that of a number of earlier investigations on relationships among the primuloid families (e.g., Anderberg and Ståhl, 1995; Anderberg, Ståhl, and Källersjö, 1998, 2000) and most recently by Källersjö, Bergqvist, and Anderberg (2000) and will therefore not be discussed further here.

Clade VIII: Styracaceae and Diapensiaceae—The relationship between Diapensiaceae and Styracaceae has strong support (94%) in the five-gene study, and both these families are monophyletic. Taxa from Styracaceae have appeared as two separate clades in some earlier studies (e.g., Morton et al., 1996), but their monophyly is supported by our data. The flowers are diplostemonous to haplostemonous in Styracaceae and haplostemonous in Diapensiaceae but sometimes with staminodes alternating with the stamens and could be considered modified diplostemonous. Diapensiaceae have often been associated with the families near Ericaceae, but the presence of a fibrous endothecium in the anthers and the lack of integumentary tapetum have been used as an argument against such a relationship. An integumentary tapetum is also missing in most Styracaceae but is otherwise present in most families. *Styrax* has bitegmic ovules, whereas other Styracaceae (except *Pamphilia* A. DC.) and Diapensiaceae have unitegmic ovules. Dickison (1993) suggested that an observed fusion of the two integuments in *Styrax* and *Pamphilia* could imply that the unitegmy in the Styracaceae originated by fusion of the two integuments of a bitegmic ancestor.

The genus *Afrostryax* Perkins and Gilg from tropical Africa was included in the Styracaceae until Baas (1972) confirmed that it belongs in the small family Huaceae. In the ordinal classification presented by APG (1998), the family Huaceae was listed as unclassified to order in the rosoid clade, and their relationship to other families is yet unclear. Analyses of *rbcL* sequences (Savolainen et al., 2000) also place this genus together with *Hua* Pierre ex DeWild, and *ndhF* data also place *Afrostryax* outside of Ericales (A. A. Anderberg, C. Rydin, and M. Källersjö, unpublished data) and hence it will not be discussed further.

Clade IX: Lecythidaceae and Sapotaceae—Our analyses of plastid data and of the combination of five genes demonstrate a sister group relationship between *Napoleonaea*, *Barringtonia*, and *Couroupita* of Lecythidaceae and the genera of Sapotaceae. The support for this relationship is low but is also implied by some anatomical and embryological synapomorphies. Both Lecythidaceae and Sapotaceae have nuclear endosperm formation, and they are also characterized by trilacunar nodes, although these character states are known also from other clades.

The Lecythidaceae clade in our analyses consists of *Napoleonaea* as sister to *Barringtonia* and *Couroupita*, and although a number of genera are not included, our result is congruent with that of Morton et al. (1997). Among other things, they concluded that *Crateranthus* Baker f. is a close relative of *Napoleonaea* and that *Asteranthos* belonged with the genera of Scytopetalaceae, which were included in Lecythidaceae. The same position of *Asteranthos* was also suggested by Appel (1996), and the position of *Foetidia* near the subfamily Planchonioidae of Lecythidaceae seems well supported by both morphology and *rbcL* data. The four aberrant genera, *Foetidia*, *Asteranthos*, *Crateranthus*, and *Napoleonaea*, had earlier been

excluded from the Lecythidaceae by Tsou (1994), who concluded that they should be treated as three separate families of uncertain position within a Theales-Ebenales complex, viz. Foetidiaceae, Asteranthaceae, and Napoleonaeaceae as outlined by Airy Shaw (1973).

Sapotaceae have unitegmic ovules, and they also have petal-opposed stamens and nuclear endosperm formation, characters they share with the families of the primuloid clade (clade VII). The unitegmic ovules in Sapotaceae could be the result of reduction of a second integument in a common ancestor or resulting from a fusion of two integuments (see above). In the Sapotaceae, we have found strong support for *Sarcosperma* as sister to the other genera, which is at odds with the classification of Pennington (1991). *Sarcosperma* differs from other genera of Sapotaceae by its frequently opposite leaves and flowers arranged in elongated racemes. *Sarcosperma* has also been treated as a separate family, Sarcospermataceae H. J. Lam, but our results do not contradict a position in Sapotaceae.

Clade X: Ericaceae, Cyrillaceae, Clethraceae, Actinidiaceae, Sarraceniaceae, and Roridulaceae—This clade more or less corresponds to the circumscription of the order Ericales by, e.g., Dahlgren (1980, 1983) and Anderberg (1992, 1993). The families all have ovules with cellular endosperm formation, which is a common character state in the enlarged Ericales, but other embryological characters such as unitegmic ovules may be synapomorphies of this clade. The vessels in all families have scalariform perforations, except in Actinidiaceae, in which simple perforations prevail, and this seems to be a synapomorphy at this level of the phylogeny. Similarities shared between *Actinidia* and Theaceae s.l., such as stamens arranged in five bundles opposite the corolla lobes and exalbuminous seeds, may be symplesiomorphic. Actinidiaceae differ from Theaceae in having raphides in the parenchymatic tissue, flowers in cymose inflorescences, and unitegmic ovules with cellular endosperm formation like the other ericoid families. Notable exceptions are the two genera *Ficalhoa* and *Sladenia*, here placed in Ternstroemiaceae, which both have flowers in dichasial cymes, but lack raphides. The monophyletic group consisting of the three families Actinidiaceae, Sarraceniaceae, and Roridulaceae is among the few in the Ericales in which the nucellus develops a hypostase, i.e., an area of dark suberized cells at the base of the embryo sac. The presence of a hypostase may be a synapomorphy for these three families, being absent in Clethraceae, Cyrillaceae, and Ericaceae, and is otherwise known only from families such as Polemoniaceae, Theaceae, Scytopetalaceae, some Sapotaceae, and perhaps some Styracaceae (Johri, Ambegaokar, and Srivastava, 1992; Dickison, 1993). In *Actinidia* and *Saurauia* of Actinidiaceae the three to five styles are more or less connate, and in *Darlingtonia* Torr., which is the sister of the two other genera of the Sarraceniaceae (Bayer, Hufford, and Soltis, 1996), the style is also branched and has separate stigmas. Other families of the clade have a solitary style and a single stigma. A branched style could be a synapomorphy for the group with Actinidiaceae and Sarraceniaceae with a subsequent change to a single style in Roridulaceae; a branched style is a parallelism in certain Theaceae s.s. The anthers in all families of the ericoid clade have a fibrous endothecium, except in Roridulaceae and in most Ericaceae where an absence of endothecium constitutes a synapomorphy for the majority of genera.

Clethraceae, Cyrillaceae, and Ericaceae are together diag-

nosed as a monophyletic group by the reduction of the seed coat, which is only one cell layer thick in the Clethraceae as well as in many Ericaceae and usually missing in Cyrillaceae. The hollow, fluted style leading to a cavity in the upper part of the ovary is another synapomorphy for the three families, and a third character diagnostic of the same clade is the presence of both micropylar and chalazal endosperm haustoria. A possible synapomorphy for Cyrillaceae plus Ericaceae is the presence of an intrastaminal nectar disc, which is lacking in Clethraceae and the other families. One species in *Purdiaea* of Cyrillaceae was originally described as a member of Clethraceae, and in spite of the similarities between this genus and *Clethra*, it is evident from our results that the Cyrillaceae are closer to Ericaceae than to Clethraceae. In the last ten years a robust hypothesis of the phylogenetic relationships in the Ericaceae has emerged. *Enkianthus* Lour. is the sister group of all other representatives of this large family, including the genera from the former families Pyrolaceae, Monotropaceae, Epacridaceae and Empetraceae, which are all derived members of Ericaceae (Anderberg, 1993, 1994; Kron and Chase, 1993; Kron, 1996). The relationships within the Ericaceae, which constitute about 40% of the genera and species of Ericales, have been thoroughly studied and will not be discussed further here.

Conclusion—We have found support for a number of clades and relationships among families, viz. Balsaminaceae-Marcgraviaceae-Pellicieraceae-Tetrameristaceae, Polemoniaceae-Fouquieriaceae, Ternstroemiaceae-Sladeniaceae-Pentaphragmataceae, Theaceae, Ebenaceae-Lissocarpaceae, Symplocaceae, Maesaceae-Theophrastaceae-Primulaceae-Myrsinaceae, Styracaceae-Diapensiaceae, Lecythidaceae-Sapotaceae, and Actinidiaceae-Roridulaceae-Sarraceniaceae-Clethraceae-Cyrillaceae-Ericaceae. The five-gene analysis shows a more resolved topology and higher support values than do either the combined plastid or the combined mitochondrial genes, which means that there are similar signals in the two data sets for relationships that are not expressed by the individual data sets alone. The plastid data give better resolution than the mitochondrial data, but the two data sets have only one conflicting group, viz. the position of *Impatiens* within Marcgraviaceae as indicated by the mitochondrial data. Several new large groups have been found with good support. However, it is clear that not even a combination of conserved genes (*atp1*, *matR*, *atpB*) with more rapidly evolving genes (*rbcL*, *ndhF*) from two genomes was sufficient to provide supported resolution between all major groups. A bold hypothesis would be that several of the groups evolved rapidly and simultaneously, resulting in the observed difficulties in finding well-supported relationships.

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