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MOLECULAR PHYLOGENETICS AND PATTERNS OF FLORAL EVOLUTION IN THE ERICALES

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The diverse and species-rich order Ericales has found considerable interest among systematists in recent years. Molecular phylogenetic studies not only have convincingly demonstrated the monophyly of the order, comprising 23 families formerly placed in three different subclasses (Asteridae, Dilleniidae, and Rosidae), but have also resolved Ericales as sister to euasterids. Most ericalean families are well circumscribed and have been or are currently subject to intrafamilial phylogenetic studies. In spite of all the attention that Ericales have received recently, there remains a major challenge, the still largely unresolved deeper nodes in the ericalean phylogeny. This study aims to improve our current knowledge of the interfamilial relationships by expanding on gene and taxon sampling and to evaluate the evolution of important floral characters in light of the resulting phylogeny. We add a nuclear region (26s rDNA) to already published data sets (nuclear: 18s rDNA; mitochondrial: *atp1*, *matR*; chloroplast: *atpB*, *ndhF*, *rbcL*, *matK*, the *rps16* intron, the *trnT-trnF* spacer, and the *trnV-atpE* spacer), for a total of 11 molecular markers that include nearly 20 kb of sequences. Our analyses, applying both maximum parsimony and Bayesian inference, resolve some of the deeper nodes in the phylogeny. Strongly supported groups, previously unrecognized or only weakly supported, include (1) a clade comprising all families except Balsaminaceae, Tetrameristaceae, Marcgraviaceae, Fouquieriaceae, Polemoniaceae, and Lecythidaceae; (2) a clade with Sapotaceae, Ebenaceae, and the primuloid families; (3) a clade with Symplocaceae, Styracaceae, and Diapensiaceae; and (4) a clade comprising the latter three families plus Theaceae, Roridulaceae, Actinidiaceae, Sarraceniaceae, Clethraceae, Cyrillaceae, and Ericaceae. At an analytical level, our results indicate that more data in the form of additional markers do improve resolution and branch support and should eventually lead to a fully resolved ericalean phylogeny. At the level of floral evolution, we demonstrate that sympetaly is a homoplasious character in the order, that a diplostemonous floral ground plan likely arose from haplostemonous flowers in Ericales, and that the combination of ovules with a single integument and cellular endosperm formation is characteristic for two of the major clades in the order.

Keywords: Asteridae, Bayesian inference, endosperm formation, Ericales, floral evolution, integument number, molecular phylogeny, androecium organization, sympetaly, 26s rDNA.

Introduction

The 23 families of the asterid order Ericales *sensu* APG II (2003) comprise 353 genera with more than 11,000 species (Stevens 2003). They display a considerable diversity in floral organization and embryological features, including actinomorphy and zygomorphy, choripetaly and sympetaly, isomery and polymery in the androecium, unitegmic and bitegmic ovules, as well as nuclear and cellular endosperm formation.

The individual families are generally well circumscribed and easily recognized on the basis of morphological features. In contrast, there are no clear-cut morphological synapomorphies supporting the monophyly of the order as a whole. The only feature shared by all investigated species is tenuinucellate ovules, but this is characteristic of asterids in general. The lack of unifying morphological features is reflected in premolecular classification systems (Dahlgren 1980; Cronquist 1981; Thorne 1992; Takhtajan 1997) in which the families now included in the Ericales were not placed together or even close to each other. In the classification system of Cronquist (1981), the 23 families were scattered in 11 orders, the majority of which were referred to the Dilleniidae, two tothe Rosidae (Balsaminaceae, Roridulaceae), and one to the Asteridae (Polemoniaceae).

The systematic position of the Ericales in the Asteridae and the monophyly of the order have been strongly supported by a series of molecular phylogenetic studies (Chase et al. 1993; Soltis et al. 2000; Albach et al. 2001*c*; Bremer et al. 2002). It is now widely agreed that Ericales consists of one of the four major lineages in the Asteridae; the other three are the Cornales, euasterids I, and euasterids II. Bremer et al. (2002) analyzed sequences for three genes and three noncoding regions for representatives of almost all asterid families and found strong statistical support for Cornales's being sister to all other asterids and Ericales's being sister to a clade

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comprising euasterids I and II. Other studies addressing the relationships among these four lineages yielded contradictory results (Olmstead et al. 2000; Soltis et al. 2000; Albach et al. 2001*c*; Hilu et al. 2003). However, none of these studies was as comprehensive as the Bremer et al. (2002) study regarding either taxon sampling or the number of markers. Also, support values for the groupings found in these studies are generally low.

Considerable efforts have also been undertaken to disentangle interfamilial relationships in the Ericales, and recent studies comprising representatives of all (Anderberg et al. 2002; Bremer et al. 2002) or a subset of the families (Soltis et al. 2000; Albach et al. 2001*c*; Geuten et al. 2004) have revealed a number of well-supported clades. However, such clades rarely encompass more than two or three families, and most of the deeper nodes in the phylogeny received low support, remained unresolved, and differed widely among studies.

Most of the ericalean families have been examined in greater detail with molecular data: Balsaminaceae (Yuan et al. 2004), Clethraceae and Cyrillaceae (Anderberg and Zhang 2002), Ericaceae (Kron and Chase 1993; Kron 1996, 1997; Kron et al. 2002*a*, 2002*b*; Li et al. 2002), Diapensiaceae (Rönblom and Anderberg 2002), Fouquieriaceae (Schultheis and Baldwin 1999), Lecythidaceae (Morton et al. 1997), Marcgraviaceae (Ward and Price 2002), Polemoniaceae (Johnson et al. 1996; Porter 1996; Porter and Johnson 1998; Prather et al. 2000), Primulaceae (Mast et al. 2001; Trift et al. 2002; Martins et al. 2003), Sapotaceae (Anderberg and Swenson 2003), Sarraceniaceae (Bayer et al. 1996), Styracaceae (Fritsch et al. 2001), Theaceae (Prince and Parks 2001), and Theophrastaceae (Källersjö and Ståhl 2003).

With this study, we add new taxa and sequences from a nuclear region (26s rDNA) to already published data sets, for a total of 11 molecular markers including nearly 20 kb of sequences and sampling all three plant genomes. The 11 markers include two nuclear genes (18s rDNA and 26s rDNA), two mitochondrial genes (*atp1* and *matR*), and seven chloroplast regions (the *atpB*, *ndhF*, *rbcL*, and *matK* genes; the *rps16* intron; and the intergenic spacers between *trnT* and *trnF* and between *trnV* and *atpE*). In addition to producing an entirely new data set (26s rDNA), we add numerous new DNA sequences to already published data sets.

The main goals of this study are (1) to improve phylogenetic resolution in the major polytomy of Ericales, (2) to test the monophyly of clades that have been found in earlier molecular studies based on smaller data sets, and (3) to evaluate the evolution of important floral characters in the Ericales. A better understanding of the ericalean phylogeny is essential for several reasons. The Ericales, as outlined above, are a species-rich and biologically extremely diverse group of plants. Several important morphological features, such as floral zygomorphy, sympetaly, isomery in the androecium, and unitegmic ovules, which are characteristic for the euasterids or large clades therein, are not constant in the Ericales. This and the position of Ericales as the sister to the euasterids make a better understanding of the interfamilial relationships in Ericales especially desirable. As such, a phylogeny would not only be helpful for understanding morphological evolution in the order itself but might also shed light on the evolutionary history of the asterids as a whole.

Material and Methods

Data Sets and Taxon Sampling

The sampling strategy for the 26s rDNA data set was to match, as closely as possible, that of the five-gene data set of Anderberg et al. (2002), in order to be able to combine the data sets. The original data set from Anderberg et al. (2002) included one to several representatives of all the ericalean families recognized by APG II (2003). Taxon sampling is somewhat denser than in the study by Anderberg et al. (2002), namely, in Fouquieriaceae (*Fouquieria*), Polemoniaceae (*Acanthogilia*, *Cantua*), and Tetrameristaceae (*Pentamerista*).

Anderberg et al. (2002) used *Cornus* (Cornaceae) as the outgroup for their analysis of Ericales. Considering that the euasterids now are strongly supported to be sister to the Ericales (jackknife [JK] = 98%; Bremer et al. 2002), we included two taxa from the euasterids, Garryaceae (*Aucuba*) and Aquifoliaceae (*Ilex*), representing euasterids I and euasterids II, respectively. Besides *Cornus*, we added another taxon from the Cornales, *Hydrangea* sp. (Hydrangeaceae), to serve as a monophyletic outgroup for Ericales and euasterids. All new sequences have been submitted to GenBank.

In total, we produced 141 new sequences, 58 of which were for the 26s data set and between 11 and 22 for each of the five markers from the Anderberg et al. (2002) study (see table 1 for taxa, voucher information, and GenBank numbers). An additional five-marker data set was assembled entirely from published sequences, including a nuclear marker (18s rDNA), a chloroplast gene (*matK*), and three chloroplast noncoding regions (the *rps16* intron and the *trnT-trnF* and *trnV-atpE* intergenic spacers). The majority of these sequences are from the Bremer et al. (2002) asterid-wide study. Taxon sampling of this five-marker data set overlaps with the six-marker data set described above but generally comprises only one representative from each family.

Taxon sampling is not fully congruent at the species level among the data sets because, in some cases, we combined the sequences of several species of the same genus (Anderberg et al. 2002). This approach has been followed widely in multigene studies (Albach et al. 2001*c*; Bremer et al. 2002; Geuten et al. 2004) and is justifiable under the assumption that the genus, from which the species are chosen, is monophyletic.

DNA Extraction, Amplification, and Sequencing

We extracted total genomic DNA from silica-dried leaf samples, using DNeasy Plant Mini Kits (Qiagen, Valencia, CA) or following the sodium dodecyl sulfate extraction protocol (Eichenberger et al. 2000). We amplified the entire 26s rDNA (ca. 3300 bp) by single polymerase chain reaction (PCR), using the N-nc26S1 and 3331rev primers by Kuzoff et al. (1998) for about half of the taxa. For the remaining ones, we amplified the whole region in two overlapping parts, using the internal primers N-nc-26S7 and 1499rev (Kuzoff et al. 1998) together with the peripheral primers. A variety of combinations of the 26s primers given in Kuzoff et al. (1998) were used for cycle sequencing. Primers used for PCR and sequencing of *matR* and *atp1* were those of Anderberg et al. (2002); primers for *ndhF* were those listed by Källersjö et al. (2000); primers for *atpB* were from Hoot et al. (1995); and

primers for *rbcL* were from Olmstead et al. (1992) and Anderberg et al. (1998). We cleaned PCR products using Ampure magnetic particles (Agencourt Bioscience, Beverly, MA). Standard cycle-sequencing techniques (Givnish et al. 2000) were used to sequence double-stranded DNA. Cycle-sequencing products were cleaned using CleanSEQ magnetic particles (Agencourt Biosience). Sequences were generated on an Applied Biosystems 3730XL DNA capillary sequencer.

Phylogenetic Analyses

We edited and assembled complementary strands in Sequencher, version 3.0 (Gene Codes, Ann Arbor, MI), which was also used to produce initial alignments for the various markers. Alignments were finalized by eye using Se-Al, version 2.0a6 (Rambaut 1996). Areas of ambiguous alignment and poly-n strings of differing lengths (only present in noncoding chloroplast regions) were omitted from all further analyses.

Phylogenetic analyses were performed using the maximum parsimony (MP) optimization as implemented in the software PAUP* (phylogenetic analyses using parsimony), version 4.0b10 (Swofford 2000), as well as Bayesian inference of phylogeny using the software program MrBayes, version 3.0b4 (Huelsenbeck and Ronquist 2001*a*). Each of the 11 individual data sets was analyzed separately and in various combinations (table 2). Individual markers and combined nuclear, chloroplast, and mitochondrial markers were analyzed using MP only; the full 11-marker data set was analyzed with both MP and Bayesian analysis (BA). To test for possible effects of the relatively large number of missing data in the 11-marker data set, we also analyzed the 11-marker data set with a reduced taxon sampling (31 instead of 63 taxa), more or less matching the sampling of the five-marker data set described above. In addition, we analyzed our new data set (26s rDNA) together with the five-gene data set of Anderberg et al. (2002). Both of these data sets were analyzed with MP as well as BA.

For MP analyses, all characters and character state transitions were weighted equally (Fitch 1971). Gaps were coded as missing characters. We applied 100 random taxon addition sequences with tree bisection reconnection (TBR) branch swapping, keeping all most parsimonious trees and the steepest descent to each heuristic search. In cases where searches did not run to completion under the described conditions because of an excessive number of most parsimonious trees, we applied the following search strategy: an initial search of 100 random addition replicates with 100 trees saved per replicate; the resulting consensus tree was used as a backbone constraint to search for trees not consistent with the initial trees. This strategy ensures that there are no shorter trees and that the strict consensus tree reflects all most parsimonious trees, even if not all trees of equal lengths have been found (Catalán et al. 1997; Davis et al. 2001; Hall et al. 2002). Relative support for the different clades was estimated using the bootstrap (Felsenstein 1985) option in PAUP*, employing a full heuristic search with 500 or 1000 replicates, a simple addition sequence, and TBR branch swapping.

In BA, a phylogeny is inferred on the basis of posterior probabilities of phylogenetic trees (Huelsenbeck and Ronquist 2001*b*). The software program MrBayes uses the Markov chain Monte Carlo (MCMC) algorithm to approximate these posterior probabilities (Huelsenbeck and Ronquist 2001*a*). Models for nucleotide substitution for use in BA (table 2) were evaluated for the combined data sets using ModelTest, version 3.06 (Posada and Crandall 1998). The data sets were partitioned by marker and codon position (for coding regions). In addition to the substitution model, we set priors that allowed for site-specific rates, partition-specific base frequencies, and partition-specific substitution rates for coding markers (first and second codon position different from third). Posterior probabilities were approximated in an initial search of more than 1 million generations via four simultaneous MCMC chains, with every hundredth tree saved. Default values were used for MCMC parameters. Of the resulting 10,000 trees, the first 2000 (burn-in) were discarded. The remaining 8000 trees were summarized in a majority-rule consensus tree, yielding the probabilities of each clade's being monophyletic. For each data set, we conducted four additional runs with identical settings but different random-starting trees. This strategy should ensure that the MCMC chains begin to explore the total tree space from different starting points in each run and is therefore a much more thorough way to explore total tree space than conducting a single run only.

We did not incorporate indel characters into any of our analyses because we were mainly interested in interfamilial relationships, and we found few unambiguously alignable indels that are informative at that level, except for the clade including Balsaminaceae, Marcgraviaceae, and Tetrameristaceae and the clade of primuloid families. Both of these clades are strongly supported on the basis of nucleotide data alone, and the coding of indel characters would most likely not have improved the resulting phylogeny, in terms of either branch support or resolution. Further, it is not possible yet to combine nucleotide and indel characters under maximum likelihood or Bayesian analyses.

Character State Mapping

We used MacClade 4.0 (Maddison and Maddison 2000) to explore character evolution and mapped selected characters onto the simplified family-level tree topology obtained from the combined 11-marker, 63-taxa BA. This tree only shows nodes with 1.0 posterior probability and is not fully resolved. We chose this tree over one of the fully resolved MP trees or the fully resolved Bayesian tree because we believe that when reconstructing character evolution it is important to use only strongly supported relationships, even if it means that character reconstructions are impossible or ambiguous in some parts of the tree. Using fully resolved but weakly supported topologies is potentially misleading. When reconstructing character evolution, we used the "soft polytomy" option in MacClade, which assumes the polytomy to represent uncertainty rather than multiple speciation (hard polytomy). Accordingly, the program reconstructs character evolution on all possible dichotomous resolutions and chooses the most parsimonious one for the character in question. As an example, corolla structure (fig. 3*A*), the polytomy including Polemoniaceae/Fouquieriaceae, Lecythidaceae, and the large clade with most of the remaining families can be resolved in three ways that result in different tree lengths: (1) Lecythidaceae sister to Polemoniaceae/Fouquieriaceae, four steps; (2)

Table 1

Voucher Information and GenBank Numbers for 11 Markers

Family, species name with author	Citation/voucher	26s	*rbcL*	*atpB*	*ndhF*	*matR*	*atp1*	18s	*rps16*	*matK*	*trnT-F*	*trnv-atpE*
Actinidiaceae:												
Actinidia arguta Miq.	Anderberg et al. 2002				AF421043	AF420991	AF420916					
Actinidia arguta Miq.	Schönenberger 616 (Z)	AY727964										
Actinidia chinensis Planch.	Albert et al. 1992		L01882									
Actinidia chinensis Planch.	Anderberg et al. 2002			AJ235382								
Actinidia kolomikta Maxim.	Bremer et al. 2002								AJ430992	AJ429279	AJ430869	AJ429640
Actinidia sp.	Soltis et al. 1997							U42495				
Aquifoliaceae:												
Ilex opaca Ait.	Soltis et al. 1999							AF206938				
Ilex repanda Griseb.	Schönenberger 743 (Z)	AY727932	AY725859	AY725920	AY725870	AY725885	AY725899					
Ilex sp.	Bremer et al. 2002								AJ431088	AJ429376	AJ430962	AJ429722
Balsaminaceae:												
Impatiens auricoma Baill.	Schönenberger 635 (Z)	AY727936		AY725922								
Impatiens capensis Meerb.	Bremer et al. 2002								AJ430993	AJ429280	AJ430870	AJ429641
Impatiens parviflora DC.	Anderberg et al. 2002				AF421060	AF421011	AF420933					
Impatiens repens Moon	Morton et al. 1997		Z80197									
Impatiens walleriana Hook.	Soltis et al. 1997							L49285				
Clethraceae:												
Clethra alnifolia L.	Bremer et al. 2002								AJ430994	AJ429281	AJ430871	AJ429526
Clethra alnifolia L.	Soltis et al. 1997							U42521				
Clethra barbinervis Sieb. & Zucc.	Anderberg et al. 2002		AF421089	AF420966	AF421047	AF420997	AF420920					
Clethra cf. *ferruginea* Ruiz & Pav.	Schönenberger 497 (Z)	AY727968										
Cornaceae:												
Cornus mas L.	Bremer et al. 2002								AJ430988	AJ429275	AJ430866	AJ429636
Cornus officinalis Sieb. & Zucc.	Soltis and Soltis 1997							U52033				
Cornus stolonifera Michx.	Schönenberger 626 (Z)	AY727930	AY725857	AY725918	AY725868	AY725883	AY725897					
Cyrillaceae:												
Cyrilla racemiflora L.	Albert et al. 1992		L01900									
Cyrilla racemiflora L.	Anderberg et al. 2002				AF421051		AF420922					
Cyrilla racemiflora L.	Bremer et al. 2002								AJ430995		AJ430872	AJ429527
Cyrilla racemiflora L.	Kron 1996							U43294				
Cyrilla racemiflora L.	Prince and Parks 2001									AF380080		
Cyrilla racemiflora L.	Savolainen et al. 2000			AJ235449								
Cyrilla racemiflora L.	Schönenberger 615 (Z)	AY727969				AY725893						
Diapensiaceae:												
Diapensia lapponica L.	Anderberg et al. 2002			AF420967		AF421001	AF420923					
Diapensia lapponica L.	Bremer et al. 2002									AJ429283	AJ430873	
Diapensia lapponica L.	Kron and Chase 1993		L12612									
Diapensia lapponica L.	Smedmark s. n. (Z)	AY727986			AY725881							
Diapensia lapponica L.	Soltis et al. 1997							L49278				
Galax urceolata (Poir.) Brummit	Anderberg et al. 2002					AF421007	AF420929					
Galax urceolata (Poir.) Brummit	Caris s. n. (Z)	AY727983		AY725936	AY725879							
Galax urceolata (Poir.) Brummit	Johnson et al. 1996									L48576		
Galax urceolata (Poir.) Brummit	Morton et al. 1997		Z80184									
Galax urceolata (Poir.) Brummit	Soltis et al. 1997							L49281				
Shortia soldanellioides (Sieb. & Zucc.) Makino	Anderberg et al. 2002		AF421105		AF421083	AF421030	AF420949					
Shortia soldanellioides (Sieb. & Zucc.) Makino	Dunlop s. n. (Z)	AY727984		AY725937								
Shortia uniflora (Maxim.) Maxim.	Nagashima s. n. (Z)	AY727985	AY725866	AY725938	AY725880	AY725895	AY725915					

Ebenaceae:												
Diospyros digyna Jacq.	Anderberg et al. 2002			AF213768	AF213731	AF421002	AF420924					
Diospyros kaki Thunb.	Bremer et al. 2002								AJ430996		AJ430874	AJ429642
Diospyros lotus L.	Kron and Chase 1993		L12613									
Diospyros lotus L.	Schönenberger 625 (Z)	AY727957										
Diospyros lotus L.	Soltis et al. 1997							U43295				
Diospyros virginiana L.	Kron et al. 2002*a*									AY145446		
Ericaceae:												
Chimaphila umbellata (L.) W. P. C. Barton	Anderberg et al. 2002		AF421087	AF420964	AF421087	AF420994	AF420917					
Chimaphila umbellata (L.) W. P. C. Barton	Kron 723 (WFU)	AY727971										
Empetrum hermaphroditum Hagerup	Li et al. 2002									AF519561		
Empetrum hermaphroditum Hagerup	Schönenberger 551 (Z)	AY727972		AY725931								
Empetrum nigrum L.	Anderberg et al. 2002		AF421091		AF421053	AF421003	AF420925					
Enkianthus campanulatus Nichols.	Anderberg et al. 2002			AF420968	AF421054	AF421004	AF420926					
Enkianthus campanulatus Nichols.	Kron and Chase 1993		L12616									
Enkianthus campanulatus Nichols.	Kron 1997									U61344		
Enkianthus campanulatus Nichols.	Schönenberger 773 (Z)	AY727970										
Enkianthus chinensis Franch.	GenBank/unpublished										AF452225	
Rhododendron ferrugineum L.	GenBank/unpublished										AF394254	
Rhododendron impeditum I. B. Balf. & W. W. Smith	Schönenberger 641 (Z)	AY727973		AY725932			AY725911					
Rhododendron tomentosum (Stokes) Harmaja	Anderberg et al. 2002		AF421101		AF421072	F421026						
Rhododendron tomentosum (Stokes) Harmaja	Kron 1997									U61335		
Vaccinium macrocarpon Ait.	Soltis et al. 1997							L49297				
Vaccinium myrtillus L.	Kron et al. 2002*b*									AF382810		
Vaccinium myrtillus L.	Schönenberger 775 (Z)	AY727974										
Vaccinium uliginosum L.	Anderberg et al. 2002		AF421107	AF420987	AF421078	AF421035	AF420953					
Fouquieriaceae:												
Fouquieria columnaris (Kellogg) Kellogg ex Curan	Johnson et al. 1999							AF003961				
Fouquieria columnaris (Kellogg) Kellogg ex Curan	Schönenberger 648 (Z)	AY727939	AY725861	AY725923	AY725873	AY725887	AY725902					
Fouquieria digueti I. M. Johnst.	Bremer et al. 2002								AJ430998	AJ429285	AJ430876	AJ429643
Fouquieria fasciculata Nash	Schönenberger 646 (Z)	AY727940	AY725862	AY725924	AY725874		AY725903					
Fouquieria splendens Engelm.	Soltis et al. 1997							L49280				
Garryaceae:												
Aucuba japonica Thunb.	Bremer et al. 2002								AJ431029	AJ429318	AJ430906	AJ429672
Aucuba japonica Thunb.	Schönenberger 653 (Z)	AY727931	AY725858	AY725919	AY725869	AY725884	AY725898					
Aucuba japonica Thunb.	Soltis et al. 1997							U42522				
Hydrangeaceae:												
Hydrangea aspera Buch.-Ham. ex D. Don	Bremer et al. 2002								AJ430990	AJ429277		AJ429638
Hydrangea hirta Sieb.	Hufford et al. 2003										AY254248	
Hydrangea macrophylla Torr.	Soltis et al. 1997							U42781				
Hydrangea sp.	Donoghue 138	AY727929	AY725856	AY725917	AY725867	AY725882	AY725896					
Lecythidaceae:												
Barringtonia asiatica (L.) Kurz	Anderberg et al. 2002				AF421044	AF420992						
Barringtonia asiatica (L.) Kurz	Bremer et al. 2002								AJ430999	AJ429286	AJ430877	AJ429644
Barringtonia asiatica (L.) Kurz	Chase 328 (K)	AY727949		AY725929			AY725906					
Barringtonia asiatica (L.) Kurz	Morton et al. 1997		Z80174									
Couroupita guianensis Aubl.	Albach et al. 2001*c*			AJ236224				AJ235993				
Couroupita guianensis Aubl.	Anderberg et al. 2002				AF421050							
Couroupita guianensis Aubl.	Morton et al. 1997		Z80181									
Couroupita guianensis Aubl.	Morton et al. 1998										AF077632	
Couroupita guianensis Aubl.	Schönenberger 576 (Z)	AY727950				AY725890	AY725907					

Table 1

(*Continued*)

Family, species name with author	Citation/voucher	26s	*rbcL*	*atpB*	*ndhF*	*matR*	*atp1*	18s	*rps16*	*matK*	*trnT-F*	*trnv-atpE*
Napoleonaea imperialis Beauv.	Anderberg et al. 2002						AF420960					
Napoleonaea imperialis Beauv.	Albach et al. 2001*c*				AJ236258							
Napoleonaea sp.	Schönenberger 672 (Z)	AY727951				AY725891						
Napoleonaea vogelii Hook. & Planch.	Morton et al. 1997		Z80173									
Napoleonaea vogelii Hook. & Planch.	Morton et al. 1998										AF077649	
Napoleonaea vogelii Hook. & Planch.	Savolainen et al. 2000			AJ235540								
Napoleonaea vogelii Hook. & Planch.	Soltis et al. 2000							AF206969				
Lissocarpaceae:												
Lissocarpa benthamii Gürke	Berry et al. 7217 (PORT)	AY727956			AY725877							
Lissocarpa guianensis Gleason	Anderberg et al. 2002		AF421094	AF420975		AF421012	AF420934					
Lissocarpa guianensis Gleason	Bremer et al. 2002									AJ429287		AJ429645
Maesaceae:												
Maesa japonica Mor. & Zoll.	Mast et al. 2001	AY727959										
Maesa tenera Mez	Anderberg et al. 1998		U96650									
Maesa tenera Mez	Anderberg et al. 2002					AF421015	AF420937					
Maesa tenera Mez	Bremer et al. 2002								AJ431000	AJ429288	AJ430878	
Maesa tenera Mez	Källersjö et al. 2000			AF213781	AF213750							
Marcgraviaceae:												
Marcgravia rectiflora Triana & Planch.	Morton et al. 1996		Z83148									
Marcgravia rectiflora Triana & Planch.	Savolainen et al. 2000			AJ235529								
Marcgravia rectiflora Triana & Planch.	Schönenberger 731 (Z)	AY727937										
Marcgravia sp.	Anderberg et al. 2002				AF421065	AF421017	AF420939					
Marcgravia sp.	Bremer et al. 2002								AJ431001	AJ429289	AJ430879	AJ429646
Norantea guianensis Aubl.	Mori 22996 (NY)	AY727938										
Norantea guianensis Aubl.	Morton et al. 1996		Z80200									
Norantea guianensis Aubl.	Ward and Price 2002										AF303475	
Norantea peduncularis Poepp. ex Wittm.	Anderberg et al. 2002			AF420978	AF421067	AF421020	AF420941					
Myrsinaceae:												
Myrsine africana L.	Anderberg et al. 1998		U96652									
Myrsine africana L.	Anderberg et al. 2002					AF421019	AF420940					
Myrsine africana L.	Bremer et al. 2002								AJ431002	AJ429290	AJ430880	AJ429647
Myrsine africana L.	Källersjö et al. 2000			AF213764	AF213751							
Myrsine africana L.	Schönenberger 654 (Z)	AY727961										
Pentaphylacaceae:												
Cleyera japonica Sieb. & Zucc.	Anderberg et al. 2002		AF421090		AF421048	AF420998						
Cleyera japonica Sieb. & Zucc.	Prince and Parks 2001									AF380078		
Cleyera japonica Sieb. & Zucc.	Chase 1690 (K)	AY727952		AY725930			AY725908					
Cleyera pachyphylla Chun & H. T. Chang	GenBank/unpublished										AF499819	
Eurya emarginata (Thunb.) Makino	Albach et al. 2001*c*							AJ235995				
Eurya handel-mazzettii H. T. Chang	GenBank/unpublished										AF499821	
Eurya japonica Thunb.	Chase 1448 (K)	AY727953										
Eurya japonica Thunb.	Morton et al. 1997		Z80207									
Eurya japonica Thunb.	Prince and Parks 2001									AF380081		
Eurya sp.	Anderberg et al. 2002			AF420969	AF421055	AF421005	AF420927					
Ficalhoa laurifolia Hiern	Anderberg et al. 2002		AF421109	AF420961	AF421079	AF421037	AF420955					
Pentaphylax euryoides Gardn. & Champ.	Anderberg et al. 2002		AF419239	AF419240	AF419241	AF419243	AF419242					
Pentaphylax euryoides Gardn. & Champ.	Bremer et al. 2002								AJ431003	AJ429291	AJ430881	AJ429648
Pentaphylax euryoides Gardn. & Champ.	GenBank/unpublished							AF320783				
Pentaphylax euryoides Gardn. & Champ.	Shui Yumin 15718 (KUN)	AY727954										
Sladenia celastrifolia Kurz	Anderberg et al. 2002		AF421108	AF420988	AF421081	AF421040	AF420959					

Sladenia celastrifolia Kurz	Bremer et al. 2002								AJ431009	AJ429297	AJ430081	AJ429654
Sladenia celastrifolia Kurz	GenBank/unpublished							AF320782				
Ternstroemia gymnathera Sprague	Anderberg et al. 2002		AF421106		AF421076	AF421033						
Ternstroemia gymnathera Sprague	Bremer et al. 2002								AJ431013		AJ430890	AJ429659
Ternstroemia gymnathera Sprague	Prince and Parks 2001									AF380109		
Ternstroemia stahlii Krug & Urb.	Axelrod 4538 (UPR)	AY727955					AY725909					
Ternstroemia stahlii Krug & Urb.	Savolainen et al. 2000			AJ235621								
Ternstroemia stahlii Krug & Urb.	Soltis et al. 2000							AF207038				
Polemoniaceae:												
Acanthogilia gloriosa (Brandegee) A. G. Day & R. Moran	Johnson et al. 1996									L48562		
Acanthogilia gloriosa (Brandegee) A. G. Day & R. Moran	Schönenberger 756 (Z)	AY727942	AY725863	AY725926	AY725875	AY725888	AY725904					
Acanthogilia gloriosa (Brandegee) A. G. Day & R. Moran	Soltis et al. 1997							L49271				
Cantua buxifolia Lam.	Schönenberger 629 (Z)	AY727943	AY725864	AY725927	AY725876	AY725889	AY725905					
Cantua pyrifolia Juss.	Johnson and Soltis 1995									L34180		
Cantua quercifolia Juss.	Johnson et al. 1999							L49276				
Cobaea scandens Cav.	Anderberg et al. 2002				AF421049	AF420999	AF420921					
Cobaea scandens Cav.	Fritsch et al. 2001										AF396217	
Cobaea scandens Cav.	Johnson et al. 1996									L48568		
Cobaea scandens Cav.	Morton et al. 1996		Z83143									
Cobaea scandens Cav.	Savolainen et al. 2000			AJ235440								
Cobaea scandens Cav.	Schönenberger 624 (Z)	AY727944										
Cobaea scandens Cav.	Soltis et al. 1997							L49277				
Polemonium caeruleum L.	Anderberg et al. 2002				AF421070	AF421023	AF420944					
Polemonium californicum Eastw.	Johnson et al. 1999							L49294				
Polemonium pauciflorum S. Watson	Schönenberger 644 (Z)	AY727941		AY725925								
Polemonium pulcherrimum Hook.	Bremer et al. 2002								AJ431004	AJ429292	AJ430882	AJ429649
Polemonium reptans L.	Olmstead et al. 1992		L11687									
Primulaceae:												
Lysimachia maxima (Knuth) St. John	Källersjö et al. 2000			AF213777								
Lysimachia nummularia L.	Mast et al. 2001										AF402443	
Lysimachia vulgaris L.	Anderberg et al. 2002		AF421095		AF421063	AF421013	AF420935					
Lysimachia vulgaris L.	Schönenberger 777 (Z)	AY727963										
Primula elatior Hill	Schönenberger 778 (Z)	AY727960					AY725916					
Primula sieboldii E. Morren	Källersjö et al. 2000			AF213787								
Primula sieboldii E. Morren	Källersjö et al. 2000		AF213799		AF213758							
Primula sikkimensis Hook.	Anderberg et al. 2002					AF421025						
Primula sp.	Johnson et al. 1999							L49295				
Primula veris L.	Bremer et al. 2002								AJ431005	AJ429293	AJ430883	AJ429650
Roridulaceae:												
Roridula gorgonias Planchon	Albach et al. 2001*c*			AJ236180	AJ236270							
Roridula gorgonias Planchon	Albert et al. 1992		L01950									
Roridula gorgonias Planchon	Anderberg et al. 2002					AF421036						
Roridula gorgonias Planchon	Bremer et al. 2002								AJ431006	AJ429294	AJ430884	AJ429651
Roridula gorgonias Planchon	M. Buzgo 412 (K)	AY727965					AY725910					
Roridula gorgonias Planchon	Soltis et al. 2000							AF207010				
Sapotaceae:												
Madhuca microphylla (Hook.) Alston	Anderberg et al. 2002		AF421096	AF420976	AF421064	AF421014	AF420936					
Madhuca microphylla (Hook.) Alston	Chase 1363 (K)	AY727947										
Manilkara zapota (L.) Van Royen	Anderberg et al. 2002					AF421016	AF420938					
Manilkara zapota (L.) Van Royen	Bremer et al. 2002								AJ431007	AJ429295	AJ430885	AJ429652
Manilkara zapota (L.) Van Royen	Kron 1997							U43080				
Manilkara zapota (L.) Van Royen	Källersjö et al. 2000		AF213793	AF213782	AF213732							

Table 1

(*Continued*)

Family, species name with author	Citation/voucher	26s	*rbcL*	*atpB*	*ndhF*	*matR*	*atp1*	18s	*rps16*	*matK*	*trnT-F*	*trnv-atpE*
Manilkara zapota (L.) Van Royen	Schönenberger 579 (Z)	AY727946										
Monotheca buxifolia (A. DC) T. D. Penn.	Anderberg et al. 2002		AF421097	AF420977	AF421066	AF421018						
Palaquium ferox H. J. Lam	Chase 1367 (K)	AY727945		AY725928								
Palaquium formosanum Hayata	Anderberg et al. 2002		AF421098		AF421068	AF421021	AF420942					
Pouteria campechiana (H. B. & K.) Baehni	Schönenberger 637 (Z)	AY727948										
Pouteria obovata (R. Br.) Baehni	Anderberg et al. 2002		AF421100	AF420981	AF421071	AF421024	AF420945					
Sarcosperma laurinum Hook. f.	Anderberg et al. 2002		AF421111	AF420989	AF421080	AF421039	AF420956					
Sarraceniaceae:												
Heliamphora minor Gleason	Schönenberger 637 (Z)	AY727966										
Heliamphora nutans Benth.	Albert et al. 1992		L02433									
Heliamphora sp.	Anderberg et al. 2002			AF420973		AF421010	AF420932					
Sarracenia psittacina Michx.	Schönenberger 627 (Z)	AY727967										
Sarracenia purpurea L.	Bremer et al. 2002								AJ431008	AJ429296	AJ430886	AJ429653
Sarracenia purpurea L.	Soltis et al. 1997							U42804				
Sarracenia flava L.	Albert et al. 1992		L01952									
Sarracenia flava L.	Anderberg et al. 2002					AF421028	AF420947					
Sarracenia flava L.	Savolainen et al. 2000			AJ235594								
Styracaceae:												
Bruinsmia styracoides Boerlage & Koorders	Anderberg et al. 2002		AF421086		AF421045							
Bruinsmia styracoides Boerlage & Koorders	Fritsch et al. 2001										AF396163	
Bruinsmia styracoides Boerlage & Koorders	Schönenberger 553 (Z)	AY727982		AY725936		AY725894	AY725914					
Halesia carolina L.	Anderberg et al. 2002			AF420972	AF421059	AF421009	AF420931					
Halesia carolina L.	Bremer et al. 2002								AJ431010	AJ429298	AJ430082	AJ429655
Halesia carolina L.	Morton et al. 1997		Z80190									
Halesia carolina L.	Schönenberger 632 (Z)	AY727981										
Halesia diptera Ellis	Johnson et al. 1999							L49284				
Styrax americanum Lam.	Johnson et al. 1999							L49296				
Styrax americanum Lam.	Kron and Chase 1993		L12623									
Styrax japonica Sieb. & Zucc.	Schönenberger 619 (Z)	AY727980										
Styrax officinalis L.	Anderberg et al. 2002			AF420984	AF421084	AF421032	AF420950					
Styrax officinalis L.	Bremer et al. 2002								AJ431011	AJ429300	AJ430888	AJ429657
Symplocaceae:												
Symplocos bogotensis Brand	Bremer et al. 2002								AJ431012	AJ429301	AJ430889	AJ429658
Symplocos paniculata Miq.	Kron 1996							U43297				
Symplocos pendula Wight	Schönenberger 559 (Z)	AY727979										
Symplocos sp.	Anderberg et al. 2002		AF421110	AF420985	AF421075	AF421038	AF420954					
Symplocos zizyphoides Stapf	Schönenberger 564 (Z)	AY727978	AY725865	AY725934	AY725878	AY725893	AY725913					
Tetrameristaceae:												
Pelliciera rhizophorae Triana & Planch.	Anderberg et al. 2002		AF421099			AF421022						
Pelliciera rhizophorae Triana & Planch.	Bremer et al. 2002								AJ431014	AJ429303	AJ430891	AJ429660
Pelliciera rhizophorae Triana & Planch.	Pennington et al. 586 (K)	AY727933			AY725871		AY725900					
Pelliciera rhizophorae Triana & Planch.	Soltis et al. 2000			AF209647				AF206983				
Pentamerista neotropica Maguire	Berry 6545 (WIS)	AY727935	AY725860	AY725921	AY725872	AY725886	AY725901					
Tetramerista sp.	Albach et al. 2001*a*				AJ400887							
Tetramerista sp.	Anderberg et al. 2002						AF420958					
Tetramerista sp.	Bremer et al. 2002								AJ431015	AJ429304	AJ430892	AJ429528
Tetramerista sp.	Coode 7925 (K)	AY727934										
Tetramerista sp.	Morton et al. 1997		Z80199									
Tetramerista sp.	Savolainen et al. 2000			AJ235623								
Tetramerista sp.	Soltis et al. 2000							AF207039				

Theaceae:												
Camellia japonica L.	Kron and Chase 1993		L12602									
Camellia japonica L.	Soltis et al. 1997							U42815				
Camellia sinensis Kuntze	Anderberg et al. 2002				AF421077	AF421034	AF420952					
Camellia sinensis Kuntze	Bremer et al. 2002								AJ431016		AJ430893	AJ429661
Camellia sinensis Kuntze	Prince and Parks 2001									AF380077		
Camellia sinensis Kuntze	Schönenberger 639 (Z)	AY727975		AY725933								
Gordonia axillaris (Roxb.) Dietr.	Anderberg et al. 2002		AF421092	AF420971	AF421058	AF421008	AF420930					
Gordonia lasianthus Ellis	Buzgo 1097 (Z)	AY727977										
Gordonia lasianthus Ellis	GenBank/unpublished										AF499807	
Gordonia lasianthus Ellis	Prince and Parks 2001									AF380085		
Laplacea alpestris W. T. Thistleton-Dyer	Anderberg et al. 2002		AF421093		AF421061							
Laplacea fruticosa Kobuski	Prince and Parks 2001									AF380088		
Schima sp.	Schönenberger 560 (Z)	AY727976					AY725912					
Schima superba Gardn. & Champ.	Anderberg et al. 2002		AF421103	AF420982	AF421073	AF421029						
Schima superba Gardn. & Champ.	GenBank								AJ431017		AJ430894	AJ429662
Schima superba Gardn. & Champ.	Prince and Parks 2001									AF380099		
Theophrastaceae:												
Clavija domingensis Urb. & Ekman	Anderberg et al. 2002					AF420995	AF420918					
Clavija domingensis Urb. & Ekman	Källersjö et al. 2000		AF213818									
Clavija eggersiana Mez	Albach et al. 2001*c*							AJ235998				
Clavija euerganea Maebr.	Källersjö et al. 2000			AF213771	AF213737							
Clavija lancifolia Desf.	Schönenberger 441 (Z)	AY727962										
Clavija spinosa (Vell.) Mez	Mast et al. 2001										AF402450	
Samolus repens (Forst.) Pers.	Anderberg et al. 2002		AF421102			AF421027	AF420946					
Samolus repens (Forst.) Pers.	Källersjö et al. 2000			AF213789								
Samolus valerandi L.	Källersjö et al. 2000				AF213760							
Samolus valerandi L.	Schönenberger 772 (Z)	AY727958										

Table 2

Data Set Characteristics

Marker	Genome	Type	No. of terminal taxa	Aligned length	Variable sites[a]	Parsimony informative sites[a]	GC content (%)	Missing sites[b] (%)	Model of nucleotide substitution
26s	Nuclear	rDNA	58	3370	672 (19.9)	460 (13.6)	56.5	8.2	
18s	Nuclear	rDNA	35	1740	220 (12.6)	112 (6.4)	49.2	44.8	
atpB	Chloroplast	Coding	62	1482	530 (35.8)	327 (22.1)	42.1	6.9	
ndhF	Chloroplast	Coding	60	2234	1172 (52.5)	823 (36.8)	31.5	11.9	
matK	Chloroplast	Coding (p.p.)	43	2023	1026 (50.7)	621 (30.7)	32.6	37.4	
rbcL	Chloroplast	Coding	63	1408	475 (33.7)	298 (21.2)	43.9	1.8	
rps16	Chloroplast	Noncoding, intron	29	1107	416 (37.6)	218 (19.7)	33.7	54.4	
trnT-trnF	Chloroplast	Noncoding, spacer	42	1790	703 (39.3)	372 (20.8)	35.2	47.8	
trnV-atpE	Chloroplast	Noncoding, spacer	42	1813	529 (29.2)	257 (14.2)	36.8	54.3	
atp1	Mitochondrial	Coding	61	1234	327 (26.5)	192 (15.6)	46.8	4.7	
matR	Mitochondrial	Coding	60	1567	547 (34.9)	234 (14.9)	52.4	7.2	
Nuclear markers	Nuclear	rDNA	59	5110	892 (17.5)	572 (11.2)	54.2	14.3	
Mitochondrial markers	Mitochondrial	Coding	62	2801	874 (31.2)	426 (15.2)	49.8	4.5	
Chloroplast markers	Chloroplast	Coding/ noncoding	63	11,857	4852 (40.9)	2916 (24.6)	36.9	30.3	
Six markers combined[c]			63	11,295	3723 (33.0)	2334 (20.7)	46.7	7.4	GTR + I + G
11 markers combined, reduced sampling[d]			31	19,485	5689 (29.2)	2923 (15.0)	43.7	6.6	GTR + I + G
11 markers combined[e]			63	19,768	6617 (33.5)	3914 (19.8)	44.5	24.4	GTR + I + G

[a] Number in parentheses is the percent of nucleotide positions in aligned sequence exclusive of ambiguous regions.
[b] Compared with the 63 taxon data set.
[c] 26s rDNA plus the five markers used in Anderberg et al. (2002).
[d] All 11 markers, taxon sampling reduced to 31 terminal taxa to match sampling of additional five markers.
[e] All 11 markers for 63 terminal taxa.

Lecythidaceae sister to the large clade, four steps; and (3) Lecythidaceae sister to the large clade plus Polemoniaceae/Fouquieriaceae, three steps. Because corolla structure can be reconstructed most parsimoniously on this latter topology (three instead of four steps), it is favored by MacClade.

Characters were scored on the basis of general literature, such as Davis (1966) and Cronquist (1981), and, whenever feasible, on original articles. In a few cases, our character scoring differs from that in earlier articles (Hufford 1992; Nandi et al. 1998) because of obvious errors, because family circumscriptions have changed (Cyrillaceae/Clethraceae), or because recent phylogenies permit the reconstruction of the plesiomorphic character state at the family level (number of integuments in Styracaceae). Characters analyzed include corolla structure, androecium organization, integument number, and type of endosperm formation.

Results

Data Set Characteristics

The combined 11-marker data set comprised 19,768 aligned positions (5110 nuclear; 11,857 chloroplast; 2801 mitochondrial). Of these, 6617 positions were variable and 3914 were parsimony informative (see table 2 for characteristics for individual markers and other combined data sets).

Parsimony Analyses

Individual MP analyses of each of the 11 markers (trees not shown; tree statistics in table 3) generally support individual families in the ingroup but fail to resolve any interfamilial relationships, with the notable exceptions of the clade comprising Balsaminaceae, Marcgraviaceae, and Tetrameristaceae (including Pellicieraceae) and the clade containing Maesaceae, Theophrastaceae, Primulaceae, and Myrsinaceae, which are supported by most individual markers. The only markers that support (bootstrap [BS] > 80%) any additional interfamilial relationships are *matK* and *ndhF*. Data from *matK* support Actinidiaceae as sister to Roridulaceae (BS = 94%); Actinidiaceae, Roridulaceae, and Sarraceniaceae as sister to Clethraceae, Cyrillaceae, and Ericaceae (BS = 88%); and Diapensiaceae as sister to Styracaceae (BS = 88%). Analysis of *ndhF* supports Balsaminaceae as sister to Tetrameristaceae (BS = 89%). There are no strongly supported conflicting relationships among the 11 individual data sets, with the exception of the anomalous positions of

Table 3

Tree Statistics from Parsimony Analyses

Marker	Length of shortest tree	No. of shortest trees	CI	RI
26s	2419	195	0.38	0.52
18s	494	6938	0.54	0.51
atpB	1345	Many	0.46	0.61
ndhF	4060	104	0.46	0.57
matK	2652	145	0.56	0.53
rbcL	1392	4350	0.46	0.57
rps16	905	2425	0.63	0.48
trnT-trnF	1540	2060	0.65	0.52
trnV-atpE	1060	352	0.64	0.48
atp1	720	Many	0.60	0.68
matR	899	Many	0.75	0.70
Nuclear markers	2946	622	0.40	0.51
Mitochondrial markers	1658	Many	0.66	0.67
Chloroplast markers	9265	142	0.49	0.56
Six markers combined[a]	11,035	96	0.48	0.56
11 markers combined, reduced sampling[b]	13,197	55	0.59	0.41
11 markers combined[c]	17,816	55	0.52	0.54

Note. CI = consistency index; RI = retention index.

[a] 26s rDNA plus the five markers used in Anderberg et al. (2002).

[b] All 11 markers; taxon sampling reduced to 31 terminal taxa to match sampling of additional five markers.

[c] All 11 markers for 63 terminal taxa.

Ternstroemia (Pentaphylacaceae; *sensu* APG II 2003) and *Bruinsmia* (Styracaceae) in the mitochondrial *atp1* topology. *Ternstroemia* is resolved as sister to *Vaccinium* (BS = 97%), embedded among other Ericaceae, and *Bruinsmia* is resolved as sister to *Cyrilla* (BS = 99%).

MP analyses of the combined nuclear markers (26s and 18s rDNA; trees not shown) and the combined mitochondrial markers (*atp1* and *matR*; trees not shown) generally find stronger support for the same groups that are also supported by the individual data sets but fail to find any additional clades with strong support. MP analysis of the combined chloroplast markers (*atpB*, *ndhF*, *matK*, *rbcL*, *rps16*, *trnT-trnF*, *trnV-atpE*; trees not shown) supports almost all families *sensu* APG II (2003) as monophyletic with bootstrap support of 87% or higher. The only exception is Pentaphylacaceae (*sensu* APG II 2003, including Ternstroemiaceae and Sladeniaceae), because *Sladenia* and *Ficalhoa* remain unresolved. In addition, chloroplast data support the following monophyletic groups comprising more than one family with bootstrap support of 80% or higher: Balsaminaceae as sister to Tetrameristaceae (BS = 90%) and this whole clade as sister to Marcgraviaceae (BS = 100%); Fouquieriaceae as sister to Polemoniaceae (BS = 88%); Diapensiaceae as sister to Styracaceae (BS = 87%); Maesaceae as sister to the other three primuloid families and Theophrastaceae as sister to Primulaceae and Myrsinaceae (all nodes BS = 100%); Actinidiaceae as sister to Roridulaceae (BS = 100%) and this clade as sister to Sarraceniaceae (BS = 88%); Cyrillaceae as sister to Ericaceae (BS = 97%) and this clade as sister to Clethraceae (BS = 100%). The latter clade is sister to the clade comprising Actinidiaceae, Roridulaceae, and Sarraceniaceae (BS = 99%). The clade comprising Balsaminaceae, Marcgraviaceae, and Tetrameristaceae is resolved as sister to the rest of the ingroup (BS = 100%). In summary, MP analyses of combined nuclear, chloroplast, and mitochondrial markers all support individual families *sensu* APG II (2003); deeper nodes remain largely unresolved, and only few interfamilial relationships are supported. The only topological conflict concerns the position of Balsaminaceae, which is resolved as sister to Marcgraviaceae by the mitochondrial data set (BS = 96%) and as sister to Tetrameristaceae by the chloroplast data set (BS = 90%).

MP analysis of the combined 11-marker data sets produced 55 trees of length 17,816 (consistency index = 0.52, retention index = 0.54). The resulting consensus tree (fig. 1*B*) is largely consistent with the results from the various analyses of individual markers or partial data sets. Strongly supported monophyletic groups not consistently found in MP analyses of partial data sets include Fouquieriaceae as sister to Polemoniaceae (BS = 100%); Ebenaceae as sister to Maesaceae, Theophrastaceae, Primulaceae, and Myrsinaceae (BS = 90%); and Symplocaceae as sister to Styracaceae and Diapensiaceae (BS = 100%). Most of the deeper nodes remain unresolved.

Bayesian Analyses

For each of the three data sets, GTR + I + G (general time reversible model allowing for a proportion of invariant sites with the rest having rates drawn from a γ distribution) turned out to be the best-fitting model (table 2). The five individual runs with different random starting trees yielded identical topologies in each case. The topology resulting from the combined 11-marker, 63-taxa data set (fig. 1*A*) is fully consistent with the topology based on the parsimony analysis (fig. 1*B*). To facilitate discussion, we have numbered major clades I through VII. The main difference between the MP tree (fig. 1*B*) and the BA topology (fig. 1A) is that the latter is better resolved, having four strongly supported (posterior probabilities [PP] = 1.0) nodes that are not present in the strict consensus MP tree (stars, fig. 1*A*). These include clade VII and two large groups within it (i.e., a clade with the primuloids, Ebenaceae, and Sapotaceae [clade III]) and as a clade comprising all the families in clade VII except for Pentaphylacaceae (clade VI). The fourth node that is not present in the MP topology resolves *Acanthogilia* as sister to the remaining members of the Polemoniaceae. The positions of Balsaminaceae, Lecythidaceae, Pentaphylacaceae, and Theaceae are not fully resolved in the combined BA. Balsaminaceae are either sister to Tetrameristaceae, as favored by chloroplast data (PP = 1.0), or sister to Marcgraviaceae, as favored by mitochondrial data (PP = 1.0). Lecythidaceae are resolved as one of the earliest diverging lineages in Ericales, either as the second- or third-branching lineage or as sister to Fouquieriaceae/Polemoniaceae. However, none of these three possibilities is strongly supported in any of the analyses. Pentaphylacaceae are members of clade VII and could be resolved either as sister to the rest of the clade, as sister to clade III, or as sister to clade VI. None of these three alternative positions is supported in any of the analyses. Finally, Theaceae are strongly supported as part of clade VI (PP = 1.0), and possible alternative positions include Theaceae as sister to the remaining members of clade VI or as sister to

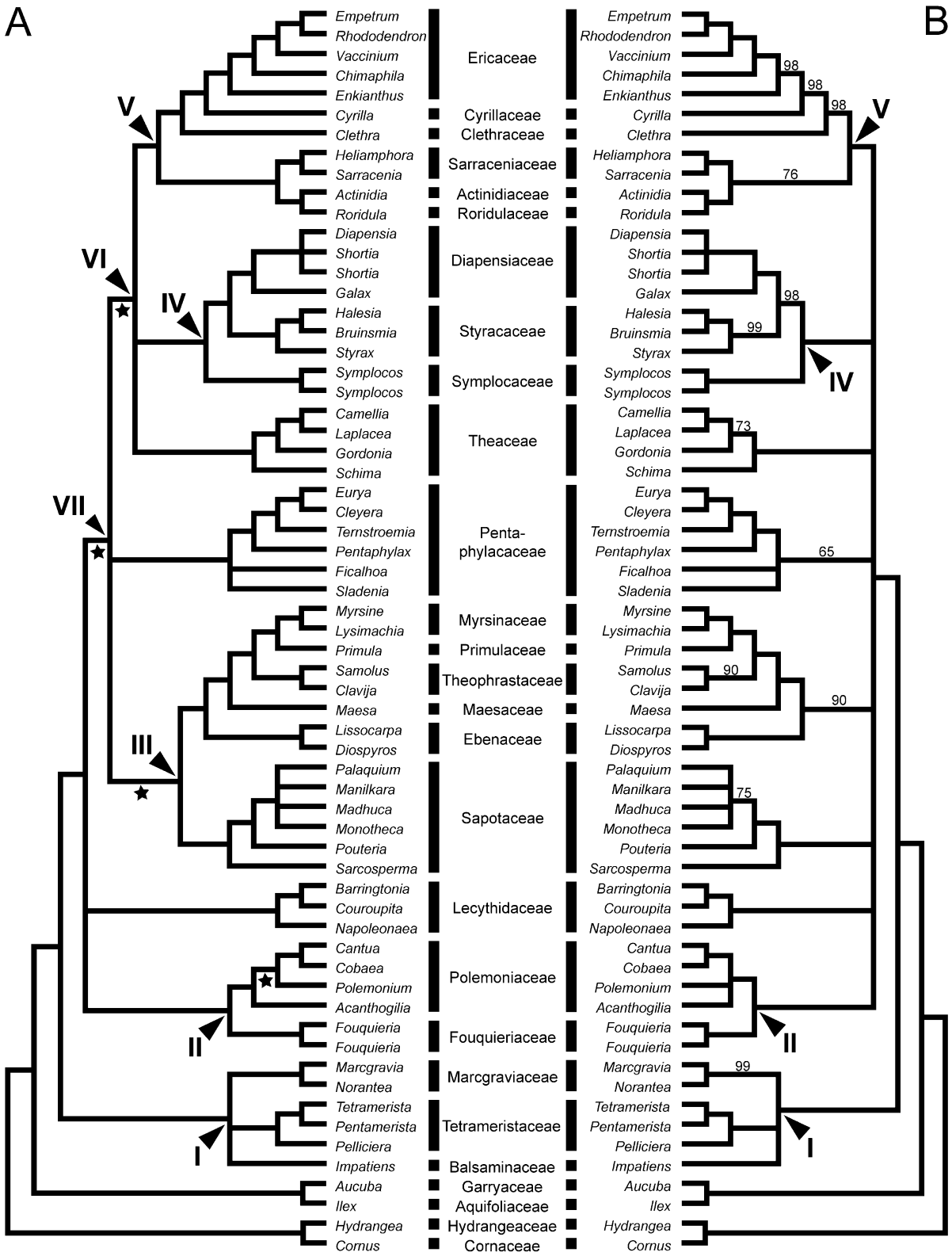

Fig. 1 Phylogenetic trees based on combined analyses of all 11 markers. Major clades referred to in the text are numbered I–VII. *A*, Topology from the Bayesian analysis: only branches with posterior probability of 1.0 are shown; others are collapsed. Asterisks indicate branches that are not present in the parsimony topology in *B*. *B*, Strict consensus topology from the parsimony analysis. Branches without support values have a bootstrap frequency of 100%.

clade IV or V. BA supports Theaceae as sister to clade IV (PP = 0.98; this node is not present in fig. 1*A* because only clades with PP = 1.0 are shown).

The resulting family-level topology from the 11-marker data set with a reduced taxon sampling (fig. 2*B*, 31 terminals, 6.6% missing data; table 2) is fully consistent with the topology based on the 63-taxa data set (fig. 2*A*, 24.4% missing data), with the only difference being two nodes (Actinidiaceae sister to Roridulaceae and Symplocaceae sister to Styracaceae/Diapensiaceae) that did not obtain full support from the BA (everything with PP < 1.0 is collapsed in the figures). The analysis of the data set combining 26s rDNA and the five genes from Anderberg et al. (2002) (fig. 2*C*, 63 terminals, 7.4% missing data) is also consistent with the topology based on the full data set but shows even fewer fully supported clades.

Character Evolution

Mapping corolla structure (sympetaly vs. choripetaly) onto the BA phylogeny (fig. 3*A*) indicates that choripetaly is plesiomorphic in the Ericales (but see "Discussion") and that sympetaly is plesiomorphic for at least clade VII (fig. 3*A*). Clades II, III, and IV are uniformly sympetalous. Other clades and even individual families are dimorphic for this character (e.g., clade V, Marcgraviaceae, and Actinidiaceae). An androecium with the stamens arranged in a single whorl (haplostemony or polyandry based on a haplostemonous organization) is plesiomorphic in the Ericales, and androecia with two whorls (diplostemony or polyandry based on a diplostemonous organization) may have evolved along the branch leading to clade VII (fig. 3*B*). Bitegmic ovules are plesiomorphic in Ericales (fig. 3*C*), and the change to unitegmic ovules occurred at least three times in the order, once in Polemoniaceae, once in Sapotaceae, and once or twice in clade VI. Cellular endosperm formation is likely to be plesiomorphic for the order (fig. 3*D*), and the switch to nuclear endosperm formation occurred after the divergence of clade I. Because of the lack of resolution in the BA phylogeny, it is unclear how many times reversals back to cellular endosperm formation occurred, but it could be just once or as many as three times.

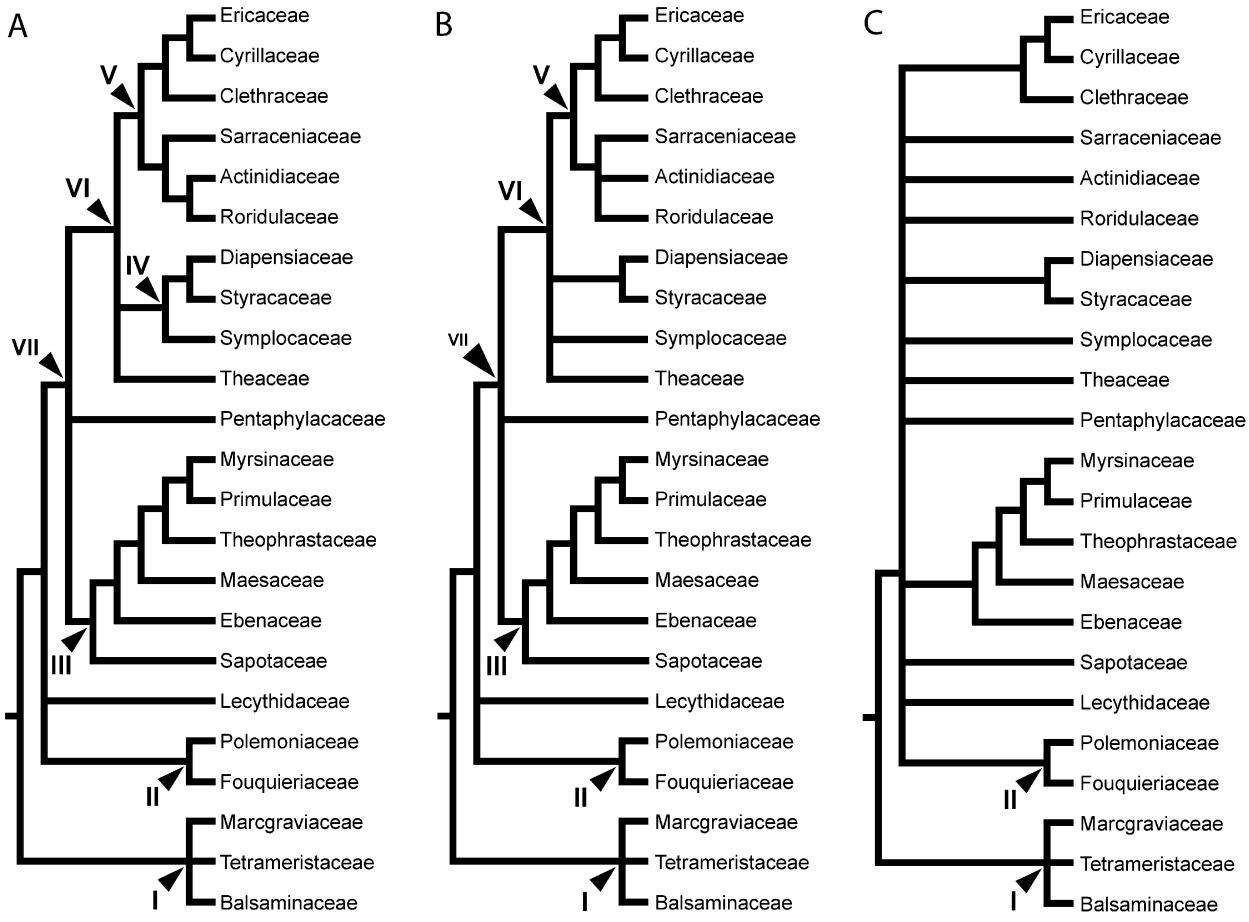

Fig. 2 Comparison of family-level topologies based on Bayesian inference of phylogeny of data sets with different levels of missing data and taxon sampling (table 2). All branches have a posterior probability of 1.0. Major clades referred to in the text are numbered I–VII. *A*, Topology resulting from analysis of the combined 11-marker data set with 63 terminal taxa (simplified, family-level tree based on the Bayesian topology in fig. 1*A*); amount of missing data is 24.4%. *B*, Topology resulting from analysis of the combined 11-marker data set with taxon sampling reduced to 31 taxa; amount of missing data is 6.6%. *C*, Topology resulting from the analysis of 26s rDNA combined with the five markers from Anderberg et al. (2002; *atpB*, *ndhF*, *rbcL*, *atp1*, *matR*) for 63 taxa; amount of missing data is 7.4%.

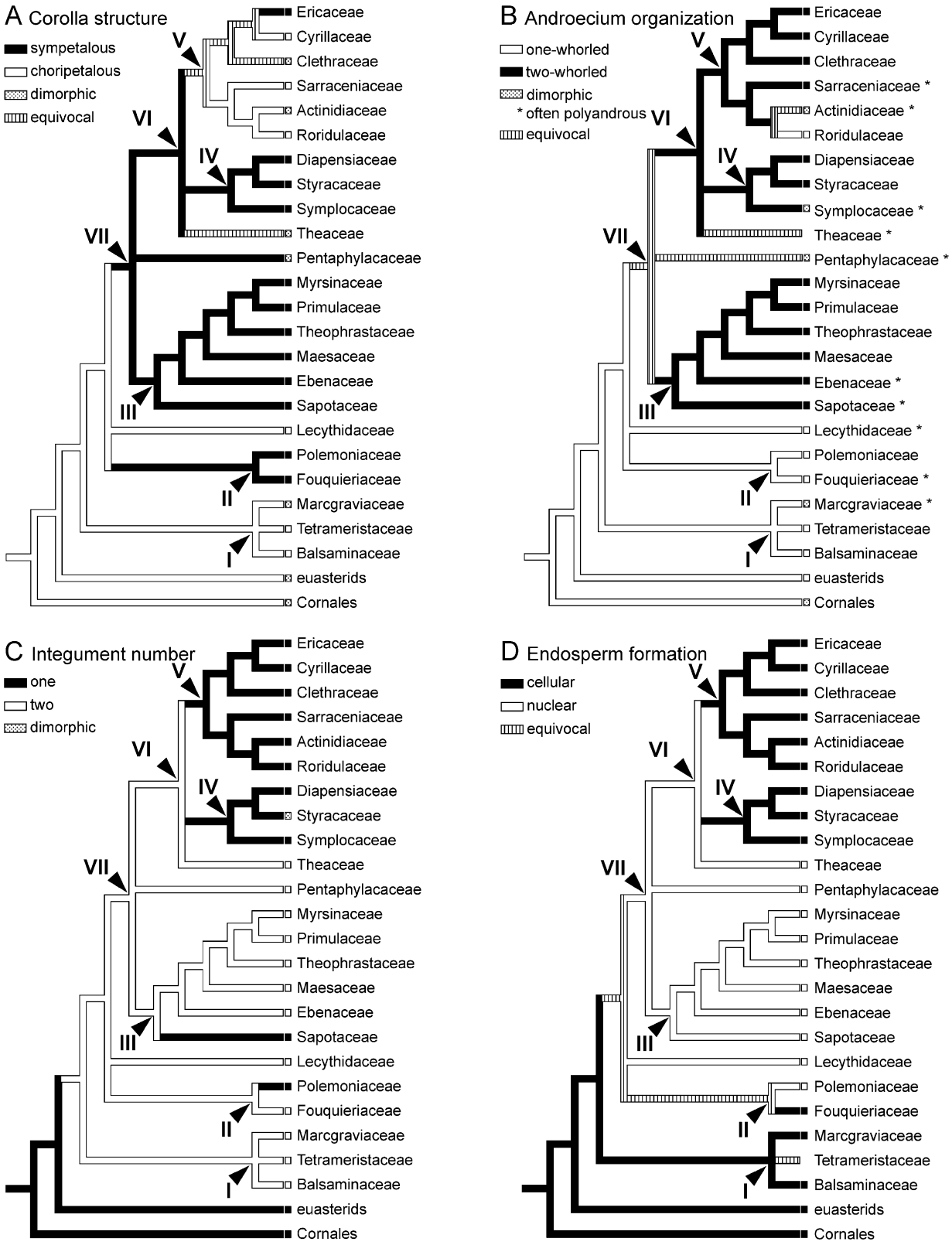

Fig. 3 Patterns of floral evolution reconstructed on the family-level topology resulting from the Bayesian analysis of the combined 11-marker data set. Dimorphic character states are indicated with a shaded square; unknown character states are indicated with the absence of a square. Major clades referred to in the text are numbered I–VII. *A*, Corolla structure: sympetalous, choripetalous. *B*, Androecium organization: one-whorled, two-whorled. *C*, Integument number: one, two. *D*, Endosperm formation: cellular, nuclear.

Discussion

Comparison with Earlier Molecular Analyses

Our results are congruent with well-supported interfamilial relationships found in earlier studies but provide statistical support for some of the deeper nodes in the ericalean phylogeny. These newly found relationships include (1) a clade comprising all families except Balsaminaceae, Tetrameristaceae, Marcgraviaceae, Fouquieriaceae, Polemoniaceae, and Lecythidaceae; (2) a clade with Sapotaceae, Ebenaceae, and the primuloid families; (3) a clade with Symplocaceae, Styracaceae, and Diapensiaceae; and (4) a clade comprising the latter three families plus Theaceae, Roridulaceae, Actinidiaceae, Sarraceniaceae, Clethraceae, Cyrillaceae, and Ericaceae. In the following discussion, branch support values for particular clades are added in parentheses in the text in the form of Bayesian posterior probabilities (PP), bootstrap values (BS), or jackknife values (JK).

The basalmost split in Ericales (fig. 1) is between clade I (PP = 1.0, BS = 100), comprising Balsaminaceae, Marcgraviaceae, and Tetrameristaceae (including Pellicieraceae), and a clade uniting the remainder of the families (PP = 1.0, BS = 100). This split had also been identified in previous studies by Källersjö et al. (1998), Anderberg et al. (2002), Bremer et al. (2002), and Geuten et al. (2004). In our combined analyses, interfamilial relationships in clade I are not resolved or are only weakly supported, apparently because of conflicting phylogenetic signals from chloroplast and mitochondrial data. Chloroplast data support Balsaminaceae as sister to Tetrameristaceae (PP = 1.0, BS = 90), whereas the combined mitochondrial genes strongly support Balsaminaceae as sister to Marcgraviaceae (PP = 1.0, BS = 96). Nuclear data are not conclusive on these relationships. In their study combining mitochondrial and chloroplast data, Anderberg et al. (2002) found Tetrameristaceae and Marcgraviaceae to form a clade (JK = 89) that in turn is sister to Balsaminaceae. Using only chloroplast data, Bremer et al. (2002) found strong support for the sister-group relationship of Balsaminaceae and Tetrameristaceae (BS = 94). Geuten et al. (2004) concluded that the closest relatives of the Balsaminaceae are the Marcgraviaceae, although their data show similar conflicting signals. In addition, while their maximum likelihood and Bayesian topologies resolve Balsaminaceae as sister to Marcgraviaceae (BS = 53, PP = 0.99), their parsimony analysis finds Balsaminaceae as sister to the clade with Marcgraviaceae + Tetrameristaceae (BS = 91). Hence, we consider the relationships in clade I as still unresolved.

Despite their remarkable disjunct distributions, the two genera of Tetrameristaceae, *Pentamerista* (Venezuela) and *Tetramerista* (Malaysia), had been recognized as closely related on the basis of morphology (Maguire et al. 1972; Bremer et al. 2002). Our analyses, which are the first to include sequence data of *Pentamerista*, fully support the sister relationship of the two genera (PP = 1.0, BS = 100). The second clade at the base of the Ericales comprises three lineages: Polemoniaceae + Fouquieriaceae (clade II), Lecythidaceae, and clade VII (PP = 1.0, unresolved in parsimony analysis). Previous studies by Anderberg et al. (2002) supported a split between clade II and all the remaining families (JK = 89), with Lecythidaceae weakly supported as sister to Sapotaceae (JK = 60). In the Bremer et al. (2002) study, Lecythidaceae was sister to Ebenaceae, but with only weak support (JK < 50). None of our analyses provides support for the exact sister-group relationship of Lecythidaceae. Clade II, comprising Fouquieriaceae and Polemoniaceae, was previously identified as a monophyletic group (JK = 72, Anderberg et al. 2002; JK = 88, Bremer et al. 2002; PP = 1.0, Geuten et al. 2004) and is strongly supported by these analyses (PP = 1.0, BS = 100).

Clade VII is strongly supported by Bayesian inference of phylogeny (PP = 1.0; fig. 1) but is not present in the strict consensus tree of the parsimony analysis and has not been found in any earlier study. At the base of clade VII is a trichotomy comprising Pentaphylacaceae, clade III, and clade VI (fig. 1*A*). Bayesian inference strongly supports clade III, comprising Sapotaceae as sister to a clade with Ebenaceae (including Lissocarpaceae) and the four primuloid families (both nodes PP = 1.0). Parsimony analysis supports the sister-group relationship of Ebenaceae and the primuloids (BS = 90) but fails to resolve the position of Sapotaceae. In Anderberg et al. (2002), the relationships of the primuloids and of Ebenaceae remain unresolved, and Sapotaceae are sister to Lecythidaceae, but with weak support (JK = 60). Sapotaceae appear as sister to the primuloids (JK = 55), and Ebenaceae are resolved as sister to Lecythidaceae, although with low statistical support (JK < 50) (Bremer et al. 2002). Relationships among the four primuloid families, with Theophrastaceae sister to a clade with Primulaceae and Myrsinaceae and Maesaceae sister to the latter three families, have been repeatedly confirmed on the basis of molecular data (Anderberg et al. 1998, 2002; Källersjö et al. 2000; Bremer et al. 2002), as they are in this study (for both nodes, PP = 1.0, BS = 100). Geuten et al. (2004) found Maesaceae (the only taxon of the primuloids in their analyses) to be strongly supported as sister to Pentaphylacaceae (represented by *Sladenia*). Further, Ebenaceae are sister to a clade with Polemoniaceae and Fouquieriaceae in their maximum likelihood topology but sister to the clade with Pentaphylacaceae and Maesaceae in their Bayesian tree. Sapotaceae were not part of the study by Geuten et al. (2004). It seems at least doubtful whether such a limited taxon sampling as used by Geuten et al. (2004) allows for unequivocal conclusions concerning the relationships in this clade.

Clade VI, the third group in clade VII, is resolved in the Bayesian topology (PP = 1.0) but not present in the parsimony strict consensus tree (fig. 1). The base of this clade is a trichotomy with Theaceae and clades IV and V, both of which are individually strongly supported by Bayesian inference (PP = 1.0) as well as parsimony analysis (BS = 100). Clade IV, comprising Symplocaceae as sister to Styracaceae and Diapensiaceae, was previously found by Bremer et al. (2002), albeit with low support (JK = 50). The sister-group relationship of Styracaceae and Diapensiaceae (PP = 1.0, BS = 98) had moderate (JK = 82; Bremer et al. 2002) to strong (JK = 94; Anderberg et al. 2002) support in earlier studies. Clade V is the ericoid group (PP = 1.0, BS = 100) that was also identified in the studies by Anderberg et al. (2002; JK = 91) and Bremer et al. (2002; JK = 90). Clade V contains six families in two well-supported subclades. The first comprises Clethraceae, Cyrillaceae, and Ericaceae (PP =

1.0, BS = 98), in which Clethraceae are sister to the two other families (PP = 1.0, BS = 98). The second includes Sarraceniaceae, Roridulaceae, and Actinidiaceae (PP = 1.0, BS = 76), in which Sarraceniaceae are sister to the two other families (PP = 1.0, BS = 100). The same topology for clade V was found by Anderberg et al. (2002) and Bremer et al. (2002). Theaceae are not resolved with respect to clades IV and V in our analysis. The strict consensus tree in Bremer et al. (2002) shows Theaceae as sister to Symplocaceae, Styracaceae, and Diapensiaceae, but with low support (JK = 52). In the study by Geuten et al. (2004), Theaceae form a clade with Symplocaceae (PP = 1.0), which conflicts with our results. Again, taxon sampling, which for clade IV and Theaceae is restricted to a single terminal per family in Geuten et al. (2004), might be a problematic issue. It has been suggested that using few terminals can lead to spurious conclusions, i.e., groupings unlikely to be recovered with denser sampling (Rydin and Källersjö 2002).

Analytical Methods and Structure of Data

The current debate concerning the validity of Bayesian support values is far from settled, and it is clear that the exact relationships between bootstrap values and posterior probabilities are complex (Suzuki et al. 2002; Cummings et al. 2003; Erixon et al. 2003) and not well understood. There are, however, two general conclusions that seem to emerge from various studies. (1) Posterior probabilities and the bootstrap frequencies behave in parallel, given sufficient data, an appropriate model of sequence evolution, and appropriate search strategies; the bootstrap values underestimate and the posterior probabilities overestimate the likelihood that any given node reflects a phylogenetic event (Suzuki et al. 2002; Cummings et al. 2003; Erixon et al. 2003). This observation is supported by empirical studies (Douady et al. 2003; Eriksson et al. 2003; Olson et al. 2003; Simmons et al. 2004) in which posterior probabilities are generally higher than bootstrap values for any given node. (2) Posterior probabilities can be higher than expected because of the selection of a model of sequence evolution that is too simple or underparameterized (Suzuki et al. 2002; Erixon et al. 2003). Despite these concerns pertaining to the explanatory power of posterior probabilities, we base the discussion of our results on the phylogenetic trees from the Bayesian analyses. One reason for this decision is that Bayesian analyses permit the incorporation of more realistic models of nucleotide substitution than parsimony analyses. In addition, the topologies of the Bayesian and the parsimony analyses are identical except for four nodes that are lacking in the strict consensus parsimony tree. The Bayesian phylogeny therefore provides a more resolved phylogenetic hypothesis for the Ericales. Finally, our approach is conservative in that the discussion is based on a "fully" supported Bayesian topology, where only clades with PP = 1.0 are considered.

A comparison of the Bayesian topologies based on our "complete" data set of Ericales (fig. 2*A*) with those based on two partial data sets (fig. 2*B*, 2*C*) provides three conclusions. (1) The addition of more data in the form of added markers improves resolution and increases the number of strongly supported clades. (2) A relatively large proportion of missing data (24.4%) does not seem to affect the topology in any unexpected way. (3) An increase in the number of taxa sampled results in better resolution and a higher number of strongly supported clades, but the increase in resolution and support is not as high as markers are added. On the basis of these three conclusions, we predict that the phylogeny of the Ericales, including its deeper nodes, eventually can be fully resolved with the help of additional markers.

Analysis of the mitochondrial *atp1* data set resulted in two topological anomalies, with *Ternstroemia* placed within Ericaceae and *Bruinsmia* as sister to *Cyrilla*. Amplification of *atp1* was done with the same total DNA extractions that were used for the amplification of other mitochondrial, chloroplast, and nuclear markers—all of which yielded the expected result of grouping *Ternstroemia* with other Pentaphylacaceae and *Bruinsmia* with other Styracaceae. Contamination of DNA samples is therefore unlikely. Processes such as horizontal gene transfer (Syvanen 1994) and lineage sorting (Pamilo and Nei 1988) can significantly alter the individual evolutionary histories of each genome (Barkman et al. 2000). Horizontal gene transfer has recently been shown to occur rather frequently, even between distantly related flowering plants (Bergthorsson et al. 2003). Four well-supported examples of horizontal gene transfer come from the mitochondrial genome, and one involves an *atp1* duplicate that has been found in *Amborella* (Barkman et al. 2000) and is interpreted to have been transferred from eudicots (Bergthorsson et al. 2003). Horizontal gene transfer could also be responsible for the anomalous positions of *Ternstroemia* and *Bruinsmia* in our *atp1* topology. We analyzed our combined data sets also without the *atp1* sequences of *Ternstroemia* and *Bruinsmia* and found the resulting topologies to be identical to the ones resulting from the complete data set. Thus, the anomalous phylogenetic signals from the two *atp1* sequences are overruled by the remainder of the data and have therefore no effect on the results presented here.

Morphological Evolution

Possible morphological synapomorphies and/or symplesiomorphies for various well-supported clades have already been outlined by Anderberg et al. (2002). Therefore, we will restrict our discussion of morphological features to clades that had previously not been recognized (clades III, IV, VI, and VII). In addition, we trace the evolution of four characters of particular interest with respect to floral evolution in the Ericales.

Clade III. Clade III includes Sapotaceae, Ebenaceae, and the four primuloid families, Maesaceae, Theophrastaceae, Primulaceae, and Myrsinaceae. The four latter families are characterized by obhaplostemonous flowers (i.e., fertile stamens are opposite the petals), free central placentation, bitegmic ovules, and nuclear endosperm formation (see also Källersjö et al. 2000; Anderberg et al. 2002). The flowers of most Ebenaceae are basically diplostemonous. In the few species with haplostemonous flowers, the fertile stamens are opposite the sepals. Sapotaceae generally have only one whorl of fertile stamens, positioned opposite the petals as in the primuloid families, and a whorl of staminodes that alternate with the fertile stamens. A whorl of staminodes is also present in

various primuloid groups. Free central placentation is clearly a synapomorphy of the four primuloid families and is absent from Ebenaceae and Sapotaceae. Ebenaceae have apical-axile and Sapotaceae have axile or basal-axile placentation. Like the primuloid families, Ebenaceae have bitegmic ovules, whereas Sapotaceae are generally reported to be unitegmic. Endosperm formation is nuclear in Sapotaceae and has been variously reported to be nuclear or cellular in Ebenaceae (Davis 1966; Yamazaki 1972; Cronquist 1981; Johri et al. 1992). At least for *Diospyros kaki*, initial nuclear endosperm formation has been reported (Fukui et al. 1991).

Clade IV. This group includes Symplocaceae, Styracaceae, and Diapensiaceae. In earlier classification systems, Symplocaceae and Styracaceae were usually thought to be closely related to Ebenaceae and Sapotaceae (Ebenales; Cronquist 1981), and a particularly close affinity of Symplocaceae and Styracaceae had been postulated by various authors (Gürke 1897; Cronquist 1981; Takhtajan 1997). However, before molecular phylogenetic analyses, Diapensiaceae have been regarded as an isolated family with affinities to Ericaceae. No one had suggested a close relationship of Symplocaceae and Styracaceae with Diapensiaceae. The three families share the combination of sympetaly, unitegmic ovules, and cellular endosperm formation (fig. 3).

Clade VI. Clade VI includes three lineages (Theaceae and clades IV and V), but relationships among them are not resolved. All families in clade VI, except for Theaceae, are characterized by unitegmic ovules and cellular endosperm. Both of these characters are otherwise rare in the Ericales and do not occur in combination elsewhere in the order (fig. 3*C*, 3*D*).

Clade VII. This clade has not been recognized before and includes all families in the order except for those in clades I and II and the Lecythidaceae (fig. 1). A possible synapomorphy for this clade is a two-whorled androecium organization, which is present in most families but subsequently lost in some (fig. 3*B*).

On tracing patterns of structural evolution. We maintain that there are four prerequisites to successfully analyze patterns of structural evolution. (1) Character evolution should be initially evaluated by tracing patterns of structural evolution on a phylogeny independent of the characters of interest (Givnish and Sytsma 1997*a*, 1997*b*; Givnish and Patterson 2000; Patterson and Givnish 2002). Whether similar patterns are found with phylogenies, including the characters of interest, is largely dependent on the nature and scope of character convergence or reversals. (2) The phylogeny must be more or less resolved, and the topology must be robust. It is preferable to maintain areas without resolution (and allow for multiple resolutions) rather than select only one of many possible topologies for analysis (as is often done). (3) The phylogeny should contain all of its major lineages. Character reconstructions become increasingly more misleading as taxa sampling declines. (4) The characters being traced must be known in as much detail as possible. This point is especially important for higher-level studies in which characters such as embryological or biochemical features, which have only been studied in one or few species of any given family, may well be polymorphic.

Obviously, these prerequisites are not always met. This analysis of Ericales largely fulfills the first three prerequisites, while prerequisite 4 is more difficult to fulfill. Even "important" characters, such as the number of integuments or petal fusion, have been studied in only a few species and often not in sufficient depth. We therefore are fully aware that the character histories we outline below are hypotheses that most likely will be adjusted as structural characters are examined in more detail and across more taxa. We discuss four floral characters because they have been used previously to define clades within Ericales and have been considered to be important at the level of Ericales and other asterids.

Corolla structure. Asterids are still commonly associated with sympetalous corollas. However, in their more recent and broader circumscriptions, sympetaly is clearly not a synapomorphy for the group, as there are various choripetalous taxa in all four major asterid lineages. In addition, there are also sporadic occurrences of sympetaly outside the asterids (some Achariaceae in Malphigiales, male flowers of Caricaceae in Brassicales, some Crassulaceae in Saxifragales, Cucurbitaceae in Cucurbitales, and Plumbaginaceae in Caryophyllales). Whether sympetaly is plesiomorphic in the asterids is currently not clear (Olmstead et al. 1993; Endress 1997).

Both choripetalous and sympetalous species are present in some cornalean families (Cornaceae, Loasaceae; Hufford 1992). In Ericales, not only do we find both choripetalous and sympetalous families, but many families or even genera are not monomorphic for this character. In clade I, Tetrameristaceae have choripetalous flowers (Cronquist 1981), whereas Marcgraviaceae can have petals distinct, partially fused, or completely fused (Ward and Price 2002). The flowers of Balsaminaceae are zygomorphic, and either the petals are distinct (*Hydrocera*) or the median petal is free, with the four remaining petals fused into lateral pairs (*Impatiens*; Grey-Wilson 1980*a*, 1980*b*). We scored Balsaminaceae as choripetalous because this partial corolla fusion seems quite different from the corolla tube formation that is generally associated with sympetaly. Lecythidaceae are either apetalous or the petals are distinct (Mori and Prance 1990; Appel 1996; Morton et al. 1997; but Frame and Durou 2001). Sympetaly defines clades II, III, and IV and Ericaceae, but sporadic occurrences of choripetaly have been reported at least for Myrsinaceae (part of *Embelia*; Walker 1940) and some Ericaceae (*Bejaria*; Kron and Chase 1993). These must be interpreted as reversals (Endress 1997). Choripetaly is apparently predominant in the clade formed by Sarraceniaceae, Roridulaceae, and Actinidiaceae. The first two families are generally considered as having distinct petals, whereas in Actinidiaceae both choripetalous and sympetalous flowers occur (Dickison 1972). Anderberg and Zhang (2002) transferred the sympetalous genus *Purdiaea* from Cyrillaceae to Clethraceae, leaving only the choripetalous genera *Cyrilla* and *Cliftonia* in the former family. In Clethraceae (i.e., in *Clethra*), both sympetalous and choripetalous flowers occur (Anderberg and Zhang 2002). The petals of the two remaining families, Theaceae and Pentaphylacaceae, are usually referred to as distinct or basally connate (Keng 1962; Cronquist 1981).

When the character states of corolla structure are mapped onto the molecular phylogeny (fig. 3*A*), it becomes apparent that sympetaly/choripetaly is homoplasious in the Ericales. The ancestral state for the order might be choripetaly, but this actually depends on how we score the outgroup, i.e., the

euasterids (scored as dimorphic; fig. 3*A*). If euasterids are scored as sympetalous, the basalmost branches in the Ericales become equivocal concerning this character. However, because many of the early-diverging groups in the otherwise predominantly sympetalous euasterids I and II, such as Icacinaceae (ordinally unplaced in euasterids I) and Cardiopteridaceae, Stemonuraceae (*sensu* Kårehed 2001), Aquifoliaceae (Aquifoliales in euasterids II), are not homogenous for this character, it is not clear whether sympetaly is actually plesiomorphic for the euasterids as a whole. If Cornales are scored as choripetalous, the basalmost node of Ericales is resolved as choripetalous, irrespective of whether euasterids are scored as sym- or choripetalous. In either case, sympetaly is the plesiomorphic character state for clade VII. Thus, the various occurrences of choripetaly, especially in clade VI, must be seen as reversals.

It appears that sympetaly is not genetically deeply rooted at this level of the asterids and that it can easily be lost and reappear (Endress 1997). Comparative studies of floral development in families where sympetaly is only weakly expressed and difficult to observe in mature flowers might eventually lead to better understanding of corolla evolution in the Ericales and in the asterids as a whole. Also, it would be interesting to know whether developmental patterns such as "early sympetaly" and "late sympetaly," as described in Erbar (1991) and Erbar and Leins (1996) for many euasterids families, can be discerned among different sympetalous groups in the Ericales.

Androecium organization. Stamen number and arrangement are important systematic characters within the Ericales. Here we distinguish between one- and two-whorled stamen arrangements. Both of these patterns can have few or numerous fertile stamens in each whorl, or one of the whorls can be represented only by staminodes, as in Theophrastaceae and some Diapensiaceae. "Haplostemony" and "diplostemony" are more narrowly defined terms, describing flowers with one or two whorls of stamens, respectively, each of which has as many stamens as petals.

In clade I, Balsaminaceae and Tetrameristaceae have haplostemonous flowers, with stamens alternating with the petals. Stamen number in Marcgraviaceae varies from haplostemony to many (Gilg and Werdermann 1925; de Roon 1970; de Roon and Dressler 1997; Dressler 2004). Using mature flowers, Gilg and Werdermann (1925) described polyandrous taxa as having the stamens arranged in a single whorl. Dressler (2004) and Endress (2003) also mention two-whorled patterns. Floral development has not yet been studied in Marcgraviaceae. For the time being, we score Marcgraviaceae as dimorphic.

In clade II, Polemoniaceae are haplostemonous throughout. In Fouquieriaceae, the flowers have 10–23 stamens that are arranged in a single whorl in mature flowers (Henrickson 1972). Ongoing studies of floral development confirm the initiation of the stamens in a single whorl (J. Schönenberger, personal observation). Lecythidaceae are characterized by complex androecia with high stamen numbers (up to 1200 in *Gustavia*), and the stamens develop centrifugally on a ring primordium (Endress 1994). On the basis of a study of floral development and anatomy, Leins (1972) interpreted the polyandrous androecium of the hexamerous flowers of *Couroupita guianensis* as derived from a single whorl of six stamens alternating with the petals, indicating a basically one-whorled organization of the androecium in Lecythidaceae.

In clade III, Sapotaceae exhibit two whorls of stamens. Hartog (1878) described the flowers of Sapotaceae as diplostemonous or obhaplostemonous. In some species, only the whorl opposite the petals is fertile, while the other whorl is staminodial (Engler 1897). Recently, floral development has been studied in the genus *Synsepalum*, in which two whorls of stamens are initiated (Caris et al. 2001). Similarly, flowers of Ebenaceae are generally reported to be diplostemonous (Gürke 1897; Cronquist 1981), and a developmental study in the family (*Diospyros kaki*) has confirmed this (Caris et al. 2001). Primuloids have only one whorl of fertile stamens, and they are positioned opposite the petals; i.e., the flowers are obhaplostemonous. Obhaplostemony is generally thought to be derived from diplostemony through the loss of the outer, episepalous stamen whorl. Many obhaplostemonous taxa do indeed have traces (e.g., in the form of staminodes) of this lost stamen whorl (Ronse Decraene and Smets 1987). In the primuloids, staminodes are present in many Theophrastaceae, in some Myrsinaceae, and in some Primulaceae (Sattler 1962; Anderberg and Ståhl 1995; Caris and Smets 2004). Maesaceae have obhaplostemonous flowers but apparently lack staminodes (Caris et al. 2000). The staminodes of *Samolus* (now in Theophrastaceae) have been interpreted as "a reminder of ancestral diplostemony" (Ronse Decraene and Smets 1995, p. 213). A two-whorled, diplostemonous pattern therefore seems to be the basic condition for the families in clade III.

In Pentaphylacaceae, the number of stamens ranges from five to many, and stamens are reported to be arranged in one or two whorls (Keng 1962). The only study of floral development in the family is Payer's (1857) description of *Visnea* as having three whorls of stamens. However, Corner (1946) reinterpreted the androecium of *Visnea* as consisting of two whorls, one of which has stamen pairs. Stamen pairs are frequently present in diplostemonous flowers (Ronse Decraene and Smets 1996). We have scored Pentaphylacaceae as dimorphic, but there is clearly a need for further study of floral development in this group.

Theaceae have many (generally >40) stamens. Early floral development has been studied in several genera, and developmental patterns of the androecium show a broad range of variation. In the flowers of *Stewartia*, five stamen fascicles are arranged in epipetalous position (Erbar 1989). Tsou (1998) distinguished two major patterns of androecium development in the family: (1) stamen primordia are arranged on a ring primordium in *Camellia*, *Polyspora*, and *Pyrenaria*, and (2) stamen primordia emerge from five separate fascicle primordia in *Hartia* and *Stewartia*. When these patterns are derived from a one- or two-whorled floral organization is currently not clear.

In clade IV, Symplocaceae are described as basically haplostemonous (Caris et al. 2002), with stamen fascicles alternating with the petals. However, Caris et al. (2002) only studied one species of *Symplocos* subg. *Hopea*, a group with stamens arranged in five fascicles. In contrast, species of subg. *Symplocos* have their stamens arranged in whorls (Brand 1907; Nooteboom 1975, 2004), and it seems likely that diplostemonous flowers are present as well. Most species in Styracaceae have twice as many stamens as petals, but higher

numbers are also present. On the basis of vascular anatomy, Dickison (1993) interpreted the flowers of Styracaceae to be of diplostemonous origin, although the stamens appear to be inserted in a single whorl in mature flowers. A study of floral development of *Styrax japonica* clearly confirmed a diplostemonous pattern (Caris et al. 2001, abstract). Diapensiaceae have one whorl of fertile stamens alternating with the petals, but most species have an additional whorl of staminodes (Rönblom and Anderberg 2002). Diapensiaceae thus are interpreted as being basically diplostemonous (Palser 1963).

In clade V, most families exhibit diplostemonous flowers. The number of stamens in Sarraceniaceae ranges from 10 to many (Macfarlane 1908; DeBuhr 1975). Shreve (1906) described the polyandrous androecium of *Sarracenia purpurea* as developing from 10 separate primary primordia, indicating a diplostemonous floral ground plan. Roridulaceae is distinct in clade V in possessing haplostemonous flowers with the stamens alternating with the petals (Dahlgren and van Wyk 1988). In Actinidiaceae, the sister group of Roridulaceae, the androecium structure is diverse, and stamen numbers range from 10 to many. Often stamens are arranged in fascicles opposite to the petals (Cronquist 1981; Takhtajan 1997). Van Heel (1987) described the stamens of *Actinidia melandra* as arranged in single whorl. Brown (1935) interpreted the flowers of *Saurauia* as diplostemonous (see also Dickison 1972). In addition, apparently diplostemonous flowers of Actinidiaceae have been described from the Late Cretaceous (*Parasaurauia*; Keller et al. 1996). We have scored Actinidiaceae as dimorphic. The remaining families of clade V (Ericaceae, Cyrillaceae, and Clethraceae) are predominantly characterized by flowers with two stamen whorls (Cronquist 1981; Kubitzki 2004; Schneider and Bayer 2004; Stevens et al. 2004).

Most euasterids, including the early-diverging lineages of both euasterids I and II (*sensu* Bremer et al. 2002; APG II 2003), such as Oncothecaceae, Icacinaceae, and Garryaceae (euasterids I) and Cardiopteridaceae and most Aquifoliaceae, Hellwingiaceae, and Phyllonomaceae (euasterids II), have haplostemonous flowers. This indicates that haplostemony is plesiomorphic for the euasterids. The flowers of most families among early-diverging Ericales have a one-whorled androecium, whereas most taxa within clade VII either are diplostemonous (Ericaceae, Styracaceae) or derive their androecia from a two-whorled organization (e.g., the obhaplostemonous flowers of the primuloid families). Using the haplostemonous euasterids to root the Ericales indicates that a haplostemony is plesiomorphic in the Ericales (fig. 3*B*). This indicates, importantly, that diplostemony or diplostemony-derived arrangement patterns have arisen within Ericales from a haplostemonous floral ground plan, a scenario that is at odds with the general belief that haplostemony cannot be reversed (for discussion, see Ronse Decraene and Smets 1995). The alternative, but considerably less parsimonious, scenario would be to have a single origin of diplostemony in this larger group of asterids, with multiple and independent shifts to haplostemony in Cornales, euasterids, and several early diverging lineages of Ericales. The idea that haplostemony cannot be reversed is probably true in groups with deeply rooted sympetaly, such as the Lamiales or Asterales. Stamens and/or stamens and petals in these asterids are synorganized in sympetalous flowers, leaving no leeway for a secondary increase in stamen number. Therefore, polyandry is absent in groups where sympetaly is well established (Endress 1997). However, sympetaly seems not to be deeply rooted or is even absent in some groups of Ericales. It therefore seems plausible that a diplostemonous floral ground plan could have evolved from a haplostemonous ancestor in the Ericales. On a more general level, the possible evolution of diplostemony from haplostemony had already been proposed by Dahlgren (1983). A diplostemonous floral ground plan is likely symplesiomorphic for clade VII, and diplostemony may have evolved along the branch to this clade (fig. 3*B*). The evolution of a diplostemonous floral organization in the Ericales also indicates that the occurrences of diplostemony are not strictly homologous across angiosperms.

Integument number. Unitegmic ovules characterize almost all members of Cornales and euasterids, and this character state is likely to be plesiomorphic in the asterids (Albach et al. 2001*b*). In Ericales, however, unitegmic and bitegmic ovules occur about equally often in reconstructions at the ordinal level (fig. 3*C*). Most families appear to be uniform for one of the two character states. The only known exceptions are Sarraceniaceae and Styracaceae. Sarraceniaceae generally are reported to have unitegmic or bitegmic ovules (Cronquist 1981; Judd et al. 2002; Kubitzki 2004). These reports, although not clearly documented, probably go back to DeBuhr (1975). He mentions both uni- and bitegmic ovules in Sarraceniaceae, but without giving any details. Shreve (1906) clearly documents *S. purpurea* to be unitegmic, and both Davis (1966) and Johri et al. (1992) indicate that Sarraceniaceae have unitegmic ovules. For the time being, we therefore scored Sarraceniaceae as unitegmic. Both uni- and bitegmic ovules are present in Styracaceae (Fritsch 2004). Bitegmic ovules are found in *Styrax*, whereas all other genera appear to be unitegmic (Dickison 1993). In spite of the predominance of unitegmic ovules in the family, Dickison (1993) argued that Styracaceae is a family that shows the evolutionary transition from bitegmic to unitegmic ovules. On the basis of our molecular phylogeny, where Styracaceae are part of a clade with otherwise unitegmic taxa, together with Diapensiaceae and Symplocaceae (clade III), it appears more likely that unitegmic ovules are in fact plesiomorphic at this level (Albach et al. 2001*b*) and that the transition is therefore likely from unitegmic to bitegmic in the family.

Mapping the number of integuments on the topology based on our combined analysis (fig. 3*C*) indicates that, in contrast to most other asterids, including the Cornales, bitegmic ovules are plesiomorphic in the Ericales. Accordingly, unitegmic ovules have reevolved at least three, maybe four, times in the order. Lineages in which unitegmic ovules evolved include clades IV and V, Sapotaceae, and Polemoniaceae (fig. 3*C*). Whether the unitegmic ovules present in members of clades IV and V evolved separately or in a common ancestor of the two clades cannot be decided on the basis of our current understanding of the group's phylogeny. Sapotaceae are generally described with unitegmic ovules (Corner 1976; Cronquist 1981; Pennington 1991; Judd et al. 2002). Detailed anatomical studies have shown this for *Manilkara zapota* (Zavaleta-Mancera and Engleman 1994) and *Mimusops elengi* (Bhatnagar and Gupta 1970). However, because Sapotaceae are embedded among bitegmic families in our phylogeny and because Engler (1897) in his treatment of the

family describes Sapotaceae as having bitegmic ovules, a closer look at ovule structure in this family is warranted. A closer examination of Polemoniaceae is also needed. The family is generally reported to possess unitegmic ovules (Davis 1966; Cronquist 1981), but an early study indicated it to be bitegmic (Schnarf 1931). These two families, like Styracaceae, may not be monomorphic with respect to number of integuments.

Endosperm formation. An asterid-wide study by Albach et al. (2001*b*) indicated that the ancestral state for endosperm formation in asterids is the cellular type, where the division of the primary endosperm nucleus is followed by wall formation. Nuclear endosperm formation, where the division of the primary endosperm nucleus is followed by free nuclear divisions (Johri et al. 1992), is thought to have arisen multiple times in the asterids (Albach et al. 2001*b*). Cellular endosperm formation in Ericales is present in clades I and II and apparently throughout clades IV and V (fig. 3*D*). In clade I, Marcgraviaceae and Balsaminaceae both have cellular endosperm. The embryology of Tetrameristaceae has not yet been studied, and the type of their endosperm formation is unknown. In clade II, Fouquieriaceae has cellular endosperm, whereas the endosperm of Polemoniaceae is nuclear. Under the assumption that cellular endosperm formation is plesiomorphic for most other major groups in the asterids, and in particular also in the euasterids, it appears that a cellular endosperm is also the plesiomorphic condition in Ericales (fig. 3*D*). Thus, it is likely that nuclear endosperm formation has arisen only once in the Ericales along the branch, giving rise to the clade comprising Lecythidaceae and clades II and VII. In this scenario, the cellular endosperm of Fouquieriaceae would be a reversal. The presence of cellular endosperm in clades IV and V must be seen as a reversal. Whether cellular endosperm has evolved separately in clade IV and V cannot be determined on the basis of our current understanding of the phylogeny.

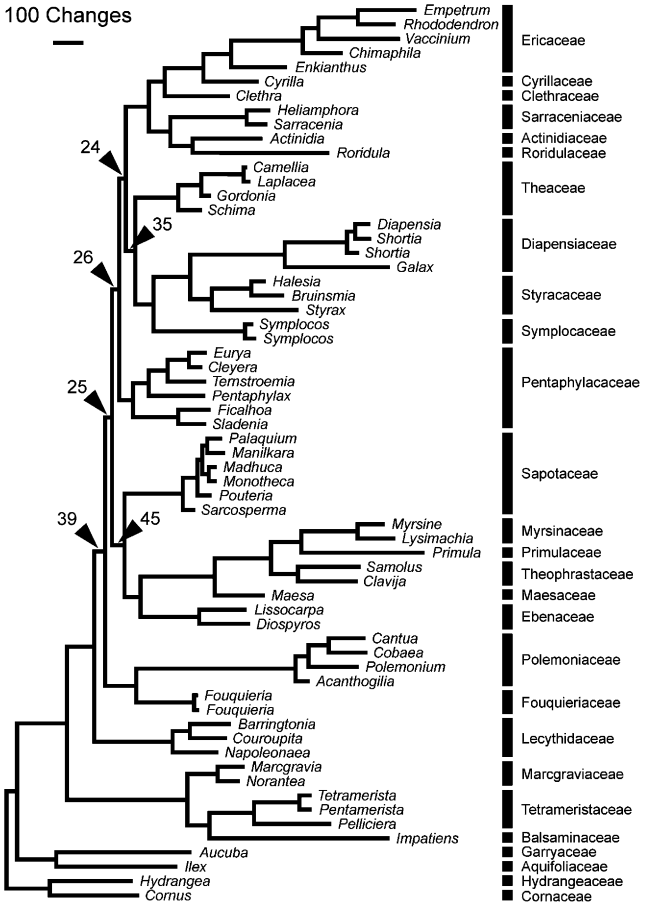

Fig. 4 Phylogram representation of one of the 55 most parsimonious trees from the maximum parsimony analysis of the combined 11-marker data set with 63 terminal taxa. Branch lengths are proportional to the number of base changes along each branch. Arrowheads and associated values (=base changes) indicate particularly short branches, i.e., branches with a low number of base changes, separating most of the deeper nodes from each other in the ericalean phylogeny.

Evolutionary History

A distinctive feature of the ericalean phylogeny are the relatively short branches among the deeper nodes (fig. 4, arrowheads), also detected by Anderberg et al. (2002) and Bremer et al. (2002). Anderberg et al. (2002) hypothesized that these short branches might be the result of a rapid radiation, i.e., that several groups branched off more or less simultaneously from the ericalean ancestral complex. We expand on that idea and discuss indirect evidence supporting this hypothesis. Within the eudicots, ericalean fossils are among the oldest, going back to the Turonian (ca. 90 Ma) in the Late Cretaceous (Magallón et al. 1999). The fossil record of the Ericales is extensive, and several families are known from the Cretaceous (Magallón et al. 1999; Schönenberger and Friis 2001). Flowers from Turonian sediments from New Jersey, assigned to *Palaeoenkianthus sayrevillensis*, are most likely related to Ericaceae (Nixon and Crepet 1993). Actinidiaceae are represented by *Parasaurauia allonensis* from the early Campanian of Georgia (Keller et al. 1996). Flowers and fruits with possible affinities to Diapensiaceae and assigned to the species *Actinocalyx bohrii* have been recovered from sediments in southern Sweden and are dated to the Santonian-Campanian (Friis 1985). From the same Swedish localities, flowers resembling those of extant Pentaphylacaceae have recently been described (*Paradinandra suecica*; Schönenberger and Friis 2001). In addition, numerous Late Cretaceous fossils with affinities to the Ericales (but unnamed or not well circumscribed) are known from various localities around the world: North America (Crepet 1996; Herendeen et al. 1999), Europe (Friis 1984; Knobloch and Mai 1986), and Asia (Takahashi et al. 1999).

As an alternative to direct fossil evidence, Bremer et al. (2004) used divergence in DNA sequence data to estimate the age of various asterid lineages. The age estimates given in Bremer et al. (2004) indicate that the crown node of the Ericales originated in the Early Cretaceous around 114 Ma, which is well in accordance with the minimum age set by the fossil record of the order. The presence of Ericaceae, Actinidiaceae, Diapensiaceae, and Pentaphylacaceae in the Late Cretaceous (90–80 Ma) indicates that there was indeed only a relatively short time span of ca. 20 Myr in the early to

mid-Cretaceous during which the main lineages could have evolved. This time span was likely considerably shorter, as Ericaceae, Actinidiaceae, and Diapensiaceae are each embedded in larger clades, indicating an even older age for the nodes giving rise to these clades and accordingly a narrower time frame for their cladogenesis, resulting in the short branches found in molecular studies.

Conclusion

At the analytical level, our results indicate that more data in the form of additional markers do improve resolution and branch support and should eventually lead to a fully resolved ericalean phylogeny. At the systematic level, these analyses present strong hypotheses for some of the deeper nodes in the ericalean phylogeny. Strongly supported groups, previously unrecognized or only weakly supported, include (1) clade VII, comprising all families except Balsaminaceae, Tetrameristaceae, Marcgraviaceae, Fouquieriaceae, Polemoniaceae, and Lecythidaceae; (2) clade III, with Sapotaceae, Ebenaceae, and the primuloid families; (3) clade IV, with Symplocaceae, Styracaceae, and Diapensiaceae; and (4) clade VI, comprising the latter three families plus Theaceae, Roridulaceae, Actinidiaceae, Sarraceniaceae, Clethraceae, Cyrillaceae, and Ericaceae. At the level of morphological evolution, we show that sympetaly, although characteristic for some of the clades, generally is a homoplasious in the Ericales. The diplostemonous floral ground plan likely arose from haplostemonous flowers along the branch, leading to clade VII. The combination of ovules with a single integument and cellular endosperm formation is characteristic for two of the major clades in Ericales. Our study also indicates the necessity of studying these morphological characters both in more structural detail and across a broader taxon sampling in order to expand, to verify, and where necessary, to correct our present knowledge on the structural evolution of the Ericales.

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