Phylogenetic Relationships of Empetraceae, Epacridaceae, Ericaceae, Monotropaceae, and Pyrolaceae: Evidence from Nuclear Ribosomal 18s Sequence Data

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Recent studies of phylogenetic relationships have indicated that the traditional recognition of Epacridaceae and Empetraceae as distinct from Ericaceae should be reevaluated. These studies used morphological data and nucleotide sequence from the chloroplast encoded *rbc*L (rubisco, large subunit) gene. They indicated that Ericaceae as presently recognized are paraphyletic and should include Epacridaceae and Empetraceae, as well as Pyrolaceae and Monotropaceae. A study of nuclear ribosomal 18s gene sequences was undertaken to test the hypothesis that Epacridaceae form a monophyletic derived group out of Ericaceae. The problematic taxa *Prionotes* and *Lebetanthus* were included because these taxa have been alternatively placed in Ericaceae and Epacridaceae. Representatives of the herbaceous (Pyrolaceae) and mycoparasitic taxa (non-chlorophyllous, Monotropaceae) were also included in the study. Taxa that represented lineages peripherally related to Ericaceae and Epacridaceae were included in order to develop a better understanding of the relationships and limits of Ericales. Parsimony analyses of 18s sequences and a combined analysis of 18s + *rbc*L sequences were performed. Results of these analyses indicate strong support for the recognition of a monophyletic Ericaceae that includes Empetraceae, Epacridaceae, Pyrolaceae, and Monotropaceae.   © 1996 Annals of Botany Company

Key words: nr18s, Empetraceae, Epacridaceae, Ericaceae, Monotropaceae, Pyrolaceae, *rbc*L

INTRODUCTION

The Ericaceae, Epacridaceae, and Empetraceae are recognized as closely related by a number of authors (Cronquist, 1981; Dahlgren, 1983; Thorne, 1992). This relationship is based on shared habit and ecology: shrubs and trees that prefer acidic soils and usually have a strong mycorrhizal association. These families also share a number of similar morphological, anatomical, and embryological characteristics such as endosperm haustoria, pollen often shed in tetrads, and the anthers inverted in development.

The close relationship of these families is emphasized by their consistent placement in the order Ericales (Table 1). Additionally, Empetraceae and Epacridaceae are consistently recognized as distinct from Ericaceae. Empetraceae have reduced perianth parts and are often wind-pollinated.

Epacridaceae are primarily Australian in distribution, however there are no unique, consistent morphological characters that distinguish this group from Ericaceae (Stevens, 1971).

Cronquist (1981) also recognized as separate families Pyrolaceae and Monotropaceae. These families contain herbaceous taxa (as compared to the usual woody habit in Ericaceae *s. st.*) and Monotropaceae are mycoparasitic, lacking chlorophyll. However, Thorne (1992) kept these taxa within Ericaceae.

Recent cladistic analysis of Ericales include studies of relationships among members of Epacridaceae, Empetraceae, and Ericaceae (including Pyrolaceae and Monotropaceae) using morphology (Anderberg, 1992, 1993; Judd and Kron, 1993). Although these studies varied in the number of taxa sampled and in the specific characters analysed they all came to the same general conclusion that Ericaceae as currently recognized are paraphyletic and that Epacridaceae and Empetraceae are derivative lineages out of Ericaceae. Anderberg (1992, 1993) and Judd and Kron (1993) also found that *Enkianthus* was sister to the remaining Ericaceae plus Epacridaceae and Empetraceae. In addition these studies indicate that recognizing Pyrolaceae and Monotropaceae as distinct from Ericaceae results in a paraphyletic Ericaceae. The inclusion of Monotropaceae and Pyrolaceae in Ericaceae has also been followed by several other investigators (Copeland, 1941; Wood, 1961; Stevens, 1971; Thorne, 1992).

Kron and Chase (1993) used *rbc*L sequence data to investigate relationships among the ericads (i.e. currently

TABLE 1. *Classifications of Ericaceae, Epacridaceae, Empetraceae and related families investigated in this study*

Family	Cronquist (1981) Order	Dahlgren (1983)	Thorne (1992)
Actinidiaceae	Theales	Ericales	Theales
Clethraceae	Ericales	Ericales	Theales
Cyrillaceae	Ericales	Ericales	Theales
Empetraceae	Ericales	Ericales	Ericales
Epacridaceae	Ericales	Ericales	Ericales
Ericaceae	Ericales	Ericales	Ericales

recognized Ericaceae, Epacridaceae, Empetraceae). Their results agree with those of Anderberg (1992, 1993) and Judd and Kron (1993) that a monophyletic Ericaceae should include Epacridaceae and Empetraceae. The *rbc*L study also found *Enkianthus* to be the sister to the remaining ericads that they sampled.

Hufford (1992) and Anderberg (1992, 1993) also analysed morphological, anatomical and embryological characters to investigate relationships of lineages more distantly related to the ericads. Hufford (1992) investigated the origins of the Asteridae (Cronquist, 1981). He found that Ericaceae are more closely related to some members of the Asteridae than to many other Dilleniidae (*sensu* Cronquist, 1981). This was supported by the results of studies based on *rbc*L sequences (Olmstead *et al.*, 1993; Chase *et al.*, 1993). Anderberg's studies (1992, 1993) of the relationships of Ericales to other orders in Cronquist's (1981) Dilleniidae found Actinidiaceae to be a suitable outgroup to the ericads. *Actinidia* is also shown to be closely related to Ericaceae *s.l.* in the analysis of *rbc*L sequences by Kron and Chase (1993).

The purpose of this study is to test whether cladistic analysis of the nuclear ribosomal (nr) 18s gene supports the hypothesis that Epacridaceae and Empetraceae are derived out of the currently recognized Ericaceae (sensu Cronquist, 1981). The relationships of the problematic genera *Prionotes* and *Lebetanthus* are included in this investigation because these genera have been considered phenetically intermediate between Epacridaceae and Ericaceae (Stevens, 1971). This study also addresses the potential of 18s data to elucidate relationships among the non-chlorophyllous Monotropaceae (Cronquist, 1981).

MATERIALS AND METHODS

Molecular data

Total DNA, representing 34 taxa, was extracted from fresh or silica gel dried (Chase and Hills, 1991) leaves using the modified CTAB method of Doyle and Doyle (1987) or, in a few cases, obtained from other workers. Taxa were chosen to represent major lineages identified by previous studies using morphology (Anderberg 1992, 1993; Judd and Kron, 1993) and *rbc*L sequences (Kron and Chase 1993; Morton, Chase and Kron, 1995). Voucher information for each taxon is listed in Table 2. The 18s gene of the nuclear ribosomal DNA was amplified using the following for each reaction: 62 μl sterile deionized water, 16 μl dNTPs mix (at 1·25 mM concentration for each dNTP), 11 μl of 10x magnesium free Taq DNA polymerase buffer, 11 μl 25 mM MgCl$_2$, 1 μl (20 $\mu$$\text{M}$) each forward and reverse 18s primers (Nickrent and Starr, 1994), and 0·5 μl of Promega Taq DNA Polymerase, 1 μl template DNA. A Coy thermocycler was programmed for the following: 94 °C for 3 min, followed by a cycle of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min with a 2 s extension time with each repeat of the cycle. This cycle was repeated 35 times. At least two PCR replicates were prepared for each taxon. The pooled amplified product was cleaned in a two-step process: (1) the pooled product was run on a 0·8 % agarose gel, stained with ethidium bromide, and the band cut out of the gel; (2) the

DNA was then cleaned again using resin (Promega Wizard PCR Prep). The cleaned double-stranded product was denatured by boiling and sequenced using the standard dideoxy technique outlined in Olmstead *et al.*, 1993. Sequencing primers were those as published in Nickrent and Starr (1994).

The nucleotide sequences of the nr 18s gene were obtained for 34 taxa (sequences are available from the author upon request). In addition, the 18s sequence of *Sarracenia* was contributed by D. Soltis, Washington State University, Pullman, WA, USA, and the 18s sequences of *Monotropa uniflora*, *Pterospora andromeda*, *Pyrola picta*, and *Sarcodes sanguinea* were made available for this analysis from D. Nickrent and A. Colwell, Southern Illinois University, Carbondale, IL, USA (see Table 2). Sequences of the chloroplast encoded *rbc*L gene were already in the possession of the author (see Kron and Chase, 1993) or were contributed by C. Morton and M. Chase, Royal Botanic Garden, Kew, UK (see Table 2). Whenever possible, the same total DNA extraction was used as template DNA for the 18s study as for the *rbc*L study.

Data analysis

Sequences were aligned visually using mungbean (obtained from GenBank, acc. X14337). Manual alignment was not difficult because single base insertion/deletions were the most common with no indels greater than two bases in length. The data were analysed using PAUP 3.1.1 (Swofford, 1993). Four searches were performed. Initial searches weighted all character-state transformations equally, gaps were treated as missing data, and only potentially informative (i.e. characters that varied by two or more bases at a position in at least two taxa) characters were used to construct the trees.

Search 1. Taxa representing major lines of evolution in Empetraceae, Epacridaceae, Ericaceae, Monotropaceae, and Pyrolaceae were analysed using *Actinidia* (Actinidiaceae) as an outgroup. The choice of *Actinidia* is supported by previous molecular (Chase *et al.*, 1993; Kron and Chase, 1993) and morphological (Anderberg, 1992, 1993; Judd and Kron, 1993) studies. The heuristic search option of PAUP was used; 100 random replicates were performed with TBR branch swapping. Using character-state transformation weighting of transitions and transversions (1:1·3) (Albert, Chase and Mishler, 1993) a subset of the population of most parsimonious trees was selected. Trees one and two steps longer than the shortest trees were also obtained (decay analysis, Mishler, Donoghue and Albert, 1991) in order to assess the relative robustness of the major branches in the trees. MacClade (Maddison and Maddison, 1993) was used to determine the number of transitions and transversions and the distribution of character-state variation within the 18s gene among the most parsimonious trees.

Search 2. The same 18s sequences of the 25 taxa in Search 1 were analysed with *Clethra* (Clethraceae) and *Cyrilla* (Cyrillaceae) as the outgroup taxa. Morphological studies (Anderberg, 1993; Judd and Kron, 1993) and traditional treatments (e.g. Cronquist, 1981) have indicated these two families as closely related to Ericaceae, Epacridaceae, and

TABLE 2. *Alphabetical list of taxa represented by 18s and/or* rbcL *sequences in this study. Taxa in bold are those analysed in the combined 18s and* rbcL *analysis. Sources with an asterisk are referred to the Appendix of* Annals of the Missouri Botanic Garden *80 (3)*, 1993

Species	Family	Voucher/source
Actinidia chinensis Planch.	Actinidiaceae	AMBG*
Arbutus menziesii Pursh	Ericaceae	RBGE 410182
Ardisia crenata Sims	Myrsinaceae	AMBG*
Bejaria racemosa Vent.	Ericaceae	AMBG*
Calluna vulgaris (L.) Hull	Ericaceae	AMBG*
Cassiope mertensiana (Bong.) G. Don	Ericaceae	AMBG*
Ceratiola ericoides Michx.	Empetraceae	AMBG*
Chamaedaphne calyculata (L.) Moench	Ericaceae	AMBG*
Chimaphila maculata (L.) Pursh	Ericaceae	Kron DNA 164, WAKE
Clethra alnifolia L.	Clethraceae	AMBG*
Cyathodes colensoi Hook.	Epacridaceae	AMBG*
Cyrilla racemiflora L.	Cyrillaceae	AMBG*
Diapensia lapponica L.	Diapensiaceae	AMBG*
Diospyros virginiana L.	Ebenaceae	AMBG*
Dracophyllum longifolium R. Br	Epacridaceae	AMBG*
Enkianthus campanulatus G. Nicholson	Ericaceae	AMBG*
Epacris impressa Labill.	Epacridaceae	AMBG*
Gaultheria eriophylla (Pers.) Sleumer	Ericaceae	AMBG*
Gaultheria miqueliana Takeda	Ericaceae	AA #1636–77
Halesia carolina L.	Styracaceae	Morton and Chase
Idria columnaris Kellogg	Fouquieriaceae	Morton and Chase
Lebetanthus myrsinites (Lam.) Duser	Ericaceae	RBGK 341059
Leucopogon fraseri A. Cunn.	Epacridaceae	AMBG*
Manilkara zapota (L.) Royen	Sapotaceae	AMBG*
Marcgravia rectiflora Triana & Planch.	Marcgraviaceae	Morton and Chase
Monotropa hypopithys L.	Monotropaceae	Kron DNA 98, WAKE
Monotropa uniflora L.	Monotropaceae	Colwell and Nickrent
Pentachondra pumila (J. R. & G. Forst.) R. Br.	Epacridaceae	AMBG*
Prionotes cerinthoides R. Br.	Ericaceae	RBGK 1984–4432
Pterospora andromeda Nuttall	Monotropaceae	Colwell and Nickrent
Pyrola rotundifolia L.	Pyrolaceae	AMBG*
Pyrola picta Smith	Pyrolaceae	Nickrent
Rhododendron hippophaeoides Balfour F. & Forrest	Ericaceae	AMBG*
Sarcodes sanguinea Torrey	Monotropaceae	Colwell and Nickrent
Sarracenia purpurea L.	Sarraceniaceae	Soltis
Stewartia malacodendron L.	Theaceae	Kron 3019, WAKE
Styrax americana Lam.	Styracaceae	AMBG*
Symplocus paniculata Miq.	Symplocaceae	AMBG*
Vaccinium macrocarpon Aiton	Ericaceae	AMBG*

Abbreviations: AMBG, Annals of the Missouri Botanic Garden; AA, Arnold Arboretum; RBGE, Royal Botanic Garden Edinburgh; RBGK, Royal Botanic Garden Kew.

Empetraceae. Trees were constructed using 100 random replicates of the heuristic search option and TBR branch swapping. A subset of trees was obtained based on transition:transversion weighting as in Search 1.

Search 3. The 18s sequences of 39 taxa were analysed. This search included the same taxa as in Searches 1 and 2 in addition to eleven taxa that represent lineages that have been suggested as related to Ericales (*sensu* Cronquist, 1981; see Table 2). A heuristic search of 100 random replicates and TBR branch swapping was performed. The trees were unrooted. As in the two previous searches transition:transversion weighting was used to select among the population of most parsimonious trees initially obtained.

Search 4. A combined data set of 18s sequences and rbcL

sequences for 27 taxa was analysed. In this analysis only taxa for which both rbcL and 18s sequences were available were used. Thus the non-chlorophyllous ericads (*Monotropa*, *Pterospora*, *Sarcodes*) were omitted from the analysis. The same search strategy was performed as in searches 1–3. Trees that were from one to three steps longer than the most parsimonious tree were obtained to assess clade robustness (decay analysis).

RESULTS

Search 1

Thirty most parsimonious trees (L = 409, c.i. = 0·616, r. i. = 0·498) were found. The strict consensus and one of the
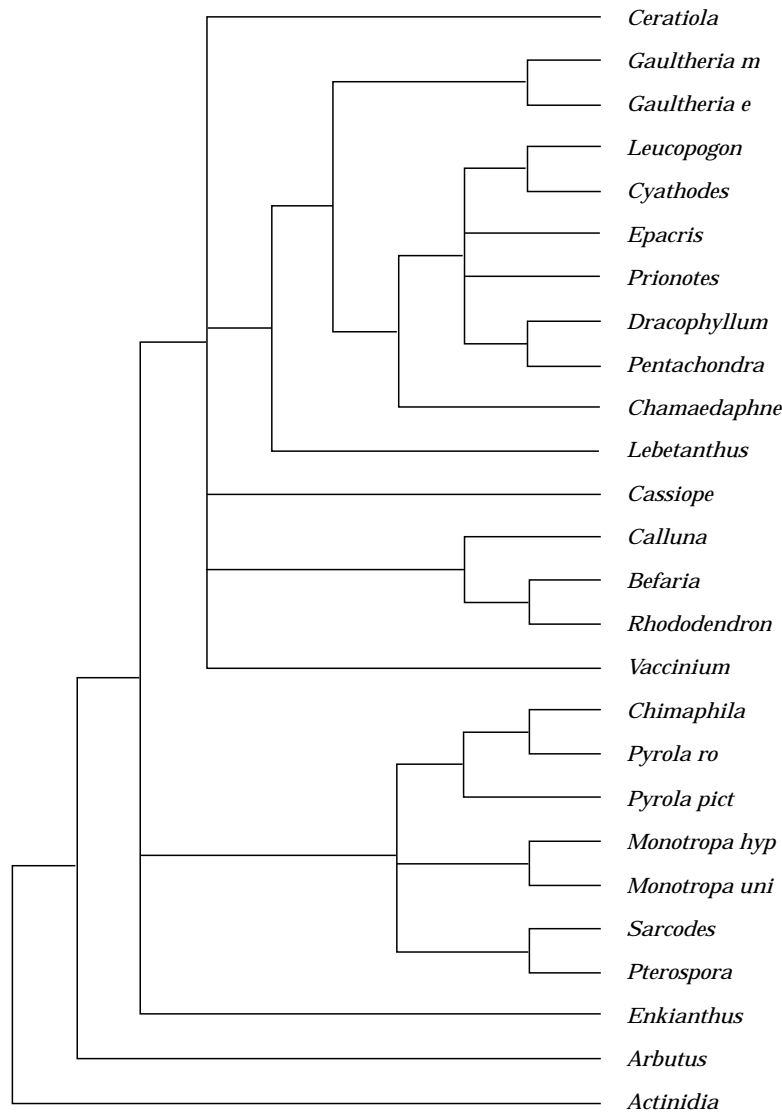
FIG. 1. Strict consensus of 30 most parsimonious trees found in Search 1. Character state transformations were equally weighted, gaps treated as missing data.

most parsimonious trees can be seen in Figs 1 and 2. MacClade (Maddison and Maddison, 1993) found 203–217 unambiguous transitions and 148–152 unambiguous transversions among the 30 most parsimonious trees. Highly variable regions are found in regions coding for both single and double stranded rRNA. One of the most variable regions corresponds to the highly variable V4 region noted in Nickrent and Starr (1994). When transversions were weighted more heavily than transitions four trees were found (Fig. 3). Upon examination these four trees were among the 30 most parsimonious trees found in the initial search. In all of the most parsimonious trees *Arbutus* is sister to the remaining ericads. The position of *Enkianthus* is unresolved in the strict consensus of the 'unweighted' trees, however the four 'weighted' trees indicate *Enkianthus* as the next branch after *Arbutus* and before the remaining ericads (see Fig. 3). The Pyrolaceae and Monotropaceae form a

clade in all most parsimonious trees. However, the strict consensus of the 30 most parsimonious trees indicates that the relationship of Pyrolaceae to the *Monotropa* clade and the *Sarcodes* + *Pterospora* clade is not resolved. This is in constrast to the 'weighted' trees that indicate Monotropaceae as paraphyletic to Pyrolaceae. The Epacridaceae is nested well within the remaining ericad clade. Within this group the relative positions of *Ceratiola* (Empetraceae), *Cassiope*, *Vaccinium*, and the *Calluna* + *Bejaria* + *Rhododendron* clade are not resolved with respect to the clade containing the epacrids. In all of the 30 most parsimonious trees *Lebetanthus* lies outside of the genera traditionally included in Epacridaceae. It is sister to *Gaultheria* + *Chamaedaphne* + Epacridaceae. *Prionotes* consistently falls within the epacrid clade in all of the most parsimonious trees.
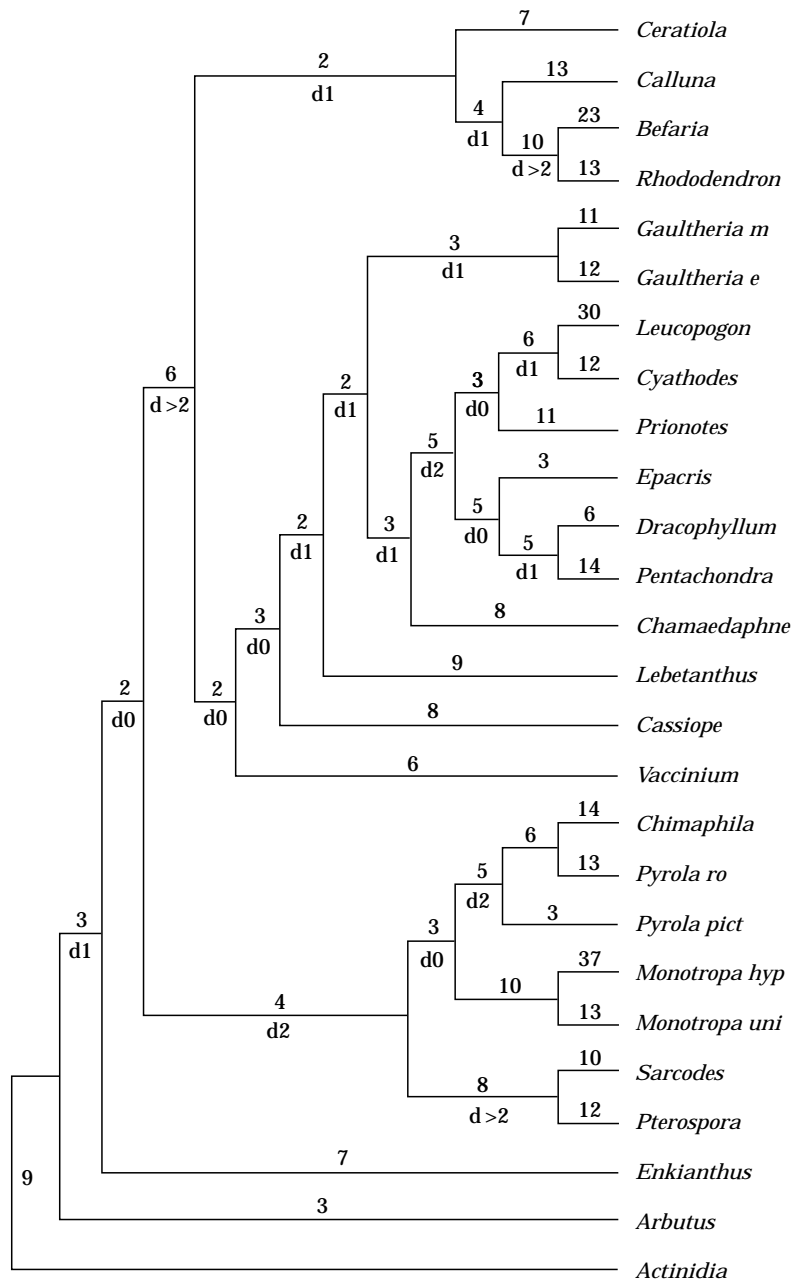
FIG. 2. One of the 30 most parsimonious trees (equal weights) found in Search 1 (L = 409, c.i. = 0·616, r.i. = 0·498). Numbers above clades represent branch lengths, numbers below clades indicate decay values.

Search 2

In this search 142 most parsimonious trees were found (L = 302, c. i. = 0·44, r. i. = 0·488). A strict consensus (Fig. 4) shows that *Enkianthus* is placed between *Clethra* and *Cyrilla* in all of these trees. Weighting of transitions and transversions selected 52 trees from among the 142 most parsimonious trees. In a strict consensus of these trees (Fig. 5) the relationships indicated among the ericad + epacrid group are the same as those of Search 1.

Search 3

The results of this search were highly unresolved (trees not shown). Of the 464 trees found (L = 453, c. i. = 0·386, r. i. = 0·465), four were selected by the weighting criterion. A strict consensus of these trees was slightly more resolved, but most of the relationships indicated are not supported by any other molecular or morphological data. This may be due to uneven sampling and would thus indicate a need for more intensive representation of Diapensiales, Ebenales, Theales, and other groups suggested as related to Ericales in future studies. Additionally, the relatively high level of homoplasy may indicate that some regions of the 18s
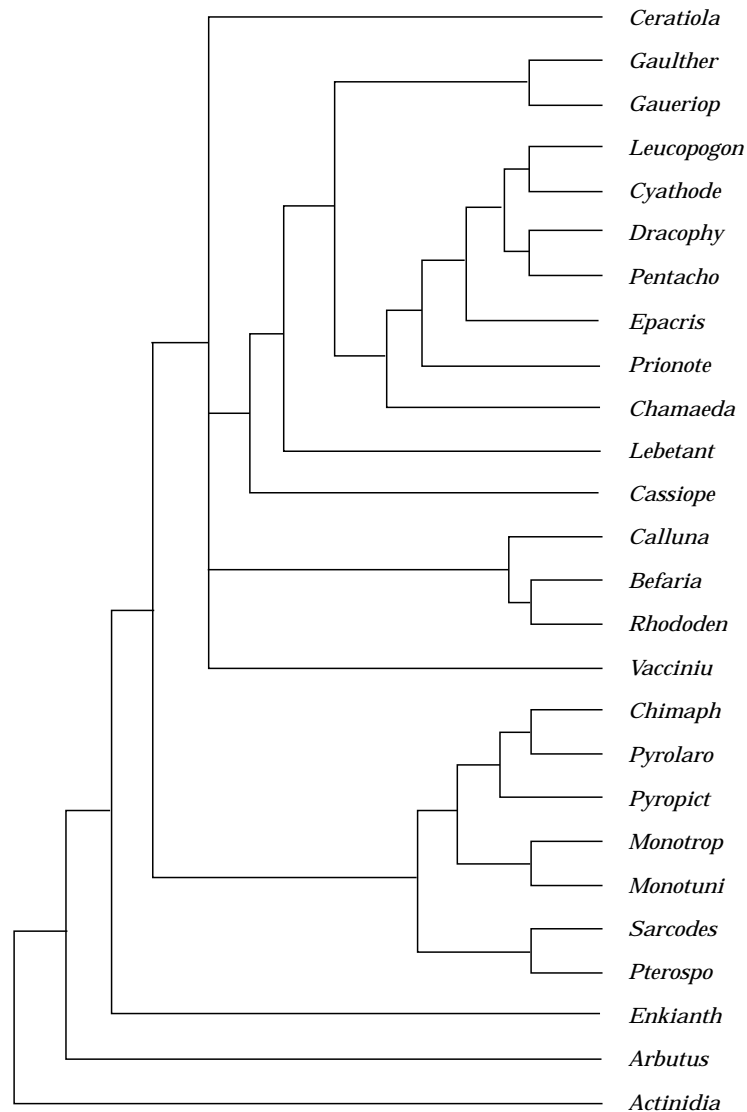
Ceratiola

Gaulther

Gaueriop

Leucopogon

Cyathode

Dracophy

Pentacho

Epacris

Prionote

Chamaeda

Lebetant

Cassiope

Calluna

Befaria

Rhododen

Vacciniu

Chimaph

Pyrolaro

Pyropict

Monotrop

Monotuni

Sarcodes

Pterospo

Enkianth

Arbutus

Actinidia

Fɪɢ. 3. Strict consensus of the four trees obtained from the 30 most parsimonious trees in Search 1 (18s data, equal weights) when transition/transversion weighting criterion is applied.

sequence are extremely variable while others are essentially invariant. Nickrent and Soltis (1995) have shown that sequence variation is not as evenly distributed in the 18s gene as it is in *rbc*L. Their analysis of 18s sequences of 62 taxa of seed plants show that regions of variability are interspersed with extremely conserved regions through the length of the 18s rDNA.

Search 4

A single most parsimonious tree (Fig. 6) was found as a result of this combined 18s + *rbc*L analysis (L = 933, c. i. = 0·439, r. i. = 0·531). In this tree *Clethra* and *Cyrilla* branch sequentially at the base of the ericad clade. The branch leading to *Clethra* + *Cyrilla* + ericads collapses in trees two steps longer. Interestingly the support for *Enkianthus* as sister to the remaining ericads is stronger in this analysis than in the *rbc*L analysis (Kron and Chase, 1993) or in Searches 1 or 2 of the 18s analyses. In the combined data

tree *Actinidia* is more distantly placed from the ericads than was indicated by the *rbc*L data alone. Relationships that are indicated in both the separate analyses of 18s and *rbc*L data are the derivation of Epacridaceae out of Ericaceae *s. st.* and the derivative position of the 'rhododendroid' clade.

DISCUSSION

Epacridaceae

In all of the 18s-based searches *Prionotes* forms a clade with the remaining Epacridaceae, excluding *Lebetanthus*. In the strict consensus of weighted trees obtained in searches 1–3 *Lebetanthus* is sister to *Gaultheria* + *Chamadaphne* + Epacridaceae (including *Prionotes*). *Prionotes* is consistently placed as sister to the epacrids in all of the weighted trees (searches 1–3). Support for the position of *Lebetanthus* is weak. Its relationship to *Gaultheria*, *Chamaedaphne* and the epacrids collapses in trees one step longer than most
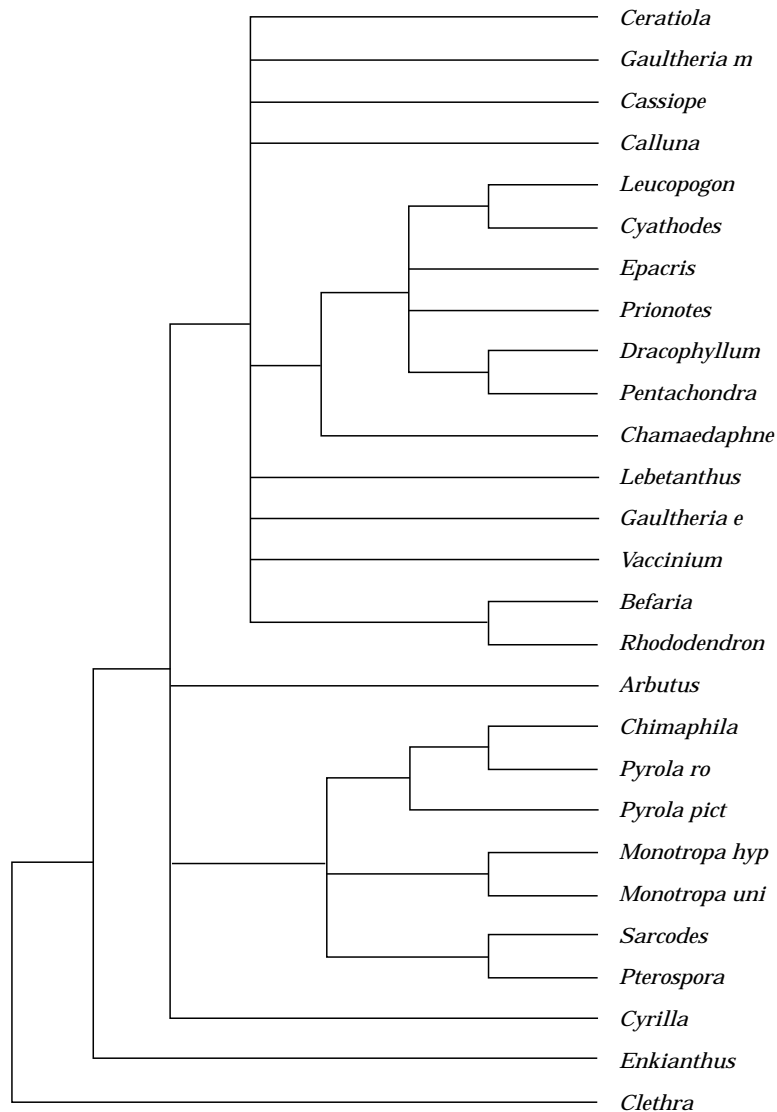
FIG. 4. Strict consensus of 142 most parsimonious trees (L = 302, c.i. = 0·44, r.i. = 0·488) found in Search 2 (18s data).

parsimonious in all searches. However, *Lebetanthus* is consistently placed outside of the 'core' epacrid clade. Stevens (1971) noted that the anatomical characters of *Lebetanthus* indicated a relationship with Epacridaceae, but that the general external morphology was quite similar to *Gaultheria*. *Chamaedaphne* is placed as sister to the epacrids in all of the most parsimonious trees found in the 18s analyses, but this relationship collapses in trees one step longer than most parsimonious in searches 1–3 as well. The general placement of Epacridaceae near the Andromedeae and Vaccinieae (*sensu* Stevens, 1971) is in agreement with the results of Anderberg's (1993) morphological study. This relationship was also indicated in the trees obtained in Kron and Chase (1993) using *rbc*L data. In the combined 18s + *rbc*L search (search 4 of this study) the relationship of the Vaccinieae (*Vaccinium*) and Andromedeae (*Chamae-daphne*, *Gaultheria*) to Epacridaceae is maintained in trees greater than three steps longer than the most parsimonious tree (Fig. 6). However the long branches that lead to the terminal taxa suggest caution in the interpretation of these results. Additionally, it is likely that *Gaultheria* is para-phyletic (e.g. Middleton and Wilcock, 1990) and in this analysis only one temperate representative of each of these highly diverse tribes (Andromedeae, Vaccinieae) is repre-sented. A future analysis of relationships will include more taxa in the Andromedeae and Vaccinieae that have a primarily southern hemispheric geographic distribution. Although the detailed relationships between the Andro-medeae, Vaccinieae, and Epacridaceae need additional investigation it is clear from this study and others (Anderberg, 1992, 1993; Chase *et al.*, 1993; Judd and Kron, 1993; Kron and Chase, 1993) that Epacridaceae are derived out of Ericaceae. The recognition of Epacridaceae as a distinct family makes Ericaceae paraphyletic and mis-represents the evolutionary history of this group. Although it can be argued that Ericaceae should be split into smaller families, thus leaving Epacridaceae recognized at the family level, the relationships in much of Ericaceae outside of
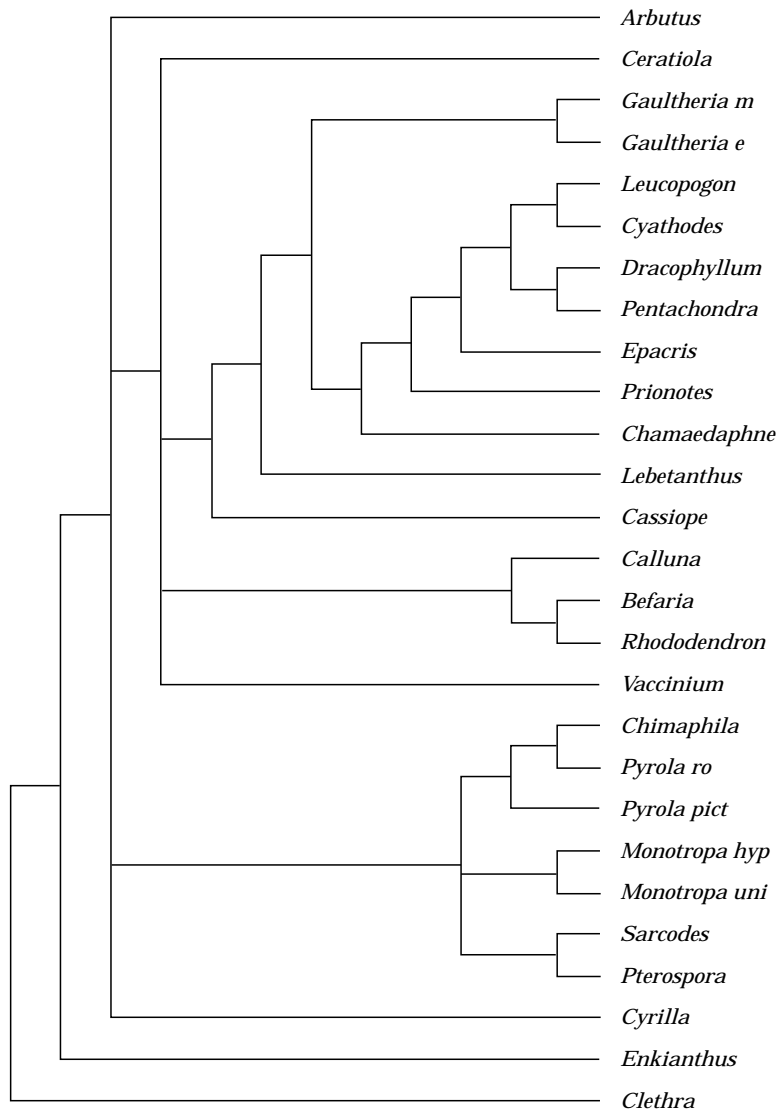
FIG. 5. Strict consensus of the 52 trees obtained from the 142 mp trees in Search 2 (18s data, equal weights) when transition/transversion weighting criterion is applied.

Epacridaceae are not sufficiently understood to formally name smaller monophyletic subsets.

Empetraceae

Anderberg (1993, 1994) has demonstrated support for the monophyly of Empetraceae (*Empetrum*, *Ceratiola*, *Corema*). This family has been segregated from Ericaceae based on the reduced perianth and wind-pollination (except in some members of *Empetrum*). In the analyses of the 18s data *Ceratiola* is consistently placed within the clade that contains *Vaccinium*, *Cassiope*, *Calluna*, *Bejaria* + *Rhododendron*, and remaining Andromedeae + Epacridaceae. Within this group the position of *Ceratiola* is unresolved in the strict consensus of any of the most parsimonious trees in searches 1 and 2 (weighted or unweighted). However, in the tree obtained in the combined 18s and *rbc*L analysis *Ceratiola* is sister to *Rhododendron* + *Calluna* (Fig. 6). This clade is maintained in

trees greater than three steps longer than most parsimonious. The relationship of *Ceratiola* to members of the Rhododendroideae (Stevens, 1971) is also suggested by Anderberg's (1993) study. Future studies of the detailed relationships among Empetraceae and the rhododendroid ericads will be investigated using the more variable *mat*K gene. Nevertheless, it is clear that Empetraceae, represented by *Ceratiola*, should be included within Ericaceae as suggested by previous studies (Anderberg, 1993; Judd and Kron, 1993; Kron and Chase, 1993).

Pyrolaceae and Monotropaceae

In all of the most parsimonious trees found in the analyses of 18s data (Figs 1–5) *Pyrola* and *Chimaphila* form a clade with the non-chlorophyllous taxa (*Monotropa*, *Pterospora*, *Sarcodes*). In those trees selected by the weighting criteria (Figs 3 and 5) the relationships indicate a paraphyletic
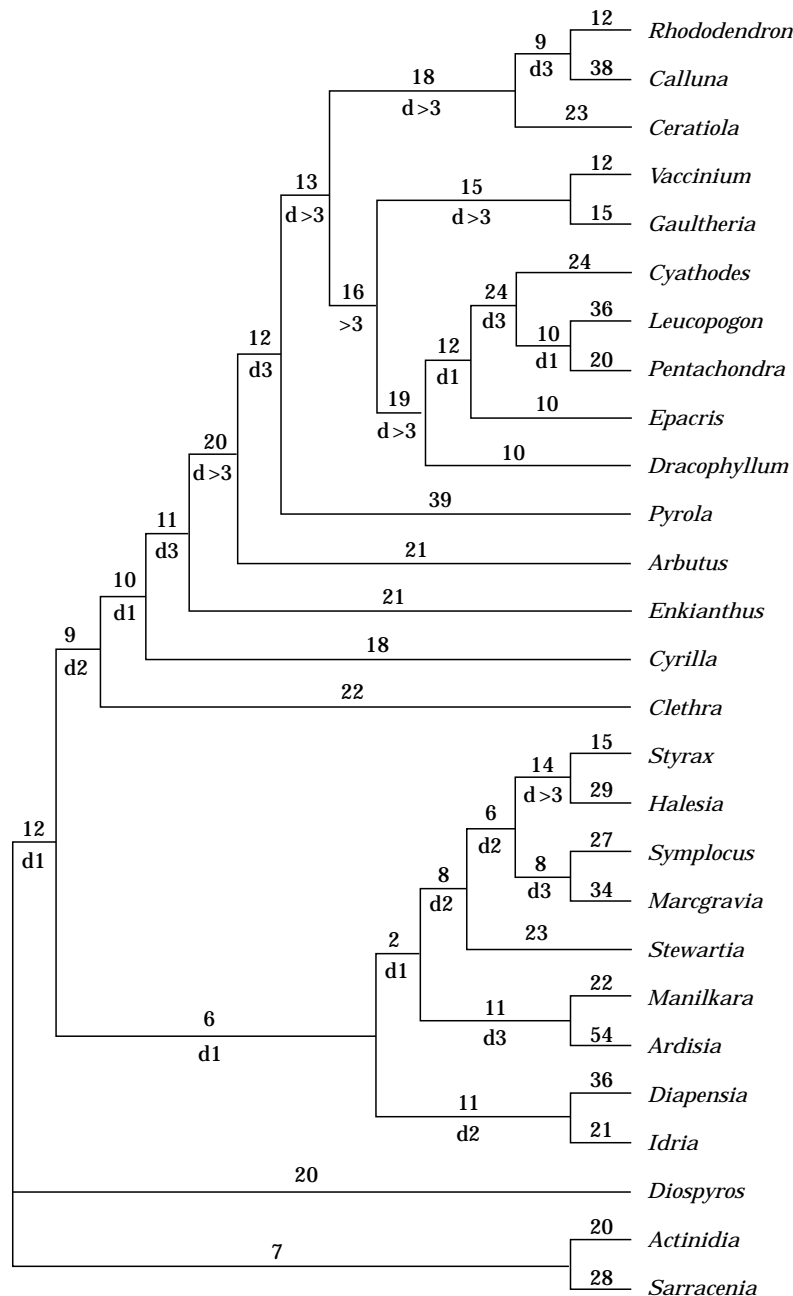
FIG. 6. Single most parsimonious tree (L = 933, c.i. = 0·439, r.i. = 0·531 obtained in Search 4 (combined 18s and *rbc*L data). Numbers above clades indicate branch lengths, numbers below indicate decay values.

Monotropaceae. The same relationship of *Pterospora* + *Sarcodes* as sister to *Monotropa* + *Chimaphila* was found by Anderberg (1993). This suggests that photosynthesis has been lost at least twice in the herbaceous Pyrolaceae + Monotropaceae group. Studies by Wolfe, Morden and Palmer (1992) have shown that in the non-chlorophyllous *Epifagus* (Scrophulariaceae) the genes involved in photo-synthesis have been lost from the chloroplast genome. If this is the case in the non-chlorophyllous ericads there would appear to be little chance of regaining the ability to photosynthesize in Pyrolaceae, as might be implied by the tree. It is also interesting to note that *Pterospora* is somewhat woody, while *Monotropa* is herbaceous, further emphasizing the general trend in the reduction from woody to herbaceous. The sister relationships between Pyrolaceae and *Monotropa* is also supported by evidence from the morphological study of Judd and Kron (1993) where *Pyrola* and *Monotropa* shared the following characters: lack of a fibre sheath in the leaves and the presence of an endothecium. The paraphyletic nature of Monotropaceae has also been suggested by Cullings and Bruns (1992) in an analysis of partial nr 28s sequences. Copeland (1941) also considered Monotropaceae to have evolved along parallel lines. Certainly more sampling is necessary before detailed relationships among the non-

T A B L E 3. *Comparison of families included in the order Ericales according to author*

Cronquist (1981)	Dahlgren (1983)	Thorne (1992)
—	Actinidiaceae	—
Clethraceae	Clethraceae	—
Cyrillaceae	Cyrillaceae	—
Empetraceae	Empetraceae	Empetraceae
Epacridaceae	Epacridaceae	Epacridaceae
Ericaceae	Ericaceae	Ericaceae
—	Diapensiaceae	—
Grubbiaceae	—	—
Monotropaceae	Monotropaceae	-in Ericaceae-
Pyrolaceae	Pyrolaceae	-in Ericaceae-
—	Roridulaceae	—

chlorophyllous taxa and Pyrolaceae can be determined. The long branches (relative to the short internal ones) leading to the terminal taxa within Pyrolaceae + Monotropaceae clade indicate that branch attraction problems may have influenced the topology. Although not addressed in this study, compensatory changes in double-stranded regions of the 18s molecule may be one of the possible sources of these long branches. These factors will be investigated in future studies that sample Monotropaceae and Pyrolaceae more intensively. However the results of the present study indicate that 18s is likely to be an informative source of phylogenetic information within this group.

Enkianthus *and* Arbutus

The 18s analyses (Figs 1 and 4) in this study are indecisive in determining whether *Enkianthus* or *Arbutus* is sister to the remaining ericads. In the combined 18s + *rbc*L analysis (Fig. 6) *Enkianthus* is sister to *Arbutus* + remaining ericads. Although this relationship is supported in trees three steps longer than the most parsimonious tree, given the large size of the data matrix, the actual percent of character-state change necessary to collapse the branch leading to *Enkianthus* is quite small. As noted in Olmstead and Palmer (1994) increased taxon sampling can increase phylogenetic information by increasing the number of potentially informative characters, previously considered autapomorphic in an analysis with fewer taxa. However, base substitution rates in 18s are significantly lower than in *rbc*L (Nickrent and Soltis, 1995) so that additional 18s sequences of taxa in the ericad lineage are unlikely to provide additional support for a sister relationship of *Enkianthus* to the rest of the ericads.

Actinidia, Clethra *and* Cyrilla

The Cyrillaceae (*Cyrilla*) and Clethraceae (*Clethra*) are often closely associated with or included in Ericales (Table 3). The single most parsimonious tree obtained from the combined 18s + *rbc*L analysis (Fig. 6) indicates *Cyrilla* as sister to the ericads, with *Clethra* branching just below *Cyrilla*. This is different from the Kron and Chase (1993) and Morton *et al.* (1995) *rbc*L studies where *Clethra* is

several nodes removed from Ericaceae *s.l.* It is also different from Anderberg's (1993) morphological study that found *Clethra* as sister to Ericaceae *s.l.*

Actinidia (Actinidiaceae) has also been closely associated with Ericales (Table 3). Cronquist (1981) and Thorne (1992) both place the Actinidiaceae in the Theales, but acknowledge the morphological similarities between *Actinidia* and Ericaceae. Dahlgren (1983) placed Actinidiaceae in his Ericales. The results of the *rbc*L study (Kron and Chase, 1993) indicate *Actinidia* as sister to Ericaceae *s.l.*, but the combined analysis of 18s + *rbc*L indicate a more distant relationship of *Actinidia* to the ericads. The relationships indicated for *Actinidia* and many of the outlying taxa included in this analysis are unresolved in trees one step longer than most parsimonious. Therefore the exact relationships of *Actinidia*, *Clethra* and *Cyrilla* to Ericaceae *s.l.* are not resolvable at this time.

CONCLUSIONS

This study indicates that nr 18s data is phylogenetically informative in the identification of Monotropaceae + Pyrolaceae clade and the placement of Empetraceae and Epacridaceae within Ericaceae *s.l.* However the lack of resolution in the results of search 3 (and to some extent search 2) indicate that the 18s rDNA sequence data did not support any consistent relationships among taxa suggested by previous studies as early branching lineages within Ericaceae *s.l.* (Anderberg, 1992, 1993; Judd and Kron, 1993; Kron and Chase, 1993) or closely related families such as the Cyrillaceae and Clethraceae (Anderberg, 1992, 1993; Chase *et al.*, 1993; Judd and Kron, 1993; Kron and Chase, 1993).

Although details of relationships differ somewhat among analyses of different data sets, it is clear that a monophyletic Ericaceae should include Epacridaceae, Empetraceae, Pyrolaceae and Monotropaceae. Future studies that might include investigation of biogeography or the evolution of mycorrhizal associations, plant pathogens, or floral development in 'traditional' Ericaceae must also take into account these derivative lineages of ericads as well.

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