

PHYLOGENETIC RELATIONSHIPS IN SAXIFRAGACEAE SENSU LATO: A COMPARISON OF TOPOLOGIES BASED ON 18S rDNA AND rbcL SEQUENCES¹

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Relationships among the morphologically diverse members of Saxifragaceae sensu lato were inferred using 130 18S rDNA sequences. Phylogenetic analyses were conducted using representatives of all 17 subfamilies of Saxifragaceae sensu lato, as well as numerous additional taxa traditionally assigned to subclasses Magnoliidae, Caryophyllidae, Hamamelidae, Dilleniidae, Rosidae, and Asteridae. This analysis indicates that Saxifragaceae should be narrowly defined (Saxifragaceae sensu stricto) to consist of ~ 30 herbaceous genera. Furthermore, Saxifragaceae s. s. are part of a well-supported clade (referred to herein as Saxifragales) that also comprises Iteoideae, Pterostemnoideae, Ribesioideae, Penthoroideae, and Tetracarpaeoideae, all traditional subfamilies of Saxifragaceae sensu lato, as well as Crassulaceae and Haloragaceae (both of subclass Rosidae), Paeoniaceae (Dilleniidae), and Hamamelidaceae, Cercidiphyllaceae, and Daphniphyllaceae (all of Hamamelidae). The remaining subfamilies of Saxifragaceae sensu lato fall outside this clade. *Francoa* (Francooideae) and *Bauera* (Baueroideae) are allied, respectively, with the rosid families Greyiaceae and Cunoniaceae. *Brexia* (Brexioideae), *Parnassia* (Parnassioideae), and *Lepuropetalon* (Lepuropetaloidae) appear in a clade with Celastraceae. Representatives of Phyllonomoideae, Eremosynoideae, Hydrangeoideae, Escallonioideae, Montinioideae, and Vahlloideae are related to taxa belonging to an expanded asterid clade (Asteridae sensu lato). The relationships suggested by analysis of 18S rDNA sequences are highly concordant with those suggested by analysis of *rbcL* sequences. Furthermore, these relationships are also supported in large part by other lines of evidence, including embryology, serology, and iridoid chemistry.

Key words: DNA sequencing; phylogeny; 18S rDNA; Saxifragaceae s. l.

The angiosperm family Saxifragaceae has putatively played a pivotal role in the evolution of major angiosperm lineages including Myrtales, Cornales, Celastrales, Gentianales, and Campanulales (e.g., Takhtajan, 1969, 1980). Until recently, however, the affinities and circumscription of Saxifragaceae were poorly understood and extremely problematic. The magnitude and diversity of systematic problems posed by Saxifragaceae have been extensively reviewed (Morgan and Soltis, 1993; Soltis et al., 1993). In this paper, we will use the traditional classification scheme of Schulze-Menz (1964) as a point of reference and refer to it as Saxifragaceae sensu lato (Saxifragaceae s. l.). Following this scheme, the family is broadly defined, consisting of 17 subfamilies (Table 1).

Recent molecular systematic studies involving the chloroplast genome or genes included therein have helped to clarify the circumscription and relationships of the morphologically diverse members of Saxifragaceae s. l. (Downie et al., 1991; Chase et al., 1993; Morgan and

Soltis, 1993; Soltis et al., 1993; Johnson and Soltis, 1994, 1995). These same studies also indicate that other subfamilies of Saxifragaceae s. l. are only distantly related to Saxifragaceae s. s. For example, Francooideae and Baueroideae are related to the rosid families Greyiaceae and Cunoniaceae, respectively; Brexioideae, Parnassioideae, and Lepuropetaloidae are closely related to Celastraceae (Fig. 1). Analyses of *rbcL* sequences (Chase et al., 1993; Morgan and Soltis, 1993; Xiang et al., 1993; Hirsch-Jetter, Soltis, and MacFarlane, in press) further suggest that representatives of other members of Saxifragaceae s. l. (Hydrangeoideae, Phyllonomoideae, Escal-

TABLE 1. Subfamilies of Saxifragaceae s. l. following Schulze-Menz (1964).

| Subfamily | No. of genera |
|-------------------|---------------|
| Saxifragoideae | 30 |
| Penthoroideae | 1 |
| Tetracarpaeoideae | 1 |
| Ribesioideae | 1 |
| Iteoideae | 1 |
| Pterostemnoideae | 1 |
| Baueroideae | 1 |
| Francooideae | 2 |
| Brexioideae | 3 |
| Lepuropetaloidae | 1 |
| Parnassioideae | 1 |
| Hydrangeoideae | 17 |
| Phyllonomoideae | 1 |
| Escallonioideae | 14 |
| Montinioideae | 2 |
| Vahlloideae | 1 |
| Eremosynoideae | 1 |

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lonioideae, Montinioideae, Vahloideae, and Eremosynoideae) are part of an expanded asterid clade (Figs. 1, 2) (Asteridae sensu lato of Olmstead et al., 1992, 1993).

Based on these data, Saxifragaceae should be narrowly defined (Saxifragaceae sensu stricto) to consist of \approx 30 genera of herbaceous perennials, a circumscription that corresponds closely both to the traditional Saxifragoideae and to recent delimitations of the family (Takhtajan, 1987; Thorne, 1992). Broad analyses of *rbcL* sequences (Morgan and Soltis, 1993; Chase et al., 1993) suggest that Saxifragaceae sensu stricto (Saxifragaceae s. s.) are closely related to several subfamilies of Schulze-Menz's Saxifragaceae s. l. (Iteoideae, Pterostemnoideae, Ribesioideae, Penthorioideae, and Tetracarpaeoideae), as well as to Crassulaceae and Haloragaceae (Rosidae), Paeoniaceae (Dilleniidae), Hamamelidaceae, Cercidiphyllaceae, and Daphniphyllaceae (Hamamelidae) (Fig. 1).

Many of the relationships suggested by *rbcL* sequences for subfamilies of Saxifragaceae s. l. are supported by other lines of evidence, particularly embryology, serology, and iridoid chemistry (Morgan and Soltis, 1993). However, some relationships suggested by *rbcL* sequences require further testing via additional phylogenetic analyses. Particularly unexpected were the relationships of subfamilies Phyllonomoideae, Montinioideae, Vahloideae, and Eremosynoideae to members of Asteridae. Also intriguing was the close relationship of Saxifragaceae s. s. to members of subclasses Dilleniidae and Hamamelidae, in addition to their relatives in Rosidae.

Comparison of phylogenetic inferences based on chloroplast and nuclear markers may provide critical insights into relationship and evolution. At lower taxonomic levels (genus and below) such comparisons have variously revealed high levels of concordance (e.g., Baldwin, 1992; Kim and Jansen, 1994; Bayer, Soltis, and Soltis, 1996) or significant discordance (e.g., Soltis and Kuzoff, 1995). At higher taxonomic levels, few such comparisons have been conducted (Nickrent and Soltis, 1995). Several recent papers have suggested that analysis of complete 18S rDNA sequences will provide resolution of relationships at the family level or above (e.g., Nickrent and Franchina, 1990; Chaw et al., 1995; Nickrent and Soltis, 1995; Kron, 1996). We therefore compared the chloroplast-based topologies for members of Saxifragaceae s. l. with those based on 18S rDNA sequences. This comparison involved a series of broad phylogenetic analyses of 18S rDNA sequences for 130 species representing all six subclasses of dicots (sensu Cronquist, 1981). The phylogenetic hypotheses generated in these analyses of 18S rDNA sequences were then compared with those based on analyses of *rbcL* sequences (Chase et al., 1993; Morgan and Soltis, 1993). Specifically, we assessed whether analyses of 18S rDNA and *rbcL* sequences both revealed: (1) the pronounced polyphyly of Saxifragaceae s. l.; (2) similar relationships of members of Saxifragaceae s. l. with diverse elements of Rosidae, Dilleniidae, Hamamelidae, and Asteridae; (3) the same narrow circumscription of Saxifragaceae; and (4) a similar suite of close relatives of Saxifragaceae s. s.

MATERIALS AND METHODS

Plant samples—We generated 18S rDNA sequences for at least one representative of each of the 17 subfamilies of Saxifragaceae s. l. (Table

2). Because 14 of the subfamilies of Saxifragaceae s. l. consist of only one or two genera (Table 1), the single 18S rDNA sequence obtained for many subfamilies represents a complete or nearly complete generic sampling. The largest subfamilies are Saxifragoideae and Hydrangeoideae, comprising \approx 30 and 17 genera, respectively; we included seven representatives of Saxifragoideae and two of Hydrangeoideae.

The numerous classifications suggested for Saxifragaceae (reviewed in Morgan and Soltis, 1993) have resulted in a long list of possible allies for the family. We therefore obtained 18S rDNA sequences for representatives of many families considered closely related to subfamilies of Saxifragaceae s. l. in traditional schemes of classification. In choosing additional families to be analyzed, we also relied heavily on broad phylogenetic analyses of *rbcL* sequences (Chase et al., 1993; Morgan and Soltis, 1993; Olmstead et al., 1993). The families possibly related to subfamilies of Saxifragaceae s. l. include Crassulaceae, Celastraceae, Cephalotaceae, Paeoniaceae, Greyiaceae, Geraniaceae, Cunoniaceae, Garryaceae, Cercidiphyllaceae, Hamamelidaceae, Gunneraceae, Rosaceae, Haloragaceae, and many families of Asteridae s. l.; representatives of all of these families were included in the analyses of 18S rDNA sequences. Of the 130 taxa (125 angiosperms plus five Gnetales) for which 18S rDNA sequences were analyzed (Table 2), 95 were generated by the authors as part of the present study. Other sequences used are from Nickrent and Soltis (1995) or are unpublished sequences kindly provided by S. Hoot, D. Nickrent, L. Johnson, J. Sweere, and R. Kuzoff.

Plant material was obtained from field collections, botanical gardens, or herbarium specimens (Table 2). In many cases, the same DNAs used earlier to sequence *rbcL* (Morgan and Soltis, 1993; Soltis et al., 1993; Olmstead et al., 1993; Chase et al., 1993; Qiu et al., 1993; Price and Palmer, 1993; Xiang et al., 1993) were used in the present study to generate 18S rDNA sequences. In those instances in which new DNAs were isolated, the methods employed are those described earlier (Soltis et al., 1991).

Amplification and sequencing—The 18S rRNA gene was amplified using *TaqI* polymerase (Promega) and the primers NS1 and C18L*. The location and base composition of these primers and the general methods for PCR amplification are given in Bult, Kallersjo, and Suh (1992). The double-stranded PCR product was subsequently purified by precipitation using 20% PEG/2.5 mol/L NaCl solution, followed by washes in 80 and 95% ethanol and resuspension in distilled H₂O (Dunn and Blattner, 1987; Morgan and Soltis, 1993).

Purified double-stranded DNAs were then used in cycle sequencing reactions that were conducted using the PRISM Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The cycle sequencing reaction mixtures contained \approx 50–60 ng of template DNA, 10% DMSO, 6.6 μ L of terminator premix, 1.5 μ L of 1.6 mmol/L primers, and the appropriate amount of water for a total volume of 15 μ L. Sequencing involved 25 cycles of denaturation for 30 s at 96°C, annealing for 15 s at 50°C, and extension for 4 min at 60°C; reactions were held at 4°C. The following sequencing primers were used: NS1, N18E, C18E, 626r, 864r, C18H, N18O, 875r, 1354r, C18J, 1575r, and C18L*. The base composition of each primer is provided in Bult, Kallersjo, and Suh (1992) or Nickrent and Starr (1994).

Following cycle sequencing, the reactions were passed through Sephadex G-50 spin columns (Centri-Sep™ columns, Princeton Separations, Adelphia, NJ) to remove unincorporated dye terminators and then dried completely in a Speed-Vac. The reaction pellets were resuspended in 3 μ L of loading buffer [three parts deionized formamide to one part of a stock solution that contains 50 mmol/L EDTA (pH 8.0) and 30 mg/mL Blue Dextran]. Automated sequencing was employed using the 373 DNA Sequencing System (Applied Biosystems, Foster City, CA); 36 samples were standardly run on each 6% acrylamide gel. The DNA sequences obtained were assembled and consensus sequences were constructed using the computer program Sequencher™ 2.1 (Gene Codes

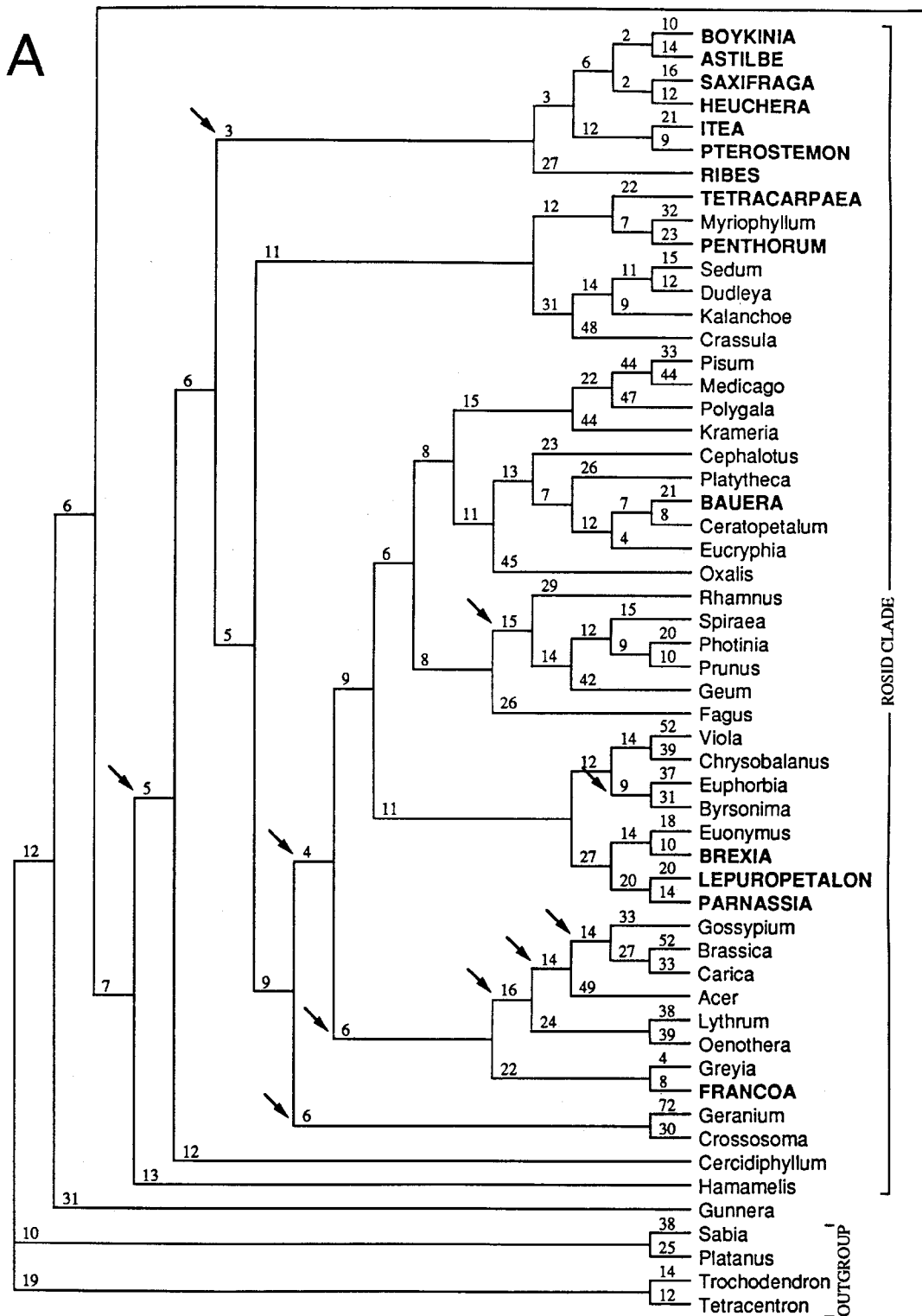


Fig. 1. (A, B) Majority-rule consensus tree constructed from 378 most parsimonious trees resulting from phylogenetic analysis of 100 *rbcl* sequences of Saxifragaceae s. l. and many other nonmagnoliid dicots (from Morgan and Soltis, 1993). This tree is identical to one of the 378 shortest trees and has a consistency index of 0.266 and a retention index of 0.46. Arrows point to nodes that did not occur in all shortest trees. Numbers above each branch indicate the number of base substitutions. Names of Saxifragaceae s. l. are shown in boldface capital letters.

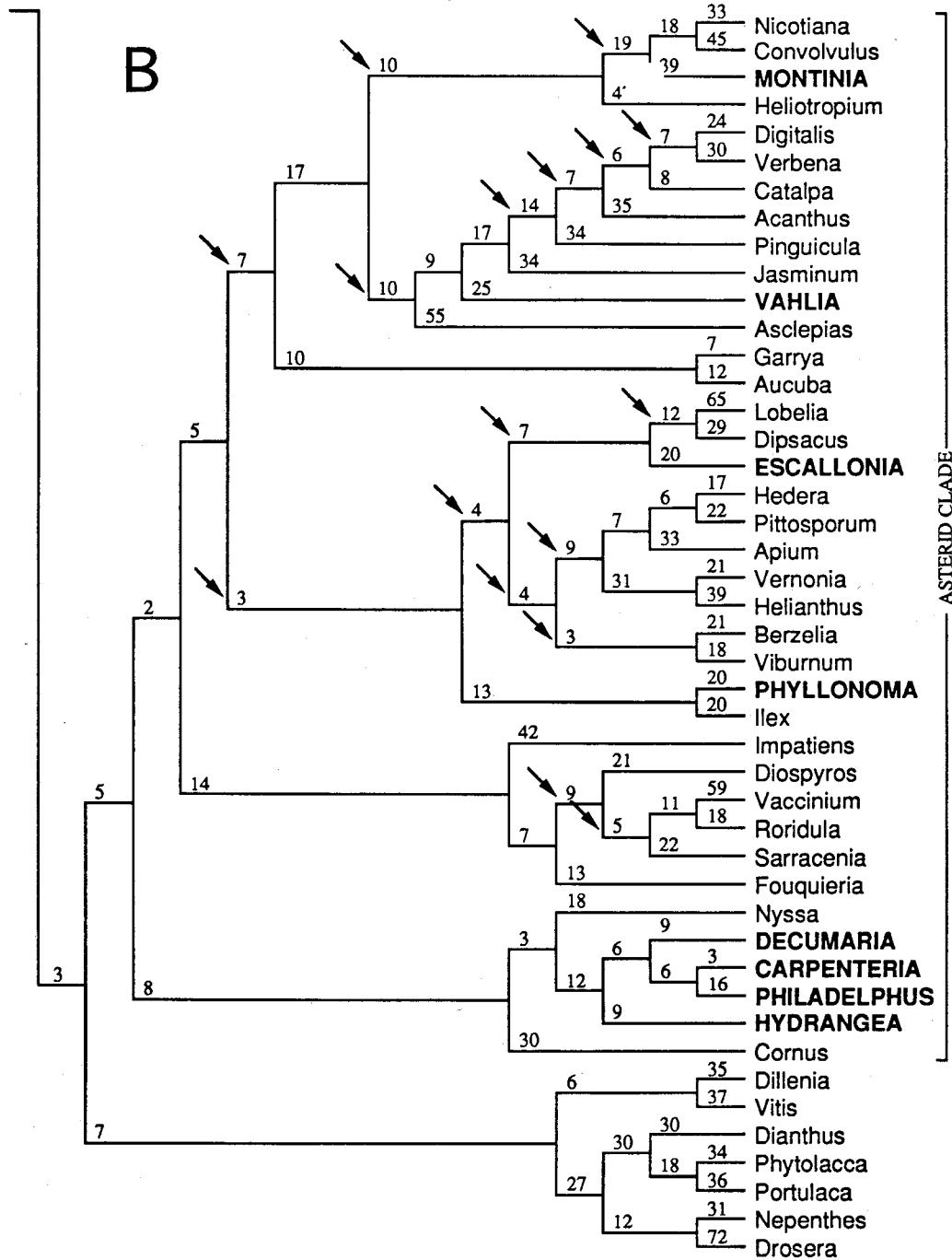


Fig. 1. Continued.

Corporation, 1994). The consensus sequences were subsequently exported to PAUP* 4.0 (D. Swofford, unpublished data).

Alignment of the 18S sequences—Alignment of sequences was straightforward and easily accomplished by eye, not only due to the highly conserved nature of 18S rDNA, but also because most of the length mutations detected involved single-base insertions or deletions (indels). Furthermore, most of the indels were confined to a few specific areas of the 18S rDNA gene. Certain small regions of 18S rDNA, such as the termini of helices E10–1, E-23–1, and 49, are prone to variation in primary sequence and length in angiosperms and confound unam-

biguous alignment over a broad taxonomic range (Nickrent and Soltis, 1995). *Glycine max* provides a convenient reference sequence because of the availability of a proposed ribosomal RNA secondary structure model (Nickrent and Soltis, 1995). Positions 230–237, 496–501, 666–672, and 1363–1369 on the 18S rDNA sequence of *Glycine max* (Eck-enrode, Arnold, and Meagher, 1985) were difficult to align across all of the taxa analyzed; these four small regions were therefore eliminated from phylogenetic analysis (as suggested by Swofford and Olsen, 1990). In addition, the extreme 5' and 3' ends of the 18S rDNA sequences were not included in the phylogenetic analysis. Positions 1–20 correspond to the forward PCR primer NS1 and therefore were not used.

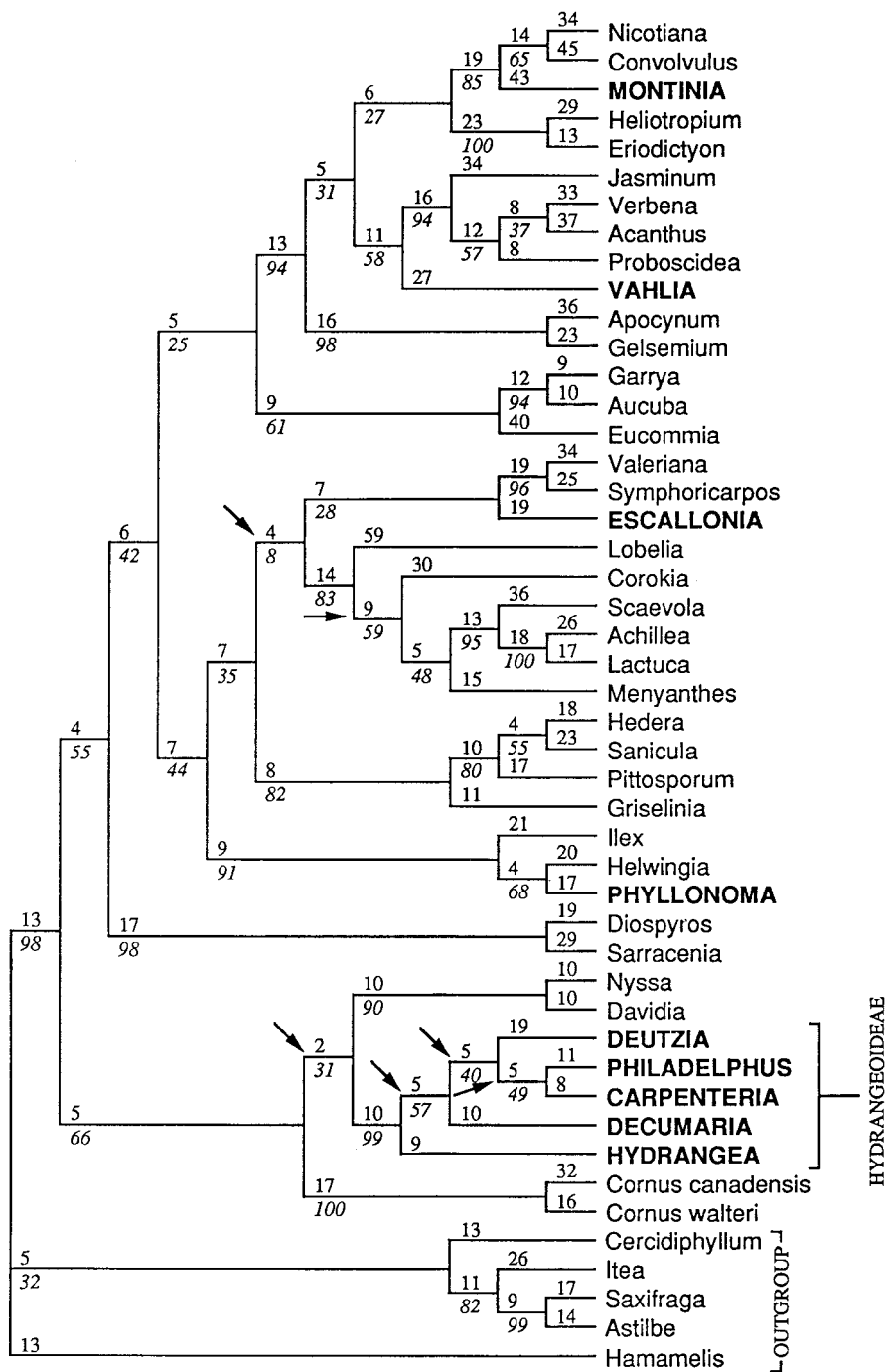


Fig. 2. Majority-rule consensus tree constructed from 36 most parsimonious trees resulting from phylogenetic analysis of *rbcL* sequences of Asteridae s. l. This tree is identical to one of the shortest trees and has a consistency index of 0.428 and a retention index of 0.486. Arrows point to nodes that do not occur in all shortest trees. Numbers above each branch indicate the number of base substitutions; numbers in italics below each branch indicate bootstrap values. Names of taxa of Saxifragaceae s. l. are shown in boldface capital letters. Modified from Morgan and Soltis (1993).

Because most of the sequences were clearly readable at, or just before, base position 41, we began analysis of our data set at position 42. At the 3' end, base positions 1751–1808 (on *Glycine max*) were often difficult to read and hence were eliminated. Due to the inclusion of alignment spacers, the total length of the data matrix is 1825 base pairs.

Two indels were detected in conserved regions not prone to insertion and deletion (Table 3). One indel, an apparent deletion of a single base pair based on outgroup comparison, is present in all higher dicots. A

second indel, an apparent insertion of one base pair, occurs in all members of a clade referred to below as Saxifragales. These indels were either scored as gaps and treated the same as other indels (see below), or considered additional characters and scored as position present (1) or absent (0).

Phylogenetic analysis—We first conducted broad phylogenetic analyses that included representatives of all 17 subfamilies of Saxifragaceae

s. l. and putative relatives, plus a broad sampling of taxa representing all six subclasses of dicots (Cronquist, 1981). Phylogenetic searches were conducted using PAUP 3.1.1 and PAUP* 4.0 (D. Swofford, 1993, Smithsonian Institution).

Because of the large number of taxa (125 ingroup plus five outgroup taxa), we used two primary search strategies in the broad analyses. The first method was a standard heuristic search performed with MULPARS, random taxon addition, and TBR branch swapping; gaps were treated as missing data (“?”). These searches were permitted to run for a week or more using either a Macintosh Quadra 650 or Sun Sparc server 600MP. These searches did not, however, find trees as short as those obtained with the method described below and will not be discussed further.

The second general search strategy was recommended by D. Swofford (personal communication) and closely follows the approach used by Maddison, Ruvolo, and Swofford (1992). We used 50–100 consecutive searches without MULPARS using random taxon addition and NNI branch-swapping. The length of the shortest trees (not the trees themselves) resulting from this search was subsequently used in the next phase of this search strategy. For each of the four separate analyses (see below) we performed 200–400 replicate searches (ten replicates required ~ 24 h of computing time) using random addition, TBR branch swapping, NCHUCK = 2, and a CHUCKLEN value based on the length of the shortest trees obtained in the initial NNI searches. This approach prevented the searches from being overwhelmed with trees. As shorter trees were found, additional searches were conducted with lower CHUCKLEN values. The shortest trees obtained in these searches were then used as starting points for subsequent searches, again with MULPARS and TBR branch swapping. These searches were permitted to run for a week or more using a Quadra 650 or Centris 650 computer; 2000–3000 trees were saved from each search. None of the searches swapped to completion.

Several different outgroups were used in the broad analyses. In one series of searches, five members of Gnetales served as the outgroup: *Ephedra sinica*, *E. torreyana*, *Gnetum nodiflorum*, *G. gnemon*, and *G. urens*. This data matrix involved 125 species of angiosperms as the ingroup; separate analyses were conducted with and without the two indels noted above (these represent data sets A and B, respectively). Two additional searches (not shown) were also conducted in which members of Gnetales were deleted and several angiosperms were used as the outgroup, all of which appear outside the clade of higher dicots to which taxa of Saxifragaceae s. l. belong (Soltis et al., 1997). In one set of searches, *Amborella*, *Nymphaea*, *Austrobaileya*, *Sassafras*, and *Ceratophyllum* served as outgroups (data set C). In another series of searches, we used *Ceratophyllum*, *Sassafras*, *Amborella*, *Saururus*, and *Lactoris* as outgroups (data set D). In these searches of data sets C and D, a total of 120 taxa served as the ingroup, and the indels noted above were not employed.

Implementing bootstrap or decay analyses is impractical with data sets of this size. To obtain an estimate of support for the 18S rDNA topologies, we applied the parsimony jackknife approach (Farris et al., 1997) to data set A (this analysis was kindly conducted by S. Farris). The jackknife is a resampling approach, similar to the bootstrap, in which a data set is resampled without replacement to generate replicate data sets. Each replicate is analyzed, and the proportion of replicates supporting a given conclusion (in this case a clade) is considered a measure of support. Jackknife percentages can therefore be interpreted similarly to bootstrap percentages. In this analysis, 1000 replicates were conducted, and a minimum jackknife value of 50 (CUT = 50) was used (i.e., only clades supported by jackknife values of 50% or greater were retained).

Following the broad analyses, it was apparent that the relationships of many Saxifragaceae s. l. were with members of Asteridae s. l. (sensu Olmstead et al., 1992, 1993). As a result, more focused analyses were subsequently conducted of this asterid clade using the findings of all of the broad analyses as a guide. Additional representatives of the Aster-

idae s. l. clade (*Linaria*, *Eucommia*, *Camellia*, *Cobaea*, *Campanula*, *Buddleja*, *Corokia*, and *Phacelia*) were also included in these analyses. These genera are part of an expanded asterid lineage in recent analyses of over 200 18S rDNA sequences (Soltis et al., 1997), as well as in broad analyses of *rbcL* sequences (Chase et al., 1993; Olmstead et al., 1993; Hibsich-Jetter et al., in press). Several regions of 18S rDNA that were difficult to align across all taxa in the broad analyses were easily aligned across only members of the expanded asterid clade. Hence, two different searches of the asterid clade were conducted. In the first search, only the following positions were excluded: 1–41, 496–501, 666–672, 1751-end (positions based on the sequence of *Glycine max*). In the second search, an attempt was made to include even those two regions that are most difficult to align (496–501; 666–672); only positions 1–41 and 1751-end were excluded.

For these analyses of Asteridae s. l., the outgroup consisted of *Saxifraga*, *Liquidambar*, *Cercidiphyllum*, and *Itea*, genera that fell outside the Asteridae s. l. clade in trees resulting from the broad searches. These genera also served as outgroups in a study of comparable scope based on *rbcL* sequences (Morgan and Soltis, 1993). To ensure that all islands (Maddison, 1991) of shortest trees were found, 100 replicate searches were performed with MULPARS using random taxon addition and TBR branch-swapping. A bootstrap analysis (Felsenstein, 1985; 100 replicates) was conducted for the asterid clade.

RESULTS

Phylogenetic analyses of all four data sets (A–D, above) yielded thousands of most parsimonious trees. It is likely that in all of these searches shorter trees are present and that classes of most parsimonious trees exist (e.g., Maddison, 1991) and were not found. Nonetheless, in all of these searches, the same relationships were suggested for members of Saxifragaceae s. l. Furthermore, these same relationships were also seen after the initial Nearest Neighbor Interchange (NNI) searches in trees that were up to 30 steps longer than the shortest trees found.

All of the broad analyses revealed: (1) a clade consisting of *Bauera* (Baueroideae), *Ceratopetalum* (Cunoniaceae), *Eucryphia* (Eucryphiaceae), and *Cephalotus* (Cephalotaceae); (2) a clade consisting of *Euonymus* (Celastraceae), *Brexia* (Brexioideae), *Parnassia* (Parnassioideae), and *Lepuropetalon* (Lepuropetaloidae); (3) *Francoa* (Francoideae) and *Greyia* (Greyiaceae) as sister taxa; (4) a “Saxifragales” clade consisting of *Boykinia*, *Sullivantia*, *Heuchera*, *Saxifraga*, *Chrysosplenium*, *Peltoboykinia* (Saxifragoideae), *Tetracarpaea* (Tetracarpaeoideae), *Pterostemon* (Pterostemnoideae), *Itea* (Iteoideae), *Penthorum* (Pentthoroideae), *Ribes* (Ribesooideae), Crassulaceae, Haloragaceae, Paeoniaceae, Cercidiphyllaceae, Hamamelidaceae, and Daphniphyllaceae; (5) an expanded asterid lineage (Asteridae s. l.) that includes representatives of several subfamilies of Saxifragaceae s. l.: *Philadelphus* and *Hydrangea* (Hydrangeoideae), *Montinia* (Montinioideae), *Vahlia* (Vahlnoideae), *Escallonia* (Escallonioideae), *Roussea* (variously placed in Escallonioideae or Brexioideae), *Eremosyne* (Eremosynoideae), and *Phyllonoma* (Phyllonomoideae). One of the 2774 shortest trees resulting from analysis of data set A (Fig. 3) is shown (length = 679 steps; CI = 0.486; RI = 0.584) to summarize the relationships of Saxifragaceae s. l. observed in broad analyses of all four data sets (A–D).

The first analysis of the asterid clade (with bases 1–

TABLE 2. Species analyzed for 18S rDNA sequence variation in this study of Saxifragaceae s. l. Species are arranged alphabetically by families (Cronquist, 1981). For members of Saxifragaceae s. l. (these are indicated by asterisks) the subfamily name is also provided. Within families, species are arranged alphabetically by genus. Unless indicated otherwise under Literature citation, the given sequence is first reported here.

| Species | Family | Voucher/source | Literature citation | Genbank | Sequence by |
|---|--------------------------|-----------------------------------|------------------------------|---------|-------------------|
| <i>Acer rubrum</i> L. | Aceraceae | <i>Soltis and Soltis 2515, WS</i> | | U42494 | Soltis |
| <i>Actinidia</i> sp. | Actinidiaceae | <i>Morgan s. n., WS</i> | | U42495 | Soltis |
| <i>Tetragonia expansa</i> Murr. | Aizoaceae | <i>Hershkovitz 111, WS</i> | | U42496 | Soltis |
| <i>Amborella trichopoda</i> Baill. | Amborellaceae | <i>Suh 44, US</i> | | U42497 | Soltis and Soltis |
| <i>Lomatium triternatum</i> (Pursh) Coult. & Rose | Apiaceae | <i>Soltis 2266, WS</i> | | U42498 | Soltis and Soltis |
| <i>Tabernaemontana divaricata</i> (L.) R. Br. | Apocynaceae | <i>Nickrent 2978, SIU</i> | | U42499 | Soltis and Soltis |
| <i>Hedera helix</i> L. | Araliaceae | <i>Plunkett 1368, WS</i> | | U42500 | Soltis and Soltis |
| <i>Saruma henryi</i> Oliver | Aristolochiaceae | <i>Qiu 91018, NEU</i> | Nickrent and Soltis, 1995 | L24417 | Nickrent |
| <i>Tagetes</i> sp. | Asteraceae | <i>Nickrent 3061, SUI</i> | | U42501 | Soltis and Soltis |
| <i>Tragopogon dubius</i> Scop. | Asteraceae | <i>Soltis 2472, WS</i> | | U42502 | Soltis and Soltis |
| <i>Austrobaileya scandens</i> C. T. White | Austrobaileyaaceae | <i>Nickrent 2953, SIU</i> | | U42503 | Nickrent |
| <i>Impatiens wallerana</i> Hook. | Balsaminaceae | <i>Johnson 95-071, WS</i> | | L49285 | Johnson |
| <i>Batis maritima</i> L. | Bataceae | <i>Ilitis 30500, WIS</i> | | U42504 | Soltis and Soltis |
| * <i>Bauera rubioides</i> Andrews | Baueraceae (Baueroideae) | <i>Kew 1977-6377</i> | | U42505 | Soltis |
| <i>Begonia metallica</i> × <i>sanguinea</i> | Begoniaceae | <i>Chase 225, NCU</i> | | U42506 | Soltis |
| <i>Podophyllum peltatum</i> L. | Berberidaceae | <i>Nickrent 2891, SIU</i> | | L24413 | Hoot |
| <i>Parmentiera cerifera</i> Seem. | Bignoniaceae | <i>Johnson 95-005, WS</i> | | L49291 | Johnson |
| <i>Bourreria succulenta</i> Jacq. | Boraginaceae | <i>J. Miller 6421, MO</i> | | U38319 | Nickrent |
| <i>Brassica hirta</i> Moench | Brassicaceae | unknown | Rathgeber and Capesius, 1990 | X17062 | |
| <i>Berzelia lanuginosa</i> (L.) Brongn. | Bruniaceae | <i>Price s. n., IND</i> | | U42508 | Soltis |
| <i>Calycanthus floridus</i> L. | Calycanthaceae | <i>Nickrent 2893, SIU</i> | | U38318 | Nickrent |
| <i>Symphoricarpos albus</i> (L.) Blake | Caprifoliaceae | <i>Olmstead s. n., COLO</i> | | U42513 | Soltis and Soltis |
| <i>Carica papaya</i> L. | Caricaceae | <i>Missouri B. G., MO</i> | | U42514 | Soltis and Soltis |
| <i>Casuarina equisetifolia</i> L. | Casuarinaceae | <i>Nickrent 2971, SIU</i> | | U42515 | Soltis |
| <i>Euonymus alatus</i> (Thunb.) Seibold | Celastraceae | <i>Nickrent 2894, SIU</i> | Nickrent and Franchina, 1990 | X16600 | Nickrent |
| <i>Cephalotus folicularis</i> Labill. | Cephalotaceae | <i>Chase 147, NCU</i> | | U42516 | Soltis |
| <i>Ceratophyllum demersum</i> L. | Ceratophyllaceae | <i>Qiu 91027, NCU</i> | | U42517 | Soltis |
| <i>Cercidiphyllum japonicum</i> Siebold & Zucc. | Cercidiphyllaceae | <i>Soltis 2540, WS</i> | | U42518 | Soltis |
| <i>Chrysobalanus icaco</i> L. | Chrysobalanaceae | <i>Fairchild Trop. G. 76-311</i> | | U42519 | Soltis |
| <i>Clethra alnifolia</i> L. | Clethraceae | <i>Kron 1884s, NCU</i> | | U42521 | Soltis |
| <i>Ipomoea hederacea</i> Jacq. | Convolvulaceae | <i>Colwell s. n., MO</i> | | U38310 | Soltis |
| <i>Aucuba japonica</i> Thunb. | Cornaceae | <i>U.S. Natl. Arb.</i> | | U42522 | Soltis |
| <i>Cornus officinalis</i> Sieb et Zucc. | Cornaceae | <i>Arnold Arb. 8156-A</i> | | U52033 | Soltis and Soltis |
| <i>Helwingia japonica</i> (Thunb.) F. Dietr. | Cornaceae | <i>Arnold Arb. 912</i> | | U42524 | Soltis and Soltis |
| <i>Crassula marnierana</i> Huber & Jacobsen | Crassulaceae | <i>Morgan 2152, WS</i> | | U42525 | Soltis |
| <i>Dudleya viscida</i> (S. Watson) Moran | Crassulaceae | <i>Huntington B. G. 62801</i> | | U42526 | Soltis |
| <i>Kalenchöe diagremontana</i> Hamet & Perrier | Crassulaceae | <i>Morgan 2151, WS</i> | | U42527 | Soltis |
| <i>Sedum rubrotintum</i> Clausen | Crassulaceae | <i>Morgan 2153, WS</i> | | U42528 | Soltis |
| <i>Crossosoma californicum</i> Nutt. | Crossosomataceae | <i>Rancho Santa Ana B. G.</i> | | U42529 | Soltis |
| <i>Ceratopetalum gummiferum</i> Small | Cunoniaceae | <i>Keller 2135, CAS</i> | | U42530 | Soltis |
| <i>Daphniphyllum</i> sp. | Daphniphyllaceae | <i>Qiu 91026, NCU</i> | | U42531 | Soltis |
| <i>Diapensia lapponica</i> L. | Diapensiaceae | <i>Hills 89018, NCU</i> | | L49278 | Johnson |
| <i>Dipsacus</i> sp. | Dipsacaceae | <i>Jansen 931, MICH</i> | | U43150 | Soltis and Soltis |
| <i>Drosera capensis</i> L. | Droseraceae | <i>Palmengarten B. G.</i> | | U42532 | Soltis |
| <i>Ephedra sinica</i> Stapf | Ephedraceae | <i>Univ. Tokyo B. G. T192-97</i> | Chaw et al. (1995) | D38242 | Chaw |
| <i>Ephedra torreyana</i> S. Wats. | Ephedraceae | <i>Gillespie 4236, US</i> | | U42414 | Swere |
| <i>Arctostaphylos uva-ursi</i> (L.) Spreng. | Ericaceae | <i>Johnson 94-085, WS</i> | | L49272 | Johnson |
| <i>Eucryphia lucida</i> Druce | Eucryphiaceae | <i>Strybing Arb. 86-0250</i> | | U42533 | Soltis |
| <i>Euphorbia pulcherrima</i> Willd. | Euphorbiaceae | <i>Soltis and Soltis 2541, WS</i> | | U42535 | Soltis |

TABLE 2. Continued.

| Species | Family | Voucher/source | Literature citation | Genbank | Sequence by |
|--|---|--|---------------------------|---------|-------------------|
| <i>Albizia julibrissin</i> Durazz. | Fabaceae | Doyle 1526, BH | | U42536 | Soltis |
| <i>Bauhinia</i> sp. | Fabaceae | Doyle s. n., MSU, no voucher | | U42537 | Soltis |
| <i>Pisum sativum</i> L. | Fabaceae | Carolina Bio.Lab., N.C. | | U43011 | Sweere |
| <i>Fouquieria splendens</i> Engelm. | Fouquieriaceae | Missouri B. G. 86-0162 | | L49280 | Johnson |
| <i>Garrya elliptica</i> Douglas ex Lind. | Garryaceae | Rancho Santa Ana B. G. 13280 | | U42540 | Soltis |
| <i>Geranium cinereum</i> Cav. | Geraniaceae | Price s. n., IND | | U42541 | Soltis |
| <i>Aeschynanthus radicans</i> Jack. | Gesneriaceae | Nickrent 2979, SIU | | U42542 | Soltis and Soltis |
| <i>Gnetum gnemon</i> L. | Gnetaceae | Gillespie 4212, US | | U42416 | Sweere |
| <i>Gnetum nodiflorum</i> Brongn. | Gnetaceae | Gillespie 4246, US | | U42415 | Sweere |
| <i>Gnetum urens</i> Aubl. Blume | Gnetaceae | Kress et al., 91-3271, US | | U42417 | Sweere |
| <i>Greyia radkofferi</i> Szyszyl. | Greyiaceae | Strybing Arb. 640406 | | U43151 | Soltis |
| * <i>Brexia madagascarensis</i> Thouars ex Ger Gawl. | Grossulariaceae (Brexioideae) | Kew 1977-14901 | | U42543 | Soltis |
| * <i>Escallonia coquimbensis</i> Reamy | Grossulariaceae (Escallonioideae) | U. Calif. B. G. 52-1333 | | U42544 | Soltis and Soltis |
| * <i>Itea virginia</i> L. | Grossulariaceae (Iteoideae) | Ware 9401, WS | | U42545 | Soltis |
| * <i>Phyllonoma laticuspus</i> (Turcz.) Engl. | Grossulariaceae (Phyllonomoideae) | Morgan 2124, WS | | U42546 | Soltis |
| * <i>Pterostemon rotundifolius</i> Ramirez | Grossulariaceae (Pterostemonoideae) | Sanchez 259, TEX | | U42547 | Soltis |
| * <i>Ribes aureum</i> Pursh | Grossulariaceae (Ribesioideae) | Soltis and Soltis 2220, WS | | L28143 | Soltis |
| * <i>Roussea simplex</i> Sm. | Grossulariaceae (Brexioideae/Escallonioideae) | Herbarium, Mauritius Sugar Industry Research Inst. | | U42548 | Soltis |
| * <i>Tetracarpaea tasmanica</i> Hook. f. | Grossulariaceae (Tetracarpaeoideae) | Jordan s. n., HO | | U42549 | Soltis |
| <i>Gunnera manicata</i> Linden | Gunneraceae | Kruckeberg s. n., WTU | | U43787 | Soltis |
| <i>Haloragas erecta</i> (Banks ex Murr.) Eichler | Haloragaceae | Chase 453 | | U42550 | Soltis |
| <i>Myriophyllum exallescens</i> Fernald | Haloragaceae | Broch 8/30/91, WS | | U42551 | Soltis |
| <i>Altingia</i> sp. | Hamamelidaceae | Royal B. G., Edinburgh 93006 | | U42552 | Soltis |
| <i>Liquidambar styraciflua</i> L. | Hamamelidaceae | Soltis and Soltis 2516, WS | | U42553 | Soltis |
| * <i>Hydrangea macrophylla</i> Torr. | Hydrangeaceae (Hydrangeoideae) | Morgan 2150, WS | | U42781 | Soltis |
| * <i>Philadelphus lewisii</i> Pursh. | Hydrangeaceae (Hydrangeoideae) | Soltis and Soltis 2411, WS | | U42782 | Soltis |
| <i>Lactoris fernandeziana</i> Phil. | Lactoridaceae | Steussy et al. 11,784, OSU | | U42783 | Soltis |
| <i>Lamium amplexicaule</i> L. | Lamiaceae | Johnson 95-001, WS | | L49287 | Johnson |
| <i>Akebia quinata</i> (Houtt.) Decne. | Lardizabalaceae | Nickrent 2945, SIU | | L31795 | Nickrent |
| <i>Sassafras albidum</i> (Nutt.) Nees. | Lauraceae | Soltis and Soltis 2518, WS | | U52031 | Soltis |
| <i>Floerkea proserpinicoides</i> Willd. | Limnanthaceae | Chase 174, NCU | | U42784 | Soltis |
| <i>Linum perenne</i> L. | Linaceae | Nickrent 2900, SIU | | L24401 | Nickrent |
| <i>Lobelia erinus</i> L. | Lobeliaceae | Jansen 989, MICH | | | Soltis and Soltis |
| <i>Malphigia coccigera</i> L. | Malphiaceae | Nickrent 2905, SIU | | L24046 | Nickrent |
| <i>Gossypium hirsutum</i> L. | Malvaceae | Alverson s. n., WIS | | U42827 | Kuzoff |
| <i>Mollugo verticillata</i> L. | Molluginaceae | Herskovitz 37, WS | | U42828 | Kuzoff |
| <i>Nepenthes</i> sp. | Nepenthaceae | Nickrent 3056, SIU | | U42787 | Soltis |
| <i>Nymphaea tuberosa</i> Paine | Nymphaeaceae | Nickrent 2906, SIU | Nickrent and Soltis, 1995 | L24404 | Nickrent |
| <i>Mirabilis jalapa</i> L. | Nyctaginaceae | Herskovitz 60, WS | | U42788 | Soltis |
| <i>Camptotheca acuminata</i> Decne. | Nyssaceae | Strybing Arb. 74-180 | | U42789 | Soltis and Soltis |
| <i>Nyssa ogeche</i> Marsh | Nyssaceae | U.S. Nat. Arb., Xiang s. n., WS | | U52032 | Soltis and Soltis |
| <i>Olea europea</i> L. | Oleaceae | Johnson 95-004, WS | | L49289 | Johnson |
| <i>Paeonia suffruticosa</i> Andr. | Paeoniaceae | Chase 486, K | | U42792 | Soltis |
| <i>Phytolacca americana</i> L. | Phytolaccaceae | Herskovitz 38, WS | | U42793 | Soltis |

TABLE 2. Continued.

| Species | Family | Voucher/source | Literature citation | Genbank | Sequence by |
|--|----------------------------------|--|---------------------------|---------|----------------------------|
| <i>Pittosporum japonicum</i> Hort. ex. C. Presl. | Pittosporaceae | <i>Rieseberg s. n.</i> , RSA | Nickrent and Soltis, 1995 | L28142 | Soltis and Kuzoff |
| <i>Platanus occidentalis</i> L. | Platanaceae | <i>Soltis and Soltis, 2514</i> | | U42794 | Sweere, Zimmer, and Soltis |
| <i>Plumbago auriculata</i> Lam. | Plumbaginaceae | <i>Nickrent s. n.</i> , SIU | | U42795 | Kuzoff |
| <i>Polygala pauciflora</i> Willd. | Polygalaceae | <i>Doyle 1567</i> , BH | | U42797 | Soltis |
| <i>Primula</i> sp. | Primulaceae | <i>Johnson 95-006</i> , WS | | L49295 | Johnson |
| <i>Knightsia excelsa</i> R. Br. | Proteaceae | Univ. of California, Santa Clara, B. G. | Nickrent and Soltis, 1995 | L24155 | Nickrent |
| <i>Ranunculus sardous</i> Crantz | Ranunculaceae | <i>Nickrent 2932</i> , SIU | Nickrent and Soltis, 1995 | L24092 | Nickrent |
| <i>Ceanothus sanguineus</i> Pursh | Rhamnaceae | <i>Morgan 2155</i> , WS | | U42799 | Soltis |
| <i>Photinia fraseri</i> Dress | Rosaceae | <i>Morgan 2131</i> , WS | | U42800 | Soltis |
| <i>Prunus persica</i> (L.) Batsch | Rosaceae | <i>E. E. Dickson, s. n.</i> , BH | | L28749 | Soltis |
| <i>Spiraea vanhouttei</i> (Briot) Zabel | Rosaceae | <i>Morgan 2130</i> , WS | | U42801 | Soltis |
| <i>Mitchella repens</i> L. | Rubiaceae | <i>Xiang s. n.</i> , WS | | U42802 | Soltis and Soltis |
| <i>Sarracenia purpurea</i> L. | Sarraceniaceae | <i>Morgan s. n.</i> , WS | | U42804 | Soltis |
| <i>Houttuynia cordata</i> Thunb. | Saururaceae | <i>Nickrent 2940</i> , SIU | Nickrent and Soltis, 1995 | L24147 | Soltis and Soltis |
| * <i>Boykinia intermedia</i> (Piper) Jones | Saxifragaceae (Saxifragoideae) | <i>Grable 11638</i> , WS | | U42806 | Soltis |
| * <i>Chrysosplenium iowense</i> Rydb. | Saxifragaceae (Saxifragoideae) | <i>Wendel s. n.</i> , ISC | | L28136 | Soltis |
| * <i>Eremosyne pectinata</i> Endl. | Saxifragaceae (Eremosynoideae) | <i>Annels and Hearn 4795</i> , PERTH | | U42807 | Soltis |
| * <i>Francoa sonchifolia</i> Cav. | Saxifragaceae (Francoideae) | <i>Soltis and Soltis 2479</i> , WS | | L28137 | Soltis |
| * <i>Heuchera micrantha</i> Douglas | Saxifragaceae (Saxifragoideae) | <i>Soltis and Soltis 1949</i> , WS | | X28139 | Soltis |
| * <i>Lepuropetalon spathulatum</i> (Muhl.) Elliott | Saxifragaceae (Lepuropetaloidae) | <i>Thomas s. n.</i> , NLU | | L28141 | Soltis |
| * <i>Montinia caryophyllacea</i> Thunb. | Saxifragaceae (Montinioideae) | <i>Williams 2833</i> , MO | | U42808 | Soltis and Soltis |
| * <i>Parnassia fimbriata</i> Banks | Saxifragaceae (Parnassioideae) | <i>Soltis and Soltis s. n.</i> , WS | | U42809 | Soltis |
| * <i>Peltoboykinia tellimoides</i> (Maxim.) Hara | Saxifragaceae (Saxifragoideae) | Nikko B. G., WS | | | Soltis |
| * <i>Penthorum sedoides</i> L. | Saxifragaceae (Penthoroidae) | <i>Hayden 2232</i> , WS | | U25660 | Soltis |
| * <i>Saxifraga integrifolia</i> Hook. | Saxifragaceae (Saxifragoideae) | <i>Soltis and Soltis 2253</i> , WS | | U42810 | Soltis |
| * <i>Saxifraga mertensiana</i> Bong. | Saxifragaceae (Saxifragoideae) | <i>Grable 11558</i> , WS | | U42811 | Soltis |
| * <i>Sullivantia oregana</i> Wats. | Saxifragaceae (Saxifragoideae) | <i>Grable 11598</i> , WS | | U42812 | Soltis |
| * <i>Vahlia capensis</i> Thunb. | Saxifragaceae (Vahlloideae) | <i>Van Wyk 10-579</i> , PRU | | U42813 | Soltis and Soltis |
| <i>Linaria vulgaris</i> P. Mill | Scophulariaceae | <i>Colwell, MO CA1</i> , SIU | | U3815 | Colwell and Nickrent |
| <i>Brunfelsia pauciflora</i> (Cham. & Schlechtend.) Benth. | Solanaceae | <i>Johnson 95-002</i> , WS | | L49274 | Johnson |
| <i>Tetracentron sinensis</i> Oliv. | Tetracentraceae | <i>Qiu 90009</i> , NCU | | U42814 | Soltis |
| <i>Trochodendron aralioides</i> Siebold & Zucc. | Trochodendraceae | <i>Qiu 90026</i> , NCU | | U42816 | Soltis |
| <i>Tropaeolum majus</i> L. | Tropaeolaceae | <i>Chase 113</i> , NCU | Nickrent and Soltis, 1995 | L31796 | Soltis |
| <i>Turnera ulmifolia</i> L. | Turneraceae | <i>Chase 220</i> , NCU | | U42817 | Soltis |
| <i>Celtis yunnanensis</i> C. K. Schneid. | Ulmaceae | <i>Qiu P90002</i> , NCU | | U42818 | Soltis |
| <i>Pilea cadierei</i> Gagnep et Guillaum | Urticaceae | <i>Nickrent 2972</i> , WS | | U42820 | Soltis |
| <i>Guaiacum sanctum</i> L. | Zygophyllaceae | <i>Anderson s. n.</i> , MICH | | U42824 | Soltis |

TABLE 3. Potentially phylogenetically informative indels located in conserved regions of 18S rDNA. Indel A is a 1-bp deletion that characterizes all higher dicots. Indel B is a 1-bp insertion that unites all members of Saxifragales. Base positions correspond to the last position given in the sequence of *Glycine max*.

| | | |
|----------------------|------------------|------|
| Indel A | | |
| <i>Glycine</i> | CCGGGTAATCTTTG- | 1529 |
| <i>Trochodendron</i> | CCGGGTAATCTTTGA | |
| Indel B | | |
| <i>Glycine</i> | TATGGCCGCTTA-GGC | 1406 |
| <i>Heuchera</i> | TATGGCGATTTAAGGC | |

41, 496–501, 666–672, 1751-end excluded) resulted in 156 trees of 679 steps (CI = 0.486; RI = 0.584). In the second analysis, with positions 496–501 and 666–672 included, 84 most parsimonious trees of length 793 were obtained (CI = 0.444; RI = 0.544). Both analyses of the asterid clade revealed the same relationships for members of Saxifragaceae s. l. Only one of the shortest trees resulting from the first of these analyses is shown (Fig. 4).

Analyses of the asterid clade reveal that *Philadelphus* and *Hydrangea* (Hydrangeoideae) are sister to an ericalean subclade that also includes Sarraceniaceae, Primulaceae, Actinidiaceae, Clethraceae, Diapensiaceae, Polemoniaceae, and several other families. The remaining members of Saxifragaceae s. l. appear as part of a “higher asterid” clade. Within the higher asterids, *Escallonia* (Escallonioidae) and *Eremosyne* (Eremosynioideae) are sisters. *Rousseia* (Escallonioidae/ Brexioidae) is in a clade with Campanulaceae and Asteraceae. *Phyllonoma* (Phyllonomoideae) and *Helwingia* (Cornaceae) are sister taxa; *Aucuba* (Cornaceae) is their sister. *Vahlia* is sister to a clade composed of Apocynaceae, Rubiaceae, Hydrophyllaceae, Boraginaceae, Solanaceae, and *Montinia* (Montinioideae).

DISCUSSION

Phylogenetic analyses of representatives of all six subclasses of dicots (Cronquist, 1981) indicate that Saxifragaceae s. l. are a polyphyletic assemblage. Representatives of the 17 subfamilies of Saxifragaceae s. l. are allied with a number of different, often distantly related, lineages representing four of Cronquist’s six subclasses of dicots (Fig. 3). The relationships seen in the shortest trees obtained in this study of 18S rDNA sequences are in close agreement with those suggested by analyses of *rbcL* sequences (Morgan and Soltis, 1993). To facilitate comparison, two *rbcL* trees from Morgan and Soltis (1993) are also presented (Figs. 1, 2); the discussion is similarly divided into several parts, each of which addresses the relationships of one or a group of the subfamilies of Saxifragaceae s. l.

Saxifragoideae and associated subfamilies and families—Saxifragoideae—It is generally agreed that the ≈ 30 genera placed in Saxifragoideae (Table 1) comprise a natural group (reviewed in Soltis et al., 1993). These taxa share many morphological, anatomical, and chemical features, and most classifications maintain this group as dis-

tinct at the familial, subfamilial, or tribal level (reviewed in Morgan and Soltis, 1993; Soltis et al., 1993). Saxifragoideae are the core of the original Saxifragaceae s. l., and some classifications define Saxifragaceae so narrowly that the family consists only of this subfamily (e.g., Takhtajan, 1987; Thorne, 1992). In contrast, other classifications (e.g., Cronquist, 1981; Dahlgren, 1983) include several additional genera in the family (e.g., *Parnassia*, *Lepuropetalon*, *Francoa*, *Eremosyne*, *Penthorum*, and *Vahlia*).

Analyses of 18S rDNA sequences support the monophyly of Saxifragoideae (Saxifragaceae s. s.) by defining a clade composed of six genera used here to represent the subfamily (Fig. 3). These analyses also suggest that five subfamilies of Saxifragaceae s. l. are closely related to Saxifragaceae s. s. (Penthoroidae, Tetracarpaeoideae, Ribesoideae, Iteoideae, Pterostemonoideae). In contrast, 11 subfamilies of Saxifragaceae s. l. (Francooideae, Baueroideae, Brexioidae, Parnassioideae, Lepuropetaloidae, Hydrangeoideae, Escallonioidae, Phyllonomoideae, Montinioideae, Eremosynioideae, Vahlloideae; Figs. 3, 4) are only distantly related to Saxifragoideae. This circumscription of Saxifragaceae s. s. is also in complete agreement with analyses of *rbcL* (Figs. 1, 2) and *matK* sequences, as well as cpDNA restriction site data (Chase et al., 1993; Morgan and Soltis, 1993; Soltis et al., 1993; Johnson and Soltis, 1995). The distribution of the loss of the *rpl2* intron also supports the monophyly of Saxifragaceae s. s. (Downie et al., 1991). Thus, several lines of evidence, derived from both the chloroplast genome and 18S rDNA sequences, suggest that Saxifragaceae should be defined as comprising only subfamily Saxifragoideae (sensu Schulze-Menz, 1964) and that other subfamilies of Saxifragaceae s. l. should be treated either as separate families or as members of other families. This narrow definition of Saxifragaceae is identical to the concept of the family proposed by Takhtajan (1987) and Thorne (1992).

Saxifragales—Analyses of 18S rDNA sequences also indicate that Saxifragaceae s. s. are part of one of the most strongly supported clades of this investigation (tentatively referred to as “Saxifragales”) (Fig. 3). “Saxifragales” are one of the few large clades exhibiting high jackknife support (73%). The monophyly of this clade is supported not only by base substitutions but also by indel B (an apparent insertion; see Table 3). This “Saxifragales” clade is also present in trees resulting from analyses of over 200 18S rDNA sequences representing all major lineages of angiosperms (Soltis et al., 1997).

In addition to Saxifragoideae (= Saxifragaceae s. s.), “Saxifragales” also contain subfamilies Iteoideae, Pterostemonoideae, Ribesoideae, Penthoroidae, and Tetracarpaeoideae of Saxifragaceae s. l. and representatives of Haloragaceae and Crassulaceae (Rosidae), Paeoniaceae (Dilleniidae), and Hamamelidaceae, Cercidiphyllaceae, and Daphniphyllaceae (Hamamelidae). Thus, this one small clade comprises representatives of three subclasses, Rosidae, Hamamelidae, and Dilleniidae, and is identical in composition to the rosid III clade revealed by the phylogenetic analysis of *rbcL* sequences of seed plants (Chase et al., 1993). Analysis of 18S rDNA sequences indicates further that families such as Cunoniaceae, Gre-

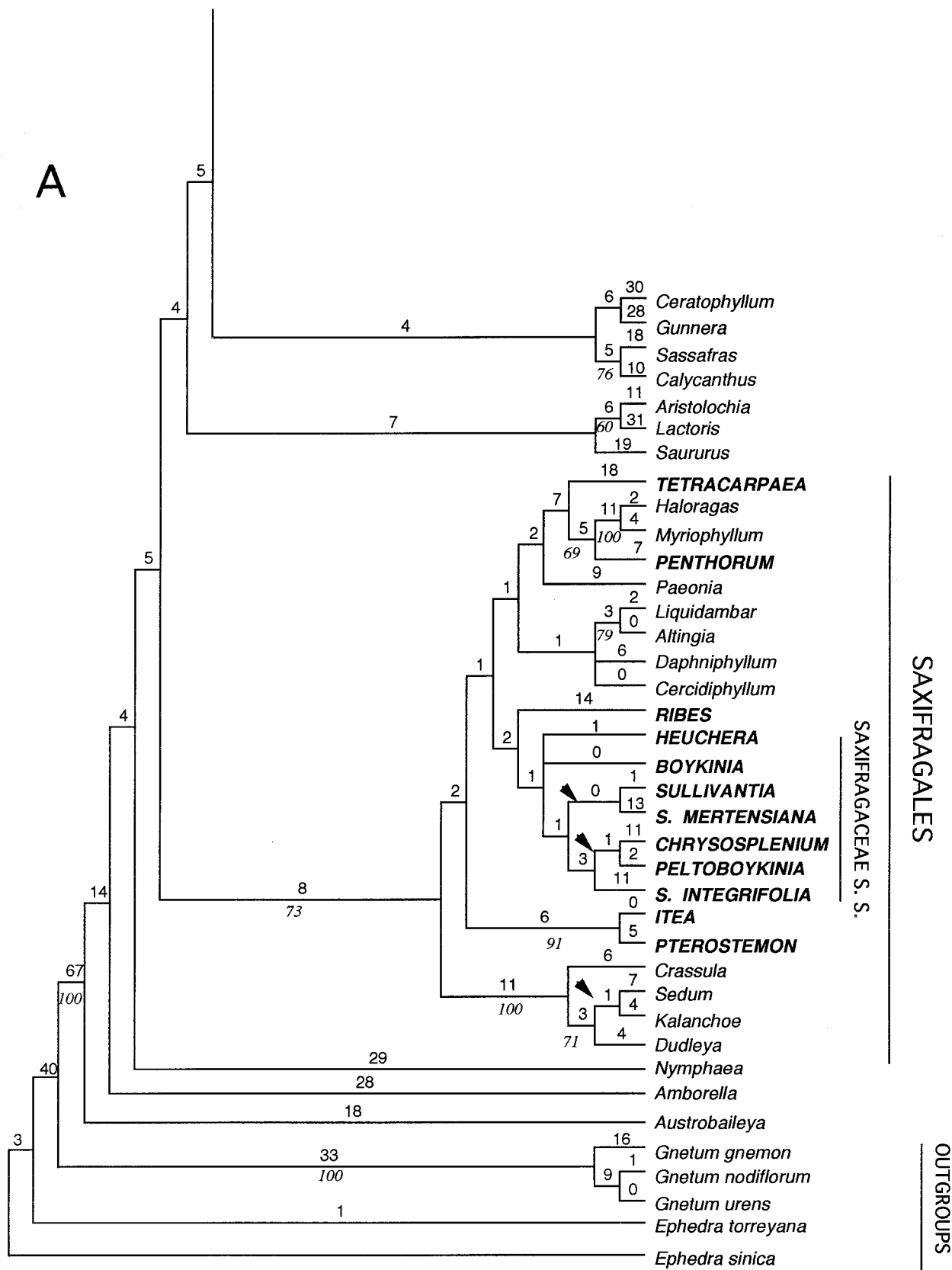


Fig. 3. (A, B, and C) Majority-rule consensus tree constructed from 2774 most parsimonious trees resulting from phylogenetic analysis of 130 entire 18S rDNA sequences. This tree is identical to one of the shortest trees and has a length of 2136 steps, a consistency index of 0.319, and a retention index of 0.566. Arrows point to nodes that do not occur in all shortest trees. Numbers above each branch indicate the number of base substitutions. Numbers below each branch indicate parsimony jackknife values (run time = 133.2 s). Names of taxa belonging to Saxifragaceae s. l. are shown in boldface capital letters.

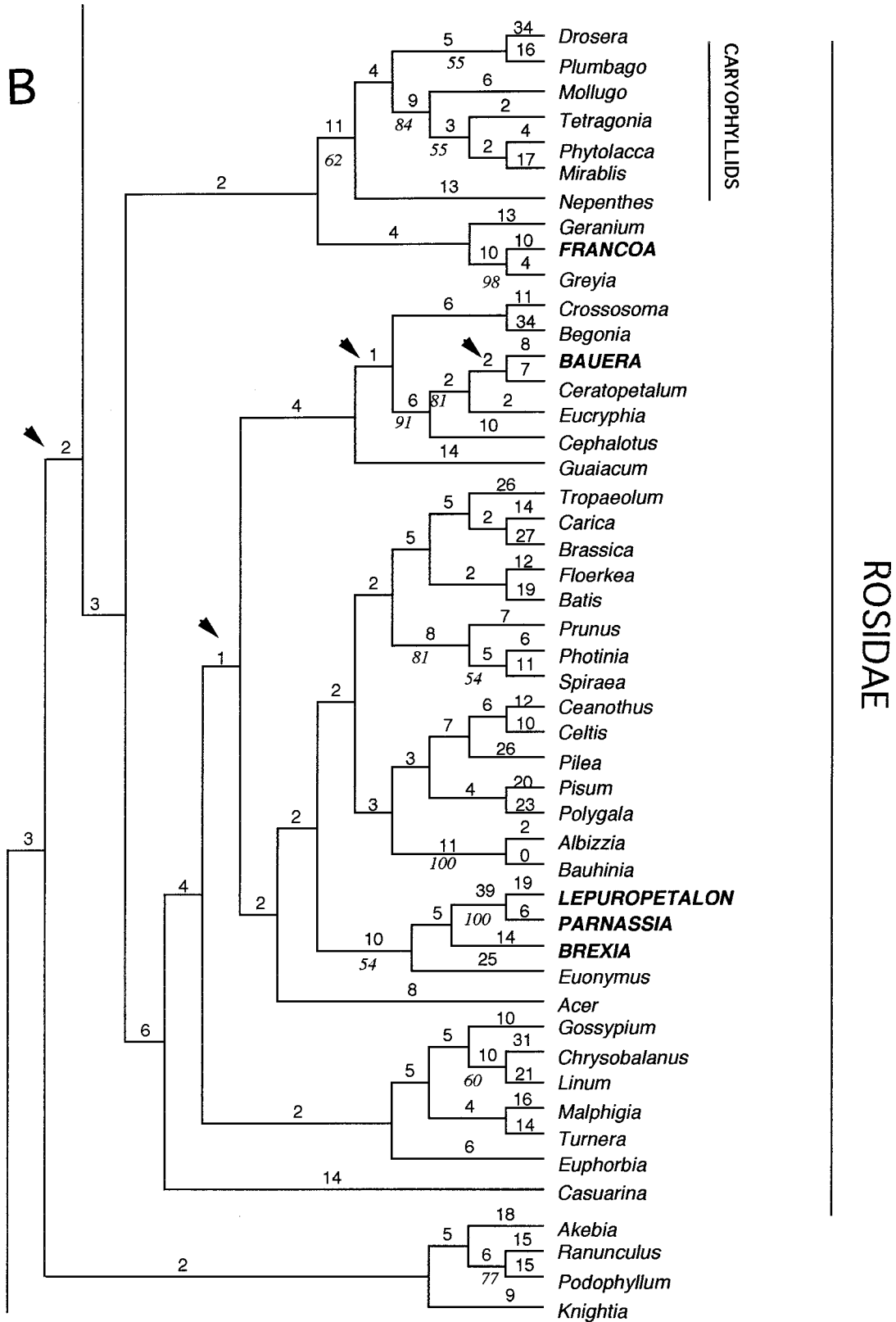


Fig. 3. Continued.

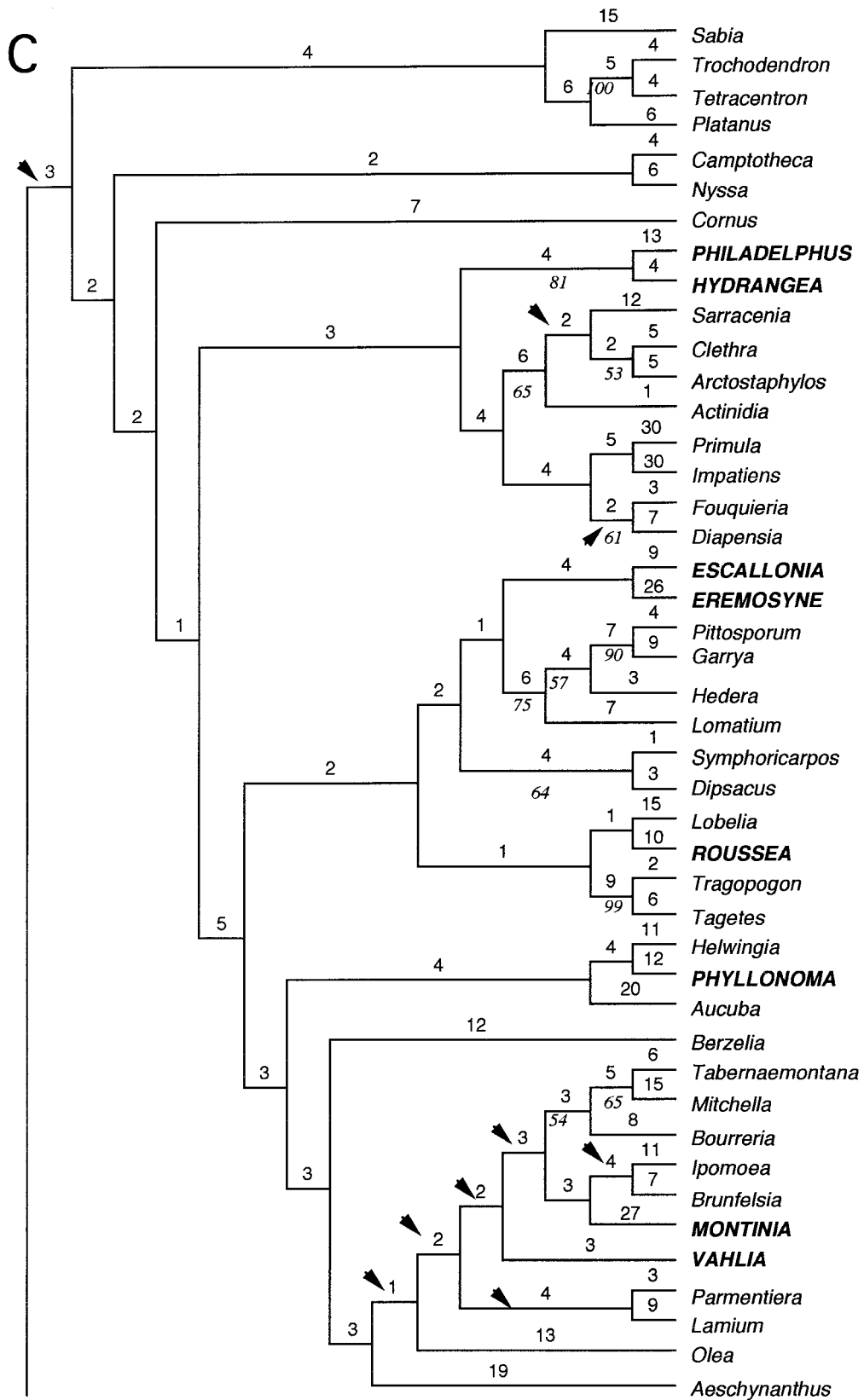


Fig. 3. Continued.

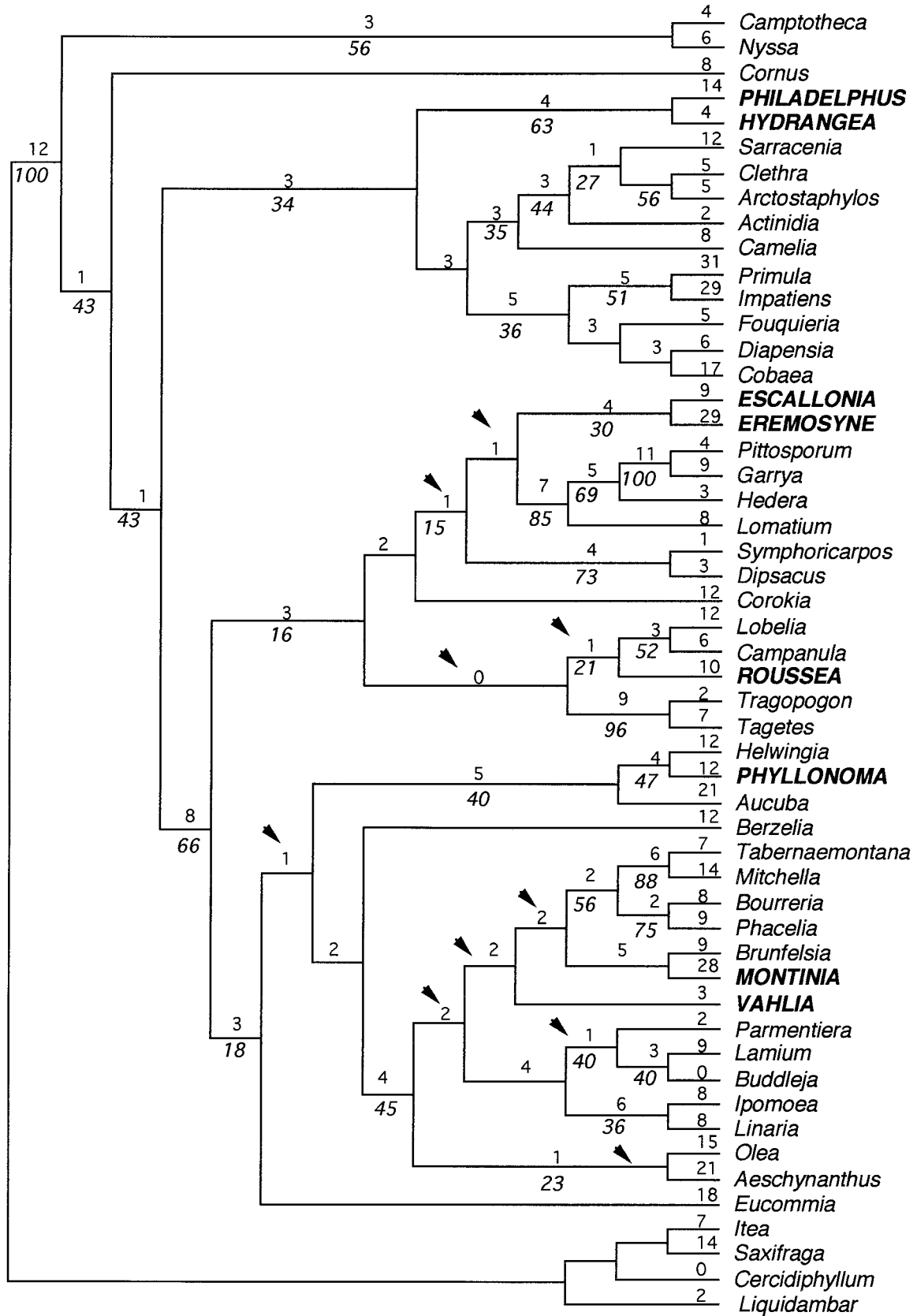


Fig. 4. One of 156 most parsimonious trees resulting from phylogenetic analysis of Asteridae s. l.; it has a length of 679 steps, a consistency index of 0.486, and a retention index of 0.584. Numbers above branches are the number of base substitutions. Numbers below branches are bootstrap values (100 replicates); only values of 15% or greater are given. Names of taxa belonging to Saxifragaceae s. l. are shown in boldface capital letters.

yiaceae, Droseraceae, Cephalotaceae, Gunneraceae, and Rosaceae, often considered closely related to Saxifragaceae s. s., are, in fact, not closely related to them (Fig. 3). These results were also obtained in analyses of *rbcL* sequences (Fig. 1) (Morgan and Soltis, 1993; Chase et al., 1993).

Although the "Saxifragales" clade is recovered by both 18S rDNA and *rbcL* sequences, this same group of taxa has not been recognized in traditional classifications. However, close relationships have been suggested among Saxifragaceae s. s., *Tetracarpaea*, *Penthorum*, Crassulaceae, *Ribes*, and *Itea* by virtually all modern authors (e.g., Dahlgren, 1980, 1983; Cronquist, 1981; Takhtajan, 1987; Thorne, 1992); the morphological, anatomical, and chemical support for their close relationship is discussed by Morgan and Soltis (1993). In contrast, the relationships of the rosid family Haloragaceae and the dilleniid family Paeoniaceae have been considered enigmatic (e.g., Cronquist, 1981), and the families of Hamamelidaceae found in this clade (Hamamelidaceae, Cercidiphyllaceae, and Daphniphyllaceae) have not been considered close relatives of Saxifragaceae s. s. The relationships of these more anomalous members of this clade are discussed below.

A close relationship between Haloragaceae and the other members of "Saxifragales" has apparently not been suggested prior to molecular phylogenetic analyses (reviewed in Morgan and Soltis, 1993). Haloragaceae are occasionally placed in Haloragales, often with another enigmatic family, Gunneraceae (Cronquist, 1981; Dahlgren, 1983). Others have placed Haloragaceae in Myrtales (Melchior, 1964; Hutchinson, 1973; Takhtajan, 1987). However, neither this analysis nor analyses of *rbcL* sequences (Chase et al., 1993; Morgan and Soltis, 1993; Conti, Litt, and Sytma, 1995) place Haloragaceae with either Gunneraceae or Myrtales. Paeoniaceae also have not been considered closely related to other members of "Saxifragales." The family has often been referred to the Ranunculales, although morphological, anatomical, palynological, and serological data (Yakovlev and Yoffe, 1957; Cave, Arnott, and Cook, 1961; Mathiessen, 1962; Sawada, 1971; Keefe and Moseley, 1978) distinguish Paeoniaceae from Ranunculaceae (reviewed in Cronquist, 1981). Cronquist allied Paeoniaceae with Dilleniaceae (see also Keefe and Moseley, 1978). Daphniphyllaceae and Hamamelidaceae have only rarely been considered close relatives of Saxifragaceae, although a close association has occasionally been suggested (e.g., Dickinson, 1989). Phylogenetic analysis of Rosidae based on morphological and chemical data (Hufford, 1992) suggests a close relationship among only Crassulaceae, *Penthorum*, and Saxifragaceae s. s., with representatives of Cercidiphyllaceae, Hamamelidaceae, and Paeoniaceae more distantly related to these taxa (Haloragaceae, Daphniphyllaceae, and several other genera of the Saxifragales clade were not included in Hufford, [1992]). In contrast, serological data are consistent with a close relationship between some hamamelids and Saxifragaceae s. s. Serological affinities between *Hamamelis* and several genera of Saxifragaceae s. s. were as high as those observed between *Ribes* and members of Saxifragaceae s. s. or between Crassulaceae and Saxifragaceae s. s. (Grund and Jensen, 1981). Clearly this "Saxifragales" clade should

be the focus of more detailed anatomical, embryological, and morphological analyses.

Subfamilies associated with other families of Rosidae—*Baueroideae*—Based on this analysis of 18S rDNA sequences, *Bauera* is most closely related to *Ceratopetalum* (Cunoniaceae) and *Eucryphia* (Eucryphiaceae) and is well separated phylogenetically from all other Saxifragaceae s. l. (Fig. 3). The clade comprising *Bauera*, *Eucryphia*, and *Ceratopetalum* is supported by a parsimony jackknife value of 81%. This placement of *Bauera* is identical to that based on *rbcL* sequences (Fig. 1) and is supported by many other lines of evidence (reviewed in Morgan and Soltis, 1993). In contrast to Engler (1890, 1928), Schulze-Menz (1964), and Hutchinson (1973), most investigators have, in fact, considered *Bauera* to be distinct from the various components of Saxifragaceae s. l. and allied with, or part of, Cunoniaceae (e.g., Cronquist, 1981; Dahlgren, 1983; Thorne, 1992). Phylogenetic analysis of morphological, anatomical, and chemical characters further indicates that *Bauera* should be included in Cunoniaceae (Hufford, 1992; Hufford and Dickinson, 1992). Furthermore, most recent workers have concluded that *Eucryphia* should be considered part of, or closely allied with, Cunoniaceae (Cronquist, 1981; Dahlgren, 1983; Takhtajan, 1987; Hufford, 1992; Hufford and Dickinson, 1992; Thorne, 1992).

Cephalotus (Cephalotaceae), a carnivorous plant, appears as sister to *Bauera*, *Ceratopetalum*, and *Eucryphia* in the 18S rDNA trees (Fig. 2). This result is strongly supported (parsimony jackknife value of 91%) and also agrees with broad analyses of *rbcL* sequences, which similarly place *Cephalotus* in a clade with *Bauera*, Cunoniaceae, and *Eucryphia* (Fig. 1).

Francooideae—In most recent classifications, Francooideae have been considered closely allied with Saxifragoideae, either as part of a narrowly defined Saxifragaceae (Cronquist, 1981; Dahlgren, 1983) or as a closely related family (Hutchinson, 1973; Takhtajan, 1987; Thorne, 1992). Analyses of 18S rDNA sequences demonstrate, however, that *Francoa* is distantly related to Saxifragoideae, as well as to other Saxifragaceae s. l., and is most closely related to *Greyia* (Greyiaceae) (Fig. 3). A sister-group relationship between *Francoa* and *Greyia* is well supported (parsimony jackknife value of 98%) and was similarly revealed by analyses of *rbcL* sequences (Fig. 1) (Morgan and Soltis, 1993; Chase et al., 1993; Price and Palmer, 1993). Chemical (Gornall, Bohm, and Dahlgren, 1979; Bohm and Chan, 1992), palynological (Erdtman, 1966; Hideux and Ferguson, 1976), morphological, and embryological data all suggest that Francooideae are distinct from Saxifragoideae and more closely related to Greyiaceae (see Morgan and Soltis, 1993).

Brexioideae*—*Parnassioideae*—*Lepuropetaloidae—Analyses of 18S rDNA sequences suggest a close relationship among *Brexia* (Brexioideae), *Parnassia* (Parnassioideae), *Lepuropetalon* (Lepuropetaloidae), and *Euonymus* (Celastraceae) (Fig. 3). The shortest 18S rDNA trees reveal that *Parnassia*—*Lepuropetalon* (parsimony jackknife value = 100%) represent a sister pair. *Euony-*

mus and *Brexia* often appear as sister taxa (parsimony jackknife value of 50%), although in the tree depicted (Fig. 3), they appear as successive sisters to *Parnassia*—*Lepuropetalon*. The 18S rDNA results again parallel those obtained in analyses of *rbcL* sequences (compare Figs. 1 and 3), which also reveal a clade comprising *Brexia*, *Euonymus*, *Parnassia*, and *Lepuropetalon* (Chase et al. 1993; Morgan and Soltis, 1993).

The many chemical (Plouvier, 1965; Jay, 1970), morphological, and embryological (Philipson, 1974, 1977; Dahlgren, 1975) features that differentiate *Brexia* from most other Saxifragaceae s. l. and suggest a close relationship between *Brexia* and Celastraceae are discussed by Morgan and Soltis (1993). *Parnassia* and *Lepuropetalon* share many morphological and palynological features that may now be viewed as synapomorphies (Spongberg, 1972; Hideux and Ferguson, 1976; Morgan and Soltis, 1993).

Roussea is a monotypic genus originally referred to Brexioidae (Engler, 1928), but later placed in Escallonioidae or Escalloniaceae (e.g., Hutchinson, 1973; Takhtajan, 1983). Analyses of 18S rDNA sequences indicate that *Roussea* is not closely related to *Brexia*, *Parnassia*, *Lepuropetalon*, or Celastraceae. Instead, it is more closely related to members of Asteridae s. l.; *Roussea* is therefore discussed in more detail below under Escallonioidae.

Subfamilies associated with Asteridae s. l.—Several analyses of *rbcL* sequences have provided evidence for an expanded Asteridae (Asteridae sensu lato; Olmstead et al., 1992, 1993; Chase et al., 1993) and have shown that several subfamilies traditionally attributed to Saxifragaceae s. l. appear to be related to members of Asteridae s. l. (Morgan and Soltis, 1993; Hibsich-Jetter, Soltis, and MacFarlane, in press): Hydrangeoideae, Phyllonomoideae, Escallonioidae, Eremosynoideae, Montinioideae, and Vahlloideae (Figs. 1, 2). Analysis of 18S rDNA sequences (Figs. 3, 4) reveals relationships nearly identical to those suggested by earlier analyses of *rbcL* sequences. Below we discuss the phylogenetic position of each of these six subfamilies in more detail.

Hydrangeoideae—The shortest 18S rDNA trees (Figs. 3, 4) place *Hydrangea*, *Philadelphus* (Hydrangeoideae), *Camptotheca*, *Nyssa* (Nyssaceae), and *Cornus* (Cornaceae) at or near the base of Asteridae s. l. *Hydrangea* and *Philadelphus* are part of a subclade that also contains Actinidiaceae, Balsaminaceae, Clethraceae, Diapensiaceae, Ericaceae, Fouquieriaceae, Primulaceae, Polemoniaceae, Sarraceniaceae, and Theaceae. These findings parallel results based on analyses of *rbcL* sequences; Hydrangeoideae, Cornaceae, and Nyssaceae appear near the base of Asteridae s. l., close to an “ericalean clade” (Chase et al., 1993; Xiang et al., 1993; many of these ericalean families are not represented, however, in Morgan and Soltis, 1993).

In analyses of *rbcL* sequences (Fig. 2), *Camptotheca*, *Nyssa*, *Cornus*, *Hydrangea*, and *Philadelphus* are part of a “cornaceous clade.” In the 18S rDNA trees, in contrast, members of this cornaceous clade do not form a monophyletic group (Fig. 4). However, bootstrap support for relationships in this portion of the 18S rDNA topology

is very low. Thus, the differences between trees based on 18S rDNA and *rbcL* sequences may simply reflect the slower rate of evolution of the former, rather than an actual discrepancy.

Long-standing disagreements surround the proper placement of the genera of Hydrangeoideae (reviewed in Morgan and Soltis, 1993). However, evidence from anatomy, embryology, morphology, chemistry, palynology, and serology not only suggests a distant relationship between Saxifragoideae (Saxifragaceae s. s.) and Hydrangeoideae (Hydrangeaceae), but also points to a close relationship of the latter to Cornaceae (Huber, 1963; Davis, 1966; Erdtman, 1966; Benschel and Palser, 1975a, b; Dahlgren, 1975; Jensen, Nielsen, and Dahlgren, 1975; Hideux and Ferguson, 1976; Krach, 1977; Gornall et al., 1979; Dahlgren, Jensen, and Nielsen, 1981; Kaplan and Gottlieb, 1982; Bohm, Nicholls, and Bhat, 1985; Xiang et al., 1993; reviewed by Morgan and Soltis, 1993). A phylogenetic analysis of morphological, anatomical, and chemical features (Hufford, 1992) also suggested a close relationship between Hydrangeoideae (Hydrangeaceae) and Cornaceae.

Phyllonomoideae—This study suggests that *Phyllonoma* (the only genus of Phyllonomoideae) falls within Asteridae s. l. (Fig. 4) and that its closest relative is *Helwingia*, a genus usually placed in Cornales (see Xiang et al., 1993). Analyses of *rbcL* sequences similarly revealed that *Phyllonoma* is allied with traditional asterids, with a particularly close relationship between *Phyllonoma* and *Helwingia* (compare Figs. 2 and 4). Not only do palynological data differentiate *Phyllonoma* from other Saxifragaceae s. l. (Erdtman, 1966; Hideux and Ferguson, 1976), but several morphological features also strongly support a close relationship between *Phyllonoma* and *Helwingia* (Johnson, 1958; Dickinson and Sattler, 1974, 1975; Hickey and Wolfe, 1975; Dickinson, 1978; reviewed in Morgan and Soltis, 1993).

Escallonioidae—Two genera of Escallonioidae, *Escallonia* and *Roussea*, are represented in the 18S rDNA analysis. Although *Escallonia* is a traditional member of this subfamily, *Roussea* has been variously referred to Brexioidae (or Brexiaceae) (e.g., Engler, 1928; Thorne, 1992), or Escallonioidae (or Escalloniaceae) (e.g., Hutchinson, 1973; Takhtajan, 1983). Although Escallonioidae are typically allied with the woody subfamilies Hydrangeoideae and Montinioideae, *Escallonia* and *Roussea* are well separated from both, based on analyses of 18S rDNA sequences (Figs. 3, 4). In this study, *Escallonia* and *Eremosyne* (Eremosynoideae) are sisters; *Roussea* appears in a clade with Lobeliaceae and Asteraceae.

Analyses of *rbcL* sequences similarly place *Escallonia* with asterids, far removed from all other members of Saxifragaceae s. l., and suggest that *Eremosyne* and *Escallonia* are closely related (Hibsich-Jetter, Soltis, and MacFarlane, in press). Morphological and embryological features support the 18S rDNA and *rbcL* topologies in separating *Escallonia* from Saxifragoideae (reviewed in Morgan and Soltis, 1993). *Roussea* was not included in previous *rbcL* analyses (Morgan and Soltis, 1993) because of the unavailability of material, but a recent *rbcL*

analysis similarly places it in Asteridae s. l. (J. Koontz and D. Soltis, unpublished data). Morphological data also suggest that *Roussea* is not closely related to Brexioideae (Al-Shammery and Gornall, 1994) and instead suggest a close relationship of *Roussea* to *Quintinia* (Ramamonjariisoa, 1980; Al-Shammery and Gornall, 1994), a genus of Escallonioideae that is also part of Asteridae s. l. based on *rbcL* sequence analyses (Xiang and Soltis, 1996).

Escallonioideae are the most morphologically diverse of the 17 subfamilies of Saxifragaceae s. l., and this diversity has complicated efforts to understand the relationships of the subfamily. Recent analyses of *rbcL* sequences have included additional genera of Escallonioideae (e.g., *Quintinia*, *Abrophyllum*) and indicate that the subfamily is polyphyletic (Xiang and Soltis, 1996). The polyphyly of Escallonioideae is further suggested by this study in that *Escallonia* and *Roussea* do not appear to be closely related. Additional studies are needed to determine the circumscriptions of Escallonioideae (Escalloniaceae) and the relationships of genera formerly placed in this subfamily or family.

Eremosynoideae—*Eremosyne*, the only member of subfamily *Eremosynoideae*, is the sister to *Escallonia* in the 18S rDNA analyses (Fig. 4). Although material of *Eremosyne* was not available for the earlier *rbcL* analysis of Saxifragaceae s. l. (Morgan and Soltis, 1993), a recent *rbcL* sequence analysis also suggests a close relationship between *Eremosyne* and *Escallonia* (Hibsch-Jetter, Soltis, and MacFarlane, in press). *Eremosyne* is poorly characterized morphologically and anatomically. However, the presence of unitegmic ovules in *Eremosyne* supports the placement of the genus within Asteridae s. l., rather than near Saxifragaceae s. s. Furthermore, Al-Shammery and Gornall (1994) suggested a close relationship between *Eremosyne* and *Escallonia* based on trichome anatomy.

Montinioideae—The closest relative of *Montinia* according to 18S rDNA sequence evidence is Solanaceae; other close relatives include Hydrophyllaceae, Boraginaceae, Rubiaceae, and Apocynaceae (Fig. 4). In the broad analysis of 18S rDNA sequences, in contrast, the closest relatives of *Montinia* are Solanaceae and Convolvulaceae, with Hydrophyllaceae, Boraginaceae, and Apocynaceae as other close relatives (Fig. 3). Based on analyses of *rbcL* sequence data (Fig. 2), *Montinia* was closely allied with Solanaceae and Convolvulaceae, with Boraginaceae and Hydrophyllaceae also close relatives (Chase et al., 1993; Morgan and Soltis, 1993). Montinioideae have been allied with a diverse array of taxa (e.g., Myrtaceae, Celastraceae, Cucurbitaceae, Onagraceae; Dahlgren, Jensen, and Nielsen, 1977), although apparently no investigator has suggested a relationship with members of Asteridae (see Morgan and Soltis, 1993). As reviewed by Morgan and Soltis (1993), however, several chemical and embryological features clearly differentiate *Montinia* from most Saxifragaceae s. l. and suggest a relationship with genera of Asteridae (Bate-Smith, 1962; Jay, 1970; Jensen, Nielsen, and Dahlgren, 1975; Dahlgren, Jensen, and Nielsen, 1977, 1981; Kaplan and Gottlieb, 1982).

Vahlioideae—*Vahlia*, the only member of Vahlioideae, has traditionally been associated with herbaceous members of Saxifragaceae. Based on rDNA sequences, *Vahlia* is well separated from Saxifragoideae and appears as the sister to the clade containing *Montinia* plus representatives of Solanaceae, Hydrophyllaceae, Boraginaceae, Rubiaceae, and Apocynaceae (Fig. 4). In analyses of *rbcL* sequences (Morgan and Soltis, 1993), *Vahlia* appears as the sister to a clade composed of *Montinia*, Solanaceae, Hydrophyllaceae, and Verbenaceae, as well as other families including Convolvulaceae, Lamiaceae, and Scrophulariaceae (Fig. 2). Thus, the closest relatives suggested for *Vahlia* by 18S rDNA and *rbcL* sequences are similar, but not identical (compare Figs. 2 and 4). Differences between the 18S rDNA and *rbcL* topologies could, in large part, reflect the lower limits of resolution of 18S rDNA sequences. *Vahlia*, like *Eremosyne*, is poorly known morphologically and anatomically.

CONCLUSIONS

Analyses of complete 18S rDNA sequences have provided nuclear-based hypotheses of relationship for members of Saxifragaceae s. l. that are highly concordant with those obtained in broad analyses of *rbcL* sequences (Chase et al., 1993; Morgan and Soltis, 1993; Olmstead et al., 1993). Both 18S rDNA and *rbcL* sequences suggest essentially identical relationships for all 17 of the subfamilies of Saxifragaceae s. l. (Schulze-Menz, 1964). Analyses of 18S rDNA, as well as *rbcL*, sequences suggest that: (1) Saxifragaceae s. l. are an extreme example of a polyphyletic angiosperm family; (2) Saxifragaceae be narrowly defined (Saxifragaceae s. s.) to consist of only ≈ 30 herbaceous genera previously treated as subfamily Saxifragoideae; (3) a Saxifragales clade be recognized that, in addition to Saxifragaceae s. s., encompasses several other subfamilies of Saxifragaceae s. l. (Iteoideae, Ribesoideae, Pterostemonoideae, Penthoroideae, Tetracarpaeoideae), as well as families of Rosidae (Crassulaceae and Haloragaceae), Dilleniidae (Paeoniaceae), and Hamamelidae (Hamamelidaceae, Daphniphyllaceae, and Cercidiphyllaceae); (4) many families traditionally considered close relatives of Saxifragaceae s. s. are only distantly related to this narrowly defined family (e.g., Cunoniaceae, Droseraceae, Cephalotaceae, Gunneraceae, Rosaceae, Greyiaceae); (5) subfamily Baueroideae is part of Cunoniaceae; (6) subfamily Francooideae is closely related to Greyiaceae; (7) Brexioideae, Parnassioideae, and Lepuropetaloidae, along with *Eunonymus* (Celastraceae), form a clade; (8) several subfamilies of Saxifragaceae s. l. (Hydrangeoideae, Phyllonomoideae, Escallonioideae, Montinioideae, Vahlioideae, Eremosynoideae) are part of an expanded asterid clade (Asteridae s. l.); and (9) evidence from embryology, iridoid chemistry, and serology often closely agrees with relationships suggested by 18S rDNA and *rbcL* sequences.

Lastly, this study is of broader systematic importance in that it further illustrates the phylogenetic potential of entire 18S rDNA sequences. Although 18S rDNA sequences clearly lack the level of resolution possible with the more rapidly evolving *rbcL*, this comparative study echoes the findings of Nickrent and Soltis (1995) that

entire 18S rDNA sequences contain sufficient information to conduct phylogenetic studies at higher taxonomic levels within the angiosperms. This conclusion is further supported by phylogenetic analyses of 18S rDNA sequences for over 200 species of angiosperms (Soltis et al., 1997), which suggest relationships among major groups of angiosperms very similar to those retrieved using *rbcL* sequence data (Chase et al., 1993). Hence, at higher taxonomic levels in the angiosperms, analysis of 18S rDNA sequences provides a nuclear-based estimate of relationship for comparison with estimates inferred from *rbcL* sequences.

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