

An ITS-Based Phylogenetic Analysis of the Perennial, Endemic Apiaceae Subfamily Apioideae of Western North America

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ABSTRACT. Phylogenetic analyses of 159 DNA sequences from the nuclear rDNA internal transcribed spacer region were conducted to evaluate the monophyly of the herbaceous, perennial genera of Apiaceae subfamily Apioideae endemic to North America (north of Mexico) and to determine the relationships of those elements that currently comprise *Cymopterus* within the group. The results of a previous phylogenetic study were equivocal in suggesting monophyly for these perennial, endemic taxa and revealed *Cymopterus* to be polyphyletic, with its species closely linked with those of *Aletes*, *Lomatium*, *Musineon*, *Oreoxis*, *Orogenia*, *Podistera*, *Pseudocymopterus*, *Pteryxia*, and *Tauschia*. Herein, we expand sampling to include comprehensive representation of *Aletes*, *Cymopterus*, *Musineon*, *Oreoxis*, *Orogenia*, *Podistera*, *Pseudocymopterus*, and *Pteryxia*, and greater representation of *Lomatium* and *Tauschia*. We also include all members of two genera not examined previously, *Glehnia* and *Oreonana*, as well as additional outgroup genera from the *Angelica* clade of the apioid superclade. Our results indicate that the perennial, endemic apioid umbellifers of North America constitute a (weakly supported) monophyletic group, with *Angelica* and the meso-American *Arracacia* clade comprising two of several possible sister groups. The two subspecies of *Glehnia littoralis* ally with *Angelica* and *Peucedanum japonicum*; *Oreonana* shows affinity with several species of *Cymopterus* and *Lomatium*. The lack of resolution in the ITS trees precludes unambiguous hypotheses of relationship among these perennial, endemic umbellifers but does show that many of these genera, where resolved, are not monophyletic. Indeed, *Cymopterus* and *Lomatium* are highly polyphyletic and permeate all major clades resolved in the molecule-derived trees. Evidence from branch lengths and low sequence divergence suggests that this group of North American umbellifers underwent rapid radiation, likely during the geoclimatic events of the Late Tertiary and Quaternary.

Cymopterus Raf. (Apiaceae subfamily Apioideae), the spring-parsleys, comprises some 35 species (Kartesz 1994) of herbaceous perennials distributed largely throughout western North America (NA). Approximately two-thirds of these species are restricted to the Great Basin region, roughly bounded by the Snake River of Idaho, Sierra Nevada of California, Colorado River of Arizona, and the Rocky Mountains of Colorado (Mathias 1965). Many species are xerophytic or semi-xerophytic, being confined to dry, sandy, or alkaline habitats. They are low in stature, possess thickened, deep taproots, and have ternately cleft, compound or dissected leaves. The inflorescence is an open compound umbel, and the flowers are mostly yellow or white, opening from very early in the spring to throughout the summer. Most distinctive, however, is their fruit, for the two marginal (lateral), and usually one or more of the three dorsal ribs, are conspicuously winged. These wings may be membranous to spongy-corky, and variously plane to wavy or corrugated. Indeed, the generic name is derived from the Greek *kyma*, a wave, and *pteron*, a wing, referring to the often undulate wings of the fruit.

Much confusion exists with regard to the proper delimitation of *Cymopterus* and its relationship to the other indigenous umbellifers of western NA (whose ranges extend from southern Canada to northern Mexico). These genera include *Aletes* J.M. Coult. & Rose, *Glehnia* F. Schmidt ex Miq., *Harbouria* J.M. Coult. & Rose, *Lomatium* Raf., *Musineon* Raf., *Neoparrya* Mathias,

Oreonana Jeps., *Oreoxis* Raf., *Orogenia* S. Watson, *Podistera* S. Watson, *Pseudocymopterus* J.M. Coult. & Rose, *Pteryxia* (Nutt. ex Torr. & A. Gray) J.M. Coult. & Rose, and *Shoshonea* Evert & Constance (Mathias 1930; Evert and Constance 1982; Downie et al. 2002). All are perennial taprooted herbs and many are similar to *Cymopterus* in their primarily low-growing and acaulescent or subcaulescent habit. *Lomatium* is the largest genus, with 78 species; *Glehnia*, *Harbouria*, *Neoparrya*, and *Shoshonea* are monospecific, and the remainder comprise some 2–6 species each (Kartesz 1994). Considered collectively, these plants present such a confusing intergradation of similar characteristics that generic delimitation is made exceedingly difficult, and in the absence of mature fruits, many species are essentially indistinguishable (Jones 1908; Mathias 1930; Theobald et al. 1963; Weber 1984; Cronquist 1997; Downie et al. 2002). Cronquist (1997) included *Oreoxis*, *Pseudocymopterus*, and *Pteryxia* within a broadly circumscribed *Cymopterus* (Hitchcock and Cronquist 1961; Cronquist 1997). Cronquist (1997) also stated that the distinction between *Cymopterus* and *Lomatium*, i.e., the conspicuously winged dorsal fruit ribs of the former, “is subject to failure.” Phylogenetic studies of these endemic and largely cordilleran genera are few and have focused almost exclusively upon *Lomatium* (Schlessman 1984; Simmons 1985; Gilmartin and Simmons 1987; Soltis et al. 1995; Soltis and Novak 1997; Hardig and Soltis 1999).

Results from a previous phylogenetic study of 85

species of NA apioid umbellifers revealed that *Cymopterus* (17 examined species) and *Lomatium* (28 examined species) are each polyphyletic, with their species intertwined with those of *Aletes*, *Musineon*, *Oreoxis*, *Orogenia*, *Podistera*, *Pseudocymopterus*, *Pteryxia*, and *Tauschia* Schldl. (Downie et al. 2002). Closely allied were three genera of central to eastern NA distribution [*Polytaenia* DC., *Taenidia* (Torr. & A. Gray) Drude, and *Thaspium* Nutt.] and *Zizia* W.D.J. Koch, the latter distributed widely across NA. Our observations of mature fruit cross-sections, as well as cladistic analysis of morphological characters, confirmed that those characteristics of the fruit used traditionally to diagnose genera (such as wing shape and composition, and the orientation of mericarp compression) are indeed highly variable, thus severely limiting their usefulness in phylogenetic estimation. The results of Downie et al. (2002) verified the close relationship among these genera, but were surprising in suggesting an evolutionary history of the group much more complicated than previously considered.

The restricted distribution of many endemic, western NA apioid genera to elevated regions of similar habitat, their similar life history and overall general habit, and overlapping patterns of fruit variation suggest that this group of umbellifers may be closely related. An obsolete stylopodium in all genera save *Podistera*, where the stylopodium is well developed as it is in most other umbellifers (Mathias and Constance 1944–1945), is a synapomorphy uniting the group (Downie et al. 2002). Further support for their monophyly comes from the shared presence of a protogynous breeding system (and associated reproductive and ecologic characteristics), atypical in a family where floral protandry prevails (Lindsey and Bell 1980; Lindsey 1982; Barrie and Schlessman 1987; Schlessman et al. 1990; Schlessman and Graceffa 2002). Protogyny is presumed derived in apioid umbellifers, a response to an early flowering season and unreliable pollinators (Schlessman and Graceffa 2002). However, the results of our prior study of the group were equivocal in confirming the monophyly of these taxa endemic to NA, owing to the lack of resolution obtained at the base of the molecular phylogenies (Downie et al. 2002).

In this paper, we augment our study of *Cymopterus* phylogeny by expanded sampling within the genus, and we consider additional material from genera that were poorly represented in our initial study (i.e., *Aletes*, *Lomatium*, *Podistera*, *Pteryxia*, and *Tauschia*). We also include representation of two genera previously unavailable to us, *Glehnia* and *Oreonana*, but whose morphologies suggest affinities to *Cymopterus* and *Tauschia*, respectively (Mathias 1928, 1930; Shevock and Constance 1979). Once more, we focus our attention on the endemic, perennial apioid genera of western NA and, as outgroups, we include additional representation

from the *Angelica* clade of the apioid superclade (Downie et al. 2001)—specifically, the perennial, circumboreal genera *Seseli* L., *Selinum* L., and *Peucedanum* L. Our major objectives are to evaluate the monophyly of the perennial, endemic genera of NA (north of Mexico) and to ascertain the phylogenetic relationships of those elements that currently comprise *Cymopterus* within this group. We continue to utilize the nuclear rDNA internal transcribed spacer (ITS) region for phylogenetic inference, given the wealth of ITS sequence data already accumulated for the Apiaceae for comparative purposes, their faster substitution rates compared to loci from the chloroplast genome (Downie et al. 2001), and their utility in resolving relationships among congeneric species of Apiaceae (Soltis and Kuzoff 1993; Downie and Katz-Downie 1996; Vargas et al. 1999).

MATERIALS AND METHODS

Taxon Sampling. Complete ITS-1 and ITS-2 sequences for 74 accessions of Apiaceae subfamily Apioideae are reported here for the first time (Table 1); these were combined with 85 previously published ITS sequences (Downie et al. 2002), for a total of 159 accessions.

Kartesz (1994), whose checklist of Apiaceae was influenced by the work of Lincoln Constance, recognized 35 species of *Cymopterus* in NA. Two species have infrageneric taxa: *C. acaulis* has five varieties; *C. panamintensis* has two. With the exception of the narrowly endemic and rarely collected *C. megacephalus* M.E. Jones, we sampled all taxa of *Cymopterus*. We also included the recently described *C. constancei* (Hartman 2000) and *C. longilobus*, the latter placed in synonymy under *Pteryxia hendersonii* by Kartesz (1994) but maintained as a distinct species of *Cymopterus* by R. Hartman (unpubl. data). Following the checklist of Kartesz (1994), we sampled all seven recognized taxa of *Aletes* (plus the Mexican *A. calicicola*; Mathias and Constance 1981), both subspecies of *Glehnia littoralis*, and all seven taxa of *Pteryxia* (*P. terebinthina* var. *calcareia* is maintained as distinct from *P. terebinthina* var. *albiflora*, based on Goodrich 1986). All species of *Musineon*, *Oreonana*, *Oreoxis*, *Orogenia*, *Podistera*, *Polytaenia*, and *Pseudocymopterus* were also included, as were 30 accessions of *Lomatium* and five species of *Tauschia*.

As outgroups, we included representation of the perennial, circumboreal genus *Angelica* and the meso-American *Arracacia* clade (i.e., *Arracacia*, *Prionosciadium*, and *Rhodosciadium*; Downie et al. 2001). Mathias (1965) described *Cymopterus* as being derived from “*Angelica*-like stock” which became adapted to drier habitats. She also noted similarities between a fossil umbelliferous fruit from a Miocene formation in Washington State and present-day *Rhodosciadium* from Mexico (Berry 1929, as cited by Mathias 1965). Results from our prior phylogenetic analysis showed that these genera are included alongside *Cymopterus* in a large, polytomous clade (Downie et al. 2002). We also considered members of the circumboreal genera *Seseli*, *Selinum*, and *Peucedanum*, for each of these genera has representatives occurring in NA. The western NA genus *Sphenosciadium* (which eventually may be transferred into *Angelica*; Katz-Downie et al. 1999) was also included, as were the NA genera *Spermolepis* and *Ciclospermum*. *Aethusa cynapium* was used to root all trees, based on the results of previous higher-level investigations where this species was sister taxon to all aforementioned taxa (Downie et al. 2000c, 2002). The 22 outgroup representatives included herein are all provisionally treated in the *Angelica* and *Arracacia* clades of the apioid superclade (Plunkett and Downie 1999; Downie et al. 2001).

Experimental Strategy. Details of the DNA extractions, PCR amplifications, and DNA sequencing are provided elsewhere (Downie and Katz-Downie 1996; Downie et al. 2000a). Total ge-

nomic DNA was obtained from ca. 20 mg of dried, leaf tissue using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) and PCR-amplified using primers "ITS4" and "ITS5" (White et al. 1990). Cycle sequencing reactions, using these same primers, were performed on the purified PCR products, using AmpliTaq DNA polymerase and fluorescent dye-labeled terminators (ABI Prism BigDye Terminator vers. 3.0 Ready Reaction Cycle Sequencing Kit; Applied Biosystems, Foster City, CA). Sequencing products were resolved by electrophoresis using Applied Biosystem's 373A DNA sequencer. Both ITS spacer regions were sequenced in their entirety on both DNA strands. All ITS-1 and ITS-2 data have been deposited in GenBank (Table 1), and the data matrix is available in TreeBASE (study accession number S1010, matrix accession number M1706).

Data Analysis. DNA sequences from both spacer regions were aligned using CLUSTAL X (Jeanmougin et al. 1998) and adjusted manually, as necessary. Sequence data from the invariant 5.8S subunit were excluded as they were unavailable for several previously published taxa owing to the manual sequencing methods used to obtain these earlier data. Uncorrected pairwise nucleotide differences ("p") were determined using PAUP* vers. 4.0 (Swofford 1998), as they are commonly provided in other angiosperm ITS studies (Baldwin et al. 1995). To facilitate analysis, taxa bearing identical sequences were combined (i.e., two accessions of *Cymopterus constancei*; two accessions of *Oreoxis trotteri*; three accessions of *Pteryxia terebinthina* var. *albiflora*; three accessions of *P. terebinthina* var. *calcarea*; two of five accessions of *Aletes macdougallii* subsp. *breviradiatus*; and the three remaining accessions of *A. macdougallii* subsp. *breviradiatus*), for a final tally of 150 terminals for the cladistic analyses. Fewer than 0.3% of all data matrix cells were scored as missing data; the sequences with missing data were obtained from GenBank and submitted by others.

The resulting data matrix was first analyzed using equally-weighted maximum parsimony (MP), with gap states treated as missing data. Alignment gaps were not scored as separate presence-absence characters because of ambiguous length mutations in several previously submitted *Lomatium* sequences as a result of compressions. MP trees were sought using the heuristic search strategies of PAUP* and the inverse-constraint approach described in Downie et al. (1998). The maximum number of MP trees was set at 20,000 and these trees were permitted to swap to completion. Bootstrap values (Felsenstein 1985) were calculated from 100,000 replicate analyses using "fast" stepwise-addition of taxa; only those values compatible with the 50% majority-rule consensus tree were recorded. Estimates of support obtained using this method are generally similar to those obtained from bootstrap analyses with branch swapping, particularly for well-supported nodes (Mort et al. 2000). The number of additional steps required to force particular taxa into a monophyletic group was examined using the constraint option of PAUP*. The g_1 statistic of skewed tree-length distribution was calculated from 10,000 random parsimony trees generated by PAUP*, and was used to assess the amount of nonrandom structure in the data (Hillis and Huelsenbeck 1992).

A maximum likelihood (ML) analysis of these ITS data was then performed. Prior to this analysis, the program Modeltest vers. 3.06 (Posada and Crandall 1998) was used to select an evolutionary model of nucleotide substitution that best fits these data. Using the hierarchical likelihood ratio test statistic, Modeltest selected the TrN+G (Tamura and Nei 1993) model (base frequencies: 0.2725, A; 0.2154, C; 0.2312, G; 0.2809, T; estimates of substitution rates: A↔C, 1; A↔G, 2.2734; A↔T, 1; C↔G, 1; C↔T, 4.5457; G↔T, 1; proportion of invariable sites = 0; gamma distribution shape parameter = 0.5930). These settings were entered into PAUP* and a heuristic search carried out using ten random addition sequence replicates and TBR branch-swapping under ML optimization. A bootstrap analysis was conducted using the neighbor-joining method and the ML parameters estimated by Modeltest. Bootstrap values were calculated from 100 replicate analyses.

Owing to its computational efficiency with large data sets, a phylogenetic analysis was also performed using MrBayes vers. 2.01 (Huelsenbeck and Ronquist 2001), a Bayesian inference pro-

gram. Starting trees were chosen at random and one million generations were run with sampling occurring every 100 generations. Tree topologies were based on the general time reversible (GTR) model of maximum likelihood (with the shape parameter of the gamma distribution inferred by Modeltest) and four simultaneous Markov Chain Monte Carlo chains to model nucleotide rate heterogeneity. Two-thousand trees were discarded as "burn-in" prior to calculating the 50% majority-rule tree. Numbers on internal branches of the tree indicate the percent of time the clade occurs among the sampled trees (i.e., the posterior probability of that clade existing; Huelsenbeck and Ronquist 2001).

RESULTS

Sequence Analysis. Alignment of 150 ITS-1 and ITS-2 sequences (representing 159 accessions) resulted in a matrix of 458 positions, with none excluded because of alignment ambiguity. Characteristics of these aligned sequences, as separate spacer regions or combined, are presented in Table 2. Thirty-seven gaps, ranging between 1 and 6 bp in size, were introduced to facilitate alignment; 33 of these were 1-bp in length, two were 2-bp in length, one was 4-bp in length, and one was 6-bp in length. Uncorrected pairwise sequence divergence values across both spacers and all taxa ranged from identity to 12.1% of nucleotides. Pairwise sequence divergence values for the 126 ingroup terminal taxa ranged from identity to 9.4% of nucleotides (the latter between *Cymopterus basalticus* and *Tauschia texana*). Pairwise distances within *Cymopterus* (n=41) ranged from identity to 7.3%; distances within *Lomatium* (n=30) ranged from identity to approximately 8.0%. Percent G + C content over both spacers ranged between 54.2 and 59.9% (mean = 57.1%). The g_1 statistic for 10,000 random trees was -0.220. This value is significantly more skewed (i.e., more negative) than random data ($g_1 = -0.09$ for 250 variable positions and 25 or more taxa; $P < 0.01$), indicating that these ITS data contain significant amounts of phylogenetic signal (Hillis and Huelsenbeck 1992). No evidence of obvious ITS length variants, representative of multiple rDNA repeat types, was observed.

Phylogenetic Analysis. MP analysis of 150 ITS-1 and ITS-2 sequences (159 accessions) resulted in the preset limit of 20,000 minimal length trees, each of 881 steps (consistency indices of 0.4654 and 0.3762, with and without uninformative characters, respectively, and a retention index of 0.6405). The strict consensus of these trees is presented in Fig. 1. Bootstrap estimates were generally low, with few values exceeding 85%.

The consensus tree revealed a monophyletic assemblage of endemic, perennial NA genera, although the clade was very weakly supported (with <50% bootstrap value). Included in this group are 126 terminals (reflecting 135 accessions) represented by 18 genera: *Aletes*, *Cymopterus*, *Harbouria*, *Lomatium*, *Musineon*, *Neoparrya*, *Oreonana*, *Oreoxis*, *Orogenia*, *Podistera*, *Polytaenia*, *Pseudocymopterus*, *Pteryxia*, *Shoshonea*, *Taenidia*, *Tauschia*,

TABLE 1. Voucher information and GenBank accession numbers for those accessions of Apiaceae subfamily Apioideae examined for nuclear rDNA ITS sequence variation. Equivalent information for those taxa considered in a previous phylogenetic study is presented in Downie et al. (2002).

Aletes calcicola Mathias & Constance—Mexico, Coahuila, Sierra de San Marcos, opposite Los Fresnos, 4–5 Apr 1969, *Pinkava et al.* 6117 (UC), ITS-1 AY146818, ITS-2 AY146884. *A. filifolius* Mathias, Constance & W.L. Theob.—USA, Texas, Culberson Co., Hunter Peak at Pine Top, Guadalupe Mountains National Park, 6 Sep 1987, *Higgins* 17453 (BRY), ITS-1 AY146819, ITS-2 AY146885. *A. macdougallii* J.M. Coult. & Rose W.L. **subsp. breviradiatus** Theob. & C.C. Tseng—USA, Utah, San Juan Co., Manti-LaSal National Forest, Notch Canyon, jct of Cottonwood Wash, 5 Jun 1985, *Atwood* 11143 (BRY), ITS-1 AY146820, ITS-2 AY146886; USA, New Mexico, San Juan Co., Chaco Culture Nat. Hist. Park, top of Chacra Mesa, 2 May 1993, *Heil* 7374 (UNM), ITS-1 AY146821, ITS-2 AY146887; USA, Utah, Garfield Co., Dixie National Forest, Death Hollow, 28 May 1989, *Franklin* 6499 (BRY), ITS-1 AY146822, ITS-2 AY146888; USA, New Mexico, San Juan Co., NM 575, 0.15 mi NNW of US 64, NW of Blanco, 10 Jul 1999, *Sun & Hartman* 649 (ILL), ITS-1 AY146823, ITS-2 AY146889. *A. macdougallii* **subsp. macdougallii**—USA, Arizona, Coconino Co., Grand Canyon, Kaibab Trail to Roaring Springs, 23 Jun 1933, *Eastwood & Howell* 986 (UC), ITS-1 AY146824, ITS-2 AY146890.

Angelica grayi (J.M. Coult. & Rose) J.M. Coult. & Rose—USA, Colorado, Garfield Co., Blair Mountain, 17 mi NNW of Glenwood Springs, 24 Aug 1991, *Vanderhorst & Palaci* 4490 (RM), ITS-1 AY146825, ITS-2 AY146891.

Cymopterus aboriginum M.E. Jones—USA, California, Mono Co., ca 5 mi E of Benton along Hwy 6, 21 Jun 1990, *Atwood* 13338 (BRY), ITS-1 AY146826, ITS-2 AY146892. *C. acaulis* (Pursh) Raf. **var. greeleyorum** J.W. Grimes & P.L. Packard—USA, Oregon, Malheur Co., McBride Creek Road, 24 May 1989, *Smithman et al.* LS-2045 (CIC), ITS-1 AY146827, ITS-2 AY146893. *C. acaulis* **var. higginsii** (S.L. Welsh) S. Goodrich—USA, Utah, Kane Co., E of Coyote Wash, 8 Apr 1993, *Chapman s.n.* (BRY), ITS-1 AY146828, ITS-2 AY146894. *C. acaulis* **var. parvus** S. Goodrich—USA, Utah, Tooele Co., Indian Rice Grass, Dunefield, NW of English village, 7 Jun 1993, *Johnson* 351 (BRY), ITS-1 AY146829, ITS-2 AY146895. *C. beckii* S.L. Welsh & S. Goodrich—USA, Utah, San Juan Co., SW of Abajo Mountains, S of the Causeway, 10 Jul 1992, *Franklin* 7564 (BRY), ITS-1 AY146830, ITS-2 AY146896. *C. cinerarius* A. Gray—USA, California, Mono Co., Sweetwater Canyon, 15 Jul 1944, *Alexander & Kellogg* 3893 (UTC), ITS-1 AY146831, ITS-2 AY146897. *C. constancei* R.L. Hartm.—USA, Colorado, Montrose Co., San Miguel and lower Dolores River drainages, Simbad Valley, 30 May 1995, *Hartman* 50943 (RM), ITS-1 AY146832, ITS-2 AY146898; USA, Wyoming, Sublette Co., Upper Green River Plain: Cretaceous Mountain, 19 May 1993, *Hartman* 37182 (RM), ITS-1 AY146833, ITS-2 AY146899. *C. corrugatus* M.E. Jones—USA, Nevada, Mineral Co., Walker River Indian Reservation, Rawhide Flats, 15 May 1988, *Tiehm* 11640 (BRY), ITS-1 AY146834, ITS-2 AY146900. *C. coulteri* (M.E. Jones) Mathias—USA, Utah, Sevier Co., Triangle Mt, Soldiers Canyon, 15 May 1990, *Thorne & Zupan* 7377 (BRY), ITS-1 AY146835, ITS-2 AY146901. *C. davisii* R.L. Hartm.—USA, Idaho, Cassia Co., N slope of Mt Harrison, 30 Jul 1983, *Cronquist* 11840 (UTC), ITS-1 AY146836, ITS-2 AY146902. *C. deserticola* Brandegee—USA, California, San Bernardino Co., 1 mi S of Kramer, 21 Mar 1958, *Raven* 11891 (UTC), ITS-1 AY146837, ITS-2 AY146903. *C. douglassii* R.L. Hartm. & Constance—USA, Idaho, Custer Co., 2 mi N of Horseheaven Pass and just W of Spring Hill VABM, Pahsimeroi Mountains, Challis National Forest, 26 Jun 1984, *Atwood* 10302 (BRY), ITS-1 AY146838, ITS-2 AY146904. *C. gilmanii* C. Morton—USA, Nevada, Clark Co., Desert Range, SE base, Desert National Wildlife Refuge, 7.3 mi from Sheep Peak summit, 17 May 1987, *Morefield et al.* 4463 (BRY), ITS-1 AY146839, ITS-2 AY146905. *C. glaucus* Nutt.—USA, Idaho, Valley Co., ridge between Fisher Creek Saddle and Black Tip along Idaho/Valley Co. line, 26 Jul 1989, *Ertter et al.* 8742 (RM), ITS-1 AY146840, ITS-2 AY146906. *C. goodrichii* S.L. Welsh & Neese—USA, Nevada, Lander Co., Toiyabe Range, 2 mi N of Bunker Hill, 16 Jul 1981, *Neese et al.* 10737 (BRY), ITS-1 AY146841, ITS-2 AY146907. *C. lapidosus* (M.E. Jones) M.E. Jones—USA, Wyoming, Uinta Co., Uinta Co Road 290, 3.7 air mi W of Lonetree, 7 Jul 1983, *Hartman* 15767 (UTC), ITS-1 AY146842, ITS-2 AY146908. *C. longilobus* (Rydb.) W.A. Weber—USA, Wyoming, Sublette Co., Wyoming Range, 15 Aug 1993, *Hartman* 43986 (RM), ITS-1 AF358482, ITS-2 AF358549. *C. macrorhizus* Buckley—USA, Texas, Tom Green Co., Grandview campground on W side of Fisher Lake, 19 Mar 1983, *Ertter & Strachan* 4690 (RM), ITS-1 AY146843, ITS-2 AY146909. *C. minimus* (Mathias) Mathias—USA, Utah, Iron Co., Cedar Breaks National Monument, just below N rim where first reached by Rattlesnake Trail, 25 Jul 1993, *Stone* 1656 (BRY), ITS-1 AY146844, ITS-2 AY146910. *C. newberryi* (S. Watson) M.E. Jones—USA, Utah, San Juan Co., 4.5 mi downstream from Bluff, 20 May 1983, *Barneby* 17896 (BRY), ITS-1 AY146845, ITS-2 AY146911. *C. panamintensis* J.M. Coult. & Rose **var. acutifolius** (J.M. Coult. & Rose) Munz—USA, California, San Bernardino Co., south side of Ord Mtn, north of Lucerne Valley, 8 Apr 1988, *Boyd et al.* 1776 (OSC), ITS-1 AY146846, ITS-2 AY146912. *C. ripleyi* Barneby—USA, Nevada, Nye Co., Stone Cabin Valley, 24 May 1995, *Holmgren & Holmgren* 12299 (BRY), ITS-1 AY146847, ITS-2 AY146913. *C. rosei* (M.E. Jones ex J.M. Coult. & Rose) M.E. Jones—USA, Utah, Sanpete Co., Hills W of Ninemile Reservoir, 8 May 1987, *Franklin* 4604 (BRY), ITS-1 AY146848, ITS-2 AY146914.

Glehnia littoralis F. Schmidt ex Miq. **subsp. littoralis**—Taiwan, Taitung Hsien, Lanyu (Orchid Island), *Liao et al.* 1235 (MO), ITS-1 AY146849, ITS-2 AY146915. *G. littoralis* **subsp. leiocarpa** (Mathias) Hultén—USA, Oregon, Tillamook Co., by the beach on the south side of Cape Kiwanda, 10 Aug 1975, *Halse* 1228 (OSC), ITS-1 AY146850, ITS-2 AY146916.

Lomatium ambiguum (Nutt.) J.M. Coult. & Rose—USA, Montana, Madison Co., ca 1.5 mi from road's end on Bear Trap Canyon Trail, 30 May 1979, *Lowry* 1867 (ILL), ITS-1 AY146851, ITS-2 AY146917. *L. bradshawii* (Rose ex Mathias) Mathias & Constance—USA, Oregon, Benton Co., NE Corvallis, W of Canterbury Circle, area around Frazier Creek, 8 Jun 1980, *Halse* 2215 (ILL), ITS-1 AY146852, ITS-2 AY146918. *L. brandegei* (J.M. Coult. & Rose) J.F. Macbr.—USA, Washington, Chelan Co., 5 mi SW of Leavenworth on Snow Creek Trail, 29 May 1977, *Lowry* 621 (ILL), ITS-1 AY146853, ITS-2 AY146919. *L. grayi* (J.M. Coult. & Rose) J.M. Coult. & Rose **var. grayi**—USA, Wyoming, Lincoln Co., Tump Range, Preacher Hollow, 16 mi NNE of Cokeville, 22 May 1993, *Hartman* 37444 (RM), ITS-1 AY146854, ITS-2 AY146920. *L. grayi* **var. depauperatum** (M.E. Jones) Mathias—USA, Utah, Juab Co., Gilson Mountains, 7 mi from Lynndyl, 7 May 1981, *Goodrich* 15335 (RM), ITS-1 AY146855, ITS-2 AY146921.

Musineon lineare (Rydb.) Mathias—USA, Utah, Cache Co., Toney Grove Trailhead Trail to Naomi Peak, 9 Jul 1989, *Hartman* 24364 (RM), ITS-1 AY146856, ITS-2 AY146922.

Oreonana clementis (M.E. Jones) Jeps.—USA, California, Tulare Co., Mt Guyot, 22 Jul 1949, *Howell* 25604 (UTC), ITS-1 AY146857, ITS-2 AY146923. *O. purpurascens* Shevock & Constance—USA, California, Tulare Co., Sequoia National Forest, Slate Mountain, along USFS trail 31E14, 26 Apr 1977, *Shevock* 5428 (BRY), ITS-1 AY146858, ITS-2 AY146924. *O. vestita* (S. Watson) Jeps.—USA, California, San Bernardino Co., Angeles National Forest, San Gabriel Mtns, between Mt Harwood and Mt San Antonio Peak, 4 May 1977, *Shevock* 5433 (BRY), ITS-1 AY146859, ITS-2 AY146925.

TABLE 1. Continued.

Oreoxis trotteri S.L. Welsh & S. Goodrich—USA, Utah, Grand Co., E of Courthouse Rock, 20 May 1985, *Welsh 23427* (BRY), ITS-1 AY146860, ITS-2 AY146926; USA, Utah, Grand Co., 13.5 mi NNW of Moab, 4 Jun 1985, *Neese & Welsh 16984* (BRY), ITS-1 AY146861, ITS-2 AY146927.

Orogenia fusiformis S. Watson—USA, Oregon, Josephine Co., flats near S boundary with Siskiyou NF, Oregon Caves National Monument, 24 May 1996, *Zika 12864* (OSC), ITS-1 AY146862, ITS-2 AY146928.

Peucedanum japonicum Thunb. ex A. Murray—cult. Botanical Conservatory Mulhouse, France (no. 97138), 8 Dec 1999, *Reduron s.n.* (Herb. Reduron), ITS-1 AF495826, ITS-2 AF495827. *P. ostruthium* (L.) W.D.J. Koch (= *Imperatoria ostruthium* L.)—Downie et al. (1998), ITS-1 U78403, ITS-2 U78463.

Podistera macounii (J.M. Coult. & Rose) Mathias & Constance—USA, Alaska, ridge W of Sheep Creek, 30 Jun 1977, *Reed et al. 6047* (BRY), ITS-1 AY146863, ITS-2 AY146929; USA, Alaska, Mountains between Essie Creek and the headwaters of Seventymile Drainage, 21 Jun 2002, *Larsen et al. s.n.* (ALA), ITS-1 AY146864, ITS-2 AY146930. *P. nevadensis* (A. Gray) S. Watson—USA, California, El Dorado Co., Lake Tahoe Basin Management Unit, Freel Peak, 27 Jul 2002, *Matson 634* (ILL), ITS-1 AY146865, ITS-2 AY146931. *P. yukonensis* Mathias & Constance—USA, Alaska, Kathul Mountain, along the Yukon River roughly halfway between Eagle and Circle, 11 Jun 2002, *Larsen & Batten 02-1263* (ALA), ITS-1 AY146866, ITS-2 AY146932.

Polytaenia texana (J.M. Coult. & Rose) Mathias & Constance—USA, Texas, Burnet Co., E of Briggs, 25 May 1985, *Barrie 1403* (RM), ITS-1 AY146867, ITS-2 AY146933.

Pseudocymopterus longiradiatus Mathias, Constance & W.L. Theob.—USA, New Mexico, Otero Co., Sacramento Mts., ca 3 mi SSW of Bent, SW side of Domingo Peak, 7 Aug 1990, *Sivinski 1546* (UNM), ITS-1 AY146868, ITS-2 AY146934.

Pteryxia davidsonii (J.M. Coult. & Rose) Mathias & Constance—USA, New Mexico, Grant Co., Bear Mountain, near Silver City, 17 Jun 1903, *Metcalfe 165* (RM), ITS-1 AY146869, ITS-2 AY146935. *P. hendersonii* (J.M. Coult. & Rose) Mathias & Constance—USA, Utah, Duchesne Co., 0.75 mi SE of Davis Lake, Uinta Mtns, 23 Aug 1996, *Huber 3502* (BRY), ITS-1 AY146870, ITS-2 AY146936. *P. petraea* (M.E. Jones) J.M. Coult. & Rose—USA, Nevada, Nye Co., Toiyabe National Forest, Toquima Range, Iron Springs, 5 Jun 1978, *Goodrich 11292* (UTC), ITS-1 AY146871, ITS-2 AY146937. *P. terebinthina* (Hook.) J.M. Coult. & Rose var. *terebinthina*—USA, Washington, Benton Co., Pit 30, between the 200 area, 17 May 1993, *McKinnon & Sackschewsky 327* (BRY), ITS-1 AY146872, ITS-2 AY146938. *P. terebinthina* var. *albiflora* (Nutt. ex Torr. & A. Gray) Mathias—USA, Wyoming, Sublette Co., Upper Green River Basin, ca 9.5 air mi NNW of La Barge, 20 May 1993, *Hartman 37374* (RM), ITS-1 AY146873, ITS-2 AY146939; USA, Utah, Daggett Co., Flaming Gorge National Recreation area, 5 Jun 1995, *Refsdal & Goodrich 3591* (RM), ITS-1 AY146874, ITS-2 AY146940. *P. terebinthina* var. *calcareo* (M.E. Jones) Mathias—USA, Wyoming, Uinta Co., 2.75 mi N of Lone-tree, 2 Jul 1999, *Goodrich 26060* (BRY), ITS-1 AY146875, ITS-2 AY146941; USA, Utah, Daggett Co., Ashley National Forest, Flaming Gorge National Recreation area, E side of Flaming Gorge Reservoir, 26 May 1992, *Goodrich 24045* (BRY), ITS-1 AY146876, ITS-2 AY146942; USA, Wyoming, Big Horn Co., Big Horn Mountains, Cold Spring Road, 26 May 1980, *Hartman & Dueholm 11224* (RM), ITS-1 AY146877, ITS-2 AY146943; USA, Wyoming, Carbon Co., ca 7 air mi NE of Savery, 14 Jun 1979, *Hartman & Coffey 8961* (RM), ITS-1 AY146878, ITS-2 AY146944. *P. terebinthina* var. *californica* (J.M. Coult. & Rose) Mathias—USA, California, Madera Co., near Whiskey Creek, 1 mi NW of Ellis Meadow, Sierra Nevada, 1 Jul 1938, *Constance 2390* (RM), ITS-1 AY146879, ITS-2 AY146945. *P. terebinthina* var. *foeniculacea* (Nutt. ex Torr. & A. Gray) Mathias—USA, Idaho, Custer Co., Morgan Creek, 10 mi N of Challis, 26 Jun 1984, *Atwood 10320* (RM), ITS-1 AY146880, ITS-2 AY146946.

Selinum candollei DC.—Downie and Katz-Downie (1996), ITS-1 U30564, ITS-2 U30565. *S. carvifolia* (L.) L.—France, Bas-Rhin, between Herbsheim and Boofzheim, 14 Aug 2001, *Reduron s.n.* (Herb. Reduron), ITS-1 AY179028, ITS-2 AY179028.

Seseli gracile Waldst. & Kit.—Katz-Downie et al. (1999), ITS-1 AF008605, ITS-2 AF009084. *S. libanotis* (L.) W.D.J. Koch—Katz-Downie et al. (1999), ITS-1 AF008603, ITS-2 AF009082. *S. tortuosum* L.—Portugal, Lisboa, Sintra Praja das Macas, cult. Botanical Conservatory Mulhouse, France (no. 98042), 2 Aug 2001, *Hildenbrand et al. s.n.* (Herb. Reduron), ITS-1 AY179031, ITS-2 AY179031.

Tauschia arguta (Torr. & A. Gray) J.F. Macbr.—USA, California, San Diego Co., Corte Madera Ranch, near Pine Valley, 22 May 1983, *Van der Werff & Clark 4571* (MO), ITS-1 AY146881, ITS-2 AY146947. *T. glauca* (J.M. Coult. & Rose) Mathias & Constance—USA, California, Trinity Co., SE of Burnt Ranch, 11 July 1990, *Spellenberg 10254* (RM), ITS-1 AY146882, ITS-2 AY146948. *T. kelloggii* (A. Gray) J.F. Macbr.—USA, California, Butte Co., SW of intersection of Black Bart Road and Forbestown Road, about 3.5 air mi SW of Forgestown, 16 Jun 1993, *Ahart 6972* (UC), ITS-1 AY146883, ITS-2 AY146949.

TABLE 2. Sequence characteristics of the two internal transcribed spacer (ITS) regions, separately and combined, for 150 terminals (159 accessions) of Apiaceae subfamily Apioideae.

Sequence characteristic	ITS-1	ITS-2	Combined (ITS-1 & ITS-2)
Length variation	206–218	220–226	427–441
No. of aligned positions	225	233	458
No. of aligned positions constant	91	93	184
No. of aligned positions parsimony-informative	89	79	168
No. of aligned positions autapomorphic	45	61	106
No. of unambiguous alignment gaps	21	16	37
No. of unambiguous gaps parsimony-informative	11	5	16
Maximum pairwise sequence divergence (%)	12.5	13.1	12.1

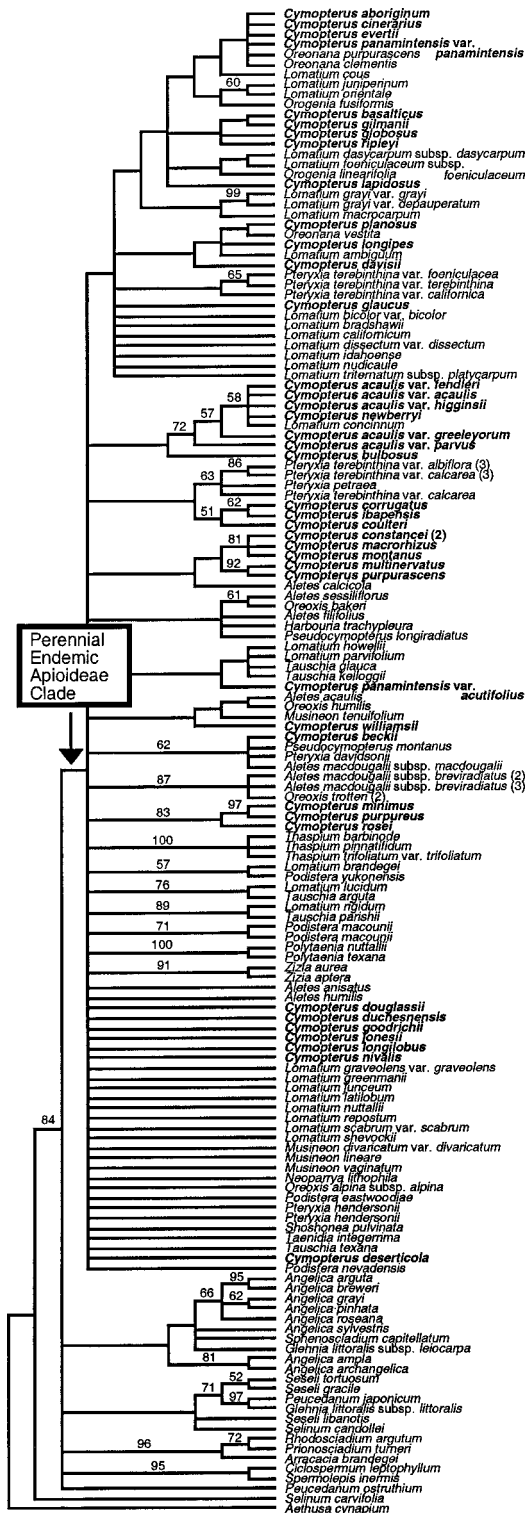


FIG. 1. Strict consensus of 20,000 minimal length 881-step trees derived from equally-weighted maximum parsimony analysis of aligned ITS-1 and ITS-2 sequences from 150 terminals (159 accessions) of Apiaceae subfamily Apioideae. Consistency indices = 0.4654 and 0.3762, with and without

Thaspium, and *Zizia*. A large basal polytomy obscures relationships among these umbellifers, yet four major clades containing six or more taxa each are apparent. The first and most distal of these clades comprises 37 taxa, representing 13 accessions of *Cymopterus*, 16 accessions of *Lomatium*, 3 accessions each of *Oreonana* and *Pteryxia*, and 2 accessions of *Orogenia*. A second clade comprises all five accessions of *Cymopterus acaulis*, plus *C. bulbosus*, *C. newberryi*, and *Lomatium concinnum*. A third clade comprises some but not all infra-specific accessions of *Pteryxia terebinthina*, plus *Pteryxia petraea*, *Cymopterus corrugatus*, *C. coulteri*, and *C. ibapensis*. The fourth major clade contains *Cymopterus constancei*, *C. macrorhizus*, *C. montanus*, *C. multinervatus*, *C. purpurascens*, and *Aletes calcicola*. Several additional clades of four to five taxa each exist, and are also of heterogeneous generic composition.

The results of the MP analysis revealed that *Cymopterus* (boldfaced in all tree figures presented herein) and *Lomatium*, the two largest genera of NA Apiaceae, are highly polyphyletic, and that their species are inextricably linked with those of the other endemic, perennial genera of the region. In addition, the genera *Aletes*, *Musineon*, *Oreonana*, *Oreocarya*, *Podistera*, *Pseudocymopterus*, *Pteryxia*, and *Tauschia* are also not monophyletic. Constraining the 41 examined taxa of *Cymopterus* to be monophyletic and rerunning the MP analysis resulted in trees 35 steps longer than those most parsimonious (Table 3). *Lomatium* is monophyletic in trees 45 steps longer than those most parsimonious. Similar analyses were performed by constraining each of the other aforementioned genera, with *Aletes*, *Glehnia*, *Oreocarya*, *Pteryxia*, and *Tauschia* each monophyletic in trees 10 or more steps greater than those most parsimonious (Table 3). In contrast, *Polytaenia*, *Thaspium*, and *Zizia* are all monophyletic in unconstrained searches.

The remaining 24 accessions examined formed a polytomy basal to this clade, and included: a monophyletic *Angelica*, with the inclusion of *Sphenosciadium* and Pacific NA *Glehnia littoralis* subsp. *leiocarpa*; the clade of *Selinum candollei*, *Seseli*, *Peucedanum japonicum*, and Pacific Asian *Glehnia littoralis* subsp. *littoralis*, the latter two taxa forming a strongly-supported sister group (97% bootstrap value); the *Arracacia* clade (*Arracacia*, *Prinosciadium*, and *Rhodosciadium*); the clade of *Ciclospermum* + *Spermolepis*; and *Peucedanum ostruthium*. Se-

uninformative characters, respectively; retention index = 0.6405. Numbers on branches represent bootstrap estimates for 100,000 replicate analyses using "fast" stepwise-addition; values <50% are not indicated. All *Cymopterus* accessions are boldfaced. Numbers in parentheses refer to numbers of accessions bearing identical DNA sequences that were combined to facilitate analysis.

TABLE 3. The number of additional steps required to force each genus to monophyly in a maximum parsimony analysis, with gap states treated as missing data.

Genus	No. of constrained accessions	Additional steps
<i>Aletes</i>	9	17
<i>Cymopterus</i>	41	35
<i>Glehnia</i>	2	11
<i>Lomatium</i>	30	45
<i>Musineon</i>	4	7
<i>Oreonana</i>	3	3
<i>Oreoxis</i>	4	15
<i>Orogenia</i>	2	1
<i>Podistera</i>	5	7
<i>Pseudocymopterus</i>	2	4
<i>Pteryxia</i>	10	14
<i>Tauschia</i>	5	19

linum carvifolia occupied the first branch above the root of the tree.

The single tree uncovered by ML analysis, under a best-fit model of DNA substitution, had a $-\ln$ likelihood score of 5856.0673 (Fig. 2A). The high-level relationships suggested by this tree are similar to those indicated by the MP analysis, in the sense that all of the major clades identified previously also occur on this tree. The lack of resolution and generally low bootstrap support values preclude unambiguous hypotheses of relationship among these clades, but do confirm that many genera are not monophyletic. Indeed, species of *Cymopterus*, *Lomatium*, *Pteryxia*, and *Aletes* are intertwined with many other genera. *Polytaenia*, *Thaspium*, and *Zizia* are all maintained as monophyletic, and unite as a clade. Outgroup relationships are also similar to those inferred by the MP analysis, with the exception that *Angelica* (with included *Sphenosciadium*) plus the closely allied *Glehnia littoralis* subsp. *leiocarpa* is sister group to the clade of NA endemics; however, this relationship is only weakly supported (<50% bootstrap value).

Relationships inferred from the Bayesian tree (Fig. 2B) are similar to, or consistent with, those estimated by MP (Fig. 1) and ML (Fig. 2A) methods. The four major clades of six or more taxa each are maintained, as is the clade comprising *Polytaenia*, *Thaspium*, and *Zizia*. Bayesian posterior clade probabilities tend to be higher than corresponding nonparametric bootstrap support values (Huelsenbeck et al. 2002), and this is reflected in our results where many branches are supported by high probability values. For example, monophyly of the perennial, NA endemic taxa is supported by a probability value of 78%, whereas in the other analyses this clade is supported by bootstrap values <50%. Once more, the large basal polytomy of in-group taxa is maintained and resolution of overall relationships is generally quite poor.

DISCUSSION

Monophyly of the Perennial, Endemic Genera of NA Apioidae. The phylogenetic results indicate that the endemic, perennial apioid genera of NA (north of Mexico) constitute a monophyletic group, albeit one that is weakly supported in all trees. The similar life history and overall general habit of many of its members, the absence of a prominent conical stylopodium in all genera except *Podistera* (where we presume it has been secondarily derived; Downie et al. 2002), and, unique among umbellifers, the shared presence of a protogynous breeding system (Schlessman and Graceffa 2002), provide additional, if not compelling, evidence of their common ancestry. In all trees except that of ML, a polytomy arises sister to this clade and comprises the following five lineages: (1) the perennial, circumboreal genus *Angelica* (plus NA *Sphenosciadium capitellatum*) and the Pacific NA *Glehnia littoralis* subsp. *leiocarpa*; (2) the Old World *Seseli*, *Selinum*, and *Peucedanum japonicum*, of which *Seseli libanotis* occurs in the eastern U.S., and the Pacific Asian *Glehnia littoralis* subsp. *littoralis*; (3) the three examined members of the Mexican and Central American *Arracacia* clade; (4) the annual genera *Spermolepis* and *Ciclospermum*, both distributed throughout the southern U.S. and other warm, temperate areas; and (5) *Peucedanum ostruthium* (= *Imperatoria ostruthium* L.), an Old World, perennial species distributed sporadically in the northeastern U.S. Sister to this polytomy in all trees is *Selinum carvifolia*, a perennial herb common in Eurasia, but restricted in NA to eastern Massachusetts. In the ML tree (Fig. 2A), the clade of *Angelica* (plus *Sphenosciadium*) and *Glehnia littoralis* subsp. *leiocarpa* is sister to a clade comprising all perennial, endemic NA genera, lending some support to Mathias' (1965) hypothesis that *Cymopterus* was derived from "Angelica-like stock." It has often been assumed that the indigenous Apiaceae of NA, of which many were initially but erroneously referred to European genera (e.g., Torrey and Gray 1840; Coulter and Rose 1888), are indeed distinct morphologically from Old World taxa (Coulter and Rose 1900; Mathias 1930, 1971; Theobald et al. 1963). The results presented herein, inferred on the basis of ITS data, support this distinction and suggest further that the perennial, endemic apioid umbellifers of NA (north of Mexico) do indeed comprise a (weakly supported) monophyletic group.

This support for monophyly of our native NA umbellifers is in contrast to the results inferred from all other molecular systematic studies to date. Prior, higher-level studies of the family using a variety of different molecular data sets, but with often very limited sampling of these indigenous NA taxa, yielded results that were equivocal in documenting the relationships among the latter (Downie and Katz-Downie 1996;

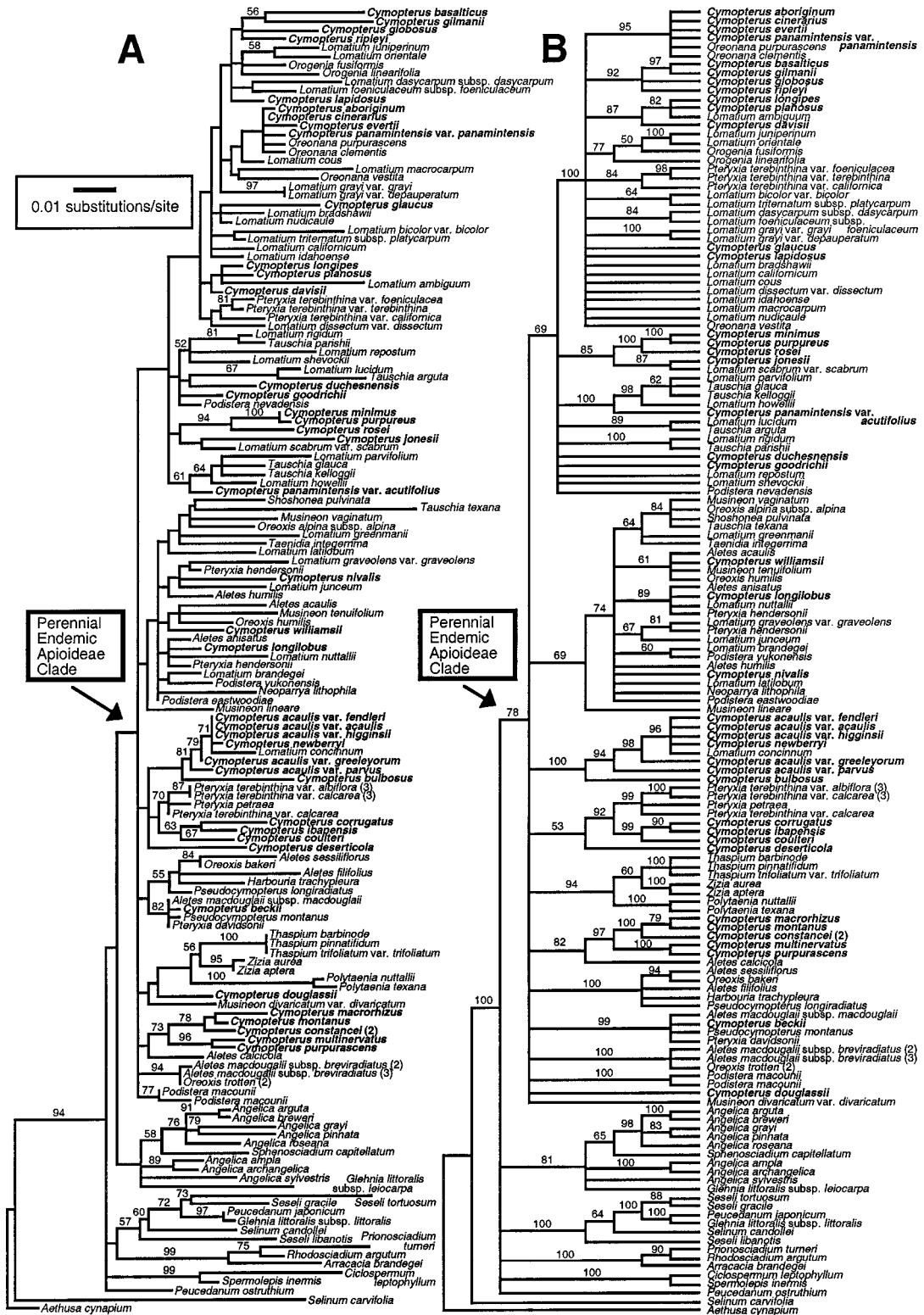


FIG. 2. A. Single tree derived from maximum likelihood analysis of aligned ITS-1 and ITS-2 sequences from 150 terminals (159 accessions) of Apioideae subfamily under a TrN+G model of nucleotide substitution. -Ln Likelihood = 5856.0673. Numbers on branches represent bootstrap estimates for 100 replicate neighbor-joining analyses using a maximum like-

Plunkett et al. 1996; Downie et al. 1998, 2000b; Plunkett and Downie 1999; Downie et al. 2002). However, given the lack of resolution and (or) low levels of branch support obtained in those clades comprising these native NA umbellifers, none of the results actually precluded this suggestion of monophyly. Because thorough taxon sampling is essential to increasing the accuracy of a phylogenetic estimate (Rannala et al. 1998; Zwickl and Hillis 2002), our current study of 159 exemplars (representing extensive taxon sampling of *Aletes*, *Cymopterus*, *Oreonana*, *Oreoxis*, *Pseudocymopterus*, *Pteryxia*, and *Musineon*, among other genera), which also includes circumboreal genera having NA representation (i.e., *Angelica*, *Seseli*, *Selinum*, and *Peucedanum*), offers the best estimate yet of phylogenetic relationships among NA umbellifers.

Nevertheless, additional study is required to evaluate the monophyly of these protogynous, NA apioid umbellifers and to elucidate their sister group, given the weak support for their monophyly and the lack of resolution in the cladograms. In particular, greater representation of Old World species is necessary, especially those of the *Angelica* clade of the apioid superclade. The low ITS sequence divergence among the western NA, meso-American, and Eurasian taxa sampled herein and the morphological similarities of many suggest that the *Angelica* clade may have diverged rapidly. Moreover, biogeographic studies of the Northern Hemisphere have suggested frequent exchanges of floristic elements of western NA with other biogeographic areas, such as eastern NA, eastern Asia, and southeastern Europe (Wen 1999), thus the assumption of monophyly of this large and widely distributed group of NA umbellifers needs to be tested further by increased sampling of plants outside of western NA. In addition, data beyond those procured from the ITS region will be required to increase the number of potentially parsimony-informative characters in order to increase resolution of the phylogenetic estimate and clarify sister group relationships.

Polyphyly of *Cymopterus*. The genus *Cymopterus*, as currently treated (Kartesz 1994), is clearly not monophyletic. Expanding the limits of *Cymopterus* to include *Oreoxis*, *Pseudocymopterus*, and *Pteryxia* (Cronquist 1997), or even placing *Lomatium* into this amalgamation as intimated by Cronquist, fares no better in yielding a group that is monophyletic upon consideration of ITS data. In fact, no historical treatment of *Cymopterus* and its allies reflects our phylogenetic results. Jones (1908) recognized a number of sections and

subgroups within a highly variable and expanded *Cymopterus* (because of "the futility of trying to divide the species into separate genera"), but none of these taxa parallels the groupings obtained in the ITS-derived trees. Given the estimates of phylogeny presented herein, and the realization that trees of much greater length than those most parsimonious are required to invoke monophyly, the genus *Cymopterus*, as presently circumscribed, is a highly artificial assemblage of species that must be abandoned in any revised system of umbellifer classification.

We are not aware of any morphological synapomorphy supporting the monophyly of *Cymopterus*. The characters used traditionally to delimit the genus are primarily those of the fruit, and these display overlapping patterns of variation with several other umbellifers endemic to western NA (Downie et al. 2002). A functional, persistent, 2-cleft carpophore, once considered a hallmark of *Cymopterus* (Mathias and Constance 1944–1945), is lacking in nearly half of its species through partial or complete adnation of its halves to the commissural surface of the mericarps and becoming deciduous with them (Hartman and Constance 1985; Cronquist 1997; Hartman 2000; Downie et al. 2002). The presence of prominent wings on all ribs of the fruit has also been used as a distinguishing character for the genus, but in several species the dorsal wings are obsolete or reduced to three inconspicuous ridges, and, thus, show similarities to fruits of *Lomatium* (Mathias and Constance 1944–1945; Hartman and Constance 1985; Goodrich 1986; Cronquist 1997; Downie et al. 2002). The lateral wings of *Cymopterus* are usually constricted at their base, but in some species they may be inflated at the base. Traditionally, mature fruits of *Cymopterus* have been described as compressed dorsally (Mathias 1930; Mathias and Constance 1944–1945), yet fruit cross-sections reveal a complex series, from fruits that are subterete or somewhat compressed laterally to those that are markedly dorsally compressed (Hartman 1985; Downie et al. 2002). The number of vittae (oil tubes), both on the commissure and in the intervals between the ribs, is another diagnostic character, but can show much variation even within a species (Downie et al. 2002). In light of the extreme polyphyly of *Cymopterus* and its complex association with other perennial, endemic genera of NA, further cladistic study of morphological characters is required. However, such an analysis must consider simultaneously all NA apioid umbellifers, and not just focus on specific genera, as monophyly of

←

likelihood model of nucleotide substitution; values <50% are not indicated. B. Bayesian inference tree. Numbers on branches represent Bayesian posterior probabilities. All *Cymopterus* accessions are boldfaced. Numbers in parentheses refer to numbers of accessions bearing identical DNA sequences that were combined to facilitate analysis.

each of these endemic, perennial genera should not be tacitly assumed.

Phylogenetic Relationships Within NA Apioideae.

Several major clades of similar composition are apparent in all trees presented herein, but each comprises species of two or more genera and most are not very well supported. Therefore, until these groups receive confirmation through additional study, we are cautious not to recognize new assemblages of taxa. We highlight some of these clades below, as well as discuss the phylogenetic placements of *Glehnia* and *Oreonana*. The phylogenetic relationships of the remaining NA endemic genera, as inferred through molecular study, have been discussed previously (Downie et al. 2002).

Six species of *Cymopterus* belong to a distinctive species group: *Phellopterus* (Nutt. ex Torr. & A. Gray) J.M. Coult. & Rose or *Cymopterus* sect. *Phellopterus* Nutt. ex Torr. & A. Gray (Coulter and Rose 1900; Mathias 1930; Hartman 2000). Its members include *C. bulbosus*, *C. constancei*, *C. macrorhizus*, *C. montanus*, *C. multinervatus*, and *C. purpurascens*. These species share large, showy bractlets that are often basally connate. All species but *C. bulbosus* are allied with one another in the molecular phylogenies, with these five species sister to *Aletes calicicola*. The separation of *C. bulbosus* from this clade is surprising, given the similarity between this species and *C. constancei*. This may very well be the only previously identified species group of *Cymopterus* that is supported, in part, by molecular evidence.

The five varieties of *Cymopterus acaulis* (vars. *acaulis*, *fendleri*, *greeleyorum*, *higginsii*, and *parvus*) form a strongly supported clade, with *C. newberryi* and *Lomatium concinnum* nested within. This clade is sister to *Cymopterus bulbosus* in all trees. In vegetative condition, plants of *L. concinnum* may easily be confused with those of *C. acaulis*; however, in flowering or fruiting condition, the species differ in flower color and fruit morphology. ITS data for *L. concinnum* were obtained from GenBank (Hardig and Soltis 1999) and its morphological features could not be confirmed.

No infrageneric classification exists for *Lomatium*, although several informal species groups have been recognized, such as the *Cynomarthrum* group (Coulter and Rose 1900), the "tuberous lomatium" group (Coulter and Rose 1888; Mathias 1938; Schlessman 1984), and the *Euryptera* group (Mastrogioseppe et al. 1985; Hartman and Constance 1988; Hardig and Soltis 1999). In the present study, the *Cynomarthrum* group (represented by *L. concinnum*, *L. junceum*, *L. nuttallii*, *L. scabrum*, and *L. triternatum* subsp. *platycarpum*), the "tuberous lomatium" group (represented by *L. ambiguum*, *L. bicolor*, and *L. cous*), and the *Euryptera* group (represented by *L. howellii*, *L. lucidum*, *L. parvifolium*, *L. repostum*, *L. rigidum*, and *L. shevockii*) are all polyphyletic, in accordance with the findings of others (e.g., Schlessman 1984; Soltis and Novak 1997). Several mem-

bers of the *Euryptera* group are closely allied with species of vegetatively similar *Tauschia* from coastal southern California.

Plants of the maritime genus *Glehnia*, initially but dubiously referred to *Cymopterus* because of their dorsally flattened fruits bearing prominent dorsal wavy wings (Gray 1860, as cited by Mathias 1928), are distributed on the sandy seashores of the northern Pacific. They have been variously treated as two subspecies or two closely allied species (Mathias 1928; Hiroe 1962). Most authors, however, recognize a single species complex comprising two subspecies (subsp. *leiocarpa*, western hemisphere; subsp. *littoralis*, eastern hemisphere). Differences in fruit characters are apparent between these taxa (Mathias 1928), but were thought not significant enough to warrant separate specific status (Hiroe 1962). However, NA *Glehnia* can be distinguished from Japanese specimens by their pattern of accumulation of secondary metabolites in the fruit (Hiraoka et al. 2002). Our analyses of ITS data reveal a separation of the subspecies, with the NA *G. littoralis* subsp. *leiocarpa* allied with *Angelica*, and the Asian *G. littoralis* subsp. *littoralis* allied with *Peucedanum japonicum*. Neither taxon allies with *Cymopterus*. The position of subsp. *leiocarpa* in the ML and Bayesian trees (Figs. 2A and 2B) is consistent with its separation from *Angelica* on the basis of its distinctive fruit morphology, whereas in the MP tree (Fig. 1) it falls within *Angelica* (but with low bootstrap support). ITS sequences from *G. littoralis* subsp. *littoralis* and *P. japonicum* are almost identical, with only one nucleotide difference between them. The latter was chosen for inclusion in this study because of its similar distribution in Pacific Asia. It also shares a similar maritime habitat. However, given the differences in habit between *G. littoralis* subsp. *littoralis* and *P. japonicum*, additional study is required to confirm their sister group relationship.

Oreonana comprises three species native to California that are adapted to montane environments characterized by low-temperature and extensive winter snow cover (Shevock and Constance 1979). These plants are cushion-forming and coarsely hairy or tomentose. Their globose to semihemispheric infructescences with numerous persistent pedicels from the staminate and sterile flowers aid in seed dispersal. In all ITS-derived trees, *O. purpurascens* and *O. clementis* are closely related; in contrast, *O. vestita* is positioned away from this group, thus, pending further study, we presume the genus is not monophyletic. *Oreonana* has been proposed to be closely related to *Tauschia* (Shevock and Constance 1979), but in all ITS trees its species ally closely with *Cymopterus* and *Lomatium*.

The lack of basal resolution among the taxa sampled coupled with the presence of many distinct (yet weakly supported) lineages in all cladograms is consistent with a pattern of rapid radiation in western NA

(Downie et al. 2002), a mode of evolution suggested previously for *Lomatium* (Soltis et al. 1995; Hardig and Soltis 1999) and *Osmorhiza* Raf. (Apiaceae; Wen et al. 2002). Single trees uncovered in the MP analysis (not shown) were similar to that tree indicated by the ML analysis (Fig. 2A) in having short internal branches and, generally, much longer terminal branches, the latter indicative of numerous autapomorphies. This unequal distribution of branch lengths suggests that this group of umbellifers originated and diversified relatively quickly and that, since their formation, its major lineages and species have persisted in isolation. Many species of *Cymopterus* and other western NA umbellifers are narrowly distributed and have strict edaphic requirements (Mathias 1930, 1965), suggesting that speciation in the group may be related to geological boundaries and edaphic shifts (Hardig and Soltis 1999). While this group of largely aridity-adapted taxa lacks a fossil record, it may be assumed that their rapid radiation accompanied late Tertiary and Quaternary geologic and climatological events that gave rise to widespread aridity and great habitat diversification in western NA that promoted speciation when new areas became available for colonization (Stebbins 1952; Mathias 1965; Delcourt and Delcourt 1993; Wen 2001). With approximately 200 species of Apioideae endemic to western NA, the bulk of which comprises *Cymopterus*, *Lomatium* and their allies, the region is recognized as a major center of differentiation for the subfamily (Mathias 1965). Rapid radiation is a common pattern of long-term evolution (Futuyma 1997); among plants, it has been reported for several other taxa endemic to continental or, specifically, western NA (e.g., Rieseberg et al. 1991; Schwarzbach and Kadereit 1995; Wojciechowski et al. 1999; Hershkovitz and Zimmer 2000). Through additional gene sequencing and further evaluation of morphological characters (F.-J. Sun, unpublished data), we hope eventually to be able to resolve the early branching patterns of these perennial, endemic NA umbellifers, although we realize that such a study poses extraordinary challenges (Fishbein et al. 2001).

Generic delimitation in the Apiaceae is often vague and arbitrary (Constance 1987; Cronquist 1997), and many species-rich genera (and especially those of the apioid superclade) are polyphyletic (Downie et al. 2000b, 2000c; Spalik et al. 2001). Unfortunately, our results do little to refute these statements. Of all the perennial, endemic apioid genera of NA considered herein, only *Polytaenia*, *Thaspium*, and *Zizia* are each resolved as monophyletic on the basis of phylogenetic analyses of ITS data. However, the latter two genera are remarkably similar in appearance and their generic limits too have been questioned (Ball 1979; Lindsey 1982; Cooperrider 1985). Alas, until we have evidence to the contrary, it appears that the vast majority of

perennial, apioid genera endemic to NA are not monophyletic. A complete reassessment of the generic limits of all of our native umbellifers is clearly required, as is a comprehensive, simultaneous analysis of both New and Old World Apiaceae.

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