

# A molecular systematic investigation of *Cymopterus* and its allies (Apiaceae) based on phylogenetic analyses of nuclear (ITS) and plastid (*rps16* intron) DNA sequences

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Considerable controversy exists with regard to the proper delimitation of *Cymopterus* (Apiaceae subfamily Apioideae) and its relationship to the other perennial endemic umbellifers of western North America. Previous molecular systematic studies using nuclear (rDNA ITS) and, for subsets of taxa, plastid (*rps16* intron and *trnF-trnL-trnT*) DNA sequences have revealed that the genus *Cymopterus* is polyphyletic, with its species inextricably linked with those of *Aletes*, *Lomatium*, *Musineon*, *Oreoxis*, *Pseudocymopterus*, *Pteryxia*, *Tauschia*, and several other genera of the region. Herein, in an effort to increase resolution of relationships, we procured *rps16* intron sequence data from 74 accessions of *Cymopterus* and its allies so that ITS and intron matrices are available for the same set of 129 taxa. These data matrices were analysed, separately and in combination, using maximum parsimony and (or)

maximum likelihood methods. For those trees exhibiting the greatest resolution, the results revealed that the perennial, endemic apioid genera of North America (north of Mexico) comprise a weakly supported monophyletic group, with *Angelica* constituting its sister lineage. *Cymopterus* is confirmed as highly polyphyletic. The *rps16* intron yielded substantially fewer parsimony informative characters than those of the ITS region and when analysed, separately or in combination, with ITS data, resulted in trees of poor resolution. Only one previously identified species group of *Cymopterus* is supported as monophyletic; this group coincides with section *Phellopterus*, and is recognised by its showy, basally connate bractlets. Many other genera endemic to western North America appear to be highly artificial assemblages of species that will likely be abandoned in any revised system of umbellifer classification.

## Introduction

The genus *Cymopterus* Raf., the spring-parsleys, comprises some 40 herbaceous perennial taxa restricted primarily to xeric habitats in western North America (Kartesz 1994, Hartman 2000). These plants are low-growing, possess thickened taproots, and have ternately cleft, compound or dissected leaves. Their most distinctive feature, however, is their fruit, for the two lateral and usually one or more of the three dorsal ribs are conspicuously winged and undulating. Rarely, the dorsal ribs may be wingless and, in such cases, the fruits resemble those of *Lomatium* (Cronquist 1997, Downie *et al.* 2002).

Much confusion exists with regard to the proper delimitation of *Cymopterus* and its relationship to the other indigenous umbellifers of western North America (Jones 1908, Mathias 1930, Theobald *et al.* 1963, Evert and Constance 1982, Weber 1984, Gilmartin and Simmons 1987, Cronquist 1997). 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. 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, Cronquist 1997, Downie *et al.* 2002). With regard to the delimitation of *Cymopterus*, extremes in treatment range from the recognition of many small, generically distinct elements (such as *Aulospermum* J.M. Coult. & Rose, *Glehnia*, *Oreoxis*, *Phellopterus* (Nutt. ex Torr. & A. Gray) J.M. Coult. & Rose, *Pseudocymopterus*, *Pteryxia* and *Rhysopterus* J.M. Coult. & Rose) to multiple sections and subgroups within a highly variable and expanded genus (Jones 1908, Mathias 1930, Mathias and Constance 1944–1945). Cronquist (1997) included *Oreoxis*, *Phellopterus*, *Pseudocymopterus* and *Pteryxia* within a broadly circumscribed *Cymopterus*.

Recently, we have carried out molecular systematic investigations of *Cymopterus* and its allies using nuclear (rDNA

ITS) and, for subsets of this group, plastid (*rps16* intron and *trnF-trnL-trnT*) sequences (Downie *et al.* 2002, Sun *et al.* 2004). Our results revealed that the perennial endemic apioid genera of North America (with the inclusion of *Polytaenia* DC., *Taenidia* (Torr. & A. Gray) Drude, *Tauschia* Schldl., *Thaspium* Nutt. and *Zizia* W.D.J. Koch) comprise a weakly supported monophyletic group, with *Angelica* and members of the meso-American *Arracacia* clade constituting two of several possible sister lineages (Sun *et al.* 2004). The lack of resolution and poor branch support obtained in the ITS trees precluded unambiguous hypotheses of relationship, but did suggest that many of these western North American genera, where resolved, are not monophyletic. *Cymopterus* is polyphyletic, with its species inextricably linked with those of many other genera of the region. Thus, it appears that the traditional emphasis placed on fruit characters in circumscribing genera within the group has led to artificial assemblages of species and, as such, a complete reassessment of the generic limits of these North American Apiaceae is in order.

To resolve relationships among the perennial endemic genera of North American Apiaceae and to better ascertain the phylogenetic placements of those elements that currently comprise *Cymopterus* within the group, herein we obtained additional sequence data from the chloroplast *rps16* intron to produce parallel ITS and intron data sets for a comprehensive sampling of 129 taxa. Previous studies have demonstrated the utility of the *rps16* intron for phylogeny estimation in Apiaceae (Downie and Katz-Downie 1999, Lee and Downie 2000, Downie *et al.* 2000, 2002), and it was hoped that this region could supply the information required to increase resolution and branch support of the resulting phylogenetic estimate.

## Materials and Methods

DNA sequence data from the chloroplast *rps16* intron were procured from 74 accessions of North American Apiaceae subfamily Apioideae (Table 1) and combined with previously published intron data for 55 taxa (Downie and Katz-Downie 1999, Downie *et al.* 2002), for a final tally of 129 accessions included in the phylogenetic analyses. ITS sequence data were already available for this same set of species (Downie *et al.* 2002, Sun *et al.* 2004). With the exception of the narrowly endemic and rarely collected *Cymopterus megacephalus* M.E. Jones, we sampled all 40 taxa of *Cymopterus* listed by Kartesz (1994). We also included the recently described *C. constancei* (Hartman 2000), and *C. longilobus*, the latter placed in synonymy under *Pteryxia* by Kartesz (1994) but maintained as a distinct species of *Cymopterus* by R Hartman (unpubl. data). Sampling of all other perennial genera endemic to North America was comprehensive or nearly so, with the exceptions of *Lomatium* and *Tauschia*, where only 20 and five accessions were included, respectively (Kartesz 1994).

We also included representatives of the perennial, circumboreal genus *Angelica* (including the monotypic genus *Sphenosciadium*; Spalik *et al.* 2004), because results of a prior phylogenetic analysis established a close relationship between this taxon and the group of endemic North

American genera (Downie *et al.* 2002, Sun *et al.* 2004). We rooted all trees with *Aethusa cynapium* L., based on the results of previous higher-level investigations where this species was sister taxon, or closely related, to all aforementioned taxa (Downie *et al.* 2000, 2002).

The experimental methods used to obtain these intron sequence data were the same as outlined previously (Downie *et al.* 2002, and references cited therein). The DNA sequences were aligned manually, facilitated by their conservative nature. GenBank accession numbers for each of these newly acquired sequences are presented in Table 1, and the data matrix is available upon request.

The resulting ITS and *rps16* intron data matrices were first analysed using maximum parsimony (MP), with gap states treated as missing data. Both separate and combined analyses were performed. MP trees were sought using the heuristic search strategies of PAUP\* (Swofford 1998), as described in Downie *et al.* (2002). The maximum number of MP trees was set at 20 000. Bootstrap values were calculated from 100 000 replicate analyses using 'fast' stepwise-addition of taxa, and only those values compatible with the 50% majority-rule consensus tree were recorded. The number of additional steps required to force particular taxa into a monophyletic group was examined using the constraint option of PAUP\*. To examine the extent of conflict among the ITS and *rps16* intron data sets, the incongruence length difference test of Farris *et al.* (1995) was implemented, using PAUP\*'s partition-homogeneity test. This test was performed with 100 partition-homogeneity test replicates, using a heuristic search option with simple addition of taxa, Tree-Bisection-Reconnection (TBR) branch swapping, and MaxTrees set at 500.

A maximum likelihood (ML) analysis of combined ITS and *rps16* intron data was then performed. The program Modeltest version 3.06 (Posada and Crandall 1998) was used to select an appropriate model of nucleotide substitution that best fits these combined data. These settings were entered into PAUP\* and a heuristic search carried out using random addition sequence and TBR branch swapping. One thousand bootstrap replicate analyses were conducted employing neighbour-joining searches with ML distance, using the ML parameters estimated by Modeltest.

## Results and Discussion

### Sequence analysis

The alignment of 129 complete ITS-1 and ITS-2 sequences (data from the intervening 5.8S region were unavailable for many taxa because of the sequencing strategies used in earlier studies) resulted in a matrix of 452 positions, with none excluded because of alignment ambiguity. Of these 452 positions, 211 (47%) were unvarying, 104 (23%) were autapomorphic, and 137 (30%) were parsimony informative. Uncorrected pairwise sequence divergence ranged from identity to 9.4% of nucleotides. Nineteen gaps, either 1bp or 2bp in size relative to the outgroup *Aethusa cynapium*, were introduced to facilitate alignment; seven of these gaps were parsimony informative.

The alignment of 129 complete *rps16* intron sequences

**Table 1:** GenBank numbers and voucher information for the 74 new accessions of North American Apiaceae subfamily Apioidae examined for chloroplast DNA *rps16* intron sequence variation. Comparable data for the 55 accessions examined previously for *rps16* intron sequence variation, and for all 129 accessions included in a previous study of nuclear rDNA ITS sequences, are presented elsewhere (Downie and Katz-Downie 1999, Downie et al. 2002, Sun et al. 2004). Herbarium acronyms are according to Holmgren et al. (1990)

Taxon	GenBank No.	Voucher Information
<i>Aletes acaulis</i> (Torr.) J.M. Coult. & Rose-2	AY372823	USA, New Mexico, Bernalillo Co., Sandia Mtns, 30 August 1946, Parker & McClintock 6504 (UTC)
<i>Aletes calcicola</i> Mathias & Constance	AY372824	Mexico, Coahuila, Sierra de San Marcos, opposite Los Fresnos, 4–5 April 1969, Pinkava et al. 6117 (UC)
<i>Aletes filifolius</i> Mathias, Constance & W.L. Theob.	AY372825	USA, Texas, Culberson Co., Hunter Peak at Pine Top, Guadalupe Mtns, National Park, 6 September 1987, Higgins 17453 (BRY)
<i>Aletes humilis</i> J.M. Coult. & Rose	AY372826	USA, Colorado, Larimer Co., Lone Pine Creek, 12 May 1996, Scully & Howell s.n. (BRY)
<i>Aletes macdougallii</i> J.M. Coult. & Rose subsp. <i>macdougallii</i>	AY372827	USA, Arizona, Coconino Co., Grand Canyon, Kaibab Trail to Roaring Springs, 23 June 1933, Eastwood & Howell 986 (UC)
<i>Aletes macdougallii</i> subsp. <i>breviradiatus</i> W.L. Theob. & C.C. Tseng-1	AY372828	USA, New Mexico, San Juan Co., Chaco Culture Nat. Hist. Park, 2 May 1993, Heil 7374 (UNNM)
<i>Aletes macdougallii</i> subsp. <i>breviradiatus</i> W.L. Theob. & C.C. Tseng-2	AY372829	USA, New Mexico, San Juan Co., NM 575, 0.15mi NNW of US 64, NW of Blanco, 10 July 1999, Sun & Hartman 649 (ILL)
<i>Aletes macdougallii</i> subsp. <i>breviradiatus</i> W.L. Theob. & C.C. Tseng-3	AY372830	USA, Utah, Garfield Co., Dixie National Forest, Death Hollow, 28 May 1989, Franklin 6499 (BRY)
<i>Aletes sessiliflorus</i> W.L. Theob. & C.C. Tseng	AY372831	USA, New Mexico, Rio Arriba Co., NW of Embudo, 1 May 1992, Hartman 13954 (RM)
<i>Angelica arguta</i> Nutt. ex Torr. & A. Gray	AY372832	USA, Wyoming, Lincoln Co., Murphy Creek, 18 July 1992, Nelson & Nelson 23524 (RM)
<i>Angelica grayi</i> (J.M. Coult. & Rose) J.M. Coult. & Rose	AY372833	USA, Colorado, Garfield Co., Blair Mtn, 17mi NNW Glenwood Springs, 24 August 1991, Vanderhorst & Palaci 4490 (RM)
<i>Angelica roseana</i> L.F. Hend.	AY372834	USA, Wyoming, Teton Co., Blue Miner Lake, 25 August 1994, Hartman 50090 (RM)
<i>Angelica sylvestris</i> L.	AY372835	Cult. UIUC from seeds obtained from Shanghai Botanic Garden, China, Downie 428 (ILL)
<i>Cymopterus aboriginum</i> M.E. Jones	AY372836	USA, California, Mono Co., c. 5mi E of Benton along Hwy 6, 21 June 1990, Atwood 13838 (BRY)
<i>Cymopterus acaulis</i> (Pursh) Raf. var. <i>greeleyorum</i> J.W. Grimes & P.L. Packard	AY372837	USA, Oregon, Malheur Co., McBride Creek Road, 24 May 1989, Smithman et al. LS-2045 (CIC)
<i>Cymopterus acaulis</i> var. <i>higginsii</i> (S.L. Welsh) S. Goodrich	AY372838	USA, Utah, Kane Co., E of Coyote Wash, 8 April 1993, Chapman s.n. (BRY)
<i>Cymopterus acaulis</i> var. <i>parvus</i> S. Goodrich	AY372839	USA, Utah, Tooele Co., Dunefield, NW of English village, W of Cedar Mtns, 7 June 1993, Johnson 351 (BRY)
<i>Cymopterus basalticus</i> M.E. Jones	AY372840	USA, Utah, Millard Co., Tunnel Springs Mtns, 20 May 2000, Tilley & Tilley 1174 (BRY)
<i>Cymopterus beckii</i> S.L. Welsh & S. Goodrich	AY372841	USA, Utah, San Juan Co., SW of Abajo Mtns, 0.7mi NE of confluence of Deep Canyon with Chippean Canyon, 6 July 1992, Franklin 7544 (MO)
<i>Cymopterus bulbosus</i> A. Nelson	AY372842	USA, Utah, Uintah Co., ESE of Vernal, 18 April 1982, Hartman 13951 (RM)
<i>Cymopterus cinerarius</i> A. Gray	AY372843	USA, California, Mono Co., Sweetwater Canyon, 15 July 1944, Alexander & Kellogg 3893 (UTC)
<i>Cymopterus constancei</i> R.L. Hartm.	AY372844	USA, Colorado, Montrose Co., SanMiguel and lower Dolores River drainages, Simbad Valley, 30 May 1995, Hartman 50943 (RM)
<i>Cymopterus corrugatus</i> M.E. Jones	AY372845	USA, Nevada, Mineral Co., Walker River Indian Reservation, 15 May 1988, Tiehm 11640 (BRY)
<i>Cymopterus coulteri</i> (M.E. Jones) Mathias	AY372846	USA, Utah, Sevier Co., Triangle Mtn, Soldiers Canyon, 15 May 1990, Thorne & Zupan 7377 (BRY)
<i>Cymopterus davisii</i> R.L. Hartm.	AY372847	USA, Idaho, Cassia Co., Mt. Harrison, 30 July 1983, Cronquist 11840 (UTC)
<i>Cymopterus deserticola</i> Brandegee	AY372848	USA, California, San Bernardino Co., 1mi S of Kramer, 21 March 1958, Raven 11891 (UTC)
<i>Cymopterus douglassii</i> R.L. Hartm. & Constance	AY372849	USA, Idaho, Custer Co., 2mi N of Horseheaven Pass, Pahsimeroi Mtns, Challis National Forest, 26 June 1984, Atwood 10302 (BRY)
<i>Cymopterus gilmanii</i> C. Morton	AY372850	USA, Nevada, Clark Co., Desert Range, Desert National Wildlife Refuge, 7.3mi from Sheep Peak summit, 17 May 1987, Morefield et al. 4463 (BRY)
<i>Cymopterus glaucus</i> Nutt.	AY372851	USA, Idaho, Valley Co., ridge between Fisher Creek Saddle and Black Tip along Idaho/Valley Co. line, 26 July 1989, Ertter et al. 8742 (RM)
<i>Cymopterus goodrichii</i> S.L. Welsh & Neese	AY372852	USA, Nevada, Lander Co., Toiyabe Range, head of Big and Kingston Creeks, c. 2mi N Bunker Hill, 16 July 1981, Neese et al. 10737 (BRY)
<i>Cymopterus ibapensis</i> M.E. Jones	AY372853	USA, Utah, Sevier Co., UT 4, W of Jct with US 89, 26 May 1982, Hartman 13978 (RM)
<i>Cymopterus lapidosus</i> (M.E. Jones) M.E. Jones	AY372854	USA, Wyoming, Uinta Co., Uinta Co. Road 290, 3.7 air mi W of Lonetree, 7 July 1983, Hartman 15767 (UTC)

Table 1 cont.

<i>Cymopterus longilobus</i> (Rydb.) W.A. Weber	AY372855	USA, Wyoming, Sublette Co., Wyoming Range, 15 August 1993, Hartman 43986 (RM)
<i>Cymopterus macrorhizus</i> Buckley	AY372856	USA, Texas, Tom Green Co., Grandview campground on W side of Fisher Lake, 19 March 1983, Erfter & Strachan 4690 (RM)
<i>Cymopterus minimus</i> (Mathias) Mathias	AY372857	USA, Utah, Iron Co., Cedar Breaks National Monument, 25 July 1993, Stone 1656 (BRY)
<i>Cymopterus newberryi</i> (S. Watson) M.E. Jones	AY372858	USA, Utah, San Juan Co., 4.5mi downstream from Bluff, 20 May 1983, Barneby 17896 (BRY)
<i>Cymopterus panamintensis</i> J.M. Coult. & Rose var. <i>acutifolius</i> (J.M. Coult. & Rose) Munz	AY372859	USA, California, San Bernardino Co., south side of Ord Mt., N of Lucerne Valley, Mojave Desert, 8 April 1988, Boyd et al. 1776 (OSC)
<i>Cymopterus purpurascens</i> (A. Gray) M.E. Jones	AY372860	USA, Arizona, Mohave Co., NE of Peach Springs, 30 March 1983, Hartman 14096 (RM)
<i>Cymopterus ripleyi</i> Bameby	AY372861	USA, Nevada, Nye Co., Stone Cabin Valley, along US Hwy 6, at Little Fish Lake turnout, 24 May 1995, Holmgren & Holmgren 12299 (BRY)
<i>Cymopterus rosei</i> (M.E. Jones ex J.M. Coult. & Rose) M.E. Jones	AY372862	USA, Utah, Sanpete Co., 2mi S of Sterling, Hills W of Ninemile Reservoir, 8 May 1987, Franklin 4604 (BRY)
<i>Glehnia littoralis</i> F. Schmidt ex Miq. subsp. <i>littoralis</i>	AY372863	Taiwan, Taitung Hsien, Lanyu (Orchid Island), Liao et al. 1235 (MO)
<i>Glehnia littoralis</i> subsp. <i>leiocarpa</i> (Mathias) Hultén	AY372864	USA, Oregon, Tillamook Co., Cape Kiwanda, 10 August 1975, Halse 1228 (OSC)
<i>Lomatium ambiguum</i> (Nutt.) J.M. Coult. & Rose	AY372865	USA, Montana, Madison Co., Bear Trap Canyon Trail, 30 May 1979, Lowry 1867 (ILL)
<i>Lomatium bradshawii</i> (Rose ex Mathias) Mathias & Constance	AY372866	USA, Oregon, Benton Co., NE Corvallis, W of Canterbury Circle, 8 June 1980, Halse 2215 (ILL)
<i>Lomatium brandegei</i> (J.M. Coult. & Rose) J.F. Macbr.	AY372867	USA, Washington, Chelan Co., 5mi SW of Leavenworth on Snow Creek Trail, 500ft above creek, 29 May 1977, Lowry 621 (ILL)
<i>Lomatium foeniculaceum</i> (Nutt.) J.M. Coult. & Rose subsp. <i>foeniculaceum</i>	AY372868	USA, Wyoming, Converse Co., Southern Powder River Basin, 12 May 1994, Nelson 30083 (RM)
<i>Lomatium grayi</i> (J.M. Coult. & Rose) J.M. Coult. & Rose var. <i>grayi</i>	AY372869	USA, Wyoming, Lincoln Co., Tunp Range, Preacher Hollow, 16mi NNE Cokeville, 22 May 1993, Hartman 37444 (RM)
<i>Lomatium grayi</i> var. <i>depauperatum</i> (M.E. Jones) Mathias	AY372870	USA, Utah, Juab Co., Gilson Mtns, 7mi from Lynndyl, 7 May 1981, Goodrich 15335 (RM)
<i>Lomatium nuttallii</i> (A. Gray) J.F. Macbr.	AY372871	USA, Wyoming, Natrona Co., Baker Cabin, 23 May 1994, Nelson 30631 (RM)
<i>Lomatium orientale</i> J.M. Coult. & Rose	AY372872	USA, Wyoming, Natrona Co., along Notches, 23 May 1994, Nelson 30536 (RM)
<i>Musineon lineare</i> (Rydb.) Mathias	AY372873	USA, Utah, Cache Co., Toney Grove Trailhead Trail to Naomi Peak, c. 1mi W of summit, 9 July 1989, Hartman 24364 (RM)
<i>Oreonana clementis</i> (M.E. Jones) Jeps.	AY372874	USA, California, Tulare Co., Mt. Guyot, Sierra Nevada, 22 July 1949, Howell 25604 (UTC)
<i>Oreonana purpurascens</i> Shevock & Constance	AY372875	USA, California, Tulare Co., Sequoia National Forest, Slate Mt., along USFS trail 31E14, 26 April 1977, Shevock 5428 (BRY)
<i>Oreonana vestita</i> (S. Watson) Jeps.	AY372876	USA, California, San Bernardino Co., Angeles National Forest, San Gabriel Mtns, 4 May 1977, Shevock 5433 (BRY)
<i>Oreoxis alpina</i> (A. Gray) J.M. Coult. & Rose subsp. <i>alpina</i>	AY372877	USA, Colorado, Saguache Co., Cochetopa Park, NN14 road, 0.5 road mi W of 17FF road, 11 July 1999, Sun & Hartman 629 (ILL)
<i>Oreoxis trotteri</i> S.L. Welsh & S. Goodrich	AY372878	USA, Utah, Grand Co., c. 13mi NW of Moab, E of Courthouse Rock, 20 May 1985, Welsh 23427 (BRY)
<i>Orogenia fusiformis</i> S. Watson	AY372879	USA, Oregon, Josephine Co., Oregon Caves National Monument, Siskiyou Mtns, 1.5 air mi WNW of Mt. Elijah, 24 May 1996, Zika 12864 (OSC)
<i>Podistera macounii</i> (J.M. Coult. & Rose) Mathias & Constance	AY372880	USA, Alaska, W of Sheep Creek, 30 June 1977, Reed et al. 6047 (BRY)
<i>Podistera nevadensis</i> (A. Gray) S. Watson	AY372881	USA, California, El Dorado Co., Lake Tahoe Basin Management Unit, Freel Peak, 27 July 2002, Matson 634 (ILL)
<i>Podistera yukonensis</i> Mathias & Constance	AY372882	USA, Alaska, Kathul Mtn, along the Yukon River roughly halfway between Eagle and Circle, 11 June 2002, Larsen & Batten 02-7263 (ALA)
<i>Pseudocymopterus longiradiatus</i> Mathias, Constance & W.L. Theob.	AY372883	USA, New Mexico, Otero Co., Sacramento Mtns, c. 3mi SSW of Bent, SW side of Domingo Peak, 7 August 1990, Sivinski 1546 (UNM)
<i>Pseudocymopterus montanus</i> (A. Gray) J.M. Coult. & Rose-2	AY372884	USA, Arizona, Pima Co., 32mi NE of Tucson via Catalina Hwy, Santa Catalina Mtns, 24 July 1965, Matthews & Matthews 456 (ILL)
<i>Ptenyxia davidsonii</i> (J.M. Coult. & Rose) Mathias & Constance	AY372885	USA, New Mexico, Grant Co., Bear Mt., near Silver City, 17 June 1903, Metcalfe 165 (RM)

Table 1 cont.

<i>Pteryxia petraea</i> (M.E. Jones) J.M. Coult. & Rose	AY372886	USA, Nevada, Nye Co., Toiyabe National Forest, Toquima Range, Iron Springs, 5 June 1978, Goodrich 11292 (UTC)
<i>Pteryxia terebinthina</i> (Hook.) J.M. Coult. & Rose var. <i>terebinthina</i>	AY372887	USA, Washington, Benton Co., Pit 30, 17 May 1993, McKinnon & Sackschewsky 327 (BRY)
<i>Pteryxia terebinthina</i> var. <i>albiflora</i> (Nutt. ex Torr. & A. Gray) Mathias-1	AY372888	USA, Wyoming, Sublette Co., Upper Green River Basin, Cretaceous Mtn/Hogsback Ridge area, 20 May 1993, Hartman 37374 (RM)
<i>Pteryxia terebinthina</i> var. <i>calcarea</i> (M.E. Jones) Mathias-1	AY372889	USA, Wyoming, Big Horn Co., Big Horn Mtns, Cold Spring Road, c. 8 air mi NE of Hyattville, 26 May 1980, Hartman & Dueholm 11224 (RM)
<i>Pteryxia terebinthina</i> var. <i>calcarea</i> (M.E. Jones) Mathias-2	AY372890	USA, Wyoming, Uinta Co., 2.75mi N of Lonetree, 2 July 1999, Goodrich 26060 (BRY)
<i>Pteryxia terebinthina</i> var. <i>californica</i> (J.M. Coult. & Rose) Mathias	AY372891	USA, California, Sonoma Co., 0.2mi W on Harrison Grade Road from junction with Stoetz Lane, 18 April 1984, Utech et al. 84-164 (RM)
<i>Pteryxia terebinthina</i> var. <i>foeniculacea</i> (Nutt. ex Torr. & A. Gray) Mathias	AY372892	USA, Idaho, Custer Co., Morgan Creek, 10mi N of Challis, 26 June 1984, Atwood 10320 (RM)
<i>Tauschia arguta</i> (Torr. & A. Gray) J.F. Macbr.	AY372893	USA, California, San Diego Co., Corte Madera Ranch, near Pine Valley, 22 May 1983, Van der Werff & Clark 4571 (MO)
<i>Tauschia kelloggii</i> (A. Gray) J.F. Macbr.	AY372894	USA, California, Butte Co., SW of intersection of Black Bart Road and Forbestown Road, about 3.5 air mi SW of Forgestown, 16 June 1993, Ahart 6972 (UC)
<i>Thaspium barbinode</i> (Michx.) Nutt.	AY372895	USA, Missouri, Montgomery Co., Graham Cave State Park, 11 May 1992, Schuette 2314 (MO)
<i>Thaspium pinnatifidum</i> (Buckley) A. Gray	AY372896	USA, Kentucky, Downie 870 (ILL)

resulted in a matrix of 1 019 positions. Here, 58 positions (from nine regions scattered throughout the intron) were excluded from further consideration due to ambiguity of alignment. These positions represented tracts of poly-A's, -G's or -T's, or indirect duplications of adjacent elements in two or more taxa. Of the remaining 961 positions, 786 (82%) were constant, 96 (10%) were autapomorphic, and 79 (8%) were parsimony informative. Twenty-seven alignment gaps, ranging between 1–51bp in size, were required. Fourteen of these gaps are autapomorphic insertions; the large, 51bp gap represented a deletion of approximately half of intron structural Domain IV in *Podistera eastwoodiae*. A comparison of the sequence characteristics of the intron's six major structural domains is presented elsewhere (Sun 2003). Eight of these 27 gaps, representing two deletions (of 1bp and 5bp) and six insertions (from 2–7bp), were parsimony informative. Uncorrected pairwise sequence divergence ranged from identity to 3.9% of nucleotides.

The combined analysis of 129 ITS and *rps16* intron sequences culminated in a matrix of 1 471 alignment positions, with the following characteristics (after excluding 58 ambiguous positions from the *rps16* intron): 997 (71%) positions constant; 200 (14%) positions autapomorphic; and 216 (15%) positions parsimony informative. Uncorrected pairwise sequence divergence values ranged from identity to 5.4% of nucleotides.

#### Phylogenetic analysis of partitioned data

Maximum parsimony analyses of separate ITS and *rps16* intron data sets each resulted in the preset limit of 20 000 minimal length trees. The strict consensus of each of these groups of trees and their measures of character fit (i.e. cladogram length and consistency, retention, and rescaled consistency indices) are presented in Figure 1. The results of the ITS analysis are very similar to those reported previously for slightly different sets of taxa (Downie *et al.* 2002, Sun *et al.* 2004). Bootstrap estimates presented in the ITS strict consensus tree are generally quite low (averaging 74%), with only 29 resolved clades having values  $\geq 50\%$ . The herbaceous perennial apioide genera endemic to North America (north of Mexico, therefore excluding the *Arracacia* clade) constitute a monophyletic group (herein, called the 'Endemic NA Clade'; Figure 1) that is sister to a clade comprising nine species of *Angelica* (including *Sphenosciadium*) and *Glehnia littoralis* subsp. *leiocarpa*. However, both of these major clades are very weakly supported, with bootstrap values  $< 50\%$ . Additional support for the monophyly of the 'Endemic NA Clade' comes from the shared presence of a protogynous breeding system, an obsolete stylopodium (in all genera except *Podistera*), flowering time, and patterns of variation in sex expression among inflorescences on the same plant (Schlessman and Barrie 2004). *Glehnia littoralis* is not monophyletic, and neither of its subspecies allies with *Cymopterus*. *Glehnia* exhibits the quintessential *Cymopterus* fruit structure, having dorsally flattened fruits and broad wavy wings, and was considered to be closely related to *Cymopterus* (Mathias 1928). Among the members comprising the 'Endemic NA Clade', a large polytomy exists yielding many weakly supported clades. Notwithstanding the

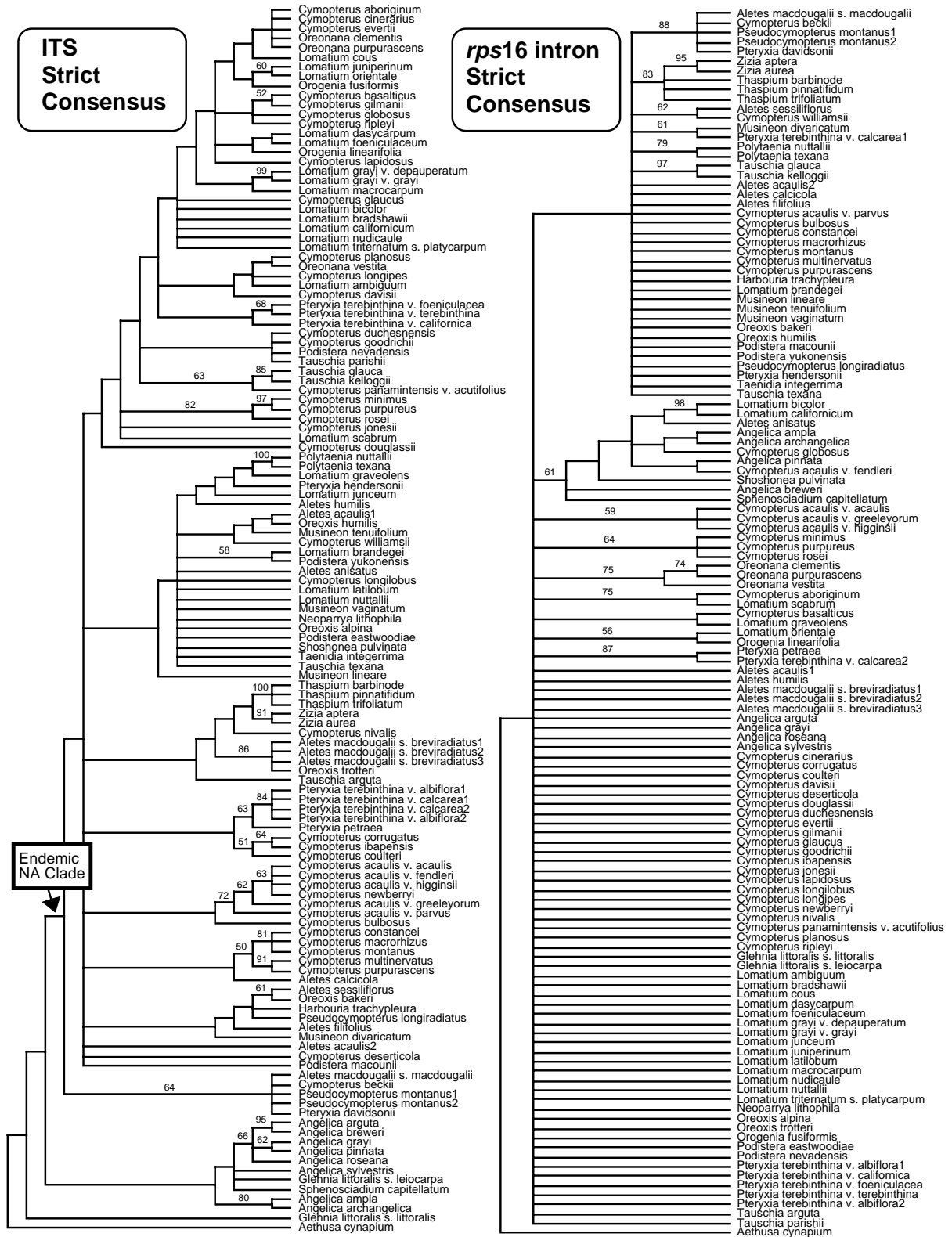
poor resolution of the ITS tree, it is evident that *Cymopterus* is not monophyletic. Species of *Cymopterus* permeate all major clades resolved in the tree. Constraining the genus to monophyly yields trees of substantially greater length (731 steps) than those without the constraint (701 steps).

The results of MP analysis of *rps16* intron sequence data yield a strict consensus tree (Figure 1) that is even less resolved than that produced using ITS sequences. Here, bootstrap values range from 56–98% (averaging 76%), but only 16 clades are resolved having values  $\geq 50\%$ . The comb-like phylogeny of the intron strict consensus tree shows only two major (albeit weakly supported) clades (of 11 and 41 taxa), with the first of these comprising five species of *Angelica* (including *Sphenosciadium*) and six members of the 'Endemic NA Clade' outlined previously (i.e. those belonging to genera *Aletes*, *Cymopterus*, *Lomatium* and *Shoshonea*). The four remaining species of *Angelica* examined, in addition to the two subspecies of *Glehnia littoralis*, form six of the many branches arising off a large polytomy. The second major clade (of 41 taxa) also does not coincide with any group occurring in the ITS tree. Within this clade, *Zizia* is monophyletic and it unites with *Thaspium*. *Polytaenia*, with its two included species, is also monophyletic. Elsewhere in this tree, the genus *Oreonana* is monophyletic. However, all other genera, where the tree is resolved, do not comprise monophyletic groups. In particular, the genus *Cymopterus* is highly polyphyletic, with its members permeating many of the resolved clades. Constraining *Cymopterus* to monophyly and rerunning the MP analysis yielded trees 27 steps longer than those without the constraint invoked.

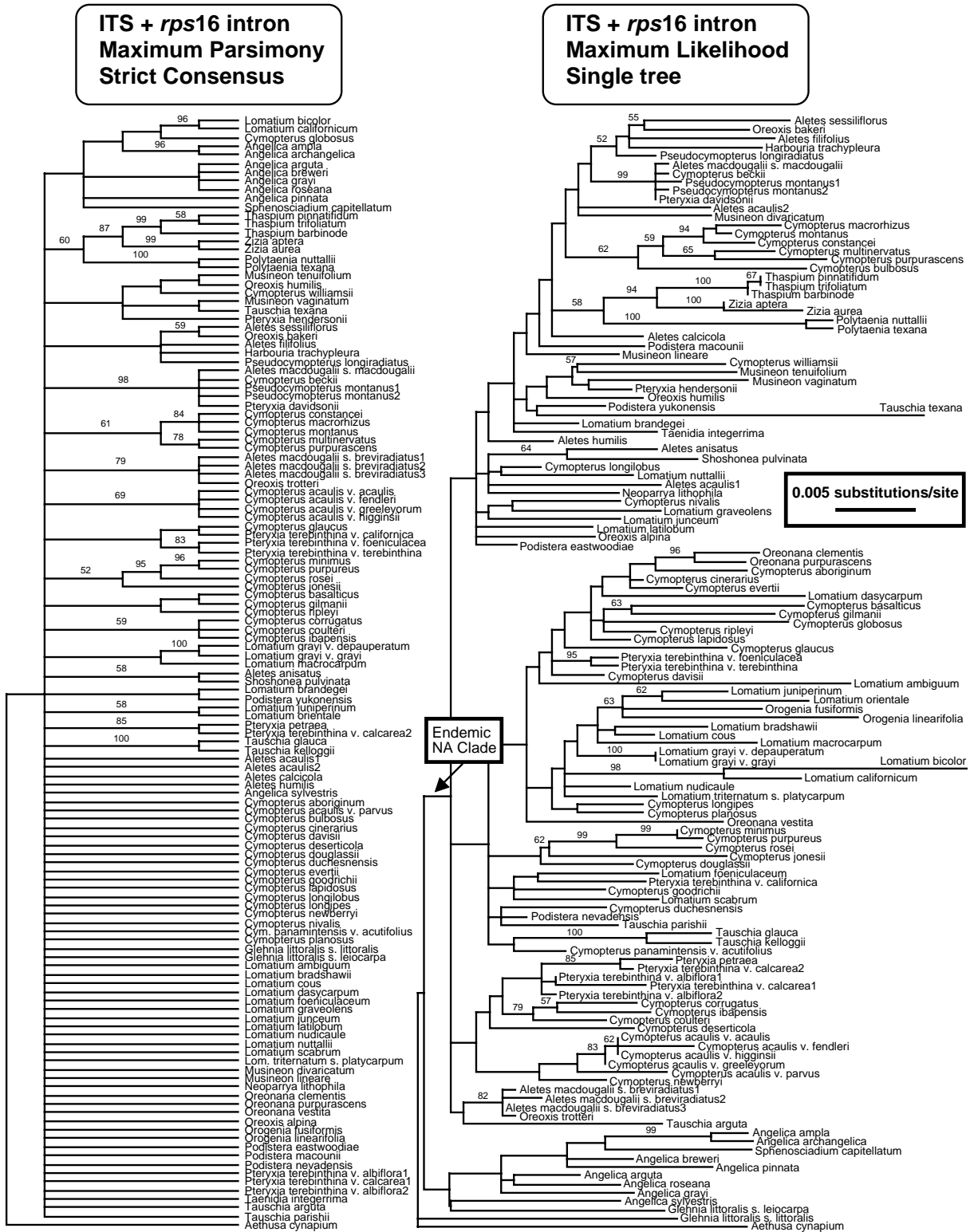
The lack of resolution in the *rps16* intron-derived strict consensus tree is undoubtedly due to a paucity of characters informative for parsimony analysis. The *rps16* intron matrix, with slightly more than double the number of aligned sites than that of the ITS matrix, contributed 79 characters that were parsimony informative (representing 8% of all included positions) whereas the ITS data set yielded 137 informative characters (representing 30% of all positions). Conflict among characters was less in the *rps16* intron tree (CI = 0.6711; RI = 0.8158) than in the ITS tree (CI = 0.4964; RI = 0.6566). The results of a partition-homogeneity test revealed that the two data sets yield significantly different phylogenetic estimates ( $P = 0.01$ ). However, a visual comparison of the ITS and intron-derived trees indicates that the discrepancies observed between them are largely attributable to poorly supported nodes. When these nodes are treated as unresolved (that is, they are collapsed to yield polytomies), both trees are similar yet show very little resolution overall.

#### Phylogenetic analysis of combined data

To increase resolution of relationships and branch support among these North American umbellifers, ITS and intron data were combined for a simultaneous analysis under MP or ML optimisation. The results of the MP analysis are presented in Figure 2. Modeltest selected the GTR+I+G model of nucleotide substitution as fitting these combined data best (base frequencies: 0.3240, A; 0.1691, C; 0.1917, G; 0.3152, T; estimates of substitution rates: A $\leftrightarrow$ C, 0.6358; A $\leftrightarrow$ G,



**Figure 1:** Strict consensus trees derived from equally-weighted maximum parsimony analyses of aligned ITS or *rps16* intron sequences from 129 accessions of Apiaceae subfamily Apioideae. ITS: Strict consensus of 20 000 minimal length 701-step trees (CI's = 0.4964 and 0.3893, with and without uninformative characters, respectively; RI = 0.6566; RC = 0.3260). *Rps16* intron: Strict consensus of 20 000 minimal length 298-step trees (CI's = 0.6711 and 0.4948, with and without uninformative characters, respectively; RI = 0.8158; RC = 0.5475). Numbers at nodes are bootstrap percentage estimates for 100 000 replicate analyses using 'fast' stepwise-addition; values <50% are not indicated. Complete taxon names are provided in Table 1 (v. = variety; s. = subspecies)



**Figure 2:** Phylogenetic trees derived from analyses of combined ITS and *rps16* intron sequences from 129 accessions of Apiaceae subfamily Apiioideae. Maximum Parsimony: Strict consensus of 20 000 minimal length 1 121-step trees (CI's = 0.4888 and 0.3591, with and without uninformative characters, respectively; RI = 0.6327; RC = 0.3093). Numbers at nodes are bootstrap percentage estimates for 100 000 replicate analyses using 'fast' stepwise-addition; values <50% are not indicated. Maximum likelihood: Single tree derived from ML analysis under the GTR+I+G model of nucleotide substitution (−Ln likelihood = 9 170.0063). Numbers at nodes are bootstrap estimates from 1 000 replicate neighbour-joining analyses under a ML model of nucleotide substitution; values <50% are not indicated. Complete taxon names are provided in Table 1 (v. = variety; s. = subspecies)



1.0044; A↔T, 0.3504; C↔G, 0.4434; C↔T, 1.9514; G↔T, 1; proportion of invariable sites = 0.4678; gamma distribution shape parameter = 0.6416). Using these parameters, a single ML tree was recovered in PAUP\*, with a  $-\ln$  likelihood score of 9170.0063 (Figure 2).

The strict consensus tree of combined data is poorly resolved, with 25 clades supported by bootstrap values  $\geq 50\%$  (averaging 80%). This average bootstrap value is higher than those obtained by the partitioned analyses (74–76%), and the number of resolved clades with bootstrap values  $\geq 50\%$  is only slightly less than that obtained in the ITS strict consensus tree (29). However, CI and RI values for the combined data tree (0.4888 and 0.6327, respectively) are less than those values calculated in the partitioned analyses, indicating greater character conflict in the former. These results suggest that the incorporation of *rps16* intron data in this study does little to bolster branch support.

The MP and ML trees of combined data (Figure 2) differ in their placement of the *Angelica* group of taxa (i.e. *Angelica*, *Sphenosciadium* and *Glehnia littoralis* subsp. *leiocarpa*), with only the ML tree showing the group as monophyletic and sister to the 'Endemic NA Clade.' Neither tree supports the monophyly of *Cymopterus* (or, for that matter, many of the other western North American genera, such as *Aletes*, *Lomatium*, *Musineon*, *Oreoxis*, *Pseudocymopterus*, *Pteryxia*, and *Tauschia*). Constraining *Cymopterus* to monophyly in subsequent MP searches produced trees of 1 157 steps, 36 steps longer than those most parsimonious. Thus, it is clear that the genus *Cymopterus*, as currently circumscribed (Kartesz 1994 or Cronquist 1997), is a highly artificial assemblage of species that must be abandoned in any revised system of umbellifer classification. The only previously identified species group of *Cymopterus* supported by this study is *Cymopterus* sect. *Phellopterus* Nutt. ex Torr. & A. Gray (Mathias 1930). This group constitutes six species (*Cymopterus bulbosus*, *C. constancei*, *C. macrorhizus*, *C. montanus*, *C. multinervatus* and *C. purpurascens*) that share large, showy bractlets that are basally connate. They also share a unique 5bp deletion in Domain III of the *rps16* intron. This species group comprises a poorly supported clade in the ML tree (62% bootstrap value), but in the MP tree (as in our previous study of ITS sequences; Sun *et al.* 2004), *C. bulbosus* is excluded from this clade. Similarly, *Cymopterus acaulis*, the nomenclatural type of the genus, consists of five not very well separated infraspecific taxa (vars. *acaulis*, *fendleri*, *greeleyorum*, *higginsii* and *parvus*). In the ML tree, these taxa comprise a monophyletic group, whereas in the MP tree, *C. acaulis* var. *parvus* is removed. These five taxa also share three *rps16* indels: a 1bp deletion and a 3bp insertion in Domain I, and a 7bp insertion in Domain IV.

A major clade resolved in both MP and ML trees of combined data (Figure 2), but not apparent when these data are analysed separately (Figure 1), is that comprising *Polytaenia*, *Thaspium* and *Zizia*. Moreover, each of these genera is resolved as monophyletic. *Polytaenia* and *Thaspium* are primarily of central and eastern North American distribution, respectively, whereas *Zizia* is distributed widely across North America (but is more common eastward). *Thaspium* and *Zizia* are morphologically similar (Cooperrider 1985, Lindsey 1982) and distinct from their

western North American allies; the union of *Thaspium* and *Zizia* with *Polytaenia*, however, is intriguing and warrants further examination. Otherwise, many of the other major clades resolved in the trees derived from combined data are also apparent in the strict consensus tree of ITS data.

In summary, based on phylogenetic analyses of nuclear (ITS) and plastid (*rps16* intron) sequences, the perennial, endemic genera of North American (north of Mexico) Apiaceae subfamily Apioideae comprise a weakly supported monophyletic group, with the circumboreal genus *Angelica* likely constituting its sister lineage. The two largest genera, *Cymopterus* and *Lomatium*, are highly polyphyletic, with elements of each inextricably linked with each other and with other genera of western North America, such as *Aletes*, *Musineon*, *Oreoxis*, *Orogenia*, *Podistera*, *Pseudocymopterus*, *Pteryxia* and *Tauschia*. The *rps16* intron provided substantially fewer parsimony informative characters than that of the ITS region and, as such, yielded a highly unresolved strict consensus tree. However, when analysed simultaneously with ITS data, one major clade resulted which was not apparent in the results of the partitioned analyses (that is, the union of *Polytaenia*, *Thaspium* and *Zizia*). Only one previously identified species group of *Cymopterus* (sect. *Phellopterus*) is supported as monophyletic. This group was recognised at the generic level (*Phellopterus*) by Coulter and Rose (1900) and Mathias (1930), and subsequently placed under *Cymopterus* by Mathias and Constance (1944–1945). Pending further study, this group may eventually again be treated as generically distinct. Otherwise, no historical treatment of *Cymopterus*, or its allies, reflects the results of the phylogenetic analyses presented herein.

The Apiaceae have been long regarded as a taxonomically problematic group, particularly so in regions that have been little explored floristically. However, it should not be assumed that our knowledge of North American Apiaceae is anywhere near satisfactory, despite over a century of systematic study. Further analysis of the relationships among this large and widely distributed, and presumably monophyletic, group of North American umbellifers is clearly required. To circumscribe genera and to increase resolution of relationships, data beyond those utilised herein will be essential. Such data will come ultimately from additional gene sequencing and the evaluation of morphological and anatomical characters. This work is currently in progress.

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## References

- Cooperrider TS (1985) *Thaspium* and *Zizia* (Umbelliferae) in Ohio. *Castanea* **50**: 116–119
- Coulter JM, Rose JN (1900) Monograph of the North American Umbelliferae. Contributions from the US National Herbarium **7**: 9–256
- Cronquist A (1997) Apiaceae. In: Cronquist A, Holmgren NH, Holmgren PK (eds), Intermountain Flora: Vascular Plants of the

- Intermountain West, U.S.A., Vol. 3, part A. The New York Botanical Garden, Bronx, New York
- Downie SR, Hartman RL, Sun F-J, Katz-Downie DS (2002) Polyphyly of the spring-parsleys (*Cymopterus*): molecular and morphological evidence suggests complex relationships among the perennial endemic genera of western North American Apiaceae. *Canadian Journal of Botany* **80**: 1295–1324
- Downie SR, Katz-Downie DS (1999) Phylogenetic analysis of chloroplast *rps16* intron sequences reveals relationships within the woody southern African Apiaceae subfamily Apioideae. *Canadian Journal of Botany* **77**: 1120–1135
- Downie SR, Watson MF, Spalik K, Katz-Downie DS (2000) Molecular systematics of Old World Apioideae (Apiaceae): relationships among some members of tribe Peucedaneae sensu lato, the placement of several island-endemic species, and resolution within the apioid superclade. *Canadian Journal of Botany* **78**: 506–528
- Evert EF, Constance L (1982) *Shoshonea pulvinata*, a new genus and species of Umbelliferae from Wyoming. *Systematic Botany* **7**: 471–475
- Farris JS, Källersjö M, Kluge AG, Bult C (1995) Testing significance of incongruence. *Cladistics* **10**: 315–319
- Gilmartin AJ, Simmons KS (1987) Relationships of *Lomatium* among other genera of Apiaceae. *Plant Systematics and Evolution* **157**: 95–103
- Hartman RL (2000) A new species of *Cymopterus* (Apiaceae) from the Rocky Mountain region, U.S.A. *Brittonia* **52**: 136–141
- Holmgren PK, Holmgren NH, Barnett LC (1990) *Index Herbariorum*. The New York Botanic Garden, New York
- Jones ME (1908) New species and notes. *Contributions to Western Botany* **12**: 1–81
- Kartesz JT (1994) A Synonymized Checklist of the Vascular Flora of the United States, Canada, and Greenland (2<sup>nd</sup> edn). Biota of the North American Program of the North Carolina Botanical Garden. Timber Press, Portland, Oregon
- Lee B-Y, Downie SR (2000) Phylogenetic analysis of cpDNA restriction sites and *rps16* intron sequences reveals relationships among Apiaceae tribes Caucalideae, Scandiceae and related taxa. *Plant Systematics and Evolution* **221**: 35–60
- Lindsey AH (1982) Floral phenology patterns and breeding systems in *Thaspium* and *Zizia*. *Systematic Botany* **7**: 1–12
- Mathias ME (1928) Studies in the Umbelliferae. I. *Annals of the Missouri Botanical Garden* **15**: 91–109
- Mathias ME (1930) Studies in the Umbelliferae. II. A monograph of *Cymopterus* including a critical study of related genera. *Annals of the Missouri Botanical Garden* **17**: 213–476
- Mathias ME, Constance L (1944–1945) Umbelliferae. In: *North American Flora*, Vol. 28B. The New York Botanical Garden, New York, pp 43–295
- Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818
- Schlessman MA, Barrie FR (2004) Protogyny in Apiaceae, subfamily Apioideae: systematic and geographic distributions, associated traits, and evolutionary hypotheses. *South African Journal of Botany* **70**: 475–487
- Spalik K, Reduron J-P, Downie SR (2004) The phylogenetic position of *Peucedanum* sensu lato and allied genera and their placement in tribe Selineae (Apiaceae, subfamily Apioideae). *Plant Systematics and Evolution* **243**: 189–210
- Sun F-J (2003) A Phylogenetic Study of *Cymopterus* and Related Genera (Apiaceae). PhD Thesis, University of Illinois at Urbana-Champaign, Urbana, USA
- Sun F-J, Downie SR, Hartman RL (2004) An ITS-based phylogenetic analysis of the perennial, endemic Apiaceae subfamily Apioideae of western North America. *Systematic Botany* **29**: 419–431
- Swofford DL (1998) PAUP\*. Phylogenetic Analysis Using Parsimony (\*and Other Methods). Version 4 Edition. Sinauer Associates, Sunderland, Massachusetts
- Theobald WL, Tseng CC, Mathias ME (1963) A revision of *Aletes* and *Neoparrya* (Umbelliferae). *Brittonia* **16**: 296–315
- Weber WA (1984) New names and combinations, principally in the Rocky Mountain flora — IV. *Phytologia* **55**: 1–6