



# Phylogenetic relationships among the perennial, endemic Apiaceae subfamily Apioideae of western North America: additional data from the cpDNA *trnF-trnL-trnT* region continue to support a highly polyphyletic *Cymopterus*

By Feng-Jie Sun and Stephen R. Downie

With 3 figures and 3 tables

## Abstract

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Considerable confusion exists with regard to the delimitation of *Cymopterus* and its relationship to the other herbaceous, perennial genera of Apiaceae subfamily Apioideae endemic to western North America north of Mexico. Previous molecular systematic studies using nrDNA ITS and cpDNA *rps16* intron sequences have shown that the genus *Cymopterus* is highly polyphyletic, with its species inextricably linked with those of *Aletes*, *Lomatium*, *Oreoxis*, *Pseudocymopterus*, *Pteryxia*, and several other genera of the region. The general lack of resolution of these trees and their overall low branch support values, however, suggested that additional data might be useful to establish monophyly of genera or identify major clades for further phylogenetic and revisionary studies. In this study, we analyze cpDNA *trnF-trnL-trnT* sequence data from 129 accessions of North American Apioideae (representing 111 species and 21 genera) using maximum parsimony, maximum likelihood and Bayesian inference methods individually and in combination with ITS and *rps16* intron sequence data available through previous phylogenetic studies for the same set of accessions. While analyses of the *trnF-trnL-trnT* region result in maximally parsimonious trees of greater branch support and less homoplasy than those generated using either ITS or *rps16* intron data sets, greatest resolution of relationships and highest branch support values are achieved when all DNA regions (representing over 3.5 kb of aligned data) are considered simultaneously. Three or four new major clades are revealed, yet none of these coincide with pre-established groups or traditionally recognized genera. The results obtained continue to suggest that *Cymopterus* is highly polyphyletic, as are most other genera endemic to western North America.

**Keywords:** Apiaceae subfamily Apioideae, *Cymopterus*, ITS, *rps16* intron, *trnF-trnL-trnT*.

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## Introduction

The genus *Cymopterus* Raf. (Apiaceae subfamily Apioideae) comprises some 35 herbaceous perennial species restricted primarily to xeric habitats in western North America (NA) north of Mexico (Kartesz 1994). Much confusion exists with regard to the proper delimitation of *Cymopterus* and its relationship to the other herbaceous, perennial apioid umbellifers indigenous to the region (reviewed in Downie et al. 2002). These genera include *Aletes* J.M. Coult. & Rose, *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, *Shoshonea* Evert & Constance, and *Tauschia* Schldl. Closely allied to these 14 western NA taxa are four other genera of primarily central to eastern NA distribution: *Polytaenia* DC., *Taenidia* (Torr. & A. Gray) Drude, *Thaspium* Nutt., and *Zizia* W.D.J. Koch. Considered collectively, the plants of western NA present such a confusing intergradation of similar characteristics that generic delimitation is exceedingly difficult, and in the absence of mature fruits many species are essentially indistinguishable (Downie et al. 2002). The recognition of infraspecific taxa is also highly problematic because of the overlapping variation in nearly all morphological characters used to differentiate among them. All aforementioned genera, however, apparently comprise a monophyletic group (Downie et al. 2002, Sun & Downie 2004, Sun et al. 2004).

Previous molecular systematic studies using nrDNA ITS (Downie et al. 2002, Sun et al. 2004), cpDNA *rps16* intron (Sun & Downie 2004) and, for a smaller set of taxa, cpDNA *trnF-trnL-trnT* (Downie et al. 2002) sequences revealed that the genus *Cymopterus* is highly polyphyletic, with its species inextricably linked with those of many other perennial, endemic apioid genera of western NA. However, the general lack of resolution of these trees, their overall low branch support values, and the relatively few informative characters obtained suggested that additional DNA sequence data might be useful to further test the monophyly of genera, as currently circumscribed (Kartesz 1994). In this study, we obtain additional sequence data from the *trnF-trnL-trnT* region and compare the results of phylogenetic analyses of these data to those trees inferred in previous studies using ITS and *rps16* intron sequences. Our major objectives are to increase resolution of relationships among the perennial, endemic Apiaceae subfamily Apioideae of western NA by considering additional sequence data for a comprehensive sampling of species and to compare the efficacy of these three loci, by way of partitioned and combined analyses, in delimiting well-supported major clades for further phylogenetic and revisionary studies. The results obtained will eventually enable us to achieve our broader goal, which is to define and delimit the various generic elements within the perennial, endemic Apiaceae subfamily Apioideae of western NA.

## Materials and methods

Sequence data from the cpDNA *trnF-trnL-trnT* (hereafter, *trnF-L-T*) region were obtained for 102 accessions of NA Apiaceae subfamily Apioideae (Table 1) and combined with previously published

Table 1. GenBank accession numbers and source and voucher information for each of the 102 accessions of Apiaceae subfamily Apioidae examined for cp-DNA *trnF-L-T* sequence variation. These data have been deposited with GenBank as separate *trnF-L* intergenic spacer, *trnL* intron, and *trnL-T* intergenic spacer sequences. GenBank and voucher information for the 27 additional accessions examined for *trnF-L-T* sequence variation are presented elsewhere (Downie et al. 2002). Herbarium acronyms are as in Holmgren et al. (1990).

Taxon	<i>trnF-L</i> spacer, <i>trnL</i> intron, <i>trnL-T</i> spacer	Source and Voucher Information
<i>Aethusa cynapium</i> L.	AY379117, AY379219, AY379321	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France, <i>Downie 337</i> (ILL)
<i>Aletes acaulis</i> (Torr.) J.M. Coult. & Rose-1	AY379118, AY379220, AY379322	USA, New Mexico, Sandoval Co., DelAgua trail along Sandia Crest, Sandia Crest National Forest, 9 Jul 1999, <i>Sun &amp; Hartman 634</i> (ILL)
<i>Aletes acaulis</i> -2	AY379119, AY379221, AY379323	USA, New Mexico, Bernalillo Co., on summit of Sandia Crest, Sandia Mtns., 30 Aug 1946, <i>Parker &amp; McClintock 6504</i> (UTC)
<i>Aletes calcicola</i> Mathias & Constance	AY379120, AY379222, AY379324	Mexico, Coahuila, Sierra de San Marcos, opposite Los Fresnos, 4-5 Apr 1969, <i>Pinkava et al. 6117</i> (UC)
<i>Aletes filifolius</i> Mathias, Constance & W.L. Theob.	AY379121, AY379223, AY379325	USA, Texas, Culberson Co., Hunter Peak at Pine Top, Guadalupe Mtns. National Park, 6 Sep 1987, <i>Higgins 17453</i> (BRY)
<i>Aletes humilis</i> J.M. Coult. & Rose	AY379122, AY379224, AY379326	USA, Colorado, Larimer Co., mountaintops on N and S sides of Lone Pine Creek, 12 May 1996, <i>Scully &amp; Howell s.n.</i> (BRY)
<i>Aletes macdougallii</i> J.M. Coult. & Rose	AY379123, AY379225, AY379327	USA, Arizona, Coconino Co., Grand Canyon, Kaibab Trail to Roaring Springs, 23 Jun 1933, <i>Eastwood &amp; Howell 986</i> (UC)
<i>Aletes macdougallii</i> subsp. <i>breviradiatus</i> W.L. Theob. & C.C. Tseng-1	AY379124, AY379226, AY379328	USA, New Mexico, San Juan Co., Chaco Culture Nat. Hist. Park, top of Chacra Mesa, 2 May 1993, <i>Heil 7374</i> (UNM)
<i>Aletes macdougallii</i> subsp. <i>breviradiatus</i> -2	AY379125, AY379227, AY379329	USA, New Mexico, San Juan Co., NM 575, 0.15 mi NNW of US 64, NW of Blanco, 10 Jul 1999, <i>Sun &amp; Hartman 649</i> (ILL)
<i>Aletes macdougallii</i> subsp. <i>breviradiatus</i> -3	AY379126, AY379228, AY379330	USA, Utah, Garfield Co., Dixie National Forest, Death Hollow, 28 May 1989, <i>Franklin 6499</i> (BRY)
<i>Aletes sessiliflorus</i> W.L. Theobald & C.C. Tseng	AY379127, AY379229, AY379331	USA, New Mexico, Rio Arriba Co., NW of Embudo, 1 May 1992, <i>Hartman 13954</i> (RM)
<i>Angelica ampla</i> A. Nelson	AY379128, AY379230, AY379332	USA, Colorado, Garfield Co., Flat Tops/White River Plateau, Main Elk Creek, 24 Jun 1990, <i>Hartman 25821</i> (RM)
<i>Angelica arguta</i> Nutt. ex Torr. & A. Gray	AY379129, AY379231, AY379333	USA, Wyoming, Lincoln Co., Murphy Creek, 18 Jul 1992, <i>Nelson &amp; Nelson 23524</i> (RM)
<i>Angelica breweri</i> A. Gray	AY379130, AY379232, AY379334	USA, California, Alpine Co., Ebbetts Pass, <i>Constance &amp; Ertter 3903</i> (UC), Cult. University of California Botanical Garden, Berkeley, L. Constance 2446

Taxon	<i>trnF-L</i> spacer, <i>trnL</i> intron, <i>trnL-T</i> spacer	Source and Voucher Information
<i>Angelica capitellata</i> (A. Gray) Spalik, Reduron & S.R. Downie	AY379210, AY379312, AY379414	USA, Nevada, Esmeralda Co., White Mtns., 27 Jul 1986, <i>Morefield 4210</i> (RM)
<i>Angelica grayi</i> (J.M. Coult. & Rose) J.M. Coult. & Rose	AY379131, AY379233, AY379335	USA, Colorado, Garfield Co., Blair Mtn., 17 mi NNW Glenwood Springs, 24 Aug 1991, <i>Vanderhorst &amp; Palaci 4490</i> (RM)
<i>Angelica pinnata</i> S. Watson	AY379132, AY379234, AY379336	USA, Wyoming, Lincoln Co., Commissary Ridge, 22 Jul 1993, <i>Hartman 41500</i> (RM)
<i>Angelica roseana</i> L.F. Hend.	AY379133, AY379235, AY379337	USA, Wyoming, Teton Co., Blue Miner Lake, 25 Aug 1994, <i>Hartman 50090</i> (RM)
<i>Angelica sylvestris</i> L.	AY379134, AY379236, AY379338	Cult. UIUC from seeds obtained from Shanghai Botanic Garden, China, <i>Downie 428</i> (ILL)
<i>Cymopterus aboriginum</i> M.E. Jones	AY379135, AY379237, AY379339	USA, California, Mono Co., ca 5 mi E of Benton along Hwy 6, 21 Jun 1990, <i>Arwood 13838</i> (BRY)
<i>Cymopterus acaulis</i> (Pursh) Raf. var. <i>acaulis</i>	AY379136, AY379238, AY379340	USA, Wyoming, Uinta Co., Basins and Mtns. of SW Wyoming, along Leavitt Creek, 18 Jun 1995, <i>Nelson &amp; Refsdal 35183</i> (RM)
<i>Cymopterus acaulis</i> var. <i>fendleri</i> (A. Gray) S. Goodrich	AY379137, AY379239, AY379341	USA, Utah, Emery Co., S of Price River, 14 May 1979, <i>Hartman 8674</i> (RM)
<i>Cymopterus acaulis</i> var. <i>greeleyorum</i> J.W. Grimes & P.L. Packard	AY379138, AY379240, AY379342	USA, Oregon, Malheur Co., McBride Creek Road, 24 May 1989, <i>Smithman et al. LS-2045</i> (CIC)
<i>Cymopterus acaulis</i> var. <i>higginsii</i> (S.L. Welsh) S. Goodrich	AY379139, AY379241, AY379343	USA, Utah, Kane Co., E of Coyote Wash, 8 Apr 1993, <i>Chapman s.n.</i> (BRY)
<i>Cymopterus acaulis</i> var. <i>parvus</i> S. Goodrich	AY379140, AY379242, AY379344	USA, Utah, Tooele Co., Dunefield, NW of English village, W of Cedar Mtns., 7 Jun 1993, <i>Johnson 351</i> (BRY)
<i>Cymopterus basalticus</i> M.E. Jones	AY379141, AY379243, AY379345	USA, Utah, Millard Co., Tunnel Springs Mtns., 20 May 2000, <i>Tilley &amp; Tilley 1174</i> (BRY)
<i>Cymopterus beckii</i> S.L. Welsh & S. Goodrich	AY379142, AY379244, AY379346	USA, Utah, San Juan Co., SW of Abajo Mtns., 0.7 mi NE of confluence Deep Canyon with Chippean Canyon, 6 Jul 1992, <i>Franklin 7544</i> (MO)
<i>Cymopterus bulbosus</i> A. Nelson	AY379143, AY379245, AY379347	USA, Utah, Uintah Co., ESE of Vernal, 18 April 1982, <i>Hartman 13951</i> (RM)
<i>Cymopterus cinerarius</i> A. Gray	AY379144, AY379246, AY379348	USA, California, Mono Co., Sweetwater Canyon, 15 Jul 1944, <i>Alexander &amp; Kellogg 3893</i> (UTC)

Taxon	<i>trmF-L</i> spacer, <i>trnL</i> intron, <i>trnL-T</i> spacer	Source and Voucher Information
<i>Cymopterus constancei</i> R.L. Hartm.	AY379145, AY379247, AY379349	USA, Wyoming, Sublette Co., Upper Green River Plain, Cretaceous Mtn., ca 0.5 air mi S to summit, 19 May 1993, <i>Hartman 37182</i> (RM)
<i>Cymopterus corrugatus</i> M.E. Jones	AY379146, AY379248, AY379350	USA, Nevada, Mineral Co., Walker River Indian Reservation, 0.8 road mi W of the reservation from Rawhide, 15 May 1988, <i>Tiehm 11640</i> (BRY)
<i>Cymopterus coulteri</i> (M.E. Jones) Mathias	AY379147, AY379249, AY379351	USA, Utah, Sevier Co., Triangle Mt., Soldiers Canyon, 3.2 mi from jet/frontage road, 15 May 1990, <i>Thorne &amp; Zupan 7377</i> (BRY)
<i>Cymopterus davisii</i> R.L. Hartm.	AY379148, AY379250, AY379352	USA, Idaho, Cassia Co., Mt. Harrison, ca 1 km below the summit, 30 Jul 1983, <i>Cronquist 11840</i> (UTC)
<i>Cymopterus deserticola</i> Brandegee	AY379149, AY379251, AY379353	USA, California, San Bernardino Co., 1 mi S of Kramer, 21 Mar 1958, <i>Raven 11891</i> (UTC)
<i>Cymopterus douglassii</i> R.L. Hartm. & Constance	AY379150, AY379252, AY379354	USA, Idaho, Lemhi Co., Salmon National Forest, E slope of Lemhi Range, 0.5 mi S of Sheep Mtns., 25 Jun 1994, <i>Markow 11117</i> (RM)
<i>Cymopterus duchesnensis</i> M.E. Jones	AY379151, AY379253, AY379355	USA, Utah, Uintah Co., ca 11 mi SW of Vernal, Twelve Mile Wash, 1 Jun 1992, <i>Goodrich 24061</i> (BRY)
<i>Cymopterus evertii</i> R.L. Hartm. & R.S. Kirkp.	AY379152, AY379254, AY379356	USA, Wyoming, Hot Springs Co., Absaroka Mtns, ca 1 mi NNW of summit of Twin Buttes, ca 35 mi W of Thermopolis, 10 Jul 1992, <i>Fertig 12964</i> (RM)
<i>Cymopterus gilmanii</i> C. Morton	AY379153, AY379255, AY379357	USA, Nevada, Clark Co., Desert Range, Desert National Wildlife Refuge, 7.3 mi from Sheep Peak summit, 17 May 1987, <i>Morefield et al. 4463</i> (BRY)
<i>Cymopterus glaucus</i> Nutt.	AY379154, AY379256, AY379358	USA, Idaho, Valley Co., ridge between Fisher Creek Saddle and Black Tip along Idaho/Valley Co. line, 26 Jul 1989, <i>Ertter et al. 8742</i> (RM)
<i>Cymopterus goodrichii</i> S.L. Welsh & Neese	AY379155, AY379257, AY379359	USA, Nevada, Lander Co., Toiyabe Range, head of Big & Kingston Creeks, ca 2 mi N Bunker Hill, 16 Jul 1981, <i>Neese et al. 10737</i> (BRY)
<i>Cymopterus ibapensis</i> M.E. Jones	AY379156, AY379258, AY379360	USA, Utah, Box Elder Co., 0.5 mi of Standrod, 21 Jun 1982, <i>Goodrich &amp; Arwood 16995</i> (BRY)
<i>Cymopterus jonesii</i> J.M. Coult. & Rose	AY379157, AY379259, AY379361	USA, Utah, Iron Co., Parowan Canyon, 6.8 air mi S of Minerville, 25 May 1993, <i>Franklin 7647</i> (BRY)

Taxon	<i>trnF-L</i> spacer, <i>trnL</i> intron, <i>trnL-T</i> spacer	Source and Voucher Information
<i>Cymopterus lapidosus</i> (M.E. Jones) M.E. Jones	AY379158, AY379260, AY379362	USA, Wyoming, Uinta Co., Uinta Co. Road 290, 3.7 air mi W of Lonetree, 7 Jul 1983, Hartman 15767 (UTC)
<i>Cymopterus longilobus</i> (Rydb.) W.A. Weber	AY379159, AY379261, AY379363	USA, Wyoming, Sublette Co., Wyoming Range, 15 Aug 1993, Hartman 43986 (RM)
<i>Cymopterus longipes</i> S. Watson	AY379160, AY379262, AY379364	USA, Utah, Uintah Co., Brownie Canyon, Uinta Mtns., ca 4.5 mi due SE of Marsh Peak, 15 Jun 1994, Huber 954 (BRY)
<i>Cymopterus macrohizus</i> Buckley	AY379161, AY379263, AY379365	USA, Texas, Tom Green Co., Grandview campground on W side of Fisher Lake, 19 Mar 1983, Errler & Strachan 4690 (RM)
<i>Cymopterus minimus</i> (Mathias)	AY379162, AY379264, AY379366	USA, Utah, Iron Co., Cedar Breaks National Monument, just below N rim where reached by Rattlesnake Trail, 25 Jul 1993, Stone 1656 (BRY)
<i>Cymopterus newberryi</i> (S. Watson) M.E. Jones	AY379163, AY379265, AY379367	USA, Utah, San Juan Co., 4.5 mi 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	AY379164, AY379266, AY379368	USA, California, San Bernardino Co., south side of Ord Mtn., N of Lucerne Valley, Mojave Desert, 8 Apr 1988, Boyd <i>et al.</i> 1776 (OSC)
<i>Cymopterus planosus</i> (Osterth.) Mathias	AY379165, AY379267, AY379369	USA, Colorado, Garfield Co., Flat Tops/White River Plateau, Main Elk Creek, 23 May 1990, Hartman & Vanderhorst 24535 (BRY)
<i>Cymopterus purpurascens</i> (A. Gray) M.E. Jones	AY379166, AY379268, AY379370	USA, Arizona, Mohave Co., NE of Peach Springs, 30 Mar 1983, Hartman 14096 (RM)
<i>Cymopterus ripleyi</i> Barneby	AY379167, AY379269, AY379371	USA, Nevada, Nye Co., Stone Cabin Valley, along US Hwy 6, at Little Fish Lake turnoff, 24 May 1995, Holmgren & Holmgren 12299 (BRY)
<i>Cymopterus rosei</i> (M.E. Jones ex J.M. Coult. & Rose) M.E. Jones	AY379168, AY379270, AY379372	USA, Utah, Sanpete Co., 2 mi 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>	AY379169, AY379271, AY379373	Taiwan, Taitung Hsien, Lanyu (Orchid Island); Liao <i>et al.</i> 1235 (MO)
<i>Glehnia littoralis</i> subsp. <i>leiocarpa</i> (Mathias) Hultén	AY379170, AY379272, AY379374	USA, Oregon, Tillamook Co., by the beach on the S side of Cape Kiwanda, 10 Aug 1975, Halse 1228 (OSC)
<i>Lomatium ambiguum</i> (Nutt.) J.M. Coult. & Rose	AY379171, AY379273, AY379375	USA, Montana, Madison Co., ca 1.5 mi from road's end on Bear Trap Canyon Trail, 30 May 1979, Lowry 1867 (ILL)
<i>Lomatium bradshawii</i> (Rose ex Mathias) Mathias & Constance	AY379172, AY379274, AY379376	USA, Oregon, Benton Co., NE Corvallis, W of Canterbury Circle, area around Frazier Creek, 8 Jun 1980, Halse 2215 (ILL)

Taxon	<i>trnF-L</i> spacer, <i>trnL</i> intron, <i>trnL-T</i> spacer	Source and Voucher Information
<i>Lomatium brandegei</i> (J.M. Coult. & Rose) J.F. Macbr.	AY379173, AY379275, AY379377	USA, Washington, Chelan Co., 5 mi SW of Leavenworth on Snow Creek Trail, 500 ft above creek, 29 May 1977, Lowry 621 (ILL)
<i>Lomatium cous</i> (S. Watson) J.M. Coult. & Rose	AY379174, AY379276, AY379378	USA, Wyoming, Sublette Co., Palmer Peak, 5 Aug 1994, Hartman 49374 (RM)
<i>Lomatium foeniculaceum</i> (Nutt.) J.M. Coult. & Rose subsp. <i>foeniculaceum</i>	AY379175, AY379277, AY379379	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>	AY379176, AY379278, AY379380	USA, Wyoming, Lincoln Co., Tump Range, Preacher Hollow, 16 mi NNE Cokeville, 22 May 1993, Hartman 37444 (RM)
<i>Lomatium grayi</i> var. <i>depauperatum</i> (M.E. Jones) Mathias	AY379177, AY379279, AY379381	USA, Utah, Juab Co., Gilson Mtns., 7 mi from Lynndyl, 7 May 1981, Goodrich 15335 (RM)
<i>Lomatium latilobum</i> (Rydb.) Mathias	AY379178, AY379280, AY379382	USA, Utah, Grand Co., SE of Moab, 13 Apr 1995, Tuby 3772 (RM)
<i>Lomatium nuttallii</i> (A. Gray) J.F. Macbr.	AY379179, AY379281, AY379383	USA, Wyoming, Natrona Co., Baker Cabin, 23 May 1994, Nelson 30631 (RM)
<i>Lomatium orientale</i> J.M. Coult. & Rose	AY379180, AY379282, AY379384	USA, Wyoming, Natrona Co., along Notches, 23 May 1994, Nelson 30536 (RM)
<i>Lomatium scabrum</i> (J.M. Coult. & Rose) Mathias var. <i>scabrum</i>	AY379181, AY379283, AY379385	USA, Utah, Millard Co., S. of Gamison, 16 May 1981, Fontken 1168 (RM)
<i>Lomatium triternatum</i> (Pursh) J.M. Coult. & Rose subsp. <i>platycarpum</i> (Torr.) Cronquist	AY379182, AY379284, AY379386	USA, Wyoming, Lincoln Co., Boulder Ridge, 22 May 1993, Hartman 37526 (RM)
<i>Musineon lineare</i> (Rydb.) Mathias	AY379183, AY379285, AY379387	USA, Utah, Cache Co., Toney Grove Trailhead Trail to Naomi Peak, ca 1 mi W of summit, 9 Jul 1989, Hartman 24364 (RM)
<i>Musineon vaginatum</i> Rydb.	AY379184, AY379286, AY379388	USA, Wyoming, Sheridan Co., Wildlife Refuge, ca 3 air mi W of Dayton, 30 Jun 1979, Hartman 9829 (BRY)
<i>Oreonana clementis</i> (M.E. Jones) Jeps.	AY379185, AY379287, AY379389	USA, California, Tulare Co., Mt. Guyot, Sierra Nevada, 22 Jul 1949, Howell 25604 (UTC)
<i>Oreonana purpurascens</i> Shevock & Constance	AY379186, AY379288, AY379390	USA, California, Tulare Co., Sequoia National Forest, Slate Mtn., along USFS trail 31E14, 26 Apr 1977, Shevock 5428 (BRY)
<i>Oreonana vestita</i> (S. Watson) Jeps.	AY379187, AY379289, AY379391	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>	AY379188, AY379290, AY379392	USA, Colorado, Saguache Co., Cochetopa Park, NN14 road, 0.5 road mi W of 17FF road, 11 Jul 1999, Sun & Hartman 629 (ILL)
<i>Oreoxis bakeri</i> J.M. Coult. & Rose	AY379189, AY379291, AY379393	USA, New Mexico, Santa Fe Co., Lake Peak, 19 Jun 1980, Hartman 11725 (RM)



Taxon	<i>trnF-L</i> spacer, <i>trnL</i> intron, <i>trnL-T</i> spacer	Source and Voucher Information
<i>Oreoxis humilis</i> Raf.	AY379190, AY379292, AY379394	USA, Colorado, Teller Co., Pikes Peak Road, 17 Jun 1980, <i>Hartman 11718</i> (RM)
<i>Oreoxis trotteri</i> S.L. Welsh & S. Goodrich	AY379191, AY379293, AY379395	USA, Utah, Grand Co., ca 13 mi NW of Moab, E of Courthouse Rock, 20 May 1985, <i>Welsh 23427</i> (BRY)
<i>Orogeria fusiformis</i> S. Watson	AY379192, AY379294, AY379396	USA, Oregon, Josephine Co., Oregon Caves National Monument, Siskiyou Mts., 1.5 air miles WNW of Mt. Elijah, 24 May 1996, <i>Zika 12864</i> (OSC)
<i>Orogeria linearifolia</i> S. Watson	AY379193, AY379295, AY379397	USA, Utah, Tooele Co., 1 mi S of Forest Service boundary along Vernon Creek, 18 Apr 1985, <i>Anwood 10700</i> (BRY)
<i>Podistera macounii</i> (J.M. Coult. & Rose) Mathias & Constance	AY379194, AY379296, AY379398	USA, Alaska, ridge W of Sheep Creek, 30 Jun 1977, <i>Reed et al. 6047</i> (BRY)
<i>Podistera nevadensis</i> (A. Gray) S. Watson	AY379195, AY379297, AY379399	USA, California, El Dorado Co., Lake Tahoe Basin Management Unit, Freel Peak, 27 Jul 2002, <i>Maison 634</i> (ILL)
<i>Podistera yukonensis</i> Mathias & Constance	AY379196, AY379298, AY379400	USA, Alaska, Kathul Mtn., along the Yukon River roughly halfway between Eagle and Circle, 11 Jun 2002, <i>Larsen &amp; Battien 02-1263</i> (ALA)
<i>Polytaenia nuttallii</i> DC.	AY379197, AY379299, AY379401	USA, Texas, Grayson Co., 8 mi W of Sherman, 26 May 1992, <i>Ulaszek 1599</i> (ILLS)
<i>Polytaenia texana</i> (J.M. Coult. & Rose) Mathias & Constance	AY379198, AY379300, AY379402	USA, Texas, Burnet Co., E of Briggs, 25 May 1985, <i>Barrie 1403</i> (RM)
<i>Pseudocymopterus longiradiatus</i> Mathias, Constance & W.L. Theob.	AY379199, AY379301, AY379403	USA, New Mexico, Otero Co., Sacramento Mtns., ca 3 mi SSW of Bent, SW side of Domingo Peak, 7 Aug 1990, <i>Shivinski 1546</i> (UNM)
<i>Pseudocymopterus montanus</i> (A. Gray) J.M. Coult. & Rose-1	AY379200, AY379302, AY379404	USA, Colorado, Rio Blanco Co., Dunckley Flat Tops, 17 Jun 1991, <i>Vanderhorst 2637</i> (RM)
<i>Pseudocymopterus montanus</i> -2	AY379201, AY379303, AY379405	USA, Arizona, Pima Co., 32 mi NE of Tucson via Catalina Highway, Santa Catalina Mtns., 24 Jul 1965, <i>Matthews &amp; Matthews 456</i> (ILL)
<i>Pteryxia davidsonii</i> (J.M. Coult. & Rose) Mathias & Constance	AY379202, AY379304, AY379406	USA, New Mexico, Grant Co., Bear Mtn., near Silver City, 17 Jun 1903, <i>Metcalfe 165</i> (RM)
<i>Pteryxia petraea</i> (M.E. Jones) J.M. Coult. & Rose	AY379203, AY379305, AY379407	USA, Nevada, Nye Co., Toiyabe National Forest, Toiyabe Range, Iron Springs, 5 Jun 1978, <i>Goodrich 11292</i> (UTC)



Taxon	<i>trmF-L</i> spacer, <i>trnL</i> intron, <i>trnL-T</i> spacer	Source and Voucher Information
<i>Pteryxia terebinthina</i> (Hook.) J.M. Coult. & Rose var. <i>terebinthina</i>	AY379204, AY379306, AY379408	USA, Washington, Benton Co., Pit 30, between the 200 area, 17 May 1993, <i>McKinnon &amp; Sackschewsky</i> 327 (BRY)
<i>Pteryxia terebinthina</i> var. <i>albiflora</i> (Nutt. ex Torr. & A. Gray) Mathias-2	AY379205, AY379307, AY379409	USA, Wyoming, Sublette Co., Upper Green River Basin, Cretaceous Mtn./Hogback Ridge area, 20 May 1993, <i>Hartman</i> 37374 (RM)
<i>Pteryxia terebinthina</i> var. <i>calcarea</i> (M.E. Jones) Mathias-1	AY379206, AY379308, AY379410	USA, Wyoming, Big Horn Co., Big Horn Mtns., Cold Spring Road, ca 8 air mi NE of Hyattville, 26 May 1980, <i>Hartman &amp; Duholm</i> 11224 (RM)
<i>Pteryxia terebinthina</i> var. <i>calcarea</i> -2	AY379207, AY379309, AY379411	USA, Wyoming, Uima Co., 2.75 mi N of Lonetree, 2 Jul 1999, <i>Goodrich</i> 26060 (BRY)
<i>Pteryxia terebinthina</i> var. <i>californica</i> (J.M. Coult. & Rose) Mathias	AY379208, AY379310, AY379412	USA, California, Sonoma Co., 0.2 mi W on Harrison Grade Road from junction with Stoetz Lane, 18 Apr 1984, <i>Utech et al.</i> 84-164 (RM)
<i>Pteryxia terebinthina</i> var. <i>foeniculacea</i> (Nutt. ex Torr. & A. Gray) Mathias	AY379209, AY379311, AY379413	USA, Idaho, Custer Co., Morgan Creek, 10 mi N of Challis, 26 Jun 1984, <i>Atwood</i> 10320 (RM)
<i>Taenidia integerrima</i> (L.) Drude	AY379211, AY379313, AY379415	USA, Illinois, Coles Co., Charleston, Lake View Park, material collected by T. Harrison, <i>Downie</i> 763 (ILL)
<i>Tauschia arguta</i> (Torr. & A. Gray) J.F. Macbr.	AY379212, AY379314, AY379416	USA, California, San Diego Co., Corte Madera Ranch, near Pine Valley, 22 May 1983, <i>Van der Werff &amp; Clark</i> 4571 (MO)
<i>Tauschia glauca</i> (J.M. Coult. & Rose) Mathias & Constance	AY379213, AY379315, AY379417	USA, California, Trinity Co., SE of Burnt Ranch, 11 Jul 1990, <i>Spellenberg</i> 10254 (RM)
<i>Tauschia kelloggii</i> (A. Gray) J.F. Macbr.	AY379214, AY379316, AY379418	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, <i>Ahart</i> 6972 (UC)
<i>Tauschia parishii</i> (J.M. Coult. & Rose) J.F. Macbr.	AY379215, AY379317, AY379419	USA, California, San Bernardino Co., 12 April 1986, <i>Boyd</i> 1762 (RM)
<i>Tauschia texana</i> A. Gray	AY379216, AY379318, AY379420	USA, Texas, Gonzales Co., 12 April 1986, <i>Barrie</i> 1435 (RM)
<i>Thaspium barbinode</i> (Michx.) Nutt.	AY379217, AY379319, AY379421	USA, Missouri, Montgomery Co., in Upland woods, Graham Cave State Park, 11 May 1992, <i>Schuette</i> 2314 (MO)
<i>Thaspium pinnatifidum</i> (Buckley) A. Gray	AY379218, AY379320, AY379422	USA, Kentucky, Cheatham Co., near Neptune, material collected by C. Baskin, <i>Downie</i> 810 (ILL)

*trnF-L-T* sequence data for 27 species (Downie et al. 2002), for a final set of 129 accessions representing 21 genera and 111 species. Data from the nrDNA ITS and cpDNA *rps16* intron were already available for the same set of 129 accessions from prior phylogenetic studies (Downie et al. 2002, Sun & Downie 2004, Sun et al. 2004). These accessions represent all currently recognized species and infraspecific taxa of *Cymopterus*, with the exceptions of the uncommon *C. panamintensis* J.M. Coult. & Rose var. *panamintensis* and the narrowly endemic and rarely collected *C. megacephalus* M.E. Jones. Five varieties have been recognized under the invalid but widely used name *C. acaulis* (Pursh) Raf. The correct name for this taxon is *C. glomeratus* (Nutt.) DC. and based on the results of multivariate analyses of 288 specimens representing the morphological variability and geographic distribution of this species complex, we proposed previously that these plants be recognized as one species, *C. glomeratus*, with no varieties (Sun et al. 2005). In the present study, however, we maintain the name *C. acaulis* so that we may refer to its five varieties and compare their relationships to those inferred in our previous molecular studies. Other than *Lomatium* and *Tauschia*, sampling of all other perennial genera endemic to NA was comprehensive or nearly so. We also included both varieties of the northern Pacific maritime species *Glehnia littoralis* F. Schmidt ex Miq. and nine species of the perennial, circumboreal genus *Angelica* L. because results of prior phylogenetic analyses supported the close relationship between these taxa and the North American endemic genera (Sun & Downie 2004, Sun et al. 2004). The western North American species *Sphenosciadium capitellatum* A. Gray is treated as *Angelica capitellata* (A. Gray) Spalik, Reduron & S.R. Downie, based on Spalik et al. (2004). All trees were rooted with *Aethusa cynapium* L., according to the results of prior molecular phylogenetic studies where this species is either the sister taxon or is closely related to a clade comprised of all aforementioned taxa (Downie et al. 2002).

The experimental methods used to obtain these *trnF-L-T* sequence data were the same as outlined previously (Downie et al. 2002 and references cited therein). The three pairs of primers described by Taberlet et al. (1991) were each used to PCR-amplify and sequence the *trnF* (GAA)-*trnL* 3' exon intergenic spacer, *trnL* intron, and *trnL* (UAA) 5' exon-*trnT* (UGU) intergenic spacer regions. Each of these three non-coding regions was sequenced in their entirety on both DNA strands, permitting unambiguous base determination in all taxa. These data have been deposited with GenBank as separate *trnF-L* intergenic spacer, *trnL* intron, and *trnL-T* intergenic spacer sequences (Table 1).

All DNA sequences were aligned manually, facilitated by their highly conservative nature. Gaps were positioned to minimize nucleotide mismatches. Sequence characteristics of each of the three *trnF-L-T* data partitions and of a matrix of combined *trnF-L-T* data were obtained. These results were compared to data matrices available for the *rps16* intron and ITS regions for the same set of 129 accessions, as well as to a matrix that included all available molecular data (*trnF-L-T*, *rps16* intron, and ITS sequences). Uncorrected pairwise nucleotide distances were determined using PAUP\* vers. 4.0b10 (Swofford 2002), as were G + C content range and mean, the numbers of constant, autapomorphic, and parsimony informative alignment positions, and the numbers and sizes of unambiguous and parsimony informative alignment gaps.

The *trnF-L-T* data matrix was analyzed initially using maximum parsimony (MP), as implemented using PAUP\* and the tree searching strategies employed by Downie et al. (2002). The maximum number of MP trees was pre-set to 20,000 and these trees were permitted to swap to completion. Bootstrap (BS) values were calculated from 100,000 replicate analyses using "fast" stepwise-addition of taxa, and only those values compatible with the majority-rule consensus tree were recorded. Unambiguous alignment gaps were incorporated into the MP analysis by scoring each parsimony informative insertion or deletion as a separate binary character. The resultant tree topologies were compared to those inferred when alignment gaps were omitted as additional characters. The number of additional steps required to force particular taxa into a monophyletic group was examined using the constraint option of PAUP\*.

Sequence data from the *trnF-L-T* matrix were then analyzed using maximum likelihood (ML). Prior to this analysis, the program Modeltest vers. 3.7 (Posada and Crandall 1998) was used to select an evolutionary model of nucleotide substitution that best fits these data, as selected by the Akaike information criterion estimator. The model settings were entered into PAUP\* and a heuristic search carried out using ten random-addition sequence replicates and subtree-pruning-regrafting branch swapping under ML optimization. One hundred BS replicate analyses were conducted using neigh-

bor-joining searches and the ML parameters estimated by Modeltest. Subsequently, Bayesian inference (BI) analysis of *trnF-L-T* sequences was conducted using the program MrBayes vers. 3.0 (Ronquist & Huelsenbeck 2003). Starting trees were chosen at random and two million generations were run with sampling occurring every 100 generations. Tree topologies were based on a model of nucleotide substitution selected by Modeltest, with the shape parameter of the gamma distribution estimated automatically by the program. Four simultaneous Markov Chain Monte Carlo chains were performed to model nucleotide rate heterogeneity. Among 20,000 trees, 2500 trees were discarded as “burn-in” before the likelihood Ln values stabilized. The posterior probability (PP) values for each bipartition of the phylogeny were determined from the remaining trees.

Previously, we reported that greater resolution of relationships among these perennial, endemic NA apioid umbellifers and higher BS support values were achieved when all available molecular evidence was combined and considered in a simultaneous phylogenetic analysis (Downie et al. 2002, Sun & Downie 2004). These studies, however, also reported significant incongruence between ITS- and *rps16* intron-derived trees. To examine the extent of conflict between the *trnF-L-T* data set and an ITS data set obtained for the same 129 accessions through previous study, the incongruence length difference (ILD) test of Farris et al. (1995) was conducted using the partition homogeneity test of PAUP\*. One hundred replicates were considered for each partition (MaxTrees set at 500), using simple-addition sequence of taxa and tree-bisection-reconnection (TBR) branch swapping. Incongruence among data sets is identified if the additive tree lengths taken from the combined matrices are greater than the sum of the tree lengths from the original data. Although questions have been raised regarding the value of this test as a criterion for deciding whether data should be combined into a single phylogenetic analysis (Yoder et al. 2001, Barker and Lutzoni 2002), it is still a widely used method of assessing data heterogeneity and combinability. An examination of potential conflict between the *trnF-L-T* and *rps16* intron data sets was not carried out. These loci are linked on a nonrecombinant chromosome and, as such, are inherited as a single linkage group.

Considering all available molecular data for the group, sequences from the *trnF-L-T*, *rps16* intron, and ITS regions were combined for simultaneous phylogenetic analyses, using the same optimality criteria and approaches outlined above. In the BI analysis of combined molecular data, 2000 trees were discarded as “burn-in” before the likelihood Ln values stabilized.

## Results

Characteristics of the 129 aligned *trnF-L-T* sequences, as separate non-coding regions and combined, are presented in Table 2. The *trnL-T* spacer region is larger than that of both the *trnF-L* spacer and *trnL* intron regions combined and contributes the greatest number of parsimony informative alignment positions and indels to the analysis. Maximum pairwise sequence divergence for the combined *trnF-L-T* data was 2.6% of nucleotides, with these divergence values greatest for the two spacer regions. Of the 2061 aligned positions in the matrix of combined *trnF-L-T* regions, 1724 (83.6%) were not variable, 199 (9.7%) were variable but uninformative, and 138 (6.7%) were parsimony informative. A total of 47 unambiguous gaps, ranging between 1 and 14 bp in size, was required for proper alignment of these sequences. Twelve of these gaps, of 1–13 bp in size, were parsimony informative. Percent G + C content for the entire *trnF-L-T* region ranged from 30.4% to 31.7%, averaging 31.1%.

MP analysis of the 2061 aligned *trnF-L-T* positions resulted in the preset maximum tree limit of 20,000 trees, each of 514 steps (consistency indices, CI, 0.7374 and 0.5574, with and without uninformative characters, respectively; retention index, RI, 0.8632). The strict consensus of these trees with accompanying BS support values is shown in Fig. 1. Of the 12 parsimony informative alignment gaps, only three map

Table 2. Sequence characteristics of the *trnF-trnL-trnT* (as partitioned and combined data sets), *rps16* intron and ITS regions, as well as all three regions combined, for 129 accessions of Apiaceae subfamily Apioideae.

	<i>trnF-L</i> spacer	<i>trnL</i> intron	<i>trnL-T</i> spacer	<i>trnF-L-T</i>	<i>rps16</i> intron	ITS	<i>trnF-L-T</i> + <i>rps16</i> intron + ITS
No. of total characters	415	565	1081	2061	1019	452	3532
Length variation (bp)	330-373	456-514	605-837	1450-1712	801-877	435-440	2743-3015
No. of constant characters	341	496	887	1724	842	211	2777
No. of autapomorphic characters	47	42	110	199	98	104	401
No. of parsimony informative characters (%)	27 (6.5)	27 (4.8)	84 (7.8)	138 (6.7)	79 (7.8)	137 (30.3)	354 (10.0)
G + C content range (%)	33.3-36.1	34.1-36.2	25.5-27.5	30.4-31.7	32.4-34.2	54.8-59.4	35.0-36.3
G + C content mean (%)	34.6	35.2	26.9	31.1	33.2	57.2	35.6
Pairwise sequence divergence range (%)	0-3.6	0-2.2	0-3.7	0-2.6	0-3.9	0-9.4	0-3.2
No. of unambiguous gaps (size in bp)	13 (2-11)	11 (1-12)	23 (1-14)	47 (1-14)	32 (1-51)	18 (1-2)	97 (1-51)
No. of unambiguous gaps parsimony informative (size in bp)	4 (6-11)	3 (1-12)	5 (2-13)	12 (1-13)	4 (2-5)	1 (1)	17 (1-13)

without homoplasy. The remaining gaps each required 2 to 9 steps to explain their distribution across all MP trees. Repeating the MP analysis with the 12 binary scored gaps included as additional characters resulted in trees 42 steps longer than those without gap characters (Length = 556 steps; CI = 0.7032 and 0.5245, with and without uninformative characters, respectively; RI = 0.8461). With the exception of slightly less resolution in Clade 1 (described below), the resultant strict consensus trees inferred with or without binary-scored gap characters are identical. Modeltest selected the K81uf+I+G model of nucleotide substitution as best fitting these *trnF-L-T* sequence data (base frequencies: 0.3477, A; 0.1378, C; 0.1250, G; 0.3895, T; estimates of substitution rates: A↔C, 1; A↔G, 0.78; A↔T, 0.1865; C↔G, 0.1865; C↔T, 0.78; G↔T, 1; proportion of invariable sites = 0.3540; gamma distribution shape parameter = 0.9940). Using these parameters, a single ML tree was recovered (not shown), with a -Ln likelihood score of 6438.793. The majority rule consensus tree of 17,500 trees derived from BI analysis of *trnF-L-T* sequences (also not shown) is fully consistent to those trees estimated using MP and ML methods. The -Ln likelihood values of these trees ranged from 6549.565 to 6649.814 (average 6594.575, standard deviation 12.700). ML BS and BI PP values are presented alongside MP BS values on the MP strict consensus tree (Fig. 1).

In all phylogenetic analyses of *trnF-L-T* data, four major clades are inferred with varying branch support (Fig. 1): Clade 1, *Cymopterus corrugatus* through *Tauschia parishii* (68% MP BS; 84% ML BS; 1.00 BI PP); Clade 2, *Lomatium orientale* through *Lomatium ambiguum* (51% MP BS; 52% ML BS; 0.76 BI PP); Clade 3, *Aletes macdougali* subsp. *macdougali* through *Taenidia integerrima* (<50% MP BS; <50% ML

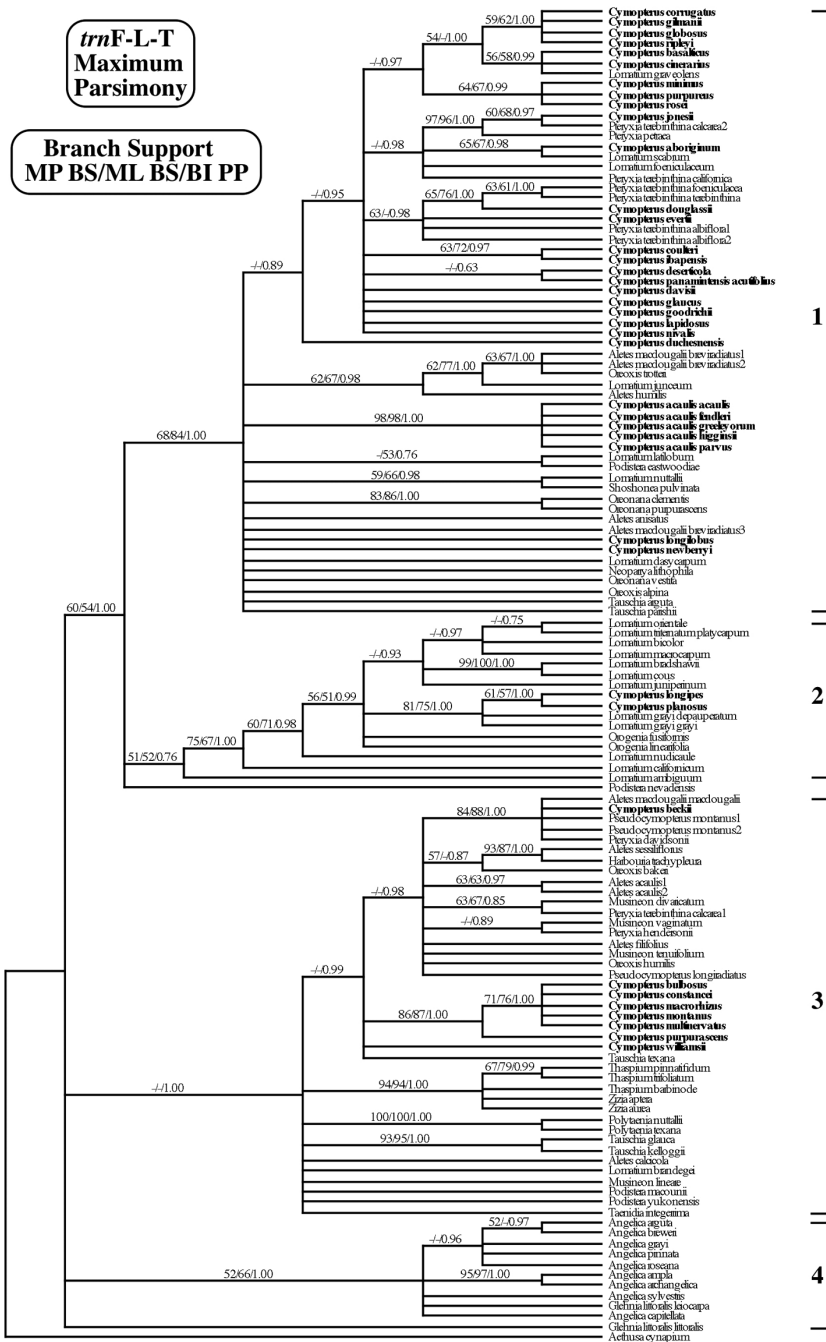


Fig. 1. Strict consensus tree of 20,000 minimal length 514-step trees derived from equally weighted MP analysis of 129 cpDNA *trnF-L-T* sequences from NA Apioideae (CI, with and without uninformative characters, 0.7374 and 0.5574; RI, 0.8632). This tree is fully consistent to those trees estimated using ML and BI methods. Therefore, numbers on branches represent support values for those clades common to all three analyses (MP BS, ML BS, and BI PP, respectively). BS support values of < 50% are indicated by "--". The 40 accessions of *Cymopterus* included in the study are boldfaced and the four major clades described in the text are circumscribed.

BS; 1.00 BI PP); and Clade 4, *Angelica arguta* through *Angelica capitellata* (52% MP BS; 66% ML BS; 1.00 BI PP). *Podistera nevadensis* comprises a trichotomy along with Clades 1 and 2 in all optimizations and is not assigned to any of these clades. The Asian Pacific *Glehnia littoralis* subsp. *littoralis* comprises an isolated lineage sister group to Clade 4 in the ML and BI trees (not shown). These results agree with those of our previous molecular systematic studies in showing that the genus *Cymopterus* is highly polyphyletic, with its members scattered among other apioid genera endemic to western NA. Moreover, each of the latter genera (i.e., *Aletes*, *Lomatium*, *Musineon*, *Oreonana*, *Oreoxis*, *Orogenia*, *Podistera*, *Pseudocymopterus*, *Pteryxia*, and *Tauschia*) is also not monophyletic. The *Phellopterus* group of *Cymopterus* (i.e., *C. bulbosus*, *C. constancei*, *C. macrorhizus*, *C. montanus*, *C. multinervatus*, and *C. purpurascens*; Clade 3) constitutes a strongly supported monophyletic group in all trees, with 86% and 87% MP and ML BS support values, respectively, and a 1.00 PP value. The five varieties of *Cymopterus acaulis* also comprise a well-supported monophyletic group (98% MP and ML BS, 1.00 PP; Clade 1), with little or no sequence divergence among them in pairwise comparisons (0-0.1% nucleotide sequence divergence). In stark contrast, conspecific members of *Pteryxia terebinthina* (5 varieties) and *Aletes macdougalii* (2 subspecies) do not ally closely, with each species having members arising in two major clades. *Thaspium* and *Zizia* unite as a strongly supported monophyletic group in all analyses, but each genus is not resolved as monophyletic. *Polytaenia* comprises a separate lineage and only weakly allies with *Thaspium* and *Zizia* (along with *Aletes calcicola*) in the ML and BI trees (<50% ML BS; 0.65 PP).

Sequence characteristics of the *rps16* intron and ITS data sets for the same group of 129 accessions are available through previous studies and are summarized in Table 2. The *rps16* intron matrix is comparable in size to that of *trnL-T* and contributes approximately the same number of parsimony informative characters. The proportion of nucleotide differences in the ITS partition was four to five times higher than either the *rps16* intron or *trnF-L-T* partitions and relative to its size the ITS region contributed the greatest percentage of informative characters to the analysis. Among all loci considered to date in Apiaceae phylogenetic studies, the ITS region is most rapidly evolving (Downie et al. 2001). The ITS region yields almost exactly the same number of parsimony informative characters as does *trnF-L-T*, despite it being only one-quarter of the size; the number of parsimony informative indels in the ITS region, however, is substantially less. An analysis of all available molecular data resulted in a matrix of 3532 aligned positions, with none excluded because of alignment ambiguities; 354 of these positions were parsimony informative (Table 2). In addition, 17 unambiguous alignment gaps were parsimony informative. Maximum pairwise sequence divergence values approached 3.2% of nucleotides in this matrix of combined molecular data.

MP analyses of the ITS and *rps16* intron data sets obtained through previous studies for the same set of 129 accessions each resulted in the preset limit of 20,000 minimal length trees (ITS tree length = 701 steps, CI with and without uninformative characters = 0.4964 and 0.3893, RI = 0.6566; *rps16* intron tree length = 300 steps, CI with and without uninformative characters = 0.6733 and 0.4948, RI = 0.8158). In the ITS strict consensus tree, 71 nodes are resolved but most of these are supported weakly (29 nodes have BS values  $\geq 50\%$ , 13 of which are  $\geq 80\%$ ). The strict consensus tree derived



from MP analysis of *rps16* intron data is much less resolved, with only 24 nodes present (17 of which have BS values  $\geq 50\%$ , only 6 of which are  $\geq 80\%$ ). In the *trnF-L-T* strict consensus tree (Fig. 1), 54 nodes are resolved; 40 of these nodes are supported by BS values  $\geq 50\%$ , 12 of which are  $\geq 80\%$ . Overall, the *trnF-L-T* matrix yields MP trees with greater BS branch support and less homoplasy than either the ITS or *rps16* intron data matrices. Similarly, BI analysis of the *trnF-L-T* matrix resulted in a more highly resolved tree with greater PP branch support than did those trees resulting from BI analyses of partitioned ITS and *rps16* intron data sets.

The results of a partition homogeneity test showed significant incongruence between the *trnF-L-T* and ITS data sets (ILD probability value = 0.01). However, by collapsing those branches with BS values  $< 50\%$ , the trees become highly consistent with respect to their major groupings. Therefore, all three data sets were combined for simultaneous molecular analyses. MP analysis of these combined molecular data resulted in the preset maximum tree limit of 20,000 trees, each of 1707 steps (CI = 0.5442 and 0.3869, with and without uninformative characters, respectively; RI = 0.6945). The strict consensus of these trees with accompanying BS support values is shown in Fig. 2. Eighty nodes are resolved, of which 39 are supported by BS values  $\geq 50\%$  and 24 are supported by BS values  $\geq 80\%$ . Repeating this analysis but with the inclusion of the 17 binary-scored alignment gaps resulted in a strict consensus tree with a topology highly consistent with that inferred previously (tree length = 1774 steps; CI = 0.5361 and 0.3835, with and without uninformative characters, respectively; RI = 0.6933). Compared with the analyses of partitioned data, greatest resolution of relationships and highest branch support are achieved when all DNA regions, representing over 3.5 kb of aligned data, are considered simultaneously.

Among a total of 20,000 trees generated in the Bayesian analysis, 2000 trees were discarded as “burn-in” and the remaining ones used to generate a majority rule consensus tree (Fig. 3). The  $-\ln$  values of these trees ranged from 16,482.244 to 16,653.266 (average 16,546.749; standard deviation 33.714). The BI consensus tree shows greater resolution of relationships and clades of higher PP values than those trees resulting from BI analyses of partitioned data — of 104 resolved nodes, 38 have a PP value of 1.00. For the ML analysis, Modeltest selected the GTR+I+G model of nucleotide substitution as best fitting these sequence data (base frequencies: A; 0.1475, C; 0.1592, G; 0.3645, T; estimates of substitution rates: A $\leftrightarrow$ C, 0.9595; A $\leftrightarrow$ G, 1.1493; A $\leftrightarrow$ T, 0.2886; C $\leftrightarrow$ G, 0.4931; C $\leftrightarrow$ T, 1.6104; G $\leftrightarrow$ T, 1; proportion of invariable sites = 0.5312; gamma distribution shape parameter = 0.6981). Using these parameters, a single ML tree was recovered by PAUP\*, with a  $-\ln$  likelihood score of 16,429.674. Relationships inferred by the ML tree are similar to those estimated using BI; clades identified with BS values  $< 50\%$  in the ML analysis are indicated on the BI tree.

The four major clades outlined in Fig. 1 resulting from MP, ML and BI analyses of *trnF-L-T* data are also retrieved on trees resulting from ML and BI analyses of all available data (Fig. 3). On the latter trees, branch support for Clades 2–4 is generally higher than those values inferred through separate ML and BI analyses of *trnF-L-T* data. In contrast, the MP strict consensus tree (Fig. 2) places Clade 2 within a paraphyletic Clade 1 and provides very weak support for the monophyly of this group; otherwise, relationships are similar among the various partitioned and combined analyses.





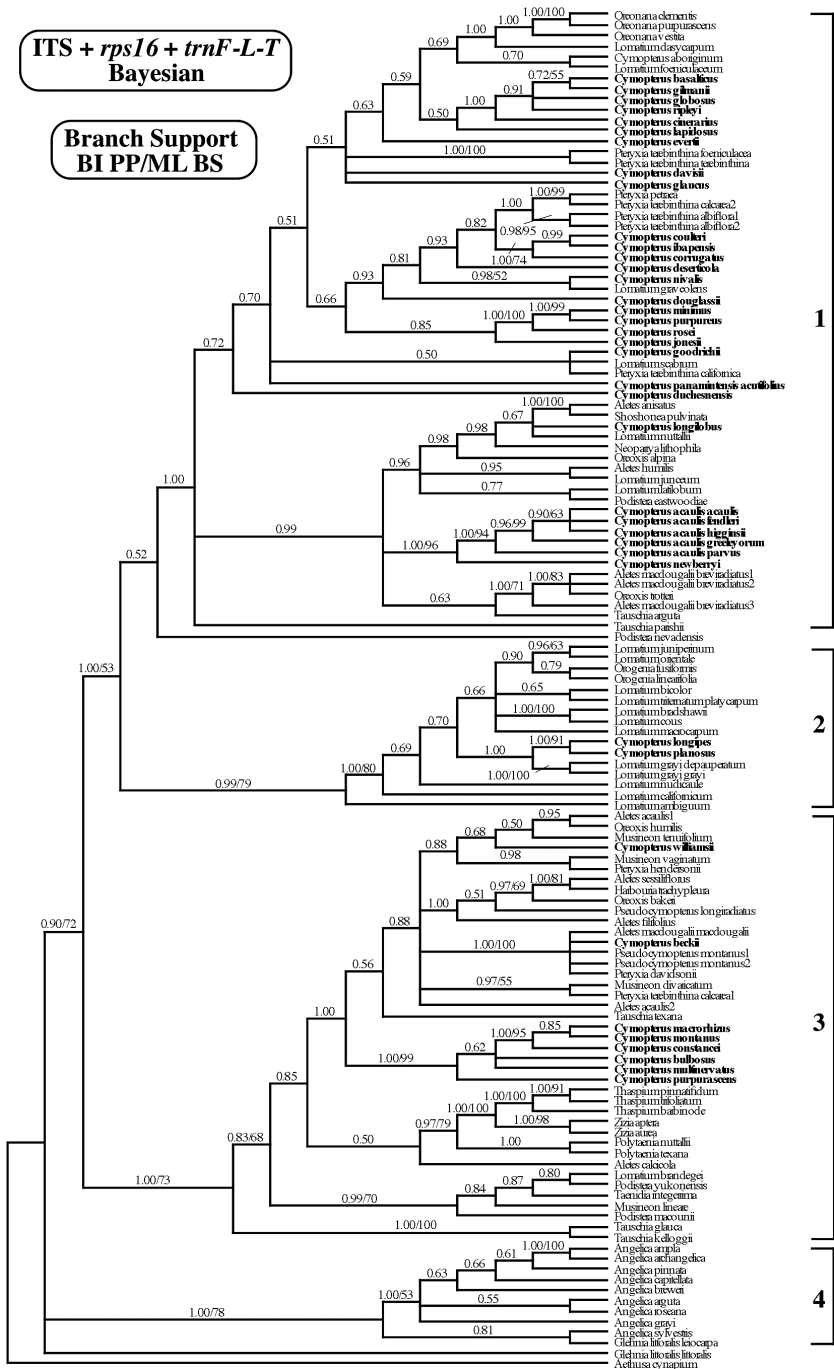


Fig. 3. Majority rule consensus tree of 18,000 trees derived from BI analyses of combined nrDNA ITS and cpDNA *rps16* intron and *trnF-L-T* sequences from 129 accessions of NA Apioideae. This tree is fully consistent with the single tree estimated using ML and a GTR+I+G model of nucleotide substitution. Numbers on branches represent BI PP and ML BS estimates, respectively; BS values <50% are not indicated. The 40 accessions of *Cymopterus* included in the study are boldfaced and the four major clades described in the text and previous figures are identified.

Table 3. The number of additional steps required to force monophyly of 13 genera in MP analyses of four data sets. The number of accessions examined for each genus is given in parentheses. Tree lengths from unconstrained MP analyses of these data sets are provided in brackets. A "0" tree length indicates that the genus is monophyletic in the unconstrained analysis of that particular data set.

Genus	ITS [L=701]	<i>rps16</i> intron [L=300]	<i>trnF-L-T</i> [L=514]	<i>trnF-L-T</i> + <i>rps16</i> intron + ITS [L=1707]
<i>Aletes</i> (11)	18	20	22	52
<i>Angelica</i> (9)	1	12	1	1
<i>Cymopterus</i> (40)	30	27	38	63
<i>Glehnia</i> (2)	4	0	3	4
<i>Lomatium</i> (20)	23	19	28	51
<i>Musineon</i> (4)	8	4	8	16
<i>Oreonana</i> (3)	3	0	0	0
<i>Oreoxis</i> (4)	14	4	16	32
<i>Orogenia</i> (2)	2	2	0	0
<i>Podistera</i> (4)	7	4	10	18
<i>Pseudocymopterus</i> (3)	3	3	3	9
<i>Pteryxia</i> (10)	13	12	27	38
<i>Tauschia</i> (5)	8	6	8	17

Again, *Cymopterus* is highly polyphyletic, as are most other genera endemic to western NA. The *Phellopterus* group of *Cymopterus* remains monophyletic, but with stronger branch support than in the *trnF-L-T* trees (MP BS 97%, ML BS 99%, BI PP 1.00). No greater resolution of relationships is obtained for *Pteryxia terebinthina* or *Aletes macdougallii*. *Polytaenia*, *Thaspium*, and *Zizia* are now each resolved as monophyletic and collectively comprise a moderately to well-supported clade (MP BS 80%; ML BS 79%; BI PP 0.97).

Constraining the 40 examined accessions of *Cymopterus* to monophyly and rerunning MP analyses of the partitioned (*trnF-L-T*, *rps16* intron, ITS) and combined data sets resulted in trees 27–63 steps longer than those minimal length trees without the constraint invoked (Table 3). Constraint analyses of 12 other genera in all but a few cases also resulted in trees of much greater length (Table 3). As examples, constraining *Aletes* (11 accessions) and *Lomatium* (20 accessions) to monophyly in MP analyses of combined molecular data required 52 and 51 additional steps, respectively. *Glehnia*, *Oreonana*, and *Orogenia* were monophyletic in some, but not all unconstrained analyses.

## Discussion

To date, studies of the phylogenetic relationships among the members of Apiaceae subfamily Apioideae endemic to western NA north of Mexico have been carried out using ITS (Downie et al. 2002, Sun et al. 2004), *rps16* intron (Sun & Downie 2004), and now *trnF-L-T* sequence data. The results of partitioned and combined phylogenetic analyses of these molecular data show that *Cymopterus*, one of the largest genera of NA Apioideae, is not monophyletic. However, this is clearly an understatement. The 40 accessions of *Cymopterus* examined herein (representing all but one of its 35 currently recognized species) comprise 19 or 20 separate branches in the BI and ML trees. In the MP analysis of combined data, trees of 63 additional steps are required to force *Cymopterus* into monophyly. *Cymopterus* is thus grossly polyphyletic, as revealed through molecular systematic studies. Similarly, the results of cladistic analyses of 54 morphological characters and 123 NA apioid taxa also reveal a highly polyphyletic *Cymopterus* (Sun 2003, Sun & Downie 2010).

The species of *Cymopterus* are linked closely with those of many other genera of western NA (i.e., *Aletes*, *Lomatium*, *Musineon*, *Oreoxis*, *Podistera*, *Pseudocymopterus*, *Pteryxia* and *Tauschia*) and, as such, each of these genera is also not monophyletic. Indeed, some of these genera are highly polyphyletic, such as *Aletes*, *Lomatium*, and *Pteryxia*. Each of these genera requires many extra steps to force their monophyly in MP analyses. Of the 14 herbaceous, perennial genera of Apiaceae subfamily Apioideae endemic to western NA, only *Oreonana* and *Orogenia* are each resolved as monophyletic in some but not all analyses (only single accessions of the genera *Harbouria*, *Neoparrya* and *Shoshonea* were included in this study). If future studies support the hypotheses presented herein, radical changes to the prevailing classification of western NA Apioideae will be necessary.

Overall, phylogenetic analyses of the *trnF-L-T* region resulted in trees of greater resolution and higher branch support than those trees generated using either ITS or *rps16* intron data sets. However, greatest resolution of relationships and branch support were achieved when all DNA regions are considered simultaneously, yet divergence estimates were still low, approaching 3.2% of nucleotides. These combined DNA regions represent over 3.5 kb of aligned data and 354 parsimony informative characters, a 40% increase in the number of informative characters relative to a previous study based on a combined analysis of ITS and *rps16* intron sequences (Sun & Downie 2004). The following relationships are revealed or are better supported through analyses of combined molecular data: (1) *Polytaenia*, *Thaspium*, and *Zizia* are each monophyletic and collectively comprise a moderately to well-supported clade. (2) *Oreonana* and *Orogenia* are each resolved as monophyletic in the *trnF-L-T* and combined molecular analyses (*Oreonana* was previously reported as monophyletic in the *rps16* intron study). (3) All six species comprising the *Phellopterus* clade are strongly supported as monophyletic. (4) There is stronger support for the *C. acaulis* clade than revealed through prior studies. The results of phylogenetic analyses of ITS data showed that *C. newberryi* is nested within *C. acaulis* (Sun et al. 2004). Such a relationship is not supported herein upon analyses of combined molecular data, instead *C. newberryi* is a strongly supported sister group to *C. acaulis*. We expect that as additional molecu-

lar data are included in future phylogenetic studies, as well as a critical evaluation of morphological and anatomical characters, further increased resolution of relationships among these taxa will be achieved.

Analyses of *trnF-L-T* and combined molecular data sets result in phylogenetic trees comprising three to four major clades. These clades, however, are variously supported depending upon the analysis, and in the MP analysis of combined data Clade 2 arises within a paraphyletic Clade 1. Clade 4, comprising all included members of the circumboreal genus *Angelica* (plus *Glehnia littoralis* subsp. *leiocarpa*), is the only major clade that coincides closely with a previously circumscribed genus. The single accession of *Glehnia littoralis* subsp. *littoralis* from Taiwan may be misidentified. Its ITS sequence matches identically to those from four accessions of *Peucedanum japonicum*, a distantly related species. *Podistera nevadensis* is not assigned to any major clade. In all trees, it comprises an isolated branch variously associated with Clades 1 and 2, and further study will be necessary to establish the closest relatives of this species. Clade 2 is comprised of 12 of the 20 accessions of *Lomatium* included in this study plus two species of *Cymopterus* and two species of *Orogenia*. Within *Lomatium*, none of the informal species groups are resolved as monophyletic (summarized in Sun et al. 2004), with some members of the *Cynomarthrum* and “tuberous lomatium” species groups falling within this major clade and others falling elsewhere. Cronquist (1997) stated that the two species of *Orogenia* are likely related to some of the smaller, white-flowered species of *Lomatium*, in spite of the differences in their fruits. Similarly, the fruit of *C. longipes* also suggests *Lomatium* (Cronquist 1997, Downie et al. 2002). Within Clades 1 and 3, several smaller clades of similar composition are apparent in all trees, but each comprises very few taxa or species from two or more genera and most are not very well supported. Those smaller clades that are well-supported, such as the one comprising *Aletes macdougalii* subsp. *macdougalii*, *Cymopterus beckii*, *Pseudocymopterus montanus* and *Pteryxia davidsonii* (MP and ML BS 100%, BI PP 1.00), represent species belonging to multiple genera and may contain plants that are quite different in fruit and flower characteristics. Therefore, until these groups receive confirmation through additional study, we are cautious not to recognize new assemblages of taxa, either formally or informally, at the present time.

The conspecific members of *Pteryxia terebinthina* (5 varieties) and *Aletes macdougalii* (2 subspecies) are not monophyletic, with their constituent members occurring distantly in all trees, whereas the five varieties of *C. acaulis* comprise a well-supported monophyletic group in all analyses. The absence of or very little sequence variation among the infraspecific taxa of *C. acaulis* supports our previous work in suggesting that no varieties be recognized in this species complex (Sun et al. 2005). Multivariate analyses of 288 specimens representing the morphological variability and geographic distribution of the *C. acaulis* complex showed that most characters previously used to recognize these varieties are highly variable within taxa and that no clearly separated clusters are revealed (Sun et al. 2005). It is surprising then that the infraspecific taxa of *P. terebinthina* also do not comprise a monophyletic group in our study, since the results of prior multivariate and phylogenetic analyses of morphological characters showed that these taxa are morphologically indistinguishable and closely related (Sun 2003, Sun et al. 2008). Similarly, Cronquist (1997) did not accept the distinction

between the two subspecies of *Aletes macdougallii* (he recognized this species under *Cymopterus macdougallii* with no infraspecific taxa), but in the trees presented herein these two subspecies are widely separated, occurring in two major clades. Further studies of the infraspecific taxa of *P. terebinthina* and *A. macdougallii* will be required.

The systematics of North American Apiaceae subfamily Apioideae, especially of its perennial, endemic members, is nowhere near satisfactory. Its two largest genera, *Cymopterus* and *Lomatium*, are grossly polyphyletic in all molecular phylogenies, with elements of each inextricably linked with each other and with other genera of western NA. Many of these genera, as described and recognized today, are ill-formed based on poor characteristics (Cronquist 1997, Downie et al. 2002, Sun et al. 2004). A complete reassessment of the generic limits of native NA umbellifers is clearly required. To delimit genera and increase resolution of relationships among these taxa, additional DNA sequence data will be necessary, as well as more critical examination and evaluation of morphological and fruit anatomical characters. It is very likely, however, that additional study will not result in monophyletic genera, as traditionally circumscribed, or new genera that can be circumscribed unequivocally using morphological data. As such, a case might be made for combining all 200 or so species of the complex into one large monophyletic genus, an extreme but possibly inevitable action. Further compounding the problem is that these plants may have originated and radiated rapidly in western NA (Downie et al. 2002, Sun et al. 2004), thus additional sequence data may not resolve early branching patterns among these perennial, endemic NA umbellifers.

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Addresses of the authors:

Feng-Jie Sun, W.M. Keck Center for Comparative and Functional Genomics, Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA; Prof. Dr. Stephen R. Downie, Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA, email: sdownie@life.uiuc.edu