Phylogenetic relationships within *Cicuta* (Apiaceae tribe Oenantheae) inferred from nuclear rDNA ITS and cpDNA sequence data

Chang-Shook Lee and Stephen R. Downie

Abstract: The genus *Cicuta* (Apiaceae tribe Oenantheae Dumort.) is the most virulently poisonous group of flowering plants native to the north temperate zone. A recent treatment recognized four species (*C. bulbifera* L., *C. douglasii* (DC.) J.M. Coult. & Rose, *C. maculata* L., and *C. virosa* L.), with *C. maculata* divided into four varieties. We present results of phylogenetic analyses of the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) locus and the region bounded by the chloroplast genes *psbI* and *trnK* 5′ exon to determine taxonomic limits and relationships among these taxa, and to assess the taxonomic status of *C. douglasii*, a polyploid thought to be derived from *C. maculata* and *C. virosa*. *Cicuta bulbifera* and *C. virosa* are each resolved as monophyletic, the latter is a sister group to all other species. Discordance between the ITS- and plastid-derived phylogenies and lack of resolution in the ITS trees preclude unequivocal hypotheses of relationship; all trees do suggest, however, that the allotetraploid *C. douglasii* is polyphyletic and possibly polytopic, with all examined accessions but one nested within *C. maculata*. This single outstanding accession is from California and, pending further study, might warrant recognition as a distinct species. The diploid *C. bulbifera* may also be of hybrid origin, as revealed by significant discordance between data sets. Within *C. maculata*, only the western North American var. *angustifolia* Hook. is resolved in the ITS trees. In the cpDNA trees, *C. maculata* var. *angustifolia* comprises a strongly supported clade with *C. maculata* var. *bolanderi* (S. Watson) G.A. Mulligan and *C. douglasii*, both of primarily western North American distribution. The eastern North American taxa, *C. maculata* vars. *maculata* and *victorinii* (Fernald) B. Boivin, also comprise a clade, sister group to *C. bulbifera*.

Key words: Cicuta, *Oenanthe*, Oenantheae, Apiaceae, nuclear rDNA ITS, chloroplast DNA, *psbI*, *psbK*, *trnQ*, *rps16* intron, *trnK*.

Résumé : Le genre *Cicuta* (Apiaceae tribu Oenantheae Dumort.) est le groupe le plus violemment poison des plantes à fleur indigènes de la zone boréale tempérée. Une présentation récente reconnaît quatre espèces (*C. bulbifera* L., *C. douglasii* (DC.) J.M. Coult & Rose, *C. maculata* L. et *C. virosa* L.), le *C. maculata* étant réparti en quatre variétés. Les auteurs présentent les résultats d'analyses phylogénétiques d'un lieu ITS de l'ADNr nucléique et de la région liée par les gènes chloroplastiques *psbl* et *trnK* exon 5′, pour déterminer les limites taxonomiques et les relations parmi ces taxons, et pour évaluer le statut taxonomique du *C. douglasii*, une polyploïdes qu'on croit dérivée du *C. maculata* et du *C. virosa*. Le *C. bulbifera* et le *C. virosa* s'avèrent chacun monophylétiques, le dernier comme groupe sœur de toutes les autres espèces. La discordance entre les phylogénies déduites des ITS et des plastes, et l'absence de résolution dans les arbres ITS empêchent toute hypothèse sans équivoque au sujet des relations; tous les arbres suggèrent cependant que le *C. douglasii* allotétraploïde est polyphylétique et possiblement polytopique, chez toutes les accessions, sauf une rattachée au *C. maculata*. Cette accession unique exceptionnelle provient de la Californie et, en attendant d'autres études, mériterait d'être reconnue comme espèce distincte. Le *C. bulbifera* diploïde pourrait également être d'origine hybride, comme le suggère une discordance significative entre les ensembles de données. À l'intérieur du *C. maculata* seule la variété nord-américaine de l'ouest, se démarque dans les arbres ITS. Dans les arbres ADNcp, le *C. maculata* var. *angustifolia* Hook. comporte un clade fortement étayé avec le *C. maculata* var. *bolandri* (S. Watson) G.A. Mulligan et le *C. douglasii*, tous deux distribuées surtout dans l'ouest nord-américain. Les taxons de l'est de l'Amérique du Nord, *C. maculata* vars. *maculata* et *victorinii* (Fernald) B. Boivin, comportent également un clade apparenté au groupe *C. bulbifera*.

Mots clés : *Cicuta*, *Oenanthe*, Oenantheae, Apiaceae, ITS de l'ADNr nucléique, ADN chloroplastique, *psbI*, *psbK*, *trnQ*, intron *rps16*, *trnK*.

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C.-S. Lee[1] and S.R. Downie.[2] Department of Plant Biology, University of Illinois at Urbana–Champaign, Urbana, IL 61801, USA.

[1]Present address: Department of Life Science, Ewha Womans University, Seoul 120-750, Korea.
[2]Corresponding author (e-mail: sdownie@life.uiuc.edu).

Introduction

The genus *Cicuta* L. (water-hemlock; Apiaceae tribe Oenantheae Dumort.) is the most virulently poisonous group of flowering plants native to the north temperate zone (Bomhard 1936; Kingsbury 1964). These plants are a menace to both livestock and humans, with many deaths recorded (Jacobson 1915; Starreveld and Hope 1975; Panter et al. 1988).

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Cicuta douglasii and *C. maculata* are especially toxic because of the high levels of cicutoxin concentrated in their tuberous roots and thickened stem bases (Mulligan 1980; James and Ralphs 1986). In the most recent taxonomic revision of the genus, Mulligan (1980) recognized four species: *Cicuta bulbifera* L., *C. douglasii* (DC.) J.M. Coult. & Rose, *C. virosa* L., and *C. maculata* L., with the latter comprising four varieties (vars. *angustifolia* Hook., *bolanderi* (S. Watson) G.A. Mulligan, *maculata*, and *victorinii* (Fernald) B. Boivin). *Cicuta virosa* is of circumboreal distribution; all other taxa are endemic to North America. Prior taxonomic treatments of the group resulted in 3–17 often poorly defined taxa (Coulter and Rose 1888, 1900; Greene 1889, 1909, 1912; Lunell 1916; Mathias and Constance 1942, 1944; Constance 1972). The genus is undoubtedly monophyletic, as revealed by phylogenetic analyses of molecular data (Downie et al. 1998, 2001; Hardway et al. 2004), the ubiquitous presence of the virulent cicutoxin and its related cicutol (Mulligan and Munro 1981), and the shared presence of unique vegetative characters, such as thickened stem bases with well-developed transverse partitions in older plants and the primary lateral veins of the well-defined leaflets usually directed to the sinuses between the teeth rather than to the tips of the teeth (Bomhard 1936; Mulligan 1980; Constance 1993; Cronquist 1997).

The characters used to delimit taxa within *Cicuta* are primarily those of the fruits, as well as leaf venation patterns, the degree of leaf division, and chromosome number and size (Mathias and Constance 1944; Mulligan 1980; Mulligan and Munro 1981). An early treatment of *Cicuta* emphasized characteristics of the roots and other subterranean organs in distinguishing species (Greene 1889). However, studies of fruit morphology and anatomy of other umbellifer genera have indicated that these characters are prone to much homoplasy and, as such, are unreliable phylogenetically (Lee and Downie 1999; Downie et al. 2004). Furthermore, these same fruit characters are often difficult to apply because their proper interpretation relies on a certain amount of expertise, and in *Cicuta* they appear to exhibit much variability (Mulligan 1980; Mulligan and Munro 1981; Cronquist 1997). It is also reasonable to presume that root and rhizome shape, as well as their degree of thickness and direction of elongation, is influenced by the nature of the substratum, and thus is not useful taxonomically (Coulter and Rose 1900; Mathias and Constance 1942; Bell and Kane 1981). Similarly, the diagnostic value of leaf venation patterns and the extent of leaf division as a means of distinguishing species in *Cicuta* appear dubious (Cronquist 1997). Plants of *C. douglasii* and *C. maculata* are often mistaken for one another, and the morphologic characters reported as being most useful in distinguishing them intergrade in areas of sympatry (Mulligan 1980; Cronquist 1997).

In contrast to their variable and overlapping morphologies, the species of *Cicuta* are cytologically distinct. *Cicuta virosa* has 22 small-sized somatic chromosomes, *C. bulbifera* has 22 medium-sized somatic chromosomes, and the four varieties of *C. maculata* each have 22 large-sized somatic chromosomes (Mulligan 1980). *Cicuta douglasii* has a chromosome count of $2n = 44$ and possesses both 22 small-sized and 22 large-sized chromosomes (Mulligan 1980). On the bases of chromosome number and size, as well as a similar morphology, it has been suggested that *C. douglasii* is of allopolyploid origin, most likely derived from progenitors similar to present-day *C. virosa* and *C. maculata* (Mulligan 1980; Mulligan and Munro 1981; Cronquist 1997).

The goal of this study was to reconstruct an estimate of phylogenetic relationships within *Cicuta* using molecular data. We examined both nuclear rDNA ITS and cpDNA *psbI–trnK* 5′ exon sequences, the latter representing a large, contiguous region heretofore not used in Apiaceae molecular systematic investigations. Consideration of data from both nuclear and chloroplast markers is important because any significant discordance of relationships between data sets may serve to identify past hybridization or introgression events (Doyle 1992; Rieseberg and Brunsfeld 1992; Soltis and Kuzoff 1995), phenomena which may have resulted in the origin of the putative allopolyploid *C. douglasii* (Mulligan 1980; Mulligan and Munro 1981). Analyses of these molecular data will permit testing of competing hypotheses of species limits and relationships proposed by Mulligan (1980) and other workers on the basis of morphology and anatomy.

Materials and methods

Taxa and outgroup selection

Eighty-seven accessions of *Cicuta* and an outgroup (*Oenanthe)* were examined for nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) and (or) chloroplast DNA (cpDNA) sequence variation (Table 1). ITS data were previously available in GenBank for eight accessions; all other ITS and most cpDNA sequences were obtained specifically for this study. A total of 70 accessions (68 *Cicuta* and 2 *Oenanthe*) was used in the analysis of ITS sequences, whereas 38 accessions (22 *Cicuta* and 16 *Oenanthe*) were used in the cpDNA study. The additional representation of the outgroup in the latter was due to the availability of these sequence data as a result of a concurrent study of *Oenanthe* phylogeny (K. Spalik and S. Downie, in preparation), as well as to provide another umbellifer genus in which to examine the efficacy of the cpDNA *psbI–trnK* 5′ exon region for resolving infrageneric relationships in Apiaceae. Twenty-one accessions (19 *Cicuta* and 2 *Oenanthe*) were common to both data sets. These accessions represent all four species of *Cicuta* recognized by Mulligan (1980), including the four varieties of *C. maculata*. The ITS trees were rooted with two species of *Oenanthe* (*O. pimpinelloides* L. and *O. sarmentosa* J. Presl ex DC.), based on results of prior molecular investigations of the family where *Oenanthe* and *Cicuta* are strongly supported sister taxa (Plunkett and Downie 1999; Downie et al. 2000*b*, 2000*c*; Hardway et al. 2004). The cpDNA-derived trees were also rooted with 16 accessions (12 species) of *Oenanthe*.

Experimental strategy

Leaf material for DNA extraction was obtained primarily from herbarium specimens, but also from plants cultivated from seed in the greenhouse. Seven of the *Cicuta douglasii* and *C. maculata* var. *bolanderi* specimens examined from herbarium DAO were annotated by and included in the cytological studies of *Cicuta* by Mulligan (Mulligan 1980; Mul-

Table 1. Accessions of *Cicuta* and *Oenanthe* from which nuclear rDNA ITS and (or) cpDNA *psbI–trnK* 5′ exon sequences were obtained, with corresponding DNA accession and GenBank reference numbers and voucher information.

Taxon	DNA accession No.	Voucher information	GenBank reference No.
Cicuta bulbifera	1529	USA, Wisconsin, Vilas Co., Allequash Lake, 24 Aug. 1996, *Crane 96-275* (ILLS 189478)	AY524706
Cicuta bulbifera	1658	USA, Illinois, Lake Co., Chain-O-Lakes State Park, 31 Aug. 1977, *Robertson & Moran 72* (ILLS 159118)	AY524707
Cicuta bulbifera	1814	USA, Illinois, Lake Co., Volo Bog, 1 Sept. 1977, *Robertson 1450* (ILLS 162965)	AY524708
Cicuta bulbifera	1919	Canada, Manitoba, Lake Winnipeg, Long Point, 13 Aug. 1982, *Shchepanek & Dugal 4821* (ILL)	AY524709
Cicuta bulbifera	1923	USA, Illinois, Will Co., Keepatau Forest Preserve, 28 Aug. 1990, *Taft 794* (ILLS 174182)	AY360233
Cicuta bulbifera	1925	USA, Illinois, Kane Co., 0.8 mi E of Fox River, South Elgin, 18 Aug. 1994, *Hill & Taft 26007* (ILLS 182654)	AY524710
Cicuta bulbifera	1935	Canada, Ontario, Kenora District, Rushing River Provincial Park, E of Kenora, 13 July 1980, *Blanz & Jones 4750* (ILL)	AY360234
Cicuta bulbifera	2283	USA, Idaho, Boundary Co., Bussard Lake, Moyie River, 19 Sept. 1992, *Taylor 13118* (UC 1731983)	AY524711
Cicuta bulbifera	2432	Canada, Saskatchewan, Prince Albert National Park, 23 Aug. 1996, *Harms 43494* (DAO 783157)	AY524712
Cicuta bulbifera	2433	Canada, Yukon, Unnamed Lake, 8 July 1983, *Cody 32443* (DAO 670762)	AY524713, DQ168959
Cicuta bulbifera	2434	Canada, Ontario, Waterloo Co., Barrie Lake, W of Galt, 6 Sept. 1961, *Gillett 10684* (DAO 453599)	AY524714, DQ168960
Cicuta bulbifera	2438	Canada, Ontario, Northumberland Co., NNW of Bewdley, 28 July 1975, *Bobbette & Bobbette 4354* (CAN 392303)	AY524715, DQ168961
Cicuta douglasii	1573	USA, Montana, Madison Co., Madison River along Bear Trap Canyon Trail, 3 Aug. 1979, *Lowry II 2811* (ILL)	AY524716
Cicuta douglasii	2279	USA, Nevada, Elko Co., N of Lanier Ranch, 12 July 1989, *Pinzl 8785* (UC 1565546)	AY524717
Cicuta douglasii	2280	USA, Montana, Madison Co., Madison River along Bear Trap Canyon Trail, 3 Aug. 1979, *Lowry II 2811* (UC 1508557)	AY524718
Cicuta douglasii	2281	USA, Oregon, Multnomah Co., Dalton Point, 1 mi NE of Bridal Veil, 8 Aug. 1994, *Halse 4821* (UC 1606455)	AY524719
Cicuta douglasii	2282	USA, Nevada, Elko Co., Bruneau River, 27 July 1992, *Pinzl 10184* (UC 1594736)	AY524720
Cicuta douglasii	2285	USA, California, Colusa Co., Lily Pond near Letts Lake, 30 June 1986, *Oswald 2176* (UC 1532347)	AY524721, DQ168962
Cicuta douglasii	2443	Canada, British Columbia, Vancouver Island, Prospect Lake, N of Victoria, 6 Aug. 1979, *Munro 2256* (DAO 266753)	AY524722, DQ168963
Cicuta douglasii	2444	Canada, British Columbia, Vancouver Island, Duncan, 6 Aug. 1979, *Munro 2254* (DAO 266755)	AY524723
Cicuta douglasii	2445	Canada, British Columbia, Vancouver Island, Nanaimo Lakes, 5 Aug. 1979, *Munro 2245* (DAO 266758)	AY524724, DQ168964
Cicuta douglasii	2446	USA, Idaho, Bonner Co., 8 mi W of Hwy. 57 on Hwy. 2 at Priest River, 31 July 1979, *Munro 2239* (DAO 266760)	AY524725
Cicuta maculata var. *angustifolia*	2428	USA, California, Santa Barbara Co., NE of Los Alamos, 27 June 1979, *Smith s.n., Mulligan & Munro 3694* (DAO 237860) [cultivated plant identified by G. Mulligan as var. *bolanderi*]	AY524726, DQ168965
Cicuta maculata var. *angustifolia*	2439	Canada, Saskatchewan, Moose Mountain Creek Valley, 2 mi E of Alameda, 26 July 1987, *Harms 38214* (DAO 732665)	AY524727
Cicuta maculata var. *angustifolia*	2440	Canada, Saskatchewan, NW shore of Little Emma Lake, 10 mi NW of Christopher Lake, 11 July 1975, *Harms 21401* (DAO 335743)	AY524728
Cicuta maculata var. *angustifolia*	2441	Canada, Saskatchewan, Besnard Lake, near Narrows Channel Bridge, 25 June 1992, *Harms 40816* (DAO 749061)	AY524729, DQ168966
Cicuta maculata var. *angustifolia*	2442	Canada, Manitoba, Riding Mountain National Park, 26 July 1979, *Wojtas 821* (DAO 296067)	AY524730
Cicuta maculata var. *bolanderi*	2286	USA, Arizona, Coconino Co., West Fork Oak Creek Canyon, 7 Aug. 1979, *Lehto & Pinkava 23986* (DAO 1565525)	AY524731, DQ168967

Table 1 (*continued*).

Taxon	DNA accession No.	Voucher information	GenBank reference No.
Cicuta maculata var. *bolanderi*	2429	USA, California, Solano Co., Suisun City, Suisun Marshes, 25 May 1977, *Fuller 20173* (DAO 153229)	AY524732, DQ168968
Cicuta maculata var. *bolanderi*	2430	USA, Arkansas, Pulaski Co., Little Rock, 6 July 1978, *Sinclair s.n., Mulligan & Junkins 3702* (DAO 237868)	AY524755
Cicuta maculata var. *maculata*	1113	USA, Wyoming, Goshen Co., Bear Creek, *Nelson et al. 33517* (RM)	AY360235
Cicuta maculata var. *maculata*	1519	USA, Illinois, Johnson Co., Bell Pond Natural Area, 21 June 1990, *Phillippe & Simon 14077* (ILLS 175985)	AY524733
Cicuta maculata var. *maculata*	1524	USA, Illinois, Johnson Co., Lower Cache River Natural Area, 25 June 1989, *Winship 1141* (ILLS 183390)	AY524734
Cicuta maculata var. *maculata*	1527	USA, Illinois, Jackson Co., Shawnee National Forest, Oakwood Bottoms, Murphysboro Ranger District, 22 Sept. 1993, *Phillippe 23124* (ILLS 181498)	AY524735
Cicuta maculata var. *maculata*	1528	USA, Illinois, Johnson Co., Cache, along Burlington Northern Railroad, 28 May 1992, *Basinger 2575* (ILLS 180620)	AY524736
Cicuta maculata var. *maculata*	1562	USA, Illinois, Johnson Co., Bell Pond Natural Area, 11 July 1990, *Phillippe & Ketzner 14181* (ILLS 172806)	AY524737
Cicuta maculata var. *maculata*	1563	USA, South Carolina, Greenwood Co., Lake Greenwood, 28 July 1993, *Horn 7333* (ILLS 191135)	AY524738, DQ168969
Cicuta maculata var. *maculata*	1564	USA, Illinois, Jackson Co., Shawnee National Forest, Oakwood Bottoms, 15 July 1993, *Phillippe 22527* (ILLS 181356)	AY524739
Cicuta maculata var. *maculata*	1567	USA, Georgia, Butts Co., SE of Jackson, off Hwy. 42, Indian Springs State Park, 17 July 1987, *Howel 0451* (ILL)	AY524740, DQ168970
Cicuta maculata var. *maculata*	1956	USA, South Carolina, Georgetown Co., Sampit River, Georgetown, 24 July 1992, *Nelson et al. 13337* (ILL)	AY524741, DQ168971
Cicuta maculata var. *maculata*	1983	USA, Michigan, Emmet Co., 3 mi SW of Mackinaw City, 14 July 1982, *Waldbauer s.n.* (ILL)	AY524742
Cicuta maculata var. *maculata*	1984	USA, Illinois, Champaign Co., railroad between Mayview and St. Joseph, 6 Oct. 1973, *Jones & Allen 2676* (ILL)	AY524743
Cicuta maculata var. *maculata*	1991	USA, Illinois, Carroll Co., Savanna Army Depot, 1 Aug. 1996, *Phillippe et al. 27993* (ILLS 200223)	AY524744
Cicuta maculata var. *maculata*	1992	USA, Illinois, Jackson Co., Shawnee National Forest, Oakwood Bottoms, 14 July 1993, *Phillippe 22409* (ILLS 181780)	AY524745
Cicuta maculata var. *maculata*	2004	USA, Illinois, Lee Co., Richardson Wildlife Foundation, W of West Brooklyn, 10 July 1997, *Phillippe & Handel 29036* (ILLS 192249)	AY524746
Cicuta maculata var. *maculata*	2005	USA, Illinois, Lake Co., Lake Forest, Hwy. 41 near Deerpath Rd., 27 July 1970, *Curtis s.n.* (ILLS 168065)	AY524747
Cicuta maculata var. *maculata*	2006	USA, Illinois, Macoupin Co., 1 mi S of Palmyra, 9 July 1982, *McKnight 2107* (ILLS 166293)	AY524748
Cicuta maculata var. *maculata*	2007	USA, Illinois, Marion Co., 1.6 mi N of Kinmundy, 14 July 1977, *Robertson 1209* (ILLS 159298)	AY524749
Cicuta maculata var. *maculata*	2008	USA, Illinois, Williamson Co., Crab Orchard National Wildlife Refuge, 6 July 1985, *Ulaszek 1140* (ILLS 174767)	AY524750
Cicuta maculata var. *maculata*	2009	USA, Illinois, Cook Co., Thornton Fractional Prairie, Calumet City, 15600 South Superior St., 23 July 1996, *Antonio et al. 7829* (ILLS 193740)	AY524751
Cicuta maculata var. *maculata*	2418	Canada, Ontario, Grenville Co., 1 mi NE of Prescott, 6 July 1960, *Dore & Staudt 17915* (DAO 453823)	AY524752
Cicuta maculata var. *maculata*	2419	Canada, Quebec, Wolfe Co., Riviere Blanche, 7 Aug. 1968, *Hamel & Brisson 15100* (DAO 634104)	AY524760
Cicuta maculata var. *maculata*	2425	USA, Illinois, Jackson Co., Marion, Howardton Rd., 19 Sept. 2002, *Lee et al. s.n.* (ILL)	AY524753
Cicuta maculata var. *maculata*	2426	USA, Illinois, Jackson Co., Marion, 0.3 mi E of Howardton, 19 Sept. 2002, *Lee et al. s.n.* (ILL)	AY524754
Cicuta maculata var. *maculata*	2435	USA, Mississippi, Oktibbeha Co., Hwy. 25, 0.9 mi S of Keaton Tower Rd., 7 June 1995, *Leidolf 1543* (DAO 706823)	AY524756, DQ168972
Cicuta maculata var. *maculata*	2436	Canada, Quebec, Vaudreuil Co., Mt. Rigaud, 24 June 1975, *Newstrom 1362* (DAO 281157)	AY524757

Table 1 (*continued*).

Taxon	DNA accession No.	Voucher information	GenBank reference No.
Cicuta maculata var. *maculata*	2437	Canada, New Brunswick, Kent Co., Carleton, Kouchibouguac National Park, Hwy. 117, 19 July 1977, *Munro & Lyons 1158* (DAO 158665)	AY524758
Cicuta maculata var. *maculata*	2450	USA, Illinois, Jackson Co., Marion, E of Howardton, Howardton Rd., wet area, 19 Sept. 2002, *Lee et al. s.n.* (ILL)	AY524759
Cicuta maculata var. *victorinii*	2447	Canada, Quebec, Portneuf Co., Saint Augustin, 22 July 1963, *Cinq-Mars 63-765* (DAO 453962)	AY524761
Cicuta maculata var. *victorinii*	2448	Canada, Quebec, Lévis Co., Saint Nicolas, 7 Aug. 1980, *Cayouette J80-80* (DAO 667789)	AY524762, DQ168973
Cicuta virosa	75	Finland; cult. UIUC from seeds obtained from University of Joensuu Bot. Gard., *Downie 75* (ILL)	U78372, DQ168974
Cicuta virosa	131	Germany; cult. UIUC from seeds obtained from University of Oldenburg Bot. Gard., *Downie 131* (ILL)	U78372, DQ168975
Cicuta virosa	426	China, Yunnan, Xiao Zhongdian, RBGE Gyaltyang Expedition, *FED 426* (E)	AY353978, AY353985
Cicuta virosa	2420	Canada, Saskatchewan, S side of Lake Athabasca, Royal Lake, 11 July 1979, *Harms et al. 25395* (DAO 333749)	AY524763, DQ168976
Cicuta virosa	2421	Canada, Saskatchewan, Clearwater River at Warner Rapids, 3 July 1984, *Harms 32564* (CAN 578648)	AY524764
Cicuta virosa	2422	Canada, Northwest Territories, Mackenzie District, Dempster Hwy., W of Peel River crossing, 15 July 1980, *Cody 27835* (DAO 398631)	AY524765
Cicuta virosa	2423	Canada, Saskatchewan, S end of Sandy Bay, Churchill River, 12 July 1973, *Harms 20076* (DAO 330832)	AY524766
Cicuta virosa	2579	Japan, Hokkaido, Kitami Prov., Notoro-ko Notoro Abashiri-shi, 26 Aug. 1974, *Furuse 6903* (MO 4013973)	AY524767, DQ168977
Cicuta virosa	2850	Denmark, Ilsø, Roldskov, dist. 11, 12 July 1967, *Holm-Nielsen s.n.* (MO 2196696)	DQ168978
Cicuta virosa	2851	Denmark, S Onsild Enge in the Skalsaa Valley, 20 km NE of Viborg, 23 June 1989, *K & S.S. Larsen 40482* (MO 4250822)	DQ168979
Cicuta virosa	2853	Russia, Mosqua Prov., Stschelkovo dist., 6 Aug. 1972, *Gogina & Thumanian 164* (MO 2350965)	DQ168980
Oenanthe aquatica (L.) Poir.	2255	Denmark, Fyn, Stævningen in Snarup Skov, *Petersen & Seberg GPL30* (ILL)	DQ168946
Oenanthe banatica Heuff.	476	Hungary; cult. UIUC from seeds obtained from the Hungarian Academy of Sciences Bot. Gard., Vácrátót, *Downie 476* (ILL)	DQ168955
Oenanthe crocata L.	40	Spain; cult. UIUC from seeds obtained from Real Jardin Botánico, Spain, *Downie 40* (ILL)	DQ168953
Oenanthe crocata L.	247	Belgium; cult. UIUC from seeds obtained from Jardin Botanique National de Belgique, *Downie 247* (ILL)	DQ168954
Oenanthe divaricata (R. Br.) Mabb.	1612	Portugal, Madeira; cult. Conservatoire botanique de la Ville de Mulhouse (No. 9316A), *Hildenbrand, Meyer & Reduron s.n.* (ILL)	DQ168952
Oenanthe fistulosa L.	165	Hungary; cult. UIUC from seeds obtained from the Univ. of Oldenburg Bot. Gard., *Downie 165* (ILL)	DQ168948
Oenanthe fistulosa L.	2254	Denmark, Sjælland, Else-engen, Sorø Sønderskov, *Petersen GPL29* (ILL)	DQ168949
Oenanthe foucaudii Tesser.	1631	France, Charente-Maritime, Soubise; cult. Conservatoire botanique de la Ville de Mulhouse (No. 9373A), *Hildenbrand, Meyer & Reduron s.n.* (ILL)	DQ168944
Oenanthe lachenalii C.C. Gmel.	1609	France, Alpes de Haute Provence, Claret; cult. Conservatoire botanique de la Ville de Mulhouse (No. 98037), *Hildenbrand, Meyer & Reduron s.n.* (ILL)	DQ168945
Oenanthe millefolia Janka	1872	Bulgaria, Stranja; cult. Conservatoire botanique de la Ville de Mulhouse (No. 98047), *Hildenbrand, Meyer & Reduron s.n.* (ILL)	DQ168943
Oenanthe peucedanifolia Pollich	1282	Germany; cult. UIUC from seeds obtained from Karl-Marx Univ., Leipzig, *Lee 24* (ILL)	DQ168956

Table 1 (*concluded*).

Taxon	DNA accession No.	Voucher information	GenBank reference No.
Oenanthe pimpinelloides L.	29	Germany; cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, *Downie 29* (ILL)	AY360251, DQ168950
Oenanthe pimpinelloides L.	273	Belgium; cult. UIUC from seeds obtained from Jardin Botanique National de Belgique, *Downie 273* (ILL)	DQ168951
Oenanthe sarmentosa J. Presl ex DC.	521	USA, California, San Mateo Co., *Plunkett 1308* (WS)	AY360252, DQ168947
Oenanthe silaifolia M. Bieb.	1873	France; cult. Conservatoire botanique de la Ville de Mulhouse (No. 98206) from seeds obtained from Conservatoire botanique national de Bailleul, *Hildenbrand, Meyer & Reduron s.n.* (ILL)	DQ168957
Oenanthe silaifolia M. Bieb.	1889	France; cult. Conservatoire botanique de la Ville de Mulhouse (No. 98206) from seeds obtained from Conservatoire botanique national de Bailleul, *Hildenbrand, Meyer & Reduron s.n.* (ILL)	DQ168958

Note: GenBank reference numbers for the cpDNA sequences are prefixed by the letters "DQ." Names of *Cicuta* taxa are according to Mulligan (1980). Herbarium acronyms are according to Holmgren et al. (1990).

ligan and Munro 1981). The keys in these papers facilitated our identification of all taxa, although in one instance there was uncertainty in the assignment of a name for a voucher specimen: a cultivated, sterile specimen of what we presumed to be *C. maculata* var. *angustifolia* (accession No. 2428) was identified as var. *bolanderi* (*Mulligan & Munro 3694*, $2n = 22$). For all accessions, total genomic DNA was obtained from about 20 mg of dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, California).

Details of PCR amplifications and purifications and the DNA sequencing strategies used to obtain these ITS data are the same as provided elsewhere (Downie and Katz-Downie 1996; Downie et al. 2000*a*; Sun et al. 2004). Simultaneous consideration of both DNA strands across the entire ITS region permitted unambiguous base determination. For one accession of *Cicuta* obtained from GenBank (*C. virosa* No. 426), sequence data from the 5.8S rDNA region were unavailable, thus the two GenBank accession numbers presented for this accession (Table 1) represent the ITS-1 and ITS-2 regions, respectively. For those accessions where the ITS-1 and ITS-2 regions were amplified and sequenced separately, 3–50 bp from near the middle of the 5.8S region could not be obtained. This portion of the 5.8S region showed no variation in the remaining accessions, hence these missing data did not affect the phylogenetic results.

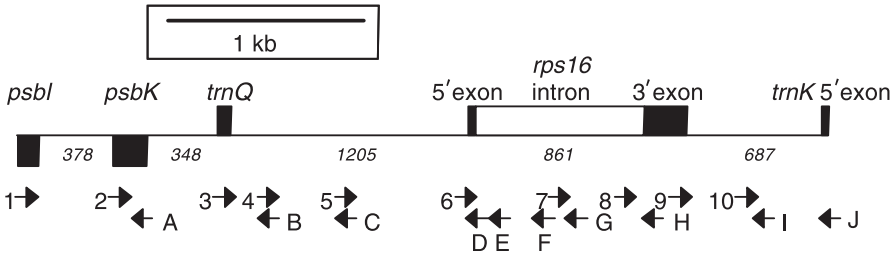
The region bounded by and including chloroplast genes *psbI* and *trnK* 5′exon, and containing genes *psbK, trnQ* and *rps16*, is 4138 bp in size in tobacco (Shinozaki et al. 1986). Also included within this region is the *rps16* intron (at 861 bp in tobacco) and four intergenic spacers (*psbI–psbK*, *psbK–trnQ*, *trnQ–rps16* 5′ exon, and *rps16* 3′ exon – *trnK* 5′ exon; Fig. 1). The sizes of these intergenic spacers and intron vary from 348 to 1205 bp in tobacco. Other than the *rps16* intron, these loci have not been considered previously for Apiaceae phylogenetic study. The strategies employed to obtain sequence data from the *rps16* intron are presented in Downie and Katz-Downie (1999). Additional primers were constructed for PCR amplification and sequencing of the regions flanking *rps16*. In total, 20 primers were used to obtain both forward and reverse sequences, with those primers anchored in the coding regions used for both PCR amplification and DNA sequencing, and those occurring within the *rps16* intron and intergenic spacers used for DNA sequencing only (Fig. 1).

Sequence comparisons and phylogenetic analyses

Nucleotide sequences of the ITS and cpDNA regions were each aligned manually; this was facilitated by their highly conserved nature. Uncorrected pairwise nucleotide distances were calculated by PAUP* version 4.0b10 (Swofford 1998). Sequence characteristics were obtained from separate ITS-1, 5.8S, and ITS-2 partitions, as well as the entire (i.e., combined ITS) region. To evaluate the relative utility of the five separate cpDNA partitions (four intergenic spacers and intron) for phylogenetic analysis, sequence characteristics of each region were compared in terms of their overall percent variability (calculated by tallying the number of autapomorphic and potentially informative alignment positions plus the number of indels divided by the total aligned length of the region $\times$ 100) and maximum pairwise sequence divergence estimates. All ITS and cpDNA sequences acquired in this study were deposited in GenBank (see Table 1 for GenBank reference numbers).

The two data matrices were each analyzed initially using maximum parsimony (MP), with gap states treated as missing data. Characters were treated as unordered and equally weighted. Heuristic MP searches were replicated 1000 times with random stepwise addition of taxa, tree-bisection-reconnection (TBR) branch swapping, and saving multiple trees with no tree limit. Bootstrap values were calculated from 1000 replicate analyses using TBR branch swapping and simple stepwise addition of taxa (the MaxTrees option was set to 1000 for the ITS analysis). The ITS data were analyzed as separate ITS-1 and ITS-2 partitions, and were also combined. To examine the extent of conflict between the ITS and cpDNA matrices for a comparable set of taxa, the incongruence length difference test of Farris et al. (1995) was implemented using the partition-homogeneity test of PAUP*. The test was performed with 1000 replicate analyses, using the heuristic search option with simple addition of taxa and TBR branch swapping. Although serious questions have been raised regarding the value of this test as a criterion for deciding whether data should be combined into

Fig. 1. Map of the 4138-bp locus of tobacco cpDNA (Shinozaki et al. 1986) showing the relative positions of genes *psbI*, *psbK*, *trnQ*, *rps16*, and (in part) *trnK*. The gene *rps16* is interrupted by an intron; only the 5′ exon of gene *trnK* is shown. Scale bar = 1 kb unit. The sizes of the four intergenic spacer regions and intron are presented in base pairs. The arrows represent the directions and approximate positions of the 20 primers used in PCR amplification and (or) DNA sequencing. Forward primers are designated 1–10; reverse primers are designated A–J. These primer sequences, written 5′ to 3′, are as follows: 1, ATTCTTCACGTCCAGGATTACGCC (“psbI”); 2, CAGCAGCTTGCCAAACAAAGGCTA (“psbK”); 3, CCCGCTATTCGGAGGTTCGA (“trnQ”); 4, TGATGGTAACATAGGTCACACCCT (“Berula L”); 5, ATTCAGCATTCCCAGAGAGTCGTG (“Berula R-2FOR”); 6, TTTAAAACGATGTGGTAGAAAGCA (“5′ exon (rps16)”); 7, TAAGAAGCACCGAAGTAATGTC (“rps16C”); 8, TTTCTCGAGCCGTACGAGGAG (“rps16-2”); 9, TTCCTTGAAAAGGGCGCTCA (“3′ exon-1”); 10, GCGTCTATGTAGTGCCAATC (“trnK-1”); A, CAAATTGCCCGAGGCCTATGCTTT (“psbK-REV”); B, AGGGTGTGACCTATGTTACCATCA (“Berula L-REV”); C, TCACGACTCTCTGGGAATGCTGAA (“Berula R”); D, ATCGTGTCCTTCAAGTCGCA (“rps16-1R”); E, AACAGAACAGATGTCGGGCCRAGA (“Berula rps16 - 2 REV”); F, TAAACGCTCGATTCCCGYGYGATA (“L-4, Lilaeopsis For2”); G, AATGGCGTTTCCTTGTTC (“rps16-CR”); H, TCGGGATCGAACATCAATTGCAAC (“3′ exon (rps16)”); I, GTTCGATACACTGTTGTC (“trnK-1R”); J, TACTCTACCGTTGAGTTAGC (“trnK”).



a single phylogenetic analysis (e.g., Yoder et al. 2001; Barker and Lutzoni 2002), it is still a widely used method of assessing data heterogeneity and combinability. The number of additional steps required to force particular taxa into a monophyletic group was examined using the constraint option of PAUP*.

The ITS and cpDNA data sets were then analyzed using maximum likelihood (ML), after using the program Modeltest vers. 3.6 (Posada and Crandall 1998) to select an appropriate model of nucleotide substitution (among 56 possible models) that best fits these data, as selected by the Akaike Information Criterion (AIC) estimator (Posada and Buckley 2004). The parameters appropriate for the chosen model were input into PAUP* and a heuristic search performed using 100 random addition sequence replicates and TBR branch swapping under ML optimization. One thousand bootstrap replicate analyses were conducted using neighbor-joining searches with ML distance estimates, using the ML parameters inferred by Modeltest.

Lastly, the ITS and cpDNA data matrices were subjected to Bayesian analyses, implemented using MrBayes version 3.0 (Ronquist and Huelsenbeck 2003). Starting trees were chosen at random and one million generations were run with sampling occurring every 100 generations. The number of substitution types was obtained from Modeltest and the gamma shape parameter (reflecting among-site rate variation) was estimated by MrBayes. Two thousand trees were discarded as “burn in” before stationarity was reached, prior to calculating the majority rule consensus tree from the remaining trees. Posterior probability values for all internal tree branches were recorded.

Results

ITS region

The alignment of DNA sequences from the combined ITS region for 68 accessions of *Cicuta* and 2 accessions of *Oenanthe* resulted in a matrix of 601 positions, with no alignment ambiguity. Of these positions, 518 (86%) were not variable, 34 (6%) were autapomorphic, and 49 (8%) were parsimony informative. Considering *Cicuta* only, 19 positions (3%) were autapomorphic and 26 positions (4%) were parsimony informative. Five gaps (indels), ranging from 1 to 2 bp in size, were required to facilitate alignment of all 70 sequences. Only one gap was parsimony informative, representing a deletion of 1 bp in the ITS-2 region in all accessions of *C. virosa* and 23 of 28 accessions of *C. maculata* var. *maculata* relative to the outgroup *Oenanthe*. This deletion represented a loss of one “G” base in a stretch of five G’s in all remaining accessions but one. Further characteristics of these aligned data, as separate ITS-1, 5.8S, and ITS-2 regions or combined, are presented in Table 2. Pairwise sequence divergence values across the entire ITS region ranged from identity to 10.2% of nucleotides; for *Cicuta* only, the maximum divergence value barely exceeded 4% (between *C. bulbifera* and *C. virosa*). Maximum intraspecific sequence divergence values for *C. bulbifera* (12 accessions), *C. douglasii* (10 accessions), *C. maculata* (38 accessions), and *C. virosa* (8 accessions) were 1.2%, 1.8%, 1.6%, and 0.2%, respectively. Maximum divergence values within *C. maculata* vars. *angustifolia* (5 accessions), *bolanderi* (3 accessions), *maculata* (28 accessions), and *victorinii* (2 accessions) were 0.2%, 0.5%, 1.2%, and identity, respectively. No evidence of ITS sequence additivity at any nucleotide site, as inferred by overlapping peaks on an electropherogram, was found.

MP analysis of the entire ITS region resulted in 3352 minimal length trees, each of 102 steps (consistency index (CI) = 0.9020 and 0.8529, with and without uninformative characters, respectively; retention index (RI) = 0.9713). Bootstrap values for nodes resolved in the strict consensus tree ranged from 60% to 100% (averaging 79%). These values were transferred to a single, arbitrarily selected, 102-step tree (Fig. 2A); bootstrap values were left off those

Table 2. Sequence characteristics of the two nuclear rDNA internal transcribed spacers (ITS-1 and ITS-2) and their intervening 5.8S region, separately and combined, for 68 *Cicuta* and two *Oenanthe* accessions.

Sequence characteristic	ITS-1	5.8S	ITS-2	Combined (ITS-1, 5.8S, ITS-2)
Length variation (bp)	209–210	163–164	225–226	598–600
No. of aligned positions	211	164	226	601
No. of aligned positions not variable	176	156	186	518
No. of aligned positions autapomorphic	12	5	17	34
No. of aligned positions potentially informative	23	3	23	49
No. of alignment gaps (indels)	3	1	1	5
No. of alignment gaps potentially informative	0	0	1	1
Maximum pairwise sequence divergence				
All 70 accessions	10.9	3.1	10.8	10.2
Cicuta accessions only	3.8	2.2	6.8	4.1

branches that collapsed in the strict consensus tree (all collapsed branches had less than 50% bootstrap support). The single informative deletion, restricted to *C. virosa* and most accessions of *C. maculata* var. *maculata*, was homoplastic (not shown). Separate MP analyses of the ITS-1 and ITS-2 data sets resulted in strict consensus trees (not shown) fully consistent with the consensus tree inferred from the simultaneous analysis of combined ITS data.

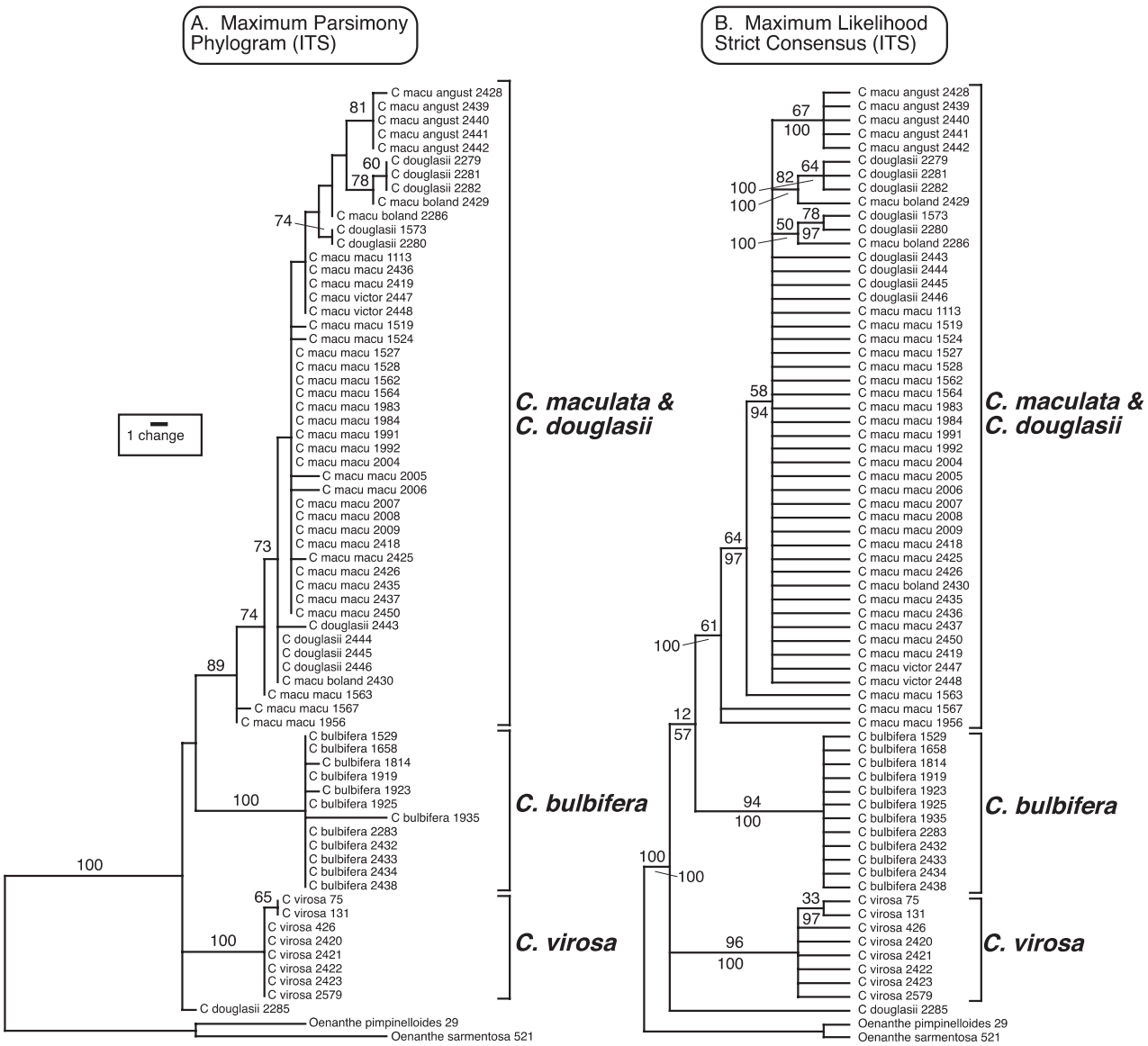
Based on the AIC estimator, Modeltest selected the TrN+G model of nucleotide substitution (Tamura and Nei 1993) as best fitting these ITS data (base frequencies: 0.2243, A; 0.2778, C; 0.2849. G; 0.2130, T; estimates of substitution rates: A↔C, 1; A↔G, 2.3620; A↔T, 1; C↔G, 1; C↔T, 4.0323; G↔T, 1; gamma distribution shape parameter = 0.2166). Using these parameters, five ML trees were recovered, each with a –ln likelihood score of 1469.07276. The strict consensus of these trees is shown in Fig. 2B. ML distance-based bootstrap values ranged from 12% to 100% (averaging 66%) on resolved branches. The results of the Bayesian analysis were nearly identical to those presented in the ML strict consensus tree, with posterior probability values ranging from 57% to 100% (averaging 92%) on the majority rule consensus tree. Support values derived from both ML and Bayesian analyses are presented on the ML strict consensus tree (Fig. 2B).

The phylogenies estimated using MP, ML, and Bayesian methods (Fig. 2) are fully consistent with one another. All trees resulted in a strongly supported, monophyletic *Cicuta* comprising four major lineages. Three of these are recognized here as the *C. virosa* clade, the *C. bulbifera* clade, and the *C. maculata* and *C. douglasii* clade (hereafter, referred to as the *C. maculata* clade). A single accession of *C. douglasii* from Colusa County, California (No. 2285) comprises a fourth lineage. The nine remaining accessions of *C. douglasii* are nested within the *C. maculata* clade. *Cicuta virosa* and *C. bulbifera* are supported most strongly, with bootstrap or posterior probability values ranging between 94% and 100% across different methods. The *C. maculata* clade is supported by values between 61% and 100% across methods. Relationships among these four lineages are equivocal, with the ML and Bayesian trees suggesting, albeit weakly, a sister group relationship between the *C. bulbifera* and *C. maculata* clades. Similarly, the model-based trees, and a subset of those trees obtained from the MP analysis, suggest that either *C. virosa* or *C. douglasii* accession No. 2285 (or both) may be the sister group to a clade consisting of *C. bulbifera* and *C. maculata*.

The *Cicuta virosa* clade comprises the eight examined accessions from eastern Asia, Europe, and Canada. The clade is supported strongly, with high bootstrap and posterior probability values and six uniquely occurring (i.e., nonhomoplastic) synapomorphies. ITS-1 data were also available for *C. virosa* accessions Nos. 2850, 2851, and 2853, and MP analysis of these data reveals that they are part of the *C. virosa* clade (results not shown). These three accessions were included in the cpDNA study. The *C. bulbifera* clade represents 12 accessions from across Canada and the United States and is also supported strongly, with six uniquely occurring synapomorphies. With the exception of a single specimen of *C. douglasii* (No. 2285), all remaining *Cicuta* accessions fell within the *C. maculata* clade. This clade, comprising *C. douglasii* and the four varieties of *C. maculata*, is supported moderately well, with 61% and 89% bootstrap support in the ML and MP analyses, respectively, and a 100% posterior probability value in the Bayesian majority rule consensus tree. Only one uniquely occurring synapomorphy supports its monophyly; the three additional substitutions occurring on this branch each represent reversals higher within the clade.

Relationships within the *C. maculata* clade are largely unresolved, a result of the low levels of sequence divergence among its included taxa. Of the four recognized varieties of *C. maculata*, only var. *angustifolia* constitutes a monophyletic group, albeit one that is moderately well supported. The five accessions of this variety, from California, Saskatchewan and Manitoba, share a single unique synapomorphy. A second synapomorphy is shared between all accessions of var. *angustifolia* and two of three accessions of *C. maculata* var. *bolanderi*. The two included accessions of *C. maculata* var. *victorinii* (both from Quebec) have identical ITS sequences to three accessions of var. *maculata* (from Wyoming and Quebec); these five accessions comprise a clade in a subset of the 3352 MP trees. All ITS trees suggest that *C. douglasii* is polyphyletic, with all examined accessions but one (No. 2285) nested within *C. maculata*. Constraining all 10 accessions of *C. douglasii* to monophyly and rerunning the MP analysis results in trees that are 10 steps longer than those without the constraint, suggesting that a signal for *C. douglasii* monophyly does not exist. Chromosome number and size information were available

Fig. 2. (A) One of 3352 minimal length 102-step trees derived from maximum parsimony analysis of 70 taxa and 601 unambiguously aligned nuclear rDNA ITS positions, with gap states treated as missing data (CI = 0.9020 and 0.8529, with and without uninformative characters, respectively; RI = 0.9713). Numbers on branches are bootstrap estimates from 1000 replicate analyses; branches without bootstrap values indicate collapses in the strict consensus of 3352 trees. (B) Strict consensus of five trees derived from maximum likelihood analysis of 70 taxa and 601 unambiguously aligned nuclear rDNA ITS positions under the TrN+G model of nucleotide substitution (–ln likelihood = 1469.07276). Numbers above branches represent bootstrap estimates calculated from 1000 replicate neighbor-joining analyses using ML distance and the parameters inferred by Modeltest. The Bayesian majority rule consensus tree was nearly identical to the ML strict consensus tree. Numbers below branches on the ML tree represent Bayesian posterior probabilities. Complete taxon names, including the ranks of infraspecific taxa that were omitted for brevity, are provided in Table 1.



for four accessions of *C. douglasii*; accession Nos. 2443, 2444, 2445, and 2446 are all $2n = 44$ (Mulligan 1980).

cpDNA *psbI–trnK* 5′ exon region

For the 38 accessions of *Cicuta* and *Oenanthe* examined, the region bounded by the chloroplast genes *psbI* and *trnK* 5′ exon ranged in size from 3387 (*C. maculata* var. *angustifolia* No. 2428) to 4218 bp (*O. divaricata* No. 1612), averaging 3394 bp in *Cicuta* and 4165 bp in *Oenanthe* (Table 3). The multiple alignment of these sequences resulted in a matrix of 4396 positions, with no alignment ambiguity. Of these aligned positions, 4149 (94.4%) were not variable, 91 (2.1%) were autapomorphic, and 156 (3.5%) were potentially informative. Fifty-seven gaps were required to align these sequences, with 38 of these potentially informative. Relative to *O. sarmentosa*, the only endemic North American species of *Oenanthe* sampled herein, these informative gaps ranged from 1 to 825 bp in size, with insertions outnumbering deletions 21:17. Twelve gaps were unique to *Cicuta*, and three additional gaps were restricted to *C. virosa*. The largest gap, of 825 bp, represented a deletion in the *trnQ–rps16* 5′ exon intergenic spacer region in all ac-

Table 3. Sequence characteristics of the cpDNA *psbI–trnK* 5′ exon region, analyzed as five separate partitions or as a combined data set (entire region), for 22 accessions of *Cicuta* and 16 accessions of *Oenanthe*.

Sequence characteristic	Entire region	*psbI–psbK*	*psbK–trnQ*	*trnQ–rps16* 5′ exon	*rps16* intron	*rps16* 3′ exon – *trnK* 5′ exon
Length variation (bp)	3387–4218	396–414	351–373	445–1273	855–866	737–760
No. of aligned positions	4396	415	379	1335	875	801
No. of aligned positions not variable	4149	392	353	1268	830	728
No. of aligned positions autapomorphic	91	10	7	33	16	21
No. of aligned positions potentially informative	156	13	19	34	29	52
No. of alignment gaps (indels)	57	5	2	20	6	24
No. of alignment gaps potentially informative	38	2	1	11	4	20
Maximum pairwise sequence divergence						
All 38 accessions	2.6	4.2	3.7	3.1	2.2	4.9
Cicuta accessions only	1.4	1.7	3.4	1.1	1.6	1.6
Oenanthe accessions only	1.4	0.8	1.4	2.0	1.0	3.1

cessions of *Cicuta* examined. The next two largest gaps (of 66 and 23 bp), also representing deletions relative to *O. sarmentosa* within the *trnQ–rps16* 5′ exon intergenic region, were each confined to several accessions of *Oenanthe*. All remaining alignment gaps were 14 bp or less in size (averaging about 5 bp). Pairwise sequence divergence values across the entire *psbI–trnK* 5′ exon region ranged from identity (between the two accessions each of *O. silaifolia* and *O. pimpinelloides*, and between *C. virosa* accession Nos. 75 and 131) to 2.6% (between *C. maculata* var. *maculata* No. 1563 and *O. foucaudii* No. 1631). Maximum pairwise sequence divergence values for both *Cicuta* and *Oenanthe* across the entire cpDNA region were identical (1.4%).

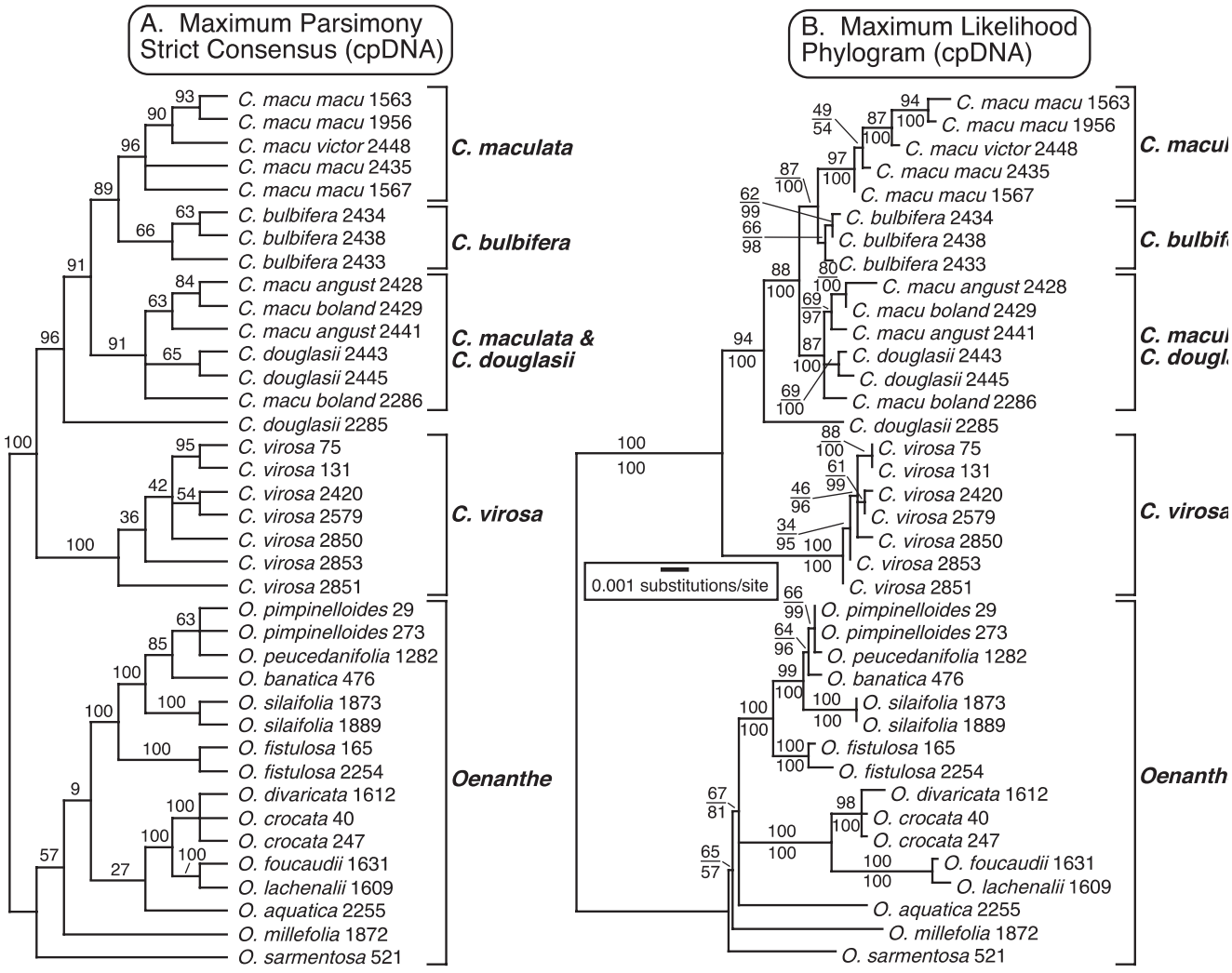
Sequence comparisons of the five separate cpDNA partitions revealed that the *rps16* 3′ exon – *trnK* 5′ exon region provided the greatest numbers of potentially informative alignment positions (52) and indels (24) and had the highest level of sequence divergence (4.9%) across all 38 accessions (Table 3). The overall percent variability of each of the five partitions (calculated by dividing the number of variable alignment positions plus the number of indels by total length of the region × 100) ranged from 5.8% (*rps16* intron) to 12.1% (*rps16* 3′ exon – *trnK* 5′ exon). Each of the four intergenic spacers had a higher overall percent variability (6.5%–12.1%) than that of the intron. The 6.5% variability for the *trnQ – rps16* 5′ exon intergenic spacer is likely deflated because of an 825 bp deletion in this region in all *Cicuta* accessions. Further sequence characteristics of these five partitions are presented in Table 3.

MP analysis of the entire cpDNA *psbI – trnK* 5′ exon region including the 38 informative gaps scored as additional binary characters resulted in eight minimal length trees, each of 329 steps (CI = 0.8906 and 0.8487, with and without uninformative characters, respectively; RI = 0.9733). The strict consensus of these trees, with accompanying bootstrap values, is presented in Fig. 3A. Bootstrap values for all resolved branches ranged from 9% to 100%, with the lowest of these values arising within the *Oenanthe* clade. Within *Cicuta*, bootstrap values ranged from 36% to 100%. All but 4 of the 38 scored alignment gaps occurred without homoplasy on each of the inferred trees: three gaps (of 1, 3, and 11 bp in size) required two steps, and one gap (of 1 bp) required three steps. The 11 bp homoplastic gap characterized both *Cicuta* (all accessions) and a clade of *Oenanthe* (*O. divaricata, O. crocata* (2 accessions), *O. foucaudii, O. lachenalii*, and *O. aquatica*). The 3 bp homoplastic gap occurred in *O. millefolia*, as well as the clade of *O. divaricata, O. crocata* (2 accessions), *O. foucaudii*, and *O. lachenalii*. While the distribution of alignment gaps supported both the monophyly of *Cicuta* and of *C. virosa*, no gaps were observed elsewhere in *Cicuta* that could have been used to bolster infrageneric relationships. In contrast, 19 gaps were nonhomoplastic in *Oenanthe*, and these were distributed on those branches of the *Oenanthe* clade identified by bootstrap values greater than 63% (Fig. 3A).

Maximum likelihood settings from the best-fit model K81uf+I selected by AIC in Modeltest were entered into PAUP* (base frequencies: 0.3501, A; 0.1436, C; 0.1589, G; 0.3474, T; estimates of substitution rates: A↔C, 1; A↔G, 1.0428; A↔T, 0.3592; C↔G, 0.3592; C↔T, 1.0428; G↔T, 1; proportion of invariable sites (I) = 0.7910). Using these values, a single ML tree was recovered, with a –ln likelihood score of 7957.60693 (Fig. 3B). ML distance-based bootstrap values ranged from 34% to 100% on resolved branches. Relationships inferred by Bayesian phylogenetic analysis were identical to those exhibited by the ML tree. Bayesian posterior clade probabilities ranged from 54% to 100%; these values are presented below the ML bootstrap values in Fig. 3B.

All phylogenetic trees inferred using cpDNA data were highly resolved and supported similar relationships. As in the ITS trees, the genus *Cicuta* is strongly supported as monophyletic. *Cicuta virosa* (7 accessions) is also monophyletic, and sister group to all other *Cicuta* species with the single accession of *C. douglasii* from California (No. 2285) the sister group of the remainder. The latter is a major clade comprising a monophyletic *C. bulbifera* and those taxa included within the *C. maculata* clade in the ITS trees. The ITS *C. maculata* clade is divided into two well-supported subclades in the cpDNA-based phylogenies. The first comprises *C. maculata* vars. *maculata* and *victorinii* and is supported by 96%–97% bootstrap values in the MP and ML analyses, and a 100% posterior probability value in the Bayesian majority rule consensus tree. The accessions therein represent plants from Georgia (1567), South Carolina (1563, 1956), Mississippi (2435), and Quebec (2448), and

Fig. 3. Phylogenies of *Cicuta* and the outgroup *Oenanthe* inferred from phylogenetic analyses of *psbI–trnK* 5′exon cpDNA sequences. (A) Strict consensus of eight minimal length 329-step trees derived from maximum parsimony analysis of 4396 unambiguously aligned nucleotide positions and 38 alignment gaps scored as additional binary characters (CI = 0.8906 and 0.8487, with and without uninformative characters, respectively; RI = 0.9733). Numbers above branches are bootstrap estimates from 1000 replicate analyses. (B) Single tree derived from maximum likelihood analysis of 4396 alignment positions under a K81uf+I model of nucleotide substitution (–ln likelihood = 7957.60693). Numbers above branches represent ML distance-based bootstrap values calculated from 100 replicate analyses. Relationships inferred by Bayesian phylogenetic analysis were identical to those exhibited by the ML tree. Numbers below branches on the ML tree represent Bayesian posterior probabilities. Complete taxon names, including the ranks of infraspecific taxa which were omitted for brevity, are provided in Table 1.



are primarily of eastern North American distribution. The second subclade comprises *C. maculata* vars. *angustifolia* and *bolanderi*, as well as *C. douglasii* (Nos. 2443 and 2445). Bootstrap values supporting this subclade range from 87%–91%, and the posterior probability value is 100%. The six accessions within this subclade were collected from western North America (Arizona, California, British Columbia, and Saskatchewan). The three accessions comprising the *C. bulbifera* clade (61%–66% bootstrap support, 98% posterior probability) are the sister group of the clade of *C. maculata* vars. *maculata* and *victorinii*. Two of three examined accessions of *C. douglasii* comprise a weakly supported monophyletic group, but considering the position of *C. douglasii* No. 2285, the species as a whole is polyphyletic.

Comparison of ITS and cpDNA phylogenies

A visual comparison of well-supported clades in the ITS and cpDNA trees indicates major discordance of several aspects of relationship within *Cicuta*. This is especially acute with regard to the placement of *C. bulbifera*. In the ITS trees, *C. bulbifera* is sister group to all other *Cicuta* accessions except *C. virosa* and *C. douglasii* No. 2285 (Fig. 2), whereas in the cpDNA trees it is nested within the *C. maculata* clade, sister group to the clade comprised of *C. maculata* vars. *maculata* and *victorinii* (Fig. 3). Results of the partition-homogeneity test on a set of 21 accessions (19 *Cicuta* and 2 *Oenanthe*) common to both data sets revealed that these matrices yield significantly different phylogenetic estimates (P = 0.001; sum of lengths for original partition = 244 steps). As a result of the significant discor-

dance between data sets, and the assumption that *C. douglasii* is likely of hybrid origin (Mulligan 1980), these data were not combined for a "total evidence" analysis. Maximum pairwise sequence divergence estimates across all 21 taxa were 8.1% and 2.3% for the ITS and cpDNA matrices, respectively. The percentage of alignment positions variable in each matrix was 11.1% (ITS) and 3.3% (cpDNA).

Discussion

Phylogeny of *Cicuta*

Cicuta bulbifera, bulbiferous or bulbet-bearing water-hemlock, is widely distributed in Canada and the United States. Its range extends from Alaska to Newfoundland in the north and from Oregon to Florida in the south (Mulligan 1980). The plants have linear or very narrowly lanceolate leaflets (up to 5 mm wide) and bulbils in the upper leaf axils for propagation. They rarely produce fruit because their flowers readily abort. On the bases of its distinctive morphology, 22 medium-sized somatic chromosomes, and results of the molecular investigations presented here, the species is monophyletic. In a subset of most parsimonious ITS trees (Fig. 2A) and in both ML strict consensus and Bayesian majority rule consensus ITS trees (Fig. 2B), the *C. bulbifera* clade is a weakly supported sister group to the *C. maculata* clade. In the cpDNA-derived phylogenies (Fig. 3), where greater resolution of relationships is achieved, *C. maculata* is paraphyletic, with the *C. bulbifera* clade sister group to a clade comprising all examined accessions of *C. maculata* vars. *maculata* and *victorinii*. This nonconcordance between cytoplasmic-based and nuclear-based phylogenies may be due to a hybridization event leading to the origin of *C. bulbifera*. Further evidence for this comes from the abortive development of flowers, the rarity of fruits seen on herbarium specimens, and the presence of bulbils for propagation, suggesting that these plants are sterile as a result of hybridization and, thus, can only reproduce asexually. As far as we are aware, *C. bulbifera* has not been examined for chromosome structure or behavior at meiosis, or even for pollen viability, which would provide information on its possible hybrid origin.

Cicuta virosa, northern water-hemlock, is circumboreal in distribution. In North America, its range extends from Alaska to Quebec, reaching south to northern British Columbia, across the northern portions of the Prairies, to northern Ontario and Quebec (Mulligan 1980). It is also characterized by a suite of fruit characters (such as, fruits a little broader than long, the corky dorsal ribs being much wider than the intervals between them, and the oil tubes within the intervals being quite small). None of these features, however, are unique to the species. Its roots are short and generally slender and fibrous, unlike the thickened roots of *C. maculata* and *C. douglasii*. The globular rootstocks grow aboveground, as they do in *C. douglasii* (Mulligan and Munro 1981). The midveins on the upper surfaces of the leaflets are scabrous and the undersurfaces of the stem leaflets are sparsely net-veined; the latter feature is also seen in *C. bulbifera*. Its leaflets are narrowly lanceolate, but not as much as *C. bulbifera*. The results of phylogenetic analyses of plastid and nuclear loci and the presence of both unique alignment gaps and 22 small-sized somatic chromosomes support monophyly of the species.

Cicuta maculata, common or spotted water-hemlock, or spotted cowbane, is the most widely distributed species of *Cicuta* in North America. It ranges from Alaska to southern Mexico, and from the Pacific to Atlantic Oceans, and occurs commonly in wet habitats (such as swamps, marshes, and meadows, and at the edges of streams and rivers) and along roadsides. Its members share the following features: 22 large somatic chromosomes, densely arranged areolae on the underside of leaflets mostly irregularly round or square in shape (rather than elongated, as in *C. douglasii*, or larger and much longer than wide, as in *C. bulbifera* and *C. virosa*; Mulligan 1980; Mulligan and Munro 1981), and oil tubes equaling to many times larger than the intervals and protruding strongly into the endosperm (Mulligan 1980). Four varieties are recognized: (1) var. *maculata*, predominantly occurring in eastern North America, (2) var. *victorinii*, confined to the freshwater tidal zone around Quebec City, (3) var. *angustifolia*, centred in western North America, and (4) var. *bolanderi*, sporadically distributed across the United States and northern Mexico, with most early collections obtained from the coastal salt marshes of the Pacific. Variety *bolanderi* is scattered throughout the southern range of *C. maculata* (Mulligan 1980).

Based on an examination of a limited number of specimens, we have observed that the fruit characters used to distinguish these varieties (such as, the shape of the fruit in cross-section, the sizes of the dorsal and lateral ribs relative to each other and to the sizes of the oil tubes, and the degree and type of constriction at the commissure) are not consistent and are difficult to interpret with any degree of certainty. Mulligan (1980) reported "a more or less gradual cline in these fruit characters from one extreme to the other [but] enough of a discontinuity to recognize four varieties." The variation in fruit morphology that we observed represents a continuum across all taxa. In the absence of fruits, it is nearly impossible to identify plants of *C. maculata* to variety. The only exception is var. *angustifolia*, which can be distinguished from the other varieties by its shorter styles and mostly narrower stem leaflets (up to five times longer than broad). The phylogenetic results presented here support, in part, these observations of morphology. Within *C. maculata*, only the western North American var. *angustifolia* is resolved in the ITS trees; in the cpDNA trees, however, the two examined accessions of this variety comprise a clade with one accession of var. *bolanderi*. It is intriguing that the cpDNA (but not the ITS) results suggest a major dichotomy within *C. maculata* that was previously unconsidered in any taxonomic treatment: one branch comprising vars. *maculata* and *victorinii* (which is sister group to *C. bulbifera*), and the other branch comprising vars. *angustifolia* and *bolanderi* (plus *C. douglasii*). This split parallels their geographic distribution, for the first group represents plants predominantly occurring in eastern North America, whereas the second represents plants primarily from western North America. While further study is in order, it appears that in *C. maculata* (with the possible exception of var. *angustifolia*) the varietal rank lacks taxonomic utility. A study of *Cicuta* morphology is currently underway (C.-S. Lee, in preparation) and the results may reveal whether vars. *bolan-*

deri and *victorinii* should be included within var. *maculata*, as suggested by their similar morphologies and the ITS trees, or whether the eastern and western members of *C. maculata* should be each recognized as distinct taxa, as inferred by the cpDNA results.

Cicuta douglasii, western water-hemlock, occurs in western North America. Its range extends from Alaska south to central California, and east to British Columbia, western Montana, Idaho, and western Nevada. The species exhibits intermediate morphology in vegetative characters between *C. maculata* and *C. virosa*, and its fruits are very similar to those of *C. virosa* (Mulligan 1980; Mulligan and Munro 1981). Such vegetative characters include the nature of the venation patterns on the lower surfaces of the leaflets and features of the rootstock and root development. Historically, it has been difficult to distinguish *C. douglasii* from *C. maculata* (Mathias and Constance 1944; Cronquist 1961, 1997; Mulligan 1980). Many early described segregates of *C. douglasii* (Greene 1889, 1912; Lunell 1916) are now included within *C. maculata*. *Cicuta purpurata* Greene (=*C. douglasii*), was erected by Greene (1889) for plants from Washington, which he stated were probably the same species as *Sium? douglasii* DC. (*C. douglasii*) from western North America. He also noticed similarities between his *C. purpurata* and other western *Cicuta* species (such as, *C. occidentalis* Greene, now submerged in *C. maculata*), but he kept them separate based on features of the rootstock and roots. Greene wrote that it had been the custom to treat *Sium? douglasii* (*C. douglasii*) as a synonym of *C. maculata*. The distribution of *C. maculata* at that time, however, was considered to be restricted to the eastern and middle states of the United States, and not occurring much westward of the Great Lakes. Coulter and Rose (1900) transferred *Sium? douglasii* into *Cicuta* without explanation and placed *C. purpurata* in synonymy under *C. douglasii*. The morphological similarity between *C. douglasii* and *C. maculata* has confused even umbellifer experts, as chromosome counts made for these species (e.g., Bell and Constance 1957, 1960; Constance et al. 1976) were attributed erroneously to the other one of the pair (Mulligan 1980).

The ranges of *Cicuta douglasii* and *C. maculata* overlap and, other than chromosome number and sizes, we can find no distinctive morphological character that would support the separation of these two species. Indeed, Constance (1993) described these species as cryptic. Both species contain large quantities of cicutoxin (the other species much less so) and are deadly poisonous (Mulligan 1980; Mulligan and Munro 1981). Cronquist (1997) has observed that the most consistent morphological difference between these species is the venation pattern of the underside of the leaves. However, he has acknowledged that "some specimens are difficult to determine by this criterion," and we concur. Both species have densely arranged veinlets, but in *C. maculata* the areolae are mostly irregularly round or square in shape, whereas in *C. douglasii*, at least according to Mulligan (1980), the areolae are more elongated. With the exception of *C. douglasii* accession No. 2285, phylogenetic analyses of both plastid and nuclear loci place all accessions of this species within the *C. maculata* clade. Furthermore, the cpDNA trees (Fig. 3) show that *C. douglasii*, *C. maculata* var. *angustifolia*, and *C. maculata* var. *bolanderi* constitute a strongly supported monophyletic group.

Origin and taxonomic status of *Cicuta douglasii*

Cicuta douglasii may be of allopolyploid origin (Mulligan 1980; Mulligan and Munro 1981; Cronquist 1997). Its chromosome count is $2n = 44$, comprised of 22 large chromosomes and 22 small chromosomes (Mulligan 1980). Previous counts of $n = 11$ for *C. douglasii* and $n = 22$ for *C. maculata* var. *bolanderi* (Bell and Constance 1957, 1960; Constance et al. 1976) are erroneous. These counts were attributed to the wrong species; specifically, *C. maculata* var. *angustifolia* and *C. douglasii*, respectively (Mulligan 1980). *Cicuta maculata* has 22 large somatic chromosomes, whereas *C. virosa* has 22 small ones. As such, and on the basis of similar morphology of certain characters, it was thought that *C. douglasii* was derived from progenitors similar to present-day *C. maculata* and *C. virosa* (Mulligan 1980; Mulligan and Munro 1981).

In the ITS study, we included four specimens of *C. douglasii* examined by Mulligan (1980) for chromosome number and size (accession Nos. 2443, 2444, 2445, and 2446). These specimens are $2n = 44$ and were collected on Vancouver Island, British Columbia, and in Idaho. (Two of these specimens were also used in the analysis of cpDNA sequences.) No evidence of additive patterns of bands was observed in these specimens. Such patterns may be associated with allopolyploidy, and would be consistent with retention of multiple rDNA loci from different parental ancestors at the time of speciation (Soltis et al. 1992; Vargas et al. 1999). However, it is clear that homogenization of rDNA or the elimination of a locus may occur quickly after allopolyploidization (Soltis et al. 1992; Popp et al. 2005), possibly explaining the nonadditive rDNA profiles observed in *C. douglasii*.

The ITS trees show that all accessions of *C. douglasii*, with the exception of accession No. 2285, are nested within the *C. maculata* clade, confirming Mulligan's suggestion that *C. maculata* is one of its parent species. In the cpDNA trees, the two accessions of *C. douglasii* also group with *C. maculata* (specifically, vars. *bolanderi* and *angustifolia*) in a well-supported clade. Chloroplast DNA is typically maternally inherited in angiosperms and permits the designation of a maternal parent in analyses of hybridization and polyploidy. As such, the maternal progenitor of *C. douglasii* appears to be *C. maculata*. In contrast, the nuclear-encoded rDNA is biparentally inherited and, thus, permits an assessment of all parental contributions to the polyploid species. In allopolyploids, however, homogenization of rDNA can occur in the direction of either of the parent species (Wendel et al. 1995). If the rDNA of a hybrid was subsequently homogenized through concerted evolution in the direction of the maternal genome, information about its paternal progenitor would be lost (e.g., Smedmark and Eriksson 2002). Such may be the case with *C. douglasii*, as both ITS and cpDNA trees suggest the same progenitor. The phylogenetic results presented here provide no evidence of a close relationship between *C. douglasii* and *C. virosa,* which would have suggested that the latter was one of the diploid progenitors of the former, as also suggested by Mulligan (1980). Low-copy nuclear genes are, with rare exception,

not subject to concerted evolution and are useful in determining the origin of allotetraploids (Sang 2002; Álvarez and Wendel 2003). Phylogenies constructed from these genes will therefore be required to confirm the parentage of *C. douglasii* (as well as that of *C. bulbifera*, discussed above).

The ITS trees suggest that *C. douglasii* is polyphyletic and, possibly, polytopic. In a subset of most parsimonious ITS trees and in the cpDNA trees, those accessions of *C. douglasii* for which chromosomal information is available comprise a monophyletic group, implying that polyploidy in these plants had a unique origin. In the ITS trees, at least two other origins of allopolyploidy can be proposed (accession Nos. 1573 and 2280 from Montana, and accession Nos. 2279, 2281, and 2282 from Nevada and Oregon), but cytological data are lacking to confirm this. Most taxonomically recognized polyploid species are of multiple origin (Soltis and Soltis 1999), with these species typically polyphyletic (Soltis et al. 1992, 2003). At the present time, the lack of resolution in the ITS trees and the unknown ploidy levels of these five additional accessions preclude definite statements on the extent of polyploid evolution in *C. douglasii*.

In the absence of cytological data, *C. douglasii* is essentially indistinguishable from *C. maculata*. In the cpDNA-derived phylogenies, all accessions of *C. douglasii* but one nest within *C. maculata*, suggesting that the maternal progenitor of *C. douglasii* is *C. maculata*. The placement of a polyploid within the clade of one of its progenitors is expected. Given its chromosome number, there is no doubt that *C. douglasii* is a tetraploid, and the presence of both 22 large and 22 small chromosomes further suggests that it is an allotetraploid. As such, *C. douglasii* would be reproductively isolated from its presumed diploid progenitors, *C. maculata* and *C. virosa*. We maintain the recognition of *C. douglasii* as a distinct species.

A new species of *Cicuta*?

In the ITS phylogenies, the accession labeled as *Cicuta douglasii* 2285, from Colusa Co., California, forms one branch of a trichotomy; in the cpDNA-derived trees, it is sister group to all other *Cicuta* taxa save *C. virosa*. The specimen presents both flowers and mature fruits, but only the uppermost portion of the plant is available. Chromosome number or size is not known. The plant was determined (by umbellifer authority L. Constance) to be *C. douglasii*. Superficially, the plant resembles *C. maculata*. However, its rounded fruits, single pinnate leaves, and primary veins on the underside of the leaflets extending into the middle of the marginal teeth and not into the sinuses between them (as they do in all other *Cicuta* species; Bomhard 1936) suggest that this plant may be *C. californica* A. Gray (Coulter and Rose 1888, 1900; Greene 1889), a species now included within *C. douglasii* (Mathias and Constance 1942). The plant was collected in shallow water in a pine forest at 3900 ft (1189 m) elevation, and its occurrence in such a habitat is also consistent with that described for *C. californica* (Bomhard 1936; Jepson 1936). To confirm its identification, additional material is required, and particularly that of the lower leaves and rootstock. An examination of its somatic chromosomes is also critical to confirm its allotetraploid nature, if it is indeed *C. douglasii*. The similar placement of accession No. 2285 in both ITS and plastid-derived trees and its unique morphology and habitat preference suggest that it might warrant recognition as a distinct species, pending further study.

Phylogenetic utility of the cpDNA *psbI-trnK* 5′exon region

The cpDNA *psbI–trnK* 5′ exon region, ranging in size between 3.4 and 4.2 kb in *Cicuta* and *Oenanthe*, is 6–7 times larger than that of the ITS region. As such, this plastid locus provided three times as many parsimony informative aligned positions and 10 times as many indels as did ITS. Thirty-eight alignment gaps were potentially informative, and all but four occurred without homoplasy on the inferred trees. Nineteen of these indels occurred within the *Oenanthe* clade. Only three indels supported relationships within *Cicuta*, and these were confined to *C. virosa*. For those 21 accessions of *Cicuta* and *Oenanthe* common to both data sets, pairwise sequence divergence estimates were 3.5 times greater for the ITS region. This corroborates earlier investigations reporting that among the several plastid and nuclear loci used to infer phylogeny of Apiaceae, the ITS region is evolving most rapidly (Downie et al. 2001).

The *psbI–trnK* 5′ exon region is easily PCR-amplified using various combinations of primers anchored in coding regions (Fig. 1). Depending upon the species, additional primers were required to obtain complete sequences, on both DNA strands, for the *rps16* intron and the two large intergenic spacer regions flanking gene *rps16*. The organization of the cpDNA *psbI–trnK* 5′ exon locus makes it technically easy to design amplification and sequencing primers because of the almost regularly spaced, conserved gene regions.

Sequence comparisons among the five separate cpDNA partitions revealed the *rps16* 3′ exon – *trnK* 5′ exon locus as being the most variable, in terms of having the greatest number of observed nucleotide substitutions and indels. Each of the four intergenic spacers had a higher overall percent variability than that of the *rps16* intron and are evolving faster (more than 1.4 times faster, on average) than the intron. Group II introns in the chloroplast genomes of land plants, such as the *rps16* intron, are excised from pre-mRNA transcripts via a series of self-catalyzed reactions and show a strong relationship between the functional importance of its secondary structural features and the probability of mutational change, with those domains, or domain portions, essential for intron-associated functions most conserved evolutionarily (Michel et al. 1989; Learn et al. 1992; Downie et al. 1996, 1998, 2000*b*; Kelchner 2002). While the numerous and well-defined conserved regions within the *rps16* intron may explain its lower rate of sequence change relative to the four intergenic spacers, the functional (and consequently structural) constraints within the latter regions and their effects on mutational processes are less understood (Kelchner 2002).

Shaw et al. (2005) examined the relative utility of 21 noncoding cpDNA sequences for phylogenetic analysis at low taxonomic levels and, of the five partitions examined here, only included data for the *rps16* intron. An assessment of rate heterogeneity among these 21 noncoding regions across a broad phylogenetic range of taxa revealed that the *rps16* intron ranked sixth (top of their "Tier 2" based on overall

qualitative usefulness) in terms of providing potentially informative characters (nucleotide substitutions and indels). Members of "Tier 2" may not be variable enough to resolve infrageneric relationships. Based on the relative comparisons presented here, the *rps16* 3′ exon – *trnK* 5′ exon region, if included by Shaw et al. (2005), would have ranked in their "Tier 1," a group of intergenic spacers that on average consistently provided the greatest number of phylogenetically informative characters across all phylogenetic lineages they tested. The continued acquisition of sequence data from the *psbI–trnK* 5′ exon region, and in particular from the *rps16* 3′ exon – *trnK* 5′ exon intergenic spacer, for assessing infrageneric relationships in Apiaceae seems worthwhile, given its higher rate of sequence evolution over other examined cpDNA loci and the ease by which these data can be obtained.

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References

Álvarez, I., and Wendel, J.F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. Mol. Phylogenet. Evol. **29**: 417–434. PMID: 14615184.

Barker, F.K., and Lutzoni, F.M. 2002. The utility of the incongruence length difference test. Syst. Biol. **51**: 625–637. doi:10.1080/10635150290102302. PMID: 12228004.

Bell, C.R., and Constance, L. 1957. Chromosome numbers in Umbelliferae. Am. J. Bot. **44**: 565–572.

Bell, C.R., and Constance, L. 1960. Chromosome numbers in Umbelliferae. II. Am. J. Bot. **47**: 24–32.

Bell, C.R., and Kane, R. 1981. An anomalous rhizomatous population of *Cicuta maculata* L. at Mountain Lake, Virginia. Castanea, **46**: 4–7.

Bomhard, M.L. 1936. Leaf venation as a means of distinguishing *Cicuta* from *Angelica.* J. Wash. Acad. Sci. **26**: 102–107.

Constance, L. 1972. Patterns in the distribution of Japanese-American Umbelliferae. *In* Floristics and paleofloristics of Asia and Eastern North America. *Edited by* A. Graham. Elsevier, New York. pp. 93–99.

Constance, L. 1993. Apiaceae. *In* The Jepson manual. Higher plants of California. *Edited by* J.C. Hickman. University of California Press, Berkeley, Calif. pp. 136–166.

Constance, L., Chuang, T.-I., and Bell, C.R. 1976. Chromosome numbers in Umbelliferae. V. Am. J. Bot. **63**: 608–625.

Coulter, J.M., and Rose, J.N. 1888. Revision of North American Umbelliferae. Wabash College, Crawfordsville, Ind. pp. 1–144.

Coulter, J.M., and Rose, J.N. 1900. Monograph of the North American Umbelliferae. Contrib. U.S. Natl. Herb. **7**: 1–256.

Cronquist, A. 1961. Umbelliferae. *In* Vascular plants of the Pacific Northwest. *Edited by* C.L. Hitchcock, A. Cronquist, M. Ownbey, and J.W. Thompson. Univ. Washington Publ. Biol., Vol. 17. pp. 506–586.

Cronquist, A. 1997. Apiaceae, the carrot family. *In* Intermountain flora: vascular plants of the Intermountain West, U.S.A. Vol. 3, part A. *Edited by* A. Cronquist, N.H. Holmgren, and P.K. Holmgren. New York Botanical Garden, Bronx, N.Y.

Downie, S.R., and Katz-Downie, D.S. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot. **83**: 234–251.

Downie, S.R., and Katz-Downie, D.S. 1999. Phylogenetic analysis of chloroplast *rps16* intron sequences reveals relationships within the woody southern African Apiaceae subfamily Apioideae. Can. J. Bot. **77**: 1120–1135. doi:10.1139/cjb-77-8-1120.

Downie, S.R., Katz-Downie, D.S., and Cho, K.-J. 1996. Phylogenetic analysis of Apiaceae subfamily Apioideae using nucleotide sequences from the chloroplast *rpoC1* intron. Mol. Phylogenet. Evol. **6**: 1–18. PMID: 8812301.

Downie, S.R., Ramanath, S., Katz-Downie, D.S., and Llanas, E. 1998. Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* intron sequences. Am. J. Bot. **85**: 563–591.

Downie, S.R., Katz-Downie, D.S., and Spalik, K. 2000*a*. A phylogeny of Apiaceae tribe Scandiceae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot. **87**: 76–95. PMID: 10636832.

Downie, S.R., Katz-Downie, D.S., and Watson, M.F. 2000*b*. A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA *rpl16* and *rpoC1* intron sequences: towards a suprageneric classification of subfamily Apioideae. Am. J. Bot. **87**: 273–292. PMID: 10675315.

Downie, S.R., Watson, M.F., Spalik, K., and Katz-Downie, D.S. 2000*c*. 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. Can. J. Bot. **78**: 506–528. doi:10.1139/cjb-78-4-506.

Downie, S.R., Plunkett, G.M., Watson, M.F., Spalik, K., Katz-Downie, D.S., Valiejo-Roman, C.M., Terentieva, E.I., Troitsky, A.V., Lee, B.-Y., Lahham, J., and El-Oqlah, A. 2001. Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. Edinb. J. Bot. **58**: 301–330. doi:10.1017/S0960428601000658.

Downie, S.R., Sun, F.-J., Katz-Downie, D.S., and Colletti, G.J. 2004. A phylogenetic study of *Perideridia* (Apiaceae) based on nuclear ribosomal DNA ITS sequences. Syst. Bot. **29**: 737–751.

Doyle, J.J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Syst. Bot. **17**: 144–163.

Farris, J.S., Källersjö, M., Kluge, A.G., and Bult, C. 1995. Testing significance of incongruence. Cladistics, **10**: 315–319.

Greene, E.L. 1889. Vegetative characters of the species of *Cicuta*. Pittonia, **2**: 1–11.

Greene, E.L. 1909. New plants from Arizona. Leafl. Bot. Obsn. Crit. **2**: 20–24.

Greene, E.L. 1912. New species of *Cicuta*. Leafl. Bot. Obsn. Crit. **2**: 236–241.

Hardway, T.M., Spalik, K., Watson, M.F., Katz-Downie, D.S., and Downie, S.R. 2004. Circumscription of Apiaceae tribe Oenantheae. S. Afr. J. Bot. **70**: 393–406.

Holmgren, P.K., Holmgren, N.H., and Barnett, L.C. 1990. Index herbariorum. New York Botanical Garden, Bronx, N.Y.

Jacobson, C.A. 1915. Water hemlock (*Cicuta*). Agricultural Experiment Station, The University of Nevada, Reno, Nev. Technical Bulletin No. 81. pp. 7–46.

James, L.F., and Ralphs, M.H. 1986. Water hemlock. Utah Sci. **47**: 67–69.

Jepson, W.L. 1936. A flora of California. Vol. 2. University of California, Berkeley, Calif.

Kelchner, S.A. 2002. Group II introns as phylogenetic tools: structure, function, and evolutionary constraints. Am. J. Bot. **89**: 1651–1669.

Kingsbury, J.M. 1964. Poisonous plants of the United States and Canada. Prentice Hall, Englewood Cliffs, N. J.

Learn, G.H., Jr., Shore, J.S., Furnier, G.R., Zurawski, G., and Clegg, M.T. 1992. Constraints on the evolution of plastid introns: the group II intron in the gene encoding tRNA-Val(UAC). Mol. Biol. Evol. **9**: 856–871. PMID: 1528110.

Lee, B.-Y., and Downie, S.R. 1999. A molecular phylogeny of Apiaceae tribe Caucalideae and related taxa: inferences based on ITS sequence data. Syst. Bot. **24**: 461–479.

Lunell, J. 1916. The vascular plants of North Dakota. VIII. Am. Midl. Nat. **4**: 467–487.

Mathias, M.E., and Constance, L. 1942. A synopsis of the American species of *Cicuta*. Madroño, **6**: 145–176.

Mathias, M.E., and Constance, L. 1944. *Cicuta. In* North American flora. Vol. 28B. The New York Botanical Garden, Bronx, N.Y. pp. 154–157.

Michel, F., Umesono, K., and Ozeki, H. 1989. Comparative and functional anatomy of group II catalytic introns—a review. Gene, **82**: 5–30. doi:10.1016/0378-1119(89)90026-7. PMID: 2684776.

Mulligan, G.A. 1980. The genus *Cicuta* in North America. Can. J. Bot. **58**: 1755–1767.

Mulligan, G.A., and Munro, D.B. 1981. The biology of Canadian weeds. 48. *Cicuta maculata* L., *C. douglasii* (DC.) Coult. & Rose and *C. virosa* L. Can. J. Plant Sci. **61**: 93–105.

Panter, K.E., Keeler, R.F., and Baker, D.C. 1988. Toxicoses in livestock from the hemlocks (*Conium* and *Cicuta* spp.). J. Anim. Sci. **66**: 2407–2413. PMID: 3049497.

Plunkett, G.M., and Downie, S.R. 1999. Major lineages within Apiaceae subfamily Apioideae: a comparison of chloroplast restriction site and DNA sequence data. Am. J. Bot. **86**: 1014–1026. PMID: 10406725.

Popp, M., Erixon, P., Eggens, F., and Oxelman, B. 2005. Origin and evolution of a circumpolar polyploid species complex in *Silene* (Caryophyllaceae) inferred from low copy nuclear RNA polymerase introns, rDNA, and chloroplast DNA. Syst. Bot. **30**: 302–313.

Posada, D., and Buckley, T.R. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. **53**: 793–808. doi:10.1080/10635150490522304. PMID: 15545256.

Posada, D., and Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics, **14**: 817–818. doi:10.1093/bioinformatics/14.9.817. PMID: 9918953.

Rieseberg, L.H., and Brunsfeld, S.J. 1992. Molecular evidence and plant introgression. *In* Molecular systematics of plants. *Edited by* P.S. Soltis, D.E. Soltis, and J.J. Doyle. Chapman and Hall, New York. pp. 151–176.

Ronquist, F., and Huelsenbeck, J.P. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics, **19**: 1572–1574. doi:10.1093/bioinformatics/btg180. PMID: 12912839.

Sang, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. Crit. Rev. Biochem. Mol. Biol. **37**: 121–147. doi:10.1080/10409230290771474. PMID: 12139440.

Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E., and Small, R.L. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am. J. Bot. **92**: 142–166.

Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.-Y., Sugita, M., Deno, H., Kamagoshira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H., and Sugiura, M. 1986. The complete nucleotide sequence of the tobacco chloroplast genome: its organization and expression. EMBO J. **5**: 2043–2049. PMID: 16453699.

Smedmark, J.E.E., and Eriksson, T. 2002. Phylogenetic relationships of *Geum* (Rosaceae) and relatives inferred from the nrITS and *trnL-trnF* regions. Syst. Bot. **27**: 303–317.

Soltis, D.E., and Kuzoff, R.K. 1995. Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). Evolution, **49**: 727–742.

Soltis, D.E., and Soltis, P.S. 1999. Polyploidy: recurrent formation and genome evolution. Trends Ecol. Evol. **14**: 348–352. doi:10.1016/S0169-5347(99)01638-9. PMID: 10441308.

Soltis, D.E., Soltis, P.S., and Tate, J.A. 2003. Advances in the study of polyploidy since *Plant Speciation.* New Phytol. **161**: 173–191.

Soltis, P.S., Doyle, J.J., and Soltis, D.E. 1992. Molecular data and polyploid evolution in plants. *In* Molecular systematics of plants. *Edited by* P.S. Soltis, D.E. Soltis, and J.J. Doyle. Chapman and Hall, New York. pp. 177–201.

Starreveld, E., and Hope, C.E. 1975. Cicutoxin poisoning (water hemlock). Neurology, **25**: 730–734. PMID: 1171406.

Sun, F.-J., Downie, S.R., and Hartman, R.L. 2004. An ITS-based phylogenetic analysis of the perennial, endemic Apiaceae subfamily Apioideae of western North America. Syst. Bot. **29**: 419–431.

Swofford, D.L. 1998. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4 edition. Sinauer Associates, Sunderland, Mass.

Tamura, K., and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. **10**: 512–526. PMID: 8336541.

Vargas, P., McAllister, H.A., Morton, C., Jury, S.L., and Wilkinson, M.J. 1999. Polyploid speciation in *Hedera* (Araliaceae): phylogenetic and biogeographic insights based on chromosome counts and ITS sequences. Plant Syst. Evol. **219**: 165–179. doi:10.1007/BF00985577.

Wendel, J.F., Schnabel, A., and Seelanan, T. 1995. Bidirectional interlocus concerted evolution following polyploid speciation in cotton (*Gossypium*). Proc. Natl. Acad. Sci. U.S.A. **92**: 280–284. PMID: 7816833.

Yoder, A.D., Irwin, J.A., and Payseur, B.A. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Syst. Biol. **50**: 408–424. doi:10.1080/106351501300318003. PMID: 12116583.