Phylogeny and biogeography of Apiaceae tribe Oenantheae inferred from nuclear rDNA ITS and cpDNA *psbI–5′trnK*$^{(UUU)}$ sequences, with emphasis on the North American Endemics clade

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Abstract: Intergeneric phylogenetic relationships within Apiaceae tribe Oenantheae were investigated using sequence data from the chloroplast DNA *psbI–5′trnK*$^{(UUU)}$ and nuclear ribosomal DNA internal transcribed spacer regions. One hundred and thirty-one accessions were examined, representing all 17 genera of the tribe and approximately one-half of its species. The cpDNA region includes four intergenic spacers and the *rps16* intron and these noncoding loci were analyzed separately to assess their relative utility for resolving relationships. Separate maximum parsimony analyses of the entire *psbI–5′trnK*$^{(UUU)}$ and ITS regions, each with and without scored indels, yielded concordant trees. Phylogenies derived from maximum parsimony, Bayesian, or maximum likelihood analyses of combined chloroplast and nuclear DNA sequences for 82 accessions were highly resolved, well supported, and consistent. Among the five noncoding loci examined, the *trnQ*$^{(UUG)}$*–5′rps16* and *3′rps16–5′trnK*$^{(UUU)}$ intergenic spacers are the most variable, with the latter contributing the greatest total number of parsimony informative characters relative to its size. The North American genera *Atrema*, *Cynosciadium*, *Daucosma*, *Limnosciadium*, *Neogoezia*, *Oxypolis*, *Ptilimnium*, and *Trepocarpus* ally with the western hemispheric and Australasian genus *Lilaeopsis* in a strongly supported North American Endemics clade that is a sister group to a clade composed primarily of Old World taxa (*Berula* sensu lato, *Cryptotaenia*, *Helosciadium*, and *Sium*). *Oxypolis* and *Ptilimnium* are not monophyletic, with the rachis-leaved members of each comprising a clade separate from their compound-leaved congeners. Dispersal-vicariance analysis suggests that the ancestors of the North American Endemics clade probably originated in Canada and the USA or in a broader ancestral area including Mexico and South America.

Key words: Apiaceae, Oenantheae, cpDNA *psbI–5′trnK*$^{(UUU)}$, nrDNA ITS, phylogeny.

Résumé : Les auteurs ont examiné les relations intergénériques au sein des Apiaceae, tribu des Oenantheae, en utilisant les données de séquences provenant de l'ADN chloroplastique *psbl–5'trnK*$^{(UUU)}$ et les régions de l'espaceur interne transcrit de l'ADN ribosomal. Ils ont examiné 133 accessions, représentant l'ensemble des 17 genres de la tribu, soit environ la moitié des espèces. La région cpADN inclut quatre espaceurs intergéniques et l'intron *rps 16*, et ils ont analysé ces lieux non codants séparément pour évaluer leur utilité relative pour résoudre les relations. Les analyses séparées de parcimonie maximale de l'ensemble du *psbl–5′trnK*$^{(UUU)}$ et des régions ITS, chacune avec ou sans indels enregistrés, conduisent à des arbres concordants. Les phylogénies déduites des analyses de parcimonie maximale, bayésienne ou de probabilité maximale sur les séquences combinées des ADN nucléiques et chloroplastiques sur 82 accessions montrent une résolution robuste, bien supportée et constante. Parmi les cinq lieux non codants examinés, les espaceurs intergéniques *trnQ*$^{(UUU)}$*–5rps16* et 3'*rps16–5'trnK*$^{(UUU)}$ sont les plus variables, le dernier fournissant le plus grand nombre total de caractères informatifs de parcimonie, compte tenu de sa dimension. Les genres nord-américains *Atrema, Cynosciadium, Daucosma, Limnosciadium, Neogoezia*, *Axypolis*, *Ptilimnium*, et *Trepocarpus*, se regroupent avec le genre *Lilaeopsis* de l'hémisphère ouest et de l'Australie dans un clade endémique nord-américain fortement supporté soit un groupe sœur constituant d'un clade comprenant des taxons de l'Ancien Monde (*Berula* sensu lato, *Cryptotaenia*, *Helosciadium* et *Sium*). Les genres *Oxypolis* et *Ptilimnium* ne sont pas monophylétiques, les membres à feuilles pinnatiséquées de chacun comprenant un clade séparé de leurs congénères à feuilles composées. L'analyse de vicariance-dispersion suggère que les ancêtres du clade des endémiques nord-américaines viennent probablement du Canada et des États-Unis, ou encore d'une région ancestrale plus large incluant le Mexique et l'Amérique du Sud.

Mots-clés : Apiaceae, Oenantheae, ADNcp *psbl–5′trnK*$^{(UUU)}$, ITS ADNnr, phylogénie.

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Introduction

Early molecular systematic studies of the higher-level relationships within Apiaceae subfamily Apioideae revealed the *Oenanthe* clade of umbellifers as a strongly supported monophyletic group (Plunkett et al. 1996; Downie et al. 1998; Plunkett and Downie 1999). This clade was subsequently recognized as tribe Oenantheae Dumort. to include the genera *Berula*, *Cicuta*, *Cryptotaenia*, *Cynosciadium*, *Helosciadium*, *Lilaeopsis*, *Limnosciadium*, *Neogoezia*, *Oenanthe*, *Oxypolis*, *Perideridia*, *Ptilimnium*, and *Sium* (taxonomic authorities for all genera of tribe Oenantheae are provided in Table 1; Downie et al. 2000, 2001; Hardway 2001). The taxonomic history of these genera is extraordinarily complex (reviewed in Hardway et al. 2004). Dumortier (1827) described Oenantheae for the genera *Aethusa* L., *Coriandrum* L., and *Oenanthe*, defined by the presence of radiately ribbed fruits. This rather heterogeneous assemblage was not recognized by later authors, nor is it supported as monophyletic by molecular studies. Plants of tribe Oenantheae, as circumscribed by Downie et al. (2000, 2001), share several attributes, such as glabrous stems and leaves, clusters of fibrous or tuberous roots, globose to broadly ovate, corky-thickened fruits, and a preference for damp, marshy, or truly aquatic habitats. Some species have a simplified vegetative morphology, where the leaves are linear, hollow, and transversely septate, apparently being derived from the rachis of a formerly pinnately compound leaf (Affolter 1985). While the tribe is strongly supported as monophyletic through molecular studies, there are no obvious morphological synapomorphies that are expressed in all of its members and all of the aforementioned features can be found in genera outside of the group (Petersen et al. 2002).

Later molecular systematic studies ascertained the limits of tribe Oenantheae by considering additional taxa whose morphologies or previous taxonomic placements (as indicated by their synonymies) suggested possible close affinities with those genera already included in the tribe. As a result, tribe Oenantheae was expanded to include *Afrocarum*, *Daucosma*, and *Trepocarpus* (Hardway et al. 2004). The East Asian species *Perideridia neurophylla* (Maxim.) T.I. Chuang & Constance, previously referable to *Pterygopleurum* Kitag., was removed from the tribe, rendering the genus *Perideridia* monophyletic and exclusively North American in distribution (Downie et al. 2004). The genus *Bifora* Hoffm., represented by the North American species *Bifora americana* (DC.) Benth. & Hook. f. ex S. Watson, was included in the tribe on the basis of *matK* sequences (Plunkett et al. 1996), but this placement was called into question when its congeners, including the nomenclatural type of the genus, were placed elsewhere (Downie et al. 1998; Plunkett and Downie 1999). Hardway et al. (2004) confirmed the separation of *B. americana* from its Eurasian congeners and its inclusion in tribe Oenantheae under the available name *Atrema americanum* DC. *Cryptotaenia* also has members that fall outside of the tribe, but its type, *Cryptotaenia canadensis* (L.) DC., is maintained within Oenantheae (Hardway et al. 2004; Spalik and Downie 2007). In total, 17 genera are recognized in tribe Oenantheae, six of which are monotypic (Table 1). Six of these genera occur exclusively within eastern and (or) south-central USA (*Atrema*, *Cynosciadium*, *Daucosma*, *Limnosciadium*, *Ptilimnium*, and *Trepocarpus*), two others (*Oxypolis* and *Perideridia*) have a greater distribution in the USA and Canada, and one (*Neogoezia*) is endemic to Mexico.

To date, phylogenetic analyses of tribe Oenantheae have been based almost exclusively upon nuclear ribosomal DNA internal transcribed spacer (ITS) sequence comparisons and restricted to either a preliminary study of intergeneric relationships of primarily Eurasian and African taxa (Hardway et al. 2004) or in-depth studies of specific oenanthoid genera (*Perideridia*, Downie et al. 2004; *Cicuta*, Lee and Downie 2006; *Berula* and *Sium*, Spalik and Downie 2006; *Cryptotaenia*, Spalik and Downie 2007; *Oxypolis* and *Ptilimnium*, Feist and Downie 2008). In the study by Hardway et al. (2004), only single exemplars of each genus native to the USA were considered, with these allying with *Neogoezia* and *Lilaeopsis* in a well supported North American Endemics clade. High rates of ITS sequence divergence among the members of this clade and its small sample size, however, precluded an accurate appraisal of relationships. Moreover, the phylogenetic placement of this clade vis-à-vis *Cicuta*, *Oenanthe*, and *Oxypolis* could not be ascertained because of conflicting tree topologies based on different methods of analyses and overall weak bootstrap (BS) support. To elucidate phylogenetic relationships within the North American Endemics clade and to ascertain its phylogenetic position within the tribe, molecular data from the more conservatively evolving chloroplast genome and denser taxonomic sampling were necessary.

The major objective of this study is to estimate intergeneric phylogenetic relationships within Apiaceae tribe Oenantheae using molecular data, with emphasis on its North American members. We utilize the cpDNA *psbI–5′trnK*$^{(UUU)}$ region (hereinafter, called *psbI–trnK*), a region comprising five noncoding loci (*psbI–psbK* intergenic spacer, *psbK–trnQ*$^{(UUG)}$ intergenic spacer, *trnQ*$^{(UUG)}$*–5′rps16* intergenic spacer, *rps16* intron, and *3′rps16–5′trnK*$^{(UUU)}$ intergenic spacer). Over the past decade, the group II *rps16* intron has been used increasingly in phylogenetic studies of both Apiaceae and other angiosperms (reviewed in Kelchner 2002), but its flanking spacer regions have been rarely considered for such a purpose (for exceptions see Hahn 2002, Lee and Downie 2006, Calviño and Downie 2007, and Calviño et al. 2008). Intergenic spacers are under less functional constraints than coding or intron regions and, therefore, should provide greater levels of variation for phylogenetic analysis (Learn et al. 1992). We examine the relative efficacy of these noncoding loci for phylogenetic inference within the tribe and compare the results obtained from phylogenetic analyses of the entire *psbI–trnK* region to those obtained using ITS sequences across a comparable set of taxa. Elucidating the phylogeny of tribe Oenantheae enables hypotheses on the biogeography of its constituent lineages. Therefore, as an additional objective, we reconstruct the biogeographic history of the tribe using dispersal-vicariance analysis (Ronquist 1997), with the primary purpose of confirming the North American origin of what has been previously called the North American Endemics clade (Hardway et al. 2004).

Table 1. Genera of Apiaceae tribe Oenantheae and their geographic distributions.

Genus	No. of species	Distributional range
Afrocarum Rauschert	1	Tropical Africa
Atrema DC.	1	USA: Ark., Okla., Tex.
Berula W.D.J. Koch	1	Africa, Asia, Europe, North America, Mexico, Central America (Guatemala)
Cicuta L.	4	Asia, Europe, North America, Mexico
Cryptotaenia DC.	4	Asia, Europe, North America
Cynosciadium DC.	1	USA: Ala., Ark., Ill., La., Mo., Miss., Okla., Tenn., Tex.
Daucosma Engelm. & A. Gray ex A. Gray	1	USA: N. Mex., Tex.
Helosciadium W.D.J. Koch	5	Asia, Europe, Africa
Lilaeopsis Greene	15	North America, Mexico, South America, Australasia
Limnosciadium Mathias & Constance	2	USA: Ark., Ill., Iowa, Kans., La., Mo., Miss., Okla., Tex.
Neogoezia Hemsl.	5	Mexico
Oenanthe L.	40	Africa, Asia, Europe, North America, Australasia
Oxypolis Raf.	7	North America
Perideridia Rchb.	13	North America
Ptilimnium Raf.	5	USA: primarily southeastern states
Sium L.	8	Asia, Europe, North America
Trepocarpus Nutt. ex DC.	1	USA: Ala., Ark., Fla., Ga., Ill., Ky., La., Miss., Mo., Okla., S. C., Tenn., Tex.

Note: Species numbers are after Pimenov and Leonov (1993), except as follows: *Atrema* (Hardway et al. 2004); *Berula* and *Sium* (Spalik and Downie 2006); *Cicuta* (Lee and Downie 2006); *Cryptotaenia* (Spalik and Downie 2007); *Cynosciadium* (Kartesz 1996); *Helosciadium* (A.C. Ronse, Z.A. Popper, J.C. Preston, and M.F. Watson, Royal Botanic Garden Edinburgh, unpublished data, 2008); and *Lilaeopsis* (Affolter 1985; Petersen and Affolter 1999; Bone 2007). Geographic distributions are from (*i*) the aforementioned references; (*ii*) USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network – (GRIN) [online], National Germplasm Resources Laboratory, Beltsville, Maryland, available from www.ars-grin.gov/cgi-bin/npgs/html/gnlist.pl?77 [accessed 3 November 2007]; or (*iii*) USDA, NRCS, The PLANTS database [online], National Plant Data Center, Baton Rouge, Louisiana available from plants.usda.gov [accessed 3 November 2007].

Materials and methods

Taxa and outgroup selection

One hundred and thirty-one accessions were examined (Table 2). These accessions represent all 17 genera of tribe Oenantheae and approximately one-half of its species (Table 1). Included as outgroups in the cpDNA phylogenetic analyses were two genera (four species) of tribe Selineae, *Selinum* L. and *Seseli* L. For the genera *Berula*, *Cicuta*, *Cryptotaenia*, *Perideridia*, and *Sium*, only a few species or intraspecific taxa of each were included, because their intrageneric relationships have been considered elsewhere with greater sampling (Downie et al. 2004; Lee and Downie 2006; Spalik and Downie 2006, 2007). Molecular systematic studies of *Lilaeopsis* and *Oenanthe* are in progress, hence sampling of these genera was also reduced (Bone 2007; K. Spalik and S. Downie, unpublished data, 2008). Otherwise, sampling of all other genera was comprehensive or nearly so. Eighty-six accessions were examined for cpDNA sequence variation and, of these, data for 76 are new. A total of 127 accessions was used in the analysis of ITS sequences, of which 68 were obtained specifically for this study. Eighty-two accessions were common to both cpDNA and ITS data sets.

The trees derived from phylogenetic analyses of ITS and combined ITS and cpDNA data sets were rooted with *Perideridia*, as previous studies of both nuclear and plastid markers supported a sister group relationship between *Perideridia* and a clade comprised of all other oenanthoid taxa (Plunkett et al. 1996; Downie et al. 1998, 2000; Plunkett and Downie 1999). The same sister group relationship was supported in this study based on cpDNA *psbI–trnK* sequences. The cpDNA-derived trees were rooted with *Selinum* and *Seseli* from tribe Selineae of the Apioid superclade (Plunkett and Downie 1999). The sister group to tribe Oenantheae has yet to be determined through previous molecular phylogenetic studies, although these results suggest it is likely a member of the Apioid superclade or a closely allied tribe, such as tribe Pleurospermeae (Downie et al. 2001). When *Physospermum cornubiense* (L.) DC. of tribe Pleurospermeae was used as an outgroup in the cpDNA analyses (results not shown), relationships within Oenantheae were identical to those inferred when members of Selineae were used to root the trees.

The name North American Endemics clade was coined to accommodate a well supported, monophyletic group of eight species native to North America: *Atrema americanum*, *Cynosciadium digitatum* DC., *Daucosma laciniatum* Engelm. & A. Gray, *Lilaeopsis occidentalis* J.M. Coult. & Rose, *Limnosciadium pinnatum* (DC.) Mathias & Constance, *Neogoezia minor* Hemsl., *Ptilimnium capillaceum* (Michx.) Raf., and *Trepocarpus aethusae* Nutt. ex DC. (Hardway et al. 2004). Six of these species are distributed exclusively in the USA; *Neogoezia minor* is endemic to Mexico. *Lilaeopsis occidentalis* is almost entirely confined to the Pacific coast of North America, whereas the genus itself is distributed more widely in the temperate regions of North and South America, with outlying species in Australia, New Zealand, Mauritius, and elsewhere (Affolter 1985; Petersen and Affolter 1999). There are, however, other species of tribe Oenantheae endemic to North America, such as all species of *Oxypolis* and all but one species of *Cicuta*, but their relationships to the North American Endemics clade have been heretofore unclear.

Table 2. One hundred and thirty-one accessions of Apiaceae tribe Oenantheae and outgroups from which cpDNA and (or) nuclear rDNA ITS sequence data were obtained, with corresponding DNA accession and GenBank reference numbers and voucher information.

Accession	DNA accession No.	Voucher information	GenBank reference No.
Afrocarum imbricatum (Schinz) Rauschert	K132	Tanzania, Iringa, Mufindi District, Igowole, *Kayombo & Kayombo 217* (MO 04672352)	ITS: AY360228
Atrema americanum DC.	1160	USA, Texas, Williamson Co., junction of US Hwy. 183 and TX Hwy. 29, 6 June 1960, *Barclay & Perdue 785* (UC 184750)	cpDNA*: EF185206; ITS: EF177699
Atrema americanum	1467	USA, Texas, Williamson Co., 4 miles (1 mile = 1.609344 km) S of Jarrell on I-35, 18 May 1988, *Nesom & Grimes 6415* (MO 3691937)	cpDNA: EF185207; ITS: AY360232
Atrema americanum	1544	USA, Texas, San Saba Co., Texas Hwy. 16, 12.2 miles S of San Saba, 27 April 1970, *Flyr 1368* (MO 2290903)	ITS: EF177700
Atrema americanum	1560	USA, Texas, Burleson Co., along Farm Rd. 60, S of Snook, 28 April 1970, *Correll & Correll 38498* (MO 2379845)	cpDNA: EF185208; ITS: EF177701
Berula erecta (Huds.) Coville subsp. *erecta* var. *erecta*	150	Germany, cult. UIUC from seeds obtained from the University of Oldenburg Botanical Garden, *Downie 150* (ILL)	cpDNA*: EF185209; ITS: U79607
Berula erecta subsp. *erecta* var. *erecta*	251	France, cult. UIUC from seeds obtained from Conservatoire et Jardins botaniques de Nancy, *Downie 251* (ILL)	cpDNA*: EF185210; ITS: U79605
Berula erecta subsp. *erecta* var. *erecta*	2257	Denmark, Sjælland, near Tuel å, 18 July 2002, *Seberg OSA 486* (ILL)	cpDNA*: EF185211; ITS: AY360231
Berula erecta subsp. *erecta* var. *incisa* (Torr.) Cronquist	503	USA, California, *Raiche & Zadnik RR50099* (UC), cult. University of California Botanical Garden, Berkeley no. 85.0288	ITS: DQ005647
Berula erecta subsp. *erecta* "*B. orientalis* Schischk."	E115	Turkey, Adapazari, *Davis & Coode 36264* (E)	ITS: DQ005655
Berula erecta subsp. *thunbergii* (DC.) B.L. Burtt	799	Ethiopia, cult. University of California Botanical Garden, Berkeley, L. Constance pers. coll. C-2453	cpDNA*: EF185212; ITS: U78369
Cicuta douglasii (DC.) J.M. Coult. & Rose	2443	Canada, British Columbia, Vancouver Island, Prospect Lake, N of Victoria, 6 August 1979, *Munro 2256* (DAO 266753)	cpDNA*: DQ168963; ITS: AY524722
Cicuta maculata L. var. *angustifolia* Hook.	2441	Canada, Saskatchewan, Besnard Lake, near Narrows Channel Bridge, 25 June 1992, *Harms 40816* (DAO 749061)	cpDNA*: DQ168966; ITS: AY524729
Cicuta maculata var. *maculata*	1563	USA, South Carolina, Greenwood Co., Lake Greenwood, 28 July 1993, *Horn 7333* (ILLS 191135)	cpDNA*: DQ168969; ITS: AY524738
Cicuta virosa L.	75	Finland, cult. UIUC from seeds obtained from the Botanical Garden of the University of Joensuu, *Downie 75* (ILL)	cpDNA*: DQ168974; ITS: U78372
Cryptotaenia canadensis (L.) DC.	817	USA, Illinois, Champaign Co., Urbana, *Downie 817* (ILL)	cpDNA*: EF185213; ITS: U79613
Cryptotaenia canadensis	1566	USA, Illinois, Alexander Co., Shawnee National Forest, 23 June 1994, *Phillippe 24778* (ILLS 184330)	cpDNA*: EF185214; ITS: DQ516351
Cryptotaenia canadensis	1570	USA, Louiseana, Avoyelles Parish, W of I-49, S of LA-115, 14 June 1990, *Thomas 118904* (ILL)	cpDNA*: EF185215; ITS: DQ516353
Cryptotaenia canadensis	1971	USA, Illinois, Alexander Co., Shawnee National Forest, 23 June 1994, *Phillippe 24833* (ILLS 184630)	cpDNA*: EF185216; ITS: EF177702
Cryptotaenia japonica Hassk.	402	China, cult. UIUC from seeds obtained from Shanghai Botanical Garden, *Downie 402* (ILL)	cpDNA*: EF185217; ITS: AY360236
Cryptotaenia japonica	574	Japan, Honshu Island, Koyosan area, *McNamara et al. 90* (UC), cult. University of California Botanical Garden, Berkeley (no. 90.0891)	cpDNA*: EF185218; ITS: U78367
Cryptotaenia thomasii (Ten.) DC.	E121	Italy, Reggio di Calabria, *Brookes et al. 5710* (E 00043297)	ITS: DQ516348

Table 2 (*continued*).

Accession	DNA accession No.	Voucher information	GenBank reference No.
Cynosciadium digitatum DC.	1552	USA, Illinois, Jackson Co., Shawnee National Forest, 23 June 1993, *Phillippe et al. 22062* (ILLS 182781)	ITS: EF177703
Cynosciadium digitatum	1571	USA, Louisiana, Madison Parish, 1 mile E of Indian Lake, 28 May 1973, *Jones 215* (ILL)	cpDNA*: EF185219; ITS: EF177704
Cynosciadium digitatum	1804	USA, Illinois, Jackson Co., Shawnee National Forest, 27 May 1993, *Phillippe 21886* (ILLS 183947)	cpDNA*: EF185220; ITS: AY360237
Cynosciadium digitatum	1985	USA, Louisiana, Morehouse Parish, 5 miles W of Bonita, *Thomas 23279* (ILL)	ITS: EF177705
Cynosciadium digitatum	1986	USA, Arkansas, Lafayette Co., 4 miles E of Red River, Hwy. 82, 24 May 1993, *Sundell et al. 10500* (ILL)	cpDNA*: EF185221; ITS: EF177706
Cynosciadium digitatum	1988	USA, Illinois, Jackson Co., Shawnee National Forest, 23 June 1993, *Phillippe et al. 22133* (ILLS 183489)	ITS: EF177707
Cynosciadium digitatum	1990	USA, Illinois, Jackson Co., Shawnee National Forest, 27 May 1993, *Phillippe 21901* (ILLS 183392)	ITS: EF177708
Daucosma laciniatum Engelm. & A. Gray	2397	USA, Texas, Kerr Co., Kerrville, 26 June 1894, *Heller 1943* (MO 2535181)	ITS: AY360238
Helosciadium crassipes W.D.J. Koch ex Rchb.	K170	France, Corse, Musella, cult. Botanical Conservatory Mulhouse no. 2048A, *Herb. Reduron s.n.*	cpDNA: EF185222; ITS: AY360239
Helosciadium nodiflorum (L.) W.D.J. Koch	317	France, cult. UIUC from seeds obtained from Jardin botanique de Caen, *Downie 317* (ILL)	cpDNA*: EF185223; ITS: EF177709
Lilaeopsis attenuata (Hook. & Arn.) Fern. subsp. *attenuata*	2666	Argentina, Corrientes, Depto Mburucuyá, Estancia Santa Teresa; cult. University of Michigan Botanical Gardens, *Affolter 115* (MICH, GA)	ITS: EF177710
Lilaeopsis brasiliensis (Glaz.) Affolter	2153	Brazil, origin unknown, C. Casselmann, 1984, material from Gitte Petersen, *Petersen GLP3* (C)	cpDNA*: EF185224; ITS: EF177711
Lilaeopsis brasiliensis	2515	Brazil, Santa Catarina, between Matos Costa and Caçador; cult. University of Michigan Botanical Gardens, *Affolter 102* (MICH, GA)	ITS: EF177712
Lilaeopsis carolinensis J.M. Coult. & Rose	2148	USA, cultivated, origin unknown; Bogner s.n., 1985, material from Gitte Petersen, *Petersen GPL4* (C)	cpDNA*: EF185225; ITS: AF466276
Lilaeopsis carolinensis	2663	Argentina, Corrientes, Depto Mburucuyá, Estancia Santa Teresa; cult. University of Michigan Botanical Gardens, *Affolter 114* (MICH, GA)	ITS: EF177713
Lilaeopsis chinensis (L.) Kuntze	2401	USA, North Carolina, New Hanover Co., W bank of Cape Fear River, 31 May 1987, *MacDougal 2068* (MO 05033977)	ITS: EF177714
Lilaeopsis macloviana (Gand.) A.W. Hill	2518	Peru, Cuzco, 15 km S of Cuzco on road to Urcos; cult. University of Michigan Botanical Gardens, *Affolter 119* (MICH, GA)	ITS: EF177715
Lilaeopsis mauritiana G. Petersen & Affolter	2150	Mauritius, Le Val Nature Park, 3 May 1992, Windeløv s.n., material from Gitte Petersen, *Petersen GPL8* (C)	cpDNA*: EF185226; ITS: AF466277
Lilaeopsis novae-zelandiae (Gand.) A.W. Hill	2152	New Zealand, cultivated, material from Gitte Petersen, *Petersen GPL9* (C)	cpDNA*: EF185227; ITS: AF466278
Lilaeopsis occidentalis J.M. Coult. & Rose	1999	USA, Oregon, Douglas Co., East Gardiner, *Hill & Dutton 32982* (ILLS 203634)	cpDNA*: EF185228; ITS: AY360242
Lilaeopsis schaffneriana (Schltdl.) J.M. Coult. & Rose subsp. *recurva* (A.W. Hill) Affolter	2947	Mexico, Sonora, Los Fresnos Cienega, 32 miles N of Cananea, 23 June 1990, *Warren*, *Anderson, & Saucedo s.n.* (ARIZ 292307)	ITS: EF177716
Limnosciadium pinnatum (DC.) Mathias & Constance	1511	USA, Louisiana, Ouachita Parish, Ouachita Wildlife Management Area, 20 May 1987, *Thomas et al. 99586* (MO 3680921)	cpDNA*: EF185229; ITS: EF177717
Limnosciadium pinnatum	2000	USA, Illinois, Champaign Co., Champaign, *Hill 30580* (ILLS 198706)	ITS: AY360243

Table 2 (*continued*).

Accession	DNA accession No.	Voucher information	GenBank reference No.
Limnosciadium pinnatum	2385	USA, Louisiana, Jackson Parish, La 34, 0.3 miles N of La 4 in Chatham, 22 May 1987, *Thomas 99634* (MO 3680952)	ITS: EF177718
Limnosciadium pinnatum	2393	USA, Arkansas, Sebastian Co., Fort Chaffee Army Base, Butler's Knob, 27 May 1989, *Thompson & Johnson C0578* (MO 4272871)	ITS: EF177719
Limnosciadium pinnatum	2395	USA, Missouri, Stoddard Co., Otter Slough Conservation Area, 31 May 2000, *Brant et al. 4380* (MO 5186226)	cpDNA*: EF185230; ITS: EF177720
Limnosciadium pumilum (Engelm. & A. Gray) Mathias & Constance	3163	USA, Louisiana, Cameron Parish, Cameron Parish Rd. 421, just E of La. 384 and S of the Calcasieu parish line, 13 April 1984, *Dutton & Taylor 1219* (CAN 495041)	ITS: EF177721
Limnosciadium pumilum	3164	USA, Texas, Brazoria Co., West Columbia, 24 March 1914, *Palmer 5003* (MO 753988)	ITS: EF177722
Neogoezia breedlovei Constance	1551	Mexico, Jalisco, Municipio of Atenguillo, 14 km E of Los Volcanes on road from Ayutla to Talpa de Allende, 27 November 1983, *Breedlove & Almeda 60575* (UC 1518421)	cpDNA*: EF185231; ITS: EF177723
Neogoezia gracilipes (Hemsl.) Hemsl.	1545	Mexico, Michoacan, Puerto del Gato, 5 km N of Zitácuaro on Hwy. 15, 26 October 1983, *Anderson 13289* (MO 3751539)	ITS: EF177724
Neogoezia gracilipes	2269	Mexico, Guerrero, Municipio of Alcozauca, Cañada de "Mini-yaa," Rancho, 22 August 1989, *Rojas et al. 49* (UC 1587310)	ITS: EF177725
Neogoezia gracilipes	2270	Mexico, Oaxaca, Nochixtlán, N of La Joya, 2 October 1993, *Panero 3614* (UC 1611523)	cpDNA*: EF185232; ITS: EF177726
Neogoezia macvaughii Constance	K70	Mexico, Jalisco, 12 km NW of Los Volcanes, 30 October 1973, *Breedlove 35768* (MO 3238958)	ITS: DQ005662
Neogoezia macvaughii	2272	Mexico, Jalisco, 49 km W of Ayutla on road to Talpa, 21 September 1983, *Anderson 12748* (MO 3751540)	cpDNA*: EF185233; ITS: EF177727
Neogoezia minor Hemsl.	1518	Mexico, Oaxaca, Ixtlán a Valle Nacional, 5 August 1981, *Trigos et al. 942* (MO 3642085)	ITS: EF177728
Neogoezia minor	2138	Mexico, Oaxaca, Sierra de San Felipe between Oaxaca and Ixtlán de Juárez, 1 August 1963, *Molseed 278* (ISU 1060)	cpDNA: EF185234; ITS: AY360244
Neogoezia minor	2273	Mexico, Oaxaca, Llano Grande, Miahuatlán, 18 October 1995, *Hinton et al. 26184* (UC 1619345)	cpDNA*: EF185235; ITS: EF177729
Neogoezia minor	2274	Mexico, Oaxaca, Cerro San Felipe summit, 9 November 1983, *Breedlove & Almeda 59951* (UC 1518420)	cpDNA*: EF185236; ITS: EF177730
Neogoezia planipetala (Hemsl.) Hemsl.	K72	Mexico, Nayarit, Municipio of Nayar, 50 km NE of Jesus Maria, 13 September 1989, *Tenorio & Flores 16030* (MO 4036088)	ITS: DQ005663
Neogoezia planipetala	2275	Mexico, Nayarit, Municipio of El Nayar, Arroyo Santa Rosa W of Santa Teresa, 21 October 1979, *Breedlove 44576* (UC 1518419)	cpDNA*: EF185237; ITS: EF177731
Oenanthe aquatica (L.) Poir.	2255	Denmark, Fyn, Stævningen in Snarup Skov, 25 July 2002, *Petersen & Seberg GPL30* (C)	cpDNA*: DQ168946; ITS: EF177732
Oenanthe banatica Heuff.	476	Hungary, cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót, *Downie 476* (ILL)	cpDNA*: DQ168955; ITS: AY360245
Oenanthe crocata L.	40	Spain, cult. UIUC from seeds obtained from Real Jardín Botánico, *Downie 40* (ILL)	cpDNA*: DQ168953; ITS: AY360246
Oenanthe peucedanifolia Pollich	1282	Germany, cult. UIUC from seeds obtained from Karl-Marx University, Leipzig, *Lee 24* (ILL)	cpDNA*: DQ168956; ITS: AY360250
Oenanthe pimpinelloides L.	29	Germany, cult. UIUC from seeds obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, *Downie 29* (ILL)	cpDNA*: DQ168950; ITS: AY360251
Oenanthe sarmentosa J. Presl ex DC.	521	USA, California, San Mateo Co., *Plunkett 1308* (WS)	cpDNA*: DQ168947; ITS: AY360252

Table 2 (*continued*).

Accession	DNA accession No.	Voucher information	GenBank reference No.
Oxypolis canbyi (J.M. Coult. & Rose) Fern.	2743	USA, South Carolina, Orangeburg Co., E side of US Hwy. 21, ca. 2 miles N of Branchville, 13 September 1985, *Nelson 4301* (NCU 538037)	cpDNA: EF185238; ITS: EF177733
Oxypolis fendleri (A. Gray) Heller	915	USA, Colorado, Rio Blanco Co., Rough Creek, 4 August 1991, *Vanderhorst 3759* (RM 616920)	ITS: AY360253
Oxypolis fendleri	2369	USA, Colorado, Chafee Co., CO Hwy. 306, 14 miles W of Buena Vista, 2 August 1973, *Haber & Given 2049* (CAN 370800)	cpDNA: EF185239; ITS: EF177734
Oxypolis fendleri	2370	USA, Wyoming, Carbon Co., Battle Creek, 15 July 1966, *Porter & Porter 10218* (DAO 456446)	ITS: EF177735
Oxypolis filiformis (Walter) Britt.	2371	USA, Louisiana, Vernon Parish, E of Drake's Creek, ca. 2 miles E of Johnsville Church and LA Hwy. 10, Kisatchie National Forest, 7 September 1987, *Thomas 101486* (DAO 574521)	cpDNA: EF185240; ITS: EF177736
Oxypolis filiformis	2713	USA, Florida, Alachua Co., Gainesville, 9 September 1987, *Alcorn 155* (FLAS 166610)	cpDNA: EF185241; ITS: EF177737
Oxypolis greenmanii Mathias & Constance	2717	USA, Florida, Bay Co., 1 miles N of US 98 on Tyndall Air Force Base, 15 September 1979, *Judd & Perkins 2439* (FLAS 174274)	ITS: EF177738
Oxypolis greenmanii	2714	USA, Florida, Bay Co., Along US Hwy. 231, 1.8 miles N of the junction with FL Rt. 388, 29 August 1980, *Judd & Perkins 2714* (FLAS 174297)	cpDNA: EF185242; ITS: EF177739
Oxypolis occidentalis J.M. Coult. & Rose	1142	USA, California, El Dorado County, Osgood Swamp, 3 August 1982, *Follette s.n.* (JEPS 82187)	cpDNA*: EF185243; ITS: AY360254
Oxypolis occidentalis	1153	USA, California, Fresno Co., Wishon Reservoir Dam, *Call 2455* (UC 282880)	cpDNA*: EF185244; ITS: EF177740
Oxypolis rigidior (L.) Raf.	1927	USA, Illinois, Vermilion Co., Windfall Hill Prairie Nature Preserve, *Phillippe et al. 19411* (ILLS 177487)	cpDNA: EF185245; ITS: AY360255
Oxypolis rigidior	1963	USA, Illinois, Lake Co., 1977, *Robertson & Moran 104* (ILLS 159308)	ITS: EF177741
Oxypolis rigidior	1964	USA, Illinois, McHenry Co., 1977, *Robertson 1505* (ILLS 162300)	ITS: EF177742
Oxypolis rigidior	1998	USA, Louisiana, Winn Parish, along LA Hwy. 126, 1.2 miles E of Jct. LA Hwy. 1233, Kisatchie National Forest, 20 September 1981, *Kessler 1877* (ILL)	cpDNA*: EF185246; ITS: EF177743
Oxypolis rigidior	2003	USA, Illinois, Lake Co., 1981, *Robertson 2640* (ILLS 166045)	cpDNA*: EF185247; ITS: EF177744
Oxypolis ternata (Nutt.) A. Heller	2735	USA, South Carolina, Horry Co., 3.8 miles S of Socastee, 25 October 1970, *Massey & Thomas 3480* (NCU 422851)	cpDNA: EF185248; ITS: EF177745
Oxypolis ternata	2738	USA, North Carolina, Pender Co., Holly Shelter Game Land, 3 October 1997, *Horn & Dirig 362* (DUKE 363865)	cpDNA: EF185249; ITS: EF177746
Perideridia americana (Nutt. ex DC.) Rchb.	1938	USA, Illinois, Shelby Co., NE of Assumption, 2 June 1981, *Shildneck 12868* (ILL)	cpDNA: EF185250; ITS: AY246910
Perideridia kelloggii (A. Gray) Mathias	778	USA, California, Sonoma Co., King Ridge Rd, 5 miles N of Cazadero, *Ornduff et al. s.n.* (UC), cult. University of California Botanical Garden, Berkeley (no. 81.0521)	cpDNA*: EF185251; ITS: U78373
Ptilimnium ahlesii Weakley & G.L. Nesom	2648	USA, South Carolina, Berkeley Co., Cooper River at the mouth of Durham Creek, 7 June 1990, *McAninch 23* (NCU 557199)	cpDNA: EF185252; ITS: EF177747
Ptilimnium capillaceum (Michx.) Raf.	2701	USA, Virginia, Lancaster Co., Bellwood Marsh, S of Rt. 3 bridge, 22 July 1994, *Weldy 849* (BRIT)	ITS: EF177748
Ptilimnium costatum (Elliott) Raf.	1646	USA, Illinois, Jackson Co., Shawnee National Forest, 20 September 1989, *Stritch 2159* (ILLS 172136)	cpDNA*: EF185253; ITS: EF177749
Ptilimnium costatum	1970	USA, Illinois, Jackson Co., Shawnee National Forest, 11 September 1989, *Stritch 2124* (ILLS 172160)	cpDNA*: EF185254; ITS: EF177750
Ptilimnium costatum	1981	USA, Louisiana, Natchitoches Parish, LA Hwy. 479 at Strange Rd. W of Goldonna in Kisatchie National Forest, 14 August 1989, *Thomas & Bell 112081* (ILL)	cpDNA*: EF185255; ITS: EF177751

Table 2 (*continued*).

Accession	DNA accession No.	Voucher information	GenBank reference No.
Ptilimnium costatum	2402	USA, Missouri, Wayne Co., Hattie's Ford Fen Area, 12 October 2001, *Brant 4857* (MO 5573699)	cpDNA*: EF185256; ITS: EF177752
Ptilimnium nodosum (Rose) Mathias	2784	USA, South Carolina, Aiken Co., Aiken, *Kress SC-7–4* (US)	cpDNA: EF185257; ITS: EF177753
Ptilimnium nodosum	2787	USA, Maryland, *Kress MG-4* (US)	cpDNA: EF185258; ITS: EF177754
Ptilimnium nuttallii (DC.) Britt.	1507	USA, Louisiana, Morehouse Parish, Tillou Baptist Church Cemetery, *Thomas 144426* (RM)	ITS: EF177755
Ptilimnium nuttallii	2165	USA, Oklahoma, Rogers Co., Claremore, 12 June 1974, *Jones 3030* (ILL)	cpDNA*: EF185259; ITS: AY360256
Ptilimnium nuttallii	2403	USA, Arkansas, St. Francis Co., by I-40, 11 miles E of Wheatley, 20 June 1976, *Kral 58316* (MO 05057831)	ITS: EF177756
Ptilimnium nuttallii	2405	USA, Mississippi, Monroe Co., ca. 3 miles W of Aberdeen, 6 June 1996, *MacDonald 9514* (MO 05082318)	ITS: EF177757
Ptilimnium nuttallii	2617	USA, Arkansas, Ashley Co., ca. 2.6 miles S of Hwy. 8 near Beech Creek, SE of Hamburg, 20 June 1986, *Thomas 97154* (WVA 114836)	cpDNA: EF185260; ITS: EF177758
Ptilimnium nuttallii	2623	USA, Illinois, Randolph Co., W of Sparta, 16 July 2003, *Feist 2510* (ILLS)	cpDNA: EF185261; ITS: EF177759
Selinum broteri Hoffmanns. & Link	1866	France, Morbihan, Guillac, cult. Botanical Conservatory Mulhouse no. 99155A, 2 August 2001, *Hildenbrand, Meyer & Reduron s.n.* (ILL)	cpDNA: EF185262
Selinum carvifolia (L.) L.	1865	France, Bas-Rhin, between Herbsheim and Boofzheim, 14 August 2001, *Reduron s.n.* (ILL)	cpDNA: EF185263
Selinum pyrenaeum Gouan	1867	France, Haut-Rhin, Vosges, Markstein, 24 July 2001, *Reduron s.n.* (ILL)	cpDNA: EF185264
Seseli tortuosum L.	1874	Portugal, Lisboa, Sintra Praja das Macas, cult. Botanical Conservatory Mulhouse no. 98042, 2 August 2001, *Hildenbrand, Meyer & Reduron s.n.* (ILL)	cpDNA: EF185265
Sium bracteatum (Roxb.) Cronk	K177	St. Helena, material provided by V. Williams (WA)	ITS: AY353982
Sium latifolium L.	1632	France, Bas-Rhin, Hultenheim, cult. Botanical Conservatory Mulhouse no. 9466, *Herb. Reduron s.n.*	cpDNA: EF185266; ITS: AY360257
Sium latifolium	2256	Denmark, Sjælland, Bromme Lillesø, 25 July 2002, *Petersen & Seberg GPL31* (C)	cpDNA*: EF185267; ITS: AY360258
Sium medium Fisch. & C.A. Mey.	2809	Kyrgyzstan, Kotshkor, *Konnov & Kotshgareva 456* (LE)	cpDNA: EF185268; ITS: DQ005674
Sium ninsi L.	K122	Japan, Tohoku distr., *Iwasaki 127* (MO 4253273)	ITS: DQ005678
Sium repandum Welw. ex Hiern	K61	South Africa, Transvaal, Kaapsche Hoop, *Rogers 9101* (G)	ITS: AY353977
Sium serra (Franch. & Sav.) Kitag.	K123	Japan, Honshu, *Tateishi et al. 14776* (MO 3883493)	ITS: DQ005681
Sium sisaroideum DC.	E132	Turkey, A9 Kars, *Davis 46661* (E)	ITS: DQ005688
Sium sisarum L.	53	Spain, cult. UIUC from seeds obtained from Real Jardín Botánico, *Downie 53* (ILL)	cpDNA*: EF185269; ITS: AY360261
Sium sisarum	83	Finland, cult. UIUC from seeds obtained from the Botanical Garden of the University of Joensuu, *Downie 83* (ILL)	cpDNA*: EF185270; ITS: AY360262
Sium sisarum	97	Hungary, cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót, *Downie 97* (ILL)	cpDNA*: EF185271; ITS: U78370
Sium sisarum	311	France, cult. UIUC from seeds obtained from Jardin botanique de Caen, *Downie 311* (ILL)	cpDNA*: EF185272; ITS: AY360259

Table 2 (*concluded*).

Accession	DNA accession No.	Voucher information	GenBank reference No.
Sium sisarum	388	Canada, Montréal, cult. UIUC from seeds obtained from Jardin botanique de Montréal, *Downie 388* (ILL)	cpDNA*: EF185273; ITS: AY360260
Sium suave Walter	12	Canada, Montréal, cult. UIUC from seeds obtained from Jardin botanique de Montréal, *Downie 12* (ILL)	cpDNA*: EF185274; ITS: AY360263
Sium suave	1494	USA, Illinois, Vermilion Co., 1991, *Morris et al. 849* (ILLS 182643)	cpDNA: EF185275; ITS: DQ005689
Sium suave	1815	USA, Illinois, Cook Co., 1998, *Feist 77* (ILLS 194650)	cpDNA: EF185276; ITS: DQ005694
Sium suave	1965	USA, Illinois, Marion Co., 1987, *Smith 1404-b* (ILLS 175302)	cpDNA: EF185277; ITS: DQ005695
Sium tenue Kom.	K63	Russia, Siberia, Primorje, *Ulanova 5981* (G 234160)	cpDNA: EF185278; ITS: DQ005706
Trepocarpus aethusae Nutt. ex DC.	1557	USA, Florida, Gadsden Co., S of US Hwy. 90 and W of Chattahoochee, 26 May 1976, *Leonard 6289* (MO 2388014)	ITS: EF177760
Trepocarpus aethusae	1660	USA, Illinois, Saline Co., US Rt. 45, E of Harrisburg levee, 7 July 1999, *Hill 31876* (ILLS 201642)	cpDNA*: EF185279; ITS: EF177761
Trepocarpus aethusae	1817	USA, Illinois, Alexander Co., Horseshoe Lake Conservation Area, 8 July 1996, *Basinger 10891* (ILLS 194558)	cpDNA: EF185280; ITS: AY360264
Trepocarpus aethusae	2129	USA, Alabama, Sumter Co., Emelle, 30 May 1972, *Kral 46903* (MO 4040089)	ITS: EF177762
Trepocarpus aethusae	2130	USA, Louiseana, Assumption Parrish, IFCO Pipe Company, 16 June 1991, *Thomas & Allen 124008* (MO 4028326)	cpDNA*: EF185281; ITS: EF177763
Trepocarpus aethusae	2131	USA, Missouri, Butler Co., off Hwy. 142, E of Cane Creek, 29 July 1993, *Hudson 79* (MO 4400853)	ITS: EF177764
Trepocarpus aethusae	2132	USA, Missouri, Mississippi Co., 5 miles SE of East Prairie, 13 May 1992, *Summers et al. 4946* (MO 4277374)	ITS: EF177765
Trepocarpus aethusae	2386	USA, Missouri, Dunklin Co., Warbler Woods Conservation Area, SE of Kennett, 8 June 1998, *Summers & Yatskievych 8622* (MO 04900271)	ITS: EF177766

Note: Eighty-six accessions were included in the cpDNA study; 57 of these were sequenced for the entire cpDNA *psbI–trnK* region (asterisks) and 29 were sequenced for the *rps16* intron – *trnK* region only. UIUC, University of Illinois at Urbana–Champaign Plant Sciences Greenhouse.

Experimental strategy

Total genomic DNAs were extracted from herbarium specimens or greenhouse-cultivated plants using a DNeasy Plant Mini Kit (QIAGEN, Valencia, Calif.). The strategies used to obtain ITS and cpDNA sequence data are presented elsewhere (Downie and Katz-Downie 1996, 1999; Spalik and Downie 2006; Lee and Downie 2006). Data were obtained for the complete ITS region (ITS 1, 5.8S rDNA, ITS 2) using a single pair of primers. The region bounded by and including chloroplast genes *psbI* and *5′trnK*$^{(UUU)}$ is 4137 base pairs (bp) in size in tobacco (Shinozaki et al. 1986). This region includes four intergenic spacers (designated herein as *psbI–psbK*, *psbK–trnQ*, *trnQ–rps16*, and *rps16–trnK*; Fig. 1) and the *rps16* intron, with the sizes of these noncoding loci varying from 347 to 1204 bp in tobacco. Twenty primers designed during our previous phylogenetic studies of *Cicuta* (Lee and Downie 2006) and other Apiaceae (Downie and Katz-Downie 1999) were used to obtain both forward and reverse sequences (Fig. 1). The entire *psbI–trnK* region was sequenced for 57 accessions (indicated by asterisks in Table 2), representing all genera of Oenantheae except the monotypic *Afrocarum* and *Daucosma*. For 29 additional accessions, data are presented for the *rps16* intron – *trnK* region only because of technical difficulties in obtaining these *psbI–rps16* data, DNAs no longer available, or consideration of these data in ongoing phylogenetic studies (M.A. Feist and S. Downie, unpublished data, 2008). All sequences obtained in this study have been deposited with GenBank (Table 2).

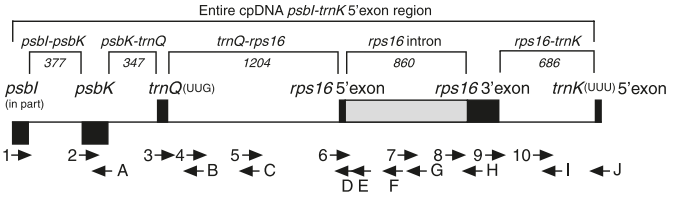
Sequence comparisons and phylogenetic analyses

Nucleotide sequences of the ITS and cpDNA regions were each aligned initially using the default pairwise and multiple alignment parameters in Clustal X (gap opening cost = 15.00, gap extension cost = 6.66, DNA transition weight = 0.50; Jeanmougin et al. 1998) then rechecked and adjusted manually, as necessary. Gaps were positioned to minimize nucleotide mismatches. Unambiguous gaps were scored as presence/absence characters using the simple indel coding method of Simmons and Ochoterena (2000). Gaps of equal length in more than one sequence were coded as the same presence or absence character state if they could not be interpreted as different duplication or insertion events. Indels of similar location but with different lengths were coded as different binary characters. Characteristics of the aligned sequences were obtained for the ITS region, each of the cpDNA intergenic spacers and intron, and the entire cpDNA *psbI–trnK* region. The latter includes the five noncoding loci plus genes *psbI* (in part), *psbK*, *trnQ*, and *rps16*. Ambiguously aligned regions were excluded from the analyses. Uncorrected pairwise nucleotide distances were calculated by PAUP* version 4.0b10 (Swofford 2002). Relative rate tests were implemented using the program RRTree version 1.1 (Robinson-Rechavi and Huchon 2000) to detect rate asymmetries of combined cpDNA and ITS regions among major clades of tribe Oenantheae.

For 29 accessions used in the analysis of the entire cpDNA *psbI–trnK* region, the portion of the matrix representing *psbI* through *rps16* 5′exon was scored as missing because it was not sequenced in these taxa. Six other smaller regions of the cpDNA sequence alignment (ranging from 42 to 668 positions) were also scored as missing because of technical difficulties in obtaining high quality sequences from these regions. Specifically, within the *rps16–trnK* intergenic spacer region, 42–500 sequence positions were scored as missing for four accessions of *Oxypolis* (Nos. 2371, 2713, 2714, and 2743; Table 2). Within the *trnQ–rps16* spacer, data for 668 alignment positions were scored as missing for *Neogoezia macvaughii* (accession No. 2272), and within the *psbK–trnQ* spacer, data for 463 alignment positions were unavailable for *Atrema americanum* (accession No. 1160).

As a means of data exploration and to assess the relative utility of the cpDNA data partitions for resolving phylogenetic relationships within Oenantheae, each of the five noncoding loci was analyzed independently using maximum parsimony (MP), as implemented by PAUP*. The results of analysis of each data partition were compared against those major clades inferred from MP analysis of sequences from the entire cpDNA *psbI–trnK* region plus binary-scored alignment gaps (because analysis of the latter matrix yielded

Fig. 1. Map of the 4137 bp locus of tobacco cpDNA (Shinozaki et al. 1986) showing the relative positions of genes *psbI*, *psbK*, *trnQ*, *rps16*, and *trnK* (in part). This map is modified slightly from that presented in Lee and Downie (2006). The gene *rps16* is interrupted by an intron and only the 5′exon of gene *trnK* is shown. The sizes of the four intergenic spacers and intron are presented in base pairs. The six cpDNA fragments used in the phylogenetic analyses are indicated by brackets. 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(“Berularps16-2REV”);
F, TAAACGCTCGATTCCCGYGYGATA(“L-4, Lilaeopsis For2”);
G, AATGGCGTTTCCTTGTTC (“rps16-CR”);
H, TCGGGATCGAACATCAATTGCAAC (“3′exon(rps16)”);
I, GTTCGATACACTGTTGTC (“trnK-1R”);
J, TACTCTACCGTTGAGTTAGC (“trnK”).



trees of greatest resolution and highest BS support overall). Comparisons were made of the number of major clades recovered in each of these analyses and their corresponding BS support values (Felsenstein 1985). Additional comparative data included the numbers of parsimony informative (PI) nucleotide positions and indels, maximum uncorrected pairwise sequence divergence estimates, the numbers and lengths of maximally parsimonious trees (MPTs), and measures of character fit. The total number of PI characters for each data partition was calculated by summing the numbers of PI nucleotide positions and PI indels. In comparing the consistency and retention indices of each cpDNA data partition (CI and RI, respectively), each group of characters was optimized onto the most parsimonious trees inferred from analysis of the entire cpDNA *psbI–trnK* plus indels matrix for a comparable set of taxa. MP analyses of the "Entire cpDNA," "ITS," and "Entire cpDNA + ITS" data sets were carried out with and without binary-scored indels as additional characters. Prior to combining the cpDNA and ITS data for simultaneous consideration, the incongruence length difference test of Farris et al. (1995) was performed using the partition-homogeneity test of PAUP* to examine the extent of conflict between the data sets. This test was executed with 100 replicate analyses, using the heuristic search option, simple stepwise addition of taxa, tree bisection–reconnection (TBR) branch swapping, and a MaxTrees setting of 15 000. The examination of potential conflict among the plastid genome data sets was not done because these loci occur on a nonrecombinant chromosome and are inherited as a single linkage group.

Heuristic MP searches were conducted for each data matrix using 100 replicate analyses, random stepwise addition of taxa, TBR branch swapping, and saving multiple trees with, initially, no set tree limit. Characters were treated as unordered and equally weighted; gap states were treated as missing data. For initial searches resulting in more than 20 000 trees, the analyses were repeated using the heuristic search strategies employed by Calviño et al. (2006) to ensure that the shortest trees have been found, even though the exact number of trees at that length is not known. BS values were calculated from 100 replicate analyses using TBR branch swapping and simple stepwise addition of taxa (for some analyses, the MaxTrees option was set to 15 000). The number of additional steps required to force particular taxa into a monophyletic group was examined using the constraint option of PAUP*.

Maximum likelihood (ML) analyses of nucleotide characters from the matrix of combined cpDNA *psbI–trnK* and ITS sequences were subsequently carried out. Modeltest version 3.7 (Posada and Crandall 1998) and the Akaike Information Criterion (AIC) estimator (Posada and Buckley 2004) were used to select the best-fit likelihood model for analysis. The parameter estimates appropriate for the chosen model were input into PAUP* and a heuristic search performed using 10 random addition sequence replicates and TBR branch swapping under ML optimization. One thousand BS replicate analyses were conducted using neighbor-joining searches with ML distance estimates, using the ML parameters estimated by Modeltest.

The matrix of combined *psbI–trnK* and ITS sequence data was also subjected to a Bayesian analysis using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Prior to analysis, MrModeltest version 2.2 (Nylander 2004) was used to select an evolutionary model of nucleotide substitution that best fits these data, as selected by the AIC estimator. The settings appropriate for the best-fit model were put into a MrBayes block in PAUP* and the priors on state frequencies and rates and variation across sites were estimated automatically from the data assuming no prior knowledge about their values. Starting trees were chosen at random and one million generations were run with sampling occurring every 100 generations. Seven hundred and fifty trees were discarded (as "burn-in") before stationarity was reached, prior to determining the posterior probability (PP) values from the remaining trees.

Biogeography

To reconstruct the optimal distributions of the ancestors of the North American Endemics clade, a dispersal vicariance analysis (DIVA) was carried out with the program DIVA version 1.1 (Ronquist 1996) using its optimize command and default option settings. A simplified, fully resolved tree of generic relationships within tribe Oenantheae, inferred from results of phylogenetic analyses of combined cpDNA *psbI–trnK* and nrDNA ITS sequences, was used to infer the biogeographic history of the group. Eight unit areas were defined: (A) North America (Canada and the USA); (B) Mexico; (C) South America; (D) Europe; (E) western and central Asia; (F) eastern Asia; (G) Australasia; and (H) Africa and St. Helena. Each genus was coded for its likely ancestral distribution and not for all of the regions in which its members presently occur, as suggested by Ronquist (1996). The likely ancestral distributions of *Berula*, *Cryptotaenia*, *Helosciadium*, *Lilaeopsis*, and *Sium* were based on results of previous or ongoing phylogenetic and biogeographic studies of each of these genera (Spalik and Downie 2006, 2007; Bone 2007). For *Oenanthe*, we assumed a broad distribution in the Old World (DEFH) and omitted its only North American member, *Oenanthe sarmentosa* C. Presl ex DC., because preliminary yet unpublished phylogenetic analyses of cpDNA and ITS data suggest its derived position within the cladograms (K. Spalik and S. Downie, unpublished data, 2008). For *Cicuta*, we assumed both broad north temperate (ADEF) and exclusively North American (A) ancestral distributions, based on the phylogenies presented in Lee and Downie (2006); the results of each analysis, however, were identical with regard to the ancestral distribution of the North American Endemics clade. Two optimizations were performed using DIVA: first, with an unconstrained number of unit areas for each ancestral node and second, with this number restricted to two areas. The rationale for the second optimization is that in an unconstrained analysis, the ancestral distributions at or near the base of the tree may be inferred to be widespread and include most or all individual unit areas inhabited by the terminals because of uncertainty (Ronquist 1996). Because we were interested in inferring the ancestral distributions if the group had more restricted (and likely realistic) distributions, we repeated the analysis by restricting the number of ancestral areas assigned to each node to two.

Results

CpDNA sequence comparisons

Sequence characteristics of each of the five noncoding cpDNA data partitions are presented in Table 3. The two smallest of these regions, *psbK–trnQ* (330–362 bp) and *psbI–psbK* (394–409 bp), had similar numbers of PI alignment positions (47–50) and PI alignment gaps (9–11) across 57 accessions. The *trnQ–rps16* region for the same 57 accessions ranged in size from 445 to 1308 bp, with the smallest fragments attributable to large deletions relative to the outgroups (e.g., *Cicuta*, 813 bp deletion; *Sium suave* Walter and *Sium latifolium* L., 333 bp deletions; *Ptilimnium costatum* (Elliott) Raf. and *Ptilimnium nuttallii* (DC.) Britt., 202 bp deletions). The *rps16* intron ranged in size from 831 to 875 bp across 86 accessions, and PI indels within this region ranged from 1 to 18 bp. Maximum pairwise sequence divergence values across these four data partitions are similar, ranging from 5.3% to 6.3% of nucleotides. The *rps16–trnK* region (at 531–778 bp in size across 86 accessions) is the most variable, with a maximum pairwise sequence divergence value of 11.6% between the outgroup *Seseli tortuosum* L. and *Oxypolis canbyi* (J.M. Coult. & Rose) Fern. Major unambiguous indels within the *rps16–trnK* region (relative to the outgroups) included a 216 bp deletion in *Ptilimnium ahlesii* Weakley & G.L. Nesom, a 98 bp deletion in *Helosciadium crassipes* W.D.J. Koch ex Rchb., a 68 bp insertion in one accession of *Atrema americanum*, and a 63 bp deletion in *Ptilimnium nodosum* (Rose) Mathias. Both *trnQ–rps16* and *rps16–trnK* intergenic spacers contribute greater numbers of total PI characters (229 and 211, respectively) than that of the *rps16* intron (157) and other loci. Proportionally, however, the *rps16–trnK* data partition had the greatest total number of PI characters relative to its overall size. It also included the largest number of ambiguously aligned nucleotide positions (257 or about 26% of the aligned *rps16–trnK* matrix).

Alignment of all cpDNA partitioned regions plus chloroplast genes *psbI* (in part), *psbK, trnQ*, and *rps16* exons (i.e., the "Entire cpDNA" region; Fig. 1; Table 3) resulted in an alignment of 4930 positions for 86 accessions. Of these, 406 positions were excluded from further analysis because of alignment ambiguities. The remaining 4524 aligned positions yielded 613 PI nucleotide characters, 40 of which occurred in coding regions. In addition, 194 unambiguous alignment gaps were inferred, of which 141 were PI. Maximum sequence divergence in pairwise comparisons reached 6.9% of nucleotides (between *Lilaeopsis carolinensis* J.M. Coult. & Rose and the outgroup *Seseli tortuosum*). Within Oenantheae, such values reached 5.2% (between *Ptilimnium nodosum* and *Oenanthe sarmentosa*). Two accessions of *Cryptotaenia canadensis* (Nos. 1570 and 1971; Table 2) had identical cpDNA sequences and were treated as one terminal in the phylogenetic analysis. Approximately 20% of the cells in the matrix were scored as missing, primarily because data from the *psbI* through *rps16* 5′exon region were not available for 29 accessions. Sequence characteristics of the "Entire cpDNA + indels" matrix are also presented in Table 3 and reflect the incorporation of 141 PI indels.

ITS sequence comparisons

Among the 127 accessions examined for ITS sequence variation, the length of the region varied from 580 to 605 bp. Eighteen species were represented by two or more accessions with each having identical DNA sequences; hence, each of these species was represented by a single terminal in the phylogenetic analysis. The ensuing matrix comprised 83 terminals. Alignment of these ITS sequences resulted in a matrix of 652 positions, with 23 excluded from further analysis because of alignment ambiguities. No cells in the matrix were scored as missing. From the remaining 629 positions, 256 were not variable, 47 were autapomorphic, and 326 were PI. Sixty-three unambiguous alignment gaps, ranging between 1 and 19 bp in size, were introduced to facilitate alignment and, of these, 33 were PI. A large, 19 bp indel was synapomorphic for the genus *Helosciadium*. Maximum sequence divergence (23.8%) was obtained between *Lilaeopsis mauritiana* and *Neogoezia macvaughii*. *Ptilimnium ahlesii* and *P. capillaceum* possessed identical ITS sequences, as did two species (three accessions) of *Oxypolis* (*O. filiformis* No. 2713 and *O. greenmanii* Nos. 2714 and 2717). The total number of PI characters for the ITS region was 359. This number is much higher than that obtained for the most variable cpDNA data partition across a comparable set of taxa.

Phylogenetic analyses

MP analysis of the entire cpDNA *psbI–trnK* region plus 141 PI indels (i.e., the "Entire cpDNA + indels" data matrix; Table 3) resulted in 11 522 minimal length trees, each of 1474 steps (CIs = 0.7551 and 0.7060, with and without uninformative characters, respectively; RI = 0.9105). Tree statistics resulting from MP analysis of these sequence data, but without the binary-scored PI indels ("Entire cpDNA" data matrix), are provided in Table 3. The strict consensus tree from the first analysis is presented in Fig. 2, with accompanying BS values resulting from analyses with and without PI indels used as additional characters. With the exception of the collapse of the branch uniting *Neogoezia* with the clade of *Atrema* + *Trepocarpus* (clade 15; Fig. 2), the results were identical in both analyses with regard to the relationships inferred among the major clades. While BS values for some clades increased upon the incorporation of indels, support for a smaller number of clades decreased slightly. Based on evidence provided by cpDNA, 17 genera or major groups of species are identified within tribe Oenantheae (Fig. 2). The genera *Limnosciadium*, *Cynosciadium*, *Lilaeopsis*, *Neogoezia*, *Atrema*, *Trepocarpus*, *Berula*, *Helosciadium*, *Cryptotaenia*, *Oenanthe*, *Cicuta*, and *Perideridia* are each strongly supported as monophyletic, with BS values ranging from 97% to 100%. The genus *Sium* is weakly supported as monophyletic (51% BS value or less) and comprises two well supported subclades: northern Holarctic (*S. suave*, *S. latifolium*, and *Sium medium* Fisch. & C.A. Mey.) and southern Palearctic (*Sium sisarum* L. and *S. tenue*; Spalik and Downie 2006). The genera *Ptilimnium* and *Oxypolis* are each not monophyletic. *Ptilimnium* comprises two clades which are identified as *Ptilimnium* I and *Ptilimnium* II, the latter including only the rachis-leaved species, *P. nodosum*. Similarly, the rachis-leaved *Oxypolis* species

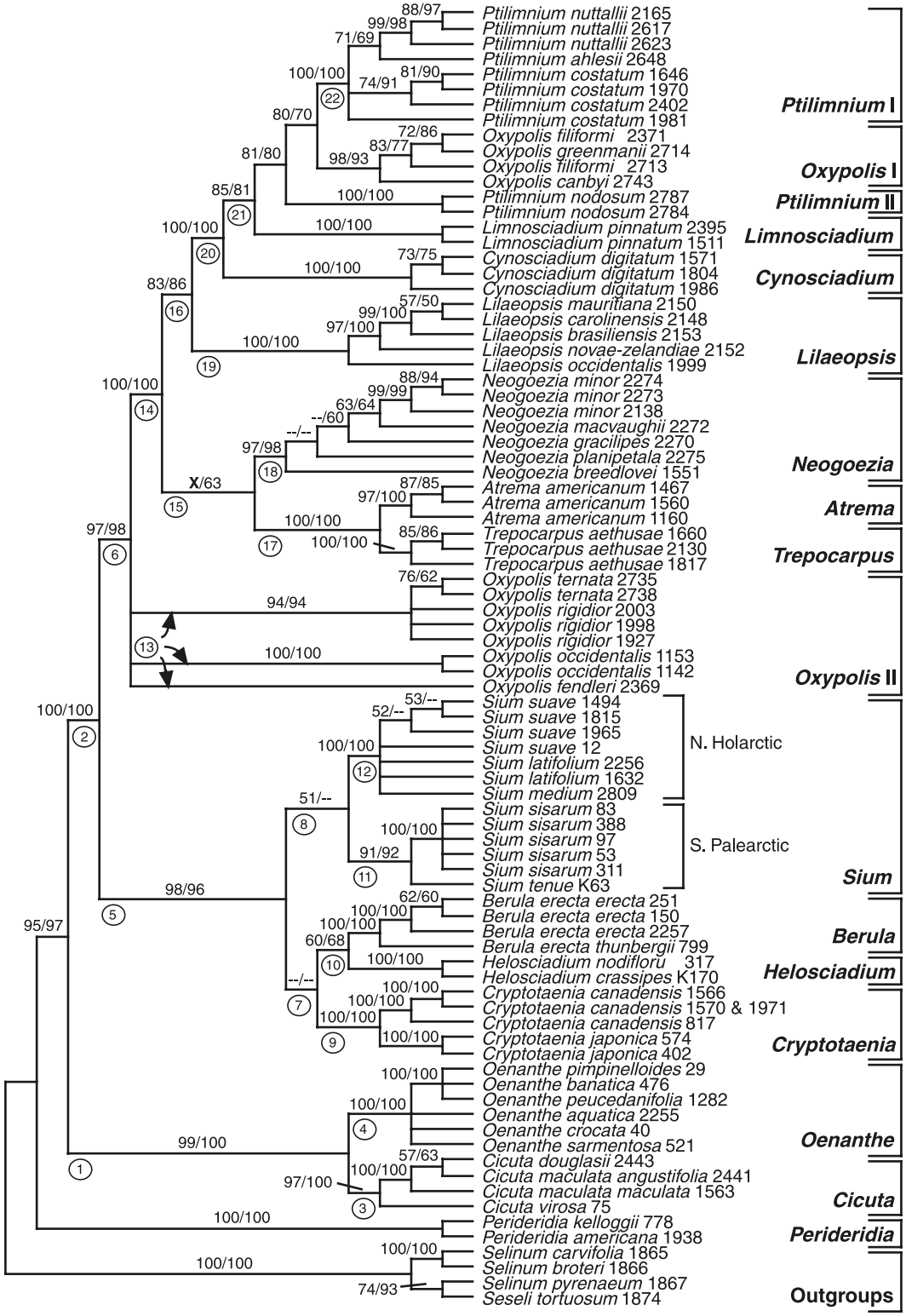
Table 3. Sequence characteristics and tree statistics for each of the partitioned and combined cpDNA data matrices analyzed in this study.

Sequence characteristics/tree statistics	Data matrix						
	psbI–psbK	*psbK–trnQ*	*trnQ–rps16*	*rps16* intron	*rps16–trnK*	Entire cpDNA	Entire cpDNA + indels
No. of accessions examined	57	57	57	86	86	86	86
No. of terminals used in the MP analysis	56	55[a]	56	85	85	85	85
Length variation (range in bp)	394–409	330–362	445–1308	831–875	531–778	3388–4272	3529–4413
Percentage cells in matrix scored as missing	0	0	0.8	0	1.0	19.8	19.6
No. of aligned positions	439	374	1580	942	1004	4930	5071
No. of positions eliminated	9	0	90	50	257	406	406
No. of positions not variable	362	306	1215	719	532	3669	3669
No. of positions autapomorphic	18	21	90	49	48	242	242
No. of positions PI	50	47	185	124	167	613	754
No. of unambiguous alignment gaps PI	11	9	44	33	44	141	141
Total no. of PI characters	61	56	229	157	211	754	754
Max. pairwise sequence divergence (%)	5.5	6.3	6.1	5.3	11.6	6.9	6.9
No. of MPTs	19 369	200	1438	>20 000	>20 000	10 346	11 522
MPT length (steps)	109	90	382	277	346	1307	1474
CI	0.6703 (0.6421)	0.7463 (0.7042)	0.7629 (0.7500)	0.6404 (0.6186)	0.6902 (0.6811)	0.6843	0.7060
RI	0.9130 (0.9014)	0.8982 (0.8743)	0.9141 (0.9078)	0.8904 (0.8797)	0.9115 (0.9077)	0.9018	0.9105

Note: The "Entire cpDNA" matrix includes the five noncoding loci plus genes *psbI* (in part), *psbK, trnQ*, and *rps16*. Binary-scored alignment gaps were included only in the analysis of the "Entire cpDNA + indels" data set and for this matrix the number of aligned positions includes the 141 PI indels. For the six other data matrices, the total number of PI characters (no. of PI nucleotide positions + no. of PI alignment gaps) is for summary purposes only and not an indication of the actual number of PI characters included in the MP analysis of each data set. Measures of character fit in parentheses were calculated by optimizing each data partition over the MPTs obtained from analyses of the "Entire cpDNA + indels" matrix across a comparable set of taxa. PI, parsimony informative; MPTs, maximally parsimonious trees; CI, ensemble consistency index excluding uninformative characters; RI, ensemble retention index.

[a]Sequence data for the single accession of *Atrema americanum* (no. 1160) were missing from this region; hence this accession was removed from the analysis.

Fig. 2. Strict consensus of 11 522 minimal length 1474-step trees derived from MP analysis of the "Entire cpDNA + indels" data matrix, which includes all coding and noncoding regions plus 141 PI alignment gaps (CIs = 0.7551 and 0.7060, with and without uninformative characters, respectively; RI = 0.9105). These results were nearly identical to those inferred when the analysis is repeated without binary-scored indel characters. Numbers above branches are BS estimates from 100 replicate analyses (with MaxTrees set to 15 000), without or with the 141 alignment gaps scored as additional characters. - -, BS values < 50%; X, demarcates a branch that did not occur in the strict consensus tree inferred without gap characters; brackets indicate genera or groups of taxa discussed in the text; circled numbers below branches correspond to the 22 clades identified in Table 4.



(*O. canbyi*, *O. filiformis*, and *O. greenmanii*) comprise a well supported clade (*Oxypolis* I), quite distant from their compound-leaved congeners. The compound-leaved *Oxypolis* species (*Oxypolis ternata* (Nutt.) A. Heller, *Oxypolis rigidior* (L.) Raf., *Oxypolis occidentalis* J.M. Coult. & Rose, and *Oxypolis fendleri* (A. Gray) Heller) comprise three branches of a tetrachotomy; this group of *Oxypolis* species is monophyletic in the BS majority rule consensus trees, albeit with weak support (54% and 56%, with and without indel characters, respectively). The group is also monophyletic upon analyses of ITS and combined cpDNA and ITS data (discussed below), thus we refer to this group as the *Oxypolis* II clade.

A summary of tree statistics resulting from MP analysis of each cpDNA data partition is presented in Table 3. Analysis of the *rps16* intron and *rps16–trnK* partitions each resulted in the preset maximum tree limit of 20 000 trees, whereas analyses of the remaining partitions resulted in a lower number of trees. Comparisons of measures of character fit, calculated by optimization of each data partition onto the MPTs inferred by analysis of the "Entire cpDNA + indels" matrix across a comparable set of taxa, revealed that the *trnQ–rps16* (CI = 0.7500, RI = 0.9078) and *rps16–trnK* (CI = 0.6811, RI = 0.9077) data matrices had the lowest levels of homoplasy. The relative utility of the five noncoding cpDNA loci in resolving phylogenetic relationships within Oenantheae was assessed further by comparing the results of MP and BS analyses of each data partition against the results obtained from MP analysis of the "Entire cpDNA + indels" matrix, because analysis of the latter yielded trees of greatest resolution and highest BS support overall. Twenty-two major clades of Oenantheae were identified on the cpDNA strict consensus tree (Fig. 2) and are described in Table 4. A comparison of BS support values for these 22 major clades obtained from partitioned and combined MP analyses of cpDNA (and ITS data, discussed below) is presented in Table 4. The *rps16–trnK* and *trnQ–rps16* data partitions recovered 14 and 16 of these major clades, respectively. The *rps16* intron partition recovered 12 major clades, whereas the two remaining data partitions each recovered only eight major clades. Only three major clades were recovered in separate analyses of all cpDNA data partitions (clades 14, 19, and 22). Clade 6 was recovered by analysis of the *rps16* intron partition only, clades 10 and 18 by analyses of *trnQ–rps16* data only, and clade 21 by analysis of the *rps16–trnK* partition only. Clades 7, 8, and 15 were not recovered in any of the partitioned analyses, yet they were each weakly supported as monophyletic when all cpDNA data were analyzed simultaneously. Among the five cpDNA data partitions, BS support values are generally the highest for the *trnQ–rps16* and *rps16–trnK* regions. The greatest resolution and highest BS support values, however, are obtained by simultaneous analysis of all data from the entire *psbI–trnK* region.

MP analysis of 629 unambiguously aligned ITS nucleotide positions plus 33 PI alignment gaps ("ITS + indels" matrix) resulted in 3451 shortest trees, each of 1381 steps (CIs = 0.4808 and 0.4585, with and without uninformative characters, respectively; RI = 0.8361). The strict consensus of these trees is presented in Fig. 3, with accompanying BS support values resulting from MP analyses with and without PI indels. The topology of this strict consensus tree is identical to the one inferred when the analysis is repeated using only nucleotide characters (tree length = 1348 steps; no. of MPTs = 10 346; CIs = 0.4681 and 0.4446, with and without uninformative characters, respectively; RI = 0.8315). The same 17 genera or groups of species identified previously occur in the ITS strict consensus tree. In addition, *Afrocarum* and the African species of *Sium*, *Sium bracteatum* (Roxb.) Cronk and *Sium repandum* Welw. ex Hiern, occur within an expanded *Berula* clade [*Berula* s.l.], in accordance with the results of Spalik and Downie (2006), and the USA endemic monotypic genus *Daucosma* is a sister group to *Limnosciadium*. Once more, the genera *Ptilimnium* and *Oxypolis* each comprise two well-supported clades. The *Ptilimnium* I clade is expanded to include the compound-leaved *P. capillaceum*. The compound-leaved *Oxypolis* species (*Oxypolis* II clade) are well supported as monophyletic, with *O. occidentalis* occurring as a sister group to a weakly supported clade consisting of *O. fendleri*, *O. rigidior*, and *O. ternata*. The ITS data recovered 18 of the 22 major clades identified on the cpDNA strict consensus tree and, in general, the incorporation of indels into the analysis of ITS sequences resulted in slightly higher BS values than when they were not included (Table 4). Clades 1, 2, 7, and 10 did not occur in the ITS strict consensus tree, and clades 6, 8, and 15 were very poorly supported.

A visual comparison of well supported clades in the cpDNA- and ITS-derived trees indicates much concordance, especially within the North American Endemics clade (clade 6). Differences between the trees involve the placement of *Helosciadium* within the Old World Endemics clade and the relative placements of *Cicuta* and *Oenanthe*. Depending upon the analysis, *Helosciadium* is either a moderately supported sister group to *Berula* (Fig. 2) or forms one branch of a trichotomy along with *Sium* and the clade of *Berula* s.l. + *Cryptotaenia* (Fig. 3). *Cicuta* is either sister group to *Oenanthe* (Fig. 2) or to the Old World Endemics clade (Fig. 3). These differences, however, are attributable to poorly supported nodes (<50% BS values). Results of a partition-homogeneity test on a set of 82 accessions common to both cpDNA and ITS data sets revealed that these matrices yield significantly incongruent phylogenetic estimates (P = 0.03). Upon the removal of the two accessions of *Helosciadium*, however, a subsequent partition-homogeneity test revealed that the data partitions are not significantly incongruent (P = 0.08), hence they were combined for simultaneous analysis. We acknowledge that serious 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 (e.g., Yoder et al. 2001; Barker and Lutzoni 2002). Since our primary objective is to ascertain relationships among the North American members of the tribe, we maintain the predominantly Eurasian genus *Helosciadium* in the analyses of combined cpDNA and ITS data.

Alignment of the entire *psbI–trnK* and ITS regions for 82 common accessions resulted in a matrix of 5582 nucleotide

Table 4. Comparison of BS support values calculated from MP analysis of combined or partitioned data, with and without their corresponding binary-coded indel matrices, for the 22 major clades of Apiaceae tribe Oenantheae identified in Fig. 2 and described here.

Clade	Entire cpDNA	Entire cpDNA + indels (Fig. 2)	*psbI–psbK*	*psbK–trnQ*	*trnQ–rps16*	*rps16* intron	*rps16–trnK*	ITS	ITS + indels (Fig. 3)	Entire cpDNA + ITS	Entire cpDNA + ITS + indels (Fig. 4)
1	99	100	n/a	85	60	84	52	n/a	n/a	97	93
2	100	100	51	n/a	95	69	73	n/a	n/a	100	100
3	97	100	n/a	n/a	57	58	94	96	98	100	100
4	100	100	100	n/a	100	99	100	100	100	100	100
5	98	96	n/a	n/a	57	*	n/a	71	74	100	100
6	97	98	n/a	n/a	n/a	*	n/a	*	*	93	89
7	*	*	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
8	51	*	n/a	n/a	n/a	n/a	n/a	*	*	58	62
9	100	100	n/a	86	*	67	67	100	99	100	100
10	60	68	n/a	n/a	53	n/a	n/a	n/a	n/a	53	53
11	91	92	80	70	99	n/a	85	74	81	98	94
12	100	100	80	n/a	100	*	99	99	100	100	100
13	n/a	n/a	n/a	n/a	96	*	n/a	92	97	95	99
14	100	100	61	*	74	*	96	99	100	100	100
15	n/a	63	n/a	n/a	n/a	n/a	n/a	58	52	67	79
16	83	86	n/a	*	n/a	n/a	80	97	97	99	99
17	100	100	91	98[a]	91	n/a	100	96	98	100	100
18	97	98	n/a	n/a	76	n/a	n/a	100	100	100	100
19	100	100	99	100	100	100	100	100	100	100	100
20	100	100	n/a	n/a	92	n/a	95	78	66	100	100
21	85	81	n/a	n/a	n/a	n/a	59	61	63	75	70
22	100	100	100	*	100	97	96	100	100	100	100

Note: Clade 1, *Cicuta* and *Oenanthe*; clade 2, All Oenantheae genera except *Cicuta*, *Oenanthe*, and *Perideridia*; clade 3, *Cicuta*; clade 4, *Oenanthe*; clade 5, Old World Endemics (*Berula* s.l., *Cryptotaenia*, *Helosciadium*, and *Sium*); clade 6, North American Endemics (*Atrema*, *Cynosciadium*, *Limnosciadium*, *Lilaeopsis*, *Neogoezia*, *Ptilimnium*, *Trepocarpus*, and *Oxypolis*, plus *Daucosma* in the ITS trees); clade 7, *Berula* s.l., *Cryptotaenia*, and *Helosciadium*; clade 8, *Sium*; clade 9, *Cryptotaenia*; clade 10, *Berula* s.l. and *Helosciadium*; clade 11, southern Palearctic *Sium* species; clade 12, northern Holarctic *Sium* species; clade 13, *Oxypolis* II (based on results of ITS and combined ITS/cpDNA data); clade 14, North American Endemics except *Oxypolis* II clade; clade 15, *Atrema, Neogoezia*, and *Trepocarpus*; clade 16, *Cynosciadium, Lilaeopsis, Limnosciadium, Ptilimnium*, and *Oxypolis* I clade, plus *Daucosma* in the ITS trees; clade 17, *Atrema* and *Trepocarpus*; clade 18, *Neogoezia*; clade 19, *Lilaeopsis*; clade 20, *Cynosciadium, Limnosciadium, Ptilimnium*, and *Oxypolis* I clade, plus *Daucosma* in the ITS trees; clade 21, *Limnosciadium, Ptilimnium*, and *Oxypolis* I clade, plus *Daucosma* in the ITS trees; clade 22, *Ptilimnium* I clade. Nodes present in the strict consensus trees but supported by BS values <50% are indicated by asterisks (*). Nodes that did not occur in the strict consensus trees are indicated as not applicable (n/a).

[a] *Atrema* was not included in this analysis

Fig. 3. Strict consensus of 3451 minimal length 1381-step trees derived from MP analysis of the "ITS + indels" data matrix, which includes 33 binary-scored alignment gaps (CIs = 0.4808 and 0.4585, with and without uninformative characters, respectively; RI = 0.8361). Numbers above branches are BS estimates from 100 replicate analyses (with MaxTrees set to 15 000), without or with the 33 alignment gaps scored as additional characters; - -, values < 50%; brackets indicate genera or groups of taxa recognized in Fig. 2 and discussed in the text. The *Berula* clade is recognized in the broad sense to include *Afrocarum*, *Sium repandum*, and *S. bracteatum* based on the results of Spalik and Downie (2006). Circled numbers below branches correspond to the 22 clades recognized in Fig. 2 and identified in Table 4; numbers in parentheses following the names of 18 species, indicate the number of accessions of that species having identical DNA sequences.

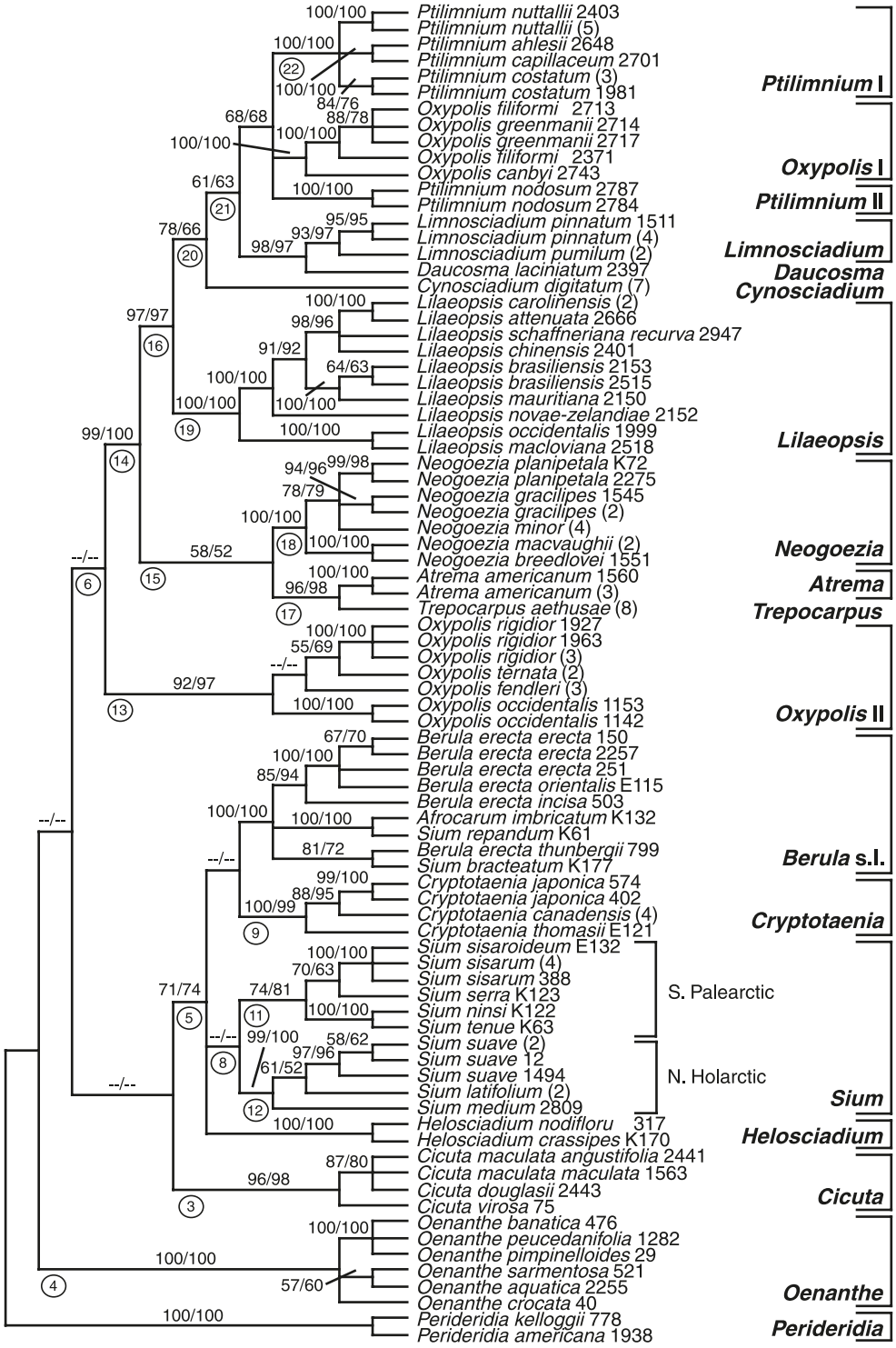
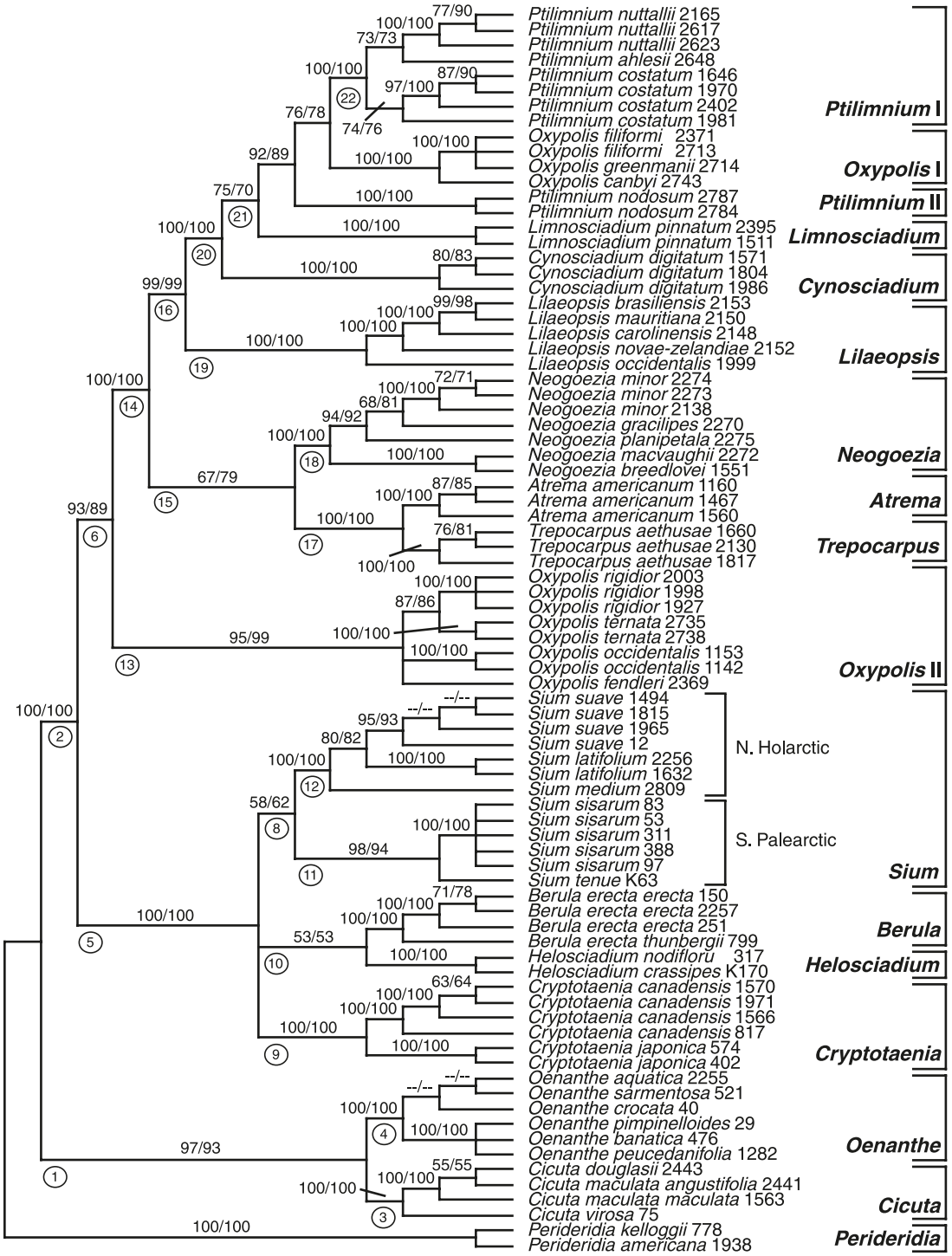


Fig. 4. Strict consensus of 768 minimal length 2617-step trees derived from MP analysis of the "Entire cpDNA + ITS + indels" data matrix which includes 157 binary-scored alignment gaps from both cpDNA and ITS regions (CIs = 0.6408 and 0.5959, with and without uninformative characters, respectively; RI = 0.8811). Numbers above branches are BS estimates from 100 replicate analyses (with MaxTrees set to 15 000), without or with the 157 alignment gaps scored as additional characters; - -, values < 50%; brackets indicate genera or groups of taxa recognized in Fig. 2 and discussed in the text; circled numbers below branches correspond to the 22 clades recognized in Fig. 2 and identified in Table 4.



positions; 429 of these were excluded because of alignment ambiguities. Of the remaining 5153 positions, 897 were PI, 281 were autapomorphic, and 3975 were not variable. The maximum pairwise sequence divergence value was 9.3% between *Lilaeopsis carolinensis* and *Ptilimnium nodosum*. Distance values were generally the highest (5%–9%) in pairwise comparisons among most members of the *Ptilimnium* I, *Oxypolis* I, *Ptilimnium* II, *Limnosciadium*, *Cynosciadium*, and *Lilaeopsis* clades, as well as in comparisons between any member of this group and those occurring in the more basally branching clades. One hundred and fifty-seven unambiguous alignment gaps were PI. MP analyses of these nucleotide data and the 157 gap characters (''Entire cpDNA + ITS + indels'' matrix) resulted in 768 minimal length trees, each of 2617 steps (CIs = 0.6408 and 0.5959, with and without uninformative characters, respectively; RI = 0.8811). The strict consensus of these trees (Fig. 4) is almost identical to the strict consensus tree inferred without the gap characters (tree length = 2433 steps; No. of MPTs = 864; CIs = 0.6247 and 0.5738, with and without uninformative characters, respectively; RI = 0.8731), but with slightly different degrees of resolution within four genera. Constraining the *Ptilimnium* I and II clades to monophyly in a subsequent MP search resulted in shortest trees just two steps longer than those without the constraint invoked (tree length = 2619 steps). Constraining all accessions of *Oxypolis* to monophyly resulted in trees 70 steps longer than those without the constraint. The same 17 genera or groups of species are resolved within tribe Oenantheae, and 21 of the 22 major clades inferred by MP analysis of the ''Entire cpDNA + indels'' matrix are recovered. BS values supporting these clades are presented in Table 4. Clade 7 (*Berula*, *Cryptotaenia*, and *Helosciadium*) did not occur in the strict consensus tree inferred from all available data. Instead, *Berula* and *Helosciadium* comprised a weakly supported clade whose relationships with *Sium* and *Cryptotaenia* were unresolved.

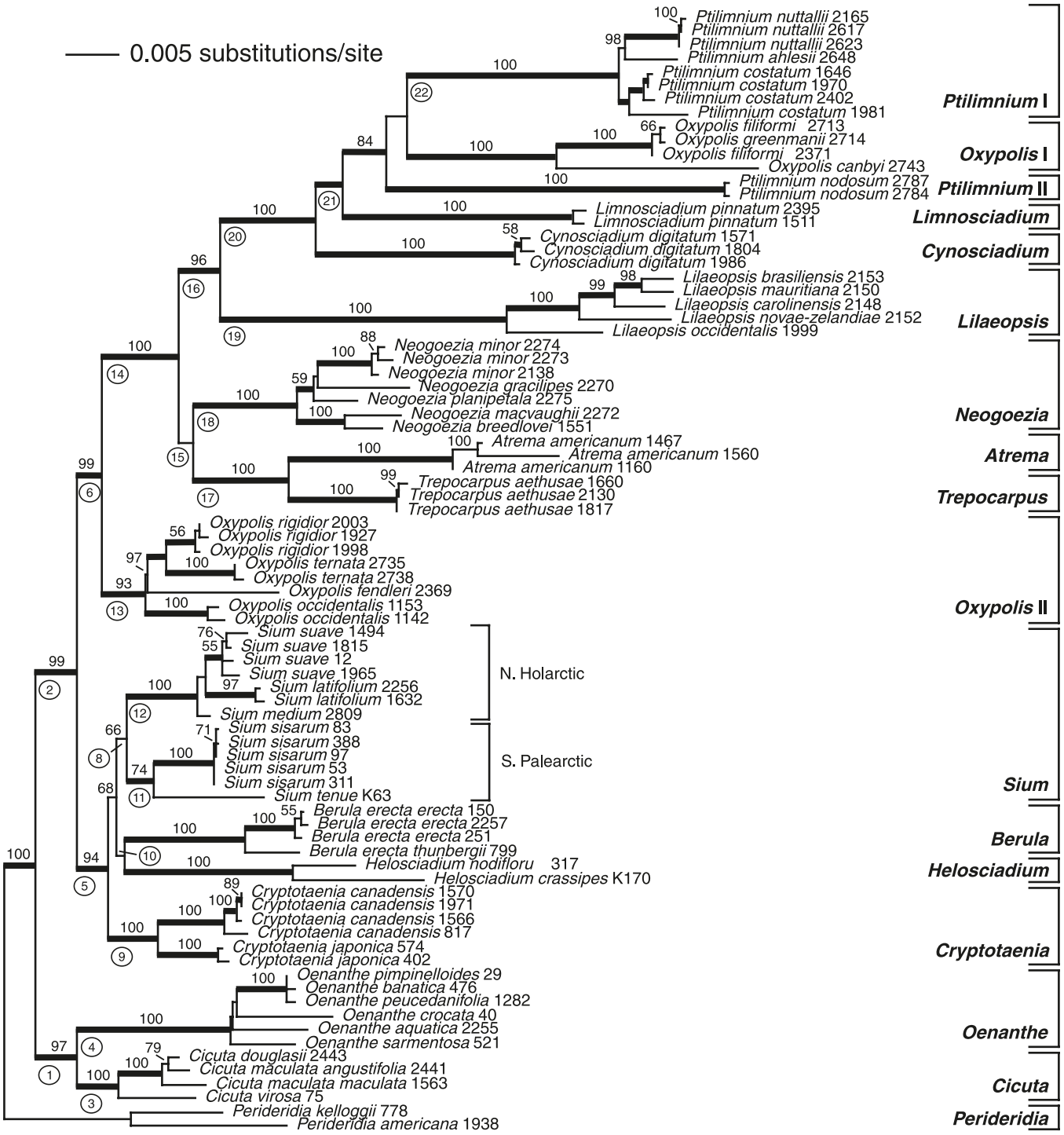
Based on the AIC estimator, Modeltest selected the GTR + I + G model of nucleotide substitution as best fitting both cpDNA and ITS sequence data in the combined matrix. Using the parameters estimated by Modeltest, a single ML tree was recovered having a –ln likelihood score of 22 208.07 (Fig. 5). MrModeltest selected the same model whose general form settings (nst = 6, rates = invgamma) were used in the Bayesian analysis. The Bayesian inference tree is fully consistent with that of the ML tree, therefore those branches supported by PP values of 1.00 are indicated on the ML tree (Fig. 5). With the exceptions of the *Oxypolis* II (93% BS, 1.00 PP) and *Sium* (66% BS, 0.89 PP) clades, all other genera or groups of species recognized previously are supported by 100% BS and 1.00 PP values. Of the 22 major clades designated previously, only clade 7 (*Berula*, *Cryptotaenia*, and *Helosciadium*) did not occur in the ML or Bayesian inference trees. In these trees, *Berula* and *Helosciadium* comprised a weakly supported clade that was a sister group to *Sium*.

The phylogenies estimated using MP (with and without scored indels), ML, and Bayesian analyses of combined cpDNA and ITS data are each highly resolved, generally well supported, and consistent. The North American genera *Atrema*, *Cynosciadium*, *Limnosciadium*, *Neogoezia*, *Oxypolis*, *Ptilimnium*, and *Trepocarpus* ally with the western hemispheric and Australasian genus *Lilaeopsis* in a strongly supported clade that is a sister group to a clade comprising primarily Old World taxa (*Berula*, *Cryptotaenia*, *Helosciadium*, and *Sium*). *Berula* and *Helosciadium* are monophyletic sister groups in all analyses of combined data; albeit their union is supported very weakly. The clade of *Berula* + *Helosciadium* is sister group to *Sium* in the ML and Bayesian trees. *Cicuta* and *Oenanthe* also unite as monophyletic and compose a sister group to all aforementioned taxa. With the exceptions of *Oxypolis* and *Ptilimnium*, all genera save *Sium* are strongly supported as monophyletic. *Sium* is weakly supported as monophyletic and comprises two well-supported subclades in all analyses: northern Holarctic and southern Palearctic (Spalik and Downie 2006). The rachis-leaved *Oxypolis* species makes up a clade (*Oxypolis* I) distant from their compound-leaved congeners (*Oxypolis* II). Similarly, the rachis-leaved *Ptilimnium nodosum* (*Ptilimnium* II) is separated from those other *Ptilimnium* species having compound leaves (*Ptilimnium* I).

Based on the results of phylogenetic analyses of combined cpDNA and ITS sequences, the following simplified, fully resolved tree of generic relationships is inferred for tribe Oenantheae: (*Perideridia*, ((*Cicuta, Oenanthe*), ((((*Berula* s.l., *Helosciadium*), *Sium*), *Cryptotaenia*), (*Oxypolis* II, (((*Atrema, Trepocarpus*), *Neogoezia*), (*Lilaeopsis*, (*Cynosciadium*, ((*Daucosma*, *Limnosciadium*), (*Ptilimnium* II, (*Oxypolis* I, *Ptilimnium* I))))))))). The phylogenetic placement of *Daucosma* as a sister group to *Limnosciadium* is inferred from the ITS trees. *Perideridia* is sister group to all other genera within the tribe based on the cpDNA trees and results of prior phylogenetic analyses (Plunkett et al. 1996; Downie et al. 1998, 2000; Plunkett and Downie 1999). *Berula* s.l. includes the monotypic genus *Afrocarum* from sub-Saharan Africa and three species of *Sium* from Africa and St. Helena: *S. repandum*, *S. bracteatum*, and *Sium burchellii* (Hook. f.) Hemsl. (Spalik and Downie 2006). While a conservative estimate of relationships would recognize *Berula* s.l., *Sium*, *Helosciadium*, and *Cryptotaenia* as a tetrachotomy because of the differing placements of *Helosciadium* in the cpDNA and ITS trees and weak support for any relationship among these genera, we use the results of the ''total evidence'' analysis, specifically the relationships suggested by ML and Bayesian analyses of combined cpDNA and ITS data, to suggest (((*Berula* s.l., *Helosciadium*), *Sium*), *Cryptotaenia*). This relationship is consistent to results obtained by MP analysis of all available data, and such a fully bifurcating tree is necessary to infer the biogeographic history of the group (Ronquist 1996).

A striking feature of all cpDNA and ITS trees is the long branches leading to distal clades *Ptilimnium* I, *Oxypolis* I, *Ptilimnium* II, *Limnosciadium*, *Cynosciadium*, and *Lilaeopsis* relative to other ingroup taxa, as seen in Fig. 5. Pairwise sequence divergence values among members of these clades are generally higher relative to comparisons between any taxa outside of this group. Moreover, 50 of the 141 PI gaps inferred in the alignment of cpDNA sequences were restricted to members of these distal clades, as were eight of the 33 PI ITS gaps. To detect rate asymmetry, ten relative rate tests were conducted. CpDNA and ITS sequences for 14 accessions were assigned to five defined lineages ((*i*), *Cicuta* and *Oenanthe*; (*ii*), *Berula*, *Cryptotaenia*, *Heloscia-*

Fig. 5. Single tree derived from ML analysis of the "Entire cpDNA + ITS" data matrix for 82 accessions of Apiaceae tribe Oenantheae under a GTR + I + G model of nucleotide substitution (–ln likelihood = 22 208.07). Branch lengths are proportional to the number of expected nucleotide substitutions per site (note scale bar). Numbers above branches represent BS estimates calculated from 1000 replicate neighbor-joining analyses using ML distance and the likelihood settings inferred by Modeltest. The relationships inferred by the Bayesian majority-rule consensus tree are wholly consistent with those of the ML tree; branches supported by Bayesian PP values of 1.00 are widened on the ML tree. Brackets indicate genera or groups of taxa recognized in Fig. 2 and discussed in the text; circled numbers below branches correspond to the major clades recognized in Fig. 2 and identified in Table 4.

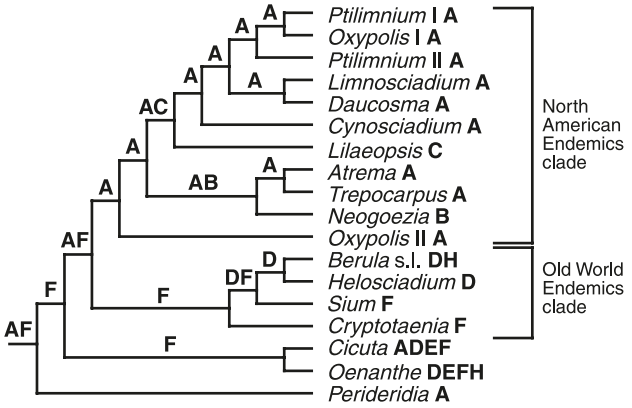


dium, and *Sium*; (*iii*), *Oxypolis occidentalis*; (*iv*), *Atrema*, *Neogoezia*, and *Trepocarpus*; and (*v*), *Cynosciadium*, *Lilaeopsis*, *Limnosciadium*, and *Ptilimnium*). *Perideridia* was used as the reference taxon (outgroup). Significant differences ($P < 0.05$) suggest that members of the *Ptilimnium* I through *Lilaeopsis* clade are each evolving faster than those of the other lineages relative to the outgroup *Perideridia*. The results of all other relative rate tests between members of the remaining groups were not statistically significant.

Biogeography

Unrestricted optimal DIVA reconstructions required 10 dispersal events (not shown). For all deep ancestral nodes, however, the reconstructions were ambiguous with each comprising two to seven individual areas. As an example, one of two reconstructed ancestral distributions of tribe Oenantheae included all individual areas except Australasia (ABCDEFH). The immediate ancestral area for the North American Endemics clade was inferred to be either North America (A) or a broader New World region encompassing North America, Mexico, and South America (ABC). The ancestral area of the Old World Endemics clade (*Berula* s.l., *Helosciadium*, *Sium*, and *Cryptotaenia*) was reconstructed as eastern Asia (F). The immediate common ancestor of the North American Endemics + Old World Endemics clades was distributed in areas AF, BCF, or ABCF, depending upon the reconstructions. With the maximum number of inferred unit areas at each node set to two, the best constrained DIVA reconstruction required 11 dispersals (Fig. 6). In this biogeographic scenario, the ancestral area for the North American Endemics clade was North America (A), whereas that of the Old World Endemics clade was eastern Asia (F). The immediate common ancestor of both of these major clades occurred widely in North America and eastern Asia (AF), with later vicariance separating the North American Endemics and Old World Endemics clades. Subsequent dispersals and vicariance events led to the present-day distributions of these taxa. The ancestral distribution of the entire tribe Oenantheae was also reconstructed as North America – eastern Asia (AF), but this hypothesis is likely unreliable in the absence of outgroups outside of Oenantheae. The ancestor of the *Cicuta* + *Oenanthe* clade was suggested to be either eastern Asia in the constrained analysis or one of four alternative solutions encompassing three to five unit areas (DEH, ADEH, DEFH, or ADEFH) in the unconstrained analysis.

Fig. 6. A dispersal-vicariance scenario of Apiaceae tribe Oenantheae, as reconstructed using the program DIVA with the maximum number of area units set to two. The phylogeny is a summary of generic-level relationships inferred by phylogenetic analyses of combined cpDNA *psbI–trnK* and nrDNA ITS sequence data. The eight unit areas are as follows: (A) North America (Canada and the USA); (B) Mexico; (C) South America; (D) Europe; (E) western and central Asia; (F) eastern Asia; (G) Australasia; and (H) Africa and St. Helena.



Discussion

Phylogenetic utility of the *psbI–trnK* region

Although the *psbI–trnK* region is approximately six to seven times larger (3388–4272 bp) than that of the ITS region (580–605 bp) across a comparable set of taxa, it contributed only twice as many PI characters (613 nucleotide positions and 141 alignment gaps) than the latter (326 nucleotide positions and 33 alignment gaps). No single cpDNA data partition, even those data partitions 1.5–2.5 times as large, contributed as many informative alignment positions as that of the ITS region. Maximum pairwise sequence divergence estimates were 6.9% for the entire cpDNA region and 23.8% for ITS. These values corroborate previous investigations reporting that among the various plastid and nuclear loci used to infer phylogeny of Apiaceae, the ITS region is evolving the most rapidly (Downie et al. 1998, 2001; Lee and Downie 2006).

While it is clear that the ITS region contributes a greater proportion of variable sites per total number of sites examined than any plastid data partition, the results of separate MP analyses of the entire cpDNA and ITS regions (excluding scored indels) demonstrated lower homoplasy (CIs excluding uninformative characters of 0.4446 and 0.6843, and RIs of 0.8315 and 0.9018, for ITS and cpDNA data sets, respectively) and higher branch support (Table 4) for the cpDNA matrix than that of ITS. It is acknowledged that the exclusion of a slightly greater percentage of alignment ambiguous sites in *psbI–trnK* can be at least partially responsible for this result. The ITS data, with or without scored indels, only recovered 18 of the 22 major clades identified on the strict consensus tree inferred from the "Entire cpDNA + indels" data matrix (Table 4). The most basally branching lineages in the ITS phylogeny are weakly supported (Fig. 3), whereas those similarly placed branches within the cpDNA-derived phylogenies are strongly supported. Moreover, the relative placements of *Cicuta* and *Oenanthe*, as well as *Helosciadium* in the Old World Endemics clade, differ from those relationships inferred using only cpDNA or combined cpDNA and ITS data. At present, the ITS region is the best marker for phylogenetic analyses of Apiaceae at low taxonomic levels because of its high rate of nucleotide substitution and the large number of sequences available in GenBank. The use of ITS sequences in phylogenetic studies has been strongly criticized because of various molecular genetic processes that may mislead phylogenetic inference (Álvarez and Wendel 2003), but these phenomena have yet to pose a serious problem for Apiaceae subfamily Apioideae (Spalik and Downie 2007). At higher taxonomic

levels in Apiaceae, we believe that more robust insights of relationships are likely to emerge from analyses of cpDNA sequences, particularly those from the *psbI–trnK* locus. These data in conjunction with the continued use of the more rapidly evolving ITS sequences are useful to resolve both basal branches and tips of the Apiaceae phylogenetic tree.

In Apiaceae tribe Oenantheae and in many other flowering plants, the cpDNA *psbI–trnK* region is approximately 4 kb in size. However, each of the noncoding loci within this region has its own tempo of evolution, thus it may not be necessary to sequence the entire *psbI–trnK* region to produce a well-resolved tree. The cpDNA *psbI–trnK* region includes three large and two small noncoding loci, of which only the *rps16* intron has been extensively characterized and used widely in phylogenetic studies to date (reviewed in Kelchner 2002). Sequence comparisons of the three largest of these loci revealed that the *trnQ–rps16* and *rps16–trnK* intergenic spacer regions are both evolving faster than that of the *rps16* intron, as assessed by their greater numbers of ambiguously aligned nucleotide positions, variable sites relative to their overall lengths, and PI characters (Table 3). The *rps16–trnK* region is evolving the fastest overall; it exhibits the highest sequence divergence estimates and has the greatest number of PI characters relative to its size. Phylogenetic analyses of all cpDNA data partitions revealed that the *rps16–trnK* and *trnQ–rps16* data sets also had the lowest levels of homoplasy and highest BS support. These two regions recovered 14 and 16 major clades, respectively, of the 22 major clades identified on the strict consensus tree inferred from MP analysis of the entire cpDNA matrix plus indels, whereas the *rps16* intron recovered 12 major clades, one of which was not recovered by the two intergenic spacer regions (Table 4). Collectively, the *trnQ–rps16* and *rps16–trnK* data partitions recovered 18 of these 22 clades. The greatest resolution and highest BS support, however, were obtained by simultaneous analysis of all data from the entire *psbI–trnK* region, including binary-scored indels. Clade 13, *Oxypolis* II, was recovered through analyses of the *trnQ–rps16* and *rps16* intron data partitions, but not through analyses of the entire cpDNA region, with or without scored indels. In contrast, this clade received strong support in the ITS analyses. In future phylogenetic studies of Apiaceae, we suggest that these noncoding loci be examined in turn, with the *rps16–trnK* region considered first and then the *trnQ–rps16* region. If further resolution of relationships is required, these data may be obtained through sequencing of the *rps16* intron and if necessary, the entire *psbI–trnK* region. While the *rps16* intron has been used widely in phylogenetic studies to date, the intergenic spacer regions flanking gene *rps16* are better candidates for phylogenetic inference.

Group II introns of the chloroplast genomes of land plants, such as that found in chloroplast gene *rps16*, show a strong relationship between the functional importance of its secondary structural features and the likelihood of mutational change, with those domains and subdomains essential for intron-associated functions most conserved evolutionarily (Downie et al. 1996, 1998, 2000; Kelchner 2002). This may explain the lower rate of sequence change of the *rps16* intron relative to the *trnQ–rps16* and *rps16–trnK* intergenic spacer regions. Shaw et al. (2005, 2007) surveyed all large noncoding regions of the chloroplast genome for their utility in interspecific phylogenetic and intraspecific phylogeographic studies and discovered that the *trnQ–rps16* and *rps16–trnK* intergenic spacer regions offer high levels of variation and, thus, are among the best choices for molecular studies at low taxonomic levels. Among the 34 noncoding regions they compared, the *trnQ–rps16* and *rps16–trnK* spacers rank second and seventh in terms of providing the greatest number of phylogenetically informative characters for low-level molecular phylogenetic studies, whereas the *rps16* intron ranked seventeenth (Shaw et al. 2007). Previously, we reported that the *rps16–trnK* region, if it had been included by Shaw et al. (2005), would have ranked in their "Tier 1," a group that on average consistently provided the greatest number of phylogenetically informative characters across all lineages they tested (Lee and Downie 2006). The highly variable nature of these loci have also been reported by Daniell et al. (2006) and Timme et al. (2007). The continued acquisition of sequence data from the cpDNA *trnQ–rps16* and *rps16–trnK* intergenic spacer regions, in conjunction with data from the nrDNA ITS region, shows great promise in resolving remaining intergeneric relationships in Apiaceae subfamily Apioideae. These plastid sequence data have already proved useful for resolving intergeneric relationships within Apiaceae subfamily Saniculoideae (Calviño and Downie 2007).

Apiaceae tribe Oenantheae phylogenetic resolutions

This and related studies of Apiaceae tribe Oenantheae represent the most comprehensive sampling of any major clade or tribe of apioid umbellifers confirmed as monophyletic on the basis of molecular systematic investigation. In this paper, we include representation of all known genera of the tribe and approximately one-half of its species, and in conjunction with recently published or on-going phylogenetic studies of specific genera within the tribe (Downie et al. 2004; Lee and Downie 2006; Spalik and Downie 2006, 2007; Bone 2007; Feist and Downie 2008; K. Spalik and S. Downie, unpublished data, 2008), nearly all of its species and infraspecific taxa have been considered. We expand sampling of the previously delimited North American Endemics clade (Hardway et al. 2004) by examining all recognized species of *Limnosciadium*, *Neogoezia*, and *Ptilimnium* and multiple accessions of the monotypic genera *Atrema*, *Cynosciadium*, and *Trepocarpus*. *Lilaeopsis* was represented by nine species from throughout its distributional range. The monotypic genus *Daucosma*, however, was only represented by a single accession. *Daucosma laciniatum* is reported from only several counties in central Texas and from one county in New Mexico (USDA, NRCS 2007) and available herbarium specimens are few and old. In the study of Hardway et al. (2004), these eight genera comprised a strongly supported North American Endemics clade whose relationships to the North American endemic genus *Oxypolis* and the more widely distributed genera *Cicuta* and *Oenanthe* were unclear. Therefore, to ascertain the phylogenetic placement of this clade within tribe Oenantheae, we included in our study all seven species of *Oxypolis* and multiple representatives of *Cicuta* and *Oenanthe*. Previous studies have already addressed phylogenetic relationships within *Cicuta* (Lee and

Downie 2006), as well as within and among Old World genera *Berula* s.l., *Cryptotaenia*, *Helosciadium*, and *Sium* (Spalik and Downie 2006, 2007). Hypotheses of relationships among these Old World genera, as inferred herein using cpDNA or combined cpDNA and ITS sequences, differ from those presented by previous studies in suggesting a novel, sister group relationship between *Berula* s.l. and *Helosciadium*. This relationship, however, is only weakly supported and, until additional studies suggest otherwise, it is best to treat the intergeneric relationships among *Berula* s.l., *Cryptotaenia*, *Helosciadium*, and *Sium* as unresolved. *Perideridia* has also been the subject of a molecular phylogenetic study (Downie et al. 2004), and research on the systematics of *Oenanthe* is currently underway (K. Spalik and S. Downie, unpublished data, 2008). Therefore, we restrict our discussion of phylogenetic resolutions within tribe Oenantheae to only those members composing the North American Endemics clade, as redefined in this study.

The North American Endemics clade, circumscribed previously on the basis of phylogenetic analysis of ITS sequences to comprise eight genera of primarily North American distribution (Hardway et al. 2004), is confirmed as monophyletic upon the inclusion of the North American endemic genus *Oxypolis*. As such, the clade is now circumscribed to include all genera of tribe Oenantheae native to North America and Mexico, save the basally branching *Perideridia*. Six of these genera are found exclusively in eastern and (or) south-central North America (*Atrema*, *Cynosciadium*, *Daucosma*, *Limnosciadium*, *Ptilimnium*, and *Trepocarpus*), *Oxypolis* is distributed more widely in North America, *Neogoezia* is restricted to Mexico, and *Lilaeopsis* is distributed in North America, Mexico, South America, and Australasia, with outlying species in Mauritius, the Kerguelen Islands, and Madagascar (Affolter 1985; Petersen et al. 2002). The optimal solution of DIVA (Fig. 6) confirms that the immediate ancestors of the North American Endemics clade originated in North America (unit area A; Canada and USA). DIVA also suggests that the ancestor of the clade of *Ptilimnium* I through *Lilaeopsis* (Fig. 6) was distributed in both North America and South America (AC), having reached South America previously from a dispersal from North America. *Lilaeopsis* originated in South America, with multiple, later dispersals from that region accounting for its present-day distribution (Bone 2007). Apparently, fruits of *Lilaeopsis* can retain their buoyancy in both fresh and salt water for many months, without a total loss of seed viability, and their dispersal by sea currents or waterfowl may have facilitated their transport to new regions (Affolter 1985).

Phylogenetic analyses of cpDNA *psbI–trnK* and nrDNA ITS sequences yield an estimate of relationships for the North American Endemics clade that is fully resolved and generally well supported, and with the exceptions of the North American endemic genera *Oxypolis* and *Ptilimnium*, all genera are monophyletic. Some species of *Oxypolis* and *Ptilimnium* have linear, terete, hollow, and transversely septate appendages known as rachis leaves, apparently being derived from a defoliated rachis of a formerly pinnately compound leaf (Affolter 1985), and it has long been questioned whether these species with highly reduced leaves should be placed in separate genera. In our study, *Oxypolis* and *Ptilimnium* are each separated into two clades, according to these differences in leaf morphology. The rachis-leaved *Oxypolis* species (*Oxypolis* I clade) have been recognized previously as the genus *Tiedemannia* DC. (de Candolle 1829), and *P. nodosum*, the sole rachis-leaved *Ptilimnium* species (*Ptilimnium* II clade), has been recognized as the genus *Harperella* Rose (Rose 1906). These classifications, however, have never been widely accepted, as differences in leaf morphology were not considered as important in generic delimitation than other features, such as those of the fruits and flowers (Coulter and Rose 1887; Mathias 1936; Easterly 1957). While our results provide compelling evidence that the name *Tiedemannia* should be resurrected for the rachis-leaved species of *Oxypolis* because the nomenclatural type of *Oxypolis* (*O. rigidior*) falls within the distantly-related compound-leaved group (*Oxypolis* II clade), they are less clear on how to treat *Ptilimnium* as trees supporting a monophyletic *Ptilimnium* are just slightly less parsimonious than those supporting the separation of *P. nodosum* from its congeners. Such nomenclatural changes must await the results of on-going ecological, morphological, and phylogeographic studies of these intriguing species of plants, two of which are federally endangered in the USA (M.A. Feist and S. Downie, unpublished data, 2008).

Sister group to the clade of *Ptilimnium* and the rachis-leaved species of *Oxypolis* is *Limnosciadium* (plus *Daucosma* in the ITS trees). Successively basal sister groups are *Cynosciadium* and *Lilaeopsis*. Many species of this assemblage have a similar internal fruit structure and a much reduced vegetative morphology (Affolter 1985; Petersen et al. 2002). All species of *Lilaeopsis* have rachis leaves. These leaves are linear, septate, hollow, and more or less terete, and show a similar developmental pattern to those of some rachis-leaved species of *Oxypolis* (Kaplan 1970). *Cynosciadium* and *Limnosciadium* have basal leaves, which have been referred to as "rachis-like," and whether or not these show a similar developmental pattern to the rachis leaves characteristic of the other taxa remains to be investigated through comparative anatomical and developmental studies (Feist and Downie 2008). Although the basal leaves of *Cynosciadium* and *Limnosciadium* are similar to rachis leaves in being linear to linear-lanceolate, septate, and entire, they differ from them in being flattened; moreover, the cauline leaves of *Cynosciadium* and *Limnosciadium* are generally palmately or pinnately divided, respectively. Rachis and rachis-like leaves are likely adaptations for existence in wet or aquatic habitats, and several of these plants spend much of their growing season at least partially submerged (Correll and Correll 1972; Godfrey and Wooten 1981; Affolter 1985; Feist and Downie 2008). The presence of rachis or rachis-like leaves may be interpreted as a synapomorphy for the *Ptilimnium* I through *Lilaeopsis* clade, with subsequent reversals on those branches leading to the *Ptilimnium* I clade (with its members characterized by having pinnately decompound leaves with filiform ultimate divisions) and *Daucosma laciniatum* (with its ternate-pinnately dissected leaves). Alternatively, true rachis leaves may have evolved in parallel in those lineages leading to the *Oxypolis* I, *Ptilimnium* II, and *Lilaeopsis* clades, as similar fistulose, sep-

tate leaves have also evolved repeatedly elsewhere in subfamily Apioideae (Affolter 1985), as well as in several independent lineages in *Eryngium* L. of Apiaceae subfamily Saniculoideae (Calviño et al. 2008).

Branch lengths leading to the most distally branching clades in the phylogenetic trees (i.e., *Ptilimnium* I, *Oxypolis* I, *Ptilimnium* II, *Limnosciadium*, *Cynosciadium*, and *Lilaeopsis*) are much longer relative to other ingroup taxa (including those of the other members of the North American Endemics clade), evidently as a result of higher rates of nucleotide substitutions and insertion/deletion events (Fig. 5). Long branch lengths were reported previously for single exemplars from eight genera of the North American Endemics clade (Hardway et al. 2004) and upon further study with increased sampling herein, it appears that these higher rates of nucleotide substitution and insertion or deletion events are restricted to all species belonging to the *Ptilimnium* I through *Lilaeopsis* clade, many of which have reduced vegetative morphologies. Among these taxa, *Lilaeopsis* exhibits the simplest vegetative morphology. Its hollow, septate, and linear to distally spatulate leaves arising from a slender, horizontal, and creeping rhizome are inconspicuous and the overall height of the plants is generally less than 20 cm (Affolter 1985). *Lilaeopsis* is also one of a few apioid umbellifers possessing simple umbels, which emerge singly from the axils of the foliage leaves. The fruit of *Lilaeopsis* also lacks a carpophore. *Lilaeopsis* may grow partially or entirely submerged, or the plants may simply be restricted to areas having soggy soils (Affolter 1985). The high degree of morphological reduction demonstrated by *Lilaeopsis* is correlated with some of the longest branches in the phylogenetic trees, supporting the paradigm that heightened rates of nucleotide substitutions represented by individual branch lengths roughly parallel increasing degrees of morphological reduction (Rothwell et al. 2004). Such morphological reduction concomitant with accelerated DNA sequence evolution has been reported for other aquatic plants, such as Podostemaceae (Les et al. 1997) and Lemnaceae (Rothwell et al. 2004).

The genus *Lilaeopsis* is extremely difficult taxonomically, as a result of its greatly reduced and generally similar vegetative morphology. In addition, the size and shape of the leaves are readily modified in response to various degrees of submergence and light intensity (Affolter 1985; Charlton 1992), and a similar degree of phenotypic plasticity is seen in several inflorescence characters (Affolter 1985). Traditionally, characters of the fruit (such as the distribution and abundance of spongy cells within the fruit) have been used to distinguish species and subdivide the genus, but these too may be extremely variable in some species (Affolter 1985). Previously, on the basis of limited sampling, the Mexican genus *Neogoezia* was implicated as the most likely sister group to *Lilaeopsis* (Petersen et al. 2002). Our results, in contrast, suggest its sister group is the clade comprising *Ptilimnium*, *Limnosciadium*, *Daucosma*, *Cynosciadium*, and the rachis-leaved species of *Oxypolis*. We refrain from discussing infrageneric relationships based on our sampling of nine of its 15 species because a molecular phylogenetic study of the genus *Lilaeopsis* was recently completed with greater sampling (Bone 2007) and a paper presenting the phylogeny and biogeography of the group is currently being prepared (T. Bone, S. Downie, and J. Affolter, unpublished data, 2008).

The three species of *Cynosciadium* and *Limnosciadium* were, at one time, treated in the genus *Cynosciadium* (Coulter and Rose 1900). Mathias and Constance (1941), however, transferred *Cynosciadium pinnatum* DC. and *Cynosciadium pinnatum* var. *pumilum* Engelm. & A. Gray [=*Cynosciadium pumilum* (Engelm. & A. Gray) J.M. Coult. & Rose] to their new genus *Limnosciadium*. While these three species are morphologically very similar, it is clear that Mathias and Constance's treatment of the group is correct because the separation of *Cynosciadium* from *Limnosciadium* is reflected in all phylogenies presented herein.

The last group within the North American Endemics clade encompasses the genera *Atrema*, *Neogoezia*, and *Trepocarpus*. Traditional taxonomic classifications treated these genera in three different tribes of subfamily Apioideae (Coriandreae, Smyrnieae, and Apieae, respectively), with *Atrema* considered a synonym of the otherwise Eurasian genus *Bifora* (Drude 1898; Pimenov and Leonov 1993). Molecular data were important in placing these genera in tribe Oenantheae and in separating *Atrema* from *Bifora* (Plunkett et al. 1996; Downie et al. 1998; Hardway et al. 2004). Constance (1987) considered *Neogoezia* as having no obvious close relatives because of its distinctive habit and simple, multiflowered umbels. However, *Neogoezia* does share characters with other members of Oenantheae, such as glabrous stems and leaves, fascicles of fleshy-tuberous roots, pinnately-compound leaves superficially resembling those of *Afrocarum* (=*Berula* s.l.), a simple umbel inflorescence like that of *Lilaeopsis*, and a preference for growing in moist areas. *Atrema* and *Trepocarpus* comprise well supported, monophyletic sister groups, and while they are similar in overall habit, their fruits are quite different and affinities to tribe Oenantheae are not immediately apparent upon consideration of morphology. *Trepocarpus aethusae* has large, oblong–linear, and prominently corky-thickened fruits and is a facultative wetland species (Wilm and Taft 1998), whereas *Atrema americanum* has smaller, subglobose fruits with filiform ribs and occurs in dry habitats.

Tribe Oenantheae was circumscribed on the basis of molecular systematic studies to include 17 genera, many of which are endemic to North America (Downie et al. 2000; Hardway et al. 2004). No prior taxonomic treatment has grouped together those genera included here in tribe Oenantheae, for in the system of classification of Apiaceae by Pimenov and Leonov (1993), modified from that of Drude (1898), these genera were distributed in three tribes and two subtribes. Moreover, while the name Oenantheae is attributed to Dumortier (1827), he only included three genera within the group, two of which are now placed in other tribes. Members of tribe Oenantheae share several attributes, such as a preference for moist or wet habitats and their associated adaptive features (e.g., spongy-thickened, globose to broadly ovate fruits, fascicled roots, and rachis or rachis-like leaves), but there are no obvious morphological synapomorphies expressed in all of its members. This is not surprising, given the fact that many tribes and clades recognized in subfamily Apioideae on the basis of molecular data cannot be delimited unambiguously using morphological or anatomical data (Downie et al. 2001). Our future plans in-

clude producing revisionary treatments for the North American members of the tribe, as well as examining a few additional (but difficult to obtain) species whose fruit and (or) vegetative morphologies suggest their possible inclusion in tribe Oenantheae.

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