Circumscription of Apiaceae tribe Oenantheae

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Previous molecular systematic investigations into the higher-level relationships of Apiaceae subfamily Apioideae have revealed a strongly supported clade recognised as tribe Oenantheae Dumort. These plants may have clusters of fibrous or tuberous-thickened roots, corky-thickened fruits, and other adaptations for existence in wet or aquatic habitats. In some species, the leaves may be finely dissected or linear-septate and much reduced. We have initiated collaborative studies to produce a comprehensive estimate of phylogeny of the tribe, but such investigations are thwarted because information on the composition of the tribe is lacking. Herein, tribe Oenantheae is circumscribed to include the following genera: *Afrocarum, Berula, Bifora (pro parte), Cicuta, Cryptotaenia (pro parte), Cynosciadium, Daucosma, Helosciadium, Lilaeopsis, Limnosciadium, Neogoezia, Oenanthe, Oxypolis, Perideridia, Ptilimnium,* *Sium*, and *Trepocarpus*. Relationships inferred from phylogenetic analyses of nuclear rDNA ITS sequences from 64 accessions representing all 17 genera reveal that four genera are not monophyletic. *Bifora* and *Cryptotaenia* have members that fall outside of the tribe; *Berula* and *Sium* each comprise two or more lineages within Oenantheae. The St Helena endemics, *Sium bracteatum* and *S. burchellii*, ally with African *Berula erecta*; this clade is sister to the African endemic species *Sium repandum* and *Afrocarum imbricatum*, and this entire group is allied closely with north temperate *Berula erecta*. Nomenclatural changes are in order, but must await further study. Representatives of eight genera native to North America comprise a monophyletic group, and results from relative rate tests suggest that this lineage is evolving much faster than any other major clade recognised within the tribe.

Introduction

In all higher-level molecular systematic studies of Apiaceae subfamily Apioideae to date, whether based on chloroplast DNA (cpDNA) restriction site comparisons or sequences from the nuclear ribosomal DNA internal transcribed spacer (ITS) region or several chloroplast loci, the 'Oenanthe clade' is revealed as a strongly supported monophyletic group (e.g. Plunkett *et al.* 1996, Downie *et al.* 1998, 2000b, Plunkett and Downie 1999). A core group of genera is recognised in many of these studies, and includes *Berula* W.D.J. Koch, *Cicuta* L., *Cryptotaenia* DC., *Helosciadium* W.D.J. Koch, *Oenanthe* L., *Oxypolis* Raf., *Perideridia* Rchb., and *Sium* L. The distinctive genera *Neogoezia* Hemsl. and *Lilaeopsis* Greene, whose affinities until recently were obscure, also belong within this clade (Plunkett *et al.* 1996, Petersen *et al.* 2002). The genera *Cynosciadium* DC., *Limnosciadium* Mathias and Constance (1944–1945), and *Ptilimnium* Raf. may be included as well, based on their similar vegetative and (or) fruit morphologies (Downie *et al.* 2000b, 2001). Thirteen genera have been recognised unequivocally as belonging to the *Oenanthe* clade.

The *Oenanthe* clade can be defined morphologically, although there are no characters that are common and unique to the group. Its members are characterised generally by having fascicled roots (which may be thickened and bear tubers) and glabrous leaves and stems. They are often associated with moist to wet habitats, and some are true aquatics. Many species, including the members of *Berula*, *Neogoezia*, *Perideridia*, and *Sium*, have pinnate leaves with primary divisions that are not further divided and are often rounded and of equal size, superficially resembling those leaves of members of the distantly related genus *Pimpinella* L. Species inhabiting moist to wet habitats, including representatives of *Helosciadium*, *Sium,* and *Oenanthe*, often have submerged leaves that are more finely dissected and have narrower lobes than those of the air-borne ones. Leaves, particularly the petioles, are sometimes succulent and the leaf lobes are reduced. In *Berula*, the lowest pair of primary divisions is absent, whereas in *Lilaeopsis* and some species of *Oxypolis* and *Ptilimnium*, all divisions are lacking and their initial number may only be inferred from the nodes (septae) extant on the leaf axis. The fruits of many members are globose to broadly-ovate and are commonly

spongy/corky thickened, facilitating dispersal in water (Affolter 1985, Wilm and Taft 1998). Indeed, Darwin (1859) was impressed by the lengthy flotation and subsequent germination abilities of *Helosciadium*. *Lilaeopsis* and *Neogoezia* have simple umbels, but this is an extremely rare feature of Apiaceae subfamily Apioideae. However, none of these attributes can be considered a synapomorphy for the clade, as each of these features can be found in genera outside of the group (Petersen *et al.* 2002). This is not surprising, given the fact that many tribes and clades recognised in subfamily Apioideae on the basis of molecular data cannot be delimited unambiguously using morphological or anatomical data (Downie *et al.* 2001).

No prior taxonomic treatment has grouped together those genera that are included here in the *Oenanthe* clade. In the system of classification of Pimenov and Leonov (1993), modified from Drude (1898), the 13 genera recognised in the group are distributed among three tribes (Apieae, Peucedaneae Dumort., and Smyrnieae Spreng.). Downie *et al.* (2000b) recognised this group as tribe Oenantheae Dumort., but stressed that their circumscription of the tribe is radically different from that proposed by Dumortier (1827) and others, such as Koso-Poljansky (1916) and Cerceau-Larrival (1962).

In this paper, we summarise the results of our efforts to identify additional members of Apiaceae tribe Oenantheae. We then report results of phylogenetic analyses of representatives of all genera comprising the tribe, placing emphasis on its African members, specifically the genera *Berula*, *Sium*, and a new addition to the tribe, *Afrocarum* Rauschert. Sequences of the ITS region were employed because they have been shown to be appropriate to assess evolutionary relationships within Apiaceae subfamily Apioideae (Downie *et al.* 2001). Given that no previous classification has grouped together those genera here recognised as constituting tribe Oenantheae, the results of this paper should facilitate further systematic activity on this widely distributed and strongly supported monophyletic group of Apiaceae.

Material and Methods

Taxonomic sampling

To ascertain the limits of Apiaceae tribe Oenantheae, ITS sequence data were procured from 100 accessions of subfamily Apioideae using standard PCR and sequencing methods (described below). These accessions included representation of the 13 genera unambiguously placed in the tribe as a result of earlier studies, plus *Bifora americana* (DC.) Benth. & Hook., provisionally included in the tribe on the basis of *matK* sequence comparisons (Plunkett *et al.* 1996). These accessions also included representation of 19 additional genera (those listed in Table 1, plus *Afrocarum*, *Daucosma* Engelm. & A. Gray ex A. Gray, and *Trepocarpus* Nutt. ex DC.) whose morphologies (or previous taxonomic placements, as indicated by their synonymies) suggested possible close affinities with those taxa already included in the tribe.

Sixty-four accessions were considered in the phylogenetic analysis (Table 2). The phylogenetic placement of the African genus *Afrocarum* was ascertained by sampling putatively allied genera *Berula* and *Sium* (Cannon 1978), from Africa and elsewhere. Sampling of the genus *Helosciadium* was comprehensive (Wolff 1927, Z. Popper and M. Watson, unpubl. data). For *Lilaeopsis* and the seven exclusively North American taxa (i.e. *Bifora americana*, *Cynosciadium*, *Daucosma*, *Limnosciadium*, *Neogoezia*, *Ptilimnium* and *Trepocarpus*), only single exemplars of each genus were included, as their phylogenetic relationships are the subject of an ongoing, concurrent study (S. Downie *et al.*, unpubl. data). Sampling of the remaining genera (*Cicuta*, *Cryptotaenia*, *Oenanthe*, *Oxypolis* and *Perideridia*) was based primarily on material availability. With the exception of *Lilaeopsis*, the nomenclatural type of each genus was included in this study.

Table 1: Taxa examined for inclusion in Apiaceae tribe Oenantheae but excluded on the basis of phylogenetic analysis of molecular data

Genus	Species examined (and synonyms)
Chamaele Miq.	*C. decumbens* (Thunb.) Makino (=*Sium decumbens* Thunb.; *Oenanthe decumbens* (Thunb.) Koso-Pol.)
Crenosciadium Boiss. & Heldr. ex Boiss.	*C. siifolium* Boiss. & Heldr. ex Boiss.
Cyclospermum Lag.	*C. leptophyllum* (Pers.) Sprague ex Britton & P. Wilson
Huanaca Cav.	*H. andina* Phil.
Lichtensteinia Cham. & Schltdl. *pro parte*	*L. interrupta* (Thunb.) Sond. (=*Oenanthe interrupta* Thunb.)
Muretia Boiss.	*M. lutea* (M. Bieb.) Boiss. (=*Sium luteum* (M. Bieb.) Spreng.)
Naufraga Constance & Cannon	*N. balearica* Constance & Cannon
Niphogeton Schltdl.	*N. ternata* (Willd. ex Schltdl.) Mathias & Constance (=*Apium montanum* Kunth)
Oreomyrrhis Endl.	*O. andicola* (Kunth) Hook. f.
Oreoschimperella Rauschert	*O. verrucosa* (J. Gay ex A. Rich.) Rauschert (=*Sium verrucosum* J. Gay ex A. Rich.)
Pimpinella L. *pro parte*	*P. siifolia* Leresche (=*Apium siifolium* (Leresche) Calest.)
	P. diversifolia DC. (=*Helosciadium pubescens* DC.)
Pterygopleurum Kitag.	*P. neurophyllum* (Maxim.) Kitag. (=*Perideridia neurophylla* (Maxim) T.I. Chuang & Constance; *Sium neurophyllum* (Maxim.) Hara)
Pternopetalum Franch.	*P. vulgare* (Dunn) Hand.-Mazz.
Sclerosciadium W.D.J. Koch ex DC. *pro parte*	*S. nodiflorum* (Schousb.) Coss. (=*Oenanthe nodiflora* Schousb.)
Spuriopimpinella (H. Boissieu) Kitag.	*S. calycina* (Maxim.) Kitag.
Stoibrax Raf.	*S. dichotomum* (L.) Raf. (=*Apium dichotomum* (L.) Calest.)

Table 2: Plant accessions from which nuclear ribosomal DNA ITS sequences were obtained, with corresponding voucher information and GenBank reference numbers. Two GenBank numbers per accession indicate separate ITS-1 and ITS-2 sequences (with no intervening 5.8S data); a single GenBank number (unless otherwise indicated) indicates a contiguous ITS-1, 5.8S, and ITS-2 sequence. Herbarium acronyms are according to Holmgren *et al.* (1990)

DNA No. or Accession Identifier	Taxon	Voucher Information	GenBank No.
132	*Afrocarum imbricatum* (Schinz) Rauschert	Tanzania, Iringa, Mufindi District, Igowole, *Kayombo & Kayombo 217* (MO)	AY360228
1444	*Afrocarum imbricatum*	Malawi, Northern Province, Mzimba District, Mzuzu, Katoto, *Pawek 5442* (MO)	AY360229
82	*Berula erecta* (Huds.) Coville	Zimbabwe, Alpes Rd near Harare, *Bayliss 10592* (MO)	AY353975 (ITS-1 only)
116	*Berula erecta*	Ethiopia, 90km S of Addis Ababa, *Ash 859* (MO)	AY353976 (ITS-1 only)
150	*Berula erecta*	Germany; cult. UIUC from seeds obtained from Univ. Oldenburg Bot. Gard., *Downie 150* (ILL)	U79607
251	*Berula erecta*	France; cult. UIUC from seeds obtained from Conservatoire et Jardins botaniques de Nancy, *Downie 251* (ILL)	U79605
2253	*Berula erecta*	Denmark, Sjælland, Sorø Sønderskov, *Petersen GPL28*	AY360230
2257	*Berula erecta*	Denmark, Sjælland, near Tuel à, *Seberg OSA 486*	AY360231
799	*Berula erecta*	Ethiopia; cult. Univ. California Bot. Gard., Berkeley, L. Constance pers. coll. C-2453 (UC)	U78369
1467	*Bifora americana* Benth. & Hook. f. ex S. Watson	USA, Texas, Williamson Co., S of Jarrell, *Nesom & Grimes 6415* (MO)	AY360232
1923	*Cicuta bulbifera* L.	USA, Illinois, Will Co., Keepatau Forest Preserve, *Taft 794* (ILLS)	AY360233
1935	*Cicuta bulbifera*	Canada, Ontario, Rushing River Provincial Park, E of Kenora, *Blanz & Jones 4750* (ILL)	AY360234
1113	*Cicuta maculata* L. var. *angustifolia* Hook.	USA, Wyoming, Goshen Co., Bear Creek, *Nelson et al. 33517* (RM)	AY360235
75	*Cicuta virosa* L.	Finland; cult. UIUC from seeds obtained from Univ. Joensuu Bot. Gard., *Downie 75* (ILL)	U78372
131	*Cicuta virosa*	Germany; cult. UIUC from seeds obtained from Univ. Oldenburg Bot. Gard., *Downie 131* (ILL)	U78372
426	*Cicuta virosa*	China, Yunnan, Xiao Zhongdian, RBGE Gyalthang Expedition, FED 426 (E)	AY353978, AY353985
817	*Cryptotaenia canadensis* (L.) DC.	USA, Illinois, Champaign Co., Urbana, *Downie 817* (ILL)	U79613
574	*Cryptotaenia japonica* Hassk.	Japan, Honshu Island, Koyosan area, *McNamara et al. 90* (UC); cult. Univ. California Bot. Gard., Berkeley (no. 90.0891)	U78367
402	*Cryptotaenia japonica*	China; cult. UIUC from seeds obtained from Shanghai Bot. Gard., *Downie 402* (ILL)	AY360236
1063	*Cynosciadium digitatum* DC.	USA, Illinois, Jackson Co., Shawnee National Forest, *Phillippe 21886* (ILLS)	AY360237
2397	*Daucosma laciniata* Engelm. & A. Gray	USA, Texas, Kerr Co., Kerville, *Heller 1943* (MO)	AY360238
62449	*Helosciadium bermejoi* (L. Llorens) Popper & M.F. Watson*	Spain, Balearic Islands, ex cult. Ville de Conservatoire botanique Mulhouse, France (E); cult RBGE (no. 19962449)	AY353979, AY353986
170	*Helosciadium crassipes* W.D.J. Koch ex Rchb.	France, Corse, Musella; cult. Conservatoire botanique de la Ville de Mulhouse (no. 2048A), Herb. Reduron s.n.	AY360239
64358	*Helosciadium inundatum* (L.) W.D.J. Koch	Sicily, Messina, *Davis & Sutton 64358* (E)	AF164822, AF164847
919	*Helosciadium nodiflorum* (L.) W.D.J. Koch	Jordan, Wadi Al-Yabis, along Jordan River, *Lahham & El-Oqlah 18* (Yarmouk Univ. Herb.)	AF164823
15108	*Helosciadium nodiflorum*	Spain, Grenada, *Charpin et al. AC15108* (E)	AY353980, AY353987
1871	*Helosciadium nodiflorum*	France, Vaucluse, Malaucène, Herb Reduron s.n.	AY360240
1870	*Helosciadium repens* (Jacq.) W.D.J. Koch	France, Haut-Rhin, Luewenheim; cult. Conservatoire botanique de la Ville de Mulhouse (no. 9463), Herb. Reduron s.n.	AY360241
1999	*Lilaeopsis occidentalis* J.M. Coult. & Rose	USA, Oregon, Douglas Co., East Gardiner, *Hill & Dutton 32982* (ILLS)	AY360242
2000	*Limnosciadium pinnatum* (DC.) Mathias & Constance	USA, Illinois, Champaign Co., Champaign, *Hill 30580* (ILLS)	AY360243

Table 2 cont.

DNA No. or Accession Identifier	Taxon	Voucher Information	GenBank No.
2138	*Neogoezia minor* Hemsl.	Mexico, Oaxaca, Sierra de San Felipe, *Molseed 278* (ISU)	AY360244
476	*Oenanthe banatica* Heuff.	Hungary; cult. UIUC from seeds obtained from Hungarian Academy of Sciences Bot. Gard., Vácrátót, *Downie 476* (ILL)	AY360245
40	*Oenanthe crocata* L.	Spain; cult. UIUC from seeds obtained from Real Jardín Botánico, *Downie 40* (ILL)	AY360246
247	*Oenanthe crocata*	Belgium; cult. UIUC from seeds obtained from Jardin Botanique National de Belgique, *Downie 247* (ILL)	AY360247
1300	*Oenanthe divaricata* (R. Br.) Mabb.	Portugal, Madeira, Levado de Norte, *Sunding s.n.*; cult. RBGE (no. 19931141)	AY360248
165	*Oenanthe fistulosa* L.	Hungary; cult. Univ. of Oldenburg Bot. Gard, *Downie 165* (ILL)	AY360249
1282	*Oenanthe peucedanifolia* Pollich	Germany; cult. UIUC from seeds obtained from Karl-Marx Univ., Leipzig, *Lee 24* (ILL)	AY360250
273	*Oenanthe pimpinelloides* L.	Belgium; cult. UIUC from seeds obtained from Jardin Botanique National de Belgique, *Downie 273* (ILL)	U78371
29	*Oenanthe pimpinelloides*	Germany; cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, *Downie 29* (ILL)	AY360251
521	*Oenanthe sarmentosa* J. Presl ex DC.	USA, California, San Mateo Co., *Plunkett 1308* (WS)	AY360252
915	*Oxypolis fendleri* (A. Gray) A. Heller	USA, Colorado, Rio Blanco Co., Rough Creek, *Vanderhorst 3759* (RM)	AY360253
1142	*Oxypolis occidentalis* J.M. Coult. & Rose	USA, California, El Dorado Co., Osgood Swamp, *Follette s.n.* (JEPS)	AY360254
1927	*Oxypolis rigidior* (L.) J.M. Coult. & Rose	USA, Illinois, Vermillion Co., Windfall Hill Prairie Nature Preserve, *Phillippe et al. 19411* (ILLS)	AY360255
1938	*Perideridia americana* (Nutt. ex DC.) Rchb.	USA, Illinois, Shelby Co., NE of Assumption, *Shildneck 12868* (ILL)	AY246910
780	*Perideridia howellii* (J.M. Coult. & Rose) Mathias	USA, California, Sonoma Co., Tin Barn Rd., *Raiche 30482* (UC), cult. UC Bot. Gard., Berkeley (no. 83.1080)	AY246959
855	*Perideridia kelloggii* (A. Gray) Mathias	USA, California, Sonoma Co., 5 mi N. of Cazadero, *Ornduff et al. s.n.* (UC); cult. Univ. of California Bot. Gard. (no. 81. 0521)	AY246962
635	*Perideridia kelloggii*	USA, California, Alameda Co., Berkeley, Univ. of California Bot. Gard., *Downie 635* (ILL)	AY246961
778	*Perideridia kelloggii*	USA, California, Sonoma Co., 5 mi N. of Cazadero, *Ornduff et al. s.n.* (UC); cult. Univ. of California Bot. Gard. (no. 81. 0521)	U78373
2165	*Ptilimnium capillaceum* (Michx.) Raf.	USA, Oklahoma, Rogers Co., Claremore, *Jones 3030* (ILL)	AY360256
177	*Sium bracteatum* (Roxb.) Cronk	St Helena, below Cuckhold's Point on west side, *Cronk 32* (E); ITS sequence supplied by J.C. Preston & Q.C.B. Cronk	AY353981, AY353988
178	*Sium bracteatum*	St Helena; material provided by V. Williams s.n. (ILL)	AY353982, AY353989
	Sium burchellii (Hook. f.) Hemsl.	St Helena; material provided by V. Williams s.n. (ILL)	AY353983, AY353990
109	*Sium frigidum* Hand.-Mazz.	China, Yunnan, Zhongdian County, Da Xu Shan, RBGE Gyalthang Expedition, FED 109 (E)	AF164842, AF164867
E	*Sium latifolium* L.	Denmark, N Sealand, *Davis s.n.* (E)	AY353984, AY353991
1632	*Sium latifolium*	France, Bas-Rhin, Hultenheim, cult. Conservatoire botanique de la Ville de Mulhouse (no. 9466), Herb. Reduron s.n.	AY360257
2256	*Sium latifolium*	Denmark, Sjælland, Bromme Lillesø, *Petersen & Seberg GPL31*	AY360258
61	*Sium repandum* Welw. ex Hiern	South Africa, Transvaal, Kaapsche Hoop, *Rogers 9101* (G)	AY353977 (ITS-1 only)
97	*Sium sisarum* L.	Hungary; cult. UIUC from seeds obtained from Hungarian Academy of Sciences Bot. Gard., Vácrátót, *Downie 97* (ILL)	U78370
311	*Sium sisarum*	France; cult. Jardin botanique de Caen, *Downie 311* (ILL)	AY360259
388	*Sium sisarum*	Canada, Montréal; cult. UIUC from seeds obtained from Jardin botanique de Montréal, *Downie 388* (ILL)	AY360260
53	*Sium sisarum*	Spain; cult. UIUC from seeds obtained from Real Jardín Botánico, *Downie 53* (ILL)	AY360261
83	*Sium sisarum*	Finland; cult. UIUC from seeds obtained from Univ. Joensuu Bot. Gard., *Downie 83* (ILL)	AY360262
12	*Sium suave* Walter	Canada, Montréal; cult. UIUC from seeds obtained from Jardin botanique de Montréal, *Downie 12* (ILL)	AY360263
1817	*Trepocarpus aethusae* Nutt. ex DC.	USA, Illinois, Alexander Co., Horseshoe Lake Conservation Area, *Basinger 10891* (ILLS)	AY360264

Methods

Details of the DNA extractions, PCR amplifications and purifications, and DNA sequencing are the same as provided elsewhere (Downie and Katz-Downie 1996, Downie *et al.* 1998, Hardway 2001). For most accessions, total genomic DNA was obtained from about 20mg of dried, leaf tissue using the Dneasy Plant Mini Kit (Qiagen Inc. Valencia, California). For the remaining accessions, the modified CTAB protocol described by Doyle and Doyle (1987) was used. The purified DNAs were PCR-amplified using primers 'ITS4' and 'ITS5' (White *et al.* 1990). Twenty-two complete ITS sequences were obtained through manual sequencing, using the dideoxy chain termination method using Sequenase (version 2.0, United States Biochemical Corporation, Cleveland, Ohio), with a-^{35}S-dATP as the labeling agent. Modifications to the sequencing protocol included denaturation of the DNA by boiling the DNA/primer/acetamide mix for 4min, followed by snap-chilling the annealing mixture for 3min in an ice water bath. Both PCR primers, and primers 'ITS2' and 'ITS3' (described in White *et al.* 1990, including modifications by Downie and Katz-Downie 1996), were used in manual sequencing reactions. Cycle sequencing reactions, using primers 'ITS4' and 'ITS5', were performed on all remaining purified PCR products using AmpliTaq DNA polymerase and fluorescent dye-labeled terminators (ABI Prism BigDye terminator vers. 3.0 Ready Reaction Cycle Sequencing Kit — Applied Biosystems, Foster City, California). Sequencing products were resolved by electrophoresis using Applied Biosystem's 377A automated DNA sequencer. All ITS sequences have been deposited in GenBank, as either separate ITS-1 and ITS-2 sequences or contiguous ITS-1, 5.8S, ITS-2 data (Table 2). For twelve accessions, sequences from 5.8S rRNA were unavailable, owing to the sequencing methods used to obtain these data. For three of these twelve accessions (*Berula erecta* nos. 82 and 116, and *Sium repandum* no. 61), sequence data were also unavailable for ITS-2, despite our repeated but unsuccessful efforts to PCR-amplify this region. Uncorrected pairwise nucleotide differences were determined using PAUP* version 4.0 (Swofford 1998), as they are commonly provided in other angiosperm studies (e.g. Baldwin *et al.* 1995).

Data analysis

The DNA sequences were aligned using CLUSTAL X (Jeanmougin *et al.* 1998). However, given the many small length differences observed among the sequences (particularly for those eight genera comprising the 'North American (NA) Endemics' clade, discussed below), a variety of costs for gap opening and gap extension was utilised. As a result of these different alignment parameters, the relative positions of several genera within the 'NA Endemics' clade changed, as did the position of this clade relative to the genera *Cicuta*, *Oenanthe*, and *Oxypolis*, when analysed using maximum parsimony. Such changes in tree topologies reflecting different cost matrices have been reported previously for Oenantheae (Petersen *et al.* 2002). We settled on using the default parameters of CLUSTAL X (specifically,

gap opening penalty = 15, and gap extension = 6.66), and reiterate that one of the major goals of this paper is to identify those genera comprising tribe Oenantheae, rather than elucidate all intergeneric relationships (particularly among the taxonomically problematic North American members of the group). The latter will be achieved in subsequent studies, by increasing the sampling of species and incorporating data from the more conservatively evolving chloroplast genome. Moreover, the relationships among *Berula*, *Afrocarum*, and the African *Sium* species, the second major goal of this paper, did not change upon consideration of different gap costs. Relative rate tests, using the method of Robinson *et al.* (1998), were implemented using the program RRTree version 1.1 (Robinson-Rechavi and Huchon 2000) to detect rate asymmetries of the ITS regions among taxa in tribe Oenantheae. The proportions of site differences were estimated using the two-parameter distance of Kimura (1980).

The resulting data matrix was first analysed using maximum parsimony (MP), with gap states treated as missing data. Characters were treated as unordered and all character transformations were weighted equally. Heuristic MP searches were replicated 1 000 times with random stepwise addition of taxa, Tree-Bisection-Reconnection (TBR) branch swapping, and saving Multiple Trees (MulTrees). Bootstrap values were calculated from 1 000 replicate analyses using TBR branch swapping and simple stepwise addition of taxa. The number of additional steps required to force particular taxa into a monophyletic group was examined using the constraint option of PAUP*. The ITS data were analysed as separate ITS-1, 5.8S, and ITS-2 regions, and combined. However, not all data sets were equivalent in their number of terminal taxa, as 5.8S and ITS-2 sequences were unavailable for twelve and three accessions, respectively. To examine the extent of conflict among the ITS-1 and ITS-2 data sets, the incongruence length difference test of Farris *et al.* (1995) was implemented using PAUP*'s partition-homogeneity test. The test was performed with 100 replicates, using the heuristic search option with simple addition of taxa, and TBR branch swapping. The complete data matrix was then analysed using maximum likelihood, after using the program Modeltest vers. 3.06 (Posada and Crandall 1998) to select an appropriate model of DNA substitution and to estimate its parameters. A heuristic search using random addition sequence and TBR branch swapping was implemented using PAUP*. One thousand bootstrap replicate analyses were conducted using neighbour-joining searches with ML distance, using the ML parameters inferred by Modeltest.

All trees were rooted with *Perideridia*. The results of previous systematic investigations of Apiaceae subfamily Apioideae based on a variety of molecular evidence reveal that the North American genus *Perideridia* is sister taxon to all other members of the tribe (Plunkett *et al.* 1996, Downie *et al.* 1998, 2000a, 2000b). In many studies, the *Komarovia* clade and tribe Pleurospermeae occur basal to tribe Oenantheae (Downie *et al.* 2001), rooting the trees with either *Komarovia* or *Pleurospermum* maintained *Perideridia* as sister taxon to all other Oenantheae genera and did little to affect ingroup tree topology (Hardway 2001).

Results

Circumscription of Oenantheae. Phylogenetic analysis of ITS sequences from 100 accessions, representing the 13 core genera of Oenantheae and 19 additional genera examined for possible inclusion in the tribe, resulted in the expansion of tribe Oenantheae by three genera (*Afrocarum, Daucosma*, and *Trepocarpus*). Representatives of the 16 remaining genera (Table 1) all fall outside of the tribe; the phylogenetic affinities of each will be discussed in a subsequent paper (K. Spalik and S. Downie, unpubl. data). *Bifora americana*, the only North American member of the genus, is confirmed as belonging to tribe Oenantheae; its congeners, *B. radians* M. Bieb. and *B. testiculata* (L.) Spreng. ex Schult., are placed in the apioid superclade (Downie *et al.* 2001). Similarly, the genus *Cryptotaenia* is polyphyletic, with *C. africana* Drude, *C. calycina* C.C. Towns., and *C. elegans* Webb ex Bolle placed outside of tribe Oenantheae, away from *C. canadensis* (L.) DC. and *C. japonica* Hassk., which are maintained in the tribe (K. Spalik and S. Downie, unpubl. data). The nomenclatural type of *Cryptotaenia* (*C. canadensis*) is included in Oenantheae, whereas the type of *Bifora* (*B. testiculata*) is not. In summary, 17 genera are recognised herein as constituting tribe Oenantheae.

Sequence analysis

Alignment of 64 ITS sequences, representing all 17 genera of tribe Oenantheae, resulted in a matrix of 633 positions, with three positions near the ITS-2–26S rRNA boundary excluded because of alignment ambiguity. Characteristics of these aligned data, as separate or combined ITS-1, 5.8S, and ITS-2 regions, are presented in Table 3. Fifty-eight unambiguous gaps, all but one ranging between one and three bp in size, were introduced to facilitate alignment. The remaining and largest gap, of 19 bp in size, characterised all accessions of *Helosciadium*. Of these 58 gaps, single bp deletions (relative to the *Perideridia* sequences) were most numerous (27), followed by single bp insertions (16) and two bp insertions (8; Figure 1). Half of these 58 gaps were restricted to sequences from the eight species comprising

the 'NA Endemics' clade (discussed below). A total of 31 gaps was parsimony informative; these were distributed almost equally between both spacer regions. Treating gaps as missing data, uncorrected pairwise sequence divergence values across the entire region ranged from identity (for several conspecific taxa) to 26.9% of nucleotides (between *Lilaeopsis occidentalis* and *Helosciadium inundatum*). The vast majority of pairwise comparisons ranged between 6% and 14%, whereas the highest divergence values were obtained among pairwise comparisons of sequences from the eight 'NA Endemics.' For the latter, these values ranged between 5.7% and 20.5% (and averaged 17%).

Phylogenetic analysis

MP analysis of combined ITS-1, 5.8S, and ITS-2 sequence data for 64 accessions of Apiaceae tribe Oenantheae resulted in 256 minimal length trees, each of 999 steps (Consistency Indices (CI's) = 0.5315 and 0.4846, with and without uninformative characters, respectively; Retention
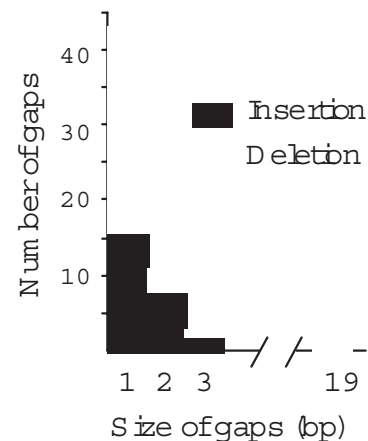
Figure 1: The number of gaps and their sizes inferred in the alignment of 64 ITS sequences of Apiaceae tribe Oenantheae. The number of insertions relative to deletions is indicated

Table 3: Comparisons among the data sets and most parsimonious (MP) trees presented in this study. The number of terminal taxa varied, for 5.8S and ITS-2 data were unavailable for 12 and 3 accessions, respectively

Data Set Characteristics and Cladogram Measures	ITS–1	5.8S	ITS–2	Combined
No. of terminals	64	52	61	64
Length variation (bp)	208–213	161–164	207–232	580–606
No. of aligned positions	224	164	245	633
No. of aligned positions excluded	0	0	3	3
No. of aligned positions constant	84	146	66	296
No. of aligned positions autapomorphic	32	8	40	80
No. of aligned positions parsimony informative	108	10	136	254
No. of unambiguous alignment gaps	27	4	27	58
No. of alignment gaps parsimony informative	15	3	13	31
Pairwise sequence divergence (range in %)	0–26.4	0–4.4	0–32.4	0–26.9
No. of MP trees	1 376	>5 000	348	256
Length of MP trees	432	27	510	999
Consistency index	0.5231	0.7037	0.5608	0.5315
Consistency index (excluding uninformative chars.)	0.4824	0.5789	0.5141	0.4846
Retention index	0.7832	0.8889	0.7887	0.7752
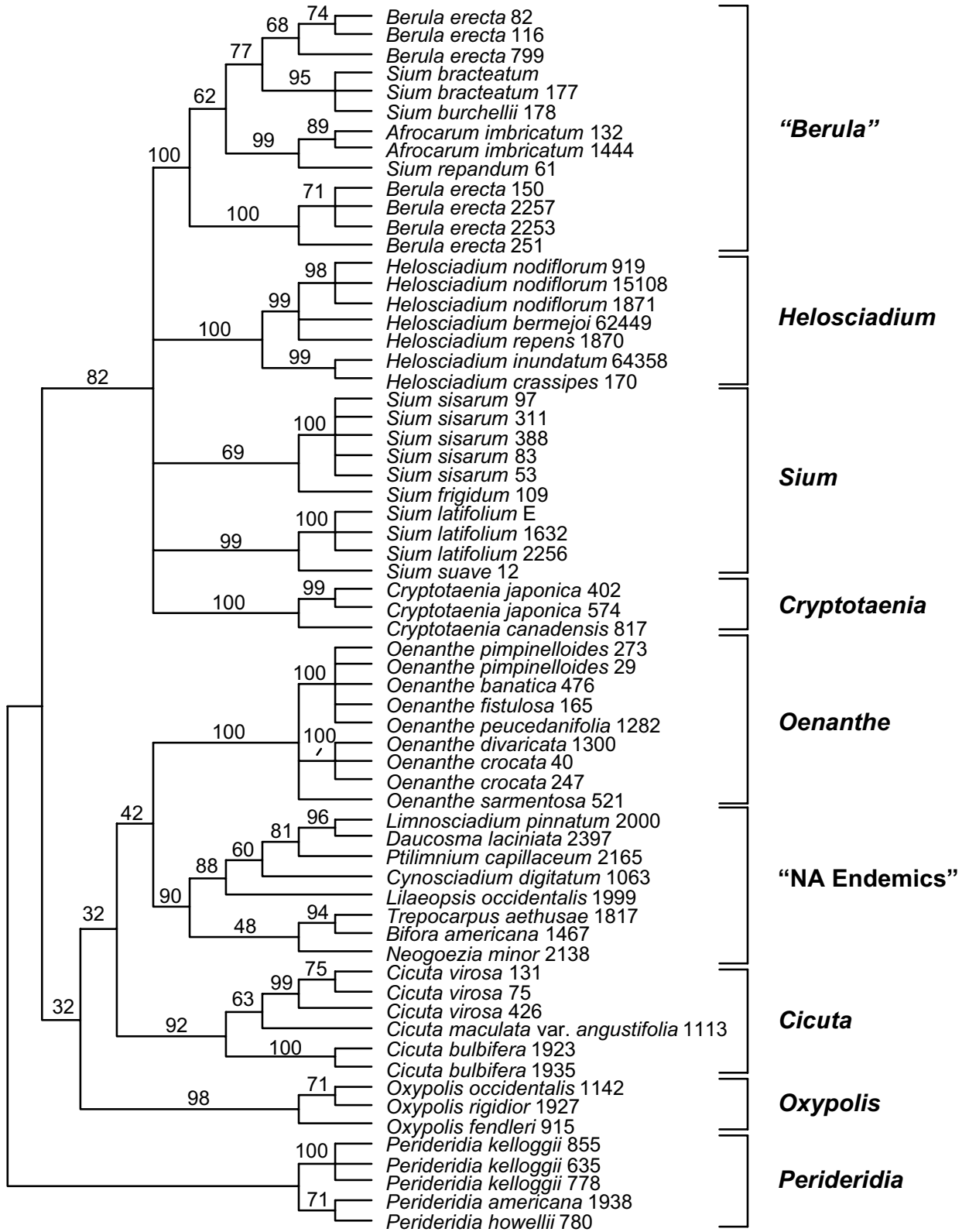
Figure 2: Strict consensus of 256 maximally parsimonious 999-step trees derived from equally weighted maximum parsimony analysis of aligned ITS-1, 5.8S, and ITS-2 sequences from 64 accessions of Apiaceae tribe Oenantheae (CI = 0.4846, excluding uninformative characters; RI = 0.7752). Numbers on branches represent bootstrap percentage estimates from 1 000 replicate analyses. Brackets indicate clade descriptors discussed in the text

Index (RI) = 0.7752 (Table 3). The strict consensus of these trees is presented in Figure 2. Separate MP analyses of the ITS-1 and ITS-2 data sets resulted in strict consensus trees (not shown) slightly less resolved but highly consistent with the strict consensus tree derived from combined data. The results of the partition-homogeneity test revealed that the two spacer regions do not yield significantly different phylogenetic estimates. Separate analysis of the 5.8S region resulted in a large polytomy, with only the group of eight 'NA Endemic' genera resolved as monophyletic. Greatest resolution of relationships was achieved when all molecular data were considered together, a result concordant to that reported from other studies of ITS data (Baldwin *et al.* 1995). Of the 31 potentially informative alignment gaps, 16 mapped without homoplasy when optimised on all minimal length trees. The largest gap, restricted to all *Helosciadium* sequences, was a 19bp deletion relative to the outgroup *Perideridia*. Other synapomorphic indels supported the monophyly of the genera *Cicuta*, *Cryptotaenia pro parte* (i.e. *C. canadensis* and *C. japonica*), *Oxypolis*, and *Perideridia*, and the species groups *Sium bracteatum* + *Sium burchellii*, *Afrocarum imbricatum* + *Sium repandum*, *Sium latifolium* + *Sium suave*, and *Trepocarpus aethusae* + *Bifora americana*.

On the basis of these results, the genera *Helosciadium*, *Cryptotaenia pro parte* (as above), *Oenanthe*, *Cicuta*, *Oxypolis*, and *Perideridia* constitute well-diagnosed groups, with supporting bootstrap values ranging between 92% and 100% and the possession of uniquely occurring indels. The genera *Berula* and *Sium* are not monophyletic. The two St Helena endemics (*Sium bracteatum* and *S. burchellii*) ally with the three *Berula* accessions from Africa (nos. 82, 116, and 799). This clade is sister to *Afrocarum imbricatum* + *Sium repandum*, two species also native to Africa, which, in turn, comprise a clade sister to the four accessions of European *Berula* examined (nos. 150, 251, 2253, and 2257). This entire clade, labeled 'Berula', comprising *Afrocarum*, *Berula* and the three *Sium* species endemic to St Helena and continental Africa, is supported strongly, with a bootstrap value of 100%. *Sium sisarum* (five accessions) + *S. frigidum* and *Sium latifolium* (three accessions) + *S. suave* comprise two distinct clades arising from a five-branched polytomy, along with the 'Berula' clade, *Helosciadium*, and *Cryptotaenia (pro parte)*.

Constraining the seven examined accessions of *Berula erecta* to monophyly and rerunning the MP analysis resulted in trees three steps longer than those most parsimonious. Constraining the ten non-African *Sium* accessions to monophyly (i.e. *Sium sisarum*, *S. frigidum*, *S. latifolium*, and *S. suave*) revealed a subset (224) of the 256 minimal length 999-step trees resulting from unconstrained analysis. Constraining all 14 *Sium* accessions to monophyly (including the African *Sium bracteatum*, *S. burchellii* and *S. repandum*) resulted in trees 26 steps longer than those most parsimonious. Based on these results, it is very unlikely that the genus *Sium*, as presently circumscribed to include the three African species, is monophyletic. In contrast, *Berula erecta* may prove to be monophyletic upon subsequent study and expanded sampling, given the many weakly supported internal branches in this portion of the tree.

The last major clade in the MP tree, labeled 'NA Endemics', comprises eight species native to North America. The genera *Cynosciadium*, *Daucosma*, *Limnosciadium*, *Ptilimnium*, and *Trepocarpus* are found exclusively in the USA, as is *Bifora americana*. *Neogoezia* is endemic to Mexico (Constance 1987). *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 a few outlying species in Australasia and elsewhere (Affolter 1985, Petersen and Affolter 1999). While we refer to this group as the 'NA Endemics' clade, we acknowledge that there are taxa outside of the clade that are also endemic to North America (such as *Oxypolis*, *Perideridia*, and all but one species of *Cicuta*). We also acknowledge that very few species of *Lilaeopsis* are actually native to North America. Therefore, we use the descriptor 'NA Endemics' for the sake of reference only.

Based on the results of the hierarchical likelihood ratio tests, Modeltest selected the TrN+G model of nucleotide substitution (Tamura and Nei 1993) as fitting these ITS data best (base frequencies: 0.2416, A; 0.2258, C; 0.2446, G; 0.2879, T; estimates of substitution rates: A↔C, 1; A↔G, 2.1879; A↔T, 1; C↔G, 1; C↔T, 4.4819; G↔T, 1; proportion of invariable sites = 0; gamma distribution shape parameter = 0.5083). Using these parameters, a single tree was recovered in PAUP*, with a –Ln likelihood score of 5805.7183 (Figure 3). A tree with identical topology (with a –Ln likelihood score of 5792.08846) was recovered using the best-fit model GTR+I+G (Rodríguez *et al.* 1990; proportion of invariable sites = 0.2017; gamma distribution shape parameter = 0.8653), selected by Modeltest's Akaike information criterion (Akaike 1974). The results of the ML analyses are similar to those inferred by MP, with the following exceptions: *Oenanthe* and *Cicuta* arise as weakly supported sister taxa; the ten non-African *Sium* accessions (*Sium sisarum*, *S. frigidum*, *S. latifolium*, and *S. suave*) unite as a weakly supported monophyletic group (with a 66% bootstrap value); and decreased internal support within the 'Berula' clade, including the near collapse of the branch uniting African *Berula*, *Sium*, and *Afrocarum*. The latter clade, however, is still supported strongly, with a 100% bootstrap value.

The presence of a five-branched polytomy in the MP tree, the many weakly supported or short basal branches in both MP and ML trees, and the rearrangement of certain taxa in the 'NA Endemics' clade depending upon the gap penalties invoked in generating the alignment, generally preclude unambiguous hypotheses of intergeneric relationship within tribe Oenantheae. Those relationships that are noteworthy include the union of *Afrocarum* with *Sium repandum*, the close affinity between the jellicos (i.e. *Sium bracteatum* and *S. burchellii*) of St Helena and African *Berula*, and the isolation of African *Sium* from its north temperate congeners, such as *Sium latifolium*, the nomenclatural type of the genus.

A striking feature of the ITS trees is the relatively long branch lengths characterising the members of the 'NA Endemics' clade, as seen in Figure 3. Sequence divergence values among the eight members comprising this clade are approximately 6–7 times higher (averaging 17%) relative to those within *Cicuta* (averaging 2.4%) or *Oenanthe* (averag-
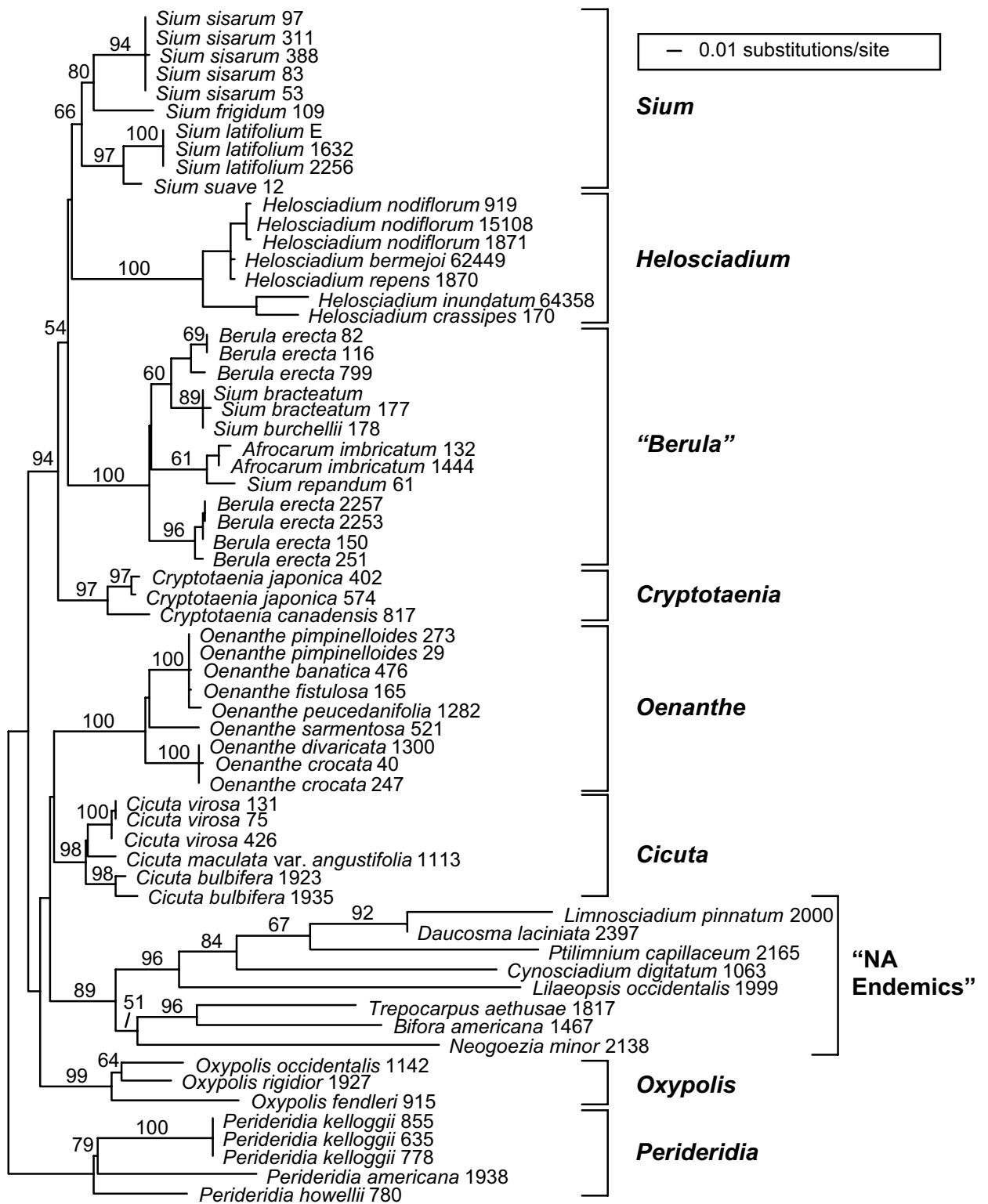
Figure 3: The single tree derived from maximum likelihood analysis of aligned ITS-1, 5.8S, and ITS-2 sequences from 64 accessions of Apiaceae tribe Oenantheae under a TrN+G model of nucleotide substitution (–Ln likelihood = 5805.7183). Numbers on branches represent bootstrap estimates for 1 000 replicate neighbor-joining analyses using a maximum likelihood model of nucleotide substitution; bootstrap percentage estimates <50% are not indicated. Brackets indicate clade descriptors discussed in the text

ing 2.8%), their putative sister taxa. Moreover, half of the 58 gaps inferred in the multiple alignment of all 64 ITS sequences were restricted to members of the 'NA Endemics' clade, as was the single small region of ambiguous alignment near the ITS-2–26S rRNA boundary excluded from the analysis. To detect rate asymmetry, 28 relative rate tests were conducted. Twelve sequences were assigned to nine defined lineages (representing one sequence from each of the nine major clades outlined in Figures 2–3, with the exception of the 'NA Endemics' clade, where four sequences were assigned). *Perideridia kelloggii* (no. 635) was used as the reference taxon (outgroup). Significant differences (P = 0.001) suggest that *Limnosciadium*, *Ptilimnium*, *Lilaeopsis*, and *Neogoezia*, the four examined sequences from the 'NA Endemics' clade, are each evolving much faster when compared to any sequence from outside of this clade. Rate differences of most other pairs of species were not statistically significant (at the 5% level). The molecular clock hypothesis for Oenantheae ITS sequences is therefore rejected.

Discussion

The circumscription and distribution of Apiaceae tribe Oenantheae

Table 4 lists the 17 genera recognised here as comprising tribe Oenantheae and their distributions. Four genera are not monophyletic. *Bifora* and *Cryptotaenia* have members that fall outside of the tribe; *Berula* and *Sium* each comprise two or more lineages within Oenantheae. The separation of *Bifora americana* from its Eurasian congeners, *B. radians* and *B. testiculata*, the latter the nomenclatural type of the genus, involves a change in nomenclature. The name

Atrema americana DC. already exists for these North American plants, but further study of North American Oenantheae is in order before such a change is implemented. The type of *Cryptotaenia*, *C. canadensis*, is maintained within the tribe, as is *C. japonica*; the latter, however, depending upon the treatment, may be recognised as a variety or subspecies of the former. Two African species of *Cryptotaenia* (*C. africana* and *C. calycina*) and the Macaronesian *C. elegans*, coinciding with Wolff's (1927) section *Afrosciadium*, are excluded from the tribe. Information on their phylogenetic placements is forthcoming (K Spalik and S Downie, unpubl. data).

The western North American monotypic genus *Shoshonea* Evert & Constance, erroneously placed in the *Oenanthe* clade on the basis of *mat*K sequence comparisons (Plunkett *et al.* 1996), belongs in the 'Angelica' clade of the apioid superclade (Downie *et al.* 1998, 2001, Plunkett and Downie 1999). These plants are caespitose-pulvinate, scaberulous, and possess a woody taproot, and are morphologically similar to several other genera of the region (Downie *et al.* 2002). They are also restricted to exposed calcareous outcroppings at high elevations (Evert and Constance 1982). Any of these features would make this genus an anomaly, if it was maintained in tribe Oenantheae. The *mat*K study of Plunkett *et al.* (1996) also placed *Cicuta* (specifically, *C. douglasii* (DC.) J.M. Coult. & Rose) in the *Angelica* clade, alongside three genera of North American distribution having affinities with *Shoshonea* (Downie *et al.* 2002). The genus *Cicuta* is unequivocally monophyletic (C. Lee and S. Downie, unpubl. data), and its position outside of tribe Oenantheae should be regarded as spurious.

Nine genera are native to North America (six exclusively to the USA), of which five are monotypic or bitypic. Three

Table 4: The composition and distribution of Apiaceae tribe Oenantheae Dumort. Species numbers are after Pimenov and Leonov (1993), except for *Berula* (Burtt 1991), *Cicuta* (Mulligan 1980), *Cynosciadium* (Mathias and Constance 1944-1945), *Helosciadium* (Wolff 1927; Z Popper and M Watson, unpubl. data), and *Lilaeopsis* (Affolter 1985, Petersen and Affolter 1999). Asterisks denote those genera that are not monophyletic as a result of this study

Genus	No. of Species	Distribution
Afrocarum Rauschert	1	Africa
Berula W.D.J. Koch*	1	Widespread
Bifora Hoffm.*	1 [a]	North America (USA)
Cicuta L.	4	3 NA; 1 Circumboreal
Cryptotaenia DC.*	2 [b]	Widespread
Cynosciadium DC.	1	North America (USA)
Daucosma Engelm. & A. Gray ex A. Gray	1	North America (USA)
Helosciadium W.D.J. Koch	5	Europe
Lilaeopsis Greene	14	New World, Australasia
Limnosciadium Mathias & Constance	2	North America (USA)
Neogoezia Hemsl.	5	Mexico
Oenanthe L.	40	Widespread
Oxypolis Raf.	7	North America
Perideridia Rchb.	13	North America
Ptilimnium Raf.	5	North America (USA)
Sium L.*	14	Widespread
Trepocarpus Nutt. ex DC.	1	North America (USA)

[a] *Bifora americana* (=*Atrema americana* DC.). *Bifora radians* and *B. testiculata* are excluded from tribe Oenantheae

[b] *Cryptotaenia canadensis* and *C. japonica*. *Cryptotaenia africana*, *C. calycina*, and *C. elegans* are excluded from tribe Oenantheae. The phylogenetic placements of *C. flahaultii* Koso-Pol., *C. polygama* C.C. Towns., and *C. thomasii* (Ten.) DC. have yet to be determined

species of *Cicuta* are also confined to North America, whereas *C. virosa* is circumboreal (Mulligan 1980). *Afrocarum* is endemic to tropical Africa, and *Helosciadium* is European in distribution. *Lilaeopsis* occurs in the temperate zones of North and South America and Australasia (Affolter 1985), as well as in Mauritius in the southwest Indian Ocean (Petersen and Affolter 1999). The remaining four genera are widely distributed, occurring in Europe, Asia, Africa, North America and, depending upon which genus, also in Central America, Australia and Australasia (Pimenov and Leonov 1993).

The 'Berula' clade

A well-supported clade in all trees (the '*Berula*' clade) contains *Berula erecta*, *Afrocarum imbricatum*, *Sium bracteatum*, *S. burchellii*, and *S. repandum*. Burtt (1991) recognised two subspecies within *Berula erecta*, and established subsp. *thunbergii* (DC.) B.L. Burtt 'with some reluctance'. These two subspecies are separated by the severity of cutting of the leaflets of the cauline leaves, with subsp. *thunbergii* having a more regular and less deeply dentate cutting than that of the typical subspecies. Moreover, subsp. *erecta*, although distributed widely in temperate Eurasia, North America, and elsewhere, does not occur in southern Africa (Burtt 1991). Geographic distribution aside, we could not satisfactorily distinguish between these subspecies, because one of our accessions from Africa had a jagged leaflet morphology just like European *Berula*. Nevertheless, it is intriguing that the African accessions of *Berula erecta* comprise a distinct clade, separate from their European counterparts. Therefore, while our results show that subsp. *thunbergii* may be a distinct taxon, the diagnostic characters used to distinguish it from the typical subspecies appear to be incorrect.

The jellicos of St Helena, *Sium bracteatum* and *S. burchellii*, unite as a monophyletic group sister to the three African *Berula* accessions. In both MP and ML trees, this entire group is sister to the clade of *Afrocarum imbricatum* and *Sium repandum*, suggesting that the St Helena endemics originated from Africa and shared an immediate common ancestor with African *Berula*. The four examined accessions of *B. erecta* from Europe comprise a strongly supported monophyletic group sister to the clade of all other aforementioned taxa. This alliance among *Berula*, *Sium*, and *Afrocarum* is not surprising. *Berula* and *Sium* are morphologically very similar and, in many early systems of classification, were treated as congeneric. The genus *Afrocarum* resembles *Sium* in several attributes (Cannon 1978), although it is generally, but erroneously, affiliated with *Carum*, under which it was first described (Cannon 1978, Townsend 1989). It is also intriguing that the jellicos of St Helena and *Sium repandum* are not immediately allied to their north-temperate congeners, such as *Sium latifolium*, the nomenclatural type of the genus.

The phylogenetic results presented here indicate that nomenclatural changes are in order, especially with regard to the monotypic genera *Afrocarum* and *Berula* vis-à-vis *Sium*. One approach is to redefine *Berula* to include *Afrocarum*, *Sium repandum*, and the two St Helena *Sium* species, but this leads to a complex genus that, pending further study, cannot readily be circumscribed morphologically.

Moreover, at least four new name combinations will be necessary. Another approach, equally unwieldy, is to recognise *Afrocarum*, *Sium repandum*, the St Helena endemics, and African *Berula* as separate genera. With the exception of the latter, each can be circumscribed unequivocally because of their distinctive morphology, but this leads to the creation of several monotypic genera, of which there are already far too many in the family (Spalik *et al.* 2001). However, before any such nomenclatural changes are implemented, further sampling and study are required, especially of *Berula erecta*.

The 'NA Endemics' clade

Based on ITS sequence data, all members of the 'NA Endemics' clade show a high level of sequence divergence, and relative rate tests suggest that this lineage is evolving much faster than any other within the tribe. This rapid divergence parallels their great morphological diversity. For example, many members of this clade (including the genus *Lilaeopsis*) exhibit a severe reduction of leaf morphology (Affolter 1985). Others, such as *Bifora americana* and *Trepocarpus*, have pinnately decompound leaves with filiform divisions. Their distinctive morphology has confused phylogenetic placement; as examples, *Neogoezia* and *Lilaeopsis*, with their simple umbels, have been treated in both subfamilies Apioideae and Hydrocotyloideae (reviewed in Constance 1987, and Peterson *et al.* 2002). Further study of the 'NA Endemics' clade is currently underway.

Taxonomic history

The taxonomic history of tribe Oenantheae Dumort. (as emended by Downie *et al.* 2000b and expanded upon here) is extraordinarily complex, especially because of the use of many longstanding names in earlier works that are now considered as synonyms. Sprengel (1820), proposing the first formal (i.e. tribal) subdivisions of subfamily Apioideae, placed *Cicuta* in tribe Smyrnieae Spreng., *Sium* in tribe Ammieae Spreng., and *Oenanthe* in tribe Pimpinelleae Spreng. Koch (1824), emphasising features of the seed face (endosperm) and mericarp ribs, moved *Oenanthe* into tribe Seseleae W.D.J. Koch and *Cicuta* into tribe Ammieae to join *Sium, Helosciadium*, and *Drepanophyllum* Hoffm. (the latter two genera being segregates of *Sium*). Dumortier (1827) refined Koch's treatment by placing *Cicuta*, *Helodium* Dumort. (= *Helosciadium*), and *Sium* in tribe Pimpinelleae subtribe Cicutinae Dumort. on the basis of the presence of flat endosperm and calyx teeth. In the same publication, Dumortier described the new tribe Oenantheae for the genera *Aethusa* L., *Coriandrum* L., and *Oenanthe*, defined by the presence of radiately ribbed fruits. This rather heterogeneous assemblage of genera was not followed by later authors, nor is it supported by molecular studies.

De Candolle (1830), following Koch's emphasis on the shape of the endosperm, as well as the distribution of vittae and other fruit anatomical characters, treated *Cicuta*, *Helosciadium*, and *Sium* (incl. *Berula*) in tribe Ammieae. He also included in this tribe, along with 18 other genera, his newly described *Cryptotaenia* DC. and *Discopleura* DC., the latter now treated in *Ptilimnium*. *Oenanthe* (including

Dasyloma DC.) and *Cynosciadium* were placed in Seseleae, with this tribe distinguished from Ammieae on the basis of the degree and direction of fruit compression. De Candolle treated his new genera *Archemora* DC. (= *Oxypolis*) and *Tiedemannia* DC. (= *Oxypolis*) in tribe Peucedaneae, *Eulophys* Nutt. ex DC. (= *Perideridia*) in tribe Smyrnieae, *Trepocarpus* Nutt. ex DC. in tribe Cumineae W.D.J. Koch, and *Crantzia* Nutt. (= *Lilaeopsis*), with its greatly reduced vegetative morphology and simple umbels, in tribe Hydrocotyleae Spreng. As such, the genera we recognise here as comprising tribe Oenantheae were scattered among six tribes!

The system of Bentham (1867) departed from all previous treatments, as he emphasised inflorescence form and the presence or absence of fruit vittae and secondary ribs. Once more, *Cicuta*, *Cryptotaenia*, and *Sium* (incl. *Berula*) were placed together in Ammieae subtribe Ammiinae Dumort.; however, *Oenanthe*, *Cynosciadium*, and *Discopleura* (= *Ptilimnium*) were treated in Seseleae subtribe Oenanthinae Benth. Also included in the latter was *Crantzia* (= *Lilaeopsis*), and eight other genera of distant affinity. *Eulophus* (= *Perideridia*) was maintained in Smyrnieae, but *Trepocarpus* was placed in tribe Caucalideae Spreng., along with *Daucus* and, surprisingly, *Bifora*. In the system of Boissier (1872), *Oenanthe* was placed in tribe Seseleae, away from *Helosciadium*, *Sium*, and *Berula* of tribe Ammieae. Drude (1898) maintained two major groups of genera, with *Cicuta*, *Cryptotaenia*, *Trepocarpus*, and *Sium* (incl. *Berula*) in Ammieae subtribe Carinae Drude, and *Oenanthe*, *Cynosciadium*, *Crantzia* (= *Lilaeopsis*), and *Discopleura* (= *Ptilimnium*) in Ammieae subtribe Seselinae Benth. *Eulophus* (= *Perideridia*) was moved to subtribe Carinae, and *Helosciadium* was included within an expanded *Apium*. *Tiedemannia* (= *Oxypolis*) was maintained in Peucedaneae. The exclusively Mexican genus *Neogoezia* was placed in Smyrnieae. Pimenov and Leonov (1993), basing their system on that of Drude, placed all but two of these genera into their large, heterogeneous tribe Apieae. *Neogoezia* and *Oxypolis* were maintained in Smyrnieae and Peucedaneae, respectively.

The detailed fruit anatomical studies of Koso-Poljansky (1916, 1917) only included some of the genera of our Oenantheae, but even so they were widely scattered among several tribes (for example, *Helosciadium* in Aethuseae Koso-Pol.; *Sium* in Crithmeae Koso-Pol.; *Oxypolis* in Peucedaneae; and *Cicuta*, *Oenanthe*, *Trepocarpus*, *Cyssopetalum* Turcz. (= *Oenanthe*), and *Ptilimnium*, along with 12 other genera now considered not very closely related, in Oenantheae Dumort.). Similarly, the novel groupings proposed by Cerceau-Larrival (1962), from her study of pollen and seedling morphology, and later adopted by Guyot (1966, 1971) in his survey of stomatal types, have done little to clarify the relationships among present-day Oenantheae. Cerceau-Larrival placed *Berula*, *Apium* (incl. *Helosciadium*), and *Sium* in her tribe Heteromorpheae, *Cryptotaenia* in her tribe Cryptotaenieae, and *Oenanthe* in a monotypic Oenantheae. None of her names, however, were validly published. In summary, no prior taxonomic treatment has unambiguously grouped together those genera defined herein as belonging to tribe Oenantheae. In recognising the tribe, Downie *et al.* (2000b) used Dumortier's (1827) name, but its circumscription is radically different from that proposed previously.

Final considerations

In this study, we considered representatives of 19 genera (beyond those recognised in Oenantheae at the onset of this study) for possible inclusion in the tribe. Of these, three (*Afrocarum*, *Daucosma* and *Trepocarpus*) represent the most recent additions to tribe Oenantheae. Two genera (*Bifora* and *Cryptotaenia*) were confirmed as polyphyletic, with some of their members having affinities outside of the tribe. The North American *Bifora americana* may be recognised as *Atrema americana*, pending further investigation. Molecular systematic studies have revealed that many genera of Apioideae are polyphyletic (Downie *et al.* 2001, Spalik *et al.* 2001); thus it is not unrealistic to assume that as material from additional species becomes available for those 16 genera examined for inclusion in the tribe but excluded on the basis of phylogenetic analysis of ITS data (such as, *Pimpinella* and its segregates), some may find affinity within tribe Oenantheae. Additional genera whose fruit and (or) vegetative morphologies suggest that they should be examined for possible inclusion in tribe Oenantheae include *Apodicarpum* Makino, *Asciadium* Griseb., *Kundmannia* Scop., *Ottoa* Kunth, and *Rutheopsis* A. Hansen & G. Kunkel. *Apium* sensu lato also merits special consideration. In the most recent worldwide treatment of *Apium*, Wolff (1927) recognised five sections, with one of these comprising those species recognised in the oenanthoid genus *Helosciadium*. Section *Apium* is taxonomically complex, and given the vegetative similarity of some of its members with those of tribe Oenantheae, it also deserves further attention. Lastly, while the ITS region provides phylogenetic resolution in tribe Oenantheae, additional sources of phylogenetic information from molecules and morphology, and denser taxonomic sampling are needed to gain a comprehensive and detailed phylogenetic understanding of Apiaceae tribe Oenantheae.

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