Molecular systematics of Old World Apioideae (Apiaceae): relationships among some members of tribe Peucedaneae sensu lato, the placement of several island-endemic species, and resolution within the apioid superclade

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Abstract: Comparative sequencing of the two internal transcribed spacer regions of nuclear ribosomal DNA was carried out to examine evolutionary relationships among representatives of Old World Apiaceae (Umbelliferae) subfamily Apioideae. Emphasis was placed on delimiting groups within the previously designated apioid superclade and clarifying relationships within and among the peucedanoid genera *Angelica*, *Ferula*, *Heracleum*, and *Peucedanum*. These spacer data, and those obtained from the chloroplast *rps*16 intron for a subset of the taxa, also enabled hypotheses on the phylogenetic placement of several narrowly distributed endemic species. The monophyly of Drude's tribe Echinophoreae is confirmed and it is sister to the Socotran endemic genera *Nirarathamnos* and *Rughidia*; the Balearic Islands endemic genus *Naufraga* allies with *Apium graveolens*; tribes Careae and Pyramidoptereae are recognized formally to be the previously designated clades "*Aegopodium*" and "*Crithmum*"; and tribes Oenantheae and Scandiceae are each expanded to include two species of *Apium*, previously attributable to *Helosciadium*, and four species of *Ferula*, respectively. Within the apioid superclade, five major lineages are recognized that are consistent with all available molecular evidence: tribe Echinophoreae, the clades "*Pimpinella*" and "*Heracleum*," and the more narrowly circumscribed clades "*Angelica*" and "*Apium*." *Angelica* and *Ferula* each comprise at least two lineages; *Heracleum* is polyphyletic if *Heracleum candicans* is retained in the genus; and *Peucedanum* is distributed in three well-separated clades with some species allied with those species of *Angelica* referred to *Xanthogalum*.

Key words: Apiaceae subfamily Apioideae, nuclear rDNA ITS, chloroplast *rps*16 intron, Umbelliferae.

Résumé : Les auteurs ont effectué un séquençage comparatif des deux régions internes de l'espaceur transcrit de l'ADN ribosomique nucléaire, afin d'examiner les relations évolutives entre les représentants des Apiaceae (Umbelliferae) de l'ancien monde. Ils se sont surtout intéressés à la délimitation des groupes au sein du superclade préalablement désigné et à la clarification des relations parmi et entre les genres peucédanoïdes *Angelica, Ferula, Heracleum*, et *Peucedanum*. Ces données sur les espaceurs, et celles obtenues à partir de l'intron chloroplastique *rps*16 pour un sous-ensemble de taxons, supportent également les hypothèses de localisation phylogénétique pour plusieurs espéces endémiques étroitement distribuées. La monophylie de la tribu Echinophorae de Drude se voit confirmée et elle est soeur des *Nirarathamnos* et *Rughidia* endémiques de l'île de Socotran; le genre *Naufraga* endémique aux îles Baléares est apparenté à l'*Apium graveolens*; on reconnait formellement les tribus Careae et Pyramidoptereae pour les clades préalablement désigné « *Aegopodium* » et « *Crithmum*; » on étend les tribus Oenantheae et Scandiceae pour inclure deux espèces d'*Apium* préalablement attribuées au genre *Helosciadium* et quatre espèces de *Ferula*, respectivement. Dans le superclade apioïde, on reconnait cinq lignées majeures qui concordent avec toutes les preuves moléculaires disponibles; on y retrouve la tribu des Echinophoreae, les clades « *Pimpinella* » et « *Heracleum* » et les clades plus étroitement circonscrits « *Angelica* » et « *Apium*. » Les *Angelica* et les *Ferula* comportent au moins deux lignées chacun. Le clade *Heracleum* est polyphylétique si on retient l' *Heracleum candicans* dans le genre, et le *Peucedanum* se distribue dans trois clades bien distincts, certaines espèces étant apparentées avec les espèces d'*Angelica* du groupe *Xanthogalum*.

Mots clés : Apiaceae sous-famille des Apioideae; ITS de l' ADNr nucléaire, intron chloroplastique *rps*16, Umbelliferae.

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Introduction

The higher level relationships within the flowering plant family Apiaceae (Umbelliferae) have been difficult to resolve, particularly within its large subfamily Apioideae. Comparison of the accounts of, for example, Koch (1824), de Candolle (1830), Bentham (1867), Boissier (1872), Drude (1898), Calestani (1905), and Koso-Poljansky (1916), which are based largely on fruit morphology and anatomy, shows widely diverging opinions on the definition and composition of its tribes and subtribes. While such focus on fruit structure has been rejected by many (Heywood 1971, 1982; Theobald 1971; Davis 1972; Cronquist 1982; Hedge et al. 1987; Shneyer et al. 1992, 1995), the highly criticized century-old system of Drude (1898), or some modification thereof (e.g., Pimenov and Leonov 1993), remains the most commonly used, as there is no widely accepted alternative. Drude (1898) divided Apiaceae into three subfamilies—Apioideae, Hydrocotyloideae, and Saniculoideae—recognizing 8 tribes and 10 subtribes within Apioideae. Molecular-systematic investigations have confirmed the monophyly of Apioideae and its sister relationship to the monophyletic subfamily Saniculoideae. These same studies have also shown that most of Drude's tribes and subtribes are not monophyletic (Downie and Katz-Downie 1996; Downie et al. 1996, 1998, 2000*a*, 2000*b*; Kondo et al. 1996; Plunkett et al. 1996*b*; Valiejo-Roman et al. 1998; Katz-Downie et al. 1999).

Tribe Peucedaneae illustrates well the problems faced with such artificial groups. Its members are characterized by a distinct dorsal flattening of the mature fruit, with the marginal ribs expanded into wing-like appendages. Drude (1898) treated the tribe in its broadest sense, recognizing three subtribes on the basis of differences in wing morphology—Angelicinae Tausch, Peucedaninae Tausch, and Tordyliinae Drude—that correspond essentially to tribes Angeliceae W.D.J. Koch, Peucedaneae Dumort., and Tordylieae W.D.J. Koch (Koch 1824; de Candolle 1830; Pimenov and Leonov 1993). The largest genera within the group are *Angelica* (110 species), *Ferula* (170 species), and *Peucedanum* sensu lato (100–120 species), and the taxonomic problems surrounding these taxa are legendary. Not only are their boundaries unclear—for example, many species have been treated in both *Angelica* and *Peucedanum,* while some (such as *Anethum graveolens*) have been referred to all three—but numerous generic segregates of uncertain taxonomic position are known (Drude 1898; Thellung 1926; Hiroe and Constance 1958; Shneyer et al. 1995). Immunological comparisons of seed-storage proteins have demonstrated the great serological heterogeneity of *Angelica* and *Peucedanum* and, in contrast, the homogeneity of *Ferula* (Shneyer et al. 1995). These same studies have also confirmed the close affinity between *Angelica* and *Peucedanum* and the separation of these genera from *Ferula*, despite the fact that *Peucedanum* and *Ferula* are often treated in the same tribe or subtribe. Taxonomic problems also surround the genus *Heracleum* (65 species) and allies, with affinities to *Ferula* suspected (Koso-Poljansky 1916; Theobald 1971). Tribe Peucedaneae is evidently nonmonophyletic (Theobald 1971; Shneyer et al. 1995; Plunkett et al. 1996*b*; Downie et al. 1998, 2000*b*), but the relationships among its major genera and many segregate taxa are far from clear.

Many early described temperate families that contain an assortment of edible, medicinal, or poisonous plants commonly have a few large genera (which comprise the greater part of the group in terms of species numbers) plus a large number of small genera, many of which are monotypic (Walters 1961; Heywood 1971). This holds true especially for subfamily Apioideae, for while several large and taxonomically complex genera exist, such as *Angelica, Ferula, Heracleum, Ligusticum, Peucedanum, Pimpinella, Pleurospermum,* and *Seseli*, so do many genera comprising only one or two species (Pimenov and Leonov 1993). The latter are particularly noteworthy, for they represent many of the aforementioned segregate genera. Whether this pattern reflects the phylogeny accurately or is simply due to taxonomic practice is also not clear. Moreover, the phylogenetic positions of many narrowly distributed endemic species, in particular those of island floras, need confirmation.

In this paper, we use the results of cladistic analyses of the chloroplast DNA (cpDNA) *rps*16 intron and nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences to ascertain relationships among available Old World representatives of Apiaceae tribe Peucedaneae sensu lato. Emphasis is placed on the large and taxonomically problematic genera *Angelica, Ferula, Heracleum*, and *Peucedanum*. We also confirm the phylogenetic positions of the Socotran endemic genera *Nirarathamnos*, *Oreofraga*, and *Rughidia*, and the Macaronesian and Mediterranean Islands endemic genera *Naufraga* and *Todaroa*. With the exception of tribes Scandiceae and Aciphylleae, where comprehensive sampling and earlier phylogenetic analyses precluded the necessity of including full representation of these monophyletic groups in this study (Mitchell et al. 1998; Lee and Downie 1999; Downie et al. 2000*a*), and the basal apioid genus *Bupleurum*, where accelerated sequence divergence has confounded alignment interpretation (Downie et al. 1998), we consider, herein, all available ITS sequences from primarily Old World Apioideae. Simultaneous analysis of these data, representing the most comprehensive analysis of Eurasian umbellifers to date, permits us to redefine the boundaries of several informal groups previously designated within the "apioid superclade" (Plunkett and Downie 1999).

Methods

Molecular tools

The utility of the nuclear rDNA ITS region for phylogenetic inference is outlined by Baldwin et al. (1995). Molecular data are also obtained from the chloroplast *rps*16 intron, given its potential for variation (Downie et al. 1996), the ease with which this region can be isolated from herbarium material using standard PCR procedures (Oxelman et al. 1997; Downie and Katz-Downie 1999), and the success others have had in using this locus for phylogenetic inference in other groups at comparable taxonomic levels (Lidén et al. 1997; Oxelman et al. 1997; Andersson and Rova 1999). For those species where sequence data are available, this group II intron varies in length from 707 to 951 bp (Oxelman et al. 1997), averaging 864 bp in Apiaceae (Downie and Katz-Downie 1999). Simultaneous consideration of nuclear and plastid DNA data can identify discrepant organismal and gene phylogenies (Rieseberg and Soltis 1991; Doyle 1992).

Accessions examined

One hundred and eighty species, representing 118 genera, were examined for nuclear rDNA ITS or cpDNA *rps*16 intron sequence variation, with 44 species common to both studies (Table 1). Thirty-five of these genera are monotypic and 13 are bitypic (Pimenov and Leonov 1993). In the ITS study, 165 complete ITS1 and ITS2 sequences were considered, of which 133 were taken from the literature (Downie et al. 1998, 2000*a*; Mitchell et al. 1998; Valiejo-Roman et al. 1998; Katz-Downie et al. 1999), 7 were obtained from unpublished data available in GenBank, and 25 are reported here for the first time (Table 1). Emphasis was placed on sampling Old World Umbelliferae, with representative species selected from the earlier analyses of Mitchell et al. (1998), Lee and Downie (1999), and Downie et al. (2000*a*). ITS sequences from Apiaceae subfamilies Hydrocotyloideae and Saniculoideae and allied families Araliaceae and Pittosporaceae were not included, owing to the difficulty of aligning them with those of Apioideae (Downie and Katz-Downie 1996; Downie et al. 1998). The apioid genus *Bupleurum* was also excluded, owing to high ITS sequence divergence (Downie et al. 1998). Included were 15 species of *Angelica* (including two accessions each of *A. acutiloba* and *A. decursiva*), eight species of *Peucedanum*, six species of *Ferula*, and four species of *Heracleum* (with two additional species of *Heracleum* included in the analysis of *rps*16 intron sequences). While these species were selected largely because of availability, they do represent many of the subdivisions within each of these genera (Boissier 1872; Drude 1898; Thellung 1926; Korovin 1947; Shishkin 1951; Pimenov 1968; Vasil'eva and Pimenov 1991).

In the *rps*16 intron study, the 22 complete sequences procured as part of this investigation were combined with 37 previously published sequences (Downie and Katz-Downie 1999; Lee and Downie 2000) for a matrix representing 59 species. The genera included were chosen with the aim of representing all three subfamilies of Apiaceae and most major lineages within Apioideae. Of the 12 major clades identified previously on the basis of phylogenetic analysis of *mat*K or *rpo*C1 intron data (Downie et al. 1996, 1998; Plunkett et al. 1996*b*), we included representation from 11. The small "*Conioselinum*" clade (group 8 of earlier investigations) was not considered.

Experimental strategy

Leaf material for DNA extraction was obtained either directly from the field, from plants cultivated from seed in the greenhouse, from accessioned plants cultivated at several botanic gardens, or from herbarium specimens. The deposition of voucher specimens is indicated in Table 1. Details of DNA extraction, PCR amplification, and DNA purification and the sequencing strategies used for both ITS and *rps*16 intron regions are the same as described previously (Downie and Katz-Downie 1996, 1999). Automated sequencing methods were used to procure all new sequence data. Simultaneous consideration of both DNA strands across both sequenced regions permitted unambiguous base determination in nearly all cases.

Sequence analysis

The new DNA sequences were added directly to ITS or intron PAUP* nexus files (version 4.0d65; D. Swofford, Smithsonian Institution, Washington, D.C.) constructed in previous studies; their respective alignments were adjusted manually as necessary. Gaps were positioned to minimize nucleotide mismatches. When gap coding was problematic or ambiguous, these regions of the alignment were excluded from the analysis. ITS boundaries corresponded to those of *Daucus carota*, which have been defined by S1 nuclease mapping (Yokota et al. 1989). Boundaries of the *rps*16 intron were determined by comparison with corresponding boundaries in tobacco, rice, mustard, and barley (Shinozaki et al. 1986; Hiratsuka et al. 1989; Neuhaus et al. 1989; Sexton et al. 1990) and consensus splice sites in other plants for group II introns (Michel et al. 1989). The determination of boundary sequences for the six conserved structural domains of the *rps*16 group II intron was based on similar boundary sequences inferred for tobacco and mustard (Michel et al. 1989; Neuhaus et al. 1989). The nucleotide sequence data reported in this study are available from GenBank (accession numbers provided in Table 1); aligned ITS or intron sequence data are available from the authors upon request.

Phylogenetic analysis

Phylogenetic analysis of the *rps*16 intron and its flanking 3′ exon sequences was carried out initially using maximum parsimony (MP) implemented using PAUP*. A finite number of shortest trees was obtained using 100 random-addition replicate searches, with tree bisection–reconnection (TBR) branch swapping, acctran optimization, and mulpars selected. Bootstrap values were calculated from 100 replicate analyses, simple-addition sequence of taxa, and TBR branch swapping. Decay analyses (Bremer 1988) were conducted until tree storage memory was exhausted. For the analysis of ITS data, MP trees were sought using the heuristic search strategies of PAUP* described in Downie et al. (1998), based on those presented in Catalán et al. (1997). Four thousand random-addition replicate searches were initiated, but no more than five of the shortest trees from each search were saved. The strict consensus of these trees was subsequently used as a topological constraint in another round of 4000 random-addition replicate searches but, in this case, only those trees that did not fit the constraint tree were saved. No additional trees were found at the length of the initial shortest trees, which suggests that the strict consensus tree adequately summarizes the available evidence, even though the exact number of trees at that length is not known. Bootstrap values (Felsenstein 1985) were calculated from 1000 replicate analyses, simple-addition sequence of taxa, and TBR branch swapping, with a set maxtree limit of 100 trees per bootstrap replicate. Distance trees for both the ITS and intron data sets were obtained from neighbor-joining (NJ) analysis (Saitou and Nei 1987) in PAUP*, using the two-parameter method of Kimura (1980). One hundred bootstrap replicates for each data set were completed. MP analyses of the reduced (i.e., 44 species) *rps*16 intron and ITS data matrices were carried out as for the separate analysis of the nonreduced intron data set, as was the analysis of the combined intron and ITS data.

Outgroups

All *rps*16 intron MP and NJ trees were rooted with *Aralia chinensis*, the only accession of Araliaceae included in the study. Phylogenetic analyses of molecular data (Plunkett et al. 1996*a*, 1997) corroborate traditional taxonomic evidence (Thorne 1973, 1992; Dahlgren 1980) in indicating that Araliaceae are closely related to Apiaceae. Molecular investigations have further revealed that the "araliaceous hydrocotyloid" genera, such as *Hydrocotyle* and *Centella*, are more closely related to Araliaceae than to other Apiaceae, whereas the hydrocotyloids *Azorella, Bolax*, and *Eremocharis* (i.e., the "*Azorella*" clade) unite as a clade that is sister to Apioideae and Saniculoideae (Plunkett et al. 1997; Downie et al. 1998, 2000*b*). Upon the removal of *Aralia, Centella*, and *Hydrocotyle*, trees rooted with *Bolax* and *Eremocharis* yielded the same internal topology as that produced when *Aralia* was used to root the trees.

ITS sequences from Araliaceae and Apiaceae subfamilies Hydrocotyloideae and Saniculoideae cannot be readily aligned with those of subfamily Apioideae (Downie and Katz-Downie 1996; Downie et al. 1998). Moreover, among the basal apioids, such as *Heteromorpha, Anginon, Glia*, and *Bupleurum*, PCR amplifications of ITS regions are either unsuccessful or yield weak products

Table 1. Accessions examined for nuclear rDNA ITS (*a*) or cpDNA *rps*16 intron – exon (*b*) sequence variation, with 44 species (*ab*) common to both studies.

		GenBank accession number	
Taxon	Source and voucher	*rps*16 intron	ITS 1, ITS 2
Aciphylla crenulata J. B. Armstr.[a]	Downie et al. 1998		
Aegokeras caespitosa (Sibth. & Sm.) Raf.[ab]	Downie et al. 1998	AF110541	
Aegopodium alpestre Ledeb.[a]	Downie et al. 1998		
Aethusa cynapium L.[ab]	Downie et al. 1998	AF110539	
Ammi majus L.[ab]	Downie et al. 1998	AF164814	
Anethum graveolens L.[ab]	Downie et al. 1998	AF110542	
Angelica acutiloba (Siebold & Zucc.) Kitag.[a]	GenBank		AJ131291
Angelica acutiloba (Siebold & Zucc.) Kitag.[a]	GenBank (as syn. *Ligusticum acutilobum*)		AB013037, AB013856
Angelica ampla A. Nelson[a]	Downie et al. 1998		
Angelica archangelica L.[ab]	Downie et al. 1998	AF110536	
Angelica arguta Nutt. ex Torr. & A. Gray[a]	Downie et al. 1998		
Angelica breweri A. Gray[a]	Downie et al. 1998		
Angelica cincta H. Boissieu[a]	Katz-Downie et al. 1999		
Angelica dahurica (Hoffm.) Franch. & Sav.[a]	Downie et al. 1998		
Angelica decurrens (Ledeb.) B. Fedtsch.[a]	Katz-Downie et al. 1999		
Angelica decursiva (Miq.) Franch. & Sav.[a]	GenBank		AJ131293
Angelica decursiva (Miq.) Franch. & Sav.[a]	Downie et al. 1998 (as syn. *Peucedanum decursivum*)		
Angelica gigas Nakai[a]	GenBank		AJ131290
Angelica polymorpha Maxim.[a]	Downie et al. 1998		
Angelica purpurascens (Avé-Lall.) Gilli[a]	Katz-Downie et al. 1999		
Angelica sachalinensis Maxim.[a]	Downie et al. 1998		
Angelica sylvestris L.[a]	Katz-Downie et al. 1999		
Angelica tatianae Bordz.[a]	Katz-Downie et al. 1999		
Anginon rugosum (Thunb.) Raf.[b]	Downie et al. 1998	AF110573	
Anisotome aromatica Hook. f. var. *pinnatisecta* Allan[a]	Downie et al. 1998		
Anthriscus caucalis M. Bieb.[ab]	Downie et al. 1998	AF110549	
Aphanopleura trachysperma Boiss.[a]	Katz-Downie et al. 1999		
Apium graveolens L.[ab]	Downie et al. 1998	AF110545	
Apium inundatum (L.) Rchb. f.[a]	Sicily, Messina, *Davis & Sutton 64358* (E)		AF164822, AF164847
Apium nodiflorum (L.) Lag.[ab]	Jordan, Wadi Al-Yabis, along Jordan River, *Lahham & El-Oqlah 18* (Yarmouk University Herbarium)	AF164820	AF164823, AF164848
Arafoe aromatica Pimenov & Lavrova[a]	Downie et al. 1998		
Aralia chinensis L.[b] [Araliaceae]	Downie et al. 1998	AF110609	
Aulacospermum anomalum (Ledeb.) Ledeb.[ab]	Katz-Downie et al. 1999	AF110558	
Aulacospermum simplex Rupr.[a]	Katz-Downie et al. 1999		
Azilia eryngioides (Pau) Hedge & Lamond[a]	Katz-Downie et al. 1999		
Balansaea glaberrima (Desf.) Maire[a]	Downie et al. 2000*a*		
Berula erecta (Huds.) Coville[ab]	Downie et al. 1998	AF164819	
Bifora radians M. Bieb.[ab]	Downie et al. 1998	AF164809	
Bifora testiculata (L.) Spreng. ex Schult.[b]	Cult. RBGE (Royal Botanic Garden Edinburgh) (No. 19970503) from cult. plant obtained from Bergius Botanic Garden, Sweden (E)	AF164817	
Bolax gummifera (Lam.) Spreng.[b]	Downie et al. 1998	AF110600	
Bubon macedonicum L.[a]	Downie et al. 2000*a* (as *Athamanta macedonica* (L.) Spreng.)		
Bunium elegans (Fenzl) Freyn[a]	Downie et al. 2000*a*		
Bupleurum fruticosum L.[b]	Spain, Jaén, Sierra de Pozo, *McBeath 2592* (E), cult. RBGE (No. 19921249)	AF110569	
Bupleurum ranunculoides L.[b]	Downie et al. 1998	AF110564	
Carlesia sinensis Dunn[a]	Downie et al. 1998		
Carum carvi L.[a]	Downie et al. 1998		
Caucalis platycarpos L.[ab]	Downie et al. 1998	AF123745	
Cenolophium denudatum (Fisch. ex Hornem.) Tutin[a]	Valiejo-Roman et al. 1998		
Centella asiatica (L.) Urb.[b]	Downie et al. 1998	AF110603	
Chymsydia colchica (Albov) Woronow ex Grossh.[a]	Downie et al. 1998		

Table 1 (*continued*).

		GenBank accession number	
Taxon	Source and voucher	*rps*16 intron	ITS 1, ITS 2
Cicuta virosa L.[ab]	Downie et al. 1998	AF164821	
Cnidiocarpa alaica Pimenov[a]	Katz-Downie et al. 1999		
Cnidium officinale Makino[a]	Downie et al. 1998		
Cnidium silaifolium (Jacq.) Simonk.[a]	Downie et al. 1998		
Conioselinum chinense (L.) Britton, Stern & Poggenb.[a]	Downie et al. 1998		
Conioselinum scopulorum (A. Gray) J.M. Coult. & Rose[a]	Katz-Downie et al. 1999		
Conioselinum tataricum Hoffm.[a]	Katz-Downie et al. 1999		
Conium maculatum L.[ab]	Downie et al. 1998	AF110546	
Conopodium bourgaei Coss.[a]	Downie et al. 2000*a*		
Coriandrum sativum L.[a]	Downie et al. 1998		
Cortia depressa (D. Don) C. Norman[a]	Katz-Downie et al. 1999		
Crithmum maritimum L.[ab]	Downie et al. 1998	AF110540	
Cryptotaenia japonica Hassk.[ab]	Downie et al. 1998	AF164818	
Daucus carota L. ssp. *sativus* (Hoffm.) Arcang.[ab]	Downie et al. 1998	AF110547	
Deverra triradiata Hochst. ex Boiss.[ab]	Downie et al. 2000*a*	AF164815	
Dicyclophora persica Boiss.[a]	Downie et al. 2000*a*		
Dystaenia takesimana (Nakai) Kitag.[a]	GenBank		AB013038, AB013857
Echinophora chrysantha Freyn & Sint.[a]	Valiejo-Roman et al. 1998		
Echinophora tenuifolia L. ssp. *sibthorpiana* (Guss.) Tutin[ab]	Downie et al. 2000*a*	AF164812	
Echinophora tournefortii Jaub. & Spach[a]	Downie et al. 2000*a*		
Elaeosticta allioides (Regel & Schmalh.) Kljuykov, Pimenov & V.N. Tichom.[a]	Downie et al. 2000*a*		
Eleutherospermum cicutarium (M. Bieb.) Boiss.[a]	Katz-Downie et al. 1999		
Endressia castellana Coincy[a]	Downie et al. 1998		
Eremocharis fruticosa Phil.[b]	Downie et al. 1998	AF110598	
Exoacantha heterophylla Labill.[a]	Katz-Downie et al. 1999		
Falcaria vulgaris Bernh.[a]	Downie et al. 1998		
Ferula communis L.[a]	Downie et al. 1998		
Ferula kingdon-wardii H. Wolff[a]	China, Yunnan, Zhongdian County, Napa Hai, RBGE Gyalthang Expedition, *FED 67* (E)		AF164824, AF164849
Ferula kokanica Regel & Schmalh.[ab]	Tadjikistan, Hushikat Gorge, *Pimenov et al. s.n.* (MW), cult. Moscow State University Botanical Garden, Russia	AF123751	AF164825, AF164850
Ferula tenuisecta Korovin ex Pavlov[a]	Valiejo-Roman et al. 1998		
Ferula tingitana L.[ab]	Cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót, *Lee 19* (ILL)	AF164808	AF164826, AF164851
Ferula violacea Korovin[a]	Valiejo-Roman et al. 1998		
Ferulago galbanifera (Mill.) W.D.J. Koch[a]	Valiejo-Roman et al. 1998		
Foeniculum vulgare Mill.[ab]	Downie et al. 1998	AF110543	
Fuernrohria setifolia K. Koch[a]	Katz-Downie et al. 1999		
Gingidia montana (J.R. Forst. & G. Forst.) J.W. Dawson[a]	New Zealand, Canterbury, RBGE Expedition to New Zealand and Australia, *ENZAT 389* (E)		AF164827, AF164852
Glia prolifera (Burm. f.) B.L. Burtt[b]	South Africa, Cape Province, Fernkloof Nature Reserve, *Barker 96A* (E), cult. RBGE (No. 19923034)	AF110572	
Grammosciadium pterocarpum Boiss.[ab]	Downie et al. 2000*a*	AF164813	
Hansenia mongholica Turcz.[a]	Katz-Downie et al. 1999		
Heracleum aconitifolium Woronow[a]	Katz-Downie et al. 1999		
Heracleum candicans Wall. ex DC.[b]	China, Yunnan, Zhongdian County, Napa Hai, RBGE Gyalthang Expedition, *FED 84* (E), cult. RBGE (No. 19952540)	AF164805	
Heracleum lanatum Michx.[ab]	Downie et al. 1998	AF110537	
Heracleum moellendorffii Hance[a]	China, Yunnan, Zhongdian County, Wu Feng Shan, RBGE Gyalthang Expedition, *FED 34* (E)		AF164828, AF164853

Table 1 (*continued*).

Taxon	Source and voucher	GenBank accession number	
		*rps*16 intron	ITS 1, ITS 2
Heracleum pastinacifolium C. Koch ssp. *incanum* (Boiss. & A. Huet) P.H. Davis[b]	Turkey A8: Çoruh, Kaçkar Dag, 15 August 1996, *K. Spalik s.n.* (WA)	AF164801	
Heracleum sphondylium L.[ab]	Downie et al. 1998	AF164800	
Heteromorpha arborescens (Spreng.) Cham. & Schltdl.[b]	Downie et al. 1998	AF110575	
Hydrocotyle rotundifolia Roxb. ex DC.[b]	Downie et al. 1998	AF110607	
Imperatoria ostruthium L.[a]	Downie et al. 1998		
Karatavia kultiassovii (Korovin) Pimenov & Lavrova[a]	Katz-Downie et al. 1999		
Komarovia anisosperma Korovin[ab]	Downie et al. 1998	AF110555	
Krubera peregrina (L.) Hoffm.[a]	Downie et al. 1998 (as *Capnophyllum dichotomum* (Desf.) Lag.)		
Laser trilobum (L.) Borkh.[a]	Katz-Downie et al. 1999		
Lecokia cretica (Lam.) DC.[a]	Downie et al. 1998		
Levisticum officinale W.D.J. Koch[a]	Downie et al. 1998		
Libanotis depressa R.H. Shan & M.L. Sheh[a]	China, Qinghai, Yushu Xian, *Ho et al. 2262* (E)		AF164829, AF164854
Lignocarpa diversifolia (Cheeseman) J.W. Dawson[a]	Mitchell et al. 1998		
Ligusticum canadense (L.) Britton[a]	Katz-Downie et al. 1999		
Ligusticum physospermifolium Albov[a]	Katz-Downie et al. 1999		
Ligusticum porteri J.M. Coult. & Rose[a]	Downie et al. 1998		
Ligusticum pteridophyllum Franch. ex Oliv.[a]	China, Yunnan, Zhongdian County, Wu Feng Shan, RBGE Gyalthang Expedition, *FED 36* (E)		AF164830, AF164855
Ligusticum scoticum L.[ab]	Downie et al. 1998	AF123756	
Lithosciadium multicaule Turcz.[a]	Cult. Moscow State University Botanical Garden, Russia		AF164831, AF164856
Malabaila secacul (Mill.) Boiss.[ab]	Katz-Downie et al. 1999	AF164802	
Monizia edulis Lowe[a]	Downie et al. 2000*a*		
Naufraga balearica Constance & Cannon[ab]	Downie et al. 2000*a*	AF164816	
Nirarathamnos asarifolius Balf. f.[ab]	Downie et al. 2000*a*	AF164811	
Oedibasis platycarpa (Lipsky) Koso-Pol.[a]	Katz-Downie et al. 1999		
Oenanthe pimpinelloides L.[ab]	Downie et al. 1998	AF110553	
Opopanax hispidus (Friv.) Griesb.[a]	Katz-Downie et al. 1999		
Oreofraga morrisiana M.F. Watson & E.L. Barclay[a*]	Yemen, Socotra, *M. Morris 4* (E)		AF164846, AF164871
Paraligusticum discolor (Ledeb.) V.N. Tichom.[a]	Downie et al. 1998		
Parasilaus asiaticus (Korovin) Pimenov[a]	Katz-Downie et al. 1999		
Pastinaca armena Fisch. & C.A. Mey.[ab]	Katz-Downie et al. 1999	AF164803	
Pastinaca sativa L.[ab]	Downie et al. 1998	AF110538	
Petagnaea saniculifolia Guss.[b]	Downie et al. 1998	AF110593	
Petroselinum crispum (Mill.) A.W. Hill[ab]	Downie et al. 1998	AF110544	
Peucedanum achaicum Halácsy[a]	Greece, N. Pelopónnisos, Vouraikos Gorge, *M. Southam s.n.* (E), cult. RBGE (No. 19912669)		AF164832, AF164857
Peucedanum caucasicum (M. Bieb.) K. Koch[a]	Katz-Downie et al. 1999		
Peucedanum cervaria (L.) Cusson ex Lapeyr.[a]	Katz-Downie et al. 1999		
Peucedanum japonicum Thunb. ex A. Murray[a]	GenBank		AJ131343
Peucedanum morisonii Besser ex Schult.[a]	Downie et al. 1998		
Peucedanum pschavicum Boiss.[a]	Katz-Downie et al. 1999		
Peucedanum terebinthaceum (Fisch. ex Trevis.) Fisch. ex Turez.[a]	Cult. UIUC (University of Illinois at Urbana—Champaign) from seeds obtained from Shanghai Botanic Garden, China		AF164833, AF164858
Peucedanum zedelmeyerianum Manden.[a]	Cult. Moscow State University Botanical Garden, Russia		AF164834, AF164859
Phlojodicarpus sibiricus (Fisch. ex Spreng.) Koso-Pol.[a]	Katz-Downie et al. 1999 (as syn. *P. popovii* Sipliv.)		
Physospermopsis kingdon-wardii (H. Wolff) C. Norman[a]	China, Yunnan, Da Xue Shan, RBGE Gyalthang Expedition, *FED 198* (E)		AF164835, AF164860
Physospermopsis rubrinervis (Franch.) C. Norman[a]	China, Yunnan, Zhongdian County, Stone Bridge Area, RBGE Gyalthang Expedition, *FED 378* (E)		AF164836, AF164861
Physospermum cornubiense (L.) DC.[ab]	Downie et al. 1998	AF110556	
Pimpinella peregrina L.[a]	Downie et al. 1998		

Table 1 (*concluded*).

Taxon	Source and voucher	GenBank accession number	
		*rps*16 intron	ITS 1, ITS 2
Pleurospermum decurrens Franch.[a]	China, Yunnan, Zhongdian County, Zhongdian, RBGE Gyalthang Expedition, *FED 327* (E)		AF164837, AF164862
Pleurospermum foetens Franch.[ab]	Katz-Downie et al. 1999	AF110559	
Pleurospermum hookeri C.B. Clarke[a]	China, Yunnan, Dechin County, Beima Shan, RBGE Gyalthang Expedition, *FED 284* (E)		AF164838, AF164863
Pleurospermum uralense Hoffm.[a]	Katz-Downie et al. 1999		
Prangos pabularia Lindl.[a]	Downie et al. 1998		
Psammogeton biternatum Edgew.[a]	India, Punjab, *R. Stewart 13791* April 1934 (US)		AF164839, AF164864
Pycnocycla aucherana Dcne. ex Boiss.[a]	Downie et al. 2000*a*		
Pyramidoptera cabulica Boiss.[a]	Katz-Downie et al. 1999		
Rhabdosciadium aucheri Boiss.[a]	Downie et al. 2000*a*		
Ridolfia segetum (L.) Moris[a]	Downie et al. 1998		
Rughidia cordata (Balf. f.) M.F. Watson & E.L. Barclay[a*]	Yemen, Socotra, *Miller et al. 10420* (E), cult. RBGE (No. 19910079)		AF164840, AF164865
Rughidia milleri M.F. Watson & E.L. Barclay[ab*]	Downie et al. 2000*a* (as *Peucedanum* sp.)	AF164810	
Sanicula canadensis L.[b]	Downie et al. 1998	AF110592	
Scaligeria moreana Engstrand[a]	Downie et al. 2000*a*		
Scandia geniculata (G. Forst.) J.W. Dawson[a]	Mitchell et al. 1998		
Scandix pecten-veneris L.[ab]	Downie et al. 1998	AF123753	
Selinum candollei DC.[a]	Downie et al. 1998		
Selinum cryptotaenium H. Boissieu[a]	China, Yunnan, Zhongdian County, Wu Feng Shan, RBGE Gyalthang Expedition, *FED 35* (E)		AF164841, AF164866
Seseli elatum L.[a]	Downie et al. 1998		
Seseli gracile Waldst. & Kit.[a]	Katz-Downie et al. 1999		
Seseli krylovii (V.N. Tichom.) Pimenov & Sdobnina[a]	Downie et al. 1998		
Seseli libanotis (L.) W.D.J. Koch[a]	Katz-Downie et al. 1999		
Seseli montanum L.[a]	Downie et al. 1998		
Seseli mucronatum (Schrenk) Pimenov & Sdobnina[a]	Katz-Downie et al. 1999		
Seseli peucedanoides (M. Bieb.) Koso-Pol.[a]	Katz-Downie et al. 1999		
Sium frigidum Hand.-Mazz.[a]	China, Yunnan, Zhongdian County, Da Xu Shan, RBGE Gyalthang Expedition, *FED 109* (E)		AF164842, AF164867
Sium latifolium L.[ab]	Downie et al. 1998	AF110552	
Smyrniopsis aucheri Boiss.[a]	Downie et al. 1998		
Smyrnium olusatrum L.[ab]	Downie et al. 1998	AF110551	
Sphaenolobium tianschanicum (Korovin) Pimenov[a]	Katz-Downie et al. 1999		
Tetrataenium rigens (DC.) Manden.[ab]	Downie et al. 1998 (as syn. *Heracleum rigens*)	AF164804	
Thapsia garganica L.[a]	GenBank		AJ007930
Thysselinum palustre (L.) Hoffm.[a]	Katz-Downie et al. 1999		
Tinguarra sicula (L.) Benth. & Hook. f.[a]	Downie et al. 2000*a*		
Todaroa aurea Parl.[a]	Canary Islands, Tenerife, Barranco del Infierno, *Dickson s.n.* (GLA)		AF164843, AF164868
Tommasinia verticillaris (L.) Bertol.[a]	Katz-Downie et al. 1999		
Tordylium aegyptiacum (L.) Lam.[ab]	Downie et al. 1998	AF164807	
Torilis arvensis (Huds.) Link[ab]	U.S.A., Illinois, Champaign Co., Urbana, *Downie 816* (ILL)	AF110548	AF164844, AF164869
Trachyspermum aethusifolium Chiou.[a]	Somalia, Bay, Qansaxdheese to Bardern, *Thulin et al. 6863* (E)		AF164845, AF164870
Trachyspermum ammi (L.) Sprague in Turrill[a]	Downie et al. 1998		
Zosima absinthifolia (Vent.) Link[ab]	Katz-Downie et al. 1999 (as syn. *Z. orientalis* Hoffm.)		AF164806

Note: Herbarium acronyms according to Holmgren et al. (1990).

*The name is not intended by the authors to be formally published in this paper; a formal description of this Socotran taxon is currently being prepared.

(Downie et al. 1998). ITS sequence data are currently available for *Heteromorpha arborescens* and several species of *Bupleurum*, the latter also exhibiting high sequence divergence. While plastid DNA data unequivocally support the basal position of the woody southern African *Heteromorpha* within Apioideae, maximum likelihood or NJ analyses of ITS data (the latter calculated with transitions weighted equally to transversions) reveal an affinity between *H. arborescens* and, surprisingly, a highly derived clade

Table 2. Sequence characteristics of the cpDNA *rps*16 intron – 3′exon and nuclear rDNA ITS regions.

Sequence characteristic	*rps*16 intron – 3′exon	ITS
Number of taxa	59	164
Nucleotide sites		
Length variation (mean) (bp)	949–1013 (973)[a]	417–446 (437)
No. of total aligned positions	1236	492
No. of aligned positions excluded	204	52
No. of aligned positions constant	651	69
No. of aligned positions parsimony informative	209	339
No. of aligned positions autapomorphic	172	32
Length variation		
No. of unambiguous alignment gaps parsimony informative	18	61
Sequence divergence (range (%))	0–13.7	0–33.6

[a] Length variation of the *rps*16 intron only is 839–903 bp (mean = 863 bp).

comprising such herbaceous and cultivated species as *Apium graveolens, Anethum graveolens*, and *Petroselinum crispum* (Downie et al. 1998; Valiejo-Roman et al. 1998). In this study, we have chosen against rooting the ITS trees with *Heteromorpha* and, instead, use the results of the current *rps*16 analysis and those of other plastid DNA studies (e.g., Downie et al. 2000*b*) to select *Physospermum* and its allies as the root.

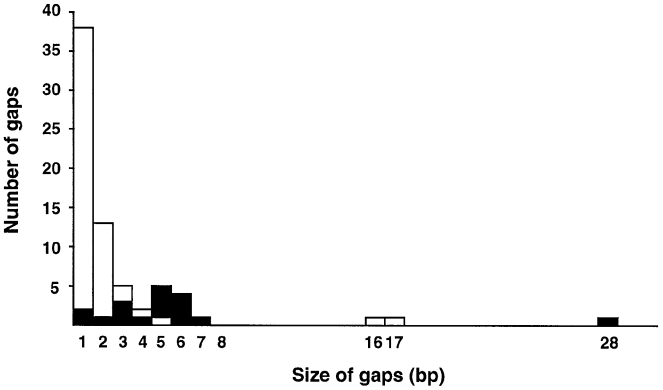
Results

*Rps*16 intron–exon sequence analysis

Among the 59 species examined, the length of the *rps*16 intron varied from 839 to 903 bp, averaging 863 bp. All sequencing reactions culminated in an additional 110 bp of sequence from the adjacent *rps*16 3′exon region, with no length variation exhibited. Alignment of all 59 intron and flanking 3′exon sequences resulted in a matrix of 1236 positions. However, owing to length mutations in some regions of the alignment confounding the interpretation of homology, 19 regions (204 alignment positions) were excluded from the analysis. These ambiguous regions ranged in size from 2 to 38 bp, averaging 11 positions each. Characteristics of the remaining 1032 alignment positions, including the number of constant, autapomorphic, and parsimony-informative sites, are presented in Table 2. Measures of pairwise sequence divergence ranged from identity (between *Anginon rugosum* and *Glia prolifera*) to 13.7% (between *Hydrocotyle rotundifolia* and *Scandix pecten-veneris*). Eighteen unambiguous alignment gaps were parsimony informative; these gaps ranged from 1 to 28 bp in size, averaging 6 bp (Fig. 1).

Like other plastid group II introns, the intron in chloroplast gene *rps*16 is characterized by six major structural domains (Michel et al. 1989). For each of these domains and across all 59 species compared, the number of aligned, excluded, constant, autapomorphic, and parsimony-informative positions, ranges in overall size and pairwise sequence divergence, and the number of unambiguous and parsimony-informative alignment gaps were calculated (Table 3). Domain I is the largest, averaging 486 bp in size, whereas domains V and VI are the smallest, ranging between 29 and 34 bp in size. The latter two domains are most conserved, with none or few informative positions, relatively low sequence divergence, and no alignment gaps. Because domains V and VI are required for intron processing (Learn et al. 1992; Clegg et al. 1994), it is not surprising that they are evolving slowly. Approximately 50% of domain II was excluded, because of alignment ambiguity, and domain III was proportionally the most variable region, as assessed by the number of autapomorphic and informative positions. Domains II and III are apparently dispensable in self-splicing introns and have been previously reported as having the highest rates of sequence change (Michel et al. 1989; Learn et al. 1992).

Fig. 1. Frequency of 18 (solid bars) and 61 (open bars) unambiguous and parsimony-informative gaps in relation to gap size inferred in the alignment of 59 cpDNA *rps*16 intron and 164 ITS sequences, respectively.



*Rps*16 intron–exon phylogenetic analysis

MP analysis of the 1032 unambiguously aligned *rps*16 intron and flanking 3′exon nucleotide positions resulted in three shortest trees. The strict consensus of these trees, each 776 steps in length, with consistency indices (CIs) of 0.6521 and 0.5345, with and without uninformative characters, respectively, and a retention index (RI) of 0.7735, is shown in Fig. 2, with accompanying bootstrap and decay values. With two exceptions, the NJ tree (not shown) was topologically identical to the MP strict consensus tree. Differences included the placement of *Physospermum cornubiense* in a clade with *Aulacospermum anomalum* and *Pleurospermum foetens* (collectively considered to be the "*Physospermum*" clade; see below) and the collapse of the (weakly supported) branch joining *Crithmum maritimum* with *Grammosciadium* and *Aegokeras*.

Table 3. Sequence characteristics of the six major structural domains of the cpDNA *rps*16 group II intron for 59 species of Apiaceae and the outgroup, *Aralia*.

Intron domain	Length (bp)		No. of aligned positions	No. of aligned positions excluded	No. of aligned positions constant	No. of aligned positions informative	No. of aligned positions autapomorphic	No. of unambiguous gaps parsimony informative	Sequence divergence (range (%))
	Variation	Average							
I	476–495	486	597	50	344	107	96	12	0–13.6
II	67–108	81	153	77	33	23	20	1	0–26.1
III	48–78	70	81	8	41	17	15	1	0–21.8
IV	126–153	137	205	69	65	46	25	4	0–30.7
V	34–34	34	34	0	32	0	2	0	0–5.9
VI	29–34	34	34	0	22	5	7	0	0–11.8

Based on the results of these analyses and on those groups erected in previous studies (Plunkett et al. 1996*b*; Downie et al. 1998, 2000*b*), 11 clades are identified in subfamily Apioideae (numbered 1–12 but excluding 8, the *Conioselinum* clade). In the context of those species examined, Apioideae are monophyletic and sister to the monophyletic subfamily Saniculoideae. Subfamily Hydrocotyloideae, on the other hand, comprises three separate lineages at the base of the trees. Considering only Apioideae and progressing upwards, group 12 (the "*Heteromorpha*" clade of previous studies) is sister to all remaining taxa, followed successively by group 11 (the "*Bupleurum*" clade), group 10 (the *Physospermum* clade of previous studies, with *Aulacospermum, Physospermum*, and *Pleurospermum* included), group 9 (the "*Komarovia*" clade), group 6 (the "*Oenanthe*" clade), group 7 (the "*Aciphylla*" clade, to which *Smyrnium* is allied), group 5 (the "*Daucus*" clade), and group 3 (the "*Apium*" clade). *Conium maculatum*, previously treated in the *Apium* clade (Downie et al. 1998), is now removed from this group (see Discussion). The "*Angelica*" (group 1), "*Crithmum*" (group 2), and "*Aegopodium*" (group 4) clades, the last represented herein by *Aegokeras* and *Grammosciadium*, comprise a trichotomy in the NJ tree (not shown), whereas groups 2 and 4 form a weakly supported clade that is sister to group 1 in the MP strict consensus tree (Fig. 2). Other phylogenetic resolutions pertinent to this study include: (*i*) the placement of *Naufraga balearica* as sister to *Apium graveolens* in the *Apium* clade; (*ii*) the placement of Socotran *Rughidia milleri* and *Nirarathamnos asarifolius* as sister to *Echinophora*; and (*iii*) a polyphyletic *Heracleum*. Other nonmonophyletic genera include *Apium* and *Bifora*. *Apium nodiflorum* falls alongside *Berula* in the *Oenanthe* clade, whereas *Bifora testiculata* allies with *Conium* (albeit with weak bootstrap support) and not with *Bifora radians*.

ITS sequence analysis

Alignment of 164 complete ITS1 and ITS2 sequences resulted in a matrix of 492 positions. *Oreofraga morrisiana* was excluded from the initial phylogenetic analysis, given its unusually short and highly divergent ITS sequence (discussed below). Of these 492 alignment positions, it was necessary to exclude 25 positions from ITS1 and 27 positions from ITS2, because of alignment ambiguities. Characteristics of these aligned sequences, including the number of constant, autapomorphic, and parsimony-informative positions, are presented in Table 2. Measures of pairwise sequence divergence across both spacer regions ranged from identity (between *Selinum cryptotaenium* and *Libanotis depressa* and between *Peucedanum japonicum* and *Angelica gigas*) to 33.6% (between *Anthriscus caucalis* and *Tordylium aegyptiacum*). Sixty-one unambiguous alignment gaps were informative for parsimony analysis; these ranged from 1 to 17 bp in size, averaging 2 bp (Fig. 1). No evidence of obvious ITS length polymorphisms within each accession, representing multiple rDNA repeat types, was observed. Sequence polymorphisms at individual nucleotide sites within individual samples were rarely encountered.

ITS phylogenetic analysis

MP analysis of 164 complete ITS1 and ITS2 sequences for subfamily Apioideae resulted in 5100 minimal-length 3061-step trees after 4000 random-addition replicate analyses (CIs = 0.2627 and 0.2524, with and without uninformative characters, respectively; RI = 0.7062). The strict consensus of these 5100 trees, rooted with those members belonging to the *Physospermum* clade (group 10), is presented in Fig. 3, with accompanying bootstrap values. A subsequent search using this consensus tree as a topological constraint failed to find any additional minimal-length trees that were not compatible with it. Ten major clades recognized in previous studies and outlined in Fig. 2 are indicated (Plunkett et al. 1996*b*; Downie et al. 1998, 2000*b*) but, unlike the results of the *rps*16 analysis, there is no resolution between apioid groups 1 and 3 (hereinafter treated collectively as group 1&3). The NJ tree (Fig. 4) is fully compatible with the results of the MP analysis with regard to the relationships among the major clades inferred. Differences between these trees occur mostly among the positions of the smaller clades within groups 1&3, 5, and 7.

Phylogenetic analysis of *rps*16 intron–exon sequences (Fig. 2), like that of the *rpl*16 intron (Downie et al. 2000*b*), supports the traditional taxonomy (Sprengel 1820; Koch 1824; de Candolle 1830; Cerceau-Larrival 1962), in suggesting a close relationship between *Physospermum* and *Pleurospermum* sensu lato (including *Aulacospermum* and *Eleutherospermum*). These genera comprise the previously delimited *Physospermum* clade (Downie et al. 2000*b*), with this group basal within Apioideae upon the exclusion of the *Bupleurum* and *Heteromorpha* clades (Downie et al. 2000*b*). As representatives of the latter two clades were not included in the ITS study, the *Physospermum* clade was used to root the trees. Progressing upwards from the base, the two Chinese *Physospermopsis* species fall sister to *Hansenia*, the latter previously treated with *Komarovia* and *Parasilaus* in

Fig. 2. Strict consensus tree of three minimal-length 776-step trees derived from equally weighted MP analysis of 59 *rps*16 intron and flanking 3′exon sequences (CIs = 0.6521 and 0.5345, with and without uninformative characters, respectively; RI = 0.7735). Numbers above the nodes are bootstrap estimates for 100 replicate analyses. Decay values for trees of one, two, and three or more steps greater than those most parsimonious are indicated by single, double, and no symbols, respectively. The numbered brackets represent those groups of Apioideae outlined in Downie et al. (1998) and discussed in the text. Abbreviations: S, Apiaceae subfamily Saniculoideae; and H, Apiaceae subfamily Hydrocotyloideae.

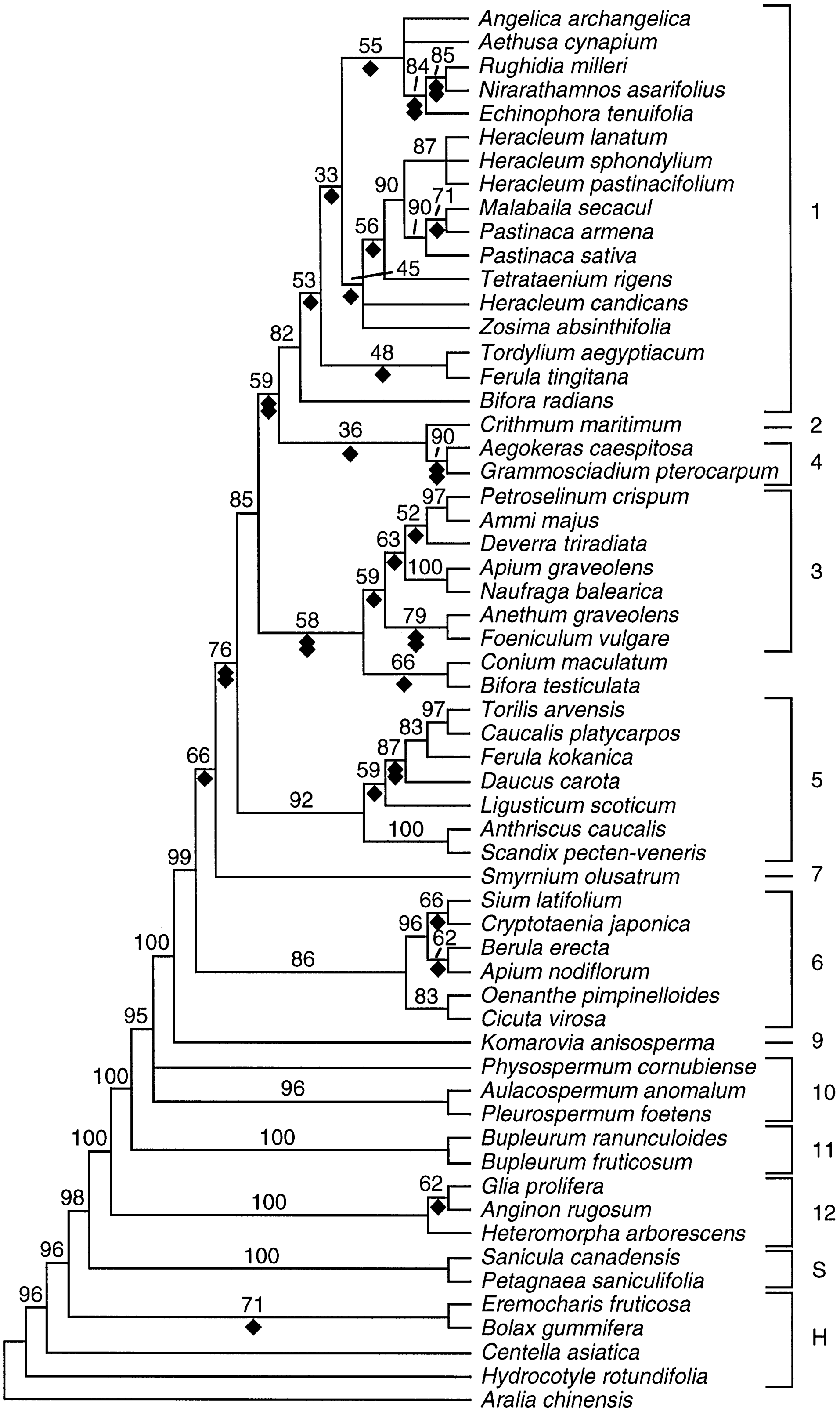


Fig. 3. Strict consensus tree of 5100 minimal-length 3061-step trees derived from equally weighted MP analysis of 164 complete ITS1 and ITS2 sequences (CIs = 0.2627 and 0.2524, with and without uninformative characters, respectively; RI = 0.7062). Numbers above the nodes are bootstrap estimates for 1000 replicate analyses (with a set maxtree limit of 100 trees per replicate); values <50% are not indicated. The numbered brackets represent those groups of Apioideae outlined in Fig. 2 and in previous investigations. Major subclades within groups 1&3, 5, and 7, all possessing bootstrap values of ≥88%, are identified. The ITS sequence for *P. japonicum* is likely misidentified (see text for further discussion).

group 9, the *Komarovia* clade (Katz-Downie et al. 1999; Pimenov et al. 1999). Next is group 7, the *Aciphylla* clade, previously delimited as comprising two related groups: the Australasian endemic genera *Aciphylla, Anisotome, Gingidia, Lignocarpa*, and *Scandia* (group 7A) and the largely Eurasian genera *Lecokia* and *Smyrnium* (group 7C). Herein we add (as group 7B) *Peucedanum terebinthaceum* and *Pleurospermum hookeri* from China and *Ligusticum scoticum*, the type of the genus *Ligusticum*. In the *rps*16 intron strict consensus tree (Fig. 2), *L. scoticum* allies with group 5, the *Daucus* clade, as it does in other studies of plastid DNA (Kondo et al. 1996; Downie et al. 1998, 2000*b*). While the members of group 7 comprise a strongly supported clade in the NJ tree (Fig. 4), the MP strict consensus tree (Fig. 3) places *Smyrnium* and *Lecokia* (group 7C) as sister to the *Daucus* clade and the remaining members of group 7. The putative sister relationship between groups 7A and 7B in both trees is supported weakly (with bootstrap values of either 53 or 67%).

The *Daucus* clade (group 5) comprises four subclades of equivocal relationship, three of which have been previously designated as Scandiceae subtribes Scandicinae Tausch (group 5A), Daucinae Dumort. (group 5B), and Torilidinae Dumort. (group 5D; Downie et al. 2000*a*). The Mediterranean and Canary Islands endemic species *Tinguarra sicula* and *Todaroa aurea* (group 5A) unite as sister taxa in the MP strict consensus tree but not in the NJ tree. In the latter, they ally with Algerian *Balansaea glaberrima* and Moroccan *Conopodium bourgaei*. The fourth subclade (group 5C) contains four of the six species of *Ferula* examined, and is designated herein as the *Ferula* subclade. In both MP and NJ trees, bootstrap values supporting clades 5A through 5D are high (87–98%). Next is group 6, the *Oenanthe* clade (with its boundaries expanded to include two species of *Apium*), followed by group 8, the *Conioselinum* clade. Progressing further up the trees, the *Crithmum* (group 2) and *Aegopodium* (group 4) clades are each well supported (bootstrap values 85–98%), with this assemblage sister to group 1&3. The sister relationship between the *Crithmum* and *Aegopodium* clades is weakly to moderately supported (with bootstrap values of 55 or 76%).

Within apioid group 1&3 (the previously delimited *Angelica* and *Apium* clades), several smaller but well-supported clades are apparent (labeled A–F in Figs. 3 and 4). Group A is a large clade of 50 taxa, representing all included members of *Angelica, Selinum,* and *Seseli* and all but one species of *Peucedanum*. With the exception of *Angelica tatianae* and *Angelica purpurascens*, the 15 remaining accessions of *Angelica* (along with *Peucedanum japonicum*) are monophyletic in the NJ tree. The species *Seseli peucedanoides*, *Cnidium silaifolium* (= *Katapsuxis silaifolia* (Jacq.) Raf. in Reduron et al. 1997), *Cnidocarpa alaica*, and *Ligusticum physospermifolium* (= *Macrosciadium physospermifolium* (Albov) V.N. Tichom. & Lavrova) comprise a strongly (100% bootstrap) supported clade. Weaker (65–69% bootstrap) support exists for the clade of *Selinum* (2 species), *Ligusticum pteridophyllum*, *Libanotis depressa*, *Cortia depressa*, *Phlojodicarpus sibiricus*, *Carlesia sinensis*, and *Seseli* (5 species). *Selinum cryptotaenium* and *L. depressa*, and *P. japonicum* and *A. gigas*, each have identical ITS sequences. Group B is a clade comprising *Heracleum*, *Malabaila*, *Pastinaca*, *Tetrataenium*, *Tordylium*, and *Zosima*. In both MP and NJ analyses, *Conium* is allied weakly with this group (with < 50% bootstrap support). Group C represents a sister relationship between the Socotran endemic species *Nirarathamnos* and *Rughidia* spp. (and Somalian *Trachyspermum aethusifolium*) and three genera of tribe Echinophoreae (*Echinophora* spp., *Dicyclophora*, and *Pycnocycla*). This relationship is supported strongly in both analyses (with 100% bootstrap values), as is the monophyly of each of their respective groups. Group D is a clade comprising *Deverra* and *Naufraga* and the cultivated species of *Ammi*, *Anethum*, *Apium*, *Foeniculum*, *Petroselinum*, and *Ridolfia*. In the NJ tree and a subset of the MP trees, *Krubera* is sister to this clade. Group E represents a clade of *Arafoe*, *Bubon*, *Pimpinella*, *Aphanopleura*, and *Psammogeton* that, in the NJ tree, is sister to all other members of group 1&3. Group F is a clade comprising *Thysselinum*, *Cenolophium*, and two of the six species of *Ferula* examined.

Of those 18 genera where two or more species were included in the ITS study, only 5 are retained as monophyletic (*Aulacospermum*, *Echinophora*, *Pastinaca*, *Physospermopsis*, and *Sium*; Table 4). The genera *Angelica*, *Apium*, *Cnidium*, *Conioselinum*, *Ferula*, *Heracleum*, *Ligusticum*, *Peucedanum*, *Pleurospermum*, *Selinum*, *Seseli*, and *Trachyspermum* are each polyphyletic, whereas *Rughidia* is paraphyletic with included *Nirarathamnos*. Pairwise sequence divergence among the 17 included accessions of *Angelica* ranges from 0.2 (between the two accessions of *A. acutiloba*) to 7.2% (between *A. dahurica* and *A. decursiva*). Constraining *Angelica* to monophyly results in trees three steps greater than those most parsimonious. *Angelica gigas* and *Peucedanum japonicum* also have identical ITS sequences, but the latter is likely misidentified (see Discussion). Of the seven remaining species of *Peucedanum* included in this study, six occur within group 1&3. Pairwise sequence divergence among these six species ranges from 1.9 (between *P. zedelmeyerianum* and *P. morisonii*) to 9.3% (between *P. pschavicum* and *P. cervaria*). Constraining these six species to monophyly results in trees 13 steps greater than those most parsimonious. Constraining *Seseli* to monophyly, with its seven included species, results in trees 25 steps greater than those most parsimonious; pairwise sequence divergence among *Seseli* accessions ranges from 3.7 to 9.8%.

The position of *Oreofraga morrisiana*, a yet to be described taxon from Socotra, could not be determined with

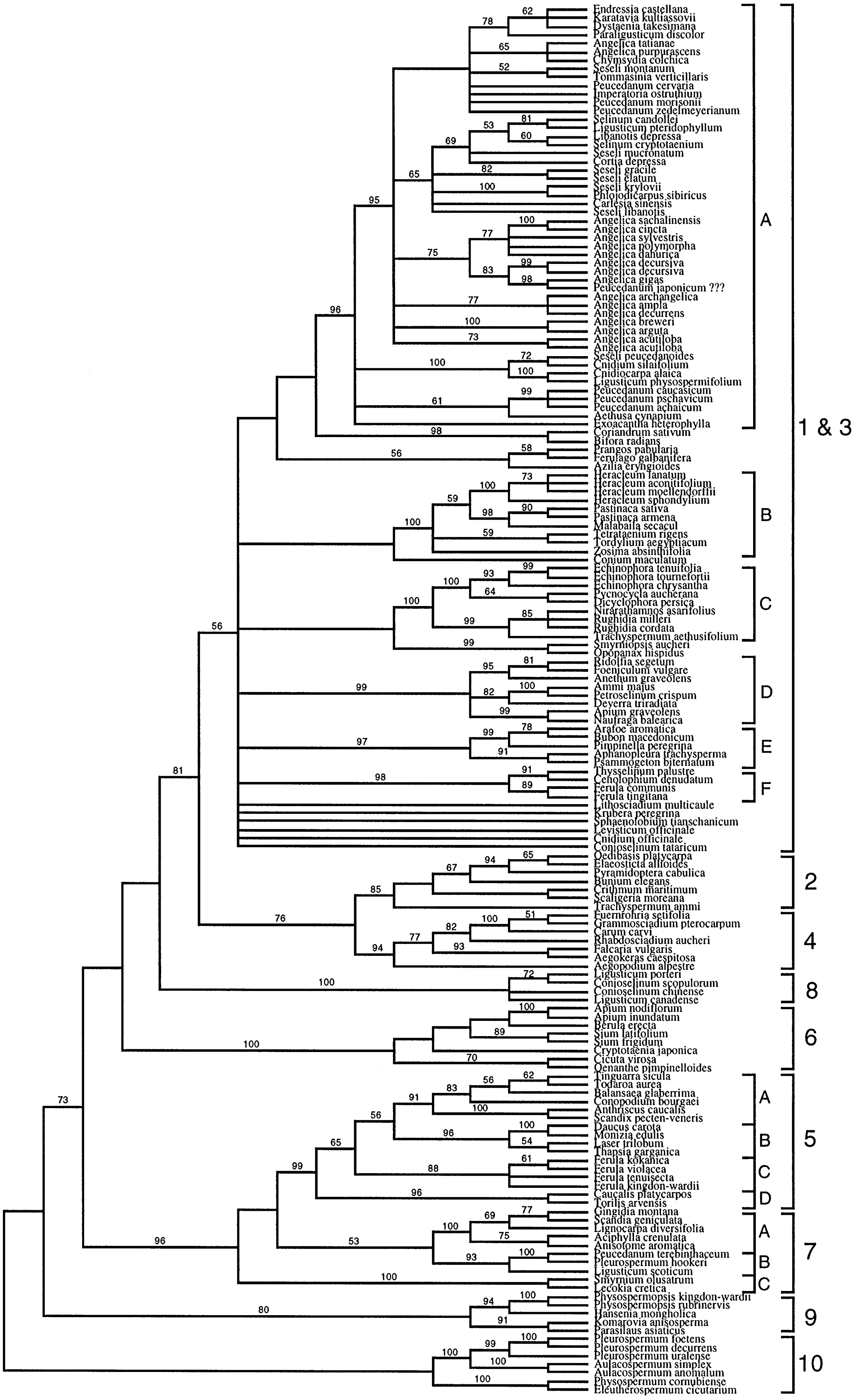

Endressia castellana
Karatavia kultiassovii
Dystaenia takesimana
Paraligusticum discolor
Angelica tatianae
Angelica purpurascens
Chymsydia colchica
Seseli montanum
Tommasinia verticillaris
Peucedanum cervaria
Imperatoria ostruthium
Peucedanum morisonii
Peucedanum zedelmeyerianum
Selinum candollei
Ligusticum pteridophyllum
Libanotis depressa
Selinum cryptotaenium
Seseli mucronatum
Cortia depressa
Seseli gracile
Seseli elatum
Seseli krylovii
Phlojodicarpus sibiricus
Carlesia sinensis
Seseli libanotis
Angelica sachalinensis
Angelica cincta
Angelica sylvestris
Angelica polymorpha
Angelica dahurica
Angelica decursiva
Angelica decursiva
Angelica gigas
Peucedanum japonicum ???
Angelica archangelica
Angelica ampla
Angelica decurrens
Angelica breweri
Angelica arguta
Angelica acutiloba
Angelica acutiloba
Seseli peucedanoides
Cnidium silaifolium
Cnidiocarpa alaica
Ligusticum physospermifolium
Peucedanum caucasicum
Peucedanum pschavicum
Peucedanum achaicum
Aethusa cynapium
Exoacantha heterophylla
Coriandrum sativum
Bifora radians
Prangos pabularia
Ferulago galbanifera
Azilia eryngioides
Heracleum lanatum
Heracleum aconitifolium
Heracleum moellendorffii
Heracleum sphondylium
Pastinaca sativa
Pastinaca armena
Malabaila secacul
Tetrataenium rigens
Tordylium aegyptiacum
Zosima absinthifolia
Conium maculatum
Echinophora tenuifolia
Echinophora tournefortii
Echinophora chrysantha
Pycnocycla aucherana
Dicyclophora persica
Nirarathamnos asarifolius
Rughidia milleri
Rughidia cordata
Trachyspermum aethusifolium
Smyrniopsis aucheri
Opopanax hispidus
Ridolfia segetum
Foeniculum vulgare
Anethum graveolens
Ammi majus
Petroselinum crispum
Deverra triradiata
Apium graveolens
Naufraga balearica
Arafoe aromatica
Bubon macedonicum
Pimpinella peregrina
Aphanopleura trachysperma
Psammogeton biternatum
Thysselinum palustre
Cenolophium denudatum
Ferula communis
Ferula tingitana
Lithosciadium multicaule
Krubera peregrina
Sphaenolobium tianschanicum
Levisticum officinale
Cnidium officinale
Conioselinum tataricum
Oedibasis platycarpa
Elaeosticta allioides
Pyramidoptera cabulica
Bunium elegans
Crithmum maritimum
Scaligeria moreana
Trachyspermum ammi
Fuernrohria setifolia
Grammosciadium pterocarpum
Carum carvi
Rhabdosciadium aucheri
Falcaria vulgaris
Aegokeras caespitosa
Aegopodium alpestre
Ligusticum porteri
Conioselinum scopulorum
Conioselinum chinense
Ligusticum canadense
Apium nodiflorum
Apium inundatum
Berula erecta
Sium latifolium
Sium frigidum
Cryptotaenia japonica
Cicuta virosa
Oenanthe pimpinelloides
Tinguarra sicula
Todaroa aurea
Balansaea glaberrima
Conopodium bourgaei
Anthriscus caucalis
Scandix pecten-veneris
Daucus carota
Monizia edulis
Laser trilobum
Thapsia garganica
Ferula kokanica
Ferula violacea
Ferula tenuisecta
Ferula kingdon-wardii
Caucalis platycarpos
Torilis arvensis
Gingidia montana
Scandia geniculata
Lignocarpa diversifolia
Aciphylla crenulata
Anisotome aromatica
Peucedanum terebinthaceum
Pleurospermum hookeri
Ligusticum scoticum
Smyrnium olusatrum
Lecokia cretica
Physospermopsis kingdon-wardii
Physospermopsis rubrinervis
Hansenia mongholica
Komarovia anisosperma
Parasilaus asiaticus
Pleurospermum foetens
Pleurospermum decurrens
Pleurospermum uralense
Aulacospermum simplex
Aulacospermum anomalum
Physospermum cornubiense
Eleutherospermum cicutarium
A
B
C
D
E
F
1 & 3
2
4
8
6
A
B
C
D
5
A
B
C
7
9
10

Fig. 4. NJ tree inferred from 164 unambiguously aligned ITS1 and ITS2 sequences from Apiaceae subfamily Apioideae. Branch lengths are proportional to distances estimated from the two-parameter method of Kimura (1980) (note scale bar). Numbers at the nodes are bootstrap estimates for 100 replicate analyses; values <50% are not indicated. The numbered brackets represent those groups of Apioideae outlined in Fig. 2 and in previous investigations. Major subclades within groups 1&3, 5, and 7 are the same as outlined in Fig. 3 (and are all supported by bootstrap values of ≥87%). The ITS sequence for *P. japonicum* is likely misidentified (see text for further discussion).

certainty. Its ITS sequence is drastically different from those of other apioid taxa and cannot be readily aligned. Moreover, it is much shorter (at 376 bp for both spacer regions) than any other known umbellifer ITS sequence. While its morphology suggests an affinity with Socotran *Rughidia* and *Nirarathamnos*, phylogenetic analysis of partially aligned sequence data places this species near the base of the tree alongside the *Physospermum* clade. However, we stress that, owing to high sequence divergence and numerous alignment gaps, its position here must be regarded as tentative.

ITS and *rps*16 intron phylogenetic comparisons

Given the disparity in size of the two data sets—there is a 3.5-fold greater number of apioid accessions in the ITS matrix than in the *rps*16 intron matrix (164 vs. 52 accessions, respectively)—comparisons between the resultant phylogenies cannot be made easily. To facilitate comparison and to examine the utility of each of these regions in providing phylogenetic information, each of the two data sets was reduced to their 44 common species and the MP and bootstrap analyses rerun.

MP analysis of the reduced ITS matrix yielded 12 minimal-length trees, each of 1436 steps (CIs = 0.4401 and 0.4080, with and without uninformative characters, respectively; RI = 0.6024; Fig. 5A). The numbers of autapomorphic and parsimony-informative positions were 61 and 284, respectively, and pairwise sequence divergence estimates ranged from 2.1 to 33.6%. Analysis of the reduced *rps*16 intron matrix yielded a single shortest tree of 464 steps (CIs = 0.7091 and 0.5545, with and without uninformative characters, respectively; RI = 0.7585; Fig. 5B). The numbers of autapomorphic and parsimony-informative positions were 145 and 126, respectively, and pairwise sequence divergence estimates ranged from 0.1 to 7.7%. Clearly, the ITS data are much more variable and demonstrate considerably more homoplasy than those of the *rps*16 intron. With respect to the relationships among the major clades, the phylogenetic resolutions inferred from the analyses of the reduced matrices were nearly identical to those of the original larger matrices. Major differences between the results of the reduced ITS and intron data sets included: (*i*) the position of group 2&4, as either sister to group 1&3 in the ITS tree (Fig. 5A) or as group 1 in the intron tree (Fig. 5B); (*ii*) the position of group 6, either as sister to group 5&7 in the ITS tree (Fig. 5A) or as group 1–5&7 in the intron tree (Fig. 5B); (*iii*) the relative positions of *Conium maculatum*, *Ligusticum scoticum*, and *Tordylium aegyptiacum*; and (*iv*) the relationships among several other species comprising apioid groups 5 and 6. Many of these differences, however, are largely attributable to poorly supported nodes. When those nodes identified by bootstrap values < 65% are treated as unresolved, the ITS and intron trees are highly consistent, with the only remaining areas of discord surrounding the relative positions of *C. maculatum* and *L. scoticum*. The intron tree provides greater resolution within group 6 and at its base, whereas in the ITS tree, group 3 is maintained with much internal resolution.

The agreement between the trees derived from the separate analyses suggested that a combined analysis of these same data would likely lead to the best estimate of phylogeny for these 44 species. MP analysis of the combined ITS and intron data set resulted in 112 minimal-length trees, each of 1929 steps (CIs = 0.4982 and 0.4272, with and without uninformative characters, respectively; RI = 0.6250; Fig. 5C). The results obtained show more resolution than either of the separate analyses and, in general, greater bootstrap support for many clades. In contrast, the areas of conflict in the separate analyses are characterized by low bootstrap values in the combined tree. Of the many relationships supported by each of these analyses, noteworthy are the sister relationship between *Naufraga balearica* and *Apium graveolens*, the placement of *A. nodiflorum* in the *Oenanthe* clade and *Ferula kokanica* in the *Daucus* clade, and the union of *Echinophora* with Socotran *Rughidia* and *Nirarathamnos*.

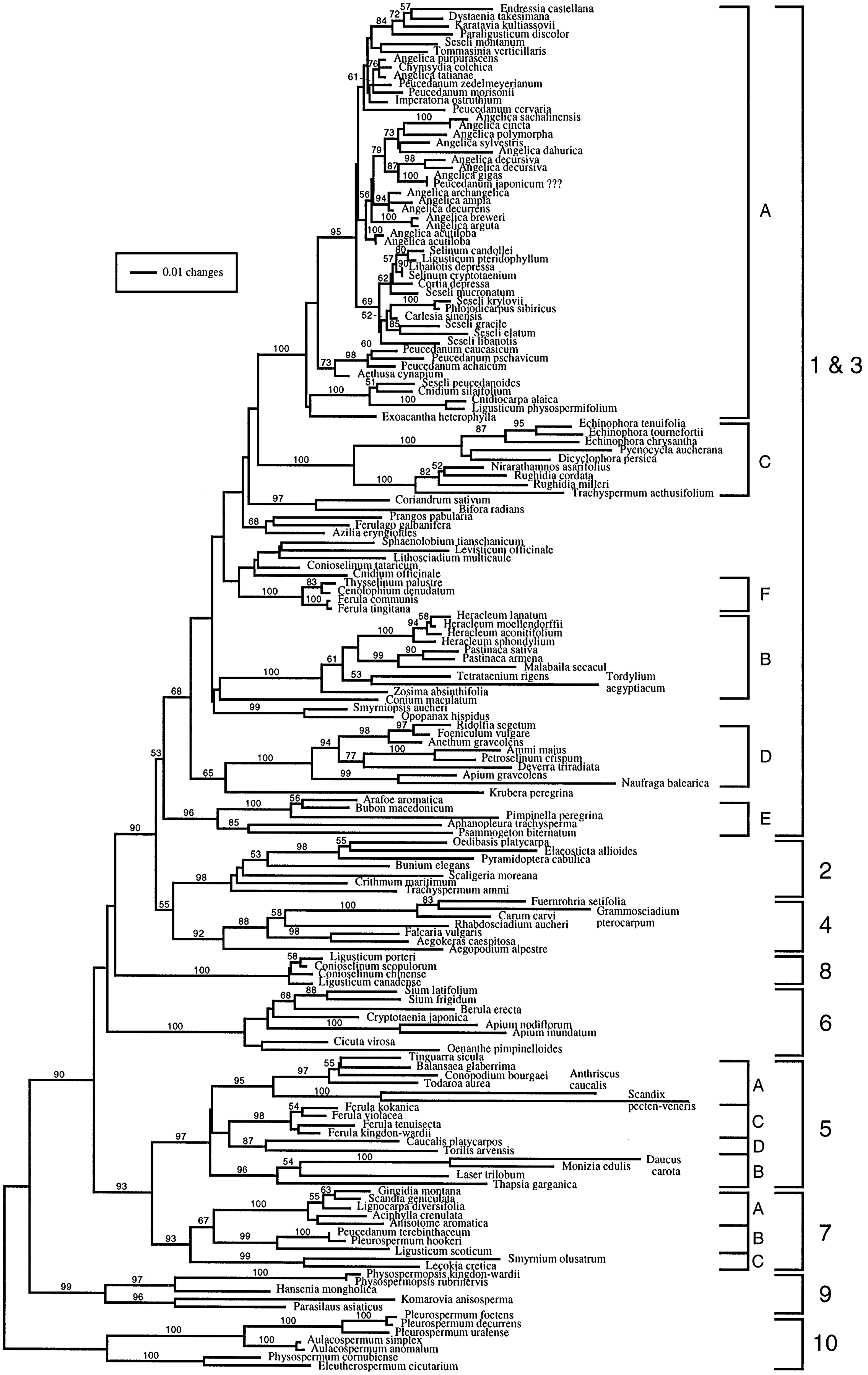
Discussion

Comparison with the treatment by Drude (1898)

As already noted in previous molecular-systematic studies, the phylogenies inferred herein provide little support for Drude's (1898) often-cited system of classification (or, for that matter, other hitherto proposed divisions of the subfamily). The apioid umbellifers display a remarkable array of morphological and anatomical modifications in their fruits, and the almost exclusive use of these characters in delimiting suprageneric groups has confounded the understanding of evolutionary relationships. In this study, Drude's tribes Apieae, Smyrnieae, and Peucedaneae, the three largest within the subfamily, are each not monophyletic, with multiple independent derivations inferred in all cladograms; tribes Scandiceae, Dauceae, and Laserpitieae are individually not monophyletic but collectively form a natural group. Tribe Coriandreae is also not monophyletic, with *Fuernrohria* placed away from *Coriandrum* and the three species of *Bifora* examined (*B. radians* and *B. testiculata* in this study and *B. americana* in Plunkett et al. 1996*b*) each occurring in separate clades. Of Drude's eight tribes, only the small distinct tribe Echinophoreae (represented here by *Echinophora, Dicyclophora*, and *Pycnocycla*) is monophyletic.

Major clades in Apioideae

Seven major lineages of Apioideae have been recognized at the tribal level on the basis of molecular evidence (Downie et al. 2000*b*). These same groups are well supported in this study and are referred to as tribes Heteromorpheae M.F. Watson & S.R. Downie (group 12, the *Heteromorpha* clade), Bupleureae Spreng. (group 11, the


0.01 changes
Endressia castellana
Dystaenia takesimana
Karatavia kultiassovii
Paraligusticum discolor
Seseli montanum
Tommasinia verticillaris
Angelica purpurascens
Chymsydia colchica
Angelica tatianae
Peucedanum zedelmeyerianum
Peucedanum morisonii
Imperatoria ostruthium
Peucedanum cervaria
Angelica sachalinensis
Angelica cincta
Angelica polymorpha
Angelica sylvestris
Angelica dahurica
Angelica decursiva
Angelica decursiva
Angelica gigas
Peucedanum japonicum ???
Angelica archangelica
Angelica ampla
Angelica decurrens
Angelica breweri
Angelica arguta
Angelica acutiloba
Angelica acutiloba
Selinum candollei
Ligusticum pteridophyllum
Libanotis depressa
Selinum cryptotaenium
Cortia depressa
Seseli mucronatum
Seseli krylovii
Phlojodicarpus sibiricus
Carlesia sinensis
Seseli gracile
Seseli elatum
Seseli libanotis
Peucedanum caucasicum
Peucedanum pschavicum
Peucedanum achaicum
Aethusa cynapium
Seseli peucedanoides
Cnidium silaifolium
Cnidiocarpa alaica
Ligusticum physospermifolium
Exoacantha heterophylla
Echinophora tenuifolia
Echinophora tournefortii
Echinophora chrysantha
Pycnocycla aucherana
Dicyclophora persica
Nirarathamnos asarifolius
Rughidia cordata
Rughidia milleri
Trachyspermum aethusifolium
Coriandrum sativum
Bifora radians
Prangos pabularia
Ferulago galbanifera
Azilia eryngioides
Sphaenolobium tianschanicum
Levisticum officinale
Lithosciadium multicaule
Conioselinum tataricum
Cnidium officinale
Thysselinum palustre
Cenolophium denudatum
Ferula communis
Ferula tingitana
Heracleum lanatum
Heracleum moellendorffii
Heracleum aconitifolium
Heracleum sphondylium
Pastinaca sativa
Pastinaca armena
Malabaila secacul
Tetrataenium rigens
Tordylium aegyptiacum
Zosima absinthifolia
Conium maculatum
Smyrniopsis aucheri
Opopanax hispidus
Ridolfia segetum
Foeniculum vulgare
Anethum graveolens
Ammi majus
Petroselinum crispum
Deverra triradiata
Apium graveolens
Naufraga balearica
Krubera peregrina
Arafoe aromatica
Bubon macedonicum
Pimpinella peregrina
Aphanopleura trachysperma
Psammogeton biternatum
Oedibasis platycarpa
Elaeosticta allioides
Pyramidoptera cabulica
Bunium elegans
Scaligeria moreana
Crithmum maritimum
Trachyspermum ammi
Fuernrohria setifolia
Grammosciadium pterocarpum
Carum carvi
Rhabdosciadium aucheri
Falcaria vulgaris
Aegokeras caespitosa
Aegopodium alpestre
Ligusticum porteri
Conioselinum scopulorum
Conioselinum chinense
Ligusticum canadense
Sium latifolium
Sium frigidum
Berula erecta
Cryptotaenia japonica
Apium nodiflorum
Apium inundatum
Cicuta virosa
Oenanthe pimpinelloides
Tinguarra sicula
Balansaea glaberrima
Conopodium bourgaei
Todaroa aurea
Anthriscus caucalis
Scandix pecten-veneris
Ferula kokanica
Ferula violacea
Ferula tenuisecta
Ferula kingdon-wardii
Caucalis platycarpos
Torilis arvensis
Daucus carota
Monizia edulis
Laser trilobum
Thapsia garganica
Gingidia montana
Scandia geniculata
Lignocarpa diversifolia
Aciphylla crenulata
Anisotome aromatica
Peucedanum terebinthaceum
Pleurospermum hookeri
Ligusticum scoticum
Smyrnium olusatrum
Lecokia cretica
Physospermopsis kingdon-wardii
Physospermopsis rubrinervis
Hansenia mongholica
Komarovia anisosperma
Parasilaus asiaticus
Pleurospermum foetens
Pleurospermum decurrens
Pleurospermum uralense
Aulacospermum simplex
Aulacospermum anomalum
Physospermum cornubiense
Eleutherospermum cicutarium
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D
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8
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A
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D
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Table 4. Genera for which two or more species were included in the phylogenetic analysis of nuclear rDNA ITS sequences.

Genus	No. of species	Sequence divergence (range (%))
Angelica[a]	15 (17 accessions)	0.2–7.2
Apium[a]	3	7.1–24.8
Aulacospermum	2	0.5
Cnidium[a]	2	11.7
Conioselinum[a]	3	1.6–14.3
Echinophora	3	3.2–5.9
Ferula[a]	6	0.2–20.5
Heracleum[a]	4	0.9–2.1
Ligusticum[a]	5	2.3–21.4
Pastinaca	2	2.6
Peucedanum[a]	8	1.9–21.2
Physospermopsis	2	0.5
Pleurospermum[a]	4	0.7–26.2
Rughidia[a]	2	6.0
Selinum[a]	2	1.4
Seseli[a]	7	3.7–9.8
Sium	2	5.7
Trachyspermum[a]	2	22.1

[a] Genus is not monophyletic based on the results of this study.

Bupleurum clade), Pleurospermeae M.F. Watson & S.R. Downie (group 10, the *Physospermum* clade), Aciphylleae M.F. Watson & S.R. Downie (group 7A, the *Aciphylla* clade), Smyrnieae Spreng. (group 7C, *Smyrnium* and *Lecokia*), Oenantheae Dumort. (group 6, the *Oenanthe* clade), and Scandiceae Spreng. (group 5, the *Daucus* clade). Within Scandiceae, three distinct yet closely related groups have also been recognized formally—subtribes Scandicinae, Daucinae, and Torilidinae—coinciding with groups 5A (the "*Scandix*" subclade), 5B (the "*Daucus*" subclade), and 5D (the "*Torilis*" subclade; Lee and Downie 1999; Downie et al. 2000*a*). To these we now add the "*Ferula*" subclade (group 5C), admittedly a rather unexpected and surprising find, as we are not aware of any morphological characters supporting this union. Subtribes Daucinae and Torilidinae constitute, to a large extent, tribe Caucalideae Spreng. (i.e., the spiny-fruited umbellifers) but with the inclusion of Laserpitieae. Subtribe Scandicineae (with included *Todaroa*) parallels tribe Scandiceae sensu Heywood and most of all subtribe Scandicinae sensu Drude. The circumscription of Oenantheae is also expanded to include two species of *Apium (A. inundatum* and *A. nodiflorum*) previously attributable to *Helosciadium* W.D.J. Koch.

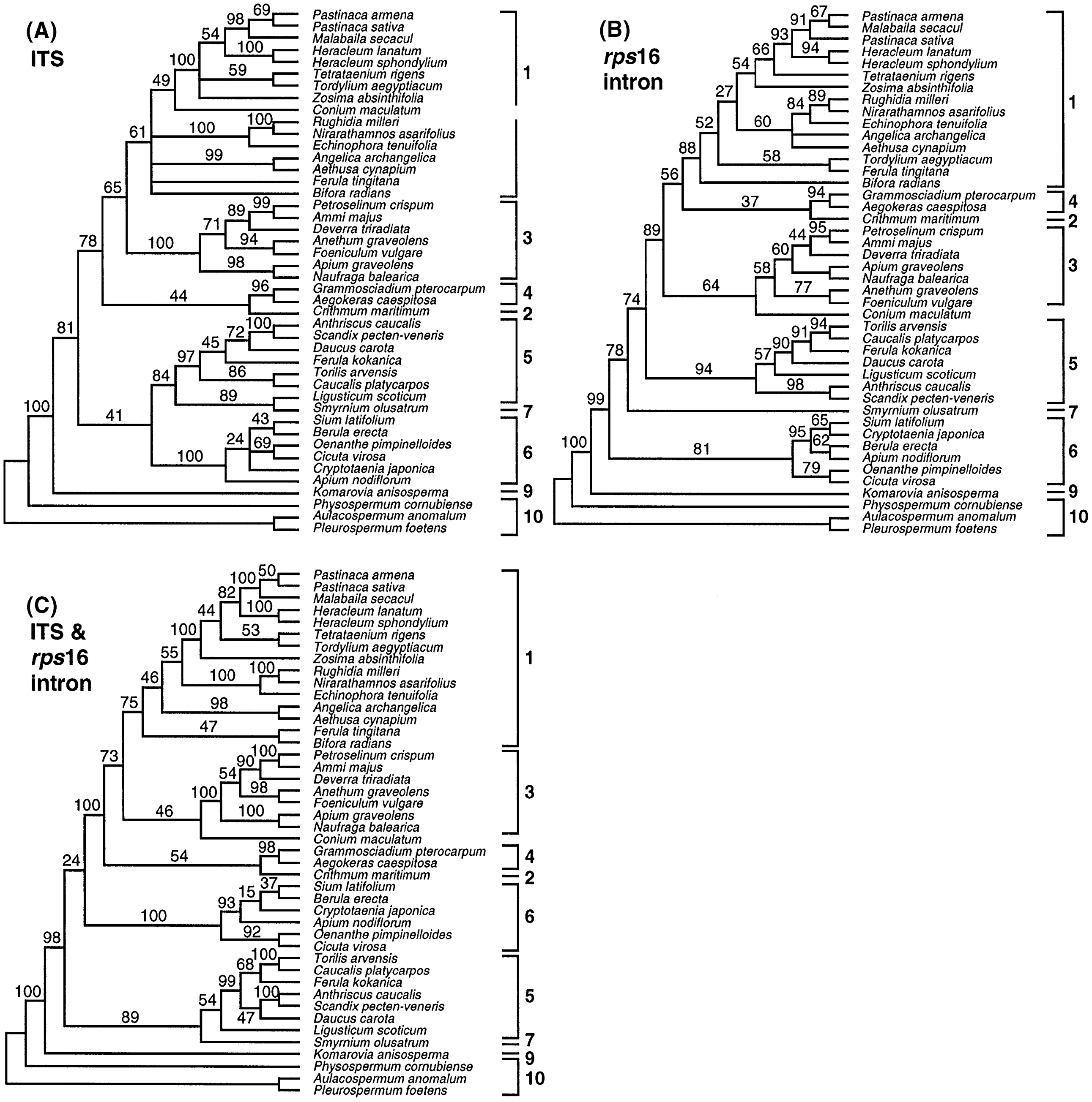
Group 8, the *Conioselinum* clade, comprises two species each of *Conioselinum* and *Ligusticum*. However, the type species for each of these genera, *C. tataricum* and *L. scoticum*, do not occur in this clade. *Conioselinum* is a small circumboreal genus of some 10 morphologically similar species, with *C. tataricum* and *C. chinense* virtually indistinguishable (Lavrova et al. 1987; Pimenov and Leonov 1993). The separation of these species in the ITS trees demands further study. *Ligusticum* sensu lato, comprising some 40–50 species, is considered highly artificial, with many of its species disposed among other genera (Pimenov and Leonov 1993; Kondo et al. 1996). Indeed, phylogenetic analysis of ITS sequences reveals its polyphyly, with its five examined species occurring in four separate clades. The monotypic *Arafoe* and *Hansenia*, both segregates of *Ligusticum* sensu lato, do not ally with any included *Ligusticum* species. We continue to recognize this group informally as the *Conioselinum* clade, despite the inclusion in this clade of the type of *Kreidion* Raf. (*Kreidion chinensis* (L.) Raf.) and the exclusion of the type of *Conioselinum*.

The position of *Ligusticum scoticum* is equivocal when the intron- and ITS-derived trees are compared. Phylogenetic analyses of plastid DNA data place this species within tribe Scandiceae, representing yet another relationship that cannot readily be supported on morphological grounds. In contrast, the ITS trees ally this species with *Peucedanum terebinthaceum* and *Pleurospermun hookeri* in group 7B. *Ligusticum scoticum* shows differences in fruit structure from other species of *Ligusticum* and, as a result, is regarded as the sole member of *Ligusticum* sensu stricto (Lavrova et al. 1983; Pimenov and Leonov 1993). Its novel association, however, with *P. terebinthaceum* [= *Kitagawia terebinthacea* (Fisch. ex Spreng.) Pimenov] and *P. hookeri* is surprising.

The genera *Hansenia, Komarovia, Parasilaus*, and *Physospermopsis* comprise group 9, the *Komarovia* clade, which is allied closely to tribe Pleurospermeae (group 10). *Pleurospermum* is a taxonomically problematic genus of some 40 species of largely Asian distribution (Pimenov and Leonov 1993) and it is not unrealistic to presume that further study of this genus (and putatively allied *Chamaesium* H. Wolff, *Hymenolaena* DC., *Korshinskya* Lipsky, *Eremodaucus* Bunge, *Molopospermum* W.D.J. Koch, and *Trachydium* Lindl.; Shneyer et al. 1992; Pimenov and Leonov 1993) might serve to establish a sister relationship between groups 9 and 10. The close relationship between *Komarovia* and *Parasilaus* is supported by immunochemical and morphological studies (Shneyer et al. 1992; Pimenov et al. 1999); their union with *Physospermopsis* and *Hansenia* is unique. The genera *Hansenia, Komarovia*, and *Parasilaus* are monotypic and, according to the NJ tree (Fig. 4), may each constitute long-evolving separate lineages. On this basis, their monotypic status is supported but, as just stated, this may reflect an artifact of sampling.

Two other well-supported groups, corresponding to the *Crithmum* (group 2) and *Aegopodium* (group 4) clades of earlier investigations (Downie et al. 1998, 2000*b*; Downie and Katz-Downie 1999; Katz-Downie et al. 1999), are consistently recognized in all analyses presented herein but have yet to receive formal recognition. In all molecular analyses to date but one (i.e., the *rpo*C1 intron study of Downie et al. 1998), these groups unite as sister taxa with low to moderate bootstrap support (55–78%). These bootstrap values, however, are less than those supporting any of the aforementioned clades that have already achieved formal recognition. Therefore, in contrast with some previous studies where both groups are treated together in an expanded *Apium* or *Aegopodium* clade (Plunkett et al. 1996*b*; Plunkett and Downie 1999; Downie et al. 2000*a*), we recognize each of these groups as distinct. The first of these, the *Crithmum* clade, includes such genera as *Bunium, Elaeosticta, Oedibasis, Scaligeria*, and *Trachyspermum* (in part) and the monotypic *Crithmum* and *Pyramidoptera*. *Trachyspermum* is a rather ill-defined genus of some 15 species (Pimenov and Leonov 1993) and its nomenclature is particularly problematic. The type of this genus, *Trachyspermum copticum* (L.)

Fig. 5. Single (B) and strict consensus (A and C) trees derived from MP analysis of separate and combined ITS and *rps*16 intron data for 44 species of Apiaceae subfamily Apioideae. Tree summary statistics are presented in the text. Numbers at nodes are bootstrap estimates for 100 replicate analyses. Groups identified in Fig. 2 and in previous phylogenetic studies are indicated.



Link, has yet to be included in any molecular analysis. The monotypic genus *Lagoecia* L. also belongs within this group. Drude (1898) treated *Lagoecia* in subfamily Saniculoideae, whereas later authors suggested its closer affinity with subfamily Apioideae (Koso-Poljansky 1916; Cerceau-Larrival 1962; Tseng 1967; Guyot 1971). Indeed, on the basis of phylogenetic analysis of *mat*K sequences, Plunkett et al. (1996*b*) ally *Lagoecia* with *Crithmum*, with this pair as sister to a clade comprising *Aegopodium, Carum*, and *Cyclospermum*. Recently procured ITS data for *Lagoecia cuminoides* L. confirm its placement alongside *Trachyspermum ammi* within the *Crithmum* clade. We now recognize this clade formally at the rank of tribe and apply the earliest name, Pyramidoptereae Boiss. Originally, Pyramidoptereae were recognized as a monotypic tribe defined by its pyramidal fruits and mericarp ribs fused to the calyx teeth. However, we can see no obvious morphological synapomorphies supporting this group as currently circumscribed.

Tribe Pyramidoptereae Boiss., Fl. or. 2: 1089. 1872.

Type genus: *Pyramidoptera cabulica* Boiss. Other included genera: *Bunium* L., *Crithmum* L., *Elaeosticta* Fenzl, *Lagoecia* L., *Oedibasis* Koso-Pol., *Scaligeria* DC., and *Trachyspermum* Link (in part).

The second group, the *Aegopodium* clade, comprises the genera *Aegopodium, Carum, Grammosciadium*, and *Rhabdosciadium* and the monotypic *Aegokeras, Falcaria*, and

Fuernrohria. Plunkett et al. (1996*b*) add *Cyclospermum*. In this clade, priority extends to *Carum*, and we now recognize the group formally at the tribal level.

Tribe Careae Baill., Hist. Pl. 7: 174, 219. 1879.

Type genus: *Carum carvi* L. Other included genera: *Aegokeras* Raf., *Aegopodium* L., *Cyclospermum* Lag., *Falcaria* Fabr., *Fuernrohria* K. Koch, *Grammosciadium* DC., and *Rhabdosciadium* Boiss.

The apioid superclade

The *Angelica*, *Crithmum*, *Apium*, and *Aegopodium* clades have been considered collectively as the apioid superclade (Plunkett and Downie 1999). This group is strongly supported as monophyletic in all analyses of cpDNA sequence or restriction site data to date, with bootstrap values ranging from 85 to 99%. Studies incorporating ITS data generally provide lesser support for this group (50–97% bootstrap values) but do confirm its monophyly. These plants are also characterized by a unique structural rearrangement of their plastid genome, where the inverted repeat segments are slightly larger or much smaller than those typically found elsewhere in the family or, in general, in any other flowering plant group (Plunkett and Downie 1999). However, while much evidence attests to its monophyly, the resolution of relationships within the superclade (with the exception of the aforementioned *Crithmum* and *Aegopodium* clades) has heretofore been poor. Since the *Angelica* and *Apium* clades were first established on the basis of plastid DNA evidence (Plunkett et al. 1996*b*; Downie et al. 1998), increased sampling has obscured their boundaries. Moreover, the results of ITS analyses have never been fully compatible with those of plastid DNA studies, with members of the *Apium* clade forming as many as five separate lineages within or at the base of the *Angelica* clade (Downie et al. 1998; Katz-Downie et al. 1999). To facilitate communication and to reconcile these groups with progress made in umbellifer molecular systematics, we now provide a more restricted view of the *Angelica* and *Apium* clades and describe several additional well-supported subclades (labeled A–F in group 1&3 in Figs. 3 and 4).

Group A

We designate this group as a reduced *Angelica* clade, as it is slightly different in composition from that originally described (Plunkett et al. 1996*b*; Downie et al. 1998). It is a large group and contains many genera of exceptional taxonomic difficulty. Included are *Angelica*, *Selinum*, and *Seseli* and all but one species of *Peucedanum*. The genus *Seseli* demonstrates generic-level problems at its boundaries with *Libanotis*, *Cortia*, *Ligusticum*, *Selinum*, *Karatavia*, and *Cnidium*, and the delimitation of its species is clouded by much overlap in morphological variation (Ball 1968; Hedge et al. 1987; Lavrova et al. 1987). The occurrence of 2-methylchromones in *Angelica*, *Libanotis*, *Peucedanum*, *Seseli*, and *Xanthogalum* (see below) may be an important marker (Hegnauer 1990). Pending further study, we refrain from treating this clade formally. However, if the group is recognized at the tribal level, the earliest available name, Selineae Spreng. (1820), would apply; this name would have precedence over Angeliceae W.D.J. Koch (1824), Seseleae W.D.J. Koch (1824), or Peucedaneae Dumort. (1827). Unfortunately, we have yet to examine *Selinum carvifolia* (L.) L., the type of the genus but, based on morphology, it should fall within this group.

The genus *Angelica* consists of some 110 species distributed throughout north-temperate regions (Pimenov and Leonov 1993). It is extremely polymorphic, showing variation in fruit anatomy, leaf morphology, and subterranean structures (Vasil'eva and Pimenov 1991). Its limits are difficult to circumscribe unambiguously, and numerous segregate genera have been recognized (e.g., *Archangelica* Hoffm., *Callisace* Fisch., *Czernaevia* Turcz. ex Ledeb., *Ostericum* Hoffm., and *Xanthogalum* Lallem.). Many treatments, however, consider *Angelica* broadly, with its various segregates placed in different subgenera and sections (Hiroe and Constance 1958; Pimenov 1968; Vasil'eva and Pimenov 1991; Pimenov and Leonov 1993). No worldwide modern treatment of *Angelica* exists, with the systems of Pimenov and colleagues being largely restricted to those species of the former Soviet Union.

The ITS phylogenies support, in part, the classificatory system of *Angelica* proposed by Pimenov (1968) and later modified by Vasil'eva and Pimenov (1991). With the exception of *A. purpurascens* and *A. tatianae* (both sect. *Xanthogalum*), the genus *Angelica*, as sampled here (including the type species *A. sylvestris* and the dubiously identified *P. japonicum*), constitutes a group of closely related species. While *A. archangelica* and *A. decurrens* (both subsect. *Archangelica*) and *A. sachalinensis* and *A. cincta* (both subsect. *Anisopleura*) each unite, the two major subdivisions within the genus (i.e., subgenera *Archangelica* and *Angelica*) are not distinguished. In the NJ tree (Fig. 4), subgenus *Archangelica* (represented by *A. archangelica* and *A. decurrens*) arises within the paraphyletic subgenus *Angelica*. The segregate genera *Archangelica* (represented by *A. archangelica*) and *Callisace* (represented by *A. dahurica*) are not supported. The close relationship between *A. decursiva* and *A. gigas* has been suggested previously (Hiroe and Constance 1958).

The species *Angelica purpurascens* and *A. tatianae*, previously included in the genus *Xanthogalum* (Shishkin 1951; Chamberlain 1972; Hedge et al. 1987), were reduced by Vasil'eva and Pimenov (1991) to *Angelica* sect. *Xanthogalum* (Lallem.) Pimenov. This transfer is not supported by the ITS results, as these two species arise away from all other *Angelica* species examined. Instead, the Caucasian *A. purpurascens* and *A. tatianae* (and the habitually similar *Chymsydia colchica*) ally, albeit with poor bootstrap support, with three species of *Peucedanum* (*P. cervaria*, *P. morisonii*, and *P. zedelmeyerianum*) and several other species, such as *Imperatoria ostruthium* and *Tommasinia verticillaris*. *Tommasinia verticillaris*, regarded as closely allied to *A. purpurascens* and *A. tatianae* and at one time even treated with them in the same section (sect. *Tommasinia* (Bertol.) Paoletti), has been referred to both *Angelica* and *Peucedanum*, whereas *Imperatoria* has been included in *Peucedanum* (Vasil'eva and Pimenov 1991; Reduron et al. 1997). Leute (1966) suggested that *Tommasinia* be submerged within *Imperatoria*. While our results confirm the distinction of *A. purpurascens* and *A. tatianae* and indicate their removal from *Angelica*, they are insufficient to unambiguously suggest an alternative placement. Our results also support the separation of *Tom-*

masinia from *Angelica* (as did the karyological investigations of Vasil'eva and Pimenov 1991) and the close affinity of *Imperatoria* to some *Peucedanum* species. The monotypic status of *Tommasinia* is maintained, although its sister relationship to *Seseli montanum*, as suggested herein, is surprising and needs confirmation. The immunochemical studies of Shneyer et al. (1995), however, do reveal high serological similarity between *Tommasinia* and *Seseli libanotis*, the only species of *Seseli* included in their study.

Peucedanum sensu lato is a widespread Old World genus of 100–120 species. It exhibits extreme polymorphism (Hiroe and Constance 1958; Solov'eva et al. 1985; Ostroumova and Pimenov 1997), with many generic segregates recognized (*Cervaria* N.M. Wolff, *Demavendia* Pimenov, *Holandrea* Reduron, Charpin & Pimenov, *Imperatoria* L., *Johreniopsis* Pimenov, *Kitagawia* Pimenov, *Leutea* Pimenov, *Oreoselinum* Hill in Scott, *Pteroselinum* (Rchb.) Rchb., *Thysselinum* Raf., *Tommasinia* Bertol., and *Xanthoselinum* Schur). Pimenov and Leonov (1993) treated the genus in the strict sense, including only those 8–10 species comprising section *Peucedanum* and separating the rest "into other natural genera," many of which are monotypic. In this study, we have included eight species of *Peucedanum*. The *P. japonicum* ITS sequence obtained from GenBank (Lee and Rasmussen 1998) is likely misidentified. This species exhibits an identical ITS sequence to that of *Angelica gigas*, simultaneously sequenced by Lee and Rasmussen (1998), and is nested within a clade comprised solely of *Angelica* species. The identity of this sequence needs confirmation, as these two species do indeed differ morphologically. *Peucedanum pschavicum* (and two other species) have been transferred to the genus *Holandrea* (Reduron et al. 1997); *P. terebinthaceum* is the nomenclatural type of *Kitagawia* (Pimenov 1986); and *P. cervaria* and *P. caucasicum* have been treated as *Cervaria rivinii* Gaertn. and *C. caucasica* (M. Bieb.) Pimenov, respectively (Pimenov 1987; Reduron et al. 1997). We have also included the putatively allied *Imperatoria ostruthium* [= *Peucedanum ostruthium* (L.) W.D.J. Koch], *Tommasinia* [= *Peucedanum verticillare* (L.) Koch ex DC.], *Thysselinum* [= *Peucedanum palustre* (L.) Moench], and *Phlojodicarpus* [= *Angelica sibirica* (Steph. ex Spreng.) Hiroe].

Our results support the separation of *P. terebinthaceum* from all other *Peucedanum* species examined, thus confirming Pimenov's (1986) removal of this species from the genus. The results also support the distinctiveness of *Phlojodicarpus sibiricus* and *Thysselinum palustre*, as these species also arise well away from any other *Peucedanum* species. *Peucedanum caucasicum, P. pschavicum*, and *P. achaicum* form a strongly supported clade that is sister to *Aethusa cynapium*. The genus *Holandrea* (= *P. pschavicum*) is not supported. Alternatively, since the type species of *Holandrea* has yet to be included in any molecular study, the possibility exists that *P. caucasicum* and *P. achaicum* may be included in *Holandrea*. Resolution among *P. cervaria, P. morisonii*, and *P. zedelmeyerianum* is poor, but it is clear that they ally closely with *Imperatoria* and several other species, such as *Tommasinia verticillaris, Chymsydia colchica, Seseli montanum*, and those species of *Angelica* attributable to *Xanthogalum*. The close relationship between *P. morisonii* (sect. *Peucedanum*) and *P. zedelmeyerianum* (sect. *Pseudoselinum*) suggests that the "true *Peucedanum* species" (i.e., those belonging to sect. *Peucedanum*; Pimenov and Leonov 1993) are in fact not really that distinct from other *Peucedanum* species. The genus *Cervaria*, with its two examined species, is polyphyletic.

Group B

The ITS study circumscribes a group that, with the addition of *Pastinaca*, coincides with Drude's Peucedaneae subtribe Tordyliinae (= tribe Tordylieae W.D.J. Koch). In contrast, the intron study removes *Tordylium aegyptiacum* (= Hasselquistia aegyptiaca L.) from this clade and allies it (with weak bootstrap support) with *Ferula tingitana*. Because of the equivocal position of *Tordylium*, we continue to recognize this group as the "*Heracleum*" clade pending further study. Monophyly of tribe Tordylieae and its separation from other peucedanoid taxa has been previously confirmed by Shneyer et al. (1995), using seed-protein immunochemistry. Of the 23 genera recognized within Tordylieae (Pimenov and Leonov 1993), we have included 6: *Heracleum, Malabaila, Pastinaca, Tetrataenium, Tordylium*, and *Zosima*. While ITS data support *Heracleum* and *Pastinaca* as each being monophyletic, the inclusion of *H. candicans* in the *rps*16 intron analysis (Fig. 2) suggests that *Heracleum* may be polyphyletic. *Heracleum candicans*, therefore, should be removed from the genus. With the exception of *Pastinaca*, all members of group B are generally characterized by fruits with thickened wing margins. They also possess a rich diversity of substituted coumarin compounds, especially those of the angular furanocoumarin type (Murray et al. 1982). *Conium maculatum* continues to evade unambiguous placement and, in this sense, remains "the odd man out" (Fairbairn 1971). All present ITS studies support its sister status or otherwise close affinity to the *Heracleum* clade, but this relationship is only poorly supported. In contrast, studies of plastid DNA (e.g., Fig. 2) place *Conium* as sister to the *Apium* clade.

Group C

The Socotran endemic genera *Nirarathamnos* and *Rughidia* (and the Somalian *Trachyspermum aethusifolium* in the ITS study) comprise a strongly supported clade. *Rughidia*, with included *Nirarathamnos*, may be paraphyletic, however (Fig. 4). This clade is sister to Drude's tribe Echinophoreae (represented herein by *Echinophora, Dicyclophora*, and *Pycnocycla*), with this relationship supported at the 100% bootstrap level. Echinophoreae are a small tribe of six genera restricted to xerophytic habitats of the Mediterranean and southwest Asia (Pimenov and Leonov 1993). The plants are characterized by an unusual inflorescence and infructescence morphology, in which both sexual differentiation of the flowers and varying degrees of fusion and induration of the bracteoles, pedicels, and mericarps occur (Hedge and Lamond 1973). The branch leading to Echinophoreae is relatively long (Fig. 4), and the clade is supported by bootstrap values of 100% in both MP and NJ analyses of intron or ITS data. Erected by Bentham (1867) and maintained by Drude (1898), we continue to recognize tribe Echinophoreae as distinct.

While the genera comprising Echinophoreae are undoubtedly closely related, their relationships to genera outside the tribe have historically been difficult to ascertain. Based on comparative palynology, Echinophoreae are considered highly advanced (Cerceau-Larrival 1962, 1971), with similarities to Scandiceae noted (Koso-Poljansky 1916; Hedge and Lamond 1973). While any similarity to tribe Scandiceae is undoubtedly due to convergence, their proposed affinity to *Rughidia* and allies is novel but not surprising. Socotra is a dry tropical island located in the Indian Ocean, east of Somalia and southeast of Yemen. The similar habit of these plants and their geographical proximity support their close relationship. Whether *Nirarathamnos, Rughidia,* and *Trachyspermum aethusifolium* are placed in an expanded Echinophoreae (which has the result of making the tribe difficult to circumscribe) or are treated as a separate but allied tribe requires further consideration.

Group D

Included in this group are the genera *Ammi, Anethum, Deverra, Foeniculum, Naufraga, Petroselinum*, and *Ridolfia* and *Apium graveolens*. Many of these genera comprise cultivated species. The stems, petioles, and (or) leaves of *Apium graveolens* (celery, celeriac), *Foeniculum vulgare* (fennel), *Petroselinum crispum* (parsley), and *Anethum vulgare* (dill) are used as food or served with it. Most taxa are monocarpic (annuals or biennials) and all have yellow or yellowish flowers, minute or obsolete sepals, and oblong fruits with filiform ridges, solitary dorsal vittae, and two commissural vittae. We designate this group as a reduced *Apium* clade or the *Apium* clade sensu stricto. In all analyses of ITS data to date, this group is supported strongly, with bootstrap values approaching or equaling 100% (e.g., Figs. 3 and 4). However, analyses of chloroplast sequence data generally result in lower bootstrap support but do confirm the monophyly of the group (e.g., Fig. 2). On the other hand, cpDNA restriction site data support the clade with a 100% bootstrap value (Plunkett and Downie 1999).

Anethum graveolens, previously referred to *Angelica, Ferula*, or *Peucedanum*, clearly belongs within the *Apium* clade sensu stricto. *Naufraga*, a monotypic genus from the Balearic Islands heretofore dubiously treated in Apiaceae subfamily Hydrocotyloideae (Constance and Cannon 1967; Pimenov and Leonov 1993), clearly allies with *Apium graveolens*. *Naufraga balearica* is a diminutive plant possessing simple umbels, petioles with a sheathing auricled base, and cauline leaves with large white stipules, and is habitually quite dissimilar to celery. The two other species of *Apium* examined, *A. inundatum* and *A. nodiflorum*, arise within tribe Oenantheae (group 6) and, consequently, should be removed from the genus. Previously, they were referred to as *Helosciadium inundatum* (L.) W.D.J. Koch and *H. nodiflorum* (L.) W.D.J. Koch and, as such, the genus *Helosciadium* should be maintained.

The *Apium* clade sensu Plunkett et al. (1996*b*) and Downie et al. (1998) cannot be circumscribed unambiguously when cpDNA- and ITS-derived trees are compared and, in those earlier studies where it did occur as monophyletic, it is supported only weakly. Previous circumscriptions of the group included such genera as *Conium*, *Krubera* (*Capnophyllum dichotomum* (Desf.) Lag.), *Pimpinella, Prangos*, and *Smyrniopsis* (Downie et al. 1998, 2000*b*; Downie and Katz-Downie 1999; Plunkett and Downie 1999), whereas the *mat*K study of Plunkett et al. (1996*b*) treated the *Apium* clade widely, incorporating genera sub-sequently separated into the *Crithmum* and *Aegopodium* clades. Pimenov and Leonov (1993) recognized 189 genera (1332–1400 species) in Apieae. This is by far the largest tribe within the family and comprises approximately half the genera within subfamily Apioideae. There is no doubt that other genera will be added to this group as a result of further study. If this clade is recognized formally at the tribal level, the name Apieae [ined.] will apply.

Group E

The genera comprising this clade, *Arafoe*, *Bubon*, *Pimpinella*, *Aphanopleura*, and *Psammogeton* and, possibly, *Registaniella* (Rechinger 1987) are characterized by hispid ovoid fruits and have been considered collectively as the “*Pimpinella*” clade (Downie et al. 2000*a*). *Pimpinella* includes some 150 species; the genera *Aphanopleura* and *Psammogeton* are more narrowly defined (with up to five species each); and *Arafoe, Bubon*, and *Registaniella* are each monotypic (Pimenov and Leonov 1993; Downie et al. 2000*a*). If further investigation supports this group, and if it is to be recognized at the tribal level, the earliest name, Pimpinelleae Spreng. (1820), should be applied. Monophyly of *Pimpinella* has been questioned repeatedly but since the type of the genus (*Pimpinella saxifraga* L.) allies with this group (Downie et al. 1998), the tribal name will stand (unless of course another genus is included bringing with it an earlier tribal name).

Group F

Group F is a small clade comprising *Thysselinum*, the monotypic genus *Cenolophium*, and two (*Ferula communis* and *F. tingitana*) of the six species of *Ferula* examined. This clade is well supported, with bootstrap values of 98 or 100% (Figs. 3 and 4). The union of *F. communis* (the type of the genus) and *F. tingitana* supports the traditional taxonomy (where both are considered in the same section); their association with *Thysselinum* and *Cenolophium*, however, is novel.

Including some 170 Old World species, *Ferula* is the largest genus in subfamily Apioideae (Pimenov and Leonov 1993). The genus is heteromorphic and varies considerably in habit and vegetative, floral, and fruit morphology. Traditionally, *Ferula* is placed alongside *Peucedanum* in the same tribe or subtribe, but the immunological studies of Shneyer et al. (1995), while demonstrating the serological homogeneity of the genus, showed that *Ferula* (and four other genera not included in our study) is serologically distinct from *Angelica* and *Peucedanum* and from all other Peucedaneae genera they examined. Indeed, *Angelica* and *Peucedanum* show similar patterns of coumarin and terpenoid distribution, whereas *Ferula* is quite different (Shneyer et al. 1995). Boissier (1872) established three sections within *Ferula*, whereas Korovin (1947) recognized six subgenera and eight sections (the latter being divided into numerous “grex” categories). In our analysis, *Ferula* is polyphyletic, with one clade (*F. communis* and *F. tingitana*, and *F. assa-foetida* in Katz-Downie et al. 1999) allied with *Thysselinum* and *Cenolophium* and the other clade (*F. kokanica, F. violacea, F. tenuisecta*, and *F. kingdon-wardii*) allied with Scandiceae. Nomenclatural changes are in order, but only after the genus and allies have undergone thorough study. The close relationship between *Ferula* and *Ferulago*, as inferred by Drude (1898), is not supported by the ITS results.

Conclusions

Comparative analysis of nuclear rDNA ITS sequences, representing the largest and most comprehensive survey of Old World umbellifers to date, confirms those lineages identified in subfamily Apioideae in previous molecular-systematic studies and redefines the boundaries of several informal groups, particularly those within the apioid superclade. Phylogenetic analysis of these ITS data, and those data obtained from the chloroplast *rps*16 intron, also enables hypotheses on the positions of several species whose phylogenetic placements have heretofore been obscure, such as the Socotran endemic genera *Nirarathamnos* and *Rughidia* and the Balearic Islands endemic genus *Naufraga*. While these nuclear- and plastid-encoded DNA sequences delimit many of the same major clades, high levels of sequence variation preclude the use of the ITS region for inferring phy-logeny across subfamilial boundaries in Apiaceae and even among basal Apioideae, whereas the *rps*16 intron, being more conserved is better suited for family-wide phylogenetic study.

Within subfamily Apioideae, nine major lineages are confirmed on the basis of molecular-systematic study; these include tribes Heteromorpheae, Bupleureae, Pleurospermeae, Aciphylleae, Smyrnieae, and Oenantheae and Scandiceae subtribes Scandicinae, Daucinae, and Torilidinae (Downie et al. 2000*a*, 2000*b*). To these we now add tribes Careae and Pyramidoptereae, which correspond to the previously designated *Aegopodium* and *Crithmum* clades, and confirm the monophyly of Drude's (1898) tribe Echinophoreae. The boundaries of both the *Angelica* and *Apium* clades are reduced, so that now both ITS and plastid DNA data support their monophyly. In addition, several other well-supported groups are recognized (e.g., the *Pimpinella* and *Heracleum* clades) but their formal recognition must await additional study. With the exception of the small specialized tribe Echinophoreae, none of Drude's (1898) tribes are retained as monophyletic in the light of molecular evidence. Drude's tribe Peucedaneae, with its members characterized by dorsally compressed fruits bearing wing-like appendages extending from the lateral ribs, is grossly unnatural, as are two of its subtribes (Angelicinae and Peucedaninae). Subtribe Tordyliinae—approximately equivalent to tribe Tordylieae and the aforementioned *Heracleum* clade—may be monophyletic, but this relationship is not supported unequivocally when intron data are considered.

We have confirmed the unnaturalness of the four largest peucedanoid genera: *Angelica, Ferula, Heracleum*, and *Peucedanum*. *Angelica* comprises at least two lineages, with one of them representing species (*A. purpurascens* and *A. tatianae*) previously attributable to the genus *Xanthogalum*. The generic segregates *Archangelica* and *Callisace* cannot be maintained. We refute hypotheses that imply monophyly of *Ferula* (Shneyer et al. 1995) and, instead, show that this genus is polyphyletic, with some species exhibiting (a rather unexpected) affinity with members of tribe Scandiceae. *Heracleum* is polyphyletic if *H. candicans* is retained in the genus; otherwise all remaining *Heracleum* species constitute a strongly supported monophyletic group. The seven species of *Peucedanum* examined are distributed in three well-separated clades. The isolated *P. terebinthaceum* has been previously referred to *Kitagawia* (Pimenov 1986); *P. achaicum, P. caucasicum*, and *P. pschavicum* constitute a strongly supported monophyletic group that is sister to *Aethusa*; and *P. cervaria, P. morisonii*, and *P. zedelmeyerianum* unite weakly with several other taxa, including *Imperatoria, Chymsydia, Tommasinia*, and those two species of *Angelica* referred to *Xanthogalum*. Within this third group, resolution of relationships is quite poor but it is clear that some species of *Angelica* are indeed closely allied with some species of *Peucedanum*, as inferred by earlier studies. In contrast, all species of *Ferula* examined are clearly isolated from this group. Our results do not support recognition of *Peucedanum* segregates *Cervaria, Holandrea*, and *Imperatoria* and provide only weak support for *Tommasinia*. The isolated position of *Thysselinum* is maintained; *Phlojodicarpus* does not exhibit close affinity with either *Angelica* or *Peucedanum*.

Clearly, the most perplexing taxonomic problems remaining in tribe Peucedaneae, if not the entire subfamily, surround the genus *Peucedanum* sensu lato and its allies. The relatively few species of the genus that we have examined exhibit complex patterns of relationship, with several generic segregates not deserving separate status. In this study, we have also excluded those New World species that were initially described in *Peucedanum* (e.g., *Lomatium*). Differences between these New World genera and *Peucedanum* are apparently not greater than those found among the diverse groups comprising Old World *Peucedanum* (Solov'eva et al. 1985), so the inclusion of these New World species may confound issues even more. Continued investigation of this complex genus and putatively allied taxa is in order, especially before further nomenclatural changes are made and other segregate genera recognized.

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