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Phylogenetic analysis of cpDNA restriction sites and *rps*16 intron sequences reveals relationships among Apiaceae tribes Caucalideae, Scandiceae and related taxa

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Abstract. Evolutionary relationships among members of Apiaceae (Umbelliferae) tribe Caucalideae Spreng. and related taxa were inferred from maximum parsimony analyses of chloroplast DNA restriction sites and *rps*16 intron sequences and the results compared to an existing phylogeny for the group based on nuclear ribosomal DNA internal transcribed spacer sequences. While these three data sets were not similar in size or composition, the relationships among the shared taxa, with few exceptions, were concordant. Three major lineages are recognized, coinciding with the previously delimited Scandiceae subtribes Daucinae Dumort. (*Agrocharis*, *Ammodaucus*, *Cuminum*, *Daucus*, *Orlaya*, *Pachyctenium*, *Pseudorlaya*), Torilidinae Dumort. (*Astrodaucus*, *Caucalis*, *Glochidotheca*, *Lisaea*, *Szovitsia*, *Torilis*, *Turgenia*, *Yabea*), and Scandicinae Tausch (*Anthriscus*, *Kozlovia*, *Myrrhis*, *Osmorhiza*, *Scandix*). Included in Daucinae is representation from tribe Laserpitieae (*Laser*, *Laserpitium*, *Melanoselinum*, *Monizia*, *Polylophium*). Daucinae and Torilidinae arise as sister taxa in the chloroplast DNA-based phylogenies, whereas in the ITS trees relationships among the three major lineages are unresolved. Unexpectedly, three species of *Ferula* ally with Daucinae and Torilidinae. The position of *Artedia* is equivocal, occurring either sister to Daucinae in the ITS trees, within Torilidinae in the intron trees, or sister to Torilidinae upon analysis of combined ITS and intron data. *Chaetosciadium trichospermum* emerges within *Torilis*, and is recognized as *Torilis trichosperma* (L.) Spreng.

Key words: Caucalideae, Scandiceae, Apiaceae, Umbelliferae, chloroplast DNA restriction sites, *rps*16 intron.

Introduction

Tribe Caucalideae Spreng., as defined by Bentham (1867) and later Boissier (1872), contains practically all of those species of Apiaceae (Umbelliferae) that have spines, hooks, tubercles, or bristly hairs on the primary and/or secondary (vallecular) ridges of their fruits. Uniquely in this group, the secondary ridges are often more strongly developed than the primary. These plants are distributed throughout Europe, the Mediterranean region, and southwestern and central Asia, with a few outlying members in North and South America and Australia. Of the 21 genera and 68 species recognized in the tribe (Heywood and Jury in Heywood 1982c), *Daucus* is the largest genus with 21 species followed by *Torilis* with 10 species.

In the most widely used revision of the family, Drude (1897–1898) redistributed these spiny-fruited plants between his divergent

Scandiceae subtribe Caucalidinae and tribe Dauceae. Drude believed that members of tribe Dauceae (e.g. *Daucus*), having spines on their prominent secondary fruit ridges, are allied to plants in tribe Laserpitieae (e.g. *Laserpitium* and *Polylophium*), whose members have fruits without spines but with primary and prominent secondary ridges. In contrast, the genera of Scandiceae subtribe Caucalidinae (e.g. *Caucalis*, *Orlaya*, and *Torilis*) are linked (by the common possession of calcium oxalate crystals in the parenchyma cells surrounding the carpophore) to those in Scandiceae subtribe Scandicinae (e.g. *Anthriscus* and *Scandix*), the latter subtribe lacking both secondary ridges and spines. Drude assumed that the secondary spinose ridges characteristic of some Caucalidinae had evolved independently from those in Dauceae. Other treatments exist for these plants, such as those proposed by Calestani (1905), Koso-Poljansky (1916, 1917), and Cerceau-Larrival (1962, 1965), but have not gained wide acceptance.

As a result of two international symposia (Heywood 1971a, Cauwet-Marc and Carbonnier 1982) and a major cooperative research program (reviewed in Heywood 1982a, c), the spiny-fruited umbellifers have received much systematic attention. The multidisciplinary research undertaken, incorporating results from the fields of scanning electron microscopy, biochemical systematics, cytology, and numerical taxonomy, culminated in changes to the prevailing taxonomy. Here Drude's Dauceae and Scandiceae subtribe Caucalidinae were reunited as tribe Caucalideae (Heywood 1968a, 1971c; Crowden et al. 1969; McNeill et al. 1969; Heywood and Dakshini 1971; Harborne and Williams 1972; Williams and Harborne 1972), and Drude's Scandiceae subtribe Scandicinae was treated at the tribal level, Scandiceae Spreng. (Heywood 1971b). Of the 21 genera provisionally recognized in Caucalideae (Heywood and Jury in Heywood 1982c), two (*Aphanopleura* and *Psammogeton*) have been excluded from the group (Pimenov and Leonov 1993, Katz-Downie et al. 1999). However, despite the wealth of available data and the multidisciplinary approaches used to analyse these data, fundamental disagreements still exist regarding the proper circumscription of tribe Caucalideae, the relationships among its members, and the delimitation of certain genera. Moreover, the relationship between Heywood's tribes Caucalideae and Scandiceae is also quite unclear.

In all phylogenetic analyses of molecular data to date, whether derived from DNA sequences of chloroplast introns or genes, chloroplast DNA (cpDNA) restriction sites, or nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequences, a close relationship between Apiaceae tribes Caucalideae and Scandiceae is apparent (Downie et al. 1996, 1998; Downie and Katz-Downie 1996; Katz-Downie et al. 1999; Plunkett et al. 1996; Plunkett and Downie 1999). However, while some trees show that Caucalideae and Scandiceae are monophyletic sister taxa, others indicate that Caucalideae is paraphyletic with Scandiceae nested within. Alternatively, the *mat*K study of Plunkett et al. (1996) shows a paraphyletic Scandiceae with included Caucalideae. Because the purpose of each of these studies was not to resolve the intergeneric relationships within tribes Caucalideae and Scandiceae but rather to infer the higher-level groupings within the family, the number of taxa sampled from each tribe was small.

We have recently addressed issues of relationship among the spiny-fruited umbellifers and related taxa by carrying out, with expanded sampling, phylogenetic analyses of nuclear rDNA ITS sequences (Lee and Downie 1999). Fifty-eight accessions, representing 18 of 21 genera of Caucalideae (*Aphanopleura*, *Psammogeton*, and the rare, monotypic *Angoseseli* were not considered) and putatively allied taxa from Heywood's Scandiceae and Drude's tribes Apieae, Laserpitieae, and Smyrnieae, were examined and analyzed using maximum parsimony, maximum likelihood, and neighbor-joining methods. The results of the maximum parsimony analysis are presented in Fig. 1. Here three major lineages are recog-

nized, designated as Scandiceae subtribes Daucinae Dumort. (1827), Torilidinae Dumort. (1827), and Scandicinae Tausch (1834; Downie et al. 2000). These taxa coincide with the previously delimited "*Daucus*," "*Torilis*," and "*Scandix*" subgroups (Lee and Downie 1999) of the "*Daucus*" clade (Plunkett et al. 1996, Downie et al. 1998, Plunkett and Downie 1999). Subtribes Daucinae and Torilidinae coincide approximately with Heywood's (1982c) tribe Caucalideae but with the inclusion of four genera of Laserpitieae and the exclusion of *Kozlovia*.

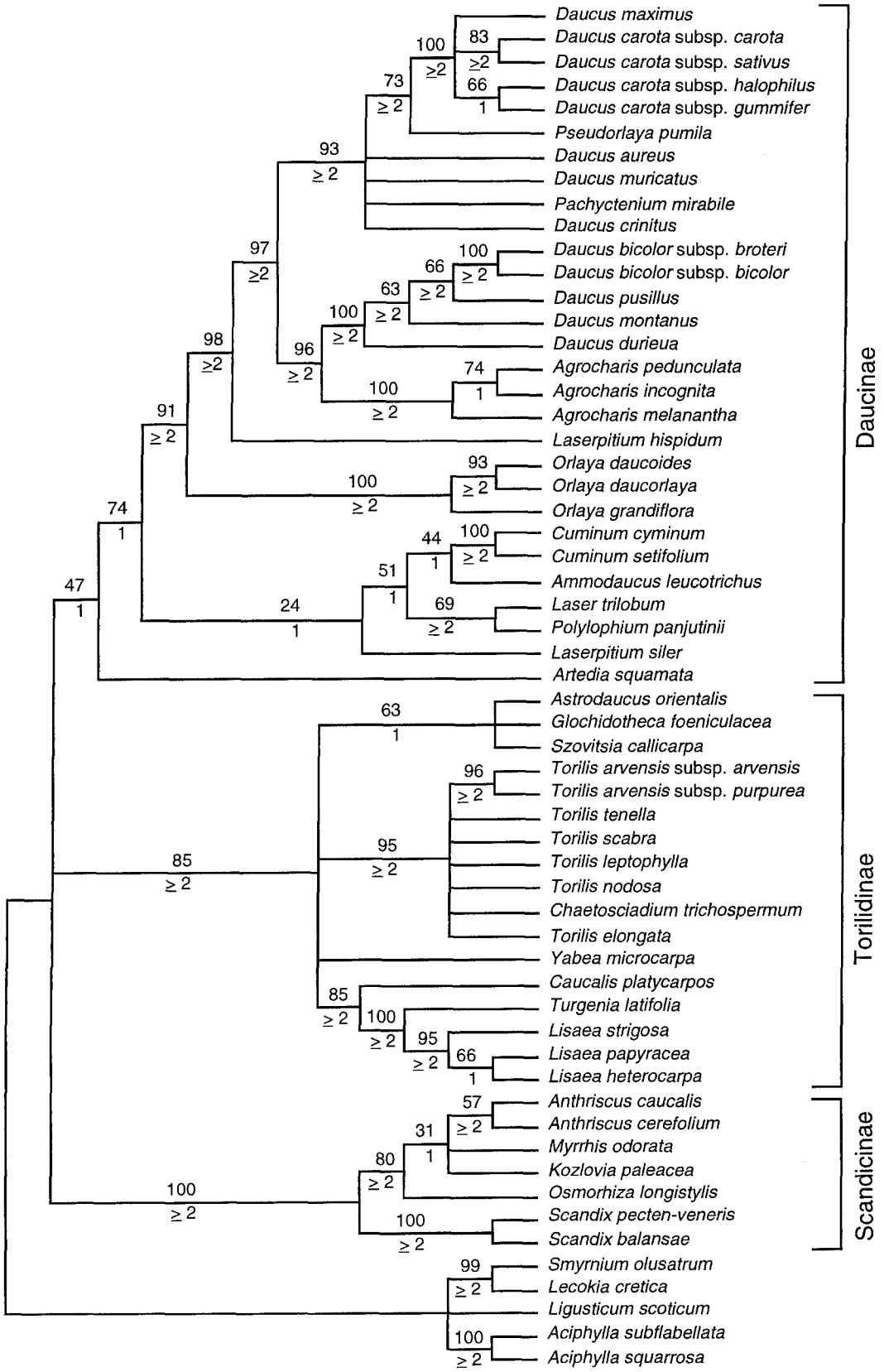
We now continue our investigations of Caucalideae phylogeny by incorporating data from the chloroplast genome. Congruence of relationship from independent lines of evidence is necessary in order to examine the robustness of earlier phylogenetic hypotheses and to identify discrepant organismal and gene phylogenies. Here we use cladistic analysis of cpDNA restriction sites and chloroplast *rps*16 intron sequences to infer historical relationships. The latter was chosen because of its potential for variation, the ease by which the region can be isolated from herbarium material, 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, Downie and Katz-Downie 1999). Our objectives are to: (1) provide a phylogenetic estimate for the group using evidence from cpDNA and to identify well-supported monophyletic groups; (2) compare the utility of cpDNA restriction sites and *rps*16 intron sequences in resolving relationships; and (3) compare the phylogenetic hypothesis obtained to that inferred using nuclear rDNA ITS sequences. Subsequent studies (manuscripts currently in preparation) deal with the cladistic analysis of morphological data, the interpretation of cytological, palynological, anatomical, morphological, and chemical character evolution in the group in light of its inferred evolutionary history, and the delimitation of genera based on these morphological and molecular data.

Materials and methods

Terminal taxa. The 52 accessions considered in this study, with corresponding source and voucher information, are listed in Table 1. Thirty-two accessions were included in the mapping of cpDNA restriction sites and 34 accessions were sequenced for the chloroplast *rps*16 intron, with 14 accessions common to both analyses. While each data set was approximately similar in size, 14 genera were included in the restriction site study (where emphasis on sampling was placed on *Daucus* and *Torilis*), whereas 27 genera were included in the intron study (where emphasis on sampling was placed on Laserpitieae and *Ferula*). The genus *Ferula*, treated in Drude's tribe Peucedaneae and thus considered distantly related to the spiny-fruited umbellifers, allies with *Torilis* in the ITS study of Valiejo-Roman et al. (1998) and, as such, was included in our investigation. These 52 accessions represent 15 of the 21 genera recognized most recently in Caucalideae (Heywood and Jury in Heywood 1982c). The six remaining genera were not included due to lack of sufficient material or, as in the case of *Aphanopleura* and *Psammogeton*, because previous studies had supported their removal from the tribe (Pimenov and Leonov 1993, Katz-Downie et al. 1999). Material from the rare, monotypic genus *Angoseseli* has yet to be included in any molecular systematic study. Additionally, we included representation from Heywood's Scandiceae and Drude's (1897–1898) tribes Apieae, Laserpitieae, and Smyrnieae, based on the results of our higher-level molecular phylogenetic analyses of the family (Downie et al. 1998) and our recent ITS studies of Caucalideae and relatives (Katz-Downie et al. 1999, Lee and Downie 1999). *Smyrnium olusatrum* (Smyrnieae) was used to root the trees.

DNA extraction. Total genomic DNA was extracted from either fresh leaf or herbarium-preserved tissue using the modified CTAB procedure of Doyle and Doyle (1987). For some taxa, the DNA was purified further by centrifugation to equilibrium in cesium chloride/ethidium bromide gradients.

Chloroplast DNA restriction site analysis. Approximately 1 μg of total genomic DNA was digested singly with each of the following 14 restriction enzymes (all recognizing 6-bp sequences except for *Nci*I which recognizes 5 bp sequences) according to the manufacturers' instructions: *Ava*I,


Daucus maximus
Daucus carota subsp. carota
Daucus carota subsp. sativus
Daucus carota subsp. halophilus
Daucus carota subsp. gummifer
Pseudorlaya pumila
Daucus aureus
Daucus muricatus
Pachyctenium mirabile
Daucus crinitus
Daucus bicolor subsp. broteri
Daucus bicolor subsp. bicolor
Daucus pusillus
Daucus montanus
Daucus durieua
Agrocharis pedunculata
Agrocharis incognita
Agrocharis melanantha
Laserpitium hispidum
Orlaya daucoides
Orlaya daucorlaya
Orlaya grandiflora
Cuminum cyminum
Cuminum setifolium
Ammodaucus leucotrichus
Laser trilobum
Polylophium panjutinii
Laserpitium siler
Artedia squamata
Astrodaucus orientalis
Glochidotheca foeniculacea
Szovitsia callicarpa
Torilis arvensis subsp. arvensis
Torilis arvensis subsp. purpurea
Torilis tenella
Torilis scabra
Torilis leptophylla
Torilis nodosa
Chaetosciadium trichospermum
Torilis elongata
Yabea microcarpa
Caucalis platycarpos
Turgenia latifolia
Lisaea strigosa
Lisaea papyracea
Lisaea heterocarpa
Anthriscus caucalis
Anthriscus cerefolium
Myrrhis odorata
Kozlovia paleacea
Osmorhiza longistylis
Scandix pecten-veneris
Scandix balansae
Smyrnium olusatrum
Lecokia cretica
Ligusticum scoticum
Aciphylla subflabellata
Aciphylla squarrosa
Daucinae
Torilidinae
Scandicinae

*Bam*HI, *Ban*II, *Bgl*II, *Cla*I, *Dra*I, *Eco*O109I, *Eco*RI, *Eco*RV, *Hin*cII, *Hin*dIII, *Nci*I, *Xba*I, and *Xho*I. DNA digests were separated electrophoretically in 1.0% agarose gels and bidirectionally transferred to Magna Charge nylon membranes (Micron Separations, Inc., Westborough, MA) for filter hybridizations to ^{32}P radiolabeled tobacco probes. Thirty-five probes (obtained from J. Palmer, Indiana University, Bloomington and described in Olmstead and Palmer 1992), covering both the LSC (large single-copy) and SSC (small single-copy) regions of the chloroplast genome, were used. The evolutionary conservatism of restriction sites in the inverted repeat region of Apiaceae cpDNAs, as noted in a concurrent but higher-level study of Apiaceae phylogeny (Plunkett and Downie 1999), suggested that this region would not supply much phylogenetic information and, therefore, was not considered. Prehybridization (2–6 hours) and hybridization (24–36 hours) were carried out at 65 °C in 2× SSC, 0.5% SDS, and 0.25% nonfat dry milk. Washing of membranes and autoradiography were as described in Olmstead and Palmer (1992). Individual nylon membranes were reused up to 17 times by stripping the hybridized probes with boiling strip solution (0.1× SSC) prior to subsequent prehybridization. Restriction site maps of the LSC and SSC regions were constructed for each accession for each of the 14 enzymes. For one taxon in each of the three recently recognized subtribes (*Daucus carota* subsp. *sativus*, *Torilis arvensis* subsp. *arvensis*, and *Scandix pecten-veneris*) and for four enzymes (*Bam*HI, *Bgl*II, *Eco*RV, *Hin*dIII), additional finer-scale hybridization probes from tobacco cpDNA were used in order to construct detailed gene maps and to survey for specific genes and introns (probes and rationale described in Downie and Palmer 1992). Fragment sizes were estimated by the inclusion of size markers by combining equimolar mixtures of phage lambda DNA digested with *Eco*RI and *Hin*dIII and with *Hin*dIII alone. One lane of tobacco cpDNA, digested with the enzyme of interest, was also included to facilitate mapping. A matrix of binary data, representing restriction site presence (1) or absence (0), was constructed. For a few taxa and enzymes (especially *Xba*I), missing data were scored as "?," a result of either partial- or non-digestion of DNA.

Amplification and sequencing of the chloroplast *rps*16 intron. For 34 accessions, a region containing the complete *rps*16 intron and about half of its flanking 3′ exon was PCR-amplified using primers "5′ exon *rps*16" (AAACGATGTGGNAGNAARCA) and "3′ exon *rps*16" (CCTGTAGGYTGNGCNCCYTT) in an equimolar ratio (primers written 5′ to 3′). In tobacco cpDNA, the *rps*16 intron is 860 bp in size (Shinozaki et al. 1986). Each set of PCR amplifications was monitored by the inclusion of positive (tobacco cpDNA) and negative (no template) controls. Details of primer design, the PCR-amplification reactions, the DNA purification and automated DNA sequencing strategies used, and the utility of this intron region for phylogeny estimation, are provided in Downie and Katz-Downie (1999). All sequencing was done using an Applied Biosystem's, Inc. (Foster City, CA) 373A Automated DNA Sequencer with Stretch upgrade. The reaction conditions were as specified by the manufacturer, with the addition of 5% dimethylsulfoxide (DMSO). Simultaneous consideration of both DNA strands across the entire region permitted unambiguous base determination in nearly all cases.

The sequences were aligned manually and gaps positioned to minimize nucleotide mismatches. When gap-coding was problematic, these regions of the alignment were excluded from the analysis. Unambiguously aligned, potentially informative gaps were few, and were not included as extra characters in the phylogenetic analysis. Pairwise nucleotide differences of unambiguously aligned positions were determined using the distance matrix option in PAUP version 3.1.1 (Swofford 1993).

Fig. 1. Strict consensus of 588 minimal length 1,035-step trees derived from equally weighted maximum parsimony analysis of nuclear rDNA ITS1 and ITS2 sequences from 58 accessions of Heywood's (1982c) Apiaceae tribe Caucalideae and related taxa using 409 unambiguously aligned nucleotide positions (CIs with and without uninformative characters = 0.466 and 0.428, respectively); RI = 0.756; Lee and Downie 1999). Numbers above the nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates; decay values are presented below. Within the ingroup, three major groups of taxa are recognized, and have been treated as Scandiceae subtribes Daucinae, Torilidinae, and Scandicinae (Downie et al. 2000)

Table 1. Accessions examined for cpDNA restriction site (†) and *rps*16 intron sequence (*) variation. These intron sequence data have been deposited with GenBank under the bracketed accession numbers. Herbarium acronyms according to Holmgren et al. (1990). RBGE = Royal Botanic Garden Edinburgh; UIUC = University of Illinois at Urbana-Champaign; UCB = Botanical Garden of the University of California, Berkeley; IPK = Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany

Taxon	Source and voucher information
Agrocharis incognita (C. Norman) Heywood & Jury*†	Africa, Kenya, Nairobi, DNA supplied by E. Knox (coll. no. 2578) [AF123730]
Anisotome aromatica Hook. f. var. *pinnatisecta* Allan*	New Zealand, South Island, Canterbury, Mt. Hutt, *Corden 29* (E), cult. RBGE (no. 19881687) [AF110550]
Anthriscus caucalis M. Bieb.*†	cult. UIUC from seeds obtained from Jardin botanique de Caen, France, *Lee 44* (ILL) [AF110549]
Anthriscus cerefolium (L.) Hoffm.†	cult. UIUC from seeds obtained from Real Jardín Botánico, Spain, *Downie 35* (ILL)
Artedia squamata L.*	Turkey, Tarsus, Namrun Plateau, *Kasapligil 6483* (UC) [AF123747]
Astrodaucus orientalis (L.) Drude*†	Iran, cult. UIUC from seeds obtained from Research Institute of Forests and Rangelands, Iran, *Lee 43* (ILL) [AF123748]
Caucalis platycarpos L.*†	cult. UIUC from seeds obtained from IPK, *Lee 29* (ILL) [AF123745]
Chaetosciadium trichospermum (L.) Boiss.*†	Jordan, Um-Qais, near Irbid, *Lahham & El-Oqlah 4* (Yarmouk University Herbarium, Jordan) [AF123740]
Cuminum cyminum L.†	cult. UIUC from seeds obtained from grocery store, *Lee 120* (ILL)
Daucus aureus Desf.†	cult. UIUC from seeds obtained from IPK, *Lee 57* (ILL)
Daucus carota L. subsp. *commutatus* (Paol.) Thell.†	cult. UIUC from seeds obtained from IPK, *Lee 96* (ILL)
Daucus carota L. subsp. *gummifer* Hook. f.†	cult. UIUC from seeds obtained from Jardin botanique de Caen, France, *Lee 47* (ILL)
Daucus carota L. subsp. *halophilus* (Brot.) Okeke†	cult. UIUC from seeds obtained from J.-P. Reduron, Mulhouse, France, *Lee 81* (ILL)
Daucus carota L. subsp. *hispanicus* (Gouan) Thell.†	cult. UIUC from seeds obtained from IPK, *Lee 84* (ILL)
Daucus carota L. subsp. *maritimus* (Lam.) Batt.†	cult. UIUC from seeds obtained from IPK, *Lee 95* (ILL)
Daucus carota L. subsp. *sativus* (Hoffm.) Arcang.*†	cult. UIUC from seeds obtained from Jardin botanique de Montréal, Canada, *Downie 386* (ILL) [AF110547]
Daucus maximus Desf.†	cult. UIUC from seeds obtained from IPK, *Lee 64* (ILL)

Daucus montanus Humb. & Bonpl.†
Daucus muricatus L.†
Daucus pusillus Michx.*†
Ferula kokanica Regel & Schmalh.*

Ferula olivacea (Diels) H. Wolff ex Hand.-Mazz.*
Ferula tenuisecta Korovin ex Pavlov*

Glochidotheca foeniculacea Fenzl*

Laser trilobum (L.) Borkh.*

Laserpitium hispidum M. Bieb.*†

Laserpitium hispidum M. Bieb.*

Laserpitium siler L.*

Ligusticum scoticum L.*

Lisaea papyracea Boiss.*
Melanoselinum decipiens (Schrad. & Wendl.) Hoffm.*
Melanoselinum decipiens (Schrad. & Wendl.) Hoffm.*
Monizia edulis Lowe*

Myrrhis odorata (L.) Scop.*
Orlaya daucoides (L.) Greuter*†

Argentina, cult. UCB (no. 94.0563)
cult. UIUC from seeds obtained from IPK, *Lee 36* (ILL)
USA, cult. UCB (no. 92.0891) [AF123729]
Tadjikistan, Hushikat Gorge, 22 July 1975, *Pimenov et al. s.n.* (MW), cult. Moscow State University Botanical Garden, Russia [AF123751]
China, Yunnan, *Chungtien et al. 790* (E), cult. RBGE (no. 19910663) [AF123750]
Uzbekistan, Angren Valley, Iertasch, 27 May 1978, *Pimenov et al. 106* (MW) [AF123752]
Turkey, Adana, *Alava 6698* (UC), DNA supplied by M. Chase (coll. no. 2922) [AF123746]
Azerbaijan, Caucasus, Vel-Veli-Chai, 1973, *Pimenov et al. s.n.* (MW), cult. Moscow State University Botanical Garden, Russia [AF123735]
cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót, *Lee 68* (ILL) [AF123731]
cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót, *Downie 120* (ILL) [AF123732]
cult. UIUC from seeds obtained from Johannes Gutenburg University, Germany, *Downie 71* (ILL) [AF123734]
USA, Massachusetts, Plymouth Co., near Mattapoisett along Buzzard's Bay, *Raiche 40411* (UC), cult. UCB (no. 84.0620) [AF123756]
Armenia, *Gambarian s. n.* (UC) [AF123744]
Portugal, Madeira, *Hutchinson s. n.* (E), cult. RBGE (no. 19970376) [AF123737]
Portugal, Madeira, cult. University of Reading, England [AF123738]
Portugal, Madeira, cult. Jardin botanico de Madeira, 21 July 1997, *F. & O. Baets 08655* (E) [AF123739]
Europe, cult. UCB (no. 89.1236) [AF123755]
cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót, *Lee 7* (ILL) [AF123733]

Table 1 (continued)

Taxon	Source and voucher information
Orlaya daucorlaya Murb.†	cult. UIUC from seeds obtained from IPK, *Downie 20* (ILL)
Orlaya grandiflora (L.) Hoffm.†	cult. UIUC from seeds obtained from Karl Marx University Botanical Garden, Leipzig, Germany, *Lee 41* (ILL)
Osmorhiza longistylis (Torr.) DC.*	USA, Illinois, Champaign Co., Urbana, Hart Woods, *Downie 738* (ILL) [AF123754]
Polylophium panjutinii Manden. & Schischk.*	W. Georgia, Caucasus, Migaria Mt., August 1990, *Daushkevich s. n.* (MW) [AF123736]
Pseudorlaya pumila (L.) Grande*†	cult. UIUC from seeds obtained from Jardin Botaniques Lisboa, Portugal, *Lee 59* (ILL) [AF123728]
Scandix pecten-veneris L.*†	cult. UIUC from seeds obtained from IPK, *Downie 27* (ILL) [AF123753]
Smyrnium olusatrum L.*†	cult. UIUC from seeds obtained from University of Oldenburg Botanical Garden, Germany, *Downie 141* (ILL) [AF110551]
Szovitsia callicarpa Fisch. & C. A. Mey.*	Azerbaijan, Moghan, *Lamond 3195* (E) [AF123749]
Torilis arvensis (Huds.) Link subsp. *arvensis*†*	USA, Illinois, Champaign Co., Urbana, Silver Creek Restaurant, *Downie 816* (ILL) [AF110548]
Torilis arvensis (Huds.) Link subsp. *purpurea* (Ten.) Hayek†	Morocco, Col du Nador, *Jury & Wilson s. n.* (RNG)
Torilis elongata (Hoffm. & Link) Samp.†	Morocco, Col du Nador, *Jury & Wilson s. n.* (RNG)
Torilis japonica (Houtt.) DC.*	cult. UIUC from seeds obtained from Jardin botanique de Nancy, France, *Downie 237* (ILL) [AF123741]
Torilis leptophylla (L.) Rchb. f.†	Asia Minor, *Anonymous s. n.* (K)
Torilis nodosa (L.) Gaertn.†	England, Isle of Wight, *Linington & Shepard s. n.* (K)
Torilis tenella (Delile) Rchb. f.†	Jordan, Ajlun, Schtafeenah, *Lahham & El-Oqlah 1* (Yarmouk University Herbarium, Jordan)
Turgenia latifolia (L.) Hoffm.*†	cult. UIUC from seeds obtained from J.-P. Reduron, Mulhouse, France, *Lee 82* (ILL) [AF123743]
Yabea microcarpa (Hook. & Arn.) Koso-Pol.*	USA, Arizona, Pima Co., *Holmgren 6772* (WTU) [AF123742]

Alignment gaps in any one sequence were treated as missing data for all taxa. Transition/transversion ratios over all maximally parsimonious trees were calculated using MacClade version 3.01 (Maddison and Maddison 1992). The *rps*16 intron and flanking *rps*16 3′ exon DNA sequences have been submitted to GenBank (accession numbers in Table 1). All data matrices are available upon request; the cpDNA restriction site matrix is presented in Lee (1998).

Phylogenetic analysis. Phylogenetic analyses of the restriction site and *rps*16 intron data sets were carried out separately (as only 14 accessions were shared between them) using the heuristic search strategies of PAUP. Twenty-five accessions were common to both the *rps*16 intron and ITS studies, and phylogenetic analyses of these reduced data sets, both separately and in combination, were also carried out. All searches were conducted with 500 random addition replicates, tree bisection-reconnection (TBR) branch swapping, with options mulpars, steepest descent, collapse, and acctran selected. Bootstrap values (Felsenstein 1985) were calculated from 100 replicate analyses, simple addition sequence of taxa, and TBR branch swapping. In order to identify weakly supported nodes, decay analyses (Bremer 1988) were conducted until tree storage memory was exhausted or 5,000 minimal length trees had been reached. We explored the effect of differentially weighting restriction site gains: losses or DNA sequence transitions: transversions. The results obtained, however, did not differ substantially from those under the assumption of equal weighting and are not reported further.

The maximum likelihood method was also applied to the 25-taxon *rps*16 intron and ITS data sets using the program fastDNAml (version 1.0.6; Olsen et al. 1994), based on the procedures of Felsenstein (1981). Maximum likelihood trees were inferred using a range of transition: transversion rate ratios between 1.0 and 2.0, randomizing the input order of sequences (jumble), and by invoking the global branch swapping search option. Empirical base frequencies were derived from the sequence data and used in the maximum likelihood calculations.

Results

Chloroplast DNA restriction site analysis. The results of the restriction site study reveal that the chloroplast genomes of the 32 examined accessions of Apiaceae are similar in gene content, gene arrangement, and structure to that of tobacco cpDNA and, thus, to the vast majority of angiosperms examined to date. No length variants in the LSC and SSC regions were detected, but since length mutations less than 200 bp could not be seen easily on our gel systems, we have likely underestimated the actual extent of this variation. CpDNA restriction site maps for four enzymes are presented in Fig. 2 for *Daucus carota* subsp. *sativus* (Scandiceae subtribe Daucinae), *Torilis arvensis* subsp. *arvensis* (subtribe Torilidinae), and *Scandix pecten-veneris* (subtribe Scandicinae). A gene map is also presented, based on our hybridization results and those results obtained using finer-scale (i.e. gene- and intron-specific) probes from a much larger study on the evolution of chloroplast genome organization and gene content in angiosperms (Downie and Palmer 1992). Within experimental limits, the gene map for *Daucus*, *Torilis*, and *Scandix* cpDNAs is the same as that for tobacco cpDNA (Shinozaki et al. 1986, as modified by Sugiura 1992 and Wolfe et al. 1992) and, likely, for all other examined Apiaceae, as all probes hybridized strongly and mapped in a colinear fashion.

A total of 688 restriction sites was identified using 14 enzymes: 291 (42.3%) were shared by two or more taxa and were potentially informative for parsimony analysis, 265 (38.5%) were unvarying, and 132 (19.2%) were unique to individual taxa. The numbers of parsimony informative, nonvariable, and autapomorphic restriction sites for each of the 14 enzymes across all 32 cleavage maps, relative to their inferred positions in either the LSC or SSC cpDNA regions, are presented in Table 2. The enzyme *Dra*I, specific for the DNA sequence TTT′AAA, cut most frequently and yielded the greatest number of potentially informative mutations. The ratio of terminal taxa (32) to parsimony informative restriction sites (291) was 1:9.1. Pairwise mean distances, calculated using PAUP, ranged from identity (among *Daucus carota* subspecies *halophilus*,

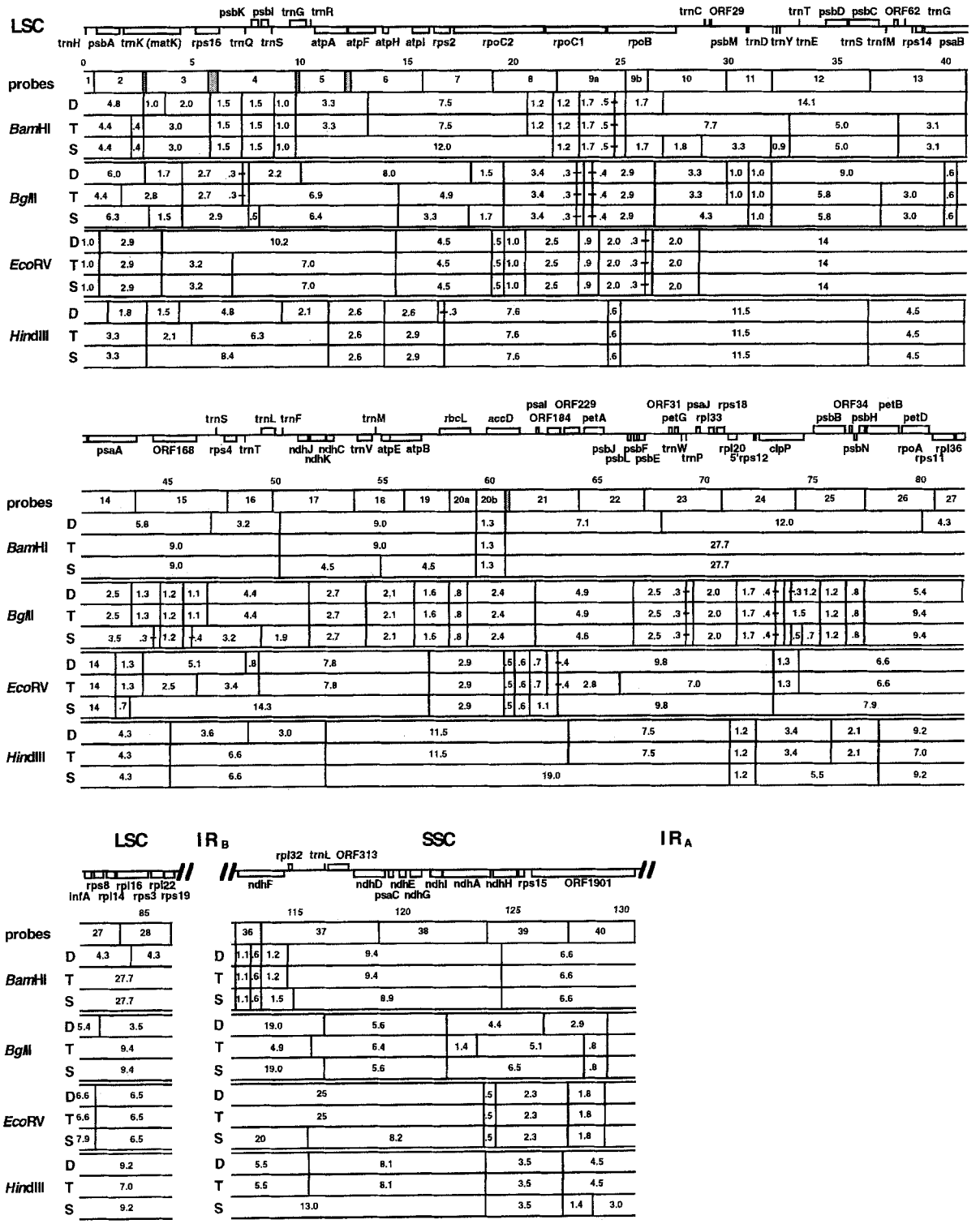


Fig. 2. Physical and gene maps of the large and small single-copy regions (LSC and SSC, respectively) of the chloroplast genomes of *Daucus carota* subsp. *sativus* (D), *Torilis arvensis* subsp. *arvensis* (T), and *Scandix pecten-veneris* (S). Maps of the two large inverted repeat regions, IR_A and IR_B, were not constructed. Chloroplast DNAs from these three taxa were each digested singly with restriction enzymes *Bam*HI, *Bgl*II,

hispanicus, and *maritimus*, and *D. maximus*) to 22.7% (between *D. pusillus* and *Scandix pecten-veneris*), the latter representing a minimum of 147 site differences. Within *Daucus*, a maximum of 64 restriction site differences were apparent (between *D. montanus* and *D. carota* subsp. *sativus*) for 9.3% mean divergence, and within *D. carota*, a maximum of seven site differences were apparent (between subspecies *commutatus* and *sativus*).

Maximum parsimony analysis of the 32 taxon × 688 character matrix resulted in two minimal length trees each of 779 steps, with consistency indices (CIs) of 0.543 (all characters) and 0.450 (excluding uninformative characters) and a retention index (RI) of 0.754. The strict consensus of these two trees, with accompanying bootstrap and decay values, is shown in Fig. 3. Three major clades are inferred: Scandiceae subtribes Daucinae (*Daucus*, *Pseudorlaya*, *Agrocharis*, *Laserpitium*, *Cuminum*, and *Orlaya*), Torilidinae (*Torilis*, *Chaetosciadium*, *Caucalis*, *Turgenia*, and *Astrodaucus*), and Scandicinae (*Anthriscus* and *Scandix*). Subtribes Daucinae and Torilidinae arise as strongly supported sister taxa (with a 100% bootstrap value). The genera *Daucus* and *Torilis* are each not monophyletic: *D. maximus* arises within the *D. carota* assemblage, and *Chaetosciadium trichospermum* occurs within *Torilis*. The three examined species of Scandicinae form a strongly supported clade sister to Daucinae + Torilidinae. Based on only three exemplars from Scandicinae, the genus *Anthriscus* is also not monophyletic. Bootstrap and decay values are generally high, ranging between 45 and 100% for the former and from one to greater than four for the latter.

Table 2. Numbers of nonvariable (N), parsimony informative (I), and autapomorphic (A) restriction sites derived from each of the 14 enzymes used to construct cpDNA maps for 32 accessions of Apiaceae tribe Caucalideae and related taxa. Location abbreviations: chloroplast genome large single copy (LSC) and small single copy (SSC) regions

Restriction enzyme	Number of restriction sites						Total
	LSC			SSC			
	N	I	A	N	I	A	
*Ava*I	18	10	8	1	2	4	43
*Bam*HI	8	14	4	1	0	6	33
*Ban*II	27	24	8	2	4	2	67
*Bgl*II	17	18	8	1	6	1	51
*Cla*I	12	19	9	1	7	2	50
*Dra*I	16	30	10	6	9	1	72
*Eco*O109I	22	12	12	6	5	1	58
*Eco*RI	19	26	6	4	6	1	62
*Eco*RV	13	10	15	2	2	3	45
*Hin*cII	17	18	5	7	2	1	50
*Hin*dIII	12	12	2	1	1	3	31
*Nci*I	29	24	5	3	1	6	68
*Xba*I	11	15	5	2	6	0	39
*Xho*I	6	8	1	1	0	3	19
Total	227	240	98	38	51	34	688

Chloroplast *rps*16 intron analysis. Among all 34 accessions examined, the *rps*16 intron ranged in size from 818 (*Artedia*) to 892 (*Chaetosciadium*) bp, and averaged 864 bp. Percentage G + C content for the intron ranged from 31.4 to 34.0%, averaging 33.0%. All sequencing reactions culminated in an additional 110 bp of sequence from the adjacent

Fig. 2 (continued)
*Eco*RV, and *Hin*dIII, and probed with the 35 indicated cloned restriction fragments from tobacco cpDNA. Additional information on gene arrangement and orientation was obtained using finer-scale probes from a much larger study on chloroplast genome organization and gene and intron content in angiosperms (Downie and Palmer 1992). Stippled boxes indicate short regions not used as probes. Restriction fragment sizes and coordinates above the physical maps are in kilobase pairs (kb), with coordinates corresponding to those of the tobacco chloroplast genome (Shinozaki et al. 1986). Within experimental limits, the gene map is the same as the tobacco map (Shinozaki et al. 1986, as modified by Sugiura 1992 and Wolfe et al. 1992) as major insertions, deletions or other structural mutations were not evident. Genes above the line are transcribed from left to right and vice versa

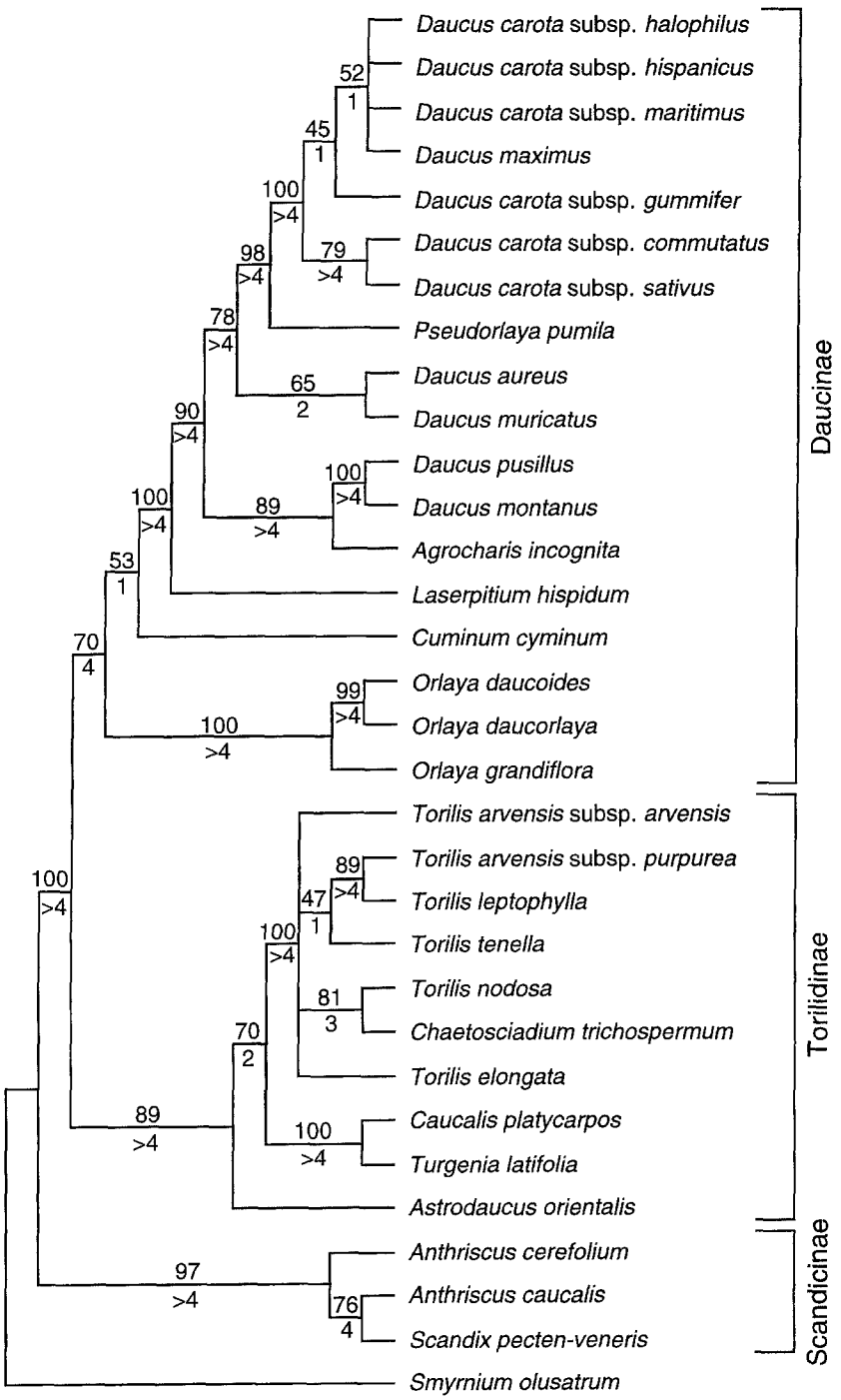


Fig. 3. Strict consensus of the two minimal length 779-step trees derived from equally weighted maximum parsimony analysis of cpDNA restriction site data (CIs = 0.543 and 0.450, with and without uninformative characters, respectively; RI = 0.754). Bootstrap percentages are provided above each branch; decay values are provided below. Three major groups of taxa are recognized, and have been designated previously as Scandiceae subtribes Daucinae, Torilidinae, and Scandicinae (Downie et al. 2000)

*rps*16 3′ exon region, with no length variation. Alignment of all 34 intron and flanking 3′ exon sequences resulted in a matrix of 1130 positions. However, due to confounding length mutations, small repetitive elements, or tracts of poly-A's and T's of variable length, it was necessary to exclude eight regions (102 alignment positions) from the analysis. These ambiguous regions ranged in size from 4 to 36 positions, averaging about 13 positions each. Of the remaining 1028 unambiguously aligned positions, 107 (10.4%) were parsimony informative, 106 (10.3%) were autapomorphic, and 815 (79.3%) were invariant. The 110-bp 3′ exon region was highly conserved, reflecting only five informative and three autapomorphic positions across all 34 taxa. The ratio of terminal taxa (34) to parsimony informative nucleotide substitutions (107) was 1:3.1. Measures of pairwise nucleotide sequence divergence ranged from 0.2% (between the two accessions each of *Laserpitium hispidum* and *Melanoselinum decipiens*) to 6.1% (between *Scandix* and the outgroup *Smyrnium*). A total of 37 unambiguous gaps was required for proper alignment. These gaps ranged in size from 1 to 46 bp (averaging 5 bp), with the largest representing a deletion in *Artedia* relative to *Smyrnium*. Seventeen gaps were potentially informative for parsimony analysis, with seven of these a single bp in size. Many more gaps were apparent, but were in those regions of the alignment excluded from the analysis.

Maximum parsimony analysis of the 34 taxon × 1028 character matrix resulted in 52 minimal length 321-step trees, with CIs of 0.782 and 0.663 (with and without uninformative characters, respectively) and a RI of 0.779. The strict consensus of these trees, with accompanying bootstrap and decay values, is presented in Fig. 4. Within this tree, bootstrap values range between 27 and 100%, and decay values are generally lower than those obtained from restriction site data. As in the analysis of restriction sites, Scandiceae subtribes Daucinae and Torilidinae form monophyletic sister groups, but this relationship is poorly supported (with a 37% bootstrap value). Included in Daucinae is representation from tribe Laserpitieae (*Melanoselinum*, *Monizia*, *Laserpitium*, *Laser*, and *Polylophium*). Sister to Daucinae + Torilidinae is a clade comprising three accessions of *Ferula*, and is designated herein as the *Ferula* clade. Subtribe Scandicinae is also monophyletic, and sister to the clade comprising Daucinae, Torilidinae, and *Ferula*. The genera *Daucus* (represented in this analysis by *D. carota* subsp. *sativus* and *D. pusillus*) and *Torilis* (*T. arvensis* subsp. *arvensis* and *T. japonica*) are each, again, not monophyletic. The two species of *Laserpitium* (*L. hispidum* and *L. siler*) also do not form a clade.

CpDNA restriction site and *rps*16 intron sequence comparisons. Comparison of phylogenies derived from separate analysis of cpDNA restriction sites (Fig. 3) and *rps*16 intron sequences (Fig. 4) reveals much concordance of relationship, despite the fact that these data sets were not parallel in construction. In each of these analyses, subtribe Daucinae is monophyletic and sister to a monophyletic Torilidinae. This group, in turn, is sister to a monophyletic Scandicinae when restriction sites are compared, or to the *Ferula* clade in the intron-based analysis. The relative placements of the 14 taxa common to both analyses are highly consistent. As examples, *Daucus carota* subsp. *sativus* unites or is very closely allied to *Pseudorlaya*, *D. pusillus* is allied with *Agrocharis*, and *Torilis* and *Chaetosciadium* unite, as does *Caucalis* and *Turgenia*.

Our results indicate that restriction site data are more variable than those of the *rps*16 intron, even though the latter matrix includes twice as many genera. In the restriction site study, 423 variable sites were scored, of which 291 were potentially parsimony-informative. In the intron study, 107 of 213 variable positions were potentially informative. The restriction site matrix provides almost three times as many informative characters as does the intron data set, with the ratio of terminal taxa to parsimony informative characters being either 1:9.1 or 1:3.1, respectively. Measures of divergence, although not directly comparable given the different types of data

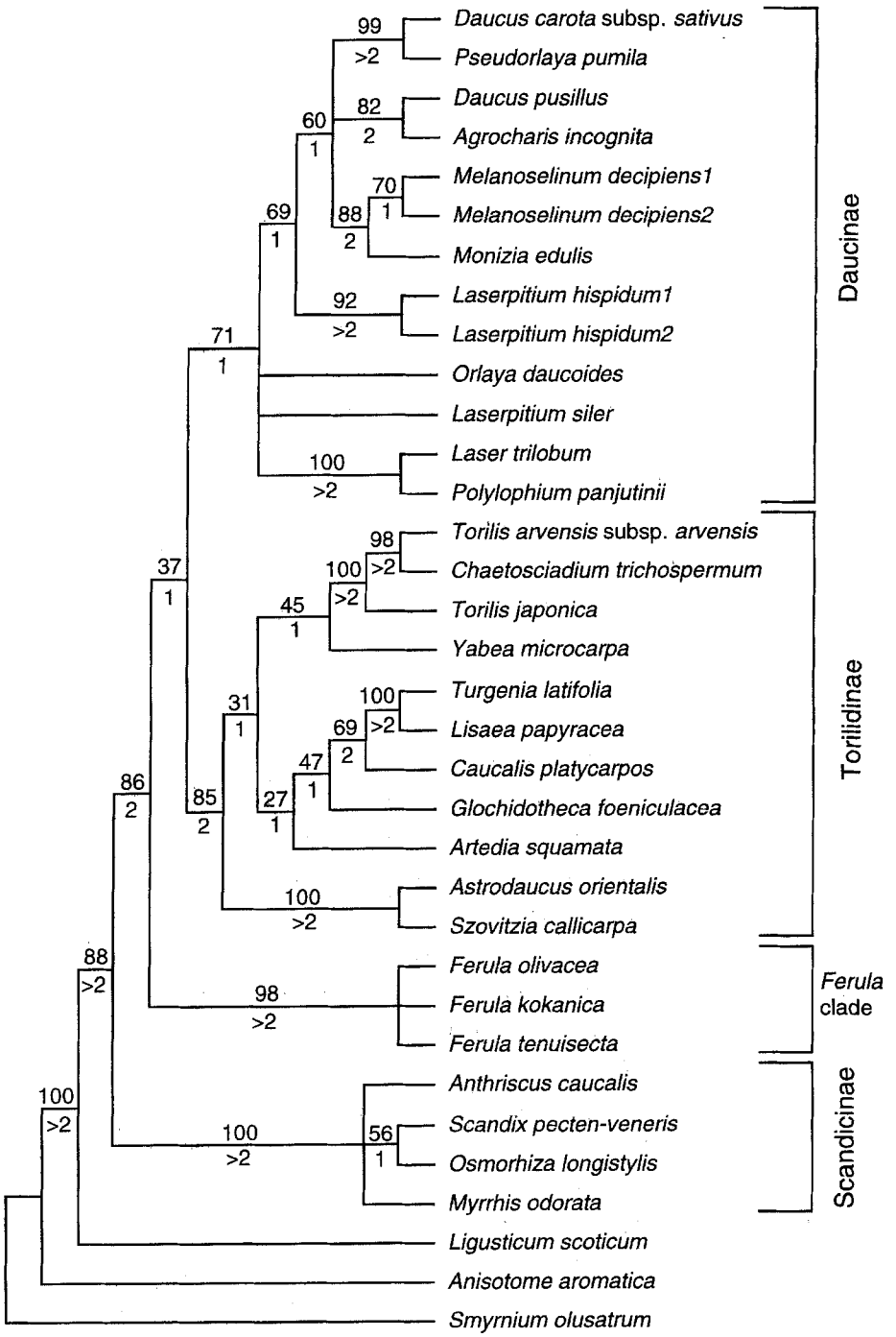


Fig. 4. Strict consensus of 52 minimal length 321-step trees derived from equally weighted maximum parsimony analysis of 34 unambiguously aligned *rps*16 intron and flanking 3′ exon sequences (CIs = 0.782 and 0.663, with and without uninformative characters, respectively; RI = 0.779). Bootstrap percentages are provided above each branch; decay values are provided below. In addition to the three major groups of taxa (Daucinae, Torilidinae, and Scandicinae), a clade comprising three species of *Ferula* is evident. The two accessions each of *Melanoselinum decipiens* and *Laserpitium hispidum* are described in Table 1

represented, are also greater in the restriction site study, with 22.7% maximum pairwise divergence vs. 6.1% when the intron sequences are compared. As a consequence of the greater number of potentially informative mutations, the branches in the strict consensus tree derived from restriction site data (Fig. 3) are generally better supported (with higher bootstrap and decay values) than those of the *rps*16 intron tree (Fig. 4). For example, in the restriction site tree, the clade of Daucinae + Torilidinae is supported with a 100% bootstrap value and a decay index > 4; in the intron tree, this clade is supported poorly, with a bootstrap value of 37% and a decay index of one. Both data sets reveal comparable levels of homoplasy, as assessed by similar RI values (0.754 or 0.779).

Chloroplast DNA and ITS sequence comparisons. A study incorporating data from the nuclear rDNA ITS region was carried out prior to the two plastid DNA studies presented herein, and the reader is referred to Lee and Downie (1999) for details of the phylogenetic analyses conducted (the results of the maximum parsimony analysis are presented in Fig. 1), evolutionary characteristics of the ITS sequences, and accession voucher information. With the exception of *Artedia*, which is sister to the Daucinae clade in the ITS tree (albeit with very weak bootstrap support; Fig. 1) or is nested within the Torilidinae clade in the *rps*16 intron tree (Fig. 4), phylogenetic analyses of each of the three data sets (ITS, *rps*16 intron, and restriction sites) reveal three major clades of similar composition (i.e. Daucinae, Torilidinae, and Scandicinae). However, the sister group relationship between subtribes Daucinae and Torilidinae, evident in the analyses of both plastid DNA data sets (Figs. 3–4), is not apparent in the ITS tree (Fig. 1).

Twenty-five accessions were common to both the ITS and *rps*16 intron data sets (as opposed to the 13 accessions shared among all three data sets), so to more readily compare the phylogenetic relationships as inferred from these nuclear- or plastid-derived data, the matrices were truncated to their taxa in common. Maximum parsimony analyses of these separate, reduced data sets resulted in a single shortest tree for the ITS matrix (Fig. 5A; tree length = 643 steps; CIs = 0.563 and 0.478, with and without uninformative characters; RI = 0.617) and 50 minimal length trees for the intron matrix (tree length = 275 steps; CIs = 0.807 and 0.669, with and without uninformative characters; RI = 0.758); the latter were used to construct a strict consensus tree (Fig. 5B). Once more, the position of *Artedia* varies depending upon DNA region analyzed, with affinities to both Daucinae and Torilidinae apparent. All other areas of discord occur within subtribe Torilidinae, and involve the relative positions of *Glochidotheca*, *Astrodaucus*, and *Szovitsia*. In the intron tree (Fig. 5B), *Glochidotheca* is sister to the clade of *Turgenia, Lisaea*, and *Caucalis*, whereas in the ITS tree (Fig. 5A), *Glochidotheca* allies with *Astrodaucus* and *Szovitsia*. The basal position of *Astrodaucus* and *Szovitsia* in subtribe Torilidinae, as inferred by the intron study, is not evident in the ITS tree.

In both the ITS- and *rps*16 intron-derived trees (Fig. 5A and 5B, respectively), branches within the Torilidinae clade, with the exception of those leading to the clade of *Turgenia*, *Lisaea*, and *Caucalis* (with 74 or 91% bootstrap values) or to the clade of *Torilis arvensis* and *Chaetosciadium* (with 100% bootstrap values), are weakly supported, with bootstrap values ranging between 28 and 66%. When those nodes characterized by bootstrap values ≤66% and a decay index of one are treated as unresolved (that is, they are collapsed to yield polytomies), the relationships among members of the Torilidinae clade are fully consistent (albeit largely unresolved), with *Artedia* being the only remaining element of discord. The general agreement between the results of these separate analyses suggested that a combined analysis would likely lead to the best estimate of phylogeny given the available data (Barrett et al. 1991, Bull et al. 1993), although we are well aware of the controversy surrounding this issue (reviewed by De Queiroz et al. 1995,

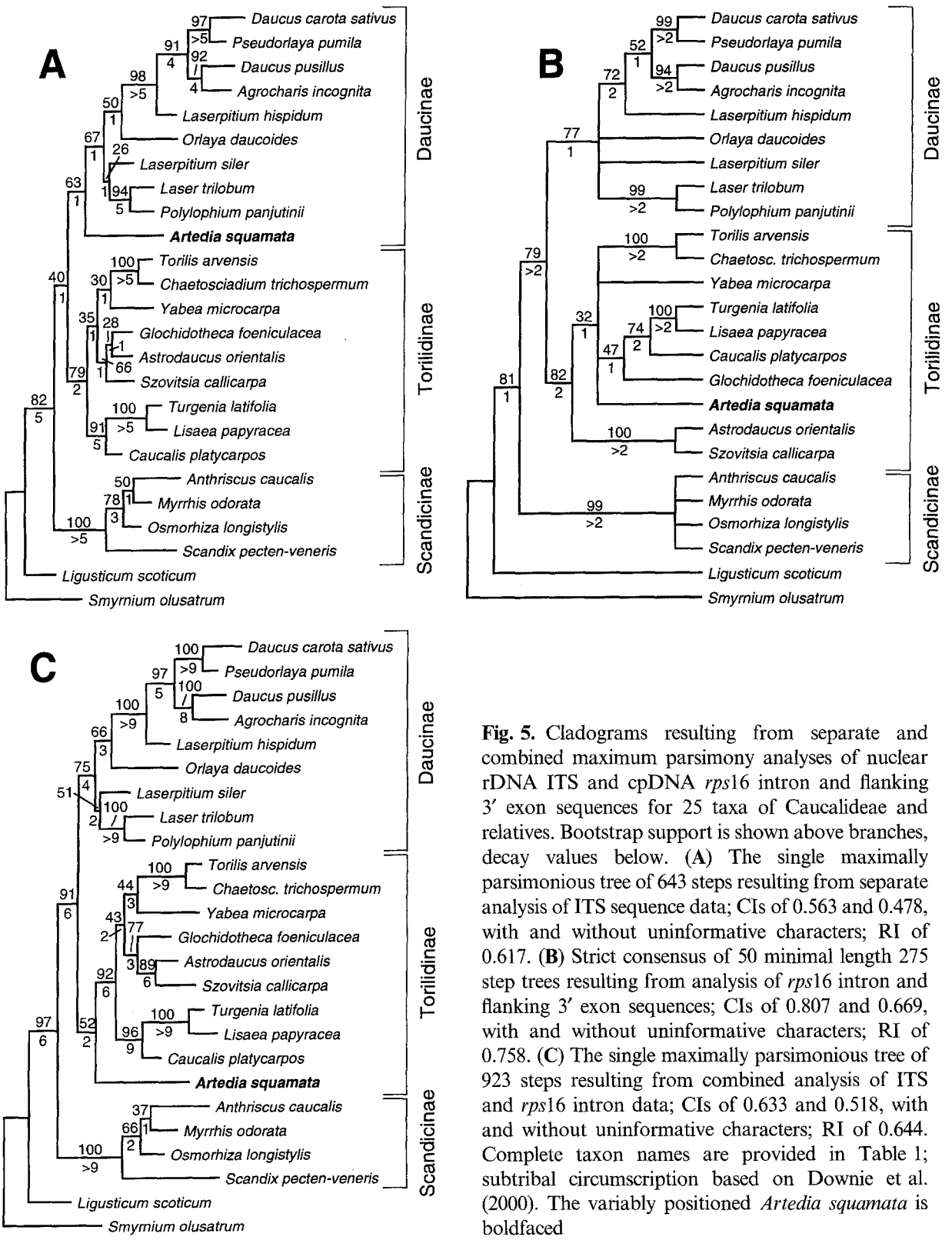


Fig. 5. Cladograms resulting from separate and combined maximum parsimony analyses of nuclear rDNA ITS and cpDNA *rps*16 intron and flanking 3′ exon sequences for 25 taxa of Caucalideae and relatives. Bootstrap support is shown above branches, decay values below. (**A**) The single maximally parsimonious tree of 643 steps resulting from separate analysis of ITS sequence data; CIs of 0.563 and 0.478, with and without uninformative characters; RI of 0.617. (**B**) Strict consensus of 50 minimal length 275 step trees resulting from analysis of *rps*16 intron and flanking 3′ exon sequences; CIs of 0.807 and 0.669, with and without uninformative characters; RI of 0.758. (**C**) The single maximally parsimonious tree of 923 steps resulting from combined analysis of ITS and *rps*16 intron data; CIs of 0.633 and 0.518, with and without uninformative characters; RI of 0.644. Complete taxon names are provided in Table 1; subtribal circumscription based on Downie et al. (2000). The variably positioned *Artedia squamata* is boldfaced

Huelsenbeck et al. 1996, Wendel and Doyle 1998). Parsimony analysis of the combined ITS and intron data sets resulted in a single most parsimonious tree of 923 steps (CIs = 0.633 and 0.518, with and without uninformative characters; RI = 0.644). The topology of this tree (Fig. 5C) is very similar to that inferred using ITS data alone, with the following exceptions: (1) *Artedia* is now sister to the Torilidinae clade; and (2) *Astrodaucus* and *Szovitsia* are monophyletic and sister to *Glochidotheca*. For those clades identified in the separate analyses that were not in conflict, bootstrap and decay values for these clades are generally higher upon consideration of all available data. To better understand what effect *Artedia* had upon the resultant phylogenetic hypothesis, this taxon was removed and the parsimony analysis of combined data rerun. Here, two maximally parsimonious trees resulted (tree length = 861 steps; CIs = 0.643 and 0.530, with and without uninformative characters; RI = 0.664) and their strict consensus, with the exception of the collapse of the branch leading to *Laserpitium siler*, was identical to the tree inferred when *Artedia* was included (figure not shown).

Long-branch attraction in parsimony analysis may affect the resulting phylogenetic hypothesis. Given that the branch leading to *Artedia* is one of the longest in the trees, maximum likelihood analyses of separate and combined data sets were carried out. However, with respect to the phylogenetic placement of *Artedia*, the results obtained using maximum likelihood (not shown) were identical to those inferred by maximum parsimony. Once more, *Artedia* arises sister to Daucinae in the ITS tree, sister to Torilidinae in the combined tree, or included within Torilidinae in the intron tree.

Discussion

Molecular characteristics of the *rps*16 intron. The chloroplast gene *rps*16, encoding ribosomal protein S16 (Neuhaus et al. 1989), is interrupted by an intron in many different land plants (Downie and Palmer 1992). This intron varies considerably in length, ranging between 707 and 951 bp in size (Oxelman et al. 1997), and our report of 818 to 892 bp for this region is consistent with these values. The intron's average G + C content of 33.0% is similar to that reported for other umbellifer *rps*16 introns (Downie and Katz-Downie 1999), and falls near the GC range reported for vascular plant chloroplast genomes in general (Palmer 1991). Like other group II introns, the *rps*16 intron is characterized by six major structural domains (Michel et al. 1989). Domains V and VI and portions of domain I are necessary for proper intron processing and are most conserved evolutionarily, whereas domains II, III, and IV can be quite variable (Learn et al. 1992, Downie et al. 1998). In this study, the smallest *rps*16 intron occurs in *Artedia* and reflects the removal of approximately half of domain IV. With the exceptions of domains V and VI, where no length mutation and a high degree of sequence conservation are apparent, all unambiguous alignment gaps and regions excluded from the analyses were distributed equally throughout the intron.

Chloroplast genome evolution. Previous studies have determined that the sizes of Apiaceae chloroplast genomes range between 140 and 155 kb, the largest being reported for *Daucus* (Debonte et al. 1984, Plunkett and Downie 1999). This variation is largely attributable to the expansion or contraction of the inverted repeat into the adjacent LSC region (Plunkett and Downie 1999). Because we did not survey for restriction site variation in the inverted repeat, estimates of genome size for Scandiceae are not available. Nevertheless, as major insertions, deletions, or other structural mutations were not evident in both LSC and SSC regions, the sizes of these single-copy regions are comparable to those reported for tobacco and many other angiosperm chloroplast genomes (Shinozaki et al. 1986, Downie and Palmer 1992). Within experimental limits, the gene map proposed in Fig. 2 for *Daucus carota* subsp. *sativus* (the common cultivated carrot), *Torilis arvensis* subsp. *arvensis*, and

Scandix pecten-veneris is also the same as that of the tobacco chloroplast genome, as all probes hybridized in a colinear manner (Shinozaki et al. 1986, as modified by Sugiura 1992 and Wolfe et al. 1992). The lack of major deletions in the restriction site maps of all other examined Apiaceae relative to these three taxa and tobacco indicates that they all possess the same complement of genes and introns in the same order.

Utility of cpDNA restriction site data. Phylogenetic analysis of separate cpDNA restriction site and *rps*16 intron sequence data sets reveals relationships that are largely consistent among the 14 accessions common to both studies. Similarly, a concurrent but higher-level study of cpDNA restriction site variation (Plunkett and Downie 1999), including representation from all three subfamilies of Apiaceae and related Araliaceae, yields trees that are congruent to those derived from the comparative analyses of chloroplast *mat*K or *rpo*C1 intron sequences across a similar group of taxa (Plunkett et al. 1996, Downie et al. 1998). While this congruence of relationship supports the robustness of the phylogenetic hypotheses inferred, it is realized that because these restriction site and sequence data are derived from the same chromosome they are inherited as a single linkage group and, hence, should have the same evolutionary history (Doyle 1992). Incongruences among the trees inferred, if they exist, may be due to several factors, such as differences in sampling or heterogeneity of evolutionary rates.

Phylogenetic studies incorporating DNA sequences have largely replaced those based on comparative restriction site mapping, largely a result of the ease of obtaining sequence data both through automated sequencing methods and PCR technology. In spite of the problems inherent in using cpDNA restriction site data for phylogenetic analysis (reviewed in Olmstead and Palmer 1994 and Mishler et al. 1996), the method has potential to yield more phylogenetically informative characters than does comparative DNA sequencing. In this study, as that of Plunkett and Downie (1999), we have found that restriction site analysis provides 3–4 times more variable characters than does DNA sequencing across a comparable array of taxa. Similar results have been obtained by Kim et al. (1992) for Asteraceae. As a consequence, the trees inferred herein using restriction site data are generally more resolved and the clades better supported than those derived from DNA sequences, with both data sets revealing comparable levels of homoplasy across the shortest trees inferred. Moreover, restriction site data appear to be better suited for resolving relationships at infrageneric levels within Apiaceae (such as those within *Daucus* and *Torilis*) than any existing DNA sequence data set. As a consequence of the greater utility of the restriction site data, we are continuing to use these data to further resolve relationships within *Daucus* (Lee and Downie, unpubl. data).

Summary of relationships. Based on phylogenetic analyses of molecular data, Apiaceae tribe Caucalideae (Heywood 1982c) comprises two major lineages, designated as Scandiceae subtribes Daucinae and Torilidinae (Downie et al. 2000). Variously associated with these two subtribes are genera belonging to Heywood's (1971b) Scandiceae (our Scandiceae subtribe Scandicinae), and while evidence from cpDNA points unequivocally to a sister group relationship between Scandicinae and the clade of Daucinae + Torilidinae, the ITS data do not. Therefore, the recognition of three distinct yet closely related groups, as proposed in an earlier investigation (Downie et al. 2000), is maintained. Because the results of our previous ITS study were largely concordant to those inferred herein on the basis of cpDNA evidence, a detailed discussion of phylogenetic relationships has already been presented (Lee and Downie 1999). Further discussion will be limited to those taxa and relationships unique to this study.

Laserpitieae. The incorporation of members of Drude's (1897–1898) Laserpitieae (i.e. *Laser*, *Laserpitium*, *Melanoselinum*, *Monizia*, and *Polylophium*) into Scandiceae subtribe Daucinae is consistent, in part, with the

classification systems of Calestani (1905) and Koso-Poljansky (1916). These systems, relying almost exclusively on anatomical characters of the mericarp, indicated a close relationship between *Laserpitium* and *Daucus* (and other taxa), treating them together in tribe Dauceae or subtribe Daucinae. Additional evidence suggesting that members of Daucinae and Laserpitieae are closely related include the presence of similar morphological characters, such as distinctive secondary ridges and dorsally compressed fruits with single rows of appendages (Tamamschjan 1947). Given that all examined members of Drude's (1897–1898) tribe Laserpitieae fall within subtribe Daucinae, its few remaining members (*Distichoselinum*, *Elaeoselinum*, *Guillonea*, *Margotia*, *Rouya*, *Thapsia*, and *Tornabenea*; Pimenov and Leonov 1993) are deservant of further study. Based on our results (e.g. Fig. 1), however, it is unlikely that Laserpitieae constitutes a monophyletic group within Daucinae.

Scandicinae. Our previous study of Caucalideae ITS sequences (Lee and Downie 1999) included five genera in Scandiceae subtribe Scandicinae (*Anthriscus*, *Kozlovia*, *Osmorhiza*, *Scandix*, and *Myrrhis*); fewer genera were included in each of the two plastid DNA studies presented herein. While monophyly of Scandicinae is supported strongly in all analyses, with bootstrap values of 97–100%, the generic level relationships within this clade differ (likely due to sample size and different genera sampled). For example, results of the *rps*16 intron analysis (Fig. 4) place *Scandix* sister to *Osmorhiza*, whereas the ITS study (Fig. 1) places *Scandix* sister to the clade of *Anthriscus*, *Myrrhis*, *Kozlovia*, and *Osmorhiza*. Moreover, the restriction site analysis (Fig. 3) reveals that *Anthriscus* is not monophyletic. Expanded sampling, incorporating ITS sequence data from all 18 genera (82 accessions) commonly treated in tribe Scandiceae, not only supports monophyly of Scandicinae (upon the exclusion of *Grammosciadium* and *Rhabdosciadium*), but also reveals that *Anthriscus* is indeed monophyletic (Downie et al. 2000).

Comparison to Drude's treatment. Drude (1897–1898) defined Scandiceae on the basis of calcium oxalate (druse) crystals in the parenchyma cells surrounding the carpophore and divided it into two subtribes, Caucalidinae and Scandicinae, according to the shape of the fruit. Both tribe Dauceae and subtribe Caucalidinae were characterized by spinose fruit ridges, with the former allied to tribe Laserpitieae, whose members have fruits without spines but with primary and prominent secondary ridges (that are often extended into wings). The secondary fruit ridges of many Caucalidinae are suppressed or less well developed than those of tribe Dauceae; members of Scandicinae lack both secondary ridges and spines. Drude assumed that the secondary spinose ridges in his presumably divergent Caucalidinae and Dauceae had evolved independently. Our results indicate clearly that Drude's Caucalidinae and Dauceae should be united, and that these spiny-fruited umbellifers constitute a closely related group, a relationship in accordance with the earlier classification systems of Bentham (1867) and Boissier (1872) and the more recent treatment of Heywood (1982c). Our results indicate further that Drude's spineless Laserpitieae allies strongly with our subtribe Daucinae, indicating that the presence of both prominent secondary ridges and strongly dorsally compressed fruits are important synapomorphies defining this clade. Considering Fig. 5C, mericarp spines are inferred to have been lost at least twice independently in Daucinae (i.e. in *Laserpitium hispidum* and the clade of *L. siler*, *Laser trilobum*, and *Polylophium panjutinii*). Additional discussion on the evolution of mericarp morphological and anatomical features in light of the group's inferred evolutionary history is forthcoming (Lee et al., ms. in prep.).

Artedia. The most striking difference between the plastid- and ITS-derived trees is the placement of the monotypic *Artedia*, with affinities to both Daucinae and Torilidinae apparent. This discordance may be the result of several factors, such as the effects of lineage

sorting or convergence, different modes of inheritance, or chloroplast capture through hybridization-introgression events (Doyle 1992, Rieseberg and Soltis 1991). At this point in time we cannot offer an explanation for this phylogenetic incongruence or to state which phylogeny, if any, most accurately reflects organismal relationships. With very few reported cases, interspecific hybridization hasn't been considered an important factor among extant umbellifers (Heywood 1982b), but this may be simply due to the lack of studies specifically set up to identify such an event. With its lateral secondary ridges developed into deeply lobed, scaly, expanded wings, *Artedia* is morphologically anomalous in the group. The fruit of *Artedia* has been considered highly specialized evolutionarily (Al-Attar 1974), yet this specialization is not reflected in many of its other characters, whether these be from flavonoids, volatile oils, stomates, seedlings, or pollen grains (Cerceau-Larrival 1962, Crowden et al. 1969, Harborne and Williams 1972, Williams and Harborne 1972, Guyot et al. 1980). Additional studies are in order.

Glochidotheca, Astrodaucus, and Szovitsia. The alliance among *Glochidotheca* [syn. *Turgeniopsis foeniculacea* (Fenzl) Boiss.], *Astrodaucus*, and *Szovitsia*, inferred on the basis of separate analysis of ITS sequences (Figs. 1 and 5A) or combined analysis of ITS and *rps*16 intron data (Fig. 5C), is surprising given the remarkable differences seen in their mericarp anatomy and in the greatly different shapes of their secondary appendages. We have observed, however, that this group can be characterized by two nonmolecular synapomorphies: the presence of curved primary hairs and the presence of peg-like projections on the surface of their secondary appendages. In all trees presented, resolution within the Torilidinae clade is poor, with many weakly supported nodes. Additional molecular data are necessary to confirm both the monophyly of *Glochidotheca*, *Astrodaucus*, and *Szovitsia* (as suggested by these morphological characters) and to better assess their placement within the subtribe.

Ferula. The placement of three species of *Ferula* alongside Daucinae and Torilidinae – a relationship proposed initially on the basis of neighbour-joining analysis of ITS data (Valiejo-Roman et al. 1998), confirmed through independent ITS sequencing (Downie, unpubl. data), and now supported using *rps*16 intron data – is intriguing. *Ferula* is a large, morphologically variable genus of some 170 species (Pimenov and Leonov 1993), considered allied to *Peucedanum* (Pimenov 1982). Indeed, our previous molecular phylogenetic investigations place *F. assa-foetida* L. and *F. communis* L. alongside *Peucedanum* and related taxa, well away from tribe Scandiceae (Downie et al. 1998, Katz-Downie et al. 1999). The monophyly of *Ferula*, supported most recently by Shneyer et al. (1995), is now brought into question. We are at a loss to explain the association between the three *Ferula* taxa included in this study and Scandiceae. No obvious morphological or anatomical characters support this relationship, nor has such an association been considered in any taxonomic work to date of which we are aware. Furthermore, both nuclear- and chloroplast-derived data point to the same relationship. Additional studies are necessary before we can accept the inclusion of *Ferula* within Scandiceae.

Chaetosciadium. In each of our analyses, the monophyly of *Torilis* is strongly supported if its boundary is expanded to include the monotypic *Chaetosciadium*. *Chaetosciadium* is characterized by mericarps that are irregularly covered with fine, long bristly hairs and with obsolete secondary ridges. These unique features led Calestani (1905) to erect the monotypic subtribe Chaetosciadieae of tribe Ligusticeae. *Chaetosciadium* and *Torilis*, however, share similar flavone distribution patterns (Crowden et al. 1969, Harborne and Williams 1972) and hairs on their primary fruit ridges (Heywood and Dakshini 1971). They also share a base chromosome number of six, a number rare in Apiaceae subfamily Apioideae where $\times = 11$ prevails (Constance et al. 1971, Moore 1971). McNeill et al. (1969) revealed the strong similarity between *Chae-*

tosciadium and *Torilis* using numerical analyses of primarily fruit, leaf, and inflorescence characters. Zohary (1972) was of the opinion that *Chaetosciadium* should probably be included within the genus *Torilis*, and on the basis of our molecular results we concur. A name for such a combination has already been published, *Torilis trichosperma* (L.) Spreng. (Umb. Spec. 142).

Daucus. Within *Daucus* the number of species varies considerably, with 21 species in seven sections recognized by Heywood (1982c). A similar situation occurs within the *D. carota* complex, with the variously delimited subspecies extremely difficult to circumscribe unambiguously (Heywood 1968b, Small 1978, St. Pierre et al. 1990). In our ITS study (Lee and Downie 1999; Fig. 1), nine species from all of Heywood's seven sections were represented, including four subspecies of *D. carota* and two subspecies of *D. bicolor*. In the cpDNA restriction site study (Fig. 3), six species were considered, including six subspecies of *D. carota*. The results of these two studies were similar in showing: (1) a close relationship between the only two New World species within the genus, *D. pusillus* and *D. montanus*; (2) an affinity between these New World taxa and the eastern tropical African genus *Agrocharis*; (3) close relationships between *D. carota* and *D. maximus*, and between *D. aureus* and *D. muricatus*; and (4) the inclusion of *Pseudorlaya pumila* within the *Daucus* clade. In our ITS study, *Pachyctenium mirabile* arises within the *Daucus* group, *D. crinitis* is allied closely with *D. aureus* and *D. muricatus*, and *D. bicolor*, *D. pusillus*, *D. montanus*, and *D. durieua* form a strongly supported clade. *D. carota* subsp. *sativus*, the domestic garden carrot, is allied strongly with *D. carota* subsp. *carota*.

Daucus maximus, recognized initially as a subspecies of *D. carota* (*D. carota* subsp. *maximus* (Desf.) Ball), was treated as a distinct species by Heywood and Saenz de Rivas (1974) and Heywood (1982c). Our restriction site study (Fig. 3) clearly positions this taxon within *D. carota* and, as such, we suggest that its subspecific status be resumed. If this is done, *D. carota* is monophyletic. On the basis of morphological and chemical data, *Pseudorlaya pumila* is very similar to *Daucus* (Harborne et al. 1969, Heywood and Dakshini 1971, Williams and Harborne 1972, Lee et al., unpubl. data), providing additional evidence for the transfer of *P. pumila* into *Daucus*. We wish, however, to examine the two remaining species of *Pseudorlaya* and additional material of *P. pumila* before nomenclatural changes are made. The position of the monotypic *Pachyctenium* has yet to be considered using cpDNA data, although on the basis of ITS sequences it is also clearly positioned within *Daucus*. The ITS phylogeny (Fig. 1) indicates a major dichotomy within *Daucus*, with *D. bicolor*, *D. pusillus*, *D. montanus*, and *D. durieua* forming one clade and all remaining examined *Daucus* species the other; a similar relationship is seen in the restriction site tree (Fig. 3), but with fewer taxa represented. The close relationship between *Agrocharis*, the only genus of Caucalideae endemic to tropical Africa (Heywood 1982c), and *Daucus* reflects the similarities observed in their fruit anatomy and morphology (Jury 1986, Lee et al., unpubl. data). Indeed, *Agrocharis* was first described as a species of *Daucus* (reviewed in Heywood 1973) and its return to the latter needs to be investigated further. Clearly, both ITS sequencing and the mapping of cpDNA restriction sites are useful in resolving interspecific relationships within *Daucus*; additional phylogenetic studies are currently being carried out in order to address the aforementioned taxonomic issues.

Conclusions

Phylogenetic analyses of cpDNA restriction site polymorphisms and *rps*16 intron sequences from representatives of Heywood's (1982c) tribe Caucalideae and related taxa yield trees largely congruent to each other and to hypotheses of relationship based on nuclear rDNA ITS sequences (Lee and Downie 1999). Three major lineages of equivocal

relationship are inferred, coinciding with the previously delimited Scandiceae Spreng. subtribes Daucinae Dumort., Torilidinae Dumort., and Scandicinae Tausch (Downie et al. 2000). Subtribes Daucinae and Torilidinae coincide with Heywood's circumscription of Caucalideae, but with the inclusion of Drude's (1897–1898) tribe Laserpitieae in the former and the transfer of *Kozlovia* to Scandicinae. Subtribe Scandicinae coincides roughly with Heywood's (1971b) circumscription of Scandiceae; further details of relationships within Scandicinae are presented in Downie et al. (2000). *Aphanopleura* and *Psammogeton*, treated previously in Caucalideae (Heywood 1982c), are distantly related to the group (Katz-Downie et al. 1999). The only major difference between the ITS- and chloroplast-derived phylogenies is the position of the morphologically anomalous *Artedia*, affiliated weakly with either the Daucinae or Torilidinae clades. Additional data are necessary to resolve the phylogenetic placement of this genus, as well as to clarify relationships within subtribe Torilidinae, particularly among the genera *Astrodaucus*, *Glochidotheca*, and *Szovitsia*. A most unusual and unexplainable find is the close relationship among three species of *Ferula* and Scandiceae in the *rps*16 intron-based analysis, a relationship reported elsewhere on the basis of nuclear rDNA ITS data (Valiejo-Roman et al. 1998). Extended molecular, morphological, and cytological studies are underway to resolve and explain this intriguing relationship. Detailed morphological and anatomical investigations are also underway, and when completed will provide insight into character evolution, including the identification of morphological synapomorphies supporting each of the major clades identified herein on the basis of molecular data.

This study demonstrates further the phylogenetic utility of chloroplast *rps*16 intron sequences, a region which has seen very little use in molecular phylogenetic studies to date. However, while both cpDNA restriction site and intron data sets suggested similar relationships for those taxa in common, greater resolution and higher branch support was achieved using restriction site data. Additional mapping studies, incorporating data from both chloroplast and nuclear genomes, and ITS sequencing are currently being pursued in order to clarify relationships within the polymorphic *Daucus* and their close relatives, such as *Pseudorlaya*, *Pachyctenium*, and *Agrocharis*.

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