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A Molecular Phylogeny of Apiaceae Tribe Caucalideae and Related Taxa: Inferences Based on ITS Sequence Data

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ABSTRACT. Since the tribe Caucalideae was recognized by Bentham and later Boissier for those species of Apiaceae (Umbelliferae) with spines, hooks, tubercles or bristly hairs on the primary and/or secondary ridges of their fruits, there has been considerable disagreement as to its proper circumscription, the relationships among its members, and the delimitation of certain genera. A recent checklist of the group recognized 68 species in 21 genera; a previous molecular systematic study, however, excluded *Aphanopleura* and *Psammogeton* from the tribe. Phylogenetic relationships among all but one of the 19 remaining genera (material from the rare, monotypic genus *Angoseseli* was not available for examination) and representatives from putatively allied tribes Scandiceae, Laserpitieae, Apieae, and Smyrnieae were inferred from nucleotide sequence variation in the internal transcribed spacer regions of 18S–26S nuclear ribosomal DNA. In all, 29 genera representing 58 taxa were examined. Phylogenies estimated using maximum parsimony, maximum likelihood, and neighbor-joining methods give trees of essentially similar topology, and reveal three major lineages of equivocal relationship: (1) *Agrocharis, Ammodaucus, Artedia, Cuminum, Daucus, Laser, Laserpitium, Orlaya, Polylophium, Pseudorlaya,* and *Pachyctenium*; (2) *Astrodaucus, Caucalis, Chaetosciadium, Glochidotheca, Lisaea, Szovitsia, Torilis, Turgenia,* and *Yabea*; and (3) *Anthriscus, Kozlovia, Myrrhis, Osmorhiza,* and *Scandix*. These groups are provisionally named the *Daucus, Torilis,* and *Scandix* subclades, respectively, of a previously delimited *Daucus* clade. The first subclade contains representatives of Drude's tribe Laserpitieae, whereas the third subclade coincides with Heywood's tribe Scandiceae. Based on those species included in the study, the genera *Daucus, Laserpitium,* and *Torilis* are each not monophyletic.

Members of tribe Caucalideae Spreng. (Apiaceae; Umbelliferae) are distributed throughout Europe, the Mediterranean region, and southwestern and central Asia, with a few outlying members in North America. Of the 21 genera and 68 species listed in the most recent checklist for the tribe (V. Heywood and S. Jury in Heywood 1982c; Table 1), *Daucus* is the largest genus with 21 species, followed by *Torilis* with 10 species. *Daucus* is also by far the most economically important member of the tribe, if not the entire family. Three species of Caucalideae are native to the New World: *Daucus montanus* Humb. & Bonpl., *D. pusillus* Michx., and *Yabea microcarpa* (Hook. & Arn.) Koso-Pol.

Classification of Apiaceae has been based largely on anatomical and morphological features of the mature fruit (Heywood and Dakshini 1971; Heywood 1982b). In most umbellifers, the dry schizocarp splits down a commissure into two one-seeded mericarps which are held together by a bifurcate carpophore. The fruit may be compressed laterally, at right angles to the commissural plane, or dorsally, parallel to the commissural plane, with varying degrees of compression evident. There are five primary, longitudinal ridges on the surface of each mericarp which contain the vascular bundles; secondary ridges, if present, occur in the valleculae, alternating with the primary ridges. Both ridge types vary considerably in their degree of development and prominence. In tribe Caucalideae, the vittae (oil ducts) usually occur in the valleculae, beneath the four secondary (vallecular) ridges.

Tribe Caucalideae, as described by Bentham (1867) and Boissier (1872), contains practically all of those species of Apiaceae that have spines, hooks, tubercles or bristly hairs on the primary and/or secondary ridges of their fruits. Uniquely in this group, the secondary ridges are often more strongly developed than the primary. Drude (1897–1898), in the most widely used monographic revision of the family, redistributed these spiny-fruited plants between his divergent Scandiceae subtribe Caucalidinae and his tribe Dauceae. Drude believed that members of tribe Dauceae, such as *Daucus* with spines on its secondary fruit ridges, were allied to plants in his tribe Laserpitieae (e.g., *Laserpitium, Polylophium*), whose members have fruits without spines but with primary and prominent secondary ridges. Based on the shared possession of calcium oxalate crystals in the parenchyma cells surround-

TABLE 1. V. Heywood and S. Jury's (in Heywood 1982c) checklist of the 21 genera and 68 species in Apiaceae tribe Caucalideae. *Glochidotheca* Fenzl replaces *Turgeniopsis* Boiss. based on Pimenov and Leonov (1993).

Genus	No. of species
Agrocharis Hochst.	4
Ammodaucus Coss. & Dur.	1
Angoseseli Chiov.	1
Aphanopleura Boiss.	5
Artedia L.	1
Astrodaucus Drude	3
Caucalis L.	1
Chaetosciadium Boiss.	1
Cuminum L.	3
Daucus L.	21
Glochidotheca Fenzl	1
Kozlovia Lipsky	1
Lisaea Boiss.	3
Orlaya Hoffm.	3
Pachyctenium Maire & Pamp.	1
Psammogeton Edgew.	1
Pseudorlaya Murb.	3
Szovitsia Fisch. & C. A. Mey.	1
Torilis Adans.	10
Turgenia Hoffm.	2
Yabea Koso-Pol.	1

ing the carpophore, his genera of Scandiceae subtribe Caucalidinae (*Ammiopsis, Astrodaucus, Caucalis, Chaetosciadium, Glochidotheca, Lisaea, Orlaya, Psammogeton,* and *Torilis*) were linked to those in his Scandiceae subtribe Scandicinae (e.g., *Anthriscus, Myrrhis, Osmorhiza,* and *Scandix*), the latter lacking both secondary ridges and spines. Drude assumed that the secondary spinose ridges characteristic of many Caucalidinae had evolved independently from those in his Dauceae. Calestani (1905) subsequently placed *Chaetosciadium* into its own subtribe in tribe Ligusticeae due to its unique long bristly fruit hairs. He also placed *Laserpitium* into tribe Dauceae, alongside *Daucus, Orlaya,* and *Artedia.* Koso-Poljansky (1916, 1917), relying primarily on anatomical characters of the mericarp, included many representatives of Drude's Laserpitieae in his Careae subtribe Daucinae, the latter subtribe placed well away from his Caucalideae. Koso-Poljansky treated the spiny-fruited plants in three major taxa: Scandiceae subtribe Scandicinae, Caucalideae, and Careae subtribe Daucinae. Cerceau-Larrival (1962, 1965), using evidence from pollen and seedling morphology, distributed these spiny-fruited plants into nine tribes, many of which were invalidly published: Artedieae, Caucalideae, Cumineae, Dauceae, Exoacantheae, Orlayeae, Torilineae, Turgenieae, and Turgeniopsideae. Needless to say, the spiny-fruited umbellifers have had a complicated taxonomic history.

As part of the increasing interest in the systematics of the Apiaceae during the 1960's and early 1970's, an international symposium on "The Biology and Chemistry of the Umbelliferae" was held at the University of Reading, England, under the auspices of the Linnean Society (Heywood 1971a). The papers presented at this symposium were multidisciplinary, bringing together for the first time such diverse fields as comparative anatomy, phytochemistry, palynology, developmental biology, and cytology (and, as such, served as the stimulus for formal cooperative systematic research in other plant groups, such as the Cruciferae, Compositae, Solanaceae, and Leguminosae; Heywood 1982a). As a consequence of the Reading symposium, a cooperative research program was established, centering mainly on tribe Caucalideae (Heywood 1982a). The research undertaken continued to be multidisciplinary, incorporating results from the then growing fields of scanning electron microscopy, biochemical systematics, and numerical taxonomy. A second symposium on the family was held in Perpignan, France in 1977 (Cauwet-Marc and Carbonnier 1982), where the progress made by this research program was discussed. With respect to the treatments of Bentham (1867), Boissier (1872), and Drude (1897–1898), major changes were made in the content and circumscription of tribe Caucalideae, with a total of 21 genera and 68 species recognized (Table 1; V. Heywood and S. Jury in Heywood 1982c). Drude's Scandiceae subtribe Scandicinae was now regarded at the tribal level, Scandiceae Spreng. (Heywood 1971b). With few exceptions (Heywood 1986; Jury 1986), there has been very little systematic work done on the spiny-fruited umbellifers as a whole since the Perpignan symposium. Despite the wealth of data available from a variety of sources, and the multidisciplinary approaches used to analyze these data, fundamental disagreements still persist regarding the proper circumscription of tribe Caucalideae, the relationships among its members, and the delimitation of certain genera.

Phylogenetic analyses of chloroplast *rpoC1, rpl16* or *rps16* intron sequences (Downie et al. 1996, 1998, and unpubl. data) or the internal transcribed spacers of nuclear rDNA (Downie and Katz-Downie 1996; Downie et al. 1998; Katz-Downie et al. 1999) reveal a close relationship between Heywood's (1971b, 1982c) tribes Caucalideae and Scandiceae. Based on these molecular studies and limited sampling, tribe Scandiceae appears to be monophyletic. The relationship, however, between these two tribes is far from clear. While some cladograms showed that Caucalideae and Scandiceae are monophyletic sister taxa, others indicated that Caucalideae is paraphyletic with Scandiceae nested within. In contrast, phylogenetic analysis of chloroplast DNA (cpDNA) *matK* sequences suggested a paraphyletic Scandiceae with included Caucalideae (Plunkett et al. 1996). Many of these molecular studies indicated a close relationship between these taxa and members of Drude's tribe Laserpitieae.

While the aforementioned molecular systematic studies were useful in demonstrating the close relationship among Apiaceae tribes Caucalideae, Scandiceae, and Laserpitieae, the intergeneric relationships within Caucalideae could not be ascertained because the number of spiny-fruited taxa examined in each study was too few. Here we present the results of an expanded investigation of intergeneric relationships in tribe Caucalideae using nuclear rDNA internal transcribed spacer (ITS) sequence data. The utility of this region in phylogenetic estimation has been reviewed by Baldwin et al. (1995). Our main objectives are: (1) to test the monophyly of Heywood's (1982c) tribe Caucalideae, particularly with regard to its relationship to tribes Scandiceae and Laserpitieae; and (2) to formulate hypotheses concerning phylogenetic relationships within Caucalideae, including the identification of major clades. The relationships inferred within Caucalideae will also be compared to those implicit in the classification system of Drude (1897–1898). This is the first of several papers reporting our results on Caucalideae phylogeny. Subsequent papers, currently in preparation, deal with cladistic analyses of cpDNA restriction sites, chloroplast *rps16* intron sequences, and morphological data.

Materials and Methods

Plant Accessions. Forty-three accessions representing 18 of the 21 genera recognized in Caucalideae by V. Heywood and S. Jury (Heywood 1982c; Table 1) were examined for nuclear ribosomal DNA ITS sequence variation (Table 2). *Psammogeton* and *Aphanopleura*, treated as members of Caucalideae by Heywood (1982c), have been recently excluded from the group (Pimenov and Leonov 1993; Katz-Downie et al. 1999). Material of the rare, monotypic genus *Angoseseli* from Angola was also excluded, due to the difficulty in obtaining fresh or adequate herbarium material for analysis. Of the 21 species recognized in *Daucus* (Heywood 1982c), nine species representing all seven sections were sampled. Fifteen accessions from putatively allied tribes Scandiceae, Laserpitieae, Smyrnieae, and Apieae were also considered (Table 2), culminating in a matrix of 58 accessions. Complete ITS1 and ITS2 sequences for 37 taxa are reported here for the first time; sequences for the remaining 21 taxa were published as a result of two earlier studies on Apiaceae phylogeny (Downie et al. 1998; Katz-Downie et al. 1999).

Previous ITS studies have revealed a close association between Caucalideae (and allied Scandiceae and Laserpitieae) and a weakly supported clade consisting of *Lecokia cretica*, *Smyrnium olusatrum*, *Ligusticum scoticum*, and several species of *Aciphylla* (Downie et al. 1998; Katz-Downie et al. 1999). Therefore, all trees computed in this study were rooted with these outgroup taxa (Table 2). Using any of these outgroups individually or in various combinations did not affect the resulting ingroup tree topology.

Experimental Strategy. Leaf material for DNA extraction was either taken from flower- and fruit-bearing plants propagated from seed in the greenhouse, sent to us as gifts, or obtained from herbarium specimens (Table 2). In several instances, extracted DNAs were supplied to us directly. All plants were identified using published keys and comparison to herbarium specimens. Voucher specimens for plants propagated in the Plant Science Laboratory greenhouses at the University of Illinois are deposited in the University's herbarium (ILL).

Total genomic DNA was extracted from either fresh leaves or herbarium material using the modified CTAB procedure of Doyle and Doyle (1987), and further purified by centrifugation to equilibrium in cesium chloride/ethidium bromide gradients. Double-stranded DNAs of the complete ITS region in each genomic DNA were amplified by the PCR (polymerase chain reaction) technique using primers "ITS5" and "ITS4" in an equimolar ratio (White et al. 1990). Details of the PCR amplifications are provided in Downie and Katz-Downie (1996). For some DNAs extracted from herbarium material, optimum amplification was achieved when the template DNA was diluted 1:100 or when the concentration of $MgCl_2$ was increased from 1.5 mmol/L to 3.0 mmol/L. Successful PCR amplifications resulted in a single DNA band correspond-

TABLE 2. Accessions of Apiaceae tribe Caucalideae and related taxa examined for nuclear rDNA ITS sequence variation. These data have been deposited with GenBank as separate ITS1 and ITS2 sequences; GenBank accession numbers for each spacer region are provided in brackets. Source information for previously published ITS data is presented in Downie et al. (1998) or Katz-Downie et al. (1999). Circumscriptions of tribes Caucalideae and Scandiceae are based on V. Heywood and S. Jury (in Heywood 1982c) and Heywood (1971b), respectively. Tribes Laserpitieae, Smyrnieae, and Apieae follow Drude (1897–1898). Herbarium acronyms according to Holmgren et al. (1990). UIUC = University of Illinois at Urbana-Champaign.

Taxon	Source and voucher information
Tribe Caucalideae	
Agrocharis incognita (C. Norman) Heywood & Jury	Kenya, Nairobi, DNA supplied by E. Knox (coll. no. 2578) [AF077793, AF077108]
Agrocharis melanantha Hochst.	Kenya, Nairobi, DNA supplied by E. Knox (coll. no. 2579) [AF077794, AF077109]
Agrocharis pedunculata (Baker f.) Heywood & Jury	Malawi, Limbe, Mpingwe Hill, *Hillard & Burtt 4131* (E) [AF077792, AF077107]
Ammodaucus leucotrichus (Coss. & Dur.) Coss. & Dur.	Spain, Canary Islands, Tenerife, *Santos-Guerra s.n.* (ORT) [AF077795, AF077110]
Artedia squamata L.	Turkey, Tarsus, Namrun Plateau, *Kasapligil 6483* (UC) [AF077799, AF077114]
Astrodaucus orientalis (L.) Drude	Iran, cult. UIUC from seeds obtained from Research Institute of Forests and Rangelands, Iran, *Lee 43* (ILL) [AF077807, AF077122]
Caucalis platycarpos L.	Downie et al. 1998 [U78364, U78424]
Chaetosciadium trichospermum (L.) Boiss.	Downie et al. 1998 [U78363, U78423]
Cuminum cyminum L.	Downie et al. 1998 [U78362, U78422]
Cuminum setifolium (Boiss.) Koso-Pol.	Afghanistan, Kandahar, Ispoli, *Hedge et al. 7083* (E) [AF077796, AF077111]
Daucus aureus Desf.	cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Lee 57* (ILL) [AF077784, AF077099]
Daucus bicolor Sibth. & Sm. subsp. *bicolor*	Israel, Judean Mtns., Har Herzel, cult. UIUC from seeds obtained from O. Cohen, *Lee 270* (ILL) [AF077791, AF077106]
Daucus bicolor subsp. *broteri* (Ten.) Okeke	Lebanon, cult. UIUC from seeds obtained from USDA acc. no. 286611, *Lee 185* (ILL) [AF077783, AF077098]
Daucus carota L. subsp. *carota*	Kazakhstan, cult. UIUC from seeds obtained from USDA acc. no. 478882, *Lee 167* (ILL) [AF077779, AF077094]
Daucus carota subsp. *gummifer* Hook. f.	cult. UIUC from seeds obtained from Jardin botanique de Caen, France, *Lee 47* (ILL) [AF077782, AF077097]
Daucus carota subsp. *halophilus* (Brot.) Okeke	cult. UIUC from seeds obtained from J.-P. Reduron, Mulhouse, France, *Lee 81* (ILL) [AF077781, AF077096]
Daucus carota subsp. *sativus* (Hoffm.) Arcang.	cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Lee 73* (ILL) [AF077780, AF077095]
Daucus crinitis Desf.	cult. UIUC from seeds obtained from Jardin Botaniques Lisboa, Portugal, *Lee 49* (ILL) [AF077786, AF077101]
Daucus durieua Lange	Israel, Samarian Desert near Sartaba, cult. UIUC from seeds obtained from O. Cohen, *Lee 271* (ILL) [AF077790, AF077105]
Daucus maximus Desf.	cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Lee 64* (ILL) [AF077778, AF077093]
Daucus montanus Humb. & Bonpl.	Argentina, cult. Botanical Garden of the University of California, Berkeley 94.0563 [AF077789, AF077104]
Daucus muricatus L.	cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany, *Lee 36* (ILL) [AF077785, AF077100]

TABLE 2. Continued.

Taxon	Source and voucher information
Daucus pusillus Michx.	cult. Botanical Garden of the University of California, Berkeley 92.0891 [AF077788, AF077103]
Glochidotheca foeniculacea Fenzl	Turkey, Adana, *Alava 6698* (UC), DNA supplied by M. Chase (coll. no. 2922) [AF077808, AF077123]
Kozlovia paleacea (Regel & Schmalh.) Lipsky	Afghanistan, Baghlan, *Podlech 21615* (NY) [AF077814, AF077129]
Lisaea heterocarpa (DC.) Boiss.	Iran, Durud, Luristan, *Koelz 15501a* (US) [AF077813, AF077128]
Lisaea papyracea Boiss.	Armenia, *Gambarian s.n.* (UC) [AF077812, AF077127]
Lisaea strigosa (Banks & Sol.) Eig	Azerbaijan, Baku to Marand, *Lamond 3884a* (E) [AF077811, AF077126]
Orlaya daucoides (L.) Greuter	cult. UIUC from seeds obtained from Hungarian Academy of Sciences, Vácrátót, *Lee 7* (ILL) [AF077797, AF077113]
Orlaya daucorlaya Murb.	Yugoslavia, Macedonia, Kuceviste, *Edmonston 27* (E) [AF077798, AF077113]
Orlaya grandiflora (L.) Hoffm.	Downie et al. 1998 [U30524, U30525]
Pachyctenium mirabile Maire & Pamp.	Libya, E Shahat, Cyrene, *Davis 50249* (E) [AF077787, AF077102]
Pseudorlaya pumila (L.) Grande	Downie et al. 1998 [U30522, U30523]
Szovitsia callicarpa Fisch. & C. A. Mey.	Azerbaijan, Moghan, *Lamond 3195* (E) [AF077809, AF077124]
Torilis arvensis (Huds.) Link subsp. *arvensis*	England, Buckinghamshire, Amersham, *Southam s.n.* (RNG) [AF077800, AF077115]
Torilis arvensis subsp. *purpurea* (Ten.) Hayek	Morocco, Col du Nador, *Jury & Wilson s.n.* (RNG) [AF077801, AF077116]
Torilis elongata (Hoffm. & Link) Samp.	Morocco, Col du Nador, *Jury & Wilson s.n.* (RNG) [AF077802, AF077117]
Torilis leptophylla (L.) Rchb. f.	Asia Minor; cult UIUC from seeds obtained from *Anonymous* (K), *Lee 107* (ILL) [AF077804, AF077119]
Torilis nodosa (L.) Gaertn.	Downie et al. 1998 [U30534, U30535]
Torilis scabra (Thunb.) DC.	Japan, Okinawa, *Beauchamp 1217* (US) [AF077805, AF077120]
Torilis tenella (Delile) Rchb. f.	Jordan, Ajlun, Schtafeenah, *Lahham & El-Oglah 1* (Yarmouk University Herbarium, Jordan) [AF077803, AF077118]
Turgenia latifolia (L.) Hoffm.	cult. UIUC from seeds obtained from J.-P. Reduron, Mulhouse, France; *Lee 82* (ILL) [AF077810, AF077125]
Yabea microcarpa (Hook. & Arn.) Koso-Pol.	USA, Arizona, Pima Co., *Holmgren 6772* (WTU) [AF077806, AF0771221]
Tribe Scandiceae	
Anthriscus caucalis M. Bieb.	Downie et al. 1998 [U79601, U79602]
Anthriscus cerefolium (L.) Hoffm.	Downie et al. 1998 [U30532, U30533]
Myrrhis odorata (L.) Scop.	Downie et al. 1998 [U30530, U30531]
Osmorhiza longistylis (Torr.) DC.	Downie et al. 1998 [U79617, U79618]
Scandix balansae Reut. ex Boiss.	Downie et al. 1998 [U79621, U79622]
Scandix pecten-veneris L.	Downie et al. 1998 [U30538, U30539]
Tribe Laserpitieae	
Laser trilobum (L.) Borkh.	Katz-Downie et al. 1999 [AF008644, AF009123]
Laserpitium hispidum M. Bieb.	Downie et al. 1998 [U78361, U78421]
Laserpitium siler L.	Downie et al. 1998 [U30528, U30529]
Polylophium panjutinii Manden. & Schischk.	Katz-Downie et al. 1999 [AF08645, AF009124]
Tribe Smyrnieae	
Lecokia cretica (Lam.) DC.	Downie et al. 1998 [U78358, U78418]
Smyrnium olusatrum L.	Downie et al. 1998 [U30594, U30595]
Tribe Apieae	
Aciphylla subflabellata W. R. B. Oliv.	Katz-Downie et al. 1999 [AF008646, AF009125]
Aciphylla squarrosa J. R. Forst. & G. Forst.	Downie et al. 1998 [U79595, U79596]
Ligusticum scoticum L.	Downie et al. 1998 [U78357, U78417]

ing to approximately 700 bp in size. Each amplified DNA fragment was electrophoresed in a 1% agarose gel, visualized with ethidium bromide, and then excised under low wavelength UV light with a sterilized scalpel. To isolate the PCR product from the agarose, the gel plugs were melted at 60°C for approximately 10 minutes and the DNA recovered and purified by using the Elu-Quik DNA Purification Kit (Schleicher & Schuell, Keene, NH). Sequencing was done manually using the dideoxy chain termination method using Sequenase (version 2.0; United States Biochemical Corp., Cleveland, OH) with α-^{35}S-dATP as the labeling agent. The sequencing protocol is further detailed in Downie and Katz-Downie (1996). Forward primers "ITS3" and "ITS5" and reverse primers "ITS2" and "ITS4" (White et al. 1990) were each used in the sequencing of each template DNA.

Phylogenetic Analysis. Only the ITS1 and ITS2 regions were included in the analysis since sequence data for the intervening 5.8S subunit were incomplete for many taxa, and those data that were available were not sufficiently variable to warrant additional sequencing. Base determination was complete and unambiguous in all cases; there were no data matrix cells scored by us as missing data. DNA sequences were aligned using CLUSTAL V (Higgins et al. 1992), adjusted manually where necessary, and imported into PAUP (version 3.1.1; Swofford 1993). Only those positions that were in obvious alignment were used in the distance calculations and phylogenetic analyses. Pairwise nucleotide differences of unambiguously aligned positions were determined using the distance matrix option in PAUP. In the phylogenetic analysis, all gaps were treated as missing data. Transition/transversion (Ts/Tv) ratios were calculated using MacClade (version 3.0; Maddison and Maddison 1992) across all maximally parsimonious trees obtained.

The ITS data were analyzed initially using maximum parsimony (MP; PAUP). All heuristic searches were conducted with 100 random addition replicates and tree bisection-reconnection branch swapping. The options mulpars, steepest descent, collapse, and acctran optimization were selected. To assess the relative support for each clade, bootstrap values (Felsenstein 1985) were calculated from 100 replicate analyses using the heuristic search strategy and simple addition sequence of the taxa. Decay analyses (Bremer 1988) with tree lengths up to two steps greater than those of the most parsimonious trees were conducted until PAUP ran out of tree storage memory. The amount of phylogenetic information in the MP analysis was estimated using the consistency (CI; Kluge and Farris 1969) and retention (RI; Farris 1989) indices. In order to assess the distribution of insertion and deletion events (indels) against a phylogeny constructed using only nucleotide substitutions, each indel was optimized visually onto one of the resultant minimal length trees.

Distance trees were constructed using the neighbor-joining (NJ) method (Saitou and Nei 1987), implemented using the NEIGHBOR program in Felsenstein's (1993) PHYLIP (version 3.572). Distance matrices were calculated using the DNADIST program of PHYLIP, and the numbers of nucleotide substitutions were estimated using Kimura's (1980) two parameter method. Transitions were weighted relative to transversions, with a Ts/Tv rate ratio of 1.6 inferred from the MP analysis used to construct the neighbor-joining tree. Rate ratios of 1.0 and 2.0 were also used. A bootstrap analysis of the data was done using 100 resampled data sets generated using the SEQBOOT program prior to calculating the distance matrices and neighbor-joining trees. PHYLIP's CONSENSE program was then implemented in order to construct a strict consensus tree.

The maximum likelihood (ML) method was also applied to these ITS data using the program fastDNAml (version 1.0.6; Olsen et al. 1994), based on the procedures of Felsenstein (1981). A maximum likelihood tree was inferred using a Ts/Tv rate ratio of 1.6, 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. Calculations of bootstrap support were computationally prohibitive and were not done.

RESULTS

Sequence Analysis. Alignment of all 58 complete ITS1 and ITS2 sequences, representing all genera of Heywood's (1982c) tribe Caucalideae except three (*Angoseseli, Psammogeton,* and *Aphanopleura*) and members from tribes Scandiceae, Laserpitieae, Apieae, and Smyrnieae, resulted in a matrix of 475 nucleotide positions. Characteristics of these aligned ITS1 and ITS2 sequences, separately and combined, are presented in Table 3. On average, the ITS1 region (at 217.5 bp in size) is slightly shorter than the ITS2 region (at 221.4 bp). Overall length variation across all 58 accessions and both spacer

TABLE 3. Sequence characteristics of the two internal transcribed spacer regions, separately and combined, in 58 accessions of Apiaceae tribe Caucalideae and related taxa.

Sequence characteristic	ITS1	ITS2	Combined (ITS1 & ITS2)
Length variation (bp)	204–221	215–226	427–445
Length mean (bp)	217.5	221.4	438.9
Sequence divergence (range in %)	0–29.9	0–31.5	0–29.4
No. total aligned positions	241	234	475
No. aligned positions excluded (and %)	48 (19.9)	18 (7.7)	66 (13.9)
No. aligned positions constant (and %)	52 (27.0)	58 (26.9)	110 (26.9)
No. aligned positions informative (and %)	117 (60.6)	124 (57.4)	241 (58.9)
No. aligned positions autapomorphic (and %)	24 (12.4)	34 (15.7)	58 (14.2)
No. unambiguous alignment gaps	12	22	34

regions ranged between 427 and 445 bp; these sizes are comparable to those values reported for other Apiaceae (Downie et al. 1998; Katz-Downie et al. 1999). Of the 475 initial alignment positions, 48 positions from ITS1 and 18 positions from ITS2 were deleted due to alignment ambiguities. In total, 66 positions (approximately 14% of the entire matrix) were excluded from subsequent analyses. Of the remaining 409 unambiguously aligned positions, 241 (58.9%) were potentially parsimony informative, 110 (26.9%) were constant, and 58 (14.2%) were autapomorphic. Thirty-four unambiguous gaps were required to optimize alignment of the 58 ITS1 and ITS2 sequences. Twenty-five of these gaps were 1 bp in length, five were 2 bp in length, one was 3 bp in length, one was 4 bp in length, one was 7 bp in length, and one was 8 bp in length. These gaps were more common in the ITS2 region (22 gaps) than in the ITS1 region (12 gaps). Of these 34 unambiguous gaps, 17 were potentially informative for parsimony analysis. No evidence of obvious ITS length variants, representing multiple rDNA repeat types, in any of the accessions analyzed was observed. Sequence polymorphisms at individual nucleotide sites within individual samples were rare. Those few sites which did exhibit polymorphisms were in regions highly G+C rich and were likely caused by compressions. These sites were in those regions of the alignment excluded from the analysis.

TABLE 4. Range in pairwise ITS sequence divergence values among those genera included in the study represented by more than one accession. Asterisks denote those genera that are not monophyletic based on the results of the phylogenetic analyses presented herein. *Anthriscus* is monophyletic in the MP and ML analyses but not in the NJ analysis.

Genus	No. of accessions examined	Sequence divergence (%)
Agrocharis	3	0.8–1.0
*Anthriscus**	2	11.4
Cuminum	2	0.8
*Daucus**	13	0–13.5
*Laserpitium**	2	12.0
Lisaea	3	0.3–0.5
Orlaya	3	2.3–5.9
Scandix	2	7.9
*Torilis**	7	0.8–6.9

In direct pairwise comparisons of unambiguous positions among all 58 accessions, sequence divergence values ranged from identity to 29.9% of nucleotides in ITS1 and from identity to 31.5% of nucleotides in ITS2 (Table 3). Comparisons of sequence pairs across both spacer regions gave divergence values ranging from identity (between *Daucus carota* subsp. *halophilis* and *D. carota* subsp. *gummifer*) to 29.4% (between *Ammodaucus leucotrichus* and *Scandix pecten-veneris*). Within Caucalideae, sequence divergence values ranged from identity to 28.0% of nucleotides. Among different species of the same genus, pairwise nucleotide divergence varied between 0.3% (between *Lisaea strigosa* and *L. papyracea*) and 13.5% (between *Daucus carota* subsp. *carota* and *D. durieua*). Sequence divergence values among other congeners are presented in Table 4.

Phylogenetic Analyses. MP analysis of the 58 combined ITS1 and ITS2 sequences resulted in 588 minimal length trees; the strict consensus of these trees, with accompanying bootstrap and decay values, is presented in Fig. 1. Each of these trees had a length of 1,035 steps, CI's of 0.466 and 0.428 (with and without uninformative characters, respectively), and a RI of 0.756. One of these 588 trees was selected arbitrarily in order to show the number of

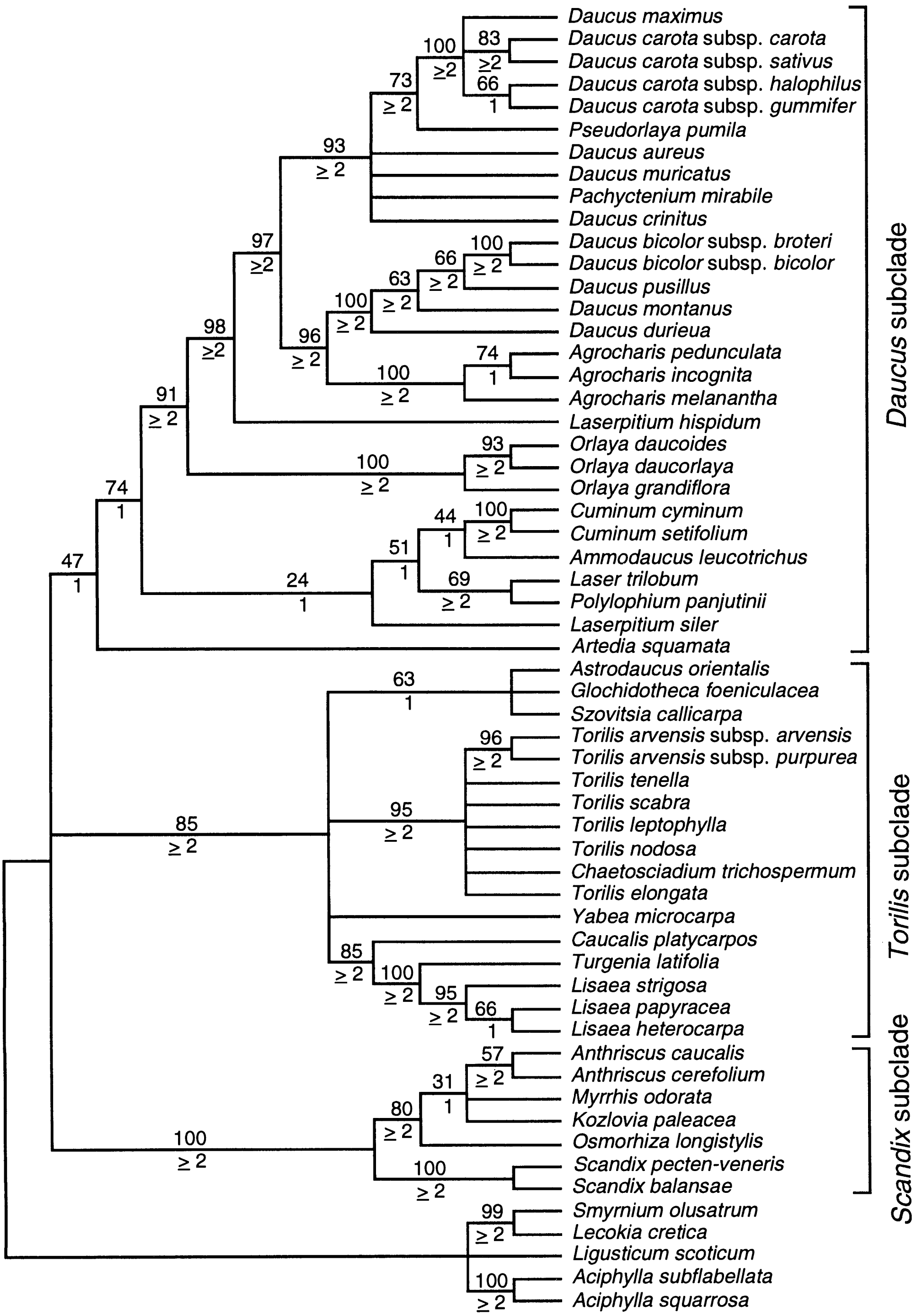

FIG. 1. Strict consensus of 588 minimal length 1,035-step trees derived from equally weighted maximum parsimony analysis of combined nuclear rDNA ITS1 and ITS2 sequences from 58 accessions of Heywood's (1982c) Caucalideae and related taxa using 409 unambiguously aligned nucleotide positions (CI's with and without uninformative characters = 0.466 and 0.428, respectively; RI = 0.756). 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 subclades are discernable and are identified by brackets.

nucleotide substitutions supporting each branch, as optimized by acctran in PAUP (Fig. 2). Thirteen of the 17 parsimony informative, unambiguous alignment gaps are consistent with this single tree (and are represented by solid bars in Fig. 2). Nine indels were inferred from the remaining four alignment gaps; three indels (labeled A, B, and C) each occur in parallel twice, and one indel (labeled D) occurs independently three times. Indels A, B, and D are each 1 bp in length; indel C is 3 bp in length, and represents a deletion in ITS2 relative to all outgroup taxa. These homoplastic indels are indicated by open bars in Fig. 2. The NJ tree, calculated with a Ts/Tv rate ratio of 1.6 based on the actual inferred frequencies determined over all 588 MP trees by MacClade, is presented in Fig. 3. On this tree, bootstrap values <20% are not indicated. The same topology resulted when Ts/Tv rate ratios of 1.0 or 2.0 were used. The best ML tree, also calculated with a Ts/Tv rate ratio of 1.6, had a log likelihood value of −5,850.52 and is presented in Fig. 4.

Phylogenetic Resolutions. Phylogenies estimated using MP, NJ, and ML methods give essentially similar topologies, with those few areas of discord noted below. In each of these trees, three major groups of taxa are discernable. The first group includes the genera *Daucus, Pseudorlaya, Pachyctenium, Agrocharis, Laserpitium, Orlaya, Ammodaucus, Cuminum, Laser, Polylophium,* and *Artedia.* The genus *Artedia,* however, is supported weakly at the base of this clade (with a bootstrap value of either 47 or 49% and a decay value of one). The second group includes *Astrodaucus, Glochidotheca, Szovitsia, Torilis, Chaetosciadium, Yabea, Caucalis, Turgenia,* and *Lisaea.* The third group, very well-supported in all trees (with bootstrap values of 100%), comprises the genera *Anthriscus, Myrrhis, Kozlovia, Osmorhiza,* and *Scandix.* In previous publications (Plunkett et al. 1996; Downie et al. 1998), the taxa examined that fell within these three groups were designated as all belonging to the *Daucus* clade. Increased sampling has achieved additional resolution, with three major clades discernable. We have named these three groups the *Daucus, Torilis,* and *Scandix* subclades, respectively (Figs. 1–4). Our third subclade coincides with Heywood's (1971b) tribe Scandiceae. The other two subclades, with the inclusion of the four Laserpitieae representatives, collectively reflect Heywood's (1982c) tribe Caucalideae. The relationships among these three subclades, however, are equivocal. In the NJ and ML trees (Figs. 3 and 4), the *Scandix* subclade is sister to the *Daucus* subclade, suggestive of a paraphyletic Caucalideae. This relationship, however, is not supported strongly. In the MP strict consensus tree (Fig. 1), the subclades form a trichotomy.

Relationships within the *Daucus* and *Torilis* subclades are largely congruent as a result of each of the phylogenetic analyses. With regard to the *Daucus* subclade, the genera *Daucus, Pseudorlaya, Pachyctenium,* and *Agrocharis* comprise a well-supported group (that is also supported by a single synapomorphic length mutation; Fig. 2); this group is sister to *Laserpitium hispidum.* This large clade, in turn, is sister to a clade comprising all three *Orlaya* exemplars. The genus *Daucus* is split, however, with one group (comprising *D. maximus, D. carota, D. aureus, D. muricatus,* and *D. crinitus*) allied with *Pseudorlaya pumila* and *Pachyctenium mirabile,* and the other group (comprising *D. bicolor, D. pusillus, D. montanus,* and *D. durieua*) allied with a monophyletic *Agrocharis.* The first of these two groups contains elements of *Daucus* sections *Daucus, Chrysodaucus* Thell., *Platyspermum* (Hoffm.) DC., and *Meoides* Lange; the second group reflects *Daucus* sections *Pseudoplatyspermum* Thell., *Leptodaucus* Thell., and *Anisactis* DC. (Heywood 1982c). *Daucus carota,* represented herein by four subspecies, is allied closely to the Mediterranean *D. maximus,* the latter at one time included as a subspecies of *D. carota* (*D. carota* subsp. *maximus* (Desf.) Ball). The two species of *Daucus* native to the New World, *D. montanus* and *D. pusillus,* ally weakly in the NJ tree (Fig. 3) but not in the other trees. These two New World species ally strongly with the eastern Mediterranean species *D. bicolor* and *D. durieua.* This clade, in turn, is sister to the *Agrocharis* clade, the latter being of eastern tropical African distribution. While our sampling of *Daucus* is incomplete, the taxa chosen do represent all seven sections recognized within the genus (Heywood 1982c). Based on these results, *Daucus* is not monophyletic as currently circumscribed.

In all phylogenetic trees, the genera *Ammodaucus, Cuminum, Laser,* and *Polylophium* comprise a clade, albeit one that is very weakly supported. In the MP and ML trees this clade is sister to *Laserpitium siler.* The monotypic *Artedia* is sister to all other taxa within the *Daucus* subclade, although this relationship is also supported by low bootstrap values (47 and 49% in the MP and NJ trees, respectively). All four species of tribe Laserpitieae included in this investigation fall within the *Daucus* subclade. *Laser trilobum* allies with *Polylophium panjutinii.* The two species of *Laserpitium,* however, do not form a clade in any tree, nor do they appear very closely related

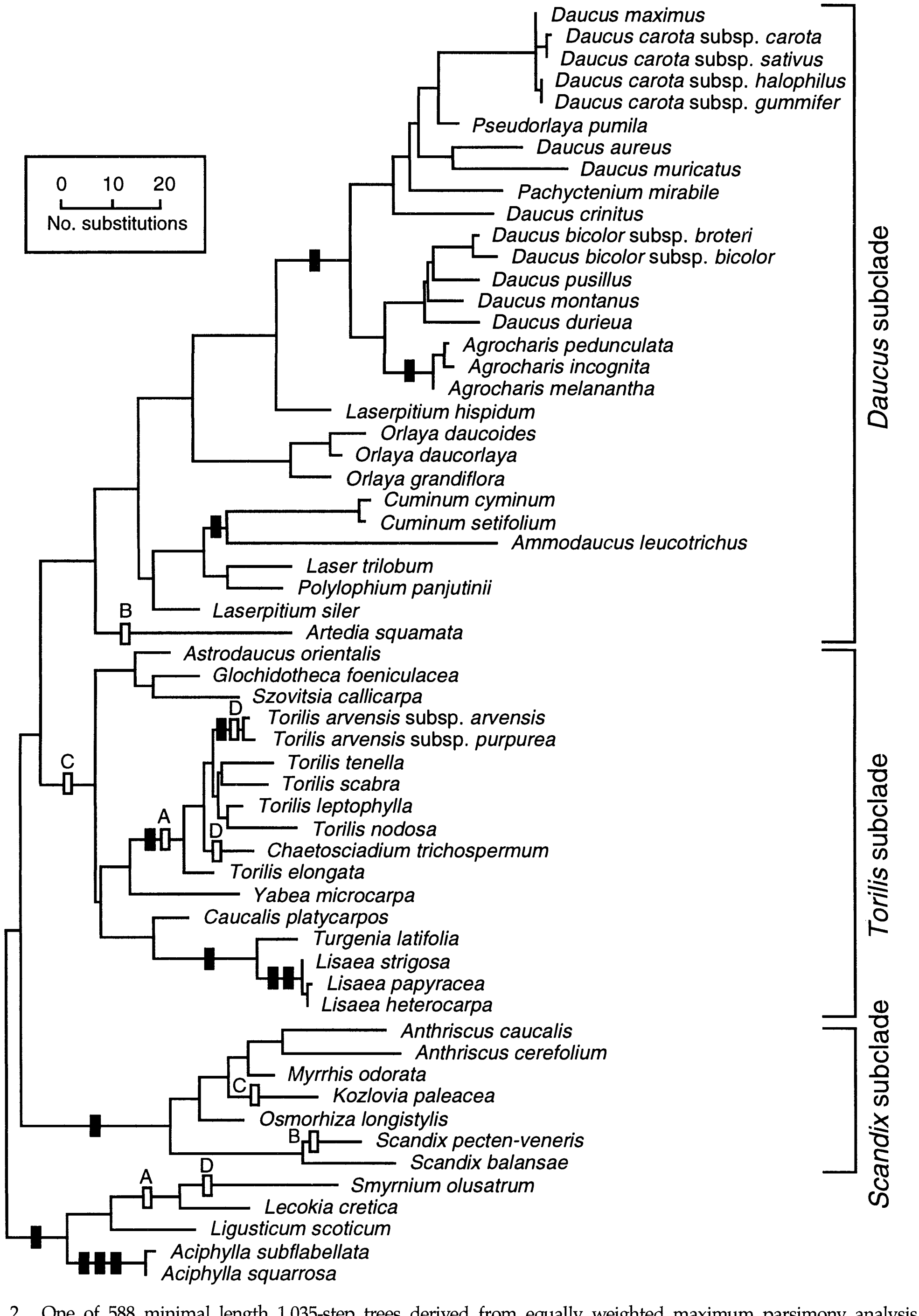

FIG. 2. One of 588 minimal length 1,035-step trees derived from equally weighted maximum parsimony analysis of combined nuclear rDNA ITS1 and ITS2 sequences from 58 accessions of Heywood's (1982c) Caucalideae and related taxa using 409 unambiguously aligned nucleotide positions (CI's with and without uninformative characters = 0.466 and 0.428, respectively; RI = 0.756). Branch lengths are proportional to the number of inferred nucleotide substitutions (acctran) occurring along them (note scale bar). The distribution of 13 synapomorphic (solid bars) and nine homoplastic (open bars) indels derived from the 17 potentially informative and unambiguous alignment gaps have been superimposed parsimoniously on the phylogram. Indels A, B, and C each occur in parallel twice; indel D occurs independently three times. Within the ingroup, the three major subclades discussed in the text are bracketed.

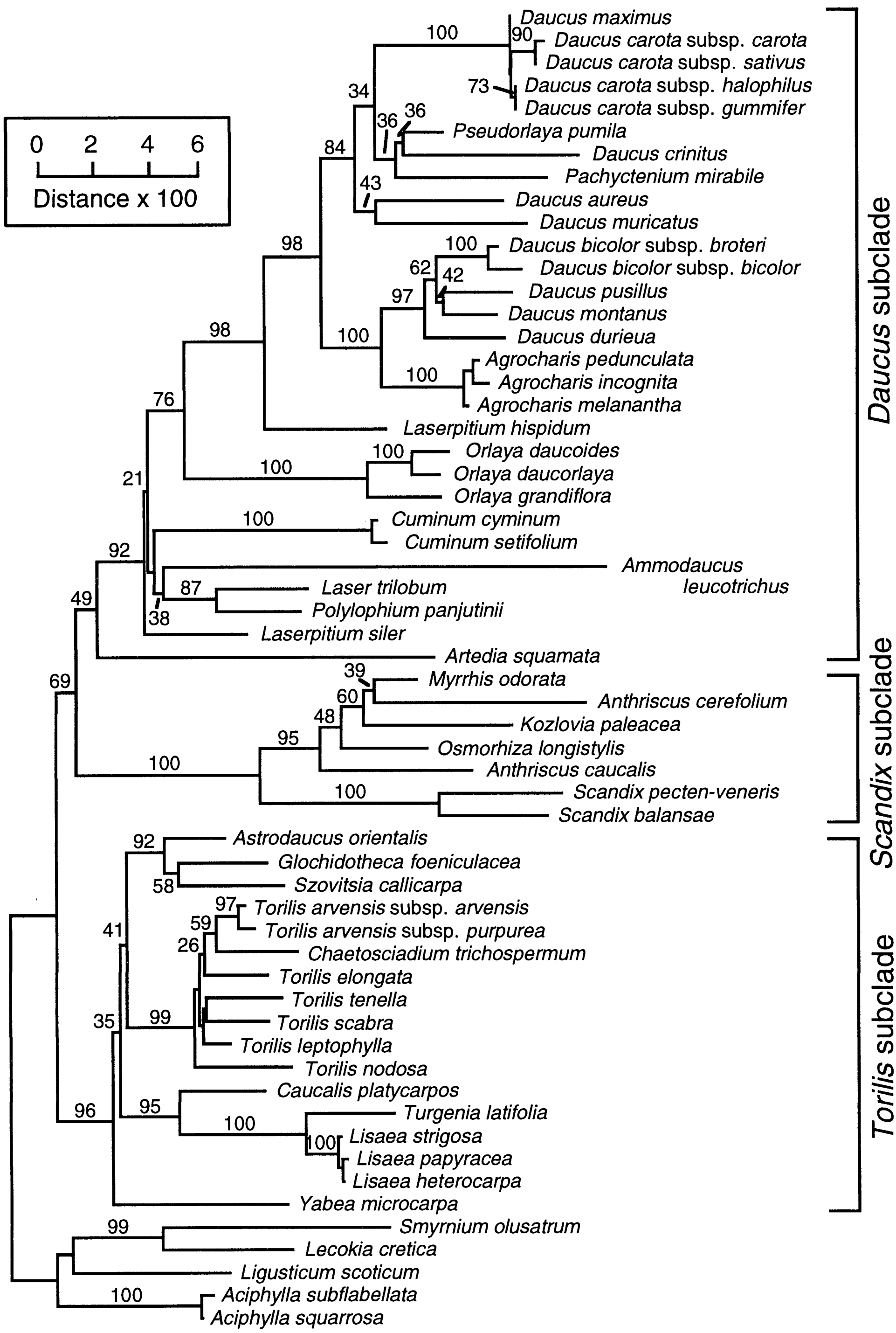

FIG. 3. Neighbor-joining tree inferred from the analysis of 58 nuclear rDNA ITS1 and ITS2 sequences from Heywood's (1982c) Apiaceae tribe Caucalideae and related taxa using a transition/transversion rate ratio of 1.6. Branch lengths are proportional to distances estimated from the two-parameter method of Kimura (scale distance is given as 100 times this value). Numbers at the nodes indicate bootstrap estimates for 100 replicate analyses; bootstrap values <20% are not indicated. Within the ingroup, the three major subclades discussed in the text are bracketed.

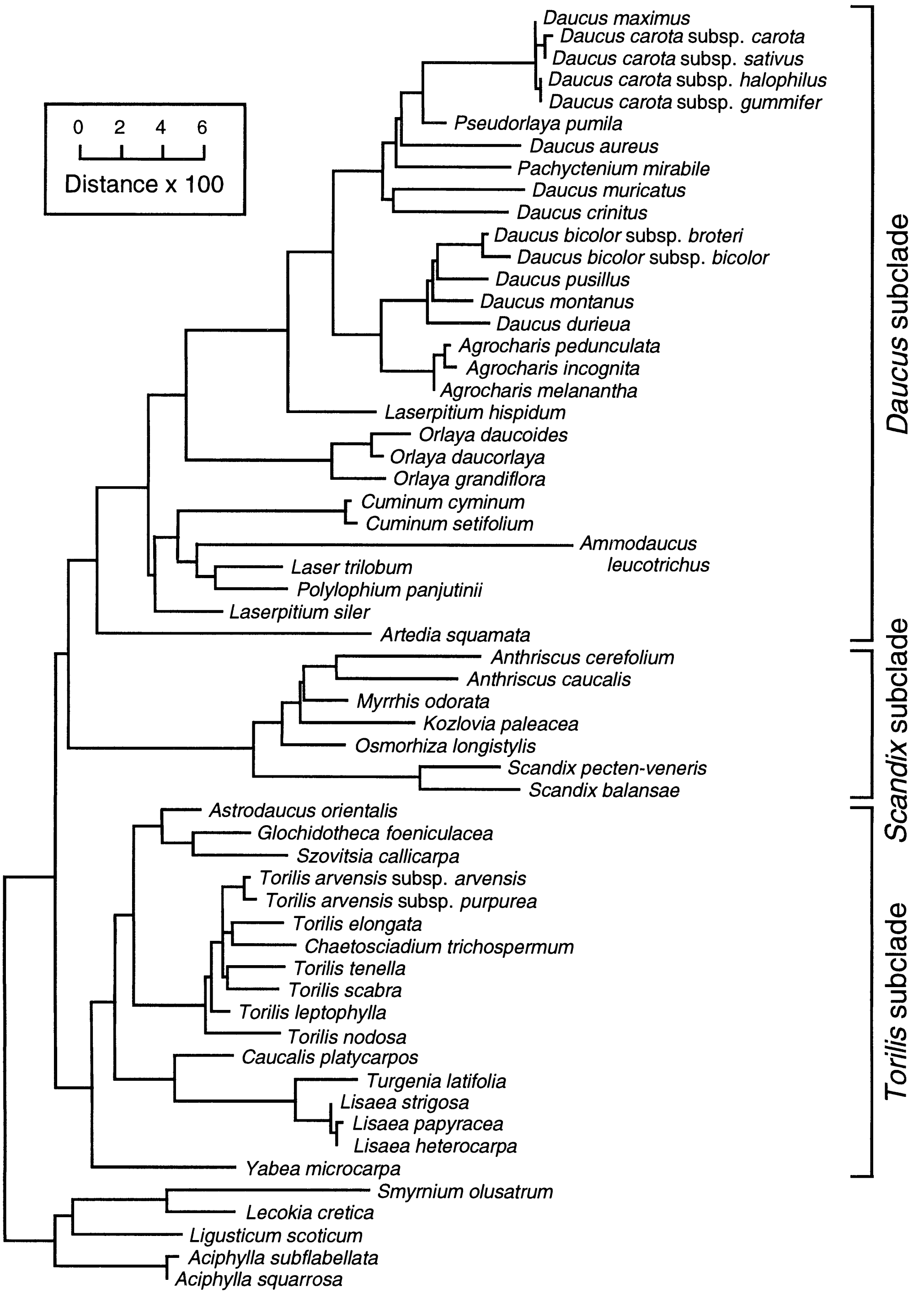

FIG. 4. Maximum likelihood tree constructed from 58 unambiguously aligned ITS1 and ITS2 sequences from Heywood's (1982c) Apiaceae tribe Caucalideae and related taxa using a transition/transversion rate ratio of 1.6 (log likelihood value −5,850.52). Branch lengths are proportional to the number of expected nucleotide substitutions per site (scale distance is given as 100 times this value). Within the ingroup, the three major subclades discussed in the text are bracketed.

to *Laser* and *Polylophium*. On this basis, the genus *Laserpitium* does not appear to be monophyletic either.

Within the *Torilis* subclade, three major lineages are evident. The first comprises *Astrodaucus, Glochidotheca,* and *Szovitsia*; the second consists of all six species of *Torilis* and *Chaetosciadium trichospermum*; and the third consists of *Caucalis, Turgenia,* and the three species of *Lisaea*. Each of these groups, especially those of the NJ tree (Fig. 3), is supported by a high bootstrap value. The *Torilis* subclade is supported by an indel (a 3-bp deletion), albeit one that is homoplastic as it also occurs in *Kozlovia* (indel C in Fig. 2). The genus *Yabea*, the remaining member of this subclade, is variably positioned depending upon the method of tree construction used. In the NJ and ML trees (Figs. 3 and 4), *Yabea* is sister to all other members of this subclade, whereas in the MP tree (Fig. 1) it arises as one branch of a polytomy. *Yabea* is the only member of this subclade endemic to the New World. The monotypic *Chaetosciadium* is included within *Torilis* in both the NJ and ML trees; the precise relationship between *Chaetosciadium* and *Torilis* in the MP tree cannot be discerned, but they are indeed very closely related. Therefore, as presently circumscribed, the genus *Torilis* is also not monophyletic.

Relationships within the *Scandix* subclade are the most variable among comparison of all phylogenies inferred. While the relationships suggested by both the MP (Fig. 1) and ML (Fig. 4) trees are consistent, they are somewhat different to those presented in the NJ tree (Fig. 3) owing to the position of *Anthriscus caucalis*. In the NJ tree, *Anthriscus* is not monophyletic. The genus *Kozlovia*, recognized either in Scandiceae (Heywood 1971b; Pimenov and Leonov 1993) or in Caucalideae (Heywood 1982c), falls within this subclade. The *Scandix* subclade is very well supported by high bootstrap values, and is characterized further by one synapomorphic length mutation (Fig. 2).

Discussion

One of our intentions in carrying out this study was to evaluate the evolutionary relationships among those plants treated by V. Heywood and S. Jury (in Heywood 1982c) in Apiaceae tribe Caucalideae. Because of its relatively small size (68 species in 21 genera), its largely Mediterranean distribution (permitting access to much research material), its complex taxonomic history, the wealth of available data (summarized in Heywood 1971a, and Cauwet-Marc and Carbonnier 1982), and the economic importance of at least some of its members (e.g., *Daucus carota* subsp. *sativus*, the common cultivated carrot, and *Cuminum cyminum*, cumin), the tribe was an obvious group to study. Moreover, the group is monophyletic upon the inclusion of Laserpitieae and Scandiceae (Downie and Katz-Downie 1996, 1998; Plunkett et al. 1996; Katz-Downie et al. 1999). Unfortunately, we have not examined material of *Angoseseli*, a rare monotypic genus of tropical Angolan distribution. This species was at one time referred to the genus *Caucalis* (Heywood 1982c).

Psammogeton *and* Aphanopleura. Based on the shared presence of fruit appendage characters and prominent primary and secondary ridges, *Aphanopleura* and *Psammogeton* were considered as belonging to tribe Caucalideae (Heywood 1982c). However, phylogenetic analysis of ITS sequences (Katz-Downie et al. 1999) supported Pimenov and Leonov's (1993) treatment in removing these genera from the tribe. The close relationship among *Aphanopleura, Psammogeton,* and *Pimpinella*, as suggested by the Katz-Downie et al. (1999) ITS phylogeny, is not surprising as some species are very similar morphologically. Indeed, many species currently recognized in *Aphanopleura* and *Psammogeton* were at one time treated as species of *Pimpinella*. We have observed that the fruits of *Aphanopleura* and *Psammogeton* do differ in some aspects from those of Caucalideae. In *Aphanopleura* and *Psammogeton*, the fruit appendages are randomly distributed, whereas in Caucalideae they are regularly distributed on the primary and secondary ridges. Moreover, the appendage characters seen in *Psammogeton* (martelliform) and *Aphanopleura* (clavate/capitellate) appear to be unique.

Caucalideae Comprises Two Major Clades. Based upon our phylogenetic results, Heywood's (1982c) tribe Caucalideae, with the exclusion of *Psammogeton, Aphanopleura,* and *Kozlovia*, comprises two major groups of taxa. We have provisionally named these groups the *Daucus* and *Torilis* subclades. Included in the *Daucus* subclade is representation of Drude's (1897–1898) tribe Laserpitieae. The three examined genera of Laserpitieae (*Laser, Laserpitium,* and *Polylophium*) do not form a monophyletic group, nor is the genus *Laserpitium* monophyletic. Variously associated with the *Daucus* and *Torilis* subclades is a third major group of taxa, provisionally called the *Scandix* subclade. *Kozlovia* is included within this group and, as such, the boundaries of this subclade reflect Heywood's (1971b) tribe Scandiceae. The relationships among these three subclades are equivocal, although there is weak support in some trees for the sister group status between the *Daucus* and *Scandix* subclades. The results of the MP analysis, however, fail to unambiguously support this relationship. These three subclades have been collectively referred to as the *Daucus* clade in earlier investigations (Plunkett et al. 1996; Downie et al. 1998).

The two major groups recognized herein in tribe Caucalideae parallel, in part, dichotomies within the tribe proposed by other workers (McNeill et al. 1969; Al-Attar 1974; Saenz de Rivas et al. 1982). McNeill et al. (1969), upon the basis of phenetic analyses of 83 primarily fruit, leaf, inflorescence, and floral characters, clustered *Orlaya* and all but two species of *Daucus* into one group, and *Torilis, Chaetosciadium, Caucalis, Turgenia,* and the two exemplars of *Daucus* section *Anisactis* (*D. durieua* Lange and *D. glochidiatus* (Labill.) Fisch. & C. A. Mey.) into another. The inclusion of *Daucus* in both groups was likely an artifact of the types of characters used and how they were scored.

Al-Attar (1974), using 12 micromorphological and anatomical fruit characters, such as spine surface structure, vascular bundle and vittae size, degree of fruit and endosperm compression, and types of appendages occurring on the primary and secondary ridges, placed the genera *Ammodaucus, Agrocharis, Astrodaucus, Daucus, Orlaya, Pseudorlaya,* and *Artedia* in one lineage, and the genera *Chaetosciadium, Caucalis, Torilis, Turgenia, Lisaea,* and *Glochidotheca* (as *Turgeniopsis*) in another. Within each lineage, these genera were arranged according to progressively increasing specialization index values, with *Artedia* and *Glochidotheca* possessing the most specialized or complex features. *Cuminum* and *Psammogeton* were regarded as basal to these two groups, as they possessed the simplest fruit structures. Starting from a slightly dorsally compressed mericarp, as seen in *Cuminum* and *Psammogeton,* evolution of mericarp morphology was thought to proceed either in the direction of progressively greater dorsal (*Ammodaucus* to *Artedia*) or greater lateral (*Chaetosciadium* to *Glochidotheca*) compression with concomitant increases in the complexity of other fruit characters. Other than Al-Attar's treatment of *Astrodaucus* alongside *Daucus* and the basal placement of *Cuminum,* the composition of each of these two lineages mirrors our *Daucus* and *Torilis* subclades. The intergeneric relationships implied, however, differ substantially from those inferred herein using ITS data.

Al-Attar's (1974) study was subsequently expanded by Saenz de Rivas et al. (1982), upon the inclusion of *Angoseseli, Exoacantha, Kozlovia, Szovitsia,* and *Yabea.* The same 12 fruit characters were considered. Here *Szovitsia* was placed alongside *Ammodaucus, Daucus,* and *Astrodaucus* in one lineage, and *Kozlovia* and *Yabea* were placed near *Torilis, Caucalis, Turgenia,* and *Glochidotheca* in the other. *Angoseseli* and *Exoacantha* fell alongside *Cuminum* and *Psammogeton* basally within the group. *Artedia* was deemed as possessing the most specialized fruit, and *Exoacantha* the least specialized. Once more, with the exception of the positioning of both *Astrodaucus* and *Szovitsia* alongside taxa which are treated in our *Daucus* subclade, the placement of *Kozlovia* near *Torilis,* and the basal placement of *Cuminum,* these results are very similar to ours in suggesting that Heywood's tribe Caucalideae comprises two distinct groups.

Comparison to Drude's Treatment. Drude's (1897–1898) treatment of Apiaceae is by far the most widely used for the family, despite it being highly criticized for using subtle or poorly defined diagnostic characters (Heywood 1982b; Pimenov and Leonov 1993; Downie et al. 1998). However, with regard to the three major subclades outlined herein, some similarities to Drude's system are evident. Drude considered his tribe Dauceae (comprising *Daucus, Artedia, Ammodaucus,* and *Exoacantha*) to have evolved from plants similar to those in his tribe Laserpitieae (such as *Laserpitium* and *Polylophium*). *Exoacantha* is now generally excluded from Caucalideae (Heywood 1982c), its removal most recently supported by molecular data (Katz-Downie et al. 1999). Although species of Laserpitieae do not have spines on their fruits, they do have both primary and prominent secondary ridges and their mericarps are strongly dorsally compressed. Many members of the *Daucus* subclade possess spines and have obvious secondary ridges, and all are characterized by dorsally compressed fruits. Our results confirm that Drude's tribe Laserpitieae is indeed very closely related to *Daucus* and allies. Along with *Ammodaucus* and *Cuminum,* three of the four Laserpitieae exemplars included in our study form a weakly supported clade that is sister to another comprising *Daucus, Orlaya, Agrocharis,* and several other genera. Additional sampling of Laserpitieae is definitely warranted, but based on those few included species it does appear that Laserpitieae and the *Daucus* subclade share an immediate common ancestor.

Additional evidence suggesting that the spiny-fruited umbellifers and Drude's Laserpitieae are closely allied comes from an interesting species we weren't able to include in this study. *Daucus laserpitioides* DC., placed by Drude in an isolated section of *Daucus*, has been treated in the genus *Laserpitium* by Koso-Poljansky (1916) as *L. daucoides* Desf. or as a separate genus, *Ctenodaucus* (Heywood and Dakshini 1971). *Daucus laserpitioides* differs from other species of *Daucus* in the structural similarity of its primary and secondary ridges (both composed of spines), and in the absence of hairs on the primary ridges (Heywood and Dakshini 1971; Okeke 1982). The secondary ridges of Laserpitieae are often extended into wings, and the fruit of *D. laserpitioides*, with its deeply serrate wings, is somewhat intermediate in structure between spiny fruits of Caucalideae and winged fruits of Laserpitieae (J.-P. Reduron, personal communication, Mulhouse, France).

Drude (1897–1898) defined his tribe Scandiceae on the basis of calcium oxalate (druse) crystals in the parenchyma cells surrounding the carpophore, and divided it into two subtribes, Scandicinae and Caucalidinae, according to the shape of the fruit. The secondary fruit ridges of some Caucalidinae are suppressed or less well developed than those of his Dauceae, and members of Scandicinae lack both secondary ridges and spines. Drude assumed that the secondary spinose ridges in Caucalidinae had evolved independently from those in his Dauceae. Drude's Scandicinae corresponds closely with our *Scandix* subclade (i.e., Heywood's 1971b Scandiceae), whereas his Caucalidinae corresponds, in part, to our *Torilis* subclade. In Caucalidinae, Drude placed *Astrodaucus, Glochidotheca, Torilis, Chaetosciadium, Lisaea*, and *Caucalis* (which included the segregate genera *Turgenia* and *Yabea*); all of these genera fall within our *Torilis* subclade. He also included *Orlaya* and *Psammogeton*; the former finds affinity with our *Daucus* subclade, whereas the latter is now excluded from the tribe (Pimenov and Leonov 1993; Katz-Downie et al. 1999). Based on our results, it can be assumed that the secondary spinose ridges seen in many members of the *Daucus* and *Torilis* subclades have arisen independently. Alternatively, these spinose ridges may have evolved only once, with subsequent multiple losses. A future paper of ours will further address this issue.

The genus *Artedia* is morphologically anomalous in the group. Its fruits are strongly dorsally compressed and its lateral secondary ridges have developed into large, deeply lobed, scaly wings; its other secondary ridges, like the primary ridges, are slender and filiform. Its placement at the base of the *Daucus* subclade is very weakly supported. In many ways, its fruit morphology and anatomy suggest affinity to tribe Laserpitieae and, to this end, its placement in this subclade alongside other Laserpitieae exemplars is consistent with this. Additional data, such as those currently being obtained from the plastid genome, may shed more light on the phylogenetic placement of this genus.

Daucus *Subclade.* The genus *Daucus* was represented in this study by nine species (including six infraspecific taxa) representing all seven sections (Heywood 1982c). The ITS phylogenies suggest a major dichotomy within the genus, with some *Daucus* species allied with *Pseudorlaya pumila* and *Pachyctenium mirabile* and others with *Agrocharis*. Evidently, the genus *Daucus* is not monophyletic. The close relationship between *Daucus* and *Pseudorlaya* is also supported by several morphological and chemical characters (Heywood and Dakshini 1971; Williams and Harborne 1972). We have identified two morphological characters that support, in part, the relationship among *Daucus, Pseudorlaya, Pachyctenium*, and *Agrocharis*. The first is the presence of a lobed primary hair base, a feature of all examined species with the exception of *Agrocharis*. The second is a strongly developed glochidiate apex of the secondary spines; this character distinguishes *Pseudorlaya, Agrocharis*, and all species of *Daucus* except *D. crinitus*. Although each of these genera is distinctive morphologically, it does appear that when material of *Pseudorlaya, Pachyctenium*, and *Agrocharis* is compared alongside a large number of *Daucus* accessions, they represent no more than extremes in the variation observed.

The taxonomic history of *Cuminum* is complex. Based upon similarity of fruit bristle structure, Boissier (1872) placed *Cuminum* alongside *Psammogeton* and *Chaetosciadium* in his tribe Caucalideae. In contrast, Drude (1897–1898) treated *Cuminum* in Apieae subtribe Carinae near *Szovitsia* and *Aphanopleura*. On the basis of flavonoid evidence, Harborne and Williams (1972) transferred *Cuminum* from Apieae to Caucalideae. Apparently, luteolin 7-glucuronosylglucoside is found only in *Cuminum cyminum, Orlaya daucorlaya*, and *O. grandiflora* and not in any other examined member of tribe Apieae. Moreover, *Cuminum* and *Daucus* share similar primary appendage characters, providing further support for the transfer of *Cuminum* to Caucalideae (Heywood and Dakshini 1971).

Drude (1897–1898) placed *Orlaya* near *Caucalis* in his subtribe Caucalidinae, in contrast to our ITS results where these genera occur in separate major clades. The placement of *Orlaya* alongside *Daucus* and allies in the *Daucus* subclade is also supported by similarities in their fruit morphology and anatomy, and patterns in their fruit flavonoid chemistry (Harborne and Williams 1972).

The placement of the four examined Laserpitieae members into the *Daucus* subclade supports, in part, the classification systems of Calestani (1905) and Koso-Poljansky (1916) where *Laserpitium* was treated alongside *Daucus* in the same tribe or subtribe. Similarly, Tamamschjan (1947) suggested an affinity between *Laserpitium* and *Daucus* based on carpological characters. The genus *Laserpitium* is not monophyletic, as evidenced by the distantly positioned *L. hispidum* and *L. siler* in each of our cladograms. *Laserpitium hispidum* is very distinctive morphologically, having both primary hairs and secondary wings on its fruits, and unlike other species of *Laserpitium* that develop both primary and secondary wings. Further systematic investigation of *Laserpitium* is in order.

Torilis *Subclade.* The alliance among *Astrodaucus, Szovitsia,* and *Glochidotheca,* as inferred by ITS data, is surprising given the remarkable differences seen in their mericarp anatomy and the shapes of their secondary appendages. As examples, *Astrodaucus* is characterized by pyramid-shaped secondary spines while its primary ridges are thread-like and inconspicuous, *Szovitsia* is characterized by unique spatulate pouches, and *Glochidotheca* is characterized by strongly laterally compressed fruits. We have observed, however, that this clade is characterized by two morphological synapomorphies: the presence of curved primary hairs, and the presence of peg-like projections on the surface of their secondary appendages.

Torilis is an extremely polymorphic genus, given the broad variation seen in both its vegetative (e.g., cauline leaves) and fruit (e.g., secondary spines) morphology. Cannon (1967, 1968) divided *Torilis* into several subspecies, all of which have been treated subsequently as different species. In this study, the monophyly of *Torilis* is strongly supported when its boundary is expanded to include *Chaetosciadium.* The monotypic *Chaetosciadium* is characterized by mericarps that are covered with fine, long bristly hairs on obsolete secondary ridges. These unique features led Calestani (1905) to erect the monotypic subtribe Chaetosciadieae in his tribe Ligusticeae. However, *Chaetosciadium* and *Torilis* share similar flavone distribution patterns (Crowden et al. 1969; Harborne and Williams 1972), base chromosome numbers ($x = 6$; Constance et al. 1971), and hairs on their primary fruit ridges (Heywood and Dakshini 1971).

Yabea microcarpa, once included in *Caucalis* (as *C. microcarpa* Hook. & Arn.), is now recognized as a distinct monotypic genus. In both NJ and ML trees (Figs. 3–4), *Yabea* arises basally within the *Torilis* subclade, but in the MP tree (Fig. 1) this position is not obvious. While *Yabea* is distinct morphologically, it has the same base chromosome number as *Torilis* and *Chaetosciadium.* This number, $x = 6$, is not known for any other genus in the *Torilis* subclade.

The genus *Caucalis* has been more extensively modified than any other genus in the tribe (Heywood 1982c). Many species which at one time had been included in *Caucalis* are now referable to other genera, such as *Torilis, Turgenia, Agrocharis, Astrodaucus, Orlaya, Yabea,* and *Angoseseli* (Cannon 1967; Heywood and Dakshini 1971; Heywood 1973, 1982c, 1986). *Caucalis* is now represented by only one species, *C. platycarpos.* The phylogenies presented herein support a strong relationship among *Caucalis, Turgenia,* and *Lisaea.* The close association between *Turgenia* and *Lisaea* reflects the remarkable similarity seen in their fruit anatomy, cotyledons, and pollen, and in their characteristic leaf morphology (Cerceau-Larrival 1962; Townsend 1964; Heywood and Dakshini 1971; Guyot et al. 1980). These two genera were at one time united under *Turgenia* (Koso-Poljansky 1916).

Scandix *Subclade.* Heywood's (1971b) tribe Scandiceae, represented by our *Scandix* subclade, comprises 17 genera and some 70–90 species, and is confined largely to southwest Eurasia. These plants have elongated, cylindrical fruits with, generally, smooth surfaces. The mericarp ribs are often inconspicuous, and the fruits may be obviously beaked. Because we have included only seven species in this study, it is premature to discuss relationships within the tribe. Nevertheless, the group is very well supported in all analyses. The monotypic genus *Kozlovia,* recognized previously in either Scandiceae (Heywood 1971b; Pimenov and Leonov 1993) or Caucalideae (Heywood 1982c), certainly belongs within the *Scandix* subclade. The genus *Kozlovia* is clearly differentiated from those genera of Caucalideae due to the presence of a fruit beak and the absence of secondary ridges, vittae, and primary hairs on the fruit commissural face. It is further distinguished by its unusual underground tuber-like stems (Rechinger 1987). Anatomically, *Kozlovia* shows similarities to *Osmorhiza,* due to the absence of vittae and the shared possession of thin-layered mesocarps.

Conclusions. Phylogenetic analyses of nuclear rDNA ITS data from representatives of Apiaceae tribes Caucalideae, Scandiceae, and Laserpitieae (collectively and previously referred to as the *Daucus* clade; Plunkett et al. 1996; Downie et al. 1998) support the recognition of three distinct groups of taxa of equivocal relationship. We have provisionally named these groups the *Daucus, Torilis,* and *Scandix* subclades until more formal nomenclature can be applied. The *Daucus* subclade contains representation of tribe Laserpitieae, whereas the *Scandix* subclade coincides with Heywood's (1971b) Scandiceae. With the exception of *Aphanopleura, Psammogeton,* and *Kozlovia*—the first two genera distantly related to the group and the third finding affinity with the *Scandix* subclade—members of Heywood's (1982c) Caucalideae are distributed between our *Daucus* and *Torilis* subclades. We are continuing our investigation of Caucalideae phylogeny by examining data from both morphology and the chloroplast genome. Congruence of relationship from independent lines of evidence is necessary in order to test the relationships proposed herein using ITS data and to identify discrepant organismal and gene phylogenies. The results from these analyses, in conjunction with those obtained from a concurrent phylogenetic study of tribe Scandiceae (S. Downie, D. Katz-Downie, and K. Spalik, unpubl. data), will be used to revise the classification of these spiny-fruited umbellifers and allies.

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