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A MOLECULAR PHYLOGENY OF APIACEAE SUBFAMILY APIOIDEAE: EVIDENCE FROM NUCLEAR RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER SEQUENCES[1]

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Phylogenetic relationships among 40 New World and Old World members of Apiaceae subfamily Apioideae, representing seven of the eight tribes and eight of the ten subtribes commonly recognized in the subfamily, were inferred from nucleotide sequence variation in the internal transcribed spacer (ITS) regions of 18-26S nuclear ribosomal DNA. Although the sequences are alignable, with only 11% of sites excluded from the analyses because of alignment ambiguity, divergence values in pairwise comparisons of unambiguous positions among all taxa were high and ranged from 0.5 to 33.2% of nucleotides in ITS 1 and from 0 to 33.2% of nucleotides in ITS 2. Average sequence divergence across both spacer regions was 18.4% of nucleotides. Phylogenies derived from ITS sequences estimated using neighbor-joining analysis of substitution rates, and maximum likelihood and parsimony methods give trees of essentially similar topology and indicate that: (1) there is little support for any existing system of classification of the subfamily that is based largely on morphological and anatomical features of the mericarp; (2) there is a major phylogenetic division within the subfamily, with one clade comprising the genus *Smyrnium* and those taxa belonging to Drude's tribes Dauceae, Scandiceae, and Laserpitieae and the other clade comprising all other examined taxa; and (3) the genera *Arracacia, Coaxana, Coulterophytum, Enantiophylla, Myrrhidendron, Prionosciadium,* and *Rhodosciadium,* all endemic to Mexico and Central America, comprise a clade but their relationships to other New World taxa are equivocal. A phylogeny derived from parsimony analysis of chloroplast DNA *rpo*C1 intron sequences is consistent with, but considerably less resolved than, relationships derived from these ITS regions. This study affirms that ITS sequences are useful for phylogenetic inference among closely related members of Apioideae but, owing to high rates of nucleotide substitution, are less useful in resolving relationships among the more ancestral nodes of the phylogeny.

Key words: Apiaceae; Apioideae; internal transcribed spacer; molecular phylogeny; nuclear ribosomal DNA.

Apiaceae (Umbelliferae) comprise ≈455 genera and some 3700 species and, although largely confined to temperate regions, are cosmopolitan in distribution (Pimenov and Leonov, 1993). It is one of the best known families of flowering plants, because of its characteristic inflorescences and fruits and the distinctive chemistry, reflected in the odor, flavor, and even toxicity of many of its members (Heywood, 1993). The division of Apiaceae into three subfamilies (Hydrocotyloideae, Saniculoideae, and Apioideae) and 12 tribes, done almost a century ago (Drude, 1898), remains the predominant system of classification for the family; however, much uncertainty exists regarding precise tribal delimitations and relationships among its constituent members.

Although the monophyly of Apiaceae is disputed—it is probably polyphyletic (Thorne, 1973; Plunkett, Soltis, and Soltis, 1992, 1994; Judd, Sanders, and Donoghue, 1994)—many features support the naturalness of subfamily Apioideae. Members of Apioideae, the typical "umbellifers," are distinguished from those in the other two subfamilies by the shared presence of compound umbels, a specialized fruit consisting of two one-seeded mericarps suspended from a common bifurcate carpophore, a soft endocarp that is sometimes hardened by woody subepidermal layers, a terminal style arising from the stylopodium, fruits without scales, an absence of stipules, the widespread but not ubiquitous occurrence of flavones, methylated flavonoids, furanocoumarins, and phenylpropenes, and a relatively distinctive insect fauna (Crowden, Harborne, and Heywood, 1969; Harborne, 1971; Hegnauer, 1971; Nielsen, 1971; Heywood, 1982; Berenbaum, 1990). Recently, phylogenetic analyses of chloroplast DNA *rbc*L sequences (Plunkett, Soltis, and Soltis, 1992) and morphological and anatomical characters (Judd, Sanders, and Donoghue, 1994) reveal that Apiaceae subfamilies Apioideae and Saniculoideae are each monophyletic and are sister taxa.

Apioideae are the largest and most taxonomically complex of the three subfamilies of Apiaceae. Existing treatments (e.g., de Candolle, 1830; Bentham, 1867; Drude, 1898; Calestani, 1905; Koso-Poljansky, 1916; Cerceau-Larrival, 1962), constructed largely on the basis of morphological and anatomical characters, give contradictory interpretations of relationship, and the tribal circumscriptions employed in each do not coincide in number or in content. Fruits of Apioideae exhibit extreme variation in overall form and detail, thus these structures have been relied upon extensively in various classifications at all taxonomic levels. Characters of the fruit include its general shape, degree of compression, the presence or ab-

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sence of wings, spines, hairs, ridges, or other outgrowths, the form and arrangement of spines and ridges, and many anatomical and embryological features. However, depending on which characters have been given greater importance, there is much diversity of treatment. De Candolle (1830), for example, primarily used criteria of the endosperm (recognizing suborders Orthospermae, Campylospermae, and Coelospermae); Bentham (1867) considered the presence or absence of oil tubes and secondary ridges in the fruit and the nature of the inflorescences (recognizing series Haplozygiae and Diplozygiae); Drude (1898) stressed fruit compression and the relative proportions of the dorsal and commissural surfaces, and the number of mericarp ribs and dorsal vittae (secretory canals); and Koso-Poljansky (1916) divided these taxa into four "legions" (Pachystereomeae, Endotaenieae, Exomestomeae, and Gymnomestomeae) based upon the distribution of crystals, vittae, and sclerenchyma and aerenchyma in the fruit walls. Many taxa are recognized solely on the basis of subtle mericarp differences, and serious doubts have been cast on the validity of using such apparently "trivial" characters (Heywood and Dakshini, 1971; Heywood, 1982). Consequently, the division of the subfamily into tribes and subtribes on the basis of fruit characteristics may not accurately depict historical relationships, and it is likely that many higher level taxa are maintained largely on considerations of tradition and convenience.

In addition to fruit characteristics, other kinds of evidence have been used to ascertain tribal relationships and composition within the subfamily. Cerceau-Larrival (1962), from her studies on the correlations between pollen morphology and the presence or absence of either round or long cotyledons, supported by evidence from inflorescences, fruits, and adult vegetative morphology, proposed a novel division of Apioideae (sensu Drude, 1898) into three subfamilies (Bupleuroideae, Endressioideae, and Apioideae) and 31 tribes. Subsequent palynological investigations (Cerceau-Larrival, 1963, 1965) culminated in several additional tribes being recognized, bringing the total number to 36! Those tribes of Drude (1898) containing taxa with more than one pollen type, but uniform with regard to cotyledon and adult vegetative morphology, were declared unnatural and divided into palynologically homogeneous units. These results departed considerably from traditional taxonomic treatments of the subfamily and have not gained much acceptance. Additional evidence has been obtained from examination of a plethora of chemical constituents and morphological and anatomical features (reviewed in Heywood, 1971a, and Cauwet-Marc and Carbonnier, 1982), and, more recently, DNA–DNA hybridization (Antonov et al., 1988), and serological (Shneyer et al., 1991, 1992) investigations. However, despite this wealth of information, there has been little speculation of phylogenetic relationships within the subfamily, apart from assignment of tribes and subtribes, and no rigorous phylogenetic analysis encompassing a broad representation of taxa at the generic level. To date, our understanding of the phylogenetic relationships within the subfamily is inadequate.

Although many have declared dissatisfaction with Drude's (1898) system of classification of Apioideae (Theobald, 1971; Davis, 1972; Hedge et al., 1987; Shneyer et al., 1992; Heywood, 1993; Pimenov and Leonov, 1993), it remains quite popular. As examples, it has been adopted with only minor modifications in the floras of Coulter and Rose (1900), Shishkin (1950–1951), Zohary (1972), Shan and Sheh (1979), and Hedge et al. (1987), and is cited widely by nonsystematists (e.g., Murray, Mendez, and Brown, 1982; Berenbaum, 1990). Moreover, many of the authors who have published papers at the occasion of two major symposia on the family (Heywood, 1971a; Cauwet-Marc and Carbonnier, 1982) present their data in the framework of Drude's system. An outline of Drude's system, indicating the eight tribes and ten subtribes circumscribed within Apioideae, is presented in Table 1.

TABLE 1. Drude's (1898) classification of Apiaceae subfamily Apioideae.

Echinophoreae	Peucedaneae
Scandiceae	Angelicinae
Scandicinae	Ferulinae
Caucalidinae	Tordyliinae
Coriandreae	Laserpitieae
Smyrnieae	Silerinae
Ammieae (=Apieae)	Elaeoselininae
Carinae	Thapsiinae
Seselinae	Dauceae

The use of biparentally inherited nuclear genes in phylogenetic estimation, in addition to chloroplast DNA (cpDNA), provides both a test of whether cytoplasmic gene flow has occurred, as well as a means of strengthening the overall phylogenetic hypothesis (Rieseberg and Soltis, 1991; Doyle, 1992). The nuclear ribosomal RNA genes (rDNAs) of higher plants are organized in long tandem repeating units (Appels and Honeycutt, 1986). Each repeat unit consists of a single transcribed region for the 18S, 5.8S, and 26S ribosomal RNAs, two small internal transcribed spacers (ITS 1 and ITS 2), and a large external nontranscribed intergenic spacer (IGS). The high copy number of rRNA genes (typically thousands per cell in plants), which are highly homogeneous within a genome (Arnheim, 1983), combined with differential rates of evolution among component subunits and spacer regions, makes the rDNA repeat unit a valuable tool for phylogenetic reconstruction at various taxonomic levels (reviewed in Hamby and Zimmer, 1992, and Baldwin et al., 1995). Sequences of the two internal transcribed spacers have proven useful for resolving relationships within and among closely related plant genera because, in general, these sequences evolve more rapidly than their flanking coding regions (e.g., Baldwin, 1992, 1993; Savard, Michaud, and Bousquet, 1993; Soltis and Kuzoff, 1993; Suh et al., 1993; Wojciechowski et al., 1993; Hsiao et al., 1994; Sang et al., 1994; Baldwin et al., 1995).

We undertook this study of Apioideae phylogeny with two broad goals in mind: (1) to demonstrate the usefulness of nuclear ribosomal DNA ITS sequences for resolving phylogenetic relationships within the subfamily; and (2) to formulate more precise hypotheses about relationships among the diverse clades comprising Apioideae. The relationships proposed here will be evaluated

TABLE 2. Collections of Apiaceae subfamily Apioideae examined for 18S–26S Nuclear Ribosomal DNA Internal Transcribed Spacer (ITS) Nucleotide Sequence Variation. ITS sequences for the accession identified as *Daucus* Yoko. in Table 4 and Figs. 1–5 were obtained from those published for *Daucus carota* by Yokota et al. (1989).

Taxon	Distribution[a]	Source and voucher
Aegopodium podagraria L.	N/O	U.S.A., Illinois, Urbana; *Downie 725* (ILL)
Aethusa cynapium L.	N/O	France, Cult. Jardin Botanique de Caen (no. 1424); *Downie 337* (ILL)
Anethum graveolens L.	N/O	France, Cult. Jardin Botanique de Caen (no. 1980); *Downie 326* (ILL)
Angelica archangelica L.	N/O	Finland, Cult. University of Joensuu Botanical Garden (no. 33); *Downie 79* (ILL)
Anthriscus cerefolium (L.) Hoffm.	N/O	Spain, Cult. Real Jardín Botánico (no. 1305); *Downie 35* (ILL)
Apium graveolens L.	N/O	France, Cult. Conservatoire et Jardins Botaniques de Nancy; *Downie 258* (ILL)
Arracacia brandegei Coult. & Rose	N	Mexico, Baja California del Sur, *Breedlove 43405* [=*Constance 2045* (UC)][b]
Arracacia nelsonii Coult. & Rose	N	Mexico, Oaxaca, *Breedlove 72434* [=*Constance 2410* (UC)]
Carlesia sinensis Dunn.	O	China, Cult. Hort. Nanjing; *Constance 2401* (UC)
Coaxana purpurea Coult. & Rose	N	Mexico, Oaxaca, *Breedlove 72745* [=*Constance 2411* (UC)][b]
Conium maculatum L.	N/O	Germany, Cult. Johannes Gutenberg University (no. 1099); *Downie 63* (ILL)
Coriandrum sativum L.	N/O	Germany, Cult. Johannes Gutenberg University (no. 1100); *Downie 65* (ILL)
Coulterophytum laxum Robins.	N	Mexico, Michoacán, *Iltis 298 & Cochrane*; [=*Constance 1650* (UC)]
Crithmum maritimum L.	O	Europe, Cult. UC Botanical Garden, Berkeley (no. 89.1222)[b]
Daucus carota L.	N/O	Germany, Cult. University of Oldenburg Botanic Garden (no. 547); *Downie 164* (ILL)
Enantiophylla heydeana Coult. & Rose	N	Mexico, Jalisco, Manantlán, *Iltis et al. 3187* [=*Constance 2251* (UC)]
Endressia castellana Coincy.	O	Switzerland, Cult. Inst. Bot. Univers. Neuchatel; *Constance 2184* (UC)
Heracleum lanatum Michx.	N	U.S.A., California, Muir Woods; *Downie 579* (ILL)
Heracleum rigens DC.	O	India, Karnataka, Mullengiri-Bababudan Hills, Chixmagalur District, P.K. *Mukherjee s.n.* [=*Constance 2274* (UC)][b]
Heracleum sphondylium L.	N/O	Finland; Cult. University of Kuopio Botanical Garden (no. 9); *Downie 433* (ILL)
Heteromorpha arborescens (Thunb.) Cham. & Schlechtd.	O	Spain, Cult. Real Jardín Botańico (no. 1330); *Downie 42* (ILL)
Laserpitium siler L.	O	Germany, Cult. Johannes Gutenberg University (no. 1112); *Downie 71* (ILL)
Lomatium dasycarpum (Torr. & Gray) Coult. & Rose	N	U.S.A., California, San Mateo Co., *Raiche 10396,* Cult. UC Botanical Garden, Berkeley (no. 81.1108)
Myrrhidendron donnell-smithii Coult. & Rose	N	Costa Rica, San José Prov., *Grantham and Parsons 0433-90,* Cult. UC Botanical Garden, Berkeley (no. 90.2637)
Myrrhis odorata (L.) Scop.	N/O	Europe, Cult. UC Botanical Garden, Berkeley (no. 89.1236)[b]
Orlaya grandiflora (L.) Hoffm.	O	France, Cult. Jardin Botanique de Caen (no. 1474); *Downie 309* (ILL)
Orlaya kochii Heywood	O	Germany, Cult. Akademie der Wissenschaften (no. 2/86); *Downie 20* (ILL)
Pastinaca sativa L.	N/O	Germany, Cult. Johannes Gutenberg University (no. 1597); *Downie 70* (ILL)
Pimpinella peregrina L.	O	Germany, Cult. Akademie der Wissenschaften (no. 29/90); *Downie 19* (ILL)
Pimpinella saxifraga L.	N/O	Germany, Cult. University of Oldenburg Botanic Garden (no. 19); *Downie 137* (ILL)
Prionosciadium sp.	N	Mexico, Colima, *Turner s.n.*; [=*Constance 2053* (UC)]
Pseudorlaya pumila (L.) Grande.	O	Germany, Cult. University of Oldenburg Botanic Garden (no. 20); *Downie 138* (ILL)
Rhodosciadium argutum (Rose) Math. & Const.	N	Mexico, Guanajuato, Xichu, *Rzedowski 41342*; [=*Constance 2371* (UC)]
Scandix pecten-veneris L.	N/O	Germany, Cult. Akademie der Wissenschaften (no. 2/77); *Downie 27* (ILL)
Selinum candollii DC.	O	India, Garhwal Himalaya, *Pradham s.n.,* Cult. UC Botanical Garden, Berkeley (no. 89.2000)[b]
Seseli montanum L.	O	France, Cult. Conservatoire et Jardins Botaniques de Nancy; *Downie 239* (ILL)
Smyrnium olusatrum L.	O	France, Cult. Jardin Botanique de Caen (no. 1492); *Downie 328* (ILL)
Torilis nodosa (L.) Gaertner.	N/O	France, Cult. Jardin Botanique de Caen (no. 1495); *Downie 322* (ILL)
Zizia aurea (L.) Koch.	N	Canada, Cult. Jardin Botanique de Montréal (no. 60); *Downie 8* (ILL)

[a] New World (N) or Old World (O) geographic distribution. N/O = Taxa endemic to the Old World but now introduced in the New World.
[b] Living material collected and provided to authors by Greg Plunkett (Washington State University).

primarily against the frequently cited system of Apiaceae classification proposed by Drude (1898).

MATERIALS AND METHODS

Ingroup taxa—Total genomic DNAs, representing 39 species (34 genera) of Apiaceae subfamily Apioideae, were isolated from fresh leaf material of one or, rarely, more individual plants using the modified CTAB procedure of Doyle and Doyle (1987) and further purified by centrifugation to equilibrium in cesium chloride-ethidium bromide gradients. The country of origin, accession, and/or voucher numbers, and geographic distributions of these taxa are provided in Table 2. Leaf material was either collected directly from the field, taken from flower- and fruit-bearing plants propagated from seed in the greenhouse, or obtained from accessioned plants cultivated at the University of California Botanical Garden, Berkeley. Vouchers for all but the latter were made and deposited at the University of Illinois Herbarium (ILL); additional information about these accessions is available upon request. All accessions were identified using published keys and comparison to herbarium specimens; many of the identifications were also confirmed by L. Constance. After collection, leaf material was placed in a plastic bag with a moist paper towel, transported on ice to the laboratory, and stored at −80°C until DNA extraction. These taxa were chosen for three primary reasons. (1) They represent seven of the eight tribes and eight of the ten subtribes of Apioideae recognized by Drude (Table 1) and, potentially, are maximally divergent evolutionarily within the subfamily (only the small Mediterranean and Near Eastern tribe Echinophoreae was not represented in this study). (2) They represent a geographically

diverse group, with 28 members endemic to the Old World and 11 endemic to the New World (Table 2). (3) Many were already the subject of an ongoing phylogenetic study using evidence from cpDNA with which the results from this study could be compared (S. Downie, unpublished data).

Outgroup taxa—Most authors, except Hutchinson (1973), agree that Apiaceae and Araliaceae are closely related (e.g., Dahlgren, 1980; Takhtajan, 1980; Cronquist, 1981; Thorne, 1992), and many have suggested an affinity between these taxa and Pittosporaceae (van Tieghem, 1884; Jay, 1969; Thorne, 1973, 1992; Dahlgren, 1980; Stuhlfauth et al., 1985; Anderberg, 1992; Judd, Sanders, and Donoghue, 1994). Initially, representatives of Apiaceae subfamilies Hydrocotyloideae (*Eremocharis fruticosa* and *Hydrocotyle bowlesioides*) and Saniculoideae (*Astrantia major, Hacquetia epipactis,* and *Petagnaea saniculifolia*), and allied families Araliaceae (*Aralia chinensis*) and Pittosporaceae (*Pittosporum tobira*) were selected as outgroups. Partial ITS sequences obtained for these taxa (not presented here but available upon request) could not be readily aligned with any Apioideae ITS sequence. Thus, these nonapioid taxa were excluded from the study.

Among all members of Apioideae included in this analysis, *Heteromorpha* is probably the earliest diverging lineage. Although the majority of Apioideae are characterized by a herbaceous habit, several members (e.g., some species of *Heteromorpha, Myrrhidendron,* and *Bupleurum*) are woody. Dawson (1971) has suggested that herbaceous Apioideae have likely evolved from montane tropical woody apioid ancestors. The wood anatomy of *Heteromorpha* (and *Pittosporum*) is much like that found in many Araliaceae. Most Apiaceae (including the woody *Myrrhidendron donnell-smithii*) have vessel elements with predominantly simple perforations, whereas *Heteromorpha, Pittosporum,* and many Araliaceae possess double perforations (Rodriguez, 1971). The basal position of *Heteromorpha* in Apioideae is also indicated in phylogenies based on *rbc*L and *mat*K sequences (Plunkett, Soltis, and Soltis, 1992), and cpDNA restriction site mutations and *rpo*C1 intron sequences (S. Downie, D. Katz-Downie, and K.-J. Cho, unpublished data; see Fig. 6). Consequently, the trees computed in this study were rooted by positioning the root along the branch connecting the putatively basal apioid genus *Heteromorpha arborescens* to the rest of the network.

PCR amplification and sequencing strategy—Double-stranded DNAs of the complete ITS regions in each genomic DNA (yielding a 3′ 18S–5′ 26S fragment) were PCR (polymerase chain reaction)-amplified using primers "ITS 5" and "ITS 4" (White et al., 1990; see below) in an equimolar ratio. These 100-μL PCR reactions contained (in order of addition) 59.0 μL of sterile water, 10.0 μL of 10 × *Taq* polymerase reaction buffer (Promega Corp., Madison, WI), 200 μmol/L of each dNTP (United States Biochemical Corp., Cleveland, OH), 1.5 mmol/L of $MgCl_2$, 2.0 Units of *Taq* DNA polymerase (Promega Corp., Madison, WI), 1.0 μmol/L of each primer, and a 1.0 μL aliquot of unquantified genomic (template) DNA. For some taxa, optimal amplification was achieved when the template DNA was diluted 1:100. Each PCR reaction cycle proceeded as follows: (1) 1 min at 94°C to denature the double-stranded template DNA; (2) 1 min at 53°C to anneal primers to single-stranded template DNA; and (3) 1 min at 72°C to extend primers. The first cycle was preceded by an initial denaturation step of 30 s at 94°C. To allow completion of unfinished DNA strands and to terminate the PCR reaction, a 10-min 72°C extension period followed completion of the 35 thermal cycles. Each set of reactions was monitored by the inclusion of positive (*Daucus carota* DNA) and negative (no template DNA) controls. Three to five microlitres of each double-stranded DNA PCR product was resolved by electrophoresis in a 3% agarose gel using 1 × TAE as the gel buffer. Successful PCR amplifications resulted in a single DNA band corresponding to ≈700 bp.

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 scalpel. The gel slice containing the DNA fragment was transferred to a 1.5-mL microcentrifuge tube and the DNA was recovered using the Elu-Quik DNA Purification Kit (Schleicher & Schuell, Keene, NH). The purified DNA was resuspended in 20 μL of sterile water; this volume was sufficient for two sequencing reactions. Sequencing was done using the dideoxy chain termination method employing Sequenase (Version 2.0; United States Biochemical Corp., Cleveland, OH) with α-^{35}S-dATP (Amersham) as the labeling agent. Modifications to the sequencing protocol included denaturation of the DNA by boiling the DNA/primer/acetamide mix for 4 min, followed by snap-chilling the annealing mixture for 3 min in an ice water bath. Forward primers "ITS 3," "ITS 3a," and "ITS 5" and reverse primers "ITS 2" and "ITS 4" were each used at least twice in the sequencing of each template DNA. All primer sequences, except "ITS 3a," which was constructed during the course of this investigation, were derived or modified from those described by White et al. (1990). Primers were synthesized by Operon Technologies, Alameda, CA or National Biosciences, Plymouth, MN. Primers "ITS 2" and "ITS 3" differed from those sequences reported in White et al. by the following underlined bases: 5′-GCT*A*CGTTCTTCATCGATGC-3′ and 5′-GCATCGATGAAGAACG*T*AGC-3′, respectively. Primer "ITS 5" differed by two italicized bases (5′-GGAAG*GA*GAAGTCGTAACAAGG-3′) and primer "ITS 4" was synthesized as reported (5′-TCCTTCCGCTTATTGATATGC-3′). These modifications were based on the availability of complete sequences for the ITS regions of *Daucus carota* (Yokota et al., 1989) and 18S sequences for *Hydrocotyle sibthorpioides* (Apiaceae subfamily Hydrocotyloideae) and *Hedera helix* (Araliaceae; Nickrent and Franchina, 1990). Primer "ITS 3a," having the sequence 5′-ACGTCTGCCTGGGTGTCAC-3′, was constructed to anneal to the 3′ end of the gene 5.8S rDNA and facilitated sequencing of the ITS 2 region.

In addition to second-strand sequencing, ambiguities were resolved using 7-deaza-dGTP or dITP in place of dGTP to prevent base compressions and hard stops, according to reaction conditions specified by the manufacturers. Reactions were separated electrophoretically in 6% polyacrylamide gels in which the xylene cyanole dye marker was run 30 cm (for a short gel) or 90 cm (for a long gel), so the entire ITS 1 or ITS 2 region could be read on both gels. Gels were dried onto Whatman 3MM paper in a vacuum dryer and then exposed to X-ray film (Kodak XAR) for 2–4 d at room temperature.

Sequence analysis—Boundaries of the coding (3′18S, 5.8S, and 5′26S rDNA) and spacer regions were determined by comparison of the DNA sequences to the corresponding boundaries in the consubfamilial *Daucus carota,* which have been defined by S1 nuclease mapping (Yokota et al., 1989). Only the ITS 1 and ITS 2 regions were included in the analysis since sequence data for the 5.8S subunit were incomplete for many taxa and those that were available were not sufficiently variable to warrant additional sequencing. DNA sequences were aligned using CLUSTAL (Higgins, Bleasby, and Fuchs, 1992) and the PILEUP program of the Genetics Computer Group (GCG) Sequence Analysis Software Package (version 7, Devereux, Haeberli, and Smithies, 1984) on a VAX computer system operated by the University of Illinois; however, a minimal amount of manual adjustment was necessary. 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 analyses, all gaps were treated as missing data. The G+C content was calculated manually for each region in each species. Transition/transversion ratios were calculated using MacClade version 3 (Maddison and Maddison, 1992). The 39 sequences reported in this study are available from GenBank (Fig. 1), but both aligned and unaligned sequences can also be obtained from the authors.

Phylogenetic analysis—The resulting data matrix (excluding ambiguous characters), together with the published ITS sequences of *Daucus*

carota (Yokota et al., 1989), was analyzed by assuming unordered character states (i.e., Fitch parsimony) using PAUP version 3.1.1 (Swofford, 1993) run on either a Macintosh Quadra 700 or Power Macintosh 8100 computer. All HEURISTICS searches were replicated 500 times with RANDOM addition sequence and TREE BISECTION-RECONNECTION (TBR) branch swapping. The options MULPARS, STEEPEST DESCENT, COLLAPSE, and ACCTRAN optimization were selected. Initially, all searches were performed using equal character weighting. Bootstrap (Felsenstein, 1985) and decay (Bremer, 1988; Donoghue et al., 1992) analyses were performed using PAUP to assess the degree of support for particular branches on the strict consensus tree. Bootstrap values were calculated from 100 replicate analyses using the HEURISTICS search strategy and SIMPLE addition sequence of the taxa. The decay index for individual clades was calculated by examining the strict consensus of all equal-length trees up to two steps longer than the shortest trees (using RANDOM addition sequence and TBR). Decay analyses with tree lengths equal to or greater than three steps longer than the most parsimonious could not be done because of computational constraints. The number of additional steps required to force particular taxa into a monophyletic group was examined using the CONSTRAINTS option of PAUP. The amount of phylogenetic information in the parsimony analyses was estimated using the consistency index (Kluge and Farris, 1969), retention index (Farris, 1989), and g_1 statistic (Hillis, 1991; Hillis and Huelsenbeck, 1992). The g_1 statistic was achieved by calculating the tree-length distribution of 10000 random parsimony trees using PAUP's RANDOM TREES selection, and was used to assess the amount of nonrandom structure in the data. Additional parsimony analyses using separate ITS 1 and ITS 2 data sets were conducted to assess the relative contributions of each spacer region to phylogenetic resolution in Apioideae. Due to limitations imposed by the data sets, the HEURISTICS search strategy with the SIMPLE addition sequence of the taxa was selected.

Gaps in the multiple alignment were incorporated into the parsimony analysis in one of two ways. First, gap positions were scored as missing data and each insertion/deletion (indel) was subsequently superimposed on one of the resulting maximally parsimonious cladograms in order to test its phylogenetic congruence with the phylogeny constructed on the basis of nucleotide substitutions. Second, each indel was scored and entered as a separate presence/absence character, while still treating gap positions as missing data (Swofford, 1993). This option, however, may actually decrease the number of equally parsimonious trees because of the redundancy involved in having two sets of scored characters for the same indel events (Wojciechowski et al., 1993).

Character-state weighted parsimony analysis, in which transversions were weighted over transitions by factors of 1.1:1, 1.4:1, or 2.5:1 using PAUP's USERTYPE STEPMATRIX command, was also implemented. In these analyses, HEURISTIC searches were conducted using SIMPLE addition and TBR branch-swapping. These methods allow for the correction of multiple substitutions and differential transition/transversion probability based on empirical observation from the data. The ratio of 1.4:1 was selected based on the actually observed frequencies in the maximally parsimonious trees; the other two ratios were arbitrarily chosen because they simply bracket this value.

In addition to parsimony analysis, distance trees were calculated using the neighbor-joining method of tree construction (Saitou and Nei, 1987), implemented using the NEIGHBOR program in Felsenstein's (1993) phylogeny inference package (PHYLIP version 3.5). Unfortunately, this method yields only a single tree and does not allow for the examination of multiple best fit trees nor for the examination of close but lesser-fitting phylogenetic hypotheses. Distance matrices were calculated using the DNADIST program of PHYLIP and the numbers of nucleotide substitutions (excluding gaps) were estimated using either the two-parameter method of Kimura (1980) or the one-parameter method of Jukes and Cantor (1969). Transversions were weighted relative to transitions, with a transition/transversion ratio of 1.4 obtained from the parsimony analysis used to construct the neighbor-joining tree. Additional weights of 1.1 and 1.8 for transitions/transversions were also used. A bootstrap analysis of these data was carried out 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 implemented to construct a strict consensus tree.

Maximum likelihood phylogeny estimation was explored utilizing the fastDNAML program (version 1.0.6; Olsen et al., 1992, 1994) based on the procedures of Felsenstein (1981). A maximum likelihood tree was inferred using a transition/transversion ratio of 2.0, randomizing the sequence addition order (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

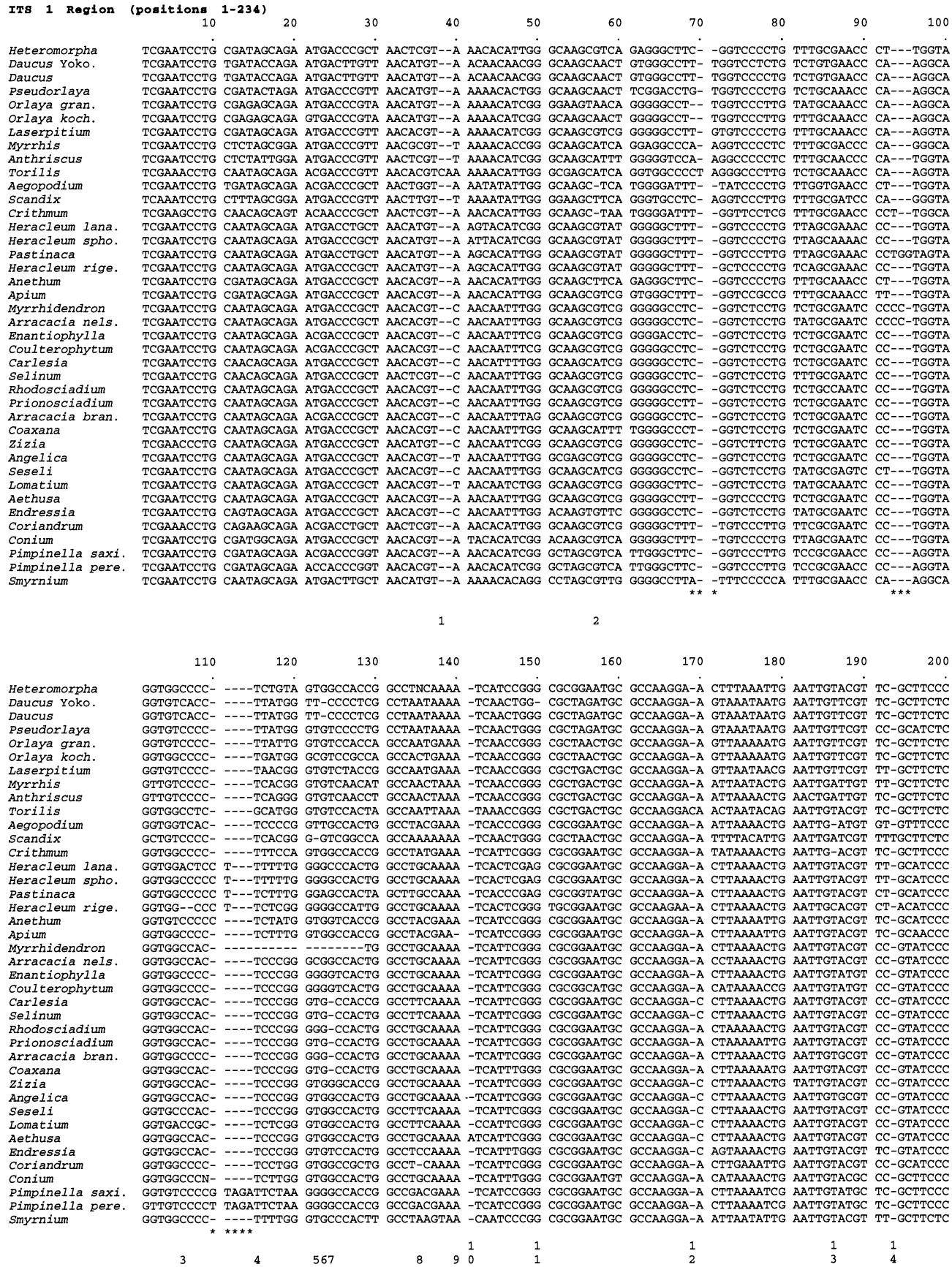
Sequence analysis—Complete and aligned DNA sequences of ITS 1 and ITS 2 for 40 taxa of Apiaceae subfamily Apioideae (including the published ITS sequences for *Daucus carota* [Yokota et al., 1989]) are provided in Fig. 1 and their characteristics summarized in Table 3. On average, ITS 2 is longer than ITS 1 by ≈6 bp.

Despite using dGTP analogues and carrying out second-strand sequencing, two areas within ITS 1 and ITS 2 were especially difficult to resolve (likely due to secondary structure derived from the high G+C content in these areas). Therefore, it is entirely plausible that the same bases may have been compressed in both directions. These compressions occurred in the multiple alignment (Fig. 1) between positions 122 and 127 (in ITS 1) and between positions 286 and 292 (in ITS 2). The first of these coincides precisely with a problematic region previously reported in *Lomatium* ITS sequences (Soltis and Kuzoff, 1993).

Proper alignment of ITS 1 and ITS 2 sequences required the introduction of 31 gaps: 21 of which were 1 bp in length, seven 2 bp in length, two 3 bp in length, and one 14 bp in length (Fig. 1). These inferred gaps were approximately equally distributed in both ITS

→

Fig. 1. Aligned DNA sequences of the ITS regions in 18S-26S nuclear ribosomal DNA from 40 representatives of Apiaceae subfamily Apioideae. Nucleotide sites are numbered 5′ to 3′ from the 18S subunit-ITS 1 boundary to the ITS 2-26S subunit boundary. The ITS 1 region ranges from position 1 to 234; the ITS 2 region extends from position 235 to 469. Sequences of the 5.8S subunit are excluded. A,C,G,T = dATP, dCTP, dGTP, dTTP, respectively; N = uncertain nucleotide state; hyphens = gaps required for alignment. Asterisks below the alignment denote ambiguous regions excluded from sequence divergence calculations and the phylogenetic analyses. Numbers, ranging from 1 to 31 and arranged vertically below the multiple alignment, identify the location of gaps used in the phylogenetic analysis; gaps of two base pairs or more in length are identified at their first position only. Complete taxon names are provided in Table 2. These data have been deposited with GenBank as separate ITS1 and ITS2 sequences under accession numbers U27578 and U30314 (*Heteromorpha*), U27589 and U30315 (*Daucus*), and U30522–U30595 (for all other taxa in their order presented). ITS sequences from *Daucus* Yoko. were obtained from Yokota et al., 1989.


ITS 1 Region (positions 1-234)
Heteromorpha TCGAATCCTG CGATAGCAGA ATGACCCGCT AACTCGT--A AACACATTGG GCAAGCGTCA GAGGGCTTC- -GGTCCCCTG TTTGCGAACC CT---TGGTA
Daucus Yoko. TCGAATCCTG TGATACCAGA ATGACTTGTT AACATGT--A ACAACAACGG GCAAGCAACT GTGGGCCTT- TGGTCCTCTG TCTGTGAACC CA---AGGCA
Daucus TCGAATCCTG TGATACCAGA ATGACTTGTT AACATGT--A ACAACAACGG GCAAGCAACT GTGGGCCTT- TGGTCCCCTG TCTGTGAACC CA---AGGCA
Pseudorlaya TCGAATCCTG CGATACTAGA ATGACCCGTT AACATGT--A AAAACACTGG GCAAGCAACT TCGGACCTG- TGGTCCCCTG TCTGCAAACC CA---AGGCA
Orlaya gran. TCGAATCCTG CGAGAGCAGA ATGACCCGTA AACATGT--A AAAACATCGG GGAAGTAACA GGGGGCCT-- TGGTCCCTTG TATGCAAACC CA---AGGCA
Orlaya koch. TCGAATCCTG CGAGAGCAGA GTGACCCGTA AACATGT--A AAAACATCGG GCAAGCAACT GGGGGCCT-- TGGTCCCTTG TTTGCAAACC CA---AGGCA
Laserpitium TCGAATCCTG CGATAGCAGA ATGACCCGTT AACACGT--A AAAACATCGG GCAAGCGTCG GGGGGCCTT- GTGTCCCCTG TTTGCAAACC CA---AGGTA
Myrrhis TCGAATCCTG CTCTAGCGGA ATGACCCGTT AACGCGT--T AAAACACCGG GCAAGCATCA GGAGGCCCA- AGGTCCCCTC TTTGCGACCC CA---GGGCA
Anthriscus TCGAATCCTG CTCTATTGGA ATGACCCGTT AACTCGT--T AAAACATCGG GCAAGCATTT GGGGGTCCA- AGGCCCCCTC TTTGCAACCC CA---TGGTA
Torilis TCGAAACCTG CAATAGCAGA ACGACCCGTT AACACGTCAA AAAACATTGG GCGAGCATCA GGTGGCCCCT AGGGCCCTTG TCTGCAAACC CA---AGGTA
Aegopodium TCGAATCCTG TGATAGCAGA ACGACCCGCT AACTGGT--A AATATATTGG GCAAGC-TCA TGGGGATTT- -TATCCCCTG TTGGTGAACC CT---TGGTA
Scandix TCAAATCCTG CTTTAGCGGA ATGACCCGTT AACTTGT--T AAAATATTGG GGAAGCTTCA GGGTGCCTC- AGGTCCCTTG TTTGCGATCC CA---GGGTA
Crithmum TCGAAGCCTG CAACAGCAGT ACAACCCGCT AACTCGT--A AACACATTGG GCAAGC-TAA TGGGGATTT- -GGTTCCTCG TTTGCGAACC CCT--TGGCA
Heracleum lana. TCGAATCCTG CAATAGCAGA ATGACCTGCT AACATGT--A AGTACATCGG GCAAGCGTAT GGGGGCTTT- -GGTCCCCTG TTAGCGAAAC CC---TGGTA
Heracleum spho. TCGAATCCTG CAATAGCAGA ATGACCCGCT AACATGT--A ATTACATCGG GCAAGCGTAT GGGGGCTTT- -GGTCCCCTG TTAGCAAAAC CC---TGGTA
Pastinaca TCGAATCCTG CAATAGCAGA ATGACCTGCT AACATGT--A AGCACATTGG GCAAGCGTAT GGGGGCTTT- -GGTCCCTTG TTAGCGAAAC CCTGGTAGTA
Heracleum rige. TCGAATCCTG CAATAGCAGA ATGACCCGCT AACATGT--A AGCACATTGG GCAAGCGTAT GGGGGCTTT- -GCTCCCCTG TCAGCGAAAC CC---TGGTA
Anethum TCGAATCCTG CGATAGCAGA ATGACCCGCT AACACGT--A AACACATTGG GCAAGCTTCA GAGGGCTTC- -GGTCCCCTG TTTGCAAACC CT---TGGTA
Apium TCGAATCCTG CGATAGCAGA ATGACCCGCT AACACGT--A AACACATTGG GCAAGCGTCG GTGGGCTTT- -GGTCCGCCG TTTGCAAACC TT---TGGTA
Myrrhidendron TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--C AACAATTTGG GCAAGCGTCG GGGGGCCTC- -GGTCTCCTG TCTGCGAATC CCCC-TGGTA
Arracacia nels. TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--C AACAATTTGG GCAAGCGTCG GGGGGCCTC- -GGTCTCCTG TATGCGAATC CCCC-TGGTA
Enantiophylla TCGAATCCTG CAATAGCAGA ACGACCCGCT AACACGT--C AACAATTTCG GCAAGCGTCG GGGGACCTC- -GGTCTCCTG TCTGCGAATC CC---TGGTA
Coulterophytum TCGAATCCTG CAATAGCAGA ACGACCCGCT AACACGT--C AACAATTTCG GCAAGCGTCG GGGGGCCTC- -GGTCTCCTG TCTGCGAATC CC---TGGTA
Carlesia TCGAATCCTG CAACAGCAGA ATGACCCGCT AACACGT--C AACATTTTGG GCAAGCATCG GGGGGCCTC- -GGTCTCCTG TCTGCGAATC CC---TGGTA
Selinum TCGAATCCTG CAACAGCAGA ATGACCCGCT AACTCGT--C AACAATTTGG GCAAGCGTCG GGGGGCCTC- -GGTCTCCTG TTTGCGAATC CC---TGGTA
Rhodosciadium TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--C AACAATTTGG GCAAGCGTCG GGGGGCCTC- -GGTCTCCTG TCTGCCAATC CC---TGGTA
Prionosciadium TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--C AACAATTTGG GCAAGCGTCG GGGGGCCTT- -GGTCTCCTG TCTGCGAATC CC---TGGTA
Arracacia bran. TCGAATCCTG CAATAGCAGA ACGACCCGCT AACACGT--C AACAATTTAG GCAAGCGTCA GGGGGCCTC- -GGTCTCCTG TCTGCGAATC CC---TGGTA
Coaxana TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--C AACAATTTGG GCAAGCATTT TGGGGCCCT- -GGTCTCCTG TCTGCGAATC CC---TGGTA
Zizia TCGAACCCTG CAATAGCAGA ATGACCCGCT AACATGT--C AACAATTCGG GCAAGCGTCG GGGGGCCTC- -GGTCTTCTG TCTGCGAATC CC---TGGTA
Angelica TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--T AACAATTTGG GCGAGCGTCG GGGGGCCTC- -GGTCTCCTG TCTGCGAATC CC---TGGTA
Seseli TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--C AACAATTTGG GCAAGCATCG GGGGGCCTC- -GGTCTCCTG TATGCGAGTC CT---TGGTA
Lomatium TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--T AACAATCTGG GCAAGCGTCG GGGGGCTTC- -GGTCTCCTG TATGCAAATC CC---TGGTA
Aethusa TCGAATCCTG CAATAGCAGA ATGACCCGCT AACACGT--A AACAATTTGG GCAAGCGTCG GGGGGCCTT- -GGTCCCCTG TCTGCGAATC CC---TGGTA
Endressia TCGAATCCTG CAGTAGCAGA ATGACCCGCT AACACGT--C AACAATTTGG ACAAGTGTTC GGGGGCCTC- -GGTCTCCTG TATGCGAATC CC---TGGTA
Coriandrum TCGAAACCTG CAGAAGCAGA ACGACCTGCT AACTCGT--A AACACATTGG GCAAGCGTCG GGGGGCTTTT -TGTCCCTTG TTCGCGAATC CC---TGGTA
Conium TCGAATCCTG CGATGGCAGA ATGACCCGCT AACACGT--A TACACATCGG ACAAGCGTCA GGGGGCTTT- -TGTCCCCTG TTAGCGAATC CC---TGGTA
Pimpinella saxi. TCGAATCCTG CGATAGCAGA ACGACCCGGT AACACGT--A AACACATCGG GCTAGCGTCA TTGGGCTTC- -GGTCCCTTG TCCGCGAACC CC---AGGTA
Pimpinella pere. TCGAATCCTG CGATAGCAGA ACCACCCGGT AACACGT--A AACACATCGG GCTAGCGTCA TTGGGCTTC- -GGTCCCTTG TCCGCGAACC CC---AGGTA
Smyrnium TCGAATCCTG CAATAGCAGA ATGACTTGCT AACATGT--A AAAACACAGG CCTAGCGTTG GGGGCCTTA- -TTTCCCCCA TTTGCGAACC CA---AGGCA
** * ***
1 2
GGTGGCCCC- ----TCTGTA GTGGCCACCG GCCTNCAAAA -TCATCCGGG CGCGGAATGC GCCAAGGA-A CTTTAAATTG AATTGTACGT TC-GCTTCCC
Daucus Yoko. GGTGTCACC- ----TTATGG TT-CCCCTCG CCTAATAAAA -TCAACTGG- CGCTAGATGC GCCAAGGA-A GTAAATAATG AATTGTTCGT TC-GCTTCTC
Daucus GGTGTCACC- ----TTATGG TT-CCCCTCG CCTAATAAAA -TCAACTGGG CGCTAGATGC GCCAAGGA-A GTAAATAATG AATTGTTCGT TC-GCTTCTC
Pseudorlaya GGTGTCCCC- ----TTATGG GTGTCCCCTG CCTAATAAAA -TCAACTGGG CGCTAGATGC GCCAAGGA-A GTAAATAATG AATTGTTCGT CC-GCATCTC
Orlaya gran. GGTGTCCCC- ----TTATTG GTGTCCACCA GCCAATGAAA -TCAACCGGG CGCTAACTGC GCCAAGGA-A GTTAAAAATG AATTGTTCGT TC-GCTTCTC
Orlaya koch. GGTGGCCCC- ----TGATGG GCGTCCGCCA GCCACTGAAA -TCAACCGGG CGCTAACTGC GCCAAGGA-A GTTAAAAATG AATTGTTCGT TC-GCTTCTC
Laserpitium GGTGTCCCC- ----TAACGG GTGTCTACCG GCCAATGAAA -TCAACCGGG CGCTGACTGC GCCAAGGA-A GTTAATAACG AATTGTTCGT TT-GCTTCTC
Myrrhis GTTGTCCCC- ----TCACGG GTGTCAACAT GCCAACTAAA -TCAACCGGG CGCTGACTGC GCCAAGGA-A ATTAATACTG AATTGATTGT TT-GCTTCTC
Anthriscus GTTGTCCCC- ----TCAGGG GTGTCAACCT GCCAACTAAA -TCAACCGGG CGCTGACTGC GCCAAGGA-A ATTAAAACTG AACTGATTGT TC-GCTTCTC
Torilis GGTGGCCTC- ----GCATGG GTGTCCACTA GCCAATTAAA -TAAACCGGG CGCTGACTGC GCCAAGGACA ACTAATACAG AATTGTACGT TC-GCTTCTC
Aegopodium GGTGGTCAC- ----TCCCCG GTTGCCACTG GCCTACGAAA -TCACCCGGG CGCGGAATGC GCCAAGGA-A ATTAAAACTG AATTG-ATGT GT-GTTTCCC
Scandix GCTGTCCCC- ----TCACGG G-GTCGGCCA GCCAAAAAAA -TCAACTGGG CGCTAACTGC GCCAAGGA-A TTTTACATTG AATTGATCGT TTTGCTTCTC
Crithmum GGTGGCCCC- ----TTTCCA GTGGCCACCG GCCTATGAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-A TATAAAACTG AATTG-ACGT TC-GCTTCCC
Heracleum lana. GGTGGACTCC T---TTTTTG GGGCCCACTG GCCTGCAAAA -TCACTCGAG CGCGGAATGC GCCAAGGA-A CTTAAAACTG AATTGTACGT TT-GCATCCC
Heracleum spho. GGTGGCCCCC T---TTTTTG GGGGCCACTG GCCTGCAAAA -TCACTCGAG CGCGGAATGC GCCAAGGA-A CTTAAAACTG AATTGTACGT TT-GCATCCC
Pastinaca GGTGGCCCCC T---TCTTTG GGAGCCACTA GCTTGCCAAA -TCACCCGAG CGCGGTATGC GCCAAGGA-A CTTAAAACTG AATTGTACGT CT-GCATCCC
Heracleum rige. GGTGG--CCC T---TCTCGG GGGGCCATTG GCCTGCAAAA -TCACTCGGG TGCGGAATGC GCCAAGAA-A CTTAAAACTG AATTGCACGT CT-ACATCCC
Anethum GGTGTCCCCC ----TCTATG GTGGTCACCG GCCTACGAAA -TCATCCGGG CGCGGAATGC GCCAAGGA-A CTTAAAATTG AATTGTACGT TC-GCATCCC
Apium GGTGGCCCC- ----TCTTTG GTGGCCACCG GCCTACGAA- -TCATCCGGG CGCGGAATGC GCCAAGGA-A CTTAAAATTG AATTGTACGT TC-GCAACCC
Myrrhidendron GGTGGCCAC- ---------- --------TG GCCTGCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-A CTTAAAACTG AATTGTACGT CC-GTATCCC
Arracacia nels. GGTGGCCAC- ----TCCCGG GCGGCCACTG GCCTGCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-A CCTAAAACTG AATTGTACGT CC-GTATCCC
Enantiophylla GGTGGCCCC- ----TCCCGG GGGGTCACTG GCCTGCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-A CTTAAAACTG AATTGTATGT CC-GTATCCC
Coulterophytum GGTGGCCCC- ----TCCCGG GGGGTCACTG GCCTGCAAAA -TCATTCGGG CGCGGCATGC GCCAAGGA-A CATAAAACCG AATTGTATGT CC-GTATCCC
Carlesia GGTGGCCAC- ----TCCCGG GTG-CCACCG GCCTTCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-C CTTAAAACTG AATTGTACGT CC-GTATCCC
Selinum GGTGGCCAC- ----TCCCGG GTG-CCACTG GCCTTCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-C CTTAAAACTG AATTGTACGT CC-GTATCCC
Rhodosciadium GGTGGCCAC- ----TCCCGG GGG-CCACTG GCCTGCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-A CTAAAAACTG AATTGTACGT CC-GTATCCC
Prionosciadium GGTGGCCAC- ----TCCCGG GTG-CCACTG GCCTGCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-A CTAAAAATTG AATTGTACGT CC-GTATCCC
Arracacia bran. GGTGGCCCC- ----TCCCGG GGG-CCACTG GCCTGCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-A CTTAAAACTG AATTGTGCGT CC-GTATCCC
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Zizia GGTGGCCAC- ----TCCCGG GTGGGCACCG GCCTGCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-C CTTAAAACTG TATTGTACGT CC-GTATCCC
Angelica GGTGGCCAC- ----TCCCGG GTGGCCACTG GCCTGCAAAA --TCATTCGGG CGCGGAATGC GCCAAGGA-C CTTAAAACTG AATTGTGCGT CC-GTATCCC
Seseli GGTGCCCAC- ----TCCCGG GTGGCCACTG GCCTTCAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-C CTTAAAACTG AATTGTACGT CC-GTATCCC
Lomatium GGTGACCGC- ----TCTCGG GTGGCCACTG GCCTTCAAAA -CCATTCGGG CGCGGAATGC GCCAAGGA-C CTTAAAACTG AATTGTACGT CC-GTATCCC
Aethusa GGTGGCCAC- ----TCCCGG GTGGCCACTG GCCTGCAAAA ATCATTCGGG CGCGGAATGC GCCAAGGA-C CTTAAAACTG AATTGTACGT CC-GTATCCC
Endressia GGTGGCCAC- ----TCCCGG GTGTCCACTG GCCTCCAAAA -TCATTTGGG CGCGGAATGC GCCAAGGA-C AGTAAAACTG AATTGTACGT TC-GTATCCC
Coriandrum GGTGGCCCC- ----TCCTGG GTGGCCGCTG GCCT-CAAAA -TCATTCGGG CGCGGAATGC GCCAAGGA-A CTTGAAATTG AATTGTACGT CC-GCATCCC
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Pimpinella pere. GTTGTCCCCT TAGATTCTAA GGGGCCACCG GCCGACGAAA -TCATCCGGG CGCGGAATGC GCCAAGGA-A CTTAAAATCG AATTGTATGC TC-GCTTCCC
Smyrnium GGTGGCCCC- ----TTTTGG GTGCCCACTT GCCTAAGTAA -CAATCCCGG CGCGGAATGC GCCAAGGA-A ATTAATATTG AATTGTACGT TT-GCTTCTC
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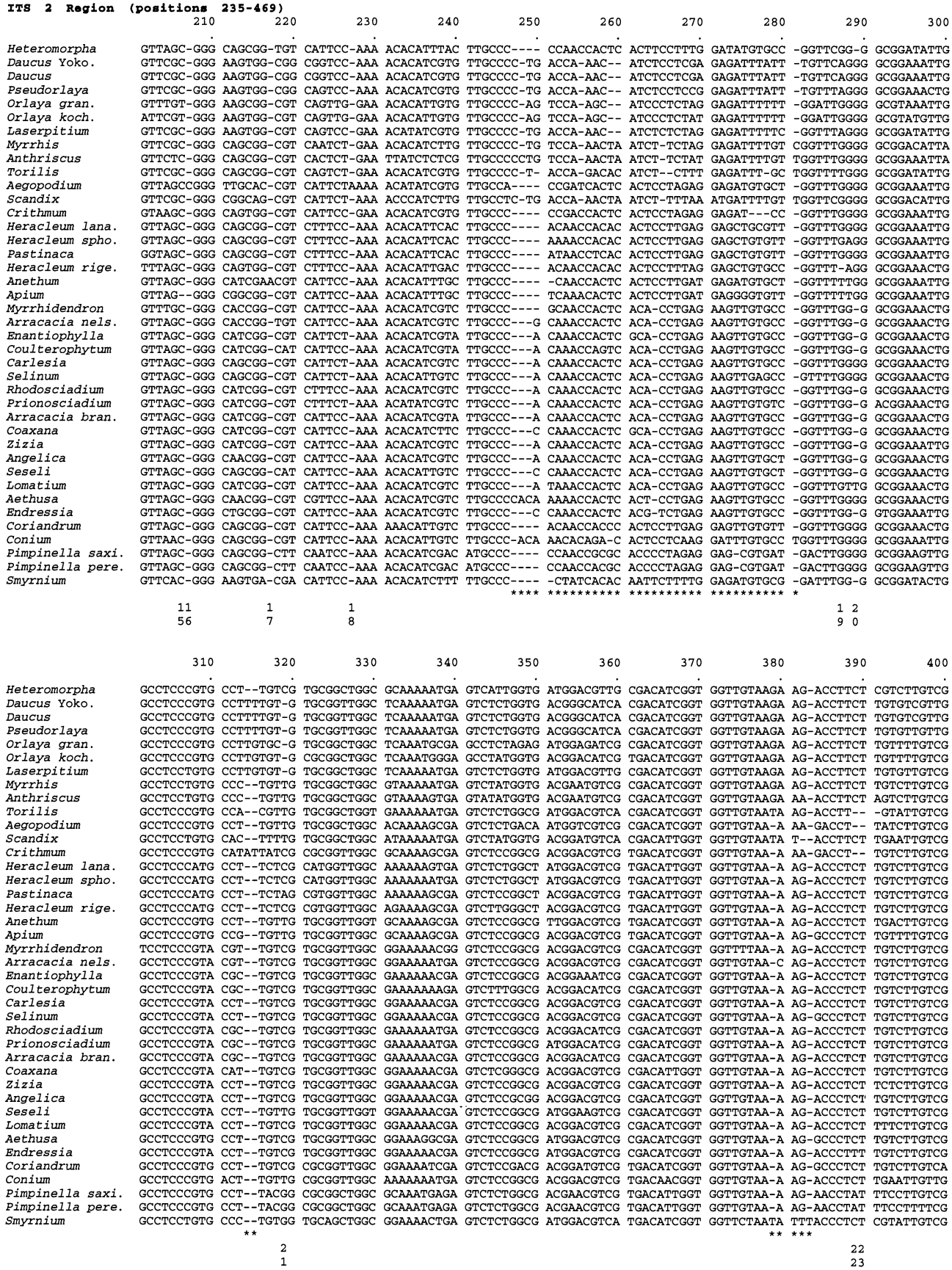

Fig. 1. Continued.

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                          410        420        430        440        450        460        469
                           .          .          .          .          .          .          .
Heteromorpha      TGTGAATGCC CGTCACCTTA GTCG-GCTCA AGGACCCT-T AGGCGC--CA CAACCTCTGT GTGCTTCGA  [438]
Daucus Yoko.      TGT--ATACC CGCCGCAGTA GGGA-ACTCG AGGGCCCT-T GGGCACAGCA AAAATTGTGT GCACTTCGA  [439]
Daucus            TGT--ATACC CGCCGCAGTA GGGA-ACTCG AGGGCCCT-T GGGCACAGCA AAAATTGTGT GCACTTCGA  [440]
Pseudorlaya       TGT--ATGCC CGCCACAGTA GGGA-ACTCG AGGGCCCT-T GGGCACTACA AGAATTGTGT GCACTTCGA  [441]
Orlaya gran.      TGT--ATGGC CGTCACCTTA GTTT-GCTCG AGGGCCCT-A TGGCACCACA AAA--TGTGT GCGCTTCAA  [438]
Orlaya koch.      TGC--GTGGC CGTCACCTTA GTTT-GCTCG AGGGCCCT-T TGGCACCATA AAA--TGTGT GTGCTTCAA  [438]
Laserpitium       TGT--ATGCC CGTCACCTCA GTCA-GCTTA AGGGCCCT-T AGGCGCAACA AAA--TGTGT GCGCTTCGA  [439]
Myrrhis           TGTGAATGTC CGTCATCTTA GAAC-GCTCA GTGACCCT-T AGCCGC--CA AAAACTTTTG GCGCTTCGA  [442]
Anthriscus        TGTGAATGTT TGTCATTTTA TAAC-GCTCA ATGACCCT-T AGGTGC--CA AAACCTTTTG GCCCTTCGA  [443]
Torilis           TGTGGAAGCC CATCCCCTCA GTTA-GCTCA AGGACCCT-T AGGCGC--CA CGAATCGTGT GCGCTTCGA  [440]
Aegopodium        CGCGAATCCC TGTCACCTTA GAGA-GCTCT AGGATCCT-T AGGCGC--CA CCCATTGTGT GCGCTTTGA  [436]
Scandix           TGTCTAAGTC TGTCACTTTA GTAAAGCTCA ATGACCCT-T AGGTGC--CA AAAACTTTTG GTGCATCTA  [442]
Crithmum          CGCGAATCCG GGTCAGGTTG GTGA-GCTCG AGGACCCT-T AGG-----CA CACATTGTGT GCGCTTCGA  [431]
Heracleum lana.   GGCGAATCCG GGTCATCTTA ACGA-GCTCC AGGACCCT-T AGGCGG--CA CACATTGTGT GCGCTTCGA  [440]
Heracleum spho.   GGCGAATCCG GGTCATCTTA ACGA-GCTCC AGGACCCT-T AGGCGG--CA CACATTGTGT GCGCTTCGA  [440]
Pastinaca         GGCGAATCCG GGTCATCTTA GCGA-GCTCC AGGACCCT-T AGGCGG--CG CACACAATGT GCGCTTCGA  [443]
Heracleum rige.   GGCGTATTCG GATCATCTTA TCGA-GCTCC AGGACCCT-T AGGCGG--CA CACATTGTGT GCGCTTCGA  [437]
Anethum           CACGAATCCT CGTCATCTAA GTGA-GCTCT AGGACCCT-T GGGCGC--TA CACAATCTGT TTGCCCTAA  [439]
Apium             CACGTAATTG TGTCATCTAA G-GA-GCTCG AGGACCCT-G AGGCGC--TA CACAATTTGT TCGCTTTAA  [435]
Myrrhidendron     CGCAAATCCT CGTCATTTTA GCGA-GCTCC AGGGCCCT-T AGGCGG--CA CACACTCTAT GCGCTTCGA  [424]
Arracacia nels.   CGCAAATCCT CGTCATTTTA GCGA-GCTCC AGGACCCT-T AGGCAG--CA CACACTGTGT GCGCTTCGA  [439]
Enantiophylla     TGCAAATCCT CGTAATTTTA GAGA-GCTCC GGGACCCT-T AGGCAG--CA CACTCTTTGT GCGCTTCGA  [437]
Coulterophytum    TGCAAATCCT CGTCATTTTA GAGA-GCTCC GGGACCCT-T AGGCAG--CA CACTCTCTGT GCGCTTCGA  [437]
Carlesia          CGCGAATCCT CGTCATCTTA GAGA-GCTCC AGGACCCT-T AGGCAG--CA CACACTCTGT GCGCTTCGA  [437]
Selinum           TGCGAATCCT CGTCATCTTA GAGA-GCTCC TGGACCCT-T AGGCAG--CA CACACTCTGT GCGCTTCGA  [437]
Rhodosciadium     TGCAAATCCT CGTCATTTTA GAGA-GCTCC AGGACCCT-T AGGCAG--CA CACACTCTGT GCGCTTCGA  [436]
Prionosciadium    TGCAAATCCT CGTCATTTTA GAGA-GCTAC GGGACCCT-T AGGCAG--CA CACACTCTGT GCGCTTCGA  [436]
Arracacia bran.   TGCAAATCCT CGTCATTTTA GAGA-GCTCC AGGACCCT-T CGGCAG--CA CACTCTCTGT GCGCTTTGA  [436]
Coaxana           TGTAAATCCT CGTCATTTTA GCGA-GCTCC AGGACCCT-T AGGCAG--CA CACACTCTGT GCGCTTCGA  [436]
Zizia             CGCGAATCCT CGTCATCTTA GCGA-GCTCC AGGACCCT-T AGGCAG--CA CACACTCTGT GCGCTTCGA  [437]
Angelica          CGCGAATCCT CGTCATCTTA GCGA-GCTCC AGGACCCT-T AGGCAG--CA CACACTCTGT GCGCTTCGA  [437]
Seseli            TGCGAATCCT CGTCATCTTA GCGA-GCTCC AGGACCCT-T AGGCAG--CA CACACTCTGT GCGCTTCGA  [437]
Lomatium          CGCGAATCCT CGTCATCTTA GCGA-GCTCC AGGACCCT-T AGGCAG--CA CACACCCTGT GCGCTTGGA  [438]
Aethusa           CGCGAATCCT CGTCATCTTA GCGA-GCTCC AGGACCCT-T AGGCGG--CA CACACTCTGT GCGCTTCGA  [442]
Endressia         TGCGAATCCT CGTCATCTTA GCGA-GCTCT AGGACCCA-T AGGCAG--CA CACACTGTGT GCGCTTTGA  [437]
Coriandrum        CGCGAATCCT AGTCATCTTA GCGA-GCTCC AGGACCCT-T AGGCG---CA CACACTCTGT GCGCTTTGA  [436]
Conium            CGCGAATCCG CGTCATCTTA GCGA-GCTCT AGGACCCT-T AGGCGA--CA CACACTCTGT GCGCTTCAA  [441]
Pimpinella saxi.  CGCGTATCC- -GTCATCTCT TAGA-GCTCT AGGACCCTCT TGGCGG--CA CACATTCTGT GCGCTCCGA  [441]
Pimpinella pere.  CGCGTATCC- -GTCATCTCT TAGA-GCTCT AGGACCCTCT TGGCGG--CA CACATTCTGT GCGCTCCGA  [441]
Smyrnium          TGTAAATGTT TGTCGCCTTA GTCA-GCTCA AGGACCCT-T AGGTGC--CA CAAATTGTGT GCGCTTTGA  [438]

                    2     2            2  2            2     2  3       3
                    4     5            6  7            8     9  0       1
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Fig. 1. Continued.

regions. Of these 31 gaps, 12 were potentially informative for phylogenetic analysis; the remainder were autapomorphic. No evidence of obvious ITS length variants within each accession examined was detected.

Alignment of all ITS 1 and ITS 2 sequence positions resulted in a matrix of 469 characters. Of these, it was necessary to delete 11 positions from ITS 1 and 42 positions from ITS 2 because of alignment ambiguities (identified by asterisks in Fig. 1). Of the remaining 416 unambiguously aligned nucleotide sites, 229 (55.0%) of these had at least two nucleotide states in two or more sequences and were potentially informative phylogenetically, 129 sites (31.0%) were unvarying, and 58 sites (13.9%) were unique to individual taxa (Table 3). Although the ITS 1 region was, on average, slightly smaller in length than ITS 2, and a greater proportion of ITS 2

TABLE 3. Sequence characteristics of the two internal transcribed spacer regions, separately and combined, in 40 taxa of Apiaceae subfamily Apioideae. Sites refer to those aligned nucleotide positions in Fig. 1.

	ITS 1	ITS 2	Combined (ITS 1 and ITS 2)
Length range (bp)	204–221	216–226	424–443
Length mean (bp)	216.3	221.8	438.0
Aligned length (bp)	234	235	469
G + C content range (%)	49.1–57.7	42.7–59.6	46.6–58.1
G + C content mean (%)	54.2	54.3	54.3
Sequence divergence (%)	0.5–33.2	0.0–33.2	0.2-30.7
Number of excluded sites (%)	11 (4.7)	42 (17.9)	53 (11.3)
Number of indels	18	13	31
Number of variable sites	152	135	287
Number of potentially informative sites (%)	121 (54.3)	108 (56.0)	229 (55.0)
Number of constant sites (%)	71 (31.8)	58 (30.1)	129 (31.0)
Number of autapomorphic sites (%)	31 (13.9)	27 (14.0)	58 (13.9)
Transitions (minimum)	269	234	503
Transversions (minimum)	195	161	356
Transitions/transversions	1.38	1.45	1.41
Skewness of tree-length distribution (g_1 value for 10000 random trees)	−0.577	−0.567	−0.167

TABLE 4. Pairwise divergence between combined ITS 1 and ITS2 nucleotide sequences from the DNAs of ten representative species of Apiaceae subfamily Apioideae. Actual numbers of unambiguous divergent sites from pairwise sequence comparisons appear below the diagonal and calculated sequence divergence values (× 100 and adjusted for missing data) appear above the diagonal. These values have not been corrected for multiple substitutions. Taxa designations are the same as in Table 2.

	Het	*Dau*	*Ant*	*Smy*	*Pas*	*Aeg*	*Api*	*Cor*	*Lom*	*Cou*
Heteromorpha	—	23.9	21.9	19.7	18.2	18.8	15.0	15.5	16.2	17.4
Daucus Yoko.	95	—	28.3	24.9	28.6	28.4	27.3	28.2	27.8	27.6
Anthriscus	88	113	—	26.3	29.2	27.3	26.2	26.9	27.2	26.8
Smyrnium	79	99	106	—	24.8	24.1	22.0	21.7	22.8	25.1
Pastinaca	73	114	118	100	—	20.7	17.0	13.4	15.8	17.9
Aegopodium	75	112	109	96	83	—	19.1	17.6	19.5	20.8
Apium	60	108	105	88	68	76	—	14.3	15.7	19.8
Coriandrum	62	112	108	87	54	70	57	—	10.9	13.7
Lomatium	65	111	110	92	64	78	63	44	—	9.4
Coulterophytum	70	110	108	101	72	83	79	55	38	—

was excluded from the study because of alignment ambiguity, both ITS regions provided approximately the same amount of information to the phylogenetic analysis.

In direct pairwise comparisons of unambiguous positions among all Apioideae accessions (using PAUP's DISTANCE MATRIX option), sequence divergence ranged from 0.5 to 33.2% of nucleotides in ITS 1 and from 0 to 33.2% of nucleotides in ITS 2. Comparison of

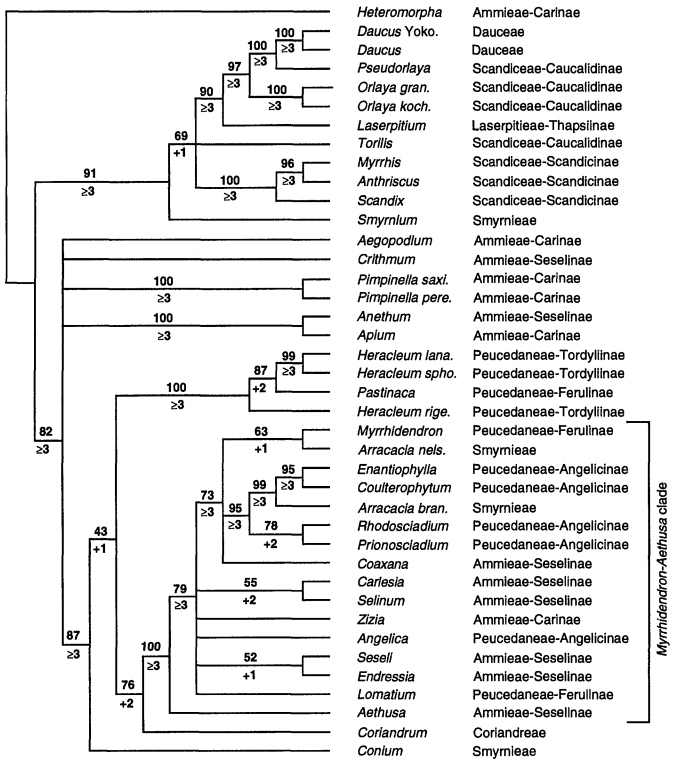

Fig. 2. Strict consensus of the 60 maximally parsimonious 900-step trees derived from equally weighted parsimony analysis of combined ITS 1 and ITS 2 sequences (CI excluding uninformative characters = 0.486, RI = 0.708). Numbers above the nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates. Branches that collapse at a tree length of one or two steps longer than the most parsimonious ones are indicated below the nodes by a +1 or +2, respectively. Decay analyses with tree lengths ≥3 steps longer than the most parsimonious trees could not be done because of computational constraints. Complete taxon names are provided in Table 2. Classification system presented is that of Drude (1898).

Apioideae sequence pairs across both spacer regions gave divergence values ranging from 0.2 to 30.7% of nucleotides and averaged 18.4%. The lowest value of 0.2% occurred between the two accessions of *Daucus carota* whose sequences varied by a single mutation at site 77 (Fig. 1). Nucleotide divergence among congeners varied between 0.7% for two species of *Pimpinella* and 8.0% for two species of *Heracleum.* In several instances, sequence divergence values between congeners were higher than they were between some intergeneric comparisons. Intergeneric sequence divergence ranged from 2.2 to 30.7% of nucleotides. The three greatest divergence values of 30.7, 30.2, and 30.0% occurred between *Scandix* and the accessions *Pimpinella peregrina, P. saxifraga,* and *Heracleum rigens,* respectively. Pairwise nucleotide divergence values between sequences of *Myrrhidendron, Arracacia* (two spp.), *Enantiophylla, Coulterophytum, Rhodosciadium, Prionosciadium, Coaxana, Carlesia, Selinum, Zizia, Angelica, Seseli, Endressia, Lomatium,* and *Aethusa,* hereafter called the *Myrrhidendron–Aethusa* clade (see Figs. 2, 4), ranged from 2.2 to 11.2%. The overall average sequence divergence value among these sixteen taxa was 6.4%. Pairwise nucleotide sequence divergence values and numbers of unambiguous divergent sites for ten of the 40 species of Apioideae examined are provided in Table 4. The average sequence divergence of 18.4% for all Apioideae pairs is about five times higher than that observed for approximately the same set of taxa on the basis of sequences obtained from the cpDNA *rpo*C1 intron (S. Downie, D. Katz-Downie, and K.-J. Cho, unpublished data).

Phylogenetic analysis—Parsimony analysis using equally weighted character states resulted in 60 maximally parsimonious topologies, whose strict consensus tree with accompanying bootstrap and decay values is shown in Fig. 2. These trees have a length of 828 steps when uninformative characters are excluded (CI = 0.486) or 900 steps when all characters are included (CI = 0.527). The consistency index value of 0.486 is higher than that of the expected value of 0.361 for 40 taxa (Sanderson and Donoghue, 1989). All trees have a retention index of 0.708.

At tree lengths of one (901) and two (902) evolutionary steps longer than the most parsimonious trees there were 985 and 7714 trees saved by PAUP, respectively.

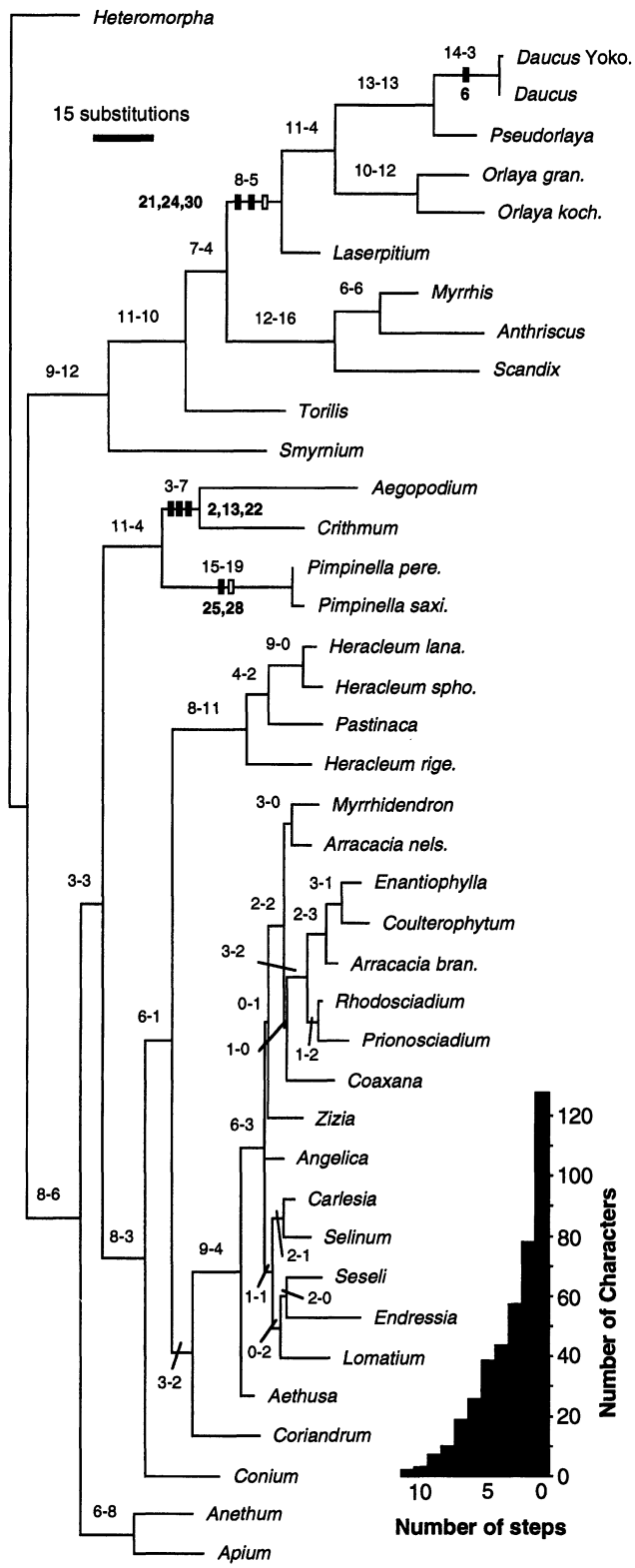

Fig. 3. One of 60 maximally parsimonious trees of 900 steps derived from parsimony analysis of ITS 1 and ITS 2 DNA sequence data using equally weighted character states. Pairs of numbers separated by a hyphen refer to numbers of transitions–transversions supporting that branch. Lengths of branches are proportional to the number of inferred nucleotide substitutions (note scale bar). The distribution of nine phylogenetically informative (i.e., synapomorphic) deletions (solid boxes) and insertions (open boxes) have been superimposed on the phylogram and are identified by boldfaced numbers corresponding to their locations in the multiple alignment (Fig. 1). The histogram (inset) summarizes the distribution of the number of inferred changes per character on this tree.

Trees three or more steps longer than the most parsimonious ones could not be examined owing to the large number of trees generated and limitations to the memory capacity of PAUP's tree buffer. Bootstrap values for the consensus clades ranged from 43 to 100%. The g_1 statistic for 10 000 random trees generated from these data was −0.62. This value is significantly more skewed (i.e., more negative) than random data ($g_1 = -0.09$ for 250 variable positions and 25 or more taxa; $P < 0.01$), indicating that these data contain significant amounts of phylogenetic signal (Hillis and Huelsenbeck, 1992).

The strict consensus of the 462 maximally parsimonious trees based on only ITS 1 sequences (CI excluding uninformative characters = 0.480, RI = 0.711) was consistent with, but considerably less resolved than, the strict consensus tree derived from the combined ITS sequences. Separate analysis of the smaller ITS 2 data set resulted in over 7000 minimal length (401 step) trees saved by PAUP (CI excluding uninformative characters = 0.509, RI = 0.725). The strict consensus tree derived from a subsequent analysis, where an arbitrary limit of 7000 trees was set, was less resolved than those generated by ITS 1 sequences alone.

One of the 60 900-step maximally parsimonious trees was arbitrarily chosen and is presented in Fig. 3 to indicate the number of transitions and transversions supporting each clade, as optimized by ACCTRAN in PAUP, and the distribution of phylogenetically informative length mutations. All but three of the 12 potentially informative length mutations were perfectly congruent (i.e., synapomorphic) with the phylogeny inferred by nucleotide substitutions. One of the three indels not congruent with the phylogeny is a 2-bp deletion (indel 31 at position 454-455 in Fig. 1) shared by *Laserpitium* and two species of *Orlaya* but not by *Pseudorlaya* or *Daucus*. This is either a reversal on the branch leading to *Daucus* and *Pseudorlaya* or it has occurred twice independently. The two others (indels 7 and 20, Fig. 1) are deletions and each map to three and four positions, respectively, on the tree. However, these deletions occur in regions of compressions (positions 124 and 289 in Fig. 1) and, thus, may be artifactual. The distribution of the number of inferred changes per character on this single tree reveals that many characters change multiple times. Sixty-seven of 416 unambiguous sites change five times or more with the average number of steps per character being 2.2 (see Fig. 3, inset).

Reanalyzing the combined ITS data set with the 31 indels included and scored as present or absent resulted in four minimal length trees each of 937 steps (CI excluding uninformative characters = 0.489). The topology of the strict consensus tree, with few exceptions, was similar to that shown in Fig. 2. Major differences include the placement of *Aegopodium* and *Crithmum* as a clade and the sister-group relationship between this group and the

two *Pimpinella* species, the recognition of *Carlesia, Selinum, Seseli, Endressia,* and *Lomatium* as a monophyletic group, and the occurrence of a trichotomy consisting of *Angelica, Zizia,* and the eight taxa comprising the *Myrrhidendron–Coaxana* clade. The relationships among the basal lineages are precisely the same as those depicted in the maximum likelihood tree (discussed below; see Fig. 5).

The average transition/transversion ratio in all ITS sequences across all 60 minimal length trees, as determined by MacClade, was 1.43. Minimal transition/transversion ratios, calculated by counting up the fewest number of each required to account for all the character states at each position, were 1.38, 1.45, and 1.41 for ITS 1, ITS 2, and combined ITS regions, respectively (Table 3). When the average observed transition/transversion ratio of 1.4 was used in a weighted parsimony analysis, three most parsimonious trees resulted. The strict consensus of these three trees differed from the consensus tree shown in Fig. 2 in uniting *Aegopodium* and *Crithmum* with the two *Pimpinella* species, placing *Torilis* as sister to the Dauceae+Laserpitieae+Scandiceae (less *Torilis*) clade, combining *Carlesia, Selinum, Seseli, Endressia,* and *Lomatium* as a monophyletic group, and placing only *Zizia* (but not *Angelica*) as sister to the *Myrrhidendron–Coaxana* clade. The same three maximally parsimonious trees were obtained when transversions to transitions were weighted either 1.1:1 or 2.5:1. The topologies of the strict consensus trees based on weighted parsimony analysis are almost precisely the same as the consensus topologies depicted in the combined nucleotide and indel analysis (discussed above) and in the maximum likelihood analysis (to be discussed below).

The two trees obtained from the neighbor-joining analysis of substitution rates calculated with either the one- or the two-parameter method differed only in their placement of *Torilis.* In the Jukes and Cantor one-parameter method (not shown), *Torilis* was sister taxon to the clade consisting of *Daucus, Pseudorlaya, Orlaya,* and *Laserpitium,* whereas in the Kimura two-parameter method (and using a transition/transversion ratio of 1.4) *Torilis* was placed basally within Dauceae+Laserpitieae+Scandiceae (Fig. 4). Neighbor-joining trees with transition/transversion ratios of 1.1, 1.4 or 1.8 were topologically identical. The results of the neighbor-joining analyses were similar to those inferred by weighted parsimony with differences occurring in the branching order of several weakly supported lineages. Unique to the neighbor-joining tree, but supported weakly, is the placement of *Conium* as sister-taxon to the *Heracleum+Pastinaca* clade, the separation of *Aegopodium* and *Crithmum,* and the union of *Lomatium* with *Angelica* and *Zizia.* With the exception of those branches within the *Myrrhidendron–Aethusa* clade, which, for the most part, are fairly short, evolutionary distances of terminal and internal branches within Apioideae were extremely heterogeneous and often quite large.

The tree obtained using the maximum likelihood method and a transition/transversion ration of 2.0 had a ln likelihood of −5087.7 (Fig. 5). This maximum-likelihood tree is similar to the trees constructed using unweighted and weighted parsimony and neighbor-joining analyses

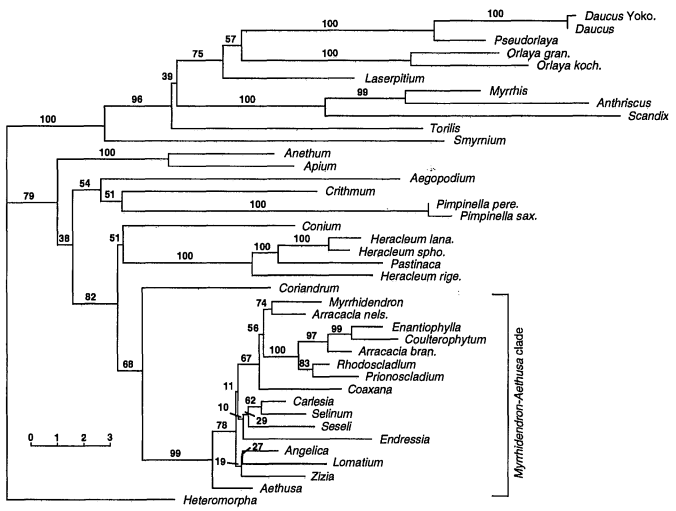

Fig. 4. Tree obtained from the neighbor-joining analysis of substitution rates estimated from the two-parameter method of Kimura (1980) for combined ITS 1 and ITS 2 sequences using a transition/transversion ratio of 1.4. Numbers above nodes indicate bootstrap estimates for 100 replicate analyses. Lengths of branches are proportional to distances; scale distance is given as 100 × value.

but, again, differs from them in those regions of the topology that were weakly supported in all the analyses.

Phylogenies estimated using neighbor-joining analysis of substitution rates, and maximum parsimony and likelihood methods reveal that, in the context of those species examined, Apioideae ITS sequences are divided into two major clades. The first of these comprises the genus *Smyrnium* (Smyrnieae) and those taxa belonging to Drude's (1898) tribes Dauceae, Laserpitieae, and Scandiceae subtribes Caucalidinae and Scandicinae. Subtribe Scandicinae is monophyletic and strongly supported in all analyses, as is the clade consisting of *Daucus, Pseudorlaya, Orlaya,* and *Laserpitium.* However, the relationship of *Torilis* (Scandiceae–Caucalidinae) to other members of Caucalidinae is equivocal. *Daucus* (Dauceae), arising from within a paraphyletic Scandiceae, exhibits a sister-group relationship with *Pseudorlaya. Laserpitium,* the only member of Laserpitieae included in this study, also arises from within Scandiceae and is sister to *Daucus+Pseudorlaya+Orlaya. Smyrnium,* one of the three generic representatives of tribe Smyrnieae examined, represents the earliest diverging lineage within this major phylogenetic division and is associated strongly with this division in all phylogenetic analyses.

The second major phylogenetic division within the subfamily comprises the remaining two genera of tribe Smyrnieae and those taxa belonging to Drude's tribes Ammieae (13 members representing two subtribes), Peucedaneae (11 members in three subtribes), and Coriandreae (one species). *Arracacia* (two species), *Coaxana, Coulterophytum, Enantiophylla, Myrrhidendron, Prionosciadium,* and *Rhodosciadium,* taxa endemic to Mexico and neighboring Central America, comprise a clade in all analyses, albeit supported by weak to moderate bootstrapping estimates. The two representatives of *Arracacia* (*A. nelsonii* and *A. brandegei*) do not form a natural group as they fall out alongside either *Myrrhidendron* or *En-*

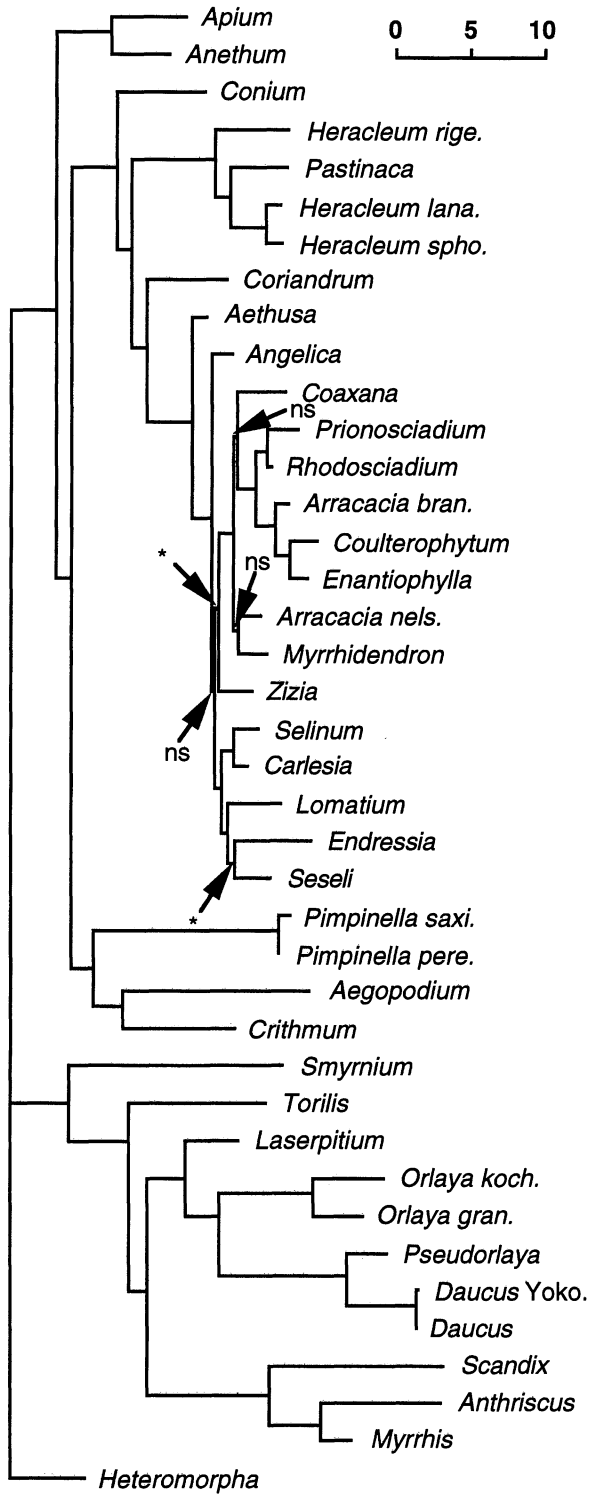

Fig. 5. Maximum likelihood tree constructed from unambiguous ITS sequences using a transition/transversion ratio of 2.0. All branch lengths, unless otherwise indicated, are significantly positive at $P < 0.01$. Two branch lengths are significantly positive at $P < 0.05$ and are indicated by a single asterisk; three branch lengths indicated by "ns" are not significantly positive. Complete taxon names are provided in Table 2. Scale distance is given as 100 × value.

antiophylla+*Coulterophytum*, respectively. Constraining the parsimony analysis so that the two *Arracacia* species are forced together produced minimal-length trees ten steps longer than those produced without the constraint. In all phylogenetic analyses, the *Myrrhidendron–Aethusa* clade, with 16 taxa, is the largest and one of the best supported. However, within this clade, relationships are poorly resolved owing to low levels of sequence divergence among several members. *Heracleum* and *Pastinaca* unite in all phylogenetic analyses to form a strongly supported clade. The one accession of *Pastinaca* examined, however, arises from within a paraphyletic *Heracleum*.

Overall, the results of the different phylogenetic analyses showed good agreement. Relationships that were strongly supported were robust to method of analysis and various weighting schemes, whereas conflicts among these methods were limited to regions of the topology that were weakly supported in all analyses. The low levels of confidence and poor resolution among the more ancestral nodes of the phylogenies are likely attributable to both homoplasy in the data and high rates of nucleotide substitution.

DISCUSSION

Evolution of Apioideae ITS sequences—The sizes and nucleotide composition of Apioideae ITS 1 and ITS 2 sequences lie within the range of those reported for most other angiosperms (reviewed in Baldwin et al., 1995). Similarly, like most other angiosperm ITS sequences, these regions have evolved primarily by point mutations, judging from the high levels of ITS sequence divergence between species and the relatively minor proportion of sites that required gaps for proper sequence alignment (Baldwin et al., 1995). Nucleotide substitutions in the ITS regions also show an apparently unequal distribution pattern among the taxa, with some of the highest numbers of substitutions occurring along the various lineages within the Scandiceae+Laserpitieae+Dauceae clade.

The lack of confidence within the branches of the *Myrrhidendron–Aethusa* clade (Figs. 2, 4) is likely due to the fairly short branch lengths between the nodes relative to the lengths of the terminal branches. Although this pattern of branch lengths could very well be an artifact of the taxonomic sample, it could also be a result of the rapid radiation of the group. The prevalence of long unbranched lineages within the Dauceae+Laserpitieae+Scandiceae+*Smyrnium* clade as well as those long branches basal to the *Myrrhidendron*+*Aethusa* clade in the other major division within Apioideae, is also likely a function of the sampling. The inclusion of additional related taxa that fall along these branches in subsequent analyses may improve the resolution among these branches.

The highly conserved sequence motif, GGCRY-(4 to 7 *n*)-GYGYCAAGGAA, located in ITS 1 and detected in published sequences from 88 species representing ten families and five subclasses of flowering plants (Cronquist, 1981; Liu and Schardl, 1994), is also seen in Apioideae ITS 1 sequences between positions 148 and 171 (Fig. 1). The 3′ portion of the motif, AAGGAA, is predicted not to be part of a base-paired stem region and is

thought to serve as a critical recognition element for rRNA processing (Liu and Schardl, 1994).

Phylogenetic utility of ITS sequences—Although ITS sequences may not provide a valuable source of intraspecific markers for population-level studies in Apiaceae (Soltis and Kuzoff, 1993), these regions appear well suited to comparisons among related species and/or closely related genera. In *Daucus carota,* there was only a single nucleotide difference between the two accessions examined in their ITS sequences, whereas sequence divergence values among congeners ranged from 0.7% (between the two species of *Pimpinella*) to 8.0% (between New World *Heracleum lanatum* and Old World *H. rigens*). These values for interspecific comparisons are higher than that obtained between species of *Lomatium* (1.5% for ITS 1 only; Soltis and Kuzoff, 1993) but are in the same range, or perhaps even lower, than those reported in other groups of angiosperms (Baldwin, 1992, 1993; Wojciechowski et al., 1993; Kim and Jansen, 1994; Sang et al., 1994).

Extensive divergence of ITS sequences between disparate pairs of Apioideae taxa raises concerns about the utility of these regions for assessing deeper level relationships within the subfamily. The robustness of a phylogenetic hypothesis can be evaluated by assessing its congruence with phylogenetic hypotheses generated from different data sets. For example, in intergeneric pairwise sequence comparisons among 26 *Astragalus* species and three outgroups, nucleotide divergence values ranged from 9.6 to 18.8% in ITS 1 and from 10.8 to 21.7% in ITS 2, yet parsimony analyses of these sequences resulted in a well-resolved phylogeny that was highly concordant with a previous cytogenetic study and a phylogeny based on cpDNA evidence (Wojciechowski et al., 1993). Among species of *Alnus, Betula,* and the outgroup *Ostrya* (Betulaceae), pairwise sequence divergence values for combined ITS regions averaged 17% but approached 25% between *Ostrya virginiana* and *Alnus maritima* (Savard, Michaud, and Bousquet, 1993). Once more, the relationships obtained from the analysis of ITS sequences agreed with those inferred using morphological data. In Asteraceae tribe Lactuceae, intergeneric sequence divergence ranged from 15.6 to 44.5% in ITS 1 and from 8.0 to 28.6% in ITS 2, and in subtribe Microseridinae pairwise intergeneric sequence divergence values reached 30% in ITS 1, 19.4% in ITS 2, and 23.6% when both ITS regions were combined (Kim and Jansen, 1994). In that study, partial incongruence between the ITS-derived phylogeny and phylogenies derived from morphological or cpDNA data was attributed to the distribution of homoplasy and/or different evolutionary constraints among

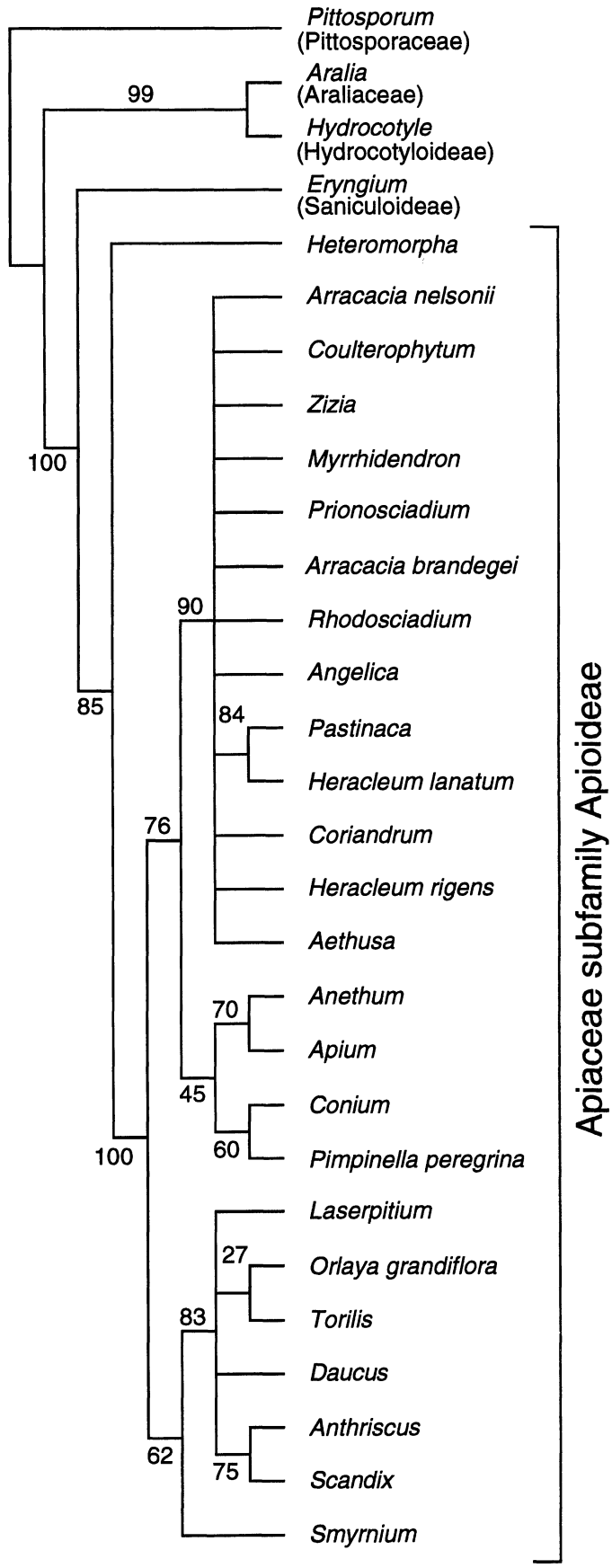

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Fig. 6. Strict consensus of the 30 maximally parsimonious 256-step trees derived from unweighted parsimony analysis of cpDNA *rpo*C1 intron sequences (CI excluding uninformative characters = 0.681, RI = 0.812). Numbers above the nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates. Complete taxon names for members of subfamily Apioideae examined are provided in Table 2. Nonapioid representatives examined include *Pittosporum tobira, Aralia chinensis, Hydrocotyle bowlesioides,* and *Eryngium planum.*

the different data sets. A phylogeny of Apioideae derived from parsimony analysis of cpDNA *rpo*C1 intron sequences (Fig. 6; S. Downie, D. Katz-Downie, and K.-J. Cho, unpublished data) is consistent with, but considerably less resolved than, relationships derived from ITS sequences even though the taxa sampled in both analyses are not wholly congruent. The substitution rate of the *rpo*C1 intron, however, is too slow to estimate the phylogeny of closely related genera. In contrast, ITS sequences are less suitable for analysis at higher levels of Apioideae diversity. Congruency between the ITS and cpDNA derived phylogenies includes: (1) the separation of subfamily Apioideae into two major clades; and (2) the association of *Smyrnium* with Dauceae+Laserpitieae+Scandiceae and not with the other Smyrnieae representatives examined.

Phylogenetic relationships within Apioideae—Phylogenies derived from ITS sequences provide very little support for Drude's (1898) widespread system of classification of the subfamily or for alternative subfamilial treatments, such as those proposed by Koso-Poljansky (1916) and Cerceau-Larrival (1962). Of the five tribes and seven subtribes recognized by Drude for which more than one generic representative was examined, only Scandiceae subtribe Scandicinae proved to be monophyletic. Additional support for a monophyletic Scandicinae is the unique occurrence of a layer of crystals in the parenchyma cells surrounding the carpophore in the mericarps of these taxa (Koso-Poljansky, 1916). Of Drude's seven tribes examined, two (Smyrnieae and Peucedaneae) are likely polyphyletic, two (Scandiceae and Ammieae) are probably paraphyletic, and the remaining three (Dauceae, Laserpitieae, and Coriandreae) are unresolved with the data at hand. The only substantive morphological differences among Drude's tribes and subtribes of Peucedaneae, Ammieae, and Smyrnieae center around the degree and type of fruit compression, the presence of secondary ribs, and the type of wing formation; however, considerable variation in these characters exists within and among these taxa.

Bentham (1867) and Boissier (1872) regarded the spiny-fruited members of Apioideae, with both primary and secondary ridges on the fruit, as comprising the tribe Caucalideae. Drude (1898), however, redistributed these spiny-fruited genera between his widely divergent Scandiceae subtribe Caucalidinae (represented in this study by *Orlaya, Pseudorlaya,* and *Torilis*) and his tribe Dauceae (represented here by *Daucus*). Drude believed that *Daucus* (and three other small genera) evolved from plants similar to those included in his tribe Laserpitieae (e.g., *Laserpitium*), whose members have fruits without spines but with primary and prominent secondary ridges, and that his genera of Caucalidinae were linked with those in his subtribe Scandicinae (represented here by *Scandix, Myrrhis,* and *Anthriscus*), whose members lack both secondary ridges and spines. Drude assumed that the secondary spinose ridges in Caucalidinae had evolved independently from those in Dauceae. On the basis of ITS nucleotide substitutions and the distribution of three uniquely occurring indels (Fig. 3), the genera *Daucus, Pseudorlaya, Orlaya,* and *Laserpitium* comprise a well-supported clade that is closely allied to a clade consisting of *Myrrhis, Anthriscus,* and *Scandix.* Thus, the molecular data support, in part, the taxonomic systems of Bentham (1867) and Boissier (1872) in that they unite the spiny-fruited members of Apioideae. They also uphold, in part, the system of Drude in establishing a relationship between *Laserpitium* and *Daucus.*

The distribution of flavonoid types within the subfamily parallels the major phylogenetic division revealed on the basis of ITS sequences. Taxa belonging to tribes Laserpitieae, Scandiceae, and Dauceae contain predominantly flavones (e.g., luteolin) in their fruits and leaves, whereas taxa belonging to tribes Coriandreae, Smyrnieae, Ammieae, and Peucedaneae contain predominantly flavonols (e.g., quercetin and/or kaempferol) (Crowden, Harborne, and Heywood, 1969; Harborne and Williams, 1972). Species of Dauceae and Scandiceae subtribe Caucalidinae also possess a much richer variation in flavonoids, including the most "highly evolved" O-methylated flavonoids, than species in any other tribe (Harborne, 1971; Harborne and Williams, 1972). The occurrence of similar yet complex compounds in Scandiceae, Laserpitieae, and Dauceae supports the view that these taxa are closely related and corroborates the morphological data in suggesting that these taxa probably represent an advanced group (or groups) within the subfamily (Harborne, 1971; Harborne and Williams, 1972).

Members of Drude's tribe Peucedaneae are generally characterized by a distinct dorsal flattening of the mature fruit with the lateral ribs expanded into wing-like appendages. It is the second largest tribe in the subfamily and includes 60 genera and some 550 species (Pimenov and Leonov, 1993). Drude (1898) recognized three subtribes on the basis of the morphology of the wings: Angelicinae (represented here by *Angelica, Coulterophytum, Enantiophylla, Prionosciadium,* and *Rhodosciadium*) are characterized by separate lateral wings, Ferulinae (Peucedaninae; *Lomatium, Myrrhidendron,* and *Pastinaca*) are characterized by closely appressed lateral wings, and Tordyliinae (*Heracleum*) are characterized by thickened wing margins. Comparative anatomical and developmental studies by Theobald (1971), however, provided strong evidence attesting to the unnaturalness of the tribe. His investigations revealed many independent derivations of peucedanoid taxa from ancestors similar to present-day members of Ammieae, Smyrnieae, and Coriandreae. As suggested by Theobald (1971), "it is quite easy to picture the evolution of dorsal flattering and wing formation as a dispersal mechanism in many independent lines from these less specialized [taxa]." The basal position of *Aegopodium, Crithmum, Pimpinella, Anethum,* and *Apium* (all Ammieae) and *Conium* (Smyrnieae) in one of the two major clades of Apioideae adds substance to this statement.

The close relationship between *Pastinaca* and *Heracleum* depicted in the ITS cladograms is reflected in many taxonomic treatments of Apioideae (e.g., Boissier, 1872; de Candolle, 1830; Calestani, 1905; Koso-Poljansky, 1916), and on the basis of comparative anatomical and developmental studies (Theobald, 1971), serological investigations (Pickering and Fairbrothers, 1971; Shneyer et al., 1991), and by the shared presence of angular furanocoumarins and their association with a distinctive insect fauna (Murray, Mendez, and Brown, 1982; Beren-

baum, 1981). It is not reflected, however, in the treatment of Drude, where the two genera are placed in different subtribes of Peucedaneae. The sampling of additional representatives of these two genera is in order to confirm the paraphyly of *Heracleum.*

Drude (1898) included in his Smyrnieae 29 genera from the Old and New World, of which three (*Arracacia, Conium,* and *Smyrnium*) have been sampled in this study. Members of this tribe were united on the basis of their round turgid mericarps, the campylospermous nature of their seeds, and the shared absence of any ridges, spines, or other outgrowths on the fruits. However, campylospermy (i.e., the presence of a deep groove on the commissural side of the seed) can also be found in both subtribes of Scandiceae, and ovoid and globose fruits are common in Ammieae and Scandiceae subtribe Caucalidinae. Serological investigations of 11 generic representatives of Smyrnieae (*Arracacia* was not included) confirm the heterogeneity of this tribe, as these taxa formed at least five distinct and distantly related taxonomic groups (Shneyer et al., 1991, 1992). Furthermore, because the genus *Smyrnium* was clearly isolated serologically from all other examined genera of *Smyrnieae*—the closest genus actually being *Myrrhis* in tribe Scandiceae—it was suggested that *Smyrnium* might be recognized best as a monotypic tribe or subtribe within Apioideae (Shneyer et al., 1992). The isolated nature of *Smyrnium* in the subfamily is also reflected in several taxonomic systems. For example, Koso-Poljansky (1916) recognized only *Smyrnium* and two other genera as belonging to tribe Smyrnieae. Hedge et al. (1987) also treated Smyrnieae in a narrow sense, recognizing only *Smyrnium* and *Smyrniopsis* in the tribe. The strongly supported association of *Smyrnium* with Dauceae+Laserpitieae+Scandiceae in all molecular analyses is a rather unexpected find.

Conium maculatum, a monotypic genus of tribe Smyrnieae, is one of few members of Apioideae that produces alkaloids (Fairbairn, 1971). Drude placed *Conium* in Smyrnieae because of its grooved endosperm, lack of crystals in the pericarp, and absence of volatile oil. Serological studies show a closer immunological affinity of this taxon with *Coriandrum* (and the closely allied *Bifora*) than to any other member of Smyrnieae examined (Shneyer et al., 1992). The ITS results, however, shed very little light on the proper phylogenetic placement of this genus.

The genera *Arracacia, Coaxana, Coulterophytum, Enantiophylla, Myrrhidendron, Prionosciadium,* and *Rhodosciadium,* all native to Mexico and/or neighboring regions of Central America, are represented only by polyploid members with known haploid chromosome numbers of 22 or, as in *Rhodosciadium* and *Prionosciadium,* 21 and 22 (Moore, 1971; L. Constance, unpublished data). These taxa have been described as "palaeopolyploids" as diploid relatives are not known (Favarger, 1967). Mathias (1965) has indicated that the Mexican highlands and Central America are one of two centers of distribution of Apioideae in the western Northern Hemisphere (the other being Pacific North America, including the Rocky Mountains); consequently, these genera may be modern derivatives of the Madro-Tertiary Geoflora (Mathias, 1965; Moore, 1971). In all phylogenetic analyses, these seven endemic New World genera comprise a clade. Their relationship to other New World taxa, however, is equivocal.

Sampling and additional study—Because the constituent tribes and subtribes of Apioideae vary considerably in number and circumscription, as do the diversity of characters defining these taxa, there was no a priori reason to exclude any representatives at the outset of this study on the basis that they might be too divergent evolutionarily. Besides, such major gaps in sampling could also weaken the resultant phylogenetic hypothesis. In light of our results, where the relationships inferred showed varying degrees of similarity to existing classification schemes of Apioideae, this was indeed a prudent approach.

In Apioideae, several Old and New World genera, such as *Seseli, Pimpinella, Daucus, Angelica, Heracleum, Lomatium,* and *Torilis,* contain a large number of species and may not represent natural groups (Heywood, 1971b). Thus, the inclusion of additional tribal/subtribal representatives in subsequent ITS studies might, perhaps, result in phylogenetic conclusions different than the ones presented here. The sampling of additional related taxa that fall along the weakly supported basal branches and long unbranched lineages, such as those from tribes Ammieae and Smyrnieae, for example, may improve the resolution at these levels.

Conclusions—The results presented here represent an initial attempt to formulate more precise hypotheses about relationships within Apiaceae subfamily Apioideae using evidence derived from nuclear ribosomal DNA ITS sequences. These results, however, must be regarded as exploratory only, as the number of representatives examined is small (relative to the ≈400 genera and some 2900 species estimated to occur within the subfamily; Pimenov and Leonov, 1993) and the taxonomic diversity of these representatives quite broad. Nevertheless, this study does provide a set of explicit hypotheses about relationships that can be tested as the data set is enlarged and more evidence, both molecular and nonmolecular, becomes available for comparative analysis.

While ITS sequences appear best suited to comparisons of congeneric species and closely related genera, and should be further explored as a promising source of nuclear phylogenetic markers within Apioideae at these levels, the high levels of sequence divergence between distantly related genera and the poor support given to many of the basal branches in the phylogenies (as ascertained by the low bootstrap and decay values) suggest that these sequences are less useful in resolving relationships among the more ancestral nodes of Apioideae phylogeny Partial concordance was observed, however, between the phylogenetic relationships proposed here and those relationships inferred on the basis of cpDNA *rpo*C1 intron sequences and, as a result, bolster our confidence in using the ITS regions to address deeper level phylogenetic questions within the subfamily.

Phylogenies derived from ITS sequences estimated using neighbor-joining analysis of substitution rates, and maximum likelihood and parsimony methods, give trees of essentially similar topology but provide little support

for Drude's (1898) widely used system of Apioideae classification or for alternative subfamilial treatments that are based largely on morphological and anatomical characters of the fruit. Despite the impressive body of Apioideae literature available, new information from molecular sources and reappraisal of traditional lines of evidence are needed before a satisfactory systematic account of the subfamily can be attempted. Developing a classification on the basis of a phylogeny estimated from a single data source, whether molecular or otherwise, is a dangerous systematic practice and must be avoided.

In order to increase resolution among the basal nodes of Apioideae phylogeny, it will also be necessary to seek information from DNA sequences evolving more slowly than those of the ITS regions. In addition to data derived from the cpDNA *rpo*C1 intron, we are examining cpDNA restriction sites and nuclear 18S ribosomal RNA sequences from the same representative taxa used in this study. Additionally, ongoing cladistic analysis of morphological data from Scandiceae, Laserpitieae, Dauceae, and Smyrnieae exemplars (B. Lee and S. Downie, unpublished data) reveals relationships similar to those estimated using molecular data. We are optimistic that the information obtained from these studies, in conjunction with additional information derived from nuclear ribosomal ITS (S. Ramanath and S. Downie, unpublished data) and cpDNA *rbc*L and *mat*K (G. Plunkett, unpublished data) sequences, will provide the resolution necessary that will lead to a thorough understanding of the historical relationships within this large and taxonomically complex subfamily of Apiaceae.

LITERATURE CITED

ANDERBERG, A. A. 1992. The circumscription of the Ericales, and their cladistic relationships to other families of "higher" dicotyledons. *Systematic Botany* 17: 660–675.

ANTONOV, A. S., K. M. VALIEJO-ROMAN, M. G. PIMENOV, AND N. A. BERIDZE. 1988. Non-equivalency of genera in Angiospermae: evidence from DNA hybridization studies. *Plant Systematics and Evolution* 161: 155–168.

APPELS, R., AND R. L. HONEYCUTT. 1986. rDNA: evolution over a billion years. *In* S. K. Dutta [ed.], DNA systematics, vol. 2, 81–135. CRC, Boca Raton, FL.

ARNHEIM, N. 1983. Concerted evolution of multigene families. *In* M. Nei and R. K. Koehn [eds.], Evolution of genes and proteins, 38–61. Sinauer, Sunderland, MA.

BALDWIN, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

———. 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. *American Journal of Botany* 80: 222–238.

———, M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence of angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

BENTHAM, G. 1867. Umbelliferae. *In* G. Bentham and J. D. Hooker [eds.], *Genera Plantarum* 1: 859–931. Reeve, London.

BERENBAUM, M. R. 1981. Patterns of furanocoumarin distribution and insect herbivory in the Umbelliferae: plant chemistry and community structure. *Ecology* 62: 1254–1266.

———. 1990. Evolution of specialization in insect-umbellifer associations. *Annual Review of Entomology* 35: 319–343.

BOISSIER, E. 1872. Umbelliferae. *In* Flora Orientalis 2, 819–1090. Georg, Genève.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

CALESTANI, V. 1905. Contributo alla systematica dell ombrellifere d'europa. *Webbia* 1: 89–280.

CAUWET-MARC, A.-M., AND J. CARBONNIER. 1982. Les Ombellifères. Actes du 2ème Symposium International sur les Ombellifères "Contributions Pluridisciplinaries à la Systématique." *Monographs in Systematic Botany from the Missouri Botanical Garden,* vol. 6. Braun-Brumfield, Ann Arbor, MI.

CERCEAU-LARRIVAL, M.-TH. 1962. Plantules et pollens d'Ombellifères leur intérêt systématique et phylogénique. *Mémoires du Muséum National d'Histoire Naturelle,* ser. B, vol. 14, 1–166.

———. 1963. Le pollen d'Ombellifères Méditerranéennes II. Tordylinae Drude. *Pollen et Spores* 5: 297–323.

———. 1965. Le pollen d'Ombellifères Méditerranéennes III. Scandicineae Drude. IV. Dauceae Drude. *Pollen et Spores* 7: 35–62.

COULTER, J. M., AND J. N. ROSE. 1900. Monograph of the North American Umbelliferae. *Contributions of the United States National Herbarium* 7: 9–256.

CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, NY.

CROWDEN, R. K., J. B. HARBORNE, AND V. H. HEYWOOD. 1969. Chemosystematics of the Umbelliferae—a general survey. *Phytochemistry* 8: 1963–1984.

DAHLGREN, R. T. 1980. A revised system of classification of the angiosperms. *Botanical Journal of the Linnean Society* 80: 91–124.

DAVIS, P. H. 1972. Umbelliferae. *In* Flora of Turkey and the East Aegean Islands, vol. 4, 256–281. University Press, Edinburgh.

DAWSON, J. W. 1971. Relationships of the New Zealand Umbelliferae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 43–61. Academic Press, New York, NY.

DE CANDOLLE, A. P. 1830. Umbelliferae. *In* de Candolle [ed.], *Prodromus systematis naturalis regni vegetabilis* 4: 55–250.

DEVEREUX, J., P. HAEBERLI, AND O. SMITHIES. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Research* 12: 387–395.

DONOGHUE, M. J., R. G. OLMSTEAD, J. F. SMITH, AND J. D. PALMER. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. *Annals of the Missouri Botanical Garden* 79: 333–345.

DOYLE, J. J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. *Systematic Botany* 17: 144–163.

———, AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19: 11–15.

DRUDE, O. 1898. Umbelliferae. *In* Engler, A. and K. Prantl [eds.], *Die natürlichen Pflanzenfamilien* 3: 63–250. Wilhelm Engelmann, Leipzig.

FAIRBAIRN, J. W. 1971. The alkaloids of hemlock (*Conium maculatum* L.) (or *Conium maculatum* L: the odd man out). *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 361–368. Academic Press, New York, NY.

FARRIS, J. S. 1989. The retention index and homoplasy excess. *Systematic Zoology* 38: 406–407.

FAVARGER, C. 1967. Cytologie et distribution des plantes. *Biological Review* 42: 163–206.

FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* 17: 368–376.

———. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

———. 1993. PHYLIP (Phylogeny Inference Package) version 3.5s. Distributed by the author. Department of Genetics, University of Washington, Seattle, WA.

HAMBY, R. K., AND E. A. ZIMMER. 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. *In* P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, 50–91. Chapman and Hall, New York, NY.

HARBORNE, J. B. 1971. Flavonoid and phenylpropanoid patterns in the Umbelliferae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 293–314. Academic Press, New York, NY.

———, AND C. A. WILLIAMS. 1972. Flavonoid patterns in the fruits of the Umbelliferae. *Phytochemistry* 11: 1741–1750.

HEDGE, I. C., J. M. LAMOND, K. H. RECHINGER, R. ALAVA, D. F. CHAM-

BERLAIN, L. ENGSTRAND, I. HERRNSTADT, C. C. HEYN, G. H. LEUTE, I. MANDENOVA, D. PEEV, M. G. PIMENOV, S. SNOGERUP, AND S. G. TAMAMSCHIAN. 1987. Umbelliferae. *In* K. H. Rechinger [ed.], Flora Iranica 162, 1–555. Akademische Druck- und Verlagsanstalt, Graz.

HEGNAUER, R. 1971. Chemical patterns and relationships of Umbelliferae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 267–277. Academic Press, New York, NY.

HEYWOOD, V. H. 1971a. The biology and chemistry of the Umbelliferae. Academic Press, New York, NY.

———. 1971b. Systematic survey of Old World Umbelliferae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 31–41. Academic Press, New York, NY.

———. 1982. General introduction to the taxonomy of the Umbelliferae. *In* A.-M. Cauwet-Marc and J. Carbonnier [eds.], Les Ombellifères. Actes du 2ème Symposium International sur les Ombellifères "Contributions Pluridisciplinaires à la Systématique," 107–112. *Monographs in Systematic Botany from the Missouri Botanical Garden,* vol. 6. Braun-Brumfield, Ann Arbor, MI.

———. 1986. The Umbelliferae—an impossible family? *Symbolae Botanicae Upsalienses* 26: 173–180.

———. 1993. Flowering plants of the world. Oxford University Press, New York, NY.

———, AND K. M. M. DAKSHINI. 1971. Fruit structure in the Umbelliferae–Caucalideae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 215–232. Academic Press, New York, NY.

HIGGINS, D. G., A. J. BLEASBY, AND R. FUCHS. 1992. CLUSTAL V: improved software for multiple sequence alignment. *Computer Applications in the Biosciences* 8: 189–191.

HILLIS, D. M. 1991. Discriminating between phylogenetic signal and random noise in DNA sequences. *In* M. M. Miyamoto and J. Cracraft [eds.], Phylogenetic analysis of DNA sequences, 278–294. Oxford University Press, Oxford.

———, AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189–195.

HSIAO, C., N. J. CHATTERTON, K. H. ASAY, AND K. B. JENSEN. 1994. Phylogenetic relationships of 10 grass species: an assessment of phylogenetic utility of the internal transcribed spacer region in nuclear ribosomal DNA in monocots. *Genome* 37: 112–120.

HUTCHINSON, J. 1973. The families of flowering plants. Oxford University Press, Oxford.

JAY, M. 1969. Chemotaxonomic researches on vascular plants XIX. Flavonoid distribution in the Pittosporaceae. *Botanical Journal of the Linnean Society* 62: 423–429.

JUDD, W. S., R. W. SANDERS, AND M. J. DONOGHUE. 1994. Angiosperm family pairs: preliminary phylogenetic analyses. *Harvard Papers in Botany* 5: 1–51.

JUKES, T. H., AND C. R. CANTOR. 1969. Evolution of protein molecules. *In* N. H. Munro [ed.], Mamalian protein metabolism, 21–123. Academic Press, New York, NY.

KIM, K.-J., AND R. K. JANSEN. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (*Krigia,* Asteraceae): additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. *Plant Systematics and Evolution* 190: 157–185.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18: 1–32.

KOSO-POLJANKSY, B. M. 1916. Sciadophytorum systematis lineamenta. *Bulletin de la Société Impériale des Naturalistes (Moscow)* 29: 93–222.

LIU, J.-S., AND C. SCHARDL. 1994. A conserved sequence in internal transcribed spacer 1 of plant nuclear rRNA genes. *Plant Molecular Biology* 26: 775–778.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade, version 3.0. Sinauer, Sunderland, MA.

MATHIAS, M. E. 1965. Distribution patterns of certain Umbelliferae. *Annals of the Missouri Botanical Garden* 52: 387–398.

MOORE, D. M. 1971. Chromosome studies in the Umbelliferae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 233–255. Academic Press, New York, NY.

MURRAY, R. D. H., J. MENDEZ, AND S. A. BROWN. 1982. The natural coumarins. Wiley and Sons, New York, NY.

NICKRENT, D. L., AND C. R. FRANCHINA. 1990. Phylogenetic relationships of the Santalales and relatives. *Journal of Molecular Evolution* 31: 294–301.

NIELSEN, B. E. 1971. Coumarin patterns in the Umbelliferae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 325–336. Academic Press, New York, NY.

OLSEN, G. J., R. OVERBEEK, N. LARSEN, T. L. MARSH, M. J. MCCAUGHEY, M. A. MACIUKENAS, W.-M. KUAN, T. J. MACKE, Y. XING, AND C. R. WOESE. 1992. The ribosomal database project. *Nucleic Acids Research* 20: 2199–2200.

———, H. MATSUDA, R. HAGSTROM, AND R. OVERBEEK. 1994. fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Computer Applications in the Biosciences* 10: 41–48.

PICKERING, J. L., AND D. E. FAIRBROTHERS. 1971. The use of serological data in a comparison of tribes in the Apioideae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 315–324. Academic Press, New York, NY.

PIMENOV, M. G., AND M. V. LEONOV. 1993. The genera of the Umbelliferae. Royal Botanic Gardens, Kew.

PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1992. Molecular phylogenetic study of Apiales (Apiaceae, Araliaceae, and Pittosporaceae). *American Journal of Botany* 79: 158 (Abstract).

———, ———, AND ———. 1994. Phylogenetic relationships within and between Araliaceae and Apiaceae based on *rbc*L and *mat*K sequence data. *American Journal of Botany* 81: 180 (Abstract).

RIESEBERG, L. H., AND D. E. SOLTIS. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* 5: 65–84.

RODRIGUEZ, R. L. C. 1971. The relationships of the Umbellales. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 63–91. Academic Press, New York, NY.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing evolutionary trees. *Molecular Biology and Evolution* 4: 406–425.

SANDERSON, M. J., AND M. J. DONOGHUE. 1989. Patterns of variation in levels of homoplasy. *Evolution* 43: 1781–1795.

SANG, T., D. J. CRAWFORD, S.-C. KIM, AND T. F. STUESSY. 1994. Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: evidence from sequences of the ITS regions of nuclear ribosomal DNA. *American Journal of Botany* 81: 1494–1501.

SAVARD, L., M. MICHAUD, AND J. BOUSQUET. 1993. Genetic diversity and phylogenetic relationships between birches and alders using ITS, 18S rRNA, and *rbc*L gene sequences. *Molecular Phylogenetics and Evolution* 2: 112–118.

SHAN, R.-H., AND M.-L. SHEH. 1979. Umbelliferae. *In* Flora Reipublicae Popularis Sinicae, vol. 55, 1–316.

SHISHKIN, B. K. 1950-1951. Umbelliferae. *In* B. K. Shiskin [ed.], Flora of the U.S.S.R., vols. 16 and 17. Akademiya Nauk, Leningrad.

SHNEYER, V. S., G. P. BORSCHTSCHENKO, M. G. PIMENOV, AND M. V. LEONOV. 1991. The serological investigation of intergeneric relationships in the subfamily Apioideae (Apiaceae). *Botanicheskii Zhurnal (Leningrad)* 76: 245–257.

———, ———, ———, and ———. 1992. The tribe Smyrnieae (Umbelliferae) in the light of serotaxonomical analysis. *Plant Systematics and Evolution* 182: 135–148.

SOLTIS, P. S., AND R. K. KUZOFF. 1993. ITS sequence variation within and among populations of *Lomatium grayi* and *L. laevigatum* (Umbelliferae). *Molecular Phylogenetics and Evolution* 2: 166–170.

STUHLFAUTH, T., H. FOCK, H. HUBER, AND K. KLUG. 1985. The distribution of fatty acids including petroselinic and tariric acids in the fruit and seed oils of the Pittosporaceae, Araliaceae, Umbelliferae, Simarubaceae and Rutaceae. *Biochemical Systematics and Ecology* 13: 447–453.

SUH, Y., L. B. THIEN, H. E. REEVE, AND E. A. ZIMMER. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA variation in Winteraceae. *American Journal of Botany* 80: 1042–1055.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic Analysis Using Parsi-

mony, version 3.1. Computer program distributed by the Illinois Natural History Survey, Champaign, IL.

TAKHTAJAN, A. 1980. Outline of the classification of flowering plants (Magnoliophyta). *Botanical Review (Lancaster)* 46: 225–359.

THEOBALD, W. L. 1971. Comparative anatomical and developmental studies in the Umbelliferae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 177–197. Academic Press, New York, NY.

THORNE, R. F. 1973. Inclusion of the Apiaceae (Umbelliferae) in the Araliaceae. *Notes from the Royal Botanic Garden, Edinburgh* 32: 161–165.

———. 1992. Classification and geography of the flowering plants. *Botanical Review* 58: 225–348.

VAN TIEGHEM, P. 1884. Sur la structure et les affinités des Pittosporées. *Bulletin de la Société Botanique de France* 31: 384–385.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and applications, 315–322. Academic Press, San Diego, CA.

WOJCIECHOWSKI, M. F., M. J. SANDERSON, B. G. BALDWIN, AND M. J. DONOGHUE. 1993. Monophyly of aneuploid *Astragalus* (Fabaceae): evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 80: 711–722.

YOKOTA, Y., T. KAWATA, Y. IIDA, A. KATO, AND S. TANIFUJI. 1989. Nucleotide sequences of the 5.8S rRNA gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. *Journal of Molecular Evolution* 29: 294–301.

ZOHARY, M. 1972. Umbelliferae. *In* Flora Palaestina, 378–453. Israel Academy of Sciences and Humanities, Jerusalem.