Molecular Systematics of Apiaceae Subfamily Apioideae: Phylogenetic Analyses of Nuclear Ribosomal DNA Internal Transcribed Spacer and Plastid rpoC1 Intron Sequences

Stephen R. Downie; Seemanti Ramanath; Deborah S. Katz-Downie; Esmeralda Llanas

American Journal of Botany, Vol. 85, No. 4. (Apr., 1998), pp. 563-591.

American Journal of Botany 85(4): 563–591. 1998.

MOLECULAR SYSTEMATICS OF APIACEAE SUBFAMILY APIOIDEAE: PHYLOGENETIC ANALYSES OF NUCLEAR RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER AND PLASTID *RPOC1* INTRON SEQUENCES[1]

STEPHEN R. DOWNIE,[2] SEEMANTI RAMANATH, DEBORAH S. KATZ-DOWNIE, AND ESMERALDA LLANAS

Department of Plant Biology, University of Illinois, Urbana, Illinois 61801

Evolutionary relationships among representatives of Apiaceae (Umbelliferae) subfamily Apioideae have been inferred from phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer (ITS 1 and ITS 2) and plastid *rpoC1* intron sequences. High levels of nucleotide sequence variation preclude the use of the ITS region for examining relationships across subfamilial boundaries in Apiaceae, whereas the *rpoC1* intron is more suitably conserved for family-wide phylogenetic study but is too conserved for examining relationships among closely related taxa. In total, 126 ITS sequences from subfamily Apioideae and 100 *rpoC1* intron sequences from Apiaceae (all three subfamilies) and outgroups Araliaceae and Pittosporaceae were examined. Phylogenies estimated using parsimony, neighbor-joining, and maximum likelihood methods reveal that: (1) Apiaceae subfamily Apioideae is monophyletic and is sister group to Apiaceae subfamily Saniculoideae; (2) Apiaceae subfamily Hydrocotyloideae is not monophyletic, with some members strongly allied to Araliaceae and others to Apioideae + Saniculoideae; and (3) Apiaceae subfamily Apioideae comprises several well-supported subclades, but none of these coincide with previously recognized tribal divisions based largely on morphological and anatomical characters of the fruit. Four major clades in Apioideae are provisionally recognized and provide the framework for future lower level phylogenetic analyses. A putative secondary structure model of the *Daucus carota* (carrot) *rpoC1* group II intron is presented. Of its six major structural domains, domains II and III are the most, and domains V and VI the least, variable.

Key words: Apiaceae; Apioideae; internal transcribed spacers; molecular systematics; phylogeny; *rpoC1* intron; Umbelliferae.

The flowering plant family Apiaceae (Umbelliferae) comprises some 300–455 genera and about ten times as many species (Cronquist, 1981; Pimenov and Leonov, 1993), and is cosmopolitan in distribution. Many of its members are readily characterized by umbellate inflorescences, specialized fruits consisting of two one-seeded mericarps suspended from a split central column (carpophore), and numerous, minute epigynous flowers. The aromatic nature of these plants, both in their foliage and fruits, has led to their common use as foods and spices. Well-known members include carrots, celery, dill, caraway, coriander, fennel, lovage, parsnips, parsley, cumin, chervil, and anise. Their distinctive chemistry also is reflected in their toxicity and widespread medicinal use (French, 1971; Babu, Kuttan, and Padikkala, 1995), with water hemlock and poison hemlock being examples of notoriously poisonous plants. Because of the common possession of several obvious characteristics, Apiaceae may claim the distinction of being "the first family of flowering plants to achieve general recognition" (Constance, 1971). Moreover, with the publication of Robert Morison's *Plantarum Umbelliferarum Distributio Nova* in 1672, the family was the first group of flowering plants to be monographed (Constance, 1971; Hedge, 1973). However, despite their large size and economic importance, three and a quarter centuries of study, and widespread recognition as a "natural" group, there is no consensus on subfamilial, tribal, and subtribal delimitations within the family and, until recently, minimal speculation on phylogenetic relationships.

The division of Apiaceae into three subfamilies (Hydrocotyloideae, Saniculoideae, and Apioideae) and 12 tribes, proposed exactly a century ago (Drude, 1897–1898), remains the predominant system of classification (Table 1). Of the three subfamilies, Apioideae are the largest and most important economically, with its members distinguished from those of the other two subfamilies by the shared possession of compound umbels, well-developed vittae (secretory canals), and free carpophores. Drude's system of Apioideae classification, like many other systems available for the subfamily, relies on a diverse array of subtle fruit differences to demarcate major taxonomic groups. Such characters include, but are not limited to, the degree and direction of fruit compression, the shape of the endosperm, the number of mericarp ribs

[1] Manuscript received 18 December 1996; revision accepted 28 July 1997.

The authors thank C. Baskin, L. Constance, R. Hartman, J. Lahham, M. Pimenov, G. Plunkett, A. Troitsky, C. Valiejo-Roman, M. Watson, and the many botanic gardens cited in the text for generously providing us with leaf and/or seed material; L. Constance for confirming some of our identifications; K.-J. Cho, B. Lee, R. Patel, J. Tovar, and W. Yang for laboratory assistance; and B. Baldwin, L. Constance, K. Spalik, J.-P. Reduron, G. Plunkett, J. Wen, and, especially, R. Olmstead for comments on an early draft of the manuscript. This work was supported by grants from the National Science Foundation (DEB-9407712) and the Campus Research Board of the University of Illinois. A portion of this paper represents a M. S. dissertation submitted by S. Ramanath to the Graduate College of the University of Illinois at Urbana-Champaign.

[2] Author for correspondence (Fax: 217-244-7246; e-mail: sdownie@uiuc.edu).

TABLE 1. Drude's (1897–1898) classification of Apiaceae (only the subtribes of Apioideae are presented).

Subfamily Apioideae	Laserpitieae
Echinophoreae	Silerinae
Scandiceae	Elaeoselininae
Scandicinae	Thapsiinae
Caucalidinae	Dauceae
Coriandreae	Subfamily Saniculoideae
Smyrnieae	Saniculeae
Apieae	Lagoecieae
Carinae	Subfamily Hydrocotyloideae
Seselinae	Hydrocotyleae
Peucedaneae	Mulineae
Angelicinae	
Ferulinae	
Tordyliinae	

and dorsal vittae, the presence or absence of wings, spines, hairs, ridges, or other outgrowths and their associated characteristics, and the distribution of calcium oxalate crystals and sclerenchyma in the fruit walls. Serious doubts have been cast on the validity of using these characters to diagnose evolutionary relationships (Theobald, 1971; Davis, 1972, Heywood, 1982; Lavrova, Pimenov, and Tikhomirov, 1983; Hedge et al., 1987; Shneyer et al., 1992). Indeed, recent cladistic analyses of nuclear ribosomal DNA internal transcribed spacers (ITS; Downie and Katz-Downie, 1996) and plastid *rpoC1* intron (Downie, Katz-Downie, and Cho, 1996), *rbcL* (Plunkett, Soltis, and Soltis, 1996a; Kondo et al., 1996), and *matK* (Plunkett, Soltis, and Soltis, 1996b) sequences provide little support for Drude's (1897–1898) system of Apioideae classification or for alternative subfamilial treatments that are based largely on morphological and anatomical characteristics of the fruit (Koch, 1824; De Candolle, 1830; Bentham, 1867; Boissier, 1872; Koso-Poljansky, 1916) or pollen and seedling morphology (Cerceau-Larrival, 1962, 1971).

While the above molecular studies were important in demonstrating the utility of particular nuclear and plastid DNA regions for inferring evolutionary history within Apiaceae (and Araliaceae) and the monophyly of subfamily Apioideae, the number of apioid taxa examined in each investigation was small. Although the taxa sampled (in all but the two *rbcL* studies) were chosen because they represented most of the tribes recognized by Drude in Apioideae, expanded sampling indicates the presence of major clades heretofore unrecognized in the subfamily and, hence, not included in these previous studies. Here we describe our recent and expanded investigations using sequences from the nuclear ribosomal DNA ITS region and chloroplast *rpoC1* intron. Our objectives are as follows: (1) to confirm the monophyly of Apioideae and to ascertain the historical relationships of the three commonly recognized subfamilies of Apiaceae; (2) to compare our results to those inferred for Apioideae using chloroplast DNA (cpDNA) *matK* sequences (Plunkett, Soltis, and Soltis, 1996b); (3) to examine congruence of relationship between nuclear (biparentally inherited) and chloroplast (maternally inherited) genome derived phylogenies; and (4) to identify major clades within Apioideae for future lower level analyses. As a fifth objective, we explore further the mode of *rpoC1* intron sequence evolution in light of its inferred secondary structure. The interpretation of morphological and chemical character evolution, a natural extension of this analysis, will be presented elsewhere (Katz-Downie et al., 1998).

MATERIALS AND METHODS

Plant accessions—One hundred and thirty-two accessions (118 species) from Apiaceae subfamily Apioideae, 15 accessions (14 species) from Apiaceae subfamilies Hydrocotyloideae and Saniculoideae, and 14 species from putatively allied families Araliaceae and Pittosporaceae (Thorne, 1973; Takhtajan, 1980; Cronquist, 1981; Judd, Sanders, and Donoghue, 1994; Plunkett, Soltis, and Soltis, 1996a, 1997), were examined for nuclear ribosomal DNA ITS and/or cpDNA *rpoC1* intron sequence variation (Table 2). With regard to the ITS sequence comparisons, only representatives of Apiaceae subfamily Apioideae were considered because a previous study revealed the difficulty in aligning these sequences with those from nonapioid taxa (Downie and Katz-Downie, 1996). Complete ITS 1 and ITS 2 sequences for 87 taxa are reported here for the first time; combining these with 39 previously published ITS sequences (Downie and Katz-Downie, 1996) culminated in a matrix of 126 accessions. With regard to the intron comparative analysis, the 70 sequences procured as part of this investigation were combined with the 30 already published (Downie, Katz-Downie, and Cho, 1996) for a data matrix of 100 taxa. Both complete ITS and *rpoC1* intron sequences are available for 65 accessions of Apioideae (Table 2).

Leaf material for DNA extraction was either collected directly from the field, taken from plants propagated from seed in the greenhouse, provided as gifts, or obtained from accessioned plants cultivated at various botanical gardens (Table 2). Field-collected or greenhouse-propagated plants were identified using published keys and compared to herbarium specimens; some identifications were confirmed by L. Constance (University of California, Berkeley).

Experimental strategy—Details of DNA extraction, the PCR (polymerase chain reaction) amplifications (including primer locations and characteristics), and the DNA purification and manual sequencing strategies used are provided elsewhere (Downie and Katz-Downie, 1996; Downie, Katz-Downie, and Cho, 1996). In summary, these previous sequence data were obtained through direct sequencing of double-stranded templates derived from the PCR procedure. With the exception of the 30 previously published *rpoC1* intron sequences, which were sequenced manually on only one strand of DNA using five primers (Downie, Katz-Downie, and Cho, 1996), all previously published and new ITS sequences and most new *rpoC1* intron sequences were sequenced in their entirety on both strands. For this study, both manual and automated sequencing methods were used. For the latter, cycle sequencing reactions were performed using the purified PCR products, AmpliTaq DNA polymerase, and fluorescent dye-labeled terminators (Perkin-Elmer Corp., Norwalk, Connecticut). The reaction conditions were as specified by the manufacturer, with the addition of 5% dimethylsulfoxide (DMSO). Each of the two PCR primers used to amplify either the entire ITS or *rpoC1* intron regions were used in the cycle sequencing. The PCR amplification conditions were as follows: 1 min at 95°C for initial denaturation followed by 25 cycles of 15 sec at 95°C for denaturation, 5 sec at 45°C for primer annealing, and 4 min at 60°C for primer extension. The sequencing products were resolved by electrophoresis using Applied Biosystem's, Inc. (Foster City, California) 373A DNA sequencing system with Stretch upgrade.

Sequence analysis and intron secondary structure—Both ITS and *rpoC1* intron DNA sequences were aligned initially using the program CLUSTAL V (Higgins, Bleasby, and Fuchs, 1992), and the resulting alignments were manually adjusted as necessary. During the final stages

of data collection, the last 30–40 ITS and intron sequences were added manually to their respective aligned data matrices. Boundaries of the coding (3′18S, 5.8S, and 5′26S ribosomal RNA) and spacer regions of ITS were determined by comparing the DNA sequences to corresponding boundaries in *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, because sequence data for the intervening 5.8S subunit were incomplete for several taxa and those that were available were not sufficiently variable to warrant their inclusion or additional sequencing.

Group II introns exhibit considerable evolutionary conservation of secondary structure and are characterized by six centrally radiating structural components (designated as domains I–VI; Michel and Dujon, 1983; Michel, Umesono, and Ozeki, 1989). The alignment of *rpoC1* intron sequences was facilitated by comparing these sequences to conserved regions documented for *rpoC1* and other group II introns (Michel, Umesono, and Ozeki, 1989). Predictions of secondary structure by the free-energy minimization method were made using MULFOLD version 2.0 (Jaeger, Turner, and Zuker, 1989; Zuker, 1989) and were used to guide the alignment of intron sequences when appropriate. Boundaries of the exon and intron regions were determined by comparison to corresponding boundaries in tobacco (Shinozaki et al., 1986) and consensus splice sites in other plants for group II introns (Michel, Umesono, and Ozeki, 1989). Only the intron sequences were included in this study, because the sequencing strategy (outlined in Downie, Katz-Downie, and Cho, 1996) effectively ignored the flanking, and presumably more conserved, exon regions.

Pairwise nucleotide differences of unambiguously aligned positions were determined using the distance matrix option in PAUP version 3.1.1 (Swofford, 1993). Insertion/deletion (indel) events were treated as missing data for all taxa. These divergence values were calculated simply as the proportion of divergent sites in each direct pairwise comparison with no provision made to account for superimposed events (multiple hits). Transition/transversion (Ts/Tv) rate ratios over a subset of the maximally parsimonious trees were calculated using MacClade version 3.01 (Maddison and Maddison, 1992). The nucleotide sequence data reported in this study have been deposited with the GenBank Data Library; accession numbers are provided in Table 2. The alignments of these sequences can be obtained directly from the authors.

Phylogenetic analysis—Phylogenetic analyses were performed on the ITS and *rpoC1* intron data matrices separately and in combination (i.e., for those 65 apioid taxa where both sequenced regions were available). All data were analyzed using Macintosh Quadra 700, Centris 650, or Power Macintosh 8100/100 AV computers. Initially, phylogenies were inferred from all data matrices using equally weighted maximum parsimony as implemented in PAUP. Due to the large number of taxa, two heuristic approaches were used. The first was similar to that described by Pryer, Smith, and Skog (1995; modified from those search strategies presented in Olmstead and Palmer, 1994), although the number of equally most parsimonious trees could not be ascertained. The length of the shortest trees was determined by initiating 500 searches each using random addition starting trees, with tree bisection-reconnection (TBR) branch swapping and MULPARS selected, but saving no more than five of the shortest trees from each search. These equally most parsimonious trees then were used as starting trees for TBR branch swapping (with MULPARS and STEEPEST DESCENT selected). In all analyses, the maximum number of trees saved was set at 12 000 and these trees were permitted to swap to completion. In order to instill greater confidence in the results of these parsimony analyses, a second heuristic approach was employed. The "inverse constraint" approach (Catalán, Kellogg, and Olmstead, 1997) uses the strict consensus tree obtained from the 12 000 trees saved in the first approach as a topological constraint. Once more, 500 random-order-entry replicate searches were initiated as above, saving no more than five trees from each search. However, in this analysis, only those trees that do not fit the constraint tree were saved. If no additional trees are found at the length of the initial 12 000 trees (as was the case here for both ITS and intron data sets), then this suggests strongly that the strict consensus tree does adequately summarize the available evidence, even though the exact number of trees at that length is not known. If additional trees of the same length are found, they should be combined with the consensus tree to produce a less resolved consensus tree and the search started over. Bootstrap values (Felsenstein, 1985) were calculated from 100 replicate analyses using a heuristic search strategy, simple addition sequence of the taxa, and TBR branch swapping. To facilitate the bootstrap analyses, a MAXTREE limit of 100 trees per replicate was set. It is realized that owing to the large size of the data sets and the restrictions imposed on the bootstrap analysis, the values obtained may not supply the best estimate of branch support.

Gaps in the alignments of the *rpoC1* intron and ITS sequences were incorporated into the parsimony analyses in one of two ways. In the intron analysis, each indel was scored and entered as a separate presence/absence (i.e., binary) character while treating gap positions as missing data (Swofford, 1993). In the ITS analysis, gap positions were retained as missing data but each indel was mapped a posteriori onto one of the resulting minimal-length cladograms in the most parsimonious way possible in an effort to ascertain their congruence with a phylogeny generated using nucleotide substitutions alone. Because most gaps in the ITS sequence alignment were single base pairs (bp), and several of these occurred in regions of compression (and, thus, may be artifactual), these gaps were not used as additional (binary-scored) characters.

Distance trees were constructed using the neighbor-joining method (Saitou and Nei, 1987) implemented using the NEIGHBOR program in Felsenstein's (1993) PHYLIP (version 3.5). Distance matrices were calculated using the DNADIST program of PHYLIP and the numbers of nucleotide substitutions were estimated using Kimura's (1980) two-parameter method. Several Ts/Tv rate ratios were used (i.e., 1.0, 1.5, and 2.0). These values represent the expected ratio of transitions to transversions. A bootstrap analysis of these data was done using 100 resampled data sets generated with the SEQBOOT program prior to calculating the distance matrices and neighbor-joining trees. PHYLIP's CONSENSE program was then used to construct a strict consensus tree.

The maximum likelihood method also was applied to these data using the program fastDNAml (version 1.0.6; Olsen et al., 1994), based on the procedures of Felsenstein (1981). Maximum likelihood trees were inferred using a range of Ts/Tv rate ratios between 1.0 and 2.0, randomizing the input order of sequences (JUMBLE), and by invoking the global branch swapping search option. The analysis was considered complete when three separate analyses (each starting with a different random number seed) produced the same highest log likelihood value. To achieve this result, seven to ten different maximum likelihood analyses were necessary for each data set. Empirical base frequencies were derived from the sequence data and used in the maximum likelihood calculations.

All trees computed were rooted with either *Pittosporum* (Pittosporaceae; *rpoC1* intron analysis) or *Heteromorpha* (Apioideae; ITS analysis). Most authors, except Hutchinson (1973), have agreed that Apiaceae and Araliaceae are closely related (e.g., Thorne, 1973; Takhtajan, 1980; Cronquist, 1981), and many have suggested an affinity between these two families and Pittosporaceae (van Tieghem, 1884; Jay, 1969; Thorne, 1973, 1992; Dahlgren, 1980; Stuhlfauth et al., 1985; Judd, Sanders, and Donoghue, 1994). Phylogenetic analyses of cpDNA *rbcL* (Plunkett, Soltis, and Soltis, 1996a) and *matK* (Plunkett, Soltis, and Soltis, 1997) sequences corroborate the inferred close relationship between Pittosporaceae and Apiales (Apiaceae+Araliaceae). The trees computed in the ITS analyses were rooted with *Heteromorpha,* one of the "basal apioids" identified in the phylogenetic analyses of *rpoC1* intron sequences (see below). *Heteromorpha* also exhibits a basal position within Apioideae in the *rbcL*- and *matK*-derived cladograms of Plunkett, Soltis, and Soltis (1996a, b). *Bupleurum* and *Anginon,* two

TABLE 2. Sources of Apiaceae subfamily Apioideae and outgroup accessions examined for nuclear ribosomal DNA ITS (*) and chloroplast DNA *rpoC1* intron (†) sequence variation. Accessions designated by both symbols (*†) were used in the phylogenetic analyses of combined ITS and *rpoC1* intron data. The ITS data have been deposited with GenBank as separate ITS 1 and ITS 2 sequences; GenBank accession numbers for both spacers and/or the *rpoC1* intron are provided in brackets. Locations of voucher specimens, where available, are provided (herbarium acronyms according to Holmgren and Keuken, 1974). UIUC = University of Illinois at Urbana-Champaign.

Taxon	Source
Apiaceae subfamily Apioideae	
Aciphylla aurea W. R. B. Oliv.*	New Zealand, Cult. Royal Botanic Garden, Edinburgh, UK (no. 19712219) [ITS: U79593, U79594]
Aciphylla crenulata J. B. Armstr.*†	New Zealand, South Island, Fiordland National Park, *Howick and Darby 1121* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 90.0616) [ITS: U78359, U78419; *rpoC1*: U72448]
Aciphylla squarrosa Forst.*	New Zealand, Cult. Royal Botanic Garden, Edinburgh, UK (no. 19781305) [ITS: U79595, U79596]
Aegopodium alpestre Ledeb.*†	Kazakhstan, Dzhungar Alatau Mtns., Lepsinsk, *Kljuykov 109* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78376, U78436; *rpoC1*: U72434]
Aegopodium podagraria L.*	USA, Illinois, Urbana, *Downie 725* (ILL) [ITS: U30536, U30537]
Aethusa cynapium L.*†	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Downie 337* (ILL) [ITS: U30582, U30583; *rpoC1*: U36278]
Aletes humilis J. M. Coult. & Rose*	USA, Colorado, Larimer Co., Powder Canyon, Grey Rock Mtn., *Hartman 11678* (RM) [ITS: U78401, U78461]
Ammi majus L.*	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Downie 308* (ILL) [ITS: U78386, U78446]
Anethum graveolens L.*†	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Downie 326* (ILL) [ITS: U30550, U30551; *rpoC1*: U36281]
Angelica ampla A. Nelson*	USA, Colorado, Garfield Co., Flat Tops/White River Plateau, *Hartman 25821* (RM) [ITS: U79597, U79598]
Angelica archangelica L.*†	Cult. UIUC from seeds obtained from Univ. Joensuu Botanical Garden, Finland; *Downie 79* (ILL) [ITS: U30576, U30577; *rpoC1*: U36279]
Angelica arguta Nutt. ex Torr. & A. Gray*	USA, California, Siskiyou Co., W. of Weed, *Raiche 364.90* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 90.2035) [ITS: U79599, U79600]
Angelica breweri A. Gray*	USA, California, Alpine Co., Ebbetts Pass, *Constance & Ertter 3903* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2446 [ITS: U78396, U78456]
Angelica dahurica Maxim.*	China, Cult. Univ. California Botanical Garden, Berkeley (no. 88.0678) [ITS: U78416, U78476]
Angelica polymorpha Maxim.*	Japan, Miyazaki, Kyushu, *McNamara et al. 264* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 90.0662) [ITS: U78415, U78475]
Angelica sachalinensis Maxim.*	Russia, Saghalin Is., Chekhov Mtn., *Pimenov & Kljuykov s.n.* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78413, U78473]
Angelica sylvestris L.*	Cult. UIUC from seeds obtained from Shanghai Botanic Garden, China; *Downie 428* (ILL) [ITS: U78414, U78474]
Anginon rugosum (Thunb.) Raf.†	South Africa, West Cape, *Batten 1018* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2399 [*rpoC1*: U72457]
Anisotome aromatica Hook.*†	Cult. Royal Botanic Garden, Edinburgh, UK (no. 19881687) [ITS: U78360, U78420; *rpoC1*: U72449]
Anthriscus caucalis Bieb.*	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Lee 44* (ILL) [ITS: U79601, U79602]
Anthriscus cerefolium (L.) Hoffm.*†	Cult. UIUC from seeds obtained from Real Jardín Botánico, Spain; *Downie 35* (ILL) [ITS: U30532, U30533; *rpoC1*: U36280]
Anthriscus sylvestris (L.) Hoffm.*	Moscow State Univ. Botanical Garden, Russia [ITS: U79603, U79604]
Apium graveolens L.*†	Cult. UIUC from seeds obtained from Conservatoire et Jardins Botaniques de Nancy, France; *Downie 258* (ILL) [ITS: U30552, U30553; *rpoC1*: U36282]
Arafoe aromatica Pimenov & Lavrova*†	Russia, N Caucasus, Krasnodar, Caucasian Reservate, Lagonaki, *Pimenov 403* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78383, U78443; *rpoC1*: U72420]
Arracacia brandegei J. M. Coult. & Rose*†	Mexico, Baja California del Sur, *Breedlove 43405* (UC); L. Constance 2045 [ITS: U30570, U30571; *rpoC1*: U36284]
Arracacia nelsonii J. M. Coult. & Rose*†	Mexico, Oaxaca, *Breedlove 72434* (UC); L. Constance 2410 [ITS: U30556, U30557; *rpoC1*: U36285]
Berula erecta (Huds.) Coville*	Cult. UIUC from seeds obtained from Conservatoire et Jardins Botaniques de Nancy, France; *Downie 251* (ILL) [ITS: U79605, U79606]
Berula erecta (Huds.) Coville*	Cult. UIUC from seeds obtained from Univ. Oldenburg Botanic Garden, Germany; *Downie 150* (ILL) [ITS: U79607, U79608]
Berula thunbergii (DC.) H. Wolff*	Ethiopia, Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2453 [ITS: U78369, U78429]
Bifora radians Bieb.*	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Lee 28* (ILL) [ITS: U78408, U78468]
Bupleurum chinense Franch.†	Cult. UIUC from seeds obtained from Shanghai Botanic Garden, China; *Downie 409* (ILL) [*rpoC1*: U72455]
Bupleurum ranunculoides L.†	Cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót; *Downie 94* (ILL) [*rpoC1*: U72456]
Capnophyllum dichotomum (Desf.) Lag.*†	Cult. UIUC from seeds obtained from Jardin Botanique National de Belgique, Belgium; *Downie 285* (ILL) [ITS: U78390, U78450; *rpoC1*: U72432]
Carlesia sinensis Dunn*	Cult. Hort. Nanjing, China; L. Constance 2401 [ITS: U30562, U30563]
Carum alpinum Benth. & Hook. f.*	Cult. UIUC from seeds obtained from Univ. Turku, Finland; *Downie 424* (ILL) [ITS: same as U78377, U78437]

TABLE 2. Continued.

Taxon	Source
Carum carvi L.*†	Cult. UIUC from seeds obtained from Univ. Kuopio Botanical Garden, Finland; *Downie 430* (ILL) [ITS: U78377, U78437; *rpoC1*: U72435]
Caucalis platycarpos L.*†	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Lee 29* (ILL) [ITS: U78364, U78424; *rpoC1*: U72437]
Chaerophyllum khorassanicum Czerniak. ex Schischk.*	Turkmenistan, Kopet Dagh Mtns., Mt. Dalancha, *Pimenov et al. 246* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78366, U78426]
Chaetosciadium trichospermum (L.) Boiss.*	Jordan, Um-Qais near Irbid, *Lahham & El-Oqlah 4* (Yarmouk Univ. Herbarium) [ITS: U78363, U78423]
Chymsydia colchica (Albov) Woronow*†	Georgia, Mt. Kvira, *Pimenov 1489* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78405, U78465; *rpoC1*: U72416]
Cicuta virosa L.*	Cult. UIUC from seeds obtained from Univ. Joensuu Botanical Garden, Finland; *Downie 75* (ILL) [ITS: U78372, U78432]
Cicuta virosa L.*†	Cult. UIUC from seeds obtained from Univ. Oldenburg Botanic Garden, Germany; *Downie 131* (ILL) [ITS: same as U78372, U78432; *rpoC1*: U72447]
Cnidium officinale Makino*	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Downie 830* (ILL) [ITS: U78388, U78448]
Cnidium silaefolium (Jacq.) Simonkai*	Cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót; *Downie 483* (ILL) [ITS: U78407, U78467]
Coaxana purpurea J. M. Coult. & Rose*	Mexico, Oaxaca, *Breedlove 72745* (UC); L. Constance 2411 [ITS: U30572, U30573]
Conioselinum chinensis (L.) B. S. P.*†	USA, California, San Mateo Co., San Bruno Mtn., *Raiche 30046,* Cult. Univ. California Botanical Garden, Berkeley (no. 83.0114) [ITS: U78374, U78434; *rpoC1*: U72452]
Conium maculatum L.*	USA, Illinois, Urbana, *Downie 739* (ILL) [ITS: U79609, U79610]
Conium maculatum L.*†	Cult. UIUC from seeds obtained from Conservatoire et Jardins Botaniques de Nancy, France; *Downie 241* (ILL) [ITS: same as U30588, U30589; *rpoC1*: U72428]
Conium maculatum L.*†	Cult. UIUC from seeds obtained from Johannes Gutenberg Univ., Germany; *Downie 63* (ILL) [ITS: U30588, U30589; *rpoC1*: U36287]
Conium maculatum L.*	Cult. UIUC from seeds obtained from Univ. Joensuu Botanical Garden, Finland; *Downie 374* (ILL) [ITS: U79611, U79612]
Coriandrum sativum L*†	Cult. UIUC from seeds obtained from Johannes Gutenberg Univ., Germany; *Downie 65* (ILL) [ITS: U30586, U30587; *rpoC1*: U36288]
Coulterophytum laxum Robins.*†	Mexico, Michoacán, 30 km E of Morelia, *Iltis 298 & Cochrane* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 1650 [ITS: U30560, U30561; *rpoC1*: U36289]
Crithmum maritimum L.*†	Europe, Cult. Univ. California Botanical Garden, Berkeley (no. 89.1222) [ITS: U30540, U30541; *rpoC1*: U72425]
Crithmum maritimum L.†	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Downie 307* (ILL) [*rpoC1*: U72424]
Cryptotaenia canadensis (L.) DC.*	USA, Illinois, Urbana, *Downie 817* (ILL) [ITS: U79613, U79614]
Cryptotaenia japonica D. Don*†	Japan, Honshu Island, Koyosan area, *McNamara et al. 90* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 90.0891) [ITS: U78367, U78427; *rpoC1*: U72445]
Cuminum cyminum L.*†	Cult. UIUC from seeds obtained from grocery store; *Lee 120* (ILL) [ITS: U78362, U78422; *rpoC1*: U72436]
Cymopterus globosus (S. Wats.) S. Wats.*	USA, Nevada, Washoe Co., N. of Sparks, *Lyons-Weiler s.n.* [ITS: U78398, U78458]
Dahliaphyllum almedae Constance & Breedlove*	Mexico, Guerrero, Puerto El Gallo-Atoyac, *Breedlove 61970* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2328 [ITS: U78395, U78455]
Daucus carota L.*†	Cult. UIUC from seeds obtained from Univ. Oldenburg Botanic Garden, Germany; *Downie 164* (ILL) [ITS: U27589, U30315; *rpoC1*: U36290]
Enantiophylla heydeana J. M. Coult. & Rose*	Mexico, Jalisco, Manantlán, *Iltis et al. 3187* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2252 [ITS: U30558, U30559]
Endressia castellana Coincy*†	Cult. Inst. Bot. Univ. Neuchatel, Switzerland; L. Constance 2184 [ITS: U30584, U30585; *rpoC1*: U72418]
Falcaria vulgaris Bernh.*†	Russia, Rostov Prov., Boguchar District, Radchenskoye, *Pimenov 25* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78378, U78438; *rpoC1*: U72433]
Ferula assa-foetida L.*†	Cult. UIUC from seeds obtained from Jardin Botanique National de Belgique, Belgium; *Downie 463* (ILL) [ITS: U78391, U78451; *rpoC1*: U72421]
Ferula communis L.*	Cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót; *Downie 112* (ILL) [ITS: U79615, U79616]
Foeniculum vulgare P. Mill.*†	Cult. UIUC from seeds obtained from National Botanic Gardens, Glasnevin, Ireland; *Downie 187* (ILL) [ITS: U78385, U78445; *rpoC1*: U72427]
Heracleum lanatum Michx.*†	USA, California, Muir Woods, *Downie 579* (ILL) [ITS: U30542, U30543; *rpoC1*: U36292]
Heracleum rigens DC.*†	India, Karnataka, Mullengiri-Bababudan Hills, Chixmagalur District, *Mukherjee s.n.* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2274 [ITS: U30548, U30549; *rpoC1*: U36293]
Heracleum sphondylium L.*	Cult. UIUC from seeds obtained from Univ. Kuopio Botanical Garden, Finland; *Downie 433* (ILL) [ITS: U30544, U30545]
Heteromorpha arborescens (Spreng.) Cham. & Schlechtd.*†	Cult. UIUC from seeds obtained from Real Jardín Botánico, Spain; *Downie 42* (ILL) [ITS: U27578, U30314; *rpoC1*: U36294]
Imperatoria ostruthium L.*†	Czechoslovakia, Krkonoschi Mtns., *Pimenov s.n.* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78403, U78463; *rpoC1*: U72417]
Komarovia anisosperma Korovin*†	Uzbekistan, Zeravschan Mtns., Urgut, *Pimenov et al. 178* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78381, U78441; *rpoC1*: U72453]

TABLE 2. Continued.

Taxon	Source
Laserpitium hispidum Bieb.*	Russia, Krasnodar, Gorjachiy Kljuch, *Ostroumova 19* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78361, U78421]
Laserpitium siler L.*†	Cult. UIUC from seeds obtained from Johannes Gutenburg Univ., Germany; *Downie 71* (ILL) [ITS: U30528, U30529; *rpoC1*: U36296]
Lecokia cretica (Lam.) DC.*†	Jordan, Ajlun, near Schtafeenah, *Lahham & El-Oqlah 7* (Yarmouk Univ. Herbarium) [ITS: U78358, U78418; *rpoC1*: U72450]
Levisticum officinale W. D. J. Koch*	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Downie 333* (ILL) [ITS: U78389, U78449]
Levisticum officinale W. D. J. Koch*	Cult. UIUC from seeds obtained from Jardin Botanique de Montréal, Canada; *Downie 387* (ILL) [ITS: same as U78389, U78449]
Levisticum officinale W. D. J. Koch*	Cult. Univ. California Botanical Garden, Berkeley (no. 82.0651) [ITS: same as U78389, U78449]
Ligusticum porteri J. M. Coult. & Rose*	USA, Colorado, Rio Blanco Co., Flat Tops/White River Plateau, *Vanderhorst 3763* (RM) [ITS: U78375, U78435]
Ligusticum scoticum L.*†	USA, Massachusetts, Plymouth Co., near Mattapoisett along Buzzard's Bay, *Raiche 40411* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 84.0620) [ITS: U78357, U78417; *rpoC1*: U72441]
Ligusticum scoticum L.*†	Cult. UIUC from seeds obtained from Jardin Botanique de Montréal, Canada; *Downie 3* (ILL) [ITS: U79591, U79592; *rpoC1*: U72440]
Lomatium californicum (Nutt.) Mathias & Constance*	USA, California, Napa Co., *Plunkett 1310* (WS) [ITS: U78397, U78457]
Lomatium dasycarpum (Torr. & A. Gray) J. M. Coult. & Rose*†	USA, California, San Mateo Co., *Raiche 10396* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 81.1108) [ITS: U30580, U30581; *rpoC1*: U72412]
Mathiasella bupleuroides Constance & Hitchcock*	Mexico, Nuevo Leon, Cerro El Viejo, *Hinton et al. 22234* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2447 [ITS: U78394, U78454]
Myrrhidendron donnell-smithii J. M. Coult. & Rose*†	Costa Rica, San José Prov., *Grantham & Parsons 0433-90* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 90.2637) [ITS: U30554, U30555; *rpoC1*: U36297]
Myrrhidendron donnell-smithii J. M. Coult. & Rose*	Costa Rica, Cartago Prov., Vulcan Irazu Crater, *Grantham & Parsons 0198-90* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 90.2276) [ITS: same as U30554, U30555]
Myrrhis odorata (L.) Scop.*†	Europe, Cult. Univ. California Botanical Garden, Berkeley (no. 89.1236) [ITS: U30530, U30531; *rpoC1*: U72438]
Notopterygium incisum Ting ex Ho-T Chang*	Cult. UIUC from seeds obtained from Shanghai Botanic Garden, China; *Downie 400* (ILL) [ITS: U78412, U78472]
Oenanthe pimpinelloides L.*†	Cult. UIUC from seeds obtained from Jardin Botanique National de Belgique, Belgium; *Downie 273* (ILL) [ITS: U78371, U78431; *rpoC1*: U72442]
Olymposciadium caespitosum (Sibth. & Sm.) H. Wolff*	Cult. Royal Botanic Garden, Edinburgh, UK (no. 19100154) [ITS: U78379, U78439]
Orlaya grandiflora (L.) Hoffm.*†	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Downie 309* (ILL) [ITS: U30524, U30525; *rpoC1*: U36298]
Orlaya kochii Heywood*	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Downie 20* (ILL) [ITS: U30526, U30527]
Osmorhiza chilensis Hook. & Arn.*†	USA, California, Alameda Co., Strawberry Canyon, Univ. California Botanical Garden, Berkeley [ITS: U78365, U78425; *rpoC1*: U72439]
Osmorhiza longistylis (Torr.) DC.*	USA, Illinois, Urbana, *Downie 738* (ILL) [ITS: U79617, U79618]
Osmorhiza occidentalis (Nutt.) Torr.*	USA, Wyoming, Sublette Co., Salt River Range, *Hartman 41878* (RM) [ITS: U79619, U79620]
Oxypolis occidentalis J. M. Coult. & Rose*†	USA, California, Kern Co., Lake Isabella, *Raiche & Zadnik RR50099* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 85.0288) [ITS: U78368, U78428; *rpoC1*: U72444]
Paraligusticum discolor (Ledeb.) V. N. Tichom.*	Kazakhstan, Dzhungar Alatau Mtns., Lepsinsk, *Kljuykov 119* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78404, U78464]
Pastinaca sativa L.*†	Cult. UIUC from seeds obtained from Johannes Gutenburg Univ., Germany; *Downie 70* (ILL) [ITS: U30546, U30547; *rpoC1*: U36299]
Perideridia kelloggii (A. Gray) Mathias*†	USA, California, Sonoma Co., N Cazadero, *Ornduff et al. s.n.* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 81.0521) [ITS: U78373, U78433; *rpoC1*: U72446]
Petroselinum crispum (P. Mill.) A. W. Hill*	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Downie 33* (ILL) [ITS: U78387, U78447]
Peucedanum decursivum Maxim.*†	Cult. UIUC from seeds obtained from Shanghai Botanic Garden, China; *Downie 359* (ILL) [ITS: U78411, U78471; *rpoC1*: U72415]
Peucedanum morisonii Bess. ex Spreng.*†	Russia, Altai Mtns., Barlak, *Ostroumova s.n.* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78406, U78466; *rpoC1*: U72414]
Physospermum cornubiense (L.) DC.*†	Ukraine, Crimea, Alikat-Bogaz Pass, *Pimenov & Tomkovich s.n.* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78382, U78442; *rpoC1*: U72454]
Pimpinella peregrina L.*†	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Downie 19* (ILL) [ITS: U30592, U30593; *rpoC1*: U36300]
Pimpinella rhodantha Boiss.†	Russia, N Caucasus, Daghestan, Charami Pass, *Pimenov s.n.* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [*rpoC1*: U72429]
Pimpinella saxifraga L.*	Cult. UIUC from seeds obtained from Univ. Oldenburg Botanic Garden, Germany; *Downie 137* (ILL) [ITS: U30590, U30591]
Prangos pabularia Lindl.*†	Kirghizia, Fergana Mtns., Urumbash, *Pimenov s.n.* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78409, U78469; *rpoC1*: U72431]

TABLE 2. Continued.

Taxon	Source
Prionosciadium turneri Constance & Affolter*†	Mexico, Colima, 20 km SSW of Colima, *Turner s.n.* (UC); L. Constance 2503 [ITS: U30568, U30569; *rpoC1*: U36302]
Pseudorlaya pumila (L.) Grande*	Cult. UIUC from seeds obtained from Univ. Oldenburg Botanic Garden, Germany; *Downie 138* (ILL) [ITS: U30522, U30523]
Rhodosciadium argutum (Rose) Mathias & Constance*†	Mexico, Guanajuato, Xichu, *Rzedowski 41342* (UC); L. Constance 2371 [ITS: U30566, U30567; *rpoC1*: U36303]
Ridolfia segetum (L.) Moris*†	Jordan, Wadi Al-Yabis, along R. Jordan, *Lahham & El-Oqlah 12* (Yarmouk Univ. Herbarium) [ITS: U78384, U78444; *rpoC1*: U72426]
Scandix balansae Reut.*	Cult. UIUC from seeds obtained from Botanischer Garten der Karl-Marx-Universität, Leipzig; *Lee 2* (ILL) [ITS: U79621, U79622]
Scandix pecten-veneris L.*†	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Downie 27* (ILL) [ITS: U30538, U30539; *rpoC1*: U36304]
Selinum candollii DC.*	India, Garhwal Himalaya, Himalaya Mtns., *Pradham s.n.*, Cult. Univ. California Botanical Garden, Berkeley (no. 89.2000) [ITS: U30564, U30565]
Seseli elatum L.*	Cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót, *Lee 13* (ILL) [ITS: U79623, U79624]
Seseli krylovii (V. N. Tichom.) Pimenov & Sdobnina*†	Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78402, U78462; *rpoC1*: U72419]
Seseli montanum L.*	Cult. UIUC from seeds obtained from Conservatoire et Jardins Botaniques de Nancy, France; *Downie 239* (ILL) [ITS: U30578, U30579]
Shoshonea pulvinata Evert & Constance*†	USA, Wyoming, Park Co., Absaroka Range, NE Logan Mtn., *Evert 10623* (RM) [ITS: U78400, U78460; *rpoC1*: U72413]
Sium latifolium L.*†	Cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót; *Downie 97* (ILL) [ITS: U78370, U78430; *rpoC1*: U72443]
Smyrniopsis aucheri Boiss.*†	Armenia, Selim Pass, *Pimenov et al. 1337* (MW), Cult. Moscow State Univ. Botanical Garden, Russia [ITS: U78393, U78453; *rpoC1*: U72430]
Smyrnium olusatrum L.*†	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Downie 328* (ILL) [ITS: U30594, U30595; *rpoC1*: U36305]
Smyrnium olusatrum L.*	Cult. UIUC from seeds obtained from Quail Botanical Gardens, CA; *Downie 343* (ILL) [ITS: U30594, U30595]
Smyrnium olusatrum L.†	Cult. UIUC from seeds obtained from Botanischer Garten der Karl-Marx-Universität, Leipzig; *Lee 113* [*rpoC1*: U72451]
Smyrnium olusatrum L.*	Cult. UIUC from seeds obtained from Univ. Oldenburg Botanic Garden, Germany; *Downie 141* (ILL) [ITS: same as U30594, U30595]
Taenidia integerrima (L.) Drude*	USA, Illinois, Lake View Park, *Downie 763* (ILL) [ITS: U78399, U78459]
Thaspium trifoliatum (L.) A. Gray*	USA, Illinois, Urbana, *Downie 744* (ILL) [ITS: U78410, U78470]
Thaspium pinnatifidum (Buckl.) A. Gray*	USA, Kentucky, *Downie 810* (ILL) [ITS: same as U78410, U78470]
Tordylium aegyptiacum (L.) Lam. var. *palaestinum* (Zoh.) Zoh.*†	Jordan, Um-Qais, near Irbid, *Lahham & El-Oqlah 11* (Yarmouk Univ. Herbarium) [ITS: U78392, U78452; *rpoC1*: U72422]
Torilis nodosa (L.) Gaertn.*†	Cult. UIUC from seeds obtained from Jardin Botanique de Caen, France; *Downie 322* (ILL) [ITS: U30534, U30535; *rpoC1*: U36306]
Trachyspermum ammi (L.) Sprague ex Turrill*†	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Downie 14* (ILL) [ITS: U78380, U78440; *rpoC1*: U72423]
Zizia aurea (L.) W. D. J. Koch*†	Cult. UIUC from seeds obtained from Jardin Botanique de Montréal, Canada; *Downie 8* (ILL) [ITS: U30574, U30575; *rpoC1*: U36307]
Zizia aurea (L.) W. D. J. Koch*	USA, Illinois, Urbana, *Downie 740* (ILL) [ITS: same as U30574, U30575]
Apiaceae subfamily Hydrocotyloideae	
Bolax gummifera Spreng.†	Cult. Royal Botanic Garden, Edinburgh, UK (no. 19361025) [*rpoC1*: U72464]
Centella asiatica (L.) Urb.†	Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2198 [*rpoC1*: U72465]
Centella erecta (L. f.) Fern.†	Cuba, La Habana, Cayo La Rosa, Laguna Ariquenabo, *Stevens et al. 23626* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2336 [*rpoC1*: U36286]
Centella erecta (L. f.) Fern.†	USA, Florida, Wakulla Co., St. Marks Wildlife Refuge, *Godfrey s.n.* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 1477 [*rpoC1*: U72466]
Didiscus pusilla DC.†	Cult. UIUC from seeds obtained from Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany; *Lee 35* (ILL) [*rpoC1*: U72469]
Eremocharis fruticosa Phil.†	Chile, Antofagasta, Quebrada Coquimbo, Taltal, *Dillon & Teillier 5082* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2382 [*rpoC1*: U72463]
Hydrocotyle bowlesioides Mathias & Constance†	Costa Rica, La Palma, *Horich s.n.* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 61.1190); L. Constance 222 [*rpoC1*: U36295]
Hydrocotyle pusilla A. Rich.†	Equador, Cult. Univ. California Botanical Garden, Berkeley, *Ornduff 9683* (UC); L. Constance 2353 [*rpoC1*: U72467]
Hydrocotyle rotundifolia Wallich.†	Cult. Missouri Botanical Garden (no. 895612) [*rpoC1*: U72468]
Klotzschia rhizophylla Urb.†	Brazil, Minas Gerais, Serra do Cipo, *Pirani 12909* (UC), Cult. Univ. California Botanical Garden, Berkeley; L. Constance 2414 [*rpoC1*: U72462]
Apiaceae subfamily Saniculoideae	
Astrantia major L. subsp. *major*†	Switzerland, *Schilling 2937,* Cult. Royal Botanic Garden, Edinburgh, UK (no. 19861407) [*rpoC1*: U72458]

TABLE 2. Continued.

Taxon	Source
Eryngium planum L.†	Cult. UIUC from seeds obtained from National Botanic Gardens, Glasnevin, Ireland; *Downie 191* (ILL) [*rpoC1*: U36291]
Hacquetia epipactis (Scop.) DC.†	Cult. Royal Botanic Garden, Edinburgh, UK (no. 19694625) [*rpoC1*: U72460]
Petagnaea saniculifolia Guss.†	Cult. Royal Botanic Garden, Edinburgh, UK (no. 19695641) [*rpoC1*: U72459]
Sanicula canadensis L.†	USA, Illinois, Urbana, *Downie 737* (ILL) [*rpoC1*: U72461]
Araliaceae	
Aralia californica S. Wats.†	USA, Alameda Co., Strawberry Canyon, Cult. Univ. California Botanical Garden, Berkeley (no. 86.1028) [*rpoC1*: U72480]
Aralia chinensis L.†	Cult. UIUC from seeds obtained from Shanghai Botanic Garden, China; *Downie 407* (ILL) [*rpoC1*: U36283]
Aralia spinosa L.†	USA, North Carolina, Carteret Co., *Bartholomew et al. 1181* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 82.2039) [*rpoC1*: U72481]
Cussonia paniculata Eckl. & Zeyh.†	Cult. Royal Botanic Gardens, Edinburgh, UK (no. 19900662) [*rpoC1*: U72470]
Dendropanax arboreus (L.) Decne. & Planch.†	Honduras, Tanpasenti, *Ornduff 8671* (UC), Cult. Univ. California Botanical Garden, Berkeley (no. 80.0232) [*rpoC1*: U72476]
Fatsia japonica (Thunb.) Decne. & Planch.†	Cult. Royal Botanic Garden, Edinburgh, UK (no. 19687549) [*rpoC1*: U72474]
Hedera helix L.†	Cult. Missouri Botanical Garden [*rpoC1*: U72477]
Kalopanax pictus (Thunb.) Nakai†	Cult. Morton Arboretum (no. 211-57); *Downie 522* (ILL) [*rpoC1*: U72473]
Oreopanax sanderianus Hemsl.†	Cult. Missouri Botanical Garden (no. 873066) [*rpoC1*: U72475]
Polyscias balfouriana (Hort. Sander) L. H. Bailey†	Cult. Royal Botanic Garden, Edinburgh, UK (no. 19697330) [*rpoC1*: U72478]
Pseudopanax arboreus (Murr.) Philipson†	Cult. Royal Botanic Garden, Edinburgh, UK (no. 19665059) [*rpoC1*: U72479]
Schefflera actinophylla (Endl.) Harms†	Cult. UIUC (no. 89172); *Downie 525* (ILL) [*rpoC1*: U72471]
Tetrapanax papyriferus (Hook.) C. Koch†	Cult. Missouri Botanical Garden (no. 863189) [*rpoC1*: U72472]
Pittosporaceae	
Pittosporum tobira (Thunb.) Ait.†	China, Cult. Missouri Botanical Garden (no. 801425) [*rpoC1*: U36301]

other "basal apioids," also were initially included in the ITS study but high nucleotide divergence in the former and the difficulty in amplifying the ITS 1 region of the latter precluded them from being used as outgroup taxa.

RESULTS

rpoC1 intron sequence analysis—Despite some 745–769 bp of sequence, including several stretches of repetitive elements, the two accessions each of *Centella erecta, Conium maculatum, Crithmum maritimum,* and *Smyrnium olusatrum* examined (Table 2) possessed identical *rpoC1* intron sequences. Thus, one representative from each of these four species was removed from subsequent analyses. Alignment of the remaining 96 intron sequences resulted in a matrix of 948 positions. A total of 50 unambiguous gaps was required for proper alignment of these sequences. These gaps ranged in size from 1 to 16 bp (Fig. 1), with the average size being ~3.4 bp. Twenty-two gaps were phylogenetically informative for parsimony analysis (Table 3). Because of alignment am-

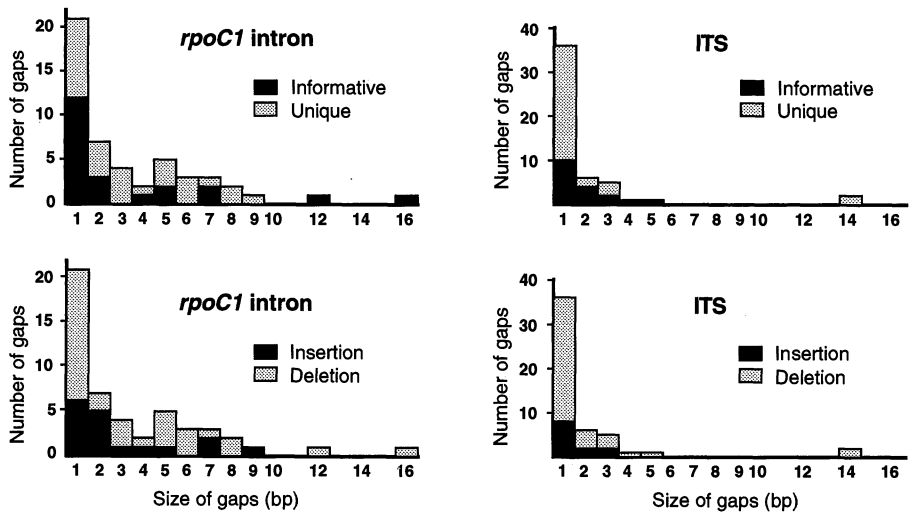

Fig. 1. Characteristics of the gaps inferred in the alignments of 96 *rpoC1* intron (left) and 95 complete ITS (right) nucleotide sequences (see also Table 3). For each DNA region, the number of gaps in each size category, the number of gaps unique (i.e., autapomorphic) vs. informative for parsimony analysis, and the distribution of inferred insertions vs. deletions are provided.

TABLE 3. Sequence characteristics of the two nuclear ribosomal DNA internal transcribed spacers, separately and combined, and chloroplast DNA *rpoC1* intron in Apiaceae subfamily Apioideae and relatives.

Sequence characteristic	*rpoC1* intron	ITS 1	ITS 2	ITS 1 and ITS 2
No. of accessions examined	96	95	95	95
Nucleotide sites				
Length variation (bp)	719–807	204–221	216–229	424–444
No. aligned	948	239	246	485
No. (and %) ambiguous	165 (17.4)	16 (6.7)	32 (13.0)	48 (9.9)
No. (and %) constant	451 (47.6)	47 (19.7)	38 (15.5)	85 (17.5)
No. (and %) autapomorphic	117 (12.3)	26 (10.9)	32 (13.0)	58 (12.0)
No. (and %) phylogenetically informative	215 (22.7)	150 (62.8)	144 (58.5)	294 (60.6)
Length variation				
No. of unambiguous alignment gaps	50	24	27	51
No. (and size range; bp) of deletions	33 (1–16)	18 (1–14)	21 (1–5)	39 (1–14)
No. (and size range; bp) of insertions	17 (1–9)	6 (1–3)	6 (1–3)	12 (1–3)
No. gaps phylogenetically informative	22	10	8	18
Sequence divergence (%)				
Subfamily Apioideae only	0–9.8	1.4–35.0	1.0–33.7	1.7–34.3
All accessions	0–12.2	1.4–35.0	1.0–33.7	1.7–34.3
G + C content (range; %)	34.1–38.3	47.9–59.3	42.7–63.8	46.6–61.5

biguities, it was necessary to ignore 12 regions from the matrix in the distance calculations and phylogenetic analyses. These ambiguous regions ranged in size from five to 31 positions and totaled 165 positions. Characteristics of these intron sequences, including overall variation in length, percentage G + C content, and the numbers of ambiguous, constant, autapomorphic, and phylogenetically informative positions are provided in Table 3. In direct pairwise comparisons of unambiguous positions among all accessions, sequence divergence values ranged from identity (between the two species each of *Centella* and *Bupleurum*) to 12.2% (between *Scandix* [Apioideae] and *Didiscus* [Hydrocotyloideae]). Among the three species each of *Hydrocotyle* and *Aralia* examined, pairwise nucleotide divergence values ranged from 1.2 to 1.4% within the former, and from 1.5 to 2.7% within the latter. Among Apioideae sequence pairs, nucleotide divergence values ranged from identity to 9.8% (between *Scandix* and *Bupleurum*), with maximum pairwise divergence values reaching 2.9, 2.2, 4.9, and 2.2%, respectively, in apioid Groups 1, 3, 5, and 6 (see Fig. 2; groups discussed below). Within Apioideae, the ratio of terminal taxa (68) to informative characters (137, excluding informative gaps) was 1:2.0. Within Araliaceae, pairwise divergence values ranged from 0.3 to 2.9% of nucleotides, and the ratio of terminal taxa (13) to informative characters (ten, excluding gaps) was 1:0.77.

rpoC1 intron phylogenetic analysis—Parsimony analysis of all unambiguously aligned positions, including the 22 informative gaps, resulted in more than 12 000 minimal length trees before the analysis terminated due to insufficient memory. In a subsequent run, the maximum number of trees saved was set at 12 000 and these trees were permitted to swap to completion. The strict consensus of these trees with accompanying bootstrap values is shown in Fig. 2. Each of the 12 000 trees had a length of 730 steps, consistency indices (CIs) of 0.640 (all characters) and 0.560 (excluding uninformative characters), and a retention index (RI) of 0.871.

One of these 730-step trees was chosen arbitrarily and is presented in Fig. 3 to indicate the distribution of the 27 indels inferred from the 22 informative alignment gaps, and the number of nucleotide substitutions supporting each branch, as optimized by ACCTRAN in PAUP. On this phylogram, 17 gaps are synapomorphic, one gap (labeled D in Fig. 3) represents a reversal, and three gaps (labeled B, C, and E) represent parallel events. In several other minimal length trees, *Myrrhidendron* allies with *Imperatoria*; in these instances, gap F is also homoplastic. These indels are widely distributed throughout the tree, supporting both basal and terminal branches. Of the 50 unambiguous gaps required for proper sequence alignment, deletions outnumbered insertions 33 to 17 (Fig. 1; Table 3). Of the 17 insertions inferred in the alignment, 13 involved perfect direct repeats of flanking sequence of either 1, 2, 3, or 7 bp and are likely to be the result of slipped-strand mispairing (Takaiwa and Sugiura, 1982; Zurawski, Clegg, and Brown, 1984). Three of the remaining four insertions of 4, 5, or 9 bp were imperfect repeats, varying from flanking sequence by one or two positions. Other repetitive motifs occurred but are in those regions of the alignment excluded from the analysis; included here were tracts of poly-As, Gs, and Ts. The number of nucleotide substitutions, reflected by the lengths of the branches in Fig. 3, shows an apparently unequal distribution pattern among the taxa. For example, within Araliaceae and many Apioideae clades branch lengths are relatively short, whereas among the hydrocotyloids (e.g., *Centella, Hydrocotyle,* and *Didiscus*) and some basal apioids (e.g., *Bupleurum*) the branches are quite long.

Reanalyzing the data without the 22 informative gaps resulted in >12 000 trees each of 702 steps (CIs of 0.634 and 0.549, with and without uninformative characters, respectively; RI = 0.857). The topology of this strict consensus tree was nearly identical to that produced when the indels are included, with the exception of the collapse of the two branches indicated by arrows in Fig. 2. The

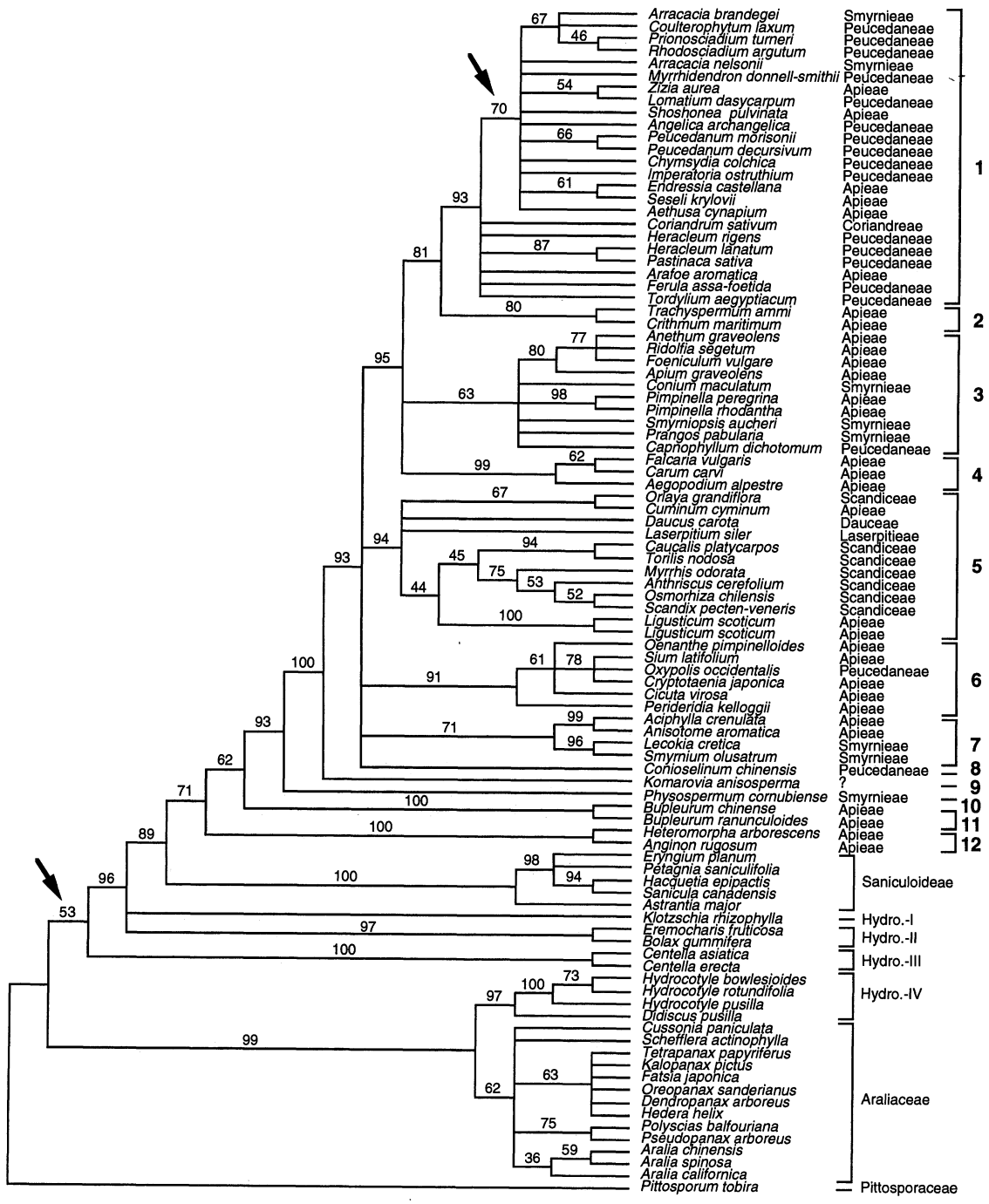

Fig. 2. Strict consensus of 12 000 730-step trees derived from equally weighted parsimony analysis of cpDNA *rpoC1* intron sequences from Apiaceae subfamily Apioideae and relatives using all unambiguously aligned positions and 22 informative gaps (CI excluding uninformative characters = 0.560, RI = 0.871). Numbers above the nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates. The two arrows indicate branches that collapse when the 22 gaps are excluded and the analysis rerun. On the basis of these results, 12 groups of Apioideae (labeled 1–12) are circumscribed; the composition and relative placement of these groups will be compared to the results of the other analyses described herein. Tribal classification of Apiaceae subfamily Apioideae is based on Drude (1897–1898); ? = tribal placement uncertain, because genus was not included in Drude's treatment; Hydro. = Apiaceae subfamily Hydrocotyloideae.

average Ts/Tv ratio among all intron sequences across 200 trees chosen randomly from the 12 000 saved trees, as determined by MacClade, was 0.94.

Distance trees obtained from the neighbor-joining analysis, estimated from the two-parameter method of Kimura (1980) with Ts/Tv rate ratios of 1.0, 1.5, or 2.0, were topologically congruent (not shown). Both the parsimony and neighbor-joining analyses recognized the same major clades (to be discussed below), although their relative positions and the relationships of taxa within each varied considerably. Similar results were obtained when the maximum likelihood method was invoked. The

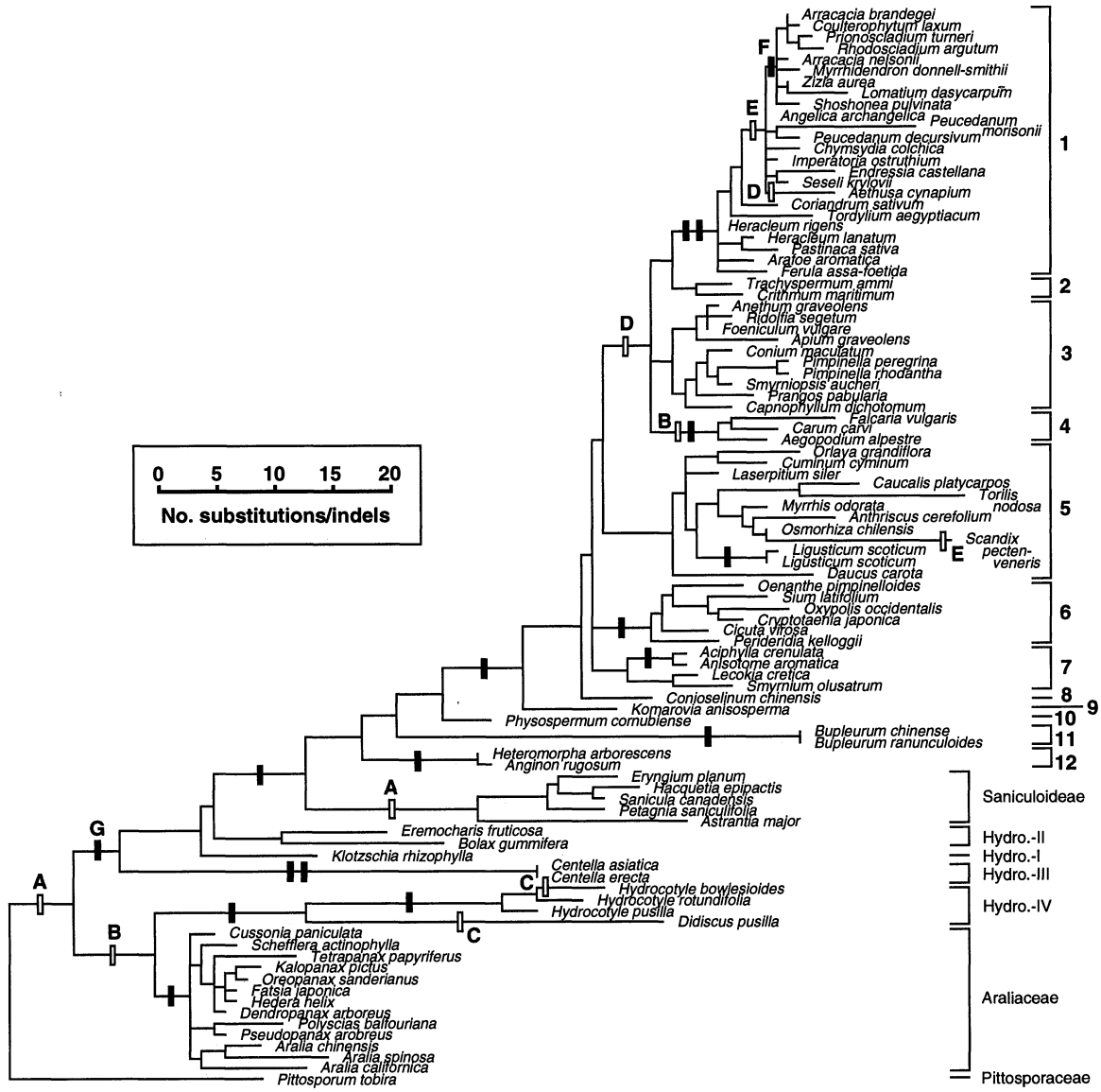

Fig. 3. One of 12 000 730-step trees derived from equally weighted parsimony analysis of cpDNA *rpoC1* intron sequences from Apiaceae subfamily Apioideae and relatives using all unambiguously aligned positions and 22 informative gaps (CI excluding uninformative characters = 0.560, RI = 0.871). Lengths of branches are proportional to the number of inferred nucleotide substitutions and indels, if present, occurring along them (note scale bar). The distribution of synapomorphic (solid bars) and homoplastic (open bars) indels are indicated; the latter are further identified by letters and are described in the text. The 12 groups of Apioideae circumscribed in Fig. 2 are indicated. Hydro. = Apiaceae subfamily Hydrocotyloideae.

best maximum likelihood tree, calculated with a Ts/Tv rate ratio of 2.0, had a log likelihood of −5652.036 (Fig. 4). Regions of the topology that were weakly supported or unresolved in the parsimony or neighbor-joining analyses were similarly supported or unresolved when the maximum likelihood method was used.

rpoC1 intron phylogenetic resolutions—Phylogenies estimated using parsimony, neighbor-joining, or maximum likelihood methods reveal that, in the context of those species examined, Apiaceae subfamily Apioideae is monophyletic. These results further suggest that subfamily Saniculoideae also is monophyletic and sister group to Apioideae. The third subfamily of Apiaceae, Hydrocotyloideae, is not monophyletic with four lineages recognized (*Klotzschia*; *Eremocharis* + *Bolax*; *Centella*; and *Hydrocotyle* + *Didiscus*; identified as Hydro. I–IV in Figs. 2–4). In all analyses, clade *Hydrocotyle* + *Didiscus* (Hydro.-IV) is strongly allied with a monophyletic Araliaceae, whereas *Klotzschia* (Hydro.-I) and the *Eremocharis* + *Bolax* clade (Hydro.-II) occur alongside Apioideae + Saniculoideae. The relationship of *Centella* (Hydro.-III), however, is equivocal. *Centella* shares a 5-bp deletion (indel G, Fig. 3) with *Klotzschia, Eremocharis, Bolax,* and all Apioideae and Saniculoideae and unites with them in the parsimony (when indels are considered; Figs. 2–3) and maximum likelihood (Fig. 4) trees. In contrast, the neighbor-joining analysis (not shown) places *Centella* (albeit with weak bootstrap support) alongside Araliaceae and the Hydro.-IV clade.

Fig. 4. Maximum likelihood tree constructed from unambiguously aligned cpDNA *rpoC1* intron sequences from Apiaceae subfamily Apioideae and relatives using a transition/transversion rate ratio of 2.0. Branch lengths are proportional to the number of expected nucleotide substitutions per site (scale distance is given as 100× this value). The 12 groups of Apioideae circumscribed in Fig. 2, based on the results of the parsimony analysis, are indicated. Hydro. = Apiaceae subfamily Hydrocotyloideae.

Within Apioideae similar groupings of taxa are consistently recognized in all trees. These groups, identified initially in Fig. 2 and presented in all subsequent tree figures, include: Group 1—the "*Angelica*" clade, a group of 24 taxa circumscribed by *Arracacia brandegei* through *Tordylium*; Group 2—the "*Crithmum*" clade, a group consisting of *Trachyspermum* and *Crithmum*; Group 3—the "*Apium*" clade, a weakly supported group consisting of *Anethum, Ridolfia, Foeniculum, Apium, Conium, Pimpinella, Smyrniopsis, Prangos,* and *Capnophyllum*; Group 4—the "*Aegopodium*" clade, consisting of *Falcaria, Carum,* and *Aegopodium*; Group 5—the "*Daucus*" clade, consisting of Drude's tribes Dauceae and Scandiceae, and genera *Cuminum, Laserpitium,* and *Ligusticum scoticum*; Group 6—the "*Oenanthe*" clade, a group consisting of *Oenanthe, Sium, Oxypolis, Cryptotaenia, Ci-*

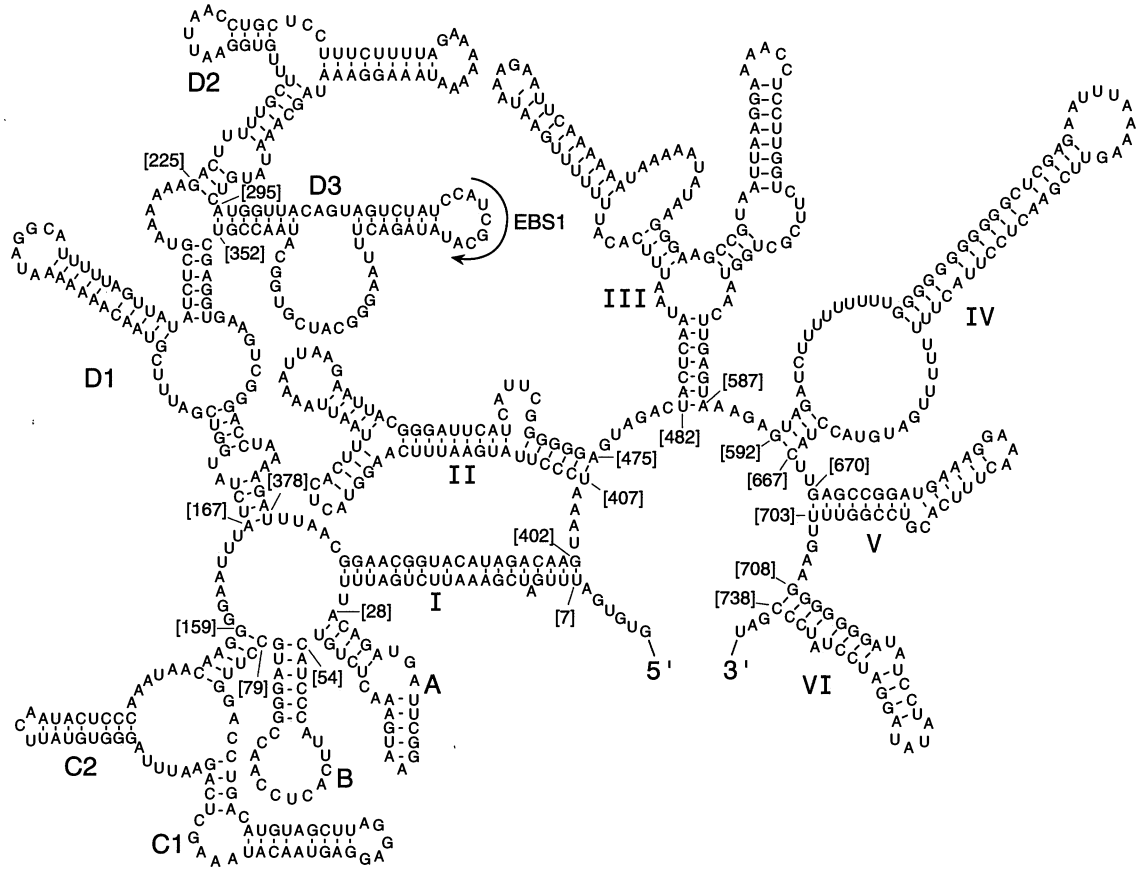

Fig. 5. Putative secondary structure model of the *Daucus carota* (carrot) cpDNA *rpoC1* group II intron. The structure consists of six major domains (I–VI) radiating from a central wheel. Domain I is divided into four subdomains (A–D) with the latter two subdomains divided further (C1 and C2, and D1 through D3, respectively). Exon binding site 1 (EBS1), common to all group II introns (Michel, Umesono, and Ozeki, 1989), consists of a stretch of six consecutive nucleotides in the D3 terminal loop and is complementary to the last six nucleotides of the 5' exon of *rpoC1* (not shown).

cuta, and *Perideridia*; and Group 7—the "*Aciphylla*" clade, a group consisting of *Aciphylla, Anisotome, Lecokia,* and *Smyrnium.* The genera *Conioselinum, Komarovia,* and *Physospermum,* each comprising separate lineages, are treated here as Groups 8 through 10, respectively. In the parsimony (Figs. 2–3) and maximum likelihood (Fig. 4) trees, *Heteromorpha* + *Anginon* (Group 12) comprise the basalmost clade within Apioideae, whereas *Bupleurum* (Group 11) holds this position in the neighbor-joining tree (not shown). The names of these groups are based, in part, on those names assigned by Plunkett, Soltis, and Soltis (1996b) for comparable groups identified in Apioideae based on phylogenetic analysis of *matK* sequences.

rpoC1 intron secondary structure—We propose a secondary structure model of the *rpoC1* intron in *Daucus carota* (carrot; Fig. 5). This reconstruction was inferred based on a consensus of group II intron secondary structures proposed by Michel, Umesono, and Ozeki (1989) and the results of the MULFOLD analysis. It should be noted, however, that only minor differences in free energy exist between this model and other conformations that can be quite different. Similarly, predictions of secondary structure by free-energy minimization for several other species of Apioideae were different from that depicted by *Daucus.* Thus, the *rpoC1* intron reconstruction presented here should be interpreted as a highly provisional estimate. In all of these inferred models, however, the conserved core structure typical of most plastid group II introns is maintained. This structure consists of six major domains (I–VI) radiating from a central wheel. Domain I is divided into four subdomains (A–D) with the latter two subdomains divided further (C1 and C2, and D1 through D3, respectively).

For each intron domain and subdomain and across all 96 sequences, the number of variable, phylogenetically informative, and ambiguous (i.e., excluded) sites, the maximum pairwise sequence divergence, the number of gaps and their size range, the location of these intron regions in the inferred secondary structure model of *Daucus carota* (Fig. 5), and their contribution to the overall composition of the alignment are provided in Table 4. Of the six major structural domains, domain I is the largest, comprising 48.6% of the total length of the alignment, whereas domains V and VI, each comprising some 3.6% of the length of the alignment, are the smallest. The most variable domains, calculated by dividing the number of variable (and unambiguously aligned) positions in each region by its size, are domains II and III with 81.2 and

TABLE 4. Sequence characteristics of the six major structural domains and various subdomains of the chloroplast DNA *rpoC1* intron in Apiaceae subfamily Apioideae and relatives.

Intron region	*Daucus carota* coordinates (see Fig. 5)	Aligned length (and % of total alignment)	No. sites ambiguous	No. sites variable (and %)	No. sites phylogenetically informative (and %)	No. gaps (and size range; bp)	Maximum pairwise sequence divergence (%)
I	7–402	461 (48.6)	38	173 (40.9)	108 (25.5)	22 (1–9)	12.4
A	28–53	28 (3.0)	0	9 (32.1)	7 (25.0)	1 (2)	
B	54–78	25 (2.6)	0	13 (52.0)	7 (28.0)	1 (8)	
C	79–159	81 (8.5)	0	29 (35.8)	17 (21.0)	2 (1-5)	
D	167–378	272 (28.7)	38	101 (43.2)	61 (26.1)	16 (1–9)	
D2	225–294	109 (11.5)	27	36 (43.9)	24 (29.3)	9 (1–9)	
D3	295–352	70 (7.4)	5	29 (44.6)	17 (26.2)	3 (1–7)	
II	407–475	105 (11.1)	36	56 (81.2)	28 (40.6)	5 (1–16)	22.1
III	482–587	187 (19.7)	77	59 (53.6)	48 (43.6)	13 (1–12)	22.2
IV	592–667	98 (10.3)	14	33 (39.3)	19 (22.6)	6 (1–7)	18.5
V	670–703	34 (3.6)	0	11 (32.4)	6 (17.6)	0	11.8
VI	708–738	34 (3.6)	0	9 (26.5)	4 (11.8)	4 (1–2)	10.7

53.6% of their positions variable, respectively. Reflecting this variability, these two domains also possessed the highest pairwise sequence divergence values (~22% of nucleotides). Domains V and VI were the least variable. With the exception of domain V, where gaps did not occur, gaps are distributed randomly throughout all intron domains and subdomains, with the greatest number (22) present in domain I. The two largest gaps, representing deletions of 12 and 16 bp, were inferred in domains III and II, respectively.

TABLE 5. ITS sequence and evolutionary characteristics of the 27 genera of Apiaceae subfamily Apioideae that were exemplified by more than one accession.

Genus	No. of accessions examined	Sequence divergence (range; %)	Monophyletic?[a]	No. of accessions included in phylogenetic analyses
Aciphylla	3	1.0–1.9	yes	1
Aegopodium	2	3.4	yes	1
Angelica	8	0.5–6.5	no	6
Anthriscus	3	6.9–11.5	yes	1
Arracacia	2	5.3	no	2
Berula	3	0.7–4.8	yes	1
Carum	2	0	yes	1
Cicuta	2	0	yes	1
Cnidium	2	11.0	no	2
Conium	4	0–1.2	yes	1
Cryptotaenia	2	3.6	yes	1
Ferula	2	0.5	yes	1
Heracleum	3	1.9–8.1	no	2
Laserpitium	2	13.7	no	2
Levisticum	3	0	yes	1
Ligusticum	3	0.2–17.3	no	2
Lomatium	2	3.1	no	2
Myrrhidendron	2	0	yes	1
Orlaya	2	7.2	yes	1
Osmorhiza	3	1.2–2.1	yes	1
Peucedanum	2	4.8	no	2
Pimpinella	2	1.0	yes	1
Scandix	2	8.4	yes	1
Seseli	3	5.5–6.0	no	2
Smyrnium	3	0	yes	1
Thaspium	2	0	yes	1
Zizia	2	0	yes	1

[a] Based on preliminary phylogenetic analysis of the entire 126-taxon data matrix. See text for further discussion.

ITS sequence analysis—The 126 accessions examined for ITS sequence variation reflect 82 genera and 114 species of Apioideae, with 27 genera represented by more than one accession (Table 5). The two accessions each of *Cicuta virosa, Myrrhidendron donnell-smithii,* and *Zizia aurea,* the three accessions each of *Levisticum officinale* and *Smyrnium olusatrum,* and two of the four accessions examined of *Conium maculatum* possessed identical ITS sequences. The following congeners also exhibited identical ITS sequences: *Carum carvi* and *C. alpinum,* and *Thaspium trifoliatum* and *T. pinnatifidum.* Parsimony analysis of the entire 126-taxon data matrix revealed that 18 of these 27 genera represent monophyletic entities (Table 5), at least on the basis of the taxa included in this study. Consequently, to facilitate analysis, only one exemplar from each of these 18 genera was retained in a reduced data matrix. In contrast, nine taxa (i.e., *Angelica, Arracacia, Cnidium, Heracleum, Laserpitium, Ligusticum, Lomatium, Peucedanum,* and *Seseli*), representing some of the most species-rich genera within the subfamily (Pimenov and Leonov, 1993), were not monophyletic; those species representing the different lineages within each genus were retained in the analysis. Thus, of the 126 accessions sequenced, only 95 were considered in subsequent phylogenetic analyses.

Alignment of all 95 ITS 1 and ITS 2 DNA sequences resulted in a matrix of 485 characters. Of these, it was necessary to delete 16 positions from ITS 1 and 32 positions from ITS 2 because of alignment ambiguities. Characteristics of these aligned ITS 1 and ITS 2 sequences, both separately and together, are presented in Table 3. On average, the ITS 1 region was slightly shorter than ITS 2. However, both spacers contributed comparable numbers of informative nucleotide substitutions and indels to the phylogenetic analysis. The ratio of terminal taxa (95) to phylogenetically informative characters for both spacers (294) was 1:3.1. In direct pairwise comparisons of unambiguous positions among all 95 apioid accessions, sequence divergence values ranged from 1.4 to 35.0% of nucleotides in ITS 1 and from 1.0 to 33.7% of nucleotides in ITS 2 (Table 3). Comparison of sequence pairs across both spacer regions gave divergence values ranging from 1.7 (between *Chymsydia* and *Peucedanum morisonii*) to 34.3% of nucleotides (between *Anthriscus*

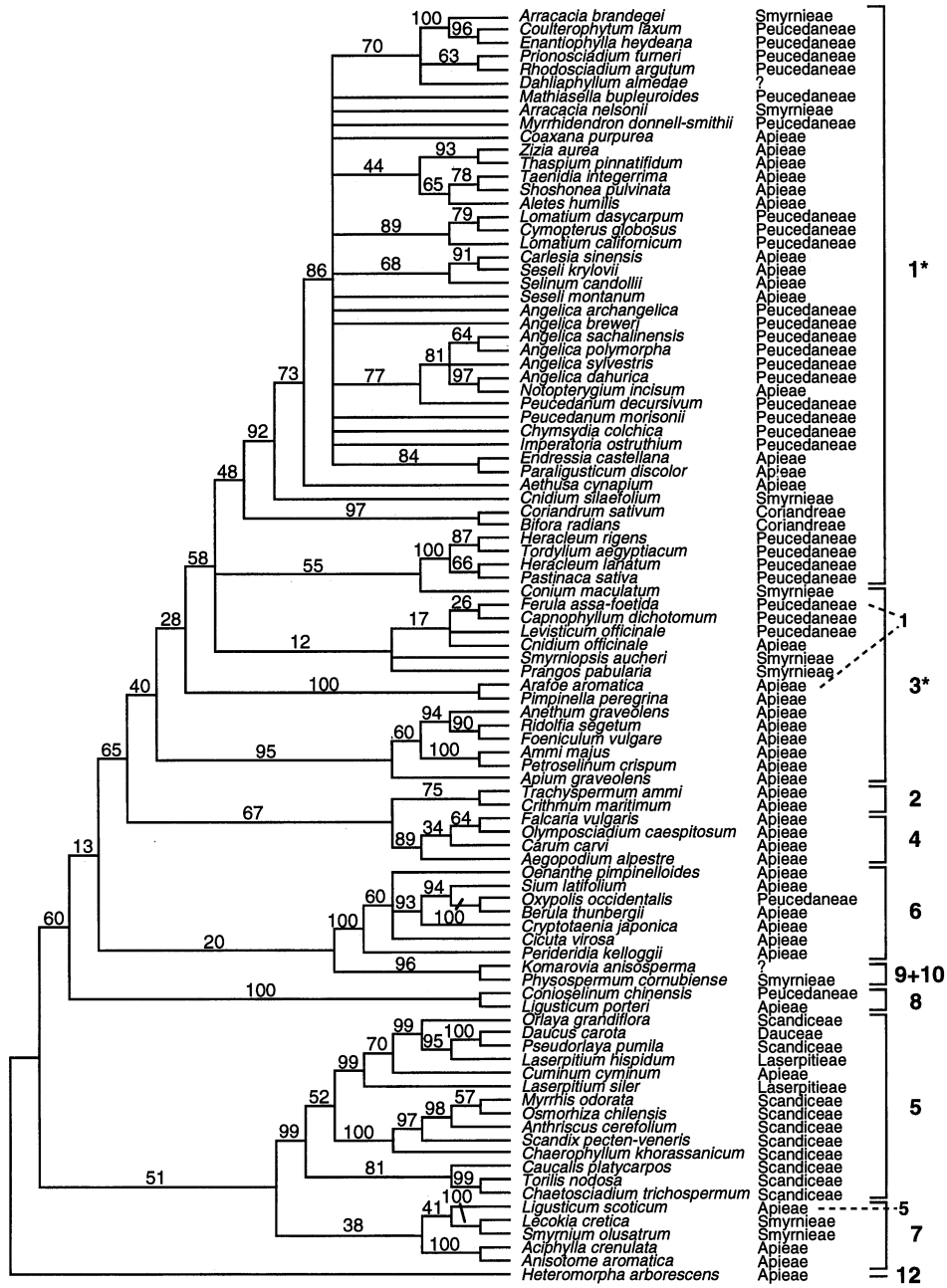

Fig. 6. Strict consensus of 12 000 2107-step trees derived from equally weighted parsimony analysis of combined nuclear ribosomal DNA ITS 1 and ITS 2 sequences from Apiaceae subfamily Apioideae using all unambiguously aligned positions (CI excluding uninformative characters = 0.314, RI = 0.649). Numbers above the nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates. Tribal classification of Apiaceae subfamily Apioideae is based on Drude (1897–1898); ? = tribal placement uncertain, because genus was not included in Drude's treatment. The various groups of Apioideae circumscribed in Fig. 2, based on the results of the parsimony analysis of cpDNA *rpoC1* intron sequences, are indicated; asterisks denote groups that are not monophyletic on this consensus tree.

cerefolium and *Tordylium*). Approximately 5% of all possible pairwise comparisons yielded divergence values ≥27.0%. Within apioid Groups 5 and 6 (see Fig. 6), sequence divergence values ranged from 6.2 to 28.4% and from 4.9 to 17.3%, respectively. Pairwise sequence divergence values among the 37 members comprising a large clade seen in Group 1, bounded by *Arracacia brandegei* and *Cnidium silaefolium* (see Fig. 6), ranged from 1.7 to 14.6%. Among congeners, nucleotide divergence varied between identity and 11.5% (Table 5). It is unlikely that the two species of *Ligusticum* examined, with a maximum pairwise sequence divergence value of 17.3%, comprise a monophyletic group (see Discussion). Fifty-one gaps, ranging between 1 and 14 bp in size, were introduced to facilitate alignment (Fig. 1; Table 3). These gaps were approximately equally distributed in both ITS regions. The majority (~73%) of these gaps were 1 bp in size. Eighteen gaps were informative for parsimony

analysis. Of the 12 putative insertions, ten represented perfect repeats of flanking sequences.

No evidence of obvious ITS length variants, representing multiple rDNA repeat types, in any of the taxa analyzed was observed. All of the double-stranded DNA PCR products obtained appeared as distinct bands in 1.0–3.0% agarose gels. Sequence polymorphisms at individual nucleotide sites within individual samples also were not commonly encountered. Despite the use of dGTP analogs (described in Downie and Katz-Downie, 1996), two areas within the ITS region were especially difficult to resolve unambiguously. These areas were highly G + C rich, resulting in compressions (and multiple bases at each position). The first of these compressed areas coincides with a problematic region reported previously in *Lomatium* ITS 1 sequences (Soltis and Kuzoff, 1993). In our analysis, a 1-bp gap (Gap A in Fig. 7) was inferred to occur in this region. The second compressed area occurred in ITS 2; this region of ambiguity was excluded from the analysis.

ITS phylogenetic analyses—The results of the analyses of combined data sets (i.e., ITS 1 and ITS 2) are presented here; separate analyses of each spacer region alone were not done. Previous studies, in Apioideae and other angiosperms, have indicated the high complementarity of spacer data and the greater phylogenetic resolution and internal support achieved in the trees when both spacers are considered together than when either spacer is treated alone (Baldwin et al., 1995; Downie and Katz-Downie, 1996). Parsimony analysis of 95 combined ITS sequences resulted in many thousands of maximally parsimonious topologies; the strict consensus of 12 000 of these trees, with accompanying bootstrap values, is presented in Fig. 6. These trees have a length of 2107 steps, CIs of 0.340 and 0.314, with and without uninformative characters, respectively, and an RI of 0.649.

One of these 12 000 trees was arbitrarily selected (Fig. 7) in order to show the number of nucleotide substitutions supporting each branch. Branch length is extremely heterogeneous with some terminal branches being quite long. Of the 51 gaps introduced to optimize alignment, deletions outnumbered insertions 39 to 12 (Table 3). All but four of the 18 informative gaps mapped without homoplasy onto this tree. Gap A (Fig. 7), a 1-bp deletion, occurs seven times, whereas gaps C (1-bp deletion) and D (3-bp deletion) each occur twice. Gap B is a reversal—a 1-bp loss that is regained in *Capnophyllum.* Because the majority of these gaps represent 1-bp deletions (Fig. 1) and some (such as gap A) occur in regions with runs of Gs and Cs, it is plausible that the same bases may have been compressed in both directions. Thus, these indels, unlike those of the *rpo*C1 intron, were not used as additional characters in the parsimony analysis. The average Ts/Tv ratio among all ITS sequences across 200 trees chosen randomly from the 12 000 maximally parsimonious trees was 1.47. Despite the high numbers of equally most parsimonious topologies, much resolution is achieved in the strict consensus tree. Major clades can be identified, and these are described below.

The neighbor-joining tree, calculated with a Ts/Tv rate ratio of 1.5 based on the inferred frequencies in the minimal length trees derived from the parsimony analysis, is presented in Fig. 8. A similar tree was obtained using a Ts/Tv rate ratio of 2.0 (not shown). When transitions were weighted equally to transversions, some shuffling of the major clades that is apparent in Fig. 8 occurred. The most drastic change here involved the repositioning of the clade containing *Anethum, Ridolfia, Foeniculum, Ammi, Petroselinum,* and *Apium* as one branch of a trichotomy at the base of the tree. The best maximum likelihood tree, calculated with a Ts/Tv rate ratio of 1.5, had a log likelihood of −11 407.132 and is shown in Fig. 9. Here, the *Anethum-Apium* group just described lies basal within the tree.

ITS phylogenetic resolutions—For comparative purposes, the major groups and lineages of Apioideae identified in Fig. 2 (based on parsimony analysis of the intron sequences) have been bracketed on the ITS trees. However, owing to the greater number of apioid taxa included in the ITS study, similar group numbers do not necessarily indicate identical composition. Moreover, several of these groups are not maintained as monophyletic in light of the ITS analyses; these groups are identified by asterisks in Figs. 6–9. Overall, the results of the three different phylogenetic analyses of ITS sequences showed much agreement in the sense that many of the same major clades occurred in all trees. Relationships that were strongly supported were robust to method of analysis, whereas conflicts appeared to be limited to regions that were weakly supported in all analyses. However, the relationships among many of these major clades as well as the relationships within each of these clades are largely equivocal.

A large clade, comprising 39 taxa extending from *Arracacia brandegei* through *Coriandrum* + *Bifora,* is uncovered in all ITS phylogenetic analyses. This group comprises a substantial portion of the "*Angelica*" clade (Group 1) identified in Fig. 2. Within this large clade, three smaller groups of taxa are evident in all analyses. The first comprises *Arracacia brandegei, Coulterophytum, Enantiophylla, Prionosciadium, Rhodosciadium,* and *Dahliaphyllum* and is extended to include *Mathiasella, Arracacia nelsonii,* and *Myrrhidendron* in the neighbor-joining (Fig. 8) and maximum likelihood (Fig. 9) trees. The second group encompasses *Zizia, Thaspium, Taenidia, Shoshonea,* and *Aletes,* and the third group comprises four of six species of *Angelica, Notopterygium,* and *Peucedanum decursivum.* In the neighbor-joining and maximum likelihood trees, this third group is allied weakly with the two remaining species of *Angelica* (*A. archangelica* and *A. breweri*) and a paraphyletic *Lomatium* (with included *Cymopterus*).

In Fig. 6, the aforementioned 39-taxon clade (i.e., *Arracacia brandegei* through *Coriandrum* + *Bifora*) forms a trichotomy with two other clades whose members have been treated in Groups 1 and 3 using *rpoC1* intron sequences. The first of these clades consists of the genera *Heracleum, Tordylium, Pastinaca,* and *Conium. Heracleum, Tordylium,* and *Pastinaca* form a strongly supported subclade in all ITS phylogenetic analyses; the genus *Heracleum,* however, is not monophyletic. *Conium maculatum,* sister taxon to the *Heracleum/Tordylium/Pastinaca* subclade, lies elsewhere in the *rpoC1* intron derived trees (Fig. 2). The last clade of the trichotomy

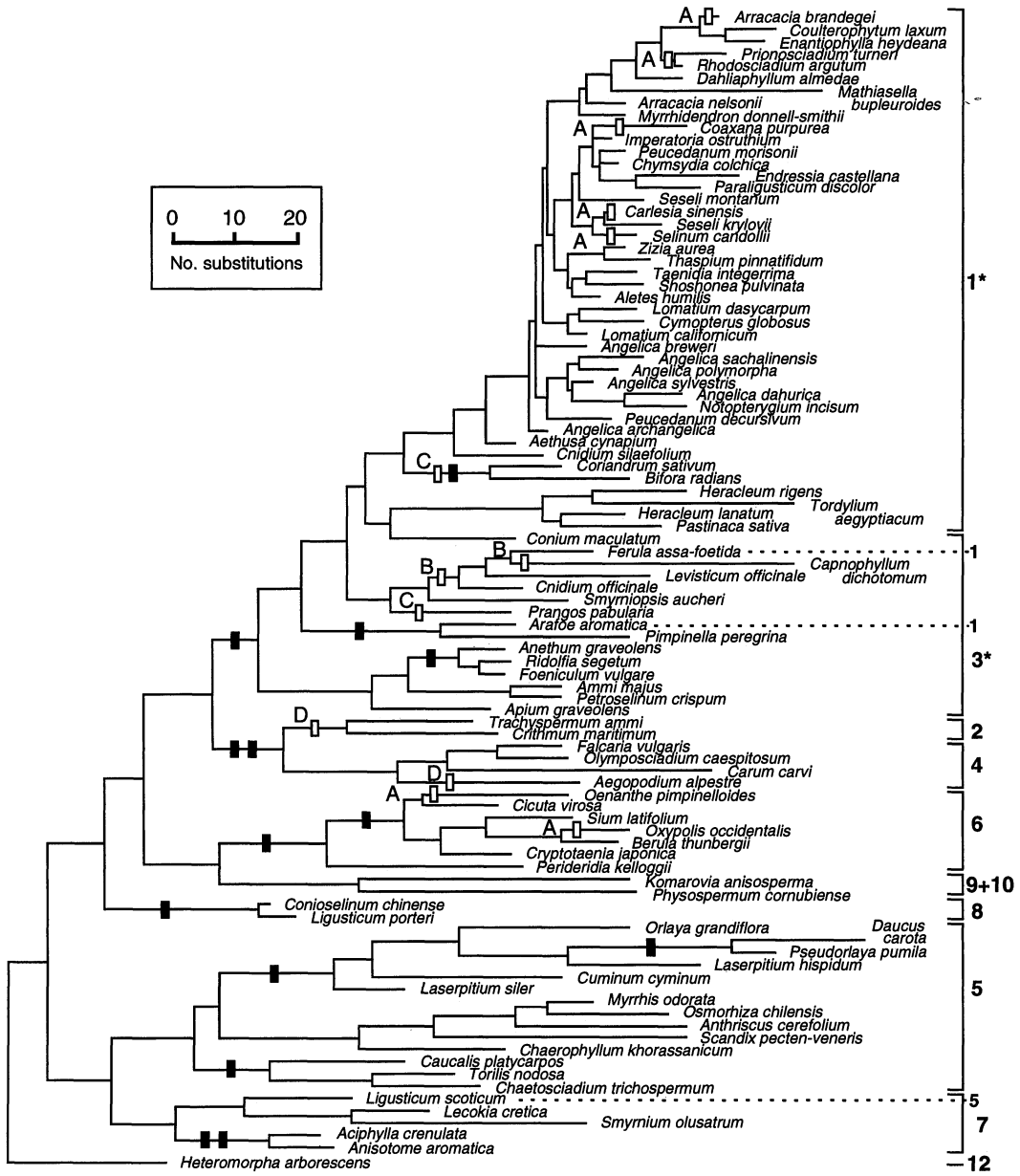

Fig. 7. One of 12 000 2107-step trees derived from equally weighted parsimony analysis of combined ITS 1 and ITS 2 sequences from Apiaceae subfamily Apioideae using all unambiguously aligned positions (CI excluding uninformative characters = 0.314, RI = 0.649). Lengths of branches are proportional to the number of inferred nucleotide substitutions occurring along them (note scale bar). The distribution of 14 synapomorphic (solid bars) and four homoplastic (open bars) indels have been superimposed on the phylogram; the latter are identified by letters A–D and are further characterized in the text. The various groups of Apioideae circumscribed in Fig. 2, based on the results of the parsimony analysis of cpDNA *rpoC1* intron sequences, are indicated; asterisks denote groups that are not monophyletic on this tree.

consists of the genera *Ferula, Capnophyllum, Levisticum, Cnidium, Smyrniopsis,* and *Prangos*; this very weakly supported clade is not present in either the neighbor-joining (Fig. 8) or maximum likelihood (Fig. 9) trees. The remaining taxa assigned to Groups 1 and 3 in the ITS trees form two major clades: (1) a clade consisting of *Arafoe* and *Pimpinella*; and (2) a clade consisting of *Anethum, Ridolfia, Foeniculum, Ammi, Petroselinum,* and *Apium.* In the results of the intron analyses (Figs. 2–4), *Arafoe* and *Pimpinella* do not ally, and the genera *Anethum, Ridolfia, Foeniculum,* and *Apium* (*Ammi* and *Petroselinum* were not included) comprise a subclade within Group 3.

The following groups of taxa are apparent in all ITS phylogenetic analyses and are similar, unless otherwise noted, to those groups recognized on the basis of *rpoC1* intron sequences: Group 2—the "*Crithmum*" clade (consisting of *Trachyspermum* and *Crithmum*); Group 4—the "*Aegopodium*" clade, with the addition of *Olymposciadium*; Group 5—the "*Daucus*" clade, with additional representatives from Drude's Scandiceae but now excluding *Ligusticum scoticum*; Group 6—the "*Oenanthe*"

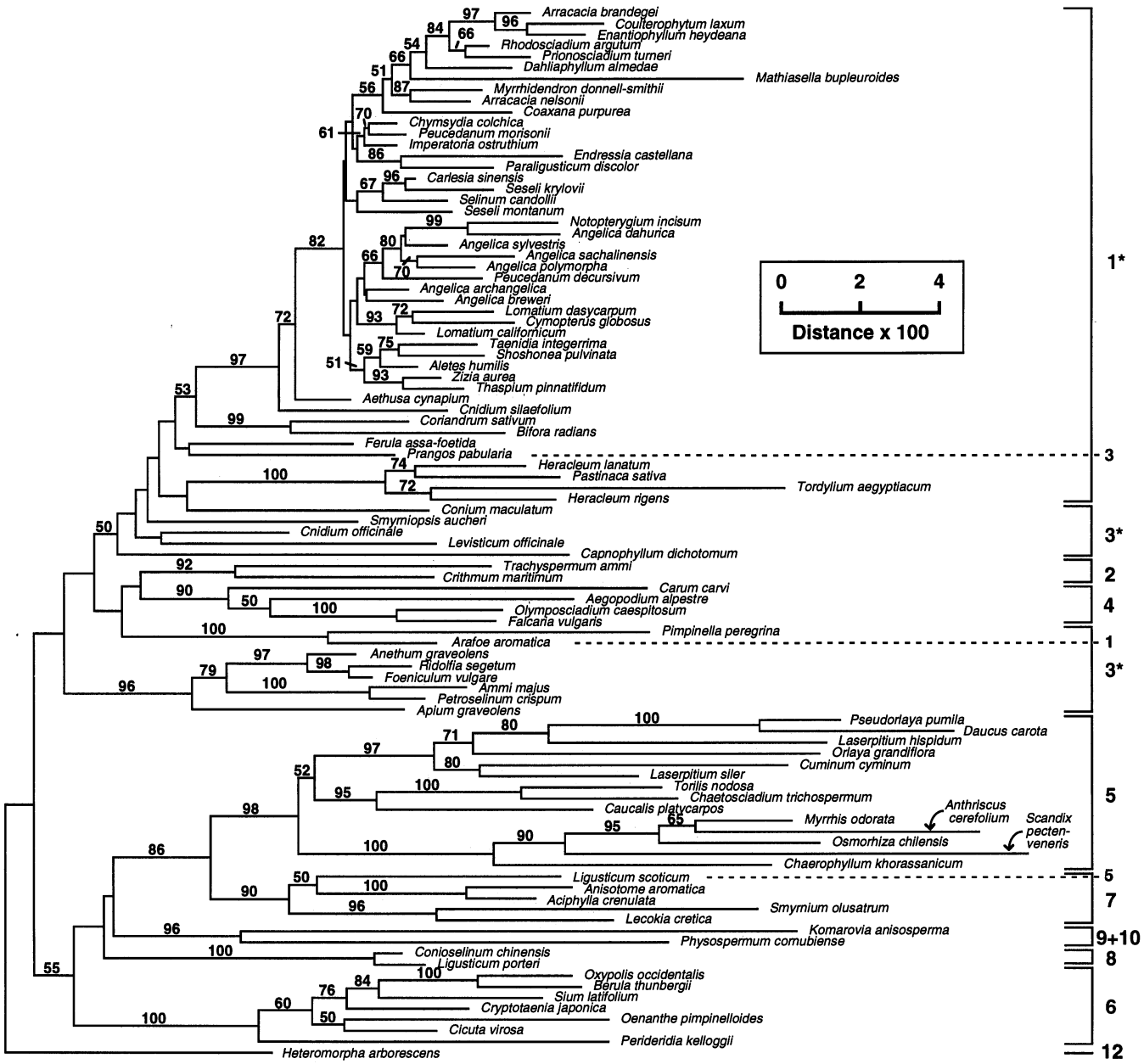

Fig. 8. Neighbor-joining tree inferred from 95 unambiguously aligned ITS 1 and ITS 2 sequences from Apiaceae subfamily Apioideae using a transition/transversion rate ratio of 1.5. Branch lengths are proportional to distances estimated from the two-parameter method of Kimura (scale distance is given as 100× this value). Numbers above nodes indicate bootstrap estimates for 100 replicate analyses; values <50% are not indicated. The various groups of Apioideae circumscribed in Fig. 2, based on the results of the parsimony analysis of cpDNA *rpoC1* intron sequences, are indicated; asterisks denote groups that are not monophyletic on this tree.

clade, with the addition of *Berula*; Group 7—the "*Aciphylla*" clade, a group whose membership is now expanded to include *Ligusticum scoticum*; and Group 8—a group formed from the union of *Conioselinum* with *Ligusticum porteri*. The genera *Komarovia* and *Physospermum*, considered separate lineages (i.e., Groups 9 and 10) in the *rpoC1* intron analyses, unite as a clade (Group 9 + 10) in the ITS trees.

Phylogenetic analyses of combined (intron + ITS) data—For those 65 accessions (60 genera) of Apioideae where both *rpoC1* intron and ITS data matrices were available, the data were combined into a single data set. Alignment of these sequence data resulted in a matrix of 1196 positions after removing all ambiguously aligned positions. Of these 1196 positions, 637 (53.3%) were constant, 367 (30.7%) were parsimony informative, and 192 (16.0%) were autapomorphic. A total of 79 gaps, ranging from 1 to 14 bp, were introduced to optimize the alignment; 23 of these gaps (nine from *rpoC1* intron and 14 from ITS) were phylogenetically informative. Pairwise sequence divergence comparisons ranged from 1.0 to 15.4% of nucleotides upon the exclusion of one ac-

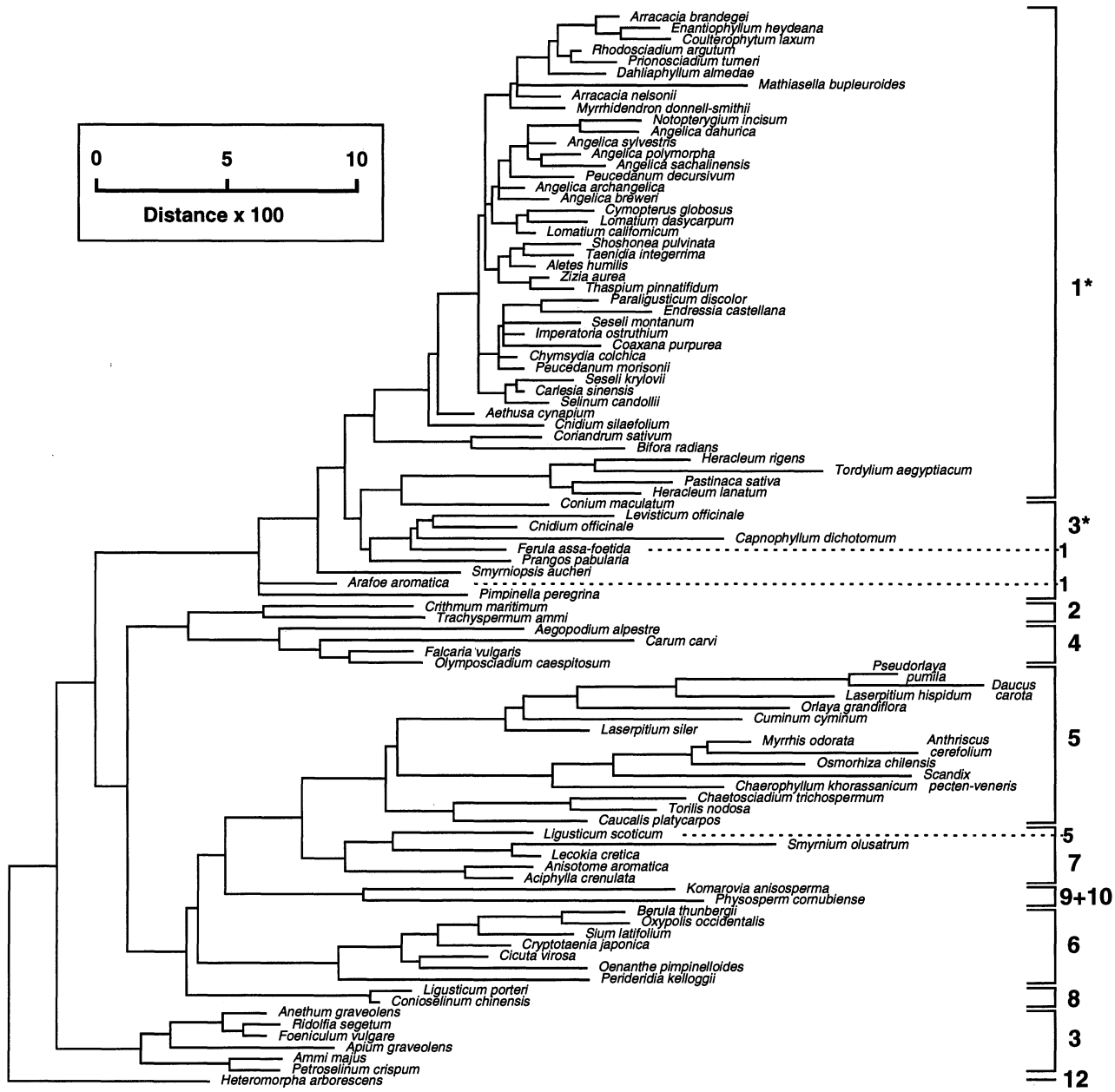

Fig. 9. Maximum likelihood tree constructed from 95 unambiguously aligned ITS 1 and ITS 2 sequences from Apiaceae subfamily Apioideae using a transition/transversion rate ratio of 1.5. Branch lengths are proportional to the number of expected nucleotide substitutions per site (scale distance is given as 100× this value). The various groups of Apioideae circumscribed in Fig. 2, based on the results of the parsimony analysis of *rpoC1* intron sequences, are indicated; asterisks denote groups that are not monophyletic on this tree.

cession each of *Conium maculatum* and *Ligusticum scoticum.*

Parsimony analysis of these combined data for 63 taxa, excluding the binary-scored informative gaps, resulted in 432 minimal-length 2067-step trees (CIs = 0.441 and 0.370, with and without uninformative characters, respectively; RI = 0.635). The strict consensus of these trees with accompanying bootstrap values is shown in Fig. 10. The average Ts/Tv ratio across all 432 trees was 1.32. A subsequent analysis, with the inclusion of the nine informative *rpoC1* intron alignment gaps (the ITS gaps were not considered), resulted in the same topology as when the gaps were excluded (CI excluding uninformative characters = 0.372; RI = 0.645; tree length = 2081 steps; number of maximally parsimonious trees = 406).

Distance trees inferred from the neighbor-joining analysis of these combined data (not shown), with Ts/Tv rate ratios of 1.0, 1.3, or 2.0, were topologically congruent to each other, and very similar (in the relative positions of the major groups) to the neighbor-joining tree constructed using only ITS sequences (Fig. 8). The best maximum likelihood tree (not shown), calculated with a Ts/Tv rate ratio of 1.3, had a log likelihood value of −13 478.395. Here apioid Groups 2 and 4, each monophyletic, formed

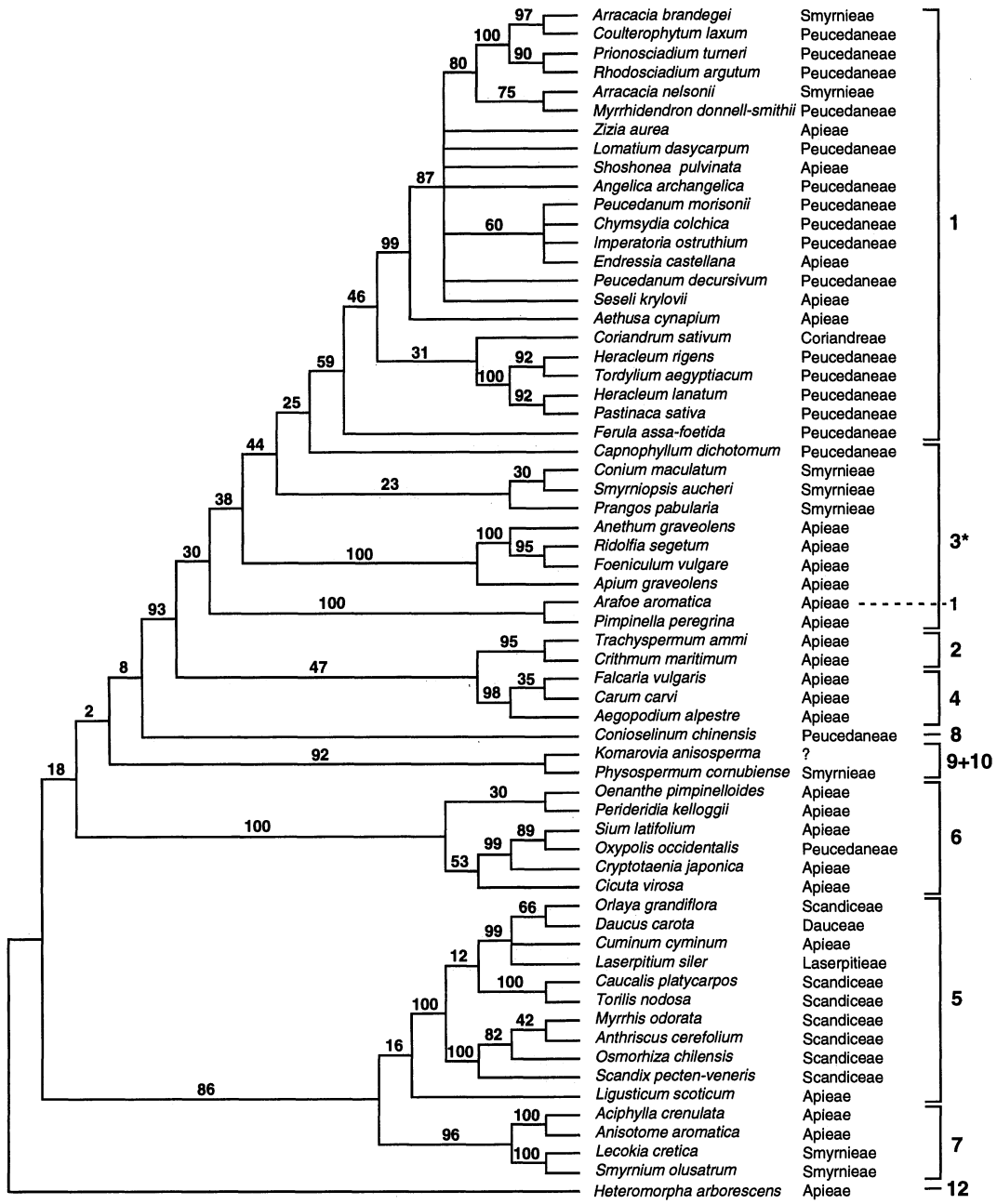

Fig. 10. Strict consensus of 432 minimal-length 2067-step trees derived from equally weighted parsimony analysis of combined cpDNA *rpoC1* intron and nuclear ribosomal DNA ITS data from Apiaceae subfamily Apioideae (CI excluding uninformative characters = 0.370, RI = 0.635). Numbers above the nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates. Tribal classification of Apioideae is based on Drude (1897–1898); ? = tribal placement uncertain, because genus was not included in Drude's treatment. The various groups of Apioideae circumscribed in Fig. 2, based on the results of the parsimony analysis of cpDNA *rpoC1* intron sequences, are indicated; asterisks denote groups that are not monophyletic on this consensus tree.

a clade that was sister group to those taxa treated in Groups 1 and 3 (as in the parsimony analysis of combined data, Fig. 10). Relationships within and between Groups 5 and 7 also were much like those depicted in Fig. 10, but with the *Torilis* + *Caucalis* clade now united with the *Myrrhis-Scandix* group. In the maximum likelihood tree, groups 9 + 10, then 6, and then 8 fall as successive clades to Groups 5 + 7. Unlike the results of the parsimony analysis of combined data (Fig. 10), where apioid Groups 8, 9 + 10, and 6 are (very weakly) associated with the major clade formed from the union of Groups 1–4, both neighbor-joining and maximum likelihood trees show a major dichotomy within Apioideae (with Groups 1–4 comprising one clade and Groups 5–10 the other).

DISCUSSION

Relationships among Drude's three subfamilies of Apiaceae—Phylogenetic analyses of chloroplast *rpoC1*

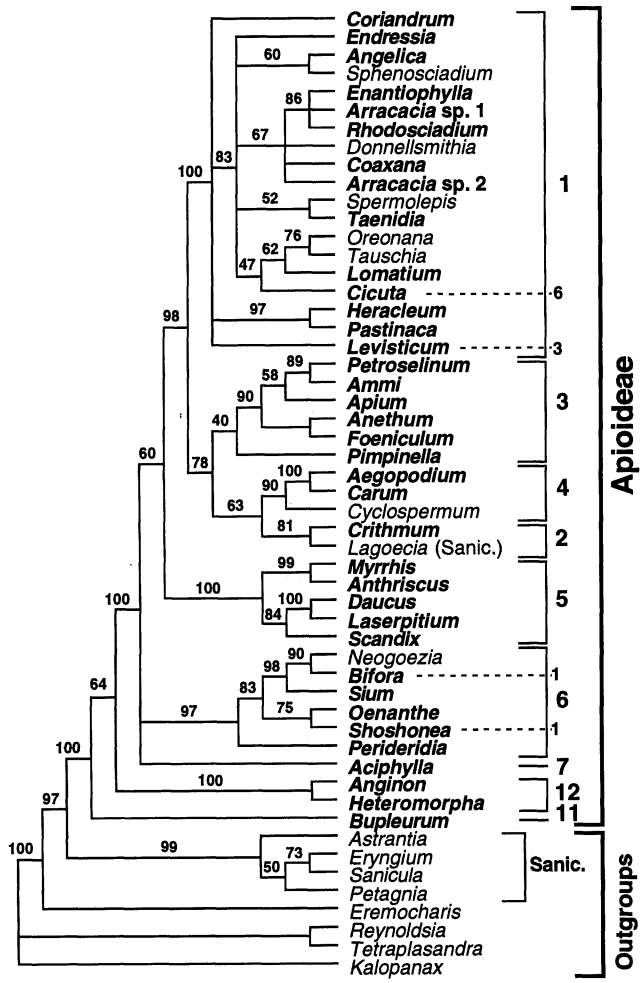

Fig. 11. Strict consensus of 902 minimal-length 709-step trees derived from parsimony analysis of partial cpDNA *matK* sequences from Apiaceae subfamily Apioideae and relatives (modified from Plunkett, Soltis, and Soltis, 1996b; CI excluding uninformative characters = 0.570, RI = 0.818). Bootstrap percentages in 1000 replicate analyses (saving no more than 100 trees per replicate) are indicated. The various groups of Apioideae circumscribed in Fig. 2, based on the results of the parsimony analysis of *rpoC1* intron sequences, are indicated. Sanic. = Apiaceae subfamily Saniculoideae. Boldfaced names indicate those taxa also examined for *rpoC1* intron and/or ITS sequence variation (this study).

intron sequences reveal that the subfamilies Apioideae and Saniculoideae of Drude (1897–1898) are each monophyletic and are sister taxa, whereas subfamily Hydrocotyloideae is not monophyletic. Within Hydrocotyloideae, the genera *Klotzschia, Eremocharis, Bolax,* and *Centella* (the latter in all trees, save the one constructed using the neighbor-joining method) arise successively immediately basal to Apioideae + Saniculoideae (Figs. 2–4). The hydrocotyloids *Hydrocotyle* + *Didiscus* (and *Centella* in the neighbor-joining analysis) are sister group to all examined Araliaceae. The monophyly of subfamilies Apioideae and Saniculoideae and their sister group status are also evident in a phylogeny of Apiales (Apiaceae + Araliaceae) based on cpDNA *rbcL* and *matK* sequence data (Plunkett, Soltis, and Soltis, 1996b, 1997; see Fig. 11).

Several members of subfamily Hydrocotyloideae, such as *Hydrocotyle, Azorella,* and *Klotzschia,* have been considered morphologically intermediate between Araliaceae and Apioideae + Saniculoideae (Harms, 1898; Baumann, 1946; Cerceau-Larrival, 1962; Shoup and Tseng, 1977). The molecular data reflect this idea with several hydrocotyloids forming a "grade" at the base of Apiaceae (Figs. 2–4). Phylogenetic analyses of Apiales based on *matK* and *rbcL* sequences, with a greater sampling of Hydrocotyloideae and Araliaceae than done here, suggest that the hydrocotyloids are polyphyletic (Plunkett, Soltis, and Soltis, 1996a, 1997). Others have hypothesized that the primarily temperate and herbaceous Apiaceae may be derived from within tropical, woody Araliaceae (Thorne, 1973; Judd, Sanders, and Donoghue, 1994). This hypothesis is not supported by our results nor by those reported by Plunkett, Soltis, and Soltis (1997); the family Araliaceae (with *Didiscus, Hydrocotyle,* and, possibly, *Centella* included) is monophyletic and sister group to all other examined Apiaceae.

Phylogenetic analyses of plastid intron and gene sequences—The robustness of a phylogenetic hypothesis can be evaluated by assessing its congruence with a hypothesis generated from a different data set. The availability of a second plastid data set for Apiaceae/Araliaceae, namely that from the gene *matK* (Plunkett, Soltis, and Soltis, 1996b), provides an opportunity to compare the results from coding (*matK*) and noncoding (*rpoC1* intron) organelle regions across the same group of plants. It is realized, however, that because these regions are located on the same chromosome they are inherited as a single linkage group and have the same history (Doyle, 1992). Data from the gene *rbcL* also are available for Apioideae (Kondo et al., 1996; Plunkett, Soltis, and Soltis, 1996a), but are not as extensive as that obtained from *matK*. The *matK* study was initiated and carried out independently from ours but many of the genera examined were the same (those 37 accessions examined for *matK,* and *rpoC1* intron and/or ITS DNA sequence variation are boldfaced in Fig. 11). In fact, much material was obtained from the same sources: the Botanical Garden of the University of California, Berkeley and the personal collections of L. Constance. Major differences in the phylogenetic hypotheses derived from these data might serve to highlight errors caused by misidentification of the plants, a common occurrence in such a problematic group, or the inadvertent PCR amplification of contaminating DNA.

Of the 1116 positions in the alignment of 53 partial *matK* sequences (44 from subfamily Apioideae sensu Drude), 711 (63.7%) were unvarying and 207 (18.5%) were parsimony informative (Plunkett, Soltis, and Soltis, 1996b). Eight gaps, of 3–9 bp, were necessary to optimize the alignment; three of these were informative. Direct pairwise comparisons among Apioideae sequences ranged from 0.1 to 9.7% of nucleotides (G. Plunkett, personal communication, Virginia Commonwealth University); the inferred Ts/Tv ratio across all 902 minimal 709-step trees was 0.94. The strict consensus of these 902 maximally parsimonious trees is illustrated in Fig. 11. In contrast, of the 948 positions in the *rpoC1* intron alignment, almost half (47.6%) were unvarying and 215

(22.7%) were parsimony informative (Table 3). Considering only Apioideae, pairwise sequence divergence values were identical to those observed for *matK,* ranging between identity and 9.8%. In both studies, the ratio of transitions to transversions was identical (0.94), and the amount of homoplasy quite similar (CI excluding uninformative characters = 0.57 and 0.55, and RI = 0.82 and 0.86, for the *matK* and *rpoC1* intron data sets, respectively). The most noteworthy difference between the two plastid regions sequenced is the sixfold greater number of unambiguous alignment gaps in the intron data matrix (50 vs. the eight inferred for *matK*). Additional gaps also were apparent in the intron matrix, but were in regions of the alignment excluded from the analysis. While these two data sets are not equivalent in the number of taxa sampled (the intron matrix is almost twice the size in this respect), they do represent the same breadth of sampling. Thus, other than the preponderance of gaps among the intron sequence comparisons (which have the potential to confound interpretation of alignment), both *matK* and *rpoC1* intron regions provide similar information to the phylogenetic analyses. To date, the use of intron sequences in published angiosperm phylogenetic studies has been limited, being restricted to two chloroplast tRNA genes (reviewed in Downie, Katz-Downie, and Cho, 1996). Introns in protein-coding chloroplast genes, such as that within *rpoC1,* can contribute valuable characters for inferring hypotheses of relationship; their use in phylogenetic analyses should be explored further as an alternative to (or in combination with) gene regions.

While the topologies of the *rpoC1* intron and *matK* cladograms agree in many ways (cf. Figs. 2 and 11, and discussed below), the positions of several genera are strikingly different. First, the monotypic *Shoshonea pulvinata,* placed alongside such taxa as *Oenanthe, Sium,* and *Perideridia* (i.e., Group 6, the "*Oenanthe*" clade) in the *matK* tree, arises within the "*Angelica*" clade (Group 1) in those trees inferred using *rpoC1* intron and/or ITS data. *Shoshonea,* a small acaulescent, caespitose-pulvinate plant endemic to western North America, is morphologically very similar to other western American genera such as *Aletes, Musineon,* and *Neoparraya* (Evert and Constance, 1982). Reflecting this similarity, the ITS trees (Figs. 6–9) indicate a close relationship among *Shoshonea, Aletes,* and, surprisingly, eastern North American *Taenidia.* Moreover, additional ITS data suggest close affinity between these three genera and *Neoparraya, Musineon,* and *Podistera* (S. Downie, unpublished data). The placement of *Shoshonea* needs to be reinvestigated.

A second point of disagreement between the *matK* tree and the cladograms presented herein involves the placement of *Cicuta* (water hemlock). While the *matK* data place the North American *Cicuta douglasii* in a clade with the morphologically divergent *Lomatium, Tauschia,* and *Oreonana* (i.e., Group 1), the ITS (with two accessions sequenced) and intron data position this genus (represented by the Eurasiatic *Cicuta virosa*) alongside members of the "*Oenanthe*" clade (Group 6). The *rbcL* analysis of Kondo et al. (1996) also reveals a close relationship among *Cicuta virosa, Oenanthe, Sium,* and *Cryptotaenia.* Evidence suggests that *Cicuta douglasii* (with 22 small and 22 large somatic chromosomes) arose from a hybridization event between *C. virosa* (2n = 22; small chromosomes) and *C. maculata* (2n = 22; large chromosomes) or some similar progenitor (Mulligan, 1980). The fruit morphology of *C. douglasii* is similar to that of *C. virosa,* and its leaves are similar to those of both *C. virosa* and *C. maculata*; these data all allude to the possible hybrid origin of *C. douglasii* from these taxa (Mulligan, 1980). Because of the strong resemblance between *C. douglasii* and *C. virosa,* and much evidence pointing to the monophyly of *Cicuta* (including the shared presence of the virulent cicutoxin; Mathias and Constance, 1942; Mulligan, 1980), additional material of *C. douglasii* should be examined in order to confirm its phylogenetic placement.

Bifora and *Coriandrum,* having ovoid-spherical, nutlike mericarps, are treated together (in tribe Coriandreae) in the classification system of Drude (1897–1898). Serological investigations corroborate the affinity between these taxa (Shneyer et al., 1992). In the ITS study, *Bifora* (represented by the European *B. radians*) and *Coriandrum* comprise a monophyletic group and occur in Group 1, the "*Angelica*" clade (*Bifora* was not included in the *rpoC1* intron study). In contrast, the *matK* phylogeny, while maintaining *Coriandrum* in the "*Angelica*" clade, shows North American *Bifora americana* alongside *Neogoezia, Sium, Oenanthe, Shoshonea,* and *Perideridia* in the "*Oenanthe*" clade (Group 6). While it is possible that *Bifora* may not be monophyletic, additional sampling is warranted to clear up this third point of disagreement between these two studies.

The fourth point of disagreement involves characterization of the most basal lineage within Apioideae. While *rbcL* (Plunkett, Soltis, and Soltis, 1996a) and most *rpoC1*-intron-based phylogenies point to *Heteromorpha* and *Anginon* as the sister group to all other apioid taxa, this position goes to *Bupleurum* in the *matK* study (Plunkett, Soltis, and Soltis, 1996b; Fig. 11). The basal position of *Bupleurum* also is evident in the neighbor-joining tree constructed using *rpoC1* intron sequences (not shown). When both *rbcL* and *matK* data are combined, the resultant cladograms show *Heteromorpha* + *Anginon, Bupleurum,* and a clade of all other examined apioids forming a trichotomy at the base of the Apioideae (Plunkett, Soltis, and Soltis, 1997). *Bupleurum,* with many of its members possessing grass-like simple leaves and parallel venation, is morphologically unusual within Apioideae. Additional data are required to resolve issues of basal branching.

Despite these four points of discordance, the *matK* phylogeny (Fig. 11) is very similar to those phylogenies inferred using *rpoC1* intron sequences (Figs. 2–4) in the sense that the same major groups are recognized in both studies. These groups include: Group 1—the "*Angelica*" clade; Group 3—the "*Apium*" clade; Group 4—the "*Aegopodium*" clade; Group 5—the "*Daucus*" clade; Group 6—the "*Oenanthe*" clade; Group 7—the "*Aciphylla*" clade, represented by only *Aciphylla* in the *matK* study; and Groups 11 and 12—the "basal apioids," represented by *Heteromorpha, Anginon,* and *Bupleurum.* Group 2, the "*Crithmum*" clade, is represented only by the genus *Crithmum* in the *matK* study. Here the apioid *Crithmum* allies with *Lagoecia,* a genus treated in Apiaceae subfamily Saniculoideae by Drude (1897–1898) but considered alongside several apioids in the systems of Koso-Poljan-

sky (1916) and Cerceau-Larrival (1962). The genus *Levisticum,* considered in Group 3 on the basis of ITS sequence variation (it was not included in the intron survey), allies with members of Group 1 in the *matK* phylogeny (Fig. 11).

Comparative analyses of nuclear ITS and plastid rpoC1 intron data—The pattern of ITS sequence evolution in Apioideae stands in stark contrast to that observed for the *rpoC1* intron across a greater diversity of taxa (Apiales), with the two spacer regions evolving much more rapidly than that of the intron. The percentage of sites that are phylogenetically informative is almost three times higher for the ITS region than for the intron (60.6 vs. 22.7%; Table 3). Although the number of aligned nucleotide sites in the intron data matrix is almost twice that of the ITS matrix (948 vs. 485 positions, respectively), the latter contributes more informative characters than does the *rpoC1* intron (294 vs. 215 characters, respectively). Similarly, maximum pairwise nucleotide divergence values are approximately three times higher in ITS sequence comparisons than in pairwise comparisons of *rpoC1* intron sequences (34.3 vs. 9.8%, respectively). Available ITS data for representatives of Apiaceae subfamilies Hydrocotyloideae and Saniculoideae and outgroups Araliaceae and Pittosporaceae could not readily be aligned with any Apioideae ITS sequence (Downie and Katz-Downie, 1996). Even within Apioideae, high ITS sequence divergence in *Bupleurum* and, possibly, *Anginon,* relative to other apioids precluded the inclusion of these taxa in the analysis. The ITS data also are more homoplastic than those data derived from the *rpoC1* intron, as reflected in their lower consistency and retention indices. Branches in the ITS phylograms, both internal and terminal, are, generally, much longer than those seen in the *rpoC1* intron trees, but many clades are supported by low bootstrap values. A difference in percentage G + C content is also apparent between the *rpoC1* intron (34.1–38.3%) and nuclear ITS sequences (46.6–61.5%). These ranges in values are comparable to those reported for other plastid introns (Shimada and Sugiura, 1991) and ITS regions in other angiosperms (Baldwin et al., 1995).

The lack of resolution within many of the major groups identified in the phylogenetic analyses of *rpoC1* intron sequences (e.g., Fig. 2), such as within the "*Angelica*" (Group 1), "*Apium*" (Group 3), and Araliaceae clades, is due to high sequence conservation among the taxa compared. Sequence divergence values in pairwise comparisons of nucleotides among the ten taxa of the "*Apium*" clade range from 0.1 to 2.2%. Among the 24 taxa in the "*Angelica*" clade, this range in sequence divergence extends from 0.1 to 2.9%. Much of this variation is concentrated towards the tips of some of the terminal branches (Figs. 3–4), and many internal branches are supported by very few nucleotide substitutions. The ratio of terminal taxa to informative characters for Apioideae and Araliaceae *rpo*C1 intron sequences is 1:2.0 and 1:0.77, respectively. Because the number of informative characters is small relative to the number of taxa examined, resolution in many parts of the resultant cladograms is not achieved.

Different rates of DNA evolution have been proposed to be influenced by generation time (Wu and Li, 1985; Schilling and Jansen, 1989; Wilson, Gaut, and Clegg, 1990; Bousquet et al., 1992; Gaut et al., 1992, 1996), with long-lived, woody species (perennials) having slower rates than annual species. Although differences in relative evolutionary rates between representative lineages were not determined in our study, it is apparent that branch lengths can vary substantially among closely related taxa possessing different life-history strategies. For example, short branch lengths are common among woody Araliaceae, whereas long branches characterize herbaceous Hydrocotyloideae (e.g., *Hydrocotyle, Didiscus, Centella*; Figs. 3–4). A similar association is also evident within subfamily Apioideae. Comprising mostly annual (and a few biennial) species, the "*Daucus*" clade (Group 5) is characterized largely by long branches in all phylogenetic analyses (Figs. 4, 8, and 9). In contrast, the herbaceous, perennial species of this clade (i.e., *Laserpitium siler, Myrrhis odorata,* and *Osmorhiza chilensis*) have some of the shortest branches. Similarly, the many short branches in the "*Angelica*" clade (Group 1) probably reflect the perennial habit of most of these plants. Differential rates of molecular evolution, however, are not always correlated with generation time (Wallace and Jansen, 1990; Jansen et al., 1991; Smith and Doyle, 1995). While many annuals (and biennials) are characterized by long branches in the phylograms presented herein (e.g., *Tordylium, Capnophyllum,* and *Carum*), so are some herbaceous perennials (e.g., *Mathiasella* and *Bupleurum*). *Mathiasella* and *Bupleurum* also are quite unusual morphologically within Apioideae (Constance and Hitchcock, 1954). A future study will more critically assess the effect of generation time and habit (and other possible factors) on variation in rates of molecular evolution in Apiaceae.

ITS and *rpoC1* intron sequence data are not useful at the same evolutionary level. The *rpoC1* intron is suitably conserved for family-wide phylogenetic study but is generally too conserved for examining relationships among recently diverged taxa. In contrast, high levels of nucleotide sequence variation reduce the utility of the two internal transcribed spacers for examining relationships among distantly related groups of Apioideae. The rapid evolution of the spacers relative to the intron might suggest that the former be most useful at lower taxonomic levels but this is not necessarily true. For example, while much resolution of relationships is achieved among the closely related (and predominantly annual) members of the "*Daucus*" clade (Group 5), the ITS sequences fail to resolve relationships among the closely related (and largely herbaceous, perennial) taxa comprising the "*Angelica*" and "*Apium*" clades (Groups 1 and 3). In general, given the taxon sampling, the ITS region is simply too small and the data too homoplastic for confident resolution at many levels within the trees.

Phylogenetic analyses of plastid *rpoC1* intron (this study), *matK* (Plunkett, Soltis, and Soltis, 1996b), or *rbcL* (Kondo et al., 1996; Plunkett, Soltis, and Soltis, 1996a) sequences provide essentially nonconflicting topologies, with those few areas of discord noted above. This topological agreement suggests that a combined analysis of these plastid sequences will lead to the best estimate of phylogeny given the available data (Bull et al., 1993).

Because our *rpoC1* intron and ITS data sets were not parallel in construction, the incongruence between them was not quantified or statistically evaluated. Instead, incongruence was evaluated by visually inspecting topologies for conflict and strength of support for individual branches. The observed discrepancies between the various ITS and intron-derived phylogenies are largely attributable to many poorly supported nodes, a result of few and/or conflicting characters. When these nodes (characterized by bootstrap values ≤25% or short branch lengths) are treated as unresolved (i.e., they are collapsed to yield polytomies), the trees are highly consistent with respect to the major groups. The relationships among many of these groups, however, are ambiguous. The trees derived from the combined (intron + ITS) analyses, upon treating poorly supported nodes as polytomies, also were not very different from those trees inferred from the separate data sets. As additional data become available, greater resolution of relationships may be achieved and regions of discordance, if they exist, may be more rigorously addressed. However, the repeated pattern of poor resolution in the same regions of the trees (e.g., within the "*Angelica*" and "*Apium*" clades) derived from either *rbcL, matK, rpoC1* intron, or ITS sequence analyses, and combined *rpoC1* intron and ITS data (Fig. 10), might reflect a real biological phenomenon—the rapid and recent radiation of these groups. If this is indeed true, additional sequence data may not lead to greater resolution of relationships.

Molecular evolution of the rpoC1 intron—Learn et al. (1992) have indicated that domain II of the intron in chloroplast gene *trnV*(UAC) has the highest rate of sequence divergence, approaching the synonymous substitution rate reported for some plastid-encoded genes. They further state that domain V and stem-loop region I-D3 of the *trnV*(UAC) intron evolve most slowly, at rates similar to those reported for nonsynonymous substitutions of several chloroplast genes. These disparate evolutionary rates appear to be tied to the functional importance of these regions in intron processing: stem-loop region I-D3 houses exon binding site 1 (EBS1), and domains V and VI are necessary for proper processing of the transcript (Michel, Umesono, and Ozeki, 1989; Learn et al., 1992). In our analysis, domains II and III were inferred to be the most variable regions, and domains V and VI the least variable (Table 4). The lower sequence conservation in domains II and III suggests that these regions may not be integral to proper functioning of the intron. In fact, domains II and III appear to be dispensable in group II introns. As examples, in the *Marchantia petB* intron, domain II is unstructured, consisting of runs of As and Ts, and in the *rpl2* intron of tobacco and *Marchantia,* like that of the *cis*-spliced *rps12* intron in these and other land plants, domain III is almost entirely absent (Michel, Umesono, and Ozeki, 1989). Most extensive pruning has occurred in the second intron of *Marchantia clpP,* where most of domain III and subdomains IA, IB, and ID1 are lacking (Kohchi et al., 1988; Michel, Umesono, and Ozeki, 1989).

Characterization of the major clades inferred within Apioideae—It is clear that phylogenies derived from nuclear ITS and/or chloroplast *rpoC1* intron sequences (this study), and *rbcL* and/or *matK* data (Kondo et al., 1996; Plunkett, Soltis, and Soltis, 1996b, 1997) provide little support for Drude's (1897–1898) often cited system of classification for the subfamily (Table 1). This is not surprising because many systematists have declared dissatisfaction with Drude's system (e.g., Theobald, 1971; Davis, 1972; Cronquist, 1982; Hedge et al., 1987; Shneyer et al., 1992; Heywood, 1993; Pimenov and Leonov, 1993). Although the umbellifers display a remarkable array of morphological and anatomical modifications of their fruits, the overuse of these characters to delimit suprageneric groups has confounded the understanding of evolutionary relationships. These modifications are suggested to be the result of adaptation to diverse ecological conditions and dispersal strategies (Theobald, 1971; Heywood, 1986; Jury, 1986).

On the basis of these molecular data, Apioideae tribes Apieae, Peucedaneae, and Smyrnieae, representing the three largest tribes in the subfamily (and accounting for approximately three-quarters of all the genera), are not monophyletic (see Figs. 2, 6, and 10). Drude's Scandiceae apparently is paraphyletic, with *Daucus* (Dauceae), *Laserpitium* (Laserpitieae), and *Cuminum* (Apieae) arising from within. The two accessions of *Laserpitium* examined for ITS sequence variation, the only representatives of Drude's tribe Laserpitieae included in this study, do not form a monophyletic group. Tribe Echinophoreae, with six genera (Pimenov and Leonov, 1993), was not represented in these molecular studies; unpublished analysis based on ITS sequences, however, reveals that *Echinophora* is nested within the "*Angelica*" clade (Group 1; C. Valiejo-Roman et al., unpublished data, Moscow State University, Russia). The status of the last remaining tribe, Coriandreae, is equivocal. While the ITS data suggest it is monophyletic, the *matK* data do not. Thus, based on the results of the phylogenetic analyses of molecular data and other empirical studies (e.g., Theobald, 1971; Shneyer et al., 1991, 1992; Shneyer, Borschtschenko, and Pimenov, 1995), an appeal is made to cease usage of Drude's tribal categories.

Of the 82 genera of Apioideae included in the phylogenetic analyses of ITS sequences, 27 were represented by more than one accession (Tables 2, 5). Of the latter, nine genera (*Angelica, Arracacia, Cnidium, Heracleum, Laserpitium, Ligusticum, Lomatium, Peucedanum,* and *Seseli*) were not monophyletic. The artificiality of many of these species-rich genera, as inferred by morphological studies, already has been expressed (e.g., Pimenov and Leonov, 1993). Generic boundaries in Apiaceae are often vague, arbitrary, and constantly fluctuating at the hands of successive investigators (Constance, 1987). Additional study of these and other species-rich genera in the subfamily Apioideae are warranted.

One of the objectives of this study was to identify major clades within Apioideae for future lower level investigations. Twelve groups of taxa are considered here based on the results of the phylogenetic analyses of *rpoC1* intron sequences. However, the integrity of several of these groups is not maintained in all analyses presented herein. For example, Group 3, recognized on the basis of the *rpoC1* intron (Fig. 2), is not monophyletic in the ITS or combined (ITS + intron) trees (Figs. 6–10), breaking

down into several smaller clades and isolated branches. Furthermore, some genera, such as *Ferula, Arafoe,* and *Prangos,* occur variously in Groups 1 and 3 depending upon the method of tree construction used and the type of sequence analyzed. While most analyses suggest a close relationship among those apioids belonging to Groups 1 through 4, and among those members belonging to Groups 5 and 7, the relationships among the remaining groups cannot be ascertained. The neighbor-joining and maximum likelihood analyses of the combined data sets do suggest, however, that a major dichotomy may exist within Apioideae, with Groups 1–4 forming one clade and Groups 5–10 forming the other. Four major clades of Apioideae (I–IV, below) are tentatively recognized pending further investigation. These clades, along with a group of "basal apioids" and several "miscellaneous" genera, are described below.

I. Groups 1, 2, 3, and 4—the "*Angelica,*" "*Crithmum,*" "*Apium,*" and "*Aegopodium*" clades. Here we treat these four groups of Apioideae as one unit (because their interrelationships cannot be resolved), and describe the several additional major subclades within: (1) A group of meso-American genera (i.e., *Arracacia, Coulterophytum, Dahliaphyllum, Donnellsmithia, Enantiophylla, Prionosciadium,* and *Rhodosciadium,* with the possible addition of *Mathiasella, Myrrhidendron,* and *Coaxana*). Many of these genera are characterized by polyploid members (Moore, 1971; L. Constance, unpublished data, University of California, Berkeley) and might possibly be modern derivatives of the Madro-Tertiary Geoflora (Mathias, 1965; Moore, 1971). (2) A group of North American genera (i.e., *Aletes, Shoshonea, Taenidia, Thaspium,* and *Zizia*). Additional ITS data for primarily western North American *Neoparraya, Musineon,* and *Podistera* indicate a close relationship among these eight genera (S. Downie, unpublished data). (3) A group of four species of *Angelica, Notopterygium,* and *Peucedanum decursivum.* These plants are primarily Asian in distribution and are allied weakly with the two other species of *Angelica* examined. Discrimination between *Angelica* and *Peucedanum* is often exceedingly difficult, because both are large, morphologically heterogeneous genera (Vasil'eva and Pimenov, 1991; Shneyer, Borschtschenko, and Pimenov, 1995). (4) A clade comprising *Heracleum, Pastinaca,* and *Tordylium.* In the *rpoC1* intron analyses, this relationship is not evident, but in the ITS cladograms these three genera comprise a well-supported group. The two examined species of *Heracleum* are not monophyletic. *Heracleum rigens* is morphologically dissimilar to *H. lanatum* and has been treated in the genus *Tetrataenium* (Mandenova et al., 1982). (5) The genera *Ammi* and *Ridolfia,* in addition to the familiar and cultivated plants *Anethum* (dill), *Apium* (celery), *Foeniculum* (fennel), and *Petroselinum* (parsley), comprise a monophyletic group. (6) The relationship of the "*Crithmum*" clade (Group 2) to other apioids is not clear. This clade is related to Group 1 in the trees inferred using *rpoC1* intron sequences (Figs. 2–4), whereas in the ITS (Figs. 6–9), *rpoC1* intron + ITS (Fig. 10), and *matK* (Fig. 11) trees the "*Crithmum*" clade is sister group to Group 4, the "*Aegopodium*" clade.

II. Group 5—the "*Daucus*" clade. Bentham (1867) and Boissier (1872) regarded the spiny-fruited members of Apioideae, with both primary and secondary (i.e., vallecular) ridges on the fruit, as members of tribe Caucalideae. Drude (1897–1898), however, redistributed these spiny-fruited genera between his widely divergent subtribe Caucalidinae (represented in this study by *Torilis, Caucalis, Orlaya,* and *Chaetosciadium*) and tribe Dauceae (e.g., *Daucus*). Drude hypothesized that the *Daucus* group evolved from plants similar to those 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 to those in his subtribe Scandicinae (represented in this study by *Anthriscus, Scandix, Osmorhiza, Myrrhis,* and *Chaerophyllum*), whose members lack both secondary ridges and spines. Drude assumed that the secondary spinose ridges in Caucalidinae had evolved independently from those in Dauceae. In our phylogenetic analyses, all data support a strong relationship among Drude's tribes Scandiceae, Dauceae, and Laserpitieae, and the genus *Cuminum.* With the exception of *Laserpitium,* the only genus of Laserpitieae examined, and those members of Drude's subtribe Scandicinae, this assemblage parallels tribe Caucalideae sensu V. Heywood and S. Jury in Heywood (1982), and in many other papers cited in Heywood (1971), and Cauwet-Marc and Carbonnier (1982).

Additional evidence suggests that tribes Caucalideae sensu Heywood (1982) and Laserpitieae may be closely related. The species *Daucus laserpitioides* DC., recognized by Drude (1897–1898) in an isolated section of *Daucus,* has been treated in the genus *Laserpitium* by Koso-Poljansky (1916; as *L. daucoides* Desf.) or as a separate genus, *Ctenodaucus* (Heywood and Dakshini, 1971). *Daucus laserpitioides* differs from other species of *Daucus* in the structural similarity of its primary and secondary ridges (both composed of spines), and in the absence of hairs on the primary ridges (Heywood and Dakshini, 1971; Okeke, 1982). The secondary ridges of Laserpitieae are often extended into wings, and the fruit of *D. laserpitioides,* with its deeply serrate wings, is somewhat intermediate in structure between typical spiny fruits of Caucalideae and winged fruits of Laserpitieae (J.-P. Reduron, personal communication, Mulhouse, France). In our ITS analyses (Figs. 6–9), *Laserpitium* forms a strongly supported clade with *Orlaya, Daucus, Pseudorlaya,* and *Cuminum*; the two examined species of *Laserpitium* are not monophyletic. These morphological and molecular data indicate that additional studies of Caucalideae phylogeny should include representation from tribe Laserpitieae.

Drude's subtribe Scandicinae (= Heywood's [1971] Scandiceae) forms a well-supported monophyletic group in our study. The relationship between this taxon and tribe Caucalideae sensu Heywood, however, is not altogether clear. With the exception of the ITS neighbor-joining tree (Fig. 8) and the tree resulting from parsimony analysis of combined (ITS + intron) data (Fig. 10), where tribes Caucalideae and Scandiceae (sensu Heywood) are each monophyletic, all other trees inferred from the phylogenetic analyses of *rpoC1* intron and ITS sequences, separately or combined, show that Caucalideae (with included *Laserpitium*) is paraphyletic with Scandiceae nested within. In contrast, results of the *matK* analysis (Plun-

kett, Soltis, and Soltis, 1996b; Fig. 11), albeit with sparser sampling, reveal a paraphyletic Scandiceae with included Caucalideae. Tribes Caucalideae and Scandiceae are, undoubtedly, very closely related. Their similar evolutionary history is manifested in the discovery of several teratological individuals of *Daucus carota* exhibiting linear fruits, similar to those seen in members of tribe Scandiceae and quite unlike those of typical *D. carota* (J.-P. Reduron, unpublished data, Mulhouse, France).

The genus *Ligusticum* has been described as a heterogeneous assemblage of not very closely related plants (Pimenov and Leonov, 1993), and cladistic analysis of *rbcL* sequences reveals, as do our results, that the genus is polyphyletic (Kondo et al., 1996). Furthermore, *L. scoticum* is an anomaly in the genus, with fruit structure unlike that of the other 40–50 species of *Ligusticum* (Pimenov and Leonov, 1993). The *rbcL* analysis of Kondo et al. (1996) places *L. scoticum* in a clade with *Osmorhiza, Daucus,* and *Torilis,* a relationship similar to that proposed herein on the basis of *rpoC1* intron sequences (Figs. 2–4). In contrast, the ITS data place *L. scoticum* in the "*Aciphylla*" clade (Group 7). In all ITS analyses, the "*Aciphylla*" clade is sister group to the "*Daucus*" clade (Group 5). When the ITS and intron data are combined (Fig. 10), *L. scoticum* is sister taxon to the "*Daucus*" clade, with this assemblage sister group to the "*Aciphylla*" clade. In light of these conflicting molecular results, additional data are necessary to resolve the phylogenetic placement of *L. scoticum.*

III. Group 6—the "*Oenanthe*" clade. Included here are *Oenanthe, Sium, Oxypolis, Berula, Cryptotaenia, Cicuta,* and *Perideridia.* With the exception of the North American *Oxypolis* (tribe Peucedaneae), Drude (1897–1898) treated these genera in tribe Apieae. The *matK* study of Plunkett, Soltis, and Soltis (1996b) also includes *Neogoezia minor* (Smyrnieae), *Bifora americana* (Coriandreae), and *Shoshonea* (Apieae) in this group (Fig. 11); the latter two genera, however, may be misplaced (discussed above). The exclusively Mexican genus *Neogoezia,* with its simple umbels and acaulescent, scapose habit, is very distinctive morphologically within Apiaceae (Constance, 1987). It does share, however, some features with other members of this clade, including glabrous stems and leaves, and clusters of tubers or tuberous roots. Moreover, all these plants generally grow in wet to moist places, such as in marshes or near streambanks.

IV. Group 7—the "*Aciphylla*" clade. Included in this clade are genera *Aciphylla, Anisotome, Lecokia,* and *Smyrnium. Aciphylla* and *Anisotome* are dioecious plants, Australasian in distribution, and very closely related to one another (Dawson, 1971). The monotypic *Lecokia* allies with *Smyrnium* in all analyses; both genera are treated in tribe Smyrnieae by Drude (1897–1898). The supposed relationship between these two clades is intriguing; additional sampling and study are required.

The "basal apioids" *Heteromorpha, Anginon,* and *Bupleurum* occupy basally divergent positions in the *rpoC1* intron (Figs. 2–4), *matK* (Fig. 11), and *rbcL* (Plunkett, Soltis, and Soltis, 1996a) cladograms. Depending upon the method of analysis used or DNA region sequenced, either *Bupleurum* or the clade of *Heteromorpha* + *Anginon* represents the earliest diverging lineage within Apioideae. The majority of Apioideae are characterized by an herbaceous habit. In contrast, *Heteromorpha* and *Anginon,* and some species of *Bupleurum,* are woody, with the wood anatomy of *Heteromorpha* much like that found in many Araliaceae (Rodríguez, 1971). Cerceau-Larrival (1962, 1971), from her studies on pollen morphology and the presence or absence of either round or long cotyledons, supported by evidence from inflorescences, fruits, and adult vegetative morphology, suggested that small-statured, perennial species with simple, entire, linear leaves, subrhomboidal pollen, and unspecialized fruit (such as *Bupleurum*), probably represent ancestral apioids. Similarly, it has been stated that herbaceous Apioideae probably evolved from montane tropical woody apioid ancestors of at least shrub to small tree dimensions (Dawson, 1971). *Heteromorpha* and *Anginon* (and one species of *Bupleurum*; Cerceau-Larrival, 1971) exemplify this type of habit and are distributed in tropical and southern Africa. In fact, some of the earliest microfossils known for Apiaceae, from the early Tertiary (Eocene), are referred to extant *Bupleurum* and *Heteromorpha* (Gruas-Cavagnetto and Cerceau-Larrival, 1982). As suggested by Plunkett, Soltis, and Soltis (1996a,b), it is likely that subfamily Apioideae originated in tropical or southern Africa from woody, simple-leaved ancestors.

The "miscellaneous" genera *Komarovia* (tribal position "*incertae sedis,*" Pimenov and Leonov, 1993), *Physospermum* (tribe Smyrnieae sensu Drude, 1897–1898), and *Conioselinum* fall as separate lineages (Groups 9 and 10, respectively) close to the "basal apioids" in the *rpoC1* intron trees (Figs. 2–4). In the ITS cladograms, however, these taxa unite as a clade (Group 9 + 10) sister to either the "*Oenanthe*" clade (Group 6, Fig. 6), or to the "*Daucus*" + "*Aciphylla*" clades (Groups 5 + 7; Figs. 8–9). Yet, when the intron and ITS are combined, the *Komarovia* + *Physospermum* clade allies, with very weak bootstrap support, with apioid Groups 1–4 (Fig. 10). Serological analyses suggest a close relationship between *Physospermum* and *Pleurospermum* (Shneyer et al., 1991, 1992), and in the *rbcL* analysis of Kondo et al. (1996), *Pleurospermum* forms a trichotomy along with *Bupleurum* at the base of Apioideae. Until more data are available, the relationships of *Komarovia* and *Physospermum* to other apioids will remain questionable.

The taxonomic placement of *Conioselinum* (plus *Ligusticum porteri* in the ITS trees; Group 8) is also equivocal. In the *rpoC1*-intron-based phylogenies estimated using parsimony (Fig. 2) or neighbor-joining (not shown) methods, *Conioselinum* forms a separate lineage (Group 8) allied with, or sister to, Groups 1–7 (the maximum likelihood tree [Fig. 4] excludes Group 6 from the latter). In the ITS analyses, *Conioselinum* and *L. porteri* unite as sister taxa (Figs. 6–9), but the relationship of this clade to other Apioideae is not clear, with several different relationships inferred depending upon type of analysis. Similar results are evident in the analyses of the combined data, with *Conioselinum* falling as sister taxon to the clades formed from the union of apioid Groups 1–4 (parsimony; Fig. 10), Groups 5, 7, 9, and 10 (neighbor-joining), and Groups 5, 6, 7, 9, and 10 (maximum likelihood). The manner of fruit development in *Conioselinum* appears to be unique within Apiaceae (Theobald, 1971) and, like the molecular results, does not point to any specific relationship.

On the basis of nuclear ITS and chloroplast *rpoC1* intron sequence comparisons, and in conjunction with hypotheses of relationships proposed using *matK* (Plunkett, Soltis, and Soltis, 1996b) and *rbcL* (Kondo et al., 1996; Plunkett, Soltis, and Soltis, 1996a) data, several major clades are provisionally recognized within Apioideae. However, the relationships among these clades, as well as the relationships among the many subclades identified within each, are largely ambiguous. Because many of the clades recognized herein contradict traditional groupings, a reevaluation of their morphological (and other) attributes is necessary in order to uncover characters diagnostic for these groups. These studies are currently in progress.

LITERATURE CITED

BABU, T. D., G. KUTTAN, AND J. PADIKKALA. 1995. Cytotoxic and antitumour properties of certain taxa of Umbelliferae with special reference to *Centella asiatica* (L.) Urban. *Journal of Ethnopharmacology* 48: 53–57.

BALDWIN, B. G., 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 on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

BAUMANN, M. G. 1946. *Myodocarpus* und die Phylogenie der Umbelliferen-Fruchte. Umbellifloren-Studien I. *Berichte der Schweizerischen Botanischen Gesellschaft* 56: 11–112.

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

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

BOUSQUET, J., S. H. STRAUSS, A. H. DOERKSEN, AND R. A. PRICE. 1992. Extensive variation in evolutionary rate of *rbcL* gene sequences among seed plants. *Proceedings of the National Academy of Sciences, USA* 89: 7844–7848.

BULL, J. J., J. P. HUELSENBECK, C. W. CUNNINGHAM, D. L. SWOFFORD, AND P. J. WADDELL. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42: 384–397.

CATALÁN, P., E. A. KELLOGG, AND R. G. OLMSTEAD. 1997. Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndhF* gene sequences. *Molecular Phylogenetics and Evolution* 8: 150–166.

CAUWET-MARC, A.-M., AND J. CARBONNIER. 1982. Les Ombellifères. Actes du 2ème Symposium International sur les Ombellifères "Contributions Pluridisciplinaires 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.

———. 1971. Morphologie pollinique et corrélations phylogénétiques chez les Ombellifères. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 109–155. Academic Press, New York, NY.

CONSTANCE, L. 1971. History of the classification of Umbelliferae (Apiaceae). *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 1–8. Academic Press, New York, NY.

———. 1987. *Neogoezia* (Apiaceae), a very distinct and elegant genus of Mexican Umbelliferae. *Opera Botanica* 92: 59–71.

———, AND C. L. HITCHCOCK. 1954. *Mathiasella,* a new genus of North American Umbelliferae. *American Journal of Botany* 41: 56–58.

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

———. 1982. Reduction of *Pseudotaenidia* to *Taenidia* (Apiaceae). *Brittonia* 34: 365–367.

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, Scotland.

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.

DOWNIE, S. R., AND D. S. KATZ-DOWNIE. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 83: 234–251.

———, ———, AND K.-J. CHO. 1996. Phylogenetic analysis of Apiaceae subfamily Apioideae using nucleotide sequences from the chloroplast *rpoC1* intron. *Molecular Phylogenetics and Evolution* 6: 1–18.

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

DRUDE, O. 1897–1898. Umbelliferae. *In* A. Engler and K. Prantl [eds.], *Die natürlichen Pflanzenfamilien* 3: 63–250. Wilhelm Engelmann, Leipzig.

EVERT, E. F, AND L. CONSTANCE. 1982. *Shoshonea pulvinata,* a new genus and species of Umbelliferae from Wyoming. *Systematic Botany* 7: 471–475.

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.

FRENCH, D. H. 1971. Ethnobotany of the Umbelliferae. *In* V. H. Heywood [ed.], The biology and chemistry of the Umbelliferae, 385–393. Academic Press, New York, NY.

GAUT, B. S., B. R. MORTON, B. C. MCCAIG, AND M. T. CLEGG. 1996. Substitution rate comparisons between grasses and palms: synonymous rate differences at the nuclear gene *Adh* parallel rate differences at the plastid gene *rbcL. Proceedings of the National Academy of Sciences, USA* 93: 10274–10279.

———, S. V. MUSE, W. D. CLARK, AND M. T. CLEGG. 1992. Relative rates of nucleotide substitution at the *rbcL* locus of monocotyledonous plants. *Journal of Molecular Evolution* 35: 292–303.

GRUAS-CAVAGNETTO, C., AND M.-TH. CERCEAU-LARRIVAL. 1982. Presence de pollens d'Ombellifères fossiles dans le Paleogene du Bassin Anglo-Parisien: premiers resultats. *In* A.-M. Cauwet-Marc and J. Carbonnier [eds.], Les Ombellifères. Actes du 2ème Symposium International sur les Ombellifères "Contributions Pluridisciplinaires à la Systématique," 255–267. *Monographs in Systematic Botany from the Missouri Botanical Garden,* vol. 6. Braun-Brumfield, Ann Arbor, MI.

HARMS, H. 1898. Araliaceae. *In* A. Engler and K. Prantl [eds.], *Die natürlichen Pflanzenfamilien* 3: 1–62. Wilhelm Engelmann, Leipzig.

HEDGE, I. C. 1973. Umbelliferae in 1672 and 1972. *Notes from the Royal Botanic Garden, Edinburgh* 32: 151–158.

———, et al. 1987. Umbelliferae. *In* K. H. Rechinger [ed.], Flora Iranica 162, 1–555. Akademische Druck-und Verlagsanstalt, Graz.

HEYWOOD, V. H. 1971. The biology and chemistry of the Umbelliferae. 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, 215–231. 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.

HOLMGREN, P. K., AND W. KEUKEN. 1974. Index herbariorum. Oosthoek, Scheltema and Holkema, Utrecht.

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

JAEGER, J. A., D. H. TURNER, AND M. ZUKER. 1989. Improved predictions of secondary structures for RNA. *Proceedings of the National Academy of Sciences, USA* 86: 7706–7710.

JANSEN, R. K., R. S. WALLACE, K.-J. KIM, AND K. L. CHAMBERS. 1991. Systematic implications of chloroplast DNA variation in the subtribe Microseridinae (Asteraceae: Lactuceae). *American Journal of Botany* 78: 1015–1027.

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.

JURY, S. L. 1986. Fruit and leaf variation in the African species of the Umbelliferae tribe Caucalideae. *Symbolae Botanicae Upsalienses* 26: 181–188.

KATZ-DOWNIE, D. S., C. M. VALIEJO-ROMAN, E. I. TERENTIEVA, A. V. TROITSKY, M. G. PIMENOV, B. LEE, AND S. R. DOWNIE. 1998. Towards a molecular phylogeny of Apiaceae subfamily Apioideae: additional information from nuclear ribosomal DNA ITS sequences. *Plant Systematics and Evolution,* in press.

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.

KOCH, W. D. J. 1824. Generum tribuumque plantarum Umbelliferarum nova dispositio. *Nova Acta Academiae Caesareae Leopoldino Carolinae Germanicae Naturae Curiosorum* 12: 55–156.

KOHCHI, T., Y. OGURA, K. UMESONO, Y. YAMADA, T. KOMANO, H. OZEKI, AND K. OHYAMA. 1988. Ordered processing and splicing in a polycistronic transcript in liverwort chloroplasts. *Current Genetics* 14: 147–154.

KONDO, K., S. TERABAYASHI, M. OKADA, C. YUAN, AND S. HE. 1996. Phylogenetic relationships of medicinally important *Cnidium officinale* and Japanese Apiaceae based on *rbcL* sequences. *Journal of Plant Research* 109: 21–27.

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

LAVROVA, T. V., M. G. PIMENOV, AND V. N. TIKHOMIROV. 1983. Description and analysis of the Umbelliferae fruit structure in the tribe Ligusticeae. *Bulletin of the Moscow Society of Naturalists, Biological Section* 88: 107–122.

LEARN, G. H., JR., J. S. SHORE, G. R FURNIER, G. ZURAWSKI, AND M. T. CLEGG. 1992. Constraints on the evolution of plastid introns: the group II intron in the gene encoding tRNA-Val(UAC). *Molecular Biology and Evolution* 9: 856–871.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade, version 3.0: Analysis of phylogeny and character evolution. Sinauer, Sunderland, MA.

MANDENOVA, I. P., J. CARBONNIER, M.-C. CARBONNIER-JARREAU, A.-M. CAUWET-MARC, M.-T. CERCEAU-LARRIVAL, M. GUYOT, D. MOLHO, AND J.-P. REDURON. 1982. Contribution a l'etude du genre *Tetrataenium* (DC.) Manden. *In* A.-M. Cauwet-Marc and J. Carbonnier [eds.], Les Ombellifères. Actes du 2ème Symposium International sur les Ombellifères "Contributions Pluridisciplinaires à la Systématique," 675–725. *Monographs in Systematic Botany from the Missouri Botanical Garden,* vol. 6. Braun-Brumfield, Ann Arbor, MI.

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

———, AND L. CONSTANCE. 1942. A synopsis of the American species of *Cicuta. Madroño* 6: 145–176.

MICHEL, F., AND B. DUJON. 1983. Conservation of RNA secondary structures in two intron families including mitochondrial-, chloroplast- and nuclear-encoded members. *EMBO Journal* 2: 33–38.

———, K. UMESONO, AND H. OZEKI. 1989. Comparative and functional anatomy of group II catalytic introns—a review. *Gene* 82: 5–30.

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.

MULLIGAN, G. A. 1980. The genus *Cicuta* in North America. *Canadian Journal of Botany* 58: 1755–1767.

OKEKE, S. E. 1982. Morphological variation of bracts, bracteoles and fruits in *Daucus* L. *In* A.-M. Cauwet-Marc and J. Carbonnier [eds.], Les Ombellifères. Actes du 2ème Symposium International sur les Ombellifères "Contributions Pluridisciplinaires à la Systématique," 161–174. *Monographs in Systematic Botany from the Missouri Botanical Garden,* vol. 6. Braun-Brumfield, Ann Arbor, MI.

OLMSTEAD, R. G., AND J. D. PALMER. 1994. Chloroplast DNA systematics: a review of methods and data analysis. *American Journal of Botany* 81: 1205–1224.

OLSEN, G. J., 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.

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. 1996a. Higher level relationships of Apiales (Apiaceae and Araliaceae) based on phylogenetic analysis of *rbcL* sequences. *American Journal of Botany* 83: 499–515.

———, ———, AND ———. 1996b. Evolutionary patterns in Apiaceae: inferences based on *matK* sequence data. *Systematic Botany* 21: 477–495.

———, ———, AND ———. 1997. Clarification of the relationship between Apiaceae and Araliaceae based on *matK* and *rbcL* sequence data. *American Journal of Botany* 84: 565–580.

PRYER, K. M., A. R. SMITH, AND J. E. SKOG. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. *American Fern Journal* 85: 205–282.

RODRÍGUEZ C., R. L. 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.

SCHILLING, E. E., AND R. K. JANSEN. 1989. Restriction fragment analysis of chloroplast DNA and the systematics of *Viguiera* and related genera (Asteraceae: Heliantheae). *American Journal of Botany* 76: 1769–1778.

SHIMADA, H., AND M. SUGIURA. 1991. Fine structural features of the chloroplast genome: comparison of the sequenced chloroplast genomes. *Nucleic Acids Res.* 19: 983–995.

SHINOZAKI, K., et al. 1986. The complete nucleotide sequence of the tobacco chloroplast genome: its organization and expression. *EMBO Journal* 5: 2043–2049.

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

———, ———, AND ———. 1995. Immunochemical appraisal of relationships within tribe Peucedaneae (Apiaceae). *Plant Systematics and Evolution* 198: 1–16.

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

SHOUP, J. R., AND C. C. TSENG. 1977. Pollen of *Klotzschia* (Umbelliferae): a possible link to Araliaceae. *American Journal of Botany* 64: 461–463.

SMITH, J. A., AND J. J. DOYLE. 1995. A cladistic analysis of chloroplast DNA restriction site variation and morphology for the genera of the Juglandaceae. *American Journal of Botany* 82: 1163–1172.

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.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony,

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

TAKAIWA, F., AND M. SUGIURA. 1982. Nucleotide sequences of the 16S-23S spacer region in an rRNA gene cluster from tobacco chloroplast DNA. *Nucleic Acids Research* 8: 2665–2676.

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 Societe Botanique de France* 31: 384–385.

VASIL'EVA, M. G., AND M. G. PIMENOV. 1991. Karyotaxonomical analysis in the genus *Angelica* (Umbelliferae). *Plant Systematics and Evolution* 177: 117–138.

WALLACE, R. S., AND R. K. JANSEN. 1990. Systematic implications of chloroplast DNA variation in the genus *Microseris* (Asteraceae: Lactuceae). *Systematic Botany* 15: 606–616.

WILSON, M. A., B. GAUT, AND M. T. CLEGG. 1990. Chloroplast DNA evolves slowly in the palm family (Arecaceae). *Molecular Biology and Evolution* 7: 303–314.

WU, C.-I., AND W.-H. LI. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. *Proceedings of the National Academy of Sciences, USA* 82: 1741–1745.

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.

ZUKER, M. 1989. On finding all suboptimal foldings of an RNA molecule. *Science* 244: 48–52.

ZURAWSKI, G., M. T. CLEGG, AND A. H. D. BROWN. 1984. The nature of nucleotide sequence divergence between barley and maize chloroplast DNA. *Genetics* 106: 735–749.