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A PHYLOGENY OF THE FLOWERING PLANT FAMILY APIACEAE BASED ON CHLOROPLAST DNA *RPL16* AND *RPOC1* INTRON SEQUENCES: TOWARDS A SUPRAGENERIC CLASSIFICATION OF SUBFAMILY APIOIDEAE[1]

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The higher level relationships within Apiaceae (Umbelliferae) subfamily Apioideae are controversial, with no widely acceptable modern classification available. Comparative sequencing of the intron in chloroplast ribosomal protein gene *rpl16* was carried out in order to examine evolutionary relationships among 119 species (99 genera) of subfamily Apioideae and 28 species from Apiaceae subfamilies Saniculoideae and Hydrocotyloideae, and putatively allied families Araliaceae and Pittosporaceae. Phylogenetic analyses of these intron sequences alone, or in conjunction with plastid *rpoC1* intron sequences for a subset of the taxa, using maximum parsimony and neighbor-joining methods, reveal a pattern of relationships within Apioideae consistent with previously published chloroplast DNA and nuclear ribosomal DNA ITS based phylogenies. Based on consensus of relationship, seven major lineages within the subfamily are recognized at the tribal level. These are referred to as tribes Heteromorpheae M. F. Watson & S. R. Downie *Trib. Nov.*, Bupleureae Spreng. (1820), Oenantheae Dumort. (1827), Pleurospermeae M. F. Watson & S. R. Downie *Trib. Nov.*, Smyrnieae Spreng. (1820), Aciphylleae M. F. Watson & S. R. Downie *Trib. Nov.*, and Scandiceae Spreng. (1820). Scandiceae comprises subtribes Daucinae Dumort. (1827), Scandicinae Tausch (1834), and Torilidinae Dumort. (1827). *Rpl16* intron sequences provide valuable characters for inferring high-level relationships within Apiaceae but, like the *rpoC1* intron, are insufficient to resolve relationships among closely related taxa.

Key words: Apiaceae; Apioideae; Hydrocotyloideae; molecular phylogeny; *rpl16* intron; *rpoC1* intron; Saniculoideae; Umbelliferae.

The flowering plant family Apiaceae Lindl. (Umbelliferae Juss.) comprises 300–455 genera and some 3000–3750 species (Constance, 1971; Pimenov and Leonov, 1993). It is cosmopolitan, being particularly abundant in the northern hemisphere. *Daucus carota* subsp. *sativus* (Hoffm.) Arcang., the common cultivated carrot, is by far its most economically important member. Other familiar vegetables, flavorings, or garnishes include angelica, anise (aniseed), caraway, celeriac, celery, chervil, coriander (cilantro), cumin, dill, fennel, lovage, parsley, and parsnip. Deadly poisonous plants include water hemlock, poison hemlock, hemlock water-dropwort, and fool's parsley. The obvious distinctive characters of many of these plants, such as herbs with hollow or pith-filled stems, pinnately divided leaves with sheathing bases, small unspecialized flowers in compound umbel inflorescences, and specialized fruits, make them easily identifiable to family (likely making them one of the first families of flowering plants to be generally recognized). However, despite their large size, widespread distribution, and economic importance, no widely acceptable modern classification is available.

The most recent treatment of the family (Pimenov and Leonov, 1993) is but an adaptation of the century-old system of Drude (1898), highly criticized for using subtle or poorly defined diagnostic characters (Heywood, 1982a). Radically different classifications exist (such as those of Koso-Poljansky, 1916, and Cerceau-Larrival, 1962), but have proved unworkable in practice and are rarely used. Drude recognized three subfamilies of Apiaceae (Apioideae, Saniculoideae, and Hydrocotyloideae), dividing each into a series of tribes and subtribes. Molecular systematic investigations have confirmed the monophyly of Apioideae and demonstrated its sister-group status to subfamily Saniculoideae, but have also shown that all of Drude's tribes (and other reclassifications of the family) are largely unsound (Downie and Katz-Downie, 1996; Downie, Katz-Downie, and Cho, 1996; Kondo et al., 1996; Plunkett, Soltis, and Soltis, 1996a, b, 1997; Downie et al., 1998; Valiejo-Roman et al., 1998; Katz-Downie et al., 1999; Plunkett and Downie, 1999). Umbellifers display a remarkable array of morphological and anatomical modifications of their fruits, many of which are adaptations for various modes of seed dispersal. Not surprisingly, these characters are prone to convergence, and their almost exclusive use to delimit suprageneric groups has confounded estimates of relationship.

Our goal over the past few years, and that of our col-

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laborators, has been to resolve the ''higher level'' relationships within subfamily Apioideae. This is necessary in order to provide the framework for ''lower level'' revisions of particular tribes and complexes of genera, so important in such a group of plants where suprageneric relationships have been largely speculative and ever changing. Eventually, this will lead to the production of a modern classification (i.e., a ''new Drude''). To achieve this goal, a variety of molecular characters have been used, such as chloroplast gene (*rbcL, matK*) and intron (*rpoC1, rps16*), and nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. A recent study examined restriction site variation of chloroplast DNA (cpDNA; Plunkett and Downie, 1999); further examination of chloroplast genomic structure is in progress (G. Plunkett and S. Downie, unpublished data). While these characters have been important in providing insight into evolutionary relationships, not all have been useful at the same hierarchical level. Moreover, because many existing data sets are not parallel in construction, opportunities to combine data have been few.

Noncoding regions of cpDNA, such as introns and intergenic spacers, tend to evolve more rapidly than coding loci, both in nucleotide substitutions and in the accumulation of insertion and deletion events (indels), presumably because they are less functionally constrained (Curtis and Clegg, 1984; Palmer, 1991; Clegg et al., 1994). Because these noncoding regions can potentially supply more informative characters than coding regions of comparable size, they have become popular for phylogenetic studies among taxa that are recently diverged. The chloroplast gene *rpl16,* encoding the ribosomal protein L16 (Posno, Van Vliet, and Groot, 1986), is interrupted by an intron in many, but not all, land plants (Campagna and Downie, 1998). In most flowering plants, this intron is ~1 kilobase in length (Campagna and Downie, 1998). Pairwise comparisons of the 17 chloroplast introns shared between tobacco and rice indicate that the *rpl16* intron is most divergent, with 64.5% sequence similarity (Downie, Katz-Downie, and Cho, 1996). Wolfe, Li, and Sharp (1987) reported that this intron has an exceptionally high rate of sequence change when *Spirodela* is compared with tobacco, and Small et al. (1998) concur that this intron is rapidly evolving, at least in the context of the seven noncoding cpDNA loci examined in a group of recently radiated tetraploid cottons. Given its large size relative to other plastid introns and potential for much variation, we have chosen to examine the historical relationships of subfamily Apioideae and allied taxa using the *rpl16* intron. Previous studies have already demonstrated the utility of this region for phylogenetic inferences in Lemnaceae (Jordan, Courtney, and Neigel, 1996), Poaceae (Kelchner and Clark, 1997), and Cactaceae (Dickie, 1996; R. Wallace, unpublished data).

In this paper, we (1) characterize the molecular evolution of the *rpl16* intron in Apiaceae and related taxa and assess its utility in estimating phylogeny, (2) present results based on phylogenetic analyses of these *rpl16* intron sequences, and (3) for a subset of the taxa, compare the phylogenetic results obtained to those inferred using *rpoC1* intron sequences. These intron data are then combined and the resultant estimate of relationship compared to phylogenies for the group inferred using other characters, such as nuclear ribosomal DNA ITS (Downie et al., 1998; Katz-Downie et al., 1999) and chloroplast *matK* (Plunkett, Soltis, and Soltis, 1996b) sequences, and chloroplast restriction sites (Plunkett and Downie, 1999). Based on consensus of relationship, we take the first steps towards a ''new Drude'' by formally recognizing seven groups of apioids at the tribal level and, in so doing, provide the requisite framework for ''lower level'' systematic study.

MATERIALS AND METHODS

Plant accessions—One hundred and nineteen species from 99 genera of Apiaceae subfamily Apioideae, five species (five genera) each from Apiaceae subfamilies Hydrocotyloideae and Saniculoideae, 11 species (ten genera) of Araliaceae, and seven species (five genera) of Pittosporaceae were examined for *rpl16* intron sequence variation (Table 1). In total, 147 species representing 124 genera were considered, with 84 of these species included in a previous phylogenetic analysis of *rpoC1* introns (Downie et al., 1998). *RpoC1* intron sequences for *Billardiera scandens* and *Bursaria spinosa* (Pittosporaceae) were procured as part of this study, for a total of 86 matching *rpl16* and *rpoC1* intron sequences (Table 1). With the exception of *Anethum graveolens* and *Crithmum maritimum,* where different accessions of the same species were examined, both *rpl16* and *rpoC1* intron data for these 86 species were obtained from precisely the same specimens.

Experimental strategy—Leaf material for DNA extraction was obtained either directly from the field, from plants cultivated from seed in the greenhouse, or from accessioned plants cultivated at several botanic gardens (Table 1). For some species, DNAs were extracted from herbarium specimens or supplied to us directly. All plants cultivated at the University of Illinois at Urbana-Champaign (UIUC), Moscow State University, and the Royal Botanic Garden Edinburgh (RBGE) are vouchered at ILL, MW, and E, respectively (herbarium acronyms according to Holmgren, Holmgren, and Barnett, 1990). Details of the DNA extraction procedures have been presented in Downie and Katz-Downie (1996). The complete *rpl16* intron from all 147 species, including portions of its flanking exons and the intergenic spacer between genes *rps3* and *rpl16,* was amplified using the polymerase chain reaction (PCR) method and primers ''rps3'' and ''L16 exon2'' in an equimolar ratio (Fig. 1). These primers were designed by comparing published *rpl16* exon2 or *rps3* sequences from tobacco, spinach, *Epifagus, Vigna,* rice, maize, and *Marchantia,* and choosing regions highly conserved among them (Ohyama et al., 1986; Shinozaki et al., 1986; McLaughlin and Larrinua, 1987; Hiratsuka et al., 1989; L. Arief, B. Entsch, and R. Wicks, unpublished data). In tobacco cpDNA, the *rpl16* intron is 1020 base pairs (bp) in size, the 3′ end of primer ''rps3'' is 377 bp upstream from the exon1-intron junction, and the 3′ end of primer ''L16 exon2'' is 18 bp downstream from the intron-exon2 junction (Shinozaki et al., 1986). Five internal primers were constructed to facilitate manual sequencing; these are labeled ''L16 exon1'' and ''intron 1–4'' in Fig. 1. All seven primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, Iowa, USA).

Details of the PCR amplification protocol and the DNA purification and sequencing strategies employed were also the same as outlined previously (Downie and Katz-Downie, 1996). Each set of PCR amplifications was monitored by the inclusion of positive (tobacco cpDNA) and negative (no template) controls. Successful PCR amplifications resulted in a single-band product of ~1400 bp. Of the 147 accessions sequenced, 37 were done so with the seven primers identified in Fig. 1 using manual sequencing methods. Here the sequence data were obtained through direct sequencing of double-stranded templates derived from the PCR procedure. The remaining species, including ten of those sequenced manually, were sequenced using an Applied Biosystem's, Inc. (Foster City, California, USA) 373A Automated DNA sequencer

with Stretch upgrade. Cycle sequencing reactions were carried out in a PTC-100 thermocycler (M. J. Research, Inc., Cambridge, Massachusetts, USA) using the purified PCR products, AmpliTaq DNA polymerase, and fluorescent dye-labeled terminators (Perkin-Elmer Corp., Norwalk, Connecticut, USA). The reaction conditions were as specified by the manufacturer, with the addition of 5% dimethylsulfoxide (DMSO). The sequencing products, after purification with Centri-Sep spin columns (Princeton Separations, Adelphia, New Jersey), were resolved by electrophoresis in 4% acrylamide gels. Sequencing primers ''L16 exon1,'' ''L16 exon2,'' and ''intron3'' (Fig. 1) were each used in the sequencing of each DNA template. All automated output was checked visually and edited for correct automated base-calling.

Sequence alignment and intron secondary structure—The DNA sequences were aligned initially using CLUSTAL W version 1.7 (Thompson, Higgins, and Gibson, 1994), copied into the data editor of PAUP version 3.1.1 (Swofford, 1993), and realigned manually. Gaps were positioned to minimize nucleotide mismatches. Consideration was also given to the probable mechanism of DNA evolution giving rise to the mutation, as described by Kelchner and Clark (1997). For example, many insertions were inferred to be the result of a single inserted direct repeat, a highly probably mutational event in noncoding DNA. Only sequence data from the *rpl16* intron were included in the analysis, because data from the *rps3-rpl16* intergenic spacer were not available for many taxa. Predictions of the *Anethum graveolens* (dill) *rpl16* intron secondary structure were made using the free-energy minimization method of MULFOLD version 2.0 (Jaeger, Turner, and Zuker, 1989; Zuker, 1989).

Pairwise nucleotide differences of unambiguously aligned positions were determined using the distance matrix option in PAUP. Alignment gaps in any one sequence 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 multiple hits. Transition/transversion (Ts/Tv) ratios over a subset of the maximally parsimonious trees were calculated using MacClade version 3.01 (Maddison and Maddison, 1992). Polytomies were arbitrarily resolved. To assess variation in levels of base substitution among sites across a subset of the maximally parsimonious trees obtained, the number of steps per four consecutive bases was estimated using MacClade. The nucleotide sequence data reported in this study have been deposited with the GenBank Data Library; accession numbers are provided in Table 1.

Phylogenetic analysis—Phylogenetic analyses were carried out on the complete 147-species *rpl16* intron data matrix and, for 86 of these species, separately and in combination with available *rpoC1* intron sequences (Table 1). The data were analyzed using Macintosh Quadra 700 or Power Macintosh computers. All trees computed were rooted with the Pittosporaceae accessions. Phylogenetic analyses of molecular data (Xiang et al., 1993; Plunkett, Soltis, and Soltis, 1996a) corroborate traditional taxonomic evidence (Jay, 1969; Dahlgren, 1980; Thorne, 1992; Judd, Sanders, and Donoghue, 1994) in suggesting that Pittosporaceae are likely sister to Apiaceae + Araliaceae.

Maximally parsimonious (MP) trees were sought using PAUP and the heuristic search strategies described in Downie et al. (1998), based on those presented in Catalán, Kellogg, and Olmstead (1997). The length of the shortest trees was obtained by initiating at least 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. The equally MP trees were then used as starting trees for TBR branch swapping. In all analyses, the maximum number of trees to be saved was set at 5000. The strict consensus of these 5000 trees was subsequently used 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, only those trees that did not fit the constraint tree were saved. As no additional trees were found at the length of the initial 5000 trees, this suggested 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. 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. Owing to the large size of the data matrix, a maxtree limit of 200 trees per replicate was set. Gaps were incorporated into the analysis by scoring each insertion or deletion as a separate presence/absence (i.e., binary) character (Swofford, 1993). The resultant topology was then compared to the one inferred when gaps were omitted as additional characters. Previous investigations of cpDNA *rpoC1* intron (Downie et al., 1998) and other noncoding sequences (e.g., van Ham et al., 1994) have revealed that indels contain much phylogenetic information and, indeed, may provide particularly clear indications of relationship.

Distance trees were constructed using the neighbor-joining (NJ) method (Saitou and Nei, 1987), implemented using the NEIGHBOR program in PHYLIP version 3.5 (Felsenstein, 1993). 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. Length mutations were not incorporated into the analysis. Two Ts/Tv rate ratios were used (1.0 and 2.0), with the former approximating the expected ratio of Ts to Tv as inferred by the MP analysis. A bootstrap analysis was done using 100 resampled data sets generated with the SEQBOOT program prior to calculating the distance matrices and NJ trees. PHYLIP's CONSENSE program was then used to construct a consensus tree.

The maximum likelihood (ML) method was also applied to these substitution data using the program fastDNAml version 1.0.6 (Olsen et al., 1994). ML trees were inferred using a Ts/Tv rate ratio of 1.0, randomizing the input order of sequences (jumble), and by invoking the global branch swapping algorithm. Empirical base frequencies were derived from the sequence data and used in the ML calculations. The ML analyses, however, could not be carried out to completion, given the large size of the data matrix and the time required to complete the global branch swapping. Despite four weeks of computer run time, none of the 12 Macintosh computers running simultaneously completed their searches or converged on the same highest (least negative) log likelihood value. After completing one round of branch swapping, the best ML tree had a log likelihood value of −8123.195.

RESULTS

Rpl16 intron sequence characterization—The sequenced *rpl16* introns varied in length from 892 bp (in *Cicuta virosa*; Apiaceae subfamily Apioideae) to 1021 bp (in *Bursaria spinosa*; Pittosporaceae). Within Apioideae, their length ranged from 892 to 982 bp (*Heracleum lanatum*). Alignment of all 147 sequences resulted in a matrix of 1444 positions. However, because of frequent length mutations of varying sizes confounding alignment interpretation, it was necessary to exclude 26 regions from the matrix in the distance calculations and phylogenetic analyses. These ambiguous regions ranged in size from one to 90 positions (averaging ~14 positions each), with several characterized by tracts of poly-A's, G's, and T's of variable length. We have taken a conservative approach to sequence alignment in excluding regions where alternative alignments are possible and that may result in conflicting phylogenetic signal. Alternating gap penalty or substitution costs were not considered. A region representing an unambiguous 92-bp deletion in all Apiaceae and Araliaceae relative to Pittosporaceae was also excluded. As a result, 452 alignment positions (or about

TABLE 1. Sources of plant material and GenBank accession numbers for the 147 species of Apiaceae, Araliaceae, and Pittosporaceae examined for cpDNA *rpl16* intron sequence variation. Asterisks denote those 86 species for which cpDNA *rpoC1* intron sequence data are also available (Downie et al., 1998). Locations of voucher specimens are provided; herbarium acronyms follow Holmgren, Holmgren, and Barnett (1990). RBGE = Royal Botanic Garden Edinburgh; UCB = Botanical Garden of the University of California, Berkeley; UIUC = University of Illinois at Urbana-Champaign.

Taxon	Source[a]	GenBank accession no.[b]
Apiaceae subfamily Apioideae		
Aciphylla crenulata J. B. Armstr.*	1	GBAN-AF094428
Aciphylla subflabellata W. R. B. Oliv.	2	GBAN-AF094429
Aegopodium alpestre Ledeb.*	1	GBAN-AF094393
Aethusa cynapium L.*	1	GBAN-AF094406
Agrocharis incognita (C. Norman) Heywood & Jury	Africa, Kenya, *Knox 2578*; cult. UIUC, *Lee 119* (ILL)	GBAN-AF094331
Anethum graveolens L.*	cult. UIUC from seeds obtained from Univ. Oldenburg Bot. Gard., Germany; *Downie 157* (ILL)	GBAN-AF094418
Angelica archangelica L.*	1	GBAN-AF094362
Anginon rugosum (Thunb.) Raf.*	1	GBAN-AF094444
Anisotome aromatica Hook. f.*	1	GBAN-AF094430
Anthriscus cerefolium (L.) Hoffm.*	1	GBAN-AF094353
Apium graveolens L.*	1	GBAN-AF094417
Arafoe aromatica Pimenov & Lavrova*	1	GBAN-AF094388
Arracacia brandegei J. M. Coult. & Rose*	1	GBAN-AF094358
Arracacia nelsonii J. M. Coult. & Rose*	1	GBAN-AF094356
Astrodaucus orientalis (L.) Drude	cult. UIUC from seeds obtained from Research Institute of Forests and Rangeland, Iran; *Lee 43* (ILL)	GBAN-AF094343
Aulacospermum anomalum (Ledeb.) Ledeb.	2	GBAN-AF094440
Azilia eryngioides (Pau) Hedge & Lamond	2	GBAN-AF094386
Bupleurum chinense DC.*	1	GBAN-AF094443
Bupleurum ranunculoides L.*	1	GBAN-AF094441
Bupleurum rotundifolium L.	cult. UIUC from seeds obtained from Jardin botanique de Caen, France; *Downie 304* (ILL)	GBAN-AF094442
Capnophyllum dichotomum (Desf.) Lag.*	1	GBAN-AF094380
Carum carvi L.*	1	GBAN-AF094392
Caucalis platycarpos L.*	1	GBAN-AF094339
Chaerophyllum temulum L.	Poland, Bot. Gard. of Warsaw Univ.; *Spalik s.n.*	GBAN-AF094354
Chaerophyllum khorassanicum Czerniak. ex Schischk.	1	GBAN-AF094355
Chaetosciadium trichospermum (L.) Boiss.	1	GBAN-AF094338
Chymsydia colchica (Albov) Woronow ex Grossh.*	1	GBAN-AF094414
Cicuta virosa L.*	1 (*Downie 131*)	GBAN-AF094423
Cnidiocarpa alaica Pimenov	2	GBAN-AF094376
Cnidium silaifolium (Jacq.) Simonk.	2	GBAN-AF094378
Conioselinum chinense (L.) B. S. P.*	1	GBAN-AF094421
Conioselinum tataricum Hoffm.	2	GBAN-AF094409
Conium maculatum L.*	1 (*Downie 63*)	GBAN-AF094385
Coriandrum sativum L.*	1	GBAN-AF094404
Cortia depressa (D. Don) C. Norman	2	GBAN-AF094403
Coulterophytum laxum B. L. Rob.*	1	GBAN-AF094361
Crithmum maritimum L.*	cult. UIUC from seeds obtained from Quail Bot. Gard., California; *Downie 345* (ILL)	GBAN-AF094391
Cryptotaenia japonica Hassk.*	1	GBAN-AF094424
Cymopterus globosus (S. Watson) S. Watson	1	GBAN-AF094365
Daucus carota L.*	1	GBAN-AF094328
Daucus pusillus Michx.	cult. UCB (no. 92.0891)	GBAN-AF094330
Eleutherospermum cicutarium (M. Bieb.) Boiss.	2	GBAN-AF094436
Endressia castellana Coincy*	1	GBAN-AF094400
Erigenia bulbosa (Michx.) Nutt.	2	GBAN-AF094433
Exoacantha heterophylla Labill.	2	GBAN-AF094407
Falcaria vulgaris Bernh.*	1	GBAN-AF094396
Ferula assa-foetida L.*	1	GBAN-AF094381
Ferula kokanica Regel & Schmalh.	Tadjikistan, Hushikat Gorge, *Pimenov et al. 166* (MW); cult. Moscow State Univ. Bot. Gard., Russia	GBAN-AF094346
Foeniculum vulgare Mill.*	1	GBAN-AF094419
Fuernrohria setifolia K. Koch	2	GBAN-AF094394
Glia prolifera (Burm. f.) B. L. Burtt	South Africa, *Barker 96A* (E); cult. RBGE (no. 19923034)	GBAN-AF094445
Glochidotheca foeniculacea Fenzl	Iraq, Sersang; *Haines W1002* (K)	GBAN-AF094345
Heracleum lanatum Michx.*	1	GBAN-AF094368

TABLE 1. Continued.

Taxon	Source[a]	GenBank accession no.[b]
Heracleum rigens DC.*	1	GBAN-AF094373
Heracleum sphondylium L.	1	GBAN-AF094369
Heteromorpha arborescens (Spreng.) Cham. & Schltdl.*	1	GBAN-AF094446
Imperatoria ostruthium L.*	1	GBAN-AF094415
Karatavia kultiassovii (Korovin) Pimenov & Lavrova	2	GBAN-AF094401
Komarovia anisosperma Korovin*	1	GBAN-AF094434
Laser trilobum (L.) Borkh.	2	GBAN-AF094335
Laserpitium hispidum M. Bieb.	cult. UIUC from seeds obtained from Hungarian Academy of Sciences Bot. Gard., Vácrátót; *Downie 120* (ILL)	GBAN-AF094332
Lecokia cretica (Lam.) DC.*	1	GBAN-AF094432
Ligusticum ferulaceum All.	cult. Moscow State Univ. Bot. Gard., Russia	GBAN-AF094379
Ligusticum physospermifolium Albov	2	GBAN-AF094377
Ligusticum scoticum L.*	1 (UCB no. 84.0620)	GBAN-AF094347
Lisaea papyracea Boiss.	Armenia, Erevan, Vokhgabert; *Gambarian s.n.* (UC)	GBAN-AF094341
Lithosciadium multicaule Turcz.	cult. Moscow State Univ. Bot. Gard., Russia	GBAN-AF094405
Lomatium californicum (Torr. & A. Gray) Mathias & Constance	1	GBAN-AF094364
Malabaila secacul (Mill.) Boiss.	2	GBAN-AF094372
Myrrhidendron donnell-smithii J. M. Coult. & Rose*	1 (UCB no. 90.2637)	GBAN-AF094357
Myrrhis odorata (L.) Scop.*	1	GBAN-AF094348
Oedibasis platycarpa (Lipsky) Koso-Pol.	2	GBAN-AF094390
Oenanthe pimpinelloides L.*	1	GBAN-AF094422
Opopanax hispidus (Friv.) Griseb.	2	GBAN-AF094410
Orlaya daucoides (L.) Greuter	Spain; cult. UIUC from seeds obtained from J.-P. Reduron, Mulhouse, France (no. 9203); *Lee 85* (ILL)	GBAN-AF094334
Orlaya grandiflora (L.) Hoffm.*	1	GBAN-AF094333
Osmorhiza chilensis Hook. & Arn.*	1	GBAN-AF094350
Osmorhiza longistylis (Torr.) DC.	1	GBAN-AF094349
Oxypolis occidentalis J. M. Coult. & Rose*	1	GBAN-AF094426
Parasilaus asiaticus (Korovin) Pimenov	2	GBAN-AF094435
Pastinaca armena Fisch. & C. A. Mey.	2	GBAN-AF094371
Pastinaca sativa L.*	1	GBAN-AF094370
Perideridia kelloggii (A. Gray) Mathias*	1	GBAN-AF094427
Peucedanum caucasicum (M. Bieb.) K. Koch	2	GBAN-AF094411
Peucedanum decursivum (Miq.) Maxim.*	1	GBAN-AF094412
Peucedanum morisonii Bess. ex Schult.*	1	GBAN-AF094413
Phlojodicarpus popovii Sipliv.	2	GBAN-AF094402
Physospermum cornubiense (L.) DC.*	1	GBAN-AF094437
Pimpinella peregrina L.*	1	GBAN-AF094387
Pleurospermum foetens Franch.	2	GBAN-AF094438
Pleurospermum uralense Hoffm.	2	GBAN-AF094439
Polylophium panjutinii Manden. & Schischk.	2	GBAN-AF094336
Prangos pabularia Lindl.*	1	GBAN-AF094382
Prionosciadium turneri Constance & Affolter*	1	GBAN-AF094359
Pseudorlaya pumila (L.) Grande	cult. UIUC from seeds obtained from Jardin Botaniques Lisboa, Portugal; *Lee 59* (ILL)	GBAN-AF094329
Pyramidoptera cabulica Boiss.	2	GBAN-AF094389
Rhodosciadium argutum (Rose) Mathias & Constance*	1	GBAN-AF094360
Ridolfia segetum (L.) Moris*	1	GBAN-AF094420
Scandix balansae Reut. ex Boiss.	1	GBAN-AF094352
Scandix pecten-veneris L.*	1	GBAN-AF094351
Seseli krylovii (V. N. Tikhom.) Pimenov & Sdobnina*	1	GBAN-AF094399
Seseli libanotis (L.) W. D. J. Koch	2	GBAN-AF094398
Shoshonea pulvinata Evert & Constance*	1	GBAN-AF094363
Sium latifolium L.*	1	GBAN-AF094425
Smyrniopsis aucheri Boiss.*	1	GBAN-AF094383
Smyrnium olusatrum L.*	1 (*Downie 343*)	GBAN-AF094431
Spermolepis inermis (Nutt. ex DC.) Mathias & Constance	2	GBAN-AF094397
Sphaenolobium tianschanicum (Korovin) Pimenov	2	GBAN-AF094416

TABLE 1. Continued.

Taxon	Source[a]	GenBank accession no.[b]
Szovitsia callicarpa Fisch. & C. A. Mey.	Azerbaijan, Moghan; *Lamond 3195* (E)	GBAN-AF094342
Thaspium pinnatifidum (Buckley) A. Gray	1	GBAN-AF094366
Thyselium palustre (L.) Raf.	2	GBAN-AF094384
Tommasinia verticillaris (L.) Bertol.	2	GBAN-AF094408
Tordylium aegyptiacum (L.) Lam.*	1	GBAN-AF094375
Torilis arvensis (Huds.) Link	U.S.A., Illinois, Champaign Co., Urbana; *Downie 816* (ILL)	GBAN-AF094337
Trachyspermum ammi (L.) Sprague in Turrill*	1	GBAN-AF094395
Turgenia latifolia (L.) Hoffm.	cult. UIUC from seeds obtained from J.-P. Reduron, Mulhouse, France; *Lee 82* (ILL)	GBAN-AF094340
Yabea microcarpa (Hook. & Arn.) Koso-Pol.	U.S.A., Arizona, Pima Co.; *Holmgren 6772* (WTU)	GBAN-AF094344
Zizia aurea (L.) W. D. J. Koch*	1 (*Downie 8*)	GBAN-AF094367
Zosima orientalis Hoffm.	2	GBAN-AF094374
Apiaceae subfamily Hydrocotyloideae		
Bolax gummifera (Lam.) Spreng.*	1	GBAN-AF094453
Centella asiatica (L.) Urb.*	1	GBAN-AF094454
Didiscus pusilla DC.*	1	GBAN-AF094456
Eremocharis fruticosa Phil.*	1	GBAN-AF094452
Hydrocotyle rotundifolia Wall.*	1	GBAN-AF094455
Apiaceae subfamily Saniculoideae		
Astrantia major L.*	1	GBAN-AF094451
Eryngium planum L.*	1	GBAN-AF094450
Hacquetia epipactis (Scop.) DC.*	1	GBAN-AF094448
Petagnaea saniculifolia Guss.*	1	GBAN-AF094449
Sanicula canadensis L.*	1	GBAN-AF094447
Araliaceae		
Aralia californica S. Watson*	1	GBAN-AF094457
Aralia spinosa L.*	1	GBAN-AF094458
Cussonia paniculata Eckl. & Zeyh.*	1	GBAN-AF094459
Dendropanax arboreus (L.) Decne. & Planch.*	1	GBAN-AF094464
Fatsia japonica (Thunb.) Decne. & Planch.*	1	GBAN-AF094466
Hedera helix L.*	1	GBAN-AF094463
Kalopanax pictus (Thunb.) Nakai*	1	GBAN-AF094467
Oreopanax sanderianus Hemsl.*	1	GBAN-AF094465
Polyscias balfouriana L. H. Bailey*	1	GBAN-AF094460
Pseudopanax arboreus (Murray) Philipson*	1	GBAN-AF094461
Schefflera actinophylla (Endl.) Harms*	1	GBAN-AF094462
Pittosporaceae		
Billardiera scandens Sm.*	cult. UIUC from seeds obtained from North Coast Regional Bot. Gard., Coffs Harbour, Australia; *Downie 633* (ILL)	GBAN-AF094471
Bursaria spinosa Cav.*	Australia, Tasmania; cult. RBGE (no. 19760574) from seeds obtained from Canberra Natl. Bot. Gard., Australia	GBAN-AF094472
Hymenosporum flavum F. Muell.	cult. UIUC from seeds obtained from North Coast Regional Bot. Gard., Coffs Harbour, Australia; *Downie 638* (ILL)	GBAN-AF094474
Pittosporum dallii Cheeseman	cult. RBGE (no. 19591283)	GBAN-AF094469
Pittosporum revolutum Aiton	cult. UIUC from seeds obtained from North Coast Regional Bot. Gard., Coffs Harbour, Australia; *Downie 829* (ILL)(UIUC 94227)	GBAN-AF094470
Pittosporum tobira (Thunb.) Aiton*	1	GBAN-AF094468
Sollya heterophylla Lindl.	cult. Missouri Bot. Gard. (no. 897138)	GBAN-AF094473

[a] Source and voucher information were previously reported in (1) Downie et al. (1998) or (2) Katz-Downie et al. (1999). When more than one accession of a species was sequenced for the *rpoC1* intron in Downie et al. (1998), the accession examined for *rpl16* intron sequence variation has been indicated here.

[b] The prefix GBAN- has been added for linking the online version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number.

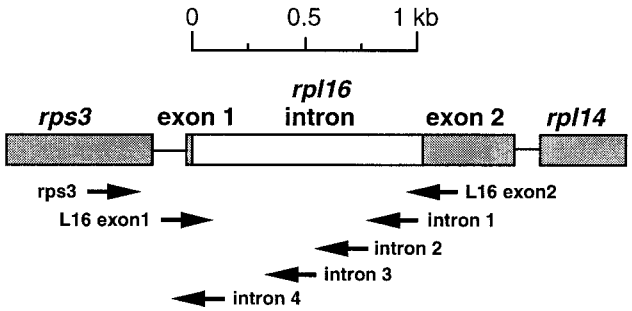


Fig. 1. Location of the 1020-bp intron S in tobacco chloroplast gene *rpl16* relative to its exons and flanking gene regions (based on Shinozaki et al., 1986). Scale is in kilobase (kb) units. The arrows represent the directions and approximate positions of the primers used in PCR amplification and/or DNA sequencing. These primer sequences, written 5′ to 3′, are as follows:
"rps3"-TTTCCTTTCGAAAAGCAATG;
"L16 exon1"-AATAATCGCTATGCTTAGTG;
"L16 exon2"-TCTTCCTCTATGTTGTTTACG;
"intron 1"-ATTATTCATTTGTATATC;
"intron 2"-TCACGGGCGAATATTKACT;
"intron 3"-TCTGATTTCTACAAYGGAGC;
"intron 4"-CGAGTCGCACACTAAGCAT.

TABLE 2. Sequence characteristics of the 147 species of Apiaceae, Araliaceae, and Pittosporaceae examined for cpDNA *rpl16* intron sequence variation.

Nucleotide sites	
No. total aligned positions	1444
No. aligned positions excluded (and %)	452 (31.3)
No. aligned positions constant (and %)	472 (32.7)
No. aligned positions parsimony informative (and %)	378 (26.2)
No. aligned positions autapomorphic (and %)	142 (9.8)
Length variation[a]	
No. unambiguous alignment gaps	90
No. deletions	71
No. insertions	19
No. unambiguous gaps parsimony informative	37
No. deletions	28
No. insertions	9
Pairwise sequence divergence (range in %)	
Subfamily Apioideae only	0–11.3
All 147 species	0–18.1

[a] Length variation relative to the outgroup *Pittosporum* (Pittosporaceae).

one-third of the entire matrix) were excluded from subsequent analyses. Characteristics of the remaining 992 unambiguously aligned positions, including the numbers of constant, parsimony informative, and autapomorphic positions, are provided in Table 2. The ratio of terminal taxa (147) to parsimony-informative nucleotide substitutions (378) is 1 : 2.6. Measures of pairwise sequence divergence ranged from identity to 11.3% across 119 accessions (99 genera) of subfamily Apioideae, and from identity to 18.1% across all 147 accessions. A total of 90 unambiguous gaps was required for proper alignment of these sequences. These gaps ranged from 1 to 92 bp, with the average size being ~7 bp; the number of gaps with respect to their size is presented in Fig. 2. Thirty-seven gaps were parsimony informative (Table 2), with three of these (including the large 92-bp deletion) distinguishing all Apiaceae and Araliaceae from Pittosporaceae. Percentage G + C content across all 147 intron sequences ranged from 28.3 to 33.2%, and averaged 30.8%.

Rpl16 intron secondary structure—A secondary structure model of the 940-bp *rpl16* intron in *Anethum graveolens* is presented (Fig. 3). This reconstruction was inferred based on consensus 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 minor differences in free energy exist between this model and other conformations that can be drastically different. These differences are particularly evident within intron domains III and IV. Therefore, this model should be interpreted as a provisional estimate. Like other group II introns, a conserved core structure is evident, consisting of six major domains (I–VI) radiating from a central wheel. Domain I is divided into several subdomains and other regions, of which we have identified subdomains IC and ID, and exon binding sites 1 and 2 (EBS 1 and EBS 2).

For each intron domain and subdomains IC and ID, the number of constant, variable, parsimony-informative, and excluded alignment positions, the maximum pairwise sequence divergence, and the number of unambiguous alignment gaps were determined (Table 3). Of the intron's six major structural domains, domain I is the largest and domains V and VI the smallest. The most variable domains, calculated by dividing the number of variable and unambiguously aligned positions in each region by its overall size, are domains III and IV, with 57.9 and 64.2% of their positions variable, respectively. Of these two, domain IV is the most variable, both in the number of unambiguous gaps inferred (26) and the high percentage of sites excluded because of alignment ambiguity (56.7%). The largest indel, a 92-bp deletion in all Apiaceae and Araliaceae, was located in this domain.

Rpl16 intron phylogenetic analysis—MP analysis of all 992 unambiguously aligned nucleotide positions and 37 informative gaps resulted in >5000 trees prior to termination of analysis. The strict consensus of 5000 of these trees, each of length 1527 steps, consistency indices (CI) of 0.553 (all characters) and 0.497 (excluding uninformative characters), and retention index (RI) of 0.845, is shown in Fig. 4. Reanalyzing the data without

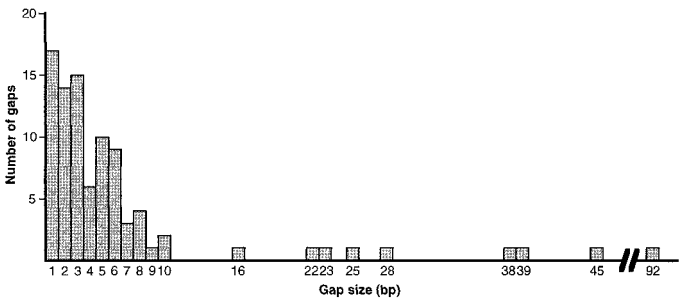


Fig. 2. Characteristics of the 90 unambiguous gaps inferred in the alignment of 147 *rpl16* intron sequences from Apiaceae, Araliaceae, and Pittosporaceae. These gaps ranged from 1 to 92 bp in size; the number of gaps in each size category is illustrated.

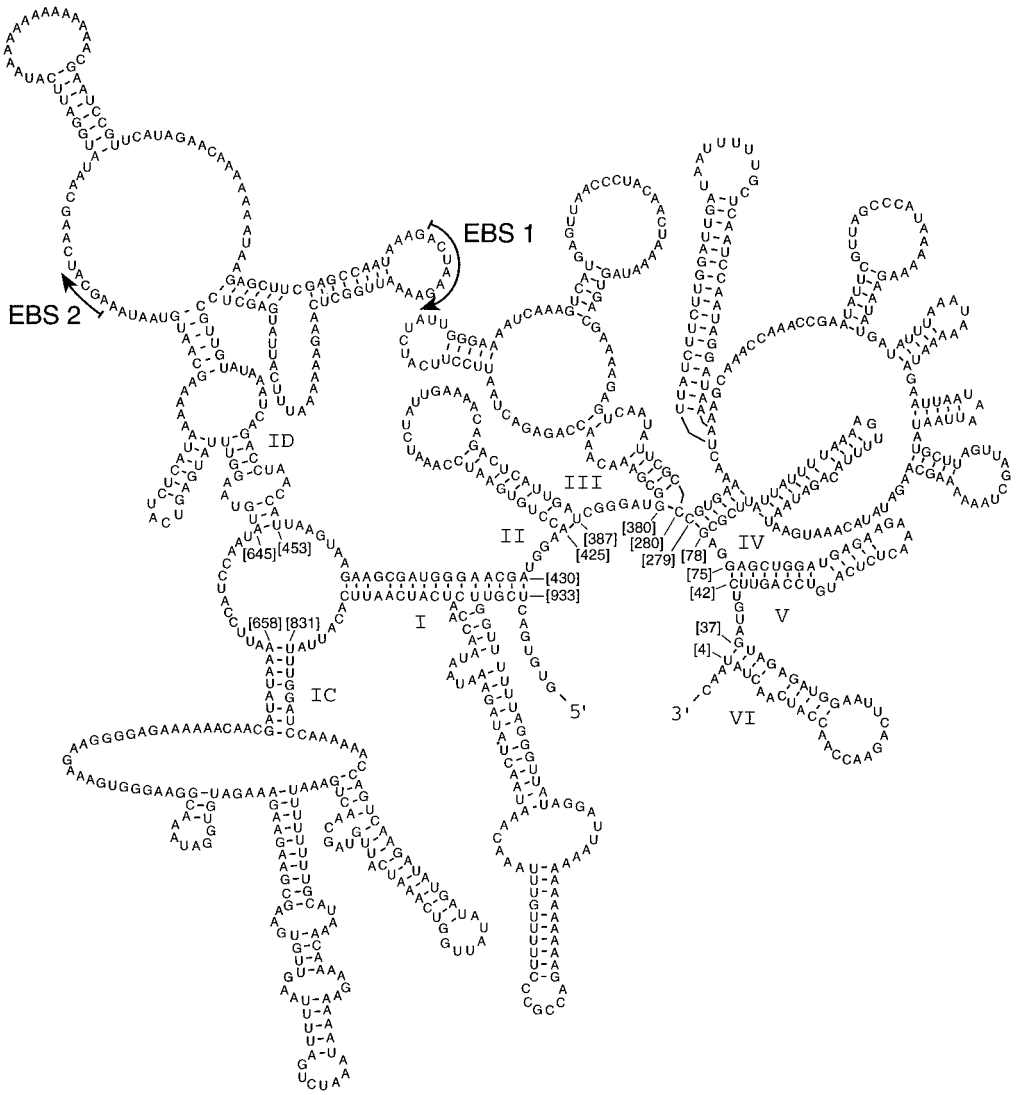


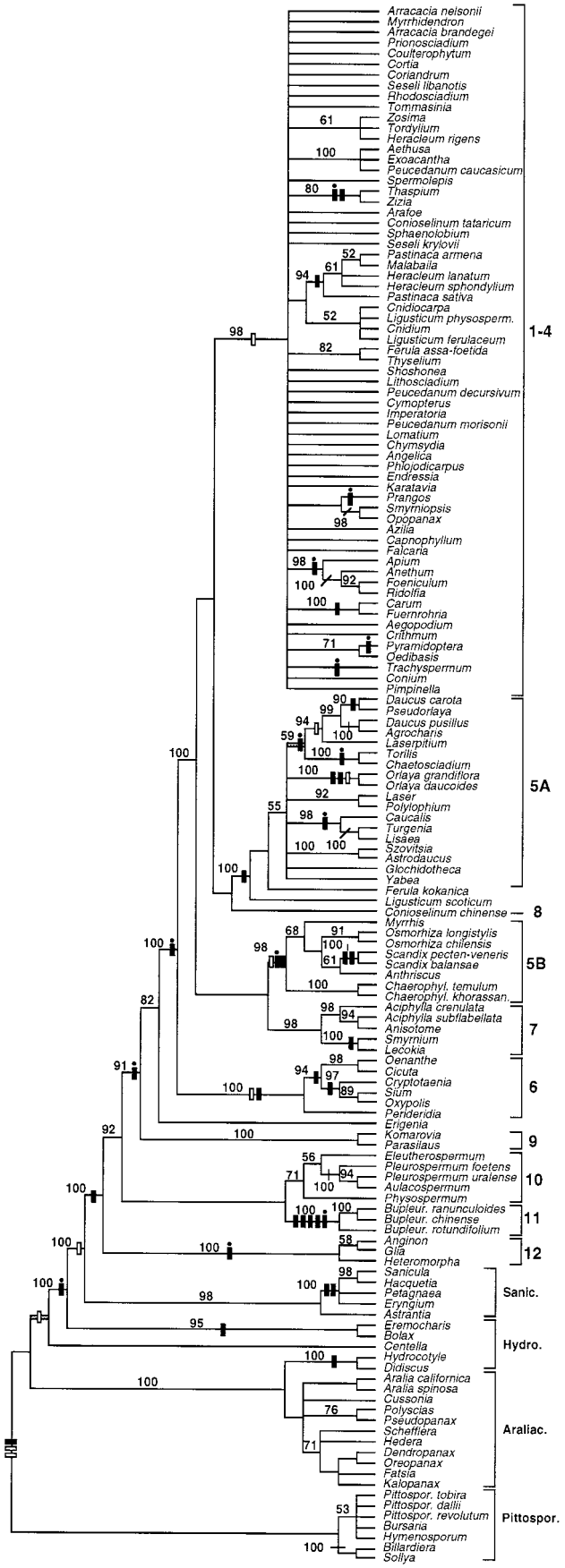
Fig. 3. Putative secondary structure model of the *Anethum graveolens* (dill) cpDNA *rpl16* intron. This model consists of six major structural domains (labeled I-VI) radiating from a central wheel. Domain I is divided into four subdomains of which only two, IC and ID, are indicated. The locations of exon binding sites (EBS) 1 and 2 are also shown. Sequence coordinates are provided in brackets and are referred to in Table 3.

the 37 scored gaps resulted in 5000 minimal length trees, each of length 1481 steps, CI's of 0.546 (all characters) and 0.486 (excluding uninformative characters), and RI of 0.836. The topology of its strict consensus tree was nearly identical to that produced when the indels are included, with the exception of the collapse of two branches (shaded in Fig. 4). The average Ts/Tv ratio among all intron sequences across 100 randomly chosen 1481-step trees, as determined by MacClade, was ~1.0.

To examine the relative variability of base substitutions

TABLE 3. Sequence characteristics of the six major structural domains and two subdomains of the cpDNA *rpl16* intron across all 147 species of Apiaceae, Araliaceae, and Pittosporaceae. *Anethum* coordinates refer to those presented in Fig. 3.

Intron region	*Anethum* coordinates	*Anethum* size (bp)	No. of aligned positions	No. of positions excluded	No. of unambiguous alignment gaps	No. of unambiguous positions	No. of positions constant	No. of positions variable	No. of positions informative	Maximum pairwise divergence (%)
I	430–933	504	687	174	45	513	235	278	206	18.8
IC	658–831	174	236	43	22	193	97	96	70	19.2
ID	453–645	193	240	50	15	190	83	107	83	20.8
II	387–425	39	78	0	1	78	56	22	14	28.2
III	280–380	101	139	25	17	114	48	66	42	22.8
IV	78–279	202	446	253	26	193	69	124	94	30.7
V	42–75	34	34	0	0	34	26	8	6	17.6
VI	4–37	34	34	0	1	34	20	14	9	17.6



across the intron, the maximum, average, and minimum numbers of inferred character-state changes per site over 100 randomly chosen 1481-step MP trees were mapped along the length of this region using a 4-bp nonoverlapping window (Fig. 5). Site variability was also considered relative to the intron's six major structural domains and subdomains IC and ID (Fig. 5). While certain regions within each domain are clearly more variable than others, this variability appears to be distributed relatively evenly over the entire length of the intron. Generally, however, the most conserved domains (i.e., domains V and VI) have the least inferred changes. Site variability is highest in several regions of domains I and IV, with the number of inferred changes occasionally surpassing 20 per four consecutive nucleotide bases.

Relative to the Pittosporaceae outgroups *Billardiera*, *Bursaria*, *Hymenosporum*, *Pittosporum*, and *Sollya*, the 37 informative gaps represent a minimum of 28 deletions and 9 insertions (Table 2). When the distribution of these gaps was optimized against any one of the 1527-step phylogenies, 46 indels are apparent; when mapped onto the strict consensus tree, as done in Fig. 4, 47 indels result. The pattern of indel distribution is consistent with the inferred phylogenies, with none of the nine insertions (open bars, Fig. 4) homoplastic. Two of these insertions are 1 bp in size, three are 2 bp in size, two are 3 bp in size, and two are 4 bp in size. Eight of these insertions involved perfect direct repeats of flanking sequence; the ninth insertion may have been the result of a 3-bp inversion of immediate, flanking sequence. Many other repetitive motifs occurred, but were in those regions of the alignment excluded from the analysis. Of the 28 remaining alignment gaps, 37 deletions (solid bars) are inferred. Three of these gaps are homoplastic, ranging between 1 and 6 bp in size, and each occurring 2–6 times (solid bars with dots).

Distance trees obtained from the NJ analysis, estimated from the two-parameter method of Kimura (1980) with Ts/Tv rate ratios of 1.0 or 2.0, were topologically congruent. The tree constructed with a rate ratio of 1.0 is presented in Fig. 6. While the ML analysis could not be completed with global branch swapping invoked, the best results obtained (not shown) were consistent with those inferred using MP and NJ methods with respect to the major clades distinguished. Within Araliaceae and many clades of Apioideae, branch lengths are quite short, whereas among the hydrocotyloids (e.g., *Bolax*, *Centella*,

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Fig. 4. Strict consensus of 5000 minimal length 1527-step trees derived from equally weighted MP analysis of 147 cpDNA *rpl16* intron sequences using 992 unambiguously aligned nucleotide positions and 37 scored gaps (CI excluding uninformative characters = 0.497, RI = 0.845). Numbers at nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates; values <50% are not indicated. Deletions are represented by dark vertical bars, homoplastic deletions by black dots above the vertical bars, and insertions by open vertical bars. The two broad horizontal, shaded lines indicate branches that collapse when the scored gaps are excluded and the analysis rerun (length of shortest trees = 1481 steps, CI excluding uninformative characters = 0.486, RI = 0.836). Sanic. = Saniculoideae; Hydro. = Hydrocotyloideae; Araliac. = Araliaceae; Pittospor. = Pittosporaceae. Complete taxon names are provided in Table 1. The numbered brackets represent those apioid groups outlined in Downie et al. (1998).

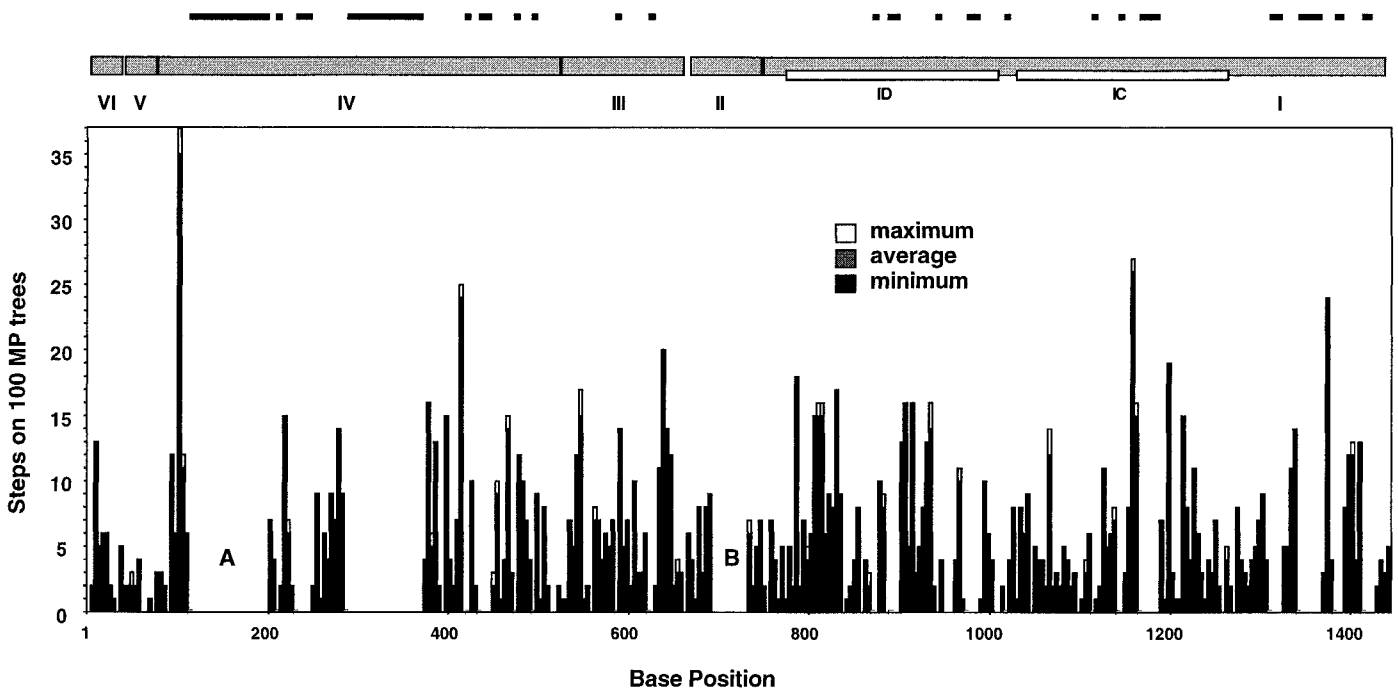


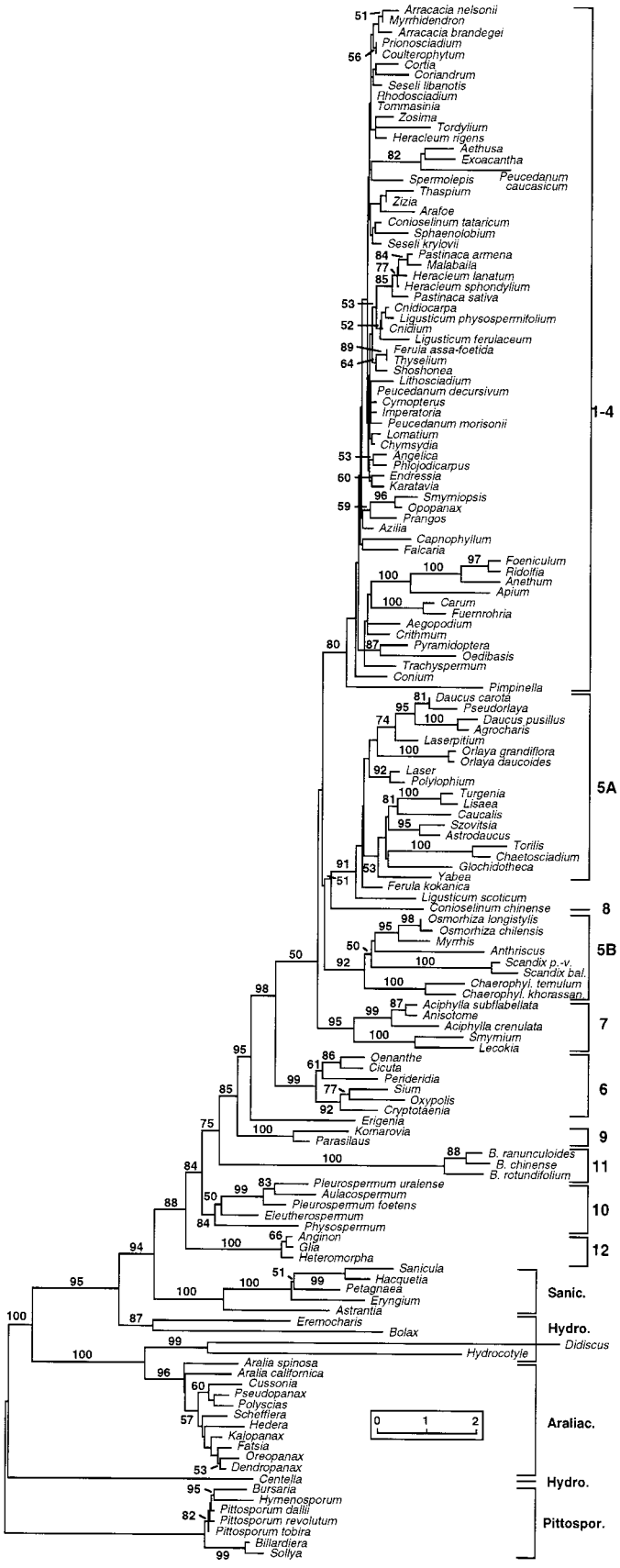
Fig. 5. Site variation over all 1444 positions from the alignment of 147 cpDNA *rpl16* intron sequences from Apiaceae, Araliaceae, and Pittosporaceae, as inferred from 100 MP trees using a window size of four consecutive bases. The approximate locations and sizes of the 22 regions excluded from the analysis because of alignment ambiguity are shown as dots and dashes at the top of the figure (their lengths are proportional to their size) relative to the intron's major structural domains. Positions of the two largest gaps are indicated (A, a 92-bp deletion in all Apiaceae and Araliaceae; B, a 39-bp insertion in *Hydrocotyle*).

Didiscus, and *Hydrocotyle*), the branches are long. While branch lengths can vary substantially among closely related taxa possessing different life-history strategies (compare predominantly woody Araliaceae vs. herbaceous Hydrocotyloideae, for example), the variation exhibited within subfamily Apioideae is not so readily explained (Downie et al., 1998).

Rpl16 intron phylogenetic resolutions—Phylogenies estimated using MP, NJ, or ML methods reveal that, in the context of those species examined, Apioideae (groups 1–12, Figs. 4 and 6) are monophyletic and sister to a monophyletic subfamily Saniculoideae. In contrast, subfamily Hydrocotyloideae is not monophyletic, with three separate lineages occurring in all trees. The first of these, *Hydrocotyle* + *Didiscus,* is sister to a monophyletic Araliaceae; the second, *Eremocharis* + *Bolax,* is sister to Apioideae + Saniculoideae; and the third, *Centella,* is variably positioned, depending upon method of analysis and whether gap scoring was used. Resolution within Araliaceae is poor. *Schefflera, Hedera, Dendropanax, Oreopanax, Fatsia,* and *Kalopanax* unite in all trees, and in the NJ tree (Fig. 6) this clade is sister to *Cussonia, Pseudopanax,* and *Polyscias.* The family Pittosporaceae is divided dichotomously, with *Pittosporum, Bursaria,* and *Hymenosporum* (the latter two genera occurring within a paraphyletic *Pittosporum,* Fig. 6) comprising one clade and *Billardiera* and *Sollya* the other.

Within Apiaceae subfamily Apioideae, similar groupings of taxa occur in all trees. These clades, identified by numbered brackets, coincide with those groups recognized on the basis of parsimony analysis of *rpoC1* intron sequences (Downie et al., 1998). The most basal elements in the subfamily belong to a well-supported clade comprising the genera *Anginon, Glia,* and *Heteromorpha* (group 12, the ''*Heteromorpha*'' clade). Progressing upwards in the trees, groups 10 (*Aulacospermum, Eleutherospermum, Physospermum,* and *Pleurospermum*; the ''*Physospermum*'' clade) and 11 (*Bupleurum*; the ''*Bupleurum*'' clade) each arise separately (Fig. 6), or unite as sister taxa (Fig. 4). In the ML tree (not shown), groups 10 and 11 form two branches of a trichotomy, the third branch representing all other members of Apioideae except group 12. Pairwise sequence divergence values in group 10 range from 1.6 to 3.9%. Next is group 9, comprising *Komarovia* and *Parasilaus* (the ''*Komarovia*'' clade), followed by the phylogenetically isolated *Erigenia.* Next, the genera *Cicuta, Cryptotaenia, Oenanthe, Oxypolis, Perideridia,* and *Sium* comprise a well-supported clade (group 6, the ''*Oenanthe*'' clade of Plunkett, Soltis, and Soltis, 1996b, and Downie et al., 1998); the relationships within this clade, however, are not consistent. While *Oenanthe* and *Cicuta* unite in all analyses, as do *Cryptotaenia, Sium,* and *Oxypolis,* their relationships to each other and to *Perideridia* are not clear. Divergence values in this clade range from 0.9 to 3.9%. Group 7, comprising *Aciphylla, Anisotome, Smyrnium,* and *Lecokia* (the ''*Aciphylla*'' clade of Plunkett, Soltis, and Soltis, 1996b, and Downie et al., 1998), arises next in the NJ (Fig. 6) and ML (not shown) trees, but is sister (albeit with poor bootstrap support) to group 5B in the MP tree (Fig. 4). In group 7, sequence divergence values vary between 0.4 and 4.3%.

Group 5, the ''*Daucus*'' clade of Plunkett, Soltis, and



Soltis (1996b) and Downie et al. (1998), consists of representatives of Drude's tribes Dauceae, Laserpitieae, and Scandiceae (the latter including subtribes Scandicinae and Caucalidinae). Within this clade, two major subgroups (5A and 5B) are recognized (Figs. 4 and 6). Subgroup 5A comprises representatives of Drude's Dauceae, Laserpitieae, and Scandiceae subtribe Caucalidinae. Subgroup 5B reflects Drude's Scandiceae subtribe Scandicinae. Subgroup 5A can be further subdivided in the NJ (Fig. 6) and ML (not shown) trees. The first group consists of *Daucus* (two species), *Pseudorlaya, Agrocharis, Laserpitium, Orlaya* (two species), *Laser,* and *Polylophium.* The genus *Daucus* is not monophyletic, with the North American *D. pusillus* allied with African *Agrocharis,* and *D. carota* allied with *Pseudorlaya.* Drude's tribe Laserpitieae, exemplified by *Laserpitium, Laser,* and *Polylophium,* is also not monophyletic. The second group consists of *Turgenia, Lisaea, Caucalis, Szovitsia, Astrodaucus, Torilis, Chaetosciadium, Glochidotheca* (= *Turgeniopsis*), and *Yabea.* In subgroup 5A, pairwise sequence divergence values among congeners range between 0.5 and 4.8%. Subgroup 5B comprises the genera *Anthriscus, Myrrhis, Chaerophyllum* (two species), *Osmorhiza* (two species), and *Scandix* (two species), and parallels Heywood's (1971) tribe Scandiceae. The last three genera are each monophyletic, but their relationships differ depending upon method of tree construction used. Among congeners, divergence values in this subgroup range between 1.2 and 5.6%. Variously associated with subgroups 5A and 5B are *Ferula kokanica, Ligusticum scoticum,* and *Conioselinum chinense.* In all analyses, *F. kokanica, L. scoticum,* and Caucalideae comprise a well-supported clade. *Conioselinum chinense* is sister to this clade in the MP and NJ trees.

All remaining species belong to groups 1–4, the ''*Angelica,*'' ''*Crithmum,*'' ''*Apium,*'' and ''*Aegopodium*'' clades, respectively, of Downie et al. (1998). Resolution here is poor, with none of these four clades distinguishable. However, six smaller clades can be inferred with varying degrees of bootstrap support and include: (1) *Aethusa, Exoacantha,* and *Peucedanum caucasicum*; (2) *Heracleum* (two species), *Pastinaca* (two species), and *Malabaila*; (3) *Apium, Anethum, Foeniculum,* and *Ridolfia*; (4) *Cnidiocarpa, Cnidium, Ligusticum ferulaceum,* and *L. physospermifolium*; (5) *Heracleum rigens, Zosima,* and *Tordylium*; and (6) *Prangos, Smyrniopsis,* and *Opopanax.* With the exception of only a few branches, such as those leading to *Peucedanum caucasicum, Apium, Oedibasis,* and *Pimpinella,* the branch lengths within apioid groups 1–4 are relatively short. In this group, pairwise sequence divergence estimates reach a maximum value of 6% (between *Peucedanum caucasicum* and *Pimpinella*).

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Fig. 6. Neighbor-joining tree inferred from 147 unambiguously aligned cpDNA *rpl16* intron sequences from representatives of Apiaceae, Araliaceae, and Pittosporaceae using a transition/transversion rate ratio of 1.0. Branch lengths are proportional to distances estimated from the two-parameter method of Kimura; scale value (at bottom of figure) is given as 100× this value. Numbers at nodes indicate bootstrap estimates for 100 replicate analyses; values <50% are not indicated.

TABLE 4. Sequence characteristics of the cpDNA *rpoC1* and *rpl16* introns, separately and combined, for 86 species of Apiaceae, Araliaceae, and Pittosporaceae.

Sequence characteristic	*rpoC1* intron	*rpl16* intron	Combined
Nucleotide sites			
No. total aligned positions	938	1404	2342
No. aligned positions excluded (and %)	158 (16.8)	433 (30.8)	591 (25.2)
No. aligned positions constant (and %)	452 (57.9)	492 (50.7)	944 (53.9)
No. aligned positions parsimony informative (and %)	192 (24.6)	308 (31.7)	500 (28.6)
No. aligned positions autapomorphic (and %)	136 (17.4)	171 (17.6)	307 (17.5)
Length variation			
No. unambiguous alignment gaps	47	72	119
No. unambiguous gaps parsimony informative	17	28	45
Pairwise sequence divergence (range in %)			
Subfamily Apioideae only	0.1–9.8	0–11.0	0.2–10.5
All 86 species	0.1–12.3	0–17.8	0.2–15.2

For those 16 genera of subfamily Apioideae where more than one species was examined, 11 are not monophyletic. These genera include *Aciphylla, Arracacia, Conioselinum, Daucus, Ferula, Heracleum, Ligusticum, Pastinaca, Peucedanum, Pleurospermum,* and *Seseli.* Of the eight tribes of Apioideae recognized by Drude, we have sampled extensively from three (Apieae, Peucedaneae, and Smyrnieae). Not surprisingly, none of these tribes are monophyletic, with multiple independent derivations inferred in all cladograms. The only suprageneric taxon in Apioideae that is maintained as monophyletic is Drude's Scandiceae subtribe Scandicinae (= tribe Scandiceae sensu Heywood, 1971).

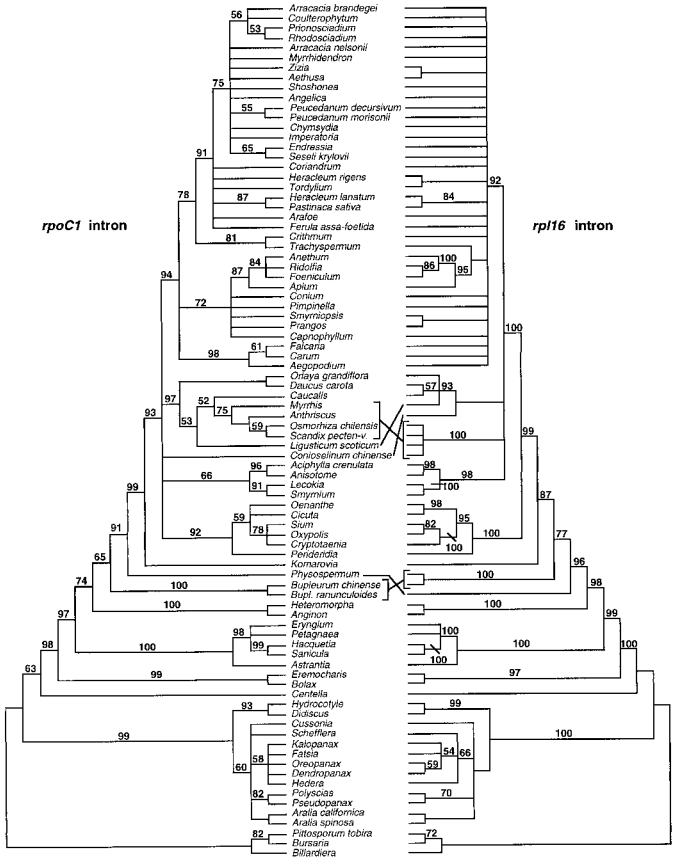

Fig. 7. A comparison of strict consensus trees derived from separate MP analyses of 86 cpDNA *rpoC1* intron (left) and *rpl16* intron (right) sequences from Apiaceae, Araliaceae, and Pittosporaceae. Complete taxon names are provided in Table 1; tree diagnostic information, such as overall length, and consistency and retention indices, is provided in text. Numbers at the nodes indicate bootstrap estimates for 100 replicate analyses; values <50% are not indicated.

Rpl16 and rpoC1 intron sequence characterization—For those 86 species where both *rpl16* and *rpoC1* intron data are available (Table 1), the data sets were analyzed separately and together using PAUP. Sequence characteristics of these separate and combined matrices are presented in Table 4. These comparisons show that sequence data from the *rpl16* intron are more variable than those of the *rpoC1* intron, as evidenced by the greater number of positions excluded due to alignment ambiguity (30.8 vs. 16.8%), the fewer invariant positions (50.7 vs. 57.9%), the greater number of positions informative for parsimony analysis (31.7 vs. 24.6%), and the greater number of unambiguous alignment gaps (72 vs. 47). Across all 86 species, pairwise sequence divergence was higher for *rpl16* than for *rpoC1* (17.8 vs. 12.3%, respectively). Within subfamily Apioideae, however, divergence values were approximately the same. In the combined matrix, pairwise sequence divergence estimates ranged between 0.2 and 15.2%.

Rpl16 and rpoC1 intron phylogenetic analysis—MP analysis of *rpoC1* intron data (with gap scoring) resulted in 5000 minimal length trees each of 683 steps before the search was terminated (CI's = 0.662 and 0.562, with and without uninformative characters; RI = 0.867). Analysis of the *rpl16* intron matrix also resulted in 5000 minimal length trees, each of 1189 steps (CI's = 0.629 and 0.546, RI = 0.842). Their strict consensus trees, with accompanying bootstrap values, are presented in Fig. 7. Major differences include: (1) the position of *Trachyspermum* (either sister to *Crithmum* in the *rpoC1* tree or sister to *Anethum, Ridolfia, Foeniculum,* and *Apium* in the *rpl16* tree); (2) the relative positions of *Bupleurum* and *Physospermum* (the former occupies a more basal position in the *rpoC1* tree, whereas the latter is basal in the *rpl16* tree); and (3) the relationships among members of the "*Daucus*" clade (group 5). The discrepancies ob-

served between these consensus trees are largely attributable to many poorly supported nodes. When these nodes (characterized by bootstrap values ≤65%) are treated as unresolved (i.e., they are collapsed to yield polytomies), the trees are consistent, with the only remaining area of discord being the relative positions of *Bupleurum* and *Physospermum.* In general, there is greater resolution at the base of the *rpl16* intron tree than that of the *rpoC1* tree (i.e., the ''*Oenanthe*'' clade is resolved in the former, its placement relative to other major clades is clearer, and greater resolution is achieved in Araliaceae). In contrast, among those apioids belonging to groups 1–4 (i.e., the clade extending from *Arracacia brandegei* to *Aegopodium*; Fig. 7), greater resolution is seen in the *rpoC1* intron tree.

The general agreement between the strict consensus trees derived from separate intron analyses suggested that a combined analysis would likely lead to the best estimate of phylogeny. Parsimony analysis of combined (*rpl16* + *rpoC1*) data (including the 45 scored informative gaps) resulted in 5000 minimal-length 1890-step trees (CI's = 0.635 and 0.545, with and without uninformative characters; RI = 0.848). Their strict consensus is presented in Fig. 8. When the analysis was repeated without the scored gaps, the 5000 minimal length trees obtained each had a length of 1831 steps, CI's of 0.631 and 0.536, and a RI of 0.837. With the exception of the collapse of two branches (illustrated by shading in Fig. 8), the topologies of these consensus trees were identical.

Rpl16 and rpoC1 intron phylogenetic resolutions—Results of the combined analysis of intron data (Fig. 8) include greater resolution and higher bootstrap support than in either of the separate analyses. Once more, subfamilies Apioideae (groups 1–12) and Saniculoideae are each monophyletic and sister taxa. Araliaceae are also monophyletic, but weakly supported with a 57% bootstrap value. The hydrocotyloids are polyphyletic, with some allied with Araliaceae (*Hydrocotyle* and *Didiscus*) and others with Apioideae and Saniculoideae (*Eremocharis* and *Bolax*). *Centella* is variably positioned, depending on whether or not gap scoring was used in the analysis. Within subfamily Apioideae, 12 major clades are discerned. These coincide with those delimited previously (Downie et al., 1998), and include: group 1—the ''*Angelica*'' clade; group 2—the ''*Crithmum*'' clade; group 3—the ''*Apium*'' clade; group 4—the ''*Aegopodium*'' clade; group 5—the ''*Daucus*'' clade, comprising subgroups 5A and 5B; group 6—the ''*Oenanthe*'' clade; group 7—the ''*Aciphylla*'' clade; groups 8, 9, 10, and 11, with *Conioselinum chinense*, *Komarovia anisosperma*, *Physospermum cornubiense*, and *Bupleurum* as their sole representatives, respectively; and group 12—the ''*Heteromorpha*'' clade. Resolution of relationships among many of these clades is poor.

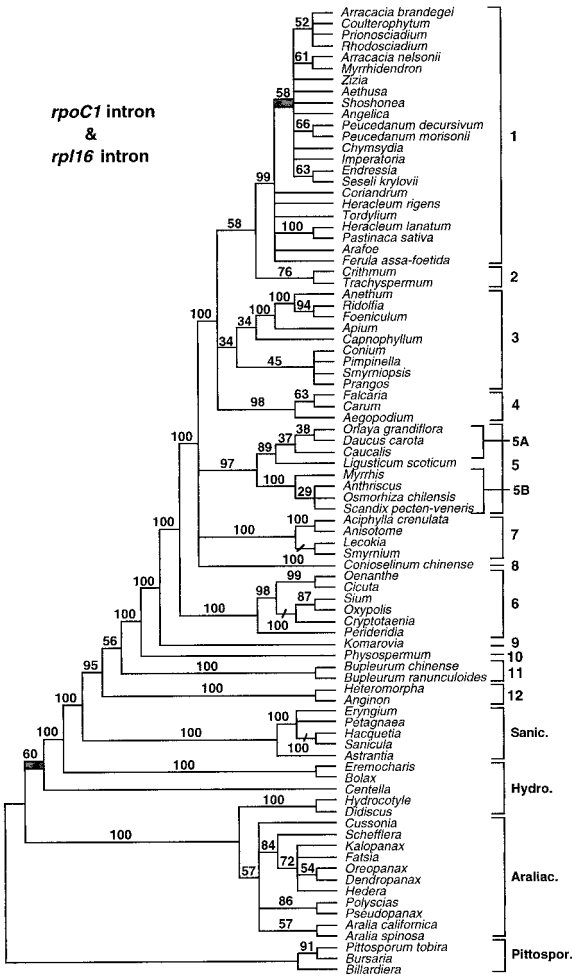

Fig. 8. Strict consensus of 5000 minimal length 1890-step trees derived from equally weighted MP analysis of 86 combined cpDNA *rpoC1* and *rpl16* intron sequences using 1751 unambiguously aligned nucleotide positions and 45 scored gaps (CI excluding uninformative characters = 0.545, RI = 0.848). The two broad horizontal, shaded lines indicate branches that collapse when the scored gaps are excluded and the analysis rerun (length of shortest trees = 1831 steps, CI excluding uninformative characters = 0.536, RI = 0.837). Numbers at nodes indicate the number of times a monophyletic group occurred in 100 bootstrap replicates.

DISCUSSION

Molecular evolution and phylogenetic utility of the rpl16 intron—The chloroplast gene *rpl16* is interrupted by an intron in many, but not all, land plants. Sequencing, PCR surveys, and blot-hybridization assays have revealed that this intron is absent from several Geraniaceae, Plumbaginaceae, and Goodeniaceae cpDNAs (Campagna and Downie, 1998). Among those species possessing an intron, it varies considerably in size, from 536 bp in *Marchantia polymorpha* (Ohyama et al., 1986) to 1411 bp in *Spirodela oligorhiza* (Posno, Van Vliet, and Groot, 1986). Evidently, this locus is able to withstand much variation in length so long as it does not fall below the minimum size (~500 bp) required for intron splicing (Doyle, Doyle, and Palmer, 1995). In most angiosperms, the *rpl16* intron is ~1 kb in size (Campagna and Downie, 1998), and its size in Apiaceae, Araliaceae, and Pittosporaceae cpDNAs (892–1021 bp) is consistent with these results.

Group II introns are excised from mRNA transcripts via a series of self-catalyzed reactions (Michel, Umesono, and Ozeki, 1989) and show a strong relationship between the functional importance of its structural features and

probability of evolutionary change (Clegg et al., 1994). Of these introns' six major structural domains, domains V and VI are required for processing of the transcript and, therefore, evolve most slowly (Learn et al., 1992). Portions of domain I, such as the region housing exon binding site 1, are also conserved evolutionarily. In contrast, domains II and III, apparently dispensable in self-splicing introns (Michel, Umesono, and Ozeki, 1989), have the highest rates of sequence change (Learn et al., 1992; Downie et al., 1998). With regard to the *rpl16* intron, domains III and IV were inferred to be the most variable and domains V and VI the least variable. Domain IV is characterized by numerous indels, and, for those positions that can be aligned unambiguously, divergence values approached 31%.

Across a comparable array of taxa, the *rpl16* intron is more variable than that of the *rpoC1* intron. This variation extends from the former possessing proportionally more informative nucleotide substitutions and length mutations to having a greater number of positions excluded from the analysis because of alignment ambiguity (Table 4). However, the amount of homoplasy in each data set is similar. Within Apioideae, sequence divergence estimates for both introns were approximately the same, whereas across basal Apiaceae, Araliaceae, and Pittosporaceae, values are much higher for *rpl16.* Despite the greater variability of the *rpl16* intron, these regions are useful at different levels. While phylogenetic analysis of *rpl16* intron sequences fail to resolve relationships among apioid groups 1–4, some resolution is achieved using *rpoC1*. In contrast, *rpl16* intron data generally provide greater resolution among basal apioids. Considered separately, *rpoC1* and *rpl16* intron sequences have little power to resolve relationships among closely related taxa. By combining these data, greater resolution and higher bootstrap support are achieved.

The *rpl16* intron has several other properties, making it attractive for comparative sequencing studies. A pair of universal primers, anchored in the exons, are sufficient to amplify the entire intron. The region is easily amplified once the PCR protocol has been optimized, and among closely related taxa the alignment of sequences is generally straightforward. Deep-level comparisons, however, result in frequent length mutations and regions of high variability. With the exception of conserved domains V and VI, variation is generally equally distributed over the length of the entire intron. Within Apiaceae, estimates of sequence divergence are comparable to those of other chloroplast intron (*rpoC1*) and gene (*matK*) sequences, but lower than that of the nuclear rDNA ITS region (Downie et al., 1998).

Apioideae phylogenetic resolutions and classification—Phylogenetic analysis of combined *rpoC1* and *rpl16* intron data supports 12 major clades within subfamily Apioideae (Fig. 8) and demonstrates patterns of relationship consistent with previously published cpDNA and ITS based phylogenies (Kondo et al., 1996; Plunkett, Soltis, and Soltis, 1996b; Downie et al., 1998; Valiejo-Roman et al., 1998; Katz-Downie et al., 1999; Plunkett and Downie, 1999). These groups are discussed below, with formal tribal recognition given to those seven clades that are consistently recognized and usually well supported in all analyses. To facilitate communication, several additional informal groups are described. However, not all present analyses support these groups as distinct, and their recognition is highly provisional.

Groups 1–4, the ''Angelica,'' ''Crithmum,'' ''Apium,'' *and* ''Aegopodium'' *clades*—Analyses of *rpoC1* intron sequences alone (Fig. 7) or in combination with *rpl16* intron data (Fig. 8) reveal the presence of four distinct yet closely allied groups of taxa, previously called the ''*Angelica*'' (group 1), ''*Crithmum*'' (group 2), ''*Apium*'' (group 3), and ''*Aegopodium*'' (group 4) clades (Downie et al., 1998). Separate analyses of *rpl16* intron sequences, however, fail to resolve these groups (Figs. 4 and 6). Upon consideration of all available molecular evidence, the ''*Angelica*'' and ''*Apium*'' clades cannot be circumscribed unambiguously, and in those studies where the latter occurs as monophyletic it is supported only weakly (Fig. 8; Plunkett, Soltis, and Soltis, 1996b; Downie et al., 1998). In the ITS studies, taxa attributed to the ''*Apium*'' clade comprise at least four lineages basal to the ''*Angelica*'' clade (Downie et al., 1998; Katz-Downie et al., 1999). The ''*Crithmum*'' clade is variously positioned, sister group to either the ''*Angelica*'' clade (Figs. 7–8) or, when ITS data are considered, to the ''*Aegopodium*'' clade (Downie et al., 1998; Katz-Downie et al., 1999). Plunkett, Soltis, and Soltis (1996b) included members of the ''*Aegopodium*'' and ''*Crithmum*'' clades in an expanded ''*Apium*'' clade; a subsequent study considered the ''*Aegopodium*'' clade as distinct, but included within it the genera *Crithmum* and *Trachyspermum,* and the saniculoid genus *Lagoecia* (Plunkett and Downie, 1999). These four groups of Apioideae have been collectively termed the ''apioid superclade'' (Plunkett and Downie, 1999), for while they unite as a strongly supported monophyletic group in all phylogenetic analyses to date, the relationships among them are equivocal.

Within the ''*Angelica*'' clade four major groups of taxa are distinguishable. Not all molecular studies, however, support these groups as distinct and their recognition here is highly provisional. The first includes a group of palaeopolyploid, meso-American genera (*Arracacia, Coulterophytum, Dahliaphyllum, Donnellsmithia, Enantiophylla, Prionosciadium,* and *Rhodosciadium,* and possibly *Coaxana, Mathiasella,* and *Myrrhidendron).* These genera are endemic to the highland regions of Mexico and neighboring Central America, one of the two centers of diversity of Apioideae in the western Northern Hemisphere (Mathias, 1965). We recognize this group as the ''*Arracacia*'' clade. The second group comprises the genera *Heracleum, Malabaila, Pastinaca, Tordylium,* and *Zosima.* While plastid DNA data do not advocate monophyly of this group, the ITS data do and with high bootstrap support (Downie et al., 1998; Katz-Downie et al., 1999). These plants are characterized generally by fruits with thickened wing margins and a rich diversity of furanocoumarins. We recognize this group as the ''*Heracleum*'' clade. A third group includes *Aletes, Cymopterus, Lomatium, Musineon, Neoparrya, Podistera, Shoshonea, Taenidia, Thaspium,* and *Zizia.* The majority of these species occur in the dry, sandy, or alkaline regions of western North America, and often at high elevations. These western members are primarily herbaceous perennials

and are frequently caespitose. Current data from the plastid genome cannot unequivocally support monophyly of this group. ITS data, however, provide weak support for this clade (S. Downie and R. Hartman, unpublished data). We have recognized this group as the ''Rocky Mountain'' umbellifers, realizing that many species extend beyond this range. A fourth group consists of *Dicyclophora, Echinophora,* and *Pycnocycla,* and their monophyly has been confirmed on the basis of ITS data (Downie, Katz-Downie, and Spalik, 2000). These taxa possess unique inflorescence and infructescence morphology and have been treated by Drude in tribe Echinophoreae. Before any of these four groups can be recognized formally, further investigations are necessary to confirm their monophyly; in the case of Echinophoreae, data from the plastid genome are required to confirm its position within the ''*Angelica*'' clade.

The ''*Crithmum*'' clade is often represented by only two taxa: *Crithmum maritimum* and *Trachyspermum ammi.* This group is recognized in studies of ITS and *rpoC1* intron sequences; separate analysis of *rpl16* intron data fail to resolve it. *Pyramidoptera* and *Oedibasis* can be added to this group, but this association is weakly supported (Katz-Downie et al., 1999). Additional evidence suggests an affinity with *Scaligeria moreana* Engstrand, *Elaeosticta allioides* (Regel & Schmalh.) Kljuykov, Pimenov & V. N. Tichom., and *Bunium elegans* (Fenzl) Freyn (Downie, Katz-Downie, and Spalik, 2000). We continue to recognize this group as the ''*Crithmum*'' clade. If further investigation supports this grouping, and if it is to be recognized at the tribal level, the earliest name Pyramidoptereae Boiss. (1871) should be applied. Within the ''*Apium*'' clade, one lineage is consistently recognized in all analyses. This group includes *Ridolfia segetum, Deverra triradiata* Hochst. ex Boiss., and *Naufraga balearica* Constance & Cannon, and the cultivated species of *Ammi, Anethum, Apium, Foeniculum,* and *Petroselinum.*

Of the ''*Angelica,*'' ''*Crithmum,*'' ''*Apium,*'' and ''*Aegopodium*'' clades, the only one that is unambiguously circumscribed in all (but the *rpl16* intron) analyses and contains more than a few members is the ''*Aegopodium*'' clade (*Aegopodium, Carum,* and *Falcaria,* in this study). ITS studies add *Aegokeras* (syn. *Olymposciadium*), *Fuernrohria, Rhabdosciadium,* and *Grammosciadium* (Downie et al., 1998; Katz-Downie et al., 1999; Downie, Katz-Downie, and Spalik, 2000), and the *matK* study of Plunkett, Soltis, and Soltis (1996b) adds *Cyclospermum.* In the *rpl16* intron trees (Figs. 4 and 6), *Fuernrohria* and *Carum* are strongly supported sister taxa, and the work of Vinogradova (1995) supports the close relationship between *Fuernrohria* and *Grammosciadium.* The *matK* study also suggests a possible affinity to the saniculoid genus *Lagoecia.* Here *Lagoecia* is allied to *Crithmum,* and both taxa are considered within the ''*Aegopodium*'' clade. If future studies indicate that *Lagoecia* is to be included within a formally described ''*Aegopodium*'' clade, its name will have priority (Lagoecieae Lange, 1880). If on the other hand it is shown that *Lagoecia* should be excluded from the group (and placed within a separate ''*Crithmum*'' clade), priority would extend to *Carum* (Careae Adanson ex Kuntze, 1904).

Group 5, the ''Daucus'' clade—Recent molecular systematic studies confirm three distinct and well-supported groups within the ''*Daucus*'' clade (Plunkett and Downie, 1999; Downie, Katz-Downie, and Spalik, 2000; Lee and Downie, 1999, and unpublished data). These three groups, coinciding herein with subgroup 5B and the two clades comprising subgroup 5A as a result of the NJ analysis (Fig. 6), have been designated as subtribes Scandicinae Tausch (1834), Daucinae Dumort. (1827), and Torilidinae Dumort. (1827), respectively, of tribe Scandiceae Spreng. (Downie, Katz-Downie, and Spalik, 2000). Included in subtribe Daucinae (the ''*Daucus*'' subclade) are the genera *Agrocharis, Ammodaucus, Cuminum, Daucus, Orlaya, Pseudorlaya,* and *Pachyctenium,* and representatives of Drude's tribe Laserpitieae (*Laser, Laserpitium, Melanoselinum, Monizia, Polylophium,* and *Thapsia*). Laserpiteae is not monophyletic, with four separate lineages arising within Daucinae. Subtribe Torilidinae (the ''*Torilis*'' subclade) includes *Astrodaucus, Caucalis, Chaetosciadium, Glochidotheca, Lisaea, Szovitsia, Torilis, Turgenia,* and *Yabea.* With the exception of Laserpitieae, Scandiceae subtribes Daucinae and Torilidinae (collectively forming group 5A) coincide closely with S. Jury and V. Heywood's (in Heywood, 1982b) circumscription of tribe Caucalideae Spreng. Subtribe Scandicinae (group 5B, the ''*Scandix*'' subclade), representing Drude's Scandiceae subtribe Scandicinae [= Heywood's (1971) tribe Scandiceae], comprises *Anthriscus, Athamanta, Balansaea, Chaerophyllum, Conopodium, Geocaryum, Kozlovia, Krasnovia, Myrrhis, Myrrhoides, Neoconopodium, Osmorhiza, Scandix, Sphallerocarpus,* and *Tinguarra.* Because the relationships among the three groups comprising the ''*Daucus*'' clade are equivocal given all available evidence, three distinct yet closely related groups have been recognized at the subtribal level (Downie, Katz-Downie, and Spalik, 2000).

Associated closely with tribe Scandiceae (the ''*Daucus*'' clade) in all but a few studies are *Ligusticum scoticum* and *Ferula kokanica.* Kondo et al. (1996), using *rbcL* sequences, placed *L. scoticum* next to *Daucus* and *Torilis.* The ITS trees, on the other hand, show *L. scoticum* (with weak bootstrap support) falling alongside *Lecokia* and *Smyrnium* in the ''*Aciphylla*'' clade (group 7); this clade is sister to group 5, the ''*Daucus*'' clade (Downie et al., 1998; Katz-Downie et al., 1999). The anomalous placement of *Ferula kokanica* near Scandiceae is just as intriguing. *Ferula* is a large, morphologically variable genus of some 170 species (Pimenov and Leonov, 1993) and, based on our results, its monophyly, although supported most recently by Shneyer, Borschtschenko, and Pimenov (1995), is brought into question. *Ferula* is thought to be allied with *Peucedanum* (Drude, 1898; Bernardi, 1979) and, in this study, *F. assa-foetida* falls within the ''*Angelica*'' clade, well away from *F. kokanica.* Valiejo-Roman et al. (1998), using ITS sequences, showed *F. tenuisecta* Korovin and *F. violacea* Korovin (each representing a different section within *Ferula*) arising within or close to the ''*Daucus*'' clade. Furthermore, ITS data for *F. kingdon-wardii* H. Wolff show that this species, together with *F. kokanica, F. tenuisecta,* and *F. violacea,* constitute a clade sister to tribe Scandiceae (S. Downie and M. Watson, unpublished data). Additional studies of *Ferula* and *Ligusticum* are

required to investigate their suspected nonmonophyly and unique phylogenetic positions.

Group 6, the ''Oenanthe'' clade—In this study, the genera *Cicuta, Cryptotaenia, Oenanthe, Oxypolis, Perideridia,* and *Sium* are treated in the ''*Oenanthe*'' clade; other studies include *Berula* (Downie et al., 1998), *Neogoezia* (Plunkett, Soltis, and Soltis, 1996b), and *Cynosciadium, Lilaeopsis,* and several species of *Apium* attributable to *Helosciadium* (S. Downie and M. Watson, unpublished data). In all analyses, this clade is very well supported, with bootstrap support values >90% and often 100%. These plants possess glabrous stems and leaves, clusters of tubers or tuberous roots, and commonly grow in moist to wet habitats. We recognize this group as tribe Oenantheae Dumort. (1827).

The taxonomic history of this group of genera is extraordinarily complex, confounded by the use of many longstanding names that are now considered synonyms, and although they share some characters in common, they are rather heterogeneous. Dumortier (1827) described tribe Oenantheae for the genera *Aethusa, Coriandrum,* and *Oenanthe,* defined by the presence of radiately ribbed fruits. This artificial assemblage was not followed by later authors, nor is it supported by molecular studies. We have used Dumortier's name, but stress that our circumscription of the tribe is radically different from his (and many others, such as Koso-Poljansky, 1916, and Cerceau-Larrival, 1962).

Group 7, the ''Aciphylla'' clade—The ''*Aciphylla*'' clade is recognized as comprising the genera *Aciphylla, Anisotome, Lecokia,* and *Smyrnium* (Plunkett, Soltis, and Soltis, 1996b; Downie et al., 1998; Katz-Downie et al., 1999; Plunkett and Downie, 1999). While phylogenetic analysis of *rpl16* intron sequences supports this clade strongly (with bootstrap values of 95 or 98%, Figs. 4 and 6), separate analyses of *rpoC1* intron (Fig. 7; Downie et al., 1998), ITS (Downie et al., 1998; Katz-Downie et al., 1999), or cpDNA restriction site data (Plunkett and Downie, 1999) do not. Furthermore, the ITS studies include *Ligusticum scoticum* alongside *Lecokia* and *Smyrnium,* but with weak bootstrap support (Downie et al., 1998; Katz-Downie et al., 1999). In all analyses, two distinct and well-supported subclades are apparent (each supported by bootstrap values of 91–100%): one comprising the Australasian endemics *Aciphylla* and *Anisotome,* and the other comprising largely Eurasian genera *Lecokia* and *Smyrnium.* Given their geographic isolation and morphological differences, we treat these subclades as separate tribes. Their union cannot readily be supported on the basis of morphological characters.

The predominantly alpine, Australasian genera *Aciphylla, Anisotome, Gingidia, Lignocarpa,* and *Scandia* constitute a closely related group (Dawson and Webb, 1982; Webb and Druce, 1984; Webb, 1986). Indeed, phylogenetic analysis of ITS sequences supports their monophyly (Mitchell, Webb, and Wagstaff, 1998; L. Radford and M. Watson, unpublished data). All are glabrous, long-lived perennials endemic to New Zealand and Australia. The genera are mostly sexually dimorphic, with *Aciphylla* and *Anisotome* dioecious and the other three usually gynodioecious (Webb, 1979). We treat this group as tribe Aciphylleae M. F. Watson & S. R. Downie.

Aciphylleae M. F. Watson & S. R. Downie, *Trib. Nov. Tribus generum dioicorum vel gynodioicorum, praesertim alpinorum, distributionis australasicae.* Type genus *Aciphylla* J. R. Forst. & G. Forst., Char. Gen. Pl.: 68 (1775). Other included genera: *Anisotome* Hook. f., *Gingidia* J. W. Dawson, *Lignocarpa* J. W. Dawson, *Scandia* J. W. Dawson.

The tribal name Smyrnieae (or subtribe Smyrniinae) has been used by virtually all authors of Apiaceae suprageneric classifications. First described by Sprengel (1820) and later modified by Koch (1824), de Candolle (1830), Bentham (1867), and Drude (1898), the size and composition of the tribe have varied considerably. Members of Drude's Smyrnieae were united on the basis of a deep groove on the commissural side of the seeds (campylospermy) and to a lesser extent on their nonelongate fruits. The artificiality of the tribe, however, has been demonstrated repeatedly (Shneyer et al., 1992; Downie and Katz-Downie, 1996; Plunkett, Soltis, and Soltis, 1996b; Downie et al., 1998; Valiejo-Roman et al., 1998). Of the 12 genera that Sprengel (1820) cited in his original publication of the tribe, only the type *Smyrnium* remains in our treatment. *Lecokia* was first included in Smyrnieae by de Candolle (1830), and it has remained there ever since. This narrow circumscription of Smyrnieae parallels, in part, the treatment of Hedge et al. (1987) where only *Smyrnium* and (the unrelated) *Smyrniopsis* are included in the tribe, and the immunochemical study of Shneyer et al. (1992) where *Smyrnium* occupies an isolated position away from all other Smyrnieae and other Apiaceae investigated (*Lecokia* was not considered). Shneyer et al. suggested that *Smyrnium* may be best treated within a monotypic tribe. Of the 29 genera in Drude's Smyrnieae, all but eight have been considered in molecular systematic investigations to date; in all of these analyses *Smyrnium* and *Lecokia* comprise a strongly supported clade. We treat this group as tribe Smyrnieae Spreng. (1820).

Group 8, the ''Conioselinum chinense'' clade—Phylogenetic analysis of ITS data indicates a close relationship among *C. chinense* (the only member of the group considered in this study), *C. scopulorum* (A. Gray) J. M. Coult. & Rose, *Ligusticum porteri* J. M. Coult. & Rose, and *L. canadense* (L.) Britton (Katz-Downie et al., 1999). *Conioselinum* and *Ligusticum* are each cosmopolitan and, based on increasing evidence, are clearly not monophyletic. Interestingly, the type species for each of these genera (*C. tataricum* and *L. scoticum*) do not occur in this clade. *Conioselinum* and *Ligusticum* are both in need of revision, and our molecular results indicate that these four species must be transferred to a new genus or genera. A tribal name cannot be assigned until a new genus is described, but it would be premature to alter the nomenclature at this stage. Pending a full revision of this group, we continue to refer to it as the ''*Conioselinum chinense*'' clade.

Group 9, the ''Komarovia'' clade—Included in the ''*Komarovia*'' clade are the genera *Komarovia* and *Parasilaus* (and *Hansenia* in Katz-Downie et al., 1999).

These monotypic genera comprise a group of distinctive umbellifers but, at present, there are insufficient data to confidently delimit a tribe. ITS studies reveal a weak association among these taxa, monotypic *Erigenia,* and members of group 10, the ''*Physospermum*'' clade. Plastid DNA data (e.g., Figs. 4 and 6) fail to support this relationship.

Group 10, the ''Physospermum'' clade—United in the ''*Physospermum*'' clade are the genera *Aulacospermum, Eleutherospermum, Physospermum,* and *Pleurospermum.* On the basis of ITS (Downie, Katz-Downie, and Spalik, 2000) and serological (Shneyer et al., 1992) studies, *Molopospermum* joins the group. ITS data (Katz-Downie et al., 1999) support this group strongly (with a 100% bootstrap value), whereas *rpl16* intron data (Figs. 4 and 6) support it only moderately (71–84% bootstrap values). We recognize this clade as tribe Pleurospermeae M. F. Watson & S. R. Downie.

Pleurospermeae M. F. Watson & S. R. Downie, *Trib. Nov. Tribus generum bracteis latis, saepe albomarginatis, sed haud omnino sic.* Type genus: *Pleurospermum* Hoffm. in Gen. Pl. Umbell., ed. 1: VIII (1814). Other included genera: *Aulacospermum* Ledeb., *Eleutherospermum* K. Koch, *Molopospermum* W. D. J. Koch, *Physospermum* Cusson ex Juss.

The majority of taxonomists have regarded *Physospermum* and *Pleurospermum* sensu lato (including *Aulacospermum, Eleutherospermum,* and several other genera) as related. Sprengel (1820) treated both in his tribe Smyrnieae, and most authors have followed this example with some adjustment of rank. *Molopospermum* has a complicated taxonomic history; Bentham (1867) was the first to treat it alongside *Physospermum,* and Cerceau-Larrival (1962) placed the monotypic (and invalidly published) Molopospermeae next to a reduced Smyrnieae (*Physospermum, Pleurospermum,* and *Smyrnium*). Krähenbühl and Küpfer (1992), using cytological evidence, agreed with the close connection between *Molopospermum* and *Physospermum.* These primarily Eurasian genera have no previous assignment to a separate group, and at present it has not been possible to delimit them on traditional characters. Because many members have broad, often white-margined bracts, we have used this morphological character in recognizing the tribe.

Group 11, the ''Bupleurum'' clade—Included here, as sole representative, is the large and widespread genus *Bupleurum.* All species examined to date form a strongly supported clade at, or near, the base of Apioideae. These plants are unusual morphologically within the subfamily, with their grass-like leaves, unique pollen and seedling characters, and showy involucel bracts (Cerceau-Larrival, 1962). We treat these plants as the monotypic tribe Bupleureae Spreng. (1820). When Sprengel (1820) described this tribe, he included *Hermas* L., *Odontites* Spreng., and *Tenoria* Spreng. alongside *Bupleurum. Hermas* is now accepted as belonging to subfamily Hydrocotyloideae, whereas the other two have long since been considered synonyms of *Bupleurum* (Pimenov and Leonov, 1993). In Sprengel's view, the conspicuous bracts and simple leaves clearly set these plants apart from all other umbellifers.

Group 12, the ''Heteromorpha'' clade—Previous studies have included *Heteromorpha* and *Anginon* in this clade and to these we now add *Glia.* Based on similar vegetative characters (Hilliard and Burtt, 1986; Winter and van Wyk, 1996) and phylogenetic analysis of chloroplast *rps16* intron sequences (Downie and Katz-Downie, 1999), *Polemannia* is also allied. These genera are all predominantly woody shrubs or small trees endemic to subsaharan Africa. The close relationship between *Anginon* and *Glia* is corroborated by the shared presence of heavily cutinized outer cell walls of the fruit epidermis, a feature not seen in other examined southern African apioids (van Wyk, Allison, and Tilney, 1997). Molecular data increasingly suggest that this clade is sister to all other apioid taxa (for an exception see Valiejo-Roman et al., 1998), consistent with the hypothesis that the subfamily may have originated in southern Africa where some of these plants, and other woody members of the family, can be found today (Plunkett, Soltis, and Soltis, 1996b; Downie and Katz-Downie, 1999). We recognize this clade as tribe Heteromorpheae M. F. Watson & S. R. Downie.

Heteromorpheae M. F. Watson & S. R. Downie, *Trib. Nov. Genera austroafricana lignosa, in xylemate cum laminis perforatis duplicibus.* Type genus: *Heteromorpha* Cham. & Schltdl. in Linnaea 1: 385 (1826). Other included genera: *Anginon* Raf., *Glia* Sond., *Polemannia* Eckl. & Zeyh.

The tribal name Heteromorpheae was previously used by Cerceau-Larrival (1962), erected on the basis of pollen and cotyledon characters. Not only is it entirely different in composition from ours, it was invalidly published. To avoid confusion, we have chosen against validating her name. At present, it is difficult to accurately delimit this tribe on solely morphological and/or anatomical characters. Nevertheless, their predominantly woody habit, generally southern African distribution, and wood anatomy like many Araliaceae (Rodríguez, 1971) set these plants apart from all other Apioideae.

Monophyly of apioid genera—In this study, 16 genera of Apioideae were represented by more than one species and, of these, five (*Bupleurum, Chaerophyllum, Orlaya, Osmorhiza,* and *Scandix*) are retained as monophyletic. The remaining genera (*Aciphylla, Arracacia, Conioselinum, Daucus, Ferula, Heracleum, Ligusticum, Pastinaca, Peucedanum, Pleurospermum,* and *Seseli*), representing some of the most species-rich genera within the subfamily, are not monophyletic. The genera *Ferula, Ligusticum,* and *Conioselinum* are polyphyletic, with *F. kokanica, L. scoticum,* and *C. chinense* allied with members of group 5A and not with their respective congeners in groups 1–4. Generic boundaries in Apiaceae are often vague, arbitrary, and fluctuating at the hands of successive investigators, and it is not unrealistic to presume that many other large genera within the subfamily are probably not monophyletic either. Therefore, the placement of these genera into each of the above designated groups must be treated as provisional until the monophyly of each has been confirmed through additional studies.

Basal phylogenetic resolutions and classification—Consistent with previous molecular systematic investi-

gations (Plunkett, Soltis, and Soltis, 1996a, b, 1997; Downie et al., 1998; Valiejo-Roman et al., 1998; Plunkett and Downie, 1999; Downie and Katz-Downie, 1999), Apiaceae subfamily Saniculoideae is monophyletic and sister to subfamily Apioideae. Drude (1898) recognized nine genera (~300 species) in subfamily Saniculoideae of which we have included five (*Astrantia, Eryngium, Hacquetia, Petagnaea,* and *Sanicula*). The genus *Lagoecia,* included in Saniculoideae by Drude but later removed by Koso-Poljansky (1916) and Cerceau-Larrival (1962), has affinity with the ''*Aegopodium*'' clade based on phylogenetic analysis of *matK* sequences (Plunkett, Soltis, and Soltis, 1996b). While saniculoid genera *Actinolema* and *Alepidea* (and putatively allied *Oligocladus* and *Arctopus*; Drude, 1898; Pimenov and Leonov, 1993) have yet to be considered in any molecular systematic study to date, support for the monophyly of Saniculoideae and its sister-group status to Apioideae is strong.

Apiaceae subfamily Hydrocotyloideae, on the other hand, is clearly not monophyletic. Of the 24–42 genera (~470 species) recognized in the subfamily (Drude, 1898; Pimenov and Leonov, 1993), 11 have been included in molecular systematic investigations: *Azorella, Bolax, Bowlesia, Centella, Didiscus, Eremocharis, Hydrocotyle, Klotzschia, Micropleura, Spananthe,* and *Xanthosia* (this study; Plunkett, Soltis, and Soltis, 1996a, 1997; Downie et al., 1998; Valiejo-Roman et al., 1998; Plunkett and Downie, 1999; Downie and Katz-Downie, 1999). Depending upon the study and included genera, up to four separate lineages can be inferred, with some of these (including the type genus *Hydrocotyle*) more closely related to Araliaceae than to other Apiaceae. In fact, *Centella, Hydrocotyle, Micropleura,* and *Spananthe* have been referred to as the ''araliaceous hydrocotyloids'' (Plunkett, Soltis, and Soltis, 1997), and to these we add *Didiscus* and *Xanthosia.* Of the two tribes and five subtribes traditionally recognized within Hydrocotyloideae (Drude, 1898), and of those where two or more genera have been sampled, none are retained as monophyletic in light of molecular systematic investigations.

While the hydrocotyloids have yet to be sampled extensively, it is interesting to note that *Azorella, Bolax,* and *Eremocharis* unite as a clade sister to Apioideae + Saniculoideae. Collectively considered ''core Apiaceae'' by Plunkett, Soltis, and Soltis (1996a, 1997), this group is strongly supported in all phylogenetic analyses to date, with bootstrap values between 95 and 100%. *Klotzschia* and *Bowlesia,* each forming separate branches but yet to be treated simultaneously in any analysis, appear to be closely allied to *Azorella* and relatives. Herein we provisionally recognize the genera *Azorella, Bolax,* and *Eremocharis* as comprising the ''*Azorella*'' clade; future studies with greater sampling should shed light on its precise composition and relationship to *Klotzschia* and *Bowlesia.* The rank assigned to the ''*Azorella*'' clade should be that of some yet to be described subfamily. We are aware that the subfamilial name Azorelloideae, erected by Cerceau-Larrival (1962) and including the genera *Azorella, Hydrocotyle, Bowlesia, Trachymene,* and *Xanthosia,* is invalid as it lacked a Latin description and, furthermore, would be illegitimate as it includes the type of an earlier validly published name at that rank (Hydrocotyloideae).

Phylogenetic analyses of *rpl16* intron data, like other studies incorporating intron and gene sequences from the chloroplast genome, fail to resolve relationships within Araliaceae (Plunkett, Soltis, and Soltis, 1996a, 1997; Downie et al., 1998). In all trees inferred, many branches are poorly supported, with low bootstrap and decay values. Moreover, the family itself (i.e., ''core Araliaceae'' sensu Plunkett, Soltis, and Soltis, 1997) is not well supported, but with the inclusion of *Hydrocotyle* and *Didiscus* bootstrap support for the clade is (or approaches) 100% (Figs. 4, 7, and 8). With the exception of *Cussonia* and *Pseudopanax,* all of our Araliaceae sampled fall within the ''*Hedera* group'' of Plunkett, Soltis, and Soltis (1996a), although this clade too is not very well supported. Additional molecular data are required to clarify relationships within Araliaceae. Regarding Pittosporaceae, the dichotomy inferred in the family based on *rpl16* intron sequences is consistent with relationships proposed using *rbcL* (Plunkett, Soltis, and Soltis, 1996a); the apparent paraphyly of *Pittosporum* (Fig. 6), however, needs to be examined further.

Conclusions—Of the eight tribes recognized within Apiaceae subfamily Apioideae by Drude (1898) and of those whose sampling has been comprehensive, none are retained as monophyletic in light of molecular evidence. Drude's three largest tribes—Apieae, Peucedaneae, and Smyrnieae—are grossly unnatural, with multiple, independent derivations inferred in all cladograms. Drude's subfamily Hydrocotyloideae is polyphyletic, with some hydrocotyloids (such as *Hydrocotyle*) allied with Araliaceae rather than Apiaceae. The ''*Azorella*'' clade, comprising the hydrocotyloid genera *Azorella, Bolax,* and *Eremocharis,* is sister to a clade comprising Apioideae and a monophyletic Saniculoideae. Thus, while three major clades can be recognized in a ''core Apiaceae'' (Plunkett, Soltis, and Soltis, 1996a, 1997), which is consistent with Drude's recognition of three subfamilies within Apiaceae, subfamily Hydrocotyloideae cannot be maintained. Instead, a subfamily encompassing *Azorella* and relatives may be erected, pending further study.

Our study of cpDNA *rpl16* intron sequences, one of only a few studies incorporating such data, adds to the existing and growing database of information on umbellifer phylogeny. While these data, like other DNA regions examined to date, are singularly insufficient to resolve relationships among closely related taxa, they do reveal insight into ''higher level'' relationships when analyzed simultaneously with data from the plastid *rpoC1* intron. Based on the results of phylogenetic analyses of combined *rpl16* and *rpoC1* intron data, and in conjunction with other molecular systematic studies of the group such as those incorporating nuclear rDNA ITS and chloroplast *matK* sequences and restriction sites, we recognize seven tribes in subfamily Apioideae: Heteromorpheae M. F. Watson & S. R. Downie *Trib. Nov.*, Bupleureae Spreng. (1820), Oenantheae Dumort. (1827), Pleurospermeae M. F. Watson & S. R. Downie *Trib. Nov.*, Smyrnieae Spreng. (1820), Aciphylleae M. F. Watson & S. R. Downie *Trib. Nov.*, and Scandiceae Spreng. (1820). Scandiceae comprises subtribes Daucinae Dumort. (1827), Scandicinae Tausch (1834), and Torilidinae Dumort. (1827). Additional clades occur within the subfamily, but further sam-

pling and study are necessary before they can be treated formally. These results, and those presented in Plunkett and Downie (1999), provide the necessary framework and explicit phylogenetic hypotheses from which future revisionary and other systematic studies can proceed.

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