TRIBES AND CLADES WITHIN *APIACEAE* SUBFAMILY *APIOIDEAE*: THE CONTRIBUTION OF MOLECULAR DATA

S. R. Downie*†, G. M. Plunkett‡, M. F. Watson§, K. Spalik**, D. S. Katz-Downie*, C. M. Valiejo-Roman††, E. I. Terentieva‡‡, A. V. Troitsky††, B.-Y. Lee*, J. Lahham§§ & A. El-Oqlah§§

Phylogenetic analyses of chloroplast gene (*rbc*L, *mat*K), intron (*rpl*16, *rps*16, *rpo*C1) and nuclear ribosomal DNA internal transcribed spacer (ITS) sequences and chloroplast DNA restriction sites, with supplementary data from variation in size of the chloroplast genome inverted repeat, have been used to elucidate major clades within *Apiaceae* (*Umbelliferae*) subfamily *Apioideae* Drude. This paper summarizes the results of previously published molecular cladistic analyses and presents a provisional classification of the subfamily based on taxonomic congruence among the data sets. Ten tribes (*Aciphylleae* M. F. Watson & S. R. Downie, *Bupleureae* Spreng., *Careae* Baill., *Echinophoreae* Benth., *Heteromorpheae* M. F. Watson & S. R. Downie, *Oenantheae* Dumort., *Pleurospermeae* M. F. Watson & S. R. Downie, *Pyramidoptereae* Boiss., *Scandiceae* Spreng. and *Smyrnieae* Spreng.) are erected or confirmed as monophyletic, with *Scandiceae* comprising subtribes *Daucinae* Dumort., *Scandicinae* Tausch and *Torilidinae* Dumort. Seven additional clades are also recognized but have yet to be treated formally, and at least 23 genera examined to date are of dubious tribal or clade placement. The utility of these different molecular markers for phylogenetic inference in *Apioideae* is compared based on maximum parsimony analyses of subsets of previously published molecular data sets. Of the six loci sequenced, the ITS region is seen to be evolving most rapidly and *rbc*L is the most conservative. Intermediate in rate of evolution are *mat*K and the three chloroplast introns; with *rpl*16 and *rps*16 evolving slightly faster than *mat*K or *rpo*C1. The analysis of restriction sites, however, provided 2–4 times more parsimony informative characters than any single DNA locus sequenced, with estimates of divergence just slightly lower than that of the ITS region. The trees obtained from separate analyses of these reduced data sets are consistent with regard to the major clades inferred and the relationships among them. Similar phylogenies are obtained by combining data or combining trees, representing the supermatrix and supertree approaches to

* Department of Plant Biology, University of Illinois, Urbana, IL 61801, USA. E-mail: {sdownie, dkatzdow}@life.uiuc.edu
† Corresponding author. Fax: 217 244 7246.
‡ Department of Biology, Virginia Commonwealth University, PO Box 842012, Richmond, VA 23284, USA. E-mail: gmplunke@vcu.edu
§ Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 9NL. E-mail: m.watson@rbge.org.uk
** Department of Plant Systematics and Geography, Warsaw University, Aleje Ujazdowskie 4, 00-478 Warsaw, Poland. E-mail: spalik@poczta.wp.pl
†† A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russia.
‡‡ Botanical Garden, Moscow State University, Moscow 119899, Russia.
§§ Department of Biological Sciences, Yarmouk University, Irbid, Jordan.

phylogenetic analysis, respectively. The inferred relationship among the tribes and informally recognized major clades within *Apioideae* is presented.

Keywords. Chloroplast intron, internal transcribed spacer, molecular systematics, nuclear rDNA, phylogeny, suprageneric classification, *Umbelliferae*.

Introduction

> 'Although *Umbelliferae* were the first family of flowering plants to achieve general recognition, after nearly three and a quarter centuries of successive and multinational effort, considerable disagreement still exists as to the proper delimitation of the family and even more uncertainty prevails as to its natural subdivisions and the criteria on which they should be erected. Clearly continued acquisition of new information and re-examination, refinement, and re-evaluation of accumulated evidence are urgently to be welcomed.'
>
> L. Constance (1971)

The higher-level relationships within *Apiaceae* (*Umbelliferae*) have been traditionally difficult to resolve, particularly within its largest subfamily *Apioideae* Drude. Comparison of the accounts of, for example, Koch (1824), de Candolle (1830), Bentham (1867), Boissier (1872), Drude (1898), Calestani (1905) and Koso-Poljansky (1916), which were erected largely on the basis of fruit morphology and anatomy, shows widely diverging opinions on the definition and composition of its tribes and subtribes. While such focus on fruit structure has been rejected by many (Heywood, 1971b, 1978a; Theobald, 1971; Davis, 1972; Cronquist, 1982; Hedge *et al.*, 1987; Shneyer *et al.*, 1992, 1995), the highly criticized century-old system of Drude (1898), or some modification thereof (Pimenov & Leonov, 1993), remains the most commonly used treatment. As such, many of the authors who published papers as a result of two international symposia on the family (Heywood, 1971a; Cauwet-Marc & Carbonnier, 1982) presented their findings in the framework of Drude's system.

Drude (1898) divided *Apiaceae* into three subfamilies (*Apioideae*, *Hydrocotyloideae* Link and *Saniculoideae* Burnett), recognizing 8 tribes and 10 subtribes within *Apioideae*. Molecular phylogenetic studies, however, while confirming the monophyly of subfamily *Apioideae*, have shown that many of its tribes and subtribes are not monophyletic (Downie & Katz-Downie, 1996; Kondo *et al.*, 1996; Plunkett *et al.*, 1996a,b, 1997; Downie *et al.*, 1996, 1998, 2000a,b,c; Valiejo-Roman *et al.*, 1998; Katz-Downie *et al.*, 1999; Plunkett & Downie, 1999). The apioid umbellifers display a remarkable array of morphological and anatomical modifications of their fruits, some of which are likely adaptations for various modes of seed dispersal (Jury, 1986). Not surprisingly, these characters are prone to convergence and their almost exclusive use to delimit suprageneric taxa has confounded attempts to identify monophyletic groups. Our goal over the past six years has been to resolve the higher-level relationships within subfamily *Apioideae*. This is necessary in order to provide the framework for lower-level revisions, as well as to interpret patterns in the evolution of the many phytochemical, anatomical, cytological, morphological and

palynological characters available for the group (such as those summarized in Heywood, 1971a, and Cauwet-Marc & Carbonnier, 1982). Although these characters have been surveyed widely, trends in their evolution and their reliability in demarcating taxonomic groups have rarely been considered outside of the framework of Drude's system (for exceptions see Plunkett *et al.*, 1996b, and Katz-Downie *et al.*, 1999). Eventually, the synthesis of these newly acquired molecular data with a re-evaluation of existing evidence will culminate in the production of a modern classification for the subfamily.

This paper has three major objectives. First, we summarize the results of a variety of previously published macromolecular analyses as they apply to clarifying the evolutionary history of subfamily *Apioideae*. To date, major clades within the subfamily have been elucidated on the basis of phylogenetic analyses of chloroplast gene (*rbc*L, *mat*K), intron (*rpl*16, *rps*16, *rpo*C1) and nuclear ribosomal DNA ITS sequences and chloroplast DNA (cpDNA) restriction sites, with supplementary data obtained from variation in size and position of the chloroplast genome inverted repeat. Based on taxonomic congruence among these previously published data sets, a provisional classification of the subfamily is proposed. Second, by focusing on subsets of these molecular data, we compare the utility of different molecular markers for phylogenetic inference within the subfamily. Third, based on separate and combined analyses of these subset data (or the trees derived from them) and the results of our earlier publications using non-reduced data sets, the phylogenetic relationships among the major clades comprising *Apioideae* are inferred. While our studies are still very much in progress, the results obtained to date provide the necessary framework and explicit phylogenetic hypotheses from which future revisions and evolutionary studies can proceed. Authorities for names at rank genus and below are given in Tables 2–5; authorities for suprageneric taxa and others not in these tables are given when first used.

Summary of Published Macromolecular Analyses

The earliest comparative studies of nucleic acids in *Apiaceae* were the DNA–DNA hybridization experiments of Valiejo-Roman *et al.* (1979, 1982) and Antonov *et al.* (1988; Table 1). The groups obtained on the basis of these hybridization data, while supporting the distinctiveness of subfamily *Apioideae* and its separation from subfamily *Saniculoideae*, stood in stark contrast to any existing system of classification available for *Apioideae*. DNA distances among the resolved apioid groups, however, were sufficiently low and the sampling too sparse to propose an alternative tribal treatment. Relationships within the family were also inferred using immunological comparisons of seed storage proteins. The earliest of these studies (Pickering & Fairbrothers, 1970) supported Drude's division of the family into three subfamilies; it also indicated that *Apioideae* are more similar serologically to *Saniculoideae* than to *Hydrocotyloideae*. Further analyses by Pickering & Fairbrothers (1971) confirmed five serological groupings for nine genera within *Apioideae*, corresponding to Drude's

TABLE 1. A summary of systematic studies of *Apiaceae* subfamily *Apioideae* incorporating macromolecular data

Reference	DNA–DNA hybridization	Systematic serology	RFLPs or restriction site mapping	ITS	*rpl*16 intron	*rps*16 intron	*rpo*C1 intron	*mat*K	*rbc*L	Chloroplast IR structure
Antonov *et al.* (1988)	●									
Debonte *et al.* (1984)			●							
Downie & Katz-Downie (1996)				●						
Downie & Katz-Downie (1999)						●				
Downie *et al.* (1996)							●			
Downie *et al.* (1998)				●			●			
Downie *et al.* (2000a)				●						
Downie *et al.* (2000b)					●		●			
Downie *et al.* (2000c)				●		●				
Katz-Downie *et al.* (1999)				●						
Kondo *et al.* (1996)									●	
Lee & Downie (1999)				●						
Lee & Downie (2000)			●			●				
Lee & Rasmussen (1998)				●						
Mitchell *et al.* (1998)				●						
Pickering & Fairbrothers (1970)		●								
Pickering & Fairbrothers (1971)		●								
Pimenov *et al.* (1999)		●		●						
Plunkett & Downie (1999)			●							
Plunkett & Downie (2000)										●
Plunkett *et al.* (1996a)									●	
Plunkett *et al.* (1996b)								●		
Plunkett *et al.* (1997)								●	●	
Radford *et al.* (2001)				●						
Shneyer *et al.* (1991)		●								
Shneyer *et al.* (1992)		●								

TABLE 1. (Continued.)

Reference	DNA–DNA hybridization	Systematic serology	RFLPs or restriction site mapping	ITS	*rpl*16 intron	*rps*16 intron	*rpo*C1 intron	*mat*K	*rbc*L	Chloroplast IR structure
Shneyer *et al.* (1995)		●								
Soltis & Kuzoff (1993)				●						
Soltis & Novak (1997)			●							
Valiejo-Roman *et al.* (1979)	●									
Valiejo-Roman *et al.* (1982)	●									
Valiejo-Roman *et al.* (1998)				●						
Vivek & Simon (1998)			●							
Vivek & Simon (1999)			●							
Watanabe *et al.* (1998)			●							

tribes *Dauceae* W. D. J. Koch, *Scandiceae* Spreng., *Coriandreae* W. D. J. Koch, *Apieae* Drude and *Peucedaneae* Dumort. Representatives of the last three tribes had the greatest protein similarity, whereas those from *Dauceae* and *Scandiceae* were each serologically distinct. These serological data also supported Drude's division of *Peucedaneae* into three subtribes: *Angelicinae* Tausch, *Peucedaninae* Tausch and *Tordyliinae* Drude. In contrast, the analyses of Shneyer *et al.* (1991, 1992, 1995), also based on immunochemical reactions of seed storage proteins but with greater sampling in tribes *Peucedaneae* and *Smyrnieae* Spreng., clearly indicated that many of the tribes and subtribes recognized by Drude do not form monophyletic groups. Subtribes *Angelicinae* and *Peucedaninae* were determined to be serologically not very well separated, whereas subtribe *Tordyliinae* was maintained as distinct.

The past several years has seen a flurry of molecular systematic activity on subfamily *Apioideae* (Table 1). Many of these studies considered DNA sequences from both coding and non-coding loci of nuclear and chloroplast genomes, with fewer studies incorporating cpDNA RFLP or structural rearrangement data. Considering only GenBank accessions of nucleotide sequence data, and excluding *Apium graveolens*, *Daucus carota* and *Petroselinum crispum* (because much of the data available for these economically important apioid species are the result of non-systematic studies), there has been a fivefold increase in the number of submitted sequences in over just the past two and a half years (from c.300 in March 1998 to c.1500 in September 2000), primarily the result of these comparative phylogenetic analyses. Considering all molecular phylogenetic studies cited in Table 1, a total of 195 apioid genera and 450 species have been examined to date, representing almost half of the 404 genera within the subfamily recognized by Pimenov & Leonov (1993).

Subfamilial relationships

The results of earlier molecular systematic investigations have confirmed the monophyly of subfamily *Apioideae* and revealed its sister group to be subfamily *Saniculoideae* (Downie & Katz-Downie, 1996, 1999; Plunkett *et al.*, 1996a, 1997; Downie *et al.*, 1998, 2000b). *Saniculoideae* are also monophyletic upon the removal of *Lagoecia* (Plunkett *et al.*, 1996b; Downie *et al.*, 2000c). However, the circumscription of the largely herbaceous subfamily *Saniculoideae* is confounded by its affinity to the woody apioid genera *Steganotaenia* Hochst. and *Polemanniopsis* B. L. Burtt (Fig. 1; Downie & Katz-Downie, 1999). The incorporation of *Steganotaenia* and *Polemanniopsis* into an expanded *Saniculoideae* (and their treatment as a separate tribe or subtribe) or their recognition as a new subfamily adjacent to *Saniculoideae* is subject to further investigation. *Hydrocotyloideae* are polyphyletic, with some genera allied with *Apioideae* plus *Saniculoideae* and other genera with *Araliaceae* (Plunkett *et al.*, 1996a, 1997; Downie *et al.*, 1998, 2000a). Upon the exclusion of the latter group (i.e. the 'araliaceous hydrocotyloids' *sensu* Plunkett *et al.*, 1997; Fig. 1),

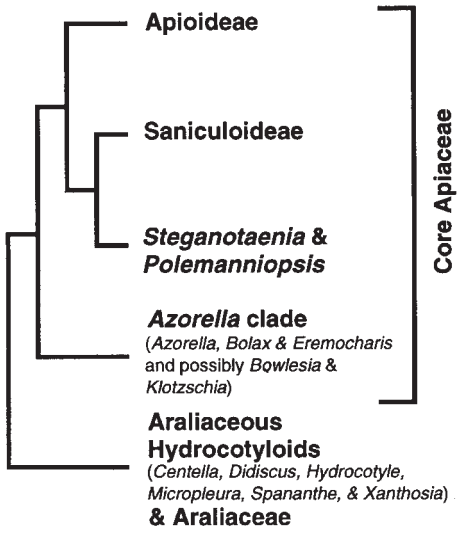


FIG. 1. Summary of relationships within core *Apiaceae* as revealed by phylogenetic analyses of molecular data (Plunkett *et al.*, 1996a, 1997; Downie & Katz-Downie, 1999; Downie *et al.*, 1998, 2000b). Members of the *Azorella* clade and the araliaceous hydrocotyloids (*Didiscus* DC. ex Hook. = *Trachymene* Rudge) are treated by Pimenov & Leonov (1993) in *Apiaceae* subfamily *Hydrocotyloideae*; the araliaceous hydrocotyloids do not comprise a monophyletic group (Plunkett *et al.*, 1997; Downie & Katz-Downie, 1999; Downie *et al.*, 2000b). *Steganotaenia* and *Polemanniopsis* (*Apioideae* and *incertae sedis*, respectively; Pimenov & Leonov, 1993), form a clade sister to *Saniculoideae* and, pending further study, may belong within this subfamily (Downie & Katz-Downie, 1999).

Apiaceae comprise a clade. Sister to *Apioideae* plus *Saniculoideae*, and collectively forming the 'core *Apiaceae* (Plunkett *et al.*, 1997)', are three genera previously attributable to *Hydrocotyloideae* (*Azorella* Lam., *Bolax* Comm. ex Juss. and *Eremocharis* Phil.). We have named this group the *Azorella* clade (Fig. 1; Downie *et al.*, 2000b), fully aware that the subfamilial name *Azorelloideae*, erected by Cerceau-Larrival (1962), is invalid. *Bowlesia* Ruiz. & Pav. and *Klotzschia* Cham. are closely related to the *Azorella* clade (Plunkett *et al.*, 1996a; Downie *et al.*, 1998; Downie & Katz-Downie, 1999); all five genera, however, have yet to be analysed simultaneously.

Tribes of Apioideae

Within *Apiaceae* subfamily *Apioideae*, ten tribes and three subtribes have been erected or supported as monophyletic as a result of previously published phylogenetic analyses of molecular data (Table 2). These include tribes *Aciphylleae* M. F. Watson & S. R. Downie, *Bupleureae* Spreng., *Careae* Baill., *Echinophoreae* Benth., *Heteromorpheae* M. F. Watson & S. R. Downie, *Oenantheae* Dumort., *Pleurospermeae* M. F. Watson & S. R. Downie, *Pyramidoptereae* Boiss., *Scandiceae*

TABLE 2. Generic composition of tribes and subtribes within *Apiaceae* subfamily *Apioideae* supported or erected on the basis of phylogenetic analyses of molecular data. Previously designated names for these clades (according to Downie *et al.*, 1998, 2000a,b,c, and Katz-Downie *et al.*, 1999) are provided in footnotes. Genera assigned to more than one suprageneric category may not necessarily be monophyletic within that group (see original papers for discussion)

Aciphylleae M. F. Watson & S. R. Downie[a]
- *Aciphylla* J. R. Forst. & G. Forst.
- *Anisotome* Hook. f.
- *Gingidia* J. W. Dawson
- *Lignocarpa* J. W. Dawson
- *Scandia* J. W. Dawson

Bupleureae Spreng.[b]
- *Bupleurum* L.

Careae Baill.[c]
- *Aegokeras* Raf.
- *Aegopodium* L.
- *Carum* L.
- *Cyclospermum* Lag.
- *Falcaria* Fabr.
- *Fuernrohria* K. Koch
- *Grammosciadium* DC.
- *Rhabdosciadium* Boiss.

Echinophoreae Benth.[d]
- *Dicyclophora* Boiss.
- *Echinophora* L.
- *Pycnocycla* Lindl.

Heteromorpheae M. F. Watson & S. R. Downie[e]
- *Anginon* Raf.
- *Dracosciadium* Hilliard & B. L. Burtt
- *Glia* Sond.
- *Heteromorpha* Cham. & Schltdl.
- *Polemannia* Eckl. & Zeyh.

Oenantheae Dumort.[f]
- *Berula* W. D. J. Koch
- *Cicuta* L.
- *Cryptotaenia* DC.
- *Cynosciadium* DC.
- *Helosciadium* W. D. J. Koch
- *Lilaeopsis* Greene
- *Limnosciadium* Mathias & Constance
- *Neogoezia* Hemsl.
- *Oenanthe* L.
- *Oxypolis* Raf.
- *Perideridia* Rchb.
- *Ptilimnium* Raf.
- *Sium* L.

Pleurospermeae M. F. Watson & S. R. Downie[g]
- *Aulacospermum* Ledeb.
- *Eleutherospermum* K. Koch
- *Molopospermum* W. D. J. Koch
- *Physospermum* Cuss.
- *Pleurospermum* Hoffm.

Pyramidoptereae Boiss.[h]
- *Bunium* L.
- *Crithmum* L.
- *Elaeosticta* Fenzl
- *Lagoecia* L.
- *Oedibasis* Koso-Pol.
- *Pyramidoptera* Boiss.
- *Scaligeria* DC.
- *Trachyspermum* Link (in part)

Scandiceae Spreng.[i]

Daucinae Dumort.
- *Agrocharis* Hochst.
- *Ammodaucus* Coss. & Durieu
- *Cuminum* L.
- *Daucus* L.
- *Laser* Borkh. ex P. Gaertn., B. Mey. & Schreb.
- *Laserpitium* L.
- *Melanoselinum* Hoffm.
- *Monizia* Lowe
- *Orlaya* Hoffm.
- *Pachyctenium* Maire & Pampan.
- *Polylophium* Boiss.
- *Pseudorlaya* (Murb.) Murb.
- *Thapsia* L.

Scandicinae Tausch
- *Anthriscus* Pers.
- *Athamanta* L. (in part)
- *Balansaea* Boiss. & Reut.
- *Chaerophyllum* L.
- *Conopodium* W. D. J. Koch
- *Geocaryum* Coss.
- *Kozlovia* Lipsky
- *Krasnovia* Schischk.
- *Myrrhis* Mill.
- *Myrrhoides* Fabr.

TABLE 2. (Continued.)

Neoconopodium (Koso-Pol.) Pimenov & Kljuykov	*Tinguarra* Parl.	*Chaetosciadium* Boiss.	*Turgenia* Hoffm.
Osmorhiza Raf.	*Todaroa* Parl.	*Glochidotheca* Fenzl	*Yabea* Koso-Pol.
Scandix L.	***Torilidinae*** Dumort.	*Lisaea* Boiss.	***Smyrnieae*** Spreng.[a]
Sphallerocarpus DC.	*Astrodaucus* Drude	*Szovitsia* Fisch. & C. A. Mey.	*Lecokia* DC.
	Caucalis L.	*Torilis* Adans.	*Smyrnium* L.

[a] *Aciphylla* clade (in part).
[b] *Bupleurum* clade.
[c] *Aegopodium* clade.
[d] *Niraratharnnos* Balf. f. and *Rughidia* ined., find affinities with this group.
[e] *Heteromorpha* clade.
[f] *Oenanthe* clade.
[g] *Physospermum* clade. *Molopospermum* is tentatively included here based on Shneyer *et al.* (1992).
[h] *Crithmum* clade.
[i] *Daucus* clade. *Artedia* L. falls in either *Daucinae* or *Torilidinae*, depending upon the study (Lee & Downie, 2000). *Ferula* (in part; Downie *et al.*, 2000c) and *Glaucosciadium* B.L. Burtt & P.H. Davis (K. Spalik, unpubl. data) may each constitute additional separate lineages within the tribe.

TABLE 3. Major clades within *Apiaceae* subfamily *Apioideae* that have yet to be treated formally. Genera assigned to a particular clade may not necessarily be monophyletic within that clade (see original papers for discussion)

***Angelica* Clade**[a]
Aethusa L.
Aletes J. M. Coult. & Rose
Angelica L.
Carlesia Dunn.
Chamaele Miq.
Chymsydia Albov
Cnidiocarpa Pimenov
Cnidium Cuss. (in part)
Coelopleurum Ledeb.
Cortia DC.
Cymopterus Raf.
Dystaenia Kitag.
Endressia J. Gay
Exoacantha Labill.
Glehnia F. Schmidt ex Miq.
Grafia Rchb.
Harbouria J. M. Coult. & Rose
Imperatoria L.
Karatavia Pimenov & Lavrova
Libanotis Haller ex Zinn
Ligusticum L. (in part)
Lomatium Raf.
Meum Hill
Musineon Raf.
Neoparrya Mathias
Notopterygium H. Boissieu
Oreonana Jeps.
Oreoxis Raf.
Orogenia S. Watson
Paraligusticum V. N. Tikhom.
Peucedanum L.
Phlojodicarpus Turcz. ex Ledeb.
Podistera S. Watson
Polytaenia DC.
Pseudocymopterus J. M. Coult. & Rose
Pteryxia (Nutt. ex Torr. & A. Gray) J. M. Coult. & Rose
Selinum L.
Seseli L.
Shoshonea Evert & Constance
Spermolepis Raf.
Sphenosciadium A. Gray
Taenidia (Torr. & A. Gray) Drude
Tauschia Schltdl.
Thaspium Nutt.
Tommasinia Bertol.
Xanthogalum Lallem.
Zizia W. D. J. Koch

***Apium* Clade**[b]
Ammi L.
Anethum L.
Apium L.
Deverra DC.
Foeniculum Hill
Naufraga Constance & Cannon
Petroselinum Hill
Ridolfia Moris

***Arracacia* Clade**[c]
Arracacia Bancroft
Coaxana J. M. Coult. & Rose
Coulterophytum B. L. Rob.
Dahliaphyllum Constance & Breedlove
Donnellsmithia J. M. Coult. & Rose
Enantiophylla J. M. Coult. & Rose
Mathiasella Constance & C. Hitch.
Myrrhidendron J. M. Coult. & Rose
Prionosciadium S. Watson
Rhodosciadium S. Watson

***Conioselinum* Clade**[d]
Conioselinum Hoffm. (in part)
Ligusticum L. (in part)

***Heracleum* Clade**[e]
Heracleum L.
Malabaila Hoffm.
Pastinaca L.
Tetrataenium (DC.) Manden.
Tordylium L.
Zosima Hoffm.

***Komarovia* Clade**[f]
Hansenia Turcz.
Komarovia Korovin
Parasilaus Leute
Physospermopsis H. Wolff

TABLE 3. (Continued.)

***Pimpinella* Clade**[g]	*Bubon* L.	*Registaniella* Rech. f.
Aphanopleura Boiss.	*Pimpinella* L.	
Arafoe Pimenov & Lavrova	*Psammogeton* Edgew.	

[a] The *Angelica* clade *sensu stricto* (Downie *et al.*, 2000c). If this clade, as currently circumscribed, were to be treated at the tribal level, the earliest name *Selineae* Spreng. would apply. However, we have yet to examine material of *Selinum carvifolia* (L.) L., the type of the genus, and until we confirm its placement we refrain from formally recognizing this clade.

[b] The *Apium* clade *sensu stricto* (Downie *et al.*, 2000c). This clade, if recognized at the tribal level, will be called *Apieae* [ined.].

[c] We name this clade after the largest and earliest described genus, *Arracacia*.

[d] Comprising two species each of *Conioselinum* and *Ligusticum* but excluding the types of each of these genera (*C. tataricum* Hoffm. and *L. scoticum* L.). However, the type of *Kreidion* Raf. (*K. chinensis* (L.) Raf.), a synonym of *Conioselinum*, is included in this clade (Downie *et al.*, 2000c).

[e] With the addition of *Pastinaca*, this group coincides with Drude's *Peucedaneae* subtribe *Tordyliinae* (or tribe *Tordylieae* W. D. J. Koch). While ITS studies support strongly the monophyly of the group, some studies of plastid DNA remove *Tordylium* from the clade.

[f] Members of this clade are allied closely to tribe *Pleurospermeae* and the genus *Erigenia* (Valiejo-Roman *et al.*, 1998; Katz-Downie *et al.*, 1999; Pimenov *et al.*, 1999). As *Pleurospermum* is taxonomically complex and surrounded by many small genera of dubious affinity, we refrain from naming this group until these genera are examined. If *Erigenia* is indeed to be included in the *Komarovia* clade (and if the latter retains its present complement of genera), the tribal name *Erigenieae* Rydb. would apply.

[g] If further investigation supports this group, and if it is to be recognized at the tribal level, the earliest name *Pimpinelleae* Spreng. should be applied, as the type of *Pimpinella* (*P. saxifraga* L.) allies with this group. *Registaniella* is included here based on its morphological similarity to *Psammogeton*.

and *Smyrnieae*, with *Scandiceae* comprising subtribes *Daucinae* Dumort., *Scandicinae* Tausch and *Torilidinae* Dumort. (Downie *et al.*, 2000a,b,c). Tribes *Aciphylleae*, *Heteromorpheae* and *Pleurospermeae* are recently described, whereas tribes *Bupleureae*, *Careae*, *Oenantheae*, *Pyramidoptereae* and *Smyrnieae*, while maintaining long-standing names, are radically different in generic composition from those groups originally recognized. Tribe *Echinophoreae* is maintained as circumscribed by Drude, although three of its six genera have yet to be included in any molecular study, and the remaining genera show affinities with the Socotran Island endemics *Nirarathamnos* and *Rughidia* (Downie *et al.*, 2000a,c). Whether these two genera are included in an expanded *Echinophoreae* or are treated as a separate taxon remains to be seen. *Scandiceae* subtribes *Daucinae* and *Torilidinae* approximate tribe *Caucalideae* Spreng., the subject of intensive multidisciplinary systematic investigations during the 1960s and 1970s (summarized in Heywood, 1978a). However, representatives of Drude's tribe *Laserpitieae* Benth. occur within subtribe *Daucinae*, and the genera *Aphanopleura*, *Kozlovia* and *Psammogeton*, treated previously in tribe *Caucalideae* (Heywood, 1978b), find affinities elsewhere (Katz-Downie *et al.*, 1999; Downie *et al.*, 2000a).

Other major clades within Apioideae

In addition to the aforementioned monophyletic tribes, seven other major clades have been recognized within subfamily *Apioideae* on the basis of phylogenetic analysis of molecular data (Downie *et al.*, 1998, 2000a,b,c). These include the *Angelica*, *Apium*, *Arracacia*, *Conioselinum*, *Heracleum*, *Komarovia* and *Pimpinella* clades, and the generic composition of each is provided in Table 3. The *Angelica* and *Apium* clades were first established on the basis of cpDNA evidence (Plunkett *et al.*, 1996b; Downie *et al.*, 1998), but increased sampling has since obscured their boundaries. Moreover, the results of the ITS-based investigations have never been fully compatible with those of the cpDNA studies, with members of the *Apium* clade forming as many as five separate lineages basal to the *Angelica* clade (Downie *et al.*, 1998; Katz-Downie *et al.*, 1999). Therefore, to reconcile these groups with recent data, a more restricted view of these clades has been put forth (Downie *et al.*, 2000c). As such, congruence is achieved in the composition of each of these clades and the bootstrap values supporting them are much higher. The phylogenetic position of the *Arracacia* clade is not clear, for in several studies it arises from within the *Angelica* clade (Plunkett *et al.*, 1996b; Downie *et al.*, 1998) and in some trees its monophyly is not very well supported (Downie *et al.*, 1998). The *Arracacia* clade may eventually be subsumed within the *Angelica* clade. The *Heracleum* clade coincides with Drude's *Peucedaneae* subtribe *Tordyliinae* (= *Tordylieae* W. D. J. Koch) and its separation from other peucedanoid taxa was confirmed previously using seed protein immunochemistry (Shneyer *et al.*, 1995). A strong serological similarity is also apparent between *Komarovia* and *Parasilaus*, two members of the *Komarovia* clade (Pimenov *et al.*, 1999).

Apioid tribes *Careae*, *Echinophoreae* and *Pyramidoptereae*, along with clades *Angelica*, *Apium*, *Arracacia*, *Heracleum* and *Pimpinella* (plus a number of genera of uncertain clade placement, see below) comprise a large assemblage of taxa termed the 'apioid superclade' (Plunkett & Downie, 1999). This superclade, confirmed as monophyletic in all analyses of molecular data to date and often supported by bootstrap values $>90\%$, comprises the previously designated *Aegopodium*, *Angelica*, *Apium* and *Crithmum* clades (Plunkett *et al.*, 1996b; Downie *et al.*, 1998). The *Aegopodium* and *Crithmum* clades are now recognized as tribes *Careae* and *Pyramidoptereae*, respectively (Downie *et al.*, 2000c). Previously, both *Careae* and *Pyramidoptereae* were considered part of an expanded *Apium* clade (Plunkett *et al.*, 1996b) or *Aegopodium* clade (Plunkett & Downie, 1999; Downie *et al.*, 2000a). While the apioid superclade is strongly supported as monophyletic, resolution of relationships among its constituent taxa is poor. However, a specific class of chloroplast genome structural rearrangement has been uncovered within the superclade and serves to strengthen support for several lineages delimited on the basis of DNA sequence data. Here, at least one expansion and seven different contractions of the junction between the large single-copy and inverted repeat (IR) regions of the chloroplast genome have been detected, each ranging in size from 1 to 16kb pairs (Plunkett & Downie, 2000). The frequency and large size of these IR junction shifts are unprecedented among angiosperms. Continued investigations on the phylogenetic utility of this molecular character in subfamily *Apioideae* are in order.

Genera of uncertain phylogenetic placement

As a consequence of the narrower circumscriptions of the *Aegopodium*, *Angelica* and *Apium* clades (Downie *et al.*, 2000c), some genera previously placed within each are left without assignment to a specific group (yet they are still retained within the apioid superclade). These include *Bifora*, *Conium*, *Coriandrum*, *Ferula*, *Levisticum* and *Prangos* (and other genera identified by asterisks in Table 4). Their inclusion would result primarily in the blurring of the boundaries of the *Angelica* and *Apium* clades. The genera *Cnidium*, *Conioselinum*, *Ferula*, *Ligusticum* and *Trachyspermum*, however, are each not monophyletic and have some members assigned to specific tribes (Table 2) or informally recognized clades (Table 3). The *Angelica* clade is a large group that contains many genera of exceptional taxonomic difficulty (such as *Angelica*, *Lomatium*, *Peucedanum*, *Selinum* and *Seseli*). Given that many large apioid genera are not monophyletic (Pimenov & Leonov, 1993; Valiejo-Roman *et al.*, 1998; Downie *et al.*, 2000c), the placement of any one genus into the *Angelica* clade (or, for that matter, any other clade heretofore recognized) must be treated as provisional until such genus is studied in detail. Considering those genera basal to the apioid superclade, *Annesorhiza* falls adjacent to *Heteromorpheae*, and *Diplolophium* and *Erigenia* fall adjacent to *Komarovia* (Downie & Katz-Downie, 1999).

TABLE 4. Genera of *Apiaceae* subfamily *Apioideae* included in molecular systematic studies but of uncertain tribal or clade placement. Asterisks (*) denote genera belonging to the apioid superclade. Genera designated as 'in part' have some members assigned to tribes (Table 2) or major clades (Table 3)

Annesorhiza Cham. & Schltdl.	*Krubera* Hoffm.*
Azilia Hedge & Lamond*	*Levisticum* Hill*
Bifora Hoffm.*	*Ligusticum* L. (in part)
Cenolophium W. D. J. Koch*	*Lithosciadium* Turcz.*
Cnidium Cuss. (in part)*	*Meum* Hill*
Conioselinum Hoffm. (in part)*	*Opopanax* W. D. J. Koch*
Conium L.*	*Prangos* Lindl.*
Coriandrum L.*	*Smyrniopsis* Boiss.*
Diplolophium Turcz.	*Sphaenolobium* Pimenov*
Erigenia Nutt.	*Thysselinum* Hill*
Ferula L. (in part)*	*Trachyspermum* Link (in part)*
Ferulago W. D. J. Koch*	

Phylogenetic Utility of Molecular Markers

We compare the utility of seven molecular data sets used for phylogenetic inference in subfamily *Apioideae*: restriction sites, ITS, *rpl*16 intron, *rps*16 intron, *rpo*C1 intron, *mat*K, and *rbc*L. While comparisons between different molecular data sets were considered in earlier papers (e.g. Plunkett *et al.*, 1997; Downie *et al.*, 1998, 2000b; Plunkett & Downie, 1999; Lee & Downie, 2000), this study is the first to offer a comparison among all seven markers. The 24–33 species considered in each data set (Table 5) represent many of the major lineages described above and, as far as possible, were included in multiple studies. However, it wasn't possible to make the data sets equitable in species composition. The *rbc*L matrix was constructed from four separate studies (Olmstead *et al.*, 1992; Chase *et al.*, 1993; Kondo *et al.*, 1996; Plunkett *et al.*, 1996a), whereas the *mat*K study (Plunkett *et al.*, 1996b) was carried out independently from those of the introns, ITS, and restriction sites. Of the 33 species included in each of the three intron studies, 30 species were shared. Here, *Ferula*, *Anthriscus*, and *Aralia* L. were each represented by one of two species; each species pair, however, is monophyletic as revealed by more comprehensive analyses (Downie *et al.*, 2000a,b,c). Ten species (18 genera) were shared among the three intron and *rbc*L matrices, and 10 species (17 genera) were shared among the intron and *mat*K data sets. Sixteen species (19 genera) were common to both *rbc*L and *mat*K analyses. The ITS study (24 species) excluded members of *Araliaceae* and *Apiaceae* subfamilies *Hydrocotyloideae* and *Saniculoideae*, as well as the basal apioids *Anginon*, *Bupleurum* and *Heteromorpha*, owing to the difficulty in aligning many of their sequences with those of other *Apioideae* (Downie & Katz-Downie, 1996; Downie *et al.*, 2000c). The restriction site study (27 species) omitted *Heracleum lanatum*, *Komarovia anisosperma*, *Physospermum cornubiense*, *Tetrataenium rigens*, *Eremocharis fruticosa* Phil. and *Petagnaea saniculifolia* Guss. because data were not available (Plunkett & Downie,

TABLE 5. Species included in separate or combined analyses of DNA data or supertree construction. Treatments of *Apiaceae* subfamily *Apioideae* based on Pimenov & Leonov (1993) and the results of previously published molecular phylogenetic studies (Tables 2, 3). The symbol '???' denotes species of uncertain tribe or clade assignment (Table 4). Voucher and source information, and GenBank accession numbers for DNA sequences, are provided in footnote references

Species	Tribe (Pimenov & Leonov, 1993)	Tribe, clade (molecular phylogenetic studies)
APIACEAE SUBFAMILY *APIOIDEAE*		
Aciphylla aurea W. R. B. Oliv.[f]	*Apieae*	*Aciphylleae*
Aegopodium podagraria L.[f,g]	*Apieae*	*Careae*
Aethusa cynapium L.[a,b,c,d,e]	*Apieae*	*Angelica* clade
Anethum graveolens L.[a,b,c,d,e,f]	*Peucedaneae*	*Apium* clade
Angelica archangelica L.[a,b,c,d,e]	*Angeliceae*	*Angelica* clade
Angelica lucida L.[f,g]	*Angeliceae*	*Angelica* clade
Anginon rugosum (Thunb.) Raf.[a,c,d,e,f,g]	*Apieae*	*Heteromorpheae*
Anthriscus caucalis M. Bieb.[b,d]	*Scandiceae*	*Scandiceae*, *Scandicinae*
Anthriscus cerefolium (L.) Hoffm.[a,c,e]	*Scandiceae*	*Scandiceae*, *Scandicinae*
Anthriscus sylvestris (L.) Hoffm.[f]	*Scandiceae*	*Scandiceae*, *Scandicinae*
Apium graveolens L.[a,b,c,d,e,f,g]	*Apieae*	*Apium* clade
Arracacia aegopodioides (Kunth) J. M. Coult. & Rose[f,g]	*Smyrnieae*	*Arracacia* clade
Bupleurum chinense DC.[a]	*Apieae*	*Bupleureae*
Bupleurum falcatum L.[f,g]	*Apieae*	*Bupleureae*
Bupleurum fruticosum L.[g]	*Apieae*	*Bupleureae*
Bupleurum ranunculoides L.[c,d,e]	*Apieae*	*Bupleureae*
Carum carvi L.[f]	*Apieae*	*Careae*
Caucalis platycarpos L.[a,b,c,d,e]	*Caucalideae*	*Scandiceae*, *Torilidinae*
Cicuta virosa L.[a,b,c,d,e,g]	*Apieae*	*Oenantheae*
Conium maculatum L.[a,b,c,d,e,g]	*Smyrnieae*	???
Coriandrum sativum L.[f,g]	*Coriandreae*	???
Crithmum maritimum L.[a,b,c,d,e,f]	*Apieae*	*Pyramidoptereae*
Cryptotaenia japonica Hassk.[a,b,c,d,e,g]	*Apieae*	*Oenantheae*
Daucus carota L.[a,b,c,d,e,f,g]	*Caucalideae*	*Scandiceae*, *Daucinae*
Donnellsmithia cordata (J. M. Coult. & Rose) Mathias & Constance[g]	*Smyrnieae*	*Arracacia* clade
Enantiophylla heydeana J. M. Coult. & Rose[f]	*Angeliceae*	*Arracacia* clade
Endressia castellana Coincy[f,g]	*Apieae*	*Angelica* clade
Ferula assa-foetida L.[c,e]	*Peucedaneae*	???
Ferula communis L.[a]	*Peucedaneae*	???
Ferula tingitana L.[b,d]	*Peucedaneae*	???
Foeniculum vulgare Mill.[a,b,c,d,e,f,g]	*Apieae*	*Apium* clade
Heracleum dulce Fisch.[g]	*Tordylieae*	*Heracleum* clade

TABLE 5. (Continued.)

Species	Tribe (Pimenov & Leonov, 1993)	Tribe, clade (molecular phylogenetic studies)
Heracleum lanatum Michx.[b,c,d,e,f]	*Tordylieae*	*Heracleum* clade
Heteromorpha arborescens (Spreng.) Cham. & Schltdl.[a,c,d,e]	*Apieae*	*Heteromorpheae*
Heteromorpha trifoliata (Wendl.) Eckl. & Zeyh.[f,g]	*Apieae*	*Heteromorpheae*
Komarovia anisosperma Korovin[b,c,d,e]	*incertae sedis*	*Komarovia* clade
Laserpitium latifolium L.[f]	*Laserpitieae*	*Scandiceae*, *Daucinae*
Levisticum officinale W. D. J. Koch [f]	*Apieae*	???
Ligusticum scoticum L.[a,b,c,d,e,g]	*Apieae*	???
Oenanthe fistulosa L.[a]	*Apieae*	*Oenantheae*
Oenanthe javanica DC.[g]	*Apieae*	*Oenantheae*
Oenanthe pimpinelloides L.[b,c,d,e]	*Apieae*	*Oenantheae*
Oenanthe sarmentosa J. Presl[f]	*Apieae*	*Oenantheae*
Osmorhiza aristata (Thunb. ex Murr.) Makino & Y. Yabe[g]	*Scandiceae*	*Scandiceae*, *Scandicinae*
Pastinaca sativa L.[a,b,c,d,e,f]	*Tordylieae*	*Heracleum* clade
Physospermum cornubiense (L.) DC.[b,c,d,e]	*Smyrnieae*	*Pleurospermeae*
Pimpinella saxifraga L.[f,g]	*Apieae*	*Pimpinella* clade
Pleurospermum camtschaticum Hoffm.[g]	*Smyrnieae*	*Pleurospermeae*
Rhodosciadium nudicaule (J. M. Coult. & Rose) Drude[f]	*Peucedaneae*	*Arracacia* clade
Scandix pecten-veneris L.[a,b,c,d,e,f]	*Scandiceae*	*Scandiceae*, *Scandicinae*
Sium latifolium L.[a,b,c,d,e]	*Apieae*	*Oenantheae*
Sium serra (Franch. & Savat.) Kitag.[g]	*Apieae*	*Oenantheae*
Sium suave Walt.[f]	*Apieae*	*Oenantheae*
Smyrnium olusatrum L.[a,b,c,d,e]	*Smyrnieae*	*Smyrnieae*
Spermolepis echinata (Nutt.) Heller[f]	*Apieae*	*Angelica* clade
Tetrataenium rigens (DC.) Manden.[b,c,d,e]	*Tordylieae*	*Heracleum* clade
Tordylium aegyptiacum (L.) Lam.[a,b,c,d,e]	*Tordylieae*	*Heracleum* clade
Torilis japonica (Houtt.) DC.[g]	*Caucalideae*	*Scandiceae*, *Torilidinae*
Apiaceae subfamily		
Hydrocotyloideae		
Bolax gummifera (Lam.) Spreng.[a,c,d,e]		
Centella asiatica (L.) Urb.[a,c,d,e]		
Centella erecta (L. f.) Fern.[g]		

TABLE 5. (Continued.)

Species	Tribe (Pimenov & Leonov, 1993)	Tribe, clade (molecular phylogenetic studies)
Eremocharis fruticosa Phil.[c,d,e,f,g]		
Hydrocotyle bowlesioides Mathias & Constance[g]		
Micropleura renifolia Lag.[g]		
Spananthe paniculata Jacq.[g]		
APIACEAE SUBFAMILY *SANICULOIDEAE*		
Eryngium bourgattii Gouan.[f,g]		
Eryngium cervantesii F. Delaroche[a]		
Lagoecia cuminoides L.[f]	*Lagoecieae*	*Pyramidoptereae*
Petagnaea saniculifolia Guss. [c,d,e,f]		
Sanicula canadensis L.[c,d,e]		
Sanicula gregari Bickn.[f,g]		
ARALIACEAE		
Aralia chinensis L.[d]		
Aralia spinosa L.[a,c,e,g]		
Kalopanax pictus Nakai[f,g]		

[a] cpDNA restriction site study (Plunkett & Downie, 1999).
[b] ITS study (Downie & Katz-Downie, 1996; Downie *et al.*, 1998, 2000c).
[c] *rpl*16 intron study (Downie *et al.*, 2000b).
[d] *rps*16 intron study (Downie *et al.*, 2000c).
[e] *rpo*C1 intron study (Downie *et al.*, 1998, 2000b).
[f] *mat*K study (Plunkett *et al.*, 1996b).
[g] *rbc*L study (Olmstead *et al.*, 1992; Chase *et al.*, 1993; Kondo *et al.*, 1996; Plunkett *et al.*, 1996a).

1999); *Sanicula canadensis* L. was also excluded but replaced with the closely allied *Eryngium cervantesii* F. Delaroche. The three intron, ITS, and restriction site data matrices shared 17 species and 20 genera (the groups of species representing *Oenanthe* and *Bupleurum* are also each monophyletic; Downie & Katz-Downie, 1999, and unpublished data), but upon consideration of *rbc*L and *mat*K, the number of shared taxa among the seven data sets decreased substantially. Analyses of combined data therefore excluded data sets *rbc*L and *mat*K. To reduce the number of terminals in the phylogenetic analysis of combined data or combined trees (discussed below), monophyletic groups of species belonging to the same genus (i.e. *Ferula*, *Anthriscus*, *Aralia*, *Oenanthe* and *Bupleurum*) were scored as a single taxon.

Experimental

Details of methodology and information on source of material, voucher deposition, and GenBank accession numbers are provided elsewhere (Plunkett *et al.*, 1996a,b; Downie & Katz-Downie, 1996, 1999; Downie *et al.*, 1996, 1998, 2000b,c; Kondo *et al.*, 1996; Plunkett &

Downie, 1999). For the ITS study, complete ITS1 and ITS2 regions were used. Each of the three intron studies included data from the entire intron region, whereas only three quarters of the *mat*K gene was sequenced (Plunkett *et al.*, 1996b). Due to missing data at both 5′ and 3′ ends of *rbc*L for some species (Kondo *et al.*, 1996), only 1382 of a total of 1428 positions were included in the multiple alignment of these sequences. For the restriction site study, data were obtained for 14 enzymes and only variable sites were scored (Plunkett & Downie, 1999). The data sets analysed herein are available upon request.

Phylogenetic analysis

All phylogenetic analyses were carried out using maximum parsimony, implemented using PAUP* version 4.0b4a (Swofford, 1998). Shortest trees for each of the eight data sets (including that of combined *rpl*16, *rps*16, and *rpo*C1 intron data) were obtained using 100 random addition replicate searches, with tree bisection-reconnection (TBR) branch swapping and ACCTRAN optimization. Bootstrap values (Felsenstein, 1985) were calculated from 100 replicate analyses, simple addition sequence of taxa, and TBR branch swapping. Pair-wise nucleotide differences of unambiguously aligned nucleotide positions were determined using the distance matrix option in PAUP* (with no provision made to account for multiple hits), with alignment gaps in any one sequence treated as missing for all taxa. To examine the extent of conflict among the three intron data sets prior to combining data, we conducted the incongruence length difference (ILD) test of Farris *et al.* (1995), as implemented by PAUP* using simple addition sequence and TBR branch swapping. Five hundred replicates were considered for each partition. The results of this partition homogeneity test revealed that the three intron matrices do not yield significantly different phylogenetic estimates and, as such, an analysis of combined data is appropriate. All trees constructed from plastid DNA data were rooted with *Aralia* (*Araliaceae*), with the exception of the *mat*K trees, which were rooted with the closely allied araliad genus *Kalopanax* Miq. (*Aralia* was unavailable for analysis). ITS trees were rooted with the apioid genus *Physospermum*, as sequences from more distantly related taxa were difficult to align.

Relationships among those genera included in the three intron, ITS, and restriction site data matrices were further explored upon simultaneous consideration of all data (the 'supermatrix' or 'total evidence' approach; Kluge & Wolf, 1993; Wiens & Reeder, 1995). Here, five data matrices were combined into a single larger matrix and analysed using maximum parsimony, with data for those taxa not included in any single analysis scored as missing. An alternative approach for simultaneous consideration of all data (as well as unique taxa) is the construction of supertrees, where the resultant trees from each of the separate analyses are combined rather than the original data used to construct them (Sanderson *et al.*, 1998). Using the method of matrix representation with parsimony (MRP; Ragan, 1992), the topologies of each of the five source (individual or consensus) trees obtained from maximum parsimony analyses of the five original data sets were translated manually into data matrices of binary characters, with question marks used for taxa not included in a given tree (Baum, 1992; Sanderson *et al.*, 1998). The binary characters from all source trees were combined into one matrix and analysed using maximum parsimony, as detailed above for the separate analyses. Bootstrap values, however, were calculated for 1000 replicate analyses; decay values were also obtained (Bremer, 1988). The resultant maximally parsimonious trees, or supertrees, were rooted with *Aralia*, having all of its states scored as '0'. We reiterate that the original data matrices or source trees do not share identical termini and that monophyletic groups of species in the same genus were treated as single terminals in these combined analyses. Therefore, while we present a strict consensus of supertrees, it is not a 'strict supertree' (Sanderson *et al.*, 1998) in the sense that it agrees with all the trees from which it was derived.

Comparative utility of molecular data

Characteristics of the eight data sets are presented in Table 6 and the single or strict consensus tree resulting from maximum parsimony analysis of each data set is presented in Fig. 2. In almost all instances, the phylogenetic results obtained from each of these reduced analyses are consistent with those of our previously published results when many more species were considered. The few areas of discord between the reduced and original analyses – most notable with regard to the *rpl*16 intron – are generally characterized by poorly supported nodes and, when collapsed, yield topologies of a similar nature. It is known that differences in species sampling may have an influence on the resulting phylogenetic hypotheses (Lecointre *et al.*, 1993; Hillis, 1996, 1998), and our results support this conclusion.

Of the six coding (*rbc*L and *mat*K) and non-coding (ITS and introns) loci sequenced, the ITS region is evolving most rapidly, as evidenced by the greater percentage of sites that are potentially parsimony informative and its higher rate of sequence change (that in some pair-wise comparisons exceeds 30% of nucleotides; Table 6). This high rate of nucleotide substitution, however, precludes the ITS region for deep-level analysis, as available sequence data for *Hydrocotyloideae*, *Saniculoideae*, and some basal *Apioideae* could not be aligned unambiguously with data from other apioid taxa. In contrast, *rbc*L is evolving most conservatively, with

TABLE 6. Characteristics of eight data sets (A–H) used in maximum parsimony analyses of selected representatives of *Apiaceae* subfamily *Apioideae* and relatives. Only variable characters were included in the restriction site study. The single or strict consensus tree resulting from each of these analyses is presented in Figs. 2A–H, respectively

	A. Restriction sites	B. ITS	C. *rpl*16 intron	D. *rps*16 intron	E. *rpo*C1 intron	F. *mat*K	G. *rbc*L	H. all 3 introns
No. of taxa	27	24	33	33	33	33	33	33
No. of total characters	778	471	1152	1083	884	1107	1382	3119
No. of eliminated characters	0	47	167	167	97	0	0	431
No. of informative characters	476	198	195	149	107	161	117	451
No. of autapomorphic characters	302	104	183	158	126	192	107	467
No. of constant characters	n/a	122	607	609	554	754	1158	1770
No. of variable characters	778	302	378	307	233	353	224	918
% informative characters[a]	61	47	20	16	14	15	8	17
% divergence (range)	3.2–26.9	2.9–33.6	0.5–14.1	0.1–14.0	0.1–11.6	0.3–10.6	0.2–5.5	0.3–13.4
No. of minimal length trees	1	2	69	8	846	224	4	8
Length of shortest tree(s)	1594	828	664	528	369	560	393	1572
Consistency index (excluding uninformative characters)	0.368	0.496	0.618	0.594	0.642	0.601	0.497	0.609
Retention index	0.552	0.605	0.751	0.774	0.789	0.784	0.696	0.762

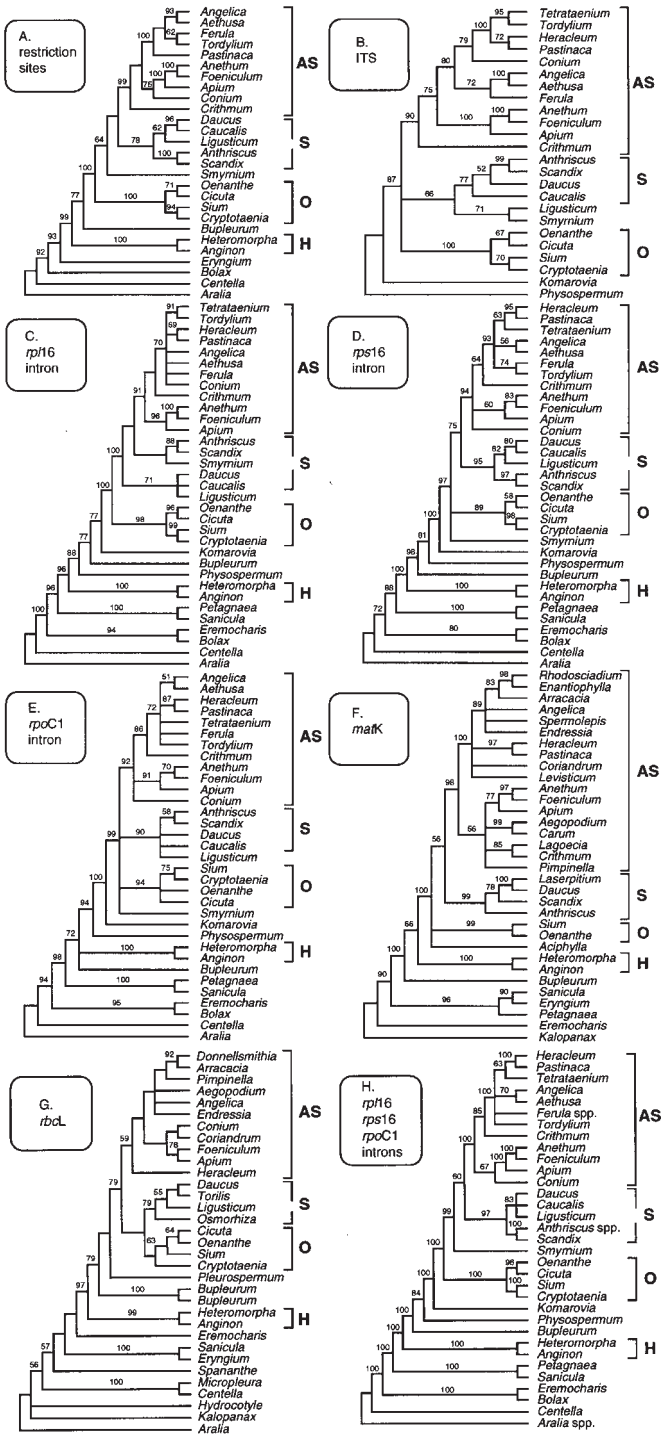
[a] No. of informative characters/(no. of total characters – no. of eliminated characters).

only 8% of all alignment positions parsimony informative and maximum pair-wise sequence divergence estimates just over 5% of nucleotides. Clearly, *rbc*L is best suited for deep-level comparisons. Intermediate in rate of evolution is *mat*K and the three chloroplast introns, with the introns *rpl*16 and *rps*16 evolving slightly faster than *mat*K or the intron *rpo*C1. The latter two regions appear to contribute similar information to the phylogenetic analysis (Downie *et al.*, 1998). The most notable difference, however, between coding and non-coding regions is the prevalence of insertions and deletions (indels) in the latter. While indels can create problems in alignment interpretation, unambiguously aligned gaps can provide an important source of phylogenetic information (Downie *et al.*, 1998; 2000b; Downie & Katz-Downie, 1999). The analysis of cpDNA restriction sites provided 2–4 times more informative characters than any single DNA region sequenced, with levels of restriction site divergence, as assessed by mean character differences, similar to that inferred for the ITS region. Only in the analysis of combined intron data did the numbers of informative characters approach those obtained through restriction site mapping, although this was at the cost of sequencing over 3kb of intron DNA for each species compared. The continued acquisition of ITS, *rps*16 intron, and *rpl*16 intron sequences for molecular systematic study in *Apioideae* seems worthwhile, given their higher rates of sequence evolution over other examined loci and the ease and rapidity by which these sequence data can be procured.

Phylogenetic resolution

The results of the phylogenetic analysis of each of the seven reduced data sets and that of combined intron data reveal a group of species previously identified as the 'apioid superclade' (AS), and with the exception of the *rbc*L tree (Fig. 2G), bootstrap support for this clade is high (90–100%). Other shared clades include those recognized previously as tribes *Scandiceae* (S; bootstrap values 77–99%), *Oenantheae* (O; 63–100%), and *Heteromorpheae* (H; 99–100%). Data from the *rpl*16 intron (Fig. 2C), however, do not resolve a monophyletic *Scandiceae*. Moreover, in those cpDNA-derived trees in which it was included, *Ligusticum* (represented by its type *L. scoticum*) falls within tribe *Scandiceae*. In some cases, this is an artifact of the reduced sample

FIG. 2. Single (tree A) and strict consensus (trees B–H) trees derived from maximum parsimony analysis of cpDNA restriction sites (A) or DNA sequence data (B–H). Tree H represents a combined analysis of the three intron data sets; the monophyletic genera *Ferula*, *Anthriscus*, and *Aralia*, each represented by one of two species in the separate analyses, were each scored as single exemplars. Tree summary statistics, including tree length, number of minimal length trees, and consistency and retention indices, are presented in Table 6. Numbers above nodes are bootstrap estimates for 100 replicate analyses; only values $>50\%$ are indicated. Complete species names are indicated in Table 5. Within subfamily *Apioideae*, the following major clades of two or more species are indicated: AS, apioid superclade; S, *Scandiceae*; O, *Oenantheae*; H, *Heteromorpheae*.


A.
restriction
sites
Angelica
Aethusa
Ferula
Tordylium
Pastinaca
Anethum
Foeniculum
Apium
Conium
Crithmum
Daucus
Caucalis
Ligusticum
Anthriscus
Scandix
Smyrnium
Oenanthe
Cicuta
Sium
Cryptotaenia
Bupleurum
Heteromorpha
Anginon
Eryngium
Bolax
Centella
Aralia
AS
S
O
H
B.
ITS
Tetrataenium
Tordylium
Heracleum
Pastinaca
Conium
Angelica
Aethusa
Ferula
Anethum
Foeniculum
Apium
Crithmum
Anthriscus
Scandix
Daucus
Caucalis
Ligusticum
Smyrnium
Oenanthe
Cicuta
Sium
Cryptotaenia
Komarovia
Physospermum
C.
*rpl*16
intron
Tetrataenium
Tordylium
Heracleum
Pastinaca
Angelica
Aethusa
Ferula
Conium
Crithmum
Anethum
Foeniculum
Apium
Anthriscus
Scandix
Smyrnium
Daucus
Caucalis
Ligusticum
Oenanthe
Cicuta
Sium
Cryptotaenia
Komarovia
Bupleurum
Physospermum
Heteromorpha
Anginon
Petagnaea
Sanicula
Eremocharis
Bolax
Centella
Aralia
D.
*rps*16
intron
Heracleum
Pastinaca
Tetrataenium
Angelica
Aethusa
Ferula
Tordylium
Crithmum
Anethum
Foeniculum
Apium
Conium
Daucus
Caucalis
Ligusticum
Anthriscus
Scandix
Oenanthe
Cicuta
Sium
Cryptotaenia
Smyrnium
Komarovia
Physospermum
Bupleurum
Heteromorpha
Anginon
Petagnaea
Sanicula
Eremocharis
Bolax
Centella
Aralia
E.
*rpo*C1
intron
Angelica
Aethusa
Heracleum
Pastinaca
Tetrataenium
Ferula
Tordylium
Crithmum
Anethum
Foeniculum
Apium
Conium
Anthriscus
Scandix
Daucus
Caucalis
Ligusticum
Sium
Cryptotaenia
Oenanthe
Cicuta
Smyrnium
Komarovia
Physospermum
Heteromorpha
Anginon
Bupleurum
Petagnaea
Sanicula
Eremocharis
Bolax
Centella
Aralia
F.
*mat*K
Rhodosciadium
Enantiophylla
Arracacia
Angelica
Spermolepis
Endressia
Heracleum
Pastinaca
Coriandrum
Levisticum
Anethum
Foeniculum
Apium
Aegopodium
Carum
Lagoecia
Crithmum
Pimpinella
Laserpitium
Daucus
Scandix
Anthriscus
Sium
Oenanthe
Aciphylla
Heteromorpha
Anginon
Bupleurum
Sanicula
Eryngium
Petagnaea
Eremocharis
Kalopanax
G.
*rbc*L
Donnellsmithia
Arracacia
Pimpinella
Aegopodium
Angelica
Endressia
Conium
Coriandrum
Foeniculum
Apium
Heracleum
Daucus
Torilis
Ligusticum
Osmorhiza
Cicuta
Oenanthe
Sium
Cryptotaenia
Pleurospermum
Bupleurum
Bupleurum
Heteromorpha
Anginon
Eremocharis
Sanicula
Eryngium
Spananthe
Micropleura
Centella
Hydrocotyle
Kalopanax
Aralia
H.
*rpl*16
*rps*16
*rpo*C1
introns
Heracleum
Pastinaca
Tetrataenium
Angelica
Aethusa
Ferula spp.
Tordylium
Crithmum
Anethum
Foeniculum
Apium
Conium
Daucus
Caucalis
Ligusticum
Anthriscus spp.
Scandix
Smyrnium
Oenanthe
Cicuta
Sium
Cryptotaenia
Komarovia
Physospermum
Bupleurum
Heteromorpha
Anginon
Petagnaea
Sanicula
Eremocharis
Bolax
Centella
Aralia spp.

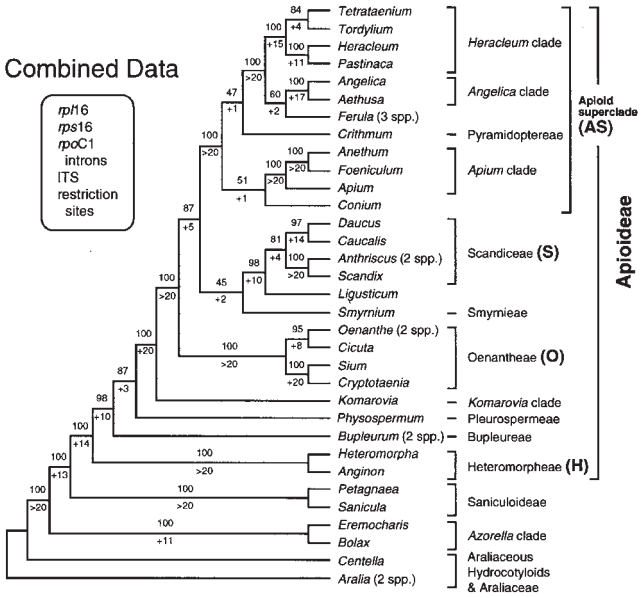


FIG. 3. Single minimal length 3929-step tree derived from maximum parsimony analysis of combined DNA sequence (*rpl*16 intron, *rps*16 intron, *rpo*C1 intron, ITS) and restriction site data (CI, excluding uninformative characters = 0. 482; RI = 0. 639). Numbers above nodes are bootstrap estimates for 1000 replicate analyses; numbers below nodes are decay values for trees up to 20 steps longer than those most parsimonious. The *Ferula*, *Anthriscus*, *Oenanthe*, *Bupleurum* and *Aralia* species examined in each of the separate analyses were not identical; however, because each group of species is monophyletic (as revealed by our earlier, more comprehensive analyses), data for each genus were combined and treated as a single exemplar. Data for genera not included in any separate analysis were scored as missing data. Complete species names are indicated in Table 5. The bracketed tribes and clades are those listed in Tables 2 and 3; the abbreviations AS, S, O and H are the same as outlined in Fig. 2.

size as its position within the clade is not supported by parsimony analyses of a much larger sample of taxa (cf. restriction sites (Plunkett & Downie, 1999), *rpl*16 intron (Downie *et al.*, 2000b), *rps*16 intron (Lee & Downie, 2000)). In other cases, *L. scoticum* does indeed ally with *Scandiceae* (Kondo *et al.*, 1996; Downie *et al.*, 1998); its position here, however, cannot be reconciled on the basis of any other kind of evidence.

The single minimal length tree derived from maximum parsimony analysis of combined DNA sequence (*rpl*16 intron, *rps*16 intron, *rpo*C1 intron, ITS) and restriction site data is presented in Fig. 3. This tree has a length of 3929 steps, a consistency index (CI; excluding uninformative characters) of 0.482, and a retention index (RI) of 0.639. Here, all clades of two or more taxa are strongly supported as monophyletic, as assessed by high bootstrap and decay values. The monophyletic *Scandiceae* is sister to *Ligusticum scoticum*. The strict consensus supertree of 16 minimal length 138-step trees constructed by the MRP method using five source trees (trees A–E in Fig. 2) and analysed using maximum parsimony is presented in Fig. 4 (CI, excluding

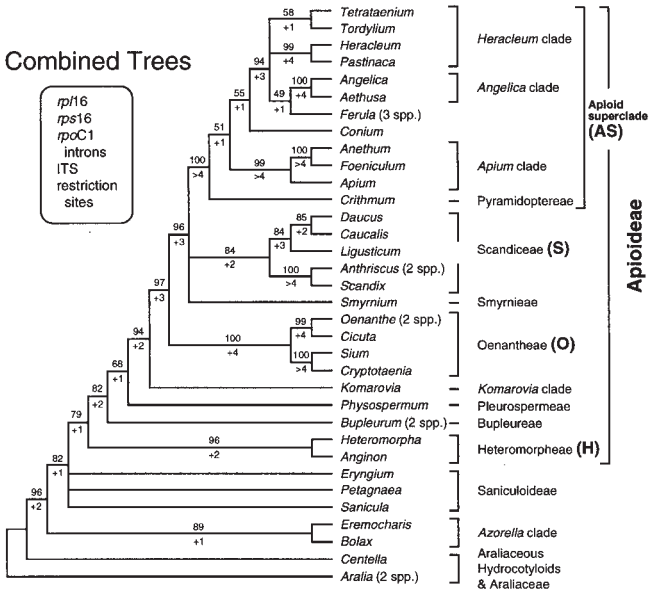


FIG. 4. Strict consensus of 16 minimal length 138-step supertrees constructed by the MRP (matrix representation with parsimony) method using five source trees (Fig. 2, trees A–E) and analysed using maximum parsimony (CI, excluding uninformative characters = 0.866; RI = 0.958). Numbers above nodes are bootstrap estimates for 1000 replicate analyses; numbers below nodes are decay values for trees up to 20 steps longer than those most parsimonious. The *Ferula*, *Anthriscus*, *Oenanthe*, *Bupleurum* and *Aralia* species sampled in each of the source trees were not equivalent, thus to reduce the number of terminals in the supertree these monophyletic groups of species were each scored as a single taxon. Taxa not included in any single source tree were coded as missing data. Complete species names are indicated in Table 5. The bracketed tribes and clades are those listed in Tables 2 and 3; the abbreviations AS, S, O and H are the same as outlined in Fig. 2.

uninformative characters = 0.866; RI = 0.958). This tree is highly consistent to that derived from the analysis of combined data (Fig. 3) and upon collapse of those nodes supported by bootstrap values less than 55% the trees are almost identical, with the only difference between them being the position of *Ligusticum*.

Phylogenetic Relationships Within Apioideae

The putative relationship among the tribes and clades so far recognized in *Apiaceae* subfamily *Apioideae* is illustrated in Fig. 5. This phylogeny was inferred based on the results of combined data and trees described above (Figs 3, 4) as well as the results of previously published molecular analyses (Plunkett *et al.*, 1996b; Downie & Katz-Downie, 1999; Plunkett & Downie, 1999; Downie *et al.*, 1998, 2000a,b,c). We emphasize that at least 23 genera included in molecular studies to date (Table 4) do not comfortably find a home within any of these clades, although the majority

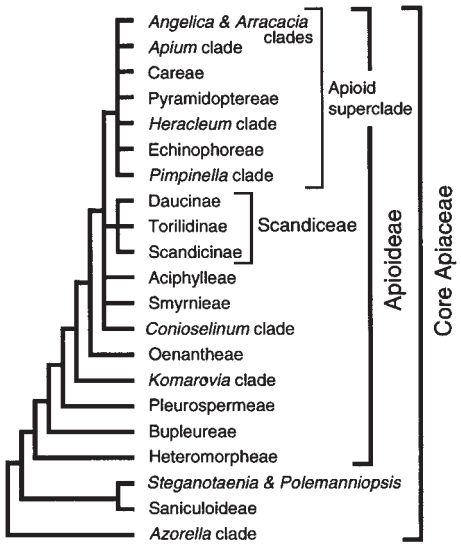


FIG. 5. Summary of relationships among the tribes (Table 2) and informally recognized clades (Table 3) of *Apiaceae* subfamily *Apioideae* as revealed by phylogenetic analyses of molecular data (this study; Downie & Katz-Downie, 1999; Downie *et al.*, 1998, 2000a,b,c). Not all genera examined to date have been assigned to a particular group (Table 4); many of these genera, however, are contained within the apioid superclade.

of these taxa fall within the apioid superclade. Their inclusion would result primarily in the blurring of the boundaries of the *Angelica* and *Apium* clades. We also point out the provisional nature of these relationships, given that only half of the approximately 400 genera recognized in *Apioideae* have been considered in molecular study. Many of the remaining genera, however, are ascribed to the large tribe *Apieae* (Pimenov & Leonov, 1993), and while it is impossible to know their affinity until they are examined, a great many will likely fall within the apioid superclade.

Heteromorpheae are sister group to all remaining *Apioideae*, in line with their woody habit, primitive wood anatomy, southern African origin, and microfossil evidence (Plunkett *et al.*, 1996b; Downie & Katz-Downie, 1999). The placement of *Heteromorpha* alongside *Anethum* and *Apium* in the ITS study of Valiejo-Roman *et al.* (1998) must be considered spurious. Successively more distally branching lineages include *Bupleureae*, *Pleurospermeae*, the *Komarovia* clade, and *Oenantheae*. The relationships among many of the remaining clades are equivocal. While the apioid superclade and tribe *Scandiceae* can each be unambiguously circumscribed on the basis of molecular data, the relationship of these two clades to *Aciphylleae*, *Smyrnieae*, and the *Conioselinum* clade is not clear.

Conclusions

Phylogenetic analyses of molecular data provide very little support for Drude's (1898) often cited system of classification of *Apioideae* or for other subfamilial

treatments that are based largely on morphology and anatomy. Drude's tribes *Apieae*, *Coriandreae*, *Dauceae*, *Laserpitieae*, *Peucedaneae*, *Scandiceae*, and *Smyrnieae* are each not monophyletic. Drude's tribes *Dauceae*, *Laserpitieae*, and *Scandiceae*, however, collectively form a monophyletic group (i.e. tribe *Scandiceae*). Of Drude's eight tribes, only the small morphologically distinct *Echinophoreae* is retained as monophyletic. Of Drude's ten subtribes and of those where sampling has been extensive (therefore, excluding the monotypic subtribe *Silerinae* Tausch and the bitypic subtribe *Elaeoselininae* Drude, both of tribe *Laserpitieae*, where material has so far been unavailable for analysis), only *Scandiceae* subtribe *Scandicinae* and *Peucedaneae* subtribe *Tordyliinae* show some resemblance to groups inferred by molecular study. Even so, the generic compositions of these clades are not identical to those proposed by Drude, with *Molopospermum*, *Grammosciadium* and *Rhabdosciadium* now removed from *Scandicinae* and *Pastinaca* added to *Tordyliinae*.

The results of these molecular phylogenetic analyses have helped to more accurately delimit the family *Apiaceae* as well as to clarify the natural subdivisions within its largest subfamily, *Apioideae*. *Apiaceae* comprise three major lineages, coinciding with subfamilies *Apioideae* and *Saniculoideae* and the *Azorella* clade, the latter representing some but not all members of Drude's subfamily *Hydrocotyloideae*. Based on taxonomic congruence among molecular data sets, ten major clades are recognized within *Apioideae* and treated at the rank of tribe, with *Scandiceae* comprising three subtribes. Each of these tribes and subtribes is strongly supported as monophyletic in many separate analyses of molecular data and, occasionally, by other lines of evidence. In naming these clades, we followed priority of validly published generic names (Pimenov & Constance, 1985) as regulated by the *ICBN* (Greuter *et al.*, 2000). Seven additional clades are identified, with formal recognition pending further study and confirmation of monophyly. Of the 195 genera examined, 23 have yet to be assigned (or are assigned only in part) to a specific group. Many of these genera fall within the apioid superclade, thus additional phylogenetic study of this large clade is warranted.

While the relationships inferred on the basis of various types of molecular markers are generally congruent, it has not been possible to delimit many of these newly circumscribed tribes and clades using obvious morphological or anatomical synapomorphies (Downie *et al.*, 2000a,b). It is hoped that future studies will add to this information, but if not, we would have to accept that the task of reclassifying *Apioideae* at the suprageneric level is to be accomplished on the basis of molecular data rather than morphology. We realize that this is a contentious issue; in the absence of evident and diagnostic morphological characters, several of the tribes recognized herein are difficult to identify or distinguish from one another. Attempts to construct a phylogeny for the entire subfamily using traditional (i.e. non-molecular) characters have proven difficult, for while cladistic analyses of morphological and anatomical data offer much insight into the phylogeny of *Scandiceae* (Spalik & Downie, 2001; Spalik *et al.*, 2001, and unpublished; Lee *et al.*, 2001), *Oenantheae* (Downie & Spalik, unpublished), and basal *Apioideae* (Roux *et al.*,

1978; Van Wyk *et al.*, 1997; Van Wyk, 2001), these data appear to be of limited value at higher taxonomic levels (Downie *et al.*, 2000a; Spalik & Downie, 2001). Such a conclusion is not surprising, given the common dissatisfaction among systematic botanists with traditional diagnostic characters used in the higher-level classification of *Apioideae* and the lack of consensus of relationships apparent among existing taxonomic treatments. Simply, many of the tribes and subtribes now recognized in *Apioideae* cannot be delimited unambiguously using morphological data and it may be unrealistic to think that morphological synapomorphies can be found for those tribes and other major clades supported by molecular studies.

Molecular systematic research on *Apioideae* has been collaborative, with participation international. These studies will continue, with emphasis on the placement of the 200 or so genera yet to be examined (including those heretofore refractory to proper phylogenetic placement) and the re-examination and refinement of the various tribes and clades recognized herein. Current and future revision of each of these major groups, incorporating both molecular and morphological data, will ultimately lead to the production of a modern classification for the subfamily.

Acknowledgements

The authors would like to express their thanks to the many individuals, botanic gardens, and herbaria who provided or loaned material during the course of our investigations and to the many individuals who provided laboratory assistance. In particular, we would like to acknowledge the contributions of Lincoln Constance and Michael Pimenov, in providing both plant material and invaluable advice. The senior author would also like to thank the Royal Botanic Garden Edinburgh for providing research facilities during his sabbatical leave, and Mark Carine and an anonymous reviewer for providing comments on an earlier draft of this manuscript. This work was supported by grants from the US National Science Foundation (DEB-9407712), the Polish Committee for Scientific Research (KBN 6 P04C 02611), and the Russian Foundation of Fundamental Research (97-04-50016).

References

Antonov, A. S., Valiejo-Roman, K. M., Pimenov, M. G. & Beridze, N. A. (1988). Non-equivalency of genera in *Angiospermae*: evidence from DNA hybridization studies. *Plant Syst. Evol.* 161: 155–168.

Baum, B. R. (1992). Combining trees as a way of combining data sets for phylogenetic inference and the desirability of combining gene trees. *Taxon* 41: 3–10.

Bentham, G. (1867). *Umbelliferae*. In: Bentham, G. & Hooker, J. D. (eds) *Genera Plantarum*, Vol. 1, pp. 859–931. London: Reeve & Co.

Boissier, E. (1872). *Umbelliferae*. In: Boissier, E. *Flora Orientalis*, Vol. 2, pp. 819–1090. Geneva: Georg.

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

CALESTANI, V. (1905). Contributo alla sistematica delle ombrellifere d'europa. *Webbia* 1: 89–280.

CANDOLLE, A. P. DE (1830). *Umbelliferae*. In: CANDOLLE, A. P. DE (ed.) *Prodromus Systematis Naturalis Regni Vegetabilis*, Vol. 4, pp. 55–250. Paris: Treüttel & Würtz.

CAUWET-MARC, A.-M. & CARBONNIER, J. (eds) (1982). *Les Ombellifères: contributions pluridisciplinaires à la systématique: actes du 2ème Symposium International sur les Ombellifères, Centre Universitaire de Perpignan, 18–21 Mai 1977*. St Louis, MO: Missouri Botanical Garden.

CERCEAU-LARRIVAL, M.-T. (1962). Plantules et pollens d'Ombellifères. Leur intérêt systématique et phylogénique. *Mém. Mus. natn. Hist. nat., Ser. B (Bot.)* 14: 1–166.

CHASE, M. W., SOLTIS, D. E., OLMSTEAD, R. G. *et al.* (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. *Ann. Missouri Bot. Gard.* 80: 528–580.

CONSTANCE, L. (1971). History of the classification of *Umbelliferae* (*Apiaceae*). In: HEYWOOD, V. H. (ed.) *The Biology and Chemistry of the Umbelliferae*, pp. 1–11. London: Academic Press.

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

DAVIS, P. H. (1972). *Umbelliferae*. In: DAVIS, P. H. (ed.) *Flora of Turkey and the East Aegean Islands*, pp. 265–538. Edinburgh: Edinburgh University Press.

DEBONTE, L. R., MATTHEWS, B. F. & WILSON, K. G. (1984). Variation of plastid and mitochondrial DNAs in the genus *Daucus*. *Amer. J. Bot.* 71: 932–940.

DOWNIE, S. R. & KATZ-DOWNIE, D. S. (1996). A molecular phylogeny of *Apiaceae* subfamily *Apioideae*: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 83: 234–251.

DOWNIE, S. R. & KATZ-DOWNIE, D. S. (1999). Phylogenetic analysis of chloroplast *rps*16 intron sequences reveals relationships within the woody southern African *Apiaceae* subfamily *Apioideae*. *Can. J. Bot.* 77: 1120–1135.

DOWNIE, S. R., KATZ-DOWNIE, D. S. & CHO, K.-J. (1996). Phylogenetic analysis of *Apiaceae* subfamily *Apioideae* using nucleotide sequences from the chloroplast *rpo*C1 intron. *Molec. Phylogenet. Evol.* 6: 1–18.

DOWNIE, S. R., RAMANATH, S., KATZ-DOWNIE, D. S. & LLANAS, E. (1998). Molecular systematics of *Apiaceae* subfamily *Apioideae*: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpo*C1 intron sequences. *Amer. J. Bot.* 85: 563–591.

DOWNIE, S. R., KATZ-DOWNIE, D. S. & SPALIK, K. (2000a). A phylogeny of *Apiaceae* tribe *Scandiceae*: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 87: 76–95.

DOWNIE, S. R., KATZ-DOWNIE, D. S. & WATSON, M. F. (2000b). A phylogeny of the flowering plant family *Apiaceae* based on chloroplast DNA *rpl*16 and *rpo*C1 intron sequences: towards a suprageneric classification of subfamily *Apioideae*. *Amer. J. Bot.* 87: 273–292.

DOWNIE, S. R., WATSON, M. F., SPALIK, K. & KATZ-DOWNIE, D. S. (2000c). Molecular systematics of Old World *Apioideae* (*Apiaceae*): relationships among some members of tribe *Peucedaneae sensu lato*, the placement of several island-endemic species, and resolution within the apioid superclade. *Can. J. Bot.* 78: 506–528.

DRUDE, C. G. O. (1898). Umbelliferae. In: ENGLER, A. & PRANTL, K. (eds) *Die Natürlichen Pflanzenfamilien*, Vol. 3 (8), pp. 63–250. Leipzig: W. Engelmann.

FARRIS, J. S., KÄLLERSJÖ, M., KLUGE, A. G. & BULT, C. (1995). Testing significance of incongruence. *Cladistics* 10: 315–319.

FELSENSTEIN, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
GREUTER, W., MCNEILL, J., BARRIE, F. R. *et al.* (eds) (2000). International Code of Botanical Nomenclature (St Louis Code). *Regnum Veg.*, Vol. 138.
HEDGE, I. C., LAMOND, J. M., RECHINGER, K. H. *et al.* (1987). *Umbelliferae*. In: RECHINGER, K. H. (ed.) *Flora Iranica*, Vol. 162, pp. 1–555. Graz: Akademische Druck und Verlagsanstalt.
HEYWOOD, V. H. (ed) (1971a). *The Biology and Chemistry of the Umbelliferae*. London: Academic Press.
HEYWOOD, V. H. (1971b). Systematic survey of Old World *Umbelliferae*. In: HEYWOOD, V. H. (ed.) *The Biology and Chemistry of the Umbelliferae*, pp. 31–41. London: Academic Press.
HEYWOOD, V. H. (1978a). General introduction to the taxonomy of the *Umbelliferae*. In: CAUWET-MARC, A.-M. & CARBONNIER, J. (eds) (1982). *Les Ombellifères: contributions pluridisciplinaires à la systématique: actes du 2ème Symposium International sur les Ombellifères, Centre Universitaire de Perpignan, 18–21 Mai 1977*, pp. 107–112. St Louis, MO: Missouri Botanical Garden.
HEYWOOD, V. H. (1978b). Multivariate taxonomic synthesis of the tribe *Caucalideae*. In: CAUWET-MARC, A.-M. & CARBONNIER, J. (eds) (1982). *Les Ombellifères: contributions pluridisciplinaires à la systématique: actes du 2ème Symposium International sur les Ombellifères, Centre Universitaire de Perpignan, 18–21 Mai 1977*, pp. 727–736. St Louis, MO: Missouri Botanical Garden.
HILLIS, D. M. (1996). Inferring complex phylogenies. *Nature* 383: 130.
HILLIS, D. M. (1998). Taxonomic sampling, phylogenetic accuracy, and investigator bias. *Syst. Biol.* 47: 3–8.
JURY, S. (1986). Fruit and leaf variation in the African species of the *Umbelliferae* tribe *Caucalideae*. *Acta Univ. Ups. Symb. Bot. Ups.* 26: 181–188.
KATZ-DOWNIE, D. S., VALIEJO-ROMAN, C. M., TERENTIEVA, E. I. *et al.* (1999). Towards a molecular phylogeny of *Apiaceae* subfamily *Apioideae*: additional information from nuclear ribosomal DNA ITS sequences. *Plant Syst. Evol.* 216: 167–195.
KLUGE, A. G. & WOLF, A. J. (1993). Cladistics: what's in a word? *Cladistics* 9: 183–199.
KOCH, W. D. J. (1824). Generum tribuumque plantarum Umbelliferarum nova dispositio. *Nova Acta Acad. Caes. Leop.-Carol. German Nat. Cur.* 12: 55–156.
KONDO, K., TERABAYASHI, S., OKADA, M., YUAN, C. & HE, S. (1996). Phylogenetic relationship of medicinally important *Cnidium officinale* and Japanese *Apiaceae* based on *rbc*L sequences. *J. Plant Res.* 109: 21–27.
KOSO-POLJANSKY, B. M. (1916). Sciadophytorum systematis lineamenta. *Bull. Soc. Imp. Nat. Moscou* 29: 93–222.
LECOINTRE, G., PHILIPPE, H., VÂN LÊ, H. L. & LE GUYADER, H. (1993). Species sampling has a major impact on phylogenetic inference. *Molec. Phylogenet. Evol.* 2: 205–224.
LEE, B.-Y. & DOWNIE, S. R. (1999). A molecular phylogeny of *Apiaceae* tribe *Caucalideae* and related taxa: inferences based on ITS sequence data. *Syst. Bot.* 24: 461–479.
LEE, B.-Y. & DOWNIE, S. R. (2000). Phylogenetic analysis of cpDNA restriction sites and *rps*16 intron sequences reveals relationships among *Apiaceae* tribes *Caucalideae*, *Scandiceae* and related taxa. *Plant Syst. Evol.* 221: 35–60.
LEE, S.-B. & RASMUSSEN, S. K. (1998). Phylogenetic analysis of rDNA internal transcribed spacer of *Angelica*, *Bupleurum* and *Peucedanum* species. Available from the Risø National Laboratory, Roskilde, Denmark, Risø-R-1091(EN).

LEE, B.-Y., LEVIN, G. A. & DOWNIE, S. R. (2001). Relationships within the spiny-fruited umbellifers (*Scandiceae* subtribes Daucinae and Torilidinae) as assessed by phylogenetic analysis of morphological characters. *Syst. Bot.* (in press).

MITCHELL, A. D., WEBB, C. J. & WAGSTAFF, S. J. (1998). Phylogenetic relationships of species of *Gingidia* and related genera (*Apiaceae*, subfamily *Apioideae*). *New Zealand J. Bot.* 36: 417–424.

OLMSTEAD, R. G., MICHAELS, H. J., SCOTT, K. M. & PALMER, J. D. (1992). Monophyly of the Asteridae and identification of its major lineages inferred from *rbc*L sequences. *Ann. Missouri Bot. Gard.* 79: 249–265.

PICKERING, J. L. & FAIRBROTHERS, D. E. (1970). A serological comparison of *Umbelliferae* subfamilies. *Amer. J. Bot.* 57: 988–992.

PICKERING, J. L. & FAIRBROTHERS, D. E. (1971). The use of serological data in a comparison of tribes in the *Apioideae*. In: HEYWOOD, V. H. (ed.) *The Biology and Chemistry of the Umbelliferae*, pp. 315–324. London: Academic Press.

PIMENOV, M. G. & CONSTANCE, L. (1985). Nomenclature of suprageneric taxa in *Umbelliferae*/*Apiaceae*. *Taxon* 34: 493–528.

PIMENOV, M. G. & LEONOV, M. V. (1993). The genera of the *Umbelliferae*: a nomenclator. Kew: Royal Botanic Gardens, Kew.

PIMENOV, M. G., SHNEYER, V. S., VALIEJO-ROMAN, K. M., TERENTIEVA, E. I & TROITSKY, A. V. (1999). *Komarovia* Korovin (*Umbelliferae*): a multidisciplinary study of a genus of uncertain taxonomic position. *Komarovia* 1: 61–73.

PLUNKETT, G. M. & DOWNIE, S. R. (1999). Major lineages within *Apiaceae* subfamily *Apioideae*: a comparison of chloroplast restriction site and DNA sequence data. *Amer. J. Bot.* 86: 1014–1026.

PLUNKETT, G. M. & DOWNIE, S. R. (2000). Expansion and contraction of the chloroplast inverted repeat in *Apiaceae* subfamily *Apioideae*. *Syst. Bot.* 25: 648–667.

PLUNKETT, G. M., SOLTIS, D. E. & SOLTIS, P. S. (1996a). Higher level relationships of Apiales (*Apiaceae* and *Araliaceae*) based on phylogenetic analysis of *rbc*L sequences. *Amer. J. Bot.* 83: 499–515.

PLUNKETT, G. M., SOLTIS, D. E. & SOLTIS, P. S. (1996b). Evolutionary patterns in *Apiaceae*: inferences based on *mat*K sequence data. *Syst. Bot.* 21: 477–495.

PLUNKETT, G. M., SOLTIS, D. E. & SOLTIS, P. S. (1997). Clarification of the relationships between *Apiaceae* and *Araliaceae* based on *mat*K and *rbc*L sequence data. *Amer. J. Bot.* 84: 565–580.

RADFORD, E. A., WATSON, M. F. & PRESTON, J. (2001). Phylogenetic relationships of species of *Aciphylla* (*Apiaceae* subfamily *Apioideae*) and related genera using molecular, morphological and combined data sets. *New Zealand J. Bot.* (in press).

RAGAN, M. A. (1992). Phylogenetic inference based on matrix representation of trees. *Molec. Phylogenet. Evol.* 1: 53–58.

ROUX, M., CARBONNIER, J. & CAUWET-MARC, A.-M. (1978). Un exemple d'analyse cladistique: le genre *Bupleurum* L. (*Umbelliferae*). In: CAUWET-MARC, A.-M. & CARBONNIER, J. (eds) (1982). *Les Ombellifères: contributions pluridisciplinaires à la systématique: actes du 2ème Symposium International sur les Ombellifères, Centre Universitaire de Perpignan, 18–21 Mai 1977*, pp. 575–592. St Louis, MO: Missouri Botanical Garden.

SANDERSON, M. J., PURVIS, A. & HENZE, C. (1998). Phylogenetic supertrees: assembling the trees of life. *Trends Ecol. Evol.* 13: 105–109.

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

SHNEYER, V. S., BORSCHTSCHENKO, G. P., PIMENOV, M. G. & LEONOV, M. V. (1992). The tribe *Smyrnieae* (*Umbelliferae*) in the light of serotaxonomical analysis. *Plant Syst. Evol.* 182: 135–148.

SHNEYER, V. S., BOTSCHTSCHENKO, G. P. & PIMENOV, M. G. (1995). Immunochemical appraisal of relationships within tribe *Peucedaneae* (*Apiaceae*). *Plant Syst. Evol.* 198: 1–16.

SOLTIS, P. S. & KUZOFF, R. K. (1993). ITS sequence variation within and among populations of *Lomatium grayi* and *L. laevigatum* (*Umbelliferae*). *Molec. Phylogenet. Evol.* 2: 166–170.

SOLTIS, P. S. & NOVAK, S. J. (1997). Polyphyly of the tuberous Lomatiums (*Apiaceae*): cpDNA evidence for morphological convergence. *Syst. Bot.* 22: 99–112.

SPALIK, K. & DOWNIE, S. R. (2001). The utility of morphological characters for inferring phylogeny in *Scandiceae* subtribe *Scandicinae*. *Ann. Mo. Bot. Gard.* (in press).

SPALIK, K., WOJEWÓDZKA, A. & DOWNIE, S. R. (2001). Delimitation of genera in *Apiaceae* with examples from *Scandiceae* subtribe *Scandicinae*. *Edinb. J. Bot.* 58 (2): 331–346.

SWOFFORD, D. L. (1998). *PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b4a*. Sunderland, MA: Sinauer Associates, Inc.

THEOBALD, W. L. (1971). Comparative anatomical and developmental studies in the *Umbelliferae*. In: HEYWOOD, V. H. (ed.) *The Biology and Chemistry of the Umbelliferae*, pp. 177–197. London: Academic Press.

VALIEJO-ROMAN, K. M., ANTONOV, A. S. & PIMENOV, M. G. (1979). DNA homology in the *Umbelliferae* of the *Apioideae* subfamily. *Dokl. Akad. Nauk SSSR* 245: 1021–1024.

VALIEJO-ROMAN, K. M., ANTONOV, A. S. & KAMALETDINOVA, M. A. (1982). Analysis of phylogenetic relationships of some *Umbelliferae* genera via DNA hybridization. *Dokl. Akad. Nauk. SSSR* 262: 749–753.

VALIEJO-ROMAN, K. M., PIMENOV, M. G., TERENTIEVA, E. I. *et al.* (1998). Molecular systematics of the *Umbelliferae*: using nuclear ribosomal DNA internal transcribed spacer sequences to resolve issues of evolutionary relationships. *Bot. Zhurn. (St Petersburg)* 83: 1–22.

VAN WYK, B.-E. (2001). A preliminary analysis of evolution of African and Madagascan *Apiaceae*. *Edinb. J. Bot*. 58 (2): 291–299.

VAN WYK, B.-E., ALLISON, I. & TILNEY, P. M. (1997). Morphological variation and phylogenetic relationships in the genus *Anginon* (*Apiaceae*). *Nord. J. Bot.* 17: 511–526.

VIVEK, B. S. & SIMON, P. W. (1998). Genetic relationships and diversity in carrot and other *Daucus* taxa based on nuclear restriction fragment length polymorphisms. *J. Amer. Soc. Hort. Sci.* 123: 1053–1057.

VIVEK, B. S. & SIMON, P. W. (1999). Phylogeny and relationships in *Daucus* based on restriction fragment length polymorphisms (RFLPs) of the chloroplast and mitochondrial genomes. *Euphytica* 105: 183–189.

WATANABE, A., ARAKI, S., KOBARI, S. *et al.* (1998). In vitro propagation, restriction fragment length polymorphism, and random amplified polymorphic DNA analyses of *Angelica* plants. *Plant Cell Rep.* 18: 187–192.

WIENS, J. J. & REEDER, T. W. (1995). Combining data sets with different numbers of taxa for phylogenetic analysis. *Syst. Biol.* 44: 548–558.

Received 27 January 2000; accepted with revision 1 October 2000