

## A PHYLOGENY OF APIACEAE TRIBE SCANDICEAE: EVIDENCE FROM NUCLEAR RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER SEQUENCES<sup>1</sup>

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The evolutionary relationships among members of Apiaceae (Umbelliferae) tribe Scandiceae and representatives of all major lineages of Apioideae (including putatively allied Caucalideae) identified in earlier molecular studies were inferred from nucleotide sequence variation in the internal transcribed spacer regions (ITS1 and ITS2) of nuclear ribosomal DNA. In all, 134 accessions representing 18 genera commonly treated in Scandiceae were analyzed. Phylogenies estimated using maximum parsimony and distance methods were generally similar and suggest that: (1) Scandiceae form a well-supported clade, consisting of the genera *Anthriscus*, *Athamanta* (in part), *Balansaea*, *Chaerophyllum*, *Conopodium*, *Geocaryum*, *Kozlovia*, *Krasnovia*, *Myrrhis*, *Myrrhoides*, *Neoconopodium*, *Osmorhiza*, *Scandix*, *Sphallerocarpus*, and *Tinguarra*; (2) *Athamanta* is polyphyletic, with *A. della-cellae* allied with *Daucus* and *A. macedonica* placed close to *Pimpinella*; and (3) *Rhabdosciadium* and *Grammosciadium* find affinity with the *Aegopodium* group of umbellifers, whereas the placement of the monotypic *Molospersmum* cannot be inferred because of its high sequence divergence. The genus *Bubon* has been restored with two new combinations, *B. macedonicum* subsp. *albanicum* and *B. macedonicum* subsp. *arachnoideum*. Scandiceae arise within paraphyletic Caucalideae, the latter comprising two major lineages whose relationships to Scandiceae are not clear. Therefore, a broad treatment of Scandiceae is proposed, with subtribes Scandicinae, Daucinae, and Torilidinae (the latter two representing the *Daucus* and *Torilis* subgroups, respectively, of recent molecular systematic investigations).

**Key words:** Apiaceae; Apioideae; molecular phylogeny; nuclear ribosomal DNA internal transcribed spacers; Scandiceae; Umbelliferae.

Apiaceae Lindl. (Umbelliferae Juss.) is one of the best known families of flowering plants. Its members include many commonly grown vegetables (e.g., carrot, parsnip, and celery/celeriac) and condiments (e.g., coriander, anise, caraway, chervil, cumin, parsley, and dill). They owe their distinctive flavor largely to diverse volatile compounds in the fruits and leaves, which not only account for their culinary use but for their wide application in medicine. The family also encompasses widespread weeds and toxic plants, including the notorious poison hemlock used in ancient Athens to execute those sentenced to death, the most famous victim being Socrates. Fortunately, such misuses of umbellifers have been rare and the Apiaceae stand out as a family of great economic importance. Despite its long taxonomic history dating back to Morison's (1672) *Plantarum umbelliferarum*, the earliest systematic study of any group of plants (Constance, 1971), the family still awaits a modern classification. The most recent treatment of umbellifers (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 (Hey-

wood, 1982a). Several alternative classifications have also been proposed (de Candolle, 1830; Bentham, 1867; Koso-Poljansky, 1916; Cerceau-Larrival, 1962), however, apart from the use of Cerceau-Larrival's by some French authors, none has gained wide recognition.

A molecular approach has contributed much to the understanding of evolutionary relationships of Apiaceae. Phylogenetic analyses of the family using chloroplast DNA (cpDNA) sequences (Downie, Katz-Downie, and Cho, 1996; Plunkett, Soltis, and Soltis, 1996; Downie et al., 1998), cpDNA restriction sites (Plunkett and Downie, 1999), and nuclear ribosomal DNA internal transcribed spacer (ITS) sequences (Downie and Katz-Downie, 1996; Downie et al., 1998; Katz-Downie et al., 1999) have revealed that two of Drude's three subfamilies, Saniculoideae and Apioideae, are each monophyletic and sister groups, while Hydrocotyloideae are polyphyletic (containing some members allied to Araliaceae and others to Apioideae and Saniculoideae). Nevertheless, the hitherto proposed divisions of subfamily Apioideae appear to be unsound, with all tribes but one seemingly polyphyletic or paraphyletic. The only tribe that has sustained the test of molecular phylogenetics is Scandiceae (Downie et al., 1998). However, only up to five genera in the group were included in each of these previous studies.

Apiaceae comprise 300–450 genera and 3000–3700 species (Constance, 1971; Pimenov and Leonov, 1993). Because of its large size, an approach to resolving the taxonomy of the family should therefore combine both a "high-level" analysis performed for a representative subset of the family as well as "low-level" revisions of par-

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TABLE 1. A comparison of classification systems of Apiaceae tribe Scandiceae. Genera included by particular authors are indicated (+). As the names and delimitations have been changing, the genera are given in their present meaning, while synonyms are provided in footnotes.

Genus	de Candolle (1830)	Benthams (1867)	Boissier (1872)	Drude (1898)	Calestani (1905)	Koso- Poljansky (1916)	Cerceau- Larrival (1962)	Heywood (1971)	Hedge et al. (1987)	Pimenov and Leonov (1993)
<i>Scandix</i> L.	+	+	+	+	+	+	+	+ <sup>a</sup>	+	+
<i>Anthriscus</i> Pers.	+	+	+	+			+	+	+	+ <sup>b</sup>
<i>Chaerophyllum</i> L.	+	+	+	+	+	+ <sup>c</sup>	+	+	+	+
<i>Grammosciadium</i> DC.	+	+ <sup>d</sup>	+	+ <sup>e</sup>	f	+ <sup>g</sup>	+	+	+	+
<i>Myrrhis</i> Mill.	+	+	+	+	+		+	+		+
<i>Geocaryum</i> Coss. <sup>h</sup>		+ <sup>d,i</sup>	+	+		+	+			
<i>Rhabdosciadium</i> Boiss.		+	+	+			+	+	+	+
<i>Myrrhoides</i> Fabr. <sup>j</sup>		+ <sup>d</sup>	+	+			+	+	+	+
<i>Osmorhiza</i> Raf.	+	+		+		+	+	+		+
<i>Sphallerocarpus</i> DC.	+	+ <sup>i</sup>		+			+	+		+
<i>Kozlovia</i> Lipsky								+		+
<i>Krasnovia</i> Schischk.								+	+	+
<i>Balansaea</i> Boiss. & Reut.				+ <sup>k</sup>		+ <sup>k</sup>		+		+ <sup>k</sup>
<i>Molopospermum</i> W. D. J. Kock	+			+				+		
<i>Tinguarra</i> Parl.		+		+				+		
<i>Neoconopodium</i> Pimenov & Kljuykov		+ <sup>d</sup>								+
<i>Athamanta</i> L.					+		+			
<i>Conopodium</i> W. D. J. Koch		+					+			
<i>Oreomyrrhis</i> Endl.	+ <sup>l</sup>	+				+				
<i>Ptychotis</i> W. D. J. Koch					+ <sup>m</sup>	+ <sup>m</sup>				
<i>Hladnikia</i> Rchb.					+ <sup>m</sup>	+ <sup>m</sup>				

<sup>a</sup> Heywood (1971) also recognized *Scandicium* (K. Koch) Thell., which is generally included in *Scandix*; <sup>b</sup> *Antrichaerophyllum* P. Fourn., a presumed hybrid between *Anthriscus* and *Chaerophyllum* recognized by Pimenov and Leonov (1993), is a synonym of *Anthriscus* (Reduron and Spalik, 1995); <sup>c</sup> split into *Golenkinianthe* Koso-Pol., *Chrysophae* Koso-Pol., and *Chaerophyllum*; <sup>d</sup> in *Chaerophyllum*; <sup>e</sup> *Caropodium* Stapf. & Wettst., now included in *Grammosciadium*, was placed by Drude (1898) in Apiaceae; <sup>f</sup> *Grammosciadium* Calestani (1905), non DC. (1829) = *Chaerophyllum*; <sup>g</sup> divided between *Falcaria* Fabr. (*Prionitis* subgen. *Grammosciadium* (DC.) Koso-Pol.) and *Stenodiptera* Koso-Pol.; <sup>h</sup> = *Biasoletia* W. D. J. Koch, = *Freyera* Rchb., = *Huetia* Boiss.; <sup>i</sup> in *Conopodium*; <sup>j</sup> = *Physocaulis* Tausch; <sup>k</sup> in *Geocaryum*; <sup>l</sup> = *Caldasia* Lag.; <sup>m</sup> in *Falcaria* (= *Prionitis* Adans. in Koso-Poljansky, 1916).

particular genera and tribes. The first aims to provide an outline of the classification and may help to formulate hypotheses on the evolution of the family, while the goal of the latter is to fill this framework with more detail and to give a deeper insight into the differentiation of these plants, including their life history strategies and evolutionary pathways of particular traits. The advantage of low-level analyses is also a smaller risk of inadequate sampling.

Scandiceae are well suited for such an approach. The tribe comprises ~20 genera with some 70–90 species largely confined to southwest Eurasia. Some of the genera are monotypic, and many have been recently revised, either regionally (Schischkin, 1950a; Tutin et al., 1968; Davis, 1972; Hedge et al., 1987) or worldwide (Engstrand, 1977; Lowry and Jones, 1984; Spalik, 1997). Although several different classifications of the tribe have been proposed (Table 1), there is a general agreement as to which genera constitute the core of the tribe (i.e., *Anthriscus*, *Chaerophyllum*, *Grammosciadium*, *Myrrhis*, *Osmorhiza*, *Rhabdosciadium*, and *Scandix*). Drude (1898) defined Scandiceae based on the crystals of calcium oxalate in the parenchyma cells surrounding the carpophore and divided it into two subtribes according to the shape of the fruit. His subtribe Caucalidinae (Caucalineae) was united with Dauceae and treated as tribe Caucalideae (Heywood, 1971; Hedge et al., 1987; Pimenov and Leonov, 1993). Molecular analyses, however, have confirmed that Scandiceae sensu Heywood (1971) and paraphyletic Caucalideae form a well-supported clade (Downie and Katz-Downie, 1996; Downie et al., 1998).

In contrast to their putative monophyly, Scandiceae are

quite diversified with respect to their life history, habit, ecology, floral morphology and umbel structure, and fruit morphology and anatomy. They may, therefore, serve as a model group to study the evolutionary biology of umbellifers, particularly the evolution of life history and reproductive strategy. Scandiceae are also of economic importance. *Anthriscus cerefolium* and *Myrrhis odorata* are used as condiments, *Chaerophyllum bulbosum* and some species of *Scandix* are occasionally eaten, and several species of *Osmorhiza* are used as food and medicine by native Americans (French, 1971). Particular attention has been drawn to *Anthriscus sylvestris*, the source of a drug used in traditional Chinese medicine (Okuyama, Sakakibara, and Shibata, 1981). These plants contain numerous active compounds (Kozawa, Morita, and Hata, 1978; Kurihara et al., 1978; Kurihara and Kikuchi, 1979; Inamori et al., 1983, 1985), some of which inhibit the proliferation of cancer cells in vitro (Ikeda et al., 1998a, b).

The position of several genera in Scandiceae is dubious as they have also been placed in other tribes. Drude (1898) underlined similarities between *Geocaryum* and *Butinia* (≡ *Conopodium* subgenus *Butinia*) from Apiaceae-Apiinae; however, he could have been referring to *Butinia capnoides*, which has also been placed in *Chaerophyllum* (Benthams, 1867; Hedge and Lamond, 1980) and eventually in a separate genus, *Neoconopodium*, within the Scandiceae (Pimenov and Kljuykov, 1987). Calestani (1905) placed *Geocaryum* in tribe Bunieae, together with *Conopodium* and *Bunium*; such a treatment was also adopted by Engstrand (1973, 1977) in his revision of *Geocaryum*, contrary to Spalik (1997) who allied it with core Scandiceae. Calestani (1905) also included several

TABLE 2. Accessions of Scandiceae and outgroups examined for nuclear rDNA ITS sequence variation. With the exception of some ITS1 (†) or ITS2 (‡) sequences, where only a single DNA strand was sequenced, all accessions were sequenced from both strands. These ITS data were deposited with GenBank as separate ITS1 and ITS2 sequences.

Accession	Source and/or voucher	GenBank accession number <sup>a</sup>
<i>Aciphylla aurea</i> W. R. B. Oliv.	Downie et al. (1998)	GBAN-U79595, GBAN-U79594
<i>Aciphylla crenulata</i> J. B. Armstr.	Downie et al. (1998)	GBAN-U78359, GBAN-U78419
<i>Aciphylla squarrosa</i> J. R. Forst. & G. Forst.	Downie et al. (1998)	GBAN-U79595, GBAN-U79596
<i>Aegokeras caespitosa</i> (Sibth. & Sm.) Raf.	Downie et al. (1998)	GBAN-U78379, GBAN-U78439
<i>Aegopodium alpestre</i> Ledeb.	Downie et al. (1998)	GBAN-U78376, GBAN-U78436
<i>Aethusa cynapium</i> L.	Downie et al. (1998)	GBAN-U30582, GBAN-U30583
<i>Anisotome aromatica</i> Hook. f.	Downie et al. (1998)	GBAN-U78360, GBAN-U78420
<i>Anthriscus caucalis</i> M. Bieb.	Downie et al. (1998)	GBAN-U79601, GBAN-U79602
<i>Anthriscus cerefolium</i> (L.) Hoffm.	Downie et al. (1998)	GBAN-U30532, GBAN-U30533
<i>Anthriscus cerefolium</i> var. <i>trichocarpa</i> Neilr.	Uzbekistan, W Tian-Shan Mtns., Chotkal Range, Aksakata Valley, 7 June 1989, <i>Spalik s. n.</i> (WA)	GBAN-AF073571, GBAN-AF073572
<i>Anthriscus kotschy</i> Boiss. & Balansa	Turkey, Niğde: Bolkar Dağ, Köpüktaş, 1 September 1997, <i>Spalik &amp; Zochowska s. n.</i> (WA)	GBAN-AF073579, GBAN-AF073580
<i>Anthriscus kotschy</i> Boiss. & Balansa†	Turkey, Niğde: Aladağlar, Demirkazık, 29 August 1965, <i>Findlay 189</i> (E)	GBAN-AF073577, GBAN-AF073578
<i>Anthriscus lamprocarpa</i> Boiss.†	Jordan, Um-Qais, near Irbid, <i>Lahham &amp; El-Oqlah 8</i> (Yarmouk Univ. Herb.)	GBAN-AF073581, GBAN-AF073582
<i>Anthriscus nitida</i> (Wahlenb.) Hazsl.†‡	France, Haut Rhin, by Lucelle River, 29 September 1991, <i>Spalik s. n.</i> (WA)	GBAN-AF073595, GBAN-AF073596
<i>Anthriscus schmalhauseni</i> (Albov) Koso-Pol.†‡	Russia, Sochi: near Staraya Macesta, 7 June 1970, <i>Vašák &amp; Vězda 314726</i> (G)	GBAN-AF073587, GBAN-AF073588
<i>Anthriscus sylvestris</i> (L.) Hoffm. subsp. <i>syvestris</i>	Downie et al. (1998)	GBAN-U79603, GBAN-U79604
<i>Anthriscus sylvestris</i> (L.) Hoffm. subsp. <i>syvestris</i> 'var. <i>abyssinica</i> A. Rich.'†‡	Ethiopia, W slope of Mt. Boruluccu, along road to Ticcio, c. 25 km SE of Asella, 6 December 1965, <i>de Wilde 9189</i> (MISS)	GBAN-AF073585, GBAN-AF073586
<i>Anthriscus sylvestris</i> (L.) Hoffm. subsp. <i>syvestris</i> 'A. <i>aemula</i> (Woron.) Schischk.'†‡	Japan, Yamagata Pref., Kaminoyama-shi, Kaminamai, Namaigawa Dam, Mt. Takahata-yama, S of Dorobu, 20 May 1994, <i>E. Hayasaka et al. 10</i> (MISS)	GBAN-AF073591, GBAN-AF073592
<i>Anthriscus sylvestris</i> (L.) Hoffm. subsp. <i>syvestris</i> 'A. <i>keniensis</i> H. Wolff'†‡	Tanzania, Arusha, Arumeru Distr., Mt. Meru, 11 January 1985, <i>Gereau 1669</i> (MISS)	GBAN-AF073583, GBAN-AF073584
<i>Anthriscus sylvestris</i> (L.) Hoffm. subsp. <i>syvestris</i> 'A. <i>yunnanensis</i> W. W. Sm.'†‡	China, eastern flank of Lichiang Range, June 1918, <i>Forrest 5914</i> (E)	GBAN-AF073589, GBAN-AF073590
<i>Anthriscus sylvestris</i> subsp. <i>alpina</i> (Vill.) Gremli†‡	France, Isère, La Grande Chartreuse, below Bovinant, October 1991, <i>Spalik s. n.</i> (WA)	GBAN-AF073593, GBAN-AF073594
<i>Anthriscus sylvestris</i> subsp. <i>fumarioides</i> (Waldst. & Kit.) Spalik‡	Yugoslavia, Serbia, May 1880, <i>Herb. Dörfler</i> (E)	GBAN-AF073575, GBAN-AF073576
<i>Anthriscus sylvestris</i> subsp. <i>nemorosa</i> (M. Bieb.) Koso-Pol.†	Turkey, Çoruh: Kaçkar Dağı, Didvake, 8 August 1996, <i>Spalik s. n.</i> (WA)	GBAN-AF073573, GBAN-AF073574
<i>Apium graveolens</i> L.	Downie et al. (1998)	GBAN-U30552, GBAN-U30553
<i>Arafoe aromatica</i> Pimenov & Lavrova	Downie et al. (1998)	GBAN-U78383, GBAN-U78443
<i>Athamanta cretensis</i> L.	France, Pont-de-Roide, fort du Lomont; 1 October 1996, <i>Reduron s. n.</i> (WA)	GBAN-AF073685, GBAN-AF073686
<i>Athamanta della-cellae</i> Asch. & Barbey ex E. A. Durand & Baratte†	Libya, Wadi Kuof, W of Beida, 28 March 1970, <i>Davis 50209</i> (E)	GBAN-AF073565, GBAN-AF073566
<i>Athamanta macedonica</i> (L.) Spreng. subsp. <i>macedonica</i>	Greece, Ioannina, Koukouli, 2 August 1981, <i>Dudley et al. 18205</i> (RNG)	GBAN-AF073541, GBAN-AF073542
<i>Athamanta turbith</i> (L.) Broth. subsp. <i>turbith</i> †	Slovenija, Bowec, 13 August 1988, <i>Gardner 4327</i> (E)	GBAN-AF073687, GBAN-AF073688
<i>Balansaea glaberrima</i> (Desf.) Maire†	Algeria, Oran: Mersal Kebir, 24 April 1953, <i>Alleizette s. n.</i> (MISS)	GBAN-AF073689, GBAN-AF073690
<i>Berula thunbergii</i> (DC.) H. Wolff	Downie et al. (1998)	GBAN-U78369, GBAN-U78429
<i>Bunium elegans</i> (Fenzl) Freyn†‡	Iran, Tehran: Abali, 20 July 1972, <i>Dini-Arazm 22458</i> (MISS)	GBAN-AF073543, GBAN-AF073544
<i>Carum carvi</i> L.	Downie et al. (1998)	GBAN-U78377, GBAN-U78437
<i>Caucalis platycarpus</i> L.	Downie et al. (1998)	GBAN-U78364, GBAN-U78424
<i>Chaerophyllum aromaticum</i> L.	Poland, Beskid Niski Mtns., Izby, 3 May 1997, <i>Spalik s. n.</i> (WA)	GBAN-AF073631, GBAN-AF073632
<i>Chaerophyllum astrantiae</i> Boiss. & Balansa‡	Turkey, Çoruh: Kaçkar Dağı, Altuparmak, 6 August 1996, <i>Spalik s. n.</i> (WA)	GBAN-AF073653, GBAN-AF073654
<i>Chaerophyllum atlanticum</i> Coss.	Morocco, near Imlil, 27 July 1980, <i>Yorks. Sch. Expl. Soc. A15</i> (E)	GBAN-AF073633, GBAN-AF073634
<i>Chaerophyllum aureum</i> L.†	Turkey, Çoruh: Kaçkar Dağı, Didvake, 9 August 1996, <i>Spalik s. n.</i> (WA)	GBAN-AF073655, GBAN-AF073656
<i>Chaerophyllum azoricum</i> Trel.	Spain, Azores, cult. Conservatoire botanique Mulhouse, France, 24 July 1996, <i>Reduron s. n.</i> (WA)	GBAN-AF073657, GBAN-AF073658

TABLE 2. Continued.

Accession	Source and/or voucher	GenBank accession number <sup>a</sup>
<i>Chaerophyllum bulbosum</i> L.	Turkey, Kars: Sarikamiş-Karakurt, 10 km S. Sarikamiş, 1 July 1988, <i>Nydegger 43552</i> (RNG)	GBAN-AF073659, GBAN-AF073660
<i>Chaerophyllum byzantinum</i> Boiss.†‡	Turkey, Bolu: Bolu-Mudurnu Abant Golu, 27 July 1988, <i>Nydegger 43891</i> (RNG)	GBAN-AF073635, GBAN-AF073636
<i>Chaerophyllum crinitum</i> Boiss.†‡	Iran, Luristan: Khali Kuh, 50–60 km from Ali-gudarz, 12–14 June 1974, <i>Rechinger 47960</i> (MISS)	GBAN-AF073661, GBAN-AF073662
<i>Chaerophyllum elegans</i> Gaudin	Switzerland, Grand-Saint-Bernard, 7 September 1996, <i>Reduron, Charpin &amp; Pimenov</i> (WA)	GBAN-AF073663, GBAN-AF073664
<i>Chaerophyllum hakkiaricum</i> Hedge & Lamond†‡	Turkey, Hakkari: Sat Mtns., 6 July 1965, <i>Fedden &amp; McColl 21</i> (E)	GBAN-AF073649, GBAN-AF073650
<i>Chaerophyllum hirsutum</i> L.	France, Haut-Rhin, Rimbach near Guebwiller, 15 September 1996, <i>Reduron s. n.</i> (WA)	GBAN-AF073665, GBAN-AF073666
<i>Chaerophyllum khorassanicum</i> Schischk.	Downie et al. (1998)	GBAN-U78366, GBAN-U78426
<i>Chaerophyllum libanoticum</i> Boiss. & Kotschy†‡	Turkey, Hatay: above Yeşilkent, 11 August 1969, <i>Darrah 569</i> (E)	GBAN-AF073637, GBAN-AF073638
<i>Chaerophyllum macropodum</i> Boiss.†‡	Iran, C. Kashan: Muteh, <i>Rechinger 46972</i> (MISS)	GBAN-AF073671, GBAN-AF073672
<i>Chaerophyllum macrospermum</i> (Spreng.) Fisch. & C. A. Mey.	Turkey, Çoruh: Kaçkar Dağı, Büyük Çay valley, 17 August 1996, <i>Spalik s. n.</i> (WA)	GBAN-AF073651, GBAN-AF073652
<i>Chaerophyllum magellense</i> Ten.†‡	Italy, Aprutii, Orfenta, August 1898, <i>Rigo 4672</i> (E)	GBAN-AF073669, GBAN-AF073670
<i>Chaerophyllum meyeri</i> Boiss. & Buhse†‡	Iran, Gilan: Assalem to Khalkhal, 29 May 1978, <i>Wendelbo &amp; Assadi 27717</i> (E)	GBAN-AF073639, GBAN-AF073640
<i>Chaerophyllum nivale</i> Hedge & Lamond†‡	Iran, Bakhtiari, Zarduh, Haftanan, 25 July 1973, <i>Riazi s. n.</i> (E)	GBAN-AF073673, GBAN-AF073674
<i>Chaerophyllum procumbens</i> (L.) Crantz	USA, Indiana, Warren Co., Fall Creek Gorge Nature Preserve, 10 May 1992, <i>Tonkovich 379</i> (ILL)	GBAN-AF073643, GBAN-AF073644
<i>Chaerophyllum tainturieri</i> Hook. ‘ <i>C. texanum</i> J. M. Coult. & Rose’	USA, Kansas, Cowley Co., 5 miles SE of Dexter, 6 June 1960, <i>Hulbert 3848</i> (ILL)	GBAN-AF073647, GBAN-AF073648
<i>Chaerophyllum tainturieri</i> Hook.†	USA, Arkansas, Bradley Co., Bradley County Park, 6 April 1984, <i>Leslie &amp; Taylor 120</i> (ILL)	GBAN-AF073645, GBAN-AF073646
<i>Chaerophyllum temulentum</i> L.	Poland, Warsaw, Botanic Garden of Warsaw University, 14 June 1986, <i>Spalik s. n.</i> (ILL)	GBAN-AF073641, GBAN-AF073642
<i>Chaerophyllum villarsii</i> W. D. J. Koch	Switzerland, Grand-Saint-Bernard, 7 September 1996, <i>Reduron, Charpin &amp; Pimenov</i> (WA)	GBAN-AF073667, GBAN-AF073668
<i>Chaetosciadium trichospermum</i> (L.) Boiss.	Downie et al. (1998)	GBAN-U78363, GBAN-U78423
<i>Cicuta virosa</i> L.	Downie et al. (1998)	GBAN-U78372, GBAN-U78432
<i>Conioselinum chinense</i> (L.) B. S. P.	Downie et al. (1998)	GBAN-U78374, GBAN-U78434
<i>Conopodium bourgaei</i> Coss.	Morocco, Chefchaouene, between Bab-Berered and Ketama, 31 May 1995, <i>Ferguson, Para, Sanchez &amp; Valdés 6413/95</i> (RNG)	GBAN-AF073691, GBAN-AF073692
<i>Conopodium ramosum</i> Costa†‡	Portugal, prov. Minho, Serra do Gerez, road to Carris, 20 July 1980, <i>Gardner &amp; Gardner</i> (RNG)	GBAN-AF073693, GBAN-AF073694
<i>Cuminum cyminum</i> L.	Downie et al. (1998)	GBAN-U78362, GBAN-U78422
<i>Daucus carota</i> L.	Downie et al. (1998)	GBAN-U27589, GBAN-U30315
<i>Deverra triradiata</i> Hochst. ex Boiss.	Saudi Arabia, Harrat al Karrah, <i>Collenette 6568</i> (E)	GBAN-AF073561, GBAN-AF073562
<i>Dicyclophora persica</i> Boiss.	Iran, N. of Borazjan, <i>Davis &amp; Bokhari 56510</i> (E)	GBAN-AF073539, GBAN-AF073540
<i>Echinophora tenuifolia</i> L. subsp. <i>sibthorpiana</i> (Guss.) Tutin	Turkey, Adana: Demirkazık Köyü to Çamardi, 12 September 1997, <i>Spalik &amp; Żochowska s. n.</i> (WA)	GBAN-AF073529, GBAN-AF073530
<i>Echinophora tournefortii</i> Jaub. & Spach	Turkey, Niğde: Ihlara, 4 September 1997, <i>Spalik &amp; Żochowska s. n.</i> (WA)	GBAN-AF073531, GBAN-AF073532
<i>Elaeosticta allioides</i> (Regel & Schmalh.) Kljuykov, Pimenov & V. N. Tikhom.†‡	Turkmenistan, SW Kopetdag, Kara-Kalinskii region, 3–4 km N of Koshtemir, 7 June 1974, <i>Nikitin &amp; Ivanov s. n.</i> (MISS)	GBAN-AF073547, GBAN-AF073548
<i>Falcaria vulgaris</i> Bernh.	Downie et al. (1998)	GBAN-U78378, GBAN-U78438
<i>Geocaryum macrocarpum</i> (Boiss. & Spruner) Engstrand	Greece, Crete, Mt. Profilas, April 1968, <i>Hedge s. n.</i> (E)	GBAN-AF073607, GBAN-AF073608
<i>Geocaryum macrocarpum</i> (Boiss. & Spruner) Engstrand†	Turkey, Antalya: mountain slope SW of Avlan Gölü, c. 80 km SW of Antalya, 28 April 1959, <i>Hennipman et al. 739</i> (BE)	GBAN-AF073605, GBAN-AF073606
<i>Grammosciadium daucooides</i> DC.†	Turkey, Ağrı: 2 km SW of Hamur, 1 June 1966, <i>Davis 44068</i> (E)	GBAN-AF073559, GBAN-AF073560
<i>Grammosciadium macrodon</i> Boiss.	Turkey, Siirt: above Sirnak, 8 May 1966, <i>Davis 42613</i> (E)	GBAN-AF073553, GBAN-AF073554

TABLE 2. Continued.

Accession	Source and/or voucher	GenBank accession number <sup>a</sup>
<i>Grammosciadium platycarpum</i> Boiss. & Hausskn.†‡	Turkey, Urfa: Sıverek, 5 km SE to Karabağçe, 20 June 1984, <i>Kaynak 455</i> (BE)	GBAN-AF073551, GBAN-AF073552
<i>Grammosciadium pterocarpum</i> Boiss.	Turkey, Ağrı: 2 km SW of Hamur, 1 June 1966, <i>Davis 44158</i> (E)	GBAN-AF073557, GBAN-AF073558
<i>Grammosciadium scabridum</i> Boiss.	Iran, Kordestan, 8 km N of Mirabad, 29 May 1978, <i>Runemark &amp; Mozaffarian 29174</i> (E)	GBAN-AF073555, GBAN-AF073556
<i>Heracleum sphondylium</i> L.	Downie et al. (1998)	GBAN-U30544, GBAN-U30545
<i>Heteromorpha arborescens</i> (Spreng.) Cham. & Schtdl.	Downie et al. (1998)	GBAN-U27578, GBAN-U30314
<i>Komarovia anisosperma</i> Korovin	Downie et al. (1998)	GBAN-U78381, GBAN-U78441
<i>Kozlovia paleacea</i> (Regel & Schm.) Lipsky	Afghanistan, Kataghan: Paigah Kotal, between Pul-i Khumri and Haibak, 5 May 1967, <i>Re-chinger 33878</i> (E)	GBAN-AF073597, GBAN-AF073598
<i>Krasnovia longiloba</i> (Kar. & Kir.) Schischk.‡	Kazakhstan, Dshungar Alatau, Mt. Bebokan, near "Dzershinskoe," 20 June 1959, <i>Goloskov 4337b</i> (MISS)	GBAN-AF073599, GBAN-AF073600
<i>Laserpitium hispidum</i> M. Bieb.	Downie et al. (1998)	GBAN-U78361, GBAN-U78421
<i>Laserpitium petrophilum</i> Boiss. & Heldr.†‡	Turkey, Adana: Aladağlar, near Emli, 6 September 1997, <i>Spalik &amp; Żochowska s. n.</i> (WA)	GBAN-AF073567, GBAN-AF073568
<i>Laserpitium siler</i> L.	Downie et al. (1998)	GBAN-U30528, GBAN-U30529
<i>Lecokia cretica</i> (Lam.) DC.	Downie et al. (1998)	GBAN-U78358, GBAN-U78418
<i>Ligusticum porteri</i> J. M. Coult. & Rose	Downie et al. (1998)	GBAN-U78375, GBAN-U78435
<i>Molopospermum peloponnesiacum</i> (L.) W. D. J. Koch	France, Mt. Lewis Pyrenees, 20 June 1987, <i>Argent ML2</i> (E)	GBAN-AF074335, GBAN-AF074336
<i>Monizia edulis</i> Lowe†‡	Madeira, cult. Madeira Botanic Garden, 21 July 1997, <i>F. &amp; O. Baets 08655</i> (E)	GBAN-AF073569, GBAN-AF073570
<i>Myrrhis odorata</i> (L.) Scop.	Downie et al. (1998)	GBAN-U30530, GBAN-U30531
<i>Myrrhoides nodosa</i> (L.) Cannon	Armenia, Megrinskii region, Shvanidzor, Mt. Tiumarants, Ehnaki-dzor, 11 June 1978, <i>Tabrizhjan s. n.</i> (MISS)	GBAN-AF073675, GBAN-AF073676
<i>Naufraga balearica</i> Constance & Cannon	Spain, Balears, Majorca, Port de Pollença, Cala de Sant Vicenc., 26 November 1994, <i>McBeath 2760</i> (E), cult. Royal Botanic Garden Edinburgh, Scotland, UK (no. 19943095)	GBAN-AF073563, GBAN-AF073564
<i>Neoconopodium capnoides</i> (Decne.) Pimenov & Kljuykov	Himalaya, near Koti Kanasar, 25 April 1894, <i>Duthie 14473</i> (E)	GBAN-AF073601, GBAN-AF073602
<i>Neoconopodium laseroides</i> (Hedge & Lamond) Pimenov & Kljuykov	Afghanistan, Taing-i-Gharu, E of Kabul, 2 April 1969, <i>Freitag 4670</i> (W)	GBAN-AF073603, GBAN-AF073604
<i>Nirarathamnos asarifolius</i> Balf. f.	Socotra, Haggier Mtns., 1 April 1996, <i>Miller &amp; Alexander 14276</i> (E)	GBAN-AF073535, GBAN-AF073536
<i>Oenanthe pimpinelloides</i> L.	Downie et al. (1998)	GBAN-U78371, GBAN-U78431
<i>Orlaya grandiflora</i> (L.) Hoffm.	Downie et al. (1998)	GBAN-U30524, GBAN-U30525
<i>Orlaya kochii</i> Heywood	Downie et al. (1998)	GBAN-U30526, GBAN-U30527
<i>Osmorhiza aristata</i> (Thunb.) Rydb.†‡	Japan, Honshu, Kyoto-fu: Kunimiyama, Kaman-yu, Maizuru-shi, 19 April 1991, <i>Takahashi 1766</i> (MISS)	GBAN-AF073609, GBAN-AF073610
<i>Osmorhiza berteroi</i> DC. (= <i>O. chilensis</i> Hook. & Arn.)	Chile, prov. Valparaíso: near Granizo, at the base of Mt. La Campana, 7 November 1978, <i>Zöllner 10097</i> (ILL)	GBAN-AF073619, GBAN-AF073620
<i>Osmorhiza berteroi</i> DC.	Downie et al. (1998)	GBAN-U78365, GBAN-U78425
<i>Osmorhiza brachypoda</i> Torr.	USA, California, Ventura Co., 4 miles from Ozena Ranger Station, 21 June 1979, <i>Seigler et al. 11324</i> (ILL)	GBAN-AF073617, GBAN-AF073618
<i>Osmorhiza claytonii</i> (Michx.) C. B. Clarke	USA, Indiana, Fountain Co., Portland Arch Nature Preserve, N of Covington, 11 May 1978, <i>Lowry 1208</i> (ILL)	GBAN-AF073615, GBAN-AF073616
<i>Osmorhiza depauperata</i> Phil.†	USA, Montana, Beaverhead Co., Red Rock Lakes National Wildlife Refuge, 10 July 1979, <i>Lowry 2351</i> (ILL)	GBAN-AF073611, GBAN-AF073612
<i>Osmorhiza depauperata</i> Phil.‡	Argentina, Tierra del Fuego, near Usuaia, 10 February 1976, <i>Zöllner 8584</i> (MISS)	GBAN-AF073613, GBAN-AF073614
<i>Osmorhiza longistylis</i> (Torr.) DC.	Downie et al. (1998)	GBAN-U79617, GBAN-U79618
<i>Osmorhiza mexicana</i> Griseb. subsp. <i>mexicana</i>	Mexico, near Zempoala National Park, 20 October 1981, <i>Warnock 2571</i> (ILL)	GBAN-AF073621, GBAN-AF073622
<i>Osmorhiza mexicana</i> subsp. <i>bipatriata</i> (Constance & Shan) Lowry & A. G. Jones	Mexico, Coahuila, Sierra del Carmen, below Campo Cinco on Madera del Carmen Road, 11 August 1981, <i>Lowry &amp; Warnock 3130</i> (ILL)	GBAN-AF073623, GBAN-AF073624
<i>Osmorhiza occidentalis</i> (Nutt.) Torr.	Downie et al. (1998)	GBAN-U79619, GBAN-U79620

TABLE 2. Continued.

Accession	Source and/or voucher	GenBank accession number <sup>a</sup>
<i>Osmorhiza purpurea</i> (J. M. Coult. & Rose) Suksd.	USA, Oregon, Clatsop Co., Lee Wooden Co. Park, 17 May 1980, Lowry 3092 (ILL)	GBAN-AF073625, GBAN-AF073626
<i>Pastinaca sativa</i> L.	Downie et al. (1998)	GBAN-U30546, GBAN-U30547
<i>Perideridia kelloggii</i> (A. Gray) Mathias	Downie et al. (1998)	GBAN-U78373, GBAN-U78433
<i>Petroselinum crispum</i> (Mill.) A. W. Hill	Downie et al. (1998)	GBAN-U78387, GBAN-U78447
<i>Peucedanum morisonii</i> Bess. ex Spreng.	Downie et al. (1998)	GBAN-U78406, GBAN-U78466
<i>Peucedanum</i> sp.	Socotra, 1992, Miller & Nyberg 11204 (E), cult. Royal Botanic Garden, Edinburgh, UK (no. 19923643)	GBAN-AF073537, GBAN-AF073538
<i>Physospermum cornubiense</i> (L.) DC.	Downie et al. (1998)	GBAN-U78382, GBAN-U78442
<i>Pimpinella peregrina</i> L.	Downie et al. (1998)	GBAN-U30592, GBAN-U30593
<i>Pseudorhiza pumila</i> (L.) Grande	Downie et al. (1998)	GBAN-U30522, GBAN-U30523
<i>Pycnocycla aucherana</i> Decne. ex Boiss.	Iran, Tehran, Kerman Darzin, Babakhanlou 23090 (E)	GBAN-AF073533, GBAN-AF073534
<i>Rhabdosciadium aucheri</i> Boiss.	Iran, Esfahan: Akhreh, Makeh-din, Kuh-evanizan, 5 August 1973, Moussavi & Satei s. n. (E)	GBAN-AF073549, GBAN-AF073550
<i>Rhabdosciadium aucheri</i> Boiss.†‡	Iran, Luristan: Brujerd, Kouhé Marrow, 18 August 1973, Moussavi & Satei s. n. (E)	GBAN-AF073549, GBAN-AF073550
<i>Scaligeria moreana</i> Engstrand†‡	Greece, Lakonia, Lakedhemona, Mt. Taygetos, 8 October 1979, Greuter & Zimmer 11489, cult. Botanical Garden Berlin-Dahlem, 22 June 1983, Schwerdfeger 14405 (MISS)	GBAN-AF073545, GBAN-AF073546
<i>Scandix balansae</i> Reut. ex Boiss.	Downie et al. (1998)	GBAN-U79621, GBAN-U79622
<i>Scandix iberica</i> M. Bieb.†‡	Jordan, Um-Qais, near Irbid, Lahham & El-Oqlah 3 (Yarmouk Univ. Herb.)	GBAN-AF073627, GBAN-AF073628
<i>Scandix pecten-veneris</i> L.	Downie et al. (1998)	GBAN-U30538, GBAN-U30539
<i>Scandix stellata</i> Banks & Sol†‡	Jordan, Ajlun, Shtafeenah, Lahham & El-Oqlah 16 (Yarmouk Univ. Herb.)	GBAN-AF073629, GBAN-AF073630
<i>Sium latifolium</i> L.	Downie et al. (1998)	GBAN-U78370, GBAN-U78430
<i>Smyrniolum olusatrum</i> L.	Downie et al. (1998)	GBAN-U30594, GBAN-U30595
<i>Sphallerocarpus gracilis</i> (Bess. ex Trevir.) Koso-Pol.	Russia, Jakutsya, 80 km S from Jakutsk, 20 August 1978, Ivanov 804 (W)	GBAN-AF073677, GBAN-AF073678
<i>Tinguarra cervariifolia</i> (DC.) Benth. & Hook. f.†‡	Spain, Canaries, La Palma, Caldera de Taburiente, La Cumbrecita, 8 October 1991, Royle 435 (BE)	GBAN-AF073681, GBAN-AF073682
<i>Tinguarra montana</i> (Webb ex H. Christ) A. Hansen & G. Kunkel†	Spain, Canaries, Tenerife, Aguamansa, los Orgarios, cult. Conservatoire botanique Mulhouse, France (no. 95179), 27 September 1996, Reduron s. n. (WA)	GBAN-AF073679, GBAN-AF073680
<i>Tinguarra sicula</i> (L.) Parl.†	Morocco, Brni Snassen, Gorges du Zegzel between Trashroute and Moulay Ahmed, 10 May 1993, Vogt 11592 & Oberprieler 6040 (BE)	GBAN-AF073683, GBAN-AF073684
<i>Torilis nodosa</i> (L.) Gaertn.	Downie et al. (1998)	GBAN-U30534, GBAN-U30535
<i>Trachyspermum ammi</i> (L.) Sprague ex Turrill	Downie et al. (1998)	GBAN-U78380, GBAN-U78440

<sup>a</sup> The prefix GBAN- has been added for linking the on-line version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number.

members of Apiaceae-APIINAE in Scandiceae, while removing some genera, like *Anthriscus* and *Myrrhoides*. He was followed by Koso-Poljansky (1916), who added genera recognized presently in tribe Echinophoreae. *Molopospermum peloponnesiacum* is the only member of Scandiceae with distinctly winged fruits and has been placed either in Smyrnieae (Bentham, 1867; Pimenov and Leonov, 1993), Apiaceae (Calestani, 1905), or in a separate tribe close to Smyrnieae (Cerceanu-Larrival, 1962). *Kozlovia* is recognized either in Scandiceae (Heywood, 1971; Pimenov and Leonov, 1993) or in Caucalideae (Heywood, 1982b; Hedge et al., 1987). Bentham (1867), admitting the similarity between *Tinguarra* and *Athamanta*, placed the former in Scandiceae and the latter in Seseleae; this treatment was also adopted by Drude (1898). *Athamanta* was transferred to Scandiceae by Cer-

ceanu-Larrival (1962) based on pollen and cotyledon morphology. Monotypic *Balansaea* was recognized in Scandiceae as a separate genus (Heywood, 1971) or included in *Geocaryum* (Drude, 1898; Pimenov and Leonov, 1993). *Balansaea* was also synonymized with *Conopodium* (Engstrand, 1973), which is usually placed in Apiaceae. Although the affinities of *Grammosciadium* and *Rhabdosciadium* to Scandiceae are rarely questioned, Hedge and Lamond (1987) indicated that both genera differ from other members of the tribe in not having a deeply sulcate endosperm. Tamamschian and Vinogradova (1969) suggested that *Grammosciadium* occupies an intermediate position between Scandiceae and Caucalideae.

The major objectives of this study were: (1) to ascertain the monophyly of Scandiceae and its relationship to the other currently recognized lineages of Apiaceae in-

ferred from molecular studies; (2) to verify the monophyly of the largest genera, i.e., *Anthriscus*, *Chaerophyllum*, and *Osmorhiza*; and (3) to ascertain the evolutionary affinities of the monotypic members, i.e., *Balansaea*, *Myrrhis*, *Myrrhoides*, *Kozlovica*, *Krasnovia*, and *Sphallerocarpus*. The reassessment of qualitative morphological characters and the interpretation of their evolution will be presented in a subsequent study.

## MATERIALS AND METHODS

**Plant accessions**—We have chosen to examine variation in nuclear ribosomal DNA (rDNA) internal transcribed spacers (ITS) sequences, regions that have been useful in estimating infrafamilial relationships in Apiaceae (Downie and Katz-Downie, 1996; Downie et al., 1998; Katz-Downie et al., 1999) as well as in other groups of angiosperms (reviewed in Baldwin et al., 1995). A total of 134 accessions representing 64 genera and 119 species was considered. Complete ITS1 and ITS2 sequences for 85 accessions representing 29 genera and 73 species are reported here for the first time (Table 2); ITS data for the remaining 49 accessions were published previously (Downie et al., 1998). The ingroup has been chosen based on the analysis of different classification systems of Scandiceae (Table 1). The most important accounts of the tribe are those of de Candolle (1830), Bentham (1867), Boissier (1872), Drude (1898), Calestani (1905), Koso-Poljansky (1916), Cerceau-Larrival (1962), Heywood (1971), Hedge et al. (1987), and Pimenov and Leonov (1993). We have omitted those sources in which Scandiceae sensu Drude (1898) were not divided into Scandicinae and Caucalidinae, as in Schischkin (1950a). Not all of the cited accounts give a complete treatment of the tribe. Moreover, older accounts (i.e., de Candolle, 1830) lack some later described genera. Boissier (1872), Calestani (1905), and Hedge et al. (1987) revised regional floras, while Heywood (1971) considered solely Old World umbellifers. The revision of Cerceau-Larrival (1962) contained merely a sketch of the system, listing some six genera in Scandiceae. The most influential classification was that of Drude (1898), and the accounts of Heywood (1971), Hedge et al. (1987), and Pimenov and Leonov (1993) generally follow it.

Moreover, the delimitations of many genera have changed since their original description. *Grammosciadium* sensu Calestani (1905), for instance, is in fact synonymous with *Chaerophyllum*. The real puzzle, due to somewhat obscure synonymy and controversial taxonomic decisions, is the classification of Koso-Poljansky (1916). He included *Osmorhiza* (as *Uraspermum*) in *Scandix* while retaining *Glycosma*, which is now recognized as a subgenus of *Osmorhiza*. He also divided *Grammosciadium* into two genera and included *Falcaria*, *Ptychotis*, and *Hladnikia* into one of them. Consequently, the comparison of classifications based solely on the lists of genera may be highly misleading. The summary presented in Table 1 is based therefore on the lists of species included in particular genera.

Representatives of some 40 currently recognized genera have been placed in Scandiceae; eight of these, however, have already been excluded from the tribe based on molecular studies (Downie and Katz-Downie, 1996; Plunkett, Soltis, and Soltis, 1996; Downie et al., 1998; Lee and Downie, 1999) and are therefore omitted from Table 1. These are *Cryptotaenia* (= *Deringa*; Koso-Poljansky, 1916), *Cuminum* (Calestani, 1905), *Falcaria* (Calestani, 1905; Koso-Poljansky, 1916, in *Prionitis*), *Petagnaea* (= *Heterosciadium*; Koso-Poljansky, 1916), *Physopermum* (Koso-Poljansky, 1916, in *Chaerophyllum*), *Rhodosciadium* (= *Velaea*; de Candolle, 1830), *Tauschia* (Koso-Poljansky, 1916), and *Yabea* (Koso-Poljansky, 1916). From among those remaining, we generally considered all genera that have been classified in Scandiceae by more than one author and included in one of the more recent treatments. Several genera have been recognized in the tribe only once, mostly by Calestani (1905) or Koso-Poljansky (1916). These are *Anisosciadium* (Koso-Poljansky, 1916), *Bunium* (Koso-Poljansky, 1916, = *Elwendia*), *Chaerophyllopsis* (Heywood, 1971), *Echinophora* (Koso-Poljansky,

1916), *Kundmannia* (Calestani, 1905), *Microsciadium* (Calestani, 1905), *Ottoa* (Bentham, 1867), *Portenschlagiella* (Calestani, 1905), *Pycnocycla* (Koso-Poljansky, 1916), *Rhopalosciadium* (Pimenov and Leonov, 1993), and *Scaligeria* (Bentham, 1867, in *Conopodium*). While most of these genera were omitted from our analysis, we did include members of *Bunium* and *Scaligeria* (sensu lato, i.e., including *Elaeosticta*) to test for the monophyly of Bunieae sensu Calestani (1905), and four accessions of tribe Echinophoreae (*Echinophora*, *Pycnocycla*, and *Dicyclophora*), whose members have not been previously analyzed. We omitted *Chaerophyllopsis*, a monotypic endemic of China recognized in Scandiceae by Heywood (1971), and *Rhopalosciadium*, transferred to Scandiceae from Caucalideae by Pimenov and Leonov (1993), as both species were not available for molecular study (the latter is known exclusively from the type collection).

In summary, the 134 accessions examined reflect 64 genera and 119 species, including 18 genera (with 67 species and 82 accessions) classified more than once in Scandiceae. Fifteen genera, including 11 of Scandiceae, were represented by more than one accession. Due to limited availability of material, different proportions of taxa from particular genera were included in the analysis. The most extensively sampled genera were *Osmorhiza*, *Anthriscus*, *Tinguarra*, and *Neoconopodium*, which included 90–100% of all currently recognized species, subspecies, and varieties, while *Geocaryum*, *Rhabdosciadium*, and *Conopodium* were not so well represented. Although only four of a maximum 20 species of *Scandix* were analyzed, these included those species retained in major taxonomic treatments (e.g., Cannon, 1968; Hedge and Lamond, 1972, 1987).

Close outgroups include members of tribes Caucalideae (i.e., *Caucalis*, *Chaetosciadium*, *Cuminum*, *Daucus*, *Orlaya*, *Pseudorlaya*, *Torilis*) and Laserpitieae (*Laserpitium*, *Monizia*). Also, representatives of all major lineages of Apioideae identified by earlier molecular studies have been included (Plunkett, Soltis, and Soltis, 1996; Downie et al., 1998). We have added the sequences of *Nirarathamnos* and *Naufraga*, monotypic endemics whose affinities are unclear, and of *Deverra triradiata*, a representative of a genus occurring in arid regions of Africa and the Arabian Peninsula. The sequence of *Heteromorpha arborescens*, a basal apioid identified by earlier studies (Rodríguez, 1971; Plunkett, Soltis, and Soltis, 1996; Downie et al., 1998), has been included to root the trees.

**DNA extraction, PCR amplification, and sequencing**—Total genomic DNAs were extracted from dried leaf or flower material using the modified hexadecyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987) or the Plant DNeasy Extraction Kit (Qiagen, Inc., Valencia, California). Some samples required further purification by centrifugation to equilibrium in cesium chloride-ethidium bromide gradients or by applying Qiagen genomic-tip purification columns. These methods allowed us to sample fairly old accessions: many specimens were over 30 yr old, while three taxa, *Anthriscus sylvestris* subsp. *fumarioides*, *Chaerophyllum magellense*, and *Neoconopodium capnoides*, were represented by century-old collections. Details of the PCR amplification reactions and sequencing strategies are provided elsewhere (Downie and Katz-Downie, 1996; Downie et al., 1998). The whole ITS region was amplified using primers ITS4 and ITS5 (White et al., 1990). For one accession (*Chaerophyllum nivale*), each spacer region was amplified separately using internal primers ITS2 and ITS3. For some samples, particularly those isolated from old herbarium material, the annealing temperature was lowered from 53°C to 46°C or even to 37°C. Each PCR product was then electrophoresed in a 1% agarose gel and stained with ethidium bromide. The DNA band was subsequently excised and eluted using the Elu-Quick DNA Purification Kit (Schleicher & Schuell, Keene, New Hampshire) or the QIAEX II Gel Extraction Kit (Qiagen). Both manual and automated sequencing strategies were used. Cycle sequencing reactions were performed using the purified PCR product, AmpliTaq DNA polymerase, and fluorescent dye-labeled terminators (Perkin-Elmer Corp., Norwalk, Connecticut).

TABLE 3. Sequence characteristics of the two nuclear rDNA internal transcribed spacers, separately and combined, for 133 representatives of Apiaceae subfamily Apioideae (*Molopospermum peloponnesiacum* was excluded from this tabulation due to alignment ambiguity). Tribe Scandiceae includes only those 72 accessions retained as a result of the phylogenetic analyses presented herein.

Sequence characteristic	ITS1	ITS2	ITS1 and ITS2
<b>Nucleotide sites</b>			
Spacer length variation (bp)	206–221	203–229	421–445
No. total aligned positions	249	250	499
No. (and %) ambiguous	10 (4.0)	14 (5.6)	24 (4.8)
No. (and %) constant	50 (20.1)	35 (14.0)	85 (17.0)
No. (and %) autapomorphic	24 (9.6)	27 (10.8)	51 (10.2)
No. (and %) parsimony informative	165 (66.3)	174 (69.6)	339 (67.9)
<b>Gaps</b>			
No. of unambiguous alignment gaps	48	44	92
No. (and size range; bp) of deletions	28 (1–8)	31 (1–17)	59 (1–17)
No. (and size range; bp) of insertions	20 (1–5)	13 (1–3)	33 (1–5)
No. of gaps parsimony informative	16	24	40
<b>Sequence divergence (mean and range in %)</b>			
All accessions	20.1 (0–32.9)	20.9 (0–36.5)	20.6 (0–33.6)
Tribe Scandiceae only	11.7 (0–22.5)	12.5 (0–22.6)	12.0 (0–21.8)

The products were resolved by electrophoresis using Applied Biosystem's, Inc. (Foster City, California) 310 and 373A DNA sequencing systems.

**Sequence analysis**—Alignment of the sequences was done manually starting from a subset of the data matrix from previously published studies (Downie and Katz-Downie, 1996; Downie et al., 1998) and consecutively adding new accessions. The initial data matrix was aligned using the program CLUSTAL V (Higgins, Bleasby, and Fuchs, 1992) and then adjusted manually when necessary. Boundaries of the coding and spacers regions were determined by comparison of the sequences to the respective boundaries in *Daucus carota* (Yokota et al., 1989). Positions with ambiguous alignment were excluded from the analysis. Pairwise nucleotide differences were determined using the distance matrix option in PAUP version 3.1.1 (Swofford, 1993). All gaps were treated as missing data, while the sequence divergence was calculated as a proportion of different sites without taking into account multiple substitutions. Transition/transversion ratios were calculated using MacClade version 3.01 (Maddison and Maddison, 1992) over a subset of the maximally parsimonious trees. The sequences reported in this study have been deposited with GenBank (Table 2), and their alignment may be obtained directly from the authors.

**Phylogenetic analysis**—The resulting data matrix was analyzed using PAUP version 3.1.1 (Swofford, 1993) or PAUP\* version 4.0.0d64 (D. Swofford, Smithsonian Institution, Washington, DC) run on Power Macintosh computers. Unordered characters states were assumed. To locate possible islands of most parsimonious trees, 500 heuristic searches were initiated using random addition starting trees, with TBR (tree bisection-reconnection) branch swapping, mulpars, and steepest descent selected. Only two shortest trees were saved from each search; these trees were subsequently used as starting trees for TBR branch swapping. This search was stopped when the number of trees reached the memory limit of 12 000. The strict consensus of these trees served as a topological constraint in a further heuristic search using the inverse constraint approach of Catalán, Kellogg, and Olmstead (1997). This time, 5000 searches were initiated, saving no more than two trees per replicate. However, in this analysis, only those trees that did not fit the constraint tree were saved. Since no additional trees, shorter or equal to those previously obtained, were found, this then suggests that the strict consensus tree satisfactorily summarizes the available evidence, even though the exact number of trees at that length is not known.

For bootstrap analysis, 100 resampled data sets were generated and, for each, 500 heuristic searches were initiated, saving no more than two

shortest trees per search, and no more than 100 trees per bootstrap replicate. The frequencies of particular groups of taxa were then calculated over the set of all trees saved. Decay values were estimated using the converse constraint method of Baum, Sytsma, and Hoch (1994). Each clade was defined as a constraint tree and a heuristic search with 100 replicates was performed with the converse of the constraint tree enforced; TBR branch swapping was selected and mulpars turned off. The decay value for the clade was calculated as the difference between the length of the shortest trees for the converse constraint of that clade and the length of the most parsimonious trees previously obtained.

Distance trees were obtained from neighbor-joining analyses, estimated with the variety of distance measures available in PAUP\* version 4.0.0d64 (such as the two- and three-parameter methods of Kimura, the Jukes-Cantor method, and maximum likelihood distance), using an IBM ThinkPad 365X computer. A bootstrap analysis was performed using 1000 resampled data sets.

## RESULTS

**Sequence analysis**—Of the 134 umbellifer accessions examined (including 18 genera with 67 species and 82 accessions classified more than once in Scandiceae), the following four pairs of accessions each had identical ITS sequences and were each represented by one terminal taxon in the phylogenetic analysis: *Rhabdosciadium aucheri* (two accessions); *Osmorhiza depauperata* (two accessions); *Chaerophyllum macrospermum* and *C. hakkiaricum*; *Athamanta cretensis* and *A. turbith*. Due to difficulty in aligning the sequence of *Molopospermum peloponnesiacum* it too was eliminated. Therefore, only 129 sequences were retained, and their alignment resulted in a matrix of 499 characters (Table 3). As the alignment of ten positions in ITS1 and 14 positions in ITS2 were ambiguous, these positions were excluded from the analysis. No evidence of obvious ITS length polymorphism within each accession examined was found.

Both spacers were similar in length; combined ITS1 and ITS2 sequences ranged between 421 and 445 bp (Table 3). The number of constant, autapomorphic, and parsimony-informative positions was similar for both spacers, and the ratio of terminal taxa (129) to informative characters across both spacers (339) was 1:2.6. The mean



TABLE 4. ITS sequence divergence of the 11 genera classified in tribe Scandiceae that were represented by more than one accession. The estimation of monophyly and the exclusion (\*\*) or part exclusion (\*) of genera from the tribe are based on results of the phylogenetic analyses presented herein.

Genus	No. of species worldwide	No. of species (and accessions) examined	Mean (and range) sequence divergence (%)	Monophyletic?
<i>Anthriscus</i>	9 <sup>a</sup>	7 (16)	5.6 (0–12.2)	yes
<i>Athamanta</i> *	5–6 <sup>b</sup>	4 (4)	17.8 (0–27.9)	no
<i>Chaerophyllum</i>	35 <sup>b</sup>	22 (23)	5.7 (0–12.5)	yes
<i>Conopodium</i> (incl. <i>Balansaea</i> )	20 <sup>b</sup>	3 (3)	7.4 (7.0–8.0)	?
<i>Geocaryum</i>	3–15 <sup>c,d</sup>	1 (2)	5.6	yes
<i>Grammosciadium</i> **	7 <sup>b</sup>	5 (5)	5.2 (1.4–10.2)	yes
<i>Neoconopodium</i>	2 <sup>e</sup>	2 (2)	6.5	yes
<i>Osmorhiza</i>	10 <sup>f</sup>	9 (12)	1.6 (0–3.5)	yes
<i>Rhabdosciadium</i> **	5 <sup>g</sup>	1 (2)	0	yes
<i>Scandix</i>	5–20 <sup>b</sup>	4 (4)	10.6 (4.4–14.6)	yes
<i>Tinguarra</i>	3 <sup>h</sup>	3 (3)	2.7 (1.4–3.4)	?

<sup>a</sup> Spalik (1997); <sup>b</sup> Pimenov and Leonov (1993); <sup>c</sup> Ball (1968); <sup>d</sup> Engstrand (1977); <sup>e</sup> Pimenov and Kljuykov (1987); <sup>f</sup> Lowry and Jones (1984); <sup>g</sup> Hedge and Lamond (1987); <sup>h</sup> Knees (1996).

sequence divergence was calculated using 133 accessions in order to account for the lack of divergence between currently recognized species, such as *Athamanta cretensis* and *A. turbith*. The mean sequence divergence value was similar for both spacers (20.1 and 20.9% for ITS1 and ITS2, respectively) and ranged from identity to 33.6% divergence for combined ITS1 and ITS2 sequences. Mean divergence for those taxa retained in Scandiceae as a result of this study was 12.0%. This value, however, may not represent the actual divergence. The greater sampling of closely related infraspecific taxa of *Osmorhiza* and *Anthriscus* than that of the more divergent *Conopodium* and *Scandix* may actually lower this value (Table 4).

Ninety-two unambiguous gaps, of either one, two, three, four, five, eight, or 17 bp, were introduced to facilitate alignment of both ITS1 and ITS2 sequences (Fig. 1; Table 3). Forty of these were phylogenetically informative for parsimony analysis. Most indels (79.3%) were 1 bp in size, with deletions outnumbering insertions 1.8:1 (Fig. 1; Table 3).

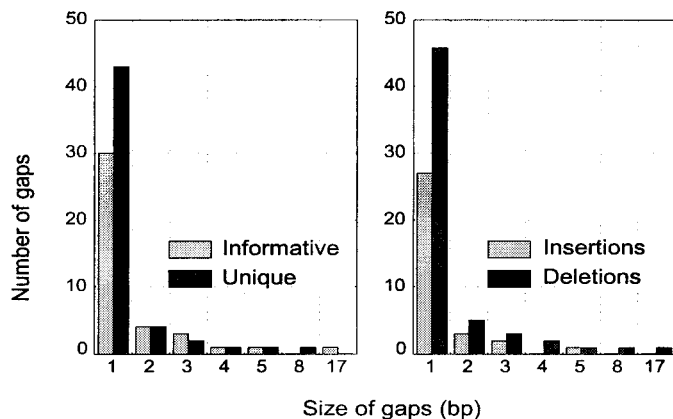


Fig. 1. Frequency of the 92 unambiguous gaps in relation to gap size, informativeness, and type (relative to the outgroup *Heteromorpha*) inferred in the alignment of combined ITS1 and ITS2 sequences for 133 accessions of Apiaceae. The number of gaps according to their size, phylogenetic signal (parsimony informative vs. autapomorphic or unique), and type (inferred insertions or deletions relative to the outgroup taxon *Heteromorpha*) is provided.

**Phylogenetic analysis**—Parsimony analysis of all 475 unambiguously aligned positions for both spacer regions resulted in more than 12 000 minimal length trees before the analysis terminated. The strict consensus of 11 900 of these trees, with accompanying bootstrap and decay values, is presented in Fig. 2. Each of these trees had a length of 2550 steps, consistency indices of 0.324 and 0.306, with and without uninformative characters, respectively, and a retention index of 0.754. Despite the high number of maximally parsimonious trees obtained, the strict consensus tree is well resolved. At the supra-generic level, many branches are supported by high bootstrap and decay values, whereas the infrageneric relationships are often ambiguous. This ambiguity, such as in *Anthriscus* and *Osmorhiza*, results from a lack of synapomorphic substitutions rather than homoplastic events. The average Ts/Tv ratio, calculated by MacClade over a set of 50 randomly chosen maximally parsimonious trees, was 1.4.

The distribution of all 40 potentially informative and unambiguous alignment gaps was optimized on one arbitrarily selected minimal length tree. As a result, 18 gaps were synapomorphic and 22 gaps were homoplastic. Overall, 83 changes are necessary to explain the distribution of these indels on the tree. The distribution of all 18 synapomorphic gaps and 14 of the 22 homoplastic gaps (identified by letters A–N) is shown in Fig. 3. For clarity, the remaining eight homoplastic gaps, which occurred only on terminal branches, were omitted. Many of these homoplastic length mutations occur in regions rich in G's and C's. The vast majority of the insertions, including autapomorphies, represent perfect repeats of flanking sequences. Two of the mapped homoplastic gaps (H and K in Fig. 3) are 3 bp in size; the remaining 12 are each 1 bp in size. Gap H occurs twice, whereas gap K has been acquired seven times. Indels, both informative and autapomorphic, were more abundant in some regions than others. For example, alignment position 57–81 in ITS1 (not shown) contains ten unambiguously aligned indels, while the beginning of ITS2 (alignment position 271–300, not shown) includes nine evident gaps, apart from those excluded due to alignment ambiguity. The longest 17-bp deletion is synapomorphic and identifies the basal branch of the *Scandix* clade (Fig. 3); this branch

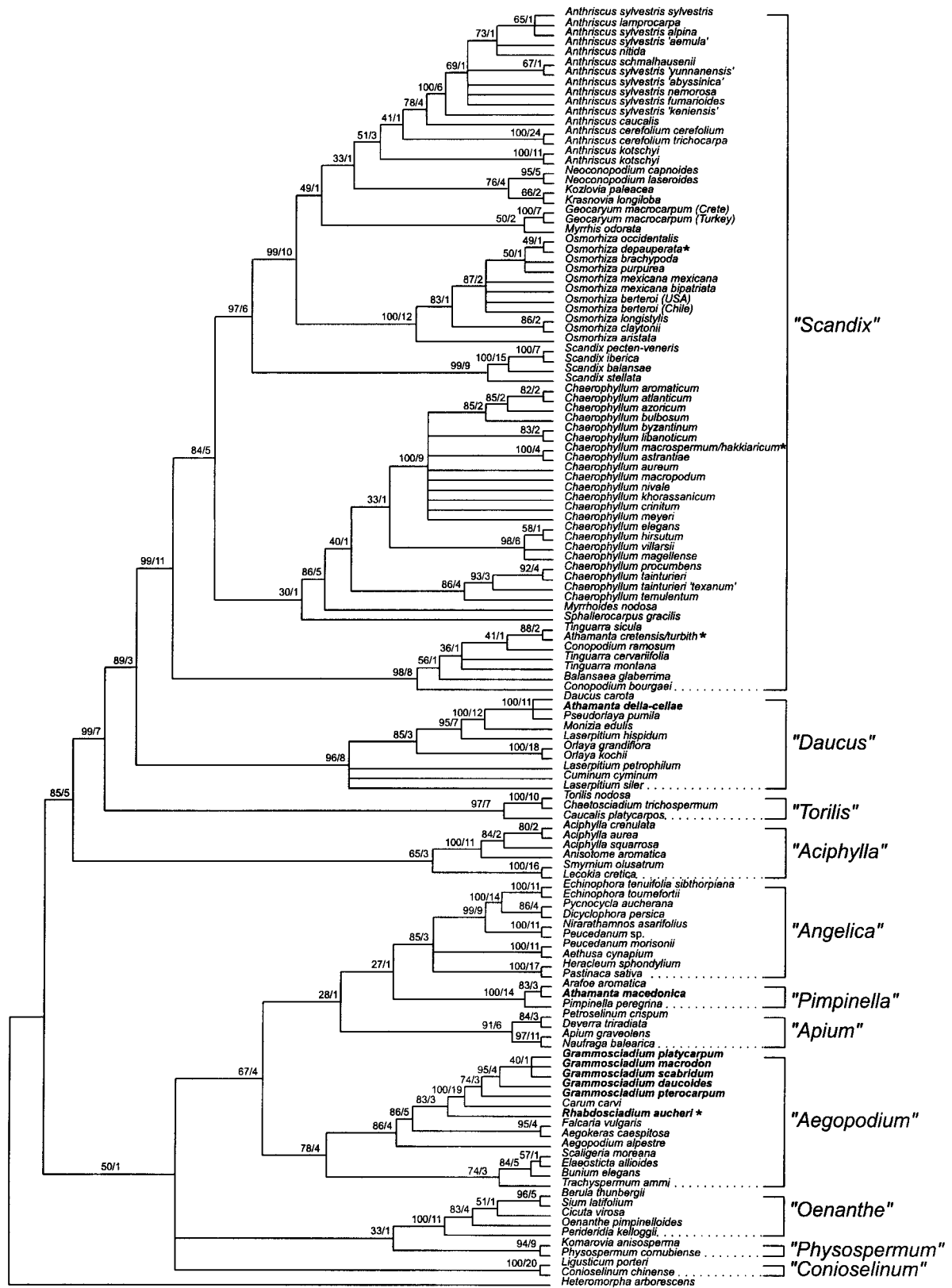


Fig. 2. Strict consensus of 11900 minimal length 2550-step trees derived from equally weighed parsimony analysis of combined ITS1 and ITS2 sequences for 133 accessions of Apiaceae (consistency index excluding uninformative characters = 0.306; retention index = 0.754). Asterisks denote the four pairs of taxa that each have identical ITS sequences. Bootstrap/decay values are placed along each node. Complete taxon names, including ranks of infraspecific taxa (which have been omitted for brevity), are provided in Table 2. Taxa previously included in Scandiceae but excluded upon the basis of this analysis are boldfaced. Brackets indicate major clades of Apiaceae. The "Scandix," "Torilis," and "Daucus" clades constitute the *Daucus* clade sensu Plunkett, Soltis, and Soltis (1996) and Downie et al. (1998).

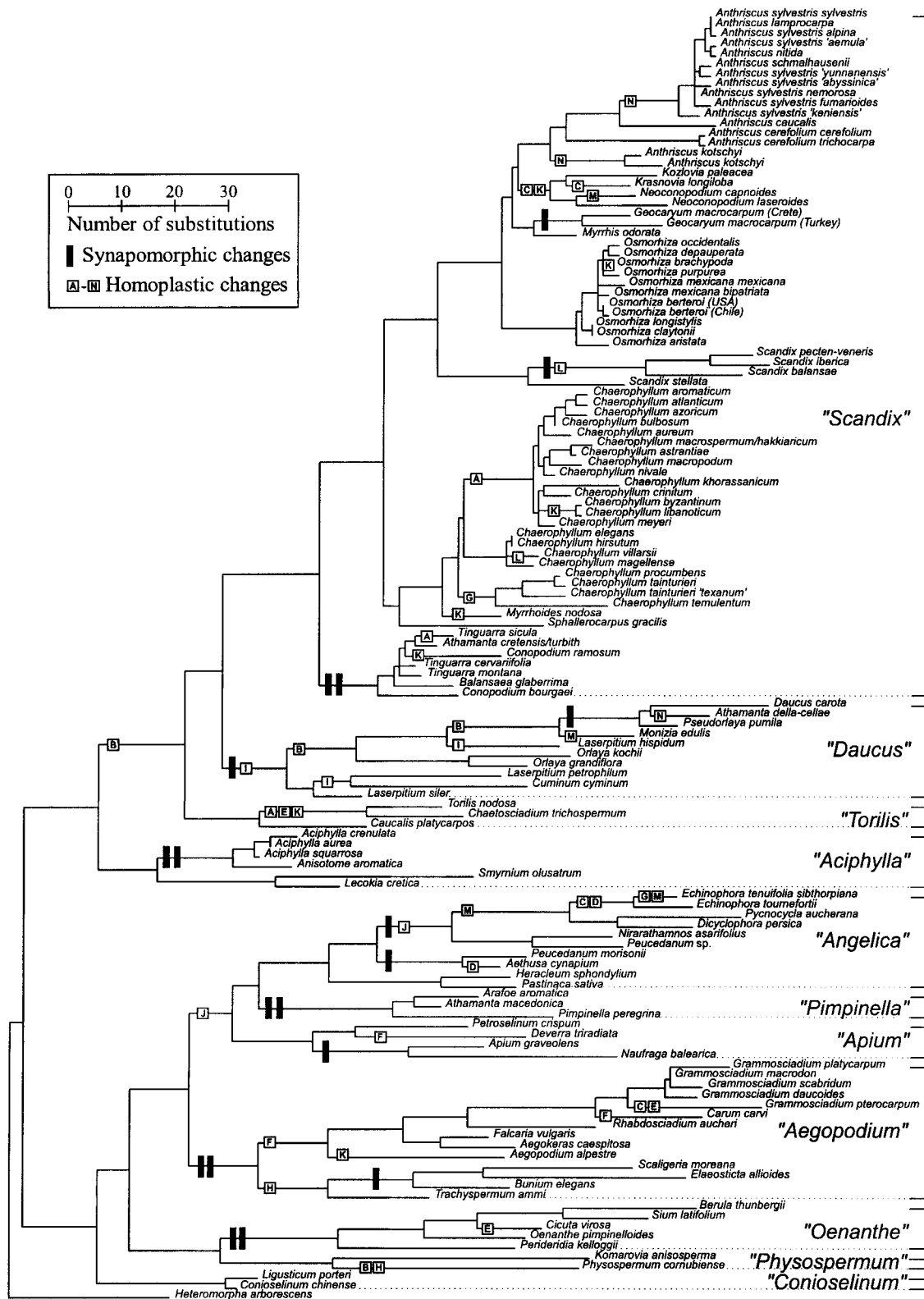


Fig. 3. Optimization of 18 synapomorphic (solid bars) and 14 of the 22 homoplastic (open squares, labeled A–N) unambiguous alignment gaps on one of the 11900 minimal length 2550-step trees derived from equally weighted parsimony analysis of combined ITS1 and ITS2 sequences. Homoplastic events are identified by similar letters. For clarity, the remaining eight homoplastic gaps, which occurred only on terminal branches, were omitted. Lengths of branches are proportional to the number of estimated nucleotide substitutions occurring along them (note scale bar). Brackets indicate major clades of Apiaceae.

comprises *Athamanta*, *Tinguarra*, *Balansaea*, and *Conopodium*.

The neighbor-joining tree, calculated using Kimura's (1980) two-parameter distance, is presented in Fig. 4. Similar trees (not shown) were obtained using other distance measures, such as Jukes-Cantor, Kimura's three-parameter, and maximum likelihood. Selecting gamma distribution as an approximation of substitution rate among sites and trying different values of the parameter  $\alpha$  did little to change the topology of the trees, other than collapsing or rearranging a few weakly supported clades.

**Phylogenetic resolutions**—Phylogenies estimated using maximum parsimony or the various distance-based procedures were very similar and all strongly supported the monophyly of Scandiceae. All major clades identified within the tribe (described below) were practically identical. The affinities of the taxa excluded from the tribe were also unequivocal. Similarly, the major clades inferred within subfamily Apioideae were the same as those identified in earlier studies; their names are presented in Figs. 2–4, following those adopted by Plunkett, Soltis, and Soltis (1996), Downie et al. (1998), and Plunkett and Downie (1999). These include the *Daucus* clade (with three subgroups, identified herein as the *Scandix*, *Daucus*, and *Torilis* subclades), the *Aciphylla* clade, the *Angelica* clade, the *Apium* clade, the *Aegopodium* clade, and the *Oenanthe* clade. Additionally, three other minor clades occur, and are identified as the *Pimpinella*, *Physospermum*, and *Conioselinum* clades. Scandiceae sensu stricto are equivalent to the *Scandix* subclade, while the *Daucus* and *Torilis* subclades constitute, to a large extent, tribe Caucalideae.

The results of the maximum parsimony analysis (Fig. 2) suggest that Scandiceae sensu stricto (the *Scandix* subclade) arise within paraphyletic Caucalideae, with the *Daucus* subclade being the closest relative and the *Torilis* subclade representing the next most basal branch. The distance-based analyses (e.g., Fig. 4) also support paraphyletic Caucalideae, but the relationship of the *Daucus* and *Torilis* subclades to each other is not clear, as the putative sister-group relationship between the *Scandix* and *Daucus* clades is very weakly supported. Although Scandiceae form a well-supported monophyletic group, several taxa previously included in the tribe are placed elsewhere. Two genera, *Grammosciadium* and *Rhabdosciadium*, fell within the *Aegopodium* clade, with the former sister to *Carum*. *Athamanta* is polyphyletic. Although the type of the genus, *A. cretensis*, is retained in Scandiceae, another European species, *A. macedonica*, clustered with *Arafoe aromatica* and *Pimpinella peregriana* in the *Pimpinella* clade. The North African *Athamanta della-cellae* was placed alongside *Daucus* and *Pseudorlaya* in the *Daucus* subclade. The positioning of the various *Athamanta* species was not only supported by high bootstrap and decay values (Fig. 2) but also by synapomorphic indels (Fig. 3). *Athamanta cretensis* and *A. turbith*, indistinguishable so far as ITS sequences are concerned, formed a strongly supported group with *Tinguarra*, *Conopodium*, and *Balansaea*; however, the phylogenetic relationships within this clade are ambiguous. In the neighbor-joining tree (Fig. 4), *Tinguarra* and *Athamanta* form a clade, denoted as the *Athamanta* group,

and arise within a paraphyletic *Conopodium*. There is little support for the monophyly of *Tinguarra*, as *T. sicula* is sister to *Athamanta cretensis*/*A. turbith* and not to the two remaining congeners. *Conopodium bourgaei* is placed as a basal branch in this clade; however, since all internal nodes in this clade are weakly supported, the rejection of monophyly of *Conopodium* may be unsound. Therefore, an additional heuristic search was initiated with *Conopodium* and *Balansaea* constrained to form a clade sister to *Athamanta* and *Tinguarra*; the shortest trees inferred in this search were only two steps longer than those obtained without the constraint invoked.

Of the 11 genera of Scandiceae represented by more than one accession, eight are monophyletic (Table 4). *Athamanta* is polyphyletic, while the monophyly of each of *Conopodium* and *Tinguarra* is dubious. With the exception of *Scandix*, sequence divergence estimates within each of the monophyletic genera were relatively low, with mean divergence values ranging from identity to 6.5% of nucleotides (Table 4).

In all analyses, the *Athamanta-Conopodium* clade (e.g., Fig. 4) is sister to all other members of Scandiceae sensu stricto. The position of the monotypic *Sphallerocarpus*, however, is equivocal. It is either placed, by itself, as the next branch up the tree (Fig. 4) or sister to *Chaerophyllum* and *Myrrhoides* (Fig. 2). The placement of *Myrrhoides* is also ambiguous, as it is either included in *Chaerophyllum* (Fig. 4) or placed as its sister taxon (Fig. 2). Nevertheless, both *Chaerophyllum* and *Myrrhoides* comprise a well-supported monophyletic group.

Seven genera of Scandiceae sensu stricto belong to a highly supported clade that is sister group to the genus *Scandix*. However, the relationships among these seven genera are unclear. Monotypic *Kozlovia* and *Krasnovia*, and both species of *Neoconopodium*, form a relatively well supported lineage, but the affinities of *Anthriscus*, *Myrrhis*, *Geocaryum*, and *Osmorhiza* are unclear. For instance, maximum parsimony (Fig. 2) favors *Myrrhis* as sister to *Geocaryum* while the distance trees (e.g., Fig. 4) ally it with *Osmorhiza*.

Infrageneric relationships within the three most extensively sampled genera—*Chaerophyllum*, *Osmorhiza*, and *Anthriscus*—are also ambiguous. Most species of *Chaerophyllum* form a highly supported but poorly resolved branch (herein called the *C. aureum* group; Fig. 4). The remaining species comprise two other major branches, herein called the *C. hirsutum* group and the *C. temulentum* group (Fig. 4). The latter group includes the North American representatives of the genus, *C. procumbens* and *C. tainturieri*. Although the monophyly of *Osmorhiza* is well supported, most taxa form a polytomous branch in the strict consensus tree (Fig. 2). It is noteworthy, however, that the basal species, *O. aristata*, is the only Old World member of the genus, while the next branch contains *O. longistylis* and *O. claytonii*, both of eastern North America. The remaining species of *Osmorhiza* constitute a poorly resolved group, which also includes *O. occidentalis*, usually placed in the monotypic subgenus *Glycosma*. Our study fails to confirm unequivocally close affinities between the two subspecies of *O. mexicana*. *Anthriscus* is likely monophyletic, although with bootstrap values of 51% or less (Figs. 2, 4) its monophyly is not strongly supported. Moreover, the re-

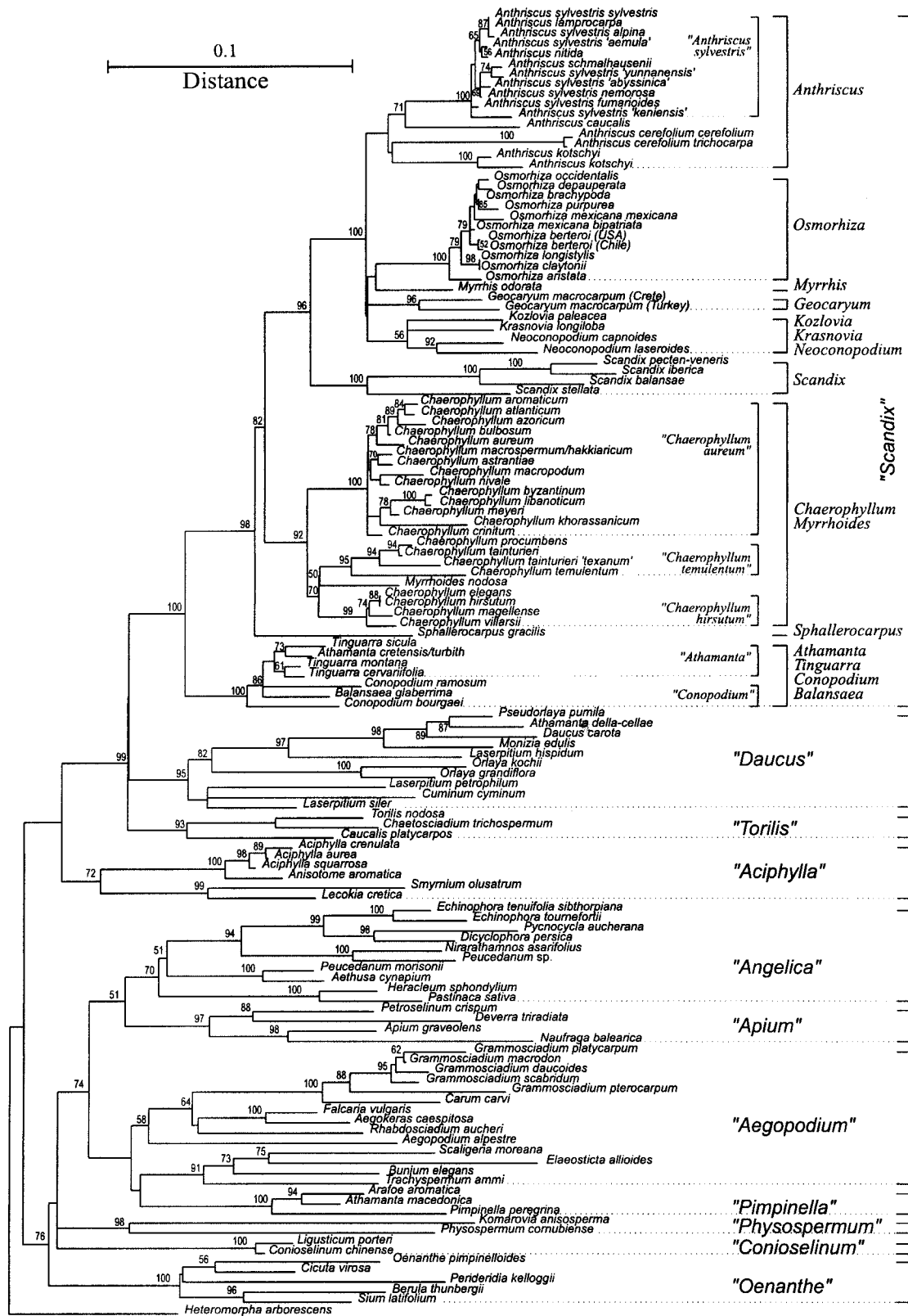


Fig. 4. Neighbor-joining tree inferred from 133 unambiguously aligned ITS1 and ITS2 sequences. Branch lengths are proportional to distances estimated from Kimura's two-parameter method (note scale bar). Numbers denote bootstrap values for particular nodes; only those > 50% are indicated. Brackets denote major clades of Apioideae. Within the *Scandix* clade, the various subclades described in the text are identified.

relationships among its species are ambiguous. Two annual species, *A. caucalis* and *A. cerefolium*, appear to be unrelated, while *A. sylvestris* is paraphyletic with *A. lamprocarpa*, *A. schmalhauseni*, and *A. nitida* arising within. A basal branch in the *A. sylvestris* clade (*A. sylvestris* subsp. *syvestris* or *A. keniensis* H. Wolff) is from Tanzania, while the European accessions of *A. sylvestris* subsp. *syvestris* unite with European *A. sylvestris* subsp. *alpina* and Levantine *A. lamprocarpa*.

A group of tuberous plants constituting Calestani's (1905) tribe Bunieae (i.e., *Geocaryum*, *Bunium*, and *Conopodium*, to which Engstrand (1973) included *Balansaea*) is not supported as monophyletic. The genera arise in at least three distant lineages. *Bunium*, together with *Scaligeria* and its segregate *Elaeosticta*, is placed close to *Trachyspermum ammi* in the *Aegopodium* clade, and *Conopodium* and *Geocaryum* belong to two separate branches within Scandiceae. *Balansaea* is indeed closely related to *Conopodium*, although monophyly of the latter has not been confirmed.

The four examined members of tribe Echinophoreae (i.e., *Echinophora*, *Pycnocycla*, and *Dicyclophora*), are sister group to the clade comprising *Nirarathamnos* and a yet to be described taxon from Socotra, tentatively considered a species of *Peucedanum* (M. Watson, Royal Botanic Garden Edinburgh, UK, unpublished data). This clade is allied with *Heracleum*, *Pastinaca*, *Aethusa*, and *Peucedanum morisonii*, all members of the *Angelica* clade. Although the *Angelica* clade is relatively well supported by high bootstrap and decay values, it is noteworthy that its members do not share any particular length mutation (Fig. 3). Deletion J, which would otherwise be synapomorphic for the whole branch including the *Apium* and *Pimpinella* clades, is lost in Echinophoreae, *Nirarathamnos*, and a *Peucedanum* species. *Monizia edulis*, an endemic of Madeira, is placed in the *Daucus* subclade. *Naufraja balearica* is sister to *Apium graveolens*, and *Deverra triradiata* is sister to *Petroselinum*. These last four genera all fall within the *Apium* clade.

## DISCUSSION

**Comparison to previous treatments**—Existing classifications of Scandiceae are generally quite similar. Drude's (1898) system, containing elements from earlier authors, such as de Candolle (1830) and Bentham (1867), is most commonly used despite its imperfections. The later accounts of Heywood (1971), Hedge et al. (1987), and Pimenov and Leonov (1993) did not introduce many changes, sometimes reverting to earlier treatments (e.g., Boissier, 1872; Bentham, 1867) with respect to the exclusion of *Molopospermum*. Notably different is the classification proposed by Koso-Poljansky (1916), based on a revision of European umbellifers by Calestani (1905). Both authors excluded several core members of Scandiceae, such as *Anthriscus*, and added some miscellaneous taxa, such as *Falcaria*, *Ptychotis*, and *Hladnikia*. The system of Cerceau-Larrival (1962) differs from all others in the inclusion of *Athamanta* and *Conopodium*.

Phylogenetic analyses of ITS sequences reveal that the majority of Scandiceae representatives examined constitute a monophyletic taxon (designated herein as the *Scandix* subclade or Scandiceae sensu stricto). This monophy-

ly is supported by relatively high bootstrap and decay values and a low average sequence divergence. Of the 18 putative ingroup genera included in this study, very few have been eliminated from the tribe. These eliminated taxa include *Grammosciadium*, *Rhabdosciadium*, and two species of *Athamanta*. The position of *Molopospermum peloponnesiacum* could not be determined with certainty; phylogenetic analyses of those ITS sequence characters that could be readily aligned with the matrix suggest an affinity with *Physospermum*. It does appear, however, that the *Molopospermum* ITS sequence is quite divergent from all other Scandiceae representatives. Those taxa that should be maintained within the tribe, as a result of this investigation, include *Anthriscus*, *Athamanta* (in part), *Chaerophyllum*, *Conopodium* (including *Balansaea*, discussed below), *Geocaryum*, *Kozlovia*, *Krasnovia*, *Myrrhis*, *Myrrhoides*, *Neoconopodium*, *Osmorhiza*, *Scandix*, *Sphallerocarpus*, and *Tinguarra*. The classification of Koso-Poljansky (1916) has found little support from these molecular data. Surprisingly, the treatment of Drude (1898) appears to be quite good, with only a few genera misplaced. The only major deficiency is his exclusion of *Athamanta* and *Conopodium* from the tribe. In this study, these taxa fall basal within Scandiceae.

### Major clades identified in Scandiceae sensu stricto—

Nine distinct clades, six of which are equivalent to generally recognized genera, are distinguished within the tribe. These clades are identified in Fig. 4.

The close relationship among *Athamanta*, *Tinguarra*, and *Conopodium* is a rather unexpected conclusion of this study as these genera appear to be morphologically distinct. *Athamanta* and *Tinguarra* are hemicryptophytes while *Conopodium* is a geophyte and, as a result, usually allied with *Bunium*. These taxa, however, share a West/Central Mediterranean distribution, unlike the remaining Scandiceae, which have an East Mediterranean/Central Asian distribution. The relationships within this clade are unresolved; better sampling of *Conopodium* may improve the resolution. It is rather unlikely that *Conopodium* is paraphyletic with regard to *Athamanta* and *Tinguarra*, as its characteristic morphology strongly suggests monophyly. The genus *Balansaea* should probably be included in *Conopodium*, as proposed by Engstrand (1973). Similarly, the monophyly of *Tinguarra* is dubious. *Tinguarra sicula* should be recognized in *Athamanta*, as in the treatment for the Flora Europaea (Tutin, 1968) but contrary to later authors (e.g., Knees, 1996). The relationship between *T. montana* and *T. cervariifolia* is also poorly supported. Based on further ITS sequence comparisons, another endemic of the Canaries, *Todaroa aurea*, is sister to *Tinguarra cervariifolia* (M. Watson, K. Spalik, and S. Downie, unpublished data), although the latter was the only species of *Tinguarra* included in that investigation. *Tinguarra montana* was originally described in *Todaroa*. *Tinguarra*, *Todaroa*, and *Athamanta* are undoubtedly closely related and, perhaps, should be recognized as a single genus. However, as revealed in this study, the delimitation of *Athamanta* is open to question, and that decision would require a better sampling of *Athamanta* combined with a closer look at the morphology and anatomy of these genera. Such a study is under way, and it

should confirm whether these two genera deserve to be kept separate or included in *Athamanta*.

The monotypic *Sphallerocarpus* appears to occupy an isolated position in Scandiceae. This isolation parallels its geographic distribution, for it occurs in Siberia, North-east China, and Korea, far removed from the center of diversity of Scandiceae in the Mediterranean region. The position of *Sphallerocarpus* varies depending upon the method of analysis used. While the results of the maximum parsimony analysis place it, albeit with weak bootstrap support, as sister to *Chaerophyllum* and *Myrrhoides* (Fig. 2), the neighbor-joining analysis places it away from this group (Fig. 4).

*Chaerophyllum* is the largest genus in Scandiceae. However, contrary to other large genera in the tribe, it has not been recently revised. The only modern account, which includes infrageneric divisions, is that of Schischkin (1950b) who recognizes three subgenera: *Nomochaerophyllum* (= *Chaerophyllum*), *Golenkinianthe*, and *Buniomorpha*. As the genus includes more than 30 species, a natural infrageneric classification would be beneficial. However, the classification of Schischkin (1950b) is not supported by the molecular data. Subgenus *Nomochaerophyllum* comprises representatives of all distinct lineages within *Chaerophyllum* identified in this study, and many of its distinguishing morphological features have been found to be homoplastic (K. Spalik and S. Downie, unpublished data). The ITS data suggest that *Chaerophyllum* comprises three distinct clades. Two clades, exemplified by *C. temulentum* and *C. hirsutum*, comprise only a few species each, while the majority belong to the *C. aureum* group (Fig. 4). The *C. temulentum* group, in addition to *C. temulentum*, includes the two American representatives of this genus: *C. tainturieri* and *C. procumbens*. The taxa constituting the *C. hirsutum* group are generally regarded as being closely related because they are so morphologically similar. The *C. aureum* group is much diversified with respect to its habit, life history, and ecology. As a consequence, it includes representation of all three subgenera of *Chaerophyllum*. *Myrrhoides* is unequivocally allied with *Chaerophyllum*, although its position here is somewhat ambiguous. The phylogeny inferred using parsimony confirms its position as a separate lineage sister to *Chaerophyllum*, while distance methods place it inside the latter, close to the *C. temulentum* group. This group includes both annual and biennial taxa somewhat similar in habit to annual *Myrrhoides nodosa*.

*Scandix* is clearly defined by its long-beaked fruits and an annual habit. Its monophyly is unambiguous. *Scandix* is sister to a large clade encompassing *Osmorhiza*, *Myrrhis*, *Geocaryum*, *Kozlovia*, *Krasnovia*, *Neoconopodium*, and *Anthriscus*. Monophyly of this group is supported by morphology (K. Spalik and S. Downie, unpublished data), and its members have already been considered closely allied (Pimenov and Kljuykov, 1987; Spalik, 1997).

Central Asiatic *Kozlovia*, *Krasnovia*, and *Neoconopodium* unite, albeit with weak support. They also share a geophytic habit. Although close relatives of *Geocaryum* have not been previously identified, this genus is also characterized by underground tubers. *Geocaryum* is taxonomically complex, with these difficulties partly ac-

counting for its poor representation in this study. Ball (1968) recognized only three species, while Engstrand (1977) has shown that the most widespread *G. cynapioides* is diversified both morphologically and in chromosome number. By carrying out a series of crosses he has shown that there is a genetic barrier among many populations once regarded as conspecific. As a result, he raised the number of species to 13. This revision, however, was based mostly on variation observed in cultivated material; he did not provide a list of representative herbarium specimens seen, material that would have made the identification of species less difficult. Both of our accessions of *Geocaryum* were originally determined as *G. cynapioides*, which according to Engstrand (1977) does not occur in the area in which they were collected. Based on their distribution and morphology, we have identified these accessions as *G. macrocarpum*, however, the high sequence divergence between them suggests that they may actually represent different species. The position of *Myrrhis* is not clearly resolved; in the strict consensus tree (Fig. 2) it allies, with weak support, with *Geocaryum*, whereas in the neighbor-joining tree (Fig. 4) it shows affinity with *Osmorhiza*. Morphologically, *Myrrhis* is more similar to *Osmorhiza* than it is to *Geocaryum*.

Although the relationships within *Osmorhiza* are mostly unresolved, some hypotheses on their evolution can be presented based on the results obtained. Lowry and Jones (1984) suggested that North America is both the center of diversity and place of origin for the genus. Although they did not provide a phylogenetic tree, their classification suggests that the most basal member within the genus might be *O. occidentalis*, the only representative of subgenus *Glycosma*. The remaining taxa constitute three lineages, i.e., sections, the typical section *Osmorhiza* containing Euroasiatic *O. aristata* and two morphologically similar eastern North American species, *O. claytonii* and *O. longistylis*. This hypothesis has not been confirmed. In contrast, we provide evidence for the Asiatic origin of the genus, as *O. aristata* is sister to all other examined species. The two other representatives of sect. *Osmorhiza*, *O. claytonii* and *O. longistylis*, constitute the next branch. Their similarity is therefore plesiomorphic. The trees inferred from these ITS data are therefore consistent with the hypothesis that the radiation of *Osmorhiza* occurred during the migration of its representatives from Asia and, in America, from north to south.

The genus *Anthriscus* was divided into three sections based on life history and habit (Spalik, 1996, 1997), with section *Anthriscus* including three annuals, *A. caucalis*, *A. cerefolium*, and *A. tenerrima*. Based on our analyses of ITS data, this section is likely polyphyletic, as *A. caucalis* and *A. cerefolium* do not appear to be closely related. Similarly, the relationships within the *A. sylvestris* group (Fig. 4), although mostly unresolved, are also somewhat different from those inferred from morphology (Spalik, 1996). The basal position of the African representatives and the close similarity of the European taxa suggest that the distribution of *A. sylvestris* in African montane "islands" are postglacial relics and that the differentiation of the taxa occurred while they migrated northwards from these refugia.

**Taxa excluded from Scandiceae and their placement—**

According to our results, *Grammosciadium* and *Rhabdosciadium* belong to the *Aegopodium* group of umbellifers. Although these two genera have consistently been placed in Scandiceae, some have underlined the differences between them and the remaining members of the tribe. Their exclusion, therefore, is not totally surprising. Drude (1898) recognized *Grammosciadium* in Scandiceae but placed *Caropodium*, a genus typified by *C. meoides* (= *Grammosciadium platycarpum*), in Apieae-Apiinae (his Ammineae-Carinae). Tamamschian and Vinogradova (1969) regarded *Grammosciadium* as occupying an intermediate position between Scandicinae and Caucalidinae, while Hedge and Lamond (1987) commented on its nonsulcate endosperm, so atypical in Scandiceae. More recently, Vinogradova (1995) transferred the genus to Apieae and suggested that *Fuernrohrria setifolia* is its closest relative. Based on phylogenetic analysis of ITS data, *Fuernrohrria* also belongs to the *Aegopodium* clade (Katz-Downie et al., 1999). *Rhabdosciadium* is a poorly known genus of which only limited material is available; this material, however, usually lacks leaves and mature fruits. The few fruits available for sectioning show a flat commissural face, as opposed to a deeply sulcate commissural face characteristic of other Scandiceae (Hedge and Lamond, 1987).

Since its validation in *Species plantarum* (Linnaeus, 1753), *Athamanta* appears to be a rather artificial assemblage of species. From among the ten names introduced in *Athamanta* by Linnaeus, only *A. cretensis*, the type of the genus, has been retained. *Athamanta sicula* is sometimes recognized in *Tinguarra* (Bentham, 1867; Knees, 1996), while two other Linnaean species now placed in *Athamanta* were originally described in *Bubon* and *Seseli* (Jarvis and Knees, 1988). This study restores *A. sicula* in the genus, but removes *A. macedonica* and *A. della-cella*.

*Athamanta macedonica* falls within the *Pimpinella* group of umbellifers, which also includes *Aphanopleura* and *Psammogeton*, recently removed from Caucalideae by Katz-Downie et al. (1999). Morphological comparisons place *Registaniella* in this clade also (Rechinger, 1987b). These genera share several morphologic features, such as hispid, ovoid fruits. *Athamanta macedonica* is generally similar in habit to some species of *Psammogeton* and *Pimpinella*, the similarity between the latter two genera having already been noted by Boissier (1872). *Pimpinella*, the largest genus in the group, includes some 150 species distributed throughout the Old World (Pimenov and Leonov, 1993). It encompasses a diverse array of species, contrary to the more narrowly defined *Aphanopleura*, *Registaniella*, and *Psammogeton*. Recently, three species of *Psammogeton* were transferred from *Pimpinella* (Rechinger, 1987a). *Pimpinella* may therefore constitute a paraphyletic aggregate of species requiring division into smaller, more natural genera. Some close relatives of *Athamanta macedonica* may eventually be identified, however, until a better understanding of the phylogeny of the *Pimpinella* clade is achieved, it seems reasonable to restore the Linnaean genus *Bubon*, typified by *Bubon macedonicum* L. As three subspecies are generally recognized in *B. macedonicum* (Tutin, 1968), two new combinations are necessary: *Bubon macedonicum*

subsp. *albanicum* (Alston & Sandwith) Spalik & S. R. Downie comb. nov. (basionym: *Athamanta albanica* Alston & Sandwith, Journ. Bot. 78: 193. 1940), and *Bubon macedonicum* subsp. *arachnoideum* (Boiss. & Orph.) Spalik & S. R. Downie comb. nov. (basionym: *Athamanta arachnoidea* Boiss. & Orph. in Boiss., Fl. or., suppl. 262. 1888).

Another species removed from *Athamanta* is *A. della-cella*, which in the present study is placed close to *Daucus*. The taxonomy of *Daucus*, albeit after some intense investigations (i.e., Small, 1978; Okeke, 1982; Heywood, 1983), is still not clear; molecular data suggest that the genus may be paraphyletic, with *A. della-cella* and several other genera of Caucalideae nested within (Lee and Downie, 1999). A detailed morphological study is necessary in order to decide whether these taxa should be included in *Daucus* or whether *Daucus* should be divided into smaller units. We therefore refrain from making a new combination that may later prove provisional.

*Molopospermum peloponnesiacum* was placed in Scandiceae by de Candolle (1830), and its retention there throughout the systems of Drude (1898) and followers reflects an inability to find a better place rather than a well-justified taxonomic decision. Creating a monotypic tribe, as did Cerceau-Larrival (1962), simply reflects that no relatives have been identified. *Molopospermum* is the only member of Scandiceae with distinctly winged fruits. Bentham (1867) placed the genus in Smyrnieae, a decision confirmed by Krähenbühl and Küpfer (1992) and followed by Pimenov and Leonov (1993). However, tribe Smyrnieae apparently represents an artificial group, as molecular analyses scatter its representatives among most major clades of Apioideae (Downie et al., 1998). This analysis has not resolved the question of taxonomic affinity of *Molopospermum*, as its sequence was too divergent to allow a reasonable alignment. Based on the analysis of partially aligned ITS data, however, an affinity to *Physospermum* may be apparent, as also suggested by Shneyer et al. (1992) based on serological data. Additional data are necessary to confirm its placement here, especially from the more conservatively evolving chloroplast genome.

**Main divisions in Apiaceae subfamily Apioideae—**

This study included representation of the *Daucus*, *Aciphylla*, *Angelica*, *Apium*, *Aegopodium*, *Oenanthe*, *Physospermum*, and *Conioselinum* clades, i.e., major lineages of Apioideae delimited on the basis of earlier molecular studies (Plunkett, Soltis, and Soltis, 1996; Downie et al., 1998). Based on the inclusion of a subset of taxa from each of these groups, the same clades were retained in this study, although their relationships differed from the earlier, more comprehensive analyses. In addition to these previously published ITS sequences, we included representation from Echinophoreae, a small tribe of Irano-Turanian distribution whose members have not been previously analyzed in a molecular systematic investigation. We also included *Bunium* and *Scaligeria* (sensu lato, i.e., including *Elaeosticta*) to test the monophyly of tribe Bunieae sensu Calestani (1905). Two new accessions of Laserpitieae, *Laserpitium petrophilum* and *Monizia edulis*, were surveyed, as well as material of *Nirarathamnos*,



*Naufraga*, and *Deverra*, genera whose affinities are largely unknown.

Echinophoreae are a small tribe comprising six genera (Pimenov and Leonov, 1993). In this study, *Echinophora*, *Pycnocycla*, and *Dicyclophora* form a monophyletic group. Sister to this group is a clade comprising the two Socotran endemics, *Nirarathamnos asarifolius* and a yet to be described species of *Peucedanum*. A detailed revisionary study of these Socotran umbellifers and their continental allies is currently being carried out by M. Watson and colleagues (Royal Botanic Garden Edinburgh, UK). All of these taxa, with the addition of *Pastinaca*, *Heracleum*, *Aethusa*, and *Peucedanum*, are nested within the *Angelica* group of umbellifers.

The *Aegopodium* group has also been enlarged based on the results of this study. It now encompasses *Grammosciadium* and *Rhabdosciadium* (both transferred from Scandiceae) and also *Bunium*, *Scaligeria*, and *Elaeosticta*, the last three forming a separate lineage together with *Trachyspermum ammi*. This entire clade is supported by two synapomorphic indels (Fig. 3). Engstrand (1977), following Calestani (1905), regarded *Bunium* as closely related to *Conopodium* and *Geocaryum*, as these genera share a geophytic habit. They appear, however, to be distantly related, with the similarities among them homoplastic. *Scaligeria* and *Elaeosticta* are sister taxa. Based on ITS1 sequence comparisons, *Scaligeria setacea* also belongs to this clade (K. Spalik and S. Downie, unpublished data). Therefore, the segregation of *Elaeosticta* from *Scaligeria*, advocated by Kljuykov, Pimenov, and Tikhomirov (1976), is optional rather than necessary; *Elaeosticta* may be satisfactorily reduced to a lower taxonomic rank, as in Rechinger (1987c). Pimenov and Kljuykov (1995) regarded *Scaligeria* and *Elaeosticta* as closely related to *Physospermum*; this affinity is not supported by ITS data.

The two new accessions of Laserpitieae, *Laserpitium petrophilum* and *Monizia edulis*, fall within the *Daucus* subgroup. This confirms earlier suggestions that Laserpitieae do not constitute a monophyletic entity but rather independent lineages differing from the remaining *Daucus* relatives in the homoplastic absence of spines (Downie et al., 1998). The monotypic genus *Naufraga* from Balears, once also present in Corsica (Gamisans et al., 1996), appears to be related to *Apium graveolens*. Another addition to the *Apium* clade is *Deverra triradiata*.

**The three subtribes of Scandiceae**—According to the phylogenies presented herein, Scandiceae sensu stricto (the *Scandix* subclade) arise within paraphyletic Caucalideae (sensu Heywood, 1971, 1982b). The strict consensus tree (Fig. 2) shows the *Daucus* subgroup being their closest relative and the *Torilis* subgroup the next basal branch. The neighbor-joining tree (Fig. 4), however, does not support this relationship as strongly. Here both the *Daucus* and *Torilis* subgroups are likely contenders for sister taxon to Scandiceae sensu stricto. All of these taxa have been considered previously as belonging to the *Daucus* group of umbellifers (Plunkett, Soltis, and Soltis, 1996; Downie et al., 1998). In this analysis, as in the studies of Plunkett and Downie (1999) and Lee and Downie (1999), the *Daucus* group encompasses three distinct lineages, albeit the relationships among them are

equivocal. Therefore, the most natural treatment seems to be the inclusion of the entire group into the tribe Scandiceae Spreng. in Roem. & Schult., Syst. Veg. 6: xlii. August–December 1820, with the division of the tribe into three subtribes: (1) Scandicinae Tausch, Flora 17: 342. 14 June 1834; (2) Torilidinae Dumort., Fl. Belg.: 81. 1827; and (3) Daucinae Dumort., Fl. Belg.: 81. 1827. These subtribes exemplify the *Scandix*, *Torilis*, and *Daucus* subgroups, respectively, of previous studies. The recognition of these three distinct yet closely related groups, the result of collapsing one of the basal nodes of the previously delimited *Daucus* clade, may achieve the stability of classification much desired by students of this important group of flowering plants.

**Molecular evolution of ITS sequences**—The ITS1 and ITS2 regions are part of the transcriptional unit of nuclear rDNA and appear to play a significant role in the maturation of nuclear rRNA. They are therefore subject to evolutionary constraints. The length of each spacer is relatively stable in angiosperms; the entire region including the intervening 5.8S rDNA is usually less than 700 bp in size (Baldwin et al., 1995). Several relatively conserved sequences have been identified in both spacers (Liu and Shardi, 1994; Hershkovitz and Lewis, 1996; Coleman and Mai, 1997), some of these apparently constituting cleavage sites (Allmang et al., 1996a, b). The ITS2 region generally possesses more constant positions than ITS1 (Hershkovitz and Zimmer, 1996; Coleman and Mai, 1997; Mai and Coleman, 1997), although no unambiguous conserved motifs shared between algae, fungi, and plants have been identified (Hershkovitz and Lewis, 1996). It is the secondary structure of ITS2 that is conserved despite wide intra- and interfamilial primary sequence divergence (Mai and Coleman, 1997). Therefore, the determination of secondary structure for both ITS regions may improve alignment of these sequences at deep levels (Coleman and Mai, 1997).

Different rates of DNA evolution relative to generation time are usually evoked to explain differences in branch length (Wu and Li, 1985; Wilson, Gaut, and Clegg, 1990; Gaut et al., 1996), with long-lived woody species likely having slower rates than annuals. Branch lengths leading to the annual and biennial members of the *Scandix* subgroup, such as *Scandix*, *Anthriscus caucalis*, *A. cerefolium*, and species within the *Chaerophyllum temulentum* clade are generally longer, but not much longer than the others. One possible reason for this is that generation time is not simply a function of life cycle, usually understood as either annual vs. perennial habit. For example, taxa from the *Anthriscus sylvestris* group are usually iteroparous (polycarpic) perennials, but they also may be semelparous (monocarpic) biennials (or even annuals). Its sister group, *A. caucalis*, is annual (or winter annual, i.e., biennial). However, the seeds of *A. sylvestris* remain viable for only a single season, while those of *A. caucalis* may persist in soil for 3–5 yr (Roberts, 1979, 1986).

## CONCLUSIONS

Subtribe Scandicinae, forming tribe Scandiceae along with subtribes Daucinae and Torilidinae, emerges as the only natural suprageneric division in subfamily Apioi-

deae, as defined by morphology and confirmed by cladistic analysis of molecular data. However, careful analysis of different accounts, including that of Drude (1898), reveals that there is no single morphological or anatomical character identifying this clade. Therefore, in the past, the included taxa were grouped intuitively, i.e., based on general similarity rather than on synapomorphies. The question is whether any evident morphological or anatomical synapomorphy can be found at all. By “evident” we mean such features that may be used to identify potential members of the clade and, to this end, a study of the morphology of Scandiceae is currently in progress. If such synapomorphies cannot be identified for this long-recognized group, then chances are they would also not be found for many other major lineages of Apiaceae, which so far have been defined only by molecular analyses. If this is the case, we would have to accept that the task of reclassifying this family at suprageneric level is to be accomplished based on molecular markers rather than on traditional taxonomic data.

The largest genera in the subtribe (*Anthriscus*, *Chaerophyllum*, and *Osmorhiza*) are likely each monophyletic, contrary to their long-recognized infrageneric divisions, which are not congruent with the molecular data. Some unexpected affinities have been revealed, particularly those among *Athamanta*, *Tinguarra*, and *Conopodium*, which collectively form the basal clade of Scandiceae. Further studies should address the question of monophyly of *Conopodium*, this monophyly supported by morphology but not by ITS data. The clade comprising *Kozlovia*, *Krasnovia*, and *Neoconopodium* also requires a detailed examination to clarify whether these genera are better kept separate or united. Monotypic *Myrrhoides* is apparently related to *Chaerophyllum*, although it is not clear whether it is nested within that genus or sister to it. *Myrrhis* and *Sphallerocarpus* seem to represent isolated lineages with no immediate relatives, thus deserving the status of monotypic genera. In the study, the genus *Bubon* is reinstated with the single species *B. macedonicum*; its close relatives may eventually be found among *Pimpinella* and allies.

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