The evolution of fruit in Scandiceae subtribe Scandicinae (Apiaceae)

Krzysztof Spalik, Aneta Wojewódzka, and Stephen R. Downie

Abstract: Evolutionary relationships among 66 representatives of the family Apiaceae, including 37 species of tribe Scandiceae subtribe Scandicinae, were inferred from separate and combined analyses of fruit morphology and anatomy and nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. Phylogenetic trees inferred from analysis of 35 fruit characters were not congruent to those derived from molecular data and, overall, had much lower bootstrap support values than the latter. Contrary to molecular data, fruit characters did not support the monophyly of subtribe Scandicinae. Fruit data do, however, corroborate the monophyly of nearly every genus within Scandicinae, the affinity of members of the "crown" clade—*Anthriscus*, *Kozlovia* (including *Krasnovia* and *Neoconopodium*), *Geocaryum*, *Myrrhis*, and *Osmorhiza*—that had been identified in previous molecular analyses, and the sister group relationship between the "crown" clade and the genus *Scandix*. Phylogenies derived from the analysis of combined ITS and fruit characters were congruent to those inferred from molecular data alone. Reconstructions of ancestral character states using the results of the combined analysis suggest that among extant Scandicinae, the fruits of *Athamanta* have retained the most plesiomorphic characters. Evolutionary tendencies that have occurred in the fruits of Scandicinae include the broadening of the vascular bundles and vittae, the thickening of the cuticle and epidermal cell wall, the origin of bristles from hairs, the appearance of a pedicel-like appendage, the development of a long beak, and lateral wings. These changes are interpreted as adaptations to fruit dispersal and seed defense.

Key words: Apiaceae, Scandiceae subtribe Scandicinae, ITS, fruit morphology.

Résumé : Parmi 66 représentants de la famille des Apiaceae, incluant 37 espèces de la tribu des Scandiceae sous-tribu Scandicinae, les auteurs ont déduit les relations évolutives, en utilisant des analyses séparées et combinées de la morphologie et de l'anatomie des fruits, ainsi que des séquences de l'espaceur interne transcrit (ITS) de l'ADN ribosomal nucléique. Les dendrogrammes phylogénétiques, obtenus de l'analyse de 35 caractères des fruits, sont incongrus par rapport à ceux dérivés des données moléculaires et, en général, montrent des valeurs de support en lacet beaucoup plus faibles que ceux-ci. Contrairement aux données moléculaires, les caractéristiques des fruits ne supportent pas la monophylie de la sous-tribu Scandicinae. Cependant, les données sur les fruits corroborent la monophylie d'à peu près tous les genres appartenant aux Scandicinae, l'affinité des membres du clade "crown"—*Anthriscus, Kozlovia* (incluant *Krasnovia* et *Neoconopodium*), *Geocaryum*, *Myrrhis* et *Osmorhiza*—qui ont été identifiés dans des analyses moléculaires précédentes, ainsi que la relation de sororité entre le clade "crown" et le genre *Scandix*. Les phylogénies dérivées de l'analyse combinée des caractères des ITS et des fruits sont congruentes avec celles obtenues à partir des données moléculaires prises isolément. Les reconstructions des caractères ancestraux, basées sur les résultats d'analyses combinées, suggèrent que parmi les Scandicinae actuelles, les fruits de l'*Athamanta* ont retenu les caractères les plus plésiomorphes. Les tendances évolutives qui se sont manifestées chez les fruits des Scandicinae incluent l'élargissement des faisceaux vasculaires et des vittae, l'épaississement de la cuticule et de la paroi des cellules épidermiques, l'origine des soies à partir des poils, l'apparence de l'appendice pédicelloïde, le développement d'un long bec, et les ailes latérales. On interprète ces changements comme des adaptations pour la dispersion des fruits et la protection des graines.

Mots clés : Apiaceae, Scandiceae sous-tribu Scandicinae, ITS, morphologie des fruits.

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Introduction

Ever since Morison's (1672) *Plantarum umbelliferarum*, fruit morphology and anatomy have been regarded as essential to the taxonomy of Apiaceae (Umbelliferae; Drude 1898; Constance 1971). Despite their general similarity, umbellifer fruits vary with respect to their external and internal features, and nearly all classification systems of the family are based on these characters. Koch (1824), for instance, divided the family into two principal groups, Multiiugatae and Pauciiugatae, on the basis of the number of ribs. He subsequently segregated these groups into 15 tribes upon consideration of fruit shape and compression and characteristics of the ribs and vittae. de Candolle (1830) stressed the importance of endosperm shape, arranging the umbellifers into three groups: Orthospermae, Campylospermae, and Coelospermae. Bentham (1867) gave priority to the number

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of mericarp ribs and two of his three major groups, Haplozygiae and Diplozygiae, are defined by the absence or presence of secondary ribs. Rompel (1895), in his influential study of umbellifer fruit anatomy, emphasized the distribution of calcium oxalate crystals. Drude (1898) considered this character among others when constructing his classification but gave priority to fruit compression and the number of ribs and vittae. For Koso-Poljansky (1916), essential features included the distribution of calcium oxalate crystals, vittae, aerenchyma, and sclerenchyma in the walls of the fruit. The only modern infrafamilial division of the family that was not built upon fruit morphology and anatomy was that proposed by Cerceau-Larrival (1962), who underlined the importance of cotyledon shape and pollen attributes.

Although those classification systems that were based on fruit morphology and anatomy used nearly the same set of characters, they differed widely, since the distribution of these characters is largely incongruent. When optimized onto phylogenies obtained using molecular evidence, many of these features are highly homoplastic (Plunkett et al. 1996; Katz-Downie et al. 1999). Contrary to those relationships implied in traditional classification systems, albeit inferred using nonphylogenetic approaches, the phylogenies estimated from diverse sets of molecular data are generally congruent with each other. Several well-supported major lineages have been identified, 10 of which are formally recognized as tribes (Downie et al. 2000*b*, 2001), with the largest tribe, Scandiceae Spreng., further divided into three subtribes: Scandicinae Tausch, Daucinae Dumort., and Torilidinae Dumort. (Downie et al. 2000*a*). Scandiceae includes members of former tribes Scandiceae, Caucalideae, and Laserpitieae (sensu Heywood 1971) and with over 120 accessions examined to date is the most extensively sampled major clade of Apiaceae (Lee and Downie 1999, 2000; Downie et al. 2000*a*).

Little regarding fruit evolution in the family is known despite the importance of fruit morphology and anatomy in umbellifer systematics. Some studies, for instance that of Cerceau-Larrival (1962), included hypothetical pathways of fruit evolution. These were, however, based on incorrect assumptions of phylogenetic relationships among taxa (Plunkett et al. 1996; Katz-Downie et al. 1999). The major objective of this paper is to assess the evolutionary history of fruit characters in Scandiceae subtribe Scandicinae. Based on a phylogeny inferred from combined morphological and molecular data sets, we reconstruct fruit morphology and anatomy at internal nodes of this tree and discuss the selective pressures that may have given rise to these changes. Scandicinae constitute a model group for such evolutionary work as molecular and morphological phylogenetic studies indicate that it is a well-defined subtribe (Downie et al. 2000*a*; Spalik and Downie 2001). Several of its included genera have been recently revised (Engstrand 1977; Lowry and Jones 1984; Spalik 1997). Of the 16 genera recognized in the subtribe as a result of our initial investigations (Downie et al. 2000*a*), five, *Krasnovia*, *Neoconopodium*, *Myrrhoides*, *Tinguarra*, and *Balansaea*, have been since reduced to synonymy (Spalik and Downie 2001; Spalik et al. 2001). The inclusion of *Tinguarra* in *Athamanta*, however, needed confirmation from additional data. Spalik and Downie (2001), upon examination of 44 vegetative, inflorescence, floral, and fruiting characters in Scandicinae, found those of the fruit to be most congruent with phylogenies inferred from molecular data indicating that phylogenetic signal is retained within these characters. Additional objectives include the examination of the taxonomic utility of fruit anatomy in subtribe Scandicinae to detect those characters that best delimit genera and to determine whether fruit anatomy and morphology confirms the inclusion of *Tinguarra* in *Athamanta*.

Materials and methods

Accessions examined

Sixty-six representatives of Apiaceae were included in the cladistic analysis of nuclear ribosomal DNA ITS sequences and fruit morphology and anatomy (Table 1). Scandiceae subtribe Scandicinae was represented by 37 species reflecting all 12 genera recognized in the subtribe on the basis of molecular studies (Downie et al. 2000*a*, 2000*b*; Spalik and Downie 2001). Subtribes Daucinae and Torilidinae were each represented by five and three species, respectively. We also considered 21 representatives of tribes Careae, Smyrnieae, and Oenantheae and the apioid superclade (including members of the *Angelica*, *Apium*, *Heracleum*, and *Pimpinella* subclades). Based on chloroplast DNA evidence, tribe Oenantheae (i.e., *Cicuta*, *Sium*, and *Oenanthe* in this study) is sister to the clade formed from all other aforementioned lineages; however, the relationships among the latter are unresolved (Downie et al. 2000*b*). We did not consider more distant outgroups (i.e., Heteromorpheae, Bupleureae, Pleurospermeae, and the *Komarovia* clade), since their ITS sequences are highly divergent; consequently, more positions would have had to be excluded from the analysis because of alignment ambiguities. Moreover, adding such distant and divergent outgroups would actually decrease the consistency of the phylogenetic estimation (Kim 1996). The set of taxa examined in this study is somewhat different from those used in our previous analyses of the subtribe (Downie et al. 2000*a*; Spalik and Downie 2001), reflecting the availability of specimens with mature fruits. We also added three taxa that were not considered in our prior studies: *Conopodium majus*, *Laserpitium prutenicum*, and *Todaroa aurea*. Voucher information for these three species is provided in Table 1; for all other taxa, references are provided therein where this information has been cited previously.

Molecular and morphological data

In the molecular analysis, we considered a subset of ITS sequences from our earlier studies (Downie and Katz-Downie 1996, Downie et al. 1998, 2000*a*; Table 1) plus the three new accessions. Details of the DNA isolation, polymerase chain reaction amplification, and sequencing procedures utilized are provided by Downie et al. (1998, 2000*a*). The sequences were aligned using CLUSTAL V (Higgins et al. 1992) and manually adjusted.

Herbarium material for the morphological analysis was obtained from Jean-Pierre Reduron (Mulhouse, France) and from the following institutions: B, BC, BM, E, ILL, KRA, KRAM, L, MO, P, W, WA (abbreviations according to Holmgren et al. 1990). Mature fruits were selected and their external characters examined using a Nikon SMZ-U dissecting microscope (8–160× magnification). For the anatomical studies, fruits were soaked in water and hand cut or embedded in paraffin and sectioned using a microtome; these dissections were then stained with phloroglucinol or safranin – fast green (Gerlach 1972) and examined using a Nikon Optiphot 2 optical microscope (40–1000× magnification). Drawings were made using a drawing tube or from photographs taken using a Nikon Microflex HFX-DX photographic system.

Table 1. Accessions of Apiaceae examined in this study, GenBank accession numbers for separate ITS 1 and ITS 2 sequences, and the matrix of morphological data.

Taxon	Source of sequence or voucher*	GenBank accession No.		Morphological characters			
		ITS1	ITS2	1–10	11–20	21–30	31–35
Aegopodium alpestre Ledeb.	b	U78376	U78436	0001000000	0011110000	5401041132	11002
Aethusa cynapium L.	a	U30582	U30583	0011000000	0011000400	5201020343	10301
Ammi majus L.	b	U78386	U78446	0011000000	0010000?00	54??030134	?1101
Anethum graveolens L.	a	U30550	U30551	0011000000	0010000300	2001020001	10310
Anthriscus cerefolium (L.) Hoffm.	a	U30532	U30533	00120001B1	1101000001	1320040000	14031
Anthriscus kotschyi Boiss. & Balansa	d	AF073579	AF073580	0012000001	1100000001	4421040000	03011
Anthriscus nitida (Wahlenb.) Hazsl.	d	AF073595	AF073596	1012000001	1100000001	3420041000	13032
Anthriscus sylvestris (L.) Hoffm.	b	U79603	U79604	1012000001	1101000001	3420041000	13022
Athamanta cretensis L.	d	AF073685	AF073686	0011100010	0013110000	2301021012	23131
Athamanta sicula L. (≡ *Tinguarra sicula* (L.) Benth. & Hook. f.)	d	AF073683	AF073684	0011100010	0011110000	340?011013	13221
Athamanta turbith (L.) Broth.	d	AF073687	AF073688	0011100010	0011110000	3301022002	23121
Bubon macedonicum L. (≡ *Athamanta macedonica* (L.) Spreng.)	d	AF073541	AF073542	0011100010	0010000000	3201121122	11210
Carum carvi L.	b	U78377	U78437	0000000000	0011000000	5401021114	11002
Caucalis platycarpos L.	b	U78364	U78424	1011112120	0013001300	5401020211	14132
Chaerophyllum aromaticum L.	d	AF073631	AF073632	0010000000	0010000000	2401012213	24341
Chaerophyllum astrantiae Boiss. & Balansa	d	AF073653	AF073654	0010000000	0011000000	2201021223	23331
Chaerophyllum aureum L.	d	AF073655	AF073656	0010000000	0011000000	3301021213	24232
Chaerophyllum bulbosum L.	d	AF073659	AF073660	0010000000	0011000000	3401020213	13211
Chaerophyllum crinitum Boiss.	d	AF073661	AF073662	0010000000	0011000000	1400001224	04241
Chaerophyllum hirsutum L.	d	AF073665	AF073666	0010000000	0010000000	1401012233	14231
Chaerophyllum macrospermum (Willd. ex Spreng.) Fisch. & C.A. Mey.	d	AF073651	AF073652	0000000000	0012000000	??01012233	13340
Chaerophyllum nodosum (L.) Crantz (≡ *Myrrhoides nodosa* (L.) Cannon)	d	AF073675	AF073676	0000000120	0013000000	2401000333	04241
Chaerophyllum procumbens (L.) Crantz	d	AF073643	AF073644	00100000A0	0010000000	2401030213	24121
Chaerophyllum tainturieri Hook.	d	AF073645	AF073646	00100000A0	0010000000	2401020343	23222
Chaerophyllum temulum L.	d	AF073641	AF073642	00100000A0	0011000000	2401000213	03221
Cicuta virosa L.	b	U78372	U78432	0000000000	0013000400	5401001341	10202
Conium maculatum L.	b	U79609	U79610	0000000000	0000??0000	5311041123	14002
Conopodium glaberrimum (Desf.) Engstrand (≡ *Balansaea glaberrima* (Desf.) Maire)	d	AF073689	AF073690	0011000000	0011000000	3311021012	03111
Conopodium majus (Gouan) Loret	e	AF336370	AF336371	0011000000	0010AA0000	4311021013	14001
Daucus carota L.	a	U27589	U30315	1011112120	0011110100	520?031003	?1101
Falcaria vulgaris Bernh.	b	U78378	U78438	0000000000	0011000000	3401021213	21011
Foeniculum vulgare Mill.	b	U78385	U78445	0001000000	0010010000	2101021???	?1320
Geocaryum macrocarpum (Boiss. & Spruner) Engstrand	d	AF073607	AF073608	1012000001	1011000000	3421041011	13012
Grammosciadium platycarpum Boiss. & Hausskn.	d	AF073551	AF073552	0000000000	0011000210	1110022223	11340
Heracleum sphondylium L.	a	U30544	U30545	0001000000	0010000110	2001031000	10320
Kozlovia capnoides (Decne.) Spalik & S.R. Downie (≡ *Neoconopodium capnoides* (Decne.) Pimenov & Kljuykov	d	AF073601	AF073602	0012A000B1	1101000000	3320141134	03022
Kozlovia longiloba (Kar. & Kir.) Spalik & S.R. Downie (≡ *Krasnovia longiloba* (Kar. & Kir.) Popov ex Schischk.)	d	AF073599	AF073600	10120001B1	1101000000	4320141002	04111
Kozlovia paleacea (Regel & Schm.) Lipsky	d	AF073597	AF073598	1012100121	1101000000	3220142001	04121

Table 1 (*concluded*).

Taxon	Source of sequence or voucher*	GenBank accession No.		Morphological characters			
		ITS1	ITS2	1–10	11–20	21–30	31–35
Laserpitium petrophilum Boiss. & Heldr.	d	AF073567	AF073568	0001111020	0011100?00	????022012	?21?1
Laserpitium prutenicum L.	f	AF336374	AF336375	0001111020	0011000000	3001021003	13201
Myrrhis odorata (L.) Scop.	a	U30530	U30531	0012100021	0111110000	2421042122	13041
Oenanthe pimpinelloides L.	b	U78371	U78431	0000000000	0010000400	430?002343	?030?
Orlaya grandiflora (L.) Hoffm.	a	U30524	U30525	1011112020	0011000C00	3101022104	13230
Osmorhiza berteroi DC.	b	U78365	U78425	1112100021	1101000000	1401040120	22141
Osmorhiza claytonii (Michx.) C.B. Clarke	d	AF073615	AF073616	1112100021	1101000000	1401041133	23141
Osmorhiza depauperata Phil.	d	AF073611	AF073612	1112100021	1101000000	1511040122	12041
Osmorhiza mexicana Griseb.	d	AF073621	AF073622	1112100021	1101000000	1511040132	22041
Pastinaca sativa L.	a	U30546	U30547	0001000000	0010000110	1001031000	10320
Petroselinum crispum (Mill.) A.W. Hill	b	U78387	U78447	0001000000	0011000000	5301031013	21102
Peucedanum cervaria (L.) Lapeyr.	c	AF008608	AF009087	0001000000	0010000010	3001021000	13320
Pseudorlaya pumila (L.) Grande	a	U30522	U30523	0001112020	0011000100	3001032114	13230
Scandix balansae Reut. ex Boiss.	b	U79621	U79622	0020100020	1111000000	1401020223	04242
Scandix iberica M. Bieb.	d	AF073627	AF073628	0020100020	1113000000	0411021222	14341
Scandix pecten-veneris L.	a	U30538	U30539	0020100120	1112000000	0311021132	04241
Scandix stellata Banks & Sol.	d	AF073629	AF073630	0021100120	11A0000000	0401040122	13241
Seseli montanum L.	a	U30578	U30579	0001100010	0010000000	5201031133	10100
Sium latifolium L.	b	U78370	U78430	0001000000	001?010400	5301011243	10002
Smyrniopsis aucheri Boiss.	b	U78393	U78453	0001000000	0011010000	5421022133	24122
Smyrnium olusatrum L.	a	U30594	U30595	0001000000	0011110?00	5511032012	24032
Sphallerocarpus gracilis (Bess. ex Trevir.) Koso-Pol.	d	AF073677	AF073678	0001000000	0011110000	4401030022	14012
Tinguarra cervariifolia (DC.) Benth. & Hook. f.	d	AF073681	AF073682	0011100010	0013110000	2401022232	13001
Tinguarra montana (Webb ex H. Christ) A. Hansen & G. Kunkel	d	AF073679	AF073680	0001100010	0011A10000	3200021040	13021
Todaroa aurea (Sol.) Parl.	g	AF336372	AF336373	0001100010	0011000010	3111?1?241	13031
Tommasinia verticillaris (L.) Bertol.	c	AF008609	AF009088	0001000000	0010000010	3001?32001	??320
Torilis nodosa (L.) Gaertn.	b	U30534	U30535	0001112120	1011001001	5320040001	1300?
Torilis trichosperma (L.) Spreng. (≡ *Chaetosciadium trichospermum* (L.) Boiss.)	a	U78363	U78423	0001113011	1011001000	3310032003	03111

Note: Synonymy is provided for those species that have recently changed generic placement. With the exception of three species, whose voucher information is provided herein, source of ITS data and voucher information and deposition are available in several previous publications. Characters are described in Table 2. Question marks denote missing data, and A, B, and C denote character states "0 or 1", "0 or 2", and "1 or 2", respectively.

*Sources of ITS sequences or voucher specimens are as follows: a, Downie and Katz-Downie (1996); b, Downie et al. (1998); c, Katz-Downie et al. (1999); d, Downie et al. (2000*a*); e, England, South Essex, Stock, 25 May 1965, *Walters & Sell* (WA); f, Poland, Klaudyn (near Pruszków), 2 August 1971, *Nowak* (WA); g, cultivated in Conservatoire Botanique Mulhouse (France) no. 95183, ex Tenerife, Barranco del Infierno, 27 September 1996, *Reduron* (WA).

Twenty qualitative (Nos. 1–20, Tables 1 and 2) and 15 quantitative (Nos. 21–35) characters were examined. Of the qualitative characters, 13 refer to the external morphology of the fruit with all but one having been considered in a previous study (Spalik and Downie 2001). The seven remaining qualitative characters, representing fruit anatomy, are new. The quantitative characters are also new for this study, and were coded as binary or multistate-ordered characters arbitrarily after examining their distributions. In total, 15 characters (Nos. 3, 14, 21–23, and 26–35) were treated as ordered. All data matrices and PAUP nexus files (Swofford 1998) used in this study can be obtained from the authors upon request.

Phylogenetic inference

Morphological and ITS data were analyzed separately and combined using both maximum parsimony and distance methods available in PAUP* version 4.0. To account for the varying number of states for the morphological characters, ranging from two to five, all characters were weighted using PAUP's scaling option and fractional weights were employed. These weights were used in both the separate analysis of morphological data as well as in the combined analysis; for the latter, each ITS position was assigned a weight of 1. The maximum parsimony analysis included heuristic searches with 500 random addition replicates and tree bisection–reconnection branch swapping. Distance methods included neighbor-joining and others depending upon the data being analyzed: Jukes–Cantor and Kimura two-parameter methods (Kimura 1980) for the ITS data set and mean and total character differences for both the morphological and combined data sets. Bootstrap support (Felsenstein 1985) was estimated using 1000 resampled data sets; in the maximum parsimony analysis, heuristic searches with simple addition sequence of taxa were employed, saving a maximum of 10 trees from each replicate. All trees were rooted with the three included members of tribe Oenantheae.

Table 2. Fruit characters used in the cladistic analysis of anatomical and morphological data.

Character No.	Character	States	Comments
1	Crown of scales (bristles) at fruit base	0, absent; 1, present	Scales (bristles) forming the crown on the pedicel at the base of fruit, if present, are distinctly longer than those that may occur along the pedicel; the presence of this crown is generally characteristic for “crown” Scandicinae, although in some species it may be reduced to single scales or even be absent; outside the subtribe, this character also occurs in Daucinae
2	Pedicel-like appendage	0, absent; 1, present	This appendage is formed when the seed does not fill the lower part of the fruit; it is synapomorphic for *Osmorhiza*, with the exception of *O. occidentalis* (not considered in the present study); its absence in the latter is considered to be a reversal (Spalik and Downie 2001)
3	Beak	[0, obsolete ↔ 1, relatively short] ↔ 2, long	Similar to the pedicel-like appendage, a distinct beak is formed when the seed does not fill the fruit entirely; a short beak occurs in many independent lineages and may also be lost repeatedly (as in some species of *Chaerophyllum*); a long beak is synapomorphic for *Scandix*
4	Primary ridge shape	0, broad rounded or obsolete; 1, filiform or winged; 2, angular (sometimes only at the top of the fruit)	This typology is simplified, accounting for variation seen in Scandicinae rather than the entire family; it is based on the ridge shape seen in transverse section of a dried mericarp; narrow obtuse or winged ridges that are clearly delimited from valleculae were scored as filiform; broadly triangular ridges that occur in a pentagonal or star-shaped pattern in “crown” Scandicinae were scored as angular; these ridges are not clearly delimited from valleculae since they are formed by the edges of the pentangle; sometimes they are extant only in the upper part of the fruit (as in *Anthriscus* and *Kozlovia*); however, after soaking fruits in water, filiform and angular ridges are not that easy to distinguish; therefore, the filiform appearance of ridges may result from the drying and contraction of the pericarp in the valleculae, whereas in the ridges it is reinforced by the vascular bundles; in the “crown” species of Scandicinae, the valleculae are only fairly contracted because of the thickened cuticle and cell walls of the epidermis; similarly, broad rounded ridges, exemplary of *Chaerophyllum*, possess valleculae (meaning in Latin “small valleys”) that are contracted when dried; yet after soaking, valleculae may be higher than the ridges (compare drawings of entire dried mericarps with the sections drawn from soaked fruits, Fig. 4).
5	Primary ridge indumentum	0, absent; 1, present	In Scandicinae, the indumentum may cover the entire fruit or be confined to the primary ridges; primary ridge indumentum refers only to those hairs or bristles that are distinctly lined along the ridges
6	Secondary ridges	0, absent; 1, present	Pronounced secondary ridges occur only in members of Daucinae and Torilidinae
7	Secondary ridge appendages	0, absent; 1, wings; 2, spines; 3, hairs	
8	Tubercles at fruit surface	0, absent; 1, present	A tubercle usually forms the base of a bristle, although the latter may be reduced to a short tooth or be completely absent (as in *Kozlovia longiloba*)

Table 2 (*continued*).

Character No.	Character	States	Comments
9	Fruit indumentum	0, absent; [1, hairs; 2, bristles]	Several species are polymorphic and include members with glabrous or hairy (bristled) fruits; in *Kozlovia longiloba*, tubercles usually lack bristles but sometimes end with short hyaline teeth that appear homologous to bristles
10	Cuticle appearance	0, dull; 1, shiny	This character is difficult to determine precisely in badly preserved herbarium specimens or when the fruits are immature; a shiny fruit appearance could be related to the thickness of the cuticle and cell wall (character No. 23); however, these characters do not exactly coincide; for example, members of *Osmorhiza* have shiny fruits, while their cuticles are not very thick
11	Cuticle texture	0, smooth or striate; 1, aculeate	An aculeate cuticle texture results from the formation of small projections above the center of the epidermal cells; in *Scandix*, they are only found close to the fruit base, with the exception of *Scandix stellata*, where the entire fruit is aculeate; the sizes of these projections are considered in character No. 24
12	Epidermis	0, uniform in color (not areolate); 1, areolate (scalariform)	An areolate epidermal appearance is due to thickened transverse cell walls (Spalik 1997); it is not always well developed and sometimes occurs only on part of the fruit
13	Vallecular vittae at fruit maturity	0, obsolete or compressed; 1, extant (not compressed)	
14	No. of intrajugal vittae per ridge	0, none ↔ 1, one ↔ 2, two ↔ 3, greater than two	Intrajugal vittae are small secretory canals that are situated in the primary ribs above the vascular bundles
15	No. of vallecular vittae per vallecula	0, one; 1, more than one (sometimes anastomosing)	This character, as well as the subsequent one, is difficult to assess in those species in which the vittae are compressed at fruit maturity
16	No. of commissural vittae	0, two; 1, more than two (sometimes anastomosing)	
17	Position of commissural vittae	0, between commissural bundles; 1, below bundles (between the bundle and the endosperm)	Commissural vittae situated below the commissural bundles were found only in those examined species of Torilidinae
18	Fruit sclerification (apart from vascular bundles)	0, absent; 1, ring around endosperm; 2, above vallecular vittae; 3, above vascular bundles and vallecular vittae; 4, between vascular bundles	Apart from the vascular bundles, different parts of the fruit may be lignified; sclerenchymatic cells may form a closed ring in the endocarp around the endosperm or be present in different parts of the mesocarp
19	Lateral primary ridges	0, not winged; 1, winged	Among Scandicinae, only *Todaroa aurea* has winged lateral primary ribs
20	Position of commissural bundles	0, distinctly lateral; 1, close to the carpophore	
21	Ratio of mericarp width (parallel to commissure) to its length	0, smaller than 0.05 ↔ 1, 0.05–0.10 ↔ 2, 0.11–0.14 ↔ 3, 0.15–0.20 ↔ 4, 0.21–0.26 ↔ 5, greater than 0.26	
22	Ratio of mericarp width (parallel to commissure) to its thickness	0, smaller than 0.3 ↔ 1, 0.31–0.45 ↔ 2, 0.46–0.60 ↔ 3, 0.61–0.79 ↔ 4, 0.80–1.00 ↔ 5 greater than 1.00	
23	Cuticle and cell-wall thickness at cell center	[0, smaller than 9 μm ↔ 1, 9–13 μm] ↔ 2, greater than 13 μm	In Scandicinae, there is no boundary between the cell wall and the cuticle; in those species with thick cuticles, the transverse cell walls of the epidermal cells are also thickened, although not as much as the outer walls; the thickening of transverse cell walls results in an areolate appearance of the epidermis (character No. 12), although the latter is also found in *Scandix* and *Osmorhiza* which have cuticles not exceeding 13 μm in thickness

Table 2 (*concluded*).

Character No.	Character	States	Comments
24	Ratio of cuticle and cell-wall thickness at cell side to that at cell center	0, smaller than 0.9; 1, equal to or greater than 0.9	
25	Angle of styles	0, smaller than 280°; 1, equal to or greater than 280°	The angle of the style is a highly variable character even within the same species; moreover, it is also influenced by fruit maturity and drying conditions; some species, particularly members of *Kozlovia*, have styles that are bent at their base and directed downwards so that they touch the fruit
26	Percent girth not protected by thickened bundles and vittae (in transverse section of mericarp)	[0, smaller than 10% ↔ 1, 11–20% ↔ 2, 21–50% ↔ 3, 51–69%] ↔ 4, equal to or greater than 70%	A transverse section through the mericarp shows vascular bundles alternating with vittae to form a ring that protects the endosperm; this character accounts for the proportion of "gaps" in this ring
27	Style length	0, smaller than 1 mm ↔ 1, 1–2 mm ↔ 2, greater than 2 mm	
28	Ratio of dorsal bundle size to mericarp size in transverse section	[0, smaller than 0.0025 ↔ 1, 0.0025–0.006] ↔ [2, 0.0061–0.025 ↔ 3, greater than 0.025]	This variable quantifies the relative bundle size; within Scandicinae, enlarged bundles are characteristic of *Chaerophyllum* and several members of *Scandix*; generally, however, this character is homoplastic; to simplify calculations, the bundles and mericarp transverse sections described in character Nos. 28–30 are treated as rectangles and their sizes calculated as width by thickness
29	Ratio of dorsal bundle thickness to its width (in transverse section of mericarp)	0, smaller than 0.027 ↔ 1, 0.0271–0.047 ↔ 2, 0.0471–0.062 ↔ 3, 0.0621–0.1 ↔ 4, greater than 0.1	
30	Ratio of lateral bundle size to commissural bundle size (in transverse section of mericarp)	0, smaller than 0.45 ↔ [1, 0.451–0.65 ↔ 2, 0.651–0.78 ↔ 3, 0.781–0.95 ↔ 4, greater than 0.95]	Enlarged commissural bundles are characteristic of *Anthriscus* although they are not unique to this genus
31	Ratio of mean epidermis cell width in valleculae to that in ridges	0, up to 1 ↔ 1, 1.01–2 ↔ 2, greater than 2	
32	Ratio of endosperm furrow depth to endosperm thickness	[0, smaller than 0.01 ↔ 1, 0.01–0.11] ↔ [2, 0.111–0.14 ↔ 3, 0.141–0.27 ↔ 4, greater than 0.27]	Although also present in other lineages of Apiaceae, the endosperm furrow at the commissural face well defines the branch of Apiaceae comprising Scandiceae; in some members of Scandiceae with dorsally flattened mericarps (e.g., *Daucus carota*), the value of this character may be quite low
33	Ratio of commissure width to mericarp width	0, smaller than 0.38 ↔ 1, 0.39–0.6 ↔ 2, 0.61–0.9 ↔ 3, greater than 0.9	Usually denoted as "commissure broad versus constricted," this character is often used in umbellifer taxonomy, although it may be quite variable even within the same genus
34	Mean fruit length	0, smaller than 4 mm ↔ 1, 4–4.9 mm ↔ 2, 5–6.9 mm ↔ 3, 7–8.9 mm ↔ 4, equal to or greater than 9 mm	
35	Ratio of endosperm thickness to its width	0, smaller than 0.5 ↔ 1, 0.5–0.8 ↔ 2, greater than 0.8	Flattening of the endosperm does not necessarily correlate with the flattening of the fruit; the latter may arise by the broadening of lateral ridges; hence, the shape of the endosperm remains unchanged

Note: Double-headed arrows indicate ordered characters. States given in brackets were subsequently merged to reduce homoplasy (see text for further discussion).

The pattern of evolution of each fruit character was assessed using MacClade version 3.7 (Maddison and Maddison 1992) and the minimal length trees resulting from maximum parsimony analysis of combined morphological and molecular data sets. For each character, retention (RI), consistency (CI), and rescaled consistency (RC) indices were calculated. For each of these indices, nearly all multistate-ordered characters had very low values; thus, some states were subsequently merged to reduce homoplasy (see Table 2). By optimizing the morphological characters onto the strict consensus tree resulting from the combined analysis, we identified those characters that are most useful in delimiting well supported clades in Scandicinae. Based on the reconstruction of character states at the internal nodes of the tree, as estimated by MacClade, we attempted to hypothesize fruit morphology and anatomy of the ancestors of all genera of Scandicinae, of some specific groups of genera, and of the entire subtribe. In those cases when MacClade provided equivocal reconstructions, we usually assumed that the loss of a character is more probable than its independent gain. However, we sometimes chose other reconstructions of ancestral states than those suggested by MacClade. For instance, Spalik (1996) noted that in *Anthriscus* bristles were lost several times so we therefore assumed that the loss of indumentum is more probable than its gain.

Results

Phylogenetic analyses

Equally weighted maximum parsimony analysis of all included ITS positions resulted in 30 minimal length trees, each of 1539 steps, with CIs of 0.425 and 0.394 (with and without uninformative characters, respectively) and a RI of 0.725. The strict consensus of these trees was well resolved (Fig. 1) with only few polytomies occurring in terminal branches. Distance trees (not shown) were generally similar in topology to those derived from the maximum parsimony analysis, and all trees were congruent to those obtained using a broader sampling of Scandicinae and outgroup genera (Downie et al. 2000*a*). In all analyses herein, tribe Scandiceae is strongly supported as monophyletic and comprises three major lineages corresponding to subtribes Scandicinae, Daucinae, and Torilidinae. Within Scandicinae, several distinct branches are identified, with eight of these equivalent to the following currently recognized genera (sensu Spalik and Downie 2001): *Anthriscus*, *Kozlovia* (including *Krasnovia* and *Neoconopodium*), *Geocaryum*, *Myrrhis*, *Osmorhiza*, *Scandix*, *Chaerophyllum* (including *Myrrhoides*), and *Sphallerocarpus*. Sister to this clade of eight genera is a polytomous clade, supported by a 97% bootstrap value, comprising *Athamanta*, *Conopodium* (including *Balansaea*), *Tinguarra*, and *Todaroa*. However, neither *Tinguarra* nor *Conopodium* is maintained as monophyletic. In a subset of the 30 trees obtained from the parsimony searches (not shown), both species of *Tinguarra* fell together. In contrast, the two examined members of *Conopodium* never formed a clade; in all trees, *Conopodium majus* is sister to *Athamanta*, while *Conopodium glaberrimum*, formerly recognized in the monotypic genus *Balansaea*, is placed one node away.

The genera *Anthriscus*, *Kozlovia*, *Geocaryum*, *Myrrhis*, and *Osmorhiza* formed a strongly supported clade (99% bootstrap value) and has been collectively recognized as "crown" Scandicinae (Spalik and Downie 2001). Within this "crown" clade, *Anthriscus* and *Kozlovia* are immediately related, but this affinity is only poorly supported (bootstrap value 28%). The genus *Kozlovia* is supported only moderately with a bootstrap value of 72%. Apart from its type, *Kozlovia paleacea*, this genus includes those members formerly treated in *Krasnovia* and *Neoconopodium* (Spalik and Downie 2001). Although the bootstrap value for *Anthriscus* is low (44%), the monophyly of this genus was confirmed previously by separate analyses of morphological data (Spalik and Downie 2001). In all molecular analyses, *Scandix* is sister to "crown" Scandicinae.

Phylogenetic analyses of the 35 fruit characters resulted in trees that were not congruent to those derived from ITS sequences. In the neighbor-joining analysis of total character differences, neither Scandiceae nor any of its three subtribes is maintained as monophyletic (Fig. 2). Members of subtribes Torilidinae and Daucinae fell together, with the former polyphyletic and arising from the latter. Members of subtribe Scandicinae occur in six different clades. In contrast, the monophyly of most genera was confirmed, but only *Scandix* and *Osmorhiza* received bootstrap support values higher than 50%. Members of "crown" Scandicinae formed a clade sister to *Scandix*, and within the former *Anthriscus* and *Kozlovia* ally as sister taxa. The infrageneric relationships within *Scandix*, *Osmorhiza*, *Kozlovia*, and *Anthriscus* were also identical or consistent to those inferred from ITS data. The close relationship among *Athamanta*, *Tinguarra*, and *Todaroa*, as inferred by the molecular analyses, was confirmed in the neighbor-joining tree. However, in those trees inferred using maximum parsimony (not shown), this clade did not occur. Instead, *Todaroa aurea* and *Tinguarra montana* allied with *Seseli montanum* and *Bubon macedonicum*. Within *Athamanta*, *Athamanta sicula* is sister to the two other species, in accordance with the molecular analyses. *Conopodium* is supported as monophyletic only in the neighbor-joining tree, but it is placed distantly from other members of Scandicinae. In the maximum-parsimony trees (not shown), this genus forms a grade at the base of the "crown" clade. *Chaerophyllum macrospermum* groups with *Grammosciadium platycarpum* in all trees inferred from morphological data, with this clade either associated with members of the apioid superclade in the neighbor-joining tree (Fig. 2) or with other *Chaerophyllum* species in the maximum parsimony trees (not shown). *Chaerophyllum nodosum*, previously recognized in the monotypic genus *Myrrhoides* (= *Physocaulis*) and recently reinstated in *Chaerophyllum* (Spalik and Downie 2001), consistently fell within the latter. In all trees, *Chaerophyllum* (save *Chaerophyllum macrospermum*) is sister to *Falcaria*.

Maximum parsimony analysis of combined ITS and differentially weighted fruit characters resulted in two minimal length trees, each of length 1766.4 steps, CIs of 0.390 and 0.362 (with and without uninformative characters, respectively), and a RI of 0.704. The topology of their strict consensus (Fig. 3) was nearly identical to that derived from parsimony analysis of ITS data (Fig. 1) but with increased resolution among the basal branches of Scandicinae and generally higher bootstrap values overall. Similar results were obtained using the neighbor-joining method. In all analyses of combined data, *Conopodium* and *Tinguarra* are each monophyletic with the latter sister to *Athamanta*.

Fig. 1. Strict consensus of 30 minimal-length 1539-step trees derived from equally weighted maximum parsimony analysis of 66 nuclear ribosomal DNA ITS sequences from Scandiceae subtribe Scandicinae and outgroups (CI = 0.394 excluding uninformative characters; RI = 0.725). Bootstrap values for 1000 replicate analyses are indicated in percent along respective nodes; only those compatible with the majority-rule consensus tree are indicated. Brackets indicate genera recognized in subtribe Scandicinae or tribal, subtribal and informal clade divisions in Apiaceae identified by Downie et al. (2000*a*, 2001). Names of informal groups are identified by double quotes to distinguish them from formally recognized taxa.

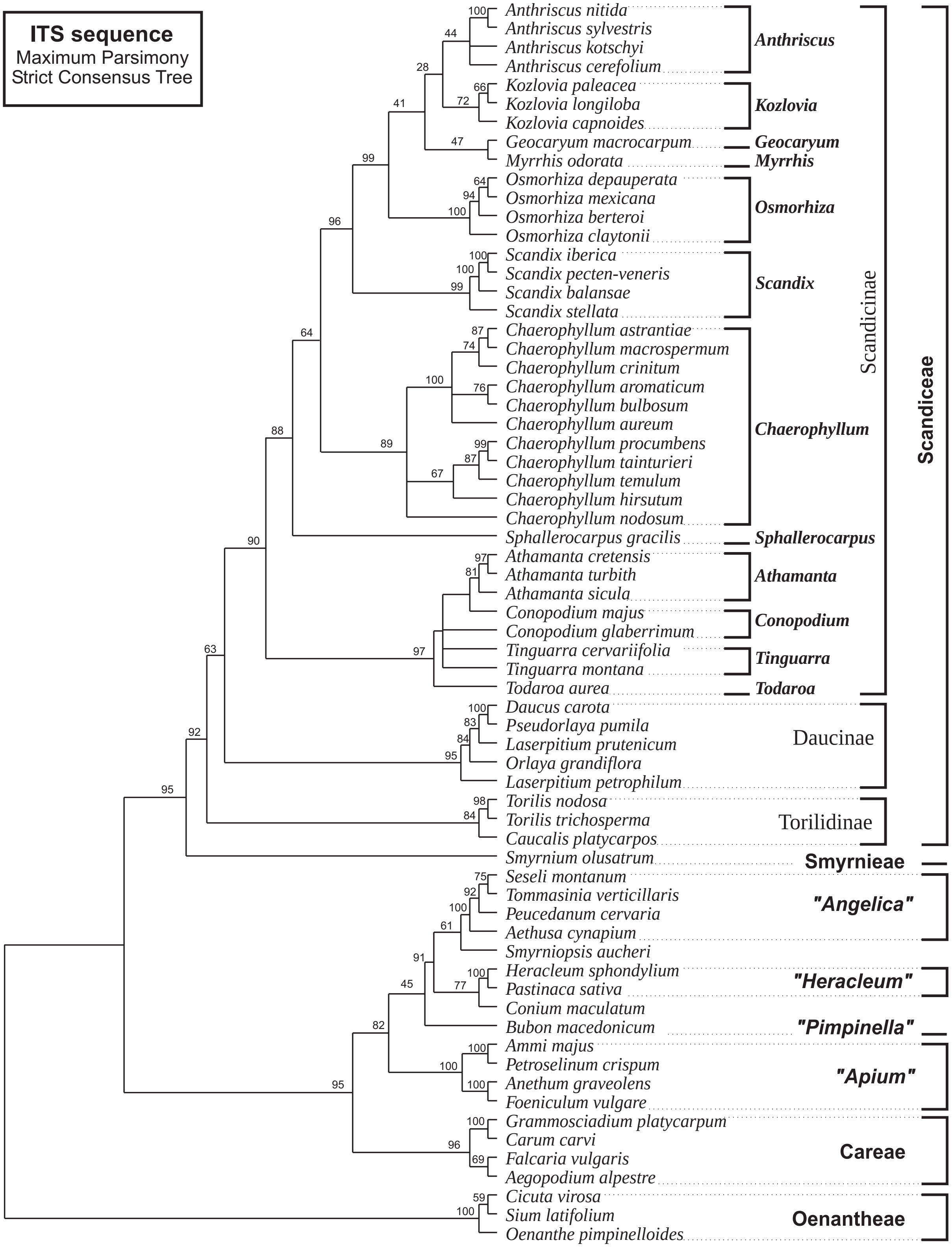


Fig. 2. Neighbor-joining tree inferred from 35 morphological and anatomical fruit characters for 66 representatives of Scandiceae subtribe Scandicinae and outgroups. Branch lengths are proportional to distances estimated using total character differences (note scale bar). Percent bootstrap values for 1000 replicate analyses are indicated along the nodes for those groups that occurred in the majority-rule consensus tree. Members of Scandicinae are bracketed in the far right of the figure.

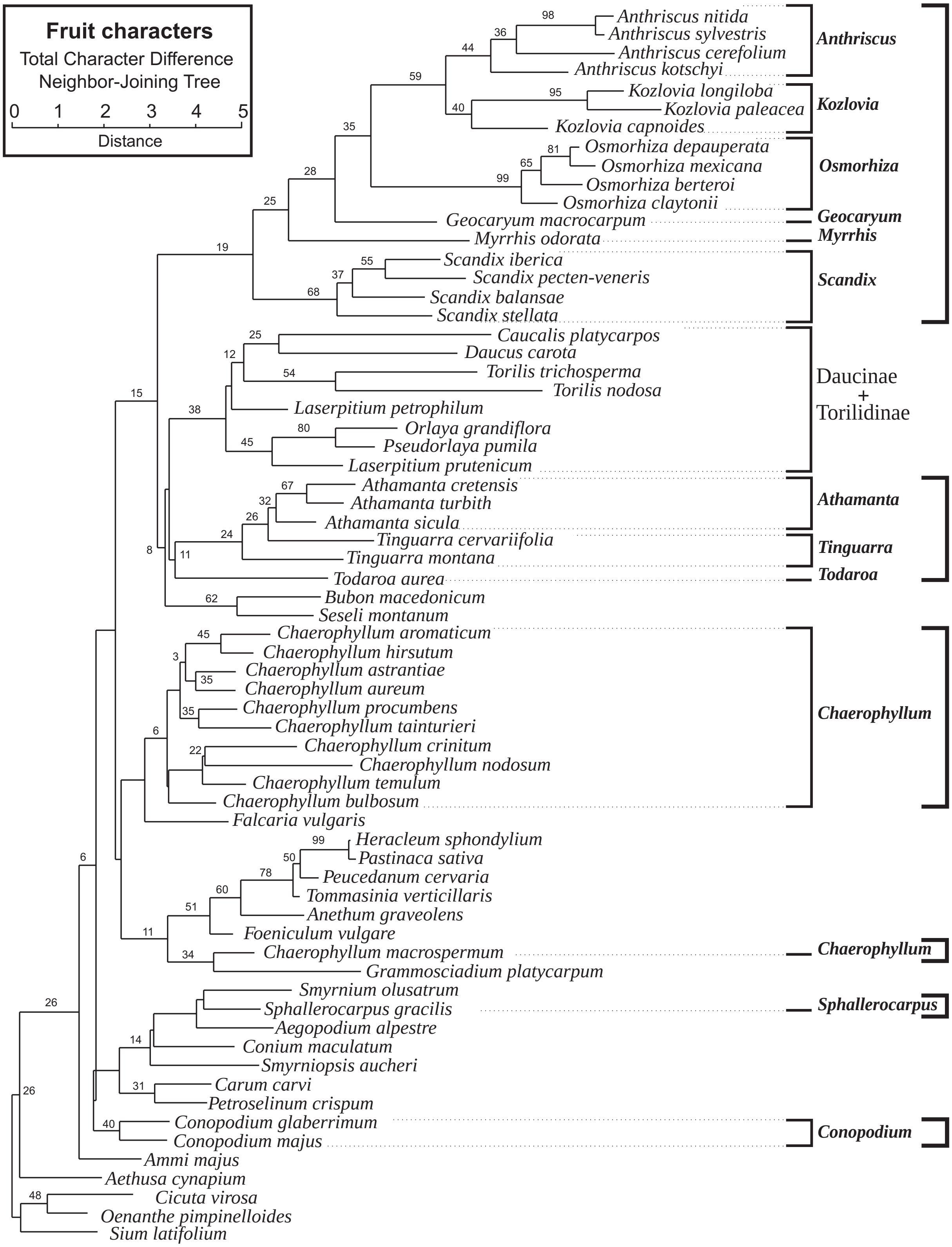
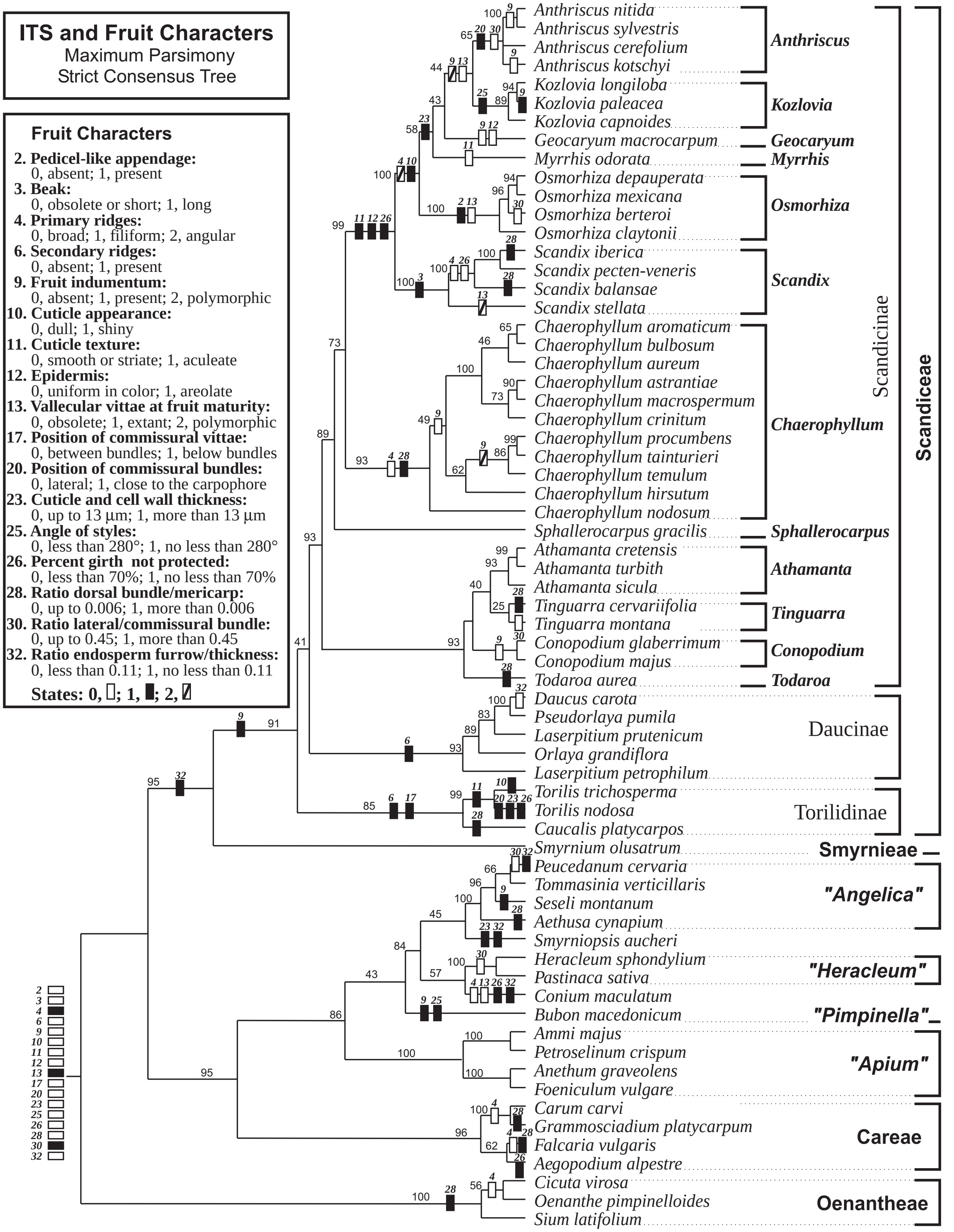


Fig. 3. Strict consensus of two minimal length trees, each of length 1766.4 steps, inferred from maximum parsimony analysis of combined morphological and ITS sequence data for 66 representatives of Scandiceae subtribe Scandicinae and outgroups (CI = 0.362 excluding uninformative characters; RI = 0.704). Numbers along nodes denote bootstrap values for 1000 replicate analyses; only those compatible with the majority-rule consensus tree are indicated. Those morphological characters and states most useful in delimiting genera and suprageneric lineages are indicated; character numbers refer to those of Tables 1 and 2. Brackets are the same as described in Fig. 1.



Taxonomic utility of fruit characters

The distribution of 17 morphological characters, nine of which had RC ≥ 0.37 (character Nos. 2, 3, 6, 9, 10, 12, 13, 17, and 20), is highlighted on the strict consensus tree resulting from maximum parsimony analysis of combined data (Fig. 3). These characters were emphasized because of their ability in defining monophyletic groups and their lower homoplasy relative to the other characters examined. Ten of these characters describe external fruit morphology. A pedicel-like appendage (character No. 2) is unique to *Osmorhiza*, while a long beak (character No. 3) is synapomorphic for *Scandix*. An aculeate and areolate fruit epidermis (character Nos. 11 and 12) unites *Scandix* and all members of "crown" Scandicinae. The latter are also characterized by angular primary ridges (character No. 4) and shiny cuticles (character No. 10), with some possessing a thickened cuticle (character No. 23) and vittae that are compressed at fruit maturity (character No. 13). Commissural bundles situated close to the carpophore (character No. 20) are characteristic for *Anthriscus*, but such bundles also occur outside of the subtribe in *Torilis nodosa*. Styles that are bent downwards (character No. 25) distinguish *Kozlovia* from closely related *Anthriscus* but are also found in *Bubon macedonicum*. A fruit indumentum, whether comprised of hairs or bristles (character No. 9), is generally characteristic for the entire tribe; however, in *Conopodium* and most species of *Chaerophyllum* the fruits are glabrous. Secondary ridges (character No. 6) with varying types of appendages (character No. 7; not shown) occur in Daucinae and Torilidinae; those examined members of the latter are also characterized by commissural vittae situated below the vascular bundles (character No. 17). In fruits of both of Daucinae and Torilidinae, as well as of some other lineages of Apiaceae, different forms of sclerification (character No. 18; not shown) may occur. This sclerification, however, is absent in subtribe Scandicinae.

Despite somewhat higher homoplasy, four other characters are also useful in defining monophyletic groups. The entire tribe as well as its sister group Smyrnieae is characterized by a grooved endosperm (character No. 32); however, this state also occurs in *Smyrniopsis aucheri*, *Peucedanum cervaria*, and *Conium maculatum*, while some members of Scandiceae reverted to an almost flat endosperm (e.g., *Daucus carota*). Commissural bundles are apparently much larger than lateral ones (character No. 30) in all members of *Anthriscus* but this feature also occurs in several other species. The size of the vascular bundles is exemplified by the ratio of dorsal bundle size to the size of the entire transverse section of the mericarp (character No. 28). Scandicinae generally have small vascular bundles, with exceptions including *Chaerophyllum* and two species of *Scandix*. Somewhat enlarged dorsal bundles characterize *Tinguarra cervariifolia* and *Todaroa aurea*. Outside the subtribe, this character is quite homoplastic. The vascular bundles and intervening vallecular vittae form a more or less continuous shield that surrounds the endosperm. However, in those members of the "crown" clade and in *Scandix stellata*, vascular bundles and vittae are reduced in size; hence, more than 70% of endosperm girth (character No. 26) is not protected. A similar pattern exists in *Conium maculatum*, *Aegopodium alpestre*, and *Torilis nodosa*.

It is noteworthy that nearly all genera of subtribe Scandicinae may be unambiguously defined based on fruit characters (Figs. 3 and 4). The only exception is *Tinguarra*, for which we did not find any characters to distinguish it from its sister *Athamanta*.

Discussion

Taxonomic utility of fruit characters

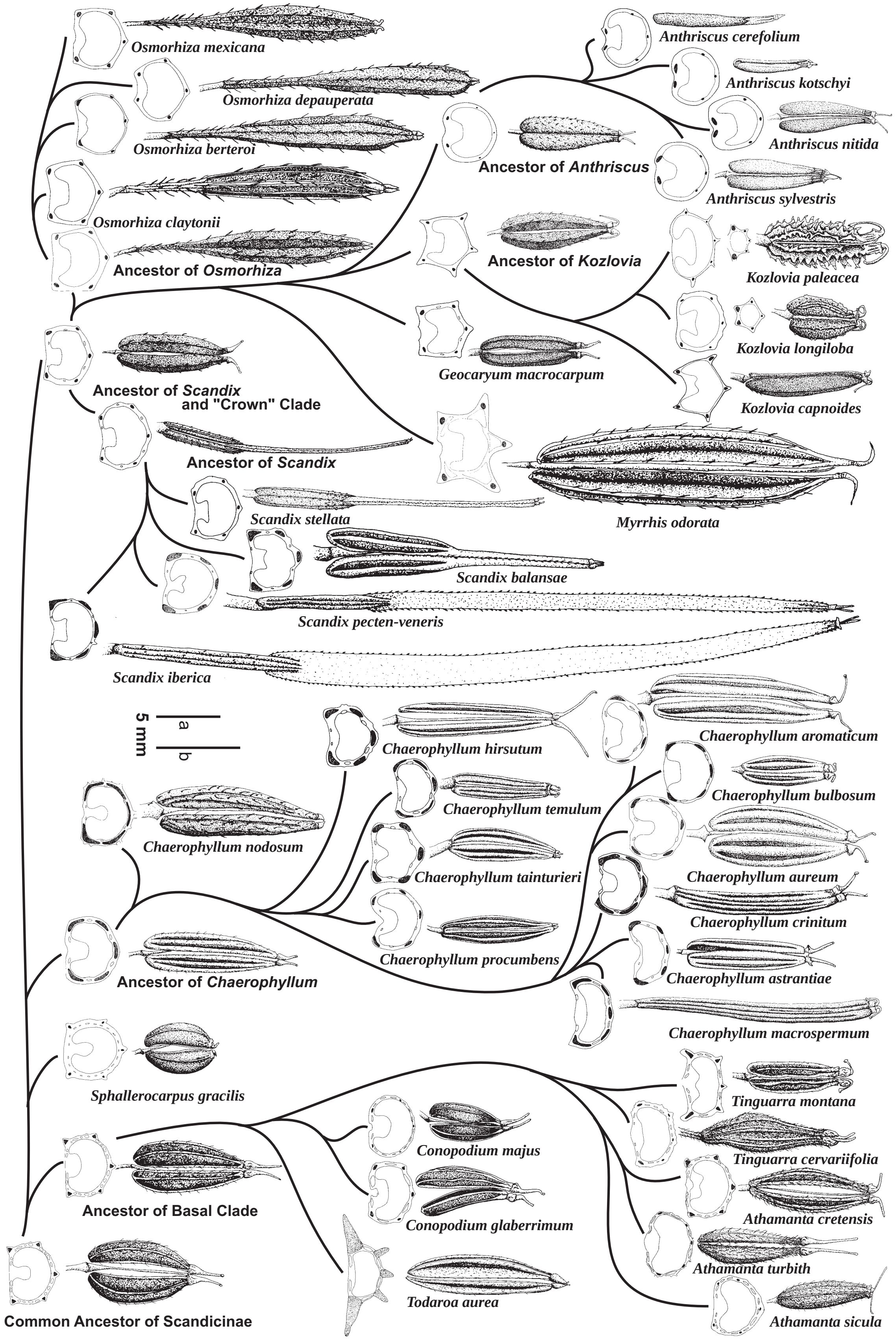
Umbellifer fruits exhibit an outstanding array of morphological and anatomical modifications, many of which are believed to constitute adaptations for various modes of seed dispersal. These characters are therefore susceptible to convergence and may constitute poor indicators of monophyletic groups. As an example, tribe Peucedaneae was defined on the basis of a distinct dorsal flattening of the mature fruit with its lateral ridges expanded into winglike appendages. Differences in the morphology of these appendages served to divide the tribe into three subtribes: Angelicinae, Ferulinae (Peucedaninae), and Tordyliinae (Drude 1898). However, as Theobald (1971) pointed out, it is easy to picture the evolution of dorsal flattening and wing formation as a dispersal mechanism in many independent lineages derived from less specialized types, and this is indeed what Downie et al. (2000*c*) inferred from their molecular analyses.

Fruit characters have also proved limiting for phylogenetic inference in subtribe Scandicinae. The trees obtained herein from analyses of morphological and anatomical data sets were not congruent to those obtained from separate analysis of ITS sequences and the relationships proposed were not as strongly supported. Moreover, the analysis of combined data produced trees that were practically identical with those inferred from molecular data alone. Although fruit characters failed to confirm the monophyly of subtribe Scandicinae, most of its included genera as well as "crown" Scandicinae were each identified as monophyletic. Moreover, the relationships within *Athamanta*, *Scandix*, *Osmorhiza*, and *Kozlovia* were identical and those within *Anthriscus* similar to those inferred from molecular data.

Nearly all genera of Scandicinae are well delimited on the basis of fruit morphology and anatomy. The exception is *Tinguarra* for it cannot be readily distinguished from its sister *Athamanta*. *Athamanta sicula* was once regarded as a member of *Tinguarra* (Bentham 1867; Drude 1898; Knees 1996), while the type of *Tinguarra*, *Tinguarra cervariifolia*, was placed by de Candolle (1830) in *Athamanta*. Both a previous study using morphological data (Spalik and Downie 2001) and this study failed to provide clear diagnostic characters in which to distinguish *Athamanta* from *Tinguarra*. Since these two genera are undoubtedly closely related and morphologically indistinguishable, they have been combined (Spalik et al. 2001) and our study supports such a treatment.

The position of *Todaroa aurea* is enigmatic. Our analysis of fruit characters places this species sister to the *Athamanta–Tinguarra* clade, while molecular data suggest an affinity that is not so straightforward. The isolated position of *Todaroa* in many trees is supported by its winged fruits, which are unique within the clade. The inclusion of *Balansaea*, exemplified here by *Conopodium glaberrimum*

Fig. 4. The evolution of fruit morphology and anatomy in Scandiceae subtribe Scandicinae. Fruit sections are not to scale. Scale bar *a* is for the whole fruits of *Myrrhis, Scandix*, and *Osmorhiza*; scale bar *b* is for all remaining species. Drawings are by A. Wojewódzka.



(≡ *Balansaea glaberrima*), in *Conopodium*, as advocated by Engstrand (1973) and adopted in this study, was confirmed by morphological data (Spalik and Downie 2001, and this study) but not by ITS data alone. The morphologically variable genus *Chaerophyllum* is well supported in molecular analyses (Downie et al. 2000*a*) but when morphology is considered the bootstrap support values are very low with one species, *Chaerophyllum macrospermum*, constantly grouped with *Grammosciadium platycarpum*. Interestingly, Koso-Poljansky (1916) placed *Chaerophyllum macrospermum* in the monotypic genus *Golenkinianthe* presumed to be related to *Grammosciadium*. This similarity is apparently homoplastic.

We have demonstrated that although fruit morphology and anatomy appear inferior to molecular data for phylogenetic inference in subtribe Scandicinae, they are still more informative than other morphological characters. Spalik and Downie (2001) found that of the 44 discrete vegetative, inflorescence, floral, and fruit characters they examined in the subtribe, those relating to the fruit were most congruent with a phylogeny inferred from molecular data. RC values for these 44 characters ranged from 0 to 0.48 (the former referring to three characters that were completely homoplastic) and averaged 0.13. The six highest RC values, all greater than 0.25, pertained to characters of the fruit. In this study, RC values for 10 characters across a comparable array of taxa exceeded 0.25 (range 0.01–1.00, mean 0.20). When combined with molecular data, fruit characters did not change the tree topology but aided to resolve terminal branches where molecular characters alone were not variable enough.

Fruit evolution in subtribe Scandicinae

Based on the distribution of morphological character changes we infer that within Scandicinae, members of *Athamanta* have fruits that have retained many plesiomorphic character states and, thus, are likely very similar to those of the hypothetical common ancestor of the subtribe (Fig. 4). These fruits were probably oblong-ovate, somewhat laterally compressed (i.e., with mericarps as broad as wide), hairy, and with filiform ridges and constricted commissures. Its endosperm was probably deeply grooved, as seen in *Smyrnium olusatrum* (which is sister to the entire tribe) or as in some earlier branching Scandicinae (such as *Sphallerocarpus gracilis* or *Chaerophyllum nodosum*). The number of vallecular vittae was most likely variable (one to four), and these were irregular and anastomosing. The reconstruction of this state in the ancestor of the subtribe depends upon the choice of outgroup. Vittae are single in most Daucinae and Torilidinae, as well as in *Todaroa*, whereas early branching Daucinae (i.e., *Laserpitium petrophilum*), *Smyrnium olusatrum*, basal Scandicinae (*Athamanta*, *Tinguarra*, most members of *Conopodium*), and *Sphallerocarpus* have fruits with numerous vittae. Noteworthy is that in young fruits of *Myrrhis odorata* two types of vallecular vittae are seen: numerous small vittae situated close to the endosperm and somewhat larger ones, one per vallecula, that are placed outwards (Drude 1898). In *Anthriscus*, vallecular vittae are usually single, but sometimes, additional ones can occur (Spalik 1997). In young fruits of *Anthriscus cerefolium* the arrangement and number of vittae is similar to that occurring in *Myrrhis* (Kowal et al. 1969).

Variation in fruit morphology and anatomy in the earliest branch of Scandicinae is minor; the number of vallecular vittae is reduced in *Todaroa* and some species of *Conopodium*, the indumentum is lost in *Conopodium*, and lateral wings occur in *Todaroa*. Fruits of these taxa are generally quite similar, with the differences among them being quantitative rather than qualitative. Many plesiomorphic characters are also retained in *Sphallerocarpus*.

In *Chaerophyllum*, vallecular vittae are reduced to one per vallecula and, along with the vascular bundles, become enlarged, particularly in *Chaerophyllum nodosum*, presumed sister to all other members of the genus. However, in some species, particularly the American *Chaerophyllum procumbens*, these structures may have undergone substantial reduction, although they remain broader than those of the common ancestor of the subtribe. Fruits of most species of the genus are glabrous; exceptions include *Chaerophyllum nodosum* that has fruits covered with stiff setose hairs (bristles) and three other species that comprise variants with pubescent fruits. These variants are, however, rare.

The most important evolutionary change that occurred in the common ancestor of *Scandix* and “crown” Scandicinae was the thickening of the exocarp cell walls. Irregular thickening of the external walls resulted in an aculeate cuticle, while the reinforcement of the transverse cell walls produced an areolate appearance to the epidermis. In some genera the entire cuticle became thickened. Members of the “crown” clade are also characterized by reduced vallecular vittae; although they have remained extant in *Myrrhis* and *Geocaryum*, in the former they are deprived of resin at fruit maturity, while in the latter they are relatively small leaving much of the endosperm girth unprotected. The common ancestor of the “crown” clade presumably had relatively narrow fruits that were distinctly pentagonal in transverse section. Such fruits are extant in *Osmorhiza*, *Geocaryum*, and *Kozlovia capnoides*. In *Kozlovia longiloba*, *Kozlovia paleacea*, and *Anthriscus*, the “overgrown” endosperm compresses the mesocarp and pushes the entire pericarp outwards; hence, the angular ridges have remained only at the top of the fruit. Therefore, mericarps of *Kozlovia capnoides*, when dissected one third above the fruit base, differ from those of *Kozlovia paleacea* and *Kozlovia longiloba*. However, when cut at two thirds above the fruit base, they look similar (Fig. 4).

The broad, thickened bundles of some *Scandix* species, and particularly *Scandix iberica*, are superficially similar to those of *Chaerophyllum*. However, in *Scandix stellata*, sister to all other examined *Scandix* species, these bundles are very small and similar to those seen among species of the “crown” clade. Moreover, because of a relatively short beak (as compared to other species of *Scandix*) and a distinctly aculeate epidermis (which is not as pronounced in the other species), its fruits are reminiscent of members of the “crown” clade. These similarities are plesiomorphic.

The evolution of fruit indumentum is obscure, as its occurrence in the subtribe is erratic, with pubescent and glabrous fruits often found in the same species (e.g., *Chaerophyllum temulentum*, *Chaerophyllum procumbens*, *Chaerophyllum tainturieri*, and *Anthriscus cerefolium*). The form of indumentum also varies considerably. Members of

Athamanta, *Tinguarra*, and *Todaroa* have fruits covered with hairs that are similar to those occurring elsewhere on the plant. Similarly, the stiff, setose hairs occurring on the fruits of *Chaerophyllum nodosum* are also indistinguishable from those arising on its stems and leaves. In contrast, those bristles covering the fruits of members of the "crown" clade, which usually arise from small tubercles, appear distinct from the indumentum on the vegetative organs. Sometimes these bristles are secondarily reduced to short teeth, as in *Kozlovia longiloba*, or they break off easily in mature fruits, as in the bristled-fruit variety of *Anthriscus cerefolium* (and other species of *Anthriscus* not considered herein); hence, these fruits are described as tuberculate rather than bristled. Bristles that often break off at fruit maturity also occur in *Myrrhis* and *Kozlovia capnoides*. Conversely, they are persistent in *Osmorhiza*, while in *Kozlovia paleacea* they are so enlarged that Heywood (1982) placed this species alongside the spiny-fruited umbellifers in the tribe Caucalideae.

This study demonstrates further that several umbellifer fruit modifications may not only have arisen independently in several lineages but are also variable at lower taxonomic levels. Many authors, including Drude (1898), Calestani (1905), and Cerceau-Larrival (1962), have already postulated independent origins for several fruit characters. These studies, however, were based on evolutionary scenarios inferred from morphological and anatomical data that are not in agreement with those relationships estimated from cladistic analyses of molecular data. Moreover, earlier workers usually refrained from interpreting these evolutionary changes in terms of adaptive shifts; such explanations are essential to our understanding of plant diversity.

Adaptive significance of fruit characters

Structural properties of the fruit result from a trade-off between its two primary functions: seed dispersal and seed protection. One may expect that fruit morphology and anatomy are subject to strong selective pressures, for it is clear that different fruit appendages reflect different dispersal strategies. Hooks and sticky hairs are usually interpreted as adaptations to epizoochory, while wings facilitate wind dispersal (Howe and Westley 1986). Strong pericarps or massive seed walls can protect seeds not only against seed eaters but also against microorganisms (fungi, bacteria) and harsh weather conditions. Grubb et al. (1998) have shown that plants with massive seed walls have significantly higher nitrogen concentrations in the embryo-cum-endosperm fraction than those with thinner walls. Moreover, other studies have suggested that even small qualitative and quantitative differences in fruit structure observed at the infraspecific level may reflect adaptive shifts to local environmental conditions. For example, in *Heterosperma pinnatum* (Asteraceae), the percentage of achenes having awns is correlated with closed vegetation, lower spring precipitation, and higher summer precipitation (Venable et al. 1998). Also, Hroudova et al. (1997) found that the achenes of two subspecies of *Bolboschoenus maritimus* (Cyperaceae) occurring in different habitats vary with respect to the development of aerenchymatic tissue that determines their buoyancy. Subspecies *compactus* occurring in temporarily flooded terrestrial habitats possesses more buoyant achenes, whereas subspecies *maritimus*, occupying littoral habitats, has less buoyant achenes. Meyer (1997) showed that achene mass variation in the North American shrub *Chrysothamnus nauseous* (Asteraceae) was under strong genetic control that was correlated with fruiting time. Additionally, those *Chrysothamnus* subspecies with the heaviest achenes are restricted to specialized edaphic environments (dunes and badlands) or late seral montane riparian communities, while subspecies that are widely distributed and occur in early seral habitats have less heavy achenes. One may surmise that the differences in fruit structure observed in Scandiceae subtribe Scandicinae also represent similar adaptive shifts.

The arrangement of vittae and vascular bundles suggests that these structures serve to protect the endosperm. The resin-filled vittae not only constitute mechanical protection but also contain active compounds that are toxic to insects (Berenbaum 1981). The broadening of vascular bundles occurs because of the development of sclerenchymatic tissue, providing a strong barrier against intruders. Phloem elements may be additionally shielded by intrajugal vittae. The presence in the epidermis of wax and a thickened cuticle constitutes both mechanical and chemical barriers that impede penetration by insects and fungi (Harborne 1993; Kerstiens 1996). The indumentum on a leaf serves as another defense mechanism (Gutschick 1999). In several species of Scandicinae, e.g., members of *Athamanta* and *Tinguarra* and *Chaerophyllum nodosum*, the indumentum of the fruit is similar to that occuring on the vegetative organs and may therefore have the same, presumably defensive, function.

In Scandicinae, it is noteworthy that different forms of fruit and endosperm protection rarely occur together. The densely hispid fruits of *Athamanta* do not have the enlarged bundles and vittae as do the naked fruits of *Chaerophyllum*. Those members of the "crown" clade characterized by a thickened cuticle usually have reduced vittae and bundles, while the presence of an indumentum is erratic. Interestingly, in some species (e.g., *Myrrhis odorata*, *Kozlovia capnoides*, and *Anthriscus*) the bristles break off easily at fruit maturity thus appearing only useful during early stages of fruit development (i.e., when the cell walls of the exocarp are still thin). Although little is known about the chemical defenses of these species, several compounds with antiproliferative activity have been found in the fruits of *Anthriscus sylvestris* (Ikeda et al. 1998). In fact, such compounds may be specifically allocated to fruits. In *Pastinaca sativa*, for instance, toxic furanocoumarins have a much higher concentration in fruits than in other parts of the plant and constitute the first line of defense against the parsnip webworm, *Depressaria pastinacella* (Zangerl et al. 1997; Zangerl and Nitao 1998). The allocation of these chemicals to fruits is correlated with the endosperm mass but also depends on the paternal genotype; its variation seems to reflect competition among seed genotypes (Zangerl and Nitao 1998).

Some members of the "crown" clade have fruits with bristles persistent at fruit maturity, seemingly to serve epizoochory. The long fruit beak of *Scandix* is usually covered with bristles and may serve to anchor the fruit on animal fur. However, *Scandix australis* and *Scandix turgida* (not considered herein) have central fruits with shorter beaks that are deprived of bristles. These fruits are therefore less exposed to removal by animals. Other fruits have heteromorphic mericarps, where the inner mericarp is smooth and the outer one is bristled (Hedge and Lamond 1972; K. Spalik, personal observation). Heterocarpy also occurs in some members of subtribes Torilidinae and Daucinae. It has been suggested that the spiny fruits are adapted for long-distance dispersal, while the naked ones are to maintain the local population (Jury 1986). Persistent bristles also occur in those members of *Osmorhiza* that are characterized by a pedicel-like appendage. This appendage may function similarly to the beak of *Scandix* in facilitating dispersal. Fruits of *Osmorhiza occidentalis* that lack this appendage, and those of *Osmorhiza mexicana* ssp. *bipatriata* in which it is poorly developed, are glabrous (Lowry and Jones 1984). The role of this bristled appendage is best demonstrated by plant population studies. Gene flow within *Osmorhiza claytonii*, *Cryptotaenia canadensis*, and *Sanicula odorata* seems to reflect differences in their dispersal abilities. It is lowest in the glabrous-fruited *Cryptotaenia canadensis* and highest in *Sanicula odorata* (Williams and Guries 1994; Williams 1994). Both *Sanicula odorata* and *Osmorhiza claytonii* have bristled fruits; however, those of *Sanicula odorata* are lighter and therefore better adapted to epizoochory than those of *Osmorhiza claytonii*.

Closely related taxa with bristled and glabrous fruits also occur in *Anthriscus*. Spalik (1996) speculated that the fruit morphology of European *Anthriscus caucalis* may have affected its dispersal abilities. The rare glabrous-fruited variety now occurs only in the northwestern Mediterranean, while the bristle-fruited variety is a common weed that has also reached Argentina, North America, India, and New Zealand (Spalik 1997). Several fruit characters promoting animal dispersal are also common to the Hawaiian and South American species of *Sanicula* and their closest North American relatives, with the adaptation to epizoochory having been lost multiple times in the western North American members of this genus (Vargas et al. 1999).

In Scandiceae subtribe Scandicinae, fruit characters provide their greatest utility at the generic level, with different patterns of fruit morphological and anatomical evolution occurring within the subtribe. In contrast, we are unable to detect a single synapomorphy supporting the monophyly of the subtribe. It is surprising how little is known about the adaptive significance of these fruit modifications. The analysis of fruit character variation on the phylogenetic tree cannot answer the question whether they actually serve dispersal or defense of seed from predation but can provide us with valuable insight into the evolution of these characters.

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References

Bentham, G. 1867. Umbelliferae. *In* Genera plantarum. Vol. 1. *Edited by* G. Bentham and J.D. Hooker. Reeve, London. pp. 859–931.

Berenbaum, M.R. 1981. Evolution of specialization in insect–umbellifer associations. Annu. Rev. Entomol. **35**: 319–343.

Calestani, V. 1905. Contributo alla sistematica delle ombrellifere d'Europa. Webbia, **1**: 89–280.

Cerceau-Larrival, M.-T. 1962. Plantules et pollens d'Ombellifères. Mem. Mus. Natl. Hist. Nat. Ser. B Bot. **14**: 1–166.

Constance, L. 1971. History of the classification of Umbelliferae (Apiaceae). *In* The biology and chemistry of the Umbelliferae. *Edited by* V.H. Heywood. Academic Press, London. pp. 1–8.

de Candolle, A.P. 1830. Umbelliferae. *In* Prodromus systematis naturalis regni vegetabilis. Vol. 4. *Edited by* A.P. de Candolle. Treüttel & Würtz, Paris. pp. 55–250.

Downie, S.R., and Katz-Downie, D.S. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot. **83**: 234–251.

Downie, S.R., Katz-Downie, D.S., and Spalik, K. 2000*a*. A phylogeny of Apiaceae tribe Scandiceae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot. **87**: 76–95.

Downie, S.R., Katz-Downie, D.S., and Watson, M.F. 2000*b*. A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA *rpl16* and *rpoC1* intron sequences: towards a suprageneric classification of subfamily Apioideae. Am. J. Bot. **87**: 273–292.

Downie, S.R., Watson, M.F., Spalik, K., and Katz-Downie, D.S. 2000*c*. 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.

Downie, S.R., Ramanath, S., Katz-Downie, D.S. and Llanas, E. 1998. Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* intron sequences. Am. J. Bot. **85**: 563–591.

Downie, S.R., Plunkett, G.M., Watson, M.F., Spalik, K., Katz-Downie, D.S., Valiejo-Roman, C.M., Terentieva, E.I., Troitsky, A.V., Lee, B.-Y., Lahham, J., and El-Oqlah, A. 2001. Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. Edinb. J. Bot. **58**: 301–330.

Drude, C.G.O. 1898. Umbelliferae. *In* Die natürlichen Pflanzenfamilien. Vol. 3(8). *Edited by* A. Engler and K. Prantl. Wilhelm Engelmann, Leipzig, Germany. pp. 63–250.

Engstrand, L. 1973. Generic delimitation of *Bunium*, *Conopodium* and *Geocaryum* (Umbelliferae). Bot. Not. **126**: 146–154.

Engstrand, L. 1977. Biosystematics and taxonomy in *Geocaryum* Cosson (Umbelliferae). University of Lund, Lund, Sweden.

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

Gerlach, D. 1972. Zarys mikrotechniki botanicznej. PWRiL, Warsaw, Poland.

Grubb, P.J., Metcalfe, D.J., Grubb, E.A.A., and Jones, G.D. 1998. Nitrogen-richness and protection of seeds in Australian tropical rainforest: a test of plant defense theory. Oikos, **82**: 467–482.

Gutschick, V.P. 1999. Biotic and abiotic consequences of differences in leaf structure. New Phytol. **143**: 3–18.

Harborne, J.B. 1993. Introduction to ecological biochemistry. Academic Press, London.

Hedge, I.C., and Lamond, J.M. 1972. *Scandix* L. *In* Flora of Turkey and the East Aegean Islands. Vol. 4. *Edited by* P.H. Davis. Edinburgh University Press, Edinburgh. pp. 325–330.

Heywood, V.H. 1971. Systematic survey of Old World Umbelliferae. *In* The biology and chemistry of the Umbelliferae. *Edited by* V.H. Heywood. Academic Press, London. pp. 31–42.

Heywood, V.H. 1982. Multivariate taxonomic synthesis of the tribe Caucalideae. *In* Les Ombellifères: Contributions Pluridisciplinaires à la Systématique. Actes du 2ème Symposium International sur les Ombellifères. *Edited by* A.-M. Cauwet-Marc and J. Carbonnier. Braun-Brumfield, Ann Arbor, Mich. Monogr. Syst. Bot. Mo. Bot. Gard. No. 6. pp. 727–736.

Higgins, D.G., Bleasby, A.J., and Fuchs, R. 1992. CLUSTAL V: improved software for multiple sequence alignment. CABIOS, **8**: 189–191.

Holmgren, P.K., Holmgren, N.H., and Barnett, L.C. 1990. Index herbariorum. New York Botanical Garden, New York.

Howe, H.F., and Westley, L.C. 1986. Ecology of pollination and seed dispersal. *In* Plant ecology. *Edited by* M.J. Crawley. Blackwell, Oxford, U.K. pp. 185–215.

Hroudova Z., Moravcova, L., and Zakravsky, P. 1997. Effect of anatomical structure on the buoyancy of achenes of two subspecies of *Bolboschoenus maritimus*. Folia Geobot. Phytotax. **32**: 377–390.

Ikeda, R., Nagao, T., Okabe, H., Nakano, Y., Matsunaga, H., Katano, M., and Mori, M. 1998. Antiproliferative constituents in Umbelliferae plants. IV. Constituents in the fruits of *Anthriscus sylvestris* Hoffm. Chem. Pharm. Bull. (Tokyo), **46**: 875–878.

Jury, S.L. 1986. Fruit and leaf variation in the African species of the Umbelliferae tribe Caucalideae. Symb. Bot. Upsal. **26**: 181–188.

Katz-Downie, D.S., Valiejo-Roman, C.M., Terentieva, E.I., Troitsky, A.V., Pimenov, M.G., Lee, B.-Y., and Downie, S.R. 1999. Towards a molecular phylogeny of Apiaceae subfamily Apioideae: additional information from nuclear ribosomal DNA ITS sequences. Plant Syst. Evol. **216**: 167–195.

Kerstiens, G. 1996. Signalling across the divide: a wider perspective of cuticular structure–function relationships. Trends Plant Sci. **1**: 125–129.

Kim, J.H. 1996. General inconsistency conditions for maximum parsimony: effects of branch lengths and increasing numbers of taxa. Syst. Biol. **45**: 363–374.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. **16**: 111–120.

Knees, S.G. 1996. *Tinguarra* Benth. & Hook. in Morocco. Lagascalia, **18**: 286–287.

Koch, W.D.J. 1824. Generum tribuumque plantarum Umbelliferarum nova dispositio. Nova Acta Acad. Caes. Leop. Carol. German. Nat. Cur. **12**: 55–156.

Koso-Poljansky, B. M. 1916. Sciadophytorum systematis lineamenta. Bull. Soc. Imp. Nat. Moscou, **29**: 93–222.

Kowal, T., Latowski, K., Macher, Z., and Pic., S. 1969. Anatomiczne cechy diagnostyczne leczniczych i trujacych gatunków *Anthriscus* Pers. Pr. Kom. Farm. Poznan. Tow. Przyj. Nauk, **7**: 39–53.

Lee, B.-Y., and 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., and 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.

Lowry, P.P., and Jones, A.G. 1984. Systematics of *Osmorhiza* Raf. (Apiaceae: Apioideae). Ann. Mo. Bot. Gard. **71**: 1128–1171.

Maddison, W.P., and Maddison, D.R. 1992. MacClade. Analysis of phylogeny and character evolution, version 3 edition. Sinauer Associates, Sunderland, Mass.

Meyer, S.E. 1997. Ecological correlates of achene mass variation in *Chrysothamnus nauseosus* (Asteraceae). Am. J. Bot. **84**: 471–477.

Morison, R. 1672. Plantarum umbelliferarum distributio nova. Sheldonian Theatre, Oxford, U.K.

Plunkett, G.M., Soltis, D.E., and Soltis, P.S. 1996. Evolutionary patterns in Apiaceae: inferences based on *matK* sequence data. Syst. Bot. **21**: 477–495.

Rompel, J. 1895. Krystalle von Calcium oxalat in der Fruchtwandt der Umbelliferen und ihre Verwerthung für die Systematik. Sitzungsber. Akad. Wiss. Wien, **104**: 417–476.

Spalik, K. 1996. Species boundaries, phylogenetic relationships and ecological differentiation in *Anthriscus* (Apiaceae). Plant Syst. Evol. **199**: 13–32.

Spalik, K. 1997. Revision of *Anthriscus* (Apiaceae). Pol. Bot. Stud. **13**: 1–69.

Spalik, K., and Downie, S.R. 2001. The utility of morphological characters for inferring phylogeny in Scandiceae subtribe Scandicinae (Apiaceae). Ann. Mo. Bot. Gard. **88**: 270–301.

Spalik, K., Wojewódzka, A., and Downie, S.R. 2001. Delimitation of genera in Apiaceae with examples from Scandiceae subtribe Scandicinae. Edinb. J. Bot. **58**: 331–346.

Swofford, D.L. 1998. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4 edition. Sinauer Associates, Sunderland, Mass.

Theobald, W.L. 1971. Comparative anatomical and developmental studies in the Umbelliferae. *In* The biology and chemistry of the Umbelliferae. *Edited by* V.H. Heywood. Academic Press, London. pp. 177–197.

Vargas, P., Baldwin, B.G., and Constance, L. 1999. A phylogenetic study of *Sanicula* sect. *Sanicoria* and *S.* sect. *Sandwicenses* (Apiaceae) based on nuclear rDNA and morphological data. Syst. Bot. **24**: 228–248.

Venable, D.L., Dyreson, E., Pinero, D., and Becerra, J.X. 1998. Seed morphometrics and adaptive geographic differentiation. Evolution, **52**: 344–354.

Williams, C.F. 1994. Genetic consequences of seed dispersal in three sympatric forest herbs: II. Microspatial genetic structure within populations. Evolution, **48**: 1959–1972.

Williams, C.F., and Guries, R.P. 1994. Genetic consequences of seed dispersal in three sympatric forest herbs. I. Hierarchical population-genetic structure. Evolution, **48**: 791–805.

Zangerl, A.R., and Nitao, J.K. 1998. Optimal defense, kin conflict and the distribution of furanocoumarins among offspring of wild parsnip. Evol. Ecol. **12**: 443–457.

Zangerl, A.R., Green, E.S., Lampman, R.L., and Berenbaum, M.R. 1997. Phenological changes in primary and secondary chemistry of reproductive parts in wild parsnip. Phytochemistry, **44**: 825–831.