

Phylogenetic analysis of nrDNA ITS sequences reveals relationships within five groups of Iranian Apiaceae subfamily Apioideae

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The flora of Iran is rich in endemic species of Apiaceae, many of which have been poorly investigated and whose phylogenetic relationships are unknown. We investigate the relationships within five genus groups of Apiaceae subfamily Apioideae native to the *Flora Iranica* region using nuclear ribosomal DNA ITS sequences. Supplementary comparative data come from morphology and, for one of these groups, petiole anatomy and palynology. These groups of Apioideae are circumscribed initially on the basis of morphological similarities and/or the results of previous molecular systematic investigations, then redefined and confirmed as monophyletic based on the results of maximum parsimony, maximum likelihood, and Bayesian analyses of ITS sequences from a broader sampling of species. These five redefined groups and their constituent genera include the *Cachrys* group (*Alococarpum*, *Azilia*, *Bilacunaria*, *Cachrys*, *Diplotaenia*, *Eriocyclus*, *Ferulago*, *Prangos*), *Cymbocarpum* group (*Cymbocarpum*, *Ducrosia*, *Kalakia*), *Ferula* group (*Dorema*, *Ferula*, *Leutea*), *Johrenia* group (*Holandrea*, *Johrenia*, *Johreniopsis*), and *Opopanax* group (*Opopanax*, *Smyrniopsis*). *Azilia eryngioides* is a sister group to all other members of the *Cachrys* group, coincident with its highly unusual morphology. The monotypic *Alococarpum* may be transferred into *Prangos* pending further study. The *Cymbocarpum* group is contained within tribe Tordylieae, the latter now expanded to include *Cymbocarpum*, and the monotypic *Kalakia* is submerged within *Cymbocarpum*. *Dorema* and *Leutea* arise from within a paraphyletic *Ferula* in tribe Scandiceae; nomenclatural changes are suggested, but must await supporting evidence from chloroplast DNA. Neither *Johrenia* nor *Johreniopsis* of the *Johrenia* group (tribe Selineae) is monophyletic, with all examined species except *Johrenia golestanica* arising from within a paraphyletic *Holandrea*. *Holandrea* may be transferred into *Johrenia* and *J. golestanica* may constitute a new, monotypic genus pending confirmation from further investigations. *Opopanax* and *Smyrniopsis*, traditionally treated in two tribes, are confirmed as monophyletic sister groups in the Apioideae superclade. They share similar morphological attributes and are closely allied to *Petroedmondia*, *Magydaris*, and *Crenosciadium*.

KEYWORDS: Apiaceae, Apioideae, Iran, morphology, nuclear ribosomal DNA ITS, palynology, petiole anatomy, phylogeny

INTRODUCTION

The varied climatic, topographic and edaphic features of Iran have resulted in a diverse flora (Zohary, 1973; Léonard, 1991–1992). Iran is a large country, with a total surface area of 1,648,000 km². Ranges in elevation extend from 26 m below sea level, on the shores of the Caspian Sea, to 5,670 m at the summit of Mt. Damavand (Firuz, 1974). Annual precipitation varies from ca. 2,000 mm in Bandar Anzali (on the coast of the Caspian Sea in northern Iran) to less than 100 mm in the central and southwest Iranian deserts. The temperature ranges from a winter low of –35°C in the northwest to a summer high of +50°C on the Persian Gulf. Phytogeographically, Iran is divided into three regions: (1) the Irano-Turanian floristic region

that covers most of the country except its most northern and southern regions; (2) the Euro-Siberian region that covers the area from the northern portion of the Alborz Mountains to the Caspian Sea; and (3) the tropical Saharo-Sindian region that includes a narrow strip in southern Iran (Zohary, 1963; Hedge & Wendelbo, 1978). Iran is rich in endemic species, with many of these occurring in its vast mountainous regions. Of the approximately 8,000 species of flowering plants in Iran, 1,724 (21.6%) are endemic (Rechinger, 1963–2001; Mozaffarian, 1994, 2003; Akhiani, 2002, 2003, 2004).

For the family Apiaceae, Iran is a major center of diversification. The country possesses one of the richest diversities of Apiaceae in the world, exceeded only by China and Turkey (Valiejo-Roman & al., 2006). A total of 363 species

and 114 genera of Apiaceae are known from Iran, of which 114 species and 12 genera are endemic (Mozaffarian, 1996; Pimenov & Leonov, 2004; Valiejo-Roman & al., 2006). In the *Flora Iranica* area (which includes Iran, Afghanistan, and the adjacent mountain regions of Iraq, Turkmenistan, and western Pakistan), about 140 genera of Apiaceae are represented. In this region, the family is poorly known; therefore, many of the accounts provided in *Flora Iranica* are provisional (Hedge & Lamond, 1987). The region contains members of several large and taxonomically complex genera, such as *Ferula* L., *Prangos* Lindl. and *Heracleum* L., and many small endemic genera whose phylogenetic affinities are obscure, such as *Alococarpum* Riedl & Kuber, *Hausknechtia* Boiss., *Azilia* Hedge & Lamond, and *Johrenia* DC. It is not clear, however, whether this high number of small, endemic genera accurately reflects phylogeny or is a result of taxonomic splitting. In general, the taxonomic confusion surrounding many genera of Apiaceae with similar morphologies has resulted in the creation of many monotypic genera when distinctive morphological characteristics are observed (Spalik & al., 2001). Within Iran, some of these endemic species have very restricted habitat requirements or narrow geographic distributions. As examples, *Hausknechtia elymaitica* Boiss. and *Azilia eryngioides* (Pau) Hedge & Lamond occur only in a few specialized niches in western Iran and *Johrenia goles-tanica* Rech. f. is restricted to Golestan National Park in northeastern Iran.

The major objective of this paper is to ascertain the taxonomic and phylogenetic relationships within five groups of genera native to the *Flora Iranica* area. Historically, these taxa were treated in Apiaceae tribes Apieae, Peucedaneae, and Smyrnieae (sensu Drude, 1898), but the results of molecular phylogenetic studies have revealed that each of these tribes is highly polyphyletic (Katz-Downie & al., 1999; Downie & al., 2000c). Thus, the phylogenetic placements of many of these Iranian taxa, several of which are narrow endemics or unusual morphologically, are also unknown or controversial. The precise composition of each of these groups and the monophyly of their constituent genera will be assessed through the results of phylogenetic analyses of nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences and, for some of the included taxa, morphological comparisons. For one of these groups, ancillary data will come from petiole anatomical and palynological studies. Additionally, the phylogenetic placements of these genus groups in Apiaceae subfamily Apioideae are inferred. These five groups represent some of the most outstanding problems of umbellifer taxonomy in Iran. We refer to these five groups of genera as the *Cachrys* group, *Cymbocarpum* group, *Ferula* group, *Johrenia* group, and *Opopanax* group. In this section, we provide a provisional circumscription of each of these groups based on histori-

cal treatments of these taxa and/or the results of previous molecular systematic investigations and discuss additional specific objectives.

Cachrys group. — In *Flora Iranica*, Hedge & Lamond (1987) recognized two morphologically similar species in *Diplotaenia* Boiss. (*D. cachrydifolia* Boiss., *D. damavandica* Mozaffarian, Hedge & Lamond). They believed that *Diplotaenia* allied closely with *Ferula* and *Prangos* by the common possession of lateral (secondary) umbels with male and hermaphrodite flowers and central (primary) umbels with hermaphrodite flowers. *Ferula* and *Prangos* (and *Ferulago* W.D.J. Koch) have a common inflorescence structure, with *Prangos* distinguished from the other two by fruit shape. *Azilia* is a morphologically unique, monotypic genus endemic to Iran and its simple to once-pinnate leaves with thick, broad segments and spiny margins are reminiscent of some *Eryngium* L. species. Previously, *A. eryngioides* was treated in *Prangos* (as *P. eryngioides* Pau), but according to Hedge & Lamond (1987) this species “has, of course, no similarity to or connection with any species of *Prangos*.” Instead, its strongly dorsally compressed fruit with thickened or winged margins and flat albumen at the commissural surface suggest its placement near such Iranian genera as *Diplotaenia*, *Dorema* D. Don, *Ferula*, *Ferulago*, and *Peucedanum* L. (Hedge & Lamond, 1987). Rechinger (1990), however, in revising Pau’s types, concluded that *Azilia* is distantly related to *Ferula* and *Peucedanum*. On the basis of previous molecular evidence, *Prangos pabularia* Lindl. and *Ferulago galbanifera* (Mill.) W.D.J. Koch comprised a clade sister group to *Azilia eryngioides* (Downie & al., 2000c). This group was poorly supported (56% bootstrap value) and, again, distantly related to *Ferula* and *Peucedanum*. Immunological studies support a close relationship between *Prangos* and *Ferulago* (and also *Bilacunaria* Pimenov & V.N. Tikhom.), but not between these genera and *Ferula* (Shneyer & al., 1995). Therefore, we provisionally recognize the *Cachrys* group as comprising the genera *Azilia* (monotypic), *Diplotaenia* (2 spp.), *Prangos* (38 spp.), *Ferulago* (45 spp.), and possibly *Bilacunaria* (4 spp.). (Numbers in parentheses here and afterwards in this section refer to numbers of species in each genus recognized by Pimenov & Leonov, 1993.) In this study, we ascertain the phylogenetic relationships among these genera. In particular, we assess the phylogenetic position of *Azilia* relative to the other members of this group. We refer to this assemblage as the *Cachrys* group because the results of our study place *Cachrys libanotis* L. within this clade. *Cachrys* L. is the oldest (Linnaean) generic name among those taxa included in the clade and *C. libanotis* is its nomenclatural type. As additional species are added in subsequent study, the name *Cachrys* will be retained whereas other potential names may be subject to change and eventual synonymization.

Cymbocarpum group. — Boissier (1872) recognized four species in *Cymbocarpum* DC. (*C. anethoides* DC., *C. erythraeum* (DC.) Boiss., *C. marginatum* Boiss., *C. wiedemanni* Boiss.), all of which are distributed in the Caucasus, Turkey, and Iran. In *Flora Iranica*, Alava (1987) transferred *C. marginatum* into the monotypic genus *Kalakia* Alava (as *K. marginata* (Boiss.) Alava) based on its thickened mericarp margins, and Rechinger (1987) recognized only two species within *Cymbocarpum* (*C. anethoides*, *C. erythraeum*). Previously, *C. marginatum* was treated in *Ducrosia* Boiss. (as *D. stenocarpa* Bornm. & Gauba), suggesting an affinity to that genus as well. Therefore, we provisionally circumscribe the *Cymbocarpum* group to include the genera *Cymbocarpum* (3 spp.), *Ducrosia* (3 spp.), and *Kalakia* (monotypic). In this study, we confirm the monophyly of *Cymbocarpum* and ascertain its relationship to *Ducrosia* and *Kalakia*.

Ferula group. — Plants of *Dorema* are monocarpic, large, possess thickened storage roots, and have large simple umbels with male flowers on the lower branches and hermaphrodite flowers on the upper branches. Twelve to sixteen species are recognized, and these are distributed primarily in the Caucasus, southern parts of central Asia, Iran, Afghanistan, and Baluchistan (Schischkin, 1951; Pimenov & Leonov, 1993). Seven species occur in Iran: *D. ammoniacum* D. Don, *D. aitchisonii* Korovin ex Pimenov, *D. gummiferum* (Jaub. & Spach) K. Korol., *D. aucheri* Boiss., *D. aureum* Stocks, *D. glabrum* Fisch. & C.A. Mey., and *D. hyrcanum* Koso-Pol. (Rechinger, 1987; Mozaffarian, 2003). *Dorema kopetdaghense* Pimenov was treated as a synonym of *D. hyrcanum* in *Flora Iranica* (Rechinger, 1987). Schischkin (1951) and Pimenov (1988) reported that *Dorema* closely resembles *Ferula* and immunological comparisons revealed that *Dorema* is serologically similar to *Ferula* and *Leutea* Pimenov (Shneyer & al., 1995). In contrast, and rather surprisingly, a recent study of ITS sequences placed *Dorema* (represented by *D. aucheri*) sister group to *Seseli mucronatum* (Schrenk) Pimenov & Sdobnina in tribe Selineae, well away from *Ferula* (Valiejo-Roman & al., 2006). *Haussknechtia* is a monotypic genus endemic to southwest Iran. These plants are highly unusual in the family because of their compressed, globose, compound umbels. Koso-Poljansky (1925, as cited in Pimenov, 1988) suggested an affinity between *Haussknechtia* and *Dorema*; Rechinger (1987) did as well, but emphasized their differences in fruit shape and inflorescence type. Recently, however, analyses of ITS sequence data showed that *Haussknechtia* is a sister group to the Iranian endemic monotypic genus *Demavendia* Pimenov in tribe Pimpinelleae (Valiejo-Roman & al., 2006). We provisionally recognize the *Ferula* group as comprising the genera *Dorema* (12 spp.), *Ferula* (170 spp.), and *Leutea* (6 spp.). In this study, we confirm the mono-

phyly of *Dorema* and assess its relationship to *Ferula* and *Leutea*.

Johrenia group. — Four species of *Johrenia* are recognized from Iran: *J. paucijuga* (DC.) Bornm., *J. aromatica* Rech. f., *J. golestanica* Rech. f., and *J. platycarpa* Boiss. (Rechinger, 1987). The diagnostic character for the genus is a thickened, spongy mericarp margin. Affinities to *Leutea*, *Peucedanum*, and *Johreniopsis* Pimenov have been proposed (Davis, 1972; Pimenov, 1987a; Rechinger, 1987; Pimenov, Kljuykov & Ostroumova, unpubl.). Salimian (2002) concluded that *J. platycarpa* should be treated within *Peucedanum*, based on examination of morphology, anatomy, and palynology. *Peucedanum* is a large, taxonomically complex genus (100–120 spp.) with many segregates (Pimenov & Leonov, 1993; Spalik & al., 2004). One of these segregates, *Holandrea* Reduron, Charpin & Pimenov, has been considered closely related to *Johrenia* and *Johreniopsis* based on molecular data and multivariate analyses of morphological characters (Pimenov, Kljuykov & Ostroumova, unpubl.). Therefore, we provisionally recognize the *Johrenia* group as comprising the genera *Johrenia* (15 spp.), *Johreniopsis* (4 spp.), and *Holandrea* (5 spp.; Reduron & al., 1997; Spalik & al., 2004). We consider *Leutea* as part of the *Ferula* group. In this study, we confirm the monophyly of *Johrenia* and ascertain its relationship to *Johreniopsis* and *Holandrea*. In particular, we reassess the relationship of the narrow endemic *J. golestanica* to its congeners using morphology, petiole anatomy, and palynology.

Opopanax group. — In *Flora Iranica*, two species are recognized in *Opopanax* W.D.J. Koch: *O. hispidus* (Friv.) Griseb. and *O. persicus* Boiss. (Rechinger, 1987). Both species have a wider distribution in Europe and Asia. *Smyrniopsis* Boiss. is monotypic (*S. aucheri* Boiss.) and endemic to the Caucasus (Tamamschian, 1987). Traditionally, these two genera were placed into tribes Peucedaneae and Smyrnieae based primarily on differences in orientation and degree of fruit compression (Drude, 1898). However, Drude's tribes Peucedaneae and Smyrnieae are each highly polyphyletic, with their members scattered throughout subfamily Apioideae (Katz-Downie & al., 1999; Downie & al., 2000c). Similar results were implicated by the serological studies of Shneyer & al. (1992, 1995). Phylogenetic analysis of molecular data reveals a sister group relationship between *Opopanax hispidus* and *Smyrniopsis aucheri* (Katz-Downie & al., 1999; Downie & al., 2000b, c). We provisionally recognize the *Opopanax* group as consisting of two genera, *Opopanax* (3 spp.) and *Smyrniopsis* (monotypic). In this study, we corroborate the monophyly of this group by considering additional material. We also provide a comparative analysis of morphological characters of *Smyrniopsis* and the two species of *Opopanax* occurring in Iran.

MATERIALS AND METHODS

Morphology. — Observations and measurements were taken from herbarium specimens and plants in the field. An examination of living plants was necessary because some species are large and poorly represented by herbarium specimens. *Dorema*, for example, may be up to 3 m in height and have a stem diameter of 10 cm or more. The morphological characters examined include those traditionally important in circumscribing genera and species, such as habit, stems (height, color, indumentums or surface sculpturing and furrowing), branching patterns, leaf segments (size, shape, margin, and surface features such as indumentum), bracts, bracteoles, rays, inflorescences, pedicels, petals (length and color), stylopodia, and mericarps. All relevant material from herbaria TARI, IRAN, TUH and the private herbarium collection of Dr. H. Akhiani (Department of Biology, Faculty of Science, University of Tehran, Iran) was examined.

Petiole anatomy. — Petiole anatomy was examined in *Johrenia paucijuga*, *J. golestanica*, and *J. aromatica*. Material collected from the field was fixed in FAA. Free-hand sections of petioles were stained with carmine (to identify the phloem) and diluted methyl green (xylem), mounted permanently on microscope slides using Canada balsam (Chamberlain, 1930), and preserved as vouchers in the private herbarium of Dr. H. Akhiani. Photographs were taken using an Olympus BX51 compound microscope and an Olympus SZX12 stereomicroscope using an Olympus DP12 digital camera. Petiole characters included cross-sectional outline shape, number of peripheral vascular bundles, and features of sclerenchyma around the vascular bundles (Pimenov & al., 1986). Herbarium voucher specimens are deposited in the aforementioned herbaria. Petiole data for *Johreniopsis seseloides* (C.A. Mey.) Pimenov and *Holandrea caucasica* (M. Bieb.) Spalik, Reduron & S.R. Downie were obtained from literature (Salimian, 2002).

Pollen morphology. — Pollen was examined from anthers collected directly in the field or from herbarium specimens from three species of *Johrenia*. Pollen grains were acetolized (Erdtman, 1969) and viewed using both an Olympus BX51 compound microscope and a Zeiss SEM-960A scanning electron microscope. Characters observed included pollen shape, length and width, exine thickness at poles and equator, and exine surface features (Punt, 1984). For each character measured, the average measurement of 100 pollen grains was determined. Palynological data for *Johreniopsis seseloides* and *Holandrea caucasica* were obtained from literature (Salimian, 2002).

DNA sequencing and species selection. — In total, 177 accessions (157 species) of Apiaceae subfamily Apioideae were examined for nrDNA ITS sequence variation (Appendix). Ninety-two of these accessions were newly sequenced; data for the remaining 85 accessions

were obtained from GenBank. The latter were chosen because preliminary phylogenetic analysis of all available ITS sequences of subfamily Apioideae in GenBank and numerous unpublished sequences (representing about 1,000 species from 250 genera) revealed that they were closely related to the five groups of Iranian genera considered herein (Downie & al., unpubl.). To date, ITS sequences comprise the most comprehensive database for Apioideae. These 1,000 accessions represent species across a broad geographic distribution and nearly all tribes and major clades of subfamily Apioideae defined on the basis of previous molecular systematic studies except those most basal within the subfamily (i.e., tribe Heteromorphae, *Annesorhiza* clade, *Lichtensteinia* clade, and several putatively basal species of uncertain position; Downie & al., 2001; Calviño & al., 2006) and those lineages within tribes Oenantheae and Scandiceae which demonstrate rapid ITS sequence evolution (Downie & al., 1998; Petersen & al., 2002; Hardway & al., 2004). Approximately two-thirds of the 157 species included in this investigation are native to Iran (Rechinger, 1987); many other species are distributed in the greater *Flora Iranica* area.

Protocols for total genomic DNA extraction, PCR-amplification using standard ITS primers, and DNA purification and sequencing strategies are presented elsewhere (Downie & Katz-Downie, 1996; Downie & al., 2000a). Cycle sequencing reactions were carried out using the purified PCR product, AmpliTaq DNA polymerase (Roche Molecular Systems, Alameda, California, U.S.A.) and fluorescent Big Dye terminators (Applied Biosystems, Foster City, California, U.S.A.). The sequencing products were resolved using an ABI 3730XL high-throughput DNA capillary sequencer. Simultaneous consideration of both DNA strands across the entire ITS region permitted unambiguous base determination.

Alignment and phylogenetic analyses. — The DNA sequences were aligned initially using the computer program CLUSTAL X (Jeanmougin & al., 1998), with default parameters for gap penalty and extension, and then adjusted manually as necessary. Gaps were positioned to minimize nucleotide mismatches. The trees were rooted with members of tribe Pleurospermeae, based on results of previous higher-level analyses of the subfamily (Downie & al., 2001). For 50 accessions obtained from GenBank, sequence data for the 5.8S rDNA region were not available. These missing data represented 6.7% of the entire matrix. Uncorrected pairwise nucleotide distances of unambiguously aligned positions were determined using the distance matrix option of PAUP* vers. 4.0b10 (Swofford, 2002). Maximum parsimony (MP) analysis of the complete data matrix was implemented using PAUP* and the search strategies described elsewhere (Downie & al., 2000a). The maximum tree limit of 20,000 minimal length trees was obtained. Bootstrap (BS) values were calculated

from one million replicate analyses using “fast” stepwise-addition of taxa and only those values compatible with a majority-rule consensus tree were recorded. The number of additional steps required to force particular taxa into a monophyletic group was examined using the constraint option of PAUP*. The data matrix was also analyzed using maximum likelihood (ML), after using the program Modeltest vers. 3.7 to choose an appropriate model of nucleotide substitution that best fits these data (Posada & Crandall, 1998), as selected by the Akaike Information Criterion (AIC) estimator (Posada & Buckley, 2004). The parameters appropriate for the chosen model were input into PAUP* and a heuristic search performed using random sequence addition and tree-bisection-reconnection branch swapping under ML optimization. One hundred BS replicate analyses were conducted using neighbor-joining searches with ML distance estimates, using the parameters inferred by Modeltest. Lastly, Bayesian analysis was conducted using the program MrBayes vers. 3.1.2 (Ronquist & Huelsenbeck, 2003). The settings appropriate for the best-fit model of nucleotide substitution, as selected by MrModeltest vers. 2.2 (Nylander, 2004) under the AIC estimator, were put into a MrBayes block in PAUP* (nst = 6; rates = invgamma). The priors on state frequencies and rates and variations across sites were estimated automatically by the program. Four Markov chains starting with a random tree were run for one million generations, sampling trees at every 100th generation. Trees from the first 100,000 generations were discarded as “burn-in” before stationarity was reached, prior to calculating the majority-rule consensus tree from the remaining trees. Posterior probability (PP) values for all internal tree branches were recorded.

RESULTS

DNA sequencing and phylogenetic resolutions.

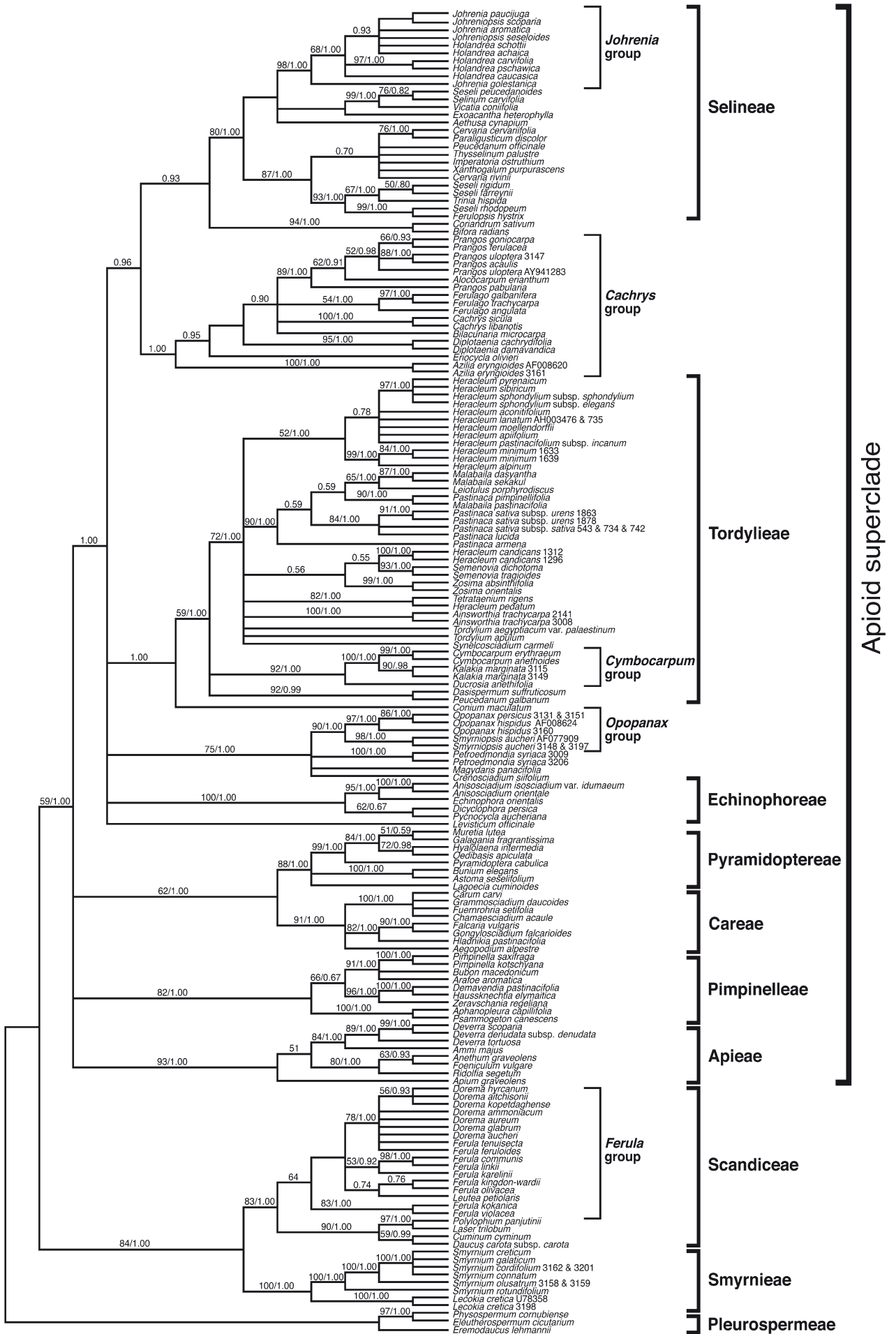
— The ITS region ranged in size from 577 bp (one accession of *Ainsworthia trachycarpa* Boiss.) to 610 bp (*Holandrea achaica* (Halácsy) Spalik, Reduron & S.R. Downie). Species with identical DNA sequences were each treated as one terminal in the phylogenetic analysis. Alignment of all ITS sequences from 177 accessions (170 terminals) resulted in a matrix of 654 positions, of which 44 were excluded from subsequent analysis because

of alignment ambiguities. The remaining 610 alignment positions yielded 200 constant, 342 parsimony informative, and 68 autapomorphic characters. Alignment gaps ranged in size between 1 and 20 bp; the vast majority of these were only a single bp in size. Maximum pairwise sequence divergence values ranged from identity to 33% (the latter between *Lagoecia* L. and the outgroup *Physospermum* Cusson).

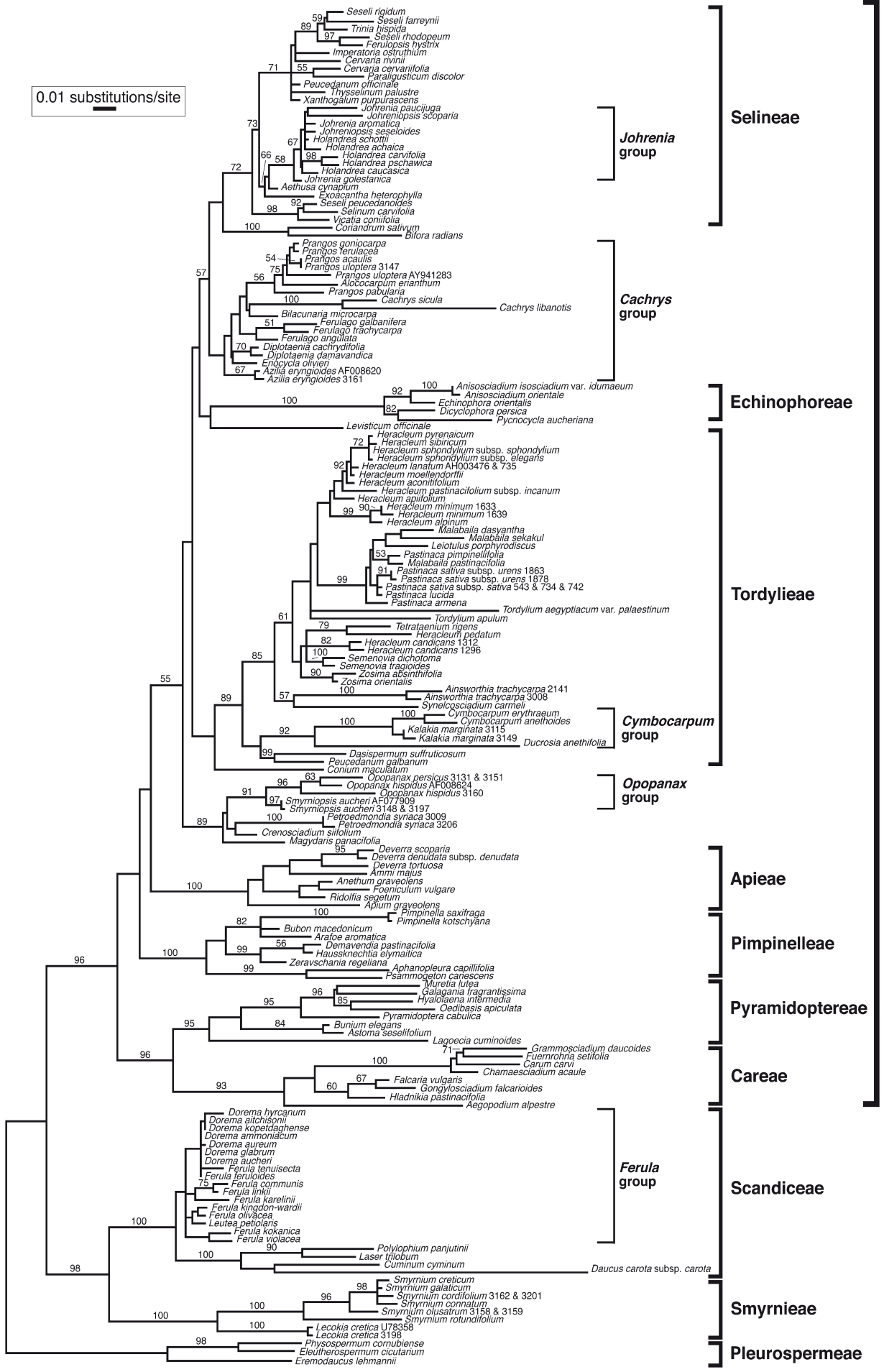
MP analysis of all unambiguously aligned positions resulted in the preset limit of 20,000 trees, each of 2,614 steps (consistency index, CI = 0.3083 and 0.2868, with and without uninformative characters, respectively; retention index, RI = 0.7642). The strict consensus of these trees, with accompanying BS values, is presented in Fig. 1. Superimposed on Fig. 1 are ten tribes recognized on the basis of previous molecular phylogenetic studies (Downie & al., 2001; Spalik & al., 2004), plus the Apioideae superclade, a large and variable group of umbellifers containing tribes and other major clades of uncertain relationship (Plunkett & Downie, 1999). Also indicated are the five groups of Iranian genera circumscribed herein, one of which is now redefined on the basis of these molecular results. Based on the AIC estimator, Modeltest selected the GTR+I+G model of nucleotide substitution as best fitting these ITS data. Using the parameters inferred by Modeltest, a single ML tree was recovered having a $-\ln$ likelihood score of 14,263.187. This tree is presented in Fig. 2 along with ML distance-based BS values and the ten tribes and clades indicated previously. Relationships inferred by the Bayesian analysis were highly consistent with those presented in the MP strict consensus tree. PP values are presented on the MP strict consensus tree (Fig. 1) for those branches common to both analyses.

The group of *Diplotaenia* (2 spp.), *Ferulago* (3 spp.), *Prangos* (6 accessions represented by 5 spp.), and the monotypic *Azilia* (2 accessions) is redefined to include *Bilacunaria microcarpa* (M. Bieb.) Pimenov & V.N. Tikhom., *Cachrys* (2 spp.), *Alococarpum erianthum* (DC.) Riedl & Kuber, and *Eriocyclus olivieri* (Boiss.) Wolff, and is referred to as the *Cachrys* group. (Numbers in parentheses here and afterwards in this section refer to numbers of species in each genus included in the phylogenetic study.) This group is variously supported, with MP and ML BS values less than 50% and a PP value of 1.00. Similarly, intergeneric relationships within this clade are generally supported poorly. The genera *Prangos* (upon the possible

Fig. 1 (overleaf). Strict consensus of 20,000 minimal-length 2,614-step trees derived from maximum parsimony analysis of nrDNA ITS sequences from 177 accessions (170 terminals) of Apiaceae subfamily Apioideae (CIs = 0.3083 and 0.2868, with and without uninformative characters, respectively; RI = 0.7642). The tree inferred by Bayesian analysis of these data was highly consistent with the MP strict consensus tree. Numbers represent bootstrap values (%) and posterior probabilities for those branches common to both analyses. Bootstrap estimates were calculated from one million replicate analyses using “fast” stepwise-addition of taxa; only those values compatible with the majority-rule consensus tree are recorded. The ten tribes recognized on the basis of previous molecular phylogenetic studies are indicated, as are the Apioideae superclade and the five groups of Iranian genera circumscribed herein.



0.01 substitutions/site



Apioideae

inclusion of *Alococarpum erianthum*, see Discussion), *Cachrys*, *Diplotaenia*, and *Ferulago* are each supported as monophyletic. The clade of *Prangos* and *Alococarpum* is variously supported as monophyletic (MP BS 89%; ML BS 56%; PP 1.00). *Prangos acaulis* (DC.) Bornm. and *P. uloptera* DC. accession no. 3147 have identical ITS sequences and the two included accessions of *P. uloptera* do not unite as monophyletic (with 3.1% nucleotide sequence variation between them). *Azilia eryngioides* is sister group to all aforementioned taxa of the *Cachrys* group.

Cymbocarpum (2 spp.), *Kalakia marginata* (2 accessions), and *Ducrosia* (1 sp.) comprise a strongly supported clade (MP and ML BS 92%; PP 1.00) which we recognized previously as the *Cymbocarpum* group. *Cymbocarpum* is monophyletic and is sister group to *Kalakia marginata* (MP and ML BS 100%; PP 1.00). Pairwise sequence divergence estimates range from 0.5% (between the two accessions of *K. marginata*) to 12.7% (between *Cymbocarpum anethoides* and *Ducrosia anethifolia* (DC.) Boiss.). *Cymbocarpum anethoides* and *C. erythraeum* show 2.2% sequence divergence; pairwise comparisons between the two species of *Cymbocarpum* and *Kalakia marginata* range from 2.8 to 3.8%. In the MP strict consensus tree (Fig. 1), the *Cymbocarpum* group comprises a trichotomy with the clade of *Heracleum* through *Synelcosciadium* Boiss. (= *Heracleum* clade of Downie & al., 2001) and the clade of two African species (*Dasispermum suffruticosum* (P.J. Bergius) B.L. Burtt and *Peucedanum galbanum* (L.) Drude). In the ML tree (Fig. 2), the *Cymbocarpum* group is a weakly supported sister group to this African clade of umbellifers (< 50% BS). In the Bayesian tree (not shown), the *Cymbocarpum* group is a strongly supported sister group to the African clade (PP 1.00). In all trees, these three lineages comprise a variously supported clade identified as tribe Tordylieae (MP BS 59%; ML BS 89%; PP 1.00).

The *Ferula* group is maintained to include *Dorema*, *Ferula*, and *Leutea*. The genus *Dorema* (7 spp.) occurs within a paraphyletic *Ferula* (9 spp.) in tribe Scandiceae in both MP and ML trees. In the Bayesian tree, these taxa form a 4-branched polytomy with the clade of *Cuminum* L., *Daucus* L., *Laser* Borkh. ex P. Gaertn., B. Mey. & Schreb., and *Polylophium* Boiss. (all members of Scandiceae subtribe Daucinae). In all trees, *Dorema* comprises a subclade with two species of *Ferula*: *F. feruloides* (Steud.) Korovin and *F. tenuisecta* Korovin ex Pavlov. Also arising from within *Ferula* but near the base of the *Ferula* group is *Leutea petiolaris* (DC.) Pimenov, the nomenclatural type

of *Leutea*. While the *Ferula* group is resolved in both MP and ML trees, it is only supported weakly. However, the *Ferula* group is distinct from Scandiceae subtribe Daucinae when differences in branch lengths are considered, with those leading to subtribe Daucinae being the longest and best supported in Scandiceae and those of the *Ferula* group being relatively short (Fig. 2). *Dorema* is monophyletic in a subset of the 20,000 maximally parsimonious trees. Interspecific sequence divergence values in *Dorema* ranged from identity (*D. glabrum*, *D. aucheri*, *D. ammoniacum*, and *Ferula feruloides* all had identical sequences, as did *D. aitchisonii* and *D. kopetdaghense*) to 1.2% (between *D. aureum* and *D. hyrcanum*). Nucleotide sequence divergence between *D. kopetdaghense* and *D. hyrcanum* was 0.9%. The monotypic genus *Hausknechtia* occurs elsewhere, sister group to *Demavendia pastinacifolia* (Boiss. & Hauskn. ex Boiss.) Pimenov in tribe Pimpinelleae, and is confirmed not to be a member of the *Ferula* group.

The species of *Johrenia* (3 spp.) comprise a clade (MP BS 98%; 58% ML BS; PP 1.00) with *Johreniopsis* (2 spp.) and *Holandrea* (5 spp.) in the previously delimited *Johrenia* group. None of these genera is monophyletic. Constraining *Johrenia* to monophyly results in trees four steps longer than those without the constraint invoked. The narrow endemic species *Johrenia golestanica* is sister group to all other members of the *Johrenia* group. Upon the exclusion of *J. golestanica*, *Holandrea* is paraphyletic and all other examined species of *Johrenia* and *Johreniopsis* arise from within it. The clade of *Johrenia*, *Johreniopsis*, and *Holandrea* is isolated from *Peucedanum officinale* L., the nomenclatural type of *Peucedanum*, and several generic segregates of *Peucedanum* s.l. (i.e., *Cervaria* Wolf, *Demavendia*, *Imperatoria* L., *Leutea*, and *Thysselinum* Raf.).

Opopanax (3 accessions representing 2 spp.) and the monotypic *Smyrniopsis* (two accessions) comprise a strongly supported monophyletic *Opopanax* group (MP BS 90%; ML BS 91%, PP 1.00). These genera are allied strongly with *Petroedmondia syriaca* (Boiss.) Tamamsch. (= *Smyrniopsis cachroides* Boiss.), *Magydaris panacifolia* (Vahl) Lange, and *Crenosciadium siifolium* Boiss. & Heldr. within the Apioideae superclade, but the relationships among these genera are unclear. The two accessions of *O. hispidus* do not unite as monophyletic and show 4.8% nucleotide sequence variation between them. Pairwise sequence divergence estimates between accessions of *Opopanax* spp. and *Smyrniopsis aucheri* range from 3.9%

Fig. 2 (previous page). Single tree inferred from maximum likelihood analysis of nrDNA ITS sequences from 177 accessions (170 terminals) of Apiaceae subfamily Apioideae (–ln likelihood = 14,263.187). Branch lengths are proportional to the number of expected nucleotide substitutions per site (note scale bar) under a GTR+I+G model of nucleotide substitution. Numbers represent bootstrap estimates calculated from 100 replicate analyses using neighbor-joining searches and maximum likelihood distance. The ten tribes recognized on the basis of previous molecular phylogenetic studies are indicated, as are the Apioideae superclade and the five groups of Iranian genera circumscribed herein.

to 5.6%. Nucleotide divergence estimates between the two accessions of *Smyrniopsis aucheri* and the two accessions of *Petroedmondia syriaca* range from 6.2% to 6.9%.

Several additional relationships and phylogenetic placements deserve comment, even though many of these taxa are not components of the Iran flora. These genera are distributed widely in Europe and Asia, while some of them also occur in North America and Africa. The genera *Ferulopsis* Kitag., *Trinia* Hoffm., and *Vicatia* DC. are new members of Apiaceae tribe Selineae (sensu Spalik & al., 2004). The large genus *Seseli* L. is not monophyletic, as reported previously (Downie & al., 1998; Spalik & al., 2004). Expanded sampling within tribe Tordylieae (i.e., the *Heracleum* clade of Downie & al., 2001) reveals polyphyly of *Heracleum* and paraphyly of *Pastinaca* L. *Heracleum candicans* Wall. ex DC. and *H. pedatum* Wight (= *Vanasushava pedata* (Wight) P.K. Mukh. & Constance) each form separate lineages away from their congeners and the type of the genus, *H. sphondylium* L. The genus *Malabaila* Hoffm. (= *Leiotulus* Ehreb.) arises from within *Pastinaca*. This clade of *Pastinaca* (with included *Malabaila* and the single accession identified in GenBank as *Leiotulus*) is well supported (MP BS 90%; ML BS 99%; PP 1.00). Tribe Tordylieae is confirmed to include *Ainsworthia* Boiss., *Semenovia* Regel & Herder, and *Synelcosciadium* (= *Tordylium* L.). *Anisosciadium* DC. is confirmed within tribe Echinophoreae. Additional members of tribe Pyramidoptereae include *Muretia* Boiss. (= *Elaeosticta* Fenzl.), *Galagania* Lipsky, *Hyalolaena* Bunge, and *Astoma* DC. Tribe Careae is expanded to include *Chamaesciadium* C.A. Mey., *Gongylosciadium* Rech. f., and *Hladnikia* Rchb., and tribe Pimpinelleae is expanded to include *Haussknechtia*, *Demavendia*, and

Zeravschania Korovin. Tribe Smyrnieae is maintained as comprising only two genera, *Smyrniium* L. and *Lecokia* DC. Analysis of six of the seven species recognized in *Smyrniium* indicates that the genus is strongly supported as monophyletic. *Eremodaucus lehmanii* Bunge is a member of tribe Pleurospermeae.

Morphology, petiole anatomy, and pollen micro-morphology. — A comparison of morphological features for four species of the *Cymbocarpum* group (*Cymbocarpum anethoides*, *C. erythraeum*, *Kalakia marginata*, *Ducrosia anethifolia*) is presented in Table 1. These species are very similar in leaf morphology, but differ in their fruits. The transfer of *C. marginatum* to the monotypic genus *Kalakia* (Alava, 1987) was based on its characteristic thickened fruit margins, yet there are also pronounced differences between the fruits of *C. anethoides* and *C. erythraeum*. In the former, the mericarps are compressed laterally, whereas in the latter they are compressed dorsally (as they are in *K. marginata*). *Cymbocarpum* and *Kalakia* show many similarities in other vegetative features (i.e., habit, stem color, stem furrows, outer petal length, and bract division). *Ducrosia anethifolia* is similar to *Cymbocarpum* and *Kalakia* in its petals and shape of leaf segments. However, *D. anethifolia* differs from the other two genera in that its mericarps are subglobular and its leaves do not wither early. In general, distinguishing among *Cymbocarpum*, *Kalakia* and *Ducrosia* using leaf characters alone is very difficult. Similarly, fruit characters, such as the orientation of fruit compression and type of fruit margin, are also not useful in separating these genera.

A comparison of selected morphological features for seven Iranian representatives from four genera provision-

Table 1. Morphological comparisons within the *Cymbocarpum* group: *Cymbocarpum anethoides*, *C. erythraeum*, *Kalakia marginata*, and *Ducrosia anethifolia*.

	<i>C. anethoides</i>	<i>C. erythraeum</i>	<i>K. marginata</i>	<i>D. anethifolia</i>
Habit	Annual	Annual	Annual	Annual or biennial
Stem color	Mostly pink throughout	Basal portion pink	Basal portion pink	Basal portion pink or green
Furrows on stem	Very small, obscure	Prominent	Prominent	Prominent
Leaf segments: shape, length × width (mm), persistence	Linear, 20–30 × 7–15, early withering	Linear, 23–30 × 10–15, early withering	Linear, 43–45 × 8–10, early withering	Linear, 100–160 × 30–40, persistent, not withering early
Bract division	Simple	Simple, sometimes divided	Simple, sometimes divided	Simple
Petal: Length (mm), color	1.5, white	1.0, white	1.0, white	1.5, white
Fruit shape	Elliptic and curved	Elliptic and boat-shaped	Oblong and flat	Ovate or orbicular and flat
Fruit surface	Whitish powder	Glabrous	Tuberous corky	Tuberous corky
Fruit compression	Lateral, moderate	Dorsal, moderate	Dorsal, strong	Dorsal, strong
Fruit margin	Thin	Thin	Thick	Thick

ally recognized in the *Cachrys* group (*Diplotaenia*, *Prangos*, *Ferulago*, *Azilia*) is presented in Table 2. Sampling of this genus group was not comprehensive but is adequate to compare critical morphological differences between *Azilia* and its generic allies, as suggested by published studies. *Diplotaenia cachrydifolia* and *D. damavandica* are morphologically very similar, with noted differences between them limited to leaf segment shape and width. Inflorescences of both species are whorled, with lateral umbels bearing male and hermaphrodite flowers and central umbels bearing only hermaphrodite flowers. *Diplotaenia* shares with *Prangos* similar leaf and inflorescence characters. Indeed, *D. cachrydifolia* is very similar to *P. ferulacea* (L.) Lindl. in leaf morphology. Their fruits, however, are quite different. In *Diplotaenia*, the fruits are strongly dorsally compressed and have prominent dorsal ribs. In *Prangos*, the fruits are moderately compressed laterally and their prominent dorsal ribs are extended into wings. *Ferulago stellata* Boiss. is very similar to the other two genera, with its similar inflorescence structure and leaf form. In contrast, *Azilia eryngioides* stands apart

from all other members of this group. Its leaf segments are orbicular to reniform and large with spiny margins and its inflorescences are paniculate with hermaphrodite umbels. *Azilia* is clearly distinguished morphologically from *Diplotaenia*, *Prangos*, and *Ferulago* (and indeed any other genus in the family), and any close ally based on morphological similarity is not apparent in our study.

A comparison of selected morphological characters for three species of *Johrenia* and one species each of *Johreniopsis* and *Holandrea* is presented in Table 3, with the primary purpose of assessing the relationship of the narrow endemic *Johrenia golestanica* to its congeners. *Johrenia golestanica* is unique in its white petals, grayish-green and finely striate stems, sub-elliptic (to sub-orbicular) fruits with non-prominent dorsal ribs, and a preference for growing in disturbed areas. *Johreniopsis* is morphologically similar to *Johrenia*. Furthermore, with its yellow petals, once- to twice-pinnate leaves, and prominent mericarp ribs, *Johreniopsis* is morphologically more similar to *Johrenia paucijuga* and *J. aromatica* than it is to *J. golestanica*. Comparisons of petiole anatomy

Table 2. Morphological comparisons within the *Cachrys* group: *Diplotaenia cachrydifolia*, *D. damavandica*, *Prangos pabularia*, *P. uloptera*, *P. ferulacea*, *Ferulago stellata*, and *Azilia eryngioides*.

	<i>D. cachrydifolia</i>	<i>D. damavandica</i>	<i>P. pabularia</i>	<i>P. uloptera</i>	<i>P. ferulacea</i>	<i>F. stellata</i>	<i>A. eryngioides</i>
Leaf segments: shape, margin	Linear to filiform, entire	Ovate, oblong, bifid or trifid, entire	Linear to filiform, entire	Linear to filiform, entire	Linear to filiform, entire	Linear to filiform, entire	Orbicular to reniform, sinuate-spinose
Leaf segments: width (mm)	< 0.5	> 0.5	0.5–2	≤ 0.5	< 0.5	< 0.5	60–70
Fruit compression	Dorsal, strong	Dorsal, strong	Lateral, slight	Lateral, slight	Lateral, slight	Dorsal, strong	Dorsal, strong
Ribs on dorsal surface of mericarp	Prominent, not winged	Prominent, not winged	Prominent, wavy winged	Prominent, wavy winged	Prominent, wavy winged	Prominent, not winged	Not prominent

Table 3. Morphological comparisons within the *Johrenia* group: *Johrenia paucijuga*, *J. aromatica*, *J. golestanica*, *Johreniopsis seseloides*, and *Holandrea caucasica*.

	<i>J. paucijuga</i>	<i>J. aromatica</i>	<i>J. golestanica</i>	<i>J. seseloides</i>	<i>H. caucasica</i>
Stem color in fruiting	Shiny yellow	Dark green	Grayish green	Green	Green
Stem furrows	Prominent	Obscure	Finely striate	Finely striate	Finely striate
Leaf segment shape	Linear	Oblong	Oblong or ovate	Linear	Rhomboid-ovoid
Leaf branching	Twice-pinnate	Once- to twice-pinnate	Once-pinnate	Once- to twice-pinnate	3–4-ternatisect
Bract and bracteole number, thickness	> 5, thickened	> 5, thickened	< 5, thin	None	None
Fruit shape, dorsal rib prominence	Elliptic, prominent	Elliptic, prominent	Sub-elliptic, not prominent	Ovate, slightly prominent	Elliptic, prominent
Petal color	Yellow	Yellow	White	Yellow	Whitish yellow
Calyx teeth	Inconspicuous	Inconspicuous	Inconspicuous	Conspicuous	Conspicuous
Habitat	Open, undisturbed	Open, undisturbed	Disturbed areas	Rocky areas	Woodlands

(Table 4; Fig. 3) and pollen grains (Table 5; Fig. 3) also support the separation of *J. golesstanica* from its congeners. Unlike the other two species of *Johrenia* where the vascular bundles are partially or wholly surrounded by sclerenchyma cells, the vascular bundles of *J. golesstanica* are without sclerenchyma tissue. Sclerenchyma at the base of the xylem, however, is present in *Johreniopsis*

seseloides and *Holandrea caucasica*. Pollen of *Johrenia*, *Johreniopsis*, and *Holandrea* is of the oval-type (sensu Cerceau-Larrival, 1971) and in *J. golesstanica* the grains are slightly smaller (in length and width) than they are in its congeners.

The morphology of eight species of *Dorema* from Iran was examined (*D. kopetdaghense* was considered

Table 4. Comparisons of petiole anatomy within the *Johrenia* group: *Johrenia paucijuga*, *Johrenia aromatica*, *Johrenia golesstanica*, *Johreniopsis seseloides*, and *Holandrea caucasica*.

	<i>J. paucijuga</i>	<i>J. aromatica</i>	<i>J. golesstanica</i>	<i>J. seseloides</i>	<i>H. caucasica</i>
Cross-sectional shape	Semi-circular, with slight furrow on adaxial side	Semi-circular, with no furrow on adaxial side	Semi-circular, with prominent furrow on adaxial side	Semi-circular, with prominent furrow on adaxial side	Semi-circular, with prominent furrow on adaxial side
Number of peripheral vascular bundles	9	9	6	13	9
Sclerenchyma above phloem	Absent	Few cells to a narrow band	Absent	Absent	Absent
Sclerenchyma at base of xylem	Few cells	Many cells, forming a thickened band	Absent	Many cells, forming a thickened band	Many cells, forming a thickened band

Data for *Johreniopsis* and *Holandrea* are from Salimian (2002).

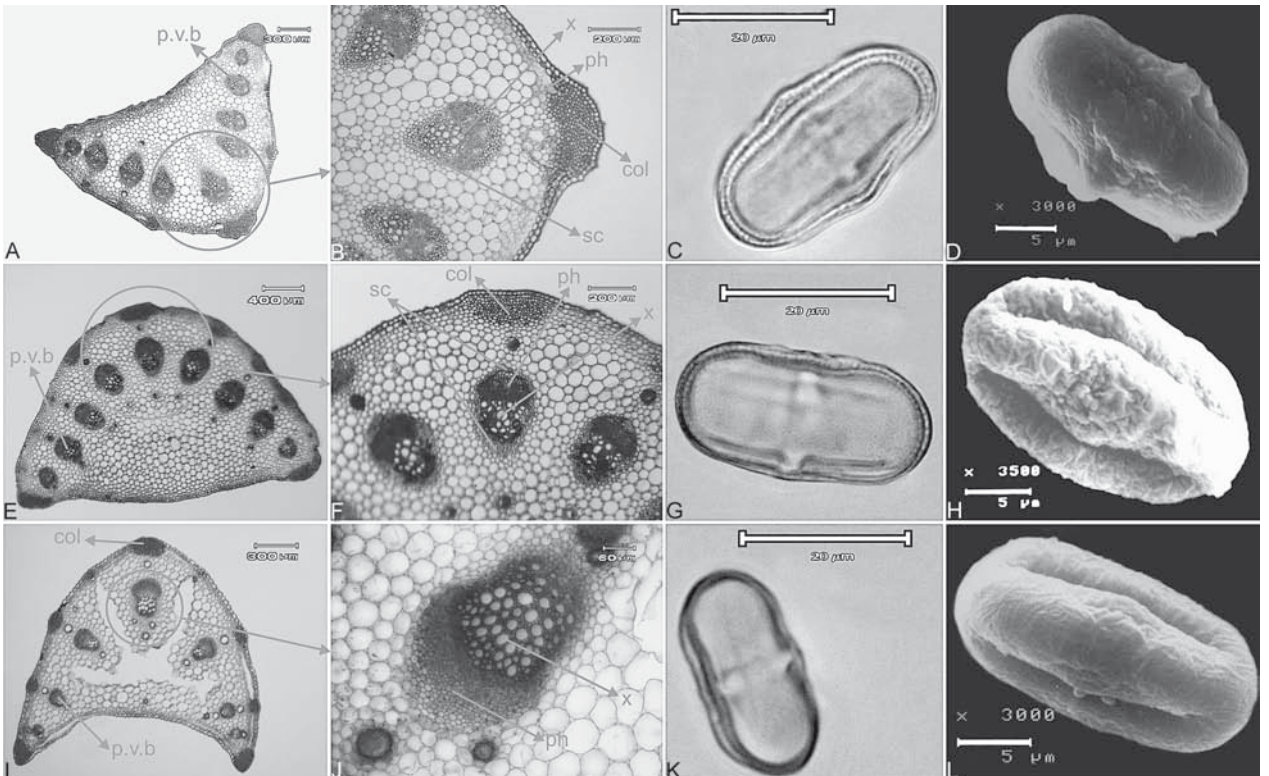


Fig. 3. Comparisons of pollen and petiole anatomy in three *Johrenia* species. Top row (left to right): *J. paucijuga* petiole anatomy (A, B), pollen grain under optical microscope (C), and pollen grain viewed under SEM (D; ×3,000). Middle row: *J. aromatica* petiole anatomy (E, F), pollen grain under optical microscope (G), and pollen grain viewed under SEM (H; ×3,500). Bottom row: *J. golesstanica* petiole anatomy (I, J), pollen grain under optical microscope (K), and pollen grain viewed under SEM (L; ×3,000). Abbreviations are as follows: col, collenchyma; ph, phloem; p.v.b., peripheral vascular bundle; sc, sclerenchyma; x, xylem.

a distinct species). All species are morphologically very similar, although each can be distinguished by several characters (results not shown). As examples, *Dorema ammoniacum* and *D. aitchisonii* are similar in their leaf segments (shape, pubescence, margin, width), peduncle and pedicel length and surface features, and having woolly fruits prior to ripening. They both differ from other species in the genus, however, in several of these attributes. *Dorema ammoniacum* and *D. aitchisonii* are separated by mericarp length, wing width, and degree of stem internode swelling (the stem is swollen in *D. aitchisonii* but not in *D. ammoniacum*). The size of the leaves in these two species shows great variation. *Dorema aucheri* has similar leaf characters to the two aforementioned species, but is separated from them by fruit size and surface texture. *Dorema kopetdaghense* and *D. hyrcanum* are morphologically distinct; the former differs in its smaller leaf segments that are lanceolate with entire margins, its pubescent immature fruits, and shorter peduncles. *Dorema glabrum* is also distinct morphologically, with its glabrous leaf segments and long pedicels. Similarities between *Dorema* and *Ferula* species are apparent and have been presented elsewhere (e.g., Schischkin, 1951; Pimenov, 1988).

A comparison of morphological features of *Opopanax hispidus*, *O. persicus*, and *Smyrniopsis aucheri* of

the *Opopanax* group is presented in Table 6. These species share several morphological attributes, such as those of the leaves and inflorescences. These features include once- or twice-pinnate basal leaves, leaf segments oblong or elliptic, acute, crenate and with cartilage at their margins, hispidus leaves, type of cork on the stem, and inflorescences of male lateral umbels and central hermaphrodite umbels. There are some obvious differences between *Opopanax* and *Smyrniopsis*, such as dorsally versus laterally compressed mericarps and solid versus hollow petioles in cross-section. The stems of *O. persicus* are smooth, whereas those of *O. hispidus* are densely hispid (especially on the basal portions), and those of *S. aucheri* are sparsely hispidus. Inflorescence shape in *O. persicus* is dense thyrsoid, in *O. hispidus* it is lax thyrsoid, and in *S. aucheri* it is conical.

DISCUSSION

The limitations of nrDNA ITS sequence data in resolving tribal-level relationships within Apiaceae subfamily Apioideae have been discussed elsewhere (Downie & al., 1998; Katz-Downie & al., 1999). Their homoplastic nature, high levels of nucleotide sequence divergence in some lineages, and small size of the region all conspire

Table 5. Comparisons of pollen grains in *Johrenia paucijuga*, *Johrenia aromatica*, *Johrenia golestanica*, *Johreniopsis seseloides*, and *Holandrea caucasica*.

	Out-line	P.L.	E.W.	P/E	Ex.P.	Ex.E	Pr.L.	C.L.
<i>J. paucijuga</i>	Oval	29.210 ± 1.844	14.010 ± 0.406	2.088 ± 0.116	1.750 ± 0.198	2.506 ± 0.393	2.045 ± 0.324	20.280 ± 1.645
<i>J. aromatica</i>	Oval	28.945 ± 1.414	14.665 ± 0.827	2.462 ± 1.979	1.505 ± 0.049	2.140 ± 0.224	2.765 ± 0.377	20.800 ± 1.428
<i>J. golestanica</i>	Oval	27.217 ± 1.409	12.919 ± 0.668	2.112 ± 0.121	1.489 ± 0.157	2.257 ± 0.342	2.222 ± 0.376	19.730 ± 0.329
<i>J. seseloides</i>	Oval	(21–)23–28	(12–)13–15	1.61–3	1.5	2.5	1.5–2	13–16(–18)
<i>H. caucasica</i>	Oval	24–28	11–14	1.92–2.27	1	2	3–5	(13–)14–17

Symbols and definitions are those of Cerceau-Larrival (1971) and Punt (1984), and are as follows: P.L., polar axis length; E.W., equatorial axis width; P/E, polar axis length/equatorial axis width ratio; Ex.P, exine thickness at poles; Ex.E, exine thickness at equator; Pr.L., porus length; C.L., colpus length. All units µm. Data for *Johreniopsis* and *Holandrea* are from Salimian (2002).

Table 6. Morphological comparisons within the *Opopanax* group: *Opopanax hispidus*, *Opopanax persicus*, and *Smyrniopsis aucheri*.

	<i>O. hispidus</i>	<i>O. persicus</i>	<i>S. aucheri</i>
Leaf segments	Oblong, elliptic, acute, crenate, with cartilage at margin	Oblong, elliptic, acute, crenate, with cartilage at margin	Oblong, elliptic, acute, crenate, with cartilage at margin
Stem surface	All parts hispidus, especially basal portions	All parts glabrous	All parts slightly hispidus
Inflorescence	Lax thyrsoid, with male lateral umbels and central hermaphrodite umbel	Dense thyrsoid, with male lateral umbels and central hermaphrodite umbel	Conical, with male lateral umbels and central hermaphrodite umbel
Fruit compression	Dorsal	Dorsal	Lateral

to reduce the utility of these data in resolving deep-level relationships within the subfamily. A glance at the trees in Figs. 1 and 2 will show poor resolution and branch support for the most basal lineages within the Apioideae superclade. Minor differences in topology are also apparent among the trees with regard to the relationships inferred among the tribes and major clades. In this study, one of our goals was to elucidate the phylogenetic placements of five groups of genera native to the *Flora Iranica* area. Based on the phylogenetic results, the *Cymbocarpum* group falls within tribe Tordylieae, the *Johrenia* group is placed within tribe Selineae, and the *Ferula* group allies with tribe Scandiceae. Unclear, however, are the tribal placements of the *Cachrys* and *Opopanax* groups. Both of these fall within the Apioideae superclade, but not in any previously recognized tribe supported strongly as monophyletic on the basis of molecular phylogenetic studies (reviewed in Downie & al., 2001). To date, phylogenies of the Apioideae inferred from ITS sequence data are generally consistent with those inferred from chloroplast markers, but until the latter are available for a broad sampling of taxa from the Apioideae superclade the relationships among its tribes and the tribal placements of the *Cachrys* and *Opopanax* groups cannot be resolved. The *Cachrys* group may constitute a new, major lineage of Apiaceae, but its recognition now as a tribe is premature in the absence of supporting plastid data. The major objective of this study was to ascertain taxonomic and phylogenetic relationships within these five genus groups. Sister group relationships of each of these five groups are not discussed in detail because of the poor resolution of relationships, limited sampling outside of these analyzed clades, and the large size and taxonomic complexity of the subfamily Apioideae. Below, we discuss the taxonomic implications of our study for each of these five genus groups.

Cachrys group. — The *Cachrys* group, as presently circumscribed, comprises a heterogeneous assemblage of genera and we are not yet aware of any obvious morphological character that would support its monophyly. Moreover, the clade is not very well supported in the MP and ML trees, nor are the relationships resolved among its constituent genera. The genera *Prangos* and *Ferulago* are large and, together with *Cachrys* and its segregates, extremely complex taxonomically. The species of *Bilacunaria* (= *Hippomarathrum* Link) was treated previously in *Cachrys* (as *C. microcarpa* M. Bieb.) and this relationship is not necessarily refuted by the ITS results because *B. microcarpa* is a weakly supported sister group to *Cachrys* in the ML tree. *Azilia eryngioides* is a sister group to all other members of this clade, coincident with its highly unusual morphology. We maintain the monotypic *Azilia* as a distinct genus given its isolated position in all ITS-derived cladograms and its unique morphology. The monotypic genus *Alococarpum* falls within the genus

Prangos and in the MP and Bayesian trees this clade is very well supported. *Alococarpum erianthum* was treated previously in *Cachrys* (as *C. eriantha* DC.), but its placement in *Prangos* seems better justified on the basis of their similar morphologies (Leute, 1987). However, before *A. erianthum* is transferred into *Prangos*, supporting evidence from cpDNA is required.

Cymbocarpum group. — Molecular and morphological studies support the close relationship among *Cymbocarpum*, *Kalakia*, and *Ducrosia*. *Cymbocarpum* is monophyletic and a strongly supported sister group to *Kalakia* in the ITS trees. These two genera are morphologically very similar and the differences noted in fruit morphology between them are just as great as those between *C. anethoides* and *C. erythraeum*. ITS sequence divergence between *Cymbocarpum* and *Kalakia marginata* is low relative to comparisons between *Cymbocarpum/Kalakia* and *Ducrosia*. The erection of the monotypic genus *Kalakia* solely on the basis of its thickened fruit margin is dubious, as similar tumid margins also occur in *Ducrosia* and elsewhere in Apioideae. Given the overall similarity between *Cymbocarpum* and *Kalakia* and their relatively low genetic divergence, we do not support *Kalakia* as a distinct, monotypic genus. Instead, we maintain three species in the Iranian genus *Cymbocarpum*. Three species also comprise *Ducrosia*, but only *D. anethifolia* was included in this study. Its other two species must be examined to confirm their taxonomic placements alongside *D. anethifolia*.

Tribe Tordylieae was circumscribed by Pimenov & Leonov (1993) to include the genera *Ainsworthia*, *Ducrosia*, *Heracleum*, *Kalakia*, *Malabaila* (incl. *Leiotulus*), *Pastinaca*, *Semenovia*, *Symphyoloma* C.A. Mey., *Tetrataenium* (DC.) Manden., *Tordylium* L. (incl. *Synelcosciadium*), *Vanasushava*, and *Zosima* Hoffm. among others, and this circumscription is corroborated by the results of phylogenetic analyses of ITS sequences. However, Pimenov & Leonov (1993) included *Cymbocarpum* in tribe Peucedaneae, as traditionally circumscribed. Given the close relationships among *Cymbocarpum*, *Ducrosia*, and *Kalakia* (= *C. marginata*), we expand the circumscription of Tordylieae to include *Cymbocarpum*. Also included in an expanded, but variously supported, tribe Tordylieae is a group of African umbellifers (Winter & al., 2008); this group is represented in our study by *Dasispermum suffruticosum* and *Peucedanum galbanum*.

Ferula group. — The recognition of *Dorema kopetdaghense* as a synonym of *D. hyrcanum*, as proposed by Rechinger (1987) in *Flora Iranica*, is not supported by our results. Sequence divergence between these two species is among the highest in infrageneric pairwise comparisons and we consider them morphologically distinct at the species level. Therefore, *Dorema kopetdaghense* and *D. hyrcanum* should be retained as separate species. Molecular

data support the monophyly of Iranian *Dorema* (upon the inclusion of *F. feruloides* and *F. tenuisecta*) and its close relationship to *Ferula* in tribe Scandiceae. Indeed, three species of *Dorema* and *Ferula feruloides* have identical ITS sequences. *Ferula feruloides* is treated in *Ferula* subgenus *Dorematoides* (Rgl. & Schmalh.) Korovin, an apparently polyphyletic group, but whose plants bear simple umbels like those of *Dorema* (Schischkin, 1951). The close relationship between *Dorema* and *Ferula* was suggested previously based on fruit anatomical, morphological, and immunological data (Schischkin, 1951; Pimenov, 1988; Shneyer & al., 1995). The placement of *D. aucheri* as a sister group to *Seseli mucronatum* in tribe Selineae, as indicated by Valiejo-Roman & al. (2006) on the basis of ITS sequence comparisons, must be regarded as spurious. In both MP and ML trees, *Dorema* arises from within a paraphyletic *Ferula*. This same relationship is supported by Kurzyna-Mlynik & al. (in press) upon further study of ITS sequences and a comprehensive sampling of *Ferula*. Therefore, we suggest that the eight species of *Dorema* from Iran be transferred into *Ferula*, but before such nomenclatural changes are implemented supporting evidence from cpDNA is required. Similarly, the genus *Leutea* may also be submerged within *Ferula*. Six species are recognized in the genus and all are almost entirely restricted to the *Flora Iranica* area. *Leutea* is a segregate of *Peucedanum* and its affinity to several other peucedanoid genera has been inferred, such as *Johrenia*. Our results indicate that *Leutea* is allied with *Ferula*, as suggested previously (Pimenov, 1987b; Shneyer & al., 1995). However, we refrain from making this transfer now because only one species of *Leutea* was included in our study and its placement within the *Ferula* subclade is unclear.

The molecular results support the placement of the monotypic genus *Hausknechtia* adjacent to *Demavendia* in tribe Pimpinelleae, well away from *Dorema* and *Opopanax* for which affinities were suggested previously (Rechinger, 1987; Pimenov, 1988; Valiejo-Roman & al., 2006). *Hausknechtia* and *Demavendia* are morphologically similar and very distinct from all examined species of *Dorema*. Recently, Valiejo-Roman & al. (2006), on the basis of ITS sequence comparisons, showed a close relationship among *Hausknechtia*, *Demavendia*, and *Zeravschania*. Such a relationship has also been supported by fruit anatomy (Pimenov & al., 2006).

Johrenia group. — Molecular, morphological, petiole anatomical, and palynological data support a distant relationship between *Johrenia golestanica* and its congeners. Thus, *J. golestanica* may constitute a new, monotypic genus pending confirmation from additional sampling of *Johrenia* and *Johreniopsis* and supporting evidence from cpDNA. These taxa are distantly related to *Peucedanum* s.str. (*P. officinale* L.), but are very closely related to

Holandrea, whose species were previously attributable to *Peucedanum* s.l. (Reduron & al., 1997; Spalik & al., 2004). In this study, *Johrenia*, *Johreniopsis*, and *Holandrea* are each not monophyletic, yet the assemblage comprises a strongly supported monophyletic group in the MP and Bayesian trees. These genera are placed in tribe Selineae along with *Peucedanum* and its segregates (*Cervaria*, *Imperatoria*, *Thysselinum*) and are distantly related to *Leutea* (tribe Scandiceae) and *Demavendia* (tribe Pimpinelleae). *Johrenia* and *Johreniopsis* constitute new members of tribe Selineae. The association of *Holandrea* with *Johrenia* and *Johreniopsis* is not surprising given their similar morphologies, thus future studies of this group must include *Holandrea*, as well. Pending further investigation, *Holandrea* may be transferred into *Johrenia*, as this genus has priority over *Johreniopsis*. However, the results of multivariate analyses of 34 morphological characters for 33 species of *Johrenia*, *Johreniopsis*, *Holandrea*, and other genera revealed that *Holandrea* should be treated as a section of *Johreniopsis* (Pimenov, Kljuykov & Ostroumova, unpubl.). Continued investigations of this group are necessary.

Opopanax group. — Historically, *Opopanax* and *Smyrniopsis* were placed in different tribes (Drude, 1898), yet molecular data revealed them to be a monophyletic group (e.g., Katz-Downie & al., 1999). Their monophyly is supported upon additional sampling. Similarly, these taxa share similar morphological attributes, especially with regard to their leaf segments and inflorescences. These genera are allied with *Petroedmondia syriaca* which was previously referable to *Smyrniopsis* (*S. cachroides*). Boissier (1872) recognized *Smyrniopsis aucheri* and *S. cachroides* in two sections; however, their recognition as distinct genera is justified based on the molecular phylogenies presented herein and the high sequence divergence values between them. In addition, there is a possible alliance of these taxa with the western European species *Magydaris panacifolia* and the taxonomically isolated, monotypic Turkish genus *Crenosciadium* Boiss. & Heldr. ex Boiss. The latter was considered by Bentham (1867) as closely allied to and possibly congeneric with *Opopanax* and these genera are treated adjacently in the *Flora of Turkey* (Davis, 1972). Further studies of the *Opopanax* group would benefit by the inclusion of *Petroedmondia*, *Magydaris*, and *Crenosciadium*.

Other relationships. — Tribe Tordylieae is one of only a few traditionally recognized higher-level taxa of Apiaceae subfamily Apioideae that has been confirmed as monophyletic on the basis of phylogenetic analysis of molecular data. Its largest genus, *Heracleum*, with 65 species (Pimenov & Leonov, 1993), is not monophyletic. The type of the genus, *H. sphondylium*, and its allies form the core *Heracleum* clade, albeit this group is not very well supported. Ten species of *Heracleum* occur in

Iran, of which we have included only one. The Chinese species *Heracleum candicans* may represent a new genus. *Heracleum pedatum* has been treated previously as *Vanasushava pedata* (Wight) P.K. Mukh. & Constance and is sister group to *Tetrataenium*. The next largest genus in the tribe, *Pastinaca*, appears paraphyletic, with *Malabaila* (= *Leiotulus*) nested within; this well supported clade is referred to as *Pastinaca* s.l., or the *Pastinaca* group (Valiejo-Roman & al., 2006), and requires further study.

In conclusion, the phylogenetic relationships within five groups of genera native to the *Flora Iranica* region have been assessed using molecular data, with supplementary comparative information coming from morphology and, for the *Johrenia* group, petiole anatomy and palynology. Given the rich diversity of Apiaceae in Iran, as well as its numerous endemic species and genera (many of which remain to be investigated using molecular data), further studies of these plants are necessary. Nomenclatural changes are suggested, but only if the relationships proposed using ITS data are corroborated by cpDNA evidence. To date, the treatment of Apiaceae in *Flora Iranica* is the best available for the region, yet many of its taxonomic accounts are still provisional (Hedge & Lamond, 1987). Our results should stimulate further detailed studies on these plants. While this manuscript was in preparation, a study appeared on the taxonomic relationships of Iranian Apiaceae using molecular data (Valiejo-Roman & al., 2006). This study included only a few exemplars of the *Cymbocarpum* and *Johrenia* groups and nothing from the *Opopanax* group; sampling of the *Cachrys* group was similar to our study. However, the one species of *Dorema* included in that investigation fell within tribe Selineae and not Scandiceae. Clearly, given the size and complexity of subfamily Apioideae in Iran, more work needs to be done to produce a modern classification of these plants.

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Appendix. List of 177 accessions (representing 157 species) of Apiaceae subfamily Apioideae used in the phylogenetic analysis of nrDNA ITS sequence data. The 85 previously published ITS sequences are indicated with GenBank reference numbers only. A single GenBank reference number for an accession indicates contiguous ITS1, 5.8S, and ITS2 data. Two GenBank reference numbers for an accession coincide with ITS1 and ITS2, with no intervening 5.8S data. For each of the 92 newly obtained sequences, voucher information, DNA accession, and GenBank reference numbers are provided.

Previously published ITS sequence data

Aegopodium alpestre Ledeb., U78376 & U78436; *Aethusa cynapium* L., U30582 & U30583; *Alococarpum erianthum* (DC.) Riedl. & Kuber, AY941264 & AY941292; *Ammi majus* L., U78386 & U78446; *Anethum graveolens* L., U30550 & U30551; *Aphanopleura capillifolia* (Regel & Schmalh.) Lipsky, DQ516368; *Apium graveolens* L., U30552 & U30553; *Arafoe aromatica* Pimenov & Lavrova, AF077874; *Azilia eryngioides* (Pau) Hedge & Lamond, AF008620 & AF009099; *Bifora radians* M. Bieb., U78408 & U78468; *Bilacnaria microcarpa* (M. Bieb.) Pimenov & V.N. Tikhom., AY941265 & AY941293; *Bubon macedonicum* L., AF073541; *Bunium elegans* (Fenzl) Freyn, AF073543 & AF073544; *Carum carvi* L., AF077878; *Cervaria rivinii* Gaertn., AF008608 & AF009087; *Chamaesciadium acaule* (M. Bieb.) Boiss., AY957495 & AY957496; *Coriandrum sativum* L., U30586 & U30587; *Cuminum cyminum* L., U78362; *Dasispermum suffruticosum* (P.J. Bergius) B.L. Burtt, AM408870; *Daucus carota* L. subsp. *carota*, AF077779; *Demavendia pastinacifolia* (Boiss. & Hausskn. ex Boiss.) Pimenov, AY911857 & AY911863; *Dicyclophora persica* Boiss., AF073539; *Eleutherospermum cicutarium* (M. Bieb.) Boiss., AF008637 & AF009116; *Eriocyclus olivieri* (Boiss.) Wolff, AY941289 & AY941317; *Exoacantha heterophylla* Labill., AF008617 & AF009096; *Falcaria vulgaris* Bernh., AF077888; *Ferula feruloides* (Steud.) Korovin, DQ379402; *Ferula kingdon-wardii* H. Wolff, AF164824 & AF164849; *Ferula kokanica* Regel & Schmalh., AF164825 & AF164850; *Ferula linkii* Webb, DQ379421; *Ferula tenuisecta* Korovin ex Pavlov, AF077890; *Ferula violacea* Korovin, AF077891; *Ferulago angulata* (Schltdl.) Boiss., AY941272 & AY941300; *Ferulago galbanifera* (Mill.) W.D.J. Koch, AF077889; *Ferulago trachycarpa* Boiss., AJ972393 & AJ972394; *Foeniculum vulgare* Mill., AY551289; *Fuernrohreria setifolia* C. Koch, AF008633 & AF009112; *Galagania fragrantissima* Lipsky, DQ422820 & DQ422839; *Grammosciadium daucoides* DC., AF073559; *Heracleum aconitifolium* Woronow, AF008625 & AF009104; *Heracleum lanatum* Michx., AH003476; *Heracleum moellendorffii* Hance, AF164828 & AF164853; *Holandrea achaica* (Halácsy) Spalik, Reduron & S.R. Downie, AF164832 & AF164857; *Holandrea caucasica* (M. Bieb.) Spalik, Reduron & S.R. Downie, AF008618 & AF009097; *Holandrea carvifolia* (Vill.) Reduron, Charpin & Pimenov, AF495828 & AF495829; *Holandrea pschawica* (Boiss.) Reduron, Charpin & Pimenov, AF008619 & AF009098; *Holandrea schottii* (Besser ex DC.) Reduron, Charpin & Pimenov, AF495830 & AF495831; *Hyalolaena intermedia* Pimenov & Kljuykov, DQ422823 & DQ422842; *Imperatoria ostruthium* L., AF077896; *Johreniopsis scoparia* (Boiss.) Pimenov, AY941274 & AY941302; *Lagoecia cuminoides* L., AF337179 & AF337187; *Laser trilobum* (L.) Borkh. ex Gaertn., AF008644 & AF009123; *Lecokia cretica* (Lam.) DC., U78358; *Leiotulus porphyrodiscus* (Stapf & Wettst.) Pimenov & Ostroumova, AY941275 & AY941303; *Leutea petiolaris* (DC.) Pimenov, AY941278 & AY941306; *Levisticum officinale* W.D.J. Koch, AF077899; *Malabaila sekakul* Boiss., AF008627 & AF009106; *Muretia lutea* (M. Bieb.) Boiss., DQ516359; *Oedibasis apiculata* (Kar. & Kir.) Koso-Pol., DQ422828 & DQ422847; *Opopanax hispidus* (Friv.) Griseb., AF008624 & AF009103; *Paraligusticum discolor* (Ledeb.) V.N. Tikhom., AF077902; *Pastinaca armena* Fisch. & C.A. Mey., AF008626 & AF009105; *Peucedanum galbanum* (L.) Drude, AM408846; *Peucedanum officinale* L., AF495820 & AF495821; *Physospermum cornubiense* (L.) DC., AF077904; *Pimpinella kotschyana* Boiss., AY581793; *Pimpinella saxifraga* L., AY581801; *Polylophium panjutinii* Manden. & Schischk., AF008645; *Prangos acaulis* (DC.) Bornm., AY941281 & AY941309; *Prangos pabularia* Lindl., U78409 & U78469; *Prangos uloptera* DC., AY941283 & AY941311; *Psammogeton canescens* (DC.) Vatke, AF008630 & AF009109; *Pycnocyclus aucheriana* Decne. ex Boiss., AF073533; *Pyramidoptera cabulica* Boiss., AF008631; *Ridolfia segetum* (L.) Moris, U78384 & U78444; *Selinum carvifolia* (L.) L., AY179028; *Semenovia dichotoma* (Boiss.) Manden., AY941287 & AY941315; *Semenovia tragioides* (Boiss.) Manden., AY941288 & AY941316; *Seseli peucedanoides* (M. Bieb.) Koso-Pol., AY179034; *Smyrniopsis aucheri* Boiss., AF077909; *Tetraenaum rigens* (Wall. ex DC.) Manden., AH003477; *Thysselinum palustre* (L.) Hoffm., AY179035; *Xanthogalum purpurascens* Avé-Lall., AF008611 & AF009090; *Zeravschania regeliana* Korovin, AY911861 & AY911867; *Zosima orientalis* Hoffm., AF008628.

Appendix. Continued.

New ITS sequence data

Ainsworthia trachycarpa Boiss., DNA No. 2141, Israel, cult. University of California Botanical Garden Berkeley, *Constance C-779* (ISU 13139), EU169242; *Ainsworthia trachycarpa* Boiss., DNA No. 3008, Jordan, *Lahham s.n.* (Yarmouk University Herbarium), EU169243; *Anisosciadium isosciadium* Bornm. var. *idumaeum* Zohary, DNA No. 1939, Jordan, *Frey & Kürschner VO 5151* (E 00042063), EU169244; *Anisosciadium orientale* DC., DNA No. 1355, Iran, *Davis & Bokhari 56241* (E 00042061), EU169245; *Astoma seselifolium* DC., DNA No. 3213, Israel, *Danin & al. 8-80* (RNG), EU169246; *Azilia eryngioides* (Pau) Hedge & Lamond, DNA No. 3161, Iran, *Ajani s.n.* (Hb. Akhani), EU169247; *Cachrys libanotis* L., DNA No. 3212, Morocco, *JMM-6083/4* (RNG), EU169248; *Cachrys sicula* L., DNA No. 3211, Morocco, *Jury & al. 16731* (RNG), EU169249; *Cervaria cervariifolia* (C.A. Mey.) Pimenov, DNA No. 3130, Iran, *Ajani 2058* (TUH), EU169250; *Conium maculatum* L., DNA No. 2998, U.S.A., Kentucky, Madison Co., Silver Creek, *Jones 3618* (ILL), EU169251; *Crenosciadium siifolium* Boiss. & Heldr., DNA No. 2410, Turkey, *Duman & Aytaç 7111* (E 00146045), EU169252; *Cymbocarpum anethoides* DC., DNA No. 3150, Iran, *Ajani 2049* (TUH), EU169253; *Cymbocarpum erythraeum* (DC.) Boiss., DNA No. 3114, Iran, *Ajani s.n.* (Hb. Akhani), EU169254; *Deverra denudata* (Viv.) Pfisterer & Podlech subsp. *denudata*, DNA No. 1942, Morocco, *Ait Lafkih & al. 4928* (E 00040889), EU169255; *Deverra scoparia* Coss. & Durieu subsp. *scoparia*, DNA No. 1941, Tunisia, *Davis & Lamond D57330* (E 00040888), EU169256; *Deverra tortuosa* (Desf.) DC., DNA No. 1943, Saudi Arabia, *Collenette 5761* (E 00040892), EU169257; *Diplotaenia cachrydifolia* Boiss., DNA No. 3116, Iran, *Ajani 955* (Hb. Akhani), EU169258; *Diplotaenia damavandica* Mozaff., Hedge & Lamond, DNA No. 3117, Iran, *Ajani 980* (Hb. Akhani), EU169259; *Dorema aitchisonii* Korovin ex Pimenov, DNA No. 3122, Iran, *Ajani 841* (Hb. Akhani), EU169260; *Dorema ammoniacum* D. Don, DNA No. 3121, Iran, *Ajani s.n.* (Hb. Akhani), EF560690; *Dorema aucheri* Boiss., DNA No. 3123, Iran, *Ajani s.n.* (IRAN), EU169261; *Dorema aureum* Stocks, DNA No. 3124, Iran, *Ajani s.n.* (IRAN), EU169262; *Dorema glabrum* Fisch. & C.A. Mey., DNA No. 3125, Iran, East Azerbaijan Province, border between Iran and Nakhjavan, after 18 Mile station, between Poldasht and Jolfa, *Ajani 900* (Hb. Akhani), EU169263; *Dorema hyrcanum* Koso-Pol., DNA No. 3126, Iran, *Ajani s.n.* (Hb. Akhani), EU169264; *Dorema kopetdaghense* Pimenov, DNA No. 3127, Iran, Khorasan Province, Tandooreh National Park, ca. 1–2 km after entrance, *Ajani 848* (Hb. Akhani), EU169265; *Ducrosia anethifolia* (DC.) Boiss., DNA No. 3113, Iran, *Ajani 2051* (TUH), EU169266; *Echinophora orientalis* Hedge & Lamond, DNA No. 1940, Turkey, *Davis 46840* (E 00042067), EU169267; *Eremodaucus lehmannii* Bunge, DNA No. 3210, Afghanistan, *Hedge & Wendelbo W 9478* (RNG), EU169268; *Ferula communis* L., DNA No. 1156, Yemen Arab Republic, *Spellenberg 7459* (UC 1492485), EU169269; *Ferula karelinii* Bunge (= *Schumannia karelinii* (Bunge) Korovin), DNA No. 3039, Pakistan, *Lamond 562* (E), EU169313; *Ferula olivacea* (Diels) H. Wolff, DNA No. 3214, China, *CLD Exp. 790*, cult. Royal Botanic Garden Edinburgh (No. 19910663) (E), EU169270; *Ferulopsis hystrix* (Bunge ex Ledeb.) Pimenov, DNA No. 3215, Russia, cult. Royal Botanic Garden Edinburgh (No. 19932301A), EU169271; *Gongylosciadium falcarioides* (Bornm. & H. Wolff) Rech. f., DNA No. 1007, Origin unknown, DNA obtained from C. Valiejo-Roman, Moscow State University, Russia, EU169272; *Haussknechtia elymaitica* Boiss., DNA No. 3128, Iran, *Ajani 942* (Hb. Akhani), EU169273; *Heracleum alpinum* L., DNA No. 2237, Switzerland, *Reduron s.n.* (Hb. Reduron), EU169274; *Heracleum apiifolium* Boiss., DNA No. 1181, Turkey, *Spalik s.n.* (WA), EU169275; *Heracleum candicans* Wall. ex DC., DNA No. 1296, China, cult. Royal Botanic Garden Edinburgh (No. 19943858), EU169276; *Heracleum candicans* Wall. ex DC., DNA No. 1312, China, cult. Royal Botanic Garden Edinburgh (No. 19952540), EU169277; *Heracleum lanatum* Michx., DNA No. 735, U.S.A., Illinois, *Downie 735* (ILL), EU169278; *Heracleum minimum* Lam., DNA No. 1633, France, *Reduron & Danton s.n.*, cult. Ville de Mulhouse Conservatoire Botanique (No. 96213), EU169279; *Heracleum minimum* Lam., DNA No. 1639, France, *Reduron s.n.* (Hb. Reduron), EU169280; *Heracleum pastinacifolium* C. Koch subsp. *incanum* (Boiss. & Huet) P.H. Davis, DNA No. 1182, Turkey, *Spalik s.n.* (WA), EU169281; *Heracleum pedatum* Wight [*Vanasushava pedata* (Wight) P.K. Mukh. & Constance], DNA No. 1455, India, *Matthew 14823* (E 00040903), EU169282; *Heracleum pyrenaicum* Lam., DNA No. 2236, France, *Reduron s.n.* (Hb. Reduron), EU169283; *Heracleum sibiricum* L., DNA No. 2265, France, *Reduron s.n.* (Hb. Reduron), EU169284; *Heracleum sphondylium* L. subsp. *elegans* (Crantz) Schübl. & Mart., DNA No. 1881, France, *Reduron s.n.* (Hb. Reduron), EU169285; *Heracleum sphondylium* L. subsp. *sphondylium*, DNA No. 1880, France, *Coudere s.n.* (Hb. Reduron), EU169286; *Hladnikia pastinacifolia* Rehb., DNA No. 3207, Slovenia, *Gardner & Gardner 2615* (RNG), EU169287; *Johrenia aromatica* Rech. f., DNA No. 3120, Iran, *Ajani s.n.* (Hb. Akhani), EU169288; *Johrenia golestanica* Rech. f., DNA No. 3119, Iran, *Ajani 2053* (TUH), EU169289; *Johrenia paucijuga* (DC.) Bornm., DNA No. 3118, Iran, *Ajani 852* (Hb. Akhani), EU169290; *Johreniopsis seseloides* (C.A. Mey.) Pimenov, DNA No. 3129, Iran, *Ajani 2057* (TUH), EU169291; *Kalakia marginata* (Boiss.) Alava, DNA No. 3115, Iran, *Ajani 2050* (TUH), EU169292; *Kalakia marginata* (Boiss.) Alava, DNA No. 3149, Iran, *Ajani 2095* (TUH), EU169293; *Lecokia cretica* (Lam.) DC., DNA No. 3198, Cyprus, *Edmondson & McClintock E2788* (E 00040979), EU169294; *Magydaris panacifolia* (Vahl) Lange, DNA No. 3208, Spain, *Gardner & Knees 4818* (RNG), EU169295; *Malabaila dasyantha* (C. Koch) Grossh., DNA No. 1862, Turkey, *Menemen & Hamzaoglu 60* (ADO), EU169296; *Malabaila pastinacifolia* Boiss. & Bal., DNA No. 1861, Turkey, *Duran & al. 5498* (ADO), EU169297; *Opopanax hispidus* (Friv.) Griseb., DNA No. 3160, Iran, *Ajani 853* (Hb. Akhani), EU169298; *Opopanax persicus* Boiss., DNA No. 3131, Iran, *Ajani 941* (Hb. Akhani), EU169299; *Opopanax persicus* Boiss., DNA No. 3151, Iran, *Ajani 2085* (TUH), EU169300; *Pastinaca lucida* Gouan, DNA No. 1879, Spain, *Hildenbrand & al. s.n.*, cult. Ville de Mulhouse Conservatoire Botanique (No. 99011), EU169301; *Pastinaca pimpinellifolia* M. Bieb., DNA No. 1864, Turkey, *Menemen & Hamzaoglu 309* (ADO), EU169302; *Pastinaca sativa* L., DNA No. 543, U.S.A., *Downie 543* (ILL), EU169303; *Pastinaca sativa* L., DNA No. 734, U.S.A., *Downie 734* (ILL), EU169304; *Pastinaca sativa* L., DNA No. 742, U.S.A., *Downie 742* (ILL), EU169305; *Pastinaca sativa* L. subsp. *urens* (Req. ex Godr.) Celak, DNA No. 1863, Turkey, *Menemen & Duran 284* (ADO), EU169306; *Pastinaca sativa* L. subsp. *urens* (Req. ex Godr.) Celak, DNA No. 1878, France, *Hildenbrand & al. s.n.*, cult. Ville de Mulhouse Conservatoire Botanique (No. 99138A), EU169307; *Petroedmondia syriaca* (Boiss.) Tamamsch. [= *Smyrniopsis cachroides* Boiss.], DNA No. 3009, Jordan, *Lahham s.n.* (Yarmouk University Herbarium), EU169308; *Petroedmondia syriaca* (Boiss.) Tamamsch. [= *Smyrniopsis cachroides* Boiss.], DNA No. 3206, Turkey, *Nydegger 42531* (RNG), EU169309; *Prangos ferulacea* (L.) Lindl., DNA No. 3146, Iran, *Ajani 2055* (TUH), EU169310; *Prangos gontiocarpa* (Boiss.) Zohary, DNA No.

Appendix. Continued.

New ITS sequence data

3152, Jordan, *Lahham & El-Oqlah 5* (Yarmouk University Herbarium), EU169311; *Prangos uloptera* DC., DNA No. 3147, Iran, *Ajani 2054* (TUH), EU169312; *Seseli farreynii* Molero & A. Pujadas, DNA No. 2243, Spain, *Hildenbrand s.n.*, cult. Ville de Mulhouse Conservatoire Botanique (No. 01115), EU169314; *Seseli rhodopeum* Velen., DNA No. 2413, Bulgaria, cult. France, Savoie, Saint-Pierre-d'Albigny, *Reduron s.n.* (Hb. Reduron), EU169315; *Seseli rigidum* Waldst. & Kit., DNA No. 2245, Bulgaria, Kaliaka, cult. France, Savoie, Saint-Pierre-d'Albigny, *Reduron s.n.* (Hb. Reduron), EU169316; *Smyrniopsis aucheri* Boiss., DNA No. 3148, Iran, *Ajani 854* (Hb. Akhani), EU169317; *Smyrniopsis aucheri* Boiss., DNA No. 3197, Iran, *Runemark & Mozaffarian 29121* (E 00040982), EU169318; *Smyrnum connatum* Boiss. & Kotschy ex Kotschy, DNA No. 3196, Turkey, *Vural 634* (E 00040989), EU169319; *Smyrnum cordifolium* Boiss., DNA No. 3162, Iran, *Ajani 2056* (TUH), EU169320; *Smyrnum cordifolium* Boiss., DNA No. 3201, Turkey, *Davis 45119* (E 00040988), EU169321; *Smyrnum creticum* Mill., DNA No. 3199, Turkey, *Möllerken s.n.* (E 00040990), EU169322; *Smyrnum galaticum* Czeaczott, DNA No. 3200, Turkey, *Baytop & Baytop 35243* (E 00040992), EU169323; *Smyrnum olusatrum* L., DNA No. 3158, France, *Gavelle s.n.* (UC 308610), EU169324; *Smyrnum olusatrum* L., DNA No. 3159, Israel, *Feinbrun & Koppel 558* (UC 002911), EU169325; *Smyrnum rotundifolium* Mill., DNA No. 3202, Italy, *Davis & Sutton D63119* (E 00040983), EU169326; *Synelcosciadium carmeli* (Labill.) Boiss., DNA No. 2142, Israel, cult. University of California Botanical Garden Berkeley, *Constance C-978* (ISU 13083), EU169327; *Tordylium aegyptiacum* (L.) Lam. var. *palaestinum* (Zohary) Zohary, DNA No. 823, Jordan, *Lahham & El-Oqlah 11* (Yarmouk University Herbarium), EU169328; *Tordylium apulum* L., DNA No. 2143, Origin unknown, cult. University of California Botanical Garden Berkeley, *Constance C-905* (ISU 13100), EU169329; *Trinia hispida* Hoffm., DNA No. 2127, Russia, *Botschkin & al. 70* (MO 04985260), EU169330; *Vicatia conifolia* DC., DNA No. 3029, Bhutan, *King 1132* (E 00059551), EU169331; *Zosima absinthifolia* (Vent.) Link, DNA No. 1973, Iraq, B. Makki 5.3, cult. University of California Botanical Garden Berkeley, *Constance C-635* (ISU 1143), EU169332.