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ORIGINAL ARTICLE

Phylogenetic position of the genus *Ferula* (Apiaceae) and its placement in tribe Scandiceae as inferred from nrDNA ITS sequence variation

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Abstract Recent molecular systematic investigations suggested that *Ferula*, an umbellifer genus of about 170 species, is polyphyletic, with its members placed in the apioid superclade and within tribe Scandiceae. We analyzed ITS sequence variation from 134 accessions of Apiaceae, including 83 accessions (74 species) of *Ferula* to ascertain the phylogenetic position of the genus within the family. Phylogenetic analyses of these data using maximum parsimony, Bayesian, and neighbor-joining methods support the monophyly of *Ferula* upon the addition of *Dorema* and *Leutea* (as *Ferula* sensu lato) and its placement in tribe Scandiceae. *Ferula* sensu is closely allied with other major lineages of Scandiceae, corresponding to subtribes Scandicinae, Daucinae, and Torilidinae. Therefore, we recognize the *Ferula* clade as subtribe Ferulinae. Another addition to tribe Scandiceae is a clade composed of genera *Glaucosciadium* and *Mozaffariania*. The three accessions of *Ferula* misplaced in the apioid superclade represent a species of *Silaum*.

Keywords Apiaceae · *Ferula* · Ferulinae · nrDNA ITS · Phylogeny · Scandiceae · Umbelliferae

Introduction

The genus *Ferula* L. includes about 170 species extending from central Asia westward throughout the Mediterranean region to northern Africa (Pimenov and Leonov 1993). Many species have been known since antiquity as sources of aromatic resins used in traditional medicine. These include asafoetida (obtained from *F. assa-foetida*, *F. aliacea* Boiss., *F. foetida*, and *F. narthex* Boiss.; authorities for species used in this study are given in the "Appendix"), sagapenum (*F. persica* Willd., *F. szowitsiana*), galbanum (*F. gummosa*), sumbul (*F. moschata*), and African ammoniacum (*F. tingitana*, *F. communis*) (Korovin 1959). Crowned with a pine cone and covered with ivy vines and leaves, a dried stalk of *Ferula* made the ancient thyrsus, a walking stick and an attribute of Dionysus and his followers Satyrs and Maenads. A plain stalk also served schoolmasters for inflicting corporal punishment on pupils (Beazley 1933). There are also speculations whether the famous spice and medicine of ancient Greece and Rome, silphium, came from a member of this genus. This plant, gathered from the wild in the coastal region of Cyrenaica (present Libya), went extinct around first century BCE, probably as a result of overexploitation and grazing (Andrews 1941).

Despite its large number of species, the genus is usually recognized as monophyletic, because its members are similar in habit and morphology. These robust, tall perennials or biennials are characterized by prominent taproots,

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stout stems, finely divided leaves with large inflated sheaths, and dorsally compressed fruits with plane commissural faces. However, there is also considerable variation in leaf division and indumentum, flowers and inflorescences, and fruit anatomy resulting in the recognition of numerous and often hardly discernible taxa. Basal leaves and mature fruits are important for correct identification; the former, however, are usually dried up before the latter are fully ripe. Because of the large size of these plants, only its lateral branches and lateral divisions of its basal leaves are usually preserved, thus making the identification of herbarium specimens difficult. With experience, most of the species can be identified from flowering material (Chamberlain and Rechinger 1987). However, the delimitation of species requires examination of complete specimens with roots, stem bases, basal leaves, inflorescence, flowers, and ripe fruits, and should be based on observations of living plants (Korovin 1947). It is not surprising, therefore, that both the infrageneric classification of *Ferula* and its phylogenetic position remain disputed.

Ferula is traditionally classified in tribe Peucedaneae subtribe Ferulinae (Drude 1897–1898; Pimenov and Leonov 1993). Similar to most traditionally circumscribed, higher level taxa within Apiaceae, molecular systematic studies have revealed that tribe Peucedaneae and its subtribes are each polyphyletic (Downie and Katz-Downie 1996; Plunkett et al. 1996). Many former members of Peucedaneae, including *Peucedanum* L. sensu lato, are now classified in tribe Selineae (Spalik et al. 2004). Phylogenetic analyses of both nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) and chloroplast DNA (cpDNA) *rps16* intron sequences suggest that *Ferula* is also polyphyletic. Of its eight species considered in previous molecular studies, three (*F. communis*, *F. tingitana*, and *F. assa-foetida*) form a clade with "*Thysselinum palustre*" and *Cenolophium denudatum* (Fisch. ex Hornem.) Tutin within the apioid superclade (Downie et al. 1998; Katz-Downie et al. 1999; Downie et al. 2000c), whereas the remaining species ally, surprisingly, with members of tribe Scandiceae (Valiejo-Roman et al. 1998; Downie et al. 2000c; Lee and Downie 2000). The accession of "*Thysselinum palustre*" used in these analyses was subsequently revealed to represent an unidentified umbellifer species rather than *T. palustre* (L.) Hoffm.; the latter and its congeners are members of tribe Selineae (Spalik et al. 2004). The affinity of *Ferula* with Scandiceae does not receive support from any line of evidence other than DNA sequences. However, the distant position of *Ferula* from other member genera of traditionally delimited Peucedaneae is confirmed by serological data. The immunochemical studies of Shneyer et al. (1995, 2003), while demonstrating the homogeneity of the genus, showed that *Ferula* is distinct from all other peucedanoid genera they included in their study.

Several large umbellifer genera are "dustbin" taxa encompassing species that do not fit elsewhere. Large genera with more than 20 species each comprise some 60% of the total number of umbellifer species (Spalik et al. 2001a). These include such genera like *Conioselinum* Hoffm., *Cymopterus* Raf., *Heracleum* L., *Ligusticum* L., *Peucedanum*, and *Seseli* L., which have each already been shown to be polyphyletic (Katz-Downie et al. 1999; Downie et al. 2000b, 2002). Therefore, the polyphyly of the large genus *Ferula* as suggested by molecular data is not a surprise. However, in contrast to the aforementioned genera, *Ferula* has long been regarded as a natural taxon. Moreover, despite the considerable phylogenetic distance between the two *Ferula* clades as suggested by previous molecular studies, we could not find any morphological character distinguishing these groups (A. Wojewódzka, University of Warsaw Botanic Garden, unpublished data).

The most comprehensive infrageneric classification of *Ferula* is that proposed by Korovin (1939, 1940, 1947); subsequently, it was modified for the *Flora of the USSR* (Korovin 1951). Korovin recognized six subgenera and eight sections, with some of these sections being divided into series or "grex" categories (Table 1). This classification was rarely adopted in extenso. In their revision of *Ferula* for the Kazakhstan flora, Safina and Pimenov (1984) rejected Korovin's subgenera and recognized 12 sections instead. Chamberlain and Rechinger (1987), in their revision of *Ferula* for *Flora Iranica*, retained only Korovin's subgenera, but refrained from referring four newly described species to any of them. Pimenov et al. (1978) carried out a phenetic cluster analysis of 90 species of *Ferula* using 33 morphological, life history, and phytochemical characters. Their results were incongruent with the classification of Korovin.

Korovin (1947) argued that fruit anatomy was of little value for the classification of *Ferula*; so, he used mostly characters of leaves and flowers to delimit infrageneric taxa. In contrast, Safina and Pimenov (1983, 1984, 1990) stressed the importance of fruit characters for circumscribing sections and inferring phylogenetic relationships. In their revision of *Ferula* for Kazakhstan, Safina and Pimenov (1984) described nine basic fruit types, with most sections they recognized being monomorphic with respect to fruit anatomy. In studies of Korovin's subgenera *Peucedanoides* (Safina and Pimenov 1983) and *Ferula* (Safina and Pimenov 1990), they provided evidence suggesting that these subgenera are highly heterogeneous with respect to fruit anatomy, with some of their respective members more similar to those of other subgenera. While demonstrating that the classification system of Korovin is artificial, the carpological studies of Safina and Pimenov

Table 1 Infrageneric classification of *Ferula* as proposed by Korovin (1947), with later additions and synonymy (Korovin 1951)

- I. Subgenus ***Scorodosma*** (Bunge) Drude
  - *F. assa-foetida* L., *F. foetida* (Bunge) Regel
- II. Subgenus ***Merwia*** (B. Fedtsch.) Korovin
  - sect. *Saprosmia* Korovin
    - *F. kelifi* Korovin (=*F. primaeva* Korovin)
  - sect. *Phacocarpa* Korovin
    - *F. botschantzevii* Korovin, *F. decurrens* Korovin, *F. lehmannii* Boiss., *F. szowitsiana* DC. (=*F. microloba* Boiss.), *F. mogoltavica* Lipsky ex Korovin
  - sect. *Discicarpa* Korovin
    - *F. litwinowiana* Koso-Pol., *F. karakalensis* Korovin
- Incertae sedis: *F. undulata* Pimenov et J.P. Baranova
- III. Subgenus ***Narthex*** (Falc.) Drude
  - sect. *Paleonarthex* Korovin
    - series *Pachycarpa* Korovin
      - *F. diversivittata* Regel et Schmalh.
    - series *Platycarpa* Korovin
      - *F. foetidissima* Regel et Schmalh., *F. tadshikorum* Pimenov, *F. eugenii* Kamelin, *F. violacea* Korovin
    - series *Neonarthex* Korovin
      - *F. gummosa* Boiss., *F. badrakema* Koso-Pol., *F. teterrima* Kar. et Kir.
- IV. Subgenus ***Ferula***
  - sect. *Phyllites* Korovin
    - *F. kokanica* Regel et Schmalh., *F. tuberifera* Korovin, *F. kelleri* Koso-Pol. (=*F. foliosa* Korovin), *F. leiophylla* Korovin, *F. samarkandica* Korovin, *F. nevskii* Korovin
  - sect. *Ferula* (= *Anatriches* Korovin)
    - *F. equisetacea* Koso-Pol., *F. koso-poljanskii* Korovin, *F. lipskyi* Korovin, *F. glauca* L., *F. communis* L., *F. tingitana* L., *F. linkii* Webb, *F. loscosii* (Lange) Willk., *F. neapolitana* Ten., *F. nuratavica* Pimenov
- V. Subgenus ***Peucedanoides*** (Boiss.) Korovin
  - sect. *Xeronarthex* Korovin
    - series *Ceratophylla* Korovin
      - *F. prangifolia* Korovin, *F. tschimganica* Lipsky ex Korovin (=*F. minkwitzae* Korovin), *F. fedoroviorum* Pimenov, *F. ceratophylla* Regel et Schmalh., *F. lapidosa* Korovin, *F. potaninii* Korovin ex Pavlov
    - series *Ovina* Korovin
      - *F. ovina* (Boiss.) Boiss. (= *F. microcarpa* Korovin, *F. pachycarpa* Korovin, *F. stylosa* Korovin), *F. dshizakensis* Korovin, *F. kyzylkumica* Korovin, *F. ferganensis* Lipsky ex Korovin, *F. dissecta* (Ledeb.) Ledeb., *F. pallida* Korovin, *F. tenuisecta* Korovin ex Pavlov, *F. xeromorpha* Korovin, *F. rubroarenosa* Korovin (= *F. mollis* Korovin)
  - sect. *Macrorrhiza* Korovin
    - series *Sumbulus* (Reinsch.) Korovin
      - *F. moschata* (Reinsch) Koso-Pol. [=*F. sumbul* (Kauffm.) Hook. f., *F. urceolata* Korovin]
    - series *Schair* Korovin
      - *F. leucographa* Korovin, *F. gypsacea* Korovin, *F. ugamica* Korovin, *F. karatavica* Regel et Schmalh., *F. oopoda* (Boiss. et Buhse) Boiss. (=*F. badhysi* Korovin), *F. tatarica* Fisch. ex Spreng., *F. varia* (Schrenk) Trautv. (=*F. schair* Borszcz.)
    - series *Clematidifolia* Korovin
      - *F. korshinskyi* Korovin, *F. clematidifolia* Koso-Pol., *F. penninervis* Regel et Schmalh., *F. olgae* Regel et Schmalh.
    - series *Lobulata* Korovin
      - *F. akitschkensis* B. Fedtsch. ex Koso-Pol., *F. songarica* Pall. ex Schult., *F. karataviensis* (Regel et Schmalh.) Korovin ex Pavlov
- VI. Subgenus ***Dorematoides*** (Regel et Schmalh.) Korovin
  - *F. schtschurowskiana* Regel et Schmalh. ex Regel, *F. feruloides* (Steud.) Korovin, *F. dubjanskyi* Korovin ex Pavlov (=*F. dshaudshamyr* Korovin)
- *Incertae sedis*: *F. karelinii* Bunge, *F. kingdon-wardii* H. Wolff, *F. olivacea* (Diels) H. Wolff, *F. renardii* (Regel et Schmalh.) Pimenov

Only the species considered in this study are listed also revealed that the general plan of fruit anatomy is similar throughout the genus.

In this paper, we use the results of phylogenetic analyses of nrDNA ITS sequences to ascertain the taxonomic position of *Ferula*, infer infrageneric relationships, and evaluate the utility of several morphological, life history, and phytochemical characters for delimiting clades. In addition, the results are compared against the traditional classification systems of *Ferula*. The genus is extensively sampled, with emphasis placed on the nomenclatural type, *F. communis*, and its putatively related species.

Material and methods

Taxon sampling

In total, 134 accessions of Apiaceae were examined for ITS sequence variation, including 83 accessions representing 74 species of *Ferula* ("Appendix"). Eighty accessions were newly sequenced for this study, including *F. communis* and its closest relatives, as estimated by morphology: *F. tingitana*, *F. glauca*, *F. neapolitana*, *F. linkii*, and *F. loscosii* (Safina and Pimenov 1990). ITS sequences from the remaining accessions were obtained from GenBank. To infer the phylogenetic position of *Ferula* within subfamily Apioideae, we selected a set of accessions representing nearly all major lineages of the subfamily (Downie et al. 2001). We included a broad sampling of Apiaceae tribe Scandiceae, because in previously published analyses some species of *Ferula* grouped with members of this tribe. Taxon names generally follow Cherepanov (1995) with some later changes (She et al. 2005). We retained *F. olgae* and *F. eugenii* as separate species, because their sequences were different from those obtained from putatively conspecific *F. penninervis* and *F. violacea*, respectively.

Laboratory procedures

Total genomic DNA was isolated from about 20 mg of dried leaf tissue using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, California, USA). The purified DNAs were PCR-amplified using primers "ITS4" and "ITS5" (White et al. 1990) or "N-nc18S10" and "C26A" (Wen and Zimmer 1996). For some accessions where the entire ITS region could not be PCR-amplified using either of these primer pairs, the ITS 1 and ITS 2 regions were each amplified separately using the primers "18S-ITS1-F" and "5.8S-ITS1-R" for ITS 1 and "ITS-3N" and "C26A" primers for ITS 2 (Spalik and Downie 2006). Details of the PCR amplifications are provided in Downie et al. (2000a). Each PCR product was electrophoresed in a 1% agarose gel, stained with ethidium bromide, then excised and eluted using a QIAquick Gel Extraction Kit (Qiagen). No obvious polymorphism (multiple bands from a single PCR product) was observed. Cycle sequencing reactions were performed using the purified PCR product, AmpliTaq DNA polymerase (Roche Molecular Systems, Alameda, California, USA), and fluorescent Big Dye terminators (Applied Biosystems, Foster City, California, USA). The products were resolved by electrophoresis using an ABI 377A automated DNA sequencer (Applied Biosystems, Foster City, California, USA). Simultaneous consideration of both DNA strands across the entire ITS region permitted unambiguous base determination. The sequences were assembled and edited using SeqMan II vers. 4.0 (Dnastar, Madison, Wisconsin, USA). All newly obtained ITS sequences have been deposited in GenBank as contiguous ITS 1, 5.8S, and ITS 2 data ("Appendix").

Sequence and phylogenetic analyses

The DNA sequences were aligned using CLUSTAL X (Jeanmougin et al. 1998), with default parameters for gap penalty and extension. The resulting matrix was edited using GeneDoc vers. 2.6.002 (Nicholas and Nicholas 1997) and adjusted manually. As in previously published studies, our initial analysis placed the accessions of *Ferula* into two disparate clades: tribe Scandiceae and the apioid superclade, the latter representing a large clade of umbellifers containing several tribes and many genera of uncertain relationship (Plunkett and Downie 1999). Resolution of relationships within Scandiceae, however, was low because of the exclusion of many potentially informative positions resulting from alignment ambiguities in outgroup accessions. Therefore, in this study, we report on the results of two separate analyses: (1) a broad analysis, encompassing a representative selection of major lineages of subfamily Apioideae and some members of the two distant clades of *Ferula*; and (2) an analysis of tribe Scandiceae, encompassing representative members of all of its subtribes and all accessions of *Ferula* that were placed in the tribe in our initial analysis (Table 2).

Table 2 Sequence characteristics of the two data matrices used in this study

Sequence characteristic	Apioideae	Scandiceae
Number of terminal taxa	49	76
Number of total aligned sites	643	636
Number of sites parsimony informative	298	227
Number of sites autapomorphic	66	105
Number of sites constant	248	304
Number of sites ambiguous	31	0

To root the phylogenetic trees in the broad analysis, we used *Physospermum cornubiense*, a member of tribe Pleurospermeae, because sequences of early branching apioid taxa (i.e. tribes Heteromorpheae and Bupleureae) cannot be unambiguously aligned with those of the members of the crown clades (Downie et al. 2000b). For the analysis of Scandiceae, outgroups comprised representatives of tribes Smyrnieae (*Smyrnium olusatrum* and *Lecokia cretica*) and Aciphylleae (*Aciphylla squarrosa* and *Anisotome filifolia*) that collectively comprised a sister group to tribe Scandiceae in previous molecular studies (Downie et al. 2000a). Data matrices have been deposited with TreeBase (study accession number S2022).

Only the alignment in the broad phylogenetic analysis presented regions of ambiguity. These regions represented 5% of the matrix and were excluded from subsequent analyses. We also excluded the 5.8S rDNA region from the model-based analyses, because these sequences were not available for several accessions obtained from GenBank. Phylogenetic analyses included neighbor-joining (NJ) and maximum parsimony (MP) methods implemented using PAUP* vers. 4.0b10 (Swofford 1998) and Bayesian inference implemented using MrBayes vers. 3.1 (Ronquist and Huelsenbeck 2003). NJ analyses were performed using several substitution models, including Jukes-Cantor, Kimura 2-parameter, and Kimura 3-parameter. MP analysis was carried out with gap states treated as missing data, characters unordered, and all character transformations equally weighted. Because the number of shortest trees in preliminary MP searches exceeded 100,000, we employed the "inverse constraint" approach of Catalán et al. (1997). One thousand heuristic searches were initiated with random stepwise addition of taxa, tree-bisection-reconnection (TBR) branch swapping, and saving multiple trees (MulTrees), with no more than 1,000 shortest trees saved per replicate. The strict consensus of these trees was used as a phylogenetic constraint in another round of 10,000 searches. This time, only those trees that did not match the constraint tree were saved. Since all of the saved trees were longer than those obtained from the initial searches, this suggests strongly that the strict consensus tree summarizes all possible shortest tree topologies. Bootstrap support (Felsenstein 1985) was estimated using 1,000 resampled data sets, with each bootstrap replicate subjected to 10 heuristic searches with random addition of sequences, TBR branch swapping, MulTrees option in effect, and saving no more than 100 trees per search. The substitution model for the Bayesian analysis was selected using MrModeltest vers. 2.2 (Nylander 2004). This analysis was carried out for 4,000,000 generations with four Monte Carlo Markov chains initiated and a sampling frequency of 100 generations. The initial 10,000 saved trees were discarded and the consensus and posterior probabilities (PP) of particular clades were calculated based on the remaining trees.

Analyses of phenotypic characters

To assess the utility of phenotypic characters for delimiting monophyletic groups in *Ferula*, we used two approaches. First, we mapped the fruit types described by Safina and Pimenov (1983, 1984, 1990) against the strict consensus tree inferred through MP analysis of molecular data. Second, we analyzed the morphological, life history, and phytochemical characters considered by Pimenov et al. (1978) in their phenetic study using MP. Molecular data were available for 61 of the 90 species of *Ferula* studied by these authors. We performed additional MP analyses for a molecular matrix representing only those 61 species and used one of the resultant trees to reconstruct the evolution of these phenotypic characters using Mesquite vers. 2.01 (Maddison and Maddison 2007).

Results

Sequence analysis

The taxa within each of the following eight groups of accessions had identical ITS sequences. As such, each group was represented by a single terminal in the phylogenetic analyses: (1) both accessions of *F. oopoda*, (2) *F. communis* 2 and *F. linkii*, (3) *F. glauca* and *F. neapolitana*, (4) *F. lehmanii* and *F. litwinowiana*, (5) *F. gummosa*, *F. badrakema*, and *F. undulata*, subsequently denoted as "*Ferula* spp. 1", (6) *F. songarica* and *F. tatarica*, (7) *F. foetida*, *F. foetidissima*, *F. kelifi*, *F. nevskii*, both accessions of *F. samarkandica*, *F. tadshikorum*, and *F. teterrima*, subsequently denoted as "*Ferula* spp. 2", and (8) *F. ceratophylla*, *F. dissecta*, *F. dshizakensis*, *F. gypsacea*, *F. karatavica*, *F. karataviensis*, *F. kyzylkumica*, *F. lapidosa*, *F. lipskyi*, all four accessions of *F. ovina*, *F. pallida*, *F. prangifolia*, *F. tschimganica*, *F. ugamica*, and *F. xeromorpha*, subsequently denoted as "*Ferula* spp. 3."

Molecular phylogenetic analyses

For the data matrix encompassing a broad sampling of taxa from subfamily Apioideae, MrModeltest with the Akaike Information Criterion selected the GTR + G + I model of nucleotide substitution as fitting these ITS data best. The Bayesian majority-rule consensus tree is presented in Fig. 1. MP searches resulted in 36 shortest trees of 1,383 steps each, consisting of two islands of 6 and 30 trees, respectively (consistency index, CI, with uninformative characters of 0.449, and retention index, RI, of 0.658).

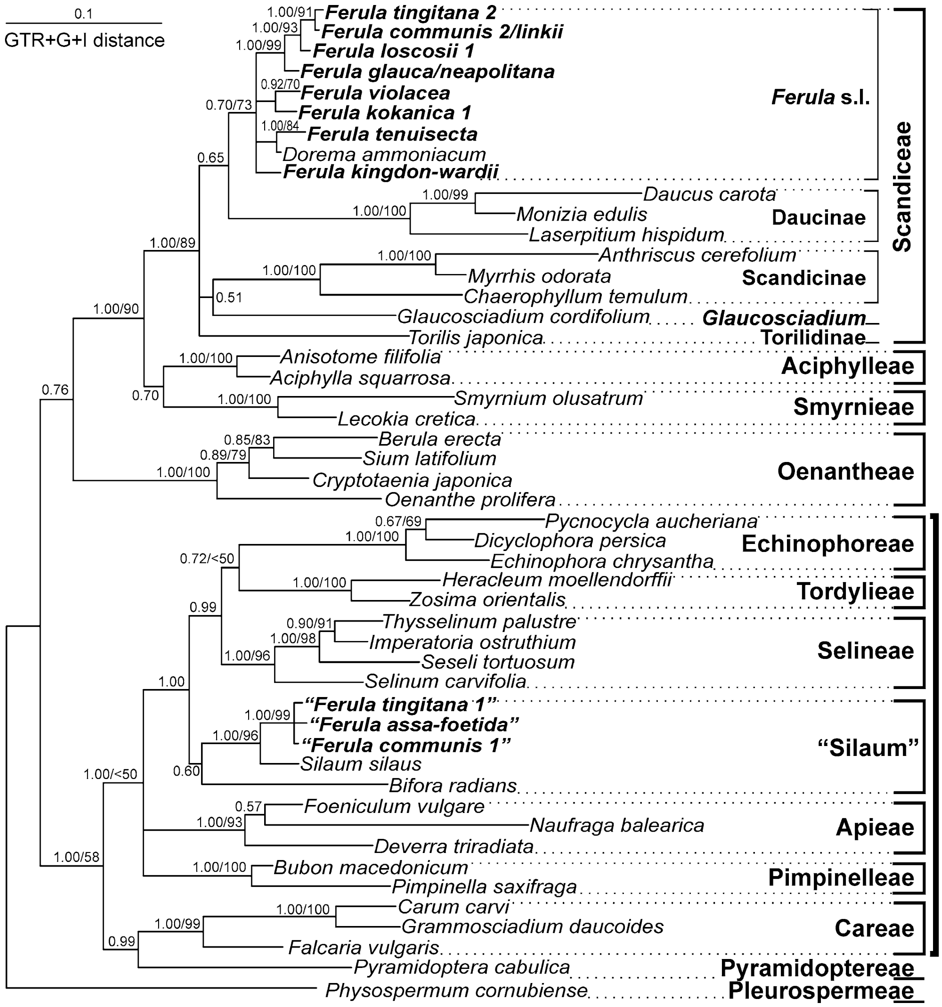


Fig. 1 Majority-rule consensus tree obtained from Bayesian analysis of 49 terminals representing *Ferula* (*boldfaced*) and most major clades of Apiaceae subfamily Apioideae using a GTR + G + I nucleotide substitution model. Posterior probabilities (PP) are given along branches. For those clades that were supported in the strict consensus of 36 minimal length 1,383-step trees obtained from maximum parsimony searches, bootstrap values (BS) are also indicated. *Numbers* following species names refer to those accessions identified numerically in the "Appendix." Accessions of *Ferula* presumed to be misidentifications are enclosed in *quotation marks*

Bootstrap values for the clades revealed in the strict consensus of these trees (not shown) are presented on the Bayesian inference tree. The major clades evident in the Bayesian and MP trees, as well as those of the NJ trees (not shown), are the same as those obtained in previous molecular systematic studies. Twelve tribes of subfamily Apioideae are identified, including tribe Scandiceae and its three subtribes: Daucinae, Scandicinae, and Torilidinae (Fig. 1). The members of *Ferula* occur in two distantly related clades. The first is denoted as "*Silaum*" and includes *F. assa-foetida*, *F. communis* 1, *F. tingitana* 1, and *Silaum silaus*. This clade received strong support from both Bayesian and MP bootstrap analyses (PP = 1.00, BS = 96%). The remaining members of *Ferula*, including additional representation of *F. communis* and *F. tingitana*, allied with representatives of tribe Scandiceae. Within Scandiceae, five major lineages are apparent, three of which represent previously recognized subtribes Daucinae, Scandicinae, and Torilidinae. The species of *Ferula* plus *Dorema ammoniacum*, the nomenclatural type of the genus *Dorema* D. Don, form a fourth clade. This clade coincides with the "*Ferula* subclade" of tribe Scandiceae in Downie et al. (2000c). Hereafter, with the addition of *Dorema* (and, see below, *Leutea*), we denote this clade as *Ferula* sensu lato (s.l.). Bayesian posterior probability and MP bootstrap support for this clade were moderate (0.70 and 73%, respectively). The fifth lineage constitutes a single species, *Glaucosciadium cordifolium*. In the Bayesian tree, the *Ferula* s.l. clade is sister group to subtribe Daucinae and *G. cordifolium* is sister group to subtribe Scandicinae; the posterior probabilities supporting these relationships, however, are low. In the MP strict consensus tree (not shown), these five lineages of Scandiceae form a polytomy.

For analyses of the Scandiceae matrix, MrModeltest with the Akaike Information Criterion selected the GTR + G model of nucleotide substitution. The Bayesian majority-rule consensus tree is presented in Fig. 2. MP searches of these data resulted in more than 900,000 shortest trees of 930 steps each (CI = 0.534, RI = 0.647). The strict consensus of these trees had a topology similar to that obtained from Bayesian searches and its branch containing the species of *Ferula* (*Ferula* s.l. clade) is presented

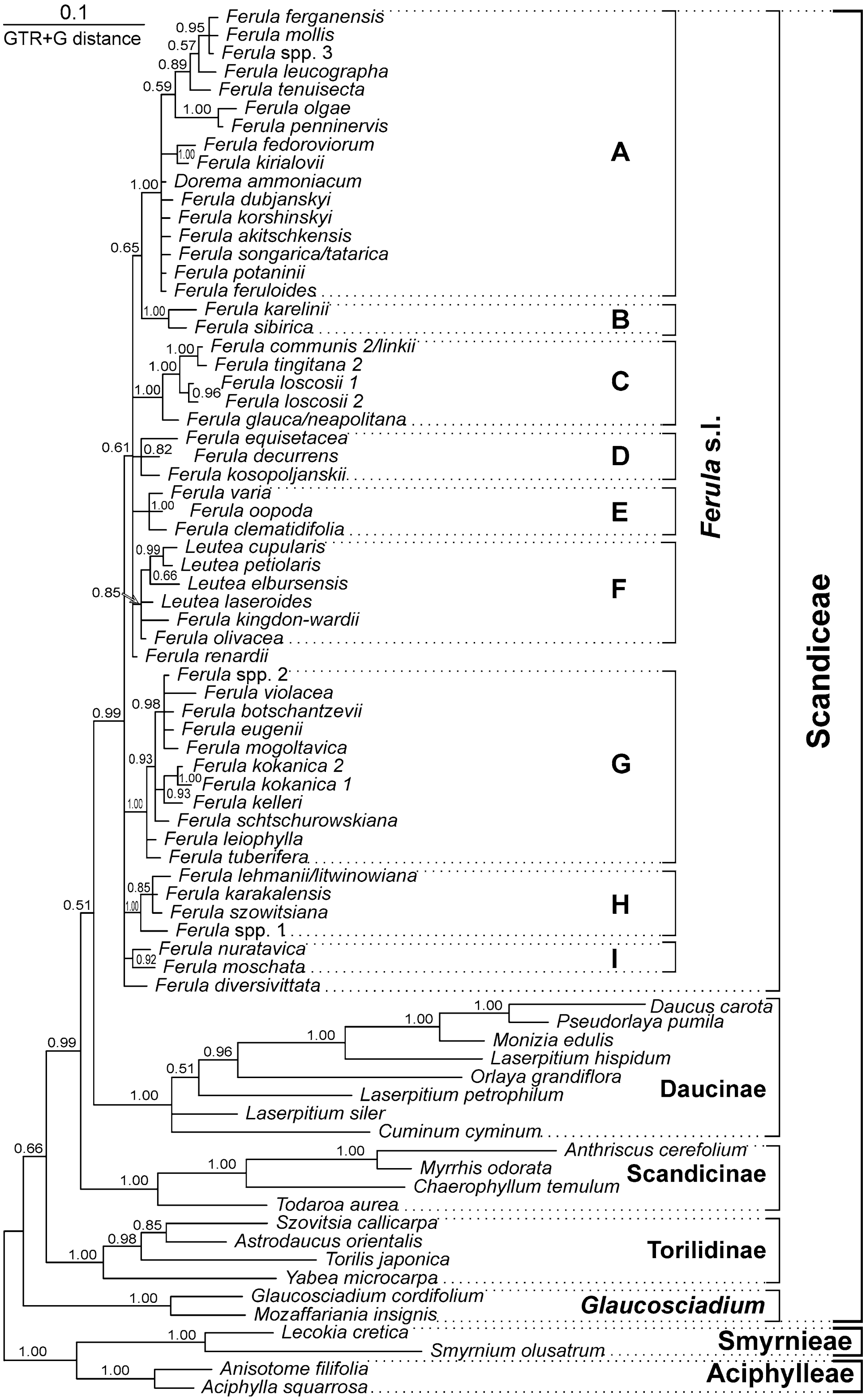
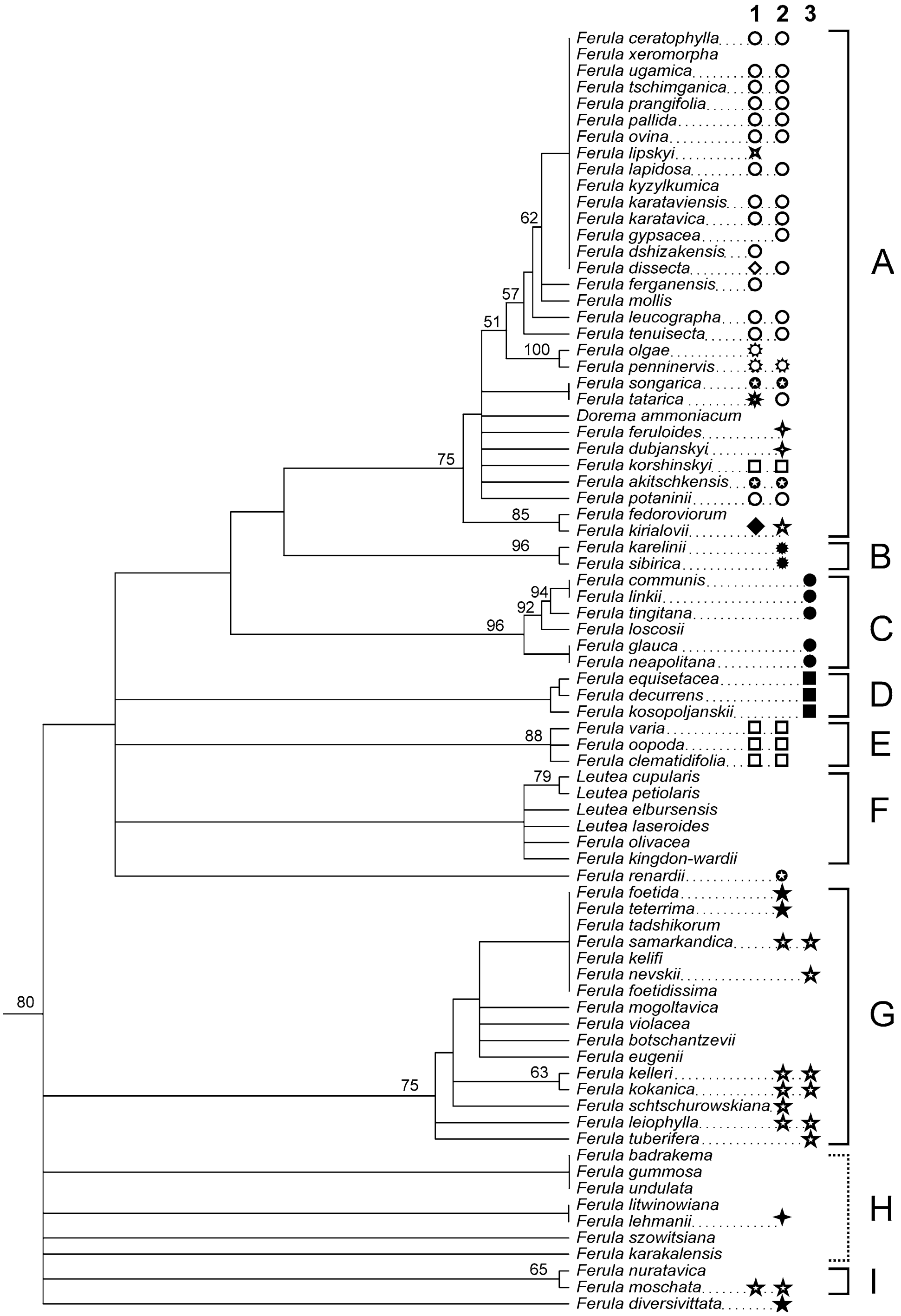


Fig. 2 Majority-rule consensus tree obtained from Bayesian analysis of 76 terminals representing *Ferula* s.l., major clades of tribe Scandiceae, and outgroups using a GTR + G substitution model. Posterior probabilities (PP) are given along branches. Terminals representing several accessions and denoted as *Ferula* spp. 1, 2, and 3, and *clades A–I* are described in the text

in Fig. 3. This branch is expanded to show all accessions of *Ferula* examined herein, including those which were combined into single terminals because of identical ITS sequences and excluding those of the "*Silaum*" clade. The topologies of the NJ trees inferred using various models of nucleotide substitutions were generally similar to one another and to the MP and Bayesian trees. In all phylogenetic analyses, the same five clades are apparent within Scandiceae, corresponding to subtribes Daucinae, Scandicinae, and Torilidinae, and the two newly identified

Fig. 3 *Ferula* s.l. clade from strict consensus of 900,000 minimal length 930-step trees obtained from MP searches. Bootstrap values are indicated along branches; values <50% are not indicated. All taxa included in the analyses are listed, with *vertical bars* joining accessions having identical ITS sequences. *Symbols* denote different fruit types recognized by Safina and Pimenov: (**1**) 1983, (**2**) 1984, and (**3**) 1990. *Groups A–I* are the same as in Fig. 2. *Group H* was inferred as monophyletic in the Bayesian analysis, but is not supported as monophyletic in the MP strict consensus tree



lineages, *Ferula* s.l. and *Glaucosciadium* B. L. Burtt et P. H. Davis, the latter also including *Mozaffariania insignis*, an endemic of Iran. All members of *Ferula* included in this matrix were grouped within *Ferula* s.l., with this clade receiving high PP (0.99) and moderate BS (80%) support. In Bayesian analyses, the *Ferula* s.l. clade is sister group to subtribe Daucinae, albeit with weak support (PP = 0.51), whereas in the MP strict consensus tree it formed a polytomy with subtribes Daucinae, Scandicinae, and Torilidinae (not shown). Within the *Ferula* s.l. clade, nine groups are identified (Figs. 2 and 3; groups A–I); with the exception of group H in the MP strict consensus tree, all groups are monophyletic. Group A encompasses 16 terminals (Fig. 2), including *Dorema ammoniacum* and *Ferula* spp. 3, the latter comprising 15 central Asiatic species having identical ITS sequences (Fig. 3). Group A allies weakly with group B (which includes only two species, *F. karelinii* and *F. sibirica*). In the MP and NJ trees, this assemblage is a weakly supported sister group to group C, a clade encompassing all Mediterranean congeners, including the type of the genus, *F. communis*. The members of group C are morphologically similar to one another. An endemic of the Canary Islands, *F. linkii*, has an ITS sequence identical to that of *F. communis.* Group C received high support from Bayesian ($PP = 1.00$) and bootstrap (96%) analyses. Clade D includes three endemics of central Asia: *F. equisetacea*, *F. decurrens*, and *F. kosopoljanskii*. Support for this clade, however, is low ($PP = 0.82$, $BP < 50\%$). Group E encompasses three Asiatic species, *F. varia*, *F. oopoda*, and *F. clematidifolia*, in a well-supported clade. Two Chinese species (*F. olivacea* and *F. kingdon-wardii*) and four species of *Leutea* Pimenov comprise group F. In the NJ trees, *F. renardii* is sister group to group F; in the Bayesian and MP trees, *F. renardii* comprises a solitary branch, and in all trees this species is not assigned to a group. Groups A–F form a weakly supported clade ($PP = 0.61$, $BS < 50\%$). Clade G encompasses 11 terminals (Fig. 2), including *Ferula* spp. 2 that is made up of seven central Asiatic species having identical ITS sequences (Fig. 3). Among its members are two broadly distributed species, *F. foetida*, a major source of asafoetida resin, and *F. kokanica*. Group H includes four terminals, with *Ferula* spp. 1 accounting for three species, including *F. gummosa*, a source of galbanum resin. Group I comprises two species, including *F. moschata* from which the resin sumbul is obtained. In both MP and Bayesian analyses, *F. diversivittata* also forms an isolated branch; in the NJ trees, *F. diversvittata* is a sister group to clade I. Although posterior probabilities for clades G–I are high (0.94–1.00), MP BS values for clades G and I are not (75 and 65%, respectively), and group H is not monophyletic in the MP strict consensus tree. Based on these results, the *Ferula* s.l. clade is expanded to include not only all examined accessions of *Ferula* falling within tribe Scandiceae, but also the nomenclatural types of the genera *Dorema* (*D. ammoniacum*) and *Leutea* (*L. petiolaris*).

Evolution of phenotypic characters

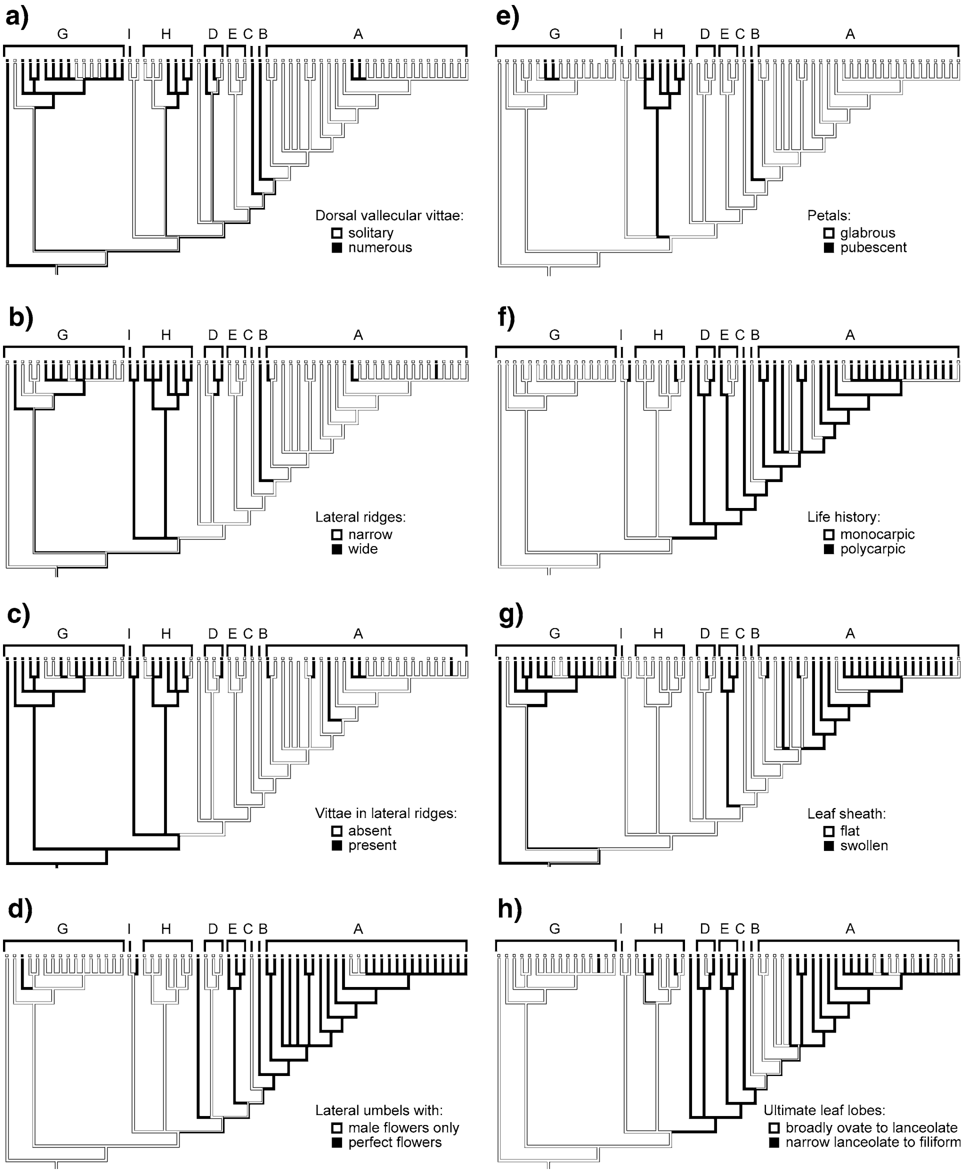
In Fig. 3, the fruit types of *Ferula* recognized by Safina and Pimenov (in 1983, 1984, and 1990, respectively) on the basis of morphology and anatomy are mapped against a branch of the strict consensus tree inferred from the MP analysis. These fruit types were defined using multiple characters and an overall general similarity of the fruits rather than by any single diagnostic or phylogenetic character. Moreover, these fruit types were neither unambiguously delimited by these authors nor were they consistently named throughout their studies. Nevertheless, we identify these fruit types by assigning each one a unique symbol. Despite this imprecise typology, several of the clades obtained from molecular analyses are well defined by fruit characters. These include groups B, C, and D, which each have a unique fruit type. The fruit type characteristic for group E occurs also in *F. korshinskyi* in group A. Clade G includes two types of fruits that also occur in other groups. Clade A is most heterogeneous with respect to fruit anatomy. The species of groups F, H, and I were poorly or not sampled by Safina and Pimenov.

Of the 33 morphological, life history, and phytochemical characters examined by Pimenov et al. (1978), six were phylogenetically uninformative (constant or autapomorphic) for the subset of 61 species for which we had molecular data. MP analyses of these phenotypic characters resulted in more than 100,000 shortest trees, and the strict consensus of these trees yielded an almost complete polytomy. In contrast, MP analyses of their respective molecular data resulted in 72 shortest trees. These trees required 245–250 steps to account for the variation in these phenotypic characters. One of the three 245-step trees was arbitrarily chosen for subsequent analyses. This tree was generally congruent to those obtained from previous analyses, with the exception of the position of *Ferula tuberifera*, which formed a sister group to its congeners, thereby making group G paraphyletic with respect to all other groups. Members of group F were not studied by Safina and Pimenov. *Ferula renardii* and *F. diversivittata* are positioned between groups D and H and sister group to group I, respectively. This tree is presented in Figs. 4 and 5 and used to reconstruct the evolution of several phenotypic characters.

All 27 informative phenotypic characters were homoplastic including those which have been previously considered as taxonomically important, such as presence of pubescence on ovary/fruit, number of dorsal vallecular vittae (Fig. 4a), width of lateral ridges (Fig. 4b), presence of vittae in lateral ridges (Fig. 4c), and structure of the hypoendocarp. Moreover, fruit characters are often variable not only among but also within major clades, as well as between closely related species. For instance, solitary dorsal vallecular vittae are characteristic but not exclusive for clade A, because two of its members have numerous dorsal vallecular vittae, whereas many members of the other clades have solitary vittae (Fig. 4a). All but three species from clade A have fruits with narrow lateral ridges (Fig. 4b). Vittae in lateral ridges were lost in the common ancestor of clades A–E and *Ferula renardii* (the terminal between clades D and H); however, this character state was independently gained in several other lineages, whereas in clades A and D some reversions occurred (Fig. 4c).

Lateral umbels composed of only male flowers are plesiomorphic for the genus (Fig. 4d). Perfect flowers occurring in these umbels are apomorphic and this character state may have been gained by the common ancestor

Fig. 4 Reconstruction of the evolution of selected characters of fruits (**a**–**c**), flowers (**d**, **e**), life history (**f**), and leaves (**g**, **h**) in the *Ferula* s.l. clade on one of 72 shortest trees obtained from MP analysis of ITS sequences for 61 species of *Ferula* chosen to minimize the number of steps in the examined phenotypic characters (see text for further explanation). *Groups A*–*I* are the same as in Figs. 2 and 3. The terminal sister group to a representative of *group I* is *F. diversivittata*, whereas the terminal between *groups D* and *H* is *F. renardii*. Phenotypic data are from Pimenov et al. (1978)



of clades A–E and *Ferula renardii* and then lost in lineages C and D and within clade A. Pubescent petals are characteristic for clade H, albeit one of its members subsequently lost this character while other lineages in other groups acquired it independently (Fig. 4e). Monocarpy is the ancestral condition in *Ferula* and polycarpy was acquired by the common ancestor of clades A–E and *Ferula renardii*; subsequently, reversals occurred in some lineages (Fig. 4f). Leaf characters were highly variable in the genus (Fig. 4g, h).

Phytochemical characters were somewhat less variable than the aforementioned morphological and life history characters, although their utility for delimiting monophyletic groups was generally poor (Fig. 5). Garlic smell indicating the presence of disulphides was common, albeit homoplastic, in clades G and H (Fig. 5a). The presence of terpenoid coumarins is plesiomorphic for *Ferula* (Fig. 5b), whereas sesquiterpene lactones (Fig. 5c) or compound volatile aromatic acids and terpenoid alcohols (Fig. 5d) were characteristic of the more derived members of the genus.

Discussion

Phylogenetic position of *Ferula*

The affinity of some members of *Ferula* with tribe Scandiceae and the polyphyly of the genus were inferred in earlier phylogenetic studies based on both chloroplast DNA (Downie et al. 2000b) and nuclear rDNA ITS (e.g., Downie et al. 2000c) sequences. In these analyses, three species—

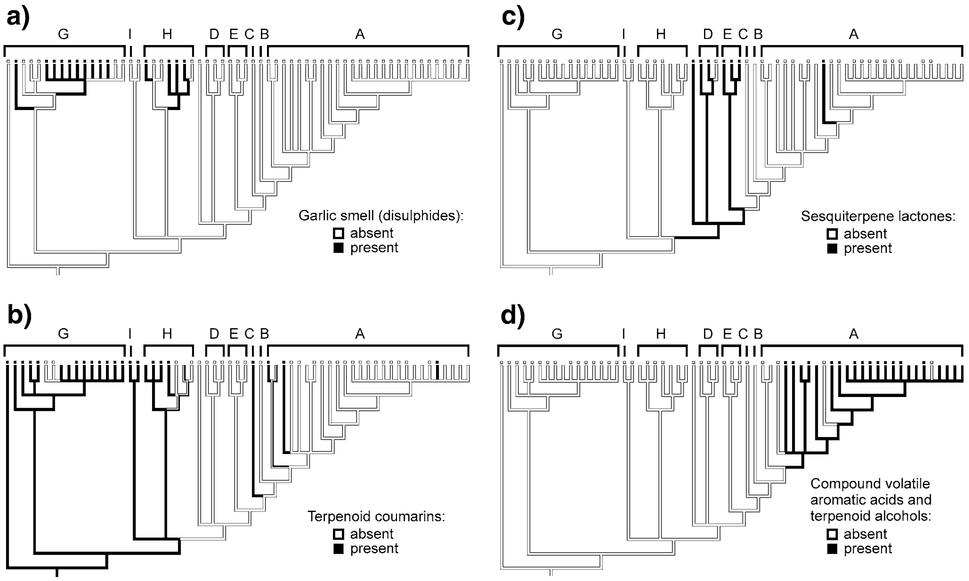


Fig. 5 Reconstruction of the evolution of phytochemical characters in the *Ferula* s.l. clade. Tree, terminals, and source of data are the same as described in Fig. 4

F. communis, *F. tingitana*, and *F. assa-foetida*—formed a clade within the apioid superclade, whereas their congeners were placed in tribe Scandiceae. The three species of the apioid superclade were allied to "*Thysselinum palustre*" and *Cenolophium denudatum* (Downie et al. 2000c). In our study, these three *Ferula* species comprise a strongly supported clade sister group to *Silaum silaus*. The newly sequenced accessions of *F. communis* and *F. tingitana*, as well as those of the closely related species *F. glauca*, *F. linkii*, *F. loscosii*, and *F. neapolitana* form a clade with all remaining congeners in tribe Scandiceae. These results strongly support the monophyly of *Ferula* s.l. (with *Dorema* and *Leutea* nested within) and suggest that these three anomalous accessions allying within the apioid superclade represent misidentifications.

The plant material previously identified as *F. communis*, *F. tingitana*, and *F. assa-foetida* used by Downie et al. (2000c) came from botanic gardens. Their seeds and the voucher specimens, while largely sterile, look similar to one another and to *Silaum silaus* and differ from the true members of *Ferula*. In our analyses, *S. silaus* is a sister species to these three disputed accessions (Fig. 1). Jean-Pierre Reduron (Mulhouse, France, personal communication) after examining some botanic garden accessions has concluded that the species commonly determined as *F. assa-foetida* and widely distributed by seed exchange is actually the Asiatic umbellifer *Silaus besseri* DC.

In this study, we did not include any new material of *F. assa-foetida* because of the uncertainty concerning this taxon. In Korovin's (1947) monograph of *Ferula* and his subsequent taxonomic revisions of the genus (Korovin 1951, 1959), this species was regarded as synonymous to *F. foetida*. However, Chamberlain and Rechinger (1987) argued that Linnaean *F. assa-foetida* is a different species that is restricted to Iran, and such a treatment was also adopted by Pimenov and Kljuykov (2002). As we did not have access to any material that could be unambiguously identified as *F. assa-foetida* sensu Chamberlain and Rechinger (1987), the phylogenetic position of this species remains uncertain. Chamberlain and Rechinger (1987) suggested that *F. assa-foetida* is closely related to *F. pseudalliacea* Rech. f., another endemic of Iran but of unknown phylogenetic position. They refer both species to Korovin's subgenus *Merwia*, retaining *F. foetida* in subgenus *Scorodosma*. The diagnosis of *F. assa-foetida* provided by these authors was based on two specimens only, including a rather incomplete type specimen in the BM-SLOANE herbarium, and this description does not allow for an unambiguous placement of this species. More material, particularly from Iran, is necessary to resolve the identity and phylogenetic affinity of *F. assa-foetida*.

Major clades within tribe Scandiceae

The results of our study clearly indicate that tribe Scandiceae comprises five major lineages, with three of them constituting subtribes Daucinae, Scandicinae, and Torilidinae. The monophyly of each of these subtribes is supported by both molecular and morphological data (Downie et al. 2000a; Spalik and Downie 2001; Spalik et al. 2001a, b). Subtribe Daucinae as currently recognized includes several members of the former tribe Caucalideae and all hitherto analyzed representatives of the former tribe Laserpitieae, whereas most remaining members of Caucalideae have been transferred to Torilidinae (Lee and Downie 1999, 2000; Lee et al. 2001). The two other major lineages within Scandiceae include *Ferula* s.l. and the *Glaucosciadium* clade, the latter including the monotypic genera *Glaucosciadium* and *Mozaffariania* Pimenov et Maassoumi.

These taxa were formerly recognized in tribe Peucedaneae (Pimenov and Leonov 1993; Pimenov and Maassoumi 2002). As traditionally delimited, tribe Peucedaneae encompasses taxa with dorsally flattened winged fruits, such as those found in *Ferula* and members of the *Glaucosciadium* clade. Flattened winged fruits are regarded as adapted to wind dispersal and experimental studies have shown that such fruits are dispersed farther than those without wings (i.e., barochoric fruits). This difference, however, is not substantial (Jongejans and Telenius 2001). Wind-dispersed fruits also occur in those members of Daucinae that were formerly recognized in tribe Laserpitieae. However, in *Ferula*, *Glaucosciadium*, and *Mozaffariania*, wings are formed on lateral primary ridges, whereas in former Laserpitieae they occur on secondary ridges. Anemochory is, therefore, homoplastic in Scandiceae.

In the broad phylogenetic analysis, encompassing a representative selection of major lineages of subfamily Apioideae, the relationships among the five lineages of Scandiceae are unresolved in the MP strict consensus tree or poorly supported in the Bayesian and NJ trees. In the analysis of Scandiceae, the *Ferula* s.l. clade is a weakly supported sister group to subtribe Daucinae. Based on morphology, Daucinae are regarded as closely related to Torilidinae, because both groups share the presence of prominent secondary ridges (Lee et al. 2001); indeed, those taxa characterized by spiny secondary ridges were once united in tribe Caucalideae (Heywood and Dakshini 1971). Although all previous molecular analyses have confirmed the placement of subtribes Daucinae and Torilidinae in tribe Scandiceae, these taxa are not sister groups, therefore rejecting the hypothesis on the apomorphic status of secondary ridges. The presumed sister group relationship between Daucinae and the *Ferula* s.l. clade is not supported by any obvious morphological synapomorphy. Therefore, instead of including the *Ferula* s.l. clade into Daucinae, we recognize it formally as subtribe Ferulinae Engl. within the tribe Scandiceae.

Glaucosciadium and *Mozaffariania*

The taxonomic affinities of *Glaucosciadium* and *Mozaffariania* are enigmatic. Both genera are monotypic and occur in the Middle East. *Glaucosciadium cordifolium* extends from Cyprus through southern Turkey to Lebanon (Hedge and Lamond 1972), whereas *Mozaffariania insignis* was described from the south-western Iranian province of Khuzestan (Pimenov and Maassoumi 2002). In the protologue of *Glaucosciadium*, Burtt and Davis (1949) failed to indicate any precise affinity of this taxon but placed it close to *Peucedanum* s.l. When describing *Mozaffariania*, Pimenov and Maassoumi (2002) regarded it as belonging to traditionally circumscribed tribes Tordylieae or Peucedaneae, but did not discuss *Glaucosciadium* as a possible relative. Apart from their geographic proximity, the two species are very similar morphologically. Both are erect, glaucous perennials with 1–2-pinnate lower leaves having broad ovate-orbicular coriaceous segments. Their upper cauline leaves are sheath-like. Their fruits are dorsally flattened with a plane commissural endosperm face. In the protologue of *Glaucosciadium*, Burtt and Davis (1949) stressed the absence of commissural vittae, whereas Pimenov and Maassoumi (2002) noted two commissural vittae in *Mozaffariania*. However, the arrangement of vittae as shown in fruit transverse sections of these two species is actually identical and the difference is purely terminological. Burtt and Davis (1949) do not consider two vittae situated in lateral ridges as commissural, whereas Pimenov and Maassoumi (2002) do.

Glaucosciadium cordifolium and *Mozaffariania insignis* differ in several characters of bracts, flowers, and fruits. The former is characterized by deciduous scale-like bracts, obsolete calyx teeth, and thin mericarp wings (Burtt and Davis 1949; Hedge and Lamond 1972), whereas in the latter the bracts are numerous and linear, the calyx teeth are small, and the mericarp wings are spongy (Pimenov and Maassoumi 2002). These differences are minor and comparable to those occurring among congeners rather than separate genera. For instance, the differences among species of *Ferula* exceed those between *Glaucosciadium* and *Mozaffariania*.

The number of genera within the Apiaceae is much inflated by the inclusion of many small or monotypic taxa (Spalik et al. 2001a). Of the 455 genera listed by Pimenov and Leonov (1993), 41% are monotypic and 26% comprise only two or three species each. As a result, 67% of all genera account for only 13% of all species. Spalik et al. (2001a) postulated that monotypic genera should only be recognized if they represent isolated lineages with no close relatives (e.g., if their sister group represents a clade of several genera) or if their inclusion into their sister taxa would much worsen the definition of the latter. The two aforementioned genera do not represent such a case. Having considered the striking morphological similarity between *Glaucosciadium* and *Mozaffariania* and their strongly supported sister group relationship as inferred from molecular data, we argue that only a single genus, *Glaucosciadium*, with two included species, should be maintained. Therefore, a new combination is necessary:

Glaucosciadium insigne (Pimenov et Maassoumi) Spalik et S.R. Downie, comb. nov.

Basionym: *Mozaffariania insignis* Pimenov et Maassoumi, Bot. Z. 87:97–100 (2002)

Dorema, *Leutea*, and segregates of *Ferula*

Traditionally, genera are delimited on the basis of morphological distance rather than phylogeny. However, morphological differences between two groups of closely related species—putative genera—do not necessarily imply that they constitute sister lineages but rather that one is nested within the other, with the former being defined based on synapomorphies and the latter on symplesiomorphies. *Dorema*, *Leutea*, and several segregates of *Ferula* illustrate this taxonomic practice well.

Like *Ferula* and *Glaucosciadium*, the genera *Dorema* and *Leutea* were traditionally placed in tribe Peucedaneae (Pimenov and Leonov 1993). Drude (1897–1898) included *Dorema* alongside *Ferula* and *Peucedanum* in his subtribe Ferulinae, and in *Peucedanum* he recognized several species that were subsequently transferred to *Leutea*, including *L. cupularis* and *L. petiolaris* considered in this study. *Dorema* includes about 12–16 species distributed in southwestern and central Asia and is regarded as closely related to *Ferula* (Rechinger 1987; Pimenov 1988). In our study, we included only the nomenclatural type of the genus, *D. ammoniacum*, and this species fell as part of a large polytomy within group A of *Ferula*. Ongoing studies of Iranian *Dorema* support this finding, for results of MP analysis of ITS sequences from five additional species of *Dorema*, including *D. aucheri*, place all species as basally branching lineages within group A (Ajani et al. 2008). These results are in contrast to those reported by Valiejo-Roman et al. (2006), where a single accession of *D. aucheri* allied as sister group to *Seseli mucronatum* and close to *Peucedanum* in tribe Selineae. As both molecular and morphological studies of *Dorema* are underway, we refrain from further discussion of the taxonomic position of this genus. It is clear, however, that *Dorema* will eventually have to be transferred into *Ferula*.

Leutea was described by Pimenov (1987) as a segregate of *Peucedanum* sect. *Juncea*. However, in the diagnosis, he placed *Leutea* closer to *Ferula* than to *Peucedanum*. The nomenclatural type of the genus, *L. petiolaris*, was originally described in *Ferula* as *F. petiolaris* DC. The genus *Leutea* is distinguished from other members of Peucedaneae by its mostly radical leaves with rigid cylindrical ultimate segments, and it differs from *Ferula* in having only hermaphroditic flowers (Pimenov 1987). Characters of leaves and flowers are variable among umbellifers and are of limited taxonomic value (Spalik and Downie 2001). In the case of *Leutea*, these characters seem to delimit a small and relatively uniform phylogenetic lineage rather than mark a major split within *Ferula* s.l. Therefore, retaining *Leutea* as a distinct genus is not justifiable and we recognize it as a taxonomic synonym of *Ferula*. The following combinations are necessary:

Ferula cupularis (Boiss.) Spalik et S. R. Downie, comb. nov.

Basionym: *Peucedanum cupulare* Boiss., Diagn. pl. or. nov. ser. 1, 6: 63. 1846

Ferula elbursensis (Mozaff.) Spalik et S. R. Downie, comb. nov.

Basionym: *Leutea elbursensis* Mozaff., Bot. Z. 88(4): 106. 2003

Ferula laseroides (Akhani) Spalik et S. R. Downie, comb. nov.

Basionym: *Leutea laseroides* Akhani Illus. Fl. Golestan Natl. Park 1: 341. 2005

We refrain from transferring the remaining members of *Leutea* to *Ferula* until their taxonomic positions are verified using molecular data.

Several segregates of *Ferula* have also been recognized. *Ferula sibirica*, included in this study (Group B, Figs. 2, 3), is sometimes placed in the monotypic genus *Soranthus* Ledeb. *Soranthus* is maintained in the floras of the Soviet Union (Korovin 1951) and China (She et al. 2005), but sunk in *Ferula* in the revision of this genus in Kazakhstan (Safina and Pimenov 1984). In our study, the sister group to *F. sibirica* is *F. karelinii*, which is often placed in the monotypic genus *Schumannia* Kuntze. Like *Soranthus*, *Schumannia* is maintained by Korovin (1951) and She et al. (2005), but sunk in *Ferula* by Safina and Pimenov (1984). *Ferula renardii* is often placed in *Talassia* Korovin along with *F. transiliensis* (the latter not included in this study). The genus *Talassia* is recognized by Korovin (1951) and She et al. (2005), but rejected by Safina and Pimenov (1984). In our analyses, *F. renardii* comprises a separate lineage whose sister group is unknown. Based on our results, none of these small segregate genera deserves recognition.

Classification systems of *Ferula* and the taxonomic value of fruit characters

Korovin (1947) based his taxonomic treatment of *Ferula* mostly on morphological characters. While acknowledging Koso-Poljansky's and Calestani's contributions of fruit anatomy to umbellifer taxonomy, he regarded these characters as useless at the infrageneric level. Consequently, his descriptions of subgenera focused primarily on characters of habit, inflorescence, leaves, and flowers, and less so on basic fruit features, such as the direction of compression and the number of vallecular vittae. His sections were defined almost exclusively upon leaf characters. Not surprisingly, none of the groups inferred in our study coincides with those infrageneric taxa recognized by Korovin (Table 1).

An alternative classification system of *Ferula* was proposed by Safina and Pimenov (1984) in their revision of the Kazakhstan members of the genus. With some modification, these authors adopted only the sectional divisions of Korovin. They also underlined the importance of fruit anatomical characters for delimiting infrageneric taxa, and many of their divisions were homogenous with respect to fruit type. Their fruit typology was based on a study of Korovin's *Ferula* subgenus *Peucedanoides* (Safina and Pimenov 1983). However, in a subsequent paper that was devoted to subgenus *Ferula*, Safina and Pimenov (1990) returned to Korovin's subgenera instead of developing their own classification system.

It is important to note that the fruit types recognized by Safina and Pimenov were based on general similarity rather than on a set of unique (and presumably apomorphic) characters. Consequently, the delimitation of these fruit types was often imprecise. For example, the fruits of *F. kirialovii* and *F. moschata* were regarded as belonging to different fruit types (Safina and Pimenov 1983) or, one year later, the same fruit type (Safina and Pimenov 1984). Because the members of *Ferula* occurring in Kazakhstan do not comprise all major clades recognized herein (groups C, D, and F are absent, while group H is represented by a single species), it is difficult to assess whether the classification system proposed by Safina and Pimenov (1984) would be better than that of Korovin (1947). Although this typology was developed intuitively rather than based on formal cladistic analyses, there is good agreement between phylogeny and fruit types, suggesting that fruit anatomy may be a valuable source of information, if not for reconstructing phylogeny then for characterization of clades inferred from molecular data.

Evolution of phenotypic characters

The conclusion that a general similarity of fruit anatomy is a better predictor of species affinity than any single fruit character is supported by the analysis of character evolution. All fruit characters analyzed are highly homoplastic (e.g., Fig. 4). Such a result is not surprising as molecular studies have already shown that morphological characters—and particularly fruit morphology and anatomy that were once regarded as crucial for elucidating evolutionary relationships among umbellifers—are of limited phylogenetic value. Early phylogenetic analyses demonstrated that all hitherto proposed classification systems of Apiaceae are incongruent with a phylogeny of the family (Downie and Katz-Downie 1996, 1999; Downie et al. 1996; Plunkett et al. 1996). At a lower taxonomic level, Spalik and Downie (2001) concluded that morphological and anatomical characters are poor phylogenetic markers for subtribe Scandicinae; interestingly, fruit characters appeared to be least homoplastic among the different vegetative, inflorescence, floral, and fruit characters they considered. Our analysis of the evolution of phenotypic characters in *Ferula* fails to indicate any clear pattern of morphological variation that would be of taxonomic use. Rather, clades may be defined based on sets of most common character states. For instance, members of clade H are usually monocarpic plants with leaves characterized by a flat sheath and ultimate lobes that are mostly broadly ovate to lanceolate. Their lateral umbels comprise male flowers only and petals are usually pubescent. Their fruits have wide lateral ridges that usually contain vittae.

Monocarpic members of *Ferula* usually have male lateral umbels, whereas those umbels of polycarpic species often contain perfect flowers (Fig. 4d, f). Lateral umbels constitute auxiliary inflorescences, as major reproductive output occurs in terminal umbels. One may hypothesize that monocarpic species limit female reproductive effort to early inflorescences, as perfect flowers in late inflorescences have a lower probability of setting fruits and resources saved for maturing those fruits would be therefore wasted. In contrast, polycarpic species may use those left-over resources for increasing survival to the next season or may store them for future reproduction. However, an alternative explanation is that this co-occurrence is the result of common ancestry rather than causal relationship.

Although phytochemistry is generally regarded as providing strong phylogenetic markers, its taxonomic value for Apiaceae has been contentious. Katz-Downie et al. (1999) analyzed the distribution of 13 classes of secondary metabolites against an ITS phylogeny of subfamily Apioideae and concluded that most of these data do not appear to be of any systematic significance. Most classes of coumarins, flavonoids, and phenylpropenes were either widely distributed within the subfamily or scattered among widely separated clades. Although the occurrence of several compounds (flavone aglycones and glycosides, for instance) was limited to certain clades, these characters were homoplastic. Phytochemical characters are also homoplastic within *Ferula*, although a certain interesting evolutionary pattern may be seen (Fig. 5). The ancestral condition seems to be the production of terpenoid coumarins that was subsequently replaced by the ability to synthesize sesquiterpene lactones in the ancestor of clades A–E plus *F. renardii*. The production of compound volatile aromatic acids and terpenoid alcohols was acquired only once within clade A and then lost in three of its lineages.

Biogeography and evolutionary radiation of *Ferula* s.l

The center of endemism of *Ferula* s.l. is Central Asia. In this paper, we adopt a broader definition of Central Asia encompassing not only the former Soviet Middle Asia (Turkmenistan, Tajikistan, Uzbekistan, and Kyrgyzstan), but also Kazakhstan, northeastern Iran, northern Afghanistan and Pakistan, northwestern India, and western China (East Turkmenistan). Floristically, this broad region is coextensive with the central and eastern portion of the Irano-Turanian region. In this area, representatives of nearly all major groups of *Ferula* occur, with the notable exception of group C [distributions were estimated based on Korovin (1947, 1951, 1959); Chamberlain and Rechinger (1987); Mukherjee and Constance (1993); Pimenov and Kljuykov (2002)]. Group C, including *F. communis* (the nomenclatural type of the genus), is distributed in the Mediterranean, reaching as far as the Canary Islands to the west (*F. linkii*) and Turkey and Israel to the east (*F. communis*). The Canarian endemic species *F. linkii* has an ITS sequence identical to the continental *F. communis*, indicating the recent origin of the former. In contrast, the Iberian *F. loscosii* comprises a separate lineage sister group to the clade of *F. communis*, *F. linkii*, and *F. tingitana* that suggests its origin through isolation in the Iberian Peninsula, one of the three most important glacial refugia in Europe (Willis 1996). As group C arises within the Central Asian lineages, a Mediterranean distribution is a derived rather than ancestral condition in *Ferula*.

Almost every group of species identified in *Ferula* s.l. includes both narrow endemics and species broadly distributed in the Irano-Turanian floristic region and often extending beyond this region to adjacent areas. These broadly distributed species include *F. ovina* (group A); *F. foetida*, *F. kokanica*, and *F. schtschurowskiana* (group G); *F. lehmannii* (group H); and *F. diversivittata*. However, upon the exclusion of these widely distributed species, there are apparent differences in the geographic distributions among members of these groups. Most members of group A are narrow endemics of Uzbekistan, Kazakhstan, or Kyrgyzstan, with some reaching Mongolia and neighboring regions of China. Apart from *F. ovina*, this group does not occur in the southern part of the Irano-Turanian region (Iran, Turkmenistan, Afghanistan, Pakistan, and northwestern India). The two species comprising group B occur in either the northern part of the Irano-Turanian region (*F. karelinii*) or adjacent southern provinces of the Circumboreal region (*F. sibirica*). The three members of group D are restricted to Pamiro-Alay in the former Soviet Middle Asia. Group F comprises species occurring in the southern part of the Irano-Turanian region, reaching as far as Yunnan (Eastern Asiatic floristic region). Members of group G occur in the former Soviet Middle Asia; however, in contrast to group A, the center of endemism for this group is situated more to the south and only a few of its included species reach Kazakhstan. Group H, with the exception of the broadly distributed *F. lehmanii*, is characterized by a southern Irano-Turanian distribution and is absent from Tajikistan, Kyrgyzstan, and Kazakhstan. Group I includes a species broadly distributed in Central Asia (*F. moschata*) and a narrow endemic (*F. nuratavica*).

From this biogeographic summary, it is evident that although the groups identified within *Ferula* by molecular data differ with respect to their distribution patterns, the radiation and diversification of the genus cannot be explained with a simple vicariance scenario. In his discussion on adaptive evolution in *Ferula*, Korovin (1940) regarded ecological conditions, particularly the availability and relative abundance of water, as crucial factors promoting diversification of the genus. Although his detailed scenarios were based on incorrect assumptions of phylogeny (e.g., an immediate link between Ferulinae and Araliaceae and the recognition of all subgenera of *Ferula* as natural groups), he was correct in identifying the major ecological factors and evolutionary tendencies shaping the diversity of *Ferula*. Contrasting climatic conditions with mild wet springs and warm dry summers promote different adaptation syndromes: mesophytic in spring and xerophytic in summer. Korovin speculated that the ancestor of *Ferula* was a monocarpic mesophyte with broad leaf lobes, with polycarpy and narrow leaf lobes resulting from adaptation to more arid conditions. These conclusions are partly supported by our analyses. Monocarpy and narrow leaf lobes are ancestral for *Ferula*; moreover, polycarpy and narrow leaf lobes often coexist (Fig. 4f, h).

Both geographic and climatic conditions of Central Asia promote radiation and diversification. These conditions include contrasting ecological regimes between lowland and montane habitats, particularly the availability of water, length of growing season, variation in soil types, and exposure to light (Korovin 1940). High mountain ranges surrounded by desert constitute more or less isolated ecological archipelagos where endemics may evolve, and in such conditions, the diversification rate may be very fast, as recently demonstrated for Andean members of *Lupinus* (Hughes and Eastwood 2006). However, in contrast to *Lupinus*, the morphological diversity of *Ferula* is low and the endemic species are distinguished based on poor diagnostic characters. Therefore, the high number of species, particularly of the central Asiatic endemics, may result from taxonomic splitting rather than rapid radiation of the genus in this region.

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Appendix

Plant accessions from which nrDNA ITS sequences were obtained are given here. Taxon names are followed by GenBank accession numbers and references or vouchers, the latter with herbarium acronyms in parentheses. Herbarium acronyms follow Holmgren et al. (1990).

Aciphylla squarrosa **J.R. Frost. et G. Frost.**—U72379, Mitchell et al. (1998). ***Anisotome filifolia*** **Cockayne et Laing**—U72375, Mitchell et al. (1998). ***Anthriscus cerefolium*** **(L.) Hoffm.**—DQ379384, Uzbekistan, Western Tian-Shan Mtns., 7 June 1989, *Spalik* (WA). ***Astrodaucus orientalis*** **(L.) Drude**—AF077807, AF077122, Lee and Downie (1999).

Berula erecta **Coville subsp.** ***erecta***—AY360230, Hardway et al. (2004). ***Bifora radians*** **M. Bieb.**—U78408, U78468, Downie et al. (1998). ***Bubon macedonicum*** **L.**—AF073541, Downie et al. (2000a).

Carum carvi **L.**—AF077878, Valiejo-Roman et al. (1998). ***Chaerophyllum temulum*** **L.**—AJ854346, Chung et al. (2005). ***Cryptotaenia japonica*** **Hassk.**—AY360236, Hardway et al. (2004). ***Cuminum cyminum*** **L.**—U78362, Downie et al. (1998).

Daucus carota **L. subsp.** ***carota***—AF077779, Lee and Downie (1999). ***Deverra triradiata*** **Hochst. ex Boiss.**—AF073561, Downie et al. (2000a). ***Dicyclophora persica*** **Boiss.**—AF073539, Downie et al. (2000a). ***Dorema ammoniacum*** **D. Don**—EF560690, Iran, *Ajani* s.n. (Hb. Akhani).

Echinophora chrysantha **Freyn et Sint.**—AF077883, Valiejo-Roman et al. (1998).

Falcaria vulgaris **Bernh.**—AF077888, Valiejo-Roman et al. (1998). ***Ferula akitschkensis*** **B. Fedtsch. ex Koso-Pol.**—DQ379385, Kazakhstan, Dzhungar Alatau, Kopal Mtns., Pendzhak, 1909, *Lipsky* (LE). ***F. assa-foetida*** **L.**—U78391, U78451, Downie et al. (1998). ***F. badrakema*** **Koso-Pol.**—DQ379388, Turkmenistan, Badkhyz, near Kepelia, 12 May 1976, *Botschantzev 696* (LE). ***F. botschantzevii*** **Korovin**—DQ379389, Tajikistan, Konki-tau Mtns., near Khozhadi village, 19 May 1960, *Nepli* (LE). ***F. ceratophylla*** **Regel et Schmalh.**—DQ379390, Kazakhstan, Syrdaria Karatau, Teksakkan Mtns., 7 July 1973, *Kamelin T-25* (LE). ***F. clematidifolia*** **Koso-Pol.**—DQ379391, Tajikistan, south part, near Tutkaul village, 12 June 1960, *Botschatnzsev and Egorova 1290* (LE). ***F. communis*** **L.**—(1) U79615, U79616 Downie et al. (1998); (2) DQ379392, Spain, Almería, Vélez-Rubio, 12 June 2004, *Sánchez-Gómez* (UZ). ***F. decurrens*** **Korovin**—DQ379393, Tajikistan, South-Tadjik depression, near Sarygor post, left bank of Kofirkash river, 4 June 1961, *Soskov and Junusov* 623 (LE). ***F. dissecta*** **(Ledeb.) Ledeb.**—DQ379394, Kazakhstan, near Balkhash lake, Arkharly Mtns., 28 May 1976, *Botschantzev and Botschantzeva 1010* (LE). ***F. diversivittata*** **Regel et Schmalh.**—DQ379395, Uzbekistan, Nuratau range, near Sintob village, 24 June 1971, *Botschantzev and Kamelin 447* (LE). ***F. dshizakensis*** **Korovin**—DQ379397, Uzbekistan, Samarkand prov., between Katta-Kurgan and mine Ingichka, 19 May 1975, *Botschantzev 280* (LE). ***F. dubjanskyi*** **Korovin ex Pavlov**—DQ379396, as *F. dshaudshamyr* Korovin, Kazakhstan, Muiun-Kum, near Ak-Zhar lake, Kotlan, 19 June 1970, *Soskov and Bukhteeva 51* (LE). ***F. equisetacea*** **Koso-Pol.**—DQ379398, Tajikistan, Gyssar range, basin of river Varzob, near Kabuty, 3 August 1973, *Kamelin* (LE). ***F. eugenii*** **Kamelin**—DQ379399, Tajikistan, Gyssar range, basin of river Varzob, Kondar gorge, 13 June 1970, *Kamelin 86* (LE). ***F. fedoroviorum*** **Pimenov**—DQ379400, Kyrgyzstan, near Naryn river, 18 July 1985, *Kluikov 201* (LE). ***F. ferganensis*** **Lipsky ex Korovin**—DQ379401, Uzbekistan, Koitash range, near Kiotash village, 28 June 1971, *Botschantzev and Kamelin 608* (LE). ***F. feruloides*** **(Steud.) Korovin**—DQ379402, Kazakhstan, Alma-Ata prov., near pass Arkhanlyk, 1952, *Maccagetov 3775* (LE). ***F. foetida*** **(Bunge) Regel**—DQ379403, Uzbekistan, Kysylkum, Bukhara prov., near Shafrikan, 19 April 1975, *Ikonnikov 25* (LE). ***F. foetidissima*** **Regel et Schmalh.**—DQ379404, Tajikistan, Upper Badakhshan, near Khorog Botanical Garden, 24 June 1966, *Kamelin* (LE). ***F. glauca*** **L.**—DQ379406, Spain, Gerona, 6 July 1923, *Augustin 4821* (KRA). ***F. gummosa*** **Boiss.**—DQ379407, Turkmenistan, Nukhur Mtns., 24 April 1913, *Samokish* (LE). ***F. gypsacea*** **Korovin**—DQ379408, Kazakhstan, south part, Alym-tau, 22 June 1973, *Kamelin 11* (LE). ***F. karelinii*** **Bunge**—EF560692, Pakistan, Makran, Hoshab to Panjgur, c. 20–30 km from Panjgur, 20 April 1965, *Lamond 562* (E). ***F. karakalensis*** **Korovin**—DQ379409, Turkmenistan, Malye Balkhany Mtns., near Akcha-Kuima, 22 May 1949, *Tarasov 25* (LE). ***F. karatavica*** **Regel et Schmalh.**—DQ379410, Kazakhstan, Karatau Mtns., near Arys river, along road Chimkent to Baidgeonsai, 16 June 1959, *Botschantzev 345* (LE). ***F. karataviensis*** **(Regel et Schmalh.) Korovin ex Pavlov**—DQ379411, Kazakhstan, between Karatau and Ulken-tau, 18 June 1959, *Pjansaeva 64* (LE). ***F. kelifi*** **Korovin** —DQ379442, as *F. primaeva* Korovin, Uzbekistan, Guvar-Baisunski massif, near village Shurab, 16 May 1979, *Kamelin and Mamedov* (LE). ***F. kelleri*** **Koso-Pol.**—DQ379405, as *F. foliosa* Korovin, Kyrgyzstan, Dzhalalabad prov., Fergana range, Ermendy river, Mt Alash-bashi, 8 July 1945, *Fedorov and Ilina 58* (LE). ***F. kingdon-wardii*** **H.Wolff**—AF164824, AF164849, Downie et al. (2000c). ***F. kirialovii*** **Pimenov**—DQ379443, as *F. pseudooreoselinum* (Regel et Schmalh.) Koso-Pol., Uzbekistan, Chatkal range, Akbulak river, 3 July 1973, *Kamelin* 2 a, b (LE). ***F. kokanica*** **Regel et Schmalh.**—(1) AF164825, AF164850, Downie et al.

(2000c); (2) DQ379412, Uzbekistan, Aktau Mtns., Ingichke, near Nurat, 26 June 1971, *Botschantzev and Kamelin 457* (LE). ***F. korshinskyi*** **Korovin**—DQ379413, Uzbekistan, Tcharvak, Arslanbai, 27 March 1916, *Lipsky* (LE). ***F. kosopoljanskii*** **Korovin**—DQ379414, Tajikistan, Upper Badakhshan, near mouth of Iazgulem river, 18 June 1968, *Ladygina* et al. (LE). ***F. kyzylkumica*** **Korovin**—DQ379415, Uzbekistan, Samarkand prov., Sangrultau Mtns., 10 June 1948, *Rodin and Arkadiev 389* (LE). ***F. lapidosa*** **Korovin**—DQ379416, Kazakhstan, Dzhambul prov., 10 km S from village Kaindy, near railway Ak-Tchulak to Ak-tobe, 30 June 1970, *Soskov and Bukhteeva* (LE). ***F. lehmannii*** **Boiss.**—DQ379418, Kazakhstan, Aktubinsk prov., Baiganin region, near Danguz-tau, 13 June 1956, *Junatov and Kuznetsov* (LE). ***F. leiophylla*** **Korovin**—DQ379420, Kazakhstan, Taldy-Kurgan prov., between Karatal river and lake Utch-Kul, near source Aishbulak, 10 June 1928, *Schiptschinsky 104* (LE). ***F. leucographa*** **Korovin**—DQ379419, Kazakhstan, Mashat-tau, Kelte-mashat, 27 July 1973, *Kamelin 26* (LE). ***F. linkii*** **Webb**—DQ379421, Cult. Conservatoire botanique de la Ville de Mulhouse, 9384, 3 November 2005. ***F. lipskyi*** **Korovin**—DQ379422, Kyrgyzstan, Dzhalalabad prov., Suzak region, sovkhoz Suzak, 28 April 1945, *Vipper* (LE). ***F. litwinowiana*** **Koso-Pol.**—DQ379423, Turkmenistan, near Uzboi, kolkhoz Dzhigurdekli, 14 May 1953, *Rustamov* (LE). ***F. loscosii*** **(Lange) Willk.**—(1) DQ379424, Spain, Huesca, Candasnos, Valcuerna, 25 April 2004, *Pérez-Collazos and Catalán* (UZ); (2) DQ379425, Spain, Albacete, Hellín, Las Minas, 28 May 2004, *Pérez-Collazos* et al. (UZ). ***F. mogoltavica*** **Lipsky ex Korovin**—DQ379429, Tajikistan, Khudjent, Mogol-tau, Tchashma-arzanok, 18 May 1924, *Popov and Vvedensky 763* (LE). ***F. moschata*** **(Reinsch) Koso-Pol.**—DQ379457, as *F. urceolata* Korovin, Tajikistan, Gyssar range, near Iskander-kul lake, 31 July 1960, *Egorova 2235* (LE). ***F. neapolitana*** **Ten .** —DQ379431, Italy, Campania di Salerno, 14 June 1913, *Pellanda 2297* (KRA). ***F. nevskii*** **Korovin**—DQ379432, Uzbekistan, Kugitangh range, near village Tengideval, 13 June 1971, *Botschantzev 111* (LE). ***F. nuratavica*** **Pimenov**—DQ379433, Uzbekistan, Aktau Mtns., sai Petyr, 14 July 1972, *Kamelin 113* (LE). ***F. olgae*** **Regel et Schmalh.**—DQ379434, Uzbekistan, Aktau Mtns., near Nurat, Ingichke, 13 July 1970, *Botschantzev 150* (LE). ***F. olivacea*** **(Diels) H. Wolff**—EF560691, China, Yunnan, Lijiang Pref.: Yulongxue Shan, Ganghoba, 4 October 1990, *CLD Exp. 790*, cult. RBG Edinburgh No. 19910663-A (E). ***F. oopoda*** **(Boiss. et Buhse) Boiss.**—(1) DQ379387, as *F. badhysi* Korovin, Turkmenistan, Badkhyz, Kzyl-Dzar ravine, near Er-Oylan-Duz lake, 27 April 1964, *Botschantzev* et al. *283* (LE); (2) DQ379435, Turkmenistan, Kopet-Dagh, Alty-Topdon gorge, 6 June 1930, *Bobrov 94* (LE). ***F. ovina*** **(Boiss.) Boiss.**—(1) DQ379436, Tajikistan, Seravshan basin, Turkestan range, gorge between villages Rarz and Pakhurd, along road to Matcha, 14 July 1960, *Egorova 1809* (LE); (2) DQ379426, as *F. microcarpa* Korovin, Kyrgyzstan, Talass valley, Igkele-tau-Aktash Mtns., Kichkeie-kapka gorge, 1970, *Kamelin 628* (LE); (3) DQ379437, as *F. pachycarpa* Korovin, Kazakhstan, Syrdaria Karatau, massif Mynzhilke, gorge Rais, 6 July 1974, *Kamelin 1595* (LE); (4) DQ379449, as *F. stylosa* Korovin, Kazakhstan, Dzhungar Alatau, Tchulak Mtns., gorge Tchulak-Dzhigde, 6 June 1956, *Goloskokov* (LE). ***F. pallida*** **Korovin**—DQ379438, Kazakhstan, South Kazakhstan, Alym-tau, 3 June 1960, *Priakhin* (LE). ***F. penninervis*** **Regel et Schmalh.**—DQ379439, Kazakhstan, Karatau Mtns., Bosturgai gorge, 19 July 1950, *Pojarkova* 234 (LE). ***F. potaninii*** **Korovin ex Pavlov**—DQ379440, Kazakhstan, near Balkhash lake, Arkharly Mtns., 28 May 1976, *Botschantzev and Botschantzeva* 1018 (LE). ***F. prangifolia*** **Korovin**—DQ379441, Uzbekistan, Ugam range, near Khumsai village, Kent-sai, 24 June 1974, *Kamelin* 1355 (LE). ***F. renardii*** **(Regel et Schmalh.) Pimenov**—DQ379444, Kyrgyzstan, Dzhalalabad prov., Arkesht village, Sary-Chilek, 10 August 1945, *Vipper* (LE). ***F. rubroarenosa*** **Korovin**—DQ379430, as *F. mollis* Korovin, Uzbekistan, Alai range, Sokh-Khashi-mardan, pass Meting-bel, 16 July 1962, *Botschantzev 186* (LE). ***F. samarkandica*** **Korovin**—(1) DQ379445, Uzbekistan, Chimgan Mtns., source of Chimganka river, 29 June 1974, *Kamelin* 1420 (LE). (2) DQ379417, as *F. latiloba*, Tajikistan, Oti-Asht basin, Topur sai, 21 June 1970, *Kamelin 257* (LE). ***F. schtschurowskiana*** **Regel et Schmalh. ex Regel**—DQ379446, Turkmenistan, Charshangin region, Kugitangh valley, between villages Kugitangh and Guardok, Albasty, 12 October 1970, *Botschantzev* 103 (LE). ***F. sibirica*** **Willd.**—DQ379447, Kazakhstan, prov. Dzeskasgan, near Zhairem, 27 July 1991, *Pimenov and Kljuykov* 133 (G). ***F. songarica*** **Pall. ex Schult.**—DQ379448, Kazakhstan, Akmolinsk prov. [Astana], near Tengyz lake, 17 August 1954, *Kalinina 1993* (LE). ***F. szowitsiana*** **DC.**—DQ379427, as *F. microloba* Boiss., Turkmenistan, central Kopet-Dagh, 14 June 1969, *Meshtsheriakov* (LE). ***F. tadshikorum*** **Pimenov**—DQ379450, Tajikistan, Saiglok range, near Sebiston village, 23 May 1979, *Kamelin and Mikhailov 380* (LE). ***F. tatarica*** **Fisch. ex Spreng.**—DQ379451, Kazakhstan, Karaganda prov., Atasuiski region, 29 July 1959, *Varivtseva 335* (LE). ***F. tenuisecta*** **Korovin ex Pavlov**—AF077890, Valiejo-Roman et al. (1998). ***F. teterrima*** **Kar. et Kir.**—DQ379452, Kazakhstan, near Balkhash lake, 5 July 1958, *Rusliaeva* (LE). ***F. tingitana*** **L.**—(1) AF164826, AF164851, Downie et al. (2000c); (2) DQ379453, Cult. Conservatoire botanique de la Ville de Mulhouse, 9521, 3 November 2005. ***F. tschimganica*** **Lipsky ex Korovin** —DQ379428, as *F. minkwitzae* Korovin, Kyrgyzstan, Syrdaria Karatau, Kzyladyr Mtns., near goarge Kysh-Kapka, 2 July 1973, *Kamelin 3-KA* (LE). ***F. tuberifera* Korovin**—DQ379454, Turkmenistan, Kugitangh range, near village Kugitangh, 13 October 1970, *Botschantzev 106* (LE). ***F. ugamica* Korovin**—DQ379455, Uzbekistan, Angren region, near village Chesh-su, left bank of Angren river, 2 June 1954, *Knorring* (LE). ***F. undulata* Pimenov et J.P. Baranova**—DQ379456, Turkmenistan, central Kopet-Dagh, 35 km S from Gueok-Tepe, near Novo-Kheirabad, 25 May 1963, *Gubanov* (LE). ***F. varia* (Schrenk) Trautv.**—DQ379386, as *F. arida* (Korovin) Korovin, Kazakhstan, Central Betpak-dala, Kok-ashik, 17 June 1960, *Grubov* (LE). ***F. violacea* Korovin**—AF077891, Valiejo-Roman et al. (1998). ***F. xeromorpha* Korovin**—DQ379458, Kazakhstan, Alymtau Mtns., 3 June 1960, *Pryakhin* et al. (LE). ***Foeniculum vulgare* Hill.**—AY581806, Tabanca et al. (2005).

***Glaucosciadium cordifolium* (Boiss.) B.L. Burtt et P.H. Davis**—DQ379459, Cult., Conservatoire botanique de la Ville de Mulhouse, 98112, 7 December 1999. ***Grammosciadium daucoides* DC.**—AF073559, Downie et al. (2000a).

***Heracleum moellendorffii* Hance**—AY548226, Choi, Choi and Jung (unpublished).

***Imperatoria ostruthium* L.**—AF077896, Valiejo-Roman et al. (1998).

***Laserpitium hispidum* M. Bieb.**—AF077898, Valiejo-Roman et al. (1998). ***L. siler* L.**—U30528, U30529, Downie and Katz-Downie (1996). ***L. petrophilum* Boiss. et Heldr.**—AF073567, Downie et al. (2000a). ***Lecokia cretica* (Lam.) DC.**—U78358, Downie et al. (1998). ***Leutea cupularis* (Boiss.) Pimenov**—AY941277, AY941305, Valiejo-Roman et al. (2006). ***L. elbursensis* Mozaff.**—AY941276, AY941304, Valiejo-Roman et al. (2006). ***L. laseroides* Akhani** (= *Laser rechingeri* Akhani)—AY974773, Valiejo-Roman et al. (2006). ***L. petiolaris* (DC.) Pimenov**—AY941278, AY941306, Valiejo-Roman et al. (2006).

***Monizia edulis* Lowe**—AF073569, Downie et al. (2000a). ***Mozaffariania insignis* Pimenov et Maassoumi**—AY941279, AY941307, Valiejo-Roman et al. (2006). ***Myrrhis odorata* (L.) Scop.**—AF077901, Valiejo-Roman et al. (1998).

Naufraga balearica Constance et Cannon—AF073563, Downie et al. (2000a).

***Oenanthe prolifera* L.**—AY691941, Kadereit and Kadereit (2005). ***Orlaya grandiflora* (L.) Hoffm.**—U30524, U30525, Downie and Katz-Downie (1996).

***Physospermum cornubiense* (L.) DC.**—AF077904, Valiejo-Roman et al. (1998). ***Pimpinella saxifraga* L.**—AY581801, Tabanca et al. (2005). ***Pseudorlaya pumila* (L.) Grande**—U30522, Downie and Katz-Downie (1996). ***Pycnocycla aucheriana* Decne. ex Boiss.**—AF073533, Downie et al. (2000a). ***Pyramidoptera cabulica* Boiss.**—AF008631, Katz-Downie et al. (1999).

***Selinum carvifolia* (L.) L.**—AY179028, Spalik et al. (2004). ***Seseli tortuosum* L.**—AY179031, Spalik et al. (2004). ***Silaum silaus* (L.) Schinz et Thell.**—EF560689, France, Bas-Rhin, between Herbsheim et Boofzheim, 14 August 2001, *Reduron* (Hb. Reduron). ***Sium latifolium* L.**—AY353984, Hardway et al. (2004). ***Smyrnium olusatrum* L.**—U30594, Downie and Katz-Downie (1996). ***Szovitsia callicarpa* Fisch. et C.A. Mey.**—AF077809, AF077124, Lee and Downie (1999).

***Thysselinum palustre* (L.) Hoffm.**—AY179035, Spalik et al. (2004). ***Todaroa aurea* (Sol.) Parl.**—AF336372, Spalik et al. (2001b). ***Torilis japonica* (Houtt.) DC.**—AY548223, Choi, Choi, and Jung (unpublished).

Yabea microcarpa (Hook. et Arn.) Koso-Pol.—AF077806, AF077121, Lee and Downie (1999).

***Zosima orientalis* Hoffm.**—AF008628, Katz-Downie et al. (1999).

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