

Taxonomy of the traditional medicinal plant genus *Ferula* (Apiaceae) is confounded by incongruence between nuclear rDNA and plastid DNA

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The aim of this study was to identify major clades in the economically important and taxonomically difficult genus *Ferula* (Apiaceae tribe Scandiceae subtribe Ferulinae) to provide a classification framework. Phylogenetic relationships among 126 of *Ferula* spp. and eight species of its sister genus *Leutea* were evaluated based on nuclear ribosomal (nr) DNA ITS and three plastid regions: the *rps16* intron, the *rpoC1* intron and the *rpoB-trnC* intergenic spacer. One hundred and fifty-three accessions were considered including type specimens of seven species. Congruence between nrDNA and plastid DNA data was assessed using a hierarchical likelihood ratio test. Phylogenetic trees were inferred using maximum likelihood and Bayesian methods. Terminals introducing topological conflict were ascertained using two approaches: identifying (1) these with significantly different positions between nrDNA and plastid DNA trees; and (2) a set of rogue taxa in combined trees that, when removed, increased tree resolution and bootstrap support. The results demonstrate significant incongruence between nrDNA and plastid DNA data that persisted after the removal of 41 terminals identified as having significantly different position in nrDNA and plastid DNA trees or after the removal of 13 terminals identified as rogue taxa. Comparison of nrDNA and plastid DNA trees suggest intensive reticulate evolution, particularly in the Irano-Turanian floristic region. Traditional classification systems of the genus are not supported by molecular data, whereas some lineages apparent in molecular trees, particularly Chinese and Mediterranean endemics, are congruent with morphological characters and/or biogeography. We select lectotypes for several infrageneric names and propose a new classification system of the genus with four subgenera and ten sections.

ADDITIONAL KEYWORDS: Ferulinae – infrageneric classification – *Leutea* – molecular phylogeny – reticulate evolution – typification.

INTRODUCTION

Among the genera of Apiaceae, *Ferula* L. is one of the largest, includes many species commonly used in traditional medicine and is a promising

source of biologically active ingredients. However, the current classification system of the genus does not match the phylogenetic trees, providing little support for research and commercial use. With many species, a wide distribution and poorly understood morphology, the genus epitomizes all the nightmares of a taxonomist. The c. 170 species of this Eurasian

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genus are distributed from the Canary Islands in the west through the Mediterranean region, Middle East and Central Asia to western China in the east and northern India in the south (Korovin, 1947; Pimenov & Leonov, 1993). The genus has not been taxonomically revised since the monograph of Korovin (1947). Regional floristic treatments resolved some critical issues but also contributed to the taxonomic confusion because they were inconsistent with one another (Korovin, 1951, 1959; Peşmen, 1972; Safina & Pimenov, 1984; Chamberlain & Rechinger, 1987; Safina & Pimenov, 1990). Substantial morphological variation in the genus has resulted in the description of multiple nearly indistinguishable species, which are sometimes represented in herbarium collections by few, poorly preserved specimens. Fruits and basal leaves are essential for correct identification (Peşmen, 1972); the latter, however, are often absent at fruit maturity in monocarpic taxa. Because of the large size of these plants, herbarium specimens usually include only lateral branches and lateral divisions of basal leaves. An experienced taxonomist may identify most species from flowering material (Chamberlain & Rechinger, 1987), but, as underlined by Korovin (1947), the circumscription of species should be based on observations of living plants because it requires examination of complete specimens with roots, stem bases, basal leaves, inflorescence, flowers and ripe fruits. Given these constraints, a taxonomic revision of *Ferula* seems currently impossible for a single taxonomist. Therefore, it would be useful to subdivide the genus into smaller, monophyletic and workable units that could be subject to partial revisions.

In his monograph, Korovin (1947) established several subgenera and sections based mostly on habit and vegetative features. Safina & Pimenov (1983, 1984, 1990) contested these infrageneric divisions and proposed an alternative classification system inferred from fruit morphology and anatomy. However, subsequent molecular studies have not supported either of these treatments, indicating that the infrageneric classification system of *Ferula* has to be constructed anew (Kurzyńska-Mitynik *et al.*, 2008; Panahi *et al.*, 2015).

Ferula was traditionally classified in tribe Peucedaneae (Pimenov & Leonov, 1993). However, immunochemical studies demonstrated that the genus is not closely related to other genera in this tribe (Shneyer, Borschtschenko & Pimenov, 1995; Shneyer, Kutjavina & Pimenov, 2003). Phylogenetic studies using nuclear ribosomal internal transcribed spacer (nrDNA ITS) sequence variation revealed that *Ferula* is placed in tribe Scandiceae and forms a clade with *Dorema* D. Don and *Leutea* Pimenov (Kurzyńska-Mitynik *et al.*, 2008). This clade was formally recognized as subtribe Ferulinae. However, the resolution of the ITS

tree was low and the infrageneric subclades received moderate internal support. Subsequent analyses using ITS and three non-coding plastid DNA (plastid DNA) sequences showed that *Leutea* is sister to *Ferula*, whereas *Dorema* is nested in it; consequently, *Dorema* was subsumed under *Ferula* (Panahi *et al.*, 2015). However, the phylogenetic signal differed significantly between nrDNA and plastid DNA markers resulting in a poor resolution of the tree at the infrageneric level. Moreover, this study included only 68 *Ferula* spp. of > 170 recognized in the genus.

Here, we further investigate the phylogenetic relationships in Ferulinae using a comprehensive sampling of species and analysing data from nuclear and plastid genomes. Our aim is the identification of major clades of *Ferula* that may be subject to partial taxonomic revisions and, therefore, provide a phylogenetically meaningful classification system of the genus.

MATERIAL AND METHODS

TAXON SAMPLING

One hundred and fifty-three samples of Apiaceae were examined for nrDNA ITS and plastid DNA sequence variation (Table S1). The ingroup comprised eight *Leutea* spp. and 126 *Ferula* spp. We obtained sequences from 72 samples for this study, with the others having been previously published (Kurzyńska-Mitynik *et al.*, 2008; Panahi *et al.*, 2015). The former genus *Dorema* that is now subsumed in *Ferula* (Panahi *et al.*, 2015) was represented by six species. The analysed samples included type material of seven species: *F. gabrielii* Rech.f., *F. kashanica* Rech.f., *F. serpentinica* Rech.f., *F. sharifii* Rech.f. & Esfand., *F. stenocarpa* Boiss. & Hausskn. ex Boiss., *F. tabasensis* Rech.f. and *Leutea nematoloba* (Rech.f.) Pimenov. Taxonomic treatment for the Middle Eastern and Central Asian members of *Ferula* generally follows the *Flora Iranica* account (Chamberlain & Rechinger, 1987) with some amendments (Czerepanov, 1995; She & Watson, 2005; Mozaffarian, 2007). The outgroups included two representatives of other major lineages of Scandiceae: *Glaucosciadium cordifolium* (Boiss.) B.L. Burt & P.H. Davis and *Thapsia thapsioides* (Desf.) Simonsen, Rønsted, Weitzel & Spalik (Panahi *et al.*, 2015). Species names with authorities, voucher information and GenBank accession numbers for the samples included in this study are listed in Table S1.

LABORATORY PROCEDURES

Total genomic DNA was extracted from c. 10–20 mg of dried plant material using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the

manufacturer's instructions. Internal transcribed spacer regions (nrDNA ITS) and three non-coding plastid DNA regions (the *rps16* intron, the *rpoC1* intron and the *rpoB-trnC* intergenic spacer) were chosen for phylogenetic reconstruction. These markers have already been used in our previous analyses of Ferulinae (Panahi *et al.*, 2015).

The ITS region was amplified using primers 'N-nc18S10' and 'C26A' (Wen & Zimmer, 1996). For some difficult DNA samples, the ITS1 and ITS2 regions were each amplified separately using primers '18S-ITS1-F' and '5.8S-ITS1-R' for ITS1 and 'ITS-3N' and 'C26A' or 'ITS4' for ITS2 (Spalik & Downie, 2006). The *rps16* intron was amplified using primers '5exonC' and '3exonR' (Calviño *et al.*, 2006) or 's16exF' and 's16exR' (Panahi *et al.*, 2015). For some samples, this region was divided into parts and amplified using a combination of the aforementioned external primers and internal primers 's16inF' and 's16inR' (Panahi *et al.*, 2015). To amplify the *rpoC1* intron and parts of the flanking exon regions, we used external primers 'C1exF' and 'C1exR' and internal primers 'C1inF' and 'C1inR' (Panahi *et al.*, 2015). The *rpoB-trnC* intergenic spacer region was amplified with primers 'rpoB' and 'trnC^{GCA}R' (Shaw *et al.*, 2005). For some samples, the external forward primer 'rpoB' was unsuccessful and was replaced with 'rpD'; additionally, internal primers 'RP', 'TP', 'rpA' and '1R' were used to overcome amplification and sequencing problems. The sequences and position of these primers and the details of PCR amplification are provided elsewhere (Panahi *et al.*, 2015).

PCR products were separated using 1% agarose gel with ethidium bromide or Midori Green DNA Stain (ABO) and purified using the QIAEX II Agarose Gel Extraction Kit (Qiagen). Sequencing from both strands was performed using Big Dye terminators (Applied Biosystems, Foster City, CA, USA). The chromatographs were assembled and edited using SeqMan Pro ver. 12 (Dnastar, Madison, WI, USA).

SEQUENCE AND PHYLOGENETIC ANALYSES

The sequences were initially aligned using the default pair-wise and multiple alignment parameters in CLUSTAL X (Larkin *et al.*, 2007) and adjusted manually using Mesquite 3.2 (Maddison & Maddison, 2017). Gaps were positioned to minimize nucleotide mismatches and treated as missing data in phylogenetic analyses. Poorly aligned regions that may interfere with phylogenetic estimation were located using trimAl v.1.2 with the *-automated1* option (Capella-Gutiérrez, Silla-Martínez & Gabaldón, 2009) and excluded from the analyses.

Congruence of the datasets was assessed using a hierarchical likelihood ratio test (hLTR) implemented in Concatenator ver. 1.7.2 (Leigh *et al.*, 2008).

Phylogenetic trees were inferred using the maximum likelihood (ML) method implemented in RAxML ver. 8.2.4 (Stamatakis, 2014) and the Bayesian inference (BI) method implemented in MrBayes ver. 3.2.6 x64 MPI (Ronquist *et al.*, 2012). Several groups of accessions had identical sequences for some markers analysed. Each of these groups was represented by a single terminal in phylogenetic analyses. In the resulting trees, these terminals were added forming a polytomy composed of zero-length branches.

ML analyses included 200 searches starting from distinct randomized maximum parsimony trees. Branch support (BS) was evaluated based on 1000 rapid bootstrap replicates. To assess whether the number of replicates was sufficient, a posteriori bootstrapping analysis was carried out with the extended majority-rule consensus tree as a criterion of convergence. All analyses were performed with GTR + G substitution model, which was most efficiently implemented and optimized for RAxML and thus recommended by the author (Stamatakis, 2014). For plastid data and combined nuclear and plastid data, partition schemes were inferred using PartitionFinder ver. 2.1.1 (Lanfear *et al.*, 2016) with branch lengths linked and BIC options.

Bayesian analyses comprised two independent runs, each with four Monte Carlo Markov chains that were run for 40 000 000 generations with a sampling frequency of 1000 generations and default priors for the parameters of nucleotide substitution model. The initial 25% of saved trees were discarded and the results were summarized on the 50% majority rule consensus tree. The effective sample size (ESS) for the estimated parameters and the convergence of the independent runs were checked using Tracer ver. 1.6.0 (Rambaut *et al.*, 2014). The model of nucleotide substitution for independent analyses of nrDNA ITS data was selected with ModelGenerator ver. 0.85 (Keane *et al.*, 2006). For the analyses of plastid data and combined nuclear and plastid data, partition schemes and substitution models were inferred using PartitionFinder comparing the models implemented in MrBayes.

Two methods were used to identify problematic terminals, i.e. those that introduce putative topological conflict or decrease branch support: (1) *compat.py* (Kauff & Lutzoni, 2002, 2003) and (2) the RogueNaRok algorithm (Aberer, Krompass & Stamatakis, 2013). The first compares support values for trees inferred from different partitions (e.g. nrDNA and plastid DNA) and identifies terminals that occur in different and well-supported clades. We therefore compared clades occurring in nrDNA and plastid DNA trees choosing the values of BS \geq 75 and PP = 1 as thresholds for ML and Bayesian analyses, respectively. In contrast, the RogueNaRok algorithm identifies a set of rogue terminals that, if pruned from the bootstrap trees, results in a consensus tree with more resolved polytomies (i.e.

containing additional bipartitions) or with increased support values. We applied this algorithm to identify rogue taxa in ML analyses of combined data. The terminals identified by this algorithm were pruned from the ML bootstrap trees, which were then summarized on the best tree. Bootstrap support values for major clades were compared to those obtained from the analyses of unpruned trees.

To check whether the terminals identified with both methods introduced incongruence between nrDNA and plastid DNA, we excluded them from the matrices and repeated the hierarchical likelihood ratio tests (hLRT) with Concatenator. The data for this study have been deposited in TreeBASE, study number 21677 (<http://purl.org/phylo/treebase/phyloids/study/TB2:S21677>).

RESULTS

SEQUENCE CHARACTERISTICS

Alignment of the sequences was relatively unambiguous. In the matrices trimmed with trimAl, the ITS region was more variable for the ingroup than the concatenated plastid DNA regions, with 25.5% variable positions as opposed to 10.0% in the latter (Table 1). Seventy-two ITS sequences were non-unique, distributed in 17 groups comprising two to 16 terminals each. In the concatenated plastid DNA data, 38 accessions were non-unique and distributed in 16 groups of two to four terminals each. However, in the matrix of combined nrDNA and plastid DNA data, only six pairs of accessions had all sequences identical. Each group of identical sequences was represented by a single terminal in subsequent phylogenetic analyses.

Hierarchical clustering of the markers based on likelihood-ratio tests concatenated all three plastid DNA regions with $P = 0.043$ and corrected $\alpha = 0.025$;

however, it failed to concatenate plastid DNA and ITS with $P < 1 \times 10^{-6}$ and corrected $\alpha = 0.017$. For ITS data, ModelGenerator with the Bayesian information criterion (BIC) selected the SYM + G nucleotide substitution model. PartitionFinder partitioned the data into ITS and plastid DNA with the SYM + G and GTR + G + I substitution models, respectively.

PHYLOGENETIC ANALYSES

Trees obtained from ML and Bayesian analyses were similar in topology; therefore, here we only show ML trees, whereas Bayesian trees are presented electronically in the Additional Supporting Information. We identify major clades with letters and their subclades with numbers; clades A–I refer to those recognized by Kurzyńska-Mitynik *et al.* (2008) and J–L are recognized here for the first time.

Separate analyses of nrDNA ITS and plastid DNA matrices of these data resulted in trees with notably different topologies. In the ITS trees, major clades of Ferulinae formed a basal polytomy (Figs 1 and S1), whereas in the plastid DNA trees relationships among those clades were better resolved, but also contained polytomies (Figs 2 and S2). However, several clades that were apparent in the ITS trees did not occur in the plastid DNA trees. Although some clades were present in the trees inferred from both datasets, numerous terminals were placed in different positions in these trees (Fig. S3). Compat.py identified 41 terminals that had significantly different positions in nrDNA and plastid DNA trees (Figs 1 and 2). Notably, the ITS tree was more congruent with the traditional taxonomic treatment of the genus than the plastid tree. For instance, all samples of *F. gummosa* Boiss. have identical ITS sequences and occurred in clade H, whereas each of them has a different plastid haplotype

Table 1. Characteristics of the datasets used in phylogenetic analyses. Apart from sequence length variation, all numbers concern matrices without ambiguously aligned positions removed using trimAl program. Numbers in parentheses refer to the ingroup (*Ferula*)

Datasets	ITS	<i>rpoB-trnC</i> spacer	<i>rps16</i> intron	<i>rpoC1</i> intron	plastid DNA	All combined
Sequence length variation	599–606	1191–1327	833–920	1045–1061	—	—
Number of unambiguously aligned positions:						
total	604	1205	835	1048	3106	3710
constant	407 (450)	1043 (1060)	749 (767)	963 (969)	2755 (2796)	3162 (3246)
autapomorphic	98 (68)	94 (79)	61 (44)	45 (40)	200 (163)	298 (231)
Parsimony informative	99 (86)	68 (66)	43 (42)	40 (39)	151 (147)	250 (233)
containing gaps	17 (15)	113 (79)	43 (41)	34 (25)	190 (145)	207 (160)
Percentage of gaps	0.2 (0.19)	0.13 (0.11)	0.15 (0.15)	0.09 (0.09)	0.12 (0.11)	0.14 (0.13)
Mean indel length [bp]	1.16 (1.14)	1.49 (1.33)	1.26 (1.25)	0.83 (0.78)	2.70 (2.66)	2.94 (2.96)
Maximum indel length [bp]	4 (4)	20 (20)	21 (21)	8 (8)	21 (21)	21 (21)

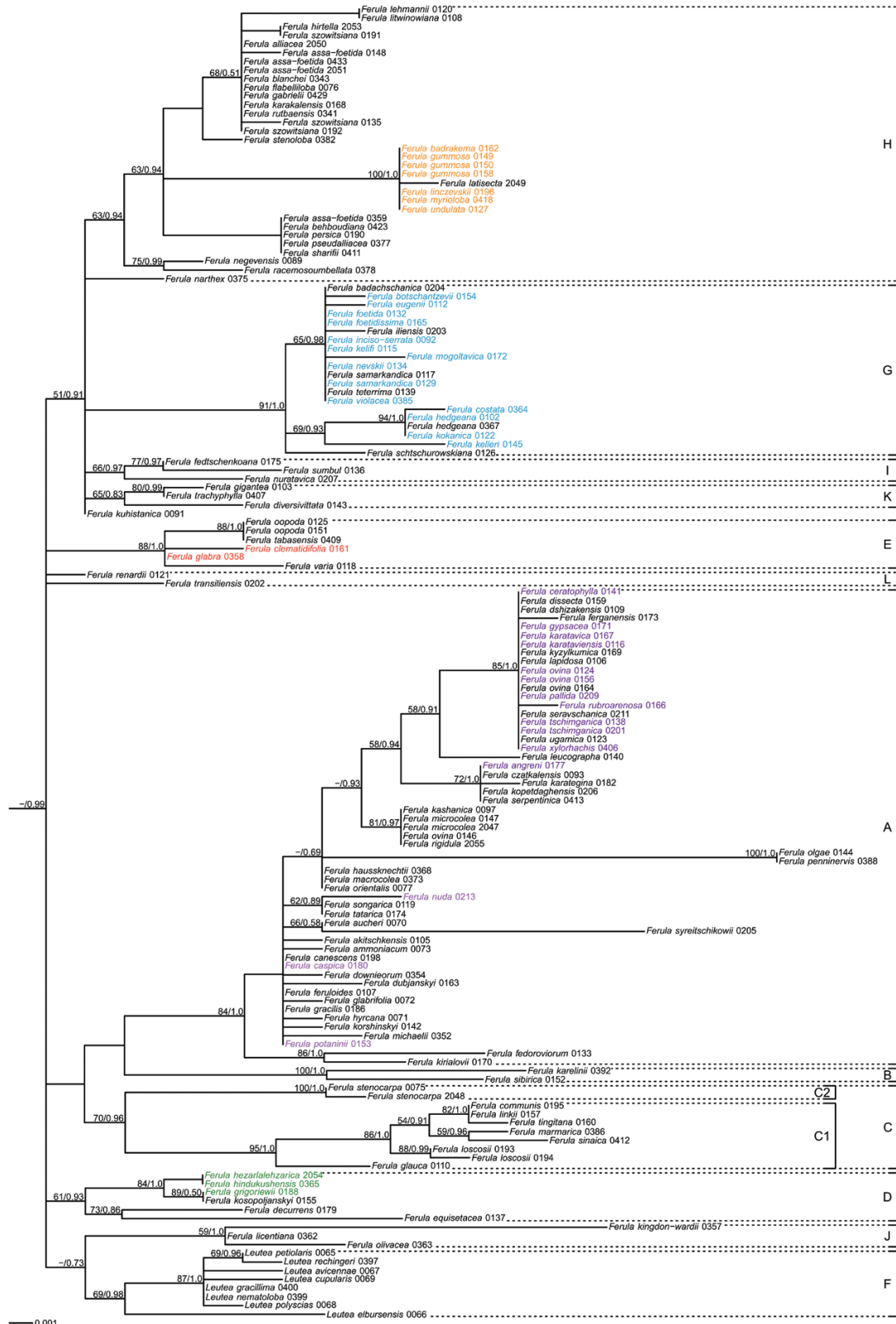


Figure 1. Maximum likelihood tree inferred from analyses of nrDNA ITS sequence data for Ferulinae. Groups of accessions with identical sequences were represented in the analyses by single terminals and then resolved to polytomies with

that was more closely related to representatives of other species than to conspecific individuals. Similar significant discrepancies occurred in *F. ovina* (Boiss.) Boiss. (clade A). Also noteworthy are the relationships among the four representatives of *F. assa-foetida* L. (clade H), although they were not supported by high BS and PP values and, therefore, these samples were not identified as conflicting by *compat.py*. Based on ITS data, terminals 0433 and 2051 are identical and were grouped with 0148, whereas sample 0359 was placed on a different branch (Fig. 1). The latter, however, had the same plastid DNA haplotype as sample 0148, whereas accessions 0433 and 2051 were placed on distant branches (Fig. 2). This example suggests that the discordance between nrDNA and plastid DNA data does not concern exclusively the terminals identified by *compat.py* as conflicting. Indeed, despite removing these 41 samples from the nrDNA and plastid DNA matrices, these datasets remained incongruent in Concatenation analyses ($P < 1 \times 10^{-6}$, corrected $\alpha = 0.017$).

Major clades in the ML and Bayesian trees of combined data (Figs 3 and S4, respectively) were almost the same as those in the nrDNA trees, whereas the relationships among these clades were more similar to those in the plastid DNA trees. *Leutea* and *Ferula* were found to be sister taxa in Ferulinae; the monophyly of each genus was strongly supported both in ML and Bayesian analyses (BS \geq 93%, PP = 1.0). In combined ML analyses of reduced data without conflicting terminals, major clades and their bootstrap support were similar to these in the tree obtained from full dataset (compare Figs 3 and S5).

In *Ferula*, there were 11 major clades in the combined ML tree (Fig. 3). All but clades H and L also occurred in nrDNA analyses (Fig. 1), whereas only four clades (B, D, L and J) were apparent in ML plastid trees (Fig. 2). In the Bayesian consensus tree from the combined data some clades collapsed into polytomies (Fig. S4).

The rogue taxa analysis using RogueNaRok identified 13 unstable terminals: six accessions belonging to clade A, one to clade E, one to clade G, four to clade H and *F. kuhistanica* Korovin, which does not have a definite placement (Fig. 3). The removal of these terminals from the dataset notably increased bootstrap support for clades E (from 69 to 87%) and H (from 12 to 49%); nevertheless, the bootstrap support for clade H remained low. The support for other clades changed only marginally (Fig. S5). Despite deleting these 13 taxa from the matrix, plastid DNA and nrDNA

datasets remained incongruent in Concatenation analysis ($P < 1 \times 10^{-6}$, corrected $\alpha = 0.017$).

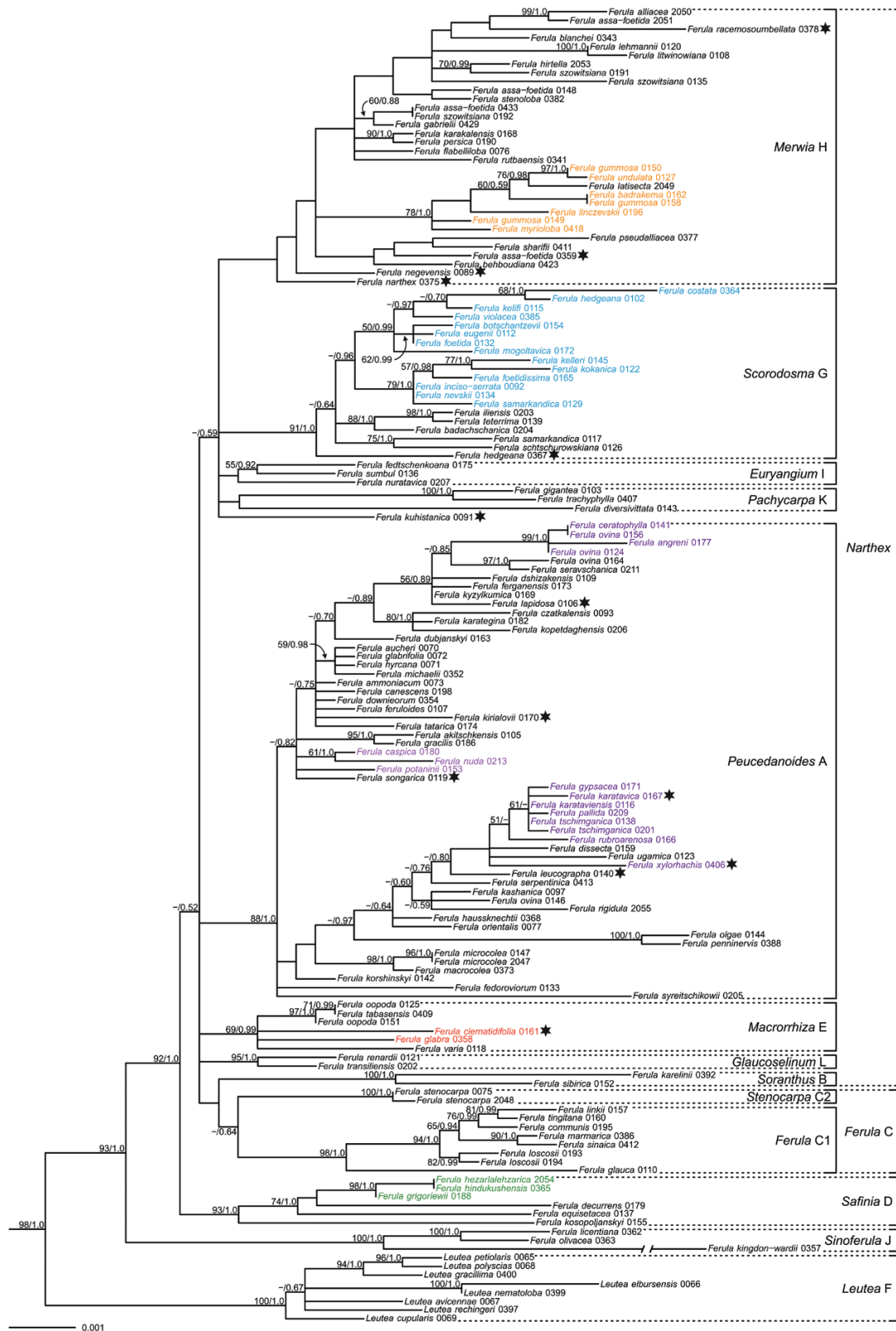
DISCUSSION

INCONGRUENCE BETWEEN nrDNA AND PLASTID DNA DATA

Several factors may have contributed to the detected incongruence between nrDNA ITS and plastid DNA data. We cannot conclusively exclude accidental contamination or human error at various stages of laboratory procedures. Due to a limited availability of plant material, samples were mostly collected from old herbarium specimens, sometimes from crumbled leaves or flowers stored in envelopes. Such materials may be accidentally contaminated with tissues of other species, e.g. those stored in the same herbarium cabinets. Additionally, some PCR primers may be more specific to the contaminant than to the nominal species. As we explained in the Material and Methods section, for some difficult DNA samples, the selected markers were amplified and sequenced in parts using various combinations of external and internal primers. Partial sequences were then assembled based on overlapping regions. These regions were usually evolutionarily conserved, particularly in the plastid DNA markers, and did not vary much among species. Therefore, a chimaeric contig from sequences originating from different species might have passed unnoticed. For the same reasons, a human error would not have been easily detected. When rechecking the data from our previous paper on Ferulinae (Panahi *et al.*, 2015), we found indeed that one sequence of the *rps16* intron (KJ660433) presumed to represent *Leutea glaucopruinosa* (Rech.f.) Akhani & Salimian was from a species of *Orlaya* Hoffm. (other plastid DNA markers from *L. glaucopruinosa* were correct and support the placement of this species in *Leutea*). Additionally, repeated sequencing for two species, *F. tadshikorum* Pimenov (*rps16* intron KJ660415) and *F. tuberifera* Korovin (*rpoB-trnC* spacer KJ660729), indicated that the DNA samples were probably contaminated with other species; therefore, these accessions were not used in the analyses presented here.

Despite the aforementioned problems, there are good reasons to conclude that the discrepancies between ITS and plastid DNA data are not artefacts but have resulted from biological processes. First, we repeated PCR and sequencing for some samples that exhibited

zero-length branches. Bootstrap support and posterior probability for nodes that also occurred in Bayesian 50% majority-rule consensus tree (see Fig. S1) are given along the branches. Accessions identified by *compat.py* as conflicting are marked with different colour for each group. Major clades are bracketed; clades A–I follow Kurzyńska-Młynik *et al.* (2008). Outgroup taxa are omitted for simplicity.



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Figure 3. Maximum likelihood tree inferred from analyses of combined nrDNA ITS and plastid *rps16* intron, *rpoC1* intron and *rpoB-trnC* intergenic spacer sequence data for Ferulinae. Accessions identified as rogue with RogueNaRok algorithm are marked with a star sign. See Figure 1 for details.

striking incongruence and got the same results. Particularly, we confirmed that the three samples of *F. gummosa* with identical nrDNA ITS sequences have different plastid haplotypes that are more closely related to those occurring in other species than to each other. Second, the discordance between plastid DNA and nrDNA was observed for DNA samples processed in two laboratories: in Warsaw and in Tehran (the latter annotated 'K' in Table S1). Third, the discrepancies occurred more often in major clades than among them suggesting that they resulted from hybridisation or introgression between closely related species as exemplified by *F. gummosa* and its close relatives.

The evolutionary processes that may result in incongruence among molecular markers are hybridization, introgression, incomplete lineage sorting and homoplastic substitutions, particularly those that have a stabilizing effect on the stem-loop secondary structure in plastid DNA sequences. This incongruence may also result from sampling error of characters or/and taxa (Salichos, Stamatakis & Rokas, 2014). However, the recorded discrepancy is more likely biological than artefactual as exemplified by *F. gummosa*. Possible hybridization and introgression resulting in discrepancy between plastid DNA and nrDNA ITS data have also been reported in several other studies of Apiaceae (Lee & Downie, 2006; Spalik, Downie & Watson, 2009; Zhou *et al.*, 2009; Bone *et al.*, 2011; Yi, Jin & Wen, 2015; Banasiak *et al.*, 2016).

Our results indicate that most intense reticulate evolution occurs among the species constituting clades A, E, G, H, I, K and L in ITS and combined data trees. These species along with members of clades B and C2 form a weakly supported superclade in plastid DNA trees (BS < 50%, PP = 0.99) comprising mostly Irano-Turanian species. Relationships in this superclade in the plastid DNA trees are fundamentally different from those occurring in the nrDNA trees. Conspecific samples more often group in the nrDNA trees than in the plastid DNA trees. The examples include *F. assa-foetida*, *F. szowitsiana* DC., *F. gummosa*, *F. samarkandica* Korovin, *F. hedgeana* Pimenov & Kljuykov *et cetera*. Moreover, the number of unique ITS sequences is rather low and several groups of species each have the same ribotype, particularly in clades A, G and H. This suggests that the exceptionally high number of species recognized in the genus is an artefact resulting from hybridization and taxonomic splitting rather than from diversification in the region. Intense reticulate evolution in *Ferula* explains why proposing an unambiguous hierarchical classification system of the genus is almost impossible. Hybridization and introgression blur boundaries among species and confound phylogenetic reconstructions, particularly when distant species exchange genetic material.

Newly described species may represent local hybrid swarms, and inadequate sampling, particularly from Central and West Asia, does not allow for a precise delimitation of taxa.

TOWARDS A NEW CLASSIFICATION SYSTEM

There is no updated and comprehensive classification system of the genus. The most important regional and worldwide treatments of *Ferula* comprising infrageneric taxa include: (1) a revision for the *Flora Orientalis* (Boissier, 1872); (2) a synopsis of the genus in Engler and Prantl's *Die natürlichen Pflanzenfamilien* (Drude, 1898); (3) the taxonomic monograph of the genus (Korovin, 1947); (4) a regional treatment for Kazakhstan (Safina & Pimenov, 1984). Korovin's classification system of *Ferula* was adopted with some amendments in subsequent regional revisions for the former Soviet Union (Korovin, 1951), Turkey and the East Aegean islands (Peşmen, 1972) and the *Flora Iranica* region (Chamberlain & Rechinger, 1987). None of these treatments agrees with molecular phylogenetic trees (Kurzyńska-Miñnik *et al.*, 2008; Panahi *et al.*, 2015; this study).

In accordance with our inference of a highly reticulated scenario of evolution in *Ferula*, when proposing a new classification system of the genus, a strict concept of monophyly, particularly in the Irano-Turanian superclade, would be misleading. This superclade is monophyletic in plastid DNA trees and collapses into a polytomy in the ITS and combined trees (Fig. 3). We recognize it as subgenus *Narthex* (Falc.) Drude with eight sections corresponding to clades A, B, E, G, H, I, K and L. In contrast, clades J, D and C1 are supported by both datasets and constitute early-diverging branches in plastid DNA trees. Accordingly, we recognize them as subgenera *Sinoferula*, *Safinia* and *Ferula*. In the latter, we also include clade C2, the affinity of which to C1 is supported by nrDNA and morphology. Below, we discuss these clades in the order from the bottom to the top of the combined tree (Fig. 3).

Clade J: subgenus Sinoferula

The monophyly of this small group is supported in all analyses (BS = 100% and PP = 1.0 in combined trees), but it has not hitherto been recognized as a natural taxon. Its included *F. licentiana* Hand.-Mazz., *F. kingdon-wardii* H. Wolff and *F. olivacea* (Diels) H. Wolff ex Hand.-Mazz. that are endemic to China and have one distinctive common feature: they are glabrous throughout, in contrast to all other Chinese congeners that are hispid, pubescent or puberulent at least on the abaxial side of leaves (She & Watson, 2005). Numerous Irano-Turanian congeners also lack indumentum

(Chamberlain & Rechinger, 1987) but the geographical ranges of these groups do not overlap. The fruits of *F. kingdon-wardii* and *F. olivacea* have prominent dorsal and lateral ribs and single vallecular and two commissural vittae, which are septate (Wang *et al.*, 2016). Similar fruits occur in *F. bungeana* Kigatawa, which was not included in our molecular analyses due to incomplete availability of plastid markers. Based on the nrDNA ITS sequence, this species is allied with clade A. The plant is, however, densely pubescent. Septate vittae also occur in clade C, mostly comprising Mediterranean taxa (Safina & Pimenov, 1990).

Clade D: subgenus Safinia

Although three species of clade D were identified by *compat.py* as introducing topological conflict, these discrepancies concerned within-group relationships and the clade was strongly supported in trees inferred from combined data (BS = 93%, PP = 1.0). It encompasses Central Asian endemics with the notable exception of *F. hezarlalehzarica* Ajani from Iran. Clade D has never been recognized as a separate taxon; however, it is reasonably well corroborated by fruit anatomy. Fruits of *F. equisetacea* Koso-Pol., *F. koso-poljanskyi* Korovin, *F. grigorievii* B.Fedtsch. and *F. decurrens* Korovin are characterized by a subcircular disposition of vittae, a development of a subepidermal layer of the ducts (these may, however, be obsolete in mature fruits of *F. grigorievii*), and the parenchymatic ribs, hypendocarp and funicle (Safina & Pimenov, 1990). However, this combination of characters is not unique in the genus as *F. lipskyi* Korovin is similar to these species (Safina & Pimenov, 1990; Safina, Ostroumova & Pimenov, 2014). *Ferula lipskyi* was placed in clade A based on ITS sequence only (Kurzyna-Mlynik *et al.*, 2008). Additional sampling from this species is necessary to confirm its distant position from the species of clade D. The similarity of *F. hindukushensis* Kitam. to *F. koso-poljanskyi* was noticed by Chamberlain & Rechinger (1987). When describing *F. hezarlalehzarica* from the Hezar and Lalezar mountains in Iran, Ajani & Ajani (2008) indicated the two former species as its closest relatives. The name of this subgenus honours botanist Lucia K. Safina for her contribution to the taxonomy of the genus.

Clade C: subgenus Ferula

This group includes two distinct subclades, C1 and C2, that have each strong support from posterior probability and bootstrap analyses, but their sister relationship is supported only in nrDNA analyses. Seven species that form clade C1 are mostly Mediterranean, including *F. communis* L., the type species of the genus. This group has long been regarded as natural, although some distantly related taxa were also usually included.

Safina & Pimenov (1990) investigated fruit anatomy of 16 representatives of subgenus *Ferula sensu* Korovin (1947) and concluded that *F. communis*, *F. tingitana* L., *F. glauca* L. and *F. linkii* Webb form a distinct group characterized by septate vittae, narrow and tangentially stretched elements of hypendocarp and a moderate sclerification of the pericarp. The most widely distributed member of this clade, *F. communis*, is diversified into local taxa that are often recognized as distinct species (Dettori *et al.*, 2016).

Based on plastid data, *F. stenocarpa*, the only representative of clade C in Iran, is closer to other Irano-Turanian taxa than to its Mediterranean relatives. Therefore, it may be of hybrid origin. This species is morphologically similar to its congeners from clade C, and this affinity has been generally recognized (Boissier, 1872; Korovin, 1947). It differs from other members of this clade in having distinctly smaller fruits, 4 mm long, as opposed to 8–18 mm (Korovin, 1947; Chamberlain & Rechinger, 1987). Based on phylogenetic analyses of ITS data, two Anatolian endemics not included in this study, *F. coskunii* H.Duman & Sağıroğlu and *F. mervynii* Sağıroğlu & H.Duman, also belong to clade C (Elibol *et al.*, 2012). Our preliminary analyses using ITS data place them close to *F. stenocarpa* (unpubl. data). These species have also smaller fruits than the Mediterranean congeners: 4–5 mm long in *F. mervynii* (Sağıroğlu & Duman, 2007) and 5–9 mm in *F. coskunii* (Duman & Sağıroğlu, 2005). We therefore recognize clades C1 and C2 as sections *Ferula* and *Stenocarpa*, comprising Mediterranean and Irano-Turanian taxa, respectively.

Irano-Turanian group: subgenus Narthex

The Irano-Turanian group includes the type species of several subgeneric names: *Narthex* (Falc.) Drude, *Scorodosma* (Bunge) Drude, *Soranthus* (Ledeb.) Drude, *Euryangium* (Kauffm.) Drude, *Peucedanoides* (Boiss.) Korovin, *Merwia* (B.Fedtsch.) Korovin and *Dorematooides* Korovin. The first four names were published simultaneously by Drude (1898), so none has priority. We therefore select *Narthex*. Drude (1898) and Korovin (1947) indicated *F. narthex* as its type, which is incorrect. Falconer (1847) described *Narthex* as a genus with a single species, *Narthex assa-foetida* ('*asafoetida*'), including Linnaean *F. assa-foetida* in synonymy. The type specimen of *F. assa-foetida* is Kaempfer's specimen from Disguun (Chamberlain & Rechinger, 1987). Falconer claimed that his *Narthex assa-foetida* was the true *asafoetida* of Kaempfer. He was, however, mistaken and described a new species. Boissier (1872) renamed this species as *F. narthex* Boiss. suggesting its placement in section *Peucedanoides* Boiss. *Narthex assa-foetida* is nevertheless synonymous with *F. assa-foetida*, not *F. narthex*.

Clade L: section Glaucoselinum (Schischk.) Pimenov

The similarity between the two species in this clade was first noticed by Korovin (1962), who segregated them from *Peucedanum* L. into *Talassia* Korovin. Pimenov (1983) transferred them to *Ferula* and placed them in a separate section but without subgeneric assignment and repeated this treatment in the subsequent revision of the genus for Kazakhstan (Safina & Pimenov, 1984). Because the validity of Korovin's *Talassia* was problematic, Pimenov (1983) used a sectional name *Glaucoselinum* that was first introduced in *Peucedanum*.

Clade E: section Macrorrhiza Korovin

This group includes five species occurring in the Irano-Turanian region; all species are glabrous (Chamberlain & Rechinger, 1987). Its support in the combined ML analyses was rather weak (BS = 69%), which is not surprising because two inclusive species were placed in significantly distant positions in plastid DNA trees. Three representatives of this clade (*F. oopoda* (Boiss. & Buhse) Boiss., *F. clematidifolia* Koso-Pol. and *F. varia* Trautv.) have been subject to carpological studies and have similar mericarp anatomy identified as type VIII (Safina & Pimenov, 1984). However, superficially similar fruits occur in *F. korshinskyi* Korovin, a member of clade A.

Clade B: section Soranthus

Ferula karelinii Bunge and *F. sibirica* Willd. form a strongly supported clade in combined analyses (BS = 100%, PP = 1.0). These species were excluded from the genus by Korovin (1947) and recognized in monospecific genera *Schumannia* Kuntze and *Soranthus* Ledeb., particularly in the regional treatments for the former Soviet Union and Asia, even in some relatively recent accounts (Schischkin, 1951; Vinogradova, 2004; She *et al.*, 2005). Their fruit morphology and anatomy lies, however, in the diversity range of *Ferula* (Pimenov & Kirillina, 1980). Both species have fruit type III based on the typology of Safina & Pimenov (1984); these authors placed *F. karelinii* in section *Merwia* (B. Fedtsch.) Koso-Pol., comprising species with fruit type II, while retaining *F. sibirica* in the monotypic section *Soranthus*.

Clade A: section Peucedanoides

This large clade encompasses species occurring in Central Asia and in the south-western part of the Irano-Turanian region. It includes all species of former *Dorema* examined for molecular markers (Panahi *et al.*, 2015) and nearly all examined species of subgenera *Peucedanoides* and *Dorematoides* that are,

however, intermingled. Its monophyly was reasonably well supported in combined analyses (BS = 88%, PP = 1.0), but most internal nodes received BS < 50%. Its two major subclades in the combined tree received no support in ITS and plastid DNA analyses.

Species formerly classified in *Dorema* have simple umbels that are arranged laterally on a flowering stem, whereas most umbellifers have compound umbels that terminate the growth of the stem. The umbels of species classified by Korovin (1947) in *Dorematoides* are proliferating; a new umbel grows in place of the central umbellule forming a pseudoverticillate inflorescence and restoring its monopodial growth. This type of inflorescence can be seen as transitory between terminal compound umbels characteristic for most *Ferula* spp. and lateral simple umbels occurring in former *Dorema*.

Korovin (1939) attributed the name *Dorematoides* to Regel and Schmalhausen. In fact, this name was introduced by Regel (1878) as group 'E. *Doremoides*' in a key to some *Ferula* spp. (with letters identifying successive groups) and repeated in a similar context in a subsequent paper (Regel & Schmalhausen, 1878). '*Doremoides*' included only *F. schtschurowskiana* Regel & Schmalh. ex Regel (*F. tschzurowskiana*). The name was unranked and without any authorship in contrast to some formal names listed in these papers. Moreover, in Regel (1878), a parallel group in 'Sectio II. *Asa foetida*' was named '*D. Juga vittata*', subsequently changed to '*D. Jugivittata*' in Regel & Schmalhausen (1878), suggesting that these groups were intended as informal and hence not validly published. Korovin (1947) corrected the name to *Dorematoides* taking into account the declension of Greek noun *dorema*, *dorematos* ('gift') and provided a Latin diagnosis. Therefore, he should be regarded as the sole author of the name at the subgeneric rank. Such an interpretation solves the problem of its typification. Vinogradova (2004) lectotypified 'section *Dorematoides* Regel & Schmalh.' with *F. caspica* M. Bieb. However, neither Regel (1878) nor Regel & Schmalhausen (1878) included *F. caspica* in group '*Doremoides*', but indicated *F. schtschurowskiana* as its sole element. Korovin (1947) placed six species in subgenus *Dorematoides* indicating the proliferating umbels as their major feature, which makes these species somewhat similar to *Dorema*. In molecular analyses, *F. schtschurowskiana* was placed in clade G, whereas *F. caspica* with two other members of subgenus *Dorematoides* [*F. feruloides* (Steud.) Korovin and *F. dubjanskyi* Korovin] were assigned to clade A that also includes former *Dorema* spp. The choice of *F. caspica* over *F. schtschurowskiana* better preserves the original meaning of *Dorematoides sensu* Korovin. Since *Dorematoides* was described by Korovin as a subgenus, the author of the combination at sectional rank is Vinogradova (2004), who published it inadvertently when citing Korovin's original name with full reference.

Clade K: section Pachycarpa

The core of clade K, *F. gigantea* B.Fedtsch. and *F. trachyphylla* Rech.f. & Riedl, was supported in all analyses. In ITS and combined ML analyses, *F. diversivittata* Regel & Schmalh. joined this group. *Ferula gigantea* and *F. diversivittata* were placed by Korovin (1947) in an unranked group (*grex*) *Pachycarpa*. *Ferula trachyphylla* was described from Afghanistan and regarded as closely allied to *F. gigantea* (Chamberlain & Rechinger, 1987), and this is confirmed by molecular data. We designate *F. gigantea* as the lectotype of *Pachycarpa* and use this name as the basionym for the sectional combination.

Clade I: section Euryangium (Kauffm.) Pimenov

The support for the monophyly of this group comes exclusively from nrDNA ITS data. Having been transferred to *Ferula* from *Euryangium* Kauffm., *F. sumbul* (Kauffm.) Hook.f. has usually been recognized in a separate subgenus (Drude, 1898), section (Pimenov, 1979) or informal *grex* (Korovin, 1947) with one or two presumed close relatives, the affinity of which has not been confirmed by molecular data. The fruit anatomy of *F. sumbul* is distinct (Safina & Pimenov, 1983); however, the other species from this clade have not yet been examined for fruit anatomical characters.

Clade G: section Scorodosma (Bunge) Boiss

Molecular data place 18 Central Asian and Iranian species in this clade. Although its monophyly is strongly supported in ITS and combined analyses (BS = 91%, PP = 1.0), the relationships among included species are poorly resolved. Many terminals were identified by compat.py as introducing topological conflict, and in the plastid DNA trees they were intermingled with some members of clade H. Conspecific samples of *F. hedgeana* and *F. samarkandica* grouped only in ITS analyses, whereas they were intermingled with other species in plastid and combined analyses. This clade includes *F. foetida* (Bunge) Regel, which had been regarded by Korovin (1947) and Chamberlain & Rechinger (1987) as having an isolated position in the genus and constituting a monospecific subgenus *Scorodosma* (Bunge) Drude. However, Safina & Pimenov (1984) suggested that its relatives include *F. iliensis* Krasn. ex Korovin and *F. teterrima* Kar. & Kir., the affinity of which is confirmed by our analyses.

Clade H: section Merwia

Species placed in clade H occur in the south-western part of the Irano–Turanian floristic region. Our results suggest that this group is subject to intense reticulate

evolution blurring the boundaries among species. This is particularly troublesome because this group comprises several economically important species including *F. assa-foetida* and *F. gummosa*.

The identity of *F. assa-foetida* is contentious. A possible source of confusion is that the exudate asafoetida is harvested from several *Ferula* spp., not only representatives of clade H, e.g. *F. alliacea* Boiss., *F. persica* Willd. and *F. narthex*, but also distant relatives including *F. foetida* (Drude, 1898; Korovin, 1959). Linnaean *F. assa-foetida* was based on material collected by Kaempfer. Chamberlain & Rechinger (1987) compared the plate in Kaempfer (1712) and the type specimen in the Sloane herbarium at BM with the available herbarium material and identified only two gatherings that are conspecific with the type. One of these is Davis & Bokhari D.56275; this specimen was also used in our molecular studies (Number 0359). In nrDNA trees, it was placed close to *F. pseudalliacea* Rech.f., in agreement with Chamberlain & Rechinger (1987), who regarded *F. assa-foetida* and *F. pseudalliacea* as closely allied and possibly conspecific. Three other samples of *F. assa-foetida* grouped with *F. alliacea*, *F. gabrielii* and other species harvested for asafoetida (Chamberlain & Rechinger, 1987).

The examined molecular markers provide conflicting results concerning the affinities of *F. gummosa*, a source of valuable galbanum. This oleo-gum-resin is subject to adulteration as the demand is higher than its production in Iran (Betti et al., 2010). Presumed adulterants include *F. assa-foetida* and *F. ammoniacum* (D. Don) Spalik et al. (Thomsen et al., 2004). Therefore, a clear circumscription of *F. gummosa* is also of considerable economic importance. In our analyses, all samples of *F. gummosa* have the same ITS sequence, which is also identical to those of *F. badrakema* Koso-Pol., *F. linczevskii* Korovin, *F. undulata* Pimenov & J.V. Baranova, and *F. myrioloba* Rech.f. In the plastid DNA tree, however, the representatives of *F. gummosa* were placed in three distant clades, sometimes with their companions from the ITS tree. These results suggest that interspecific boundaries in this group are unclear; therefore, presumed adulterants as assessed through phytochemical studies may reflect a hybrid origin of harvested populations. Chamberlain & Rechinger (1987) suggested a close proximity of *F. gummosa*, *F. myrioloba* and *F. badrakema*, which is confirmed by molecular trees. Pimenov & Kljuykov (1996) synonymized *F. badrakema* under *F. gummosa* and also reinstated *F. galbaniflua* Boiss. & Buhse in this group. Chamberlain & Rechinger (1987) regarded *F. latisecta* Rech.f. & Aellen and *F. undulata* as closely related if not conspecific, also in agreement with molecular data. However, they placed *F. latisecta* and *F. gummosa* in separate infrageneric divisions.

CONCLUSIONS

The main aim of our study, to divide *Ferula* into smaller, monophyletic and workable units that may be subject to partial revisions, has only partly been accomplished. Although subgenera *Sinoferula*, *Safinia* and *Ferula* seem to be natural units, the most species-rich subgenus *Narthex* is difficult to resolve, possibly due to reticulate evolution blurring boundaries among species and sections. Our study suggests that multigene approach will be necessary to elucidate hybridization patterns and circumscribe species in this economically important genus.

TAXONOMIC TREATMENT

Ferula L., *Sp. Pl.* 1: 246. 1753.

Lectotype: *Ferula communis* L. (Hitchcock & Green, 1929: 140).

Subgenus Sinoferula Spalik, Puchałka & M.Panahi, *subgen. nov.*

Diagnosis: Plants entirely glabrous, endemic to China.

Etymology: from *sino-*, ‘Chinese’, and *Ferula*.

Type: *Ferula kingdon-wardii* H.Wolff.

Species included: *F. kingdon-wardii* H.Wolff, *F. licentiana* Hand.-Mazz., *F. olivacea* (Diels) H.Wolff ex Hand.-Mazz.

Subgenus Safinia Spalik, M.Panahi & Puchałka, *subgen. nov.*

Diagnosis: Subepidermal layer of secretory ducts well developed in mericarp including marginal ribs (wings); ribs, hypendocarp and funicle parenchymatic. *Etymology*: dedicated to Lucia K. Safina to acknowledge her contribution to the taxonomy of *Ferula*.

Type: *Ferula equisetacea* Koso-Pol.

Species included: *F. decurrens* Korovin, *F. equisetacea* Koso-Pol., *F. grigorievii* B.Fedtsch., *F. hezarlalezharica* Ajani, *F. hindukushensis* Kitam., *F. koso-poljanskyi* Korovin.

Subgenus Ferula

Section Ferula

≡ section *Anatriches* Korovin, Ill. Monogr. *Ferula* 9. 1947; lectotype (designated here): *Ferula communis* L.

Species included: *F. communis* L., *F. glauca* L., *F. linkii* Webb, *F. loscosii* (Lange) Willk., *F. marmarica*

Asch. & Taub. ex Asch. & Schweif., *F. sinaica* Boiss., *F. tingitana* L.

Section Stenocarpa Puchałka & Spalik, *sect. nov.*

Diagnosis: Fruit c. 4–9 mm long (as opposed to 8–18 mm in section *Ferula*). Irano–Turanian.

Etymology: from the specific epithet of its type.

Type: *Ferula stenocarpa* Boiss. & Hausskn. ex Boiss.

Species included: *F. coskunii* H.Duman & Sağıroğlu, *F. mervynii* Sağıroğlu & H.Duman, *F. stenocarpa* Boiss. & Hausskn. ex Boiss.

Subgenus Narthex (Falc.) Drude in Engl. & Prantl, *Nat. Pflanzenfam.* 3(8): 229. 1898.

Type: *Narthex assa-foetida* (L.) Falc. (≡ *F. assa-foetida* L.).

= subgenus *Merwia* (B. Fedtsch.) Korovin, Ill. Monogr. *Ferula* 8. 1947. *Type*: *Merwia androssowii* B. Fedtsch. (= *F. litwinowiana* Koso-Pol.).

= subgenus *Scorodosma* (Bunge) Drude in Engl. & Prantl, *Nat. Pflanzenfam.* 3(8): 230. 1898. *Type*: *Scorodosma foetidum* Bunge (≡ *F. foetida* (Bunge) Regel).

= subgenus *Soranthus* (Ledeb.) Drude in Engl. & Prantl, *Nat. Pflanzenfam.* 3(8): 230. 1898. *Type*: *Soranthus meyeri* Ledeb. (= *F. sibirica* Willd.).

= subgenus *Peucedanoides* (Boiss.) Korovin, Ill. Monogr. *Ferula* 9. 1947. *Lectotype*: *Ferula orientalis* L. (Vinogradova, 2004).

= subgenus *Dorematoides* Korovin Ill. Monogr. *Ferula* 10. 1947. *Lectotype*: *Ferula caspica* M.Bieb. (Vinogradova, 2004).

= subgenus *Euryangium* (Kauffm.) Drude in Engl. & Prantl, *Nat. Pflanzenfam.* 3(8): 230. 1898. *Type*: *Euryangium sumbul* Kauffm. (≡ *F. sumbul* (Kauffm.) Hook.f.).

Section Glaucoselinum (Schischk.) Pimenov, *Biol. Nauki* 12: 77. 1983.

Type: *Peucedanum transiliense* Herder (≡ *F. transiliensis* (Herder) Pimenov).

Species included: *F. renardii* (Regel & Schmalh.) Pimenov, *F. transiliensis* (Herder) Pimenov.

Section Macrorrhiza Korovin, Ill. Monogr. *Ferula* 10. 1947.

Lectotype (designated here): *Ferula oopoda* (Boiss. & Buhse) Boiss.

Species included: *F. clematidifolia* Koso-Pol., *F. glabra* Rech.f. & Riedl, *F. oopoda* (Boiss. & Buhse) Boiss., *F. tabasensis* Rech.f., *F. varia* Trautv.

Section Soranthus (Ledeb.) Pimenov in Safina & Pimenov, *Feruly Kazakhstana* 37. 1984.

Type: *Soranthus meyeri* Ledeb. (= *F. sibirica* Willd.).

Species included: *F. karelinii* Bunge, *F. sibirica* Willd.

Section Peucedanoides Boiss., *Fl. Or.* 2: 983. 1872.

Lectotype: *Ferula orientalis* L. (Vinogradova, 2004)
= section *Xeronarthex* Korovin, Ill. Monogr. *Ferula* 9. 1947. Lectotype (designated here): *Ferula orientalis* L.

= section *Dorematooides* (Korovin) V.M.Vinogr. in Tzvelev, *Fl. Vostochnoi Evropy* 11: 389. 2004. Lectotype: *Ferula caspica* M.Bieb. (Vinogradova, 2004).

Species included: *F. akitschkensis* B.Fedtsch. ex Koso-Pol., *F. ammoniacum* (D.Don) Spalik & al., *F. angreni* Korovin, *F. aucheri* (Boiss.) Piwczynski & al., *F. canescens* (Ledeb.) Ledeb., *F. caspica* M.Bieb., *F. ceratophylla* Regel & Schmalh., *F. czatkalensis* Pimenov, *F. dissecta* (Ledeb.) Ledeb., *F. downieorum* Spalik & al., *F. dshizakensis* Korovin, *F. dubjanskyi* Korovin, *F. fedoroviorum* Pimenov, *F. ferganensis* Lipsky ex Korovin, *F. feruloides* (Steud.) Korovin, *F. glabrifolia* M.Panahi & al., *F. gracilis* (Ledeb.) Ledeb., *F. gypsacea* Korovin, *F. haussknechtii* H.Wolff ex Rech.f., *F. hyrcana* (Koso-Pol.) Puchalka & al., *F. karatavica* Regel & Schmalh., *F. karataviensis* (Regel & Schmalh.) Korovin ex Pavlov, *F. karategina* Lipsky ex Korovin, *F. kashanica* Rech.f., *F. kirialovii* Pimenov, *F. kopetdaghensis* Korovin, *F. korshinskyi* Korovin, *F. kyzylkumica* Korovin, *F. lapidosa* Korovin, *F. leucographa* Korovin, *F. macrocolea* Boiss., *F. michaelii* M.Panahi & al., *F. microcolea* (Boiss.) Boiss., *F. nuda* Spreng., *F. olgae* Regel & Schmalh., *F. orientalis* L., *F. ovina* (Boiss.) Boiss., *F. pallida* Korovin, *F. penninervis* Regel & Schmalh., *F. potaninii* Korovin ex Pavlov, *F. rigidula* Fisch. ex DC., *F. rubroarenosa* Korovin, *F. seravschanica* Pimenov & J.V.Baranova, *F. serpentinica* Rech.f., *F. songarica* Pall. ex Schult., *F. syreitschikowii* Koso-Pol., *F. tatarica* Fisch. ex Spreng., *F. tschimganica* Lipsky ex Korovin, *F. ugamica* Korovin ex Baranov, *F. xylorhachis* Rech.f.

Section Pachycarpa (Korovin) Banasiak, M.Panahi & Spalik, *comb. & stat. nov.*

Basionym: *Ferula* grex *Pachycarpa* Korovin, Ill. Monogr. *Ferula* 8. 1947.

Lectotype (designated here): *Ferula gigantea* B.Fedtsch.

Species included: *F. diversivittata* Regel & Schmalh.,

F. gigantea B.Fedtsch., *F. trachyphylla* Rech.f. & Riedl.

Section Euryangium (Kauffm.) Pimenov, *Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol* 84: 107. 1979.

Type: *Euryangium sumbul* Kauffm. (= *F. sumbul* (Kauffm.) Hook.f.).

Species included: *F. fedtschenkoana* Koso-Pol., *F. nuratavica* Pimenov, *F. sumbul* (Kauffm.) Hook.f.

Section Scorodosma (Bunge) Boiss., *Fl. Or.* 2: 983. 1872.

Type: *Scorodosma foetidum* Bunge (= *F. foetida* (Bunge) Regel).

= section *Palaeonarthex* Korovin, Ill. Monogr. *Ferula* 9. 1947. Lectotype: *Ferula foetidissima* Regel & Schmalh. (Pimenov & Baranova, 1979).

= section *Phyllites* Korovin, Ill. Monogr. *Ferula* 9. 1947. Lectotype (designated here): *Ferula kokanica* Regel & Schmalh.

= section *Saprosmia* Korovin, Ill. Monogr. *Ferula* 8. 1947. Lectotype (designated here): *Ferula kelifi* Korovin.

Species included: *F. botschantzevii* Korovin, *F. costata* Korovin ex Nasir, *F. eugenii* Kamelin, *F. foetida* (Bunge) Regel, *F. foetidissima* Regel & Schmalh., *F. hedgeana* Pimenov & Kljukov, *F. iliensis* Krasn. ex Korovin, *F. inciso-serrata* Pimenov & J.V.Baranova, *F. kelifi* Korovin, *F. kelleri* Koso-Pol., *F. kokanica* Regel & Schmalh., *F. mogoltavica* Lipsky ex Korovin, *F. nevskii* Korovin, *F. samarkandica* Korovin, *F. schtschurowskiana* Regel & Schmalh. ex Regel, *F. teterrima* Kar. & Kir., *F. violacea* Korovin.

Section Merwia (B. Fedtsch.) Koso-Pol., *Byull. Obshch. Estetvoisp. Voronezhsk. Gosud. Univ.* 1: 38. 1925.

Type: *Merwia androssowii* B. Fedtsch. (= *F. litwinowiana* Koso-Pol.).

= section *Discicarpa* Korovin, Ill. Monogr. *Ferula* 8. 1947. Lectotype (designated here): *Ferula litwinowiana* Koso-Pol.

= section *Neonarthex* Korovin, Ill. Monogr. *Ferula* 9. 1947. Lectotype (designated here): *Ferula galbaniflua* Boiss. & Buhse (= *F. gummosa* Boiss.).

= section *Phacocarpa* Korovin, Ill. Monogr. *Ferula* 8. 1947. Lectotype (designated here): *Ferula lehmannii* Boiss.

Species included: *F. alliacea* Boiss., *F. assa-foetida* L., *F. badrakema* Koso-Pol., *F. behboudiana* (Rech.f. & Esfand.) D.F.Chamb., *F. blanchei* Boiss., *F. flabelliloba* Rech.f. & Aellen, *F. gabrielii* Rech.f., *F. gummosa* Boiss., *F. hirtella* Boiss., *F. karakalensis* Korovin, *F. latisecta*

Rech.f. & Aellen, *F. lehmannii* Boiss., *F. linczevskii* Korovin, *F. litwinowiana* Koso-Pol., *F. myrioloba* Rech.f., *F. narthex* Boiss., *F. negevensis* Zohary, *F. persica* Willd., *F. pseudalliacea* Rech.f., *F. racemosoumbellata* (Gilli) Rech.f., *F. rutbaensis* C.C.Towns., *F. sharifii* Rech.f. & Esfand., *F. stenoloba* Rech.f., *F. szowitsiana* DC., *F. undulata* Pimenov & J.V. Baranova.

Incertae sedis: *F. kuhistanica* Korovin.

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REFERENCES

- Aberer AJ, Krompass D, Stamatakis A. 2013.** Pruning rogue taxa improves phylogenetic accuracy: an efficient algorithm and webservice. *Systematic Biology* **62**: 162–166.
- Ajani Y, Ajani A. 2008.** A new species of *Ferula* (Umbelliferae) from southern Iran. *Edinburgh Journal of Botany* **65**: 425–431.
- Banasiak Ł, Wojewódzka A, Baczyński J, Reduron J-P, Piwczyński M, Kurzyna-Młynik R, Gutaker R, Czarnocka-Cieciura A, Kosmala-Grzechnik S, Spalik K. 2016.** Phylogeny of Apiaceae subtribe Daucinae and the taxonomic delineation of its genera. *Taxon* **65**: 563–585.
- Betti G, Casabianca H, Schmidt M, Thomsen M, Omidbaigi R. 2010.** *Ferula gumosa*: study on the phytochemical variability and the occurrence of adulterations. *Iranian Journal of Pharmaceutical Research* **3**: 9.
- Boissier PE. 1872.** Umbelliferae. In: *Flora Orientalis*, Vol. 2. Geneva, Basel: H. Georg, 819–1090.
- Bone TS, Downie SR, Affolter JM, Spalik K. 2011.** A phylogenetic and biogeographic study of the genus *Lilaeopsis* (Apiaceae tribe Oenantheae). *Systematic Botany* **36**: 789–805.
- Calviño CI, Tilney PM, Wyk BE, Downie SR. 2006.** A molecular phylogenetic study of southern African Apiaceae. *American Journal of Botany* **93**: 1828–1847.
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009.** trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics (Oxford, England)* **25**: 1972–1973.
- Chamberlain DF, Rechinger KH. 1987.** *Ferula* L. In: Hedge IC, Lamond JM and Rechinger KH, eds. *Umbelliferae, Flora Iranica*, Vol. 162. Graz: Akademische Druck- und Verlagsanstalt, 387–426.
- Czerepanov SK. 1995.** *Vascular plants of Russia and adjacent states*. Cambridge: Cambridge University Press.
- Dettori CA, Loi MC, Brullo S, Arguimbau PFi, Tamburini E, Bacchetta G. 2016.** The genetic diversity and structure of the *Ferula communis* L. complex (Apiaceae) in the Tyrrhenian area. *Flora* **223**: 138–146.
- Drude CGO. 1898.** Umbelliferae. In: Engler A and Prantl K, eds. *Die natürlichen Pflanzenfamilien*, Vol. 3 (8). Leipzig: Wilhelm Engelmann, 63–250.
- Duman H, Sağıroğlu M. 2005.** A new species of *Ferula* (Apiaceae) from south Anatolia, Turkey. *Botanical Journal of the Linnean Society* **147**: 357–361.
- Elibol Z, Menemen Y, Sağıroğlu M, Duman H. 2012.** A molecular phylogenetic study on some Turkish *Ferula* L. (Apiaceae) species using nrDNA ITS sequences. *Pakistan Journal of Botany* **44**: 589–594.
- Falconer H. 1847.** Description of the asafoetida plant of Central Asia. *Transactions of the Linnean Society of London* **20**: 285–291.
- Hitchcock AS, Green ML. 1929.** Standard-species of Linnean genera of Phanerogamae (1753–54). In: *International Botanical Congress, Cambridge (England) 1930. Nomenclature. Proposals by British botanists*. London: Wyman & Sons, 111–199.
- Kaempfer E. 1712.** *Amoenitatum exoticarum*. Lemgo: Meyer.
- Kauff F, Lutzoni F. 2002.** Phylogeny of the Gyalectales and Ostropales (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. *Molecular Phylogenetics and Evolution* **25**: 138–156.
- Kauff F, Lutzoni F. 2003.** *Compat.py – a program to detect topological conflict between supported clades in phylogenetic trees*. Available at: <http://lutzonilab.org/downloadable-programs/>
- Keane TM, Creevey CJ, Pentony MM, Naughton TJ, McInerney JO. 2006.** Assessment of methods for amino acid matrix selection and their use on empirical data shows that *ad hoc* assumptions for choice of matrix are not justified. *BMC Evolutionary Biology* **6**: 29.
- Korovin EP. 1939.** Glavneishie linii v sistematike roda *Ferula* (Tourn.) L. *Bulletin de la Societe des Naturalistes de Moscou, s. biologique*. **48**: 5–15.
- Korovin EP. 1947.** *Generis Ferula (Tourn.) L. monographia illustrata*. Tashkent: Academiae Scientiarum UzRSS.
- Korovin EP. 1951.** *Ferula* L. In: Schischkin BK, ed. *Flora SSSR*, Vol. 17. Moscow, Leningrad: Izdatelstvo Akademii Nauk SSSR, 62–142.
- Korovin EP. 1959.** *Ferula* L. In: Vvedensky AI, ed. *Flora Uzbekistana*, Vol. 4. Tashkent: Izdatelstvo Akademii Nauk Uzbekskoi SSR, 399–438.
- Korovin EP. 1962.** Novye rody i vidy iz semeistva zontichnyh flory Kazakhstana. *Trudy Instituta Botaniki, Akademiya Nauk Kazakhskoi SSR* **13**: 242.
- Kurzyna-Młynik R, Oskolski AA, Downie SR, Kopacz R, Wojewódzka A, Spalik K. 2008.** Phylogenetic position of the genus *Ferula* (Apiaceae) and its placement in tribe Scandiceae as inferred from nrDNA ITS sequence variation. *Plant Systematics and Evolution* **274**: 47–66.

- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B.** 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34**: 772–773.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG.** 2007. Clustal W and Clustal X version 2.0. *Bioinformatics (Oxford, England)* **23**: 2947–2948.
- Lee C-S, Downie SR.** 2006. Phylogenetic relationships within *Cicuta* (Apiaceae tribe Oenantheae) inferred from nuclear rDNA ITS and cpDNA sequence data. *Canadian Journal of Botany* **84**: 453–468.
- Leigh JW, Susko E, Baumgartner M, Roger AJ.** 2008. Testing congruence in phylogenomic analysis. *Systematic Biology* **57**: 104–115.
- Maddison WP, Maddison DR.** 2017. *Mesquite: a modular system for evolutionary analysis. Version 3.2.* Available at: <http://mesquiteproject.org>.
- Mozaffarian V.** 2007. Umbelliferae. In: Assadi M, Maassoumi AAR, Khatamsaz M, eds. *Flora of Iran, Vol. 54.* Tehran: Research Institute of Forests and Rangelands, 1–596.
- Panahi M, Banasiak Ł, Piwczyński M, Puchalka R, Oskolski AA, Spalik K.** 2015. Phylogenetic relationships among *Dorema*, *Ferula* and *Leutea* (Apiaceae, Scandiceae, Ferulinae) inferred from nrDNA ITS and cpDNA noncoding sequences. *Taxon* **64**: 770–783.
- Peşmen H.** 1972. *Ferula* L. In: Davis PH, ed. *Flora of Turkey and the East Aegean Islands, Vol. 4.* Edinburgh: Edinburgh University Press, 440–453.
- Pimenov MG.** 1979. Obzor vidov sektiis *Euryangium* (Kauffm.) M.Pimen. roda *Ferula* L. *Byulleten Moskovskogo Obshchestva Ispytatelei Prirody Otdel Biologicheskii* **84**: 106–111.
- Pimenov MG.** 1983. Sektsia *Glaucoselinum* (Schischk.) M.Pimen. roda *Ferula* L. (Umbelliferae). *Biologicheskii Nauki* **12**: 74–79.
- Pimenov MG, Baranova IV.** 1979. A synopsis of species of the section *Palaonarthex* Korov. genus *Ferula* L. (in Russ.). *Byulleten Moskovskogo Obshchestva Ispytatelei Prirody Otdel Biologicheskii* **84**: 82–92.
- Pimenov MG, Kirillina NA.** 1980. The carpology of *Soranthus*, *Ladyginia*, *Eriosynaphe* and *Schumannia* in connection with the problem of the taxonomic limits of the genus *Ferula* (Apiaceae). *Botanicheskii Zhurnal* **65**: 1756–1766.
- Pimenov MG, Kljuykov EV.** 1996. Taxonomic and nomenclatural notes on some *Ferula* (Umbelliferae) species of Iran, Turkmenistan, Tadzhikistan and Afghanistan. *Botanicheskii Zhurnal* **81**: 58–62.
- Pimenov MG, Leonov MV.** 1993. *The genera of the Umbelliferae.* Kew: Royal Botanic Gardens.
- Rambaut A, Suchard MA, Xie D, Drummond AJ.** 2014. *Tracer v1.6.* Available at: <http://beast.bio.ed.ac.uk/Tracer>
- Regel E.** 1878. Plantae regiones Turkestanicas incolentes secundum specimina sicca a Regelio et Schmalhauseno determinatae. *Trudy Imperatorskago S.-Peterburgskago Botanicheskago Sada* **5**: 577–620.
- Regel E, Schmalhausen JT.** 1878. *Ferula foetidissima* Rgl. et Schmalh. *Gartenflora* **27**: 195–199.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP.** 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- Safina LK, Ostroumova TA, Pimenov MG.** 2014. Carpology of the species of *Ferula* subgen. *Merwia* (Umbelliferae–Apiioideae) and some taxonomic implications. *Nordic Journal of Botany* **33**: 140–150.
- Safina LK, Pimenov MG.** 1983. Karpoanatomicheskiye osobennosti vidov roda *Ferula* podroda *Peucedanooides* (Apiaceae) w svyazi s sistematikoy roda. *Botanicheskii Zhurnal* **68**: 730–739.
- Safina LK, Pimenov MG.** 1984. *Feruly Kazakhstana.* Alma-Ata: Izdatelstvo “Nauka” Kazakhskoy SSR.
- Safina LK, Pimenov MG.** 1990. Carpology of the species of type subgenus of the *Ferula* and some problems of their systematics. *Feddes Repertorium* **101**: 135–151.
- Sağiroğlu M, Duman H.** 2007. *Ferula mervynii* (Apiaceae), a distinct new species from northeast Anatolia, Turkey. *Botanical Journal of the Linnean Society* **153**: 357–362.
- Salichos L, Stamatakis A, Rokas A.** 2014. Novel information theory-based measures for quantifying incongruence among phylogenetic trees. *Molecular Biology and Evolution* **31**: 1261–1271.
- Schischkin BK.** 1951. *Flora SSSR, Vol. 17.* Moscow, Leningrad: Izdatelstvo Akademii Nauk SSSR.
- Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL.** 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analyses. *American Journal of Botany* **92**: 142–166.
- She M, Pu F, Pan Z, Watson MF, Cannon JFM, Holmes-Smith I, Kljuykov EV, Phillippe LR, Pimenov MG.** 2005. Apiaceae (Umbelliferae). In: *Flora of China* Editorial Committee, ed. *Flora of China, Vol. 14.* St Louis: Missouri Botanical Garden, 1–205.
- She M, Watson MF.** 2005. *Ferula* Linnaeus. In: *Flora of China* Editorial Committee, ed. *Flora of China, Vol. 14.* St Louis: Missouri Botanical Garden, 174–180.
- Shneyer VS, Borschtschenko GP, Pimenov MG.** 1995. Immunochemical appraisal of relationships within the tribe Peucedaneae (Apiaceae). *Plant Systematics and Evolution* **198**: 1–16.
- Shneyer VS, Kutyavina NG, Pimenov MG.** 2003. Systematic relationships within and between *Peucedanum* and *Angelica* (Umbelliferae–Peucedaneae) inferred from immunological studies of seed proteins. *Plant Systematics and Evolution* **236**: 175–194.
- Spalik K, Downie SR.** 2006. The evolutionary history of *Sium sensu lato* (Apiaceae): dispersal, vicariance, and domestication as inferred from ITS rDNA phylogeny. *American Journal of Botany* **93**: 747–761.
- Spalik K, Downie SR, Watson MF.** 2009. Generic delimitations within the *Sium* alliance (Apiaceae tribe Oenantheae) inferred from cpDNA *rps16-5 trnK(UUU)* and nrDNA ITS sequences. *Taxon* **58**: 735–748.

- Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)* **30**: 1312–1313.
- Thomsen M, Schmidt M, Betti G, Casabianca H, Omidbaigi R. 2004.** *Ferula gummosa* Boiss.: phytochemical variability in Iran. International Congress on Natural Products Research. Phoenix, USA, P272.
- Vinogradova VM. 2004.** Apiaceae Lindl. (Umbelliferae Juss.). In: Tzvelev NN, ed. *Flora Vostochnoi Evropy, Vol. 11*. Moscow, St Petersburg: KMK, 315–437.
- Wang X-W, Liu M, Ru J, Wang J-R, Wang Y-T. 2016.** Morphological and anatomical characteristics of the fruit of Chinese *Ferula* and related taxa in Apiaceae [in Chinese]. *Acta Prataculturae Sinica* **25**: 81–93.
- Wen J, Zimmer EA. 1996.** Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* **6**: 167–177.
- Yi TS, Jin GH, Wen J. 2015.** Chloroplast capture and intra- and inter-continental biogeographic diversification in the Asian–New World disjunct plant genus *Osmorhiza* (Apiaceae). *Molecular Phylogenetics and Evolution* **85**: 10–21.
- Zhou J, Gong X, Downie SR, Peng H. 2009.** Towards a more robust molecular phylogeny of Chinese Apiaceae subfamily Apioideae: additional evidence from nrDNA ITS and cpDNA intron (*rpl16* and *rps16*) sequences. *Molecular Phylogenetics and Evolution* **53**: 56–68.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Bayesian majority-rule consensus tree inferred from analyses of nrDNA ITS sequence data of Ferulinae. Groups of accessions with identical sequences were represented in the analyses by single terminals and then resolved to polytomies with zero-length branches. Posterior probabilities are given along the branches. Accessions identified by *compat.py* as conflicting are marked with a different colour for each group. Major clades are bracketed; clades A–I follow [Kurzyna-Mitynik et al. \(2008\)](#). Outgroup taxa are omitted for simplicity.

Figure S2. Bayesian majority-rule consensus tree inferred from analyses of combined plastid *rps16* intron, *rpoC1* intron and *rpoB-trnC* intergenic spacer sequence data of Ferulinae. See Figure S1 for details.

Figure S3. Comparison of topologies of maximum likelihood trees inferred from nrDNA ITS data (left) and concatenated plastid DNA data (right). See [Figures 1 and 2](#) for details.

Figure S4. Bayesian majority-rule consensus tree inferred from analyses of combined nrDNA ITS and plastid *rps16* intron, *rpoC1* intron and *rpoB-trnC* intergenic spacer sequence data for Ferulinae. See [Figure S1](#) for details.

Figure S5. Maximum likelihood tree inferred from analyses of combined nrDNA ITS and plastid *rps16* intron, *rpoC1* intron and *rpoB-trnC* intergenic spacer sequence data for Ferulinae with the exclusion of 41 accessions indicated by *compat.py* as introducing topological conflict. Groups of accessions with identical sequences were represented in the analyses by single terminals and then resolved to polytomy with zero-length branches. Bootstrap support and posterior probability for nodes that also occurred in Bayesian majority-rule consensus tree are given along the branches.

Table S1. Representatives of Scandiceae subtribe Ferulinae and outgroups examined in this study with respective GenBank reference numbers. Collector's data in bold face indicate the type specimens of respective species. Newly obtained sequences are identified with an asterisk behind the reference number.