



# Phylogenetic position of *Dorema* within *Ferula* (Apiaceae)

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## Abstract

*Dorema* (Apiaceae) includes 12 species distributed in the Irano-Turanian region. The genus differs from other genera of subfamily Apioideae in having paniculate inflorescences with simple umbels. Recently, molecular phylogenetic analyses placed six species of *Dorema*, including the generitype, in *Ferula*, thus subsuming the former into the synonymy of the latter. The remaining six congeners have not been yet included in molecular analyses. Here, we fill this gap by determining the phylogenetic position of these species. Because of a low resolution in some parts of the *Ferula* tree in earlier studies, alongside standard set of markers—nuclear nrDNA ITS and plastid *rps16* and *rpoC1* introns and the *rpoB-trnC* intergenic spacer—we additionally assessed the utility of nrDNA ETS sequences in resolving infrageneric relationships in the genus. We included 43 ingroup species representing major infrageneric divisions recognised in the most recent taxonomic revision. Although we were not successful in obtaining all markers for all newly studied species, they were placed with high support in one clade within section *Peucedanoides* together with formerly studied congeners in all maximum likelihood and Bayesian analyses of nuclear, plastid, and combined data sets. The addition of the ETS marker to the combined phylogenetic analyses confirmed the most recent classification of *Ferula* with increased branch support in some parts of the tree. However, many branches still remained unresolved. In particular, the monophyly of former *Dorema* within section *Peucedanoides* as suggested by morphology has not been definitely confirmed. A new combination and two *nomina nova* are proposed.

**Keywords** Ferulinae · nrDNA ITS and ETS · *rpoB-trnC* intergenic spacer · *rpoC1* intron · *rps16* intron

## Introduction

*Dorema* D. Don (Apiaceae Lindl.) includes 12 species distributed in semi-deserts, steppes and dry mountain slopes in the Irano-Turanian region (Pimenov 1988). The genus is well distinguished from other genera of the subfamily Apioideae by the presence of paniculate inflorescences with simple umbels. Due to dorsally flattened mericarps (single-seeded parts of a fruit constituting a dispersal unit), *Dorema* was traditionally classified in the tribe Peucedaneae, while its affinity to *Ferula* L. (Drude 1898; Pimenov

& Leonov 1993) was supported due to the presence of fibrous or prosenchymatic lignified hypendocarp, i.e. the inner layer of the pericarp (Drude 1898; Korovin 1939; Pimenov 1988). The close relationship between *Dorema* and *Ferula* was subsequently confirmed by immunological studies, although the placement of these two genera in Peucedaneae Dumort. was questioned (Shneyer et al. 1995). Recent phylogenetic studies, based on nuclear and plastid markers, firmly established the position of both genera far from *Peucedanum* L. (currently a member of Selineae Spreng.) in tribe Scandiceae Spreng. and clearly showed the position of *Dorema* within *Ferula* (Ajani et al. 2008; Kurzyna-Młynik et al. 2008; Panahi et al. 2015; Piwczyński et al. 2018). In a new classification system of the genus *Ferula* (Panahi et al. 2018), the studied species of *Dorema* were placed in the section *Peucedanoides* Boiss. of subgenus *Narthex* (Falc.) Drude together with some species of *Ferula* (*F. caspica* M. Bieb., *F. feruloides* (Steud.) Korovin) having unusual arrangement of compound umbels: a central umbellet is replaced by successive compound umbel resulting therefore in pseudovercillate,

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'proliferating' inflorescence. Interestingly, the inflorescence morphology was considered one of the most important criteria in distinguishing infrageneric units in *Ferula* in its traditional taxonomic system (Korovin 1939, 1947). However, low resolution within the section *Peucedanoides* in molecular analyses allowed neither to confirm whether members of *Dorema* form a clade nor to indicate their closest relatives. Moreover, only six species of *Dorema* were molecularly analysed (Panahi et al. 2018). The remaining six were either included in *Ferula* based solely on the morphology with two species synonymised under a single species name (*F. namanganica* Sennikov  $\equiv$  *D. microcarpum* Korovin, *F. sabulosa* (Litv.) Sennikov  $\equiv$  *D. sabulosum* Litv. and  $=$  *D. karataviense* Korovin; Sennikov and Tojibaev 2021) or were left as *incertae sedis* (*D. badhysi* Pimenov, *D. balchanorum* Pimenov and *D. kopetdaghense* Pimenov).

The infrageneric relationships within *Ferula* have usually been inferred using ITS sequence variation and/or three plastid markers: *rps16* and *rpoC1* introns and the *rpoB-trnC* intergenic spacer. However, even the combined analyses left many internal parts of the phylogenetic tree of *Ferula* unresolved. Recently, Piwczyński et al. (2018) examined the utility of three potentially highly variable pDNA regions, *trnH-psbA*, *trnS-trnG*, and *atpB-rbcL* intergenic spacers, for resolving relationships within the genus *Ferula*. They found that combined pDNA markers provided a low number of parsimony informative sites, even lower than ITS alone. Moreover, pDNA and ITS phylogenetic trees were incongruent. The cause of this incongruence might be manifold, including hybridisation, a lack of a phylogenetic signal, or homoplastic substitutions (Panahi et al. 2015, 2018). These examples show that highly variable molecular markers are necessary to increase the resolution of the phylogenetic tree of the genus. One of the markers potentially having strong phylogenetic signal is nuclear ribosomal DNA external transcribed spacer (ETS) (Logacheva et al. 2010; Yi et al. 2015). This marker, likewise as ITS, is a part of the rDNA cistron, which occurs in high-copy numbers in the genome and is subject to homogenisation (Poczai and Hyvönen 2010). In Apiaceae, the ETS region has been shown to evolve faster and to have higher number of parsimony informative sites than ITS (Logacheva et al. 2010). Therefore, this marker is the most prospective candidate that may help to increase the resolution of the phylogenetic tree of *Ferula*.

The aim of our study was (1) to determine molecular phylogenetic position of all species of *Dorema* and to ascertain whether their paniculate inflorescence, a unique trait among apioid umbellifers, is synapomorphic and (2) to assess the utility of ETS sequences in resolving infrageneric relationships in *Ferula*.

## Materials and methods

### Taxon sampling

The genus *Dorema* was subsumed into *Ferula* based on molecular analysis of six species (Panahi et al. 2015) out of 12 recognised by Pimenov (1988). In spite of the morphological similarity of the unsampled species, Panahi et al. (2015) refrained from their formal inclusion in *Ferula* until their placement is confirmed with molecular data. Three of these species were recently included into *Ferula* based exclusively on their morphology (Sennikov and Tojibaev 2021). Here, we sampled herbarium material for all six *Dorema* species that have never been included in the molecular analyses: *D. badhysi*, *D. balchanorum*, *D. kopetdaghense*, *D. microcarpum* ( $\equiv$  *F. namanganica* in Sennikov and Tojibaev 2021), *D. karataviense* ( $=$  *F. sabulosa* in Sennikov and Tojibaev 2021) and *D. sabulosum* ( $\equiv$  *F. sabulosa* in Sennikov and Tojibaev 2021). For these samples, we attempted to obtain nuclear rDNA ITS and ETS, plastid *rpoB-trnC* intergenic spacer, and two plastid introns: *rps16* and *rpoC1*, which were used in previous studies of the genus (Panahi et al. 2015, 2018). We supplemented these sequences with ITS and plastid data for 36 species of *Ferula* (39 accessions) and a representative of *Leutea* Pimenov from GenBank representing all infrageneric groups recognised by Panahi et al. (2018) and additionally sequenced ETS marker for these accessions. Finally, our matrix consisted of 43 ingroup taxa (46 accessions) and two outgroup species from subtribe Daucinae Dumort.: *Laserpitium gallicum* subsp. *paradoxum* (O.Bolòs & Font Quer) P.Monts. and *Thapsia garganica* L. (Table S1).

### DNA isolation and sequencing

Total genomic DNA was extracted from ca. 20 mg of dried plant material using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.) following the manufacturer's instructions. The PCR reactions, primers description and sequencing protocol for obtaining ITS and three plastid markers for newly sampled *Dorema* species are provided in details elsewhere (Panahi et al. 2015). To PCR-amplify the ETS marker, we used the universal primers for Apiaceae, 18S-ETS and Umb-ETS (Logacheva et al. 2010). Each 20- $\mu$ L polymerase chain reaction (PCR) was prepared using 1  $\times$  PCR buffer, 0.2 mM of deoxynucleoside triphosphates (dNTPs), 0.2  $\mu$ M of each primer, 2.5 mM of MgCl<sub>2</sub>, 0.2 U of Taq DNA polymerase (ThermoScientific) and 1  $\mu$ L of DNA template. The PCR included the initial denaturation at 95 °C for 5 min, followed by 30 cycles comprising 1 min of denaturation at 95 °C, 40 s of annealing at 64 °C and 45 s of extension at 72 °C. The final extension at 72 °C lasted for 5 min. Each PCR product was subsequently electrophoresed

using 1% agarose gel and stained with GelRed (Biotium) to determine whether the amplification was successful. We considered a PCR product appropriate for sequencing if no obvious polymorphism (multiple bands from a single PCR product) was observed. The PCR product was then purified using Ampure (1:1.8) and sequenced using fluorescent Big Dye terminators (Applied Biosystems, Foster City, CA, USA). The sequencing products were precipitated using 0.3 M sodium acetate, washed with 80% ethanol, dried, and then resolved using an automated DNA sequencer at the Laboratory of Molecular Biology Techniques, Adam Mickiewicz University (Poznań, Poland) or Genomed (Warsaw, Poland). The sequences were assembled and edited using SeqMan II v. 4.0 (DNASTAR, Madison, WI, USA). All newly obtained sequences were deposited in GenBank (Table S1).

### Phylogenetic analyses

DNA sequences were aligned using MUSCLE v.3.8.31 (Edgar 2004) using a graphical interface in Seaview v.4.6.1 (Gouy et al. 2010), separately for each marker. The resulting matrices were corrected manually if necessary. In the case of plastid markers, we also manually excluded long autapomorphic indels and parts of the alignment with ambiguous site homologies (e.g. long mono- or dinucleotide repeats). Subsequently, we concatenated the alignments into five data sets: nuclear markers only (ITS and ETS), two data sets containing plastid markers only (*rpoB-trnC*, *rpoC1* and *rps16*) with and without manual elimination of ambiguous sites (full and trimmed thereafter), and two concatenated alignments including nuclear and full or trimmed plastid data sets. With these five data sets, we tested the influence of our alignment manipulation on phylogenetic relationships as well as the putative influence of incongruence between nuclear and plastid data sets, recognised in earlier studies (Panahi et al. 2018), on the placement of analysed *Dorema* species.

Phylogenetic analyses were performed using the maximum likelihood (ML) method implemented in RAxML v.8.2.12 (Stamatakis 2014) and the Bayesian inference (BI) implemented in MrBayes v.3.2.7 (Ronquist et al. 2012). Optimal partitioning schemes and substitution models were inferred with PartitionFinder v.1.1.1 (Lanfear et al. 2012, 2014) using the corrected Akaike information criterion (AICc). The search algorithm was set to 'all' to choose among all possible partitioning combinations. In the case of ML analyses, we fixed model substitution to GTR+G (GTR is the only model implemented in RAxML) and searched only for the best partitioning scheme with PartitionFinder (models = GTR+G). Although GTR+G+I model, with the proportion of invariable sites, is also available in RAxML, GTR+G is preferred due to the redundancy of the I

parameter, which is not independent of the alpha parameter of the gamma distribution. MrBayes supports more models than RAxML, although it is also restricted to the subset of all possible general time-reversible models. To analyse this particular subset, we set models = mrbayes in PartitionFinder. All analyses were run using models with branch lengths linked among partitions (branchlengths = linked).

All ML analyses consisted of 2000 independent searches starting from distinct randomised maximum parsimony trees, and the branch support (BS) was evaluated based on 2000 standard non-parametric bootstrap replicates. In Bayesian analyses, two independent runs were executed simultaneously, each with four Monte Carlo Markov chains with 10 000 000 generations, a sampling frequency of 1000 generations, and all priors set to default. The initial 25% of saved trees were discarded as burn-in and the results were summarised 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 v.1.7.1 (Rambaut et al. 2018).

## Results

### Sequence characteristics

For the six newly examined species, we obtained six ITS and four ETS sequences (Table S1). With respect to plastid sequences, we obtained all markers for *D. microcarpum* ( $\equiv$  *F. namanganica*), two (*rpoB-trnC* and *rps16* intron) for *D. balchanorum* and *D. karataviense* ( $\equiv$  *F. sabulosa*), and we failed to sequence plastid markers for *D. badhysi*, *D. sabulosum* ( $\equiv$  *F. sabulosa*), and *D. kopedaghense*.

The ETS region was the shortest albeit most variable among the analysed markers (Table 1). For example, the second most variable marker, ITS had 5.6% parsimony informative positions (34/608), while ETS had 9.7% (46/474). The combined alignment of nuclear markers had 7.3% parsimony informative sites (80/1082). Five accessions forming two groups (*Ferula hyrcana* (Koso-Pol.) Puchałka & al. 0071, *D. balchanorum* KEIB\_AP\_00802 and *F. ovina* (Boiss.) Boiss. 0124, *F. ovina* 0156, *F. tschimganica* Lipsky ex Korovin 0201) shared identical sequences. Each group was represented by a single terminal in the subsequent maximum likelihood phylogenetic analyses of nuclear data. Plastid markers were longer, less variable and had more indels than nuclear markers did (Table 1). The manual removal of indels and ambiguous sites had little effect on the number of parsimony informative positions. The concatenated plastid data sets had 32 and 30 parsimony informative sites in full and trimmed alignments, respectively. Each species analysed had an unique plastid haplotype.

**Table 1** Characteristics of the data sets used in the phylogenetic analyses. Numbers in parentheses refer to the trimmed data sets

Data set	ITS	ETS	<i>rpoB-trnC</i> spacer	<i>rps16</i> intron	<i>rpoC1</i> intron	Nuclear DNA	Plastid DNA	All combined
Sequence length variation:	599–604	422–465	1191–1251 (1189–1221)	833–885 (833–885)	1046–1061 (1036–1050)	603–1069	2057–3172 (2055–3139)	603–4229 (603–4207)
Number of aligned positions:								
Total	608	474	1346 (1237)	951 (927)	1077 (1051)	1082	3374 (3215)	4456 (4297)
Constant	521	380	1276 (1168)	907 (883)	1033 (1011)	901	3216 (3062)	4117 (3963)
Autapomorphic	53	48	56 (55)	38 (38)	32 (30)	101	126 (123)	227 (224)
Parsimony informative	34	46	14 (14)	6 (6)	12 (10)	80	32 (30)	112 (110)
Containing gaps	19	54	193 (83)	122 (98)	43 (18)	492	1392 (1232)	3866 (3707)
Mean indel length	1.14	1.51	11.20 (7.07)	6.40 (5.98)	4.53 (3.03)	2.93	9.42 (8.67)	12.43 (13.02)
Maximum indel length	5	25	40 (20)	29 (29)	10 (8)	474	1077 (1051)	3848 (3689)

All characteristics are calculated for the ingroup: *Ferula* + *Leutea* + *Dorema*

PartitionFinder chose separate substitution models for ITS and ETS in the nuclear data set for both ML and BI analyses (Table 2). For BI, SYM + G and GTR + G were selected for ITS and ETS, respectively. Both full and trimmed plastid data sets were partitioned identically for ML analyses with *rpoC1* and *rps16* forming one partition, and *rpoB-trnC* having separate substitution model. The partitioning for BI analysis, on the other hand, resulted in two different divisions for full and trimmed data sets (Table 2). For the former, all markers were treated as separate partitions, while for the latter, *rpoC1* and *rps16* formed one partition. In general, the GTR model was selected for these partitioning schemes, although with various combinations of G and I parameters (Table 2). The same difference between full and trimmed data sets was also obtained for concatenated nuclear and plastid markers data sets (Table 2).

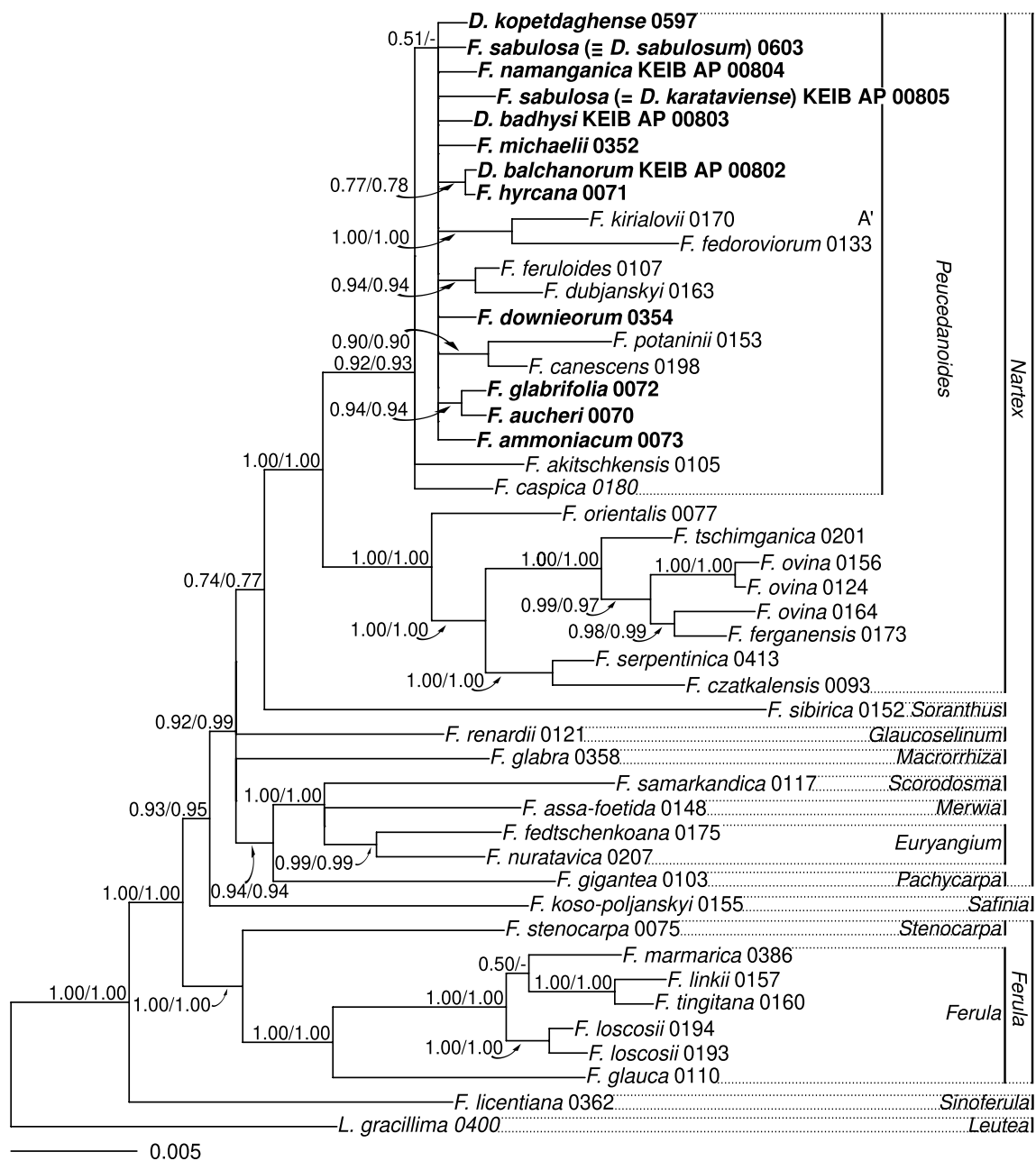
### Phylogenetic position of the six species formerly described in *Dorema*

The incongruence between plastid and nuclear genomes recognised in *Ferula* did not influence the placement of the analysed species (Figs. 1, 2 and Fig. S1). The six analysed species were grouped in one clade with formerly studied congeners in the analyses of nuclear, plastid, and combined data sets. In the most informative combined analyses, all species were placed within well-supported clade representing section *Peucedanoides* (PP = 1.0/1.0, BS = 87/84%, for manually trimmed and full data sets, respectively; Figs. 1, 2). However, they did not form a monophyletic group, but they were intermingled with other species of *Ferula* in one highly supported clade (clade A') within the section (PP = 0.92/0.93, BS = 97/96%). In general, the relationships in this clade were unresolved or poorly supported.

**Table 2** Best-fit partitioning schemes and substitution models inferred from PartitionFinder based on the AICc criterion and used in subsequent ML and BI analyses

Data set	Best partitioning schemes for MrBayes/RAxML	Substitution models for MrBayes
Nuclear	(ITS) (ETS)/(ITS) (ETS)	(SYM + G) (GTR + G)
Plastid (full)	( <i>rpoB-trnC</i> ) ( <i>rpoC1</i> ) ( <i>rps16</i> )/( <i>rpoB-trnC</i> ) ( <i>rpoC1</i> , <i>rps16</i> )	(GTR + G) (GTR + I) (GTR + G)
Plastid (trimmed)	( <i>rpoB-trnC</i> ) ( <i>rpoC1</i> , <i>rps16</i> )/( <i>rpoB-trnC</i> ) ( <i>rpoC1</i> , <i>rps16</i> )	(GTR + G) (GTR + I)
All combined (full)	(ITS) (ETS) ( <i>rpoB-trnC</i> ) ( <i>rpoC1</i> ) ( <i>rps16</i> )/(ITS) (ETS) ( <i>rpoB-trnC</i> ) ( <i>rpoC1</i> , <i>rps16</i> )	(SYM + G) (GTR + G) (GTR + I + G) (GTR + I + G) (GTR + I)
All combined (trimmed)	(ITS) (ETS) ( <i>rpoB-trnC</i> ) ( <i>rpoC1</i> , <i>rps16</i> )/(ITS) (ETS) ( <i>rpoB-trnC</i> ) ( <i>rpoC1</i> , <i>rps16</i> )	(SYM + G) (GTR + G) (GTR + I) (GTR + I + G)

In the case of RAxML, only the best partitioning schemes are shown as the substitution model was fixed to GTR + G in all analyses



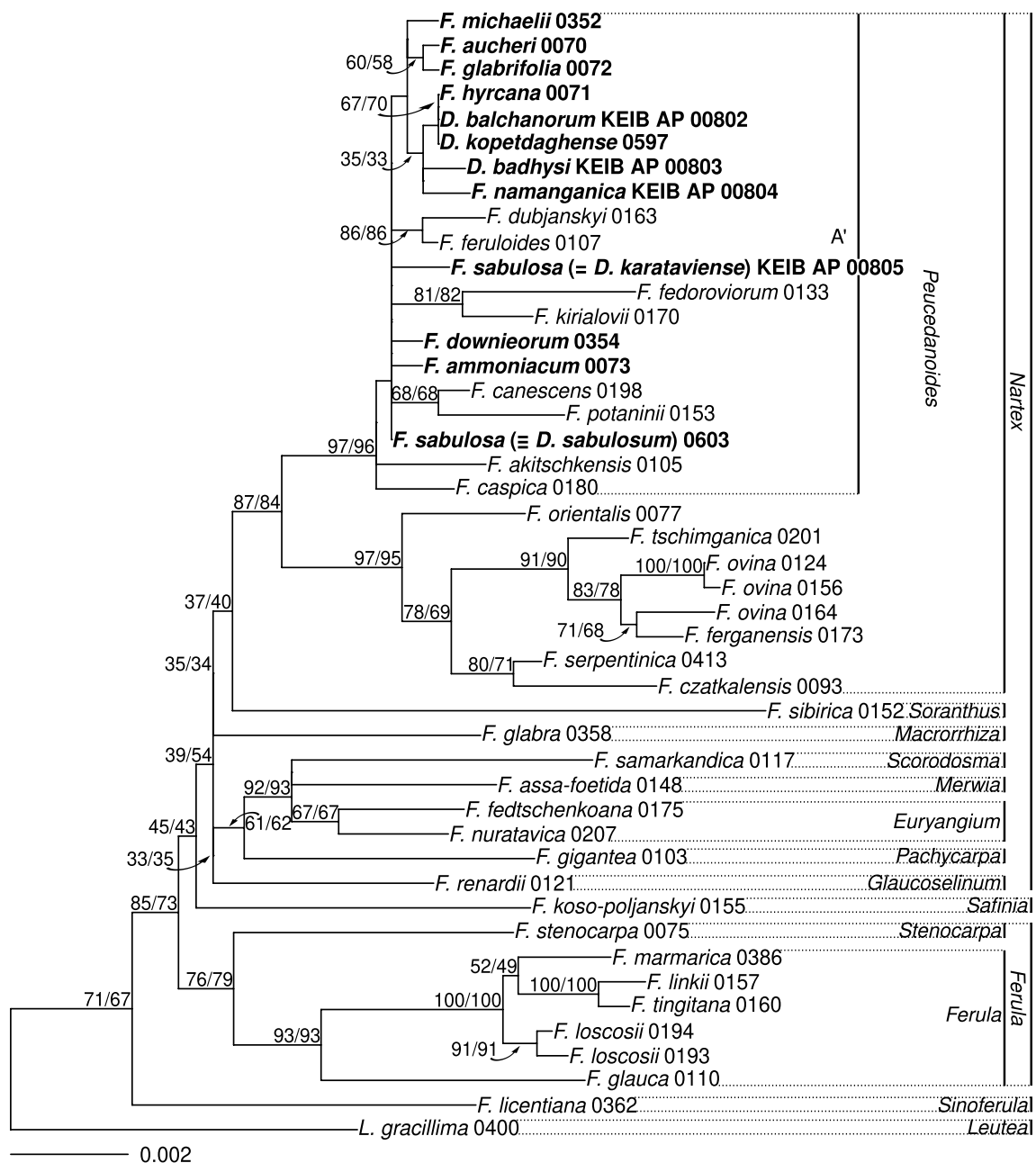
**Fig. 1** The Bayesian 50% majority-rule consensus tree of 46 representatives of Ferulinae inferred from analysis of combined nuclear (nrDNA ITS and ETS) and trimmed plastid sequence data (*rpoB-trnC* intergenic spacer, *rps16*, and *rpoC1* introns). Posterior probability values are given along branches for both combined analyses, i.e. with full and trimmed plastid data. Members of former *Dorema* are indicated in boldface. Major clades representing subgenera (*Ferula*,

*Leutea*, *Narthex*, *Safinia*, and *Sinoferula*) and sections (*Euryangium*, *Ferula*, *Glaucoselinum*, *Macrorrhiza*, *Merwia*, *Pachycarpa*, *Peucedanooides*, *Scorodosma*, *Soranthus*, and *Stenocarpa*) are bracketed and named according to Panahi et al. (2018). Clade A' comprising all species from former *Dorema*, is also marked. For simplicity, outgroup taxa are omitted

### Phylogenetic relationships within *Ferula*

The single representative of the genus *Leutea* was sister to all studied *Ferula* species including the six newly analysed species of *Dorema* (Figs. 1, 2). Monophyly of *Ferula* was strongly supported in BI analyses and moderately in ML

analyses (PP = 1.0/1.0, BS = 71/67%). *Ferula licentiana* Hand.-Mazz., a representative of subgenus *Sinoferula* Spalik, Puchalka & M. Panahi, was sister to the remaining congeners. Subgenus *Ferula* was strongly supported in BI trees and moderately in ML (PP = 1.0/1.0, BS = 76/79%). *Ferula koso-poljanskyi* Korovin, representing subgenus



**Fig. 2** The maximum likelihood tree of 46 representatives of Ferulinae inferred from analysis of combined nuclear (nrDNA ITS and ETS) and trimmed plastid sequence data (*rpoB-trnC* intergenic

spacer, *rps16* and *rpoC1* introns). Bootstrap support values are given along branches for both combined analyses, i.e. using full and trimmed plastid data. See Fig. 1 for details

*Safinia* Spalik, M.Panahi & Puchałka, was a sister in both BI and ML analyses (PP = 0.93/0.95, BS = 45/43%) to the members of subgenus *Narthex*, which in turn was well supported by BI and poorly by ML method (PP = 0.92/0.99, BS = 39/54%). Within the subgenus *Narthex*, four sections, *Euryangium* (Kauffm.) Pimenov, *Merwia* (B.Fedtsch.) Koso-Pol., *Pachycarpa* (Korovin) Banasiak, M.Panahi & Spalik, and *Scorodosma* (Bunge) Boiss., were clustered in

a group highly supported in BI analyses and moderately in ML trees (PP = 0.94/0.94, BS = 61/62%). Moreover, *Euryangium*, *Merwia*, and *Scorodosma* formed a highly supported clade (PP = 1.0/1.0, BS = 92/93%). The remaining sections within the subgenus *Narthex*—*Glaucoselinum* (Schischk.) Pimenov, *Macrorrhiza* Korovin, and *Soranthus* (Ledeb.) Pimenov—had an uncertain position or were poorly supported by both BI and ML analyses.

## Discussion

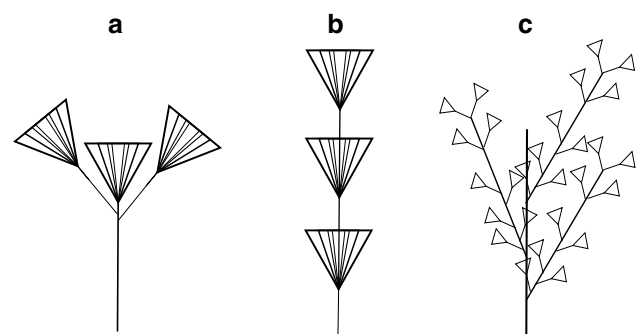
### Phylogenetic position of *Dorema*

In spite of being described at the beginning of the nineteenth century by Scottish botanist D. Don, a comprehensive taxonomical treatment of the genus *Dorema* had not been accomplished until the monograph by M.G. Pimenov (Pimenov 1988). This author critically summarised all available information about the genus that was spread out across various floras, historical publications, and monographs. Importantly, knowing that herbarium material for *Dorema* is often incomplete, he also collected information from the field. He argued that the genus comprised 12 species, for which he generated a simple phenogram (although without providing technical details) based on four vegetative traits of stem and leaves and 12 generative traits of inflorescence, flowers, and fruits. The results were used to propose an infrageneric classification. Pimenov divided the genus into two sections: *Dorema* (four species) and *Microcarpa* Pimenov (eight species), which division constitutes the only hitherto proposed hypothesis on infrageneric relationships within *Dorema*. We included all species from both sections in the present study.

One of the main arguments for the separation of *Dorema* as a distinct genus was the presence of simple umbels arranged in a panicle-like inflorescence. Pimenov in his monograph emphasised several times the uniqueness of this feature among Apiaceae. For example, he criticised Koso-Poljansky (1925) for the inclusion of *Hausknechtia elymaitica* Boiss. into *Dorema* (*Dorema elymaitica* (Boiss.) Koso-Pol.) arguing that this species is only superficially similar to *Dorema* because of the compression of compound umbels into capitulum-like structures (Pimenov 1988). Contrary to the morphological arguments, molecular analyses showed unequivocally that *Dorema* is nested within *Ferula* (Panahi et al. 2015). The present study corroborated this result for all 12 species of *Dorema* recognised by Pimenov (1988), including six analysed for the first time. All species were placed in a well-supported clade corresponding to section *Peucedanoides* according to the new classification of *Ferula* (Panahi et al. 2018; Figs. 1, 2 and Fig. S1). This section, except *Dorema*, includes mostly species previously placed by Korovin (1947) in subgenera *Peucedanoides* and *Dorematooides* Korovin. Interestingly, within the section, highly supported clade A' was formed comprising all 12 species formerly placed in *Dorema* (Figs. 1, 2) and three species—*F. dubjanskyi* Korovin, *F. feruloides* and *F. caspica*—which have proliferating inflorescences: a new compound umbel grows in place of a central umbellet forming therefore a pseudoverticillate inflorescence and restoring its

monopodial growth. This type of inflorescence might be considered as transitory between terminal compound umbels typical for most *Ferula* species and paniculate inflorescence with lateral simple umbels characteristic for *Dorema* (Fig. 3). According to Ajani & Claßen-Bockhoff (2021), simple umbels of *Dorema* are homologous to umbellets, while the long branches with simple umbels are homologous to compound umbels. They speculated that this unique inflorescence architecture not only allows for the increase in the number of umbellets per umbel but also enhances their wide spatial exposition along primary and secondary branches forming a conspicuous floral display, which is adaptive in arid environments characterised by short flowering season and pollinator limitation.

Despite using a highly variable ETS marker, the relationships within the clade A' remained unresolved. Our analyses did not give a definite answer whether the species formerly placed in *Dorema* form a monophyletic group. However, the species from Pimenov's section *Microcarpa*, formed a clade in some analyses, although with poor support. For example, in maximum likelihood trees based on the combined nuclear and plastid data sets, a clade comprising eight species of which seven are from sect. *Microcarpa* is apparent (Fig. 2, Fig. S1). These species are distinguished by several characters such as glabrous ovaries, glabrous dorsal side of petals and the lack of secretory canals in ripe mericarps (Pimenov 1988). The only species from Pimenov's sect. *Microcarpa* that did not cluster in this group is *F. downieorum* Spalik & al. ( $\equiv$  *D. aureum* Stocks), while the only addition to this group from former sect. *Dorema* is *F. michaelii* M.Panahi & al. ( $\equiv$  *D. aitchisonii* Korovin ex Pimenov). The four remaining species are intermingled with other species of *Ferula* and did not form a clade in any analysis. Likely, there is no



**Fig. 3** An umbel architecture in section *Peucedanoides*: **a** main compound umbel and two lateral ones in the form of dichasium (e.g. *F. canescens*, *F. potaninii* in clade A'), **b** 'proliferous' inflorescence; a new compound umbel grows in place of a central umbellet forming a pseudoverticillate inflorescence and restoring its monopodial growth (in clade A': *F. dubjanskyi*, *F. feruloides* and *F. caspica*), and **c** paniculate inflorescence with simple umbels arranged along the branches; this type characterizes all species formerly described in *Dorema*

sufficient variation in the used markers to place all *Dorema* species in one or several well-supported groups. Therefore, our results cannot be used, for example, to answer questions concerning synonymy of *D. sabulosum* and *D. karataviense* postulated by Sennikov and Tojibaev (2021).

### The utility of nrDNA ETS marker to resolve phylogenetic relationships within *Ferula*

Generally, the addition of the ETS marker to the combined phylogenetic analyses confirmed the recent classification system of *Ferula* based on molecular data (Panahi et al. 2018) and increased support for some internal branches. In particular, the monophyly of subgenus *Narthex* is well supported by BI reinforcing the taxonomic system proposed by Panahi et al. (2018). Similarly, the division of the subgenus *Ferula* into two sections, *Stenocarpa* Puchałka & Spalik and *Ferula*, was strongly confirmed in BI and ML analyses (Figs. 1, 2). In Panahi et al. (2018), this relationship was poorly supported by combined marker analyses, most likely because of contradictory phylogenetic signals placing *F. stenocarpa* Boiss. & Hausskn. ex Boiss. as a sister to the representatives of section *Ferula* only in ITS analysis. The close relationship of these two sections is also supported by morphology as they were formerly placed in sect. *Anatriches* Korovin, nom. illeg. ( $\equiv$  sect. *Ferula*) based on leaf morphology—linear-lanceolate ultimate segments of leaves (Korovin 1947).

In former studies, the relationships among three sections, *Euryangium*, *Scorodosma*, and *Merwia*, were unresolved (Figs. 3 and S5 in Panahi et al. 2018). Here, they form one strongly supported clade (Figs. 1, 2). The taxonomy of these three sections has practical significance, because their members such as *F. assa-foetida* L., *F. gummosa* Boiss., and *F. sumbul* (Kauffm.) Hook.f. are sources of gum resins rich in biologically active chemical compounds of potential medicinal importance.

As exemplified by the present study, the ETS marker is highly variable (Table 2) and easy to sequence even from old herbarium materials, thus constituting a useful supplement to the ITS marker. Both markers can be applied for the tentative placement of scarce herbarium specimens or new species of *Ferula* on the phylogenetic tree of the genus. Over ten species of *Ferula* have been described since 2000 (e.g. Brullo et al. 2018, Pimenov and Kljuykov 2013, Ahmad 2013, Akalin et al. 2020) but few, to our knowledge, used ITS as a supplementary source of evidence alongside morphology and anatomy (e.g. Ma et al. 2019; Tuncay et al. 2023). However, even in combination with plastid markers, ETS and ITS do not provide a sufficient phylogenetic signal to answer many questions concerning phylogenetic relationships within *Ferula* as exemplified by the present study. New genomic methods

such as genome skimming or capture-based target enrichment are necessary to answer these questions.

### Taxonomic treatment

*Ferula badhysiensis* Puchałka, Spalik & Piwczyński, **nom. nov.**  $\equiv$  *Dorema badhysi* Pimenov, *Opred. Rast. Sred. Azii* 7: 379. 1983, non *Ferula badhysi* Korovin, *Ill. Monogr. Ferula* 67. 1947.—TYPE: Afghanistan, Badghis, 14–16 May 1885, *Aitchison 462* (holotype: LE; isotype: K000685784).

*Etymology*: The specific epithet *badhysi* is not available in *Ferula* because of *F. badhysi*, a species described from Turkmenistan and at present synonymised with *F. oopoda*. To keep the reference to Badghis (Badhys), a province in Afghanistan from which the species was described, we propose the epithet *badhysiensis*, i.e. from Badhys, retaining the original spelling of the name.

*Ferula balchanorum* (Pimenov) Puchałka, Spalik & Piwczyński, **comb. nov.**  $\equiv$  *Dorema balchanorum* Pimenov, *Opred. Rast. Sred. Azii* 7: 378. 1983.—TYPE: Turkmenistan, Uly Balkan, northern slope, ascent to Mt Arlan, Pat'ma, 10 May 1975, *Pimenov et al. 351* (holotype: MW barcodes MW0594061 and MW0594063).

*Ferula kopetdagi* Spalik & Puchałka, **nom. nov.**  $\equiv$  *Dorema kopetdaghense* Pimenov, *Opred. Rast. Sred. Azii* 7: 378. 1983, non *Ferula kopetdagensis* Korovin, *Ill. Monogr. Ferula*, 61. 1947.—TYPE: Turkmenistan, Kopet Dag, Firuza ravine, 21 May 1972, *MG Pimenov 151* (holotype: MW barcodes MW0594064 and MW0594067; isotypes: LE, MW barcodes MW0594065, MW0594066).

*Etymology*: The substitute epithet also refers to the Kopet Dag, from where the species was described.

### Information on electronic supplementary material

**Online Resource 1.** The maximum likelihood and Bayesian 50% majority-rule consensus trees inferred from analyses of nuclear data, full and trimmed plastid data, and combined nuclear and full plastid data.

**Online Resource 2.** Accessions of *Dorema*, *Ferula*, and outgroups from which nrDNA and pDNA sequence data were obtained, with corresponding voucher information and GenBank reference numbers.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00606-023-01857-z>.



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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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