Brief Report

Phylogenetic Structure and Marker Congruence in Central Asian *Ferula* (Apiaceae): Insights from Nuclear ITS2 and Plastid psbA–trnH Data

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Phylogenetic Structure and Marker Congruence in Central Asian *Ferula* (Apiaceae): Insights from Nuclear ITS2 and Plastid psbA–trnH Data

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Abstract

The genus *Ferula* (Apiaceae) is taxonomically challenging because of morphological plasticity, incomplete lineage sorting, and documented discordance between nuclear and plastid datasets. To test marker congruence at the regional scale, we analysed five *Ferula* species from Central Kazakhstan using the nuclear ITS2 and plastid psbA–trnH loci. Sequences were aligned with MUSCLE and analysed in MEGA X using Maximum Likelihood with 1000 bootstrap replicates and model selection based on the Akaike Information Criterion. Both loci yielded largely congruent topologies, and the two-locus consensus recovered species-level relationships without strongly supported cytonuclear conflict, unlike some previously reported *Ferula* lineages. These results support the utility of ITS2 and psbA–trnH for regional phylogenetic studies in *Ferula* and provide additional molecular evidence for species relationships in Central Asian representatives of Ferulinae.

Keywords: *Ferula*; Apiaceae; phylogeny; ITS2; psbA–trnH; marker congruence; Kazakhstan

1. Introduction

The genus *Ferula* L. comprises one of the most taxonomically complex lineages in Apiaceae, particularly across the Irano–Turanian region, where morphological convergence, ecological plasticity, and uncertain species boundaries complicate systematics [1–8]. More broadly, phylogenetic studies of Apiaceae have shown that generic delimitations and tribal relationships are best resolved through combined nuclear and plastid evidence [9–18]. Recent plastome-scale studies have further refined deep relationships in Apiaceae and revealed lineage-specific patterns that are not always apparent in single-marker analyses [19–23]. Within *Ferula*, however, incongruence between nrDNA and plastid datasets has been reported in several lineages, suggesting the possible influence of introgression, hybridisation, or incomplete lineage sorting [3,5,6]. At the same time, ITS2 and plastid barcode regions such as psbA–trnH remain among the most useful markers for species-level plant identification and regional phylogenetic studies [24–30].

Beyond its phylogenetic interest, *Ferula* is a genus of considerable ecological and practical importance in Central Asia and Kazakhstan. The genus comprises more than 180 species, with one of its principal centres of diversity located in Middle Asia and Kazakhstan, where 52 species have been recorded [31,32]. Species of *Ferula* occupy a broad ecological range, from lowland deserts to foothills and mountain belts, and show particularly high diversity in arid and montane landscapes of the region [31,32]. In addition to their systematic importance, members of the genus are valued as aromatic, resin-bearing, essential-oil, forage, honey, and medicinal plants, and several species have a long history of traditional use in Central Asia and neighbouring regions [33–36]. Representative habit images of the analysed species are shown in Figure 1.

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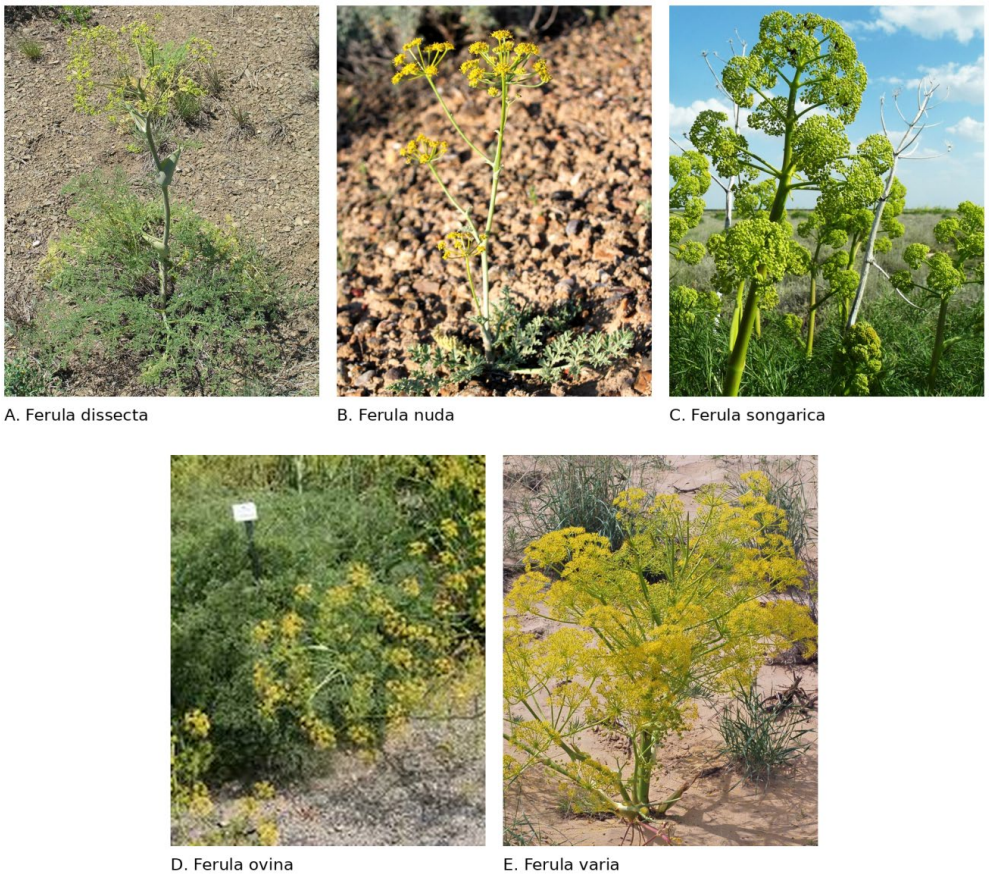

Figure 1. Representative habit images of the analysed species from Central Kazakhstan: (A) *Ferula dissecta*, (B) *Ferula nuda*, (C) *Ferula songarica*, (D) *Ferula ovina*, and (E) *Ferula varia*.

Against this background, the present study evaluates whether nuclear ITS2 and plastid psbA–trnH provide congruent phylogenetic signals in five *Ferula* species sampled in Central Kazakhstan.

2. Materials and Methods

2.1. Sampling and Taxon Coverage

Five *Ferula* species were sampled in Central Kazakhstan, and *Peucedanum officinale* was used as an outgroup. The study focused on two loci that had complete taxon representation across all sampled species: the nuclear ITS2 region and the plastid psbA–trnH intergenic spacer. A summary of sampled taxa, sequence metadata and the reference outgroup is provided in Table 1. Detailed best-match information for each newly generated sequence is provided in Supplementary Table S1.

Table 1. Sample metadata and sequence-deposition status for taxa included in the phylogenetic analysis.

Species	Sample code(s)	Collection locality	Sequence length (bp)	GenBank accession	Outgroup
Ferula dissecta	ITS2: 2,1; psbA–trnH: 4,1	Northern Balkhash region, Kazakhstan	ITS2: 390; psbA–trnH: 194	ITS2: pending; psbA–trnH: pending	No
Ferula nuda	ITS2: 2,2; psbA–trnH: 4,2	Gulshat area, Kazakhstan	ITS2: 336; psbA–trnH: 253	ITS2: pending; psbA–trnH: pending	No

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Ferula songarica	ITS2: 2,5; psbA–trnH: 4,5	Koktas area, Kazakhstan	ITS2: 319; psbA–trnH: 254	ITS2: pending; psbA–trnH: pending	No
Ferula ovina	ITS2: 2,4; psbA–trnH: 4,4	Torangylyk area, Kazakhstan	ITS2: 342; psbA–trnH: 230	ITS2: pending; psbA–trnH: pending	No
Ferula varia	ITS2: 2,3; psbA–trnH: 4,3	Torangylyk area, Kazakhstan	ITS2: 318; psbA–trnH: 233	ITS2: pending; psbA–trnH: pending	No
Peucedanum officinale	Reference outgroup	Reference outgroup	Reference sequences from GenBank	ITS2: AH012690.2; psbA–trnH: JF807609.1	Yes

2.2. Sequence Alignment and Phylogenetic Reconstruction

Sequence alignment was performed with MUSCLE [38]. Phylogenetic analyses were conducted in MEGA X [39] using the Maximum Likelihood method with 1000 bootstrap replicates. Bootstrap support followed the general non-parametric resampling framework introduced for phylogenetic inference [40]. Model choice was guided by likelihood-based model selection procedures [41], following standard practice in molecular phylogenetics [42,43].

2.3. Marker Congruence Assessment and Data Deposition

Congruence between the nuclear and plastid datasets was assessed by direct comparison of Maximum Likelihood tree topologies and node support values. The majority-rule two-locus consensus was used to evaluate whether the principal species-level relationships were supported by both loci. The ITS2 and psbA–trnH sequences generated in this study formed the basis of all phylogenetic analyses.

3. Results

3.1. Phylogenetic Reconstruction Based on ITS2 and psbA–trnH

Maximum Likelihood reconstruction of the ITS2 and psbA–trnH datasets resolved the analysed *Ferula* species into well-defined lineages. Both ITS2 and psbA–trnH yielded well-resolved Maximum Likelihood trees with moderate to high bootstrap support, as shown in Figure 2A,B. The ITS2 topology recovered clear separation among the analysed *Ferula* species, as shown in Figure 2A. The plastid psbA–trnH tree showed a similar species-level arrangement, as shown in Figure 2B. The majority-rule two-locus consensus stabilised the principal interspecific relationships and did not reveal any strongly supported conflict between nuclear and plastid signals, as shown in Figure 2C.

3.2. Phylogenetic Congruence Analysis

Phylogenetic congruence between nuclear ITS2 and plastid psbA–trnH datasets was assessed through direct comparison of tree topologies and node support values, as illustrated in Figure 3. Both markers recovered the same overall grouping pattern, and no strongly supported conflicting clades were detected. Minor differences in branch resolution likely reflect locus-specific evolutionary rates and the contrasting inheritance of nuclear and plastid genomes. The absence of well-supported topological conflict indicates a high level of cytonuclear congruence in the studied taxa. This contrasts with previously documented cases of incongruence in parts of *Ferula* [3,5,6], but supports the view that multilocus approaches can recover stable regional patterns when sampled taxa represent comparatively coherent evolutionary units [7,8,14–23].

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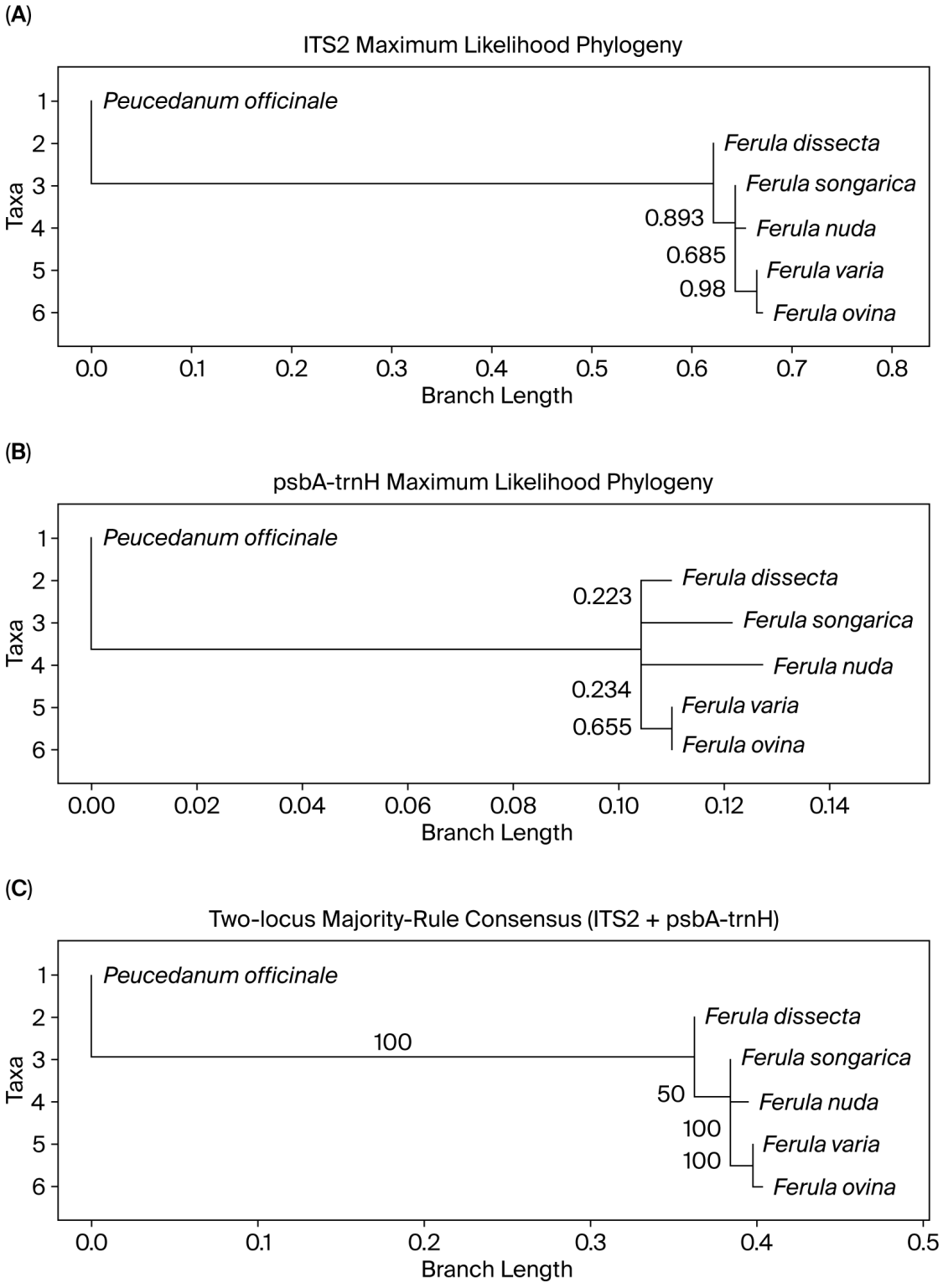

Figure 2. Maximum Likelihood phylogenies inferred from ITS2 (A) and psbA–trnH (B), together with the two-locus majority-rule consensus tree (C). Bootstrap values are shown at the nodes. *Peucedanum officinale* was used as the outgroup.

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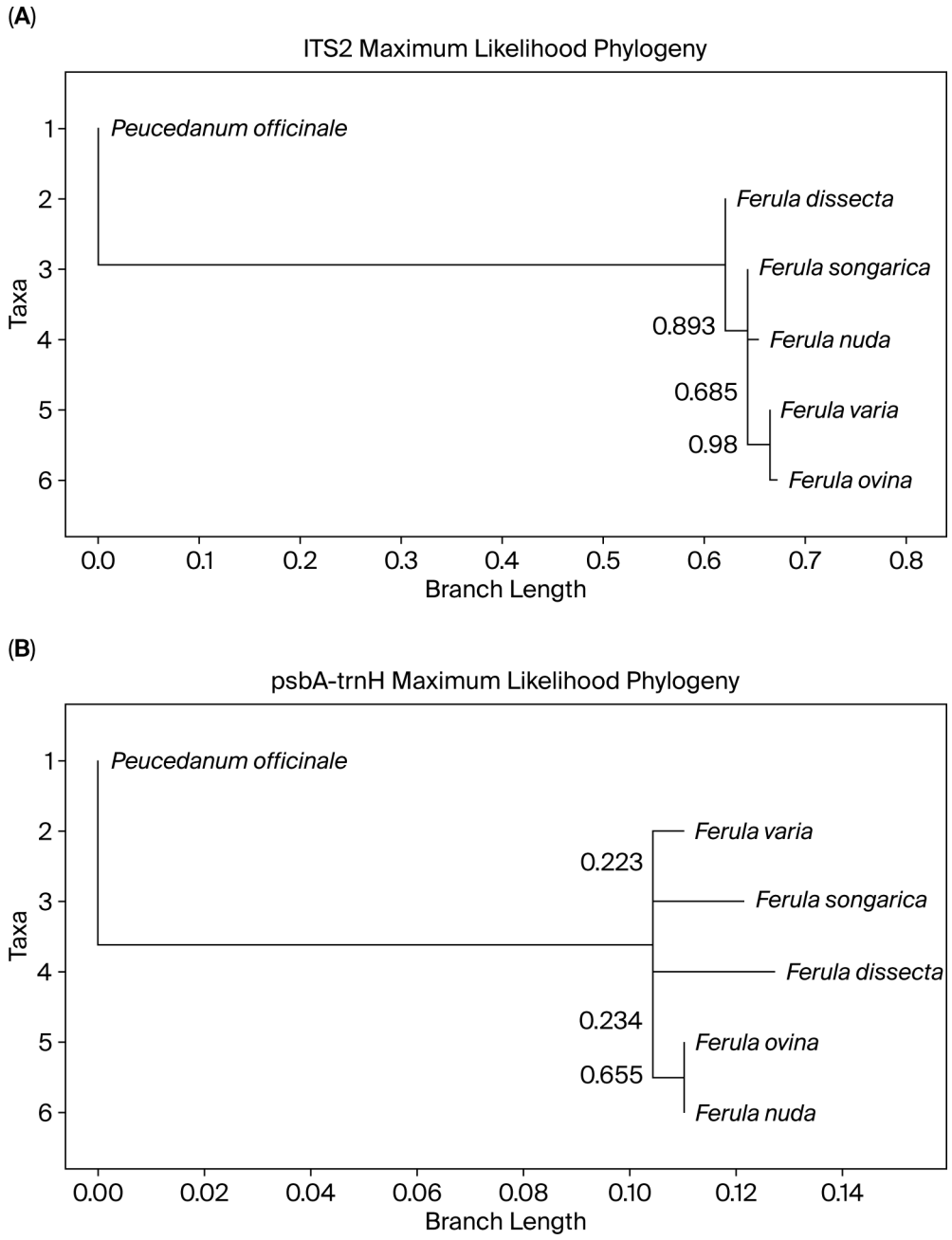

Figure 3. Comparison of Maximum Likelihood phylogenies inferred from the nuclear ITS2 dataset (A) and the plastid psbA–trnH dataset (B). Bootstrap support values (≥50%) are shown at the nodes. *Peucedanum officinale* was used as the outgroup.

4. Discussion

The congruent signal recovered from ITS2 and psbA–trnH suggests that phylogenetic structure in the sampled Central Kazakhstan lineages is not strongly distorted by recent introgression or hybridisation. This differs from reports of nuclear–plastid discordance in other parts of *Ferula* [3,5,6] and suggests that the regional species pool analysed here may represent comparatively stable evolutionary units.

The results also reinforce the methodological value of combining a variable nuclear marker with a plastid spacer, as recommended in plant barcoding and regional phylogenetic studies [24–30]. At a broader comparative level, the present findings fit well within modern views of Apiaceae evolution derived from combined-marker and plastome-based datasets [9–22,37]. Thus, the study provides both a regional phylogenetic hypothesis for *Ferula* in Central Kazakhstan and a small-scale test case

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demonstrating that carefully selected nuclear and plastid loci can yield coherent species-level signal in a taxonomically difficult genus.

It should be noted that the present study is based on a limited number of loci and taxa. Future studies incorporating additional nuclear and plastid markers, broader taxon sampling, and complete public sequence accession data may further refine the phylogenetic relationships within *Ferula*.

5. Conclusions

The combined analysis of ITS2 and psbA–trnH resolves species-level relationships in the sampled *Ferula* taxa with sufficient support for a short-format phylogenetic study. The absence of major incongruence highlights the stability of the analysed lineages and supports the continued use of ITS2 and plastid spacers as core markers for regional *Ferula* systematics [24–30]. More broadly, these results are consistent with current phylogenetic understanding of Apiaceae and Ferulinae [1–22,37].

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org: Table S1: Detailed summary of the ITS2 and psbA–trnH sequences generated in this study and used for phylogenetic analyses, including sample codes, sequence lengths, best database matches, best-hit accession numbers, and identity percentages.

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Data Availability Statement: The sequence metadata are included in the article and supplementary material. GenBank accession numbers for the newly generated ITS2 and psbA–trnH sequences are pending and will be added before publication. Alignment and tree files can be supplied as supplementary files during submission or on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

MUSCLE	Multiple Sequence Comparison by Log-Expectation
MEGA X	Molecular Evolutionary Genetics Analysis X
nrDNA	nuclear ribosomal DNA
AIC	Akaike Information Criterion

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