

New data for systematics of the genus *Zeravschania* (Apiaceae) and related taxa based on the nrDNA (ITS) sequences

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Abstract. All 13 species of the genus *Zeravschania* were studied in molecular and morphological analyzes. The molecular study showed that *Zeravschania* is a clearly paraphyletic taxon including monotypic genera *Demavendia* and *Hausknechtia*. Further phylogenetic, anatomical and morphological studies are needed to confirm taxonomic position of these genera. *Zeravschania afghanica* confirms its placement in the genus *Zeravschania*, not in *Cephalopodium* or *Selinum*. *Dichoropetalum knappii* confirms its placement in the genus *Dichoropetalum*, not in *Zeravschania*.

1 Introduction

The genus *Zeravschania* Korovin (Apiaceae) was described as a monotypic genus with a type species *Z. regeliana* Korovin distributed in mountainous Central Asia [1]. Seven species were included in *Zeravschania* from other genera such as *Peucedanum* L. [2, 3], *Eleutherospermum* K. Koch [4] and *Selinum* L. [5]. Five more species were described as new to science: *Z. khorasanica* Kljuykov & Lyskov, *Z. kopetdaghensis* Pimenov & Kljuykov, *Z. latifolia* Pimenov & Kljuykov, *Z. podlechii* Kljuykov & Ukrainsk., and *Z. scabrifolia* Pimenov [2, 6, 7]. All members of the genus are perennial polycarpic plants distributed in Iran, Afghanistan, and Central Asia (Pamir-Alai mountainous region). The greatest diversity of species is founded in northeastern Iran and northern Afghanistan. *Zeravschania* to date includes 13 species, but nevertheless the question of the genus composition remains debatable. Species of the genus *Zeravschania* have quite homogeneous morphological features. According to results of molecular studies [9, 10] three studied species namely *Z. aucheri* (Boiss.) Pimenov, *Z. membranacea* (Boiss.) Pimenov, and *Z. regeliana* form a clade with strong support. Close relationships of *Zeravschania* and monotypic genera *Demavendia* Pimenov and *Hausknechtia* Boiss. were also showed in this study.

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The main purpose of our study was to carry out a comparative morphological and molecular analysis of 13 species of *Zeravschania* and to identify their diagnostic features.

2 Material and Methods

Specimens from the following herbaria were revised: E, G, LE, M, MW, TARI, and W. Individual samples were collected by the authors from expeditions to southwest Asia. Some species were represented by two to three samples from different regions.

The set of taxonomically important characters for Apiaceae following was examined Kljuykov et al. [11]. Carpological data are partially taken from previously published materials [3, 6–8, 12].

As a molecular marker in the molecular-phylogenetic analysis, we have used internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA). DNA extraction, amplification and sequencing the spacers (ITS1, 2) were conducted according to Valiejo-Roman et al. [9]. ITS sequences were aligned using MUSCLE 3.6 [13] and then manually adjusted in BioEdit 7.2.5 [14]. Newly obtained sequences were deposited to the GenBank.

Molecular- phylogenetic trees have been obtained by two methods: Bayesian inference (BI) and maximum parsimony (MP) methods. Maximum parsimony analysis was performed using PAUP* version 4.0b08 [15] with TBR branch swapping and equal weighing of characters; gaps were treated as missing data. Bayesian analysis of molecular data was performed using the program MrBayes version 3.2.6 [16].

3 Results and discussion

We determined ITS sequences for 27 samples of 13 *Zeravschania* species. In the molecular-phylogenetic analysis, we additionally included 93 ITS sequences (representing 70 genera) that represent nearly all tribes of subfamily Apioideae. *Pleurospermum uralense* Hoffm. was used as out-group based on the results of the previous phylogenetic study of the subfamily Apioideae [9]. The emphasis of our samplings focused on the species of *Zeravschania* plus their closest congeners *Demavendia pastinacifolia* (Boiss. et Hausskn.) Pimenov, *Haussknechtia elymaitica* Boiss., *Johrenia* DC., *Dichoropetalum* Fenzl and *Cephalopodium* Korovin which chosen through results of several previous phylogenetic studies [2–5, 8, 9].

The alignment of 120 ITS sequences resulted in a matrix of 438 positions after excluding ambiguous positions. 93 characters are constant, 46 characters are variable but parsimony-uninformative, and 299 (68%) characters are parsimony-informative.

Molecular-phylogenetic trees generated by different methods (MP and BI) possessed similar topology, so only a Bayesian tree is provided with indication of posterior probabilities and bootstrap support for the maximum parsimony tree (Fig. 1).



Fig. 1. Consensus tree obtained from Bayesian analysis of nrDNA ITS sequences from 120 accessions (representing 70 genera) of Apiaceae subfamily Apioideae. The tree inferred by Bayesian analysis of these data was highly consistent with the MP strict consensus tree. Bayesian posterior probabilities ≥ 0.95 are shown above branches. Bootstrap values $>60\%$ from the parsimony analyses are shown below branches. “-” indicates node not supported in MP analysis.

The study of the nrITS region shows that all analyzed species of *Zeravschania*, *Demavendia*, and *Hauscknechtia* together with critical samples formed a well-supported “Zeravschania” clade (I). All investigated species of *Zeravschania* are clearly differentiated, each species forming a strongly supported discrete cluster, which is consistent with the results of morphological studies [2, 5–8]. The species of *Zeravschania* are very similar morphologically, but differ in minor characteristics. Analysis of morphological characters showed that the most important taxonomic characters for the identification species of *Zeravschania* are as follows: life form (inflorescence form); presence of solid remains of dead leaves at the base of stem; presence of pubescence of stems and leaves; dissection of leaf blades; shape of terminal lobes of leaves; number of rays in umbels (extreme states); length of umbel rays; presence of calyx teeth; color of petals. Shape and number of bracts and bracteoles and fruit features are rather uniform within the genus and are important primarily for the diagnosis of the genus *Zeravschania*. There is insignificant variability in the shape and size of mericarps, and their ribs. In *Z. latifolia*, a hypoderm of 1–2 layers of small cells is developed under the exocarp. In *Z. aucheri*, the mesocarp consists entirely of lignified cells with porous membranes, while in species *Z. afghanica* Pimenov, *Z. ferulifolia* (Gilli) Pimenov and *Z. scabrifolia*, lignified cells with porous membranes are developed only in the marginal ribs.

The monotypic genera *Hauscknechtia* and *Demavendia* are placed within the “Zeravschania” clade (I), however these genera pose significant morphological differences from *Zeravschania* species. Based on morphological data, Pimenov et al. [3] considered that *Hauscknechtia* and *Demavendia* are better treated as separate monotypic genera. So the genus *Zeravschania* is not monophyletic, as it was reported previously [3, 9] and we consider *Zeravschania* as a paraphyletic taxon. Thus, further phylogenetic, anatomical and morphological studies are needed to confirm taxonomic position of *Hauscknechtia* and *Demavendia*.

The critical sample considered as *Demavendia pastinacifolia* from East Khorasan (MW), has identical nrITS sequences to *Z. stricticaulis* (Rech.f.) Pimenov. Thus, the genus *Demavendia* is distributed only in West and Central Iran [2] and is not found in the East Khorasan.

The species *Z. knappii* (Bornm.) Pimenov et Kljuykov included in clade (II) among several species of *Dichoropetalum*. *Zeravschania knappii* and *Dichoropetalum palimbioides* (Boiss.) Pimenov et Kljuykov have nearly identical nrDNA sequences. Thus, this result is in accordance with results of morphological study by Kljuykov et al. [8].

Cephalopodum badachschanicum Korovin and *Cephalopodum hissaricum* Pimenov form a highly supported monophyletic group (Fig. 1). According nrDNA data the remaining sample of *Cephalopodum afghanicum* (Rech. f. & Riedl) Pimenov&Kljuykov (= *Selinum afghanicum* Rech. f. & Riedl) fell into the “Zeravschania” clade is distinct from the members of the “Cephalopodum” clade (III) and *Selinum carvifolia* Gilib. et DC. (type species), that is accordance with studies by Kljuykov et al. [8] and Degtjareva et.al. [5]. Our results support the taxonomic placement *C. afghanicum* to the genus *Zeravschania* with a new combination *Z. afghanica* Pimenov.

The present results are preliminary and have needed further careful morphological, carpological and molecular investigation with another markers (nrDNA, cpDNA).

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