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Систематические обзоры и новые таксоны

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**ANOTHER LOOK ON *SELINUM AFGHANICUM* AND ON AFFINITY  
OF *CEPHALOPODUM* (UMBELLIFERAE)**

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The systematic position of two presumably related Apiaceae-Apioideae taxa, the rare and poorly known Afghan species *Selinum afghanicum* and the genus *Cephalopodium*, is clarified based of nrITS DNA sequence data. In the molecular phylogenetic tree, these taxa were revealed as not allies. *Selinum afghanicum* is nested among *Zeravschania* species within Pimpinelleae Clade; its position is consistent with morphology that allows us to transfer it to the genus *Zeravschania*. After this transfer, *Zeravschania* numbers 12 species with a center of diversity in Iran and Afghanistan. Two *Cephalopodium* species are nested within a poorly resolved clade together with *Ferula* species; it contradicts morphological, especially carpological, characters and is more consistent with the phytogeographic specificity of the genera.

**Key words:** DNA sequencing, nrDNA ITS, Apiaceae, *Zeravschania*

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*Selinum afghanicum* Rech. f. & Riedl is a rare, little known and true enigmatic species of Afghan flora. It was described by Rechinger and Riedl (1963) on the basis of very limited (type – Edelberg 1922 (C, W)) and incomplete (“mericarpia valde immatura”) herbarium collection. One more species from the region, *S. duriusculum* Rech. f. & Riedl, was described side by side with *S. afghanicum*. Later *S. duriusculum* was transferred by Leute (1970) to *Ligusticum* L., whereas *S. afghanicum* was left by Leute (1970) and Rechinger (1987) in traditional *Selinum* s.l., but with a significant reserve. K. H. Rechinger wrote (in our translation from German) that “the generic attribution of *S. afghanicum* to *Selinum* in common use is not fixed. This species resembles other species of *Selinum* in the leaf shape and division. There is a certain similarity in habit and leaf form to monotypic genus *Pyramidoptera*, endemic to Afghanistan, which, however, is completely different in the form and structure of the fruits”. Currently, the assignment of the species to *Selinum* looks like a complete anachronism, since all former “*Selinum*” from Middle Asia have been transferred to the independent genera *Sphaenolobium* Pimenov (1975) and *Karatavia* Pimenov & Lavrova (Lavrova et al., 1987), and most of the Himalayan ones – to a satellite genus of *Selinum*, namely *Oreocome* Edgew. (Pimenov et al., 2001). As a result, the genus *Selinum* is completely absent throughout the vast region of Asia (with a small exception of the eastern slope of the Ural Mts.)

In an attempt to find an adequate place for *Selinum afghanicum* in the classification of Asian Umbelliferae, Pimenov and Kljuykov (2002) compared it with *Cephalopodium* Korovin, a little-known genus which was early recorded only from Middle Asia (Tajikistan and Uzbeki-

stan); one species of the latter (*C. badashchanicum* Korovin) was recently found in Afghanistan, near Tajikistan border (Pimenov et al., 2014). In their opinion, these two taxa, *Selinum afghanicum* and *C. badashchanicum*, are morphologically similar, including fruit structure and especially habitual characters by a presence of specific agglomeration of rigid, enlarged, dry petiole remains of died leaves of the preceding years near a stem base. As a result, *Selinum afghanicum* was transferred to *Cephalopodium* as its third species. Of course, a similarity in such rigid petiole remain agglomeration may be considered as an adaptation to the life in very arid conditions of Tajik and Afghan Badakhshan, but carpological data are more valuable in the Umbelliferae taxonomy, and could not be ignored. It is needed to note, however, that the mericarps of both *S. afghanicum* and *C. badachshanicum* have rather simple structure, without any specific features. Such fruits are usually classified as non-specialized, and they are known in several other taxa, never regarded as closely related (*Haussknechtia* Boiss., *Rupiphila* Pimenov & Lavrova, *Sison* L., *Carum carvi* L., *Petroselinum* Hill, *Falcaria* Fabr., *Paulita* Soják, *Seselopsis* Schischk., *Sphaenolobium* Pimenov and others). Therefore an independent occurrence of similar mericarp features in *S. afghanicum* and *C. badachshanicum* cannot be ruled out. Besides, there are some additional differences in life-form, stylopodium, calyx teeth and spermoderma cell characters between *C. badachshanicum* and *S. afghanicum*.

In order to broaden the taxonomic and phylogenetic basis of *Selinum afghanicum* systematics, we turned to the use of modern molecular methods. These methods, in close interaction with morphology, allow in many cases to solve or at least get closer to solving many complex taxonomic problems, in particular in the Umbelliferae. Molecular phylogenetic studies of Umbelliferae were mainly concentrated on analyses of internal transcribed spacer of nuclear ribosomal DNA (nrDNA ITS) sequence data (Downie et al., 2010; Banasiak et al., 2013). For this reason, we sequenced and analyzed ITS to address questions of relationship of *Selinum afghanicum*.

## MATERIAL AND METHODS

Herbarium materials from GOET, MW, TASH and W herbaria were studied. The nrDNA ITS sequences of *Selinum afghanicum* (4 accessions), *Cephalopodium badachshanicum* (4 accessions), *C. hissaricum* (one accession from a single known species locality) and *Oreocome hindukushensis* Pimenov & Kljuykov (one accession) were generated. Total DNA was extracted from fruit or leaf tissue with the NucleoSpin plant isolation kit (Macherey-Nagel, Düren, Germany) following protocol of the manufacturer. The procedure used for amplification of ITS region is the same as described in Valiejo-Roman et al. (2006). Amplification products were purified using the DNA Cleanup Mini kit (Evrogen, Moscow, Russia). Direct sequencing was performed using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and a BigDye Terminator Cycle Sequencing Ready Reaction kit. Newly obtained sequences were deposited in GenBank (see Appendix). No sequence variation among accessions was found in both *Selinum afghanicum* and *Cephalopodium badachshanicum*, therefore they are represented by single terminals in our tree.

An initial set of taxa for comparison with newly obtained sequences was determined using the BLAST option of the GenBank database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). This search suggested that the most similar to *Selinum afghanicum* were species of *Zeravshania* from Pimpinelleae Clade, whereas two species of *Cephalopodium* show similarity with *Ferula* from Scandiceae Clade in the currently adopted molecular phylogeny of Apiaceae (Downie et al., 2010). The final dataset was designed to cover representatives related to the studied species based on molecular data (members of Pimpinelleae and Scandiceae Clades) and morphology (species of *Oreocome* and *Selinum* from Selineae Clade) based on results of Valiejo-Roman et al. (2006), Pimenov et al. (2008), Banasiak et al. (2013), Degtjareva et al. (2018) and Pimenov et al. (2018). In total, 100 accessions, including the outgroup (*Physospermum cornubiense* DC.), were sampled in the present analysis. To infer relationships, the Bayesian analysis was per-

formed using MrBayes version 3.2.6 (Ronquist et al., 2012) with the GTR+G model of nucleotide substitutions, which was selected by AIC in MrModeltest version 2.3 (Nylander, 2004). Twenty five million generations were run in two independent analyses each with four chains. One tree was saved every 1000 generations, and the first 50 trees were discarded as burn-in. The remaining trees were used to build a majority-rule consensus tree to assess the Bayesian posterior probabilities (PP).

## RESULTS AND DISCUSSION

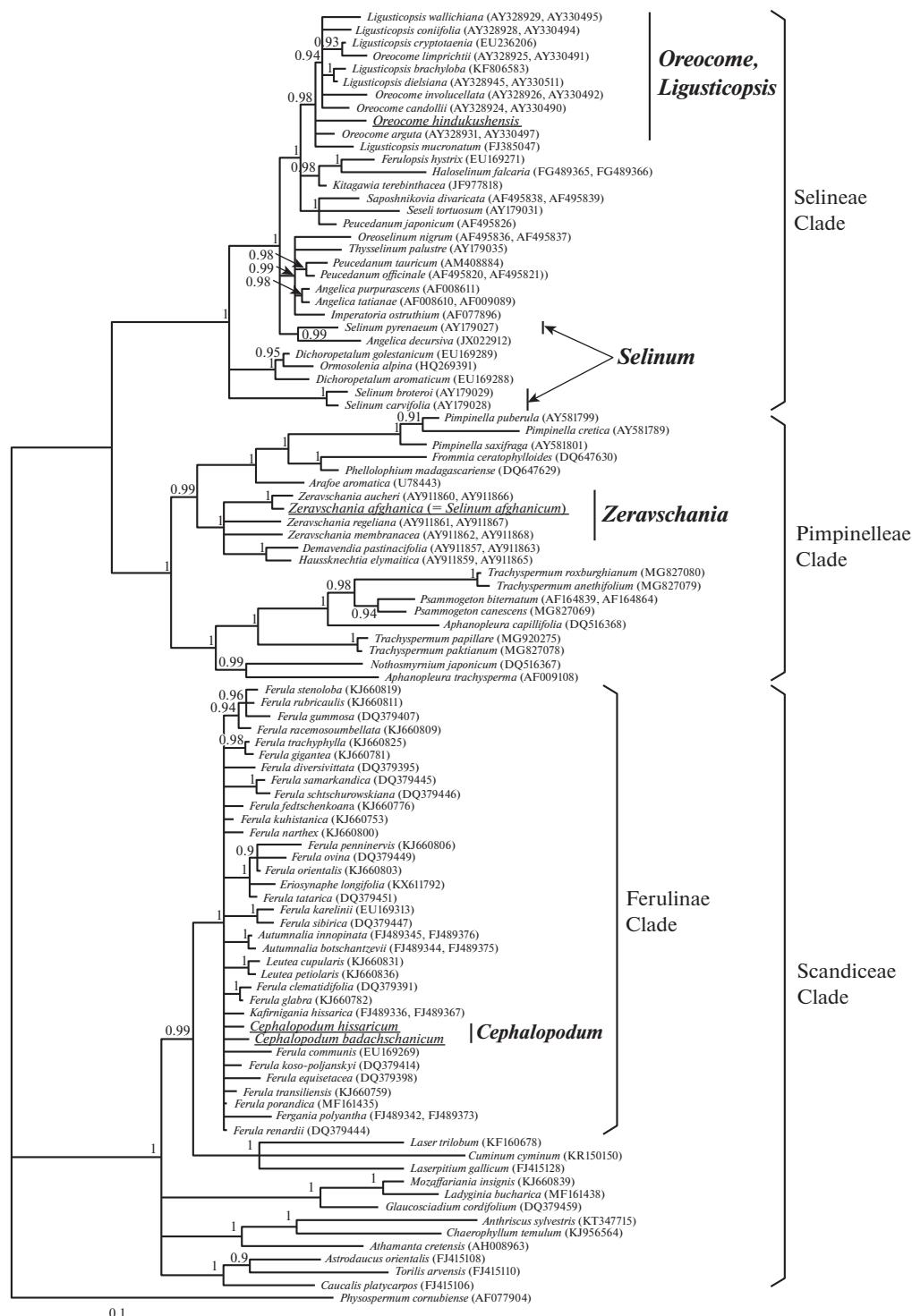
The Bayesian analysis of the ITS sequences resulted in a phylogenetic tree (see Figure) that forced us to make some corrections to the concept of the relationship of taxa, which has been formed solely on the basis of morphology (mainly carpology). *Selinum afghanicum* is deeply submerged in one of the subclades of Pimpinelleae Clade, made up of species of *Zeravschania*, *Demavendia* and *Haussknechtia*. Two species of *Cephalopodium* occupy their position among species of *Ferula* (Ferulinae Clade) in Scandiceae Clade. In contrast to some other parts of Pimpinelleae Clade, there is little resolution of relationships among species of *Cephalopodium*, *Ferula* and its satellite genera (for example, *Leutea*, *Autumnalia*) within Ferulinae Clade. Moreover, two species of *Cephalopodium* do not group together and do not form a clade with any other species; their exact placement is still unclear. Both *Oreocome* and *Selinum* are members of Selineae Clade and show affinity neither to *Selinum afghanicum*, nor to *Cephalopodium*. As a consequence of this, changes in the taxonomic interpretation of the generic affiliation of *Selinum afghanicum* as well as of the intended relationship of *Cephalopodium* became necessary.

### *Selinum afghanicum*

The placement of *Selinum afghanicum* in the molecular tree contrasts with all hypotheses proposed based on morphology. Our results of the nrITS data phylogenetic analyses clearly show that *Selinum afghanicum* cannot be placed in the genus *Cephalopodium* as was proposed by Pimenov and Kljuykov (2002). Close relationship between *Selinum afghanicum*, by analogy with some other *Selinum* species of South and South-Eastern Asia, and the genus *Oreocome* Edgew. suggested previously by Pimenov et al. (2001) and Pimenov and Kljuykov (2009) was also not confirmed. The studied species of *Oreocome* (*O. candellei*, *O. limptichtii*, *O. involucellata*, *O. hindukuschenis*) occupy their position in large Selineae Clade. It is obvious that specific agglomeration of dry petiole remains of died leaves at stem base is not significant as a character at generic level.

According to our results, *Selinum afghanicum* is closely related to *Zeravschania*, *Demavendia* and *Haussknechtia*. The fruit structure of *Haussknechtia*, *Demavendia* and *Zeravschania* are similar (Pimenov et al., 2007), and it is similar to that of *S. afghanicum*, that supports the naturalness of the clade and possibility to transfer *S. afghanicum* to one of these genera. Among them, the genus *Zeravschania* is the most similar to *S. afghanicum*, especially some its species, distributed in the region of “Flora Iranica”. Although the species of *Zeravschania* have not such compact agglomeration of rigid dry petiole remains in lower stem part as *Cephalopodium*-has, the stem base in *Zeravschania* is also densely covered by petiole remains. In addition, *Selinum afghanicum* and species of *Zeravschania* have similar bracts and bracteoles, inconspicuous calyx teeth, conical or short conical stylopods, filiform dorsal mericarp ribs, spermoderm of small cells (in *Cephalopodium* – of large cells), broad commissure, and solitary vallecular secretory ducts.

*Zeravschania* Korovin (type species – *Z. regeliana* Korovin) was described by Korovin (1948) as a monotypic genus of Pamir-Alai, close to *Scaligeria* DC (in modern interpretation to *Elaeosticta* Fenzl) based on plants without mature fruits. It was later modified in a taxon with several species earlier attributed to *Peucedanum* s. ampl. (Pimenov, 1987, 1988; Pimenov et al., 2007; Pimenov, Kljuykov, 2013); Salimian and Akhani (2003) proposed to transfer the species *Peucedanum pastinacifolium* Boiss into *Zeravschania*. However, this species differs in



some anatomical and morphological characters from accepted species of *Zeravschania*. It was earlier separated into an independent genus *Demavendia* Pimenov (Pimenov, 1987). The only molecular study of *Zeravschania* based on the ITS sequences (Valiejo-Roman et al., 2006) showed its affinity to *Demavendia* and *Haussknechtia*. According to classification of Apioideae genera based on the ITS sequences (Downie et al., 2010; Wang et al., 2014), the genus belongs to Pimpinelleae Clade together with *Aphanopleura*, *Arafoë*, *Demavendia*, *Haussknechtia*, *Nothosmyrnum*, *Opsicarpium*, *Pimpinella*, *Psammogeton* and some other taxa.

At present, *Zeravschania* includes 11 species, distributed in SW and Middle Asia and in Transcaucasia. The highest species diversity is observed in Iran (5 species, 4 of them are endemic) and Afghanistan (3 species); two species were found in Tajikistan, and one species in each of these countries: Armenia, Azerbaijan, Turkmenistan, Uzbekistan, and Pakistan. With the transfer of *S. afganicum* to *Zeravschania*, these figures will change – the total diversity in the genus will reach 12 species, including 4 species in Afghanistan, three of which are endemic.

### *Cephalopodium*

According to ITS sequence data analyses a position of two *Cephalopodium* species in the system of Umbelliferae should be changed completely. Both species, *C. badachschanicum* and *C. hissaricum*, fall into large Ferulinae clade. As additional Apiaceae species from Middle and Central Asia were included in molecular studies, more and more taxa fell within Ferula clade, sometimes they are quite different in morphological characters. In this group, molecular data better correlate with geographical origin than with morphological character. This complex problem is waiting for its solution based on more complete material.

The topology of the Ferulinae Clade in our present tree as well as in previous publications with somewhat different species sets (Kurzyna-Młynik et al., 2008; Elibol et al., 2012; Degtjareva et al., 2018; Piwczyński et al., 2018) seems to be low resolved, very compact, i.e. containing the most similar sequences. In other words, its inner structure is poorly supported. The mutual arrangement of many clades, corresponding to one species or group of few species, remains unresolved in this tree. *Cephalopodium badachschanicum* occupies isolated position and does not group with other taxa, whereas *C. hissaricum* groups together with *Kafirnigania hissarica* (Korovin) Kamelin & Kinzik (former *Peucedanum hissaricum* Korovin), but with very low support (posterior probability 0,6), so this clade was collapsed to polytomy in the present tree (see Figure). The proximity of two *Cephalopodium* species is poorly supported; their ITS sequence possess species-specific nucleotide substitutions only and do not contain genus-specific nucleotide substitutions in contrast to some satellite genera related to *Ferula*, e.g. *Leutea* and *Autumnalia*. One specific nucleotide substitution for *Kafirnigania hissarica* and *Cephalopodium hissaricum* is observed. Additionally, some members of Ferulinae Clade have an insertion of TGA nucleotides near the end of ITS2. This insertion has not been found in any other taxon of Umbelliferae investigated, so it may be regarded as a group-specific character. Within Ferulinae, this insertion is absent in *Cephalopodium badachschanicum* (but not in *C. hissaricum*), *Ferula porandica*, *F. communis*, and species of *Leutea*. Although taxonomic significance of this insertion should be evaluated on more representative sampling of Ferulinae

←  
**Fig. 1.** The Bayesian tree obtained from analysis of 100 nrITS sequences. GenBank accession numbers ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) are cited after taxon names. Branch lengths are proportional to the number of the expected nucleotide substitutions. Bayesian posterior probabilities are indicated above nodes. Nodes with support values below 0.9 are not shown. Newly studied species are underlined.

**Рис. 1.** Молекулярно-филогенетическое дерево, полученное методом Байеса по результатам анализа 100 нуклеотидных последовательностей ITS ядерной рибосомной ДНК. После названий таксонов указаны их номера в генбанке ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). Числа около узлов представляют значения апостериорной вероятности. Узлы с поддержкой менее 0,9 не показаны. Вновь изученные виды и подчеркнуты.

Clade, presence/absence of insertion provides an additional difference between two *Cephalopodium* species.

The position of *Cephalopodium* in Ferulinae Clade is a new phylogenetic hypothesis for this genus, but the relationship between its two species is still unclear. *Cephalopodium hissaricum* remains to be a little-known and now inaccessible species, and even morphological (carpological) characters of it are partly unknown.

#### TAXONOMIC CONCLUSION

***Zeravshania afghanica*** (Rech. f. & Riedl) Pimenov & Degtjareva, comb. nov.

≡ *Selinum afghanicum* Rech. f. & Riedl in Biol. Skr. 13(4) (Symb. Afghan. 5): 126. 1963.

**References:** Leute, 1970: 510; Rechinger, 1987: 361, tab. 293; Podlech, 2012: 197; Breckle, Hedge & Rafiqpoor, 2013: 139.

≡ *Cephalopodium afghanicum* (Rech. f. & Riedl) Pimenov & Kluykov, Candollea 57: 268, fig. 1C, 2C, map 3, 3. 2002.

**Type:** AFGHANISTAN. “Central-Afghanistan: Deh Kundi, 2900 m, 11.06.1949, *Edelberg 1922*” (lectotype W! [W 1988-00100040]; designated here by Pimenov; isolectotype C! [C10008552]).

**Distribution:** Afghanistan (Centr.: Bamyan, Wardak, Ghazni; SE: Urozgan).

#### Appendix

Source of nrITS sequences used in the present study and GenBank accessions numbers.

***Cephalopodium badachschanicum*** Korovin: Tajikistan, Badakhshan, valley of Pjanj river, 3 km below Choshtkandez settlement, 04 viii 2013, M.G. Pimenov et al. 48 (MW), MK424582; Tajikistan, Badakhshan, valley of Pjanj river, Shidz settlement, 30 vii 1981, M.G. Pimenov et al. 1008 (MW), MK424583; Tajikistan, Badakhshan, valley of Pjanj river, near Dekh settlement, M.G. Pimenov et al. 1005 (MW), MK424584; Tajikistan, Darvaz ridge, valley of Obi-Kharek, 30 vi 1973, M.G. Pimenov 1011 (MW), MK424585. ***Cephalopodium hissaricum*** Pimenov: Uzbekistan, Hissar ridge, valley of Tupolang river, left bank of the river Taminda, below Taminda settlement, 10 vii 1948, A.D. Pjataeva 887 (TASH), MK424581; ***Oreocome hindukushensis*** Pimenov & Kluykov: Pakistan, E-Hindukush, Sai valley, 01 ix 1993, Schickhoff 2035 (GOET), MK424590. ***Selinum afghanicum*** Rech.f. & Riedl: Afghanistan, Ghazni, O. Anders 3940 (W), MK424586; Afghanistan, Kabul, valley of Maidan, K.H. Rechinger 35986 (W), MK424587; Afghanistan, Deh Kundi, L. Edelberg 1922 (W), MK424588; Afghanistan, Doab, Darreh-i Shikar between Doab and Butola, K.H. Rechinger 16733 (MW), MK424589.

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## ИНОЙ ВЗГЛЯД НА СИСТЕМАТИКУ *SELINUM AFGHANICUM* И РОДСТВО *CEPHALOPODUM* (UMBELLIFERAE)

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Сравнительное молекулярное исследование нуклеотидных последовательностей ITS ядРНК редкого и мало изученного афганского вида *Selinum afghanicum* и видов рода *Cephalopodium*, к которому этот вид *Selinum* ранее предлагалось отнести, показало, что эти таксоны не являются близко родственными. *S. afghanicum* по молекулярным данным более близок к роду *Zeravshania*, что не противоречит морфологическим признакам. На основании совокупности данных предложено перенести *S. afghanicum* в род *Zeravshania*. После этого переноса род *Zeravshania* насчитывает 12 видов с центром разнообразия в Иране и Афганистане. Род *Cephalopodium*, состоящий из 2 видов, в молекулярном дереве оказался в плохо разрешенной кладе рода *Ferula*, что находится в некотором противоречии с морфологическими, особенно карпологическими признаками и более соответствует фитогеографической специфике родов.

**Ключевые слова:** секвенирование ДНК, ITS яд-рДНК, Apiaceae, *Zeravshania*

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