

MOLECULAR AND MORPHOLOGICAL REVISION REVEALS DIFFERENT EVOLUTION PATTERNS IN *ALLIUM* SECT. *OREIPRASON* AND SECT. *FALCATIFOLIA* (AMARYLLIDACEAE)

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Background

In 2008–2013, we performed a large molecular and morphological revision of the *Allium saxatile* M. Bieb. group, which is forming a nomenclatural core of sect. *Oreiprason* F. Herm. (Seregin et al., 2015, in press). Former taxonomic circumscription of *Allium saxatile* s.l. widely distributed from Italy to China was disputable with a number of accepted species ranging from 3 to 7. We studied ca. 2000 herbarium specimens in line with morphological revision, made thorough nomenclatural study of all available names (Sennikov, Seregin, 2014, 2015, in press), and successfully sampled 86 accessions of the *A. saxatile* group and a few related species to reconstruct a molecular phylogeny. We sequenced the internal transcribed spacer (ITS) from ribosomal nuclear DNA, as well as the plastidic *trnL-trnF* and *trnL-rpl32* regions.

We revealed that the monophyletic *A. saxatile* group consists of 15 entities united in two geographically sound clades («European» and «Siberian»). Two yellow-flowered species *A. obliquum* and *A. petraeum* surprisingly nested within the *A. saxatile* clade both in ITS and cpDNA analyses. The eldest names within the group, i.e. *A. saxatile* and *A. globosum* M. Bieb. ex DC. from Caucasus, represent genetically identical populations and should be synonymised. We found that there is a clear lack of valid names for distinct species in the *A. saxatile* group. We established five new species (*A. austrodanubiense* nom. prov., *A. schistosum* nom. prov., *A. cretaceum* nom. prov., *A. montanostepposum* nom. prov., *A. kirilovii* nom. prov.), a new hybrid (*A. ×agarmyschicum* nom. prov.), and raised a variety to species level (*A. rubriflorum* nom. prov.).

All species from the *A. saxatile* group are diploids. Due to this, we consider geographical isolation to be the main driving force of speciation. Some genetically close taxa are very distinct morphologically. For instance,

tall *A. obliquum* L. has yellow flowers and wide flat leaves, whereas its close relatives from *A. montanostepposum* — *A. cretaceum* complex with purple flowers and terete leaves were formerly treated as «*A. globosum*».

Study of cpDNA fragments had shown that nine species of the «European» clade (SE Europe, Crimea, and Caucasus) originated from a single ancestor. Chloroplast DNA of *A. austrodanubiense* retains some important ancestral traits similar to those of Siberian and Middle Asian species from related sections. Most probably the ancestor of the «European» clade arrived to Balkan Peninsula as a result of a single long dispersal event.

Material and methods

In 2014, we performed further molecular survey for a complete revision of sect. *Oreiprason*. For most samples, the nuclear DNA ITS region was amplified using ITS-SF and ITS-SR primers.

We mainly worked with DNA material from herbarium specimens, some of them were dated back to the first half of 20th century. Once we were lucky to make a successful amplification of ITS from an 88-years old specimen, but this is somewhat exceptional. Success rate in amplification and further sequencing for specimens collected in 1920–30s was 26% growing gradually up to 94% for specimens collected in 2000s.

All together, we sampled DNA from 89 specimens and made 351 ITS amplifications with varying level of DNA in order to find appropriate conditions of PCR for old specimens. As a result, we got complete ITS sequences from 50 specimens and two partial sequences. 275 amplifications and 22 sequences failed.

Almost all specimens with successful ITS sequences were appropriate for cpDNA fragments sequencing. We used *rpl32-trnL* and *trnL-trnF* regions for phylogeny reconstruction (44 successful amplifications for each fragment).

Key results

1. Recircumscription of sect. *Oreiprason*. Surprisingly, many slender species of sect. *Oreiprason* known to occur in Middle Asia are definitely closely related to robust species from sect. *Falcatifolia* N. Friesen. This section was introduced for *A. carolinianum* DC. and *A. platyspathum* Schrenk with large flat falcate leaves. Later on, Fritsch & Friesen (2009) transferred tall *A. hymenorrhizum* Ledeb. and similar *A. kaschianum* Regel to sect. *Falcatifolia* on genetic ground.

Species like *A. filifolium* Regel, *A. kokanicum* Regel, *A. caricoides* Regel, *A. alexandrae* Vved. are more or less similar to «*A. globosum*» in their gross-morphology, but show similar ITS and cpDNA fragments with abovementioned species from sect. *Falcatifolia*. The most intricate issue that sect. *Oreiprason* and sect. *Falcatifolia* are not closely related in the general tree of the genus *Allium* (Friesen et al., 2006).

Thereby, monophyletic sect. *Oreiprason* s.str. includes two groups — large *A. saxatile* group as circumscribed by Seregin et al. (2015, in press) (i.e. including *A. petraeum* and *A. obliquum*) and its probable sister *A. tianschanicum* Rupr. s.l. All other species should be excluded from this section. Form of leaf cross-sections should not be considered as a phylogenetically important character, because there are plants with terete, hollowed and wide flat leaves both in sections *Oreiprason* and *Falcatifolia*.

2. New species in sect. *Oreiprason* s.str. Further sampling made us sure that we need recognition and formal description of several new entities in sect. *Oreiprason* s.str. For instance, at least three clear genetic entities of monophyletic origin exist in *A. petraeum* Kar. et Kir. We confirmed yellow-flowered *A. petraeum* s.str. from Dzungarian Alatau and Trans-Ili Alatau. Red-flowered plants of *A. petraeum* s.l. were reported earlier by Egorova (1977), but she did not recognize them taxonomically. Such plants grow in Dzungarian Alatau only and form a monophyletic clade. Yellow-flowered plants of the southern lineage were sampled from several localities in Central Tian Shan (Kazakhstan and China).

We splitted *A. dshungaricum* Vved. into two species earlier. The second member of this lineage was described from a single locality in Eastern Tian Shan, China. Now we confirmed this species from another locality in Central Tian Shan (Kazakhstan). Plants from Tarbagatai and Dzungarian Alatau may represent different groups (additional material is needed). Also, we discovered the third member in the *A. cretaceum* — *A. montanostepposum* complex which is a rare species formed on SE fringes of *A. montanostepposum* range. We sampled two specimens of this entity from Tarbagatai and Sary Chelek Lake.

A. tianschanicum Rupr. was described from Tian Shan, but consequently reported from Pamiro-Alai. It has purplish flowers whereas plants from Alai and adjacent ridges have somewhat yellowish flowers. Their monophyletic origin and clear genetic delimitation confirmed that they represent a previously neglected species new to science (known as *A. pamiri* Vved. ined.). Relations of *A. tianschanicum* and *A. saxatile* M. Bieb. are unresolved.

3. Genetic differentiation in sect. *Falcatifolia*. ITS variation within newly circumscribed sect. *Falcatifolia* is fairly low comparing with such in sect. *Oreiprason*. All species are forming tight core clade with two early diverged sister clades — *A. talassicum* sensu Vved. and *A. platyspathum* s.l. Within the core clade there are both widely distributed species and narrow endemics. Species with large ranges like *A. hymenorrhizum* (from Altai to Turkey), *A. carolinianum* (from Nepal to Mongolia), and *A. kokanicum* (from Pamiro-Alai to Dzungarian Alatau) have been sampled from many localities and has almost no ITS variation. Lineages with narrow endemics are notably variable and need further sampling, proper morphological characterization, and perhaps further splitting on both morphological and genetic ground (*A. alexandrae*, *A. filifolium*, etc.).

Some rare species were sampled only once. *Allium kastekii* Vved., *A. clausum* Vved. and one unknown species show uncertain positions within sect. *Falcatifolia*. They might represent separate sections or intersectional hybrids, but for proper decision additional sampling is vitally important.

Tobias Herden (University of Osnabrück) performed in BEAST age analysis of sect. *Oreiprason* ITS tree in order to reveal time of splitting of proposed ancestors (only mean figures are given below). The first splitting in sect. *Oreiprason* probably happened ca. 12.0 Mya; splitting of the European clade in the *A. saxatile* group took place ca. 8.4 Mya, while splitting within *A. tianschanicum* s.l. occurred ca. 4.8 Mya. In contrast, the core clade of sect. *Falcatifolia* is certainly younger. Age of speciation within it could be estimated as 1.4 Mya.

Genetic uniformity and striking morphological variability of younger sect. *Falcatifolia* contrast with long genetic distances between morphologically similar «old» species in sect. *Oreiprason*. Unfortunately, we know little about chromosome numbers in sect. *Falcatifolia*, although *A. carolinianum* is reported to be a tetraploid. We suppose that in the latter section other evolutionary mechanisms (like hybridization and probably polyploidization) are employed in speciation rather than clear geographical isolation within sect. *Oreiprason*.

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