

OXYTROPIS AZIZII (FABACEAE-FABOIDEAE) AS A NEW COMBINATION SUPPORTED BY MOLECULAR AND MORPHOLOGICAL EVIDENCE

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Abstract

Based on molecular and morphological evidence, our results clearly indicate that *Astragalus azizii* should be reclassified as a member of the genus *Oxytropis*, representing a distinct species closely related to *O. lapponica*. In our molecular examination of the *Astragalus* sect. *Brachylobium*, we analyzed both the isotype and holotype of *A. azizii* and found that they were positioned among *Oxytropis* species in the nrDNA ITS-inferred phylogram. Through a detailed dissection of the flower, we observed the mucronate appendage on the keel petal — a diagnostic trait of the genus *Oxytropis*.

Keywords: *Astragalus*; Iran; nrDNA ITS; *Oxytropis*; Phylogeny

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گونه *Oxytropis azizii* به عنوان ترکیب جدیدی براساس شواهد مولکولی و ریخت‌شناسی

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چکیده: براساس شواهد مولکولی و ریخت‌شناسی، نتایج ما به صراحت نشان می‌دهند که گونه‌ی

Astragalus azizii به عنوان عضوی از جنس *Oxytropis* رده‌بندی می‌شود و گونه‌ای مجزا و خویشاوند

نزدیکی از *O. lapponica* است. در حین بررسی‌های ما بر روی بخش *Brachylobium* از جنس گون،

نمونه‌های تیپ و ایزوتیپ گونه‌ی *A. azizii* آنالیز شد و درخت تبارزایی حاصل از توالی‌های nrDNA

ITS نشان داد که این گونه در میان گونه‌های *Oxytropis* قرار می‌گیرد. تشریح گل بادقت انجام گرفت و

زائده‌ی نوک نیز انتهای بر روی گلبرگ ناو مشاهده شد (صفت شاخص جنس *Oxytropis*).

INTRODUCTION

De Candolle (1802) was the first to separate the genus *Oxytropis* DC. from *Astragalus* L. using two morphological traits: the presence of a mucronate keel and bilocular pod, resulting from inversion of the ventral suture rather than the dorsal one. Even though De Candolle's treatment still prevails, these differences are so subtle that they can easily be overlooked. Consequently, many *Oxytropis* species have been misidentified as *Astragalus*, and vice versa. Here are some examples: *Oxytropis cabulica* (Boiss.) Boiss., *O. immersa* (Baker) Bunge ex Lipsky, *O. lapponica* (Wahlenb.) J.Gay, *O. luteocaerulea* (Baker) Ali, *Astragalus beketowii* (Krasn.) B.Fedtsch., *A. caulescens* (Gontsch.) Abdusal., *A. dutreuilii* (Franch.) Grubov & N.Ulziykh and *A. yazdii* (Vassilcz.) Podlech & Maassoumi (Vassilczenko 1984; Podlech & Zarre 2013; Maassoumi 2013). In all of these cases, the newly established names have retained their basionym epithet.

The genus *Oxytropis* comprises 608 accepted species distributed across temperate and subarctic regions of the Northern Hemisphere (POWO 2025). Central Asia represents the main biodiversity center of the genus, harboring between 153 and 166 species (Malyshev 2008). Molecular studies have clearly demonstrated that *Oxytropis* constitutes a monophyletic lineage, forming the sister group to either *Astragalus* crown clade (Wojciechowski 2005; Moghaddam & Kazempour-Osaloo 2020) or Coluteoid clade (Duan & al. 2021; Li & al. 2025).

Astragalus azizii Maassoumi was first described based on specimens collected from the north slope of Mount Sabalan in Ardebil Province (Maassoumi 1989). Subsequently, in August 2019 and 2020, this species was recorded from the same locality as a candidate for inclusion in Iran's Red List of threatened species (Bidarlord & al. 2022). In the course of our phylogenetic study on *A. sect. Brachylobium* Boiss., *A. azizii* was one of those investigated species (Podlech & Zarre 2013; Maassoumi 2018). Initially, leaf samples of the isotype specimens, preserved at HCAT (Thiers 2023), were examined for phylogenetic analysis. When we realized that the species belongs to the genus *Oxytropis*, we conducted our further molecular and morphological investigations on the holotype preserved at TARI (Thiers 2023).

MATERIALS AND METHODS

Morphological observation

The holotype specimen [A. Javanshir 1260, TARI] (Fig. 1A) was examined, and dissection slides of flower parts were prepared for stereomicroscopy.

Molecular taxon sampling

The ITS region of nuclear ribosomal DNA (nrDNA ITS) was utilized to reconstruct the phylogenetic relationships of *Oxytropis azizii*. A total of 27 accessions representing 21 species of *Oxytropis* and four species of *Astragalus* were included in phylogenetic analyses. 25 sequences were retrieved from GenBank. Seven nrDNA ITS sequences of *Oxytropis* were retrieved from the Sequence Read Archive (SRA) in GenBank by mapping them to the nrDNA ITS consensus sequence of *Oxytropis* species using Bowtie2 v3.2.0 (Langmead & Salzberg 2012) with the default setting, as implemented in Geneious Prime 2019.1.3 (Kearse & al. 2012; www.geneious.com). *Caragana grandiflora* DC. and *Colutea persica* Boiss. were chosen as outgroups based on previous studies (Kazempour-Osaloo & al. 2003, 2005). All the sampled taxa, as well as their voucher specimens and GenBank accession numbers, are listed in Table 1.

DNA extraction, nrDNA ITS amplification and sequencing

Total genomic DNA was extracted from herbarium specimens using the modified 2×CTAB procedure of Doyle & Doyle (1987). The universal ITS primers, AB101 and AB102 (Douzery & al. 1999) were used to amplify the nrDNA ITS for the two accessions of *O. azizii*. PCR reactions were performed in a 20 mL volume, containing 7 mL deionized water, 10 mL of 2× Taq MasterMix RED (Amplicon, cat. no. 180301; 150 μM Tris-HCl pH 8.5, 40 μM (NH₄)₂SO₄, 3.0 μM MgCl₂, 0.4 μM dNTPs, 0.05 units/μL Amplicon Taq DNA polymerase, inert red dye and a stabilizer), 0.5 mL of each primer (10 pmol/mL), 1 mL of DMSO, and 1 mL of template DNA (20 ng/mL). PCR procedures were 4 min at 94 °C, followed by 35–38 cycles of 94 °C for 50 s, annealing at 53 °C for 45 s, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. PCR products were checked by electrophoresis in 1% agarose gels with Tris-borate-EDTA (TBE) buffer (pH 8.0) and stained with RedSafe™ Nucleic Acid Staining Solution. Each set of reactions was monitored by the inclusion of a negative (no template) control, and then the gels were visualized under UV light. PCR products were sent for Sanger sequencing at Pishgam Inc. (Tehran, Iran).

Phylogenetic analyses

nrDNA ITS sequences were aligned with MAFFT online version 7 (Kato & al. 2019) under default parameters and then manually adjusted in BioEdit v7.0.9.0 (Hall, 1999). The analyses employed maximum likelihood (ML, 10000 bootstrap replicates)

and Bayesian inferences (ngen = 5,000,000, samplefreq = 100, nruns = 2). According to ModelTest-NG v0.1.7 (Darriba & al. 2019), implemented in raxmlGUI v2.0.16 (Edler & al. 2021), the best nucleotide substitution model was GTR+G. ML analysis using IQ-Tree v2.1.2 (Minh & al. 2020),

and Bayesian inference performed with MrBayes v3.2.7 (Ronquist & al. 2012) were both carried out on CIPRES portal (Miller & al. 2010). The resulting trees were visualized using iTOL v7.3 (Letunic & Bork 2021).

Table 1. Voucher information and GenBank accession numbers for taxa included in this study (newly generated sequences indicated by *)

Species	Voucher information	Accession number
<i>Astragalus daenensis</i> Boiss.	GenBank	AB231099
<i>A. daenensis</i>	GenBank	KX954930
<i>A. penetratus</i> Maassoumi	GenBank	AB231100
<i>A. yazdii</i> (Vassilcz.) Podlech & Maassoumi	GenBank	LC278543
<i>Caragana grandiflora</i> DC.	GenBank	AB051905
<i>Colutea persica</i> Boiss.	GenBank	AB051907
<i>Oxytropis azizii</i> (Maassoumi) Maassoumi, Mirvaziri & Kaz.Osaloo	Iran: Javanshir 1260 (Holotype, TARI)	*LC896413
<i>O. azizii</i>	Iran: A. Javanshir 1260 (Isotype, HCAT)	*LC896414
<i>O. carinthiaca</i> Fischer-Oost. [= <i>O. lapponica</i> (Wahlenb.) Gay]	GenBank	ERR14010471
<i>O. deflexa</i> (Pall.) DC.	GenBank	LC213345
<i>O. deflexa</i>	GenBank	LC213346
<i>O. deflexa</i>	GenBank	MK802452
<i>O. deflexa</i>	GenBank	SRR26765397
<i>O. glabra</i> DC.	GenBank	LC213353
<i>O. glabra</i>	GenBank	LC213354
<i>O. helvetica</i> Scheele	GenBank	ERR14012678
<i>O. heratensis</i> Bunge	GenBank	LC213360
<i>O. lapponica</i> (Wahlenb.) Gay	GenBank	ERR5554997
<i>O. lapponica</i>	GenBank	ERR14012457
<i>O. lapponica</i>	GenBank	SRR26754487
<i>O. lapponica</i>	GenBank	LC213388
<i>O. montana</i> (L.) DC.	GenBank	SRR26754348
<i>O. riparia</i> Litv.	GenBank	LC213426
<i>O. savellanica</i> Boiss.	GenBank	LC213435
<i>O. savellanica</i>	GenBank	LC213434
<i>O. savellanica</i>	GenBank	LC213433
<i>O. sojakii</i> Vassilcz.	GenBank	LC213442

RESULTS AND DISCUSSION

Morphological comparison

Examination of the floral dissection of *O. azizii* clearly revealed that the type specimen possesses a short mucro at the apex of the keel petal, as illustrated in Fig.1B:c, which represents a diagnostic trait of the genus *Oxytropis* (Maassoumi 2013, 2023). The species can be

easily distinguished from the related species *O. lapponica* by the morphological characters shown in Table 2. *Oxytropis lapponica* previously included several varieties, all of which have since been synonymized under *O. lapponica*, as they were considered insufficiently distinct from the type.

Table 2. Morphological comparison of *Oxytropis azizii* and *O. lapponica*.

Morphological characters	<i>O. azizii</i>	<i>O. lapponica</i>
Stipule		
length (mm)	3	4–10
vestiture	with appressed black and white hairs	sparsely pilose
Leaflet		
length (mm)	2–4	5–15
width (mm)	1–2	2–3
number	7–9 pair	17–37
Bracts		
length (mm)	ca. 2	2.5–6
vestiture	with black hairs	with appressed white and black hairs
Calyx		
length (mm)	ca. 3.5–4	6–7
teeth (mm)	ca. 1	1.5–3
teeth shape	subulate	lanceolate-subulate
Corolla	dark violet	pale purple
standard		
length (mm)	8	8–12
keel		
length (mm)	ca. 6.5	7–8
mucrone (mm)	<1	2–2.5
Pod		
shape	oblong	cylindric, ovoid, or narrowly cylindric
stipe (mm)	ca. 1	1.5–2

Phylogenetic analysis

The phylogenetic tree based on nrDNA ITS is given in Fig. 2. In this analysis, two accessions of *O. azizii* were clustered in a clade with three accessions of *O. lapponica*. Notably, the two species differ at a single polymorphic site at position 120 in the nrDNA ITS1 region.

According to Shahi-Shavvon & al. (2017), *Oxytropis* has undergone a recent rapid radiation in Iran. Consequently, genetic differentiation among *Oxytropis* species is generally low, and even limited sequence divergence may indicate species-level separation. In this context, the minor molecular variation observed, together with the distinct morphology of *O. azizii*, is sufficient to support their recognition as a separate species.

Taxonomic treatment

Oxytropis azizii (Maassoumi) Maassoumi, Mirvaziri & Kaz.Osaloo, **comb. nov.**

Fig 1.

Basionym: *Astragalus azizii* Maassoumi, Mitt. Bot. Staatss. München 28: 504. 1989.

Type: Iran, Ardebil, N. slope of Mount Sabalan, 3500–3800 m, 30.7.1987, *Aziz Javanshir 1260* (Holotype: TARI; Isotype: HACT (Herbarium Agriculture College University Tabriz).

Amended description: Perennial, acaulescent, ca. 7–12 cm tall; caudex divided. Stipules very short, ca. 3 mm long, covered with appressed black and white

hairs, at the base ca. 1.5 mm adnate to the petiole and jointed to one another. Leaves ca. 4–5 cm long; petiole 2 cm, both petiole and rachis covered with appressed black and white hairs; leaflets 7–9 pairs, narrow-ovate, 2–4 mm long and 1–2 mm broad, acute; upper surface toward the margin covered with some scattered hairs; lower surface densely covered with white appressed hairs. Inflorescence densely spike-like; peduncle ca. (3–)6–9 cm long, with appressed black and white hairs. Bract ca. 2 mm long, with black hairs. Flowers numerous; pedicel deflexed, densely covered with appressed black-and-white hairs. Calyx campanulate ca. 3.5–4 mm long; the teeth subulate ca. 1 mm long, with black hairs. Corolla dark violet. Standard ca. 8 mm long; the limb obovate or orbicular, ca. 5 mm broad, emarginate at the apex, and abruptly attenuate at the base. Wing ca. 7.5 mm long; the limb oblong, shortly dilated toward the apex, emarginate at the apex, at the base auriculate, auricle ca. 1.5 mm long; the limb minutely longer than the claw. Keel ca. 6.5 mm long; the limb oblique-elliptic, mucronulate. Ovary densely covered with black hairs, at the base with a stipe 1.5 mm. Pods oblong, pendulous, ca. 12 mm long and 4.5–5 mm broad, densely vested with appressed black hairs, toward the apex terminating in a short beak ca. 1 mm long, at the base stipitate; stipe ca. 1 mm long, ventrally carinate, dorsally applanate or shortly sulcate, unilocular. Seeds numerous, ca. 1 mm long, reniform.



Fig.1. *Oxytropis azizii*. A, Photograph of holotype (TARD); B, Illustration of holotype: a, standard; b, wing; c, keel bearing a short mucro (marked with arrow) [adopted from Illustrated guide to the genus *Astragalus* in Iran. vol. 1: plate 59. (Maassoumi 1990)].

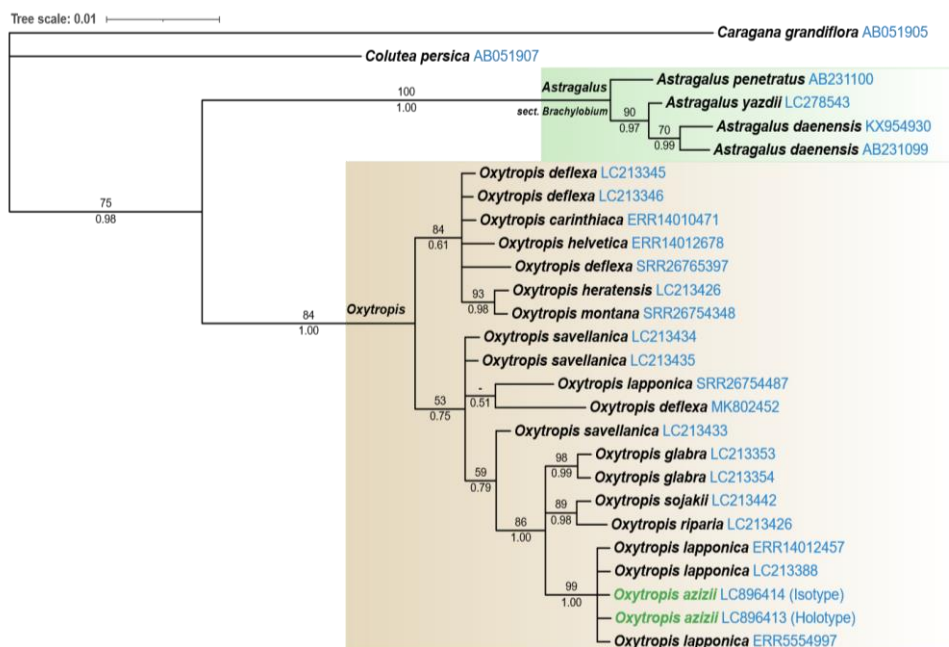


Fig. 2. 50% majority-rule consensus tree obtained using Bayesian inference. Numbers shown above and below the branches represent maximum likelihood bootstrap values and posterior probabilities, respectively (values below 50% are not displayed). GenBank accession numbers are provided alongside each species name.

Conservation status

According to Bidarlord & al. (2022), this taxon is restricted to a single population with an area of occupancy (AOO) of 0.25 km² in the upper alpine zone of Mount Sabalan. They estimated the fertile adults to be around 500 individuals, thereby qualifying this species as Critically Endangered (CR) under IUCN guidelines.

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