

ASTRAGALUS SECT. IRANIA (FABACEAE): A NEW MONOTYPIC SECTION FROM IRAN

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Based on morphological and molecular phylogenetic data, here we describe *Astragalus* sect. *Irania* Maassoumi, Mahmoodi & Bagheri as a new section in the immense genus *Astragalus*. Description and diagnosis are given. Morphologically, the new section is closely related to sect. *Stereothrix* Bunge, which is widely distributed in Elburz and Zagros Mountains ranges. The new section differs from sect. *Stereothrix* in characters of the inflorescence, color of petals and stipules. But in the molecular phylogenetic analysis, it is surprisingly placed among the thorny *Astragalus* species. Type of the new section is *A. issatissensis* Maassoumi, Mahmoodi, which is so far known only from the highest elevations of Shirkuh Mt. above 3800 m a.s.l in Yazd province.

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Key words: New section; *Astragalus issatissensis*; Shirkuh; alpine; Yazd; Iran

بخشه **Astragalus sect. Irania**: بخشه جدید و تک‌گونه‌ای از ایران

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در این مقاله براساس داده‌های تبارزایی مولکولی و ریخت‌شناسی بخشه *Astragalus* sect. *Irania* به‌عنوان آرایه‌ای جدید و تک‌گونه‌ای متعلق به سرده گون (تیره باقلائیان) معرفی می‌شود. شرح کامل بخشه و ویژگی‌های افتراقی آن از آرایه‌های مشابه ارائه شده است. از نظر ریخت‌شناسی، نزدیک‌ترین خویشاوند این بخشه جدید *sect. Stereothrix* Bunge است که به‌صورت نسبتاً گسترده‌ای در رشته کوه‌های البرز و زاگرس پراکنش دارد. بخشه جدید از نظر ویژگی‌های گل‌آذین، رنگ گلبرگ‌ها و گواشوارک با بخشه *sect. Stereothrix* متفاوت است. اما در تحلیل‌های تبارزایی مولکولی در کمال تعجب در میان گون‌های خاردار قرار می‌گیرد. نمونه تیپ این بخشه متعلق به گونه *A. issatissensis* است که تاکنون فقط در منطقه آبی شیرکوه یزد در ارتفاع ۳۸۰۰ متری مشاهده شده است.

INTRODUCTION

Astragalus L. is the largest genus of flowering plants with over 2900 species (Podlech & Zarre, 2013). Due to the large size of the *Astragalus*, infrageneric classification can be very helpful for better

understanding of the diversity within this genus (Zarre & Podlech, 2001). In first conclusive subgeneric classification of the genus, Bunge described about 791 species in 9 subgenera and 102 sections (Bunge, 1868 & 1869). Despite some differences and changes, in

almost all subsequent taxonomic treatments, infrageneric classification continued as a common practice (Boissier, 1872; Rydberg, 1929; Barneby, 1964; Goncharov, 1965; Maassoumi, 1998; Podlech & Zarre, 2013; Maassoumi, 2016). In Iran, this genus consisted about 840 species (Ghahremaninejad & al., 2020) in 80 different small, medium and large size sections, which are abundant through all Iranian territory especially in mountainous region. Shirkuh Mountain situated as an isolated mountain in central flatted part of Iran in Yazd province (4050 m a.s.l. at the highest peak; fig. 1). It has recently been identified as an area of endemism together with the Kerman Mountains (Noroozi & al., 2019). Over the years, several botanical excursions have been made to Yazd province and Shirkuh Mt., which has led to the discovery of numerous new Taxa such as *Astragalus jesdianus* Boiss. & Buhse, *A. mercklinii* Boiss. & Buhse, *A. schirkuhicus* Bornm., *A. ischredensis* Bunge, *A. lycioides* Boiss., *A. melanocalyx* Boiss. Also more recently several new endemic species such as *A. yazdii* (Vassilcz.) Podlech & Maassoumi, *A. mehrizianus* Podlech & Maassoumi, *A. darrehbidensis* Podlech & Zarre, *A. nowroozi* Podlech & Zarre, *A. neo-sytinii*

Ranjbar have been described from this province. A total of 57 *Astragalus* species are recorded from Yazd province, 41 (72 %) of which are endemic to Iran (table 1). Among endemics, 30 species are present in the Shirkuh Mt., showing the high rate of diversity, endemism and speciation of the genus in Shirkuh Mt. (Mahmoodi & al., 2009). Also there are 13 species of flowering plants endemic to Shirkuh Mt. confirming that Shirkuh Mt. are a biodiversity hotspot with a rich local endemism (Noroozi & al., 2020). Among collected materials by second author (MM) during a field trip to alpine zone of Shirkuh Mt. in early July 2012, *Astragalus issatissensis* Maassoumi & Mahmoodi was originally described as a new species in sect. *Stereothrix* Bunge (Mahmoodi & al., 2013). Also recently a monotypic genus *Yazdana* A. Pirani & Noroozi (Type: *Yazdana shirkuhensis* A. Pirani & Noroozi), belonging to Caryophyllaceae, was described (Noroozi & al., 2020) from material collected during the same field trip. Here a new section is described as a new monospecific section for the genus *Astragalus* based on molecular phylogenetic and morphological data. *Astragalus issatissensis* is designed as the type of the section.

Table 1. Checklist of *Astragalus* species recorded in Yazd province.

Species	Present in Shirkuh Mt.	Endemic of Iran
<i>A. yazdii</i> (Vassilcz.) Podlech & Maassoumi		*
<i>Astragalus albispinus</i> Širj. & Bornm.	*	*
<i>Astragalus anserinifolius</i> Boiss.	*	*
<i>Astragalus argyroides</i> Beck		
<i>Astragalus bakaliensis</i> Bunge		
<i>Astragalus calliphysa</i> Bunge	*	*
<i>Astragalus calliphysa</i> Bunge		*
<i>Astragalus callistachys</i> Buhse		*
<i>Astragalus campylanthus</i> Boiss.		*
<i>Astragalus cephalanthus</i> DC.	*	*
<i>Astragalus compactus</i> Lam.		
<i>Astragalus dactylocarpus</i> subsp. <i>acinaciferus</i> (Boiss.) E.Ott		
<i>Astragalus daenensis</i> Boiss.	*	*
<i>Astragalus darrehbidensis</i> Podlech & Zarre	*	*
<i>Astragalus fasciculifolius</i> Boiss.		
<i>Astragalus filicaulis</i> Fisch. & Mey. ex Karel.		
<i>Astragalus glaucacanthus</i> Fisch.	*	*
<i>Astragalus griseus</i> Boiss.	*	*
<i>Astragalus horridus</i> Boiss.	*	*
<i>Astragalus impexus</i> Podlech	*	*
<i>Astragalus iranicus</i> Bunge		
<i>Astragalus ischredensis</i> Bunge	*	*

Table 1. Continued.

<i>Astragalus issatissensis</i> Maassoumi & Mahmoodi	*	*
<i>Astragalus jesdianus</i> Boiss. & Buhse	*	*
<i>Astragalus johannis</i> Boiss.	*	*
<i>Astragalus kahiricus</i> DC.		
<i>Astragalus kirrindicus</i> Boiss.		
<i>Astragalus ledinghamii</i> Barneby		*
<i>Astragalus longistylus</i> Bunge	*	*
<i>Astragalus lycioides</i> Boiss.	*	*
<i>Astragalus lycioides</i> Boiss.	*	*
<i>Astragalus macropelmatus</i> Bunge subsp. <i>macropelmatus</i>		
<i>Astragalus mehrizianus</i> Podlech & Maassoumi	*	*
<i>Astragalus melanocalyx</i> Boiss.	*	*
<i>Astragalus melanodon</i> Boiss.	*	*
<i>Astragalus mercklinii</i> Boiss. & Buhse		
<i>Astragalus microphysa</i> Boiss.	*	*
<i>Astragalus multijugus</i> DC.	*	*
<i>Astragalus myriacanthus</i> Boiss.	*	*
<i>Astragalus neosytinii</i> Ranjbar		*
<i>Astragalus nowroozii</i> Podlech & Zarre		*
<i>Astragalus ovinus</i> Boiss.		
<i>Astragalus ovoideus</i> Širj. & Rech.f.		*
<i>Astragalus podolobus</i> Boiss. & Hohen.		
<i>Astragalus pseudobrachystachys</i> Širj. & Rech.f.		
<i>Astragalus pseudoshebarensis</i> Podlech	*	*
<i>Astragalus reuterianus</i> Boiss.		*
<i>Astragalus rhodosemius</i> Boiss. & Hausskn.	*	*
<i>Astragalus sclerocladus</i> Bunge		*
<i>Astragalus siliquosus</i> subsp. <i>siliquosus</i>		
<i>Astragalus spachianus</i> Boiss. & Buhse	*	*
<i>Astragalus supervisus</i> Sheld.	*	*
<i>Astragalus tenuiscapus</i> Freyn & Bornm.	*	*
<i>Astragalus trachyacanthos</i> Fisch.	*	*
<i>Astragalus vanillae</i> Boiss.		*
<i>Astragalus yazdii</i> (Vassilcz.) Podlech & Maassoumi	*	*

MATERIAL AND METHODS

Study area

Shirkuh Mt. is an isolated mountain in central part of Iranian Plateau that reaches 4050 m a.s.l. at the highest peak. Geographically it is located between

31°22'48" to 31°52'48" N latitude and 53°42'36" to 54°25'48" E longitude (fig. 1). It has Mediterranean Xeric continental climatic condition, which is surrounded by Mediterranean Desertic continental (Djamali & al., 2011).

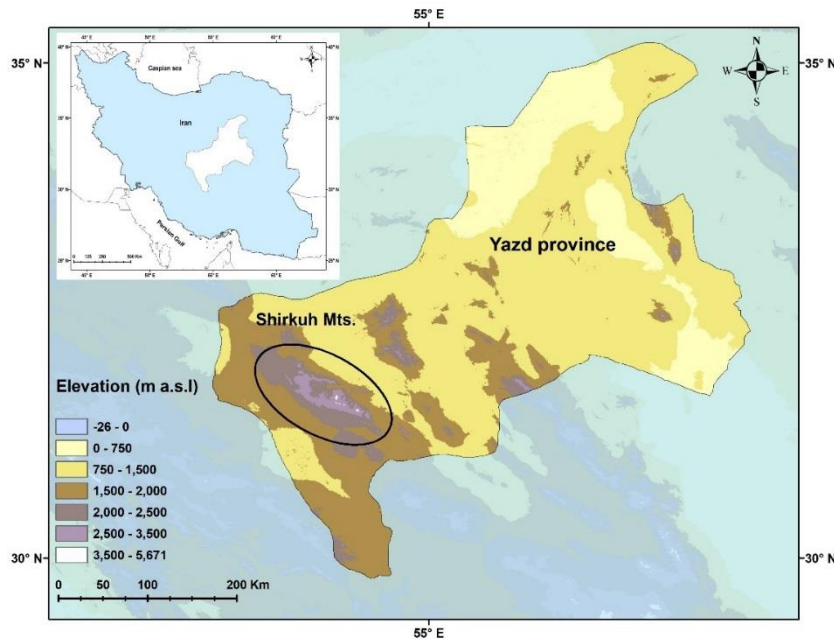


Fig. 1. Topographic map of Yazd province showing the location of the Shirkuh Mt.

Plant material

Plant material of the new taxon was collected in early July 2012 during a botanical expedition conducted by the second author to the alpine zone of Shirkuh Mt. in Central Iran. All the herbarium specimens are deposited in TARI. Herbarium codes used follow Thiers (2020).

DNA extraction, PCR amplification and molecular phylogenetic analyses

Total DNA from *Astragalus issatissensis* was extracted from the type herbarium specimens using the Plant Mini DNA Kit according to the instructions of the manufacturer, and used directly in PCR amplification. The complete nrDNA ITS region (ITS1, 5.8S and ITS2) was amplified using the primers ITS-A and ITS-B (Blattner 1999). The PCR conditions for the nrDNA ITS followed Bagheri & al. (2017). PCR conditions and primers for the *matK* followed Wojciechowski & al. (2004). Sequences from the species in *A. sect. Stereothrix* and its closely related taxa from other sections and also outgroups downloaded from GenBank and were used for the phylogenetic analysis. The examined taxa and GenBank accession numbers of specimens used are listed in table 2. The sequences were manually aligned together with 42 sequences of a dataset of *Astragalus* and one *Oxytropis* species as outgroup. All datasets (ITS and *matK* as well as combined) were analyzed in Bayesian phylogenetic analyses were performed using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). The sequence evolution model (SYM+G for the ITS and GTR+I+G for the combined dataset) were chosen following the Akaike Information

Criterion (AIC). BI with two times four Markov Chain Monte Carlo (MCMC) analyses run for 4 million generations, with a sampling trees every 1000 generations. The first 25% of trees were discarded as burn-in.

RESULTS AND DISCUSSION

After preliminary analyses of ITS (fig. 2) and *matK* (not shown) sequences, ILD test (Farris & al. 1994) did not provide significant difference between the outcomes of these two datasets. Hence, we combined the ITS and *matK* sequences. In obtained BI trees in both of ITS (fig. 1) and combined dataset (fig. 3), *A. issatissensis* has a distinct position and strongly separated from the main clade of taxa belonging to sect. *Stereothrix* and its relatives such as sect. *Hypoglottidei* and sect. *Malacothrix*. After detailed morphological reexamination of *A. issatissensis* samples and the materials in the herbarium TARI, we figured out that it does not fit in any known sections present in Iran and adjacent region (Ghahremaninejad & Behçet, 2003). We concluded it should be excluded from sect. *Stereothrix* (fig. 4). Therefore, here we present a description of Sect. *Irania* as a new section based on molecular phylogenetic and morphological data. Surprisingly, in both trees, the taxa belonging to thorny species of *Astragalus* are placed along with the section *Irania*, morphologically the last taxa (thorny species) by having spiny habit, are easily distinguishable from our new section. Morphological affinities of *A. sect. Irania* with *A. sect. Stereothrix* and other relative sections are presented in table 3.

Table 2. Informations of studied taxa in molecular phylogenetic analysis.

Species	Section	DNA source	accession No. of ITS	accession No. of <i>matK</i>
<i>A. aduncus</i>	<i>Onobrychoidei</i>	GenBank	AB727507.1	KX955059.1
<i>A. alaschanus</i>	<i>Oroboidei</i>	GenBank	KX954889.1	KX955208.1
<i>A. alopecias</i>	<i>Alopecuroidei</i>	GenBank	AB741272.1	JF409729.1
<i>A. alyssoides</i>	<i>Hololeuce</i>	GenBank	AB727527.1	KX955061.1
<i>A. annularis</i>	<i>Annulares</i>	GenBank	AB051912.1	KX955062.1
<i>A. australis</i>	<i>Hemiphragmium</i>	GenBank	KX954898.1	KX955067.1
<i>A. beckii</i>	<i>Malacothrix</i>	GenBank	KX954902.1	KX955071.1
<i>A. brachyodontus</i>	<i>Ornithopodium</i>	GenBank	AB727530.1	AB727537.1
<i>A. capito</i>	<i>Stereothrix</i>	GenBank	AB051996.1	KX955086.1
<i>A. caspicus</i>	<i>Adiaspastus</i>	GenBank	AB052025.1	MK958484.1
<i>A. cephalanthus</i>	<i>Microphysa</i>	GenBank	AB052027.1	MK958485.1
<i>A. compactus</i>	<i>Rhacophorus</i>	GenBank	AB231136.1	KX955091.1
<i>A. daenensis</i>	<i>Brachylobium</i>	GenBank	AB051963.1	KX955095.1
<i>A. echinops</i>	<i>Alopecuroidei</i>	GenBank	AB741278.1	AB741318.1
<i>A. facetus</i>	<i>Oroboidei</i>	GenBank	KX954942.1	KX955105.1
<i>A. glaucacanthos</i>	<i>Poterion</i>	GenBank	AB052017.1	KX955114.1
<i>A. glumaceus</i>	<i>Hymenostegis</i>	GenBank	AB231102.1	MK958492.1
<i>A. griseus</i>	<i>Malacothrix</i>	GenBank	KX954954.1	KX955117.1
<i>A. hemiphaca</i>	<i>Oroboidei</i>	GenBank	KX954959.1	KX955220.1
<i>A. herbertii</i>	<i>Hypoglottidei</i>	GenBank	MN812587	MN812603
<i>A. hymenostegis</i>	<i>Hymenostegis</i>	GenBank	KX955124.1	AB231103.1
<i>A. hypoglottis</i>	<i>Hypoglottidei</i>	GenBank	KX954964.1	KX955125.1
<i>A. issatissensis</i>	<i>Irania</i>	Holotype, 98654, TARI	MW342759	MW362137
<i>A. kaufmannii</i>	<i>Hemiphragmium</i>	GenBank	KX954970.1	KX955131.1
<i>A. ledinghamii</i>	<i>Stereothrix</i>	GenBank	AB051997.1	-
<i>A. leucothrix</i>	<i>Stereothrix</i>	GenBank	KX894515.1	-
<i>A. lunatus</i>	<i>Ornithopodium</i>	GenBank	AB727533.1	AB727540.1
<i>A. macrostachys</i>	<i>Malacothrix</i>	GenBank	AB051990.1	KX955143.1
<i>A. macrourus</i>	<i>Malacothrix</i>	GenBank	AB231124.1	KX955144.1
<i>A. microcephalus</i>	<i>Rhacophorus</i>	GenBank	KX894517.1	KX955147.1
<i>A. nurensis</i>	<i>Hypoglottidei</i>	GenBank	MN812588	MN812604
<i>A. penetratus</i>	<i>Brachylobium</i>	GenBank	AB231100.1	KX955162.1
<i>A. perpexus</i>	<i>Stereothrix</i>	GenBank	AB051998.1	-
<i>A. pish-chakensis</i>	<i>Hypoglottidei</i>	GenBank	MN812591	MN812607
<i>A. plagiophacos</i>	<i>Plagiophaca</i>	GenBank	AB231132.1	-
<i>A. rimarum</i>	<i>Hypoglottidei</i>	GenBank	AB231124.1	KX955144.1
<i>A. saganlugensis</i>	<i>Hypoglottidei</i>	GenBank	MN812598	MN812614
<i>A. spachianus</i>	<i>Malacothrix</i>	GenBank	KX955028.1	KX955187.1
<i>A. sphaeranthus</i>	<i>Stereothrix</i>	GenBank	MN812599	MN812615
<i>A. submitis</i>	<i>Anthylloidei</i>	GenBank	AB052009.1	KX955194.1
<i>A. vegetus</i>	<i>Onobrychoidei</i>	GenBank	AB727525.1	KX955203.1
<i>Oxytropis aucheri</i>	-	GenBank	AB051908.1	KM387602.1

Table 3. Comparison of morphological features of *Astragalus* sect. *Irania* with closely related sections.

Character/section	<i>Irania</i>	<i>Stereothrix</i>	<i>Hypoglottidei</i>	<i>Malacothrix</i>	<i>Oroboidei</i>	<i>Rhacophorus</i>
Hairs on vegetative parts	two kidns (short and long), only white, longer hairs uncinata	two kidns (short and long), black and white, all hairs straight	one kidns, black and white, all hairs straight (some species are glabrous)	one kidns or two kidns (short and long), black and white, all hairs straight	one kidns, black and white, all hairs straight	one kind, only white, all hairs straight
Stipules	adnate to petioles, very shortly vaginate-connate behind stem	adnate to petioles, very shortly vaginate-connate behind stem	free from the petiole	adnate to petioles, very shortly vaginate-connate behind stem or free from each other	free from the petiole	adnate to petiole, free from each other
Leaflets	imparipinnate, imbricate	imparipinnate, imbricate	imparipinnate, few and remote	imparipinnate, remote	imparipinnate, few and remote	paripinnate, few and
Leaflets margin	red, cartilaginous	green	green	green	Green	green
Peduncle	thin, terminal	thick, axillary	thin, axillary	thick, axillary	thin, axillary	absent
Inflorescence	spike, terminal, conical to capitate	Spike, spherical to cylindrical	raceme or spike, cylindrical	raceme, cylindrical	raceme, cylindrical	synflorescens
Calyx tube	funnel shape	infundibular	tubular	tubular	campanulate	turbinate
Calyx teeth	setaceous, equal to slightly longer than tube	setaceous, longer than tube	subulate to narrowly triangular, shorter than tube	subulate, shorter than tube	± narrowly triangular, shorter than tube	subulate to triangular, equal to longer than tube
Corolla	dark purple with white venation	violet or yellowish	pale blue to white	usually yellow, rarely purple	small, yellow to purplish	pale violet

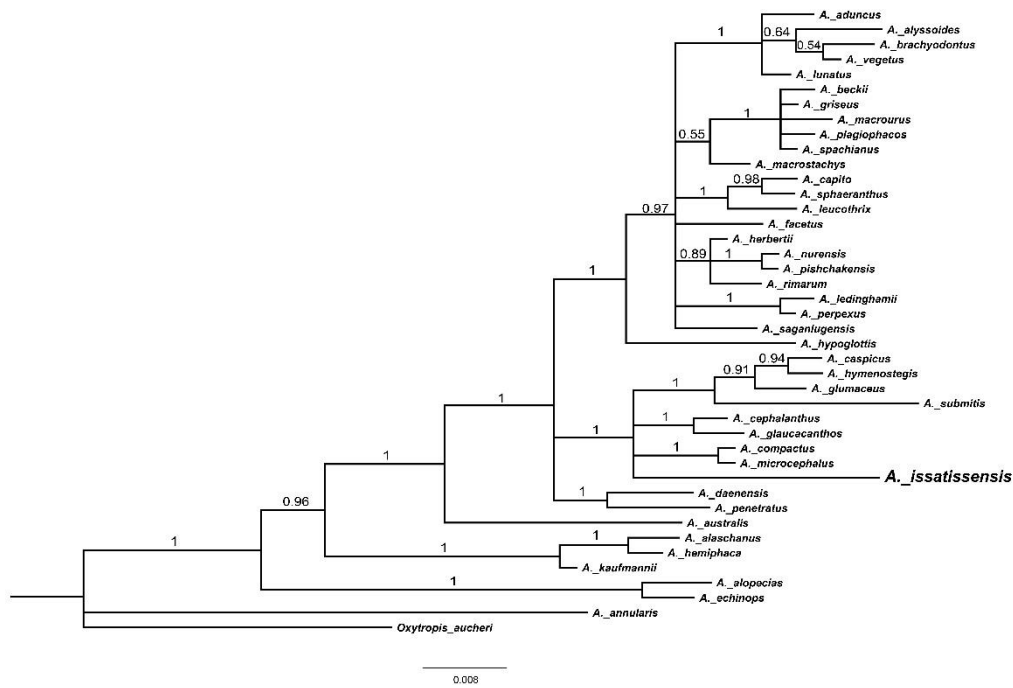


Fig. 2. Phylogenetic tree obtained from a BI analysis of the ITS sequences. Numbers above branches provide Bayesian posterior probabilities (pp).

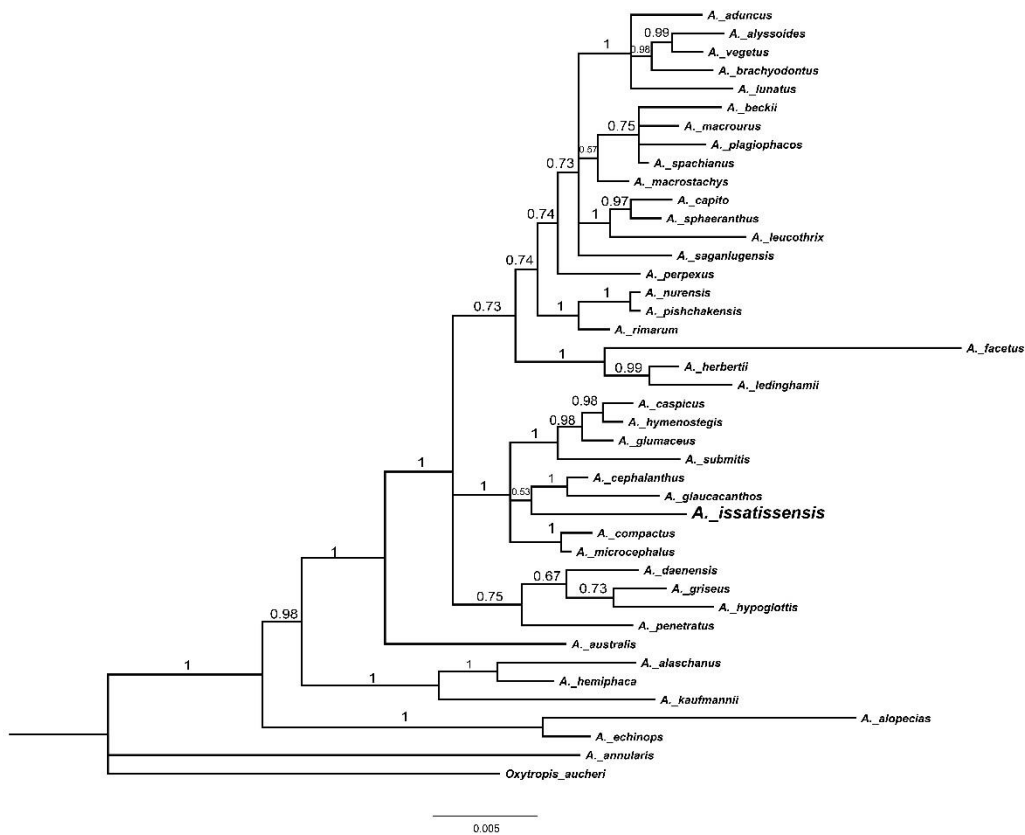


Fig. 3. Phylogenetic tree obtained from a BI analysis of the combined ITS and *matK* sequences. Numbers above branches provide Bayesian posterior probabilities (pp).

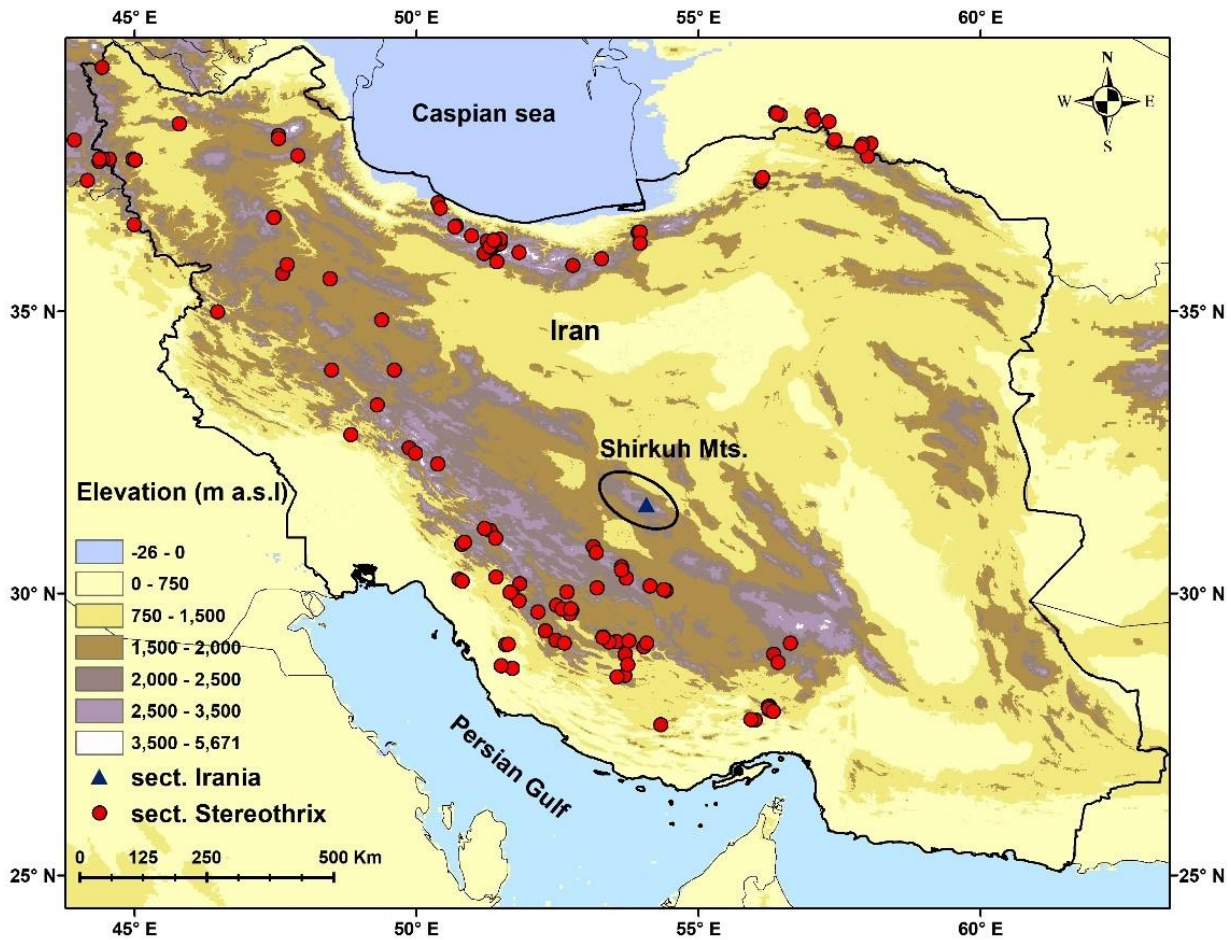


Fig. 4. Distribution of sect. *Irania* (*Astragalus issatissensis*) and sect. *Stereothrix* in Iran.

Taxonomic treatment

Astragalus Sect. *Irania* Maassoumi, Mahmoodi & Bagheri, **sect. nov.** (fig. 5)

Type: *A. issatissensis* Maassoumi & Mahmoodi, Willdenowia 43: 264 (2013).

Iran, Yazd province, Taft, Deh-e Balla Village, Shirkuh Mt., 31°37'04"N, 54°03'55"E, 3800-3900 m, 4 Jul 2012, Mahmoodi & Noroozi 98654 (TARI!).

Section *Irania* differs from sect. *Stereothrix* Bunge, in inflorescence dense, conical-capitate (not spherical to cylindrical); flowers dark purple with narrow white veins (not violet or yellowish), stipules shortly adnate to the petiole and shortly jointed to each other (not highly adnate to petiole); from sect. *Rhacophorus* Bunge, leaves imparipinnate (not paripinnate), terminal leaflets similar to laterals (not changes to spine), inflorescence dense spike (not synflorescens); from sect. *Anthylloidei* DC., calyx funnel shape (not tubular), claw in wings and keels free from staminal tube (not

shortly adnate to staminal tube), calyx in fruiting time cylindrical (not inflated); from sect. *Hymenostegis* Bunge, herbaceous, dense caespitose (not lax and suffruticous at the base), flowers dark purple (not yellow or violaceous), bracts narrowly lanceolate (not widely ovate).

Plant herbaceous, small, prostrate. Petiole and peduncle densely covered with two kinds of hairs, short hairs erect, long hairs spreading to ascendance, clearly uncinatate or shortly curved tip. Stipules densely hairy, adnate to the petiole, very shortly vaginate-connate behind stem. Petioles equaling the limb; leaflets imbricate, folded, both sides covered with spreading hairs, margin red, cartilaginous. Peduncle thin covered with short ascending hairs. Inflorescence conical-capitate, flowers numerous. Bracts narrowly lanceolate, pilose, inner side glabrous. Calyx funnel shape, pilose; teeth equaling to minutely longer than tube. Flowers dark purple with narrow white veins.



Fig. 5. Type specimens of sect. *Irania* (*Astragalus issatissensis*) from TARI.

Distribution and habitat

As currently known, the section is endemic to the alpine zone of Shirkuh Mt., growing on limestone screes at 3800-3950 m a.b.s (possibly up to 4075 m); while its related section, *Astragalus* sect. *Stereothrix* is distributed in Turkey, Iran, Ukraine, Turkmenistan, Armenia, Iraq, Syria and Lebanon (Podlech & al., 2013; Mahmoodi & al., 2013).

Conservation status

The new section exists only in the highest elevations of Shirkuh Mt. at two very close localities. Mahmoodi & al. (2013) evaluated it as Critically Endangered (i.e., facing an extremely high risk of extinction in the wild).

Etymology

Astragalus Sect. *Irania* is named after territory of Iran, the country where the new taxon was discovered.

Conclusion

In this study *Astragalus* Sect. *Irania* comprising the sole species *A. issatissensis* from the high alpine zone of the Shirkuh Mt, is introduced as a new section for the genus. Finding a new section of *Astragalus* and a new monotypic genus (Yazdana) in a single trip shows the urgent need to study the flora and vegetation of Shirkuh Mt., especially in alpine and nival zone. On the other hand, the altitudinal distribution of endemic plants in Shirkuh Mt. has a special pattern and percentage of endemism is high, which is probably due to the isolation and allopatric speciation (Noroozi & al., 2020). Considering global warming and the increasing destructive impact of human on natural ecosystems, it is likely that some valuable plant species, as genetic resources, will become extinct before they have chance to be discovered. Since there is no conservation management program in Shirkuh Mt., we strongly recommend that the area be announced as protected.

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