

## A NEW SPECIES OF ASTRAGALUS SECT. HYPOGLOTTIDEI (FABACEAE) FROM IRAN

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*Astragalus kazempourii* is described as a new species from Firuzkuh in Tehran province, Iran. Morphological data and phylogenetic analyses of the nrDNA internal (ITS) and external transcribed spacer (ETS) regions places this species within sect. *Hypoglottidei* as sister of *Astragalus parvarensis*. The new species differs morphologically from *A. parvarensis* by having a short stem densely covered with basifixed hairs, short peduncles and a long calyx. A distribution map of the new species is provided and the habitat condition of the taxon and close relatives, as well as the conservation status of *A. kazempourii* are discussed. The phylogenetic analyses conducted also support the recent transfer of *Astragalus bojnurdensis* from *A. sect. Brachylobium* to sect. *Hypoglottidei*, which was in accordance with the morphological characters of this species.

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**Key words:** Fabaceae; *Astragalus*; phylogeny; new taxon; Iran

گونه جدیدی از بخشه **Hypoglottidei** سرده گون (تیره باقلاییان) از ایران

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در این مقاله *Astragalus kazempourii* به عنوان گونه جدیدی از فیروزکوه در استان تهران شرح داده شده است. داده‌های ریخت‌شناسی و تجزیه و تحلیل تبارزایی بر پایه توالی‌های ITS و ETS هسته‌ای ریبوزومی (nrDNA) این گونه را در بخشه *Hypoglottidei* و به عنوان گروه خواهری *A. parvarensis* قرار می‌دهد. گونه جدید از نظر ریخت‌شناسی، به واسطه داشتن ساقه کوتاه پوشیده از کرک‌های ساده مترکم، دمگل‌آذین کوتاه و

کاسه بلند از گونه خواهری خود *A. parvarensis* متمایز است. نقشه پراکنش *A. kazempourii* ارائه و شرایط زیستگاهی این گونه و خویشاوندان نزدیک آن و همچنین وضعیت حفاظتی آن بحث شده است. با در نظر گرفتن صفات متمایزکننده بخشه *Hypoglottidei* در مقایسه با بخشه *Brachylobium* به عنوان آرایه بسیار نزدیک به آن، و نیز بر اساس شواهد مولکولی انتقال گونه *A. bojnurdensis* از بخشه *Brachylobium* به بخشه *Hypoglottidei* مورد تأیید قرار می‌گیرد.

## INTRODUCTION

*Astragalus* L. (Fabaceae), with about 3000 species, is known as the largest genus of flowering plants (Podlech & Zarre 2013). A review of the literature revealed that 240 sections are estimated in the genus, of which only few, including *A. sect. Hypoglottidei* DC., occur in both the Old and New World (Podlech & Zarre 2013). *Astragalus hypoglottis* L. was selected as the type species by de Candolle (1825) and later was chosen as the lectotype by Podlech for this section (Podlech 1990). *Astragalus sect. Hypoglottidei* with a total of 46 species belongs to the medium-sized sections of the genus in the Old World (Mahmoodi & al. 2012). Turkey and Iran with 19 and 10 *Hypoglottidei* species, respectively, are the countries with the highest numbers of occurring taxa (Podlech & Zarre 2013). The prominent and diagnostic characters of *A. sect. Hypoglottidei* are: (1) a tubular calyx with filiform teeth, which are shorter than the tube, (2) long and erect petals and (3) distinctly incised wing apices. Based on Kazempour Osaloo & al. (2003, 2005) the most closely related section to *sect. Hypoglottidei* is *sect. Brachylobium* Boiss., which clearly differs from the latter, by different states for the above mentioned characters. Here we provide a description and the taxonomic position for a newly discovered *Astragalus* taxon from Iran. Furthermore, to define its phylogenetic relationship with the closely related taxa, we included it in a molecular phylogenetic analysis

## MATERIALS AND METHODS

### Field collection and morphology

In the summer of 2013 and spring of 2016, during botanical expeditions to the Tehran and Semnan provinces in northeastern Iran, several specimens belonging to *A. sect. Hypoglottidei* were collected. Comparison with the *Astragalus* materials in TARI and MSB herbaria including the holotype specimen of *A. parvarensis* Podlech & Sytin and review of the relevant taxonomic literature (Podlech & al. 2010, 2012; Podlech & Zarre 2013) led us to conclude that these specimens closely resemble *A. parvarensis* but differ from it by several diagnostic morphological features of the stem, peduncles, calyx and indumentum.

### DNA extraction and molecular analyses

Total genomic DNA of the examined taxa of *Astragalus* was extracted from leaf materials using the DNeasy Plant Mini Kit (Qiagen). The nuclear ribosomal DNA internal transcribed spacer (ITS) region, including the spacers ITS1 and ITS2 together with the 5.8S rRNA gene lying in between, and the 5' external transcribed spacer (ETS) region upstream of the 18S rDNA were PCR amplified using the primers ITS-A and ITS-B (Blattner 1999) for the former and ETS-cis2F and 18S-ETS (Riahi & al. 2011) for the latter. Both regions were directly Sanger sequenced on an ABI 3730 XL using the amplification primers. Forward and reverse sequences from each individual were manually checked and assembled into individual contigs. As no indications for intra-individual polymorphisms in ITS and ETS were found in the contigs derived from direct sequencing, we did not clone amplicons.

The ITS and ETS sequences were manually aligned together with 21 sequences of species from other closely related sections such as *Malacothrix*, *Stereothrix*, *Brachylobium*, as well as *Oxytropis aucheri* Boiss. that was defined as outgroup. As both sequenced regions belong to the nrDNA cistron and evolve mostly together, we concatenated the sequences into a single data matrix. MODELTEST 3.7 (Posada & Crandall 1998) was used to test different models of sequence evolution and the SYM+I model was selected by the Akaike information criterion. Bayesian inference (BI) was conducted in MRBAYES 3.1 (Ronquist & Huelsenbeck 2003) running two times four chains for 2 million generations under the appropriate DNA evolution model, sampling a tree every 1000 generations. Convergence of the analyses was evaluated and the first 25% of the trees were discarded as burn-in. A maximum parsimony (MP) analysis was conducted in PAUP\* 4.0a152 (Swofford 2002) using the heuristic search algorithm. To test clade, support a bootstrap analysis with 500 bootstrap resamples was conducted. A list of all the taxa and voucher specimens used for the molecular analysis, as well as GenBank accession numbers are given in table 1.

Table 1. Information of examined species in molecular analysis.

Species	DNA source	GenBank accession No. for ITS	GenBank accession No. for ETS
<i>A. aegobromus</i>	GenBank	AB051953.1	JF409728.1
<i>A. alopecias</i>	GenBank	AB741272.1	JF409729.1
<i>A. annularis</i>	GenBank	AB051912.1	JF409730.1
<i>A. bojnurdensis</i>	383, TARI	MN812585	MN812601
<i>A. bojnurdensis</i>	738, TARI, Holotype	MN812586	MN812602
<i>A. echinops</i>	GenBank	AB741278.1	JF409737.1
<i>A. herbertii</i>	39851, TARI, Holotype	MN812587	MN812603
<i>A. nurensis</i>	51549, TARI	MN812588	MN812604
<i>A. parvarensis</i>	52233, MSB, Holotype	MN812589	MN812606
<i>A. parvarensis</i>	80598, TARI	MN812590	MN812605
<i>A. pish-chakensis</i>	75471, TARI	MN812591	MN812607
<i>A. pish-chakensis</i>	51407, TARI, Holotype	MN812592	MN812608
<i>A. kazempourii</i>	100649, HUI	MN812593	MN812609
<i>A. kazempourii</i>	100825, HUI	MN812594	MN812610
<i>A. kazempourii</i>	105495, HUI	MN812595	MN812611
<i>A. kazempourii</i>	98137, TARI	MN812596	MN812612
<i>A. kazempourii</i>	100636a, TARI, Holotype	MN812597	MN812613
<i>A. saganlugensis</i>	98373, TARI	MN812598	MN812614
<i>A. sphaeranthus</i>	10230, TARI	MN812599	MN812615
<i>A. sphaeranthus</i>	96975, TARI	MN812600	MN812616
<i>O. aucheri</i>	GenBank	AB051908.1	JF409764.1

## RESULT

### Phylogenetic analyses

The alignment of the included sequences had a length of 879 base pairs (ITS: 607 bp, 54 parsimony informative characters; ETS: 272 bp, 32 parsimony informative characters). Parsimony analysis resulted in a single tree of 196 steps length (not shown) with a consistency index (CI) of 0.893 and a retention index (RI) of 0.915. The parsimony and the Bayesian phylogenetic analyses (fig. 1) resulted in trees with identical topologies except for *A. saganlugensis* Trautv. that in MP is sister to all other included taxa of sect. *Hypoglottidei* while in BI it is sister to *A. bojnurdensis*, *A. parvarensis* and *A. kazempourii*. However, support values are for both topologies rather low. MP and BI analyses agree on the specimens of *A. parvarensis* forming the sister group of *A. kazempourii*. This close relationship is supported by the overall high similarity between both species; considering rather small genetic differences between closely related taxa in the rapidly evolving genus *Astragalus* (Bagheri & al. 2017). Maassoumi (2018) transferred *A. bojnurdensis* from *A. sect. Brachylobium* to *A. sect. Hypoglottidei*. We included this species in our molecular phylogenetic analyses, the result strongly confirm the position of *A.*

*bojnurdensis* within *A. sect. Hypoglottidei*.

### Taxonomic treatment

*Astragalus kazempourii* Bagheri, Maassoumi & Mahmoodi **sp. nov.** figs 2-3.

Type. Iran. Tehran province: Firuzkuh, 25 km on the road toward Semnan, 1.5 km after Goorsefid village, N 35°43'40.9'', E 53°02'18.6'', 2356 m, 26 May 2016, Mahmoodi & Bagheri 100636 (holotype TARI, isotypes HUI, TARI).

Perennial herb, 10–20 cm tall, laxly caespitose, caudex much divided, caulescent. Densely covered with rigid, appressed to spreading, white hairs 0.2–0.6 mm long, mixed with few black hairs. Stems 3–7 cm, erect, slender, and densely covered with short and long appressed white hairs. Stipules yellowish, ca. 4–5 mm long, triangular, free from petiole, connate with the stem up to 1 mm, sparsely to densely covered with appressed to spreading white and few black hairs, ciliate at the margin. Leaves compound, 1–3 cm; petiole 0.5–1.5 cm, like the rachis slender, sparsely covered with subappressed white hairs. Leaflets (5–) 7–10 pairs, narrowly elliptic, 3–5 × 0.5–1.5 mm, subacute, densely covered with subappressed to spreading, rigid hairs. Racemes subglobose, becoming elongate,

densely 10–16-flowered. Peduncles 4–8 cm long, densely covered in lower part with predominantly white appressed hairs, in upper part below the inflorescence with few black appressed hairs. Bracts narrowly ovate, acute, pale greenish, 2–2.5 mm long, with black and white appressed hairs on outer surface. Calyx 7–12 mm, chartaceous to membranous, cylindric, covered with appressed to subappressed white hairs up to 1.2 mm long, mixed with black hairs mostly in upper parts; teeth subulate, 3 mm long, shorter than the tube, mostly covered with white hairs mixed with few black hairs. Petals lilac to purplish, standard 13–14 × 7.5 mm, ovate to oblong, retuse, at the base cuneately narrowed to a claw. Wings 11 mm;

blades elliptic to oblong, apex slightly emarginate or rotundate, 7 × 2.5 mm; auricle ca. 1 mm, claw 5–6 mm. Keel 10–11.5 mm; blades oblique, oblong, acute; auricle 0.5 mm. Ovary ca. 8 mm, shortly stipitate, hairy, stigma capitate. Stamens ca. 10 mm, diadelphous, 9 + 1, the connate stamens free from each other in the upper 2–3 mm. Style glabrous. Fruit a legume, ca. 10–15 × 5 mm, stipitate, stipe glabrous, oblong to ovoid, densely covered with long spreading white hairs ca. 2.5 mm long, mixed with very short erect black hairs, dorsally and ventrally carinate. Seeds 1–2 in each locule, 3 × 2 mm long, brownish. See also Table 2 for the comparison of characteristic traits.

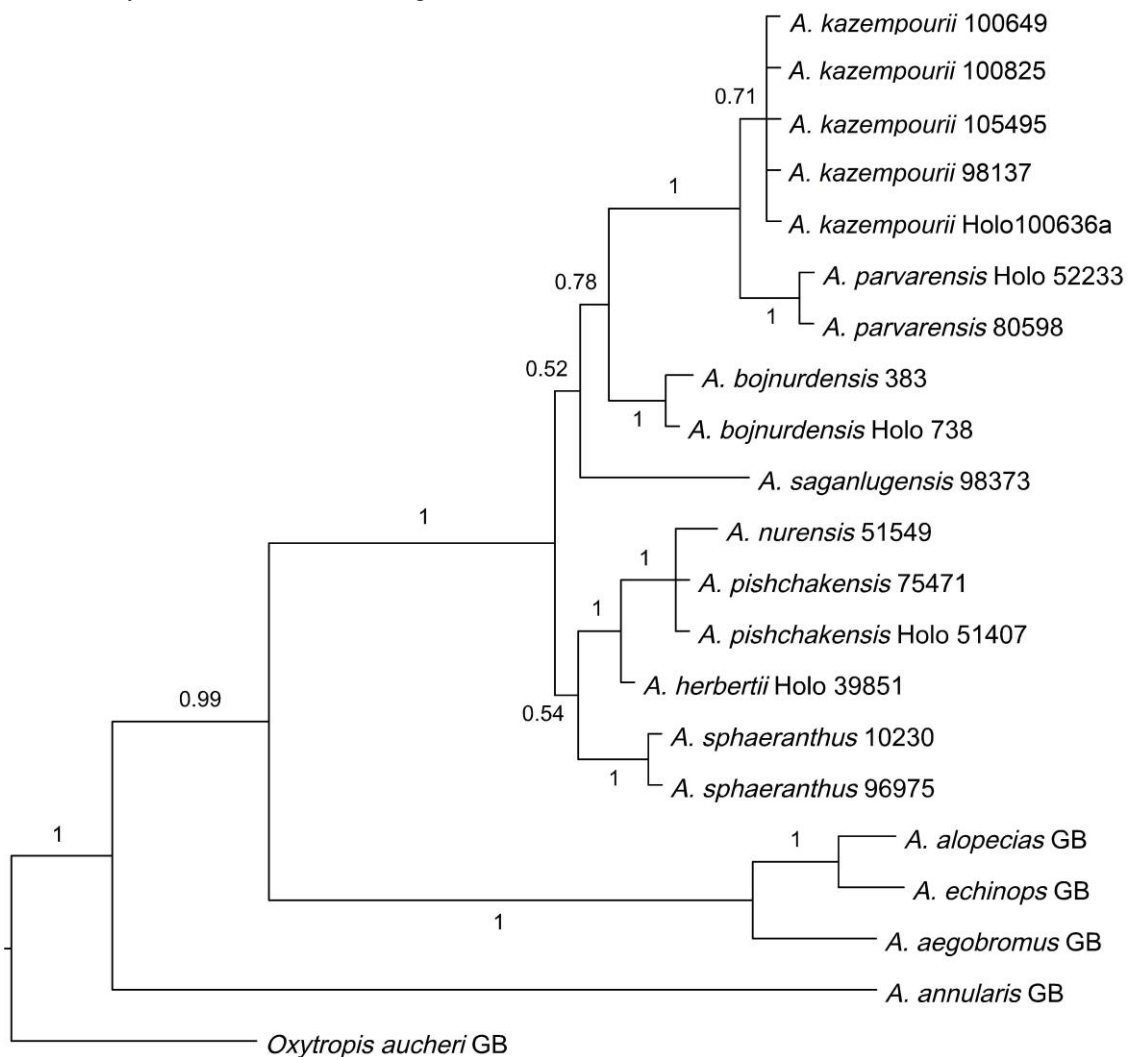


Fig. 1. Phylogenetic tree derived from a Bayesian phylogenetic analysis of combined sequence data of the nuclear rDNA ITS and ETS regions. Numbers along branches provide Bayesian posterior probabilities (pp). Sequences of species names followed by 'GB' were obtained from GenBank.

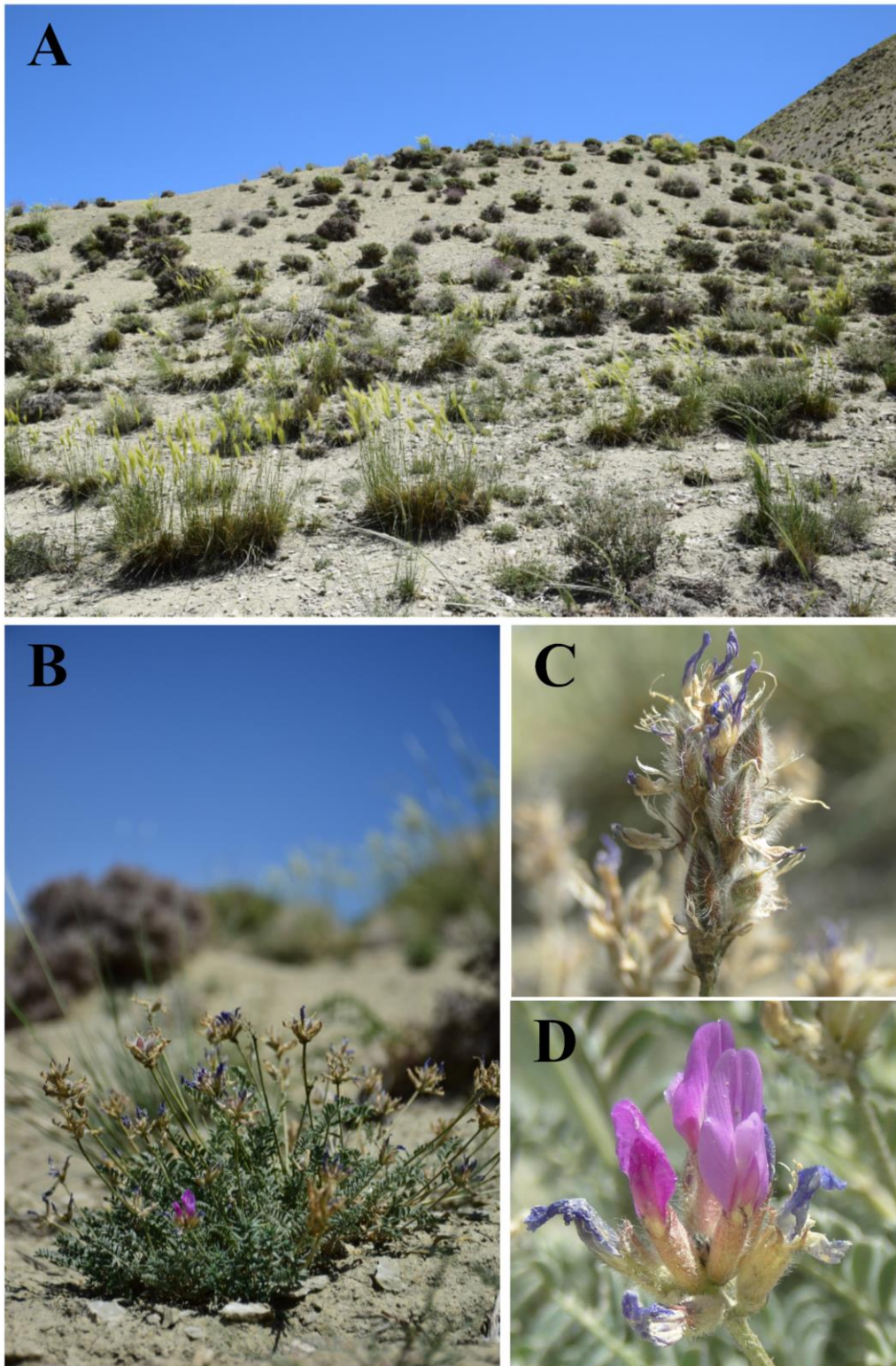


Fig. 2. *Astragalus kazempourii*: a. Habitat; b. Habit; c. Fruits; d. Inflorescence. (Photographs taken at the type locality, 26 May 2016, by M. Mahmoodi).

**Flowering:** May to June.

**Fruiting:** June to July.

**Etymology:** The specific epithet “kazempourii” was chosen in honour of Prof. Dr. Kazempour, who is a specialist on the molecular phylogeny of Legumes in Iran.

**Additional specimens seen (Paratypes):** Iran. Tehran province: 35 km from Semnan to Firuzkuh, N 35°43'37'', E 53°02'20'', 2400 m, 07 Jun 2013, Bagheri 98137 TARI, HUI; Tehran province: Firuzkuh, 16 km on the road toward Semnan, 5 km after Seranza village, N 35°44'52.5'', E 52°57'03.3'', 2200 m, 26 May 2016, Mahmoodi & Bagheri 100649, 100824,

100825 TARI, HUI; Tehran province: Firuzkuh, 25 km on the road toward Semnan, 1.5 km after Goorsefid village, N 35°43'40.9'', E 53°02'18.6'', 2356 m, 26 May 2016, Mahmoodi & Bagheri 105493, 105494, 105495 TARI, HUI.

*Astragalus kazempourii* is morphologically close to *A. parvarensis* and *A. bojnurdensis*, which are endemic to Iran, but there are clear differences in indumentum, stem length, peduncle length, calyx length and texture, calyx teeth length, petal shape and length. The morphological characters of these species are compared in table 2.

Table 2. Diagnostic differences among *Astragalus kazempourii*, *A. parvarensis* and *A. bojnurdensis*.

Species / Characteristic	<i>A. kazempourii</i>	<i>A. parvarensis</i>	<i>A. bojnurdensis</i>
Stem length	3-7 cm	18 cm	1.5 cm
Indumentum on vegetative parts	basifixed	subbasifixed	basifixed
Peduncles length	4-8 cm	6-20 cm	1-3.5 cm
Peduncles hairs	appressed	appressed	spreading
Rachis hairs	appressed	appressed with few ascending hairs, partly glabrescent	spreading
Calyx length	7-12 mm	8-9 mm	10 mm
Calyx hairs	appressed	appressed with few ascending hairs	spreading
Calyx texture	chartaceous to membranous	chartaceous	hyaline-membranous
Calyx teeth	3-4 mm, shorter than the tube	3 mm, shorter than the tube	5 mm, as long as the tube
Standard	13-14×5.5 mm	15×5 mm	10×3.5 mm
Wings	11 mm; lamina 7×2.5 mm	12 mm, lamina 6×2 mm	8 mm; lamina 4×1 mm

**Distribution and habitat:** Distribution of *Astragalus kazempourii* is restricted to a small area in central Alborz mountain range between the provinces of Tehran and Semnan (fig. 4). It has hitherto been found three times at 2200–2400 m altitudinal range. *Astragalus kazempourii* belongs to the high mountain steppe zone and mostly grows on gypsum containing soils. Most frequently associated species in the observed localities of the new species are: *Alyssum linifolium* Stephan ex Willd., *Astragalus brunsianus* Bornm., *A. aegobromus* Boiss. & Hohen., *A. citrinus* Bunge, *A. iranicus* Bunge, *A. masenderanus* Bunge, *A. mesoleios* Boiss. & Hohen., *A. podolobus* Boiss., *Cousinia aggregata* DC., *Crucianella gilanicum* Trin., *Eryngium billardierei* F. Delaroché, *Glaucium elegans* Fisch. & C.A.Mey., *Hyoscyamus niger* L., *Krascheninnikovia ceratoides* subsp. *ceratoides* var.

*latifolia*, *Matthiola farinosa* Bunge ex Boiss., *Noaea mucronata* (Forssk.) Asch. & Schweinf., *Psathyrostachys fragilis* (Boiss.) Nevski, *Pseudotrachydium depressum* (Boiss.) Pimenov & Kljuykov, *Stipa caucasica* Schmalh., *Taraxacum roseum* Bornm., *Tragopogon graminifolius* DC., *T. sp.*, *Scorzonera sp.*, *Papaver sp.*, and *Allium sp.*

**Conservation status:** *Astragalus kazempourii* is known so far as an endemic of only few localities within a distance of 10 km in the eastern part of Tehran province. There are no protection plans for the areas where the new species grows and also because of its vicinity to several villages, there is frequent overgrazing as a destructive factor in those areas. Consequently, following the IUCN Red List criteria (IUCN, 2012) *A. kazempourii* is categorized as Critically Endangered: CR, B1b (iii) +B2 (ii).

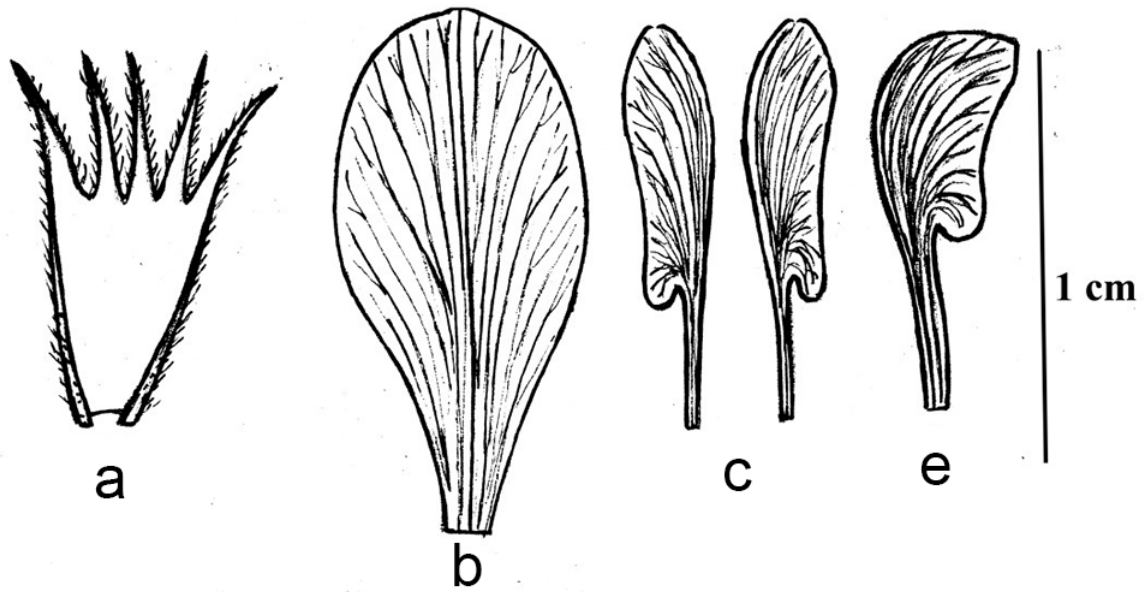


Fig. 3. Hand drawing of *Astragalus kazempourii* flowers: a. Calyx, b. Standard, c. Wings, d. Keel.

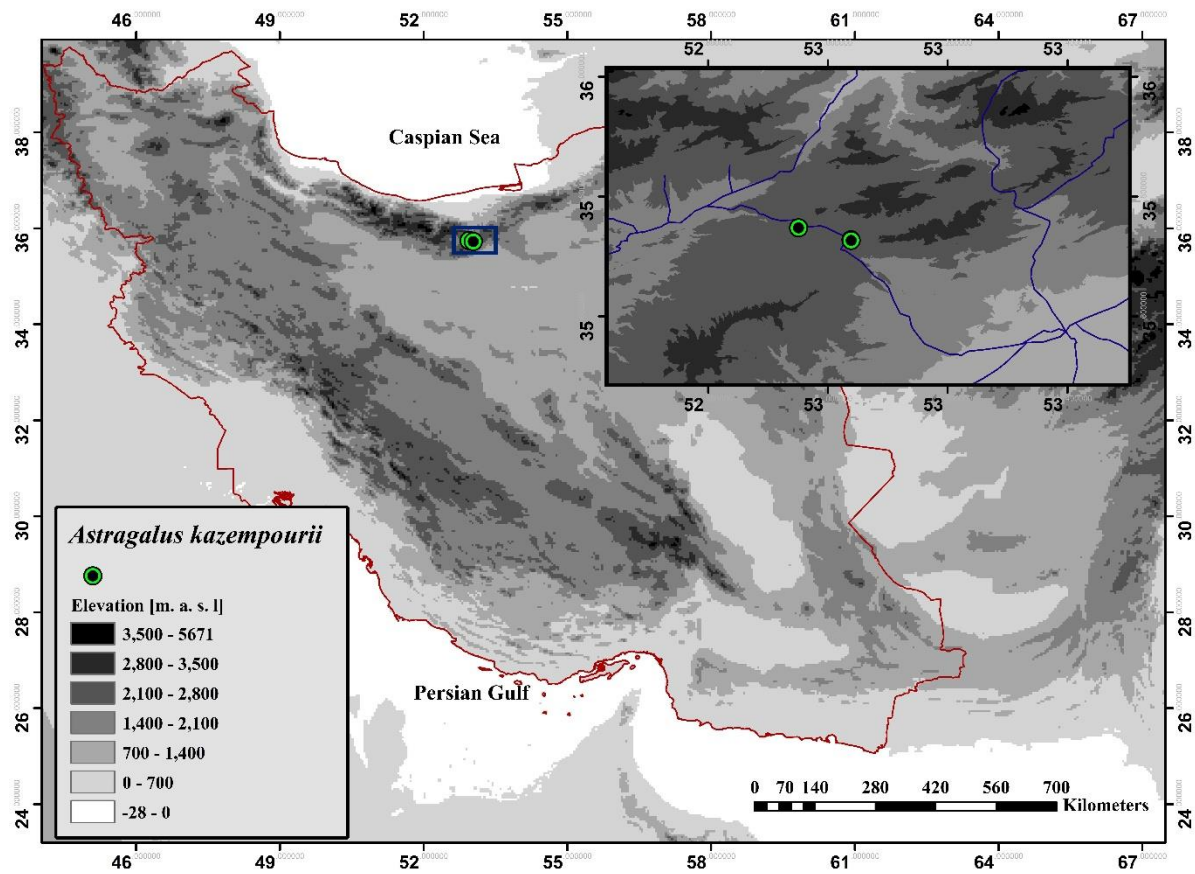


Fig. 4. Distribution map of *Astragalus kazempourii*.

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