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A NEW SPECIES OF ASTRAGALUS SECTION DISSITIFLORI (FABACEAE) FROM IRAN; EVIDENCE FROM MORPHOLOGICAL AND MOLECULAR DATA

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Astragalus lignipes (Fabaceae), a new species of sect. *Dissitiflori* will be described here based on morphological and molecular data (ITS and *matK* sequences). Our Maximum Parsimony and Bayesian Phylogenetic Inference analyses show that the new species is most closely related to *A. aestimabilis*, *A. aucheri* and *A. xiphidium*. It is characterized by long woody basal stems, chartaceous bracts and long pedicels. We provide information on the distribution and habitat of the new species with taxonomic notes about it and its sister taxa. We also assess the conservation status of the new species according to IUCN criteria as Critically Endangered.

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Key words: Fabaceae; taxonomy; Flora of Iran; Zagros Mountains; Isfahan province; Iran

یک گونه جدید از گون از بخشه Dissitiflori (تیره بقولات) از ایران با استفاده از شواهد ریخت‌شناسی و مولکولی

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Astragalus lignipes به عنوان یک گونه جدید از بخشه Dissitiflori براساس داده‌های ریخت‌شناسی و مولکولی (توالی‌هایITS و matK) شرح داده می‌شود. تحلیل‌های تبارزایی بیشینه صرفه‌جویی و بیزین ما نشان می‌دهد که گونه جدید، خویشاوند نزدیک گونه‌های A. aestimabilis, A. aucheri و A. xiphidium است. این گونه، به داشتن ساقه‌های قاعده‌ای بلند چوبی، براکته‌های کاغذی، و دمگل‌های بلند متمایز می‌شود. اطلاعاتی در خصوص پراکنش و زیستگاه گونه جدید به همراه نکات تاکسونومیک این گونه و خویشاوندان نزدیک آن ارائه شده است. همچنین وضعیت حفاظتی گونه جدید، مطابق معیارهای IUCN با درجه "در بحران انقراض" ارزیابی می‌شود.

INTRODUCTION

Astragalus L. (Fabaceae) is the largest genus of flowering plants, comprising about 2400 species in the Old and 500 in the New World (Podlech & Zarre 2013). *Astragalus* has its largest diversity in South-western and Central Asia, the Sino-Himalayan region, western North America along the Rocky Mountains and in the Andes in South America (Lock & Simpson 1991; Maassoumi 1998). In his comprehensive account of the genus, Candolle (1825) validly established sect.

Dissitiflori including originally 15 species. Podlech (1990), "lectotypified" this section by *Astragalus virgatus* Pallas. This section became through time one of the largest sections of the genus by increasing its species number to 166 (Podlech & Zarre 2013). The members of sect. *Dissitiflori* are distributed in Central and eastern Europe, South-western and South-eastern Asia (Podlech & Zarre 2013; Maassoumi 1998). Sect. *Dissitiflori*, as well as other morphologically similar sections, such as sect. *Ornithopodium* Bunge and sect. *Onobrychoidei* DC., is widely distributed in Europe and Asia (Podlech & Zarre 2013; Maassoumi 1998). Members of sect. *Dissitiflori* are perennial plants, with bifurcate hairs, imparipinnate leaves, flowers organized in loose racemes, flowers with short pedicle and legumes with bilocular valves (Bunge 1868). Recently several new species of this section were described (Ghahreman & al. 1996; Maassoumi & al. 2000; Ghahremani-Nejad 2005; Zarre & al. 2005; Maassoumi, 2007; Ranjbar 2007; Souzani & al. 2009). Finally, Podlech & Zarre (2013) recognized 20 species for the flora of Iran within this section including 15 endemics. In order to assess phylogenetic relationships of this section and its allies, Sheikh Akbari Mehr & al. (2012) published an nrDNA ITS analysis and Sheikh Akbari Mehr & al. (2016) a plastid DNA analysis of this group. According to their studies, sect. *Dissitiflori* is paraphyletic, becoming monophyletic with the enclosure of some members of sections *Corethrum* Bunge, *Erioceras* Bunge and *Cytisodes* Bunge (Sheikh Akbari Mehr & al. 2016). In addition, Bartha & al. (2013) studied molecular phylogenetic relationships in the European members of this section with emphasis on reticulate speciation among this group. Here we describe and illustrate a newly discovered species from Iran based on morphological and molecular data highlighting its taxonomic relationships with some related species in *A.* sect. *Dissitiflori*.

MATERIAL AND METHODS

Field collection and examined taxa

During the field expeditions in 2012 and 2018 by the first author to Fereydunshahr, Isfahan province in Central Zagros, some interesting specimens of the genus *Astragalus* were collected from few small populations. Basing on diagnostic morphological features, they resulted to belong to sect. *Dissitiflori*. After careful comparison with the taxa listed in *Flora Iranica* (Podlech & al. 2010, 2012), *Flora of Iran* (Maassoumi 2018) and other relevant literature (Podlech & Zarre 2013), as well as with herbarium specimens in B, E, G, HUI, K, M, MSB, P, SFAHAN, TARI and W including type materials, it became clear that our specimens were possibly to attributed to a new species of *Astragalus*, which is described here. Within this section, *A. aestimabilis* Podlech morphologically is the most similar, as it shares some relevant features such as woody branching at the base, shape and type of hairs in stipules, loosely, few flowerd in racemes, tubular calyx and size of petals. In addition, we analyse our populations by a nuclear and plastid molecular dataset to determine their phylogenetic position in sect. *Dissitiflori*.

Molecular analysis

Total genomic DNA of five individuals of the presumed new taxon and the isotype of *A. aestimabilis* were extracted from leaf materials using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. For the phylogenetic analyses, we used as a nuclear DNA region the nuclear ribosomal internal transcribed spacer region (ITS) and for the chloroplast the gene maturase K (*mat*K) in 24 taxa (table 1). For both species the ITS region, including the spacers ITS1 and ITS2 together with the 5.8S rRNA gene lying in between, was PCR amplified using the primers ITS-A and ITS-B (Blattner 1999), and *mat*K was PCR amplified using the primers provided in (Wojciechowski & al. 2004). Amplicons were Sanger sequenced on an ABI 3730 XL. Forward and reverse sequences from each individual were manually checked and assembled into individual contigs. Sequences were obtained from the GenBank nucleotide database except for presumed new taxon and *A. aestimabilis.* For the presumed new taxon, we analyzed five individuals. The sequences were manually aligned together with 28 sequences of a dataset of *Astragalus* and one *Oxytropis* species as outgroup. MODELTEST 3.7 (Posada & Crandall 1998) was used to test different models of sequence evolution and the HKY+Imodel was selected by Akaike information criterion (AIC). Phylogenetic analyses were performed using the Bayesian inference (BI) and Maximum Parsimony (MP). BI was calculated using MrBayes v.3.1 (Ronquist & Huelsenbeck 2003) with two times four Markov Chain Monte Carlo (MCMC) analyses run for 2.5 million generations, with a sampling frequency of one in every 1000 generations. The initial 25% of the trees were discarded as burn-in. MP analysis was conducted in PAUP* 4.0a163 (Swofford 2002) using the heuristic search algorithm with 500 random addition sequences. To test clade support, a bootstrap analysis with 1000 bootstrap resamples with the heuristic search algorithm without random sequence additions was conducted.

Table 1. Information of examined species in molecular analysis.

Species	DNA source	GenBank accession No. for ITS	GenBank accession No. for matK
A. aestimabilis	1939, Isotype, W	MK584618	MK603206
A. annularis	GenBank	AB051912.1	KX955062.1
A. argyroides	GenBank	AB721936.1	AB727543.1
A. aucheri	GenBank	AB721937.1	KM387659.1
A. baraftabensis	GenBank	AB721942.1	LC129307.1
A. brachyodontus	GenBank	AB727530.1	AB727537.1
A. caprinus	GenBank	KX954920.1	KX955087.1
A. dendroproselius	GenBank	AB721952.1	LC129293.1
A. dictyolobus	GenBank	AB741277.1	AB741316.1
A. echinops	GenBank	AB741278.1	AB741318.1
A. goktschaicus	GenBank	AB727515.1	LC129315.1
A. husseinovii	GenBank	AB721939.1	LC129308.1
A. jodostachys	GenBank	AB727532.1	AB727539.1
A. juladakensis	GenBank	AB721950.1	LC129295.1
A. lignipes	98155a, Holotype, TARI	MK584619	MK603207
A. lignipes	98155b, TARI	MK584620	MK603208
A. lignipes	98155c, TARI	MK584621	MK603209
A. lignipes	98155d, TARI	MK584622	MK603210
A. lignipes	98155e, TARI	MK584623	MK603211
A. melanocalyx	GenBank	AB721941.1	LC129298.1
A. pravitzii	GenBank	AB721944.1	AB727544.1
A. ruscifolius	GenBank	AB721945.1	AB727545.1
A. saadatabadensis	GenBank	AB721946.1	LC129292.1
A. sitiens	GenBank	AB721947.1	LC129305.1
A. teheranicus	GenBank	AB727523.1	LC129314.1
A. viridis	GenBank	AB721953.1	KM387668.1
A. xiphidium	GenBank	AB721949.1	LC129296.1
Oxytropis aucheri	GenBank	AB051908.1	KM387602.1

RESULTS AND DISCUSSION

Molecular results

Since the initial separate analyses of ITS and *mat*K (figs not shown here) sequences, ILD test (Farris & al. 1994) did not provide significant difference between the results of these two markers, we combined the ITS and *mat*K sequences of 15 species of sect. *Dissitiflori* (the new species was included with five individuals), four species from closely related sections, four species from sections only distantly related to sect. *Dissitiflori*, and one outgroup sequence of *Oxytropis*. This combined dataset was an alignment with the length of 1802 base pairs and 191 variable characters of which 99 were parsimony informative. Parsimony analysis resulted in six equally parsimonious trees 235 steps long with a consistency index (CI) of 0.877 and a retention index (RI) of 0.907. In the BI (fig. 3) and MP trees (fig. 4) and specimens of *A. aestimabilis*, *A. aucheri*, *A. husseinovii* and *A. xiphidium* form a clade together with presumed new taxon. All sequenced individuals of the presumed new species shared identical ITS and *mat*K sequences. The sequences of presumed new taxon are clearly different from the respective sequences of the other species within this clade. Generally, molecular differences (correlating to the branch lengths in the BI tree) within sect. *Dissitiflori* are pronounced in comparison to other groups of *Astragalus* from the same area (Bagheri & al. 2017), which indicates differences in speciation rates among different groups of *Astragalus*.

Taxonomic Treatment

Astragalus lignipes Akhavan & Maassoumi **sp. nov**. figs. 1 & 2.

Typus: Iran. Isfahan province: West of Isfahan, Fereydunshahr, Tatashvilli Mountain, 32° 56′ 11”N, 50° 07′ 19”E, 2600 m, 28 May 2012, Akhavan & Bagheri 98155a (holotype TARI, isotypes SFAHAN, HUI).

Paratypes: Iran, Isfahan province: West of Isfahan, Fereydunshahr, Tatashvilli Mountain, 32° 56′ 01”N, 50° 07′ 25”E, 2580 m, 22 May 2018, Akhavan & Bagheri 22242 (TARI, SFAHAN, HUI).

Similar to *A. aestimabilis* Podlech, but 40-60 cm tall (vs. 15-35 cm); stems of the current year up to 20 cm long (vs. 2-7 cm); stipules triangular, subulate-acuminate, 4-6 mm long (vs. ca. 3 mm); leaves 3-4 cm long (vs. 4-7 cm); leaflets in 3-4 pairs (vs. 4-6 pairs), sparsely hairy (vs. rather densely hairy); peduncles 8-10 cm long (vs. 9-14 cm); bracts chartaceous, 3-4 mm long (vs. whitish-membranous, 1.5-2 mm); pedicels 3-5 mm long (vs.1-2 mm); legumes 20-26 mm long (vs.15-17 mm). Similar to *A. argyroides* Beck, but 40-60 cm tall (vs. 30-40 cm); leaflets in 3-4 pairs (vs. 4-6(-7)); peduncles 8-10 cm long (vs. 1-7 cm); bracts chartaceous, 3-4 mm long (vs. whitish-membranous, 2.5-3 mm); pedicels 3-5 mm long (vs.1-2 mm); legumes 20-26 mm long (vs.40-50 mm). Similar to *A. aucheri* Boiss., but stipules 4-6 mm long (vs. 1-2.5 mm); leaves 3-4 cm long (vs. 4-6 cm); peduncles 8-10 cm long (vs. 7-20 cm); bracts chartaceous, 3-4 mm long (vs. membranous, 2-3 mm); pedicels 3-5 mm long (vs.1-1.5 mm); legumes 20-26 mm long (vs.50-60 mm). Similar to *A. xiphidium* Bunge, but 40-60 cm tall (vs. up to 35 cm); stems of the current year up to 20 cm long (vs. 3-12 cm); stipules 4-6 mm long (vs. 2-3 mm); leaflets on both sides sparsely hairy (vs. densely hairy); bracts chartaceous, 3-4 mm long (vs. membranous, 1-2 mm); pedicels 3-5 mm long (vs. c. 1 mm); legumes 20-26 mm long (vs.50-60 mm).

Plant 40-60 cm tall, suffruticose, covered in vegetative parts with medifixed, appressed mostly white hairs. Rootstock branched. Stems at the base woody, branched, stems of the current year up to 20 cm long. Stipules triangular, subulate-acuminate, 4-6 mm long, shortly adnate to the petiole, densely covered with black hairs mixed with few white hairs. Leaves 3-4 cm long; petiole 0.5-1cm long, densely covered with black and white hairs. Leaflets in 3-4 pairs, narrowly oblong to linear, 8-22 × 1.5-3 mm long, on both sides sparsely covered with white appressed hairs 0.5-1 mm long. Peduncles 8-10 cm long, with white appressed, medifixed hairs in upper parts mixed with black, medifixed hairs. Racemes loosely 4-9-flowered, after anthesis elongated; axis densely covered with appressed to subappressed black and white hairs. Bracts triangular, chartaceous, 3-4 mm long, covered with black and white hairs. Pedicels 3-5 mm long, covered with black and white spreading hairs. Calyx 9-12 mm long, tubular, gibbous at the base, densely covered with short, subappressed to spreading black hairs mixed with few long white hairs; teeth subulate, 2-3 mm, white hairy on inner side as well as mostly black and few white hairs on outer side. Standard petals bluish to greenish in fresh stage, but pale yellowish in dry stage, wings yellowish-white and keel greenish. Standard c. 20 mm long; blade oblong to obovate, 6-7 mm wide, rounded, emarginated at apex, constricted below the middle, sub-angularly passing into the cuneate claw. Wings 17-19 mm long; blades elliptic to narrowly oblong, subacute, 12 × 3 mm long; auricles 0.5-1 mm long, claws 9-10 mm long. Keel 15-17 mm long; blades obliquely elliptic-curved, subacute, 5-6 × 3-4 mm; auricles very short, claw 12 mm long. Ovary sessile, hairy with mostly black and a few white hairs. Legumes sessile, elongated, slightly curved, 20-26 mm long, 2-3 mm thick, 4-5 mm wide, keeled ventrally, slightly grooved dorsally, bilocular; valves coriaceous, covered with very dense bifurcate subappressed to spreading black and white hairs. Seeds 4-5 in each locule, brownish, 2.5-4 × 2 mm.

Etymology: The specific epithet “lignipes” refers to the woody caudex of the new taxon.

Phenology: Flowering time is in May and fruiting time in June or later.

Distribution, habitat and taxonomic remarks: The new species is endemic to west of Iran in the Isfahan province. Its distribution is limited to a mountainous and steppe zone (Tatashvilli Mountain) of central Zagros in Fereydunshahr where it occurs at elevations above 2500 m, bordering the provinces of Isfahan and Chaharmahal va Bakhtiari. The associated species in the type locality of *Astragalus lignipes* are: *Astragalus adscendens* Boiss. & Hausskn. (main species), *Centaurea aucheri* (DC.) Wagenitz, *Lactuca orientalis* (Boiss.) Boiss., *Stachys acerosa* Boiss., *Euphorbia decipiens* Boiss. & Buhse, *Onosma microcarpa* DC. etc.

According to our results, the most similar species to the new species is *Astragalus aestimabilis*, which is also an endemic species of west Iran, Kurdistan province, and known only from its type locality. Based on the shape of legumes, Maassoumi (2018) included the species along with *A. dendroproselius*, *A. kharvanensis*, *A. viridis* and *A. juladakensis* in the sect. *Corethrum*, but the molecular phylogenetic results have not supported this position. Therefore, in this study, all of these species are considered in the sect. *Dissitiflori*.

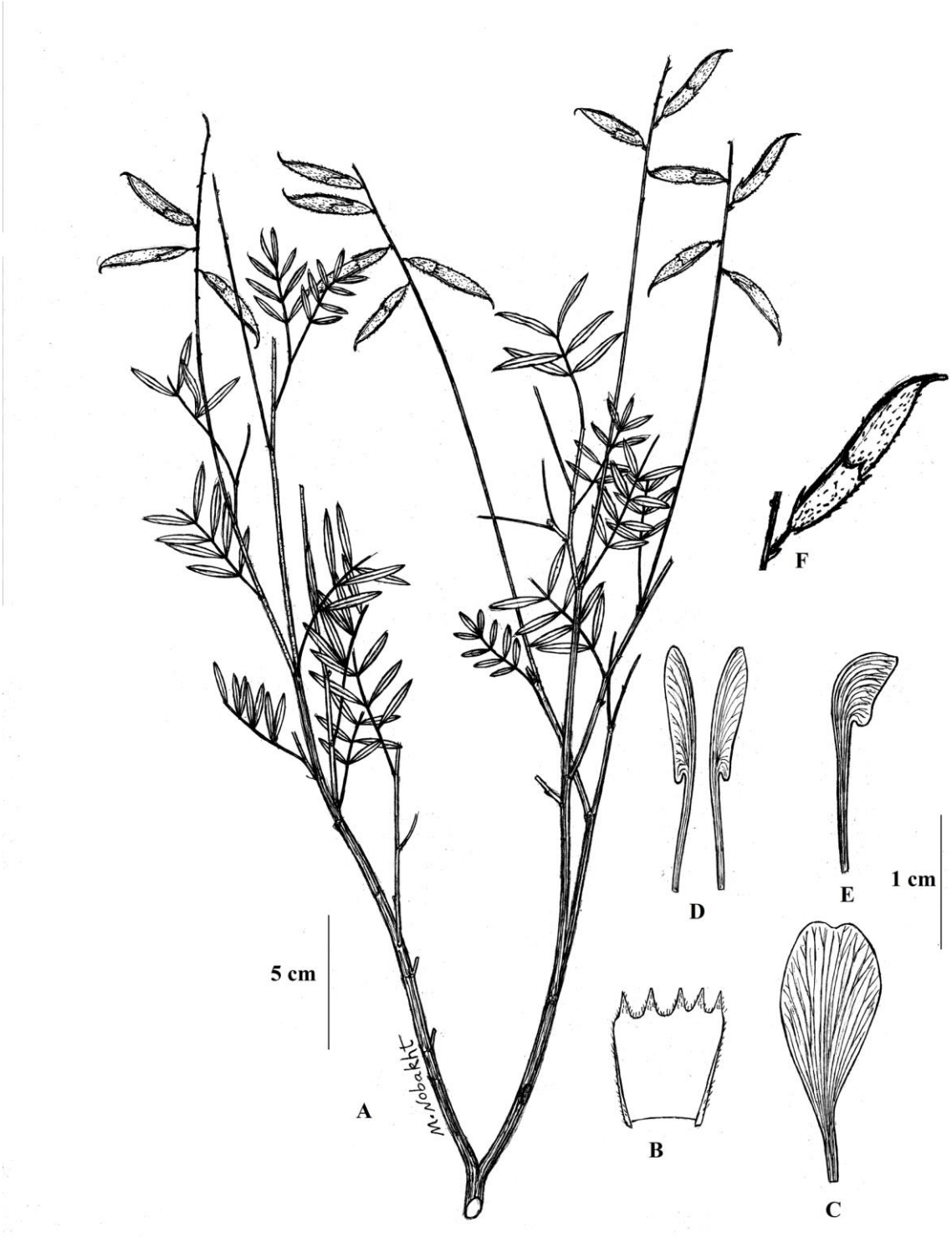

Fig. 1. *Astragalus lignipes*. A, Habit; B, inner side of calyx; C, standard; D, wings; E, Keel; F, fruit. (Drawing is based on the type specimen).

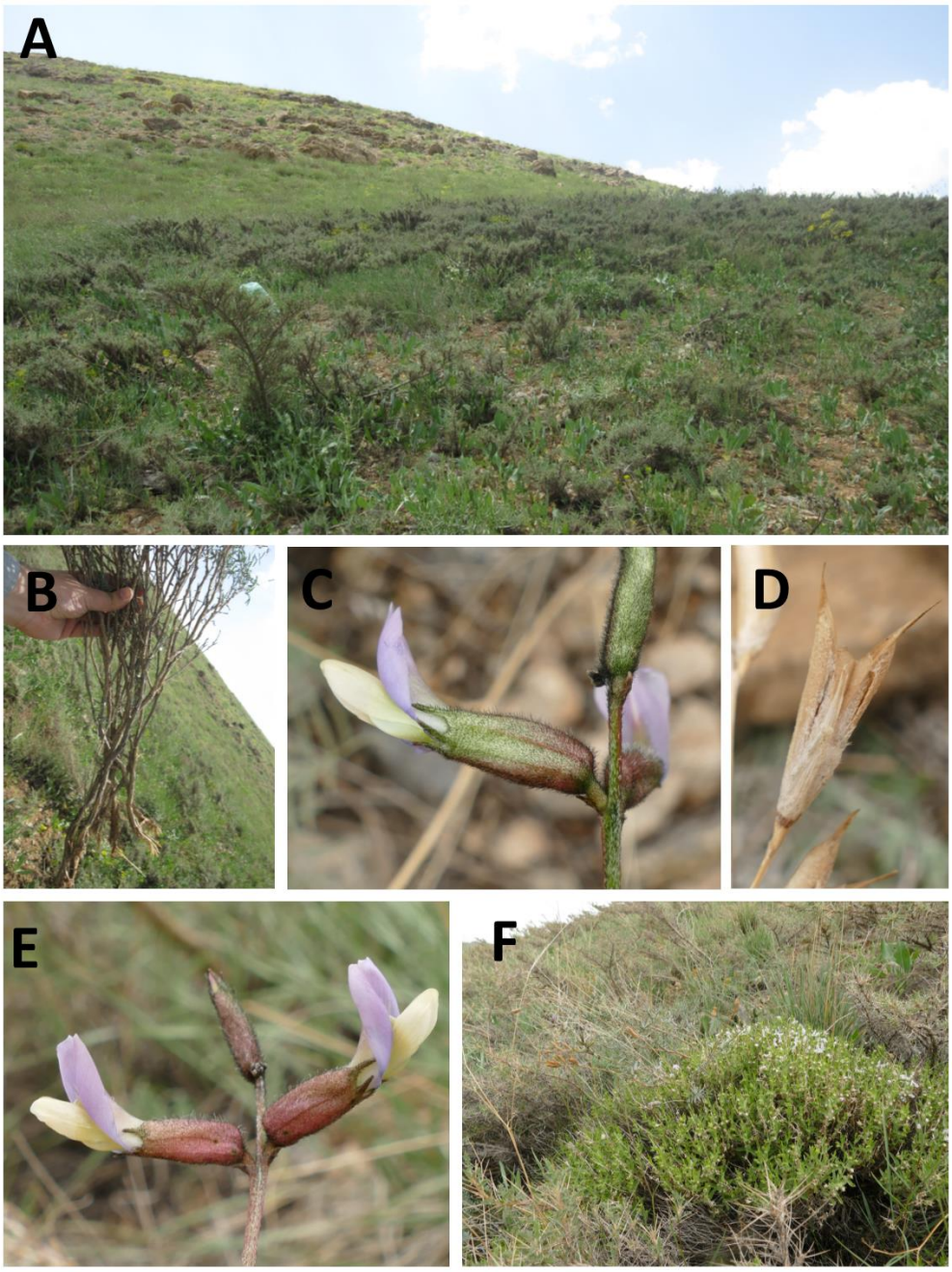

Fig. 2. *Astragalus lignipes*. A, Habitat; B, habit with woody caudex; C & E, inflorescence; D, fruit in dried stage; F, habit in natural setting.

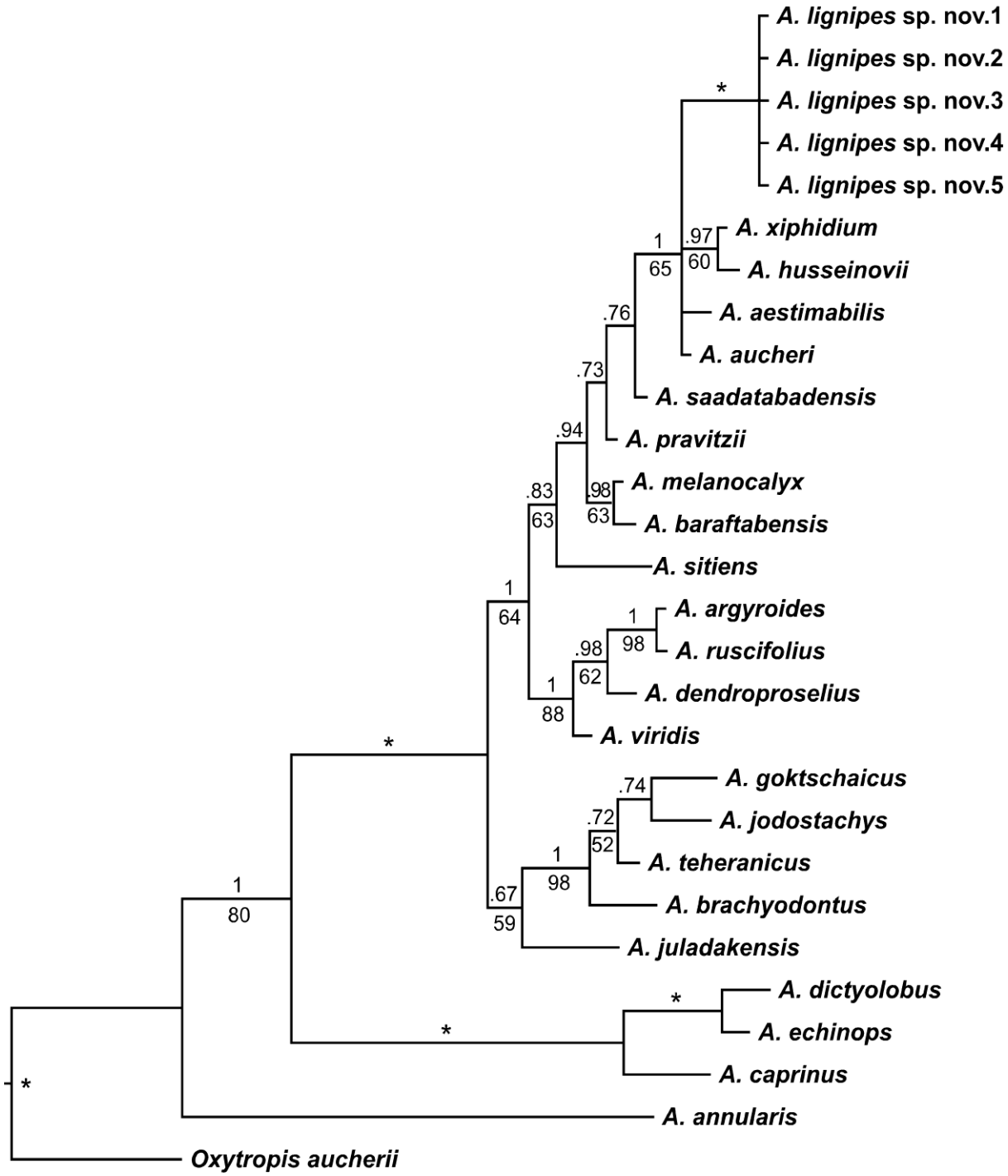

Fig. 3. Phylogenetic tree obtained from a BI analysis of the combined ITS and *mat*K sequences. Numbers above branches provide Bayesian posterior probabilities (pp), those below them are bootstrap support (bs) values from a parsimony analysis. Asterisks indicate branches with 1.0 pp and 100% bs.

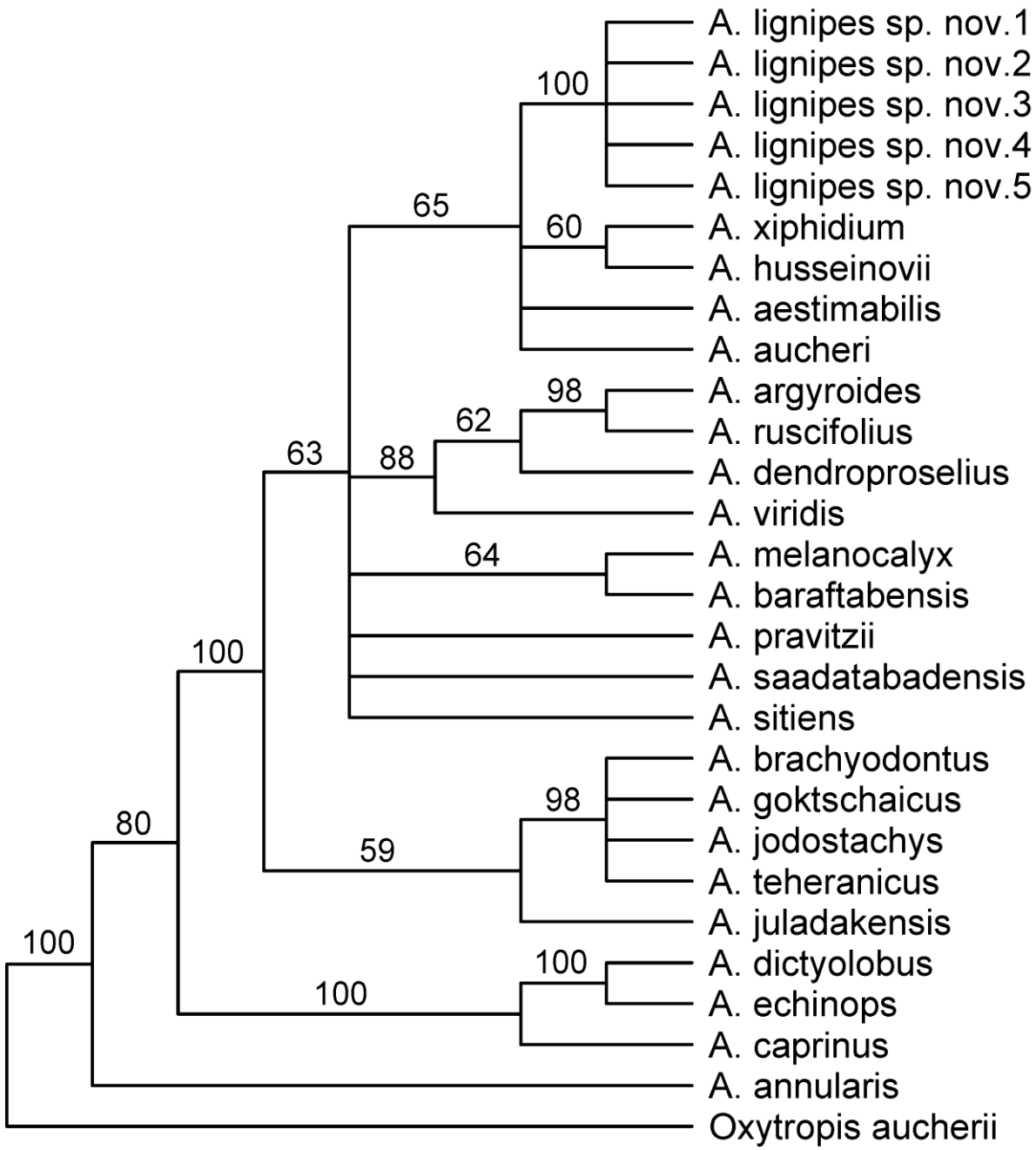

Fig. 4. Strict consensus of six equally parsimonious trees based on the combined ITS and *mat*K dataset. Numbers along branches provide bootstrap support values ≥50% derived from 1000 bootstrap resamples.

The new species is a rare and narrow endemic found in Isfahan province, while the similar taxa, *A. argyroides*, *A. aucheri* and *A. xiphidium*, have a much wider distribution. *Astragalus argyroides* occurs in Azerbaijan, Turkey, and nearly all parts of Iran, *A. aucheri* occurs in eastern Turkey, Armenia as well as western Iran and *A. xiphidium* is distributed in Azerbaijan, Georgia, and north west Iran. The main characters to distinguish these species (Podlech & al. 2010, 2012; Podlech & Zarre 2013) are listed in table 2.

Conservation status: Astragalus lignipes is known just from few sites within a distance of less than 2 km in the

Tatashvilli Mountain of central Zagros. Since this area is not protected and also due to evident overgrazing of this mountain area, the new species is strongly at risk. During our field trips to the type locality in recent years from 2012 to 2018, we have found only 15 individuals. Each population consists of only 1-4 individuals, the total number of individuals are estimated to be less than 100, in consideration of possible undiscovered stands. Regarding to the very small number of individuals and scattered distribution, we evaluate this species as Critically Endangered (CR); according to the IUCN Red List criteria (IUCN 2012).

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Table 2. Diagnostic morphological characters of *Astragalus lignipes* compared to most closely relative taxa: *A. aestimabilis, A. argyroides, A. aucheri* and *A. xiphidium.*

Characters	***A. aestimabilis***	***A. argyroides***	***A. aucheri***	***A. lignipes***	***A. xiphidium***
Plants height (cm)	15–35	30–40	20–50	40–60	Up to 35
Stems of the current year (cm)	2–7	3–20	5–20	up to 20	3–12
Leaves (cm)	4–7	3–5	4–6	3–4	3–6
Leaflets (pairs)	4–6	(3–)4–6	(2–)3–4	3–4	3–6
Leaflets hairs	on both sides rather densely covered with white hairs 0.8-1 mm	on both sides loosely to rather densely covered with white hairs 0.8–1.2 mm	covered on upper side loosely, on underside densely with hairs 1–1.5 mm	on both side sparsely covered with white appressed hairs 0.5–1 mm	on both sides ± densely appressed hairy
Peduncle (cm)	9–14	1–7	7–20	8–10	6–10
Peduncle indumentum	rather densely to densely hairy, glabrescent with age	loosely to rather densely hairy	loosely to rather densely hairy	appressed medifixed hairs	rather densely hairy
Bracts (mm)	whitish membranous, 1.5-2	whitish-membranous, 2.5–3	membranous, 2–3	chartaceous, 3–4	membranous, 1–2
Pedicels (mm)	1–2	1–2	1–1.5	3–5	c. 1
Calyx (mm)	12–14	11–13(–15)	10–13	9–12	13–15
Legumes (mm)	ellipsoid-oblong, 15–17	linear, slightly to strongly upcurved (30–) 40–50	linear, 50–60	elongated, 20–26	narrowly linear, 50–60
Distribution	endemic to Iran (Kordestan province)	Azerbaijan, Turkey, Iran (almost all parts of the country)	Turkey, Armenia, and Iran (Kordestan and Lorestan provinces)	endemic to Iran (Isfahan province)	Georgia, Azerbaijan, Iran (East Azerbaijan, and Zanjan provinces)

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