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Research

Astragalus oksutdagensis (Fabaceae), a new species from Turkey

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This study describes the new species *Astragalus oksutdagensis* (*A.* sect. *Eustales*) from Kayseri, Turkey. The species is compared morphologically to the two similar species *A. flavescens* and *A. vestitus*, considering also pollen micromorphology. The geographical distribution of *A. oksutdagensis*, *A. vestitus* and *A. flavescens* is mapped. The phylogenetic relationships between the new species and other closely related species in the genus are inferred based on DNA data from the internal transcribed spacer regions (ITS) and it is shown that *A.* sect. *Eustales* is monophyletic but closely related to *A.* sect. *Tragacantha*. The new species is assessed as critically endangered (CR) following the IUCN Red List Categories and Criteria.

Keywords: *Astragalus*, *Eustales*, phylogeny, taxonomy, Turkey

Introduction

Astragalus L. is the largest genus of vascular plants with approximately 2900 species. It has two main diversity centers, i.e. Eurasia (with ca 2400 species) and America (ca 500 species) (Chaudhary et al. 2008, Zarre and Azani 2013). The genus is represented by 483 taxa in Turkey, and these have been classified into 63 sections (Aytaç et al. 2012, Taeb and Uzunhisarcıklı 2012, Dinç et al. 2013, Karaman Erkul and Aytaç 2013, Çeçen et al. 2016, Karaman Erkul et al. 2016, Dönmez and Uğurlu Aydin 2018, Hamzaoğlu 2020).

Astragalus sect. *Eustales* Bunge is represented by two species, and both are endemic to Turkey (Chamberlain and Matthews 1970, Davis et al. 1988, Aytaç 2000, Podlech and Zarre 2013). The species of *A.* sect. *Eustales* are dwarf spiny cushion-forming shrublets, densely covered with bifurcate hairs. Their inflorescence is very shortly pedunculated, 2–8-flowered, with widely ovate bracts, campanulate to tubular calyx slightly inflated in fruit, and yellow, densely hairy petals (Podlech and Zarre 2013).

Many *Astragalus* species have similar features, and the complexity of morphological characters make it difficult to distinguish them. In that sense, DNA barcoding based on standardized DNA markers is an advantageous tool for species identification (Zhang and Jiang 2020). In recent years, these markers have been widely used in species discrimination and for conservation of endangered species (Kress et al. 2005, Chen et al. 2010, China Plant BOL Working Group 2011, Kress 2017). Especially, the nuclear ribosomal DNA

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(nrDNA) internal transcribed spacer region (ITS) has become widely used for such purposes. There are several studies using ITS regions as molecular evidence to describe new *Astragalus* species, including some recent studies (Pahlevani et al. 2020, Akhavan et al. 2020, El-Ghani et al. 2021).

During a field trip near Öksüt Mountain (Kayseri) in June 2015, H. Duman collected some unusual specimens of *Astragalus*. A detailed morphological examination of the collected specimens revealed that they belong to *A.* sect. *Eustales*, but they are distinct from both known species of the section. This study aims 1) to describe these plants as a new species, *Astragalus oksutdagensis*, based on morphological data and results of phylogenetic analyses 2) to distinguish micromorphological differences of the new species from *A. vestitus* and *A. flavescens* (sect. *Eustales*).

Material and methods

The collected specimens were checked with Chamberlain and Matthews (1970), Davis et al. (1988), Aytaç (2000) and Podlech and Zarre (2013). They were also compared with the type specimens and other representative collections at GAZI, KNYA and MSB herbaria, and images of type specimens from BM, G, GOET, HBG, JE, K, TUB, W, WE and ZT. The abbreviations of plant name authors follow Brummitt and Powell (1992) and IPNI 2020 (<www.ipni.org>) was followed for the plant name authors.

To evaluate molecular data, leaf samples of *Astragalus oksutdagensis* were obtained from its natural habitat. Moreover, leaves of the other two species of *A.* sect. *Eustales* (*A. vestitus* and *A. flavescens*) were taken from GAZI herbarium

Figure 1. (a) Holotype of *Astragalus oksutdagensis* sp. nov. (b) standard, (c) wing, (d) keel, (e) calyx, (f) ovary, (g) staminal tube, (h) fruit (scale bars: 1 cm).

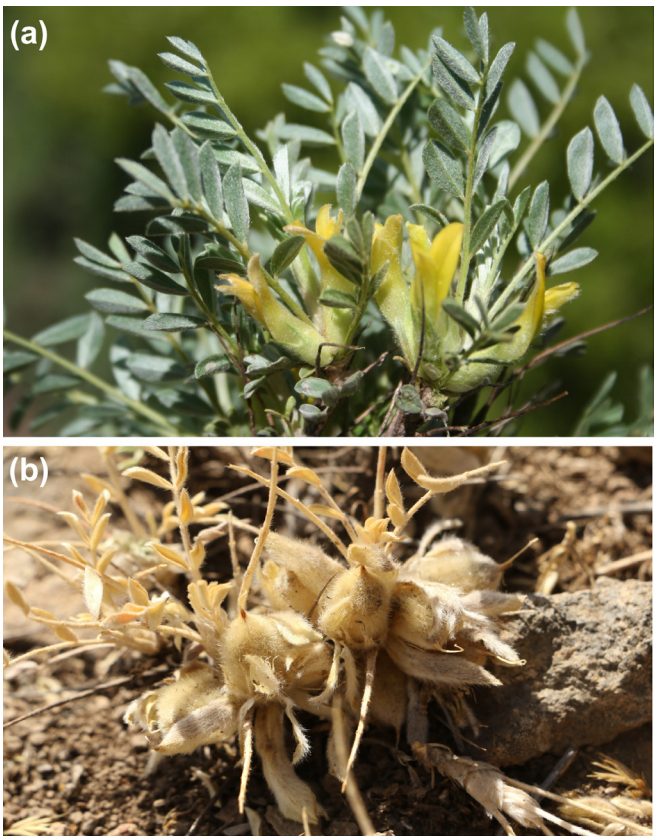

Figure 2. Habit of *Astragalus oksutdagensis* in its natural habitat. (a) Flower, (b) fruit. Photographed by H. Duman.

(GAZI 6455, GAZI 2320, GAZI 10226). Total genomic DNA was extracted by using the plant DNA extraction kit (MACHEREY–NAGEL). Amplification of the ITS region (ITS1+5.8S+ITS2) was carried out using the primer pairs of Hsiao et al. (1995). PCR amplifications were performed in a total volume of 20 µl containing 2.5 µl 5× Hot FirePol Blend PCR Mix (Solis Biodyne) (15 mM $MgCl_2$), 0.4 µl each primer pair, 1 µl template DNA and 15.7 µl water. PCR reactions were performed with a Thermo cycler (MultiGENE, Cleaver Scientific Ltd) by optimized cycling parameters as: 5 min at 95°C for initial denaturation, followed by 30 cycles of 30 s at 95°C for template denaturation, 30 s for annealing, and 90 s at 72°C for extension and 10 min at 72°C for final extension. After checking all the products on an agarose gel (2%), purification and sequencing of the samples were done by RefGEN Biotechnology Company (Ankara). The obtained sequences were checked before analysis by Finch Tv software ver. 1.4.0-manufactured by Geopiza Research Team (Patterson et al. 2004–2006). The MUSCLE (multiple sequence comparison by log expectation) tool (Edgar 2004) of the MEGA (molecular evolutionary genetics analysis) ver. 7.0.9 software (Kumar et al. 2016) was used for alignment of the sequences. Furthermore, MEGA was used to find the best substitution model for reconstructing the species phylogeny. The program suggested GTR (general time reversible) (Nei and Kumar 2000) as the best substitution model to evaluate the sequences, and the maximum likelihood method based on GTR model with bootstrap test analysis was hence used for constructing the phylogenetic tree. Sequences of some previously studied spiny *Astragalus* from different sections native to Turkey (*Tragacantha* DC., *Chronopus* Bunge, *Adiaspastus* Bunge, *Macrophyllium* Bunge, *Pterophorus* Bunge, *Rhacophorus* L., *Acanthophace* Bunge, *Hymenostegis* Bunge, *Hymenocoleus* Bunge), and some representative species of the related genera *Oxytropis* DC. and *Colutea* L. as outgroups, were retrieved from NCBI data bank in addition to the study samples to provide an evolutionary perspective.

The pollen and leaflet material of *A. vestitus* (10 226) and *A. flavescens* (6455) were taken from GAZI herbarium. Pollen morphology was investigated with light microscopy (LM) and scanning electron microscopy (SEM). Pollen slides were prepared using the Wodehouse (1935) technique for LM. The following characters of at least 30 pollen grains were measured and analysed with a Leica DM 500 digital imaging system LM. For the preparation of SEM pictures, dried pollen grains, leaflets (upper and inner sides) and seeds were transferred on aluminium stubs using double-sided adhesive tape, and coated with gold particles in a sputter-coater (Ceter et al. 2013). Morphological observations were made in a Jeol JSM 6490LV SEM at Kastamonu University SEM Laboratory. Terminology was adopted from Salgado-Labouriau (1982), Faegri and Iversen (1992), Punt et al. (2007), Hesse et al. (2009), Punt and Hoen (2009), Ceter et al. (2013), Halbritter et al. (2018) and shape classification follows that of Erdtman (1969) based on P/E ratio.

Results

Taxonomy

Astragalus oksutdagensis H. Duman & Karaman sp. nov. (Fig. 1, 2).

Diagnosis

A species similar to *A. vestitus* and *A. flavescens*. It differs from *A. vestitus* by the usually absent peduncle, or up to 3 mm long if present (versus 5–15 mm long), racemes 1–3-flowered (versus 3–10-flowered), pedicels 0.5–3 mm long (versus flowers subsessile), bracts caducous (versus persistent), calyx 11–15 mm long (versus 16–18 mm long), teeth subulate – narrowly triangular, 2–5 mm long (versus narrowly triangular, 5–7 mm long), standard 20–25 mm long, with acute blade 8–9 mm wide and a narrower claw 3–4 mm wide (versus 18–21 mm long, with obtuse blade 8–9 mm wide and claw 5–6 mm wide), ovary densely covered with straight cream or pale-yellow hairs (versus densely covered with curled pale brownish hairs) (Table 1).

It differs from *A. flavescens* by having stipules 8–10 mm long, densely hairy (versus 5–7 mm long, sparsely or, especially toward the tip ± densely hairy), petiole 1–3 cm long (versus 0.5–1.0 cm long), terminal spine shorter than the

Table 1. Comparison of diagnostic characteristics of *Astragalus oksutdagensis*, *A. vestitus* and *A. flavescens*.

Character	*A. oksutdagensis*	*A. vestitus*	*A. flavescens*
Stipules	hyaline-membranous, 8–10 mm long, densely hairy	membranous, 12–18 mm long, densely hairy	hyaline-membranous, 5–7 mm long, sparsely or, especially toward the tip ± densely hairy
Leaves	2–10 cm long; petiole 1–3 cm long, terminal spine shorter than the uppermost leaflets	2–10 cm long; petiole 1.5–2.0 cm long, terminal spine shorter than the uppermost leaflets	1.5–4.0 cm long; petiole 0.5–1.0 cm long, terminal spine 1.5–2.0 times longer than the uppermost leaflets
Leaflets	3–7 pairs, 5–13 × 2–5 mm	5–7 pairs, 5–8 × 2–4 mm	3–5 pairs, 3.0–6.0 × 1.5–3.0 mm
Leaflets hairs	symmetrically to asymmetrically bifurcate, subappressed, thin, 1.5–2.0 mm long.	asymmetrically bifurcate, partly flexuose, partly straight, tangled, 1.0–1.5 mm long.	asymmetrically to strongly asymmetrically bifurcate, flexuose, tangled and straight, ca 1 mm long.
Peduncle	absent (very rarely present, up to 3 mm long)	5–15 mm long	up to 0.5 cm long
Racemes	1–3-flowered	3–10-flowered	2–8-flowered
Flowers	pedicellate, 0.5–3.0 mm long	subsessile	subsessile
Bracts	5–6 × 8–9 mm, caducous	6–13 mm long, persistent	3–6 mm long, persistent
Calyx	11–15 mm long, tubular	16–18 mm long, tubular	8–9 mm long, campanulate
Calyx hairs	very densely covered with flexuose, tangle hairs ca 0.5 mm long and with subbasifixed, subappressed to ascending hairs 1–2 mm long	very densely covered with flexuose, tangle hairs ca 0.5 mm long and with subbasifixed, subappressed to ascending hairs 2–3 mm long	densely covered with subbasifixed, subappressed to ascending, pale brownish yellowish hairs ca 1 mm long
Calyx teeth	subulate – narrowly triangular, 2–5 mm long	narrowly triangular, 5–7 mm long	subulate, 3–4 mm long
Standard	20–25 mm long; blade acute, ca 8–9 mm wide, with a narrower claw from the blade.	18–21 mm long; blade elliptic, ca 8–9 mm wide, rounded, with a widely cuneate claw nearly as long as the blade	11–14 mm long; blade widely elliptic, ca 8 × 7 mm, subacute, with a widely cuneate claw nearly as long as the blade
Wings	17–20 mm long; blades narrowly oblong, obtuse, 10.0–12.0 × 2.0–2.5 mm; auricle 1 mm long, claw 8–13 mm long	16–20 mm long; blades narrowly oblong, obtuse, 7.0–9.0 × 2.0–2.5 mm; auricle ca 1 mm long, claw 9–11 mm long	10–13 mm long; blades narrowly obovate, acute, 5.0–7.0 × 2.0–2.5 mm; auricle ca 0.8 mm long, claw 5–7 mm long
Keel	15–18 mm long; blades narrowly elliptic, straight, subacute, 8–9 × 2–3 mm; auricle 0.5 mm long, claw 9–10 mm long	15–18 mm long; blades narrowly elliptic, straight, subacute, 7–8 × 3 mm; auricle 0.5 mm long, claw 9–10 mm long	9–12 mm long; blades elliptic, with slightly curved lower and upper edge, acute, 4.5–5.5 × 2.5–3.0 mm; auricle minute, claw 5–7 mm long
Ovary	densely covered with straight cream-pale yellow hairs	densely covered with curled pale brownish hairs	densely covered with curled pale brownish hairs
Legume	oblong-elliptic, 12–14 × 8–9 mm, beak 7–8 mm long	oblong-ovate, 12 – 14 × 4–6 mm, beak 7–8 mm long	–
Seed	light brown, reniform spotted or not, 2–3 × 3–4 mm	brown, reniform, 3 × 2 mm	

uppermost leaflets (versus terminal spine 1.5–2 times longer than the uppermost leaflets), leaflets 5–13 × 2–5 mm (versus 3.0–6.0 × 1.5–3.0 mm), peduncle absent, rarely present, up to 3 mm (versus up to 5 mm), racemes 1–3-flowered (versus 2–8-flowered), flowers with pedicels 0.5–3.0 mm long (versus flowers subsessile), bracts caducous (versus persistent), calyx 11–15 mm long, tubular (versus 8–9 mm long, campanulate), standard 20–25 mm long, blade acute, ca 8–9 mm wide, with a narrower claw 3–4 mm wide (versus 11–14 mm long, blade subacute, ca 8 × 7 mm, with a widely cuneate claw nearly as long as the blade and 5–6 mm wide), and ovary densely covered with straight cream-pale yellow hairs (versus densely covered with curled pale brownish hairs) (Table 1).

Type: Turkey, Kayseri: Develi, Öksüt Mountain, west of Keltepe, steppe, 1750–1800 m a.s l., 4 Jun 2015, H. Duman 10663 (holotype: GAZI; isotype: AKSU). Paratype: ibid., 28 Jul 2020, H. Duman 10664 (GAZI).

Description

Dwarf, cushion-forming spiny shrubs, branched at base, with white or yellowish hairs, becoming brown with age. Stems up to 12 cm long, covered with asymmetrically bifurcating hairs 1–2 mm long; enveloped by stipules, under the stipules bifurcate-hairy. Stipules membranous, 8–10 mm long, adnate to the petiole for 4–5 mm, vaginate-connate behind the stem often up to the top, covered with asymmetrically bifurcating, subappressed to ascending, very thin and 0.5–1.0 mm long hairs. Leaves 2–10 cm long; petiole 1–3 cm long, covered with symmetrically to asymmetrically bifurcating, subappressed, thin, 1.5–2.0 mm hairs, spiny; terminal spine shorter than the uppermost leaflets. Leaflets in 3–7 pairs, elliptic to obovate, 5–13 × 2–5 mm, obtuse to subacute, covered by symmetrically to asymmetrically bifurcating, subappressed, thin, 1.5–2.0 mm long hairs. Peduncle absent (very rarely present, ca 3 mm long), densely hairy like the rachis. Racemes 1–3-flowered. Bracts caducous, membranous, 8–9 × 5–6 mm, widely ovate, hairy. Flowers pedicellate, 0.5–3 mm.

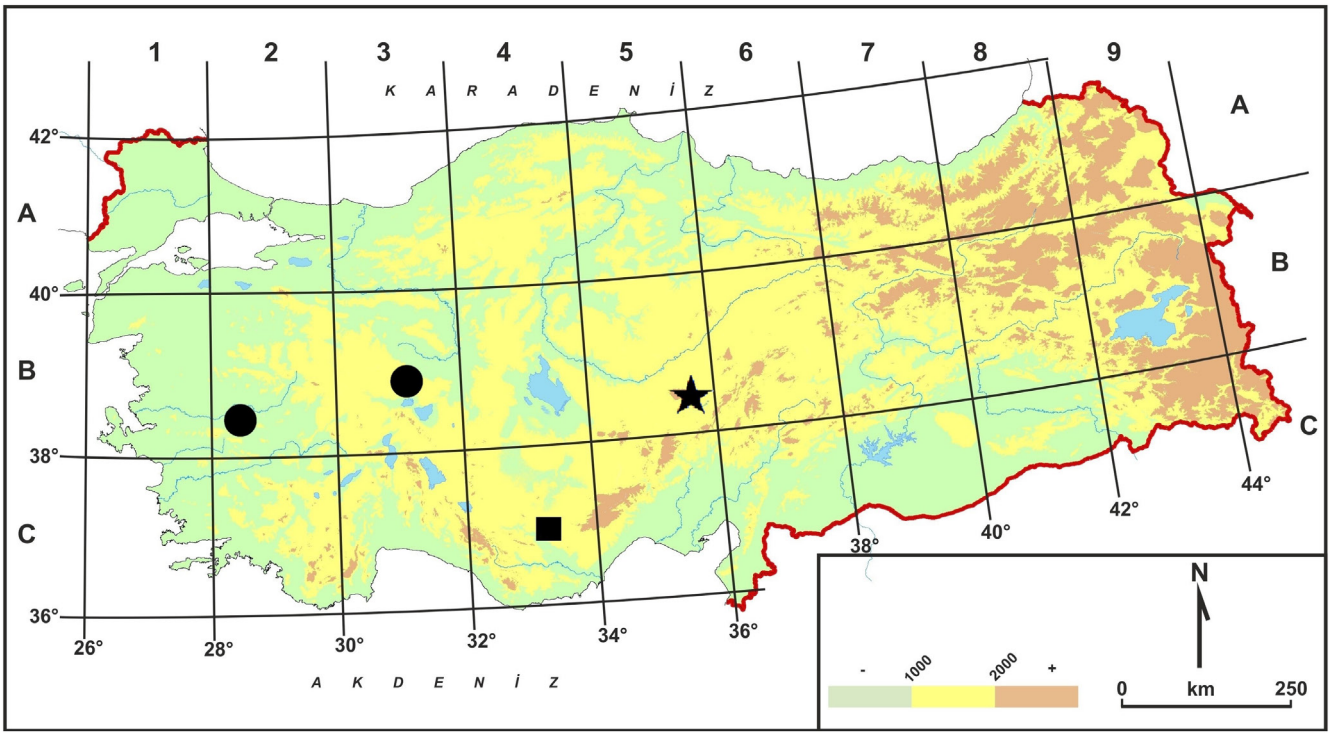

Figure 3. Distribution of *Astragalus oksutdagensis* (★), *A. vestitus* (■) and *A. flavescens* (●).

Bracteoles similar to the bracts, 4–5 mm long. Calyx 11–15 mm long, tubular, obliquely cut at the mouth, very densely covered by flexuose, tangle, ca 0.5 mm long hairs and subbasifixed, subappressed to ascending, 2–3 mm long hairs; teeth subulate – narrowly triangular, 2–5 mm long, densely hairy on inner side. Petals yellow, throughout densely covered with subappressed, strongly asymmetrically bifurcating hairs up to 2 mm long. Standard 20–25 mm long; blade acute, ca 8–9 mm wide, with a claw narrower than the blade. Wings 17–20 mm long; blades narrowly oblong, obtuse, 10.0–12.0 × 2.0–2.5 mm; auricle 1 mm long, claw 8–13 mm long. Keel 15–18 mm long; blades narrowly elliptic, straight, subacute, 8–9 × 2–3 mm; auricle 0.5 mm; claw 9–10 mm long. Stamen-tube nearly truncate at the mouth. Ovary subsessile, ellipsoid, densely covered with straight cream to pale-yellow hairs; style glabrous. Legumes 12–14 × 8–9 mm, oblong-elliptic, bilocular, leathery, hairy, with a 7–8 mm long beak. Seeds light brown, triangular-reniform, spotted or not, 2–3 × 3–4 mm.

Phenology

Flowering from June to July, fruiting from July to August.

Etymology

The plant takes its scientific name from Öksüt Mountain (Kayseri), which is the collection area.

Suggested Turkish name

Turkish name of this species is suggested as 'Öksütgeveni' according to the guidelines by Menemen et al. (2013).

Distribution and habitat

Astragalus oksutdagensis is currently known only from Öksüt Mountain in Develi district, Kayseri, Turkey (Fig. 3). It grows in steppe and oak forest at an elevation of 1650–1800 m a.s.l. Some of the associated species are *Festuca valesiaca* Schleich. ex Gaudin, *Phlomis capitata* Boiss., *Bromus tomentellus* Boiss., *Astragalus microcephalus* Willd., *Thymus sipyleus* Boiss., *Daphne oleoides* Schreb., *Marrubium parviflorum* Fisch. & C.A. Mey., *Stipa lessingiana* Trin. & Rupr., *Stipa holosericea* Trin., *Asphodeline damascena* (Boiss.) Baker subsp. *damascena*, *Acantholimon acerosum* (Willd.) Boiss. and *Campanula stricta* Wall. var. *alidaghensis* Damboldt.

Conservation status

Astragalus oksudagensis is known from a single location; its extent of occurrence (EOO) and area of occupancy (AOO) is approximately 24 km^2 (criterion B1, B2); number of mature individuals less than 2500 (D1); number of locations is only one (a), and estimated continuing decline (b) in area of occupancy (ii), quality of habitat (iii), number of mature individuals (v). The main threats are the ongoing opencast mining activities and heavy grazing in this area. As a result,

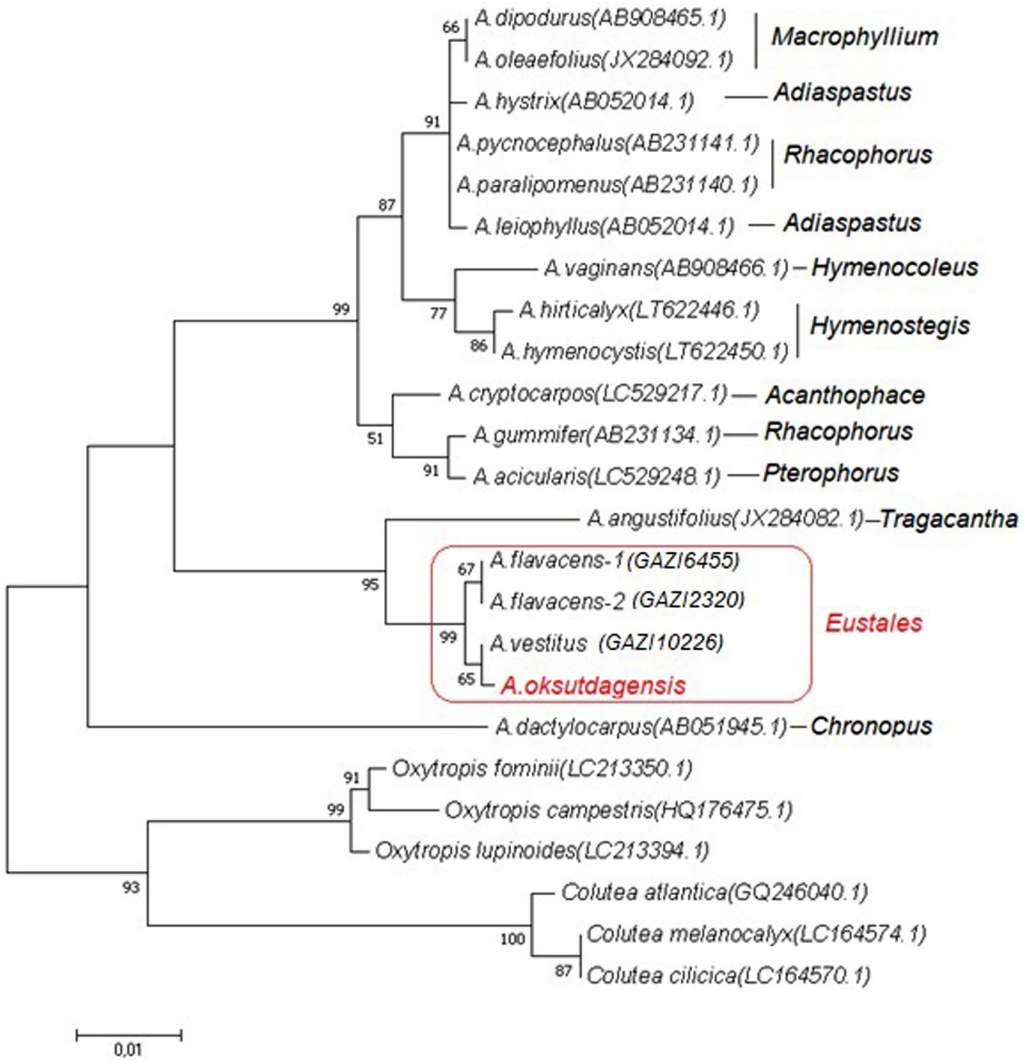

Figure 4. Phylogenetic tree based on ITS and inferred by maximum likelihood based on the GTR model. Initial tree(s) for the heuristic search were obtained automatically by applying the maximum parsimony method. Bootstrap values are shown next to the branches (burn under 1000).

this new species is assessed as 'Critically Endangered: CR B1ab (ii,iii,v) + B2ab (ii,iii,v)' (IUCN 2019).

Key to species of *Astragalus* sect. *Eustales*

1. Leaves 2–10 cm long; terminal spine shorter than uppermost leaflets; calyx tubular……2
   – Leaves 1.5–4.0 cm long; terminal spine 1.5–2 times longer than the uppermost leaflets; calyx campanulate……*A. flavescens*
2. Stipules 12–18 mm long; peduncle distinct, 5–15 mm long; raceme 3–10-flowered; bracts persistent; calyx teeth 5–7 mm long; standard blade obtuse……*A. vestitus*
   – Stipules 8–10 mm long; peduncles absent (very rarely present, ca 3 mm long); racemes 1–3-flowered; bracts caducous; calyx teeth 2–5 mm long; standard blade acute……*A. oksutdagensis*

Examined specimens

***A. flavescens* Boiss.**

Turkey, Afyon: Emirdağ, 15 km N of Bolvadin, 1100 m a.s.l., 9 Jul 1978, Ehrendorfer & al. 787-18-3 (MSB!); İscehisar, Karakaya village, calcareous *Cistus* scrub, 1150–1200 m a.s.l., 2 Aug 1993, Aytaç 6455 (GAZI!); Kocatepe, subalpine *Astragalus* steppe, 1850 m a.s.l., 19 Jul 1983, Vural 2320; Sandıklı, Kumalar Dağı, 1250 m a.s.l., 4 Aug 1993, Aytaç 6502 (GAZI!). Izmir: 10–11 km NE of Ödemiş on 7th km of the road from Birgi to Salihli, 2 Jul 1989, Boratynski, Tomlik & Zielinski 8125 (MSB!); Tmoleus circa Bozdagh, Jul 1842, Boissier (MSB003985!, G00445654!, G00386597!, G00386598!, GOET2888!, HBG520419!, TUB011076!, JE00010644!, JE00010646!, W0025477!, WE0025477!, ZT00010106!); Yaila de Bozdagh (Tmolus occidental), 22 Jul 1854, Balansa 215 (MSB!, G00776737!); Salihli-Odemiş,

Table 2. Accession numbers of the sequences of ITS gene regions of the taxa that used to construct phylogenetic tree as outgroups from NCBI database.

Taxon	NCBI accession numbers (ITS regions)	References
Astragalus dipodurus	AB908465.1	Safar et al. 2014
Astragalus vaginans	AB908466.1	
Astragalus pycnocephalus	AB231141.1	Osaloo et al. 2005
Astragalus paralipomenus	AB231140.1	
Astragalus leiophyllus	AB052014.1	
Astragalus hirticalyx	LT622446.1	Bagheri et al. 2017
Astragalus hymenocystis	LT622450.1	
Astragalus oleaefolius	JX284092.1	Samad et al. 2014
Astragalus angustifolius	JX284082.1	
Astragalus hystrix	AB052014.1	Osaloo et al. 2003
Astragalus dactylocarpus	AB051945.1	
Astragalus gummifer	AB231134.1	
Astragalus acicularis	LC529248.1	Khalili et al. 2021
Astragalus cryotocarpos	LC529217.1	
Colutea melanocalyx	LC164574.1	Moghaddam et al. 2017
Colutea cilicica	LC164570	
Colutea atlantica	GQ246040.1	Ahlquist and Wojciechowski 2010
Oxytropis campestris	HQ176475.1	Archambault and Strömvik 2011
Oxytropis lupinoides	LC213394.1	Shahi Shavvon et al. 2017
Oxytropis fominii	LC213350.1	

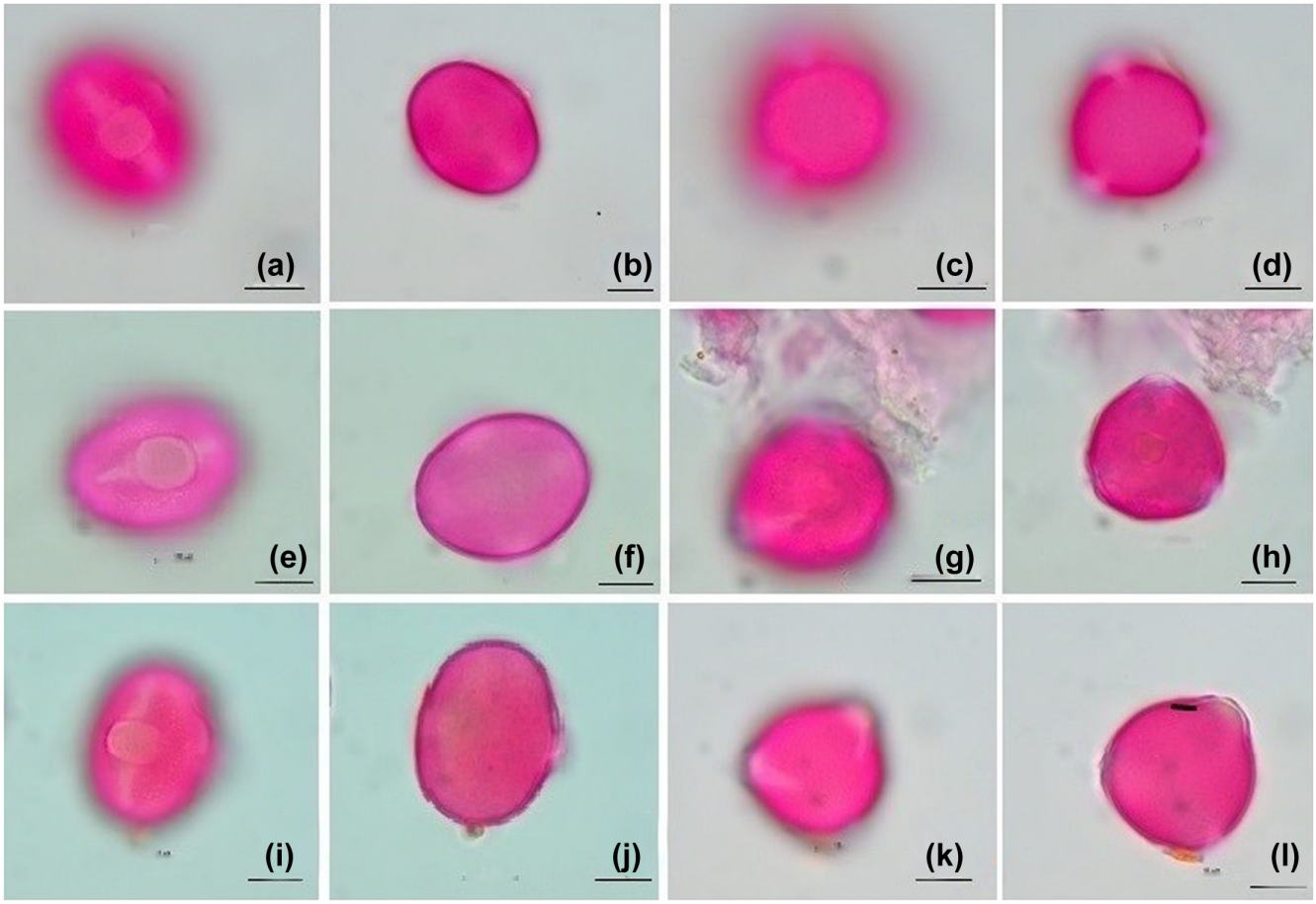

Figure 5. Pollen grain LM microphotographs of *A. oksutdagensis.* (a–b) equitorial view, (c–d) polar view), *A. vestitus* (e–f) equitorial view, (g–h) polar view) and *A. flavescens* (i–j) equitorial view, (k–l) polar view. Scale bars: a–l: 10 µm.

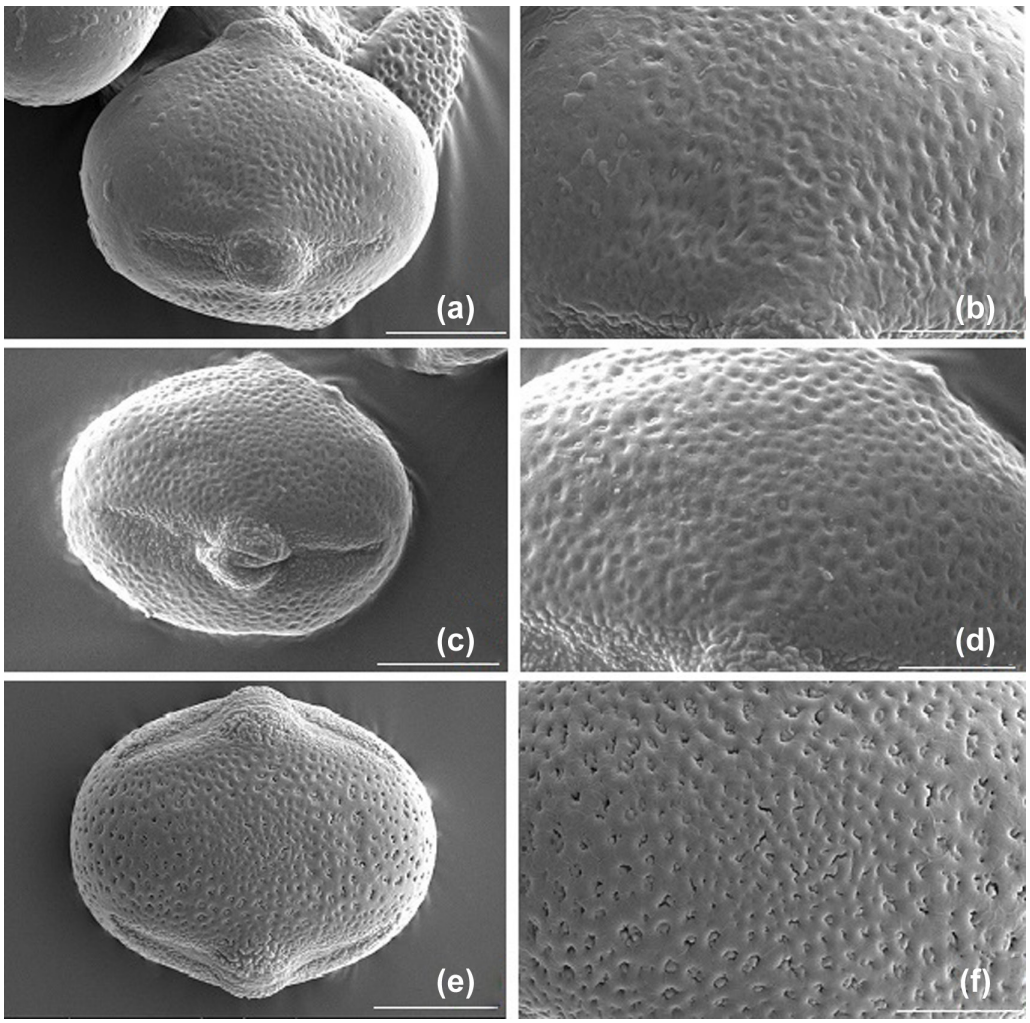

Figure 6. Pollen grain SEM microphotographs of *A. oksutdagensis* (a) general view, (b) ornamentation), *A. vestitus* (c) general view, (d) ornamentation) and *A. flavescens* (e) general view, (f) ornamentation. Scale bars: (a–c–e) 10 µm, (b–d–f) 5 µm.

unterhalb Bozdağköy, 1120 m a.s.l., 6 Jun 1989, Nydegger 44081 (MSB!).

A. vestitus Boiss. & Heldr.

Turkey, Konya: Karaman, Kılbasan, Karadağ, volcanic steppe, 1620 m a.s.l., 3 Jul 2007, O.Tugay 4914, Ertuğrul & Vural (KNYA!); Karaman, Kılbasan Town, Karadağ, volcanic steppe, 1590 m a.s.l., 29 May 2008, O.Tugay (KNYA!); Kılbasan to Radar, Karadağ, 1590 m a.s.l., 29 Jun 2008, Vural 10226 (GAZI!); mt. Karadagh Isauriae, 1380 m a.s.l., 21 Jun 1845, Heldreich 930 (BM000884308!, G00445620!, G0077674!, K001094444!, K001094443!, MSB!, W1889-0018562!, W0026144!, WU0068413!).

Phylogenetic study

ITS sequences were obtained as a total of 611 bp in length, and the overall mean genetic divergence was calculated as 0.035 among the studied species of *Astragalus*. Furthermore, the overall mean genetic divergence among the species of *A.* sect. *Eustales* was calculated as 0.005, which means that they are genetically very close to each other.

Although all members of *A.* sect. *Eustales* were phylogenetically similar to each other, *A. flavescens* was separated from the other two species (with parsimony informative sites). Moreover, *A. vestitus* and *A. oksutdagensis* were genetically more similar to each other than to *A. flavescens*. As a result of these similarities, the phylogenetic tree indicated that *A.* sect. *Eustales* is divided into two branches. One of the main branches is composed of two samples of *A. flavescens* from a different area in Turkey, and the other branch is composed of *A. vestitus* and *A. oksutdagensis*.

Other spiny *Astragalus* species native to Turkey were also included in the analysis to figure out the phylogenetic position of *A.* sect. *Eustales* within the genus. Generally, these species were divided into two main clades. *Astragalus dactylocarpus* (sect. *Chronopus*) formed a clade of its own, while all other studied species formed the other clade. *Astragalus angustifolius*

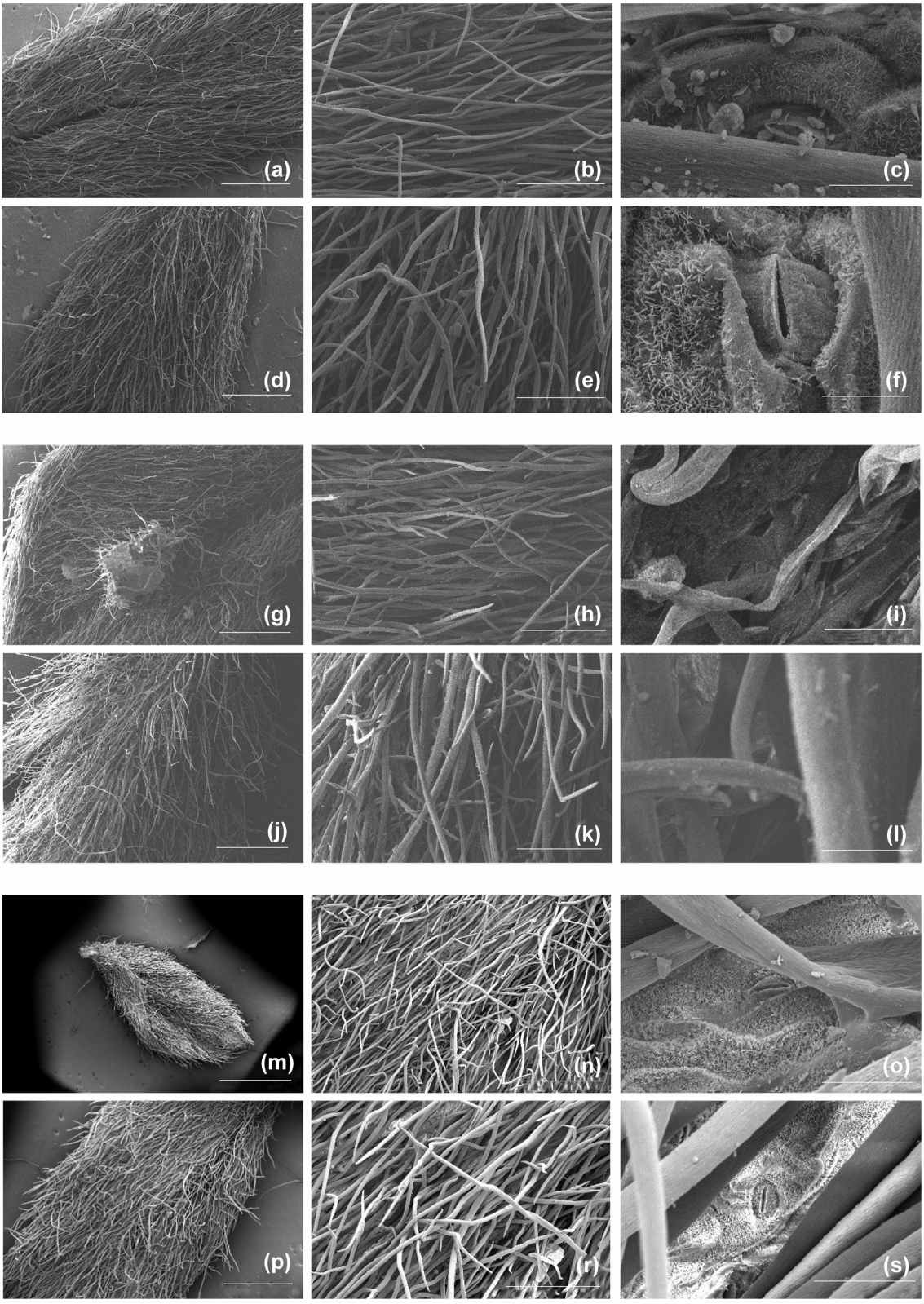

Figure 7. Leaflet SEM microphotographs of *A. oksutdagensis.* (a–c) adaxial surface, (d–f) abaxial surface), *A. vestitus* ((g–i) adaxial surface, (j–l) abaxial surface) and *A. flavescens* ((m–o) adaxial surface, (p–s) abaxial surface. Scale bars: (a, d, g, j, m, p) 2 mm, (b, e, h, k, n, r) 500 µm, (c, f, i, l, o, s) 30 µm.

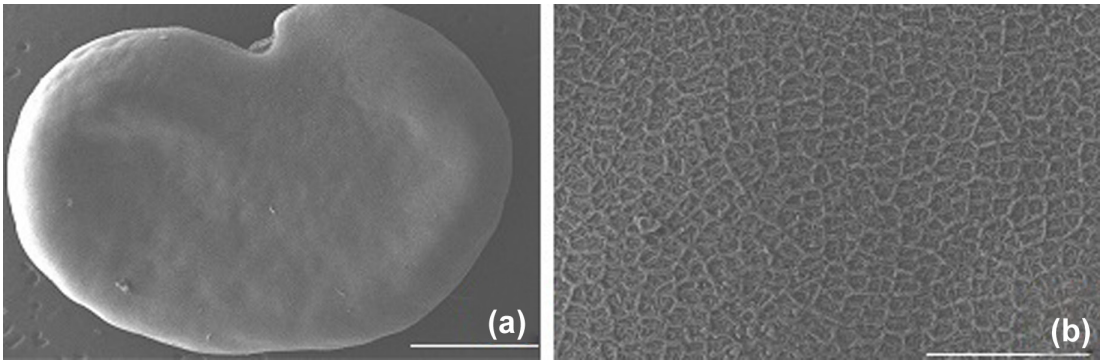

Figure 8. Seed SEM microphotographs of *A. oksutdagensis.* (a) general view, (b) ornamentation. Scale bars: (a) 1 mm, (b) 50 µm.

(sect. *Tragacantha*), which is morphologically the most similar species, was positioned in the same clade with *A.* sect. *Eustales* and they together formed a main branch separated from the other species with high (95) bootstrap values (Fig. 4). All other spiny *Astragalus* spp. belonging to six different sections formed a separate clade (Fig. 4, Table 2).

Pollen morphology

Pollen grains were observed to be trizonocolporate, radially symmetrical and isopolar in *Astragalus oksutdagensis*, *A. flavescens* and *A. vestitus* (*A.* sect. *Eustales*). The pollen shape of *A. oksutdagensis* is subprolate (ratio P/E 1.23 ± 0.06), polar view (P) 33.25 ± 1.95 µm, equatorial view (E) 27.11 ± 1.33 µm. Amb circular, ornamentation psilate-perforate at polar region, reticulate–perforate at equatorial region. Colpus thin and tall (Clg 23.72 ± 1.34 µm, Clt 4.52 ± 0.60 µm), operculate, operculum membrane has granulate ornamentation. Pore suboblate, Plg 8.49 ± 0.96 µm, Plt 9.84 ± 0.85 µm. Apocolpium 12.69 ± 2.24 µm and mezocolpium 19.58 ± 1.62 µm.

The pollen shape of *A. vestitus* is prolate-spheroidal (1.13 ± 0.05), P 31.10 ± 2.72 µm, E 27.56 ± 1.46 µm. Amb semitriangular, ornamentation perforate. Colpus thin and tall (Clg 24.76 ± 2.85 µm, Clt 5.42 ± 1.50 µm), operculate, operculum membrane has granulate ornamentation. Pore spheroidal, Plg 9.41 ± 1.48 µm, Plt 9.29 ± 1.18 µm. Apocolpium 11.56 ± 1.35 µm and mezocolpium 19.16 ± 0.90 µm.

The pollen shape of *A. flavescens* is subprolate (1.32 ± 0.06), P 36.6 ± 1.56 µm, E 27.83 ± 1.17 µm. Amb semitriangular, ornamentation microreticulate. Colpus thin and tall (Clg 28.12 ± 1.45 µm, Clt 4.13 ± 0.78 µm), operculate, operculum membrane has granulate ornamentation. Pore suboblate, Plg 7.90 ± 0.83 µm, Plt 10.09 ± 0.83 µm. Apocolpium 14.43 ± 0.84 µm and mezocolpium 20.82 ± 0.93 µm (Fig. 5, 6).

Leaflet morphology

Leaflets of *Astragalus oksutdagensis*, *A. vestitus* and *A. flavescens* have simple, distantly striate hairs (Fig. 7). Both the upper and lower surfaces of the leaflets have dense epiticular wax. Leaflets are amphistomatic and the stomata are mesomorphic.

Seed morphology

Astragalus oksutdagensis seed is light brown, triangular-reniforme, spotted or not, 2–3 × 3–4 mm. The shape is spheroidal and reniforme. The surface ornamentation is reticulate. Periclinal walls of testa cells were found to be convex around the hilum and concave in the other parts of the seed (Fig. 8).

Discussion

Among the sections of *Astragalus* with spiny members, *A.* sect. *Eustales* is morphologically most similar to *A.* sect. *Tragacantha*, as they both share a spiny leaf rachis, not inflated flowers and leaflets with bifurcate hairs. The close relationship of the two sections is also supported by the results of our phylogenetic analysis. *Astragalus* sect. *Eustales* differs from sect. *Tragacantha* by having stipules connate (often entirely) (versus shortly connate), bracteoles widely ovate (versus linear) and racemes 2–8-flowered (versus 1–2-flowered). *Astragalus oksutdagensis* is closely related to *A. vestitus* and *A. flavescens* with spiny rachis, bifurcate hairs on leaflets, yellow flowers and standard hairy at the adaxial surface. However, it can be easily distinguished from the most similar species *A. vestitus* by the lack of peduncle (or very rarely with a short one up to ca 3 mm long) and flower features (Table 1).

When the pollen morphology of the three species is examined, there are differences among them in terms of pollen shape, amb, ornamentation and pore structure. However, no distinctive characters were found in leaflet morphology to distinguish the three species.

Several authors have studied the seed surface ultrasculpture of *Astragalus*. Engel (1990) reported that *Astragalus* spp. have reticulate, multireticulate, and foveolate and multifoveolate seed surface sculpture. Ekici et al. (2005) noted regulate–granulate pitted seed surfaces for *Astragalus ovalis* Boiss. & Balansa (*A.* sect. *Ammodendron* Bunge). Vural et al.

(2008) and Shemetova et al. (2018) studied the morphology of seeds from 48 species of the sections *Onobrychoidei* DC., *Uliginosi* Gray and *Ornithopodium* Bunge, and found two main types of seed surface ultrasculpture: rugose and rugose–reticulate. The shape of the *A. oksutdagensis* seed is spheroidal and reniforme. The surface ornamentation is reticulate. As can be inferred from studies on different sections and types, when the ornamentation type is examined in detail, there are significant differences among sections or even species, which may be used as important diagnostic characters.

Although morphological differences does not always reflect phylogenetic relationships, *A. oksutdagensis* differs both morphologically and phylogenetically from all other members of *A.* sect. *Eustales*. Moreover, our results support the monophyly of *A.* sect. *Eustales*, which seems to be a sister group to *A.* sect. *Tragacantha*.

The phylogenetic analyses showed that the new species and *A. vestitus* are sister taxa within the clade formed by *A.* sect. *Eustales* (Fig. 4-with a bootstrap value of 65). Even if *A. flavescens* also belongs to the same section as *A. vestitus* and *A. oksutdaghensis*, it is phylogenetically distict from both species (Fig. 4-with a bootstrap value of 99).

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Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.kh189326m> (Karaman Erkul et al. 2021).

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