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## Astragalus aybarsii a new species of sect. Onobrychoidei DC. (Fabaceae) from Turkey

Hayri DUMAN<sup>®</sup>, Zeki AYTAÇ<sup>\*</sup><sup>®</sup>, Funda ÖZBEK<sup>®</sup>

Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

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Abstract: Astragalus aybarsii H. Duman & Aytaç (Fabaceae) is described and illustrated as a new species from Kastamonu province (Turkey). The new species grows in open areas of Pinus brutia Ten. forests. Diagnostic and morphological charactersof the new species are compared with its close relatives, A. setulosus Boiss. & Balansa and A. eubrychioides Boiss. Also, some notes on the ecology, biogeography, conservation status and the pollen morphological and leaflet micromorphological features of the new species are presented. The geographical distribution of this new species and its allied species is mapped.

Key words: Astragalus, Kastamonu, Leguminosae, Onobrychoidei, taxonomy, Turkey

#### 1. Introduction

Astragalus L. is the largest genus of flowering plants in the world. According to the International Plant Names Index (IPNI), the number of taxa is 5,2501. Also, according to Maassoumi (1998), this genus is represented with eight subgenera, 245 sections and 2,530 taxa (subspecies and varieties), 102 of them are annual and 2,428 are perennial in the old world. Also, according to Podlech and Zarre (2013), the number of taxa of this genus in the old world is 2,398 attributed to 136 sections. In Turkey, this genus includes 63 sections and 459 taxa, so its endemism rate is 51% (Ekici et al., 2015; Aytaç et al., 2020; Tunçkol et al., 2020).

The section Onobrychoidei DC. is the largest section without the spiny group and it comprises 29 species, 13 taxa of which are endemic for Turkey, indicating an endemism rate of 45% (Ekici et al., 2015). There are some reports concerning the pollen morphology and leaflet micromorphology of the family Fabaceae. The pollen morphological properties of 45 specimens belonging to 29 species of the genus Astragalus from section Onobrychoidei in Turkey were investigated using light microscopy (LM) and scanning electron microscopy (SEM) (Pınar et al., 2009). The pollen grains of 15 species belonging to section Hololeuce Bunge were studied, which are growing in Turkey (Ceter et al., 2013). The researchers reported that especially exine sculpturing is significant for discriminating the taxa from each other. Also, Al-Ghamadi et al. (2013) noticed that pollen grains are the most useful features in the 13 Astragalus species they examined from Saudi Arabia. Metin et al. (2018) recorded that the ornamentation of trichomes is a distinctive character for the taxonomy of the Astragalus victoriae Podlech & Agerer-Kirchhoff and A. melanophrurius Boiss. studied and endemic to Turkey.

In the Astragalus genus, chromosome numbers change to 2n = 16 to 64. Martin et al. they confirm in their study that, the number of chromosomes in some parts of the Astragalus sections varies between 2n = 16 and 64 (Martin et al., 2019).

During the fieldwork, some interesting Astragalus specimens were collected by first author around the Kastamonu province. After the literature and herbaria studies, it was understood that they belong to the sect. Onobrychoidei. After thorough investigations of these specimens, we decided that it is a new taxon for science.

## 2. Materials and methods

Some specimens were collected from Hanözü village (Kastamonu) by the first author in 2018 and 2019. These specimens were checked with the Flora of Turkey and the East Aegean Islands (Chamberlain and Matthews, 1970; Davis et al., 1988), Flora Europeae (Tutin et al., 1981) and other relevant resources of the section Onobrychoidei (Podlech and Zarre, 2013; Ekici et al., 2015), and also compared with specimens in the herbaria GAZİ, ANK, AEF, HUB, EGE and LE.

In the present study, the pollen morphology of the new taxon was investigated using LM and SEM. The pollen

<sup>1</sup> IPNI (2020). International Plant Names Index [online]. Website http://www.ipni.org [accessed 18 May 2020].

<sup>\*</sup> Correspondence: zaytac@gazi.edu.tr



morphological characteristics of its closely related species, A. setulosus and A. eubrychioides, were studied within the other species in the section Onobrychoidei by Pınar et al. (2009). Therefore, our results regarding the pollen grains of A. aybarsii were compared with the findings of its allied species according to Pınar et al. (2009). At first, pollen slides were prepared using the Wodehouse technique (1965). Pollen grains were stained with glycerin-jelly plus safranin with slight heating of the slide, then a cover slip was placed on it. These preparations were investigated and measured using an Olympus CX31 LM with an ocular micrometer. Measurements were based on at least 30 pollen grains for each of the pollen morphological features. Minimum-maximum range, mean and standard deviation (SD) values of these characteristics of the new taxon were given. For the SEM studies, dried pollen grains were transferred onto stubs and then coated with gold. They were observed and photographed with a JEOL JSM 6060 SEM at the SEM laboratory of Faculty of Science at the Gazi University, Ankara, Turkey. The terminology adopted is in accordance with Faegri and Iversen (1992), Punt et al. (2007), Pinar et al. (2009) as well as Ceter et al. (2013). The pollen-shape class, based on the ratio of polar axis to equatorial axis (P/E), was identified using Erdtman's system (Erdtman, 1969).

The micromorphologies (hairs, stomata, and surfaces) of the leaflets in the new species and the closely related species of *A. setulosus* and *A. eubrychioides* were examined

with SEM. Both surfaces of the leaflet were mounted onto stubs and coated with gold and then they were investigated and photographed with a JEOL JSM 6060 SEM at the SEM laboratory of the Faculty of Science at the Gazi University. The terminologies of Barthlott (1981) and Karaman et al. (2009) were followed for leaflet micromorphological characteristics.

Authors of botanical names were abbreviated according to Authors of Plant Names (Brummitt and Powell, 1992).

The specimens were deposited in the GAZI herbarium.

## 3. Results

# 3.1. *Astragalus aybarsii* H. Duman & Aytaç sp. nov., bala geveni, (Figures 1 and 2).

**Type:** TURKEY. A5 Kastamonu: Hanönü to Taşköprü 3. km, 500 m, in open areas of *Pinus brutia* forest, 26.vi.2019, *H. Duman* 10558 (**Holo**: GAZI; **iso**: HUB, ANK).

**Diagnosis:** The new species is similar to *Astragalus setulosus* and *A. eubrychioides*. But it differs from *A. setulosus*, with short stipules (3–4 mm long, not 4–12 mm long), bracts 3–4 mm long (not 6–11 mm long), obovate leaflets (not elliptic) and stipitate ovary (not sessile). Also it is distinguished from *A. eubrychioides* with short stipules (3–4 mm long [not (5–) 7–8 mm long], distinctly long peduncle (5–10 cm, not ca. 4 cm long), calyx 10–12 mm long (not 5–6 mm long) and short calyx teeth (3–4 mm, not 4.5–9 mm long) and leaflets 4–10 pairs not 10–20 and stipitate ovary (not sessile).



Figure 1. Habitus of A. aybarsii in nature.



Figure 2. Habitus *A. aybarsii*. A: habitus; B1: upper surfcae of leaflet; B2: lower surface of leaflet; C: flowers; D: dissected calyx; E: standart; F1 and F2: wings; G: keel; H: stamens; I: ovary; J: fruit; K: seed.

**Description**: *Plants* 15–30 cm long. *Rootstock* divided with short branches. *Stems* mostly several, 5–15 cm long, erect, densely covered with subappresed to appressed white, sometimes rarely black hairs at base. *Stipules* 3–4

mm long, greenish, narrowly triangular, free from the petiole, densely asymmetrical bifurcate white hairs at margins with long white hairs. *Leaves* 7–15 cm long, petiole 1.5–2 cm long, with the rachis densely covered with

subappresed to spreading long hairs like the stem. Leaflets in (4-) 6–10 pairs, 6–10 × 4–6 mm, obovate (sterile branch leaflets smaller), rounded to subacute, densely covered with submedifixed to asymmetrically bifurcate appressed hairs on both surfaces. Peduncle 5-10 cm long, hairy like the stem. Racemes first globose-oblong then cylindrical, 10-15 flowered. Bracts 3-4 mm long, greenish, narrowly triangular to lanceolate, covered with asymmetrically bifurcate, subappresed to appressed white. Pedicels absent to  $\pm 1$  mm long, white hairy. Calyx 10–12 mm long, tubularcampanulate, covered with asymmetrically bifurcate, subappresed to appressed white hairs to  $\pm$  appressed black hairy on dorsal side; teeth 3-4 mm long, sparsely long spreading white hairy. Corolla bluish to violet (sometimes cream when dry materials), glabrous. Standards  $16-18 \times$ 6-10 mm, tongue-like narrowed toward the emarginate; wings 13-14 mm long, narrowly obovate; keel 11-12 mm long, obliquely obovate. Stamens including in keel. Ovary stipitate densely white hairy. Stylus hairy at the base. Legume stipitate (1–1.5 mm long), oblong,  $10-12 \times 3-4$ mm, bilocular; valves densely covered with asymmetrically bifurcate, appressed hairs, partly glabrescent with age, with 1.5-2 mm curved beaked. Seeds 2-2.5 mm long, reniform, brownish and smooth.

Flowering and fruiting time: July to August.

## 3.2. Palynology

The pollen grains of *A. aybarsii* are trizonocolporate, radially symmetrical and isopolar. Their shape is

subprolate (P/E: 1.18) with the polar axes  $30.72-38.4 \mu m$  and the equatorial axes  $26.88-32.64 \mu m$ . Colpi are long and narrow with clear margins (Clg  $26.88-30.96 \mu m$ , Clt  $3.12-4.8 \mu m$ ). The aperture membrane is granulate. Pores are lalongate or circular (Plg  $6.72-9.6 \mu m$ , Plt  $8.64-11.52 \mu m$ ). The exine is subtectate and has a thickness of  $0.92-1.25 \mu m$ . The ectexine is thicker than the endexine. Exine sculpturing is microreticulate-perforate in the meridional and polar optical sections. The intine has a thickness of  $0.48-0.96 \mu m$  (Table 1, Figure 3).

## 3.3. Leaflet micromorphology

The leaflets of A. aybarsii, A. setulosus and A. eubrychioides are amphistomatic. Stomata are usually mesomorphic. The number of stomata in 1 mm<sup>2</sup> are 122.65  $\pm$  28.17 on the adaxial surface and  $125.65 \pm 29.61$  on the abaxial surface of leaflets in A. aybarsii. A total of 125.65 ± 14.8 stomata in 1 mm<sup>2</sup> were observed on the leaflet adaxial surface and a total of 128.64  $\pm$  28.17 stomata in 1 mm<sup>2</sup> were observed on the abaxial part of A. setulosus and also the stoma number was found to be 146.59 ± 38.23 on the adaxial surface and  $167.53 \pm 18.73$  on the abaxial surface in 1 mm<sup>2</sup> on the leaflets of A. eubrychioides. In these species, the stoma number on the adaxial surface is less than on the abaxial part of leaflets. The trichomes are bifurcate and their ornamentation is striate-verrucate and psilateverrucate. In three species the adaxial and abaxial surfaces of the leaflets contain densely epicuticular waxes (Figures 4 and 5).

**Table 1.** Pollen morphological data of *Astragalus aybarsii, A. setulosus* and *A. eubrychioides* (values in  $\mu$ m; minimum, maximum, mean  $\pm$  standard deviation).

Species/characters		A. aybarsii	A. setulosus (Pınar et al., 2009)	A. eubrychioides (Pınar et al., 2009)
	Min.	30.72	27.3	27.1
Polar axes	Max.	38.4	42.1	35.4
	Mean	34.83 ± 1.87	35.4	31.4
Equatorial axes	Min.	26.88	20.3	20.3
	Max.	32.64	30.7	28.1
	Mean	29.48 ± 1.17	24.3	22.1
Pollen shape		Subprolate	Prolate	Prolate
Aperture type		Trizonocolporate	60% trizonocolporate 40% spiraperturate	Trizonocolporate
Ornamentation		Microreticulate-perforate	Microreticulate	Microreticulate
Column (Cl)	Colpus length (Clg)	29.26 ± 1.1	30.5	27.4
Colpus (CI)	Colpus width (Clt)	$3.92 \pm 0.33$	3.1	2.1
	Pore length (Plg)	$8.58 \pm 0.58$	6.3	4.2
Pore (PI)	Pore width (Plt)	$10.2 \pm 0.66$	6.3	4.3
Exine thickness		$0.95 \pm 0.01$	2	2
Intine thickness		$0.77 \pm 0.21$	0.4	0.3



Figure 3. SEM micrographs of pollen grains of *Astragalus aybarsii*. A–B: equatorial view and exine ornamentation; C–D: polar view and exine ornamentation.

#### 3.4. Distribution, habitat, and ecology

The new species grows on sandy soil which is in open areas of *Pinus brutia* Ten. forest, together with *Berberis crataegina* DC., *Reseda lutea* L. var. *lutea*, *Helianthemum canum* (L.) Baumg., *Dianthus varankii* Hamzaoğlu & Koç (local endemic), *Astragalus spruneri* Boiss, *Astragalus andrachneifolius* Fenz., *Astragalus karamasicus* Boiss. & Bal. (endemic), *Oxytropis pallasii* Pers., *Dorycnium graecum* (L.) Ser., *Hedysarum varium* Willd., *Onobrychis tournefortii* (Willd.) Desv. (endemic), *Verbascum pseudovarians* Hub.–Mor. (endemic), *Salvia cyanescens* Boiss. & Bal. (endemic). The type location of new species has a typical Mediterranean microclimate in the Euro-Siberian phytogeographic region (Figure 6).

#### 3.5. Conservation status

The new species is known only one location. Extent of occurence (EOO) and area of occupancy (AOO) are less than 1 km<sup>2</sup>. Area of occupancy (AOO) and habitat quality continuing decline, for these ArcGIS and spatial analysis

programs were used. Because road construction activities present in this area, this new species is considered "critically endangered" CR B1ab(iii,v) + 2ab(iii,v) (IUCN, 2017).

#### 3.6. Etymology

The plant takes its scientific name from the first author' son. The Turkish name of the new species was suggested as "bala geveni" (Menemen et al., 2016).

#### 4. Discussion

The members of this genus widely grow in different habitats. On the contrary, some of them choose special habitats, such as *Astragalus yildirimli* Aytaç & Ekici and *A. antiochianus* Post. This new species especially grows on sandy habitats. *A. setulosus* and *A. antiochianus* are distributed in the Central Anatolia (Iran-Turan) and Mediterranean regions, while *A. aybarsii* grows as an enclave of the Mediterranean in the Black Sea region. *A. aybarsii* is distinguished from the related species by its

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**Figure 4.** SEM micrographs of adaxial surfaces of leaflet. *Astragalus aybarsii*: A–D; *A. setulosus*: E–H; *A. eubrychioides*: I–L. A, E, I: general view; B, F, J: higher magnification of surface; C, G, K: trichome; D, H, L: stoma.



**Figure 5.** SEM micrographs of abaxial surfaces of leaflet. *Astragalus aybarsii*: A–D; *A. setulosus*: E–H; *A. eubrychioides*: I–L. A, E, I: general view; B, F, J: higher magnification of surface; C, G, K: trichome; D, H, L: stoma.



Figure 6. Distribution map of *A. aybarsii* ★, *A. eubrychioides* ▲ and *A. setulosus* ■ in Turkey.

smaller stipules and with its stipitate ovary. On the other hand, while it looks like *A. eubrychoides*, it differs with its longer calyx and calyx teeth. Although it resembles *A. setulosus* in calyx structure and calyx teeth, it is separated by the shortness of its bracts. The comparison of the diagnostic characteristics of *A. aybarsii, A. eubrychoides* and *A. setulosus* was given in Table 2.

The pollen grains of A. aybarsii and the closely related species, A. setulosus and A. eubrychioides, are isopolar and radially symmetrical. The aperture type is trizonocolporate in A. aybarsii in this study. It was also found to be trizonocolporate in A. eubrychioides, whereas the pollen grains were detected to be 60% trizonocolporate and 40% spiraperturatein A. setulosus by Pinar et al. (2009). Although, Pinar et al. (2009) generally observed the trizonocolporate aperture type, some heteromorphic pollen grains were encountered, such as tetrapantocolporate, spiraperturate, tricolpodiporate as well as paratrizonocolporate in the Astragalus taxa they studied. Similarly, Ceter et al. (2013) detected trisyncolporate and tricolpate pollen grains as well as trizonocolporate in the section Hololeuce. Moreover, the researchers defined three different pollen shapes, prolate-spheroidal, subprolate and prolate in their investigated Astragalus species. In our study the pollen shape is subprolate in A. aybarsii (P/E: 1.18). Pınar et al. (2009) reported that the pollen grains of A. setulosus (P/E: 1.46) and A. eubrychioides (P/E: 1.42) were subprolate, but these values were evaluated as prolate by Erdtman (1969). Therefore, the shape of pollens shows differences between the new species and the two closely related species. A. avbarsii has subprolate pollen grains, whereas the pollen shape is prolate in A. setulosus and A. eubrychioides. Exine sculpturing shows differences between the new species and its closely related two species. It is microreticulateperforate in the equatorial and polar optical sections in A. aybarsii, but microreticulate ornamentation was detected on the two regions of pollen grains belonging to A. setulosus and A. eubrychioides (Pınar et al., 2009). Whereas, Akan et al. (2005) determined only microreticulate ornamentation in the Alopecuroidei section of the species and likely in the Hymenostegis section (Bagheriet al., 2019). Various ornamentation types, such as reticulate and perforate sculpturing at the polar section and perforate, reticulate, microreticulate, perforate-granulate, microreticulateperforate, microrugulate-perforate, microrugulategranulate-perforate, microreticulate, microreticulategranulate ornamentation at the meridional section were reported besides microreticulate by Ceter et al. (2013).

The seeds structure is the same form in new species as some of sections of genus *Astragalus* (Shemetova et al., 2018).

It was observed that there was no significant variation in the species studied, except for the number of stomata for the leaflet micromorphology. *A. aybarsii* has the least stomata (122.65  $\pm$  28.17 on the adaxial surface and 125.65  $\pm$  29.61 on the abaxial surface), while *A. setulosus* has the

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Characters	A. aybarsii	A. eubrychoides	A. setulosus
Plant	15–30 cm long	at least 30 cm long	6–35 cm long
Stipule	3–4 mm long, greenish, narrowly triangular	(5–) 7–9 mm long, yellowish (membranous), triangular	4–12 mm long, greenish, closely triangular
Leaves	7–15 cm long	4–8 cm long	3–15 cm long
Leaflets	(4–) 6–10 pairs, 6–10 mm long, obovate	7–20 pairs, 8–20 mm long, narrowly elliptic to oblong	(4–) 5–11 pairs, 4–23 mm long, elliptic
Peduncles	5–10 cm long	4–10 cm long	3–20 cm long
Inflorescence	10–15 flowered, first oblong then cylindrical	30–50 flowered cylindrical	10–15 flowered, short globe-like to ovoid
Bracts	3–4 mm long, narrowly triangular to lanceolate	5–7 mm long, closely triangle-shaped	6–11 mm long, triangular to linear- acute
Calyx	10–12 mm long, tubular-campanulate; teeth 3–4 mm long, sparsely white hairy on inner side	5–6 mm long, shortly tubular; teeth linear-acute, 2–3 mm long, sparsely hairy on inner side	9–15 mm long, tubular-campanulate; teeth 4.5–9 mm long, subulate, sparsely hairy on inner side
Standard	16–18 mm long, emarginate tip	15 (-20) long, emarginate tip	18–20 mm long, slightly emarginated tip
Wing	13–14 × 6–9 mm, blades narrowly obovate	$13-18 \times 3-4$ mm, blades ± oblong	13–15 × 6–9 mm, blades narrowly obovate
Keel	11–12 mm long,	14–18 mm long,	12–13 mm long,
Ovary	Stipitate	Sessile	Sessile
Fruit	Stipitate, $8-10 \times 3-4$ mm, oblong, appressed bifurcate hairy; beak 2–2.5 mm long	sessile, $8-10 \times 2-3$ mm, oblong, appressed bifurcate hairy, partly glabrescent soon; beak 1–2 mm long	sessile, 10–12 × 2.5–3 mm, oblong, appressed bifurcate hairy; beak 1.5– 2.5 mm long
Seeds	2–2.5 mm long, brownish, smooth	3–4 mm long, smooth	2-3.5 mm long, brownish, smooth

Table 2. Comparison of the diagnostic characteristics of A. aybarsii, A. eubrychoides and A. setulosus.

most numerous ones (146.59  $\pm$  38.23 and 167.53  $\pm$  18.73, respectively). The trichomes are bifurcate and striate-verrucate and psilate-verrucate ornamented in these species examined.

Key for distinguishing the section members whose flower parts are hairy in Turkey:

- 1- Calyx teeth 2-4 mm; bracts at most 7 mm ......2
- Calyx teeth 4.5-7 mm; bracts 6-11 mm .... A. setulosus
- 2- Stipule 3-4 mm; ovary stipitate ...... A. aybarsii
- 2– Stipule (5–) 7–9 mm; ovary sessile .... A. eubrychioides

## **Examined specimens**

*A. setulosus:* Sakarya, by the road Geyve–Taraklı, c. 2 km from Taraklı to Geyve, 520 m, 23.5.1990, Lampinen 7795 (photo MSB!); Ankara: Akyarma pass, 33 km SE Gerede toward Ankara, 1540 m, 6.7.1983, Nydegger, 18017 (MSB!); Kayseri: E' Side of the Araplı between Yeşilhisar and Niğde, 1260 m, 14.6.1987; Develi–Yahyalı 15. km, steppe, 1350 m, 2.6.2001, Aytaç 7173 (GAZI!); Ereğli,

Kıraman village, 1800 m, 23.7.1995, steppe, Z. Aytaç 7225 (GAZI!); Akyokuş, Keçilidere district, 1100 m, 14.6.1980, H. Dural 665 (KNYA!); Belören, Hadim, 10 km N Hadim, 1370 m, 28.7.1992, Nydegger 46879 (MSB!); İçel: Mut, Büyükeğri mountain, 1500 m, 24.7.1977, M. Vural 443 (ANK!, E!); Ulukışla–Darboğaz road, 13.6.1991, Gemici 6084 et al. (EGE!); 8 km S Çamardı against Pozantı, 1450 m, 27.6.1989, Nydegger 44278 (M, MSB!).

*A. eubrychioides*: Kars: Akçay–Cumaçay 10. km, steppe, 20.7.2001, 1800 m, M. Ekici 2602 et al. (GAZI!); Van: Çiçekli–Bahçesaray 21. km, steppe, 2050 m, 7.6.2001, M. Ekici 2321 et al. (GAZI!); Ağrı–Van junction, steppe, 1700 m, 9.6.2001, M. Ekici 2403 et al. (GAZI!).

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