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Astragalus nurhakdagensis (sect. *Hololeuce* Bunge / Fabaceae), a new species from Turkey

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Abstract: *Astragalus nurhakdagensis*, a new species from the south of Turkey was described, illustrated, and compared to the closest taxa *Astragalus hirsutus*, *A. dumanii* and *A. cataonicus* from which it is set apart by a longer calyx length (13–14 mm) and calyx teeth (7–9 mm, longer than tube), white corolla colour, longer bracts (13–15 mm) and black hairy stripes below the stem nodes. In *A. hirsutus,* the calyx length (6–10 mm) and calyx teeth are much smaller (2–3 mm long, shorter than tube), with yellow corolla colour and without black hairy stripes. It also differs from *A. dumanii* with its longer stipules 9–13 mm (not 5–8 mm), bracts 13–15 mm (not 4–6 mm), calyx length 13–14 mm (not 7–10 mm) and calyx teeth 7–9 mm (not 2–4 mm), and also with spreading hairs (not adpressed) on the calyx and 7–9 pairs of leaflets (not 3–7 pairs). It is also differentiated from *A. cataonicus* with a white corolla colour (not lilac to pink), longer stipule 9–13 mm (not 5–7 mm), bracts 13–15 mm (not 6–12 mm), and longer calyx teeth 7–9 mm (not 5–7 mm). Prolate (*A. nurhakdagensis*, *A. hirsutus* and *A. cataonicus*) and subprolate (*A. dumanii*) shapes were determined in the tricolporate pollen grains. Perforate, granulate, and reticulate ornamentation types were observed in the pollen grains in SEM. The seed shapes were reniform–globose in *A. nurhakdagensis* and *A. dumanii*, whereas they were reniform in *A. hirsutus* and *A. cataonicus*. Surface ornamentation of the seeds differed slightly: rugulate in *A. nurhakdagensis* and *A. hirsutus*, reticulate-rugulate in *A. dumanii* and reticulate-striate in *A. cataonicus*. Taxonomic description, micrographs of seeds and pollen surfaces and geographical distribution of the new species were provided. Conservation status was discussed.

Key words: *Astragalus*, Leguminosae, taxonomy, sect. *Hololeuce*, Turkey

1. Introduction

Astragalus L., the most abundant member of the Fabaceae family in the world, is known as a taxonomically difficult genus (Podlech, 1986). This genus has a very wide distribution area, including nearly 3000 taxa, extending from Asia and Europe to America (Podlech and Zarre, 2013). In Turkey, it grows mainly in dry habitats on steppes throughout the East and Central Anatolian Regions and includes at least 479 species (Aytaç, 2000; Podlech and Zarre, 2013; Aytaç et al., 2020). The majority of this number (51%) belongs to the Irano-Turanian phytogeographical region (Aytaç, 2000). High mountainous areas with hard-to-reach terrain are the habitat of many members of this genus, and these narrow habitats shelter some hitherto unidentified species (Uzun et al., 2009). After the old-world revision of the genus *Astragalus* edited by Podlech and Zarre (2013), many new *Astragalus* species from Turkey have been described for plant science. This means that the semi-isolated Anatolia still serves as the speciation centre of the genus *Astragalus*. The newly described taxa from Turkey are as follows: *Astragalus unalii* (Çeçen et al., 2016), *A. topalanense* (İlçim and Behçet, 2016), *A. ihsancalisii* (Dönmez and Aydın, 2018), *A. sertavulensis* (Aytaç et al., 2020), *A. bartinense* (Tunçkol et al., 2020) and *A. aybarsii* (Duman and Aytaç, 2020).

An unusual *Astragalus* specimen was encountered in a flora survey conducted at Nurhak Mountain, which constitutes the highest peak of Kahramanmaraş province (Killi Hill, 3071 m) (Uzun et al., 2019). Nurhak is a district located in Kahramanmaraş province between the Irano-Turanian and Mediterranean regions of Turkey. At first glance in the terrain, it was thought that the plant specimen was *Astragalus dumanii* Ekici & Aytaç because of its general appearance and corolla color, but the diagnostic keys of the *Flora of Turkey* failed to determine the specimens (Davis et al., 1988; Aytaç, 2000). Regional floras of the neighbouring

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countries (Townsend and Guest, 1974; Podlech, 1999; Podlech et al., 2001), *European Flora* (Chater, 1968) and the old-world checklist (Podlech and Zarre, 2013) also remained inconclusive. After a detailed examination of the specimens in the plant laboratory using the revisions of the relevant sections (Ekici and Ekim, 2004; Podlech and Zarre, 2013; Ekici et al., 2015), as well as comparisons with many *Astragalus* specimens (cited in the appendix) in GAZI, ANK, HUB and KASOF (Kahramanmaraş Sütçü İmam University) herbaria (Thiers, 2020), it was realized that the specimens apparently belong to a new species and were not described previously.

According to Chamberlain and Matthews (1970), section *Hololeuce* Bunge in the *Flora of Turkey* was represented by 20 species within the boundaries of Turkey, 16 of which were considered endemic. After that, Ekici and Ekim (2004) recognised 15 species, which belong to this section in Turkey. Sect. *Hololeuce* Bunge (incl. sect. *Chlorosphaerus* Bunge) comprises scapose or very shortly caulescent, perennial herbs. Leaves imparipinnate; leaflets bifurcate-hairy; stipules free or united below, free from or adnate to the petioles. Inflorescence a dense, pedunculate, many-flowered spike. Flowers ebracteolate or with 1- or 2-min bracteoles. Calyx black and white, bifurcate, or simple-hairy, not inflated. Legumes usually slightly longer than the calyx, bilocular, and several-seeded.

New specimens were well fitted above characteristics, but the diagnostic features, such as flower structures, corolla color and dimensions, stem indumentum, peduncle length and hairiness, stipule and bract lengths, and the proportional comparison of calyx teeth with tubes have proved to be distinctness within the section. The first noted difference was that the calyx teeth were clearly longer than the calyx tubes compared to other species in the section. Among the closest species, calyx teeth, as long as the calyx tube in *A. cataonicus* Bunge, are much shorter in *A. dumanii* Ekici & Aytaç and *A. hirsutus* Vahl.

In the present study, the new taxon belonging to sect. *Hololeuce* Bunge was described, illustrated, and compared with the closest species in the section. Accordingly, with this study, the number of taxa increased to 16. In addition, the micromorphological characters of their seeds and pollen were compared by using scanning electron microscopy (SEM).

2. Materials and methods

Plant materials of the new taxon were collected from Nurhak Mountain (Kahramanmaraş, South Anatolia, Turkey) in 2020 (Figure 1). The morphological data and the detailed photographs used in the identification were obtained by the authors using a photo-stereoscopic microscope (Leica APO8) (Figure 2, 3). Voucher specimens were stored in the herbaria of the Kahramanmaraş Sütçü İmam University (KASOF) and Gazi University (GAZI) (Thiers, 2020). Morphological features were also observed on fresh materials in the field. *Astragalus hirsutus*, *A. dumanii* and *A. cataonicus* in the sect. *Hololeuce* Bunge, which show the closest taxonomic features with the new species, were included in the study. Herbaria samples of *Astragalus hirsutus* (*Aytaç* 2655 & *H. Duman*), *A. dumanii* (*H. Yıldırım* 3075 and *A. Duran* 7681), and *A. cataonicus* (*M. Ekici* 2038) given in the appendix were used for morphological comparison.

The plant names mentioned here follow the *International Plant Names Index* ([1]IPNI 2020).

Pollen materials (anthers) were gathered from living plants in the field. Observations were examined by applying Erdtman's acetolysis method (1952) and photographed with EVO LS10 SEM. Size values were based on the measurements of 30 pollen grains. Characters such as polar axis (P), equatorial diameter (E), P/E ratio, shape in polar view, shape in equatorial view, colpus length/width and aperture types were examined. The shape of pollen grains, based on the ratio of polar axis to equatorial diameter (P/E), was identified according to Erdtman's (1952) pollen-shape classes. In the present study, the pollen morphology of the new taxon was revealed for the first time. The pollen morphological characteristics of *A. hirsutus, A. dumanii* and *A. cataonicus* were already studied within the other species in the section *Hololeuce* by Ceter et al. (2013). Therefore, the results regarding the pollen grains of *A. nurhakdagensis* were compared with the findings of its allied species according to Ceter et al. (2013).

SEM studies: Scanning electron microscopic (SEM) examination was carried out on the outer surfaces of the seeds and pollen grains of *Astragalus nurhakdagensis* (*holo. Tülücü* 46 & *Uzun, para. Tülücü* 98 & *Uzun*), *A. hirsutus* (*Aytaç* 2655 & *H. Duman*), *A. dumanii* (*A. Duran* 7681) and *A. cataonicus* (*M. Ekici* 2038). Seed and pollen samples were mounted on metal stubs using double-sided adhesive tape and coated with gold before observation with the EVO LS10. Scanning was performed at different magnifications (100 ×, 1000 × and 5000 × for seeds, and 5000 × and 15,000 × for pollen grains) and the micrographs were obtained. Pollen surface terminology followed Barthlott (1981), Punt et al. (2007), Halbritter et al. (2018), as well as Ceter et al. (2013). Seed shape and surface terminology followed Vural et al. (2008) and Shemetova et al. (2018). In addition, the axis parameters were measured in the photo-stereoscopic microscope with the help of the digital measurement system.

Numerical analysis: For morphometric comparisons, species characteristics of four taxa of *Astragalus* were

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

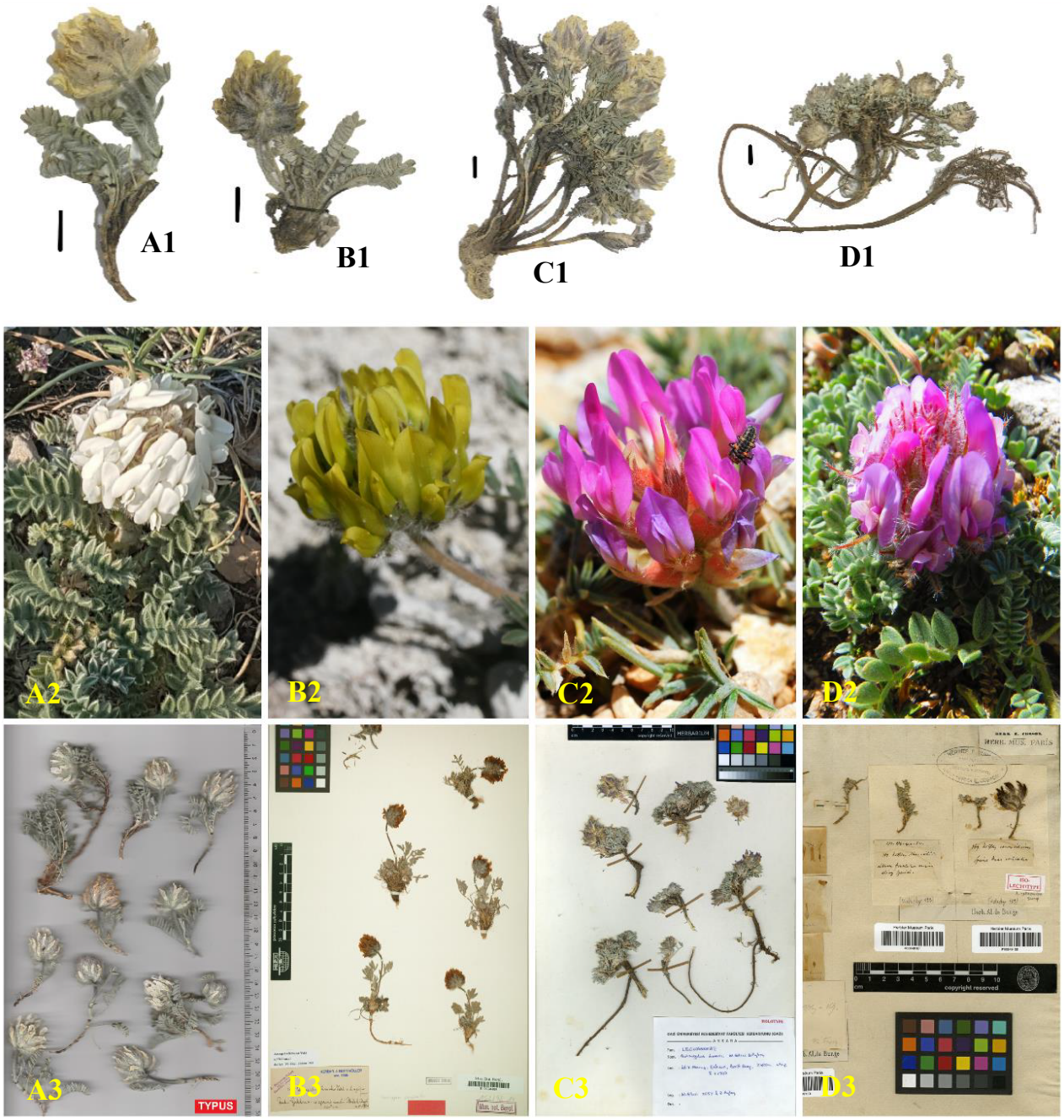

Figure 1. *Astragalus nurhakdagensis* (A1 and A2 from field, A3 *holo*. *Tülücü* 46 & *Uzun*). *A. hirsutus* (B1 and B2 from field, B3 Bornmüller, J., 2103 (B). *A. dumanii* (C1 and C2 from field, C3 *holo. Ekici & Aytaç* 24382) and *A. cataonicus* (D1 and D2 from field, photo by Işık Kavalcı, D3 *lecto*. *Kotschy* 169, P00649150), (Scale bars= 1 cm for A1, B1, C1 and D1).

transferred to an excel file (Table 1). Then, statistical analyses were performed for the twenty most distinctive quantitative characters based on standard, calyx, bract, stipule, peduncle, wings, keel, and P/E values. We used UPGMA (unweighted pair-group methods) clustering method (Romesburg, 2004) and principal component analysis (PCA) based on the Euclidean index to determine the relationships of these species (Jolliffe, 2002). Also, one-way ANOVA (several sample test) was calculated to determine whether the characters are statistically significant in delimitation of the species. Principal component and cluster analyses as powerful tools to support taxonomic identification (Marramà and Kriwet, 2017). In several studies, cluster analysis data can yield

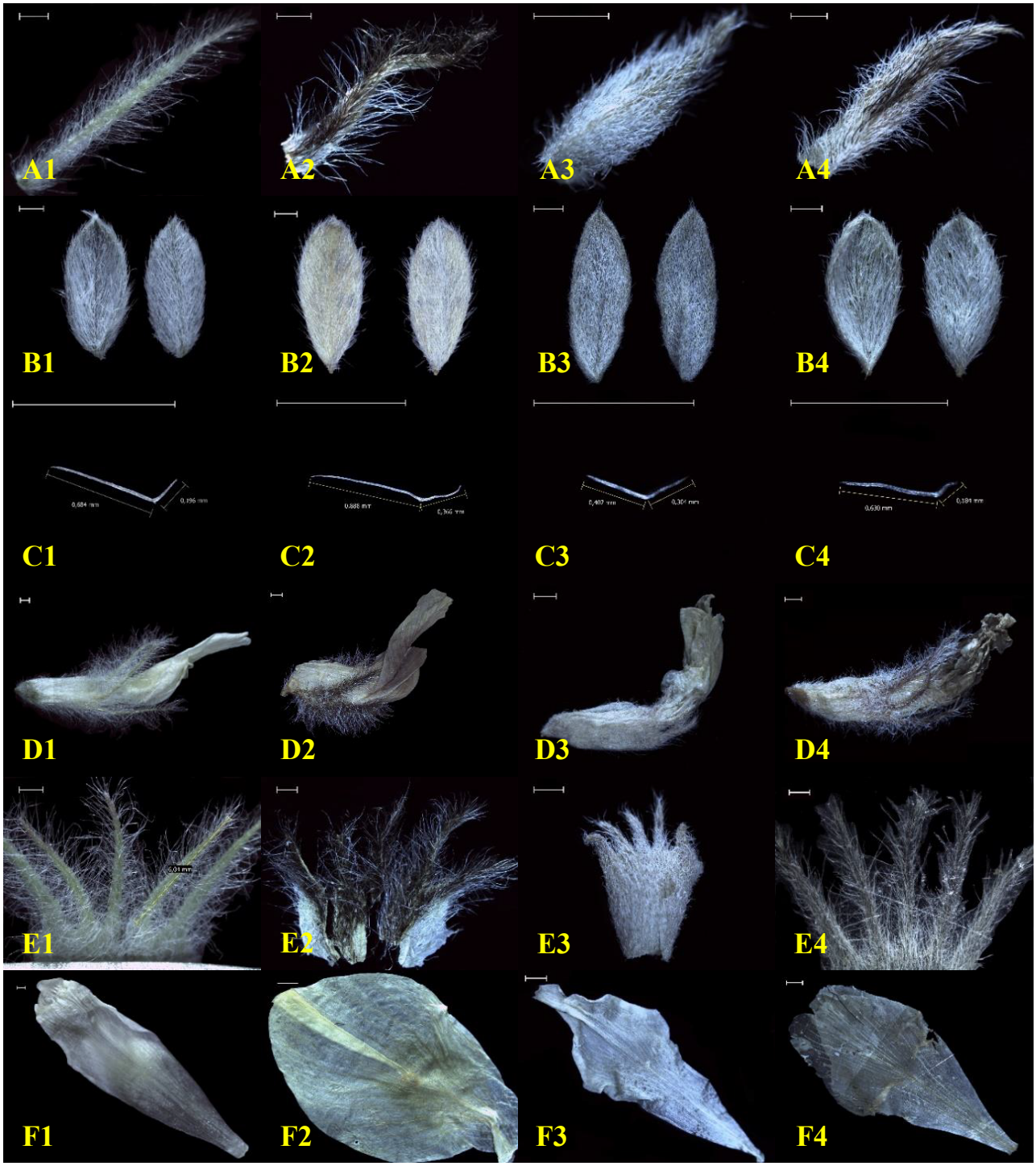

Figure 2. (1) *Astragalus nurhakdagensis* (*holo*. *Tülücü* 46 & *Uzun*), (2) *A. hirsutus* (*Aytaç* 2655 & *H. Duman*), (3) *A. dumanii* (*H. Yıldırım* 3075 and *A. Duran* 7681), (4) *Astragalus cataonicus* (*M. Ekici* 2038). A: bract, B: leaflet (adaxial and abaxial surface), C: leaf trichome, D: corolla and calyx, E: calyx teeth, F: standard, (Scale bars= 1 mm).

similar trees with the morphological classification of taxa (Açar and Satıl, 2019; Dirmenci et al., 2019; Arabaci et al., 2021; Fırat and Selvi, 2021). Analyses were performed using with PAST (paleontological statistics) software package for education and data analysis version 4.03 (Hammer et al., 2001).

Table 1. List of morphological characters used in statistical analysis.

No	Characters	No	Characters
1	Standard Min length	**11**	Bract Max length
2	Standard Max length	**12**	Stipule Min length
3	Standard Min width	**13**	Stipule Max length
4	Standard Max width	**14**	Peduncle Min length
5	Calyx Min length	**15**	Peduncle Max length
6	Calyx Max length	**16**	Wings Min length
7	Calyx teeth Min length	**17**	Wings Max length
8	Calyx teeth Max length	**18**	Keel Min length
9	Calyx teeth / tube ratio	**19**	Keel Max length
10	Bract Min length	**20**	P/E value

The distinguishing features selected as a result of these methods can be used as a guide in the differentiation of species and may be beneficial in eliminating characters that do not have taxonomic value in identification keys. It will also do a hash evaluation of many more features, increasing the precision of the range of features determined (Kucharczyk et al., 2012).

3. Results

3.1. *Astragalus nurhakdagensis*

Uzun, Aytaç & Tülücü *sp. nov.* [Sect. *Hololeuce* Bunge] Nurhak geveni, (Figures 1–6).

Type: Turkey, B6 Kahramanmaraş: Nurhak Mountain, 21 km from the centre of Nurhak, 2000 m, steppe, calcareous rocks, 17 June 2020, *Tülücü* 46 & *Uzun* (holo. GAZI, iso. KASOF, ANK); B6 Kahramanmaraş: Nurhak Mountain, 21 km from the centre of Nurhak, 2000 m, steppe, calcareous rocks, 30 June 2020, *Tülücü* 98 & *Uzun* (para. KASOF, GAZI).

Diagnosis: The species is closely related to *A. hirsutus, A. dumanii* and *A. cataonicus,* but differing from *A. hirsutus* by having black hairy stripes below the nodes on stems (not without black hairy stripes below the nodes); white-cream color of corolla (not yellow); stipules white and/or black hairy (not only white); number of flowers 20–30 (not 10–20); bracts 13–15 mm long (not 6–10 mm); calyx 13–14 mm long (not 6–10 mm); calyx teeth 7–9 mm long (not 2–3 mm); and standard 22–23 mm long (not 14–18 mm). It was distinguished from *A. dumanii* by having stipules 9–13 mm long (not 5–8 mm); leaflets 7–9 pairs (not 3–7 pairs); bracts 13–15 mm long (not 4–6 mm); covered with white and black sub–bifurcate hairs (not only white); having a longer calyx 13–14 mm long (not 7–10 mm); calyx teeth 7–9 mm long (not 2–4 mm); spreading hairs (not appressed) on the calyx and leaflets; and standard 22–23 mm long (not 15–18 mm). It also differs from *A. cataonicus* by white (not lilac to pink) corolla, longer stipules 9–13 mm (not 5–7 mm); and longer bracts 13–15 mm (not 6–12 mm).

Description: Perennial, procumbent; 2–5 cm, acaulescent to shortly caulescent. **Stem** when present up to 2 cm long, densely covered with unequally bifurcate white and black hairs, black hairy stripes surround the stem below the nodes, and woody caudex branched. **Stipules** 9–13 mm long, triangular to narrowly triangular, acuminate at the apex (acumen 5–8 mm long, base 4–5 mm long), base shortly connate around the stem and attached to the petiole with 1/4–1/2 of its length, acumens free, densely covered with spreading black and/or white ± sub-bifurcate hairs, sometimes also with dense black hairs near the base, internodes prominent 1.0–2.0 cm long. **Leaves** 2.5–4.5 cm long, mostly longer than peduncles. **Petiole** 10–15 mm long, appressed to sub-spreading hairy. **Leaflets** in 7–9 pairs, narrowly elliptic, acute at the apex, mostly folded, 5.5–9 × 2.5–3.5 mm, on both sides densely covered with dense silvery subadpressed to spreading unequally bifurcate white hairs (long arm 0.5–1 mm, short arm 0.2–0.4 mm), also some black bifurcate hairs at the lower part of the leaflets. **Peduncles** 2–4 cm long, erect, as long as or shorter than leaves, densely sub-bifurcate (long arm 0.8 mm, short arm 0.06 mm long), black and white hairy. **Racemes** globose to ovoid, dense, 20–30 flowered spike, 3 × 3 cm, not elongating in fruit. **Bracts** 13–15 × 0.4–0.5 mm, linear-lanceolate, membranous, covered with long spreading, dense white and sparse black sub-bifurcate hairs. **Bracteoles** absent. **Pedicels** up to 1 mm long. **Calyx** 13–14 mm long, campanulate-tubular, covered with mostly dense and long (2–3 mm) spreading sub-bifurcate white hairs, rarely with also shorter (up to 1.5 mm) sparse black hairs at base of tube, and with very few short (up to 1 mm) white and black sub-bifurcate hairs; calyx teeth (6–)7–9 mm long, linear, longer than calyx tube, spreading sub-bifurcate, dominantly white and also black hairs. **Corolla** white to cream (brownish-yellowish when dry). **Standard** 22–23 × 8 mm, rhombic-obovate, emarginate at the apex, without clearly differentiated claw, gradually narrowed at the base, glabrous. **Wings** 15–16 mm long (claw 7–8 mm, lamina 8–9 mm, auricle 1 mm), glabrous. K**eel** 12–13 mm long (claw 8 mm, lamina 6 mm), glabrous. **Ovary** ± stipitate, elliptic, white hairy. **Legume** 6–7 × 1.5–3.0 mm, narrowly elliptic, ± stipitate with a straight beak 3–4 mm long, bilocular with 4 seeds in each locule, densely covered with appressed to subappressed white sub-bifurcate hairs. **Seed** reniform–globose (1.3–1.5 × 1.6–1.8 mm), **color** dark greenish brown.

Flowering: May–June and fruiting in June–July.

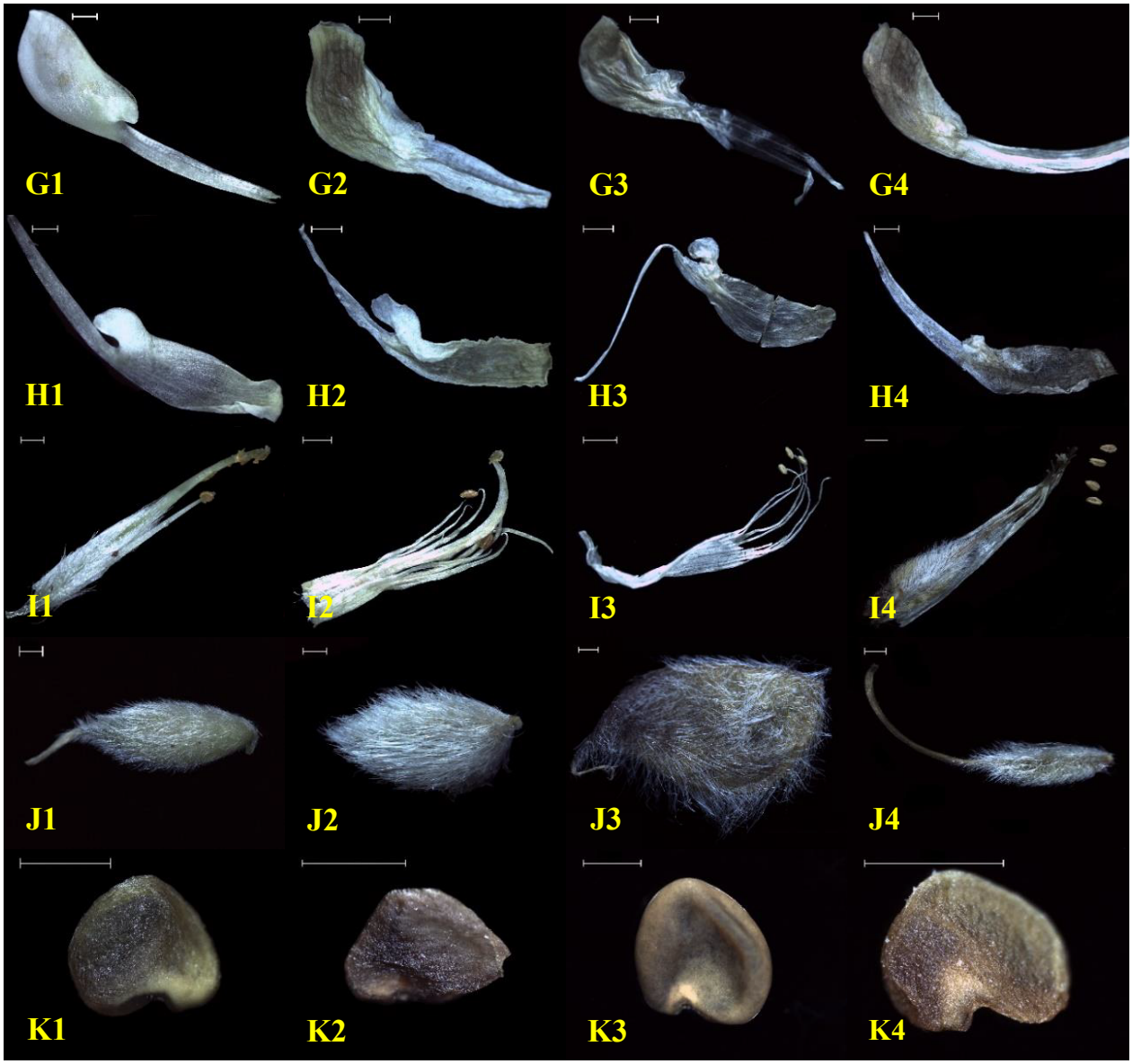

Figure 3. (1) *Astragalus nurhakdagensis* (*holo*. *Tülücü* 46 & *Uzun* and *para. Tülücü* 98 & *Uzun*), (2) *A. hirsutus* (*Aytaç* 2655 & *H. Duman*), (3) *A. dumanii* (*H. Yıldırım* 3075 and *A. Duran* 7681), (4) *A. cataonicus* (*M. Ekici* 2038). G: keel, H: wing, I: pistil and stamen, J: pod, K: seed, (Scale bars= 1 mm).

3.2. Palynology

Pollen grains of the studied taxa of *Astragalus* in sect. *Hololeuce* showed variation in their morphological characters, as already mentioned by Ceter et al. (2003). As the general characteristic of the studied taxa, the pollen grains are isopolar and radially symmetric. Pollen grains ranges between 29.60–45.11 µm in polar axis and 20.05–32.25 µm in equatorial diameters. Circular in polar view. Their colpi are long with clear margins. Perforate, granulate, and reticulate ornamentation types were observed in meridional and polar sections of the pollen grains of the studied taxa. Aperture type is tricolporate in all taxa (Table 2, Figure 4).

Pollen size: Pollen measurements were made on the pollen obtained by acetolysis method. According to the size classes of the pollen grains (Erdtman, 1952), 5 groups present: very small (<10 µm), small (10–25 µm), medium (26–50 µm), large (51–100 µm) and very large (>101 µm). All taxa in this paper were in medium in size. Largest-sized pollen was seen in *A. nurhakdagensis* (45.11 µm), *A. dumanii* (32.93 µm) and *A. cataonicus* (32.49 µm), respectively. The smallest-sized pollen was observed in *A. hirsutus* (29.60 µm). The P/E ratio varies between 1.27 and 1.62. The lowest P/E ratio was in *A. dumanii*, while the highest ratio was in *A. cataonicus.*

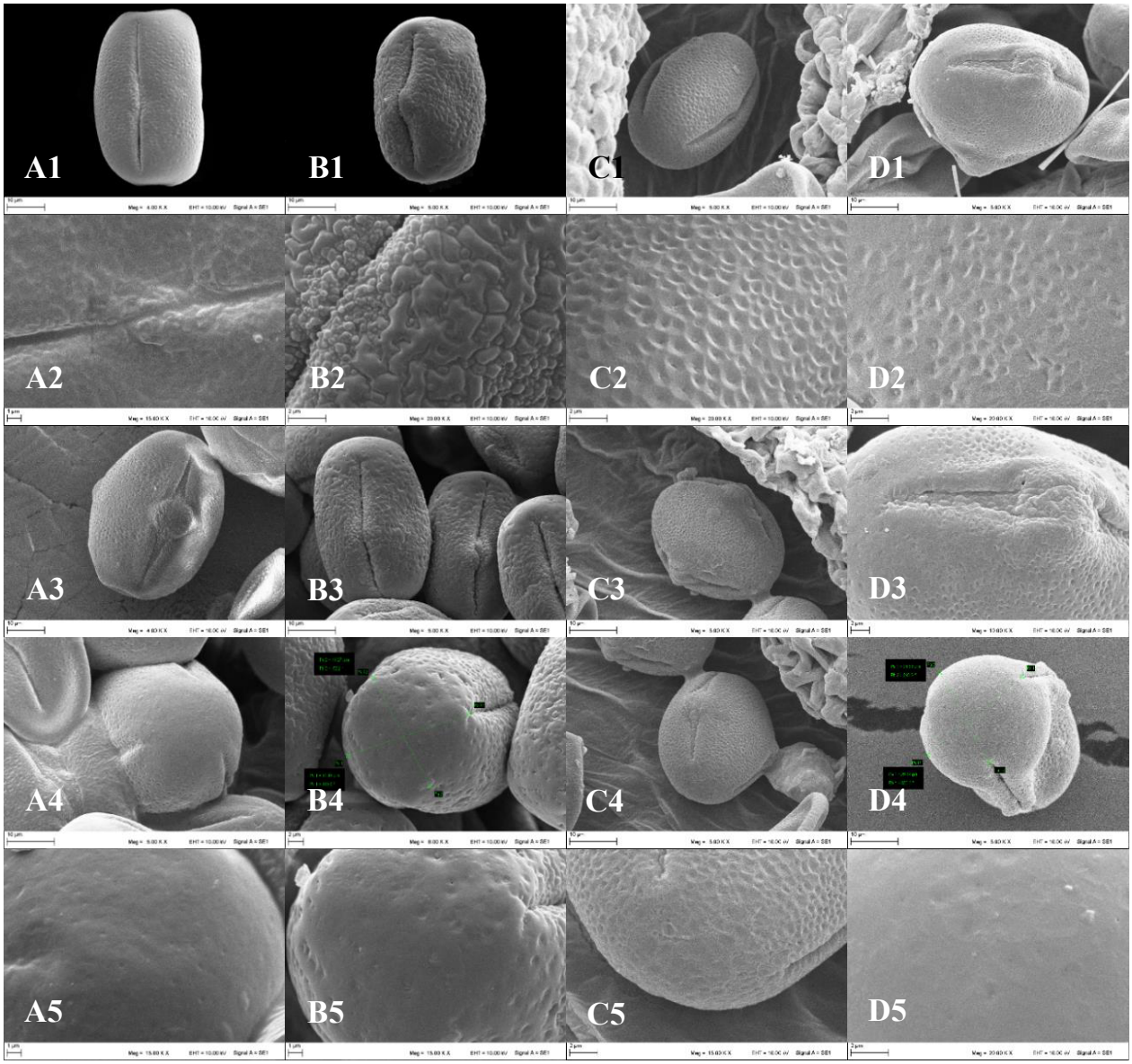

Figure 4. SEM micrographs; equatorial (A1-3, B1-3, C1-3, D1-3) and polar views (A4-5, B4-5, C4-5, D4-5) of pollen grains of *Astragalus nurhakdagensis* from *holo*. *Tülücü* 46 & *Uzun* (A1-5), *A. hirsutus* from *Aytaç* 2655 & *H. Duman* (B1-5), *A. dumanii* from *H. Yıldırım* 3075 (C1-5) and *A. cataonicus* from *M. Ekici* 2038 (D1-5).

Pollen shape: The shape of pollen grains was determined using the P/E ratios according to Punt et al. (2007). Two different pollen shapes were observed according to the pollen shape classes. Prolate grains (1.33–2.00) were observed in *A. nurhakdagensis* (1.40) *A. hirsutus* (1.45) and *A. cataonicus* (1.62), while subprolate grains (1.14–1.33) were observed in *A. dumanii* (1.27).

Aperture: The pollen grains of the studied taxa show a tricolporate type of aperture. Usually, three pores and three colpi are present that are regularly spaced around either the edge or the equator of the pollen grains, which are radially symmetrical and isopolar. The range of colpi length is 24.05–36.70 µm. The smallest colpus length was observed in *A. hirsutus* while the largest colpus length was observed in *A. nurhakdagensis*. The range of colpus width of the taxa is 4.41–5.00 µm. The largest colpus width was observed in *A. cataonicus* and the narrowest colpus width was found in *A. nurhakdagensis.* The aperture membrane is perforate-granulate in *A. nurhakdagensis,* microreticulate-granulate and perforate-granulate in *A. hirsutus,* reticulate in *A. dumanii* and microreticulate-perforate in *A. cataonicus* (Ceter et al., 2013).

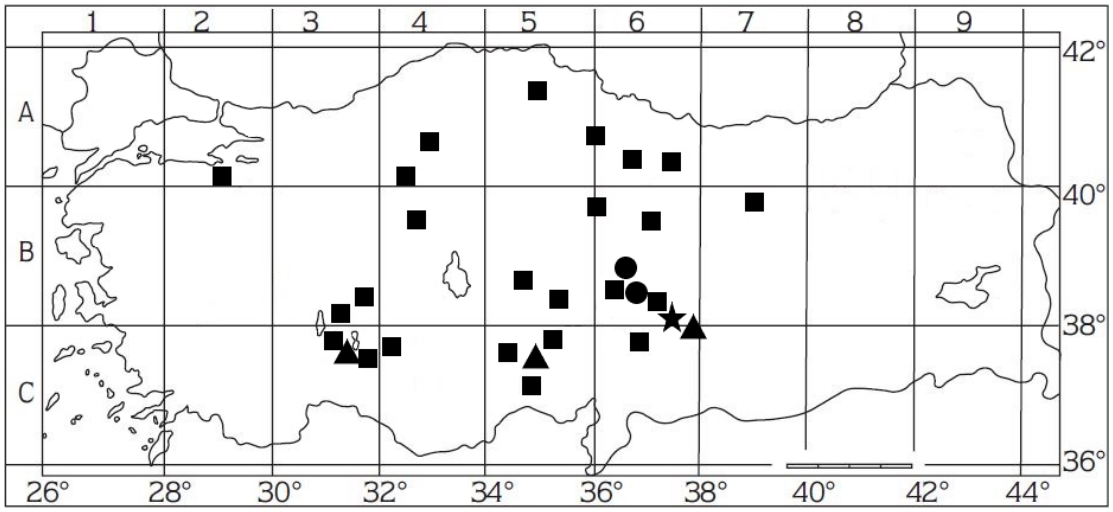

Figure 5. Distribution in Turkey, *Astragalus nurhakdagensis* (★), *A. hirsutus* (■), *A. dumanii* (●) and *A. cataonicus* (▲).

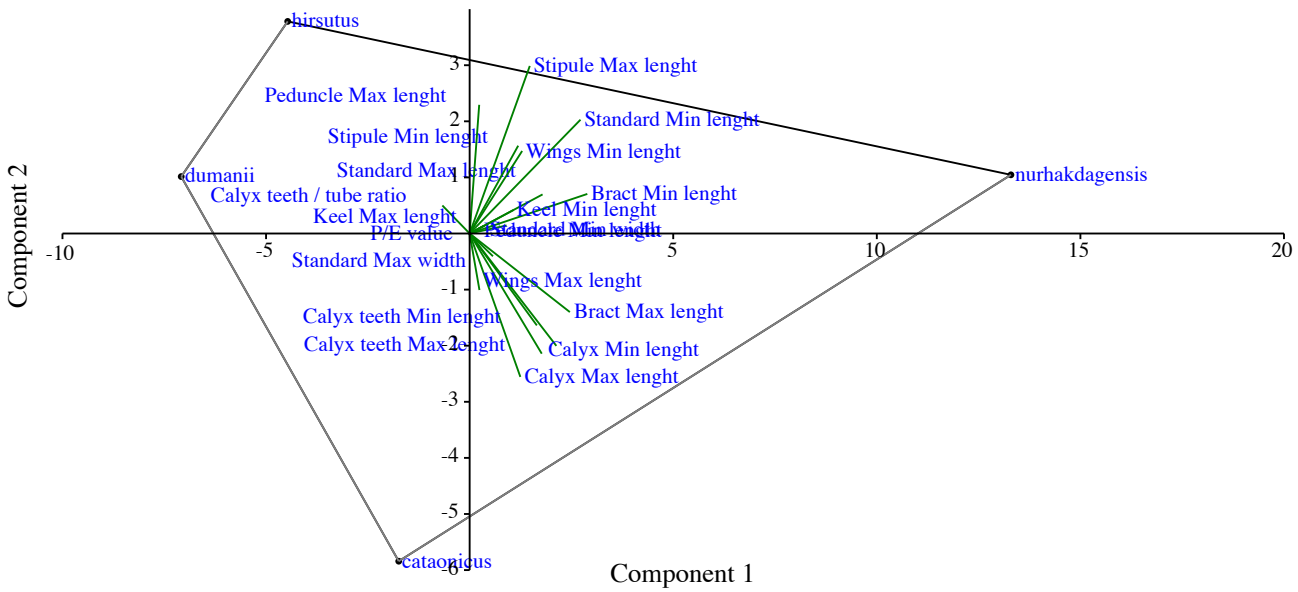

Figure 6. PCA (Principal component analysis) scatter plot according to morphological quantitative characters.

3.3. Etymology

The plant takes its scientific name from the local name 'Nurhak Dağı (= mountain)', which is the collection area. The Turkish name of new species has been suggested as 'Nurhak geveni' according to Menemen et al. (2016).

3.4. Habitat and ecology

Astragalus nurhakdagensis grows on calcareous rocky slopes in steppe vegetation together with some endemic species such as; *Ebenus laguroides* Boiss. var. *laguroides*, *Marrubium globosum* Montbret & Aucher ex Benth. subsp. *globosum*, *Salvia caespitosa* Montbret & Aucher ex Benth., *Bellevalia gracilis* Feinbrun., *Astragalus aduncus* Willd., *Astragalus lineatus* Lam. var. *longidens* (Freyn) Matthews, *Silene montbretiana* Boiss., *Silene marschallii* C.A. Mey. subsp. *marschallii*, *Fritillaria pinardii* Boiss. and *Allium scorodoprasum* L. subsp. *rotundum* (L.) Stearn.

3.5. Distribution and conservation status

It is known only from the type locality, in the transition zone of the Eastern Mediterranean and Eastern Anatolian Regions (Upper Euphrates River basin) and endemic to Turkey (Figure 5). Phyto-geographically, it belongs to the Irano-Turanian element. The entire known population comprises less than 50 individuals and has an area of occupancy (AOO) and extent of occurrence (EOO) smaller than 10 km^2. Consequently, the threat category of this new taxon has been assessed as critically endangered

Table 2. Pollen morphological data of *Astragalus nurhakdagensis, A. hirsutus*, *A. dumanii,* and *A. cataonicus* (mean values in µm).

Characters / Species		***A. nurhakdagensis***	***A. hirsutus***	***A. dumanii***	***A. cataonicus***
Collector ID.		(*holo. Tülücü* 46 & *Uzun*)	(*Aytaç*, 4908)	(*M. Ekici* 2057 & *Aytaç*)	(*M. Ekici* 2038)
Collected Date		17/6/2020	23/6/1992	8/10/2008	23/7/1997
Pollen grains		Mean ± Std.dev.			
Polar axis		45.11 ± 3.41	29.60 ± 1.68	32.93 ± 1.68	32.49 ± 1.79
Equatorial diameter		32.25 ± 2.21	20.40 ± 1.58	25.83 ± 2.10	20.05 ± 1.89
P / E		1.40	1.45	1.27	1.62
Pollen shape		Prolate	Prolate	Subprolate	Prolate
Aperture type		Tricolporate	Tricolporate	Tricolporate	Tricolporate
Ornamentation	Meridional section	Perforate-granulate	Microreticulate-granulate (90%) and perforate-granulate (10%)	Reticulate	Microreticulate-perforate
	Polar section	Perforate	Perforate	Reticulate	Perforate
Colpus (Cl)	Colpus length (Clg)	36.70 ± 4.02	24.05 ± 1.59	25.15 ± 2.15	25.13 ± 2.11
	Colpus width (Clt)	4.41 ± 0.66	4.77 ± 0.55	4.97 ± 0.65	5.0 ± 0.68
Pore (PI)	Pore length (Plg)	10.13 ± 0.96	6.13 ± 0.55	7.12 ± 0.80	7.03 ± 0.85
	Pore width (Plt)	9.44 ± 2.21	7.03 ± 1.23	7.87 ± 0.90	7.92 ± 0.91

[CR 2ab (ii, iii)] according to the International Union for Conservation of Nature ([2]IUCN 2019).

Goat-grazing and marble quarrying are the two main threats to the conservation of this new species. In addition, an increase is not expected in the future projection for the population of this species due to insufficient fertilization and that the fruits that do not hold enough seeds. Therefore, seed banking, micro-propagation, and cultivation in botanical gardens are highly recommended for the ex-situ conservation efforts. Searching for more populations in similar habitats is also needed.

3.6. Statistical evaluation

The results of the analysis shed light on the relationships among the studied species. In addition, the received dendrograms allowed not only to show the similarity within the *Astragalus* species studied but to suggest the most valuable traits that could be used to distinguish species. In the PCA of the morphological characters (Figure 6, Table 3), the first component explains a significant part of the variation between species (75.89%). The next two components explain respectively 15.35% and 8.76% of the variation. Together, these three components explain the entire variation, and the percentage of variation explained by each component is around the expected value. Eigenvalues and % variances of the components of taxa are given in Table 3. According to the one-way ANOVA test, the use of quantitative characters in the delimitation of the species was found to be statistically significant ($p < 0.05$; F 18.19, p 3.5E-18).

The results obtained from the statistical methods were evaluated, and some inferences were made below. According to the UPGMA clustering method, *Astragalus hirsutus* was much closer to *A. dumanii* and *A. cataonicus*, and these three formed a separate cluster. On the other hand, *A. nurhakdagensis* was related to these species but formed a separate cluster on its own (Figure 7). This result reveals that the new taxon is quite distinct from these three. According to PCA analyses, quantitative characters such as minimum and maximum lengths of standard, calyx (also its teeth), stipule, bract, and peduncle are the most explainable features in distinguishing the species from each other.

According to the analyses given here and our evaluations for the section *Hololeuce* (Ekici and Ekim, 2009), *A. nurhakdagensis* can be clearly distinguished from all species in the section, especially with its very long bracts (13–15 mm) and calyx teeth (7–9 mm). Also, the calyx teeth are significantly longer than the tube, while in other species they are either equal or mostly much shorter.

[2] IUCN (2019). International Union for Conservation of Nature [online]. Website http://www.iucnredlist.org/documents/ RedListGuidelines.pdf [accessed 15 Sept. 2020].

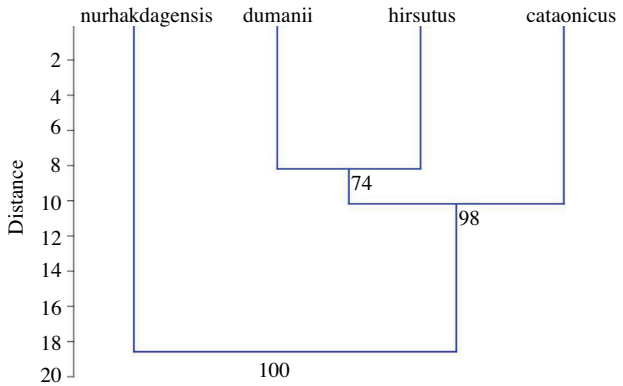

Figure 7. UPGMA clustering method (Euclidean Boot N 1000).

4. Discussion

A. nurhakdagensis, A. hirsutus, A. cataonicus, and *A. dumanii* form a group within the *Hololeuce* section. They are morphologically similar. However, when they are evaluated micro-morphologically, they can be easily distinguished by hairiness, leaflets, stipules, bracts and flower structures. While the peduncle length is up to 6 cm in *A. hirsutus*, it does not exceed 2 cm in *A. cataonicus* and *A. dumanii*, and it varies between

2-4 cm in *A. nurhakdagensis*. It was understood from its synonyms that *A. hirsutus* cannot fully complete its phylogeny taxonomically (Chamberlain 1970; Ekici, 2004). Of these four taxa, the corolla color is yellow only in *A. hirsutus*, while it is white or lilac or purple in the others.

At first glance, *A. nurhakdagensis* superficially looks like *A. dumanii* with its white corolla and *A. hirsutus* with its stance in the field, but when examined in detail, *A. nurhakdagensis* is well distinguished from all the other species of the section (*Hololeuce*) by the combination of a longer standard (22–23 mm), calyx length (13–14 mm), calyx teeth (7–9 mm long) and calyx teeth-tube ratio (teeth noticeably longer than tube), stipules (9–13 mm) and bracts (13–15 mm), also with black hairy stripes below the stem nodes, white having corolla color and spreading hairs on the calyx (Table 4).

The new species forks perfectly from the *A. hirsutus* group according to the diagnostic key of the *Hololeuce* sect. (Ekici and Ekim, 2004). In the *Flora of Turkey*, *A. hirsutus* was placed in the section *Chlorosphaerus*, but, later, it was evaluated in the *Hololeuce* section by Chamberlain because of its stipule structure (Chamberlain, 1970). These two sections are distinguished from each other with their stipule structure. While the stipule is adnate to the petiole in *Chlorosphaerus*, it is free from the petiole in *Hololeuce*.

Revised diagnostic key:

1- Corolla yellow ..... *A. hirsutus*

1- Corolla white, lilac to purple

Table 3. PCA eigenvalues according to morphological quantitative characters.

PC	Eigenvalue	% variance
1	83.3048	75.8900
2	16.8498	15.3500
3	9.6164	8.7604

2- Standard 22-23 mm long; bracts 13-15 mm long .... *A. nurhakdagensis*

2- Standard 12-18 mm long; bracts 4-8 (-12) mm long

3- Calyx teeth as long as tube, 5-7 mm; leaflets obovate to elliptic ..... *A. cataonicus*

3- Calyx teeth distinctly shorter than tube, 2-4 mm; leaflets linear to linear-oblong ..... *A. dumanii*

The seed shape, color, size, surface sculpture, and hilum position are taxonomically informative in *Astragalus* and are often of remarkable importance in species delimitation (Vural et al., 2008; Shemetova et al., 2018). According to the specimens: the seed shape of *A. nurhakdagensis* and *A. dumanii* were reniform–globose, whereas *A. hirsutus* and *A. cataonicus* were reniform (Figure 8, Table 5). Seed surface ornamentation differs slightly among these taxa investigated: rugulate in *A. nurhakdagensis,* wavy rugulate in *A. hirsutus,* reticulate-rugulate in *A. dumanii,* and reticulate-striate in *A. cataonicus.*

5. Conclusion

According to morphological observations, palynological and statistical analyses, seed statistics, indumentum characteristics, the new species is a characteristic member of sect. *Hololeuce*. The new species has a close relationship with *A. dumanii, A. hirsutus* and *A. cataonicus* according to UPGMA cluster analysis. It has many similarities among the group. On the other hand, dimensions of flower parts, color of corolla, and also stem indumentum can be useful to differ these four species from each other at first glance. At the same time, the new taxon can also be distinguished in terms of pollen and seed characteristics, as well as having slightly different sculpture patterns of the seeds than the others. Quantitative features allow a clear delimitation in the separation of *Astragalus* species studied, and other qualitative characters also support this distinction. Minimum bract and standard length emerged as the two most important characters in differentiating new species from the others according to PCA.

With the new current species from Turkey, the species number of the genus is raised to 480 (endemism 67%) according to Aytaç et al. (2020) and the number of taxa belonging to sect. *Hololeuce* Bunge has been raised to 16

Table 4. Diagnostic morphological characters of *A. nurhakdagensis, A. hirsutus, A. dumanii,* and *A. cataonicus.*

Morphological characters	***A. nurhakdagensis***	***A. hirsutus***	***A. dumanii***	***A. cataonicus***
Collector ID.	(*holo. Tülücü* 46 & *Uzun* and *para. Tülücü* 98 & *Uzun*)	(*Aytaç* 2655 & *H. Duman*)	(*M. Ekici* 2057 & *Aytaç*)	(*M. Ekici* 2038)
Stem indumentum	white hairy and with black hairy stripes below the nodes	white hairy	white hairy	densely covered with white hairs
Leaflets	7–9 pairs	3–10 pairs	3–7 pairs	4–8 pairs
Stipules	9–13 mm, very shortly adnate to the petiole (2 mm)	7–11 mm, adnate to the petiole for 4–6 mm	5–8 mm, free from the petiole	5–7 mm, free from the petiole
Stipule indumentum	spreading black and/or white hairy	adpressed white hairy	spreading white hairy	adpressed white, sometimes also with black hairs near the base
Petal color	white-cream	yellow	white, lilac to purple	lilac to purple
Raceme	20–30 flowered	(7–)10–20 flowered	10–25 flowered	20–40 flowered
Peduncle	2–4 cm, white and black hairy	1–6 cm, white and black hairy	0.5–1.5 cm, densely white hairy	0.5–2 cm, densely white-hairy
Bracts	13–15 mm long, spreading long dense white and sparsely black hairs	6–10 mm, long spreading long white hairs or sometimes with white and black hairs	4–6 mm long, appressed white or predominantly white hairs	6–12 mm long, densely adpressed white hairy, rarely with predominantly black hairs
Calyx	13–14 mm long	6–10 mm long	7–10 mm long	10–14 mm long
Calyx teeth/tube	7–9 mm long and longer than tube	2–3 mm long and shorter than tube	2–4 mm long and shorter than tube	5–7 mm long and as long as tube
Calyx indumentum	covered with mostly, dense and long (2–3 mm) spreading sub-bifurcate white hairs, rarely with also shorter (up to 1.5 mm) sparse black hairs at the bottom of the calyx tube, and with few short (up to 1 mm) white and black sub-bifurcate hairs	densely covered with spreading strongly asymmetrically bifurcate white hairs 2–3 mm and with short black and white hairs	covered with adpressed black and white hairs	densely villous with long spreading, sub-bifurcate and short, adpressed white hairs, sometimes with also some adpressed black hairs
Standard	22–23 × 8 mm	14–18 × 6–8 mm	15–18 × 5–7 mm	13–18 × 6–8 mm

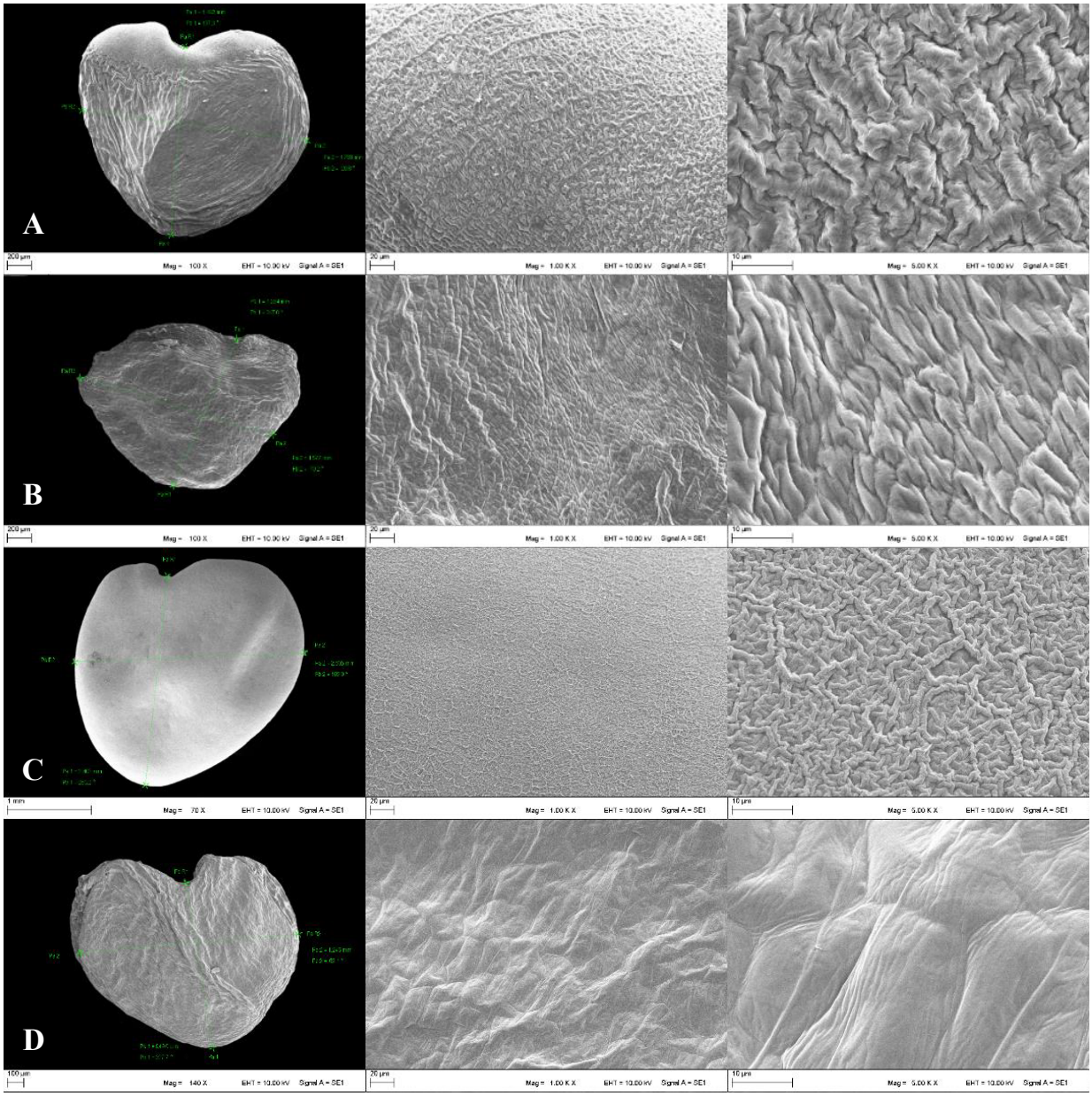

Figure 8. SEM micrographs of *Astragalus nurhakdagensis* from *para. Tülücü* 98 & *Uzun* (A), *A. hirsutus* from *Aytaç* 2655 & *H. Duman* (B), *A. dumanii* from *H. Yıldırım* 3075 (C) and *A. cataonicus* from *M. Ekici* 2038 (D). Panels; lateral view of entire seed (left), seed sculptures at magnification 1.00 KX (middle), seed sculptures at magnification 5.00 KX (right).

(endemism 69%) according to Ekici and Ekim (2004). With the addition of this new species, eleven taxa (ten species) are now endemic for sect. Hololeuce in Turkey.

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Table 5. Seed morphological data of *Astragalus nurhakdagensis, A. hirsutus*, *A. dumanii,* and *A. cataonicus.*

Characters / Species		***A. nurhakdagensis***	***A. hirsutus***	***A. dumanii***	***A. cataonicus***
Collector ID.		(*para. Tülücü* 98 & *Uzun*)	(*Aytaç* 2655 & *H. Duman*)	(*A. Duran* 7681)	(*M. Ekici* 2038)
Seed	Shape	reniform–globose	reniform	reniform–globose	reniform
	Length (mm)	1.3–1.5	1.0–1.3	2.2–2.5	0.9–1.2
	Width (mm)	1.6–1.8	1.5–1.7	2.3–2.6	1.2–1.5
	Surface ornamentation	rugulate	wavy rugulate	reticulate-rugulate	reticulate-striate
	Color	dark greenish brown	dark brown	light brown	light greenish brown

specimens used in the present study. Special thanks to Dr. Funda Özbek for the seed surface evaluations and to Işık Kavalcı for permission to use photographs of the terrain for *A. cataonicus*.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix

Additional specimens examined:

Astragalus hirsutus: Turkey, [**B2**] Bursa; Ulu Dağ., rocky slopes, c. 2300 m, 7.1978, Polunin 15006 (E, photo!). [**B5**] Kayseri; Gemerek, Çat village, steppe, c. 1540 m, 8.6.1980, T. Ekim 4942 (ANK!) – Alidağ, 1700 m, steppe, 24.6.1997, M. Ekici 1974 & H. Akan (GAZI!) - Nevşehir: 12 miles [S] from Ürgüp in the Akköy direction, 1600 m, 21.5.1965, Coode & Jones 1277 (E). [**B6**] Kahramanmaraş: Göksun, Çardak, Berit Dağı, Arpa Çukuru Yaylası, steppe, 2500-2600 m, 3 June 1989, Aytaç 2655 & H. Duman (GAZI!) – Göksun; Binboğa mt., above Karlı Y. [Yaylak], 2500 m, 15.7.1952, Davis 20032, Dodds & Çetik (K) – Göksun; Kaman mt., 2000 m, 20.6.1981, B. Yıldız 3026 (HUB!) – Kayseri: Pınarbaşı; Tersakan village, Hınzır mt., Üçkuyular, 1850 m, 18.5.1980, N. Çelik 1113 (HUB!) – Pınarbaşı; above Kaynar, 1750 m, 18.5.1980, N. Çelik 1074 (ANK!) – Sarız; Yalak, between Körkuyu and Sıçak, 2400-2600 m, 21.7.1992, stony places, Z. Aytaç 5438 & H. Duman (GAZI!) – above Pınarbaşı, 1600 m, 7.7.1996, M. Ekici 1915 & H. Akan (GAZI!) – ibid., 7.6.1997, M. Ekici 1948 (GAZI!) – Sivas: ad Sivas, 1892, Bornmüller 3330 (B, E, K, W).

Astragalus dumanii: Turkey, [**B6**] Sivas, Gürün-Kangal yolu, Tecer ayırımı, Tecere 37 km kala, Marn-jipsli topraklar, 1565 m, 16.7.2014, H. Yıldırım 3075! – Kayseri; Sarız, Yalak (Yeşilkent), Binboğa Dağı, 2700 m, 7.8.2007, A. Duran 7681! – Kayseri Binboğa mt., between Körkuyu and Sıçak plateau, stony places, 2400-2600 m, 21.7.1992, Z. Aytaç 5434 & H. Duman (GAZI!). [**C6**] Kahramanmaraş: Berit Dağı, 2300-2400 m, 8.9.1997, M. Ekici 2057 & Z. Aytaç (holotype: GAZI!).

Astragalus cataonicus: Turkey: [**C3**] Isparta: distr. Sütçüler, Dedegöl mt. above Dedegöl, 2700-2800 m, 3.8.1949, Davis 16010A (ANK, E photo!, K, P) – ibid., 2400 m, 23.7.1997, M. Ekici 2038 (GAZI!, MSB). [**C5**] Niğde: Alpes Bulgar Dagh, in summon jugus inter Gisyl teppe et Koschan, 2350 m, Kotschy 158 (K) & 169 (P, STU) – Sivas: in summon Karababa dicto montis Ak-dagh, 2700 m, 1.8.1889, Bornmüller 1023 (B), 1023b (W). [**B/C6**] Adıyaman: in cacum. Karalea dicto montis Ak-dagh, 2700 m, 1.8.1889, Bornmüller 1023 (B) – in monte Ak Dagh (=Aryly Tash) inter urbem Malatija et vicum Kjachta, 2600-2670 m, 17.7.1910, Handel-Mazzetti 2354 (B).