Kastamonu Uni., Orman Fakültesi Dergisi, 2025, 25(3): 392-408 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1845409

Palynological Study of Species of *Astragalus* L. sect. *Hymenostegis* Bunge and *Hymenocoleus* Bunge (Fabaceae) in Türkiye

Seher KARAMAN[1], Oktay BIYIKLIOĞLU[2*], Şeyma BOYAR[1], Talip ÇETER[2]

[1]Aksaray University, Faculty of Science and Letters, Department of Biology, Aksaray, TÜRKİYE
[2]Kastamonu University, Faculty of Science, Department of Biology, Kastamonu, TÜRKİYE
*Corresponding Author: oktayman@gmail.com

Received Date: 08.02.2025 Accepted Date: 23.06.2025

Abstract

Aim of study: Astragalus is one of the largest genus of the Fabaceae family. Morphological, molecular and genetic studies as well as palynological studies are being used to solve the taxonomic problems of this genus, which has a wide distribution worldwide.

Material and method: In this study, the pollen morphology of 14 species belonging to the *Hymenostegis* and *Hymenocoleus* sections distributed in Türkiye was examined by light and electron microscopy.

Main results: In all taxa examined, pollen grains were detected as monad, radially symmetrical and apolar type. While the aperture types were determined as tricolporate in all species examined, only 5% trisyncolpate and 95% tricolporate aperture types were found in *A. gueruenensis* and *A. ciloensis.*

Research highlights: Ornamentation types vary between the polar to equatorial region. Eight different ornamentation types have been identified, consisting of different combinations of psilate, perforate, microreticulate and reticulate ornamentation elements. Ornamentation in the equatorial and polar areas, P/E ratio and shape, Aperture type and pore structure are the most valuable variables to distinguish between *Hymenostegis* and *Hymenocoleus* section types.

Keywords: *Astragalus*, Numerical Analysis, Pollen Grains, Türkiye

Türkiye'deki *Astragalus* L. Cinsi *Hymenostegis* Bunge ve *Hymenocoleus* Bunge Seksiyonlarina Ait Türlerin Palinolojik Yönden İncelenmesi

Öz

Çalışmanın amacı: Astragalus cinse, Fabaceae familyasının en büyük üyelerinden biridir. Dünya genelinde geniş bir yayılış olanı olan bu cinsin taksonomik problemlerinin çözülmesi için morfolojik, moleküler ve genetik çalışmaların yanı sıra Palinolojik çalışmalardan destek alınmaktadır.

Materyal ve yöntem: Bu çalışma ile Türkiye'de yayılış gösteren *Hymenostegis* ve *Hymenocoleus* seksiyonlarına ait 14 türün polen morfolojisi ışık ve elektron mikroskobu ile incelenmiştir.

Temel sonuçlar: Polen taneleri monad, radyal simetrik ve izopolardır. Tüm incelenen türlerde apertür trikolporat olarak belirlenirken, *A. gueruenensis* ve *A. ciloensis'*te sadece %5 oranında trisinkolpat ve %95 oranında trikolporat apertür tipleri bulundu.

Araştırma vurguları: Ornamentasyon tipleri kutup bölgesi ve ekvatoral bölge arasında değişmektedir. Psilat, perforat, mikroretikülat ve retikülat süsleme elemanlarının farklı kombinasyonlarından oluşan sekiz farklı süsleme tipi tanımlanmıştır. Ekvator alanı ve kutup alanındaki süsleme, P/E oranı ve şekli, Apertür tipi ve por yapısı *Hymenostegis* ve *Hymenocoleus* kesit türlerini ayırmak için en değerli değişkenlerdir.

Anahtar Kelimeler: *Astragalus*, Sayısal Analiz, Polen, Türkiye

Citation (Atıf): Karaman, S., Biyiklioglu, O., Boyar, S., & Ceter, T. (2025). Palynological Study of Species of *Astragalus* L. sect. *Hymenostegis* Bunge and *Hymenocoleus* Bunge (Fabaceae) in Türkiye. *Kastamonu University Journal of Forestry Faculty*, 25 (3), 392-408.

Introduction

Astragalus L. (Fabaceae), one of the largest genera of flowering plants in terms of number of species, is represented by 3156 Turks and 255 sections in the world (Lock & Simpson, 1991; Khan et al., 2023). In South Asia and Türkiye, 490 taxa belonging to *Astragalus* are distributed (Aytaç & Ekici, 2012; Özhatay et al., 2013; Özhatay et al., 2017; Özhatay et al., 2019).

Astragalus section *Hymenostegis* was first named by Bunge (1868–1869) in his revision under the subgenus *Calyphysa*. The origin and diversification center of this section is Iran. This section includes about 80 species and is distributed in Iran. However, some species are distributed in Türkiye, Armenia, Georgia, and Iraq. The members of sect. *Hymenostegis* Bunge is characterized by many-flowered racemes, large papery bracts, and tubular-inflated calyces. The section is represented by 13 species, including *Astragalus ciloensis* Podlech, *A. velenovskyi* Nabelek, *A. gueruenensis* Podlech, and *A. narmanensis* Karaman & Aytaç, which are endemic to Türkiye (Podlech & Zarre, 2013; Karaman Erkul et al., 2016; Bagheri et al., 2019).

Section *Hymenocoleus* shared *Astragalus vaginans* DC., which is endemic to Türkiye. This species was separated from the section *Hymenostegis* by imparipinnate (not paripinnate) leaves and forming wide cushions (Chamberlain & Matthews, 1970). Section *Hymenostegis* was subdivided by Zarre & Podlech (1996) into two subsections: subsect. *Hymenocoleus* (Bunge) Podlech & Zarre includes only the Turkish endemic *A. vaginans* and subsect. *Hymenostegis* includes 13 species. Karaman Erkul et al. (2016) kept *Hymenocoleus* at the sectional level.

Recently, pollen morphology has been one of the primary sources of data used in systematic studies for the separation of closely related species. In this context, pollen morphology of *Onobrychis* (Avcı et al. 2013), *Oxytropis* (Çeter et al., 2013a), *Genista* (İşgör et al., 2012), *Trigonella* (Pınar et al., 2013), *Trifolium* (Altın et al., 2024b), *Lathyrus* (Kahraman et al., 2012), and *Vicia* (Binzat et al., 2014) taxa distributed in Türkiye have been examined in detail, and their contribution to the systematics of taxa has been revealed.

Although *Astragalus* pollen morphology is generally similar, many studies have reported that many characters, such as aperture structure, pollen size, and exine ornamentation, are reliable taxonomically. Khan et al. (2022a) used UPGM and PCA analyses using pollen morphology characters in the taxonomic distinction of *Astragalus* species. Bilgi et al. (2024), in their study on nine different *Astragalus* sections, reported that pollen morphology provided reliable characters in distinguishing taxa at the species level but was insufficient in distinguishing sections.

Many studies have been carried out to reveal the pollen morphology of the genus *Astragalus*. However, more studies are recommended to understand the differences and similarities between taxa (Perveen & Qaisar, 1998; Simons & Chinnappa, 2004; Akan et al., 2005; Ekici et al., 2005; Dane et al., 2007; Oskouian et al., 2007; Pınar et al., 2009a; Çeter et al., 2013b; Al-Ghamadi et al., 2013; Bagheri et al., 2017; Metin et al., 2018; Atasagun et al., 2018; Bagheri et al., 2019; Tunçkol et al., 2020; Özbek et al., 2021; Khan et al., 2022a; Khan et al., 2022b; Khan et al., 2023; Altın et al., 2024a; Bilgi et al., 2024). Hayrapetyan & Elbakyan (2017) studied eight *Astragalus* species including, *A. lagopoides, A. sosnowskyi*, and *A. uroniolimneus* from Armenia. Bagheri et al. (2019) studied twenty-two taxa of section *Hymenostegis* from Iran whose four taxa (*A. hirticalyx* Boiss. & Kotschy, *A. rubrostriatus* Bunge, *A. uroniolimneus* Boiss. and *A. zohrabi* Bunge) spread in Türkiye as well.

In this research, the pollen morphology of fourteen species of *Astragalus* section *Hymenostegis* and *Hymenocoleus* was observed under LM (light microscope) and SEM (scanning electron microscope), and the pollen morphology of eight species was reported for the first time. The aim of this study is to determine the contributions of pollen morphological characters in the systematic differentiation of species and sections of *Astragalus* taxa distributed in Türkiye. Thus, it reveals the importance of palynological features in *Astragalus* taxonomy.

Material and Methods

Plant Material

The materials were used in the present study were collected from their habitat during the flowering period. All collected samples were identified using relevant literature (Rzazade, 1954; Chamberlain & Matthews, 1970; Townsend, 1974; Yakovlev et al., 1996; Zarre & Podlech, 1996; Maassoumi, 1998; Aytaç, 2000; Maassoumi, 2003; Podlech & Zarre, 2013). The plant specimens were deposited at Gazi University Herbarium (GAZI) and Aksaray University Herbarium (AKSU). Collectors and habits are shown in Table 1, and Figure 1 and the localities of the taxa are given in Figure 2.

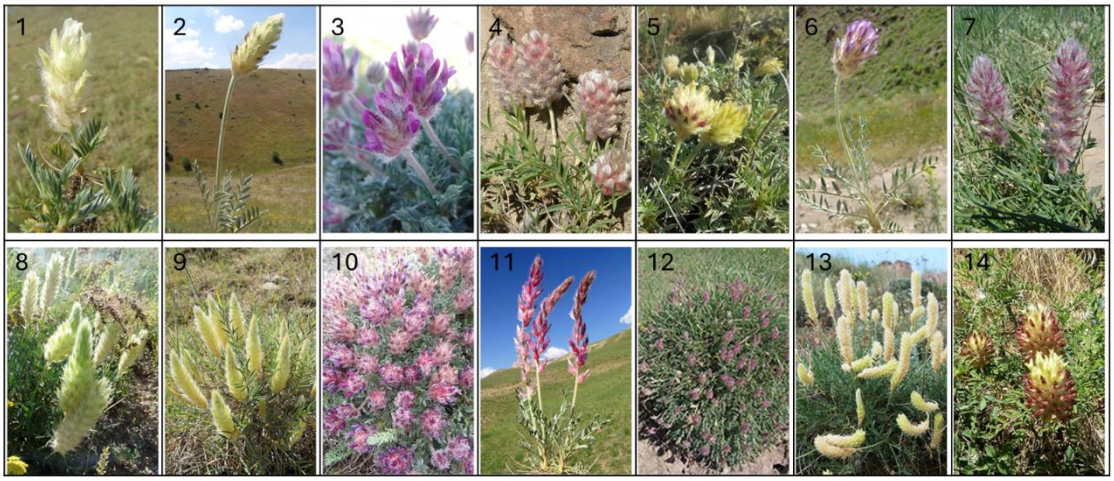

Figure 1. Habits of the taxa of section *Hymenostegis* and *Hymenocoleus* from Türkiye

1: *A. hymenocystis*; 2: *A. lagopoides*; 3: *A. hirticalyx*; 4*: A. trifoliastrum*; 5: *A. gueruenensis*; 6: *A. ciloensis*; 7*: A. zohrabi*; 8: *A. sosno*wskyi; 9: *A. velenovskyi*; 10: *A.uraniolimneus*; 11: *A. rubrostriatus*; 12: *A. qoturensis;* 13: *A. narmanicus;* 14: *A. vaginans*.

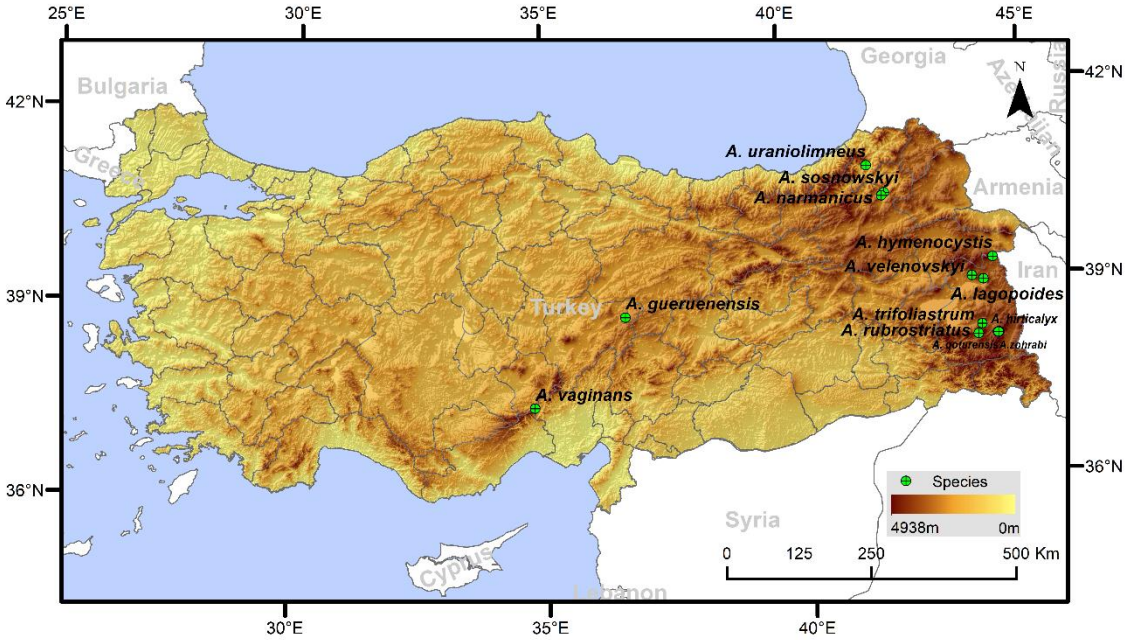

Figure 2. Distribution map of *Astragalus* species used in the pollen analysis

Table 1. Investigated *Astragalus* species of sect. *Hymenostegis* and *Hymenocoleus* from Türkiye

Section	Taxa	Localities and Voucher
Hymenostegis Bunge	*A. hymenocystis* Fisch. & C.A.Mey.	Van: Tendürek pass, volcanic rocky slopes, 2644 m, 12.07.2011, S. Karaman 2635
	A lagopoides Lam.	Van: Muradiye, vicinity of Şeytan bridge, steppe, 1730 m, 18.06.2011, S. Karaman 2576.
	A. hirticalyx Boiss. & Kotschy	Van: Hoşap-Başkale road, Güzeldere pass, steppe, 2616 m, 21.06.2011, S. Karaman, F. Taeb & İ. Kaval 2599.
	A. zohrabi Bunge	Van: Güzeldere pass, steppe, 2650 m, 22.06.2013, S. Karaman 2751
	A. sosnowskyi Grossh.	Erzurum: Narman− Oltu road, 5 km from Narman, roadside, 1327 m, 16.06.2011, S. Karaman 2569
	A. velenovskyi Nabelek	Van: Erciş, between Deliçay and Pay villages, steppe, 2000 m, 23.06.2013, S. Karaman 2759.
	A. trifoliastrum Hub.-Mor. & Matthews	Van: Gürpınar, between Hamurkesen−Işıkpınar villages, steppe, 2155 m, 13.07.2011, S. Karaman 2650
	A. uraniolimneus Boiss.	Artvin: Yusufeli, Ziyaret mountain, Narlık plateau, alpine meadows, 2630 m, 23.07.2013, S. Karaman 2797
	A. gueruenensis Podlech	Sivas: 46. km from Gürün to Pınarbaşı, roadsides and steppe, 1700 m, 16.07.2013, S. Karaman 2777
	A. ciloensis Podlech*	Van: Gürpınar, Güzeldere pass, steppe, 2760 m, 13.07.2011, S. Karaman 2652
	A. qoturensis Podlech	Van: between Başkale-Hoşap, Güzeldere pass, steppe, 2760 m, 13.07.2011, S. Karaman 2653
	A. rubrostriatus Bunge	Van: Gürpınar, between Kırkgeçit- Taşınocak, steppe, 2207 m, 22.06.2011, S. Karaman 2603
	A.narmanicus Karaman&Aytaç	Erzurum: Between Narman and Oltu, steppe, 1916 m, 08.07.2014, S. Karaman 2897 & Z. Aytaç
Hymenocoleus Bunge	*A. vaginans* DC. *	Niğde: Ulukışla, Çiftehan, Horoz village, near the gardens, 1200-1350 m, 28.06.2013, S. Karaman 2776

*Endemic species

Pollen Analysis

Pollen slides were prepared according to Wodehouse (1935) technique for light microscopy study (LM). The pollen grains from dried flowers were poured on a clean slide with a clean dissecting needle, and 1-2 drops of ethyl alcohol were dropped on it. Next, it was mixed by heating until it evaporated. Then, one drop of saffron-containing glycerin-gelatin was added to the pollen, mixed with a pin, and covered with a coverslip. The acquired preparation was inverted and left for 1 day. The preparations were examined with 10x ocular, 100x lens, and immersion oil using Leica DM3000 digital photomicrograph system (Germany), and their photos were taken. The scored features including polar axis (P), equatorial diameter (E), intine thickness, exine thickness, colpus length (Clg), colpus width (Clt), por length (Plg), and por width (Plt). There are 30 pollen grains were measured for each species. Minimum, maximum, average values and standard deviations were determined (Pınar et al., 2009b; Kendir et al., 2015). For the study with Scanning Electron Microscope (SEM), the preparations from dry flower samples were placed on aluminium stabs with double-sided tape and subjected to 150 mA current in vacuum for 5 minutes in the coating device. Their photos were taken with Geol JSM 6490LV electron microscope at the Turkish Petroleum Corporation Research Center SEM Laboratory. During the SEM photography, at least one image in which the pollen's identification was visible and at least one image in which the ornamentation was evident was taken for each pollen. Terminology was evaluated according Faegri & Iversen (1975), Punt et al. (2007) and Halbriter et al. (2018), and shape

classification follows the study by Erdtman (1969) based on P / E ratio.

Numerical Analysis

The pollen morphology of 14 taxa belonging to the genus *Astragalus* which found in Türkiye are examined in the present study. Fifteen characteristics (P, E, P/E, Clg, Clt, Plg, Plt, exine thickness, intine thickness, polar area ornamation, equatorial area ornamation, aperture type, apaocolpium, mesocolpium, and pollen shape) of pollen morphology were determined, and a data set was prepared with these characteristics. Both continuous and discrete data have been standardized for use in cluster analysis. Standardized data were used in the phenogram and UPGMA analysis with NTSYS-pc software (Numerical Taxonomy and Multivariate Analysis System, Version 2.1). In addition, three dimensional projections in space phenogram were created using NTSYS-pc version 2.02 (Applied Biostatistic, Exeter Software, Setauket, New York, USA) and Minitab softwares.

Results

The pollen morphology of 14 species belongs to sect. Hymenostegis and Hymenocoleus were studied using light (LM) and scanning electron microscopy (SEM). The measured and described pollen features are given in Table 2, and the pollen microphotographs taken with LM and SEM are given in Figures 3, 4, 5, and 6.

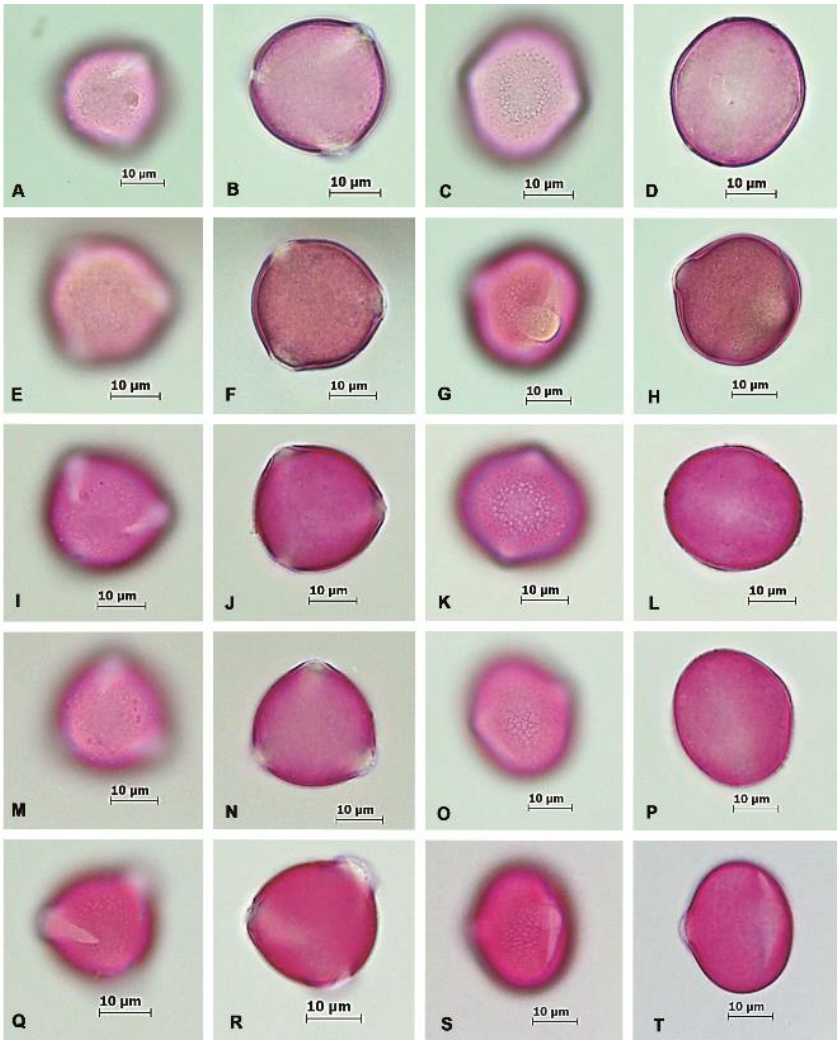

Figure 3. Light microscope pollen images of *Astragalus*

A-D: *A. hymenocystis*; E-H: *A. lagopoides*; I-L: *A. hirticalyx;* M-P: *A. zohrabi*; Q-T: *A. sosnowskyi*. (First column: ornamentation detail in polar view; Second column: optical cross-section in polar view; Third column: ornamentation/aperture detail in equatorial view; Fourth column: optical cross-section in equatorial view)

Table 2. Pollen morphological features of sect. *Hymenocystis* and *Hymenocoleus* (Measurement in µm)

	Section	Polar axis (P)			Equatorial axis (E)			P / E ratio and shape	Aperture type	Exine ornamentation		Colpus(Cl)		Pore (Pl)		Exine thickness	Intine thickness	Apocolpium	Mesocolpium
		Min.	Mean	Max.	Min.	Mean.	Max.			Polar area, aperture surround	Equatorial area	Clg	Clt	Plg	Plt				
1	*A. hymenocystis*	29.0	30.2	31.8	26.1	27.0	28.7	1.12 / Spheroidal	Tricolporate	Microreticulate	Reticulate	27.9±3.5	4.2±0.9	6.5±1.1	7.1±1.5	1.4±0.3	0.52±0.2	11.8±1.0	20.3±1.0
2	*A. lagopoides*	28.5	29.5	30.5	28.4	29.0	29.7	1.02 / Spheroidal	Tricolporate	Psilate-perforate	Microreticulate	25.1±2.3	4.0±0.4	7.2±0.5	8.7±0.9	1.2±0.2	0.55±0.2	11.0±1.1	20.2±0.5
3	*A. hirticalyx*	26.2	28.6	30.7	24.4	26.1	27.4	1.1 / Spheroidal	Tricolporate	Microreticulate	Reticulate	24.7±2.2	3.7±0.5	6.0±0.7	6.8±1.2	1.2±0.2	0.55±0.2	10.9±1.2	20.0±1.2
4	*A. zohrabi*	29.8	30.9	31.6	25.4	27.2	28.7	1.14 / Subprolate	Tricolporate	Psilate-perforate	Reticulate	25.1±1.9	4.2±1.2	7.5±1.6	8.0±1.5	1.4±0.1	0.58±0.1	12.4±1.2	20.2±1.0
5	*A. sosnowskyi*	25.4	28.1	29.8	22.8	24.4	25.7	1.15 / Subprolate	Tricolporate	Psilate	Microreticulate	23.5±2.5	3.9±0.2	7.3±1.5	8.0±1.5	1.3±0.1	0.51±0.2	7.8±0.8	17.2±1.0
6	*A. velenovskyi*	27	29.33	30.83	23.5	24.4	25.5	1.2 / Subprolate	Tricolporate	Psilate-perforate	Reticulate	21.9±2.1	3.96±0.8	6.6±0.8	9.1±0.9	1.53±0.1	0.4±0.2	11.5±0.7	18.8±0.7
7	*A.uraniolimneus*	23.1	24.7	26.55	22.1	23.7	25.1	1.04 / Spheroidal	Tricolporate	Perforate	Microreticulate	23.6±2.7	3.6±0.7	5.8±0.9	7.3±2.3	1.1±0.2	0.5±0.1	8.7±0.9	18.4±0.7
5	*A.trifoliastrum*	24.1	27.4	30.2	18.9	23.4	27.0	1.18 / Subprolate	Tricolporate	Perforate	Microreticulate	21.1±3.9	2.7±1.1	6.2±1.9	6.7±2.1	1.1±0.1	0.6±0.05	11.4±1.3	21.3±1.4
9	*A. gueruenensis*	28	30.1	31.83	24.33	27	30	1.12 Spheroidal	95% Trizonocolporate 5% Trisyncolporate	Psilate-perforate	Perforate	26.5±2.4	4.1±0.8	7.7±0.6	9.3±1.7	1.12±0.1	0.55±0.1	11.9±1.1	22.8±1.0
10	*A. ciloensis*	29.3	31.0	32.8	26.4	28.4	29.7	1.09 / Spheroidal	95% Trizonocolporate 5% Trisyncolporate	Psilate-perforate	Perforate	24.9±2.9	4.9±0.7	6.2±1.9	8.3±0.3	1.2±0.2	0.5±0.1	11.4±1.3	22.0±1.2
11	*A. rubrostriatus*	29.0	30.4	32.0	24.9	26.4	27.2	1.15 / Subprolate	Tricolporate	Psilate	Reticulate	23.9±2.2	3.9±0.6	6.5±1.3	7.5±1.3	1.4±0.1	0.7±0.2	11.7±0.8	20.8±1.4
12	*A.qoturensis*	25.6	28.7	31.1	23.9	27.7	29.7	1.04 / Spheroidal	Tricolporate	Psilate-perforate	Microreticulate	27.5±0.3	4.9±0.3	9.2±1.1	9.9±2.2	1.3±0.2	0.5±0.1	12.3±0.8	21.9±1.3
13	*A. narmanicus*	29.2	31.2	32.8	25.1	29.2	30.9	1.07 / Spheroidal	Tricolporate	Perforate	Reticulate	27.1±0.6	4.6±0.2	8.1±0.8	9.8±1.1	1.1±0.3	0.5±0.1	11.1±0.7	21.3±1.0
14	*A. vaginans*	28.7	29.7	31.0	24.9	25.4	25.7	1.17 / Subprolate	Tricolporate	Psilate-perforate	Microreticulate	24.6±2.1	3.8±0.9	7.3±0.7	7.1±1.5	1.2±0.1	0.5±0.2	8.1±0.7	17.9±1.1

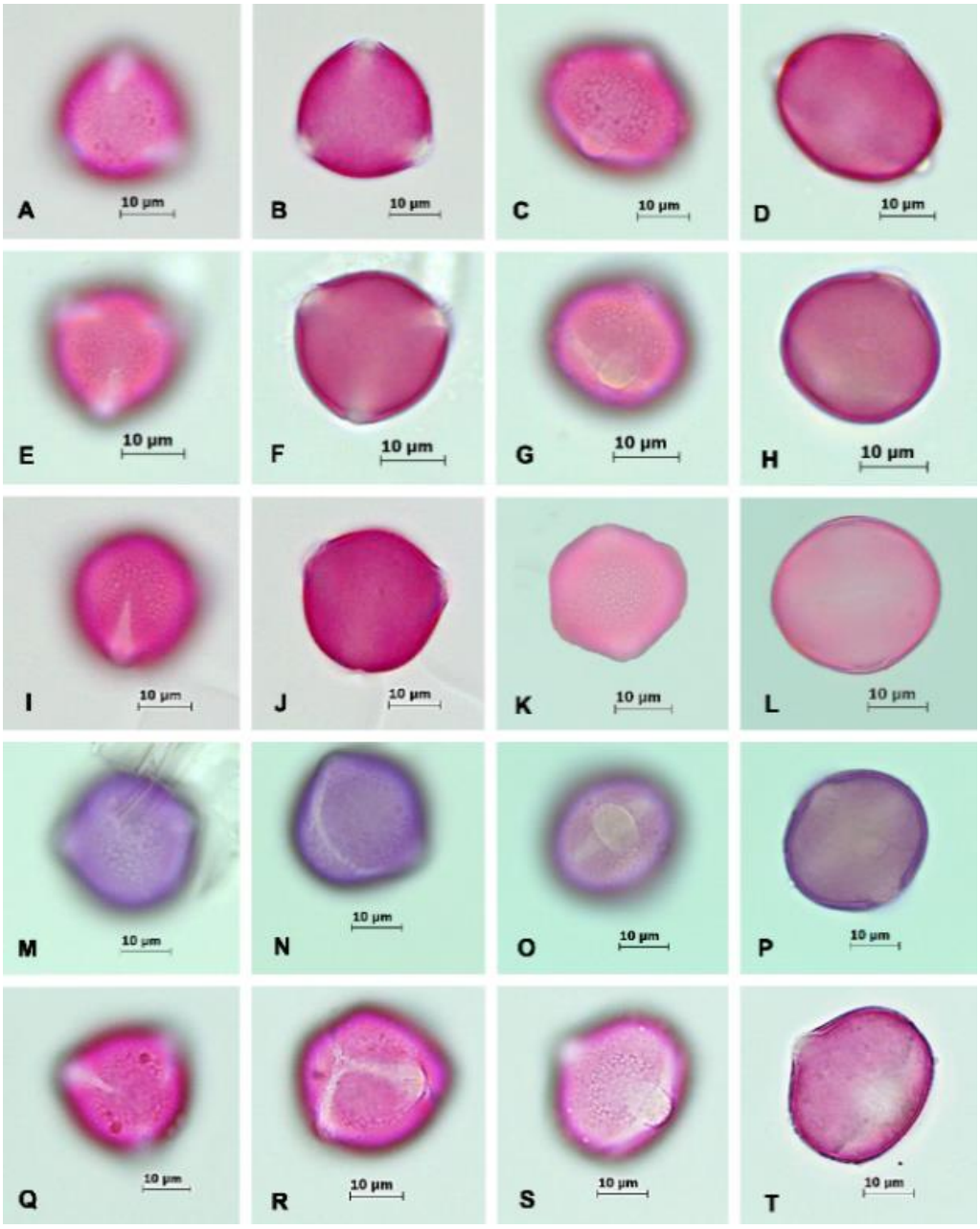

Figure 4. Light microscope pollen images of *Astragalus*

A-D: *A. velenovskyi*; E-H: *A.uraniolimneus*; I-L: *A.trifoliastrum*; M-P: *A. gueruenensis*; Q-T: *A. ciloensis*. (First column: ornamentation detail in polar view; Second column: optical cross-section in polar view; Third column: ornamentation/aperture detail in equatorial view; Fourth column: optical cross-section in equatorial view)

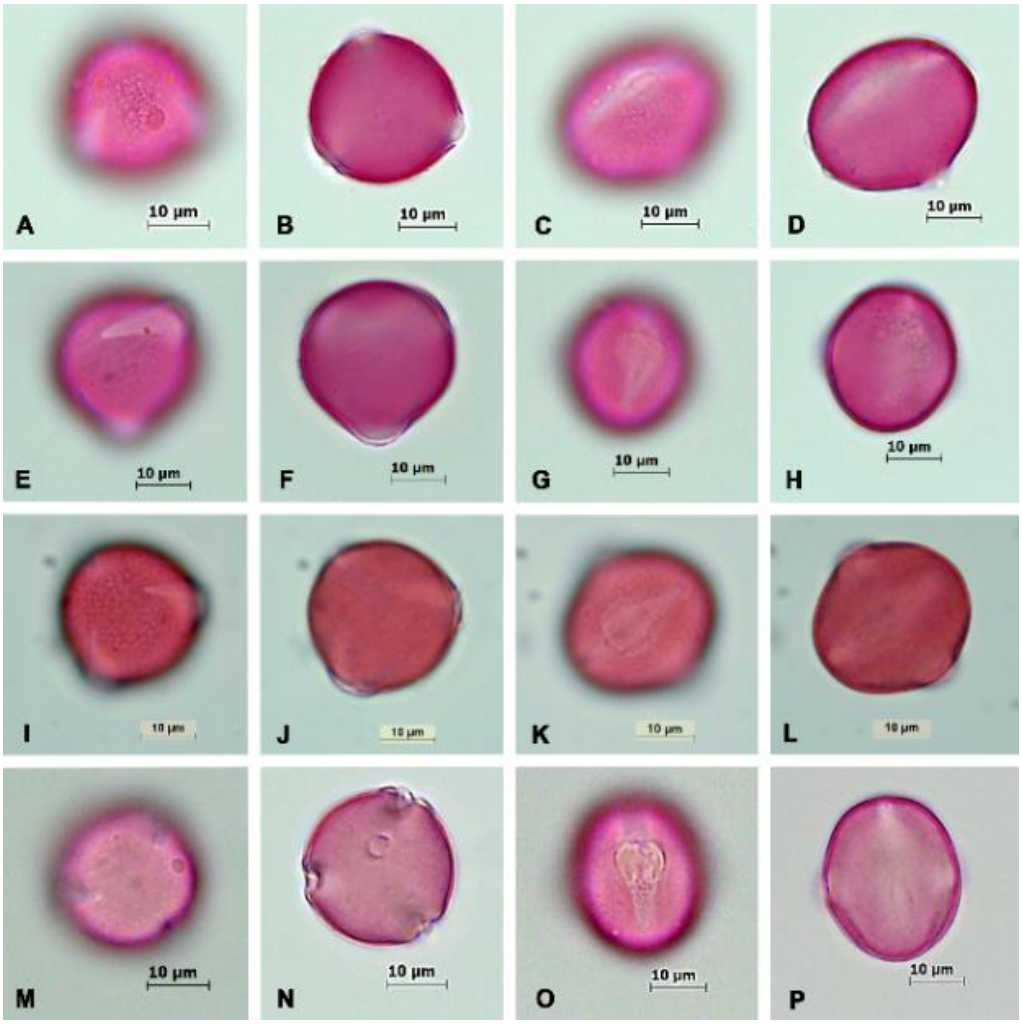

Figure 5. Light microscope pollen image of *Astragalus*

A-D; *A. rubrostriatus*. E-H; *A. qoturensis*. I-L; *A. narmanicus*. M-P; *A. vaginans*. (First column; ornamentation detail in polar view. Second column; optical cross-section in polar view. Third column; ornamentation/aperture detail in equatorial view. Fourth column; optical cross-section in equatorial view)

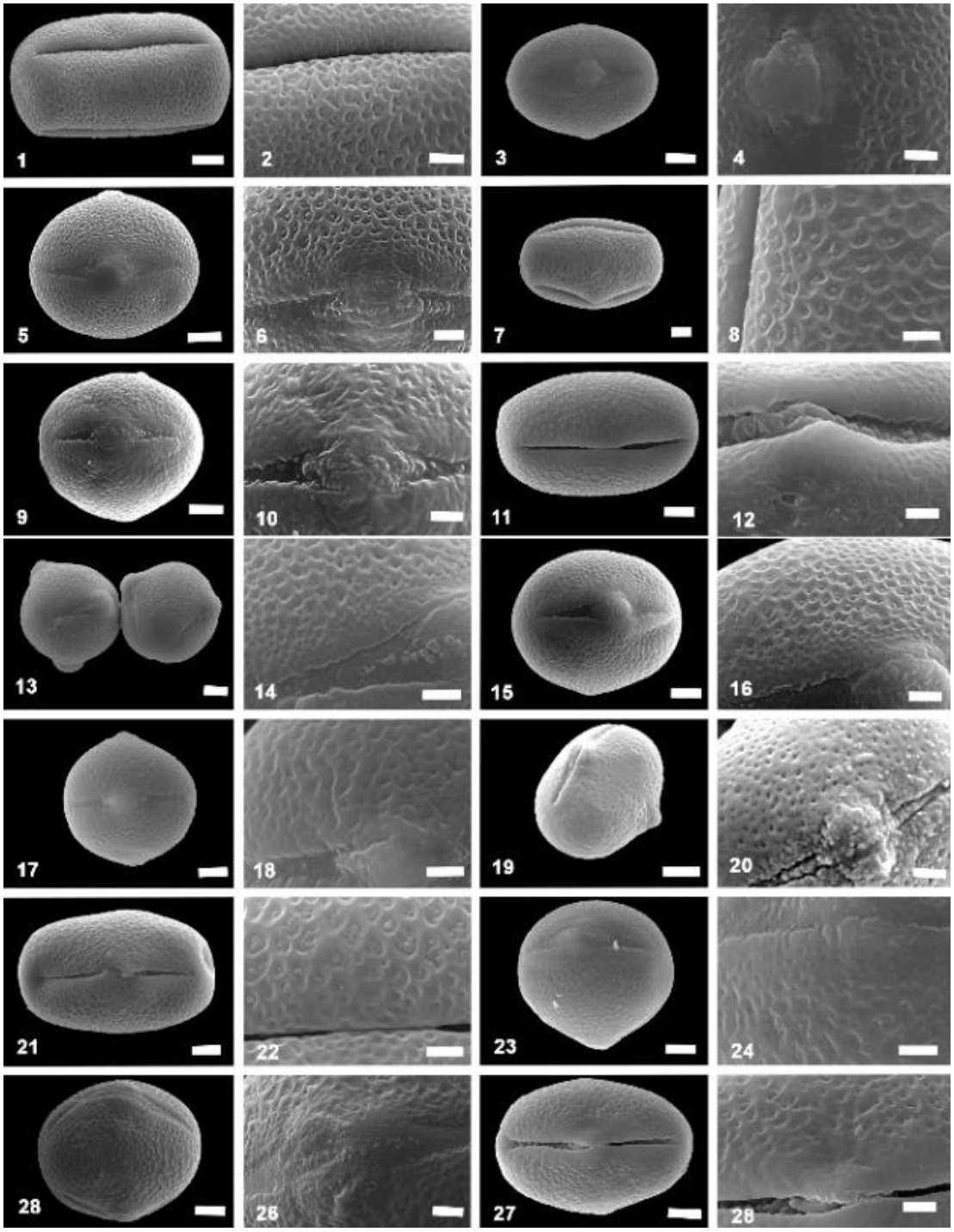

Figure 6. Scanning electron microscope pollen images of *Astragalus*
1-2: *A. hymenocystis*; 3-4: *A. lagopoides*; 5-6: *A. hirticalyx;* 7-8: *A. zohrabi*; 9-10: *A. sosnowskyi*; 11-12: *A. velenovskyi*; 13-14: *A. uraniolimneus*; 15-16: *A. trifoliastrum;* 17-18: *A. gueruenensis*; 19-20: *A. ciloensis*; 21-22: *A. rubrostriatus*; 23-24: *A. qoturensis*; 25-26: *A. narmanicus;* 27-28: *A. vaginans* (Scale bar 5 µm for 1st and 3rd column. 2 µm for 2nd and 4th column)

Pollen Size, Shape and Symmetry

The pollen grains were determined as monad, radially symmetric, and isopolar in all of the studied species. The pollen of all the taxa studied is medium size. The polar axis length was determined between 23.1 and 32.8 µm. and the equatorial diameter length was between 18.9 and 30.9 µm. The smallest pollen was detected in *A. trifoliastrum* with an average polar axis length of 27.4 µm and an average equatorial diameter length of 23.4 µm. while the largest pollen was detected in

A. narmanicus with an average polar axis length of 31.2 µm and an average equatorial diameter length of 29.2 µm. The pollen shape was determined as semitriangular-circular in the polar view and spheroidal-ellipsoidal in the equatorial view. The P/E ratio was determined between 1.02 and 1.18. Based on this ratio, two pollen shapes were identified: spheroidal (*A. hymenocystis*. *A. lagopoides*. *A. hirticalyx*. *A. uraniolimneus*. *A. gueruenensis*. *A. ciloensis*. *A. qoturensis*. *A. narmanicus*) and subprolate (*A. zohrabi*. *A. sosnowskyi*. *A. velenovskyi*. *A. trifoliastrum*. *A. rubrostriatus*. *A. vaginans*).

Pollen Apertures

Aperture type was determined as tricolporate in all the studied species, and only 5% trisyncolpate and 95% tricolporate aperture types were found in *A. gueruenensis* and *A. ciloensis* species. The aperture is operculate, and the operculum membrane has granulate ornamentation in all the species. The colpus has a long and narrow shape with acute end. Colpus length was determined between 21.1 ± 3.9-27.9 ± 3.5 µm, and colpus width was determined between 2.7 ± 1.1-4.9 ± 0.7 µm. The porus occurs as endoaperture and is located in the middle part of the colpus. Porus was determined as laterally alongated (lalongate), and the shape is oblate-spheroidal, suboblate, or oblate. Pore length was determined between 5.8 ± 0.9-9.2 ± 1.1 µm, and pore width was determined between 6.7 ± 2.1-9.9 ± 2.2 µm.

Exine Ornamentation and Intine Thickness

Ornamentation type varies between polar region-aperture surroundings and equatorial area. In general, the ornamentation elements in the equatorial area (mesocolpium) are composed of more prominent and wide-structured elements (reticulate, microreticulate), and it transforms or disappears into less prominent elements around the aperture and polar region (microreticulate, perforate to psilate). In the studied species, eight different ornamentation types have been identified as different combinations of psilate, perforate, microreticulate, and reticulate ornamentation elements in polar region-aperture surroundings and equatorial areas. These ornamentation types are Type I: Microreticulate at polar area-aperture surround and reticulate at equatorial area (mesocolpium), determined for *A. hyrticalyx* and *A. hymenocystis*; Type II: Psilate-perforate at polar area-aperture surround and microreticulate at equatorial area (mesocolpium), determined for *A. lagopoides*, *A. qoturensis* and *A. vaginans*; Type III: Psilate-perforate at polar area-aperture surround and reticulate at equatorial area (mesocolpium), determined for *A. zohrab*i and *A. velenovskyi*; Type IV: Psilate at polar area-aperture surround and microreticulate at equatorial area (mesocolpium), determined for *A. sosnowskyi*; Type V: Perforate at polar area-aperture surround and microreticulate at equatorial area (mesocolpium), determined for *A. uraniolimneus* and *A. trifoliastrum*; Type VI: Psilate-perforate at polar area-aperture surround and perforate at equatorial area (mesocolpium), determined for *A. gueruenensis* and *A. ciloensis*; Type VII: Psilate at polar area-aperture surround and reticulate at equatorial area (mesocolpium), determined for A. *rubrostriatus*; Type VIII: Perforate at polar area-aperture surround and reticulate at equatorial area (mesocolpium), determined for *A. narmanicus*.

The exine of the studied species was pertectate. Its thickness was between 1.1 ± 0.1-1.53 ± 0.1 µm, and intine thickness was between 0.4 ± 0.2-0.7 ± 0.2 µm.

Numerical Analysis

The determined qualitative and quantitative characteristics were scored for the numerical analysis. Our results are generally arising congruent with UPGMA clustering and PCA analysis in suggesting two main branches and three groups, which included the distribution of fourteen species studied (Figure 7). UPGMA results show that *A. trifoliastrum* is separated in the initial major clade (Group III) in the dendrogram, and all the remaining 13 species are found in other major clades (Group I and II). Group I includes eight species: *A. hirticalyx, A. hymenocystis, A. gueruenensis, A. ciloensis, A. narmanicus, A. lagopoides, A. uraniolimneus, A. qoturensis*. Group II includes five species: *A. zohrabi, A. sosnowskyi, A. velenovskyi, A. rubrostriatus,*

A. vaginans, and Group III only includes *A. trifoliastrum*. All the taxa of Group I have spheroidal pollen shape, and Group II are subprolate. The pore structure, P/E ratio, pollen shape, aperture type, and the ornamentations in equatorial area and polar area are the most significant variables (Table 2). The Clg, Clt, Plg, Plt, exine and intine thickness were secondary important characteristics. PCO analysis distinguished the species into three groups (Figure 8 and 9).

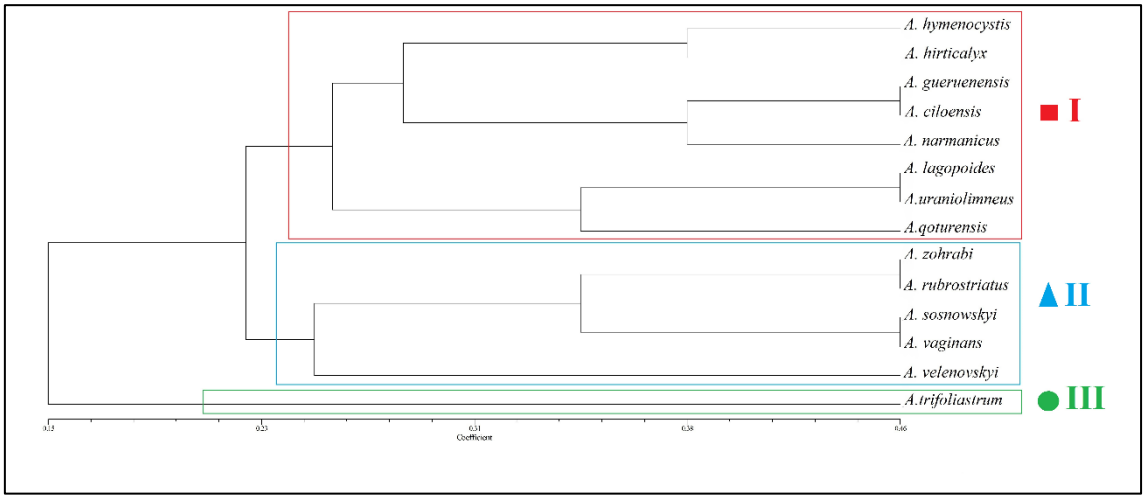

Figure 7. The phenogram obtained by evaluating pollen data from UPGMA analysis in NTSYS software.

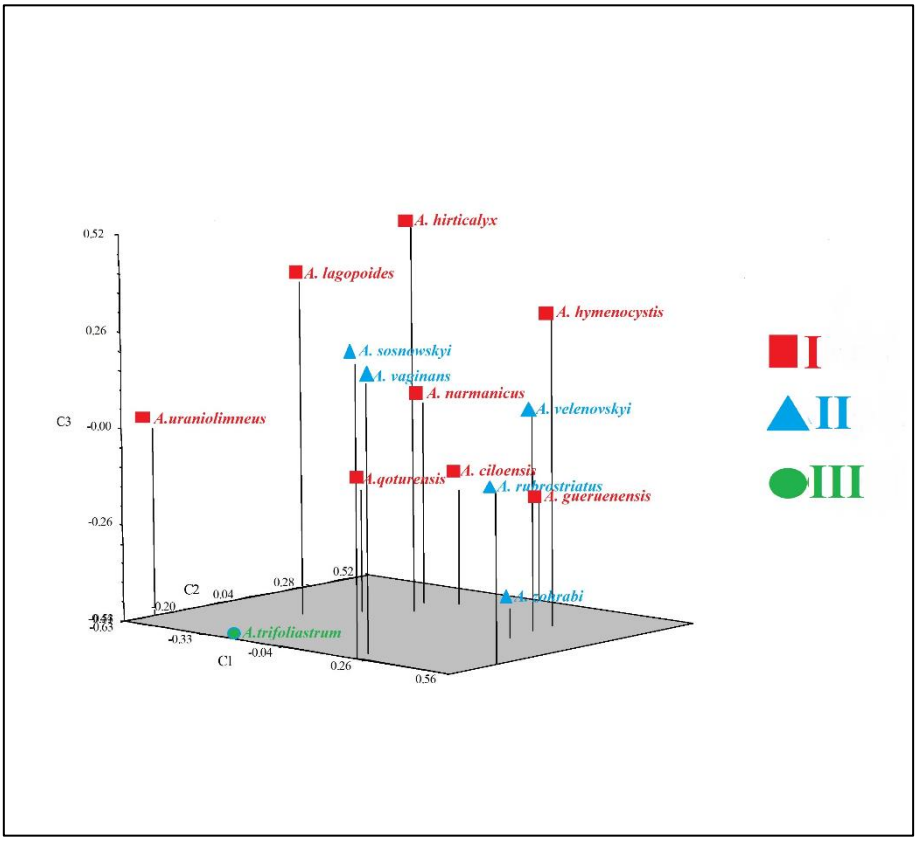

Figure 8. The three-dimensional phenogram obtained from the basic components analysis (PCO) of pollen data in NTSYSpc software

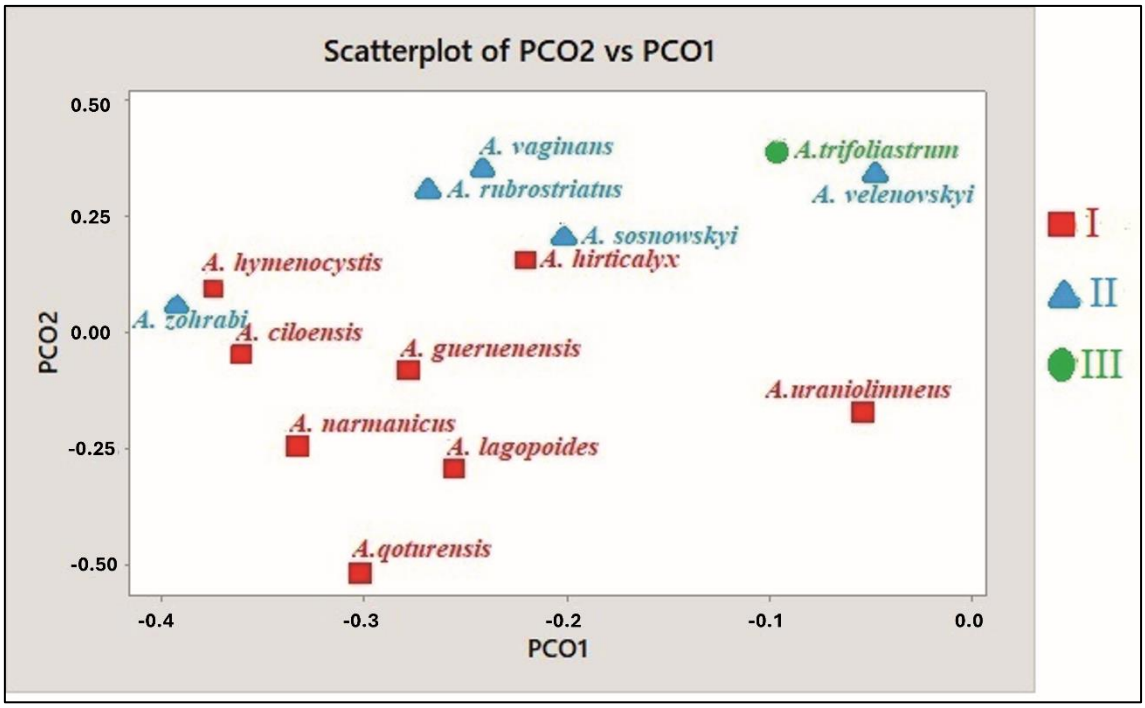

Figure 9. The two-dimensional phenogram obtained in Minitab software from basic components analysis (PCO) of pollen data.

Discussion

The studies which were carried out for these species show that the pollen characteristics are common to many *Astragalus* genus (Perveen & Qaisar, 1998; Akan et al., 2005; Pınar et al., 2009a; Çeter et al., 2013b; Al-Ghamadi et al., 2013; Hayrapetyan & Elbakyan, 2017; Bagheri et al., 2019; Bilgi et al., 2024) many different breeds of Fabaceae family (Avcı et al., 2013; Çeter et al., 2013a; İşgör et al., 2012; Pınar et al., 2013; Kahraman et al., 2012; Binzat at al., 2014)

Pollen grains of the *Astragalus* genus generally have tricolporate type aperture, but some taxa may have different aperture types such as trypantocolporate, tetrapantocolporate, spiraperturate, tricolpate, and tetrazonocolporate (Pınar et al., 2009a; Çıtak et al., 2016). In addition, some taxa may contain pollen grains with two different aperture types. In the study conducted by Bilgi et al. (2024), it was reported that the pollen of *A. emarginatus* in the sect. *Dasyphyllium* and *A. pendulus* in the sect. *Incani* were of tricolporate and trisyncolporate types. Similar results were stated for aperture type in section *Onobrychoidei* (Pınar et al., 2009a) and section *Alopecuroidei* (Akan et al., 2005). Additionally, tricolporate and trisyncolporate aperture types were observed in section *Hololeuce* (Çeter et al., 2013b). Hayrapetyan and Elbakyan (2017) observed trizonocolporate aperture in *A. lagopoides*, *A. sosnowskyi*, and *A. uraniolimneus*, which were also examined in this study. The aperture type stands out as a taxonomically reliable and distinctive character.

Perveen & Qaisar (1998) determined prolate-spheroidal, subprolate, and prolate pollen shapes for *Astragalus* species belonging to different sections in the flora of Pakistan. Al-Ghamadi et al. (2013) determined prolate-spheroidal, subprolate, prolate, and perprolate pollen shapes in 13 *Astragalus* taxa from Saudi Arabia. The pollen shape is subprolate or prolate-spheroidal in *Alopecuroidei* (Akan et al., 2005); prolate, subprolate, and prolate-spheroidal in *Onobrychoidei* (Pınar et al., 2009a); and prolate–spheroidal, subprolate, or prolate in *Hololeuce* (Çeter et al., 2013b). As a result of our study, the shape of pollen grains is spheroidal (prolate-spheroidal) for eight species and subprolate for six species in the studied specimens. Similar pollen shape was observed for *A. lagopoides*, *A. sosnowskyi*, and A. *uraniolimneus* by Hayrapetyan &

Elbakyan (2017). However, in the current study, the polar axis lengths and equatorial diameter were determined to be larger than the data obtained in the study conducted by Hayrapetyan & Elbakyan (2017). It is thought that the reason for this is due to the different examination methods (Erdtman, 1969; Wodehouse, 1935).

Pollen morphology, particularly exine ornamentation, has been used to distinguish *Astragalus* species at the sectional level (Khan et al., 2022a). Akan et al. (2005) examined the pollen morphology of 18 taxa belonging to section *Alopecuroidei*. Microreticulate ornamentation was reported in all taxa examined. In a study conducted in section Onobrychoidei, various ornamentation types were detected as microreticulate, reticulate and regulate (Pınar et al., 2009a). Oskouian et al. (2007) defined mostly reticulate and seldomly verrucate ornamentation in 37 *Astragalus* species belonging to *Malacothrix*, *Hypoglottidei*, *Plagiophaca and Stereothrix* sections. Khan et al (2022a) reported that the exine ornamentation of pollen grains in 12 different *Astragalus* taxa varied from reticulate to microreticulate.

In addition, as found in the present study, many studies have shown that ornamentation varies in many taxa in the equatorial and polar regions. Çeter et al. (2013b) found reticulate and perforate ornamentation in the polar regions of pollen grains in the *Hololeuce* section, while perforate, reticulate, microreticulate, perforate-granulate, microreticulate-perforate, microrugulate-perforate, micro-rugulate-micro-reticulate, granular-perforate, and micro-reticulate-granular ornamentation were found to be dominant in equatorial regions. Similarly, in a study conducted by Al-Ghamadi et al. (2013) on 16 different *Astragalus* taxa, microreticuate, reticulate and perforate types of ornamentation were detected in the equatorial view, while psilate, perforate and scabrate types of ornamentation were detected in the polar view. In the study conducted by Khan et al. (2024) on the *Aegacantha* section, reticulate, microperforate, and perforate ornamentations were detected in the polar regions of the pollen, while reticulate, microreticulate, perforate-granulate, perforate, and regulate type ornamentations were determined in the equatorial regions. While Bagheri et al. (2019) defined the ornamentation type of *A. hirticalyx*, *A. rubrostriatus*, *A. uroniolimneus*, and *A. zohrabi* as microreticulate, the present study defines *A. hirticalyx* as microreticulate in the polar region and as reticulate in the equatorial region; *A. rubrostriatus* as psilate in the polar region and reticulate in the equatorial region, *A. uroniolimneus* as perforate in the polar region and as microreticulate in the equatorial region, *A zohrabi* as psilate-perforate in the polar region and as reticulate in the equatorial region. Ornamentation type is an important character that can be used in the taxonomy of the *Astragalus* genus. However, since ornamentation shows regional diversity even on the same pollen, a detailed study is necessary.

The phenogram which was obtained by evaluating pollen data from UPGMA analysis in NTSYS software shows three distinct groups of species; Group I, II, and III (Figure 7, 8 and 9).

In Group I, the pollens of *A. gueruenensis* and *A. ciloensis* differed from the others with aperture type (95% tricolporate- 5% trisyncolporate), ornamentation of polar area (psilate-perforate), equatorial area (perforate), and Plg and intine thickness. *A. narmanicus* is located close to these two species, but it differs from them with aperture type percentage (100% tricolporate), polar (perforate) and equatorial area (reticulate), and Plg thickness. *A. hymenocystis* and *A. hirticalyx* were at the close clads due to their similarity in polar (microreticulate), equatorial (reticulate) area, and intin thickness. *A. lagopoides* and *A. uraniolimneus* were at the close clads due to their similarity in equatorial area (psilate-perforate and perforate), Clg, Clt, Plt, and exine thickness. *A. qoturensis* looks different due to Clt, Plt, and exine thickness. However, due to its similarity to *A. lagopoides* and *A. uraniolimneus* in terms of other characteristics, it remained close to these species.

In Group II, *A. zohrabi* and *A. rubrostriatus* show similarities in polar (psilate-perforate and psilate) and equatorial area (microreticulate) and exine thickness. *A. sosnowskyi* and *A. vaginans* are similar in

terms of polar (psilate and psilate-perforate) and equatorial (microreticulate) area, Clg, exine, and intine thickness. The pollen characteristics of *A. velenovskyi* is similar to these two species. Equatorial area (reticulate), Clt, exine, and intine thickness are distinct from them.

Many genetic and molecular studies have been carried out to solve the problems in *Astragalus* taxonomy. Ateş (2017) examined the phylogenetic relationships among the species of the *Astragalus* genus using data obtained from cpDNA regions belonging to sections *Hymenocoleous*, *Hymenostegis*, *Poterion*, *Macrophyllium*, *Halicacabus* and *Megalocystis*. According to cpDNA data, the endemic *A. vaginas*, the only member of the *Hymenocoleous* section, has always been reported to be positioned differently, although it is close to the species in the *Hymenostegis* section. Similarly, in the study conducted by Bagheri et al. (2017) with ITS and ycf1 sequences, it was shown that *A. vaginas* is close to the species in the *Hymenostegis* section. However, this species could not be positioned differently in the studies conducted on the ITS region (Alvarez & Wendel, 2003; Blattner, 2004). In the current study, *A. vaginans* looked different but not contrasting to *Hymenostegis* section. Therefore, *A. vaginans* should continue to be recorded as a different section named Hymenocoleous.

Endemic *A. trifoliastrum* Group III was observed to be slightly more distant to the taxa in Group I and Group II. In *A. trifoliastrum* taxon in Group III, a different clade was included because polar axis, P / E ratio, and Clt characteristics differ completely from the other 13 species. As a result of molecular analyses by Ateş (2017), species of section *Hymenostegis* were grouped together, and *Hymenostegis* section formed a mono phyletic group. However, *A. trifoliastrum* was positioned in a sub-branch different from the other species of *Hymenostegis* section with bootstrap value of 51. These molecular analyses results match the phenogram obtained by evaluating the pollen data.

PCO has distinct groups of species. *A. zohrabi* might be placed in Group I according to PCO (Figure 8) while it groups clearly within Group II based on UPGMA, which is the main difference between PCO and UPGMA analyses.

The grouping of taxa based on pollen characteristics in UPGMA and PCoA provides some correlations with species relationships obtained through molecular phylogenetic analyses of ITS and *ycf1* sequences results (Bagheri et al., 2017).

PCO and UPGMA analyses reveal that pollen morphology can be used to distinguish taxa. However, as reported by Bilgi et al. (2024), no significant contribution of pollen morphology to the distinction of sections was detected.

Conclusion

Pollen morphology of 14 species in *Hymenostegis* and *Hymenocoleous* sections of the genus *Astragalus* found in Türkiye was examined in detail by light microscope and scanning electron microscope. PCO and UPGMA analyses reveal that pollen shape and exine ornamentation are the most effective palynological characters in *Astragalus* taxonomy. Additionally, traits such as polar axis length, equatorial diameter, colpus length and width, and pore length and width were found to contribute significantly. When evaluated together with the existing literature, it is seen that palynological characters are parallel to genetic and molecular methods in taxonomic distinction. It is evaluated that anatomy, pollen morphology, and molecular data, along with morphological characteristics, would yield better results in solving the systematic problems of the species. This study supports that pollen morphology can contribute to solving problems in *Astragalus* taxonomy.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: S.K.; Investigation: S.K., T.Ç., Ş.B.; Material and Methodology: S.K., T.Ç., O.B.; Visualization: S.K., Ş.B.; Writing-Original Draft: S.K; Writing-review & Editing: S.K., T.Ç., O.B., Ş.B., All authors

have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This work was supported by the Scientifc and Technological Research Council of Türkiye (TÜBİTAK) under Grant [TBAG-110 T 911].

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