**Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 14(2), 616-624, 2024**
***Journal of the Institute of Science and Technology, 14(2), 616-624, 2024***

**ISSN: 2146-0574, eISSN: 2536-4618**

**Biyoloji / *Biology*** **DOI: 10.21597/jist.1424706**

**Araştırma Makalesi / *Research Article***

**Geliş tarihi / *Received*: 23.01.2024** **Kabul tarihi / *Accepted*: 25.03.2024**

**Atıf İçin:** Altın, F., Akan, H. ve Çeter, T**.** (2024). Şanlıurfa'da Yayılış Gösteren Bazı *Astragalus* L. Türlerinin Palinolojik Olarak İncelenmesi. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 14(2), 616-624.

***To Cite:*** Altın, F., Akan, H. & Çeter, T. (2024). Palynological Investigation of Some *Astragalus* L. Species Distributed in Şanlıurfa. *Journal of the Institute of Science and Technology*, 14(2), 616-624.

# Şanlıurfa'da Yayılış Gösteren Bazı *Astragalus* L. Türlerinin Palinolojik Olarak İncelenmesi

Fatma ALTIN[1], Hasan AKAN[1*], Talip ÇETER[2]

**Öne Çıkanlar:**
- Fabaceae familyası
- *Astragalus* cinsi
- Polen çalışmaları

**Anahtar Kelimeler:**
*Astragalus*
- Palinoloji
- Şanlıurfa

**ÖZET:**

Bu çalışmada, Şanlıurfa ilinde yayılış gösteren Fabaceae familyasından *Astragalus* cinsine ait 5 türün (*Astragalus guttatus* Banks & Sol., *A. cretaceus* Boiss., *A. caprinus* L. subsp. *caprinus*, *A. brachystachys* DC. ve *A. ancistrocarpus* Boiss. & Hausskn.) polen morfolojisi ışık ve elektron mikroskopları ile incelenmiştir. Çalışma sonucunda taksonlara ait polenlerin monad, radyal simetrili ve izopolar oldukları tespit edilmiştir. Çalışılan tüm türlerin polenleri trizonokolporat olup kolpus uzunlukları (Clg)16,5-27,62 µm, kolpus genişlikleri (Clt) 2,15-4,16 µm, por uzunlukları (Plg) 6,69-9,32 µm, por genişlikleri (Plt) 6,69-11,44 µm arasında tespit edilmiştir. Polar eksen uzunlukları 19,23-33,66 µm arasında, ekvatoral eksen çapları 14,95-28,61 µm olarak ölçülmüştür. Ornamentasyon polar bölge ve apertür çevresinde psilat, psilat-perforat, psilat-mikroretikülat ve mikroretikülat olarak saptanırken ekvatoral bölgede mikroretikülat olarak belirlenmiştir. Çalışma sonucunda polen şekli, polen büyüklüğü ve yüzey ornamentasyonu gibi karakterlerin türler arasında farklılık gösterdiği ve türlerin sistematik ayırımında taksonomik değere sahip olabilecek karakterler olduğu tespit edilmiştir.

## Palynological Investigation of Some *Astragalus* L. Species Distributed in Şanlıurfa

**Highlights:**
- Fabaceae family
- *Astragalus* genus
- Pollen studies

**Keywords:**
- *Astragalus*
- Palinology
- Şanlıurfa

**ABSTRACT:**

In this study, pollen morphologies of 5 species (*Astragalus guttatus* Banks & Sol., *A. cretaceus* Boiss., *A. caprinus* L., *A. brachystachys* DC. and *A. ancistrocarpus* Boiss. & Hausskn.) belonging to the *Astragalus* genus of the family Fabaceae, which are distributed in Şanlıurfa province, were exemined by light and electron microscope. As a result of the study, it was determined that the pollen of the taxa were monad, radial symmetrical and isopolar. Pollen of all studied species are trizonocolporate, colpus length (Clg) 16.5-27.62 µm, colpus width (Clt) 2.15-4.16 µm, pore length (Plg) 6.69-9.32 µm, pore width (Plt) was detected between 6.69-11.44 µm. Polar axis length was measured between 19.23-33.66 µm and equatorial axis diameter was measured as 14.95-28.61 µm. Ornamentation was determined as psilate, psilate-perforate, psilate-microreticulate, microreticulate in the polar region and aperture surround the while microreticulate in the equatorial region. As a result of the study, it was determined that the characters such as pollen shape, pollen size and surface ornamentation differ between species and these characters have taxonmic value in the systematic separation of species.

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This study was produced from Fatma Altın's Master's thesis.

## INTRODUCTION

The genus *Astragalus* L., has about 3000 taxa (Podlech & Zarre, 2013) in the world. *Astragalus* which is the most common genus in Türkiye, is represented by 62 sections and 484 taxa in the Flora of Türkiye (Chamberlain & Matthews, 1970; Davis et al., 1988; Podlech, 1999; Duman & Akan, 2003; Aytaç et al., 2012; Karaman Erkul, 2022; Özhatay et al. 2017; 2019). 245 of the taxa are endemic and the endemism rate is 51% (Aytaç et al., 2012, Podlech & Zarre, 2013; Uzun et al., 2019; Karaman Erkul, 2022). The taxon number increase by adding new species (İlçim & Behçet, 2016; Aytaç et al., 2020; Tunçkol et al., 2020; Karaman Erkul, 2022). One of the reasons for the high rate of endemism belonging to the genus in our country is that the members of *Astragalus* grow in the high mountains of Anatolia and one of the gene centers is Türkiye. It spreads on forest, steppe and mountain slopes at altitudes of 1300-3500 meters in the Eastern Anatolia and Central Anatolia Regions of Türkiye, and at an altitude of 1300-2300 meters in the Central Aegean and Taurus Mountains. Members of this genus are frequently encountered in the steppes of mountainous regions (Akan et al., 2007).

In studies on genera belonging to Fabaceae family, it has been stated that pollens are stenopalinous in terms of some characters, and these characters do not show significant differences between different genera of the family. It has been determined that the pollen distribution unit, which is one of these characters, is commonly monad, and tetrad or polyad pollen distribution units are found only in some genera of the Mimosoideae subfamily. Isopolar and radial symmetric pollen types has been reported in different studies. It has been reported that the aperture type is also commonly trizonocolporate, rarely the trisincolporate aperture type or the tricolpate aperture type. It has been stated that features such as aperture length and width, pollen shape, apocolpium, mesocolpium, polar axis length, equatorial axis diameter and surface ornamentation show differences between species, therefore they are important characters that can be used in the systematic differentiation of species. (İşgör et al., 2012; Avcı et al., 2013; Çeter et al., 2013a; Kahraman et al., 2013; Nassiri-Semmani et al., 2014; Pınar et al., 2014; Antonio-Domingues et al., 2018; Altın et al., 2021; Bahadur et al., 2022,).

Pollens have a unique characters for each species and that's why it become important at the studies of plant taxonomy. In this study, pollens were generally determined as monad, isopolar and radial symmetrical. Pollens of the genus are commonly trizonocolporate, rarely tricincolporate pollens have been reported. Pollen shape can have shapes ranging from suboblate to perprolate. It has been reported that different ornamentation types such as psilate, perforate, reticulate, microreticulate and rugulate can occur in different combinations in polar region, equatorial region and aperture circles. (Simons & Chinnappa, 2004; Akan et al., 2005; Ekici et al., 2005; Dane et al., 2007; Oskouian et al., 2007; Pınar et al., 2009 ; Çeter et al., 2013b; Osman et al., 2014; Karaman Erkul et al. 2017, Atasagun et al., 2018; Metin et al., 2018; Bagheri et al., 2019; Özbek et al., 2021; Khan et al., 2022a, Khan et al., 2022b).

In this study, some members of *Astragalus*, which is distributed in Şanlıurfa and has been studied palynologically, by light microscopy and scanning electron microscopy. It is aimed to contribute to the completion of the palynological data of the *Astragalus* species distributed in Türkiye.

## MATERIALS AND METHODS

### Speciemens Investigated

The study material consists of the species *Astragalus guttatus, A. cretaceus, A. caprinus* subsp. *caprinus, A. brachystachys* and *A. ancistrocarpus*, which are distributed in Şanlıurfa (Table 1). As the main source for the identification of these collected plants; Davis (1965-1985), Davis et al. (1988), Guner et al. (2000), Podlech & Zarre (2013) were used. The specimens are kept in HARRAN herbarium.

For pollen studies, taxa were collected from different regions and flower samples were also studied. Pollen morphology of taxa was examined in detail under light and scanning electron microscopy.

**Table 1.** Species name of *Astragalus* members and Localities

| Species name | Localities |
|---|---|
| ***Astragalus guttatus*** | Şanlıurfa-Viranşehir 40. km, 22.04.2008, MNM 1339 & Akan;<br>Şanlıurfa–Viranşehir 43. km, 07.05.2008, MNM 1403 & Akan. |
| ***A. cretaceus*** | Birecik; Zeytinbahçe-Abdallı, 04.05.2008, MNM 1375 Akan & Balos. |
| ***A. caprinus* subps. *caprinus*** | Şanlıurfa-Bozova, Nergisli village, 29.04.2007, MNM 1215 &Akan;<br>Bozova, Tektaş village, 13.05.2006, MNM 1088 &Akan;<br>Birecik to Şanlıurfa 15 km, 03.06.2007, MNM 1319;<br>Birecik; Çiftlik village, 04.05.2008, MNM 1374 &Akan |
| ***A. brachystachys*** | C6 Şanlıurfa: Birecik; Çiftlik village, 04.05.2008, MNM1374 &Akan |
| ***A. ancistrocarpus*** | Şanlıurfa-Mardin road, Osmanbey campus, 27.03.2007, MNM 1149 &Akan;<br>Viranşehir road; Osmanbey campus, 06.05.2008, 500 m, steppe, MNM 1389 & Akan. |

## Palynological Analyses

Pollen preparations were prepared according to the protocol specified in the Wodehouse (1935) method. Pollen grain transferred on a clean slide with the help of a sterile dissection needle, stained with safranin, and they were examined in a light microscope with Leica DM3000 digital imaging system. For each pollen character evaluated, measurements were taken from 30 different pollen and their averages were recorded.

Pollen samples were appropriately placed on aluminum staples with the help of double-sided adhesive tape, coated with gold using the Cressington Sputter Coater device, and microphotographs were taken with a Quanta FEG 250 model Scaning Electron Microscope (SEM). Pollen surface morphology and ornamentation analysis of the taxa were made using SEM microphotographs and relevant literature (Faegri ve Iversen 1975, Ertdman 1969 ve Punt vd. 2007).

## RESULTS AND DISCUSSION

As a result of pollen examinations in the studied taxa, main pollen characteristics were determined as radially symmetrical, isopolar and trizonocolporate. Pollen shape is determined as prolate-spheroidal, subprolate or prolate. Polar axis measured between 19.23 – 33.6 µm, and equatorial diameter is 18.06-28.61 µm. Amb shape is circular, the diameter of the apocolpium was measured between 12.55-18.41 µm and the mesocolpium between 11.99-22.58 µm. Ornamentation was determined as psilate, psilate-perforate, psilate-microreticulate, microreticulate in the polar region aperture surround the while microreticulate in the equatorial region. The colpus is thin, long, operculate, and the operculum membrane has granulate ornamentation. Porus are laterally elongated (lalongate) with suboblate shape (Table 2, Figures 1-5).

***Astragalus guttatus*** Banks & Sol.

Pollen grain are radially symmetrical, isopolar, trizonocolporate. Pollen shape is determined as subprolate. Polar axis measured between 19.23-22.70 µm, and equatorial diameter is 13.76-20.14 µm. Amb shape is circular, the diameter of the apocolpium was measured as 12,55 µm and the mesocolpium as 11.99 µm. Ornamentation was determined as psilate-microreticulate in the polar region aperture surround the while microreticulate in the equatorial region. The colpus is thin, long (Clg 14-18 µm, Clt 3.8 - 8.6 µm) operculate, and the operculum membrane has granulate ornamentation. Porus are laterally elongated (lalongate) with suboblate shape (Plg 3.79-8.6 µm , Plt 5.95-10.23 µm).

**Table 2.** Pollen characteristic features of studied *Astragalus* species

| Taxa / *Takson adı* | Polar axis (P) *Polar eksen* | | | Equatorial diameter (E) *Ekvatoral çap* | | | P / E ratio and pollen shape *P/E oranı ve polen şekli* | Aperture *Apertur* |
|---|---|---|---|---|---|---|---|---|
| | Min. | Ort. | Max. | Min. | Ort.. | Max. | | |
| *A. guttatus* | 19.23 | 20.38 | 22.70 | 14.95 | 17.30 | 20.14 | 1,17 / Subprolate | Trizonocolporate |
| *A. cretaceus* | 28.16 | 33.6 | 38.2 | 21.5 | 26.0 | 27.58 | 1,29 / Subprolate | Trizonocolporate |
| *A. caprinus* subsp. *caprinus* | 26.75 | 28.80 | 30.4 | 23.58 | 26.26 | 28.61 | 1,09 / Prolate-sferoidal | Trizonocolporate |
| *A.brachystachys* | 28.66 | 30.22 | 31.81 | 18.06 | 19.6 | 21.38 | 1.52 / Prolate | Trizonocolporate |
| *A.ancistrocarpus* | 28.73 | 31.48 | 33.66 | 25.41 | 27.08 | 28.51 | 1.16 / Subprolate | Trizonocolporate |

* Clg: Colpus width, Plg: Pore width, * Clt: Colpus length, Plt: Pore length

**Table 2.** Pollen characteristic features of studied *Astragalus* species (continued)

| Taxa / *Takson adı* | Ornamentation *Ornemantasyon* | | Colpus (Cl) *Kolpus* (Cl) | | Porus (Pl) *Por (Pl)* | | Ekzine *Ekzin* | İntine *intin* | Apocolpium *Apokolpium* | Mesocolpium *Mezokolpiyum* |
|---|---|---|---|---|---|---|---|---|---|---|
| | Polar area and aperture souronding | Equatorial area | Clg | Clt | Plg | Plt | | | | |
| *A. guttatus* | Psilate-microreticulate | Microreticulate | 16.5 | 3.11 | 7.5 | 6,9 | 0.53 | 0,513 | 12.25 | 11.99 |
| *A. cretaceus* | Psilate-perforate | Microreticulate | 27.62 | 4.16 | 9.32 | 10.69 | 0.55 | 0.58 | 13.95 | 17.06 |
| *A. caprinus* subsp. *caprinus* | Psilate | Microreticulate | 23.89 | 2.93 | 9.11 | 10.49 | 0.59 | 0,55 | 15.5 | 19.43 |
| *A.brachystachys* | Psilate | Microreticulate | 22.5 | 2.15 | 6.69 | 7.3 | 0.74 | 0.69 | 12.68 | 15.55 |
| *A.ancistrocarpus* | Microreticulate | Microreticulate | 24.74 | 2.9 | 9.09 | 11.44 | 0.57 | 0.43 | 18.41 | 22.58 |

* Clg: Colpus width, Plg: Pore width, * Clt: Colpus length, Plt: Pore length

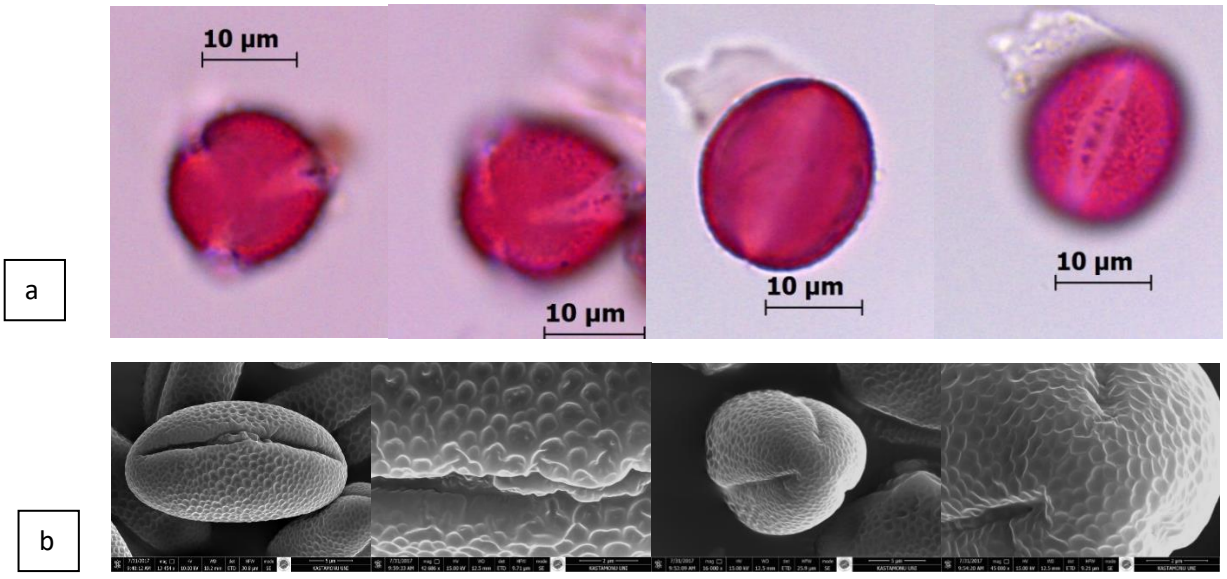


**Figure 1**. *A.guttatus.* a: Light microscope mirophotograph, b: SEM microphotograph

***Astragalus cretaceus*** Boiss.

Pollen grain are radially symmetrical, isopolar, trizonocolporate. Pollen shape is determined as subprolate. Polar axis measured between 28.16 – 32.20 µm, and equatorial diameter is 21.5 – 27.58 µm. Amb shape is triangular, the diameter of the apocolpium was measured as 13.95 µm and the mesocolpium as 17.06 µm. Ornamentation was determined as psilate-microreticulate in the polar region aperture surround the while microreticulate in the equatorial region. The colpus is thin, long (Clg 23.5-30.44 µm, Clt 32.5-6.6 µm) operculate, and the operculum membrane has granulate ornamentation. Porus are laterally elongated (lalongate) with suboblate shape (Plg 6.9-15.83 µm, Plt 9.5-13.08 µm).

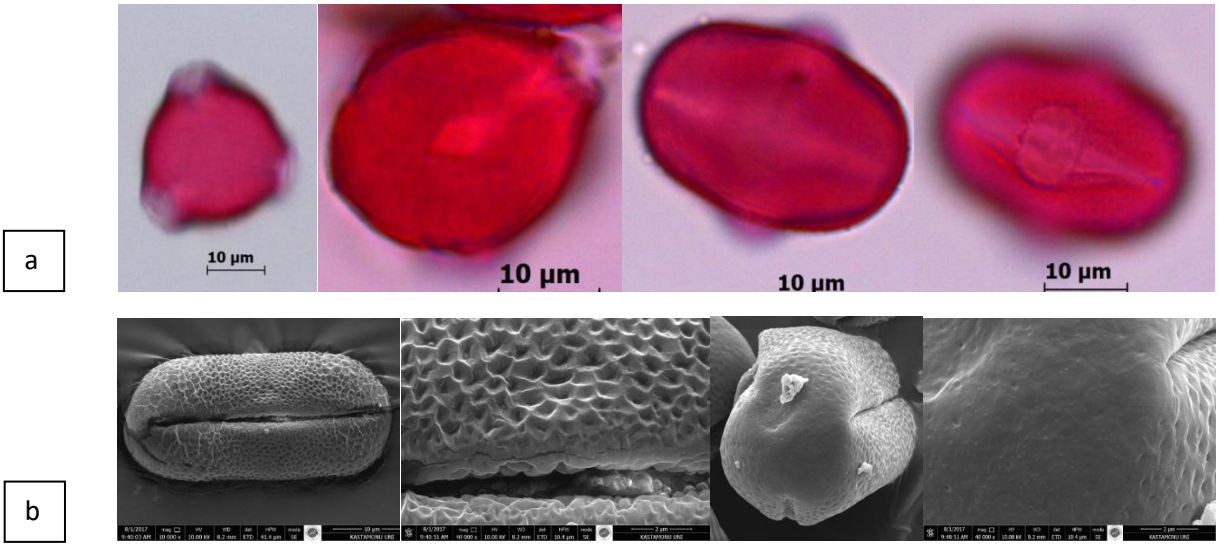


**Figure 2**. a: Light microscope photos of *Astragalus cretaceus*, b: SEM photos

***Astragalus caprinus*** L. subsp. ***caprinus***

Pollen grain are radially symmetrical, isopolar, trizonocolporate. Pollen shape is determined as subprolate-spheroidal. Polar axis measured between 26.75-30.33 µm, and equatorial diameter is 23.58-28.61 µm. Amb shape is semi-triangular, the diameter of the apocolpium was measured as 15.22 µm and the mesocolpium as 19.49 µm. Ornamentation was determined as psilate in the polar region aperture surround the while microreticulate in the equatorial region. The colpus is thin, long (Clg 20.77-25.66 µm, Clt 1.83-3.86 µm) operculate, and the operculum membrane has granulate ornamentation. Porus are laterally elongated (lalongate) with suboblate shape (Plg 7.45-10.8 µm, Plt 8.66-13.26 µm).

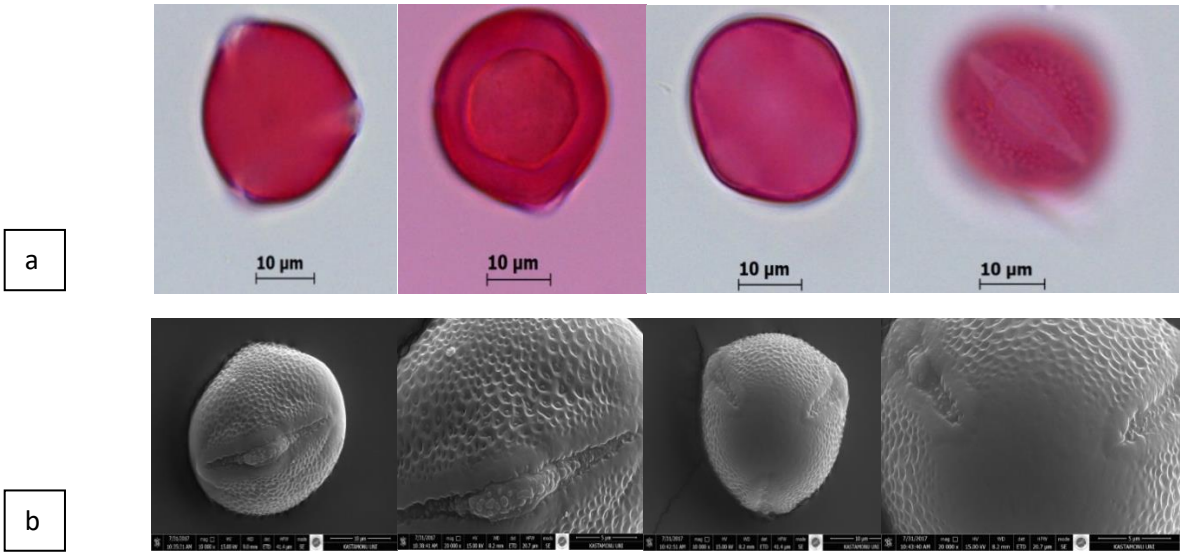


**Figure 3**. a. *Astragalus caprinus* L. subsp. *caprinus* light microscope photographs b. SEM photos

***Astragalus brachystachys*** DC.

Pollen grain are radially symmetrical, isopolar, trizonocolporate. Pollen shape is determined as prolate. Polar axis measured between 29.45-31.55 µm, and equatorial diameter is 18.18-21.38 µm. Amb shape is circular, the diameter of the apocolpium was measured as 12.68 µm and the mesocolpium as 15.5 µm. Ornamentation was determined as psilate in the polar region aperture surround the while microreticulate in the equatorial region. The colpus is thin, long (Clg 20.48-25.04 µm, Clt 1.1-2.6 µm)

operculate, and the operculum membrane has granulate ornamentation. Porus are laterally elongated (lalongate) with oblate-spheroidal shape (Plg 7.45-10.8 µm, Plt 8.66-13.26 µm).

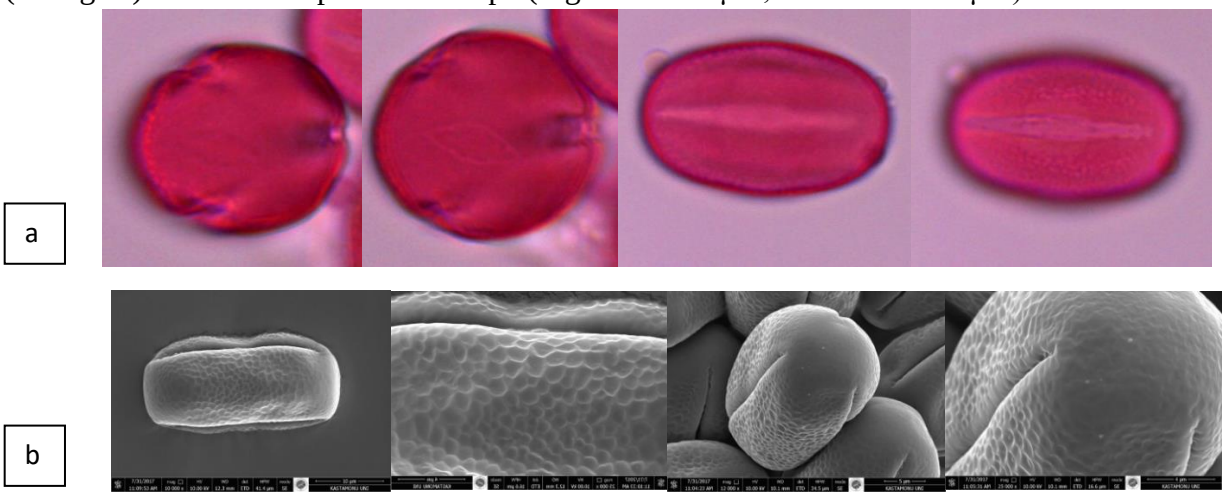


**Figure 4:** a. Light microscope photographs of *Astragalus brachystachys* species, b. SEM photos

***Astragalus ancistrocarpus*** Boiss. & Hausskn.

Pollen grain are radially symmetrical, isopolar, trizonocolporate. Pollen shape is determined as subprolate. Polar axis measured between 28.73-33.66 µm, and equatorial diameter is 25.41-28.51 µm. Amb shape is circular, the diameter of the apocolpium was measured as 18.41 µm and the mesocolpium as 22.58 µm. Ornamentation was determined as microreticulate in both the polar region aperture surround and equatorial region. The colpus is thin, long (Clg 23.33-25.66 µm, Clt 2.2-4.3 µm) operculate, and the operculum membrane has granulate ornamentation. Porus are laterally elongated (lalongate) with suboblate shape (Plg 6.5-17.25 µm, Plt 8.83-15.38 µm).

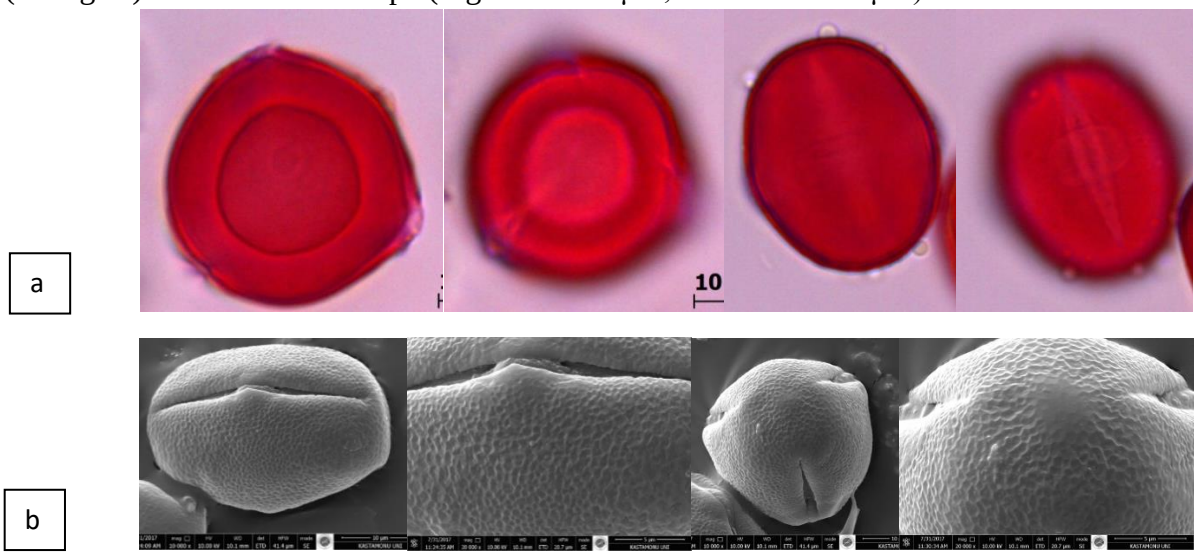


**Figure 5:** a. Light microscope photographs of *Astragalus ancistrocarpus* species, b. SEM photos

With this study, some members of *Astragalus* were investigated palynologically. Pollens of all studied *Astragalus* taxa were determined as radial symmetric, isopolar and trizonocolporate. Studies on both different *Astragalus* taxa (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) and in studies on different genera of the 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) in terms of polarity and symmetry of pollen reveals that they mostly exhibit similar characteristics. Hayrapetyan & Elbakyan (2017) determined the

aperture type as trizonocolporate for the different *Astragalus* species they studied. Likewise, Akan et al. (2005) in species belonging to the Hololeuce section and Pınar et al. (2009a) determined the trizonocolporate aperture type for the species belonging to the Onobrychoidei section. According to Çeter et al. (2013b) in addition to trizonocolporate ornamentation, rarely trisyncolporate aperture type was determined in species belonging to the Hololeuce section.

Prolate-spheroidal (*A. caprinus* subsp. *caprinus*), subprolate (*A. guttatus, A. cretaceus, A. ancistrocarpus*) and Prolate (*A. brachystachys*) pollen forms were determined in the pollen of the studied taxa. Similarly, subprolate, prolate and prolate-spheroidal pollen forms were determined in some *Astragalus* species belonging to "Flora of Pakistan" (Perveen & Qaisar, 1998) and taxa belonging to Onobrychoidei (Pınar et al., 2009) and Alopecuroidei (Akan et al., 2005) sections in "Flora of Turkey". Al-Ghamadi et al. (2013) determined a perprolate pollen shape in addition to the above-mentioned pollen shapes in the pollen of 13 *Astragalus* species distributed in the "Flora of Saudi Arabia".

Ornamentation in *Astragalus* taxa is microreticulate in the equatorial region, microreticulate (*A. ancistrocarpus*), psilate-microreticulate (*A. guttaus*), psilate (A. *caprinus* subsp. *caprinus, A. brachystachys*), Psilate-perforate (*A. cretaceus*) around the polar region and aperture.) ornamentation types were determined. Akan et al. (2005) Microreticulate ornamentation in the *Alopecuroidei* section of the genus *Astragalus*. Pınar et al. (2009a) determined microreticulate, reticulate and rugulate ornamentation in species belonging to the Onobrychoidei section. Bagheri et al. (2019) determined microreticulate ornamentation in 22 species belonging to the Hymenostegis section. Oskouian et al. (2007) determined mostly reticulate and rarely verrucate ornamentation in 37 species belonging to Malacothrix section. Al-Ghamadi et al. (2014) identified 6 different ornamentation types as reticulate, reticulate-psilate, reticulate-scabrate, microreticulate-perforate, microreticulate-psilate, and perforate-psilate in 13 different *Astragalus* species. According to Çeter et al. (2013b) Reticulate and perforate in the polar region in species belonging to the Hololeuce section, perforate in the equatorial region, reticulate, microreticulate, perforate-granulate, microreticulate-perforate, microrugulate-perforate, microrugulate-microreticulate, granulate-perforate, microreticulate- granulate

## CONCLUSION

As results of many studies on the *Astragalus* genus, no differences were detected between pollen in terms of aperture type, polarity and symmetry, and the pollen of all studied species was found to be trizonocolporate, isopolar and radially symmetrical. However, the polar axis, equatorial diameters, pollen shapes, colpus and pore measurements and ornamentation types of the studied species showed significant differences. Pollen shape was determined as prolate-spheroidal, subprolate and prolate. Ornamentation was determined as psilate, psilate-perforate, psilate-microreticulate, microreticulate in the polar region and aperture surround the while microreticulate in the equatorial region.

It has been determined that pollen morphological features such as polar axis, equatorial diameter, pollen shape and pollen surface ornamentations, differ between species and can be used in the differentiation of species.

## ACKNOWLEDGEMENTS

The authors would like to thank to Harran University for financial support (Project no 17074).

### Conflict of Interest

The article authors declare that there is no conflict of interest between them.

### Author's Contributions

The authors declare that they have contributed equally to the article.

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