Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 11(4): 2525-2536, 2021
Journal of the Institute of Science and Technology, 11(4): 2525-2536, 2021

ISSN: 2146-0574, eISSN: 2536-4618

Biyoloji / *Biology* **DOI: 10.21597/jist.962219**

Araştırma Makalesi / *Research Article*

Geliş tarihi / Received: 04.07.2021 **Kabul tarihi / Accepted: 08.09.2021**

To Cite: Özbek F, Ekici M, Büyükkartal HN, Pınar NM, 2021. Anatomy, Palynology and Micromorphology of the Genus *Astragalus* L. (Fabaceae) Section *Uliginosi* Gray in Turkey. Journal of the Institute of Science and Technology, 11(4): 2525-2536.

Anatomy, Palynology and Micromorphology of the Genus *Astragalus* L. (Fabaceae) Section *Uliginosi* Gray in Turkey

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ABSTRACT: The anatomical, palynological and leaflet and fruit micromorphological properties of two *Astragalus* L. species, namely *A. falcatus* Lam. and *A. odoratus* Lam. attributed to the section *Uliginosi* Gray (Fabaceae) from Turkey were considered via light (LM) and scanning electron microscopies (SEM) in order to determine their these characteristics and to assess the diagnostic value of these properties as taxonomic evidence. The leaflets type in terms of the anatomy, the type of stomata and trichomes, the shape and aperture of pollen grains and also the surface ornamentation of the fruits and trichomes are similar in these two species. Anatomical features such as the shape of the stem and petiole, the absence or presence of pith cavity in the stem and the number of vascular bundles in the petiole possess a systematic importance. Bifurcate trichomes are present on both surfaces of the leaflets. The ornamentation of trichomes is verrucate. The surface pattern of fruits is reticulate. The pollen grains are isopolar, radially symmetrical, trizonocolporate and prolate-spheroidal. Exine sculpturing is found to be a valuable characteristic for separating these species.

Keywords: *Astragalus*, *Uliginosi*, anatomy, pollen, micromorphology

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Bu çalışma Funda Özbek'in Yüksek Lisans tezinden üretilmiştir.

Makalenin bir kısmı 5-7 Mayıs 2011 tarihlerinde Düzce'de düzenlenen "Ekoloji Sempozyumu'nda" poster; 21-27 Şubat 2011 tarihlerinde düzenlenen "Biosystematics Congress'de" poster ve 18-21 Kasım 2014 tarihlerinde Malezya'da düzenlenen "2. International Conference on Advances in Plant Sciences (ICAPS)'da" sözlü olarak sunulmuştur.

INTRODUCTION

Astragalus L. is one of the largest genera of flowering plants with about 2530 taxa worldwide. This genus is classified into 8 subgenera and approximately 245 sections (Maassoumi, 1998) and also recently according to Podlech and Zarre (2013) 2398 species and 136 sections. One-third of them are known to grow in America, and are called the New World species, and the remainder are known to grow in the continents of Asia, Europe and Africa, and are called the Old World species. The genetic center of the genus seems to be Eurasia. The number of this genus is particularly quite high in Central Asian steppes, Iran and Turkey. This genus is represented with 63 sections and 459 taxa indicating an endemism rate of 51% in Turkey (Ekici et al., 2015; Duman et al., 2020). The members of the genus *Astragalus* generally grow in a steppeic formation (in dry climates) and they are found just as much in the Irano-Turanian phytogeographic region of Holarctic world.

At first, Bunge (1868, 1869) comprehensively classified this genus into 10 subgenera for its species growing in the Old World. Podlech (1982) reduced the number of eight perennial subgenera in Bunge's system (1868, 1869) to only 2 groups as subgenus *Astragalus* and subgenus *Cercidothrix* Bunge. Later annual species were transferred to the subgenus *Trimeniaeus* Bunge (Podlech, 1994). The number of Bunge's subgenera was then reduced to 8 when the species from 2 subgenera were transferred into the others (Maassoumi, 1998). The section *Uliginosi* belongs to the subgenus *Cercidothrix*. *Cercidothrix* had been considered to be monophyletic (Podlech, 1998). However, molecular studies showed that this subgenus is polyphyletic. Within this subgenus only the sections *Ammodendron* Bunge and *Incani* DC. are monophyletic. As section *Uliginosi* includes *A. fragrans* Willd., which is the member of section *Acmothrix* Bunge, this section is a paraphyletic group (Kazempour et al., 2003).

The section *Uliginosi* is represented in Turkey with 2 species, namely *Astragalus falcatus* Lam. and *A. odoratus* Lam.; in Iran with 1; in the former USSR with 7; in Azerbaijan with 2; in Armenia with 2; in Syria with 1; in China with 3 and in Japan with 2 (Gontscharov et al., 1946; Chamberlain and Matthews, 1970; Maassoumi, 1998)

There are some reports of the family Fabaceae based on anatomy (Metcalfe and Chalk, 1957; Çobanoğlu, 1989; Haddad and Barnet, 1989; Evren and Çobanoğlu, 1993; Pirani et al., 2006; Mehrabian et al., 2007; Saghi et al., 2015), palynology (Tewari and Nair, 1979; Perveen and Qaiser, 1998; Simons and Chinnappa, 2004; Akan et al., 2005; Ekici et al., 2005; Pınar et al., 2009; Al-Ghamadi et al., 2013; Çeter et al., 2013; Bagheri et al., 2019) and micromorphology (Vural et al., 2008; Karaman et al., 2009; Mourad and Sharawy, 2010; Grohar et al., 2016; Metin et al., 2018). Mehrabian et al. (2007) studied the petiole anatomy of 24 *Astragalus* species belonging to the section *Incani* DC. from Iran and detected that petiole anatomy was an appropriate characteristic for taxonomical differentiation at species level. Haddad and Barnett (1989) investigated the 14 species of European spiny *Astragalus* in terms of petiole anatomy and they found two major groups of species according to anatomical properties. Pirani et al. (2006) examined the spine anatomy of 35 species of the genus *Astragalus* and these researchers detected that this character was suitable for restricting the small natural groups and species even if it was not helpful for delimiting sections in spiny *Astragalus*. The pod morphological and anatomical studies of 26 *Astragalus* taxa distributed in Egypt were carried out with light and scanning electron microscopies and also numerically investigated the inter- and the infra-specific relationships by Mourad and Sharawy (2010). Saghi et al. (2015) studied on anatomical properties of the leaflet and petiole of 20 *Astragalus* species within the section *Caprini* distributed in Iran and they reported that variations in these characters were not useful for delimitation and identification of this section's species.

Pınar et al. (2009) examined the palynology of 29 *Astragalus* species within the section *Onobrychoidei* DC. using LM and SEM from Turkey. The pollen of 15 *Astragalus* taxa of section *Hololeuce* Bunge from Turkey were studied by Çeter et al. (2013). The researchers reported that especially exine sculpturing was significant for discriminating the taxa each other. Also, Al-Ghamadi et al. (2013) noticed that pollen grains were the most useful features in their examined 13 *Astragalus* species, which were growing in Saudi Arabia. The pollen morphology of 22 species of *Astragalus* attributed to the section *Hymenostegis* Bunge distributed in Iran were studied by Bagheri et al. (2019) and they stated that although there were low variations in pollen grains between the species, the differences occured between the sections based on the pollen properties.

Karaman et al. (2009) investigated the micromorphology of leaflet belonging to the genus *Oxytropis* DC. in Turkey and it was determined that this character had a taxonomic value for supporting relationship between the examined taxa. The leaflet trichome micromorphology on the genus *Mimosa* L. was found to be diagnostic due to showing differentiation among the taxa by Grohar et al. (2016). Metin et al. (2018) studied the micromophologies of leaflet, pollen and seed of *Astragalus victoriae* Podlech & Agerer-Kirchhoff and *A. melanophrurius* Boiss. endemic to Turkey and stated that the surface pattern of trichomes was a distinctive feature in taxonomy of their studied species.

The anatomical, palynological and leaflet and fruit micromorphological characteristics of the section *Uliginosi* have not been investigated before. The objectives of present research are to examine and determine the anatomy, palynology, and leaflet and fruit micromorphologies of this section in Turkey and to evaluate the taxonomic importance of these features.

MATERIALS AND METHODS

Plant Materials

Research samples were collected from their various localities in Turkey at flowering and fruiting periods (Table 1) within the scope of Gazi University Research Fund (05/2010-54) and deposited at Gazi University, Faculty of Science Herbarium (GAZI).

Table 1. The localities data of the investigated *Astragalus* specimens

Species	Locality	Voucher Number
Astragalus falcatus	A9 Kars: Kağızman-Cumaçay 26. km, roadsides, 1800 m, 14.07.2012; 11.06.2010	*M.Ekici* 3148 *F.Özbek* 1007
Astragalus odoratus	B5 Kayseri: Erciyes University campus, 1100 m, 31.05.2011	*F.Özbek* 1023
	B6 Kayseri: Bünyan-Pınarbaşı 20. km, roadsides, 1491 m, 09.07.2002	*M.Ekici* 3024
	B9 Van: Y.Y.U., Faculty of education garden, 1640 m, 10.06.2010	*F.Özbek* 1001

Anatomical Analyses

The plant materials were also kept in 70% alcohol. Anatomical studies were made on the transverse sections of the stem, leaflet, petiole, and fruit using the paraffin method (Johansen, 1944) and by free hand. Free-hand sections were taken from the leaflet surface and some of the stem. Paraffin sections were stained with safranin-fast green and mounted using Entellan. Other sections were stained with Floruoglycine-HCl and then mounted with 10% glycerine. Photographs were taken with a Leica DM1000 digital photomicrograph system.

Micromorphological Analyses

The micromorphologies of the leaflet and fruit were investigated by SEM. Both surfaces of the leaflet and fruit were put on stubs, covered with gold coating and then photomicrographs were taken with a JEOL JSM 6060 SEM. The terminology was adopted from Karaman et al. (2009) and Mourad and Sharawy (2010) for leaflet and fruit micromorphological characteristics.

Palynological Analyses

Pollen slides were prepared according to Wodehouse's method (1935) for LM. Pollen grains were stained with glycerin-jelly plus safranin. The prepared pollen slides were investigated and measured using an Olympus CX31 Light Microscope with an ocular micrometer. Measurements were performed for at least 30 pollen grains for each pollen morphological property. For SEM studies, pollen were placed on stubs and then coated with gold. They were investigated and photographed with a JEOL JSM 6060 SEM. The terminologies of Erdtman (1969), Faegri and Iversen (1992), Punt et al. (2007) and also Pınar et al. (2009) were used.

RESULTS AND DISCUSSION

The representatives of the section *Uliginosi* are different in terms of the anatomical, palynological and micromorphological features of leaflet and fruit. Our findings are consistent with the molecular results of Kazempour Osaloo et al. (2003) and Barneby's hypothesis (1964) that *A. falcatus* is not closely related to other members of the Old World section group. Kazempour Osaloo et al. (2003) stated that the section *Uliginosi* is a paraphyletic group. The Eurasian *A. falcatus* unites with the North American *A. oreganus* Nutt. ex Torr. & A. Gray (and *A. canadensis* L. of section *Uliginosi*), rather than with Eurasian *A. odoratus*.

Anatomical and Micromorphological Characteristics

Stem anatomy

In transverse section, the stem of *A. falcatus* is subcircular like a wave structure with the cells of epidermis protruding outward in shape and there is a pith cavity in the centre of the stem whereas the stem of *A. odoratus* is ovate and there are large parenchymatic cells in the pith (Figure 1A, E). The epidermis is composed of single layer of rectangular cells with thick-walled and it is covered with a cuticle layer of 8–10 μm thickness in *A. falcatus* and 5–7 μm thickness in *A. odoratus*. The collenchyma tissue found just below the epidermis is 6–9 layered cells on the corners and 1–2 layered cells between the corners in *A. falcatus*, while it is 4–7 layered cells on the corners and 2–3 layered cells between the corners in *A. odoratus* (Figure 1B, F). The chlorenchyma tissue, which is between the corners under the collenchyma, is composed of 4–5 layers in *A. falcatus* and 2–3 layers in *A. odoratus* of parenchymatous cells having many chloroplasts. Multilayered cortex has oval parenchymatous cells. The endodermis is located above the vascular bundles (Figure 1C, G). Vascular bundles are of the collateral type and they are larger at the corners than the others. The sclerenchymatical ring is situated above the phloem. The cambium is distinguishable and 2–4 layered cells. The xylem consists of trachea and tracheids (Figure 1D, F).

Leaflet anatomy and micromorphology

In transverse section, the leaflets of *A. falcatus* and *A. odoratus* have upper and lower epidermis covered by a thin cuticle consisting of uniseriate, thin-walled and differently-sized cells. The leaflets of two species are isobilateral, whereas the leaves in this family are usually dorsiventral and less frequently isobilateral (Metcalfe and Chalk, 1957) and also amphistomatic (Figure 2A, E). The palisade tissue under the upper epidermis is 2–3 layered cells, above the lower epidermis is 2 layered cells. The thickness of mesophyll is 110.25 ± 7.16 μm in *A. falcatus*, whereas in *A. odoratus* it is 168.34 ± 15.32 μm. There is a large median vascular bundle. Vascular bundles are surrounded by squarish or big parenchymatic cells. Small lateral bundles on the sides mostly vertically transcurrent (Figure 2B, F). Stomata are anomocytic and they are more abundant on the abaxial surface of the epidermis. They are placed at the same level as the epidermis in *A. falcatus* and slightly below the level of the epidermal cells in *A. odoratus*. Metcalfe

and Chalk (1957) stated that the hairs are non-glandular and equally or unequally two armed in species of *Astragalus*. The findings of this study are consistent with theirs. Trichomes are bifurcate, 0.3–0.5 mm long, verrucate, 3.32 ± 0.42 wart in 100 μm^2; 3–4 on the adaxial surface and 8.21 ± 2.06 mm^2 on the abaxial surface in *A. falcatus*. Similarly in *A. odoratus*, trichomes are bifurcate, 0.3–0.5 mm long, verrucate, 2.35 ± 0.45 wart in 100 μm^2; 3–4 on the adaxial surface and 13.75 ± 2.25 mm^2 on the abaxial surface (Figures 2C, D, G, H, 3, 4).

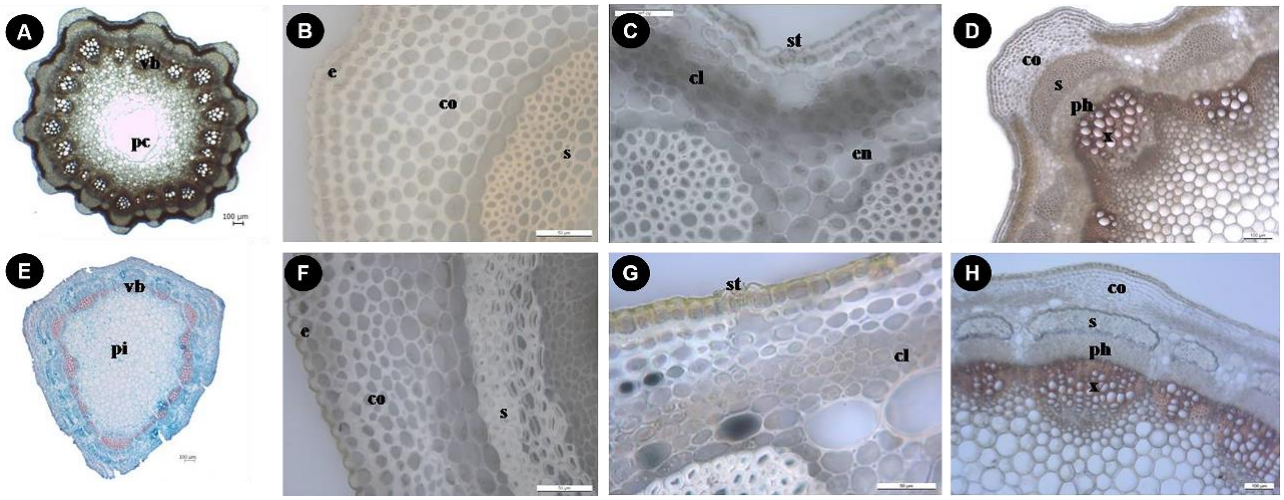

Figure 1. Transverse sections of the stem of *Astragalus falcatus* (A–D) and *A. odoratus* (E–H). cl: chlorenchyma, co: collenchyma, en: endodermis, e: epidermis, pc: pith cavity, ph: phloem, pi: pith, s: sclerenchyma, st: stoma, x: xylem, vb: vascular bundle

Karaman et al. (2009) were found to be various ornamentation in trichomes of *Oxytropis*. The common type was striate-verrucae, but in *O. persica* Boiss. and *O. engizekensis* H. Duman and Vural different surface patterns were determined between adaxial and abaxial surfaces and between the other taxa. In our study, only verrucate ornamentation on the trichome surface was observed in two species.

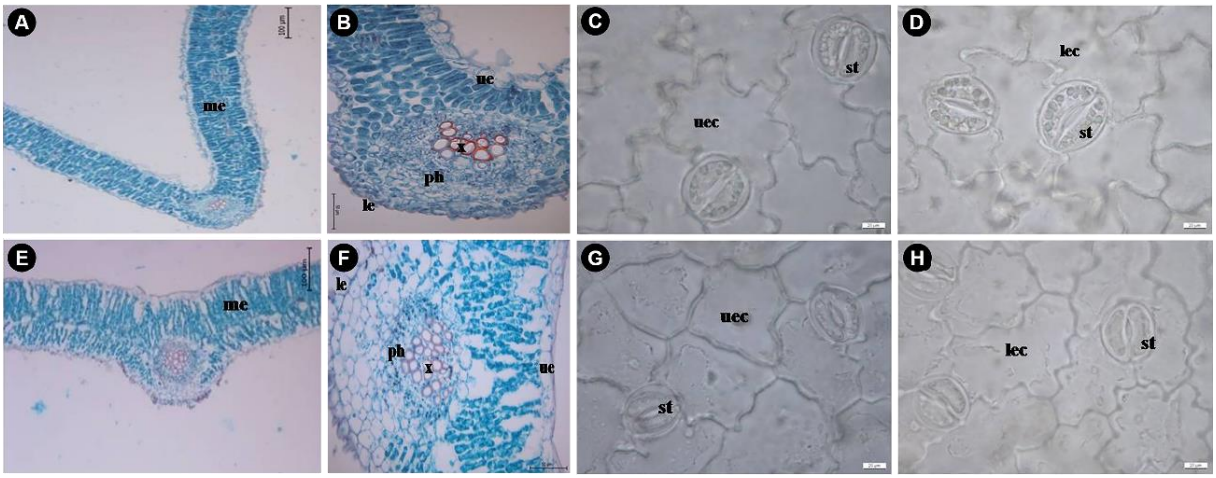

Figure 2. Transverse and surface sections of the leaflet of *Astragalus falcatus* (A–D) and *A. odoratus* (E–H): general view (A, E), the midrib (B, F), stomata on leaflet adaxial surface (C, G), stomata on leaflet abaxial surface (D, H). le: lower epidermis, lec: lower epidermis cell, me: mesophyll, ph: phloem, st: stoma, ue: upper epidermis, uec: upper epidermis cell, x: xylem

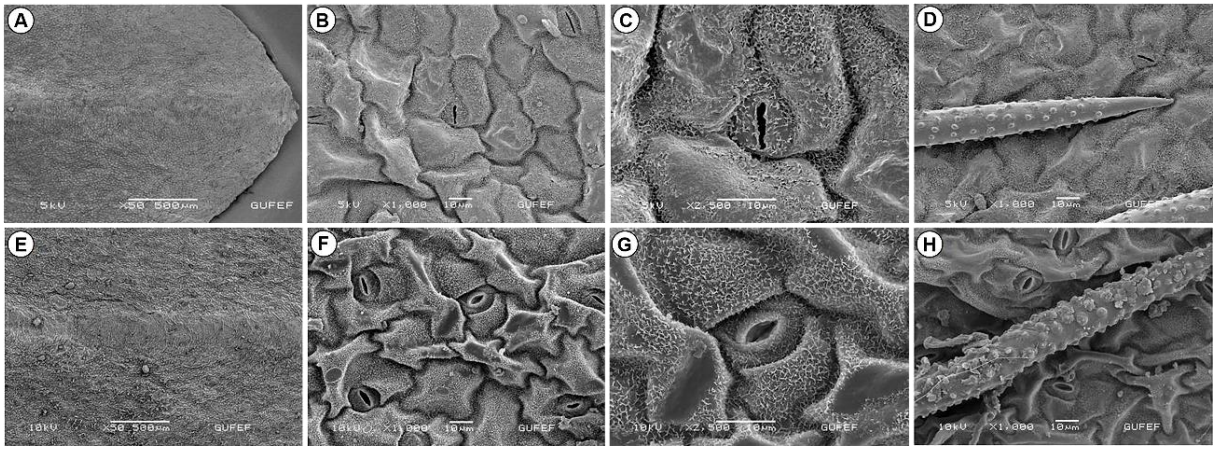

Figure 3. Scanning electron microscopy micrographs of adaxial surfaces of leaflets of *Astragalus falcatus* (A–D) and *A. odoratus* (E–H): general view (A, E), higher magnification of surfaces (B, F), stoma (C, G), parts of trichome (D, H)

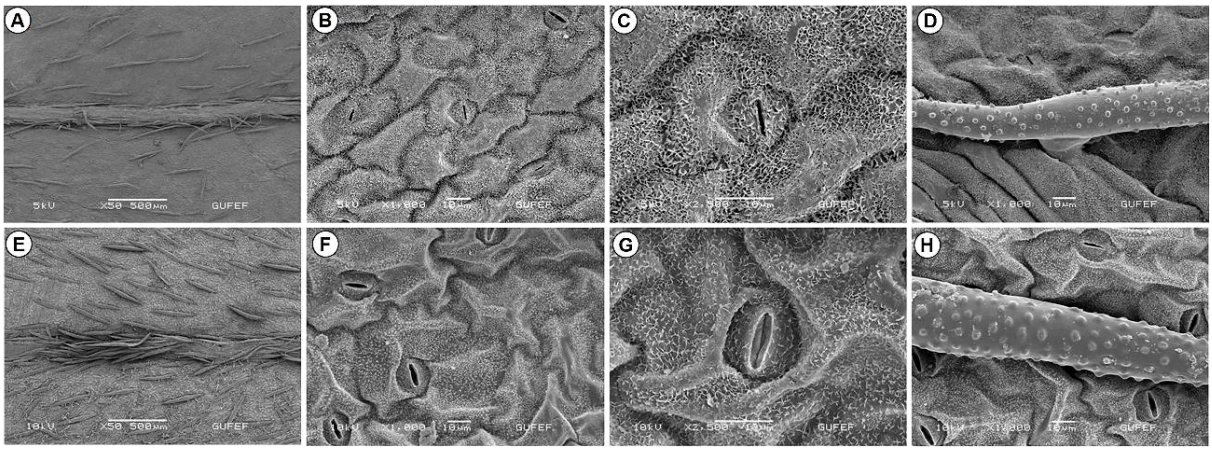

Figure 4. Scanning electron microscopy micrographs of abaxial surfaces of leaflets of *Astragalus falcatus* (A–D) and *A. odoratus* (E–H): general view (A, E), higher magnification of surfaces (B, F), stoma (C, G), parts of trichome (D, H)

Petiole anatomy

In transverse section, the petiole of *A. falcatus* is subcircular like a wave structure in that the cells of epidermis are protruding outward, while it is U-shaped in *A. odoratus* (Figure 5A, C). Haddad and Barnett (1989) reported that the variations in petiole outline and vascular bundles number between the their studied spiny *Astragalus*. It was found that the outline of petiole varied from subcircular to semiangular and the vascular bundles ranged from 5 to 11. Also, Mehrabian et al. (2007) recognized that the shape of cross section of the petiole varied from elliptic-ovate (*A. fridae* Rech. f., *A. homandicus* Maassoumi & Podlech), to broadly ovate (*A. punctatus* Bunge, *A. subalpinus* Boiss. & Buhse) to orbicular (*A. gudrunensis* Boiss & Hausskn., *A. supervisus* (Kuntze) Sheld.). The epidermis is formed of one layer of circular to rectangular shaped cells. The chlorenchyma tissue, between the large vascular bundles, is composed of 2–4 layers of parenchymatous cells having many chloroplasts. The cortex consists of 7–10 layers (*A. odoratus*) and 5–9 layers (*A. falcatus*) of differently-sized oval or amorphous parenchymatous cells. There are four large and four small vascular bundles in *A. falcatus*, whereas *A. odoratus* has three large and two small vascular bundles. The 4–6 layered sclerenchyma cells are located above the phloem (Figure 5B, D).

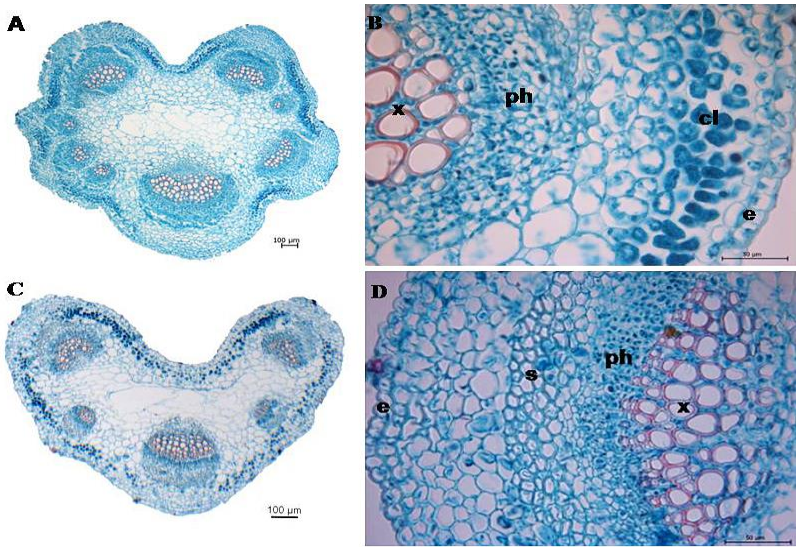

Figure 5. Transverse sections of the petiole of *Astragalus falcatus* (A, B) and *A. odoratus* (C, D): general view (A, C), enlargement showing internal structure (B, D). cl: chlorenchyma, e: epidermis, ph: phloem, s: sclerenchyma, x: xylem

Fruit anatomy and micromorphology

In transverse section, the fruits of *A. falcatus* and *A. odoratus* show the pericarp to have the following characteristics. The exocarp is composed of thin-walled, rectangular epidermis cells with a cuticle. The mesocarp consists of 3–7 layers (*A. falcatus*) and 5–7 layers (*A. odoratus*) of thin-walled parenchymatous cells of different sizes. Collateral vascular bundles are embedded into the mesocarp. The endocarp is composed of 2–4 layers (*A. falcatus*) and 4–6 layers (*A. odoratus*) of radial and tangential elongated sclerenchymatic cells. The inner epidermis is a single layer on the endocarp (Figure 6A, E). The surface ornamentation of the fruit is reticulate in two species. The thickness of the muri is 6.09 ± 0.38 µm, lumen size is 23.81 ± 5.73 µm in *A. falcatus*, while the thickness of the muri is 6.65 ± 0.42 µm, lumen size is 17.24 ± 2.15 µm (Figure 6B, F). There are few anomocytic stomata and ornamented verrucate, 4.71 ± 0.48 wart (*A. falcatus*) and 4.09 ± 0.5 wart (*A. odoratus*) in 100 µm^2, bifurcate trichomes (Figure 6C, D, G, H).

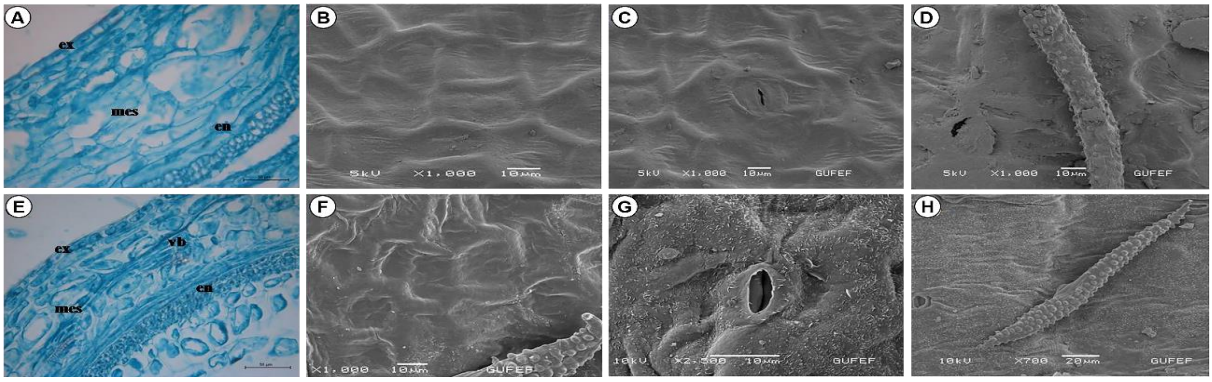

Figure 6. Fruit anatomy and micromorphology of *Astragalus falcatus* (A–D) and *A. odoratus* (E–H): transverse section of the fruit showing pericarp structure (A, E), reticulate sculpture under SEM (B, F), stoma under SEM (C, G), parts of trichome under SEM (D, H). en: endocarp, ex: exocarp, mes: mesocarp, vb: vascular bundle

In terms of fruit anatomy no significant differences are found between the two species. Our results are consistent with those of Mourad and Sharawy (2010). Moreover they noticed not only reticulate but also irregular reticulate, scalariform and rugose ornamentation in their studied *Astragalus* species. In contrast them, the fruit surface only shows reticulate ornamentation in our investigated two species.

Pollen Morphological Characteristics

The pollen grains of *A. falcatus* and *A. odoratus* are isopolar and radially symmetrical. Simons and Chinappa (2004) stated that the aperture type of *Astragalus* is generally tricolporate and also Akan et al. (2005) found only tricolporate apertures in the section *Alopecuroidei* DC. Our results are congruent with these researchers' findings. Pollens of the *Uliginosi* section are homogeneous and they are tricolporate.

The shape of them is prolate-spheroidal with the polar axis 24.53 ± 0.8 µm and the equatorial axis 21.82 ± 0.9 µm in *A. falcatus*. In *A. odoratus*, the sizes of the polar axis and equatorial axis are 26.50 ± 0.94 µm and 22.70 ± 0.78 µm, respectively. Colpi are long and narrow with clear margins. Pores are lalongate in *A. falcatus*, whereas they are 50% circular and 50% lalongate in *A. odoratus*. The membrane of the apertures is granulate. The exine is subtectate; the ectexine is thicker than the endexine. The exine ornamentation of pollen grains of the two species is different. In *A. falcatus* the ornamentation is microreticulate in the meridional and polar optical sections and it is 90% microreticulate, 5% microrugulate and 5% perforate in equatorial optical section; 95% microreticulate-perforate, 5% perforate in polar optical section in *A. odoratus* (Figures 7, 8). While the general ornamentation in *Astragalus* species is microreticulate (Simons and Chinappa, 2004), the studied *A. odoratus* shows considerable variation in ornamentation type. The heteromorphy in pollen grains is based on some variations in ornamentation type (Mukherji, 1951; Nair and Kaul, 1965; Sharma, 1967; İnceoğlu, 1973). Oskouian et al. (2007) commonly observed reticulate exine sculpturing and seldom verrucate, Pınar et al. (2009) found microreticulate, but rarely reticulate or rugulate sculpturing in the equatorial region, psilate and perforate sculpturing in the polar region, and Çeter et al. (2013) noticed reticulate, perforate in polar section and perforate, reticulate, microreticulate, perforate-granulate, microreticulate-perforate, microrugulate-perforate, microrugulate-microreticulate, granulate-perforate, microreticulate-granulate sculpturing in equatorial region. Exine sculpturing on equatorial and polar optical sections has a systematic value for distinguishing taxa from each other. The detailed pollen morphological characters of two species are given Table 2.

Table 2. Pollen morphological data of *Astragalus falcatus* and *A. odoratus* (values in µm)

Species/ Characters		***Astragalus falcatus***	***Astragalus odoratus***
Polar Axes	Min.	23.07	24.6
	Max.	26.7	28.3
	Mean	24.5	26.5
Equatorial Axes	Min.	19.5	20.9
	Max.	23.3	24.2
	Mean	21.8	22.7
P/E ratio, Pollen Shape		1.12, Prolate-spheroidal	1.16, Prolate-spheroidal
Aperture Type		Trizonocolporate	Trizonocolporate
Ornamentation	Meridional	Microreticulate	90% Microreticulate, 5% Microrugulate, 5% Perforate
	Polar	Microreticulate	95% Microreticulate-perforate, 5% Perforate
Colpus (Cl)	Clg	20.97 ± 0.69	20.81 ± 0.78
	Clt	4.1 ± 0.35	5.11 ± 0.63
Pore (Pl)	Plg	6.56 ± 0.34	7.05 ± 0.55
	Plt	8.14 ± 0.33	8.43 ± 0.47
Exine thickness		0.43 ± 0.02	0.63 ± 0.09
Intine thickness		0.37 ± 0.02	0.47 ± 0.05

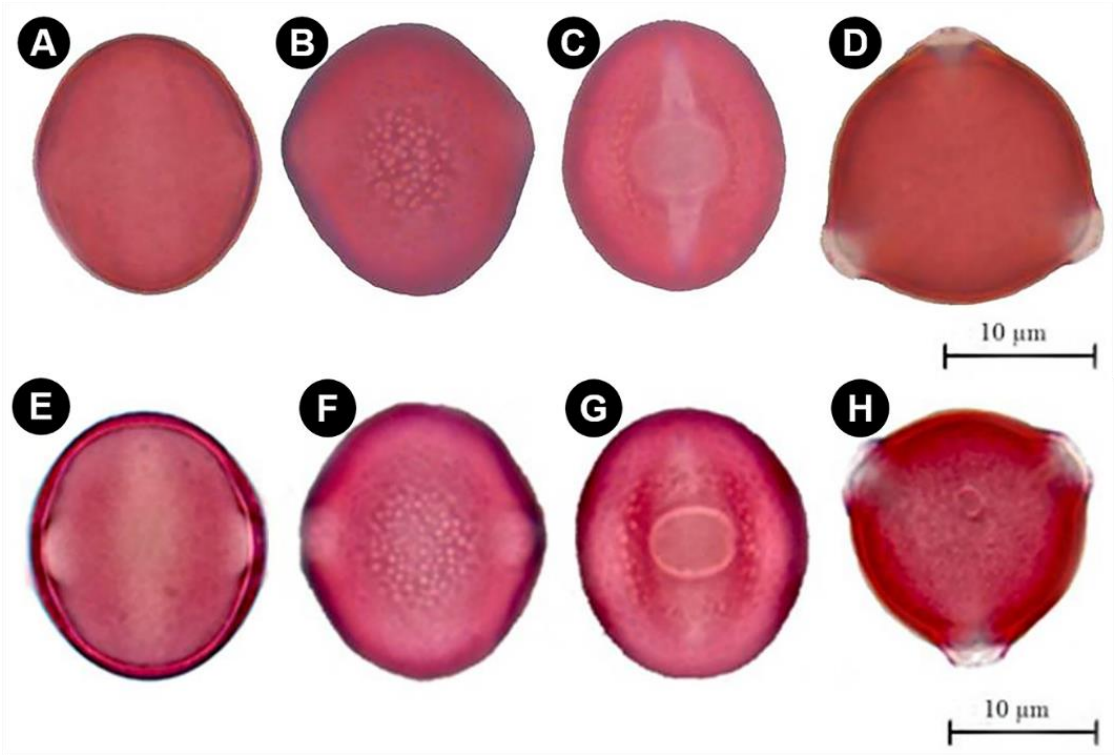

Figure 7. LM micrographs of pollen grains of *Astragalus falcatus* (A–D) and *A. odoratus* (E–H): equatorial view (A, E), ornamentation (B, F), apertures (C, G), polar view (D, H)

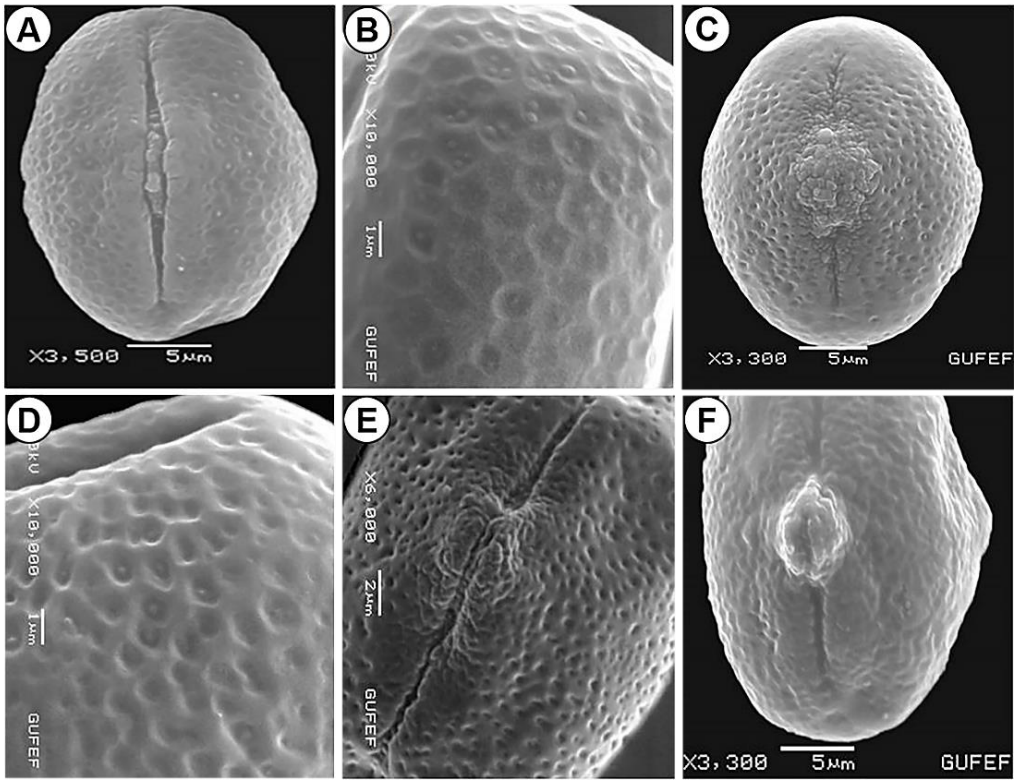

Figure 8. SEM micrographs of pollen grains of *Astragalus falcatus* (A, B) and *A. odoratus* (C–F): equatorial view and apertures (A, C), microreticulate ornamentation (B, D), perforate ornamentation (E), rugulate ornamentation (F)

CONCLUSION

The shape of stem and petiole, the number of vascular bundles in petiole and exine sculpturing at equatorial and polar regions of pollen grains have showed variations among the our studied species. Therefore, according to our findings from anatomy, palynology and micromorphology of 2 species of

the genus *Astragalus* belonging to the section *Uliginosi*, the stem and petiole anatomy and also exine ornamentation are valuable characteristics for distinguishing the species. Similarly, Haddad and Barnett (1989), Mehrabian et al. (2007), Pınar et al. (2009) and also Çeter et al. (2013) observed differences in these properties in their investigated *Astragalus* taxa and stated the importance of them for separating the taxa. As there is no significant differences in the micromorphologies of leaflet and fruit between the two species, these properties are not useful for the discrimination of species at species rank.

ACKNOWLEDGEMENTS

The authors thank to Gazi University Research Fund (05/2010-54) for its financial contribution.

Conflict of Interest

The 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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