MELLIFERA RESEARCH ARTICLE

Micromorphological Characters of Pollen, Leaflet and Seed of *Astragalus victoriae* and *Astragalus melanophrurius* Endemic to Turkey

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Received: 13th September, 2018; accepted: 15th October,2018; published: 30th November,2018

A B S T R A CT

In this study, palynological, seed and leaflet micromorphological properties of *Astragalus victoriae* and *Astragalus melanophrurius* species were investigated in detail using light microscopy (LM) and scanning electron microscopy (SEM). The aim of this study is to determine the contribution of pollen, leaflet and seed morphology characters in distinguishing between these two closely related species. Study results indicate that pollen of species are radial symmetric and isopolar. Aperture type is trizonocolporate. Pollen shape is determined as prolate-spheroidal for *Astragalus melanophrurius* and subprolate for *Astragalus victoriae*. Ornamentation type is observed as perforate at polar area and aperture surround, microreticulate at the other sides of pollen for *A. melanophrurius* while ornamentation determined as rugulate at polar area and aperture surround, microreticulate at the other sides of pollen for *Astragalus victoriae*. Seeds shape are reniform, the colour is light brown and surface ornamentation determined as psilate in light microscope for both species while reticulate-rugulate with thin and irregular muri determined in *Astragalus victoriae* and reticulate surface ornamentation with thick and regular muri in *Astragalus melanophrurius* in SEM micrograph. Leaflet observed as elliptic-ovate, emarginated with dens single celled trichomes on lower surface for *A. melanophrurius* while narrowly elliptic, apiculate, with densely single-celled trichomes on lower surface leaflet observed for *A. victoriae*. Trichomes have scabrate ornamentation at *A. melanophrurius* and striate ornamentation at *A. victoriae*. On the lower surface of the leaflet of *A. victoriae* 240 stomas and on the upper surface 263 stomas were detected in 1 mm^2. Wile on the lower surface of the leaflet of *A. melanophrurius* 170 stomas and on the upper surface182 stomas were detected in 1 mm^2. According to results pollen shape, pollen surface ornamentation, seed surface ornamentation, leaflet shape, trichome ornamentation, number of stomata per 1 mm^2 determined as important micromorphological character for systematical delimination of two endemic species.

Keywords: *Astragalus*, pollen, leaflet, seed morphology

Introduction

Fabaceae is a large family of Fabales, which includes the shrubs and tree species and most of the herbaceous plants. According to recent studies, Fabaceae family includes 727 genera and 19325 taxa [1].

Fabaceae is the third largest family of Angiospermae after the family Compositae and Orchidaceae in the world [2].

There are approximately 136 sections and 2400 taxa of the *Astragalus* belonging to Fabaceae family in the world. The gene center is Eurasia. Especially, Central Asian steppe, Iran and Turkey are places where the genus shows wide spread. There are 63 sections and 480 taxa belonging to the genus *Astragalus* in Turkey [3].

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Astragalus victoriae Podlech Et Agerer-Kirchhoff and *A. melanophrurius* Boiss. are endemic for Turkey. IUCN Red List category of *A.victoriae* is CR (Critically Endangered) and *A. melanophrurius* is NT (Near Threatened) [4] . *A.victoriae* is belongs to Christianophyta, *A. melanophrurius* belongs to *Astragalus* section. The *Christianophyta* section is monotypic [5].

Some of the thorny species of *Astragalus* are used in the production of "Kitre" called "Gum Tragacantha". In our country, especially *A. microcephalus* Willd. [6] have been obtained.Various species of *Astragalus* spp. is the main source of nectar in the mountains [7]. The studies show that, *Astragalus* species are the main plant which honey bees benefit as pollen, nectar and propolis sources [8, 9].

The pollen morphology of sections *Ammodendron* Bunge, *Onobrychoidei* DC., *Allopecuroidei* DC. and *Hololeuce* Bunge and *Poterion* Bunge of the *Astragalus* and *Oxytropis* distributed in Turkey was studied in detail [10, 11, 12, 13, 14,15].

Domestic and foreign scientists are collecting *A.victoriae* and *A. melanophrurius* specimens from Aksaray-Konya and around them in Turkey. However, due to similarities like cream - white flowers and habitus, they are mixed each other and it is difficult to diagnose these two species. For this reason, it is extremely important to reveal the distinctive features of these two types which spread in similar areas and endemic for Turkey.

In this study, palynological, seed and leaflet morphology studies were carried out on the two close endemic *Astragalus* species *Astragalus victoriae* and *Astragalus melanophrurius* of Turkey. The aim of the study is to compare and systematically separate this similar two species from each other with regard to pollen, leaflet and seed morphology.

Materials and Methods

Plant Material

Astragalus victoriae and *A. melanophrurius* Boiss. were collected and used both with flowers and fruits between April and October of 2017.

Aksaray: 32 km from Aksaray to Nevşehir, road sides, 1252 m, 16.05.2017, H.Metin 1089 & S.Karaman.

Aksaray: Aksaray-Adana road, arround the Karacaören village, road sides,, 1132 m, 15.06.2017 , S. Karaman 3043 & H. Metin.

Seed Micromorphology

For the character analysis of the seeds, 20 different samples were measured. The weights of 50 seeds were measured and the averages were taken. The microphotographs of the seeds were taken with Leica S8APO digital imaging stereomicroscope. Measurement of each character taken from 20 different seed. In addition, in order to investigate the seed surface in detail, seed of taxa replaced on aluminium stabs and examined with scanning electron microscope (SEM).

Leaf Surface Micromorphology

The microphotographs of the leaf were taken with Leica S8APO digital imaging stereomicroscope. The leaf of taxa replaced on aluminium stabs and examined with scanning electron microscope (SEM).

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Palynological studies

Pollen slides were prepared according to the Wodehouse [16] method and examined with light Leica DM3000 Digital Imaging Microscope. For each character measurement taken from 20 different pollen. In addition, in order to investigate the pollen in more detail, we have worked with scanning electron microscope (SEM) at Kastamonu University SEM Laboratory.

Results and Discussion

A. victoriae is close to *A.melanophrurius*, but differs it by having 14-19 pairs, narrowly elliptic , 13-18 (-20) x 3.5 mm, apiculate leaflets (not 17-27 pairs, ovate to elliptic, 5-13x5-8 mm, emarginate); glabrous style (not hairy in lower third); bracts 9-11 mm (not 5-7 mm); pedicels 1 mm (not 2-2.5 mm) and calyx inflated at maturity (not inflated) (Figure 1).

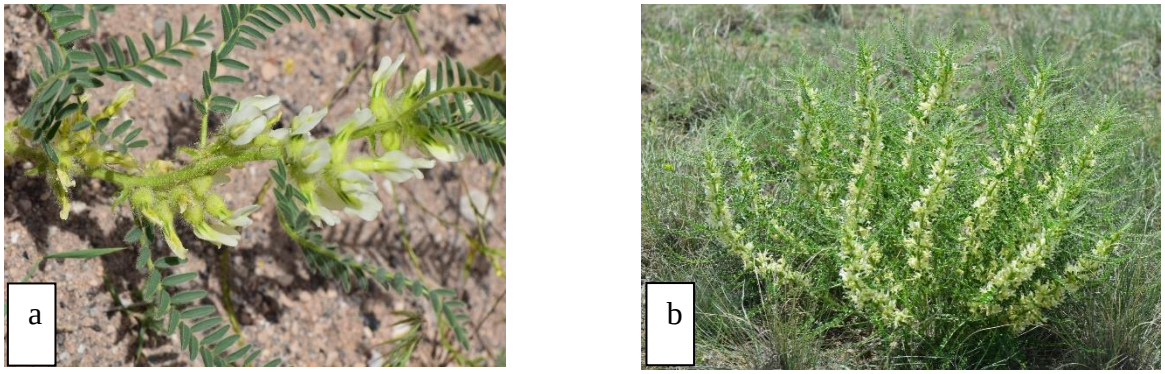

Figure 1. Habit of *Astragalus victoriae* (a) and *Astragalus melanophrurius* (b)

The pollen of *Astragalus melanophrurius* is radial symmetric and isopolar. Aperture type is trizonocolporate. Ornamentation type is perforate at polar area and aperture surround, microreticulate at the other sides of pollen. Polar axis (P) measured as 28.7 ± 1.5 µm, equatorial axis (E) 25.3 ± 1.4 µm and pollen shape is determined as prolate-spheroidal (P/E; 1.13).

Colpus is long-acute, operculate and operculum membrane granulat

Length of colpus (Clg) is measured as 22.3 ± 1.2 µm and latitude of colpus (Clt) is 3.6 ± 0.3 µm. (Figure 2).

The pollen of *Astragalus victoriae* is radial symmetric and isopolar. Aperture type is trizonocolporate. Ornamentation type is rugulate at polar area and aperture surround, microreticulate at the other sides. Polar axis (P) measured as 28.0 ± 1.35 µm, equatorial axis (E) 23.2 ± 1.2. Pollen shape is determined as subprolate (P/E; 1.204). Colpus is long-acute, opreculate and operculum membrane granulat. Clg measured as 19.0 ± 1.1 µm and Clt is 2.3 ± 0.4 µm (Figure 3).

Karaman Erkul et al. (2017) similarly determined radial symmetric, isopolar, trizonocolporate aperture type, prolate shape in *A. bruguieri* and subprolate shape *A. ruselli* belong to section *Poterion*. Pollen ornamentation determined as microreticulate in *A. ruselli* and perforate at polar area and microreticulate at equatorial area in *A. bruguieri*. Pinar et al. (2009) studied morphology of the genus *Astragalus* L. of the section Onobrychoidei

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DC. distributed in Turkey. Pollen of taxa observed as 3-colporate, prolate, subprolate, and prolate-spheroidal. Ornamentation is usually microreticulate, rarely reticulate or rugulate in the equatorial area and psilate and perforate in the polar area. Çeter et al. (2013) studied pollen morphology of 15 taxa belonging to the section Hololeuce Bunge of genus *Astragalus* L. The pollen grains of Hololeuce taxa observed as radially symmetric, isopolar, prolate–spheroidal, subprolate or prolate operculate and trizonocolporate. Pollen grains show reticulate, perforate polar section and perforate, reticulate, microreticulate, perforate–granulate, microreticulate–perforate, microrugulate–perforate, microrugulate–microreticulate, granulate–perforate, microreticulate–granulate at equatorial area. Study results emphasize that especially pollen surface ornamentation on polar section and meridional section has systematic value to distinguish taxa from each other.

Seeds shape are reniform. The colour is light brown. Surface ornamentation determined as psilate in light microscope, reticulate-rugulate with thin and irregular muri in *Astragalus victoriae* while with thick and regular muri in *Astragalus melanophrurius*in SEM micrograph. Sizes are ranging between 3.33-3.78x2.50-2.76 mm for *Astragalus victoriae* and 3.27-3.99x2.32-2.58 mm for *Astragalus melanophrurius* (Figure 4). Karaman Erkul et al. (2017) observed similar seed shape, size, colour and ornamentation for *A. bruguieri* and *A. ruselli*.

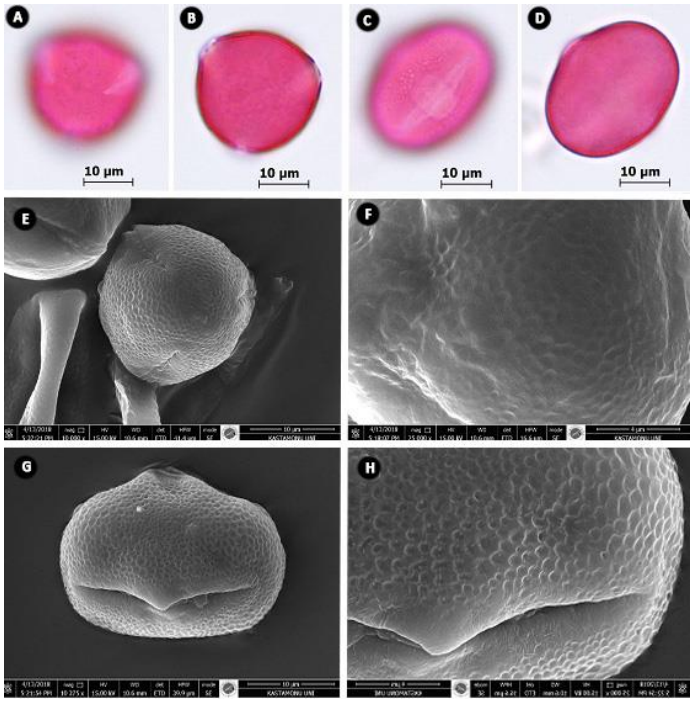

Figure 2. Pollen micrograph of *A. melanophrurius.* A-D; Light micrograph, E-H; SEM micrograph, E-F; Polar view and ornamentation, G-H; Equatorial view and ornamentation.

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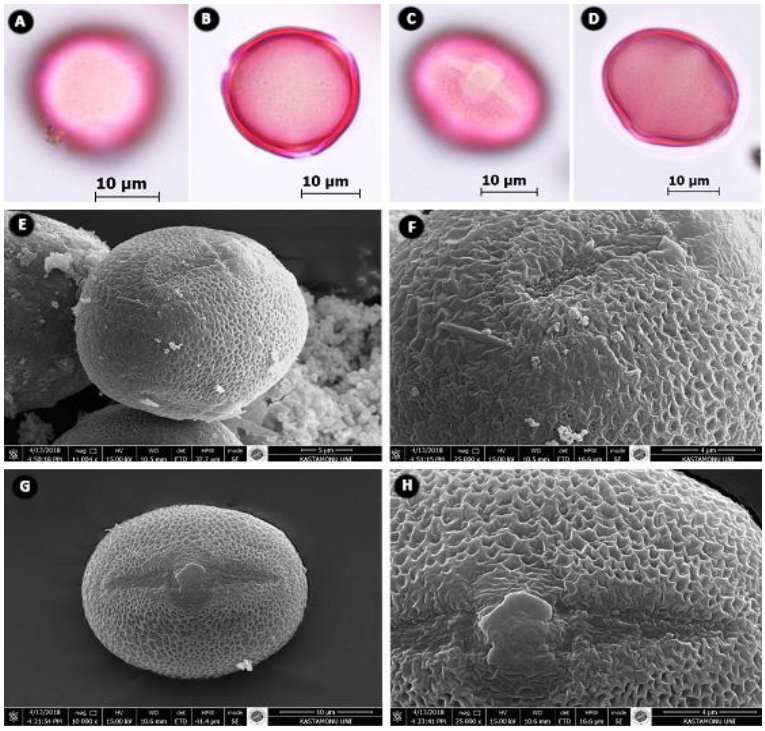

Figure 3. Pollen micrograph of *A. victoriae.* A-D; Light micrograph. E-H; SEM micrograph, E-F; Polar view and ornamentation, G-H; Equatorial view and ornamentation

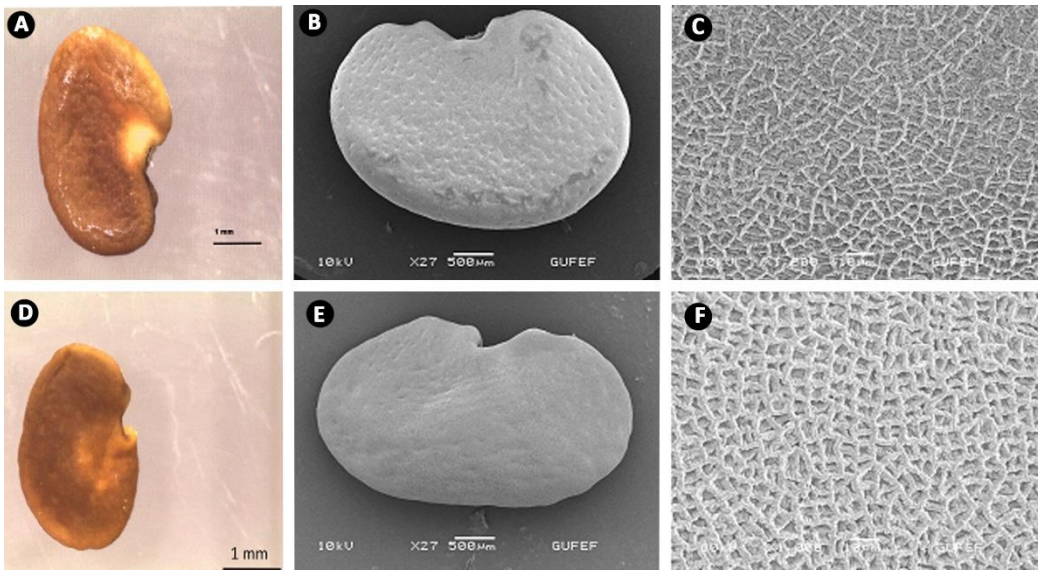

Figure 4. Seed of *A. victoriae* and *A.melanophrurius.* A-C: *A. victoriae* A; LM micrograph, B; General view with SEM, C; Ornamentation with SEM, D-F: *A.melanophrurius*, D; LM micrograph, E; General view with SEM, F; Ornamentation with SEM,

Leaflets are amphistomatic in terms of stoma, and the number of stoma on the upper and lower surface may show differences. The stomata are usually mesomorphic on the lower surface and on the upper surface. On the lower surface of the leaflet of *A. victoriae* 240 stomas and on the upper surface 263 stomas were detected in 1 mm^2. Wile on the lower surface of the leaflet of *A. melanophrurius* 170 stomas and on the upper surface182 stomas were detected in 1 mm^2.

A. melanophrurius Leaflet observed as elliptic-ovate, 5-13 x 5-8 mm, emarginate, densely single-celled trichomes were found on the lower surface of the leaflet and upper surface is glabrous while *A. victoriae* Leaflet observed as narrowly elliptic, 13-18(-20)x3.5 mm, apiculate, densely single-celled trichomes were found on the lower surface of the leaflet and upper surface is glabrous. Trichomes have scabrate ornamentation at *A. melanophrurius* and striate ornamentation at *A. victoriae.*

Very dense epicuticular wax is present on the lower and upper surfaces of the leaves in both species (Figure 5).

Fruit character is very important in distinguishing two species. The fruit of *A. victoriae* is widely ovoid to subglobose and included in the calyx and strong coriaceous, fruit of *A. melanophrurius* is narrowly ellipsoid, not included in the calyx and leather like (Figure 9).

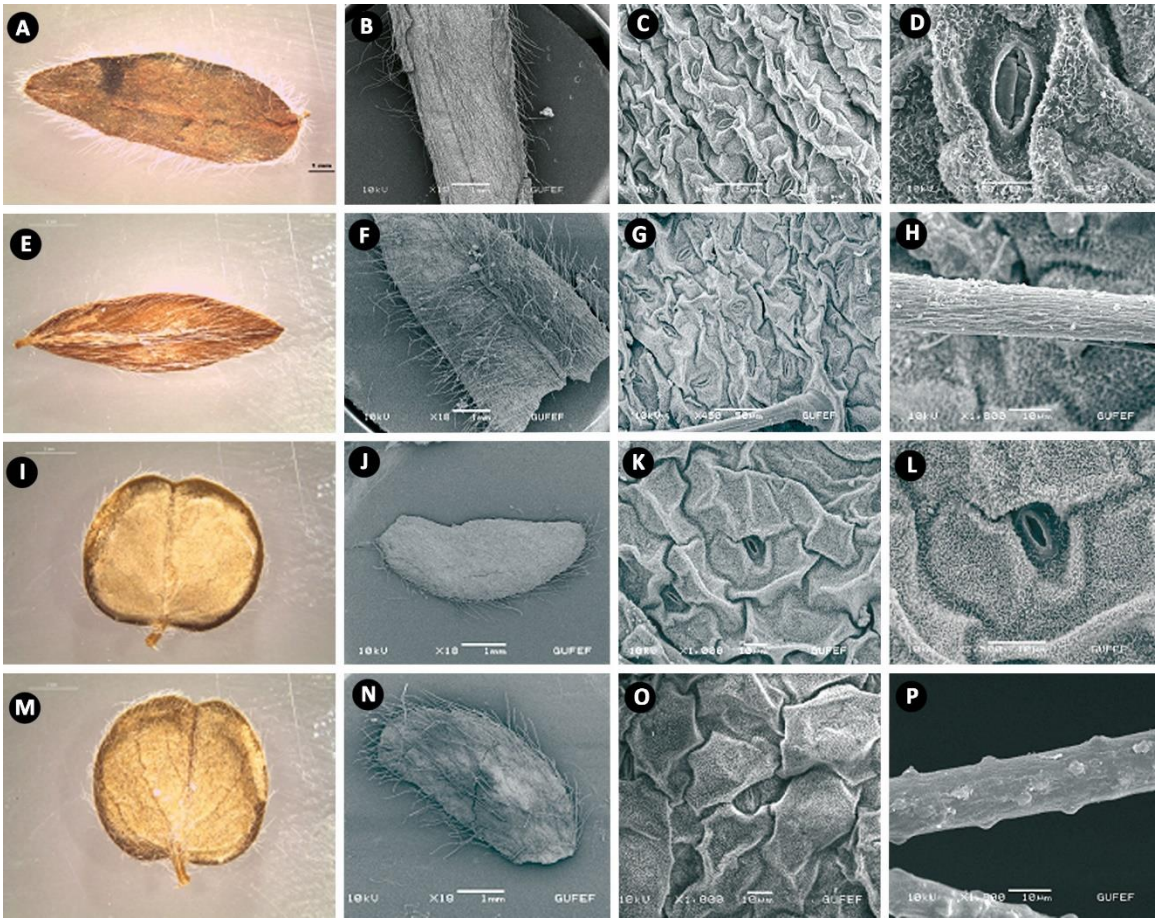

Figure 5. LM and SEM micrograph of *Astragalus* leaflets. A-H: *A. victoriae,* A-D; Upper surface, E-H; Lower surface, I-P: *A.melanophrurius,* I-L; Upper surface, M-P; Lower surface.

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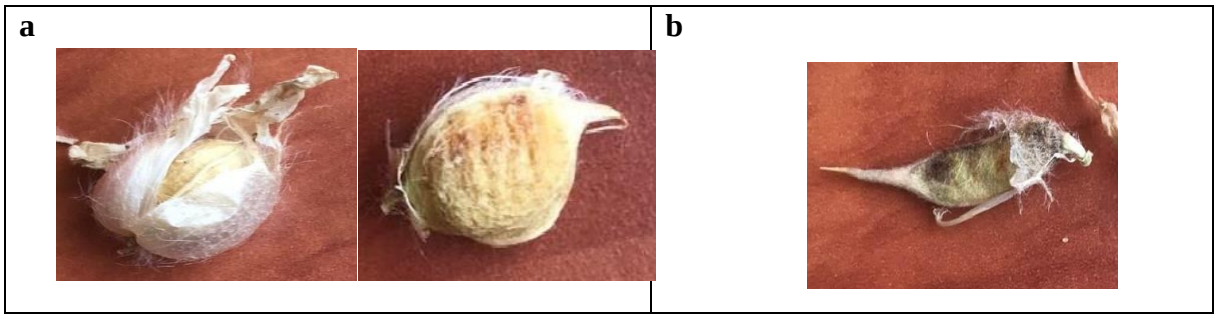

Figure 6. Legume of *A. victoriae* (a) and *A.melanophrurius* (b)

Conclusion

Morphologically, the characters such as being the plants decumbent or erect, leaflet shape, fruit shape, fruit is in calyx or not are can distinguish the two species at first. Seed size, colour, weight, surface ornamentation, pollen size, shape and surface ornamentations were identified as important and distinguishing characters for systematics of studied taxa. There was no significant difference between the two species in terms of seed characteristics. The leaflet sizes, shape, and having striate-granulate ornamentation on the trichomes at *A. melanophrurius*, striate ornamentation at *A. victoriae* is distinctive. Pollen size, shape and surface ornamentation are described as important and distinctive characters for the systematic study of the examined taxa.

Acknowledgement:

This study was supported by Scientific Research Projects Unit (Project code: 2017-027) from Aksaray University and TUBITAK (Project code: TBAG-110 T 911).

Türkiye'ye endemik *Astragalus victoria* ve *Astragalus melanophrurius* türlerinin polen, yaprakcık ve tohum mikromorfolojik karakterlerinin incelenmesi

Öz: Bu çalışmada, Türkiye'ye endemik *Astragalus victoriae* ve *Astragalus melanophrurius* türlerinin polen morfolojisi ile tohum ve yaprakçık mikromorfolojisi ışık mikroskobu (LM) ve taramalı elektron mikroskobu (SEM) ile ayrıntılı olarak çalışılmıştır. Çalışma sonuçları iki türün de polenlerinin radial simetrili, izopolar ve trikolporat olduğunu göstermektedir. Polen şekli *A. melanophrurius* polenlerinde prolat sferoidal ve *A. victoriae* polenlerinde subprolat olarak belirlenmiştir. *A. melanophrurius* polenlerinde ornamentasyon kutup bölgesi ve apertür çevresinde perforat diğer kısımlarda mikroretikülat olarak belirlenirken, *A. victoriae* polenlerinde kutup bölgesi ve apertür çevresinde rugulat diğer kısımlarda mikroretikülat olarak belirlenmiştir. Her iki türün tohumları böbrek şekilli, açık kahve renginde ve yüzeyleri ışık mikroskop fotoğraflarında psilat olarak belirlenmiştir. SEM fotoğraflarında ise *A. victoriae* tohum yüzey ornametasyonu ince ve düzensiz muriye sahip rugulat-retikülat olarak belirlenirken *A. melanophrurius* tohum yuzey ornamentasyonu kalın ve düzenli muriye sahip retikülat olarak belirlenmiştir. Yaprakçıklar *A. melanophrurius*'ta eliptik-

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ovat, emarjinat ve alt tarafı yoğun tek hücreli basit tüylü olarak belirlenirken, *A. victoriae*'de dar eliptik, apikulat ve at tarafı yoğun tek hüreli tüylu olarak saptanmıştır. Tüy yüzeyi *A. melanophrurius*'ta skabrat ve *A. victoriae*'de sitriat olarak belirlenmiştir. Bu sonuçlara göre polen şekli, polen yüzey ornamentasyonu, tohum yüzey ornamentasyonu, yaprakçık şekli, trikom yüzey ornamentasyonu ve birim alandaki stoma sayısı iki türün sistematik ayırımında kullanılabilecek onemli mikromorfolojik karakterler olarak belirlenmiştir.

Anahtar Kelimeler: *Astragalus*, polen, yaprakçık, tohum morfolojisi

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