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Research Article

Morpho-anatomical and palynological properties of endemic *Astragalus tmoleus* var. *bounacanthus* (Fabaceae)*

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ABSTRACT

Astragalus tmoleus Boiss. var. *bounacanthus* (Boiss.) Chamberlain is a member of the *Pterophorus* Bunge section of the Fabaceae family and consists of the spiny cushion-forming shrub. In this study, morphological, anatomical and pollen properties of the plant, an endemic species for Turkey, have been investigated in detail. Morphological characters such as the shape of paripinnate leaves, stipules and bracts, the flower number in the inflorescence, the stenonychioid type of standard and the reniform type of seed can be helpful to distinguish *A. tmoleus* var. *bounacanthus*. The stem, leaf and rachis characteristics of the plant were examined in anatomical studies. There is a secondary growth in stem anatomy. The number of vascular bundles is 15-18 in a circle. The shape of the transversal section of the rachis is polygonal or almost orbicular. The sclerenchyma tissue is located on the vascular bundles in the rachis. *A. tmoleus* var. *bounacanthus* has equifasial leaves. The stomata type is anomocytic and occurs on both leaflet epidermises. The pollen grains of *A. tmoleus* var. *bounacanthus* have tricolpate aperture and they are subprolate-shaped. While the pollen exine sculpturing is microreticulate, the seed surface sculpturing is a prominent undulate-ridged pattern formed by irregular cells. Scanning electron microscopy (SEM) is used to determine pollen and seed morphology.

Keywords: Anatomy, *Astragalus tmoleus* var. *bounacanthus*, Morphology, Pollen, Seed

1. INTRODUCTION

The genus *Astragalus* L. (Fabaceae) which is one of the largest genera of vascular plants in the world, is distributed in semi-arid steppe regions. It is represented by c. 3000 species and more than 250 sections. Turkey is one of the most important centers of distribution of the genus *Astragalus* and it is represented by 476 taxa, 64 sections. It has high endemism rates, with a value of 51% in Turkey [1-4].

Astragalus species are economically important because most of them produce the valuable tragacanth gum and selenium. Tragacanth gum is recorded Tragacantha or Gummi Tragacantha in the name codex and pharmacopoeias [5]. The genus *Astragalus* is also ecologically important because most of the provide pillows and play a very important role in the prevention of erosion. However, they are consumed in mountainous and rural areas as firewood and animal feed [6]. The roots of some *Astragalus* species represent very old and well-known drugs in traditional medicine for the treatment of nephritis, diabetes, and uterine cancer and as antiperspirant, diuretic, and tonic. In Turkish folk medicine, the aqueous extracts of various *Astragalus* species are used to treat leukemia as well as for wound healing [7].

A. tmoleus Boiss. is an element of the Mediterranean phytogeographical mainly distributed on stony slopes of the southwest and central Anatolia region. It is a member of the section *Pterophorus* and it grows on stone slopes of central and south-west Anatolia in 800-2000 m [1]. *A. tmoleus* is represented by two varieties (var. *tmoleus* and var. *bounacanthus* (Boiss.) Chamberlain in the Flora of Turkey. Both of varieties are used by chewing the resin for toothache, abdominal pain in cattle and small ruminants [8]. A previous study by Pirdal et al. (1991) reported the morphology, anatomy and ecology of *A. tmoleus* var. *tmoleus* [9].

In this study, morphological, anatomical, palynological and seed surface properties of endemic *A. tmoleus* var. *bounacanthus* were investigated in detail for the first time.

2. MATERIALS AND METHODS

2.1. Plant Material

A. tmoleus var. *bounacanthus* was collected during the flowering and fruiting periods (July-September, 2011) from Eskişehir (Karacaşehir-Eşenkara köyü, 39º 42’ 42’’K ve 30º 25’ 40’’D) province of Turkey. Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskisehir (ESSE 14578), Turkey.

2.2. Microscope Studies

Macromorphological observations (20 samples) were carried out under a binocular stereomicroscope, Olympus SZX12 with a drawing tube. Live material was stored in 70% ethanol for anatomical studies. All sections were made manually and taken from the middle regions of the plant. All sections (60 sections) were embedded in glycerin-gelatine and mounted on microscope slides with Canadian Balsam. Olympus BX51 binocular light microscope and camera were used. Photographs were taken. For the SEM study, the pollen (more than 50 pollen grains) and seed specimens (10 specimens) were mounted onto SEM stubs using double-sided adhesive tape and coated with gold. Photographs were taken with the electron microscope (Zeiss EVO 50).

3. RESULTS

3.1. Morphology

Astragalus tmoleus var. *bounacanthus* is a lax cushion-forming shrub, 10-32 cm. Leaf rachis is 1.9-5 cm, spiny, straight or slightly incurved and haired in lower parts. Leaflets are in (3-) 4-6 (-7) pairs,

Figure 1. *A. tmoleus* Boiss. var. *bounacanthus* (Boiss.) Chamberlain in natural

5-13 x 2.5-5 mm, elliptic, spine-tipped, densely adpressed simple pilose-tomentolleus, cuneate in the base. Stipules are 9-14 x 4-7 mm, ± ovate-lanceolate, tomentellous. Flowers are sessile and they are found in 2-3 per leaf axil. The inflorescence is globose to cylindrical, 2.3-3 cm in diameter and 20-40 flowered. Bracts are ovate-lanceolate, orbicular, bilobed and 8-12 x 5-8 mm and tomentellous. Bracteoles are 7-12 x 1.5-3(-4) mm, linear, densely white pilose, resembling the calyx lobes. The calyx is 10-15 mm,

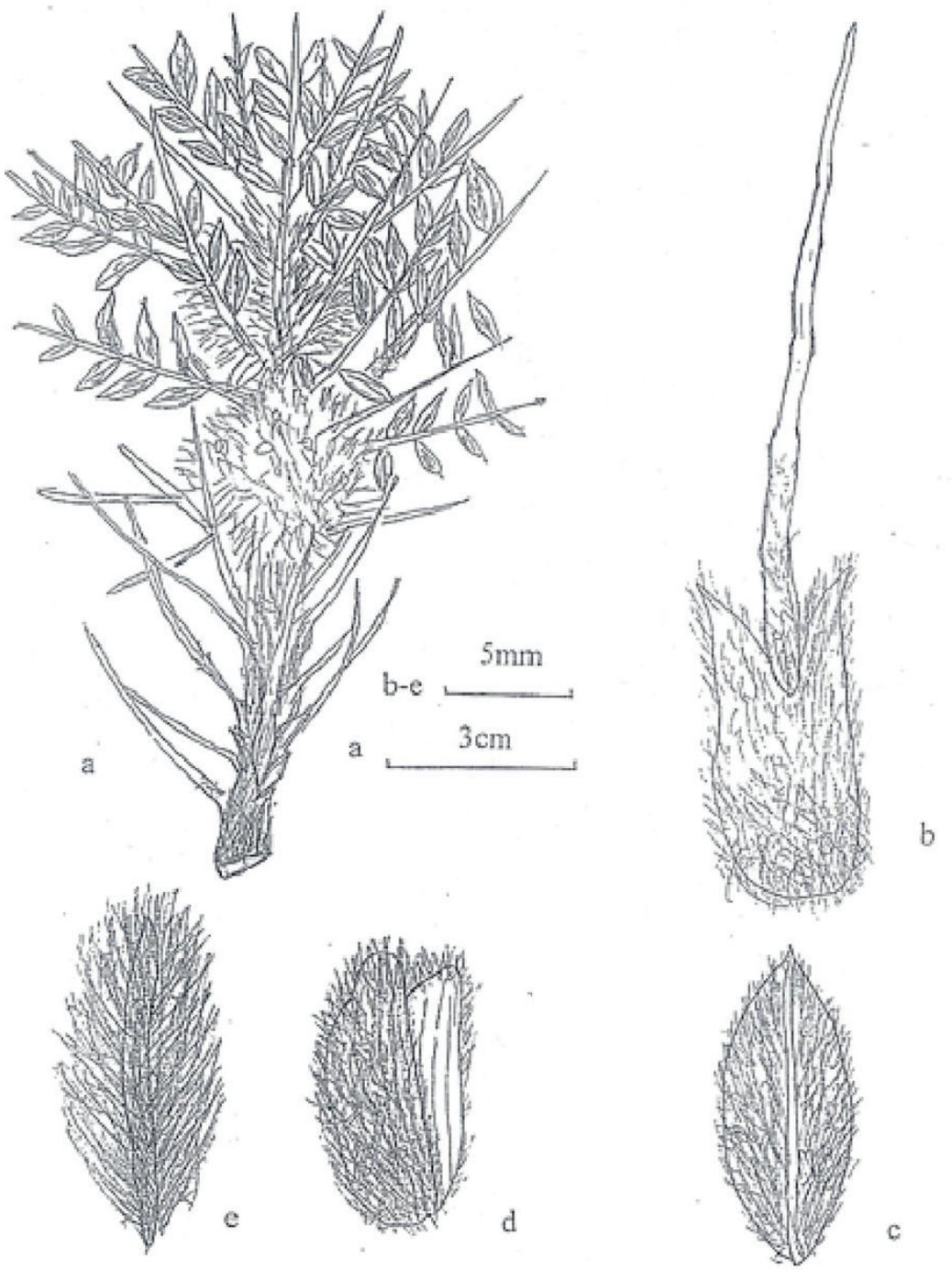

Figure 2. *A. tmoleus* var. *bounacanthus* a. plant b. stipul, c. leaflet, d. bract, e. bracteol

densely white pilose to villous. The lobes of calyx are divided to the base by apparently forming a 2-4 mm tube. Corolla is rosa-pink. The standard is stenonychioid type, 12-18.5 mm, spathulate, obtuse-rotundate in the apex. The wings are 12-18 mm, oblong-triangular in the above and obtuse in the apex. The keels are 12-17 mm, falcate and lobed. The stamen is 11-18 mm, diadelphous. The ovary is 4-6 x 1.8-2.5 mm, ± elliptic, densely long and white-haired. Legumens are 5-7 x 2-5 mm, ovate, densely

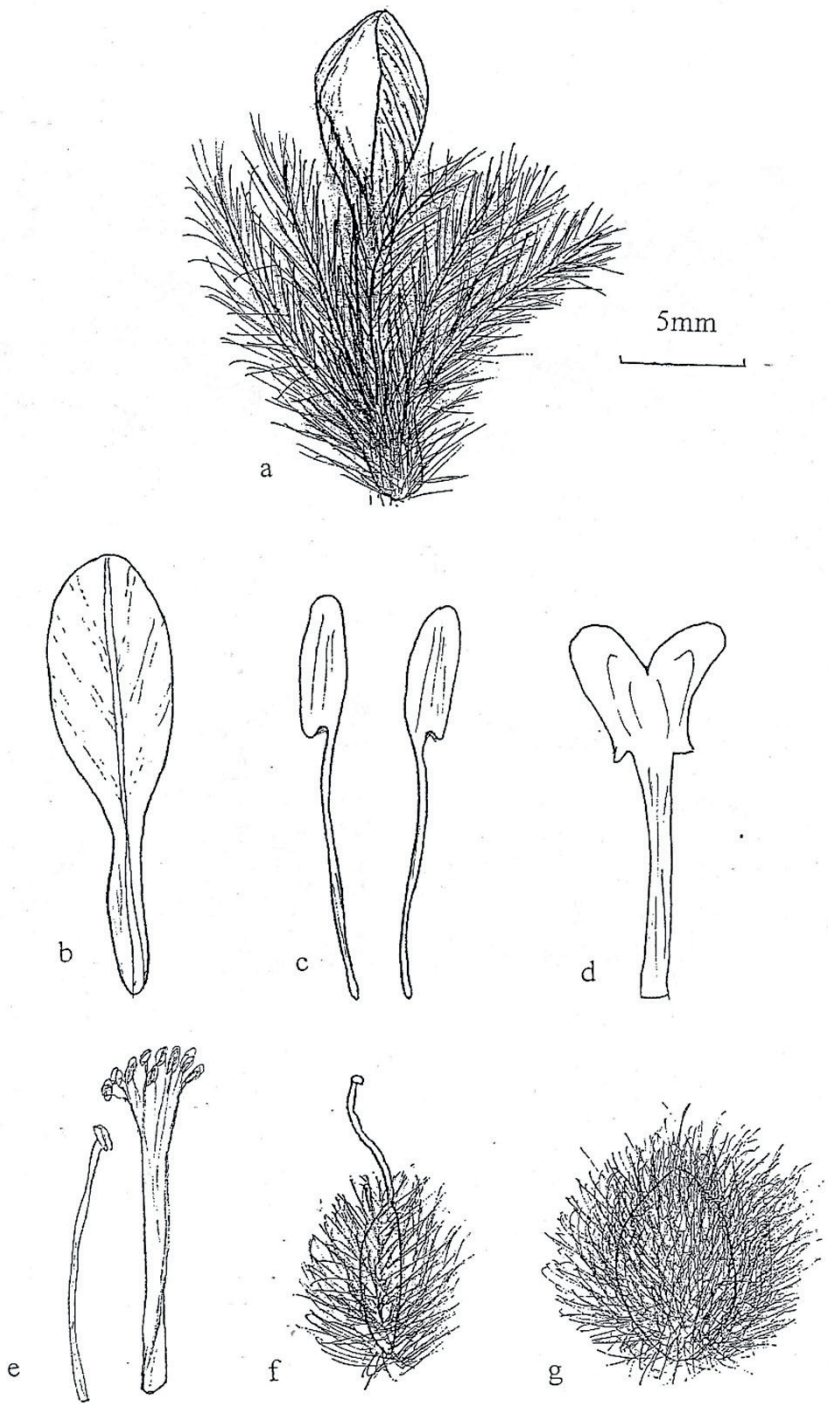

Figure 3. *A. tmoleus* var. *bounacanthus* a. flower, b. standard c. wings d. keel e. diadelphous stamens f. ovarium g. fruit

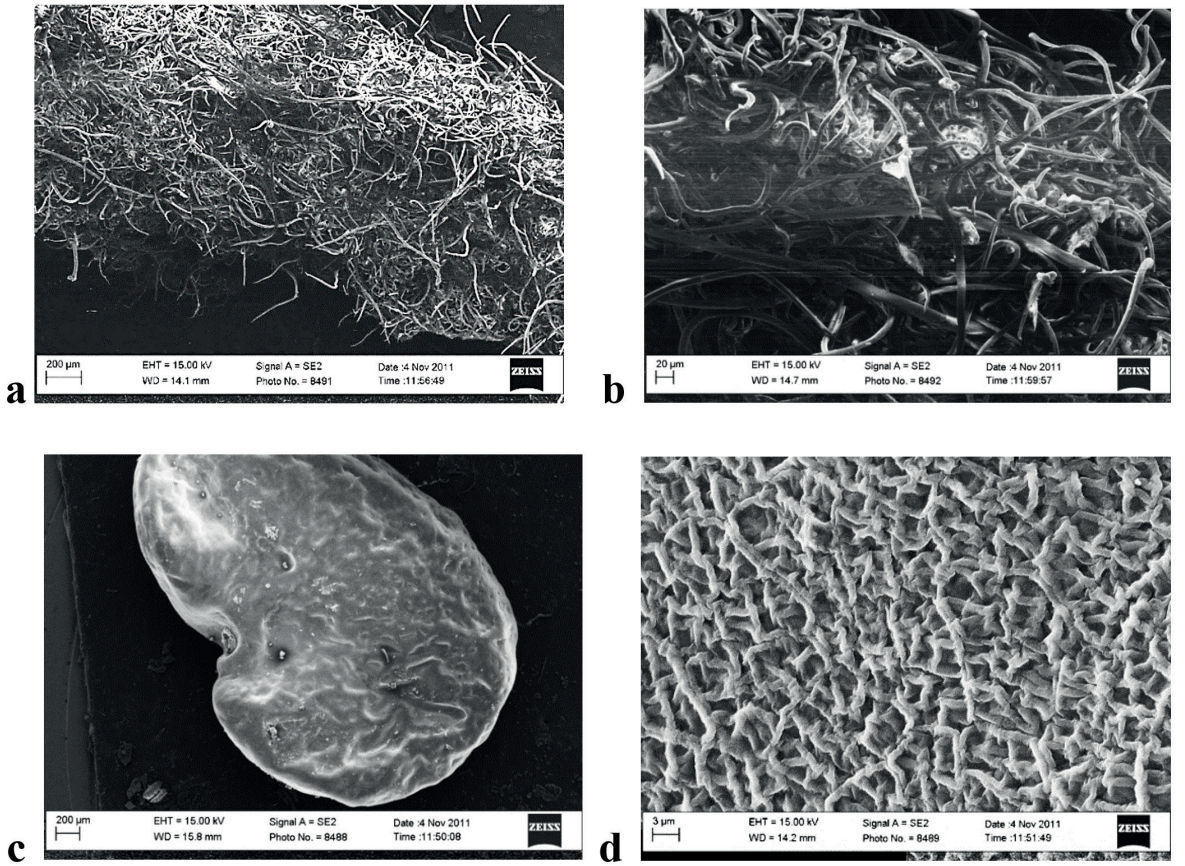

Figure 4. a-b Leaflet upper surface view; c-d Seed and seed surface of *A. tmoleus* var. *bounacanthus* at SEM

long and white-haired. The seeds are (2-) 2.5-3.8 x 1.5-2.5 mm, yellowish-brown and reniforme shaped. The seed surface has a prominent undulate-ridged pattern formed by irregular cells (Figures 1-4).

3.2. Pollen Morphology

The pollen grains are radially symmetrical, isopolar, and have a tricolporate aperture. Polar axis 23.8-34 µm, equatorial axis 17.7-29.3 µm. P/E: 1.16-1.34 µm. The shape of pollen grains is subprolate. Colpi is narrow and long. Clg (13.4-) 18.5–25 µm, Clt (0.2-) 0.4- 0.6 (-1) µm. Exine sculpturing is microreticulate (Figure 5).

3.3. Anatomy

3.3.1. Stem

Transverse sections taken from the middle part of the stem were observed as follows.

The epidermis is composed of a single layer of almost small, square arranged cells. The upper surface is covered with a thick cuticle. Covering trichomes consists of the non-glandular type which is simple, dense and long. The collenchyma tissue is located under the epidermis and occurs from 6-8 layered. The primary cortex is composed of parenchyma cells, 7-15 layered in between the collenchyma and vascular bundle. The shape of these cells is oval-rectangular and they include starch. The secondary cortex is composed of sclerenchyma cells, which are located on the phloem tissue of vascular bundles. The number of vascular bundles is 15-18 in a circle. However, 3-4 vascular bundles are also present in between parenchyma and collenchyma cells. A group of 2-3 stone cells is located in between sclerenchyma and phloem. The shape of phloem cells is irregular-polygonal. The cambium is 4-7-layered, with flattened cells and thin walls. There are secondary xylem under the cambium and primary xylem faces

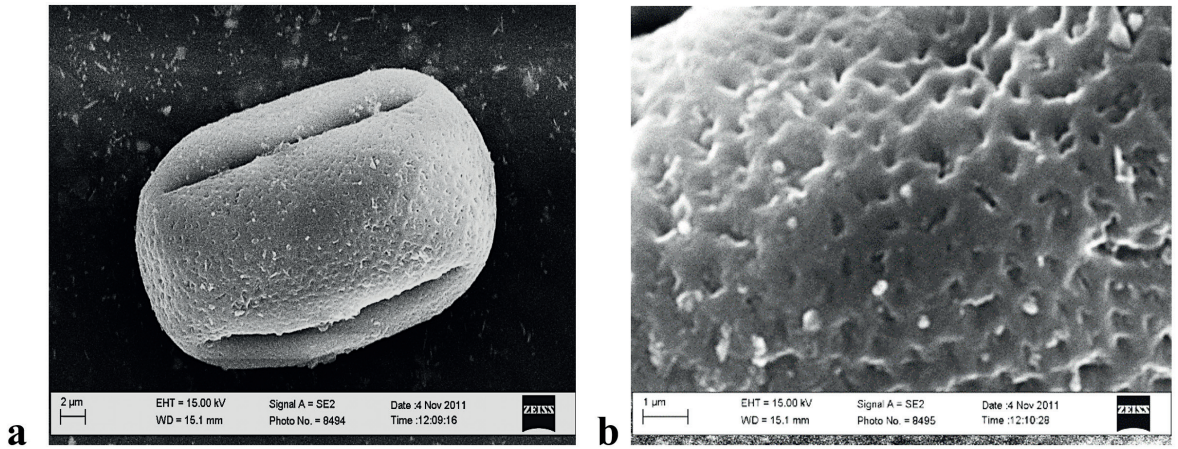

Figure 5. SEM of pollen. a. general view b. ornamentation

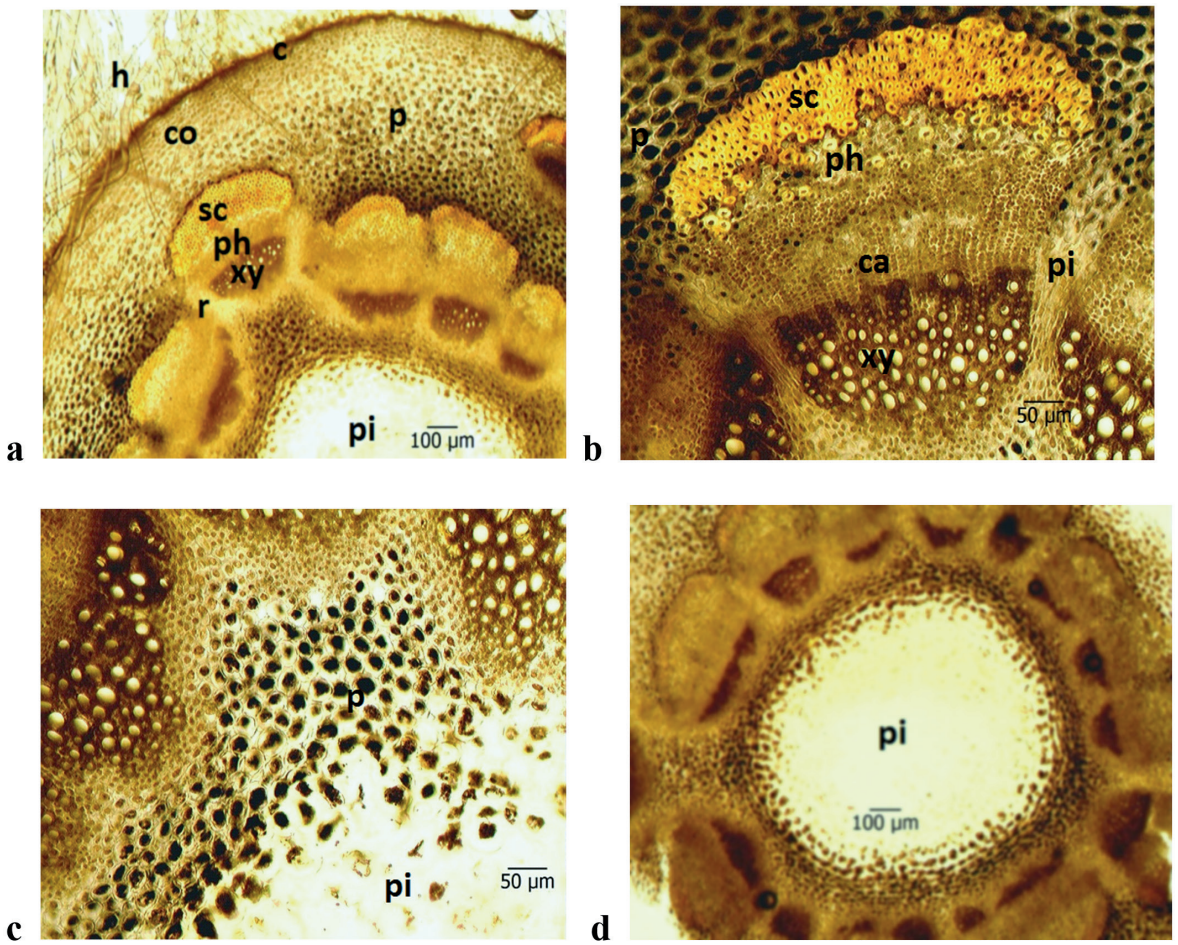

Figure 6. Transverse section of stem. a-general view b-vascular bundle c-d pith; cu-cutikula, co-collenchyma ph-phloem ca-cambium xy-xylem p-parenchyma pi-pith r-ray sc-sclerenchyma h-hair

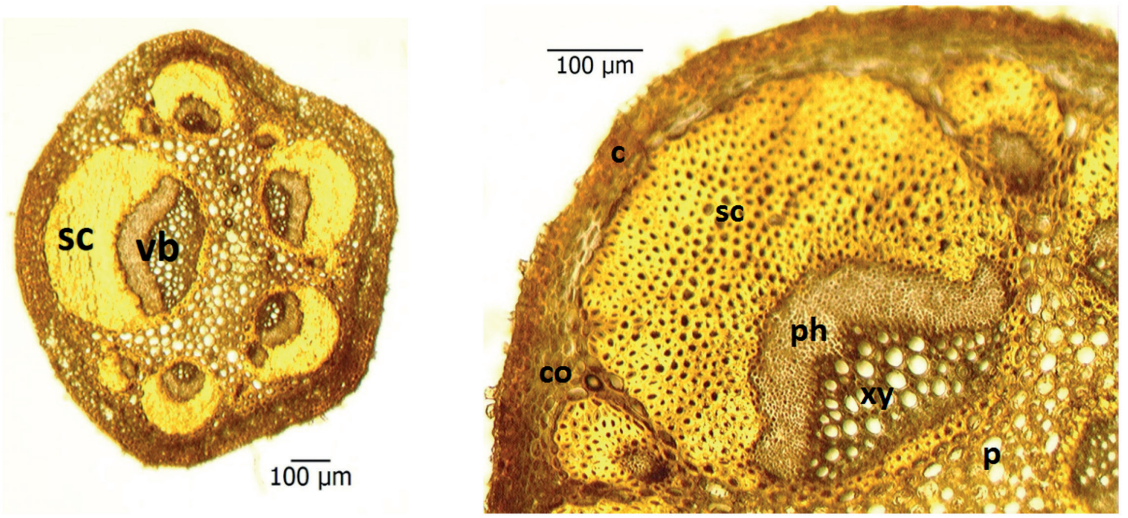

Figure 7. Transverse section of rachis. a-general view b-vascular bundle; co-collenchyma sc-scleranchyma p- parenchyma ph-phloem vb-vascular bundle xy-xylem

toward the pith. Rays are 3-6 (10) layered. The pith consists of large parenchyma cells including abundant starch. After a few layered parenchyma cells, they are rented in the middle stems and large tragacanth spaces in the pith section are occurred (Figure 6).

3.3.2. Rachis

The shape of the transversal section of the rachis is polygonal or almost orbicular. The uppermost of the rachis is a thick cuticle layer and there is an epidermis with one-layered cells under the cuticle. The collenchyma tissue is located under the epidermis and it consists of 3-5-layered and transversally oval-rectangular-shaped cells. The vascular bundles are located one big and 8-14 small at the top and lateral ways of the section after the collenchyma. The vascular bundles are surrounded by broadly and multilayers scleranchymatic cells on the phloem while the sclerenchyma cells are several layers under the xylem. They are polygonal shaped. The vascular bundles are arranged as phloem on the outside and xylem on the inside. The vascular bundles with together sclerenchyma tissue are almost rounded shaped. Pith is composed of parenchymatic cells with a thick wall and it covers a narrow area (Figure 7).

3.3.3. Leaflet

The upper and lower epidermises consist of uniseriate oval and rectangular cells in the transverse section. The upper walls are thicker than the lower and lateral walls. Both epidermises are covered with a thick cuticle and included covering long trichomes. The stomata type is anomocytic and occurs on both leaflet epidermises. The leaflets are the equifacial type which consists of 2 layers palisade parenchyma cells, on the adaxial and abaxial surfaces of the leaflets. The spongy tissue with a row of flattened cells or often indistinct. The midrib region is protruding and bigger than the others. There is also an 8-12 small vascular bundle in lateral regions. The vascular bundles are of the collateral type and surrounded by a parenchymatic bundle sheath. A group of scleranchymatic cells is found below the phloem and they are thick-walled and polygonal shaped. The xylem occupies a narrow area and it faces toward the adaxial epidermis and is surrounded by the phloem. The phloem is between sclerenchyma tissue and xylem and it faces towards the abaxial epidermis. The collenchyma cells are located in between the lower epidermis and bundle sheath while the palisade parenchyma is located in between the upper epidermis and bundle sheath (Figure 8).

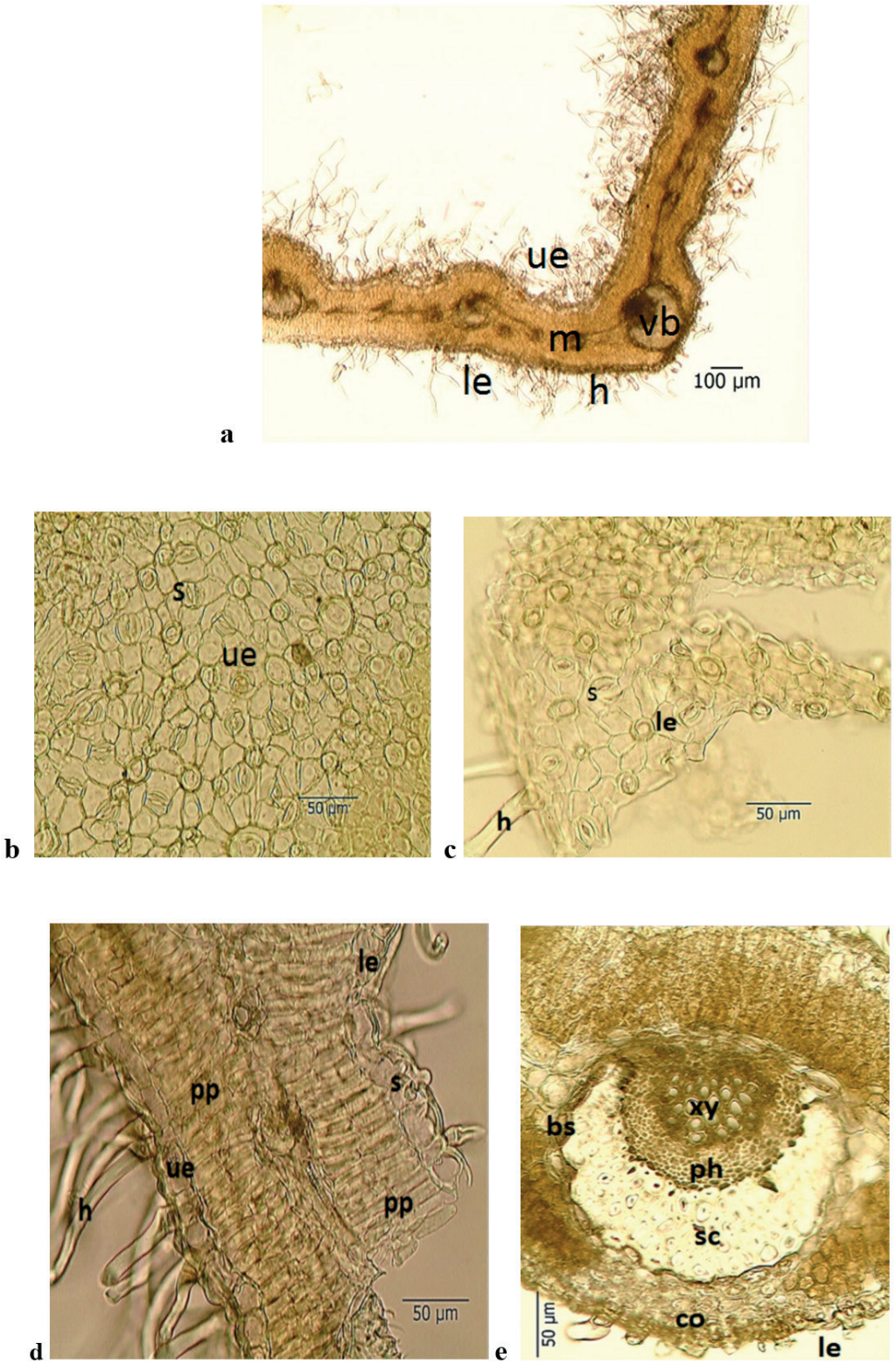

Figure 8. Transverse section of leaflet. a-general view b-upper surface c-lower surface d-mesophyll, e-midrib, bs-bundle sheat h-hair co-collenchyma s-stomata ue-upper epidermis le-lower epidermis pp-palsade parenchyma m-mesophyll ph-phloem sc- sclerenchyma xy-xylem.

4. DISCUSSION

A. tmoleus is a member section of *Pterophorus* and it is represented by two endemic varieties (var. *tmoleus* and var. *bounacanthus*) in the Flora of Turkey [1]. According to Flora, varieties separate from the shape of bracts. The shape of bracts is linear in var. *tmoleus* while it is ovate-lanceolate to orbicular shaped and bilobed in var. *bounacanthus*. In this study, morphological, anatomical and palynological properties of *Astragalus tmoleus* var. *bounacanthus* have been investigated in detail. Morphological results have been compared to the Flora of Turkey in Table 1 and some differences were observed. For example, the lengths of the leaflet and stipule are more than Flora of Turkey. Also, the length of the bracts, wing, keel, stamen, ovary, legume and seed characteristics were described for the first time in this study. All morphological images were drawn and the deficiencies in the Flora were completed.

A study is present about *A. tmoleus* var. *tmoleus* [9]. When the morphological part of this study is examined, it is seen that it is a literal translation of the plant definition in the Flora of Turkey. In conclusion, I have not formed a positive opinion about the reliability of Pirdal et al.'s study [9].

Table 1. Morphological characters of Astragalus tmoleus var. bounacanthus based on the present study and Flora of Turkey

	Astragalus tmoleus* var. *bounacanthus	**Flora of Turkey**
Leaf rachis	1.9-5 cm	2-5 cm
Leaflets	(3-) 4-6 (-7) çift	3-6 (-7) çift
	5-13 x 2.5-5 mm	4-8 mm
Stipul	9-14 x 4-7 mm	10 mm
Flower number	20-40	20-40
Bract	8-12 x 5-8 mm	-
Bracteol	7-12 x 1.5-3 (-4) mm	8-12 mm
Calyx	10-15 mm	10-15 mm
Corolla	Rose-pink	Rose-pink
Standart	12-18,5 mm	15-18 mm
Wings	12-18 mm	-
Keels	12-17 mm	-
Stamen	11-18 mm	-
Ovary	4-6 x 1,8-2,5 mm	-
Legumen	5-7 x 2-5 mm	-
Seed	(2-)2,5-3,8 x 1,5-2,5 mm	-

A. tmoleus in Flora of Turkey is defined as a species close to *A. trojanus.* However, the differences of inflorescence and the shape of bracts in species are reported. Furthermore, the leaflets of *A. tmoleus* are pilose-tomentolleus while the leaflets *A. trojanus* are pilose or glabrous. The seeds of some *Astragalus* species such as *A. tmoleus* var. *bounacanthus* are generally reniforme shaped and seed surface is rugulate or undulate-ridges [4,10-13].

The pollen grains in the Fabaceae family are simple or united. Generally, they are tricolpate, sometimes 2, 4 or 6 aperture and variable shape from peroblate to prolate. In Turkey, pollen grains of *Astragalus* species are generally stenolapynous and radial symmetry, isopolar, tricolpate and they show microreticulate ornamentation [3,11,13-18]. Our pollen findings are similar to the above evidences.

Astragalus species are xeromorphic plants that grow on stone slopes. The presence of a thick cuticula in the leaflet, dense trichomes and the lack of stomata or located at epidermis level stomata and fewness of sponge parenchymatic cells in leaflets, cushing-forming shrubs are characteristic for *A. tmoleus* var. *bounacanthus*. These features are the peculiar properties of the xeromorph plants [19]. As a result of the anatomic studies, *A. tmoleus* var. *bounacanthus* shows the characteristics of the xeromorph plants. Anomocytic types of stomata are characteristics for the Fabaceae family and this type is observed in this study [20].

Some anatomical (stem, rachis and leaflets) studies have been reported on various *Astragalus* species [9-10,21-23]. Pirdal et al. [9] are reported anatomical properties of root, stem, rachis and leaflets in endemic *A. tmoleus* var. *tmoleus*. In another study, Uysal [10] investigated stem, rachis and leaflets anatomy properties of endemic *A. trojanus.* The primary cortex structure, sclerenchyma tissue on the phloem and the presence of amylum in the piths of *A. tmoleus* var. *tmoleus* and *A trojanus* are consistent with our results.

The rachis anatomy features in an earlier studies [9-10,21-23] are similar to our findings as the shape of rachis, collenchyma tissue under the epidermis, small-big vascular bundles, vascular bundles surrounded by scleranchymatic cells, pith in a narrow area.

Leaflet anatomical properties of Pirdal et al. [9] and Uysal [10] are also supported by our leaflet anatomical results as mentioned in equifacial leaflets, anamocytic stomata, the structure of vascular bundles and the lackness of sponge parenchyma. Metcalfe and Chalk [20] recorded equifacial type in *Astragalus* species. *A. tmoleus* var. *bounacanthus*'s leaves have the same mesophyll structure.

Pirani et al. [21] had been studied the petiole anatomy of 35 *Astragalus* species in *Rhacophorus* section while Mehrabian et al. [23] had been investigated the petiole anatomy of 24 *Astragalus* species in the *Incani* section. Researchers in both studies reported two petiole types. The first type of petioles has a narrow pith area that consists of thick-walled parenchyma cells and vascular bundles are surrounded by broadly a sclerenchyma cell group. The second type has a large pith area which consists of thin-walled parenchyma cells and vascular bundles are surrounded by narrowly a sclerenchyma cell group. Our samples are similar to the first type.

5. CONCLUSION

In the present study, morphological, anatomical and pollen characteristics of endemic *A. tmoleus* var. *bounacanthus* have been investigated for the first time in this study. In morphological studies, in detail description of the plant was given were supported by drawings. Morphological characters such as the shape of paripinnate leaves, stipules and bracts, the flower number in the inflorescence, the stenonychioid type of standard and the reniform type of seed can be helpful to distinguish *A. tmoleus* var. *bounacanthus.* While the stem, leaf and rachis characteristics of the plant were examined in anatomical studies, the pollen structure of the plant was determined in palynological studies. The data obtained at this stage enrich the information about the these features of the members of the Fabaceae family and can be used in comparative studies, both between the species of the genus *Astragalus* and also with molecularly related genera.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: AK; Design: AK; Supervision: AK; Materials: AK; Data Collection and/or Processing: AK; Analysis and/or Interpretation: AK; Literature Search: AK; Writing: AK; Critical Reviews: AK.

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Conflict of interest

The author declared that there is no conflict of interest.

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