

ANATOMICAL AND MICROMORPHOLOGICAL DIFFERENTIATION IN THE GENUS *MOLTZIA* LEHM IN TURKEY

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Abstract

The genus *Moltkia* Lehm., is represented by *Moltkia coerulea* (Willd.) Lehm., and *Moltkia aurea* Boiss in Turkey. The aim of this study is to determine the anatomical and micromorphological characteristics of the 2 species, and to compare these features with each other. For the anatomical studies, transverse sections of stem and leaves, and the surface sections of leaves were studied. For the micromorphological studies, the pollens were examined using light and scanning electron microscope (SEM), and the nutlets were examined using stereo-microscope and SEM. After the studies, it was pointed out that they are very distinct from each other in terms of the anatomical and micromorphological features. According to the results obtained from anatomical studies, there are lots of differences between the two species considering the structure of the cortex, vascular cambium and endodermis of the stems. The vascular bundle and upper and lower surface of the leaves are also noticeably different in 2 species. The 2 taxa are also readily distinguishable in terms of the pollen characteristics such as pollen shape, the outline in equatorial and polar view, polarity, aperture type and distribution and tectum ornamentation. In addition, while the nutlet shape is similar in the taxa, its surface is verrucate in *M. coerulea*, but tuberculate in *M. aurea*.

Introduction

The family Boraginaceae is distributed throughout the tropical, subtropical, and temperate regions of the world. The centers of the highest diversity in the North Temperate Zone are the Irano-Turanian and Mediterranean regions and in the tropics, the centers of the highest diversity are Central America and northern and central South America (Al-Shehbaz, 1991). The family Boraginaceae comprises of about 131 genera and 2500 species, mainly annual, bi-annual or perennial herbs, sub-shrubs and shrubs, some trees and a few lianes. The members of the family grow mainly on dry, cliffy and sunny habitats (Retief & Vanwyk, 1997).

Based on the gynoecium characters, the family of Boraginaceae is divided into four subfamilies which are Cordioideae, Ehretioideae, Heliotrpioideae and Lithospermae (Gürke, 1894). The genus *Moltkia* Lehm., is included in the tribe Lithospermae according to tribal classification of the subfamily Boraginoideae (Candolle, 1846; Baillon, 1888; Riedl, 1997). *Moltkia* is close to the genera *Echium* L, *Onosma* L, within the Lithospermae.

Many taxonomical studies on the members of the family "Boraginaceae" have been carried out in Turkey and in other parts of the world. These studies were focused on anatomical, palynological and micromorphological features of the family members along with external morphology. While some of these studies exhibit the features of only one species alone, the other studies are comparative and they show the taxonomic significance of these features in the large genera of the family (Akçin & Engin, 2001; Akçin, 2004; Akçin & Engin, 2005; Akçin, 2007a; Binzet & Orcan, 2003; Binzet & Akçin, 2009a).

The stems, leaves and inflorescences of Boraginaceae were characterized by the presence of rough hairs on their surfaces (Heywood, 1993). Trichome morphology is of immense significance in classification at all the levels of

the family. The trichome types on the epidermis have played an important role in the modern re-classification of the family. Similarly, leaf epidermis and the leaf cross-sectional anatomy provide extensive taxonomical data. Many studies have recognized and reported the unmistakable taxonomic importance of epidermal characteristics such as the shape and the size of the epidermal cells, the type of trichomes and the type of stomata (Taia, 2006).

The electron microscopy provides new characters for taxonomic studies and the use of scanning electron micrographs is now common in the taxonomic literature (Stace, 1989). Especially, the nutlet shape and surface along with pollen shape and ornamentation are commonly studied using electron microscopy in the family Boraginaceae (Scheel *et al.*, 1996; Bigazzi & Selvi, 1998; Bigazzi *et al.*, 2006). The nutlet shape and its surface provide valuable taxonomic data for supra and infrageneric classification in Boraginaceae family (Riedl, 1978). The detailed comparative investigations on the nutlet micromorphologies of the problematic genus *Onosma* in Turkey show that these characters have taxonomic value in interspecific classification as well (Akçin, 2007b; Binzet & Akçin, 2009b). Similarly, previous palynotaxonomic studies on the *Boraginaceae* were shown to be valuable in delimiting genera and also in the study of the evolutionary trends within the whole family (Clarke *et al.*, 1979). The pollen morphology and its surface ornamentation, for example, are useful in separating *Maharanga* taxa from *Onosma* (Ning *et al.*, 1995).

The two species of the genus, *Moltkia coerulea* (Willd.) Lehm., and *Moltkia aurea* Boiss., grow in Turkey. The aim of this study is to determine anatomical and micromorphological features of the 2 species and to establish the taxonomic importance of these characters in segregation of the species.

Materials and Methods

The plants were collected from various districts of Turkey between 1995-2008. The investigations were made on three populations for each species. The collection data for the examined specimens are given in Table 1. Some plant samples belonging to the both taxa were fixed in 70% alcohol. Anatomical observations were performed on transverse sections of stem and leaves, and surface sections of leaves. The transverse sections were

painted with basic fuchsin and covered with glycerin-gelatin (Vardar, 1987). For each structure, at least, 30 preparations were observed, and their photographs were taken with Olympus BX-50 microscope. Anatomical assessments were made by means of observations under the microscope and using the photographs. The stomatal index was calculated according to Meidner & Mansfield (1968). Features similar in both species were not emphasized when presenting the anatomical results.

Table 1. Studied materials of *Moltkia* species.

Species	Locality	Collection data
<i>M. coerulea</i>	B4 AKSARAY: Ekicek Dağı etekleri, Çavdarlı Köyü üstü, tarla kenarı, 1250 m, 02.06.1996	M.Dinç 95 ^{b,c,e} (KNYA)
	C4 KONYA: Çumra-Bozkır yolu 3. km, tarla kenarı, 1100 m, 15.05.2009	S. Doğu & M. Dinç 1998 ^{a,b,c,d} (KNYA)
	C4 KONYA: Bozkır-Seydişehir yolu 17. km, step, 1200m, 06.06.2009	S.Doğu 2002 ^{a,c,d} (KNYA)
<i>M. aurea</i>	C4 Karaman: Sarıveliler, Uğurlu Köyü, Yassıkır Mevkii, step, 1600 m, 14.04.2008	S. Doğu 1841 ^{b,c,d} (KNYA)
	C4 KONYA: Konya-Beyşehir yolu 22. km, açıklıklar, 1300-1350m, 27.05.2009	M.Dinç & S.Doğu 3131 ^{a,b,c,d} (KNYA)
	C4 Karaman: Ermenek-Karaman yolu 5.km, step, 1550 m, 06.06.2009	S.Doğu 2008 ^{a,b,c,d} (KNYA)

^aThe specimens used for the anatomical studies

^bThe specimens used for the pollen studies using SEM

^cThe specimens used for the pollen studies using light microscope

^dThe specimens used for the nutlet studies using stereo microscope

^eThe specimens used for the nutlet studies using SEM

Palynological investigations were made by both light microscope and scanning electron microscope. For light microscope studies, the pollen slides were prepared according to the Wodehouse technique (Wodehouse, 1965). Pollen grains were dissected from herbarium specimens and then they were placed on a clean microscope slide. Glycerin-gelatin with basic fuchsin was placed on pollens and allowed to melt and mixed by a clean pin to get scattered pollen grains. All measurements were determined on at least 30 pollen grains. For SEM study, pollen grains were mounted directly on stubs with single-side adhesive tape and coated with gold. The grains were examined by SEM, and the best representatives were photographed both in general and in detail. Pollen terminology follows (Faegri & Iversen, 1989) and (Punt *et al.*, 2007). Nutlets were first observed with a stereomicroscope to ensure that they were of normal size and maturity. For nutlet length and width, 30 samples of each taxon were taken and measured. Mature nutlets were also mounted directly on aluminum stubs and coated with gold, after which they were observed and photographed as in the palynological study.

Results

Stem anatomy: The transverse section taken from the stem is observed as follows: The epidermis is composed of a single layer of rectangular, ovoid and occasionally quadrangular cells. It is covered by cuticle. There are robust multicellular hairs on the epidermis. The collenchyma tissue consisting of 3-4 layer of ovoidal cells is located underneath the epidermis. The cortex tissue is present under the collenchyma. In *Moltkia coerulea*, it is

narrow and composed of 2-4-layered flattened quadrangular and ovoidal cells with thin walls. In *Moltkia aurea*, it is wide and composed of 7-9 layered more or less orbicular cells with thick walls. Underneath the cortex the endodermis is present. While it is very distinct and composed of 1-3-layered flattened and quadrangular cells which are conspicuously different from those within the cortex in *Moltkia coerulea*, it is less distinguishable and composed of 1-layered ovoidal cells which are similar to those within the cortex *Moltkia aurea*. The phloem ring located between endodermis and xylem is composed of 5-6 layers of cells. Although the cambium between the phloem and xylem is indistinguishable in *Moltkia coerulea*, it is readily distinguishable, thick and composed of 3-4 layered and radially prolonged cells in *Moltkia aurea*. In the centre of the stem, there is a large pith region. The pith consists of large and cylindrical parenchymatic cells. Their walls are almost as thick as the walls of parenchymatic cells within the cortex in *Moltkia coerulea*, but they are thinner than the walls of parenchymatic cells within the cortex in *Moltkia aurea*. The paranchymatic cells in the pith region have intercellular schizogen gap (Fig. 1 and Table 2).

Leaf anatomy: The transverse section of the lamina, mid-rib and surface preparations of both epidermises revealed the following elements: In transverse section, the upper and lower epidermises comprise of uniseriate, oval, quadrangular and rectangular cells. However, lower epidermal cells are larger than the upper ones. Both epidermises are covered with a thick cuticle. There are setiform trichomes on both epidermises. Mid-rib is triangle

shaped and includes larger vascular bundle which has richer xylem and phloem elements as compared to the other parts of the mesophyll. The walls of xylem parenchyma cells are intricated in *Moltkia aurea*, but those are not intricated in *Moltkia coerulea*. There is 2-3 layered collenchyma located below both epidermises within the mid-rib. The vascular bundles are present below the collenchyma. It is collateral and surrounded by a parenchymatic bundle sheath. Parenchymatic bundle sheaths in mid-rib are more distinct than those in the other parts of the mesophyll in both species. In addition, although it is more distinct and composed of more or less orbicular and rectangular cells in *Moltkia coerulea*, it is less distinct

and composed of orbicular, ovoidal, quadrangular and rectangular cells below, irregular shaped with intricated walls above in *Moltkia aurea*. Leaves are isolateral, that is, spongy parenchyma is present between the palisade parenchyma located under both epidermises. However, each palisade tissue is composed of 2-3 layered cylindrical cells which have abundant chloroplasts, the lower palisade cells are shorter than the upper ones. The spongy tissue has 4-5 layered orbicular or quadrangular cells which have a few chloroplasts. Palisade parenchyma cells are 2-layered under the upper epidermis. Spongy parenchyma cells are 3-4 layered under the lower epidermis (Fig. 2 and Table 2).

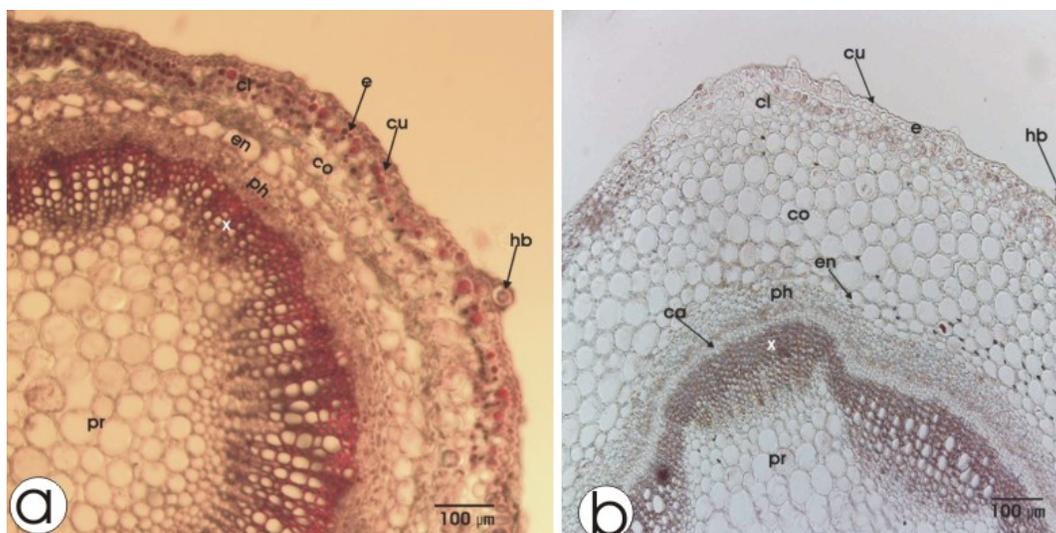


Fig. 1. Transverse sections of the stems. *Moltkia coerulea* (a) and *M. aurea* (b). e=epidermis; cu= cuticle; hb=hair base; cl=collenchyma; co=cortex; en=endodermis; ph=phloem; ca= cambium; x=xylem; pr=pith region.

Table 2. Differential anatomical characters of *Moltkia* species in Turkey.

	<i>Moltkia coerulea</i>	<i>Moltkia aurea</i>
Stem		
Cortex	Narrow and composed of 2-4-layered flattened quadrangular and ovoidal cells with thin walls	Wide and composed of 7-9 layered more or less orbicular cells with thick walls
Vascular cambium	Indistinguishable	Distinguishable, thick, composed of 3-4 layered and radially prolonged cells
Endodermis	Composed of 1-3-layered cells. The cells are flattened and quadrangular, conspicuously differ from cortex cells	Less distinguishable, composed of 1-layered cells. The cells are ovoidal, similar to cortex cells
Leaf		
Vascular bundle	The walls of xylem parenchyma cells are not intricated. The vascular bundle sheath is more distinct and composed of more or less orbicular and rectangular cells	The walls of xylem parenchyma cells are intricated. The vascular bundle sheath is less distinct and composed of orbicular, ovoidal, quadrangular and rectangular cells below, irregular shaped with intricated walls above.
Upper surface	with sparse setiform hairs; epidermal cells are larger, long, wide, have thick anticlinal walls; the guard cells are long wide; the number of epidermal cells is 92 ± 9 per mm^2 , that of stomata is 26 ± 5 per mm^2 ; stomatal index is 28.2	with sparse setiform hairs; epidermal cells are smaller, long, wide, have thick anticlinal walls; the guard cells are smaller, long wide; the number of epidermal cells is 196 ± 8 per mm^2 , that of stomata is 27 ± 3 per mm^2 ; stomatal index is 12.1.
Lower surface	with sparse setiform hairs; epidermal cells are larger, long, wide, and have thick and heterogeneously thickened anticlinal walls; the guard cells are larger, long, wide; the number of epidermal cells is 88 ± 7 per mm^2 , that of stomata is 14 ± 4 per mm^2 ; stomatal index is 13.8	with sparse setiform hairs; epidermal cells are smaller, long, wide, and have druse crystals and thick but not heterogeneously thickened anticlinal walls; the guard cells are smaller, long, wide; the number of epidermal cells is 220 ± 10 per mm^2 , that of stomata is 38 ± 4 per mm^2 ; stomatal index is 14.7

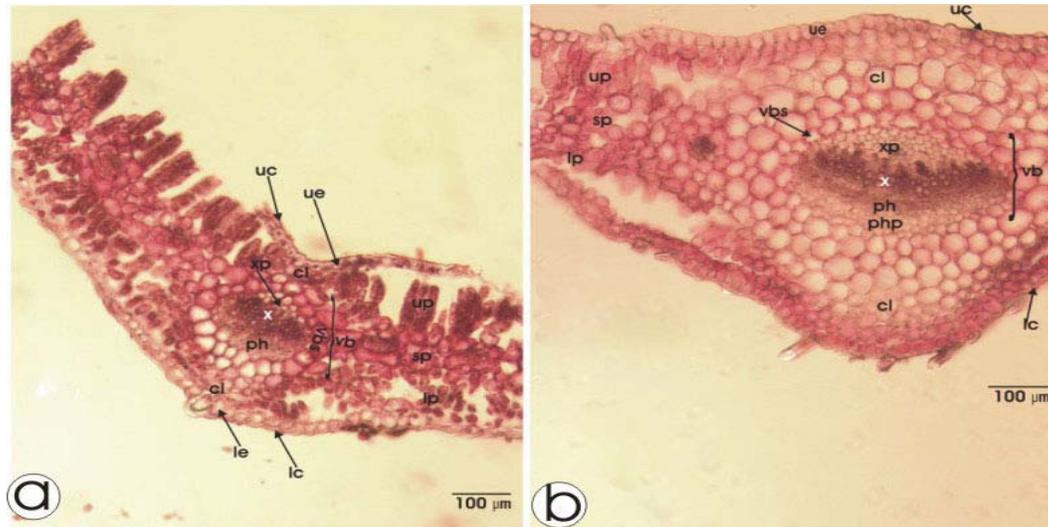


Fig. 2. Transverse sections of the leaves. *Moltkia coerulea* (a) and *M. aurea* (b). uc=upper cuticle; lc=lower cuticle; ue=upper epidermis; cl=collenchyma; x=xylem; ph=phloem; up=upper palisade parenchyma; lp=lower palisade parenchyma; sp=spongy parenchyma; le=lower epidermis; xp= xylem parenchyma; php= phloem parenchyma; vb= vasculer bundle; vbs=vasculer bundle sheet.

The features of the leaves surfaces of *Moltkia coerulea* are as follows: Leaves are amphistomatic with anomocytic to anisocytic stomata but lower surface has fewer stomata than the upper one. There are some setiform hairs on the upper epidermis. The upper epidermal cells are 95-130 μm long, 40-60 μm wide and they have thick anticlinal walls. The guard cells belonging to the upper stomata are 45-55 μm long and 25-27 μm wide. The number of epidermal cells is 92 ± 9 per mm² and that of stomata is 26 ± 5 per mm² on the upper epidermis. Stomatal index is 28.2 for the upper epidermis. The lower epidermal cells are 100-150 μm long, 44-62 μm wide and they have heterogeneously thickened anticlinal walls. The guard cells belonging to the lower stomata are 57-70 μm long and 18-24 μm wide. The number of epidermal cells is 88 ± 7 per mm² and that of stomata is 14 ± 4 per mm² on the lower epidermis. Stomatal index is 13.8 for the lower epidermis (Fig. 3 and Table 2).

The features of the leaves surfaces of *Moltkia aurea* are as follows: Leaves are amphistomatic with anomocytic to anisocytic stomata but lower surface has fewer stomata than the upper one. There are some setiform hairs on the upper epidermis. The upper epidermal cells are 60-90 μm long, 20-30 μm wide and they have thick anticlinal walls. The guard cells belonging to the upper stomata are 40-43 μm long and 10-13 μm wide. The number of epidermal cells is 196 ± 8 per mm² and that of stomata is 27 ± 3 per mm² on the upper epidermis. Stomatal index is 12.1 for the upper epidermis. The lower epidermal cells are 60-110 μm long, 20-28 μm wide and they have thick anticlinal walls. The guard cells belonging to the lower stomata are 34-43 μm long and 13-17 μm wide. The number of epidermal cells is 220 ± 10 per mm² and that of stomata is 38 ± 4 per mm² on the lower epidermis. Stomatal index is 14.7 for the lower epidermis (Fig. 3 and Table 2).

Pollen characteristics: The genus *Moltkia* in Turkey shows a wide variation in tectum ornamentation, polarity, the shape of grains and number and shape of apertures.

Pollen grains of *Moltkia coerulea* are small sized, heteropolar, erect to suberect, 8-9-zonocolporate, rarely 10-zonocolporate, prolate-spheroidal to subprolate in shape. The outline is pyriform in equatorial view and circular to round triangular in polar view. In equatorial view, one pole is about 3 times broader than the other; the long sides are slightly concave, the poles are convex, the broad one is obtuse, and the narrow one is acute. The dimension ranges from 14 to 16 μm in polar length, 10-12 μm (widest part up to 14 μm) in equatorial width. Colpus is rather short, 6.0-8.2 μm in length and narrow, more or less parallel sided, sharply narrowed towards poles, the membrane is regularly granular basically rhombic in outline. Porus is small, circular in outline, 0.5-1.0 μm in length and width. P/E ratio is 1.6-1.95. Tectum ornamentation is psilate throughout. Exine is thin, 0.40-0.60 μm in thickness. Intine thickness is between 0.60-0.80 (Fig. 4a & c and Table 3).

Pollen grains of *Moltkia aurea* are small sized, isopolar, semierect, 10-pantocolporate, rarely 12-pantocolporate, oblate-spheroidal to prolate-spheroidal in shape. The outline is widely elliptic in equatorial and polar view, dimension ranges are 10.0-12.2 μm in polar length, 10.2-12.1 μm in equatorial width. Colpus is rather short, 6.2-8.3 μm in length and narrow, 0.5-2.3 μm in width, more or less parallel sided, sharply narrowed towards poles, the membrane is regularly granular. Porus is small, circular in outline, 0.5-1 μm in length and width. P/E ratio is 0.94-1.12. Tectum ornamentation is rugulate. Exine is thin, 0.40-0.60 μm in thickness. Intine thickness is between 0.60-0.80 (Fig. 4b & d and Table 3).

Nutlet characteristics: The size and the shape of the nutlet are similar in the two species but the species differ from each other in terms of the surface ornamentation. Nutlets of *Moltkia coerulea* are light-brown, 3.70-4.35 mm long, 2.75-3.30 mm ovoid-triangular in outline. The surface is verrucate (Fig. 5a & c). Nutlets of *Moltkia aurea* are pale-grey, 3.90-4.30 mm long, 3.00-3.70 wide, ovoid-triangular in outline. The surface is tuberculate (Fig. 5b & d).

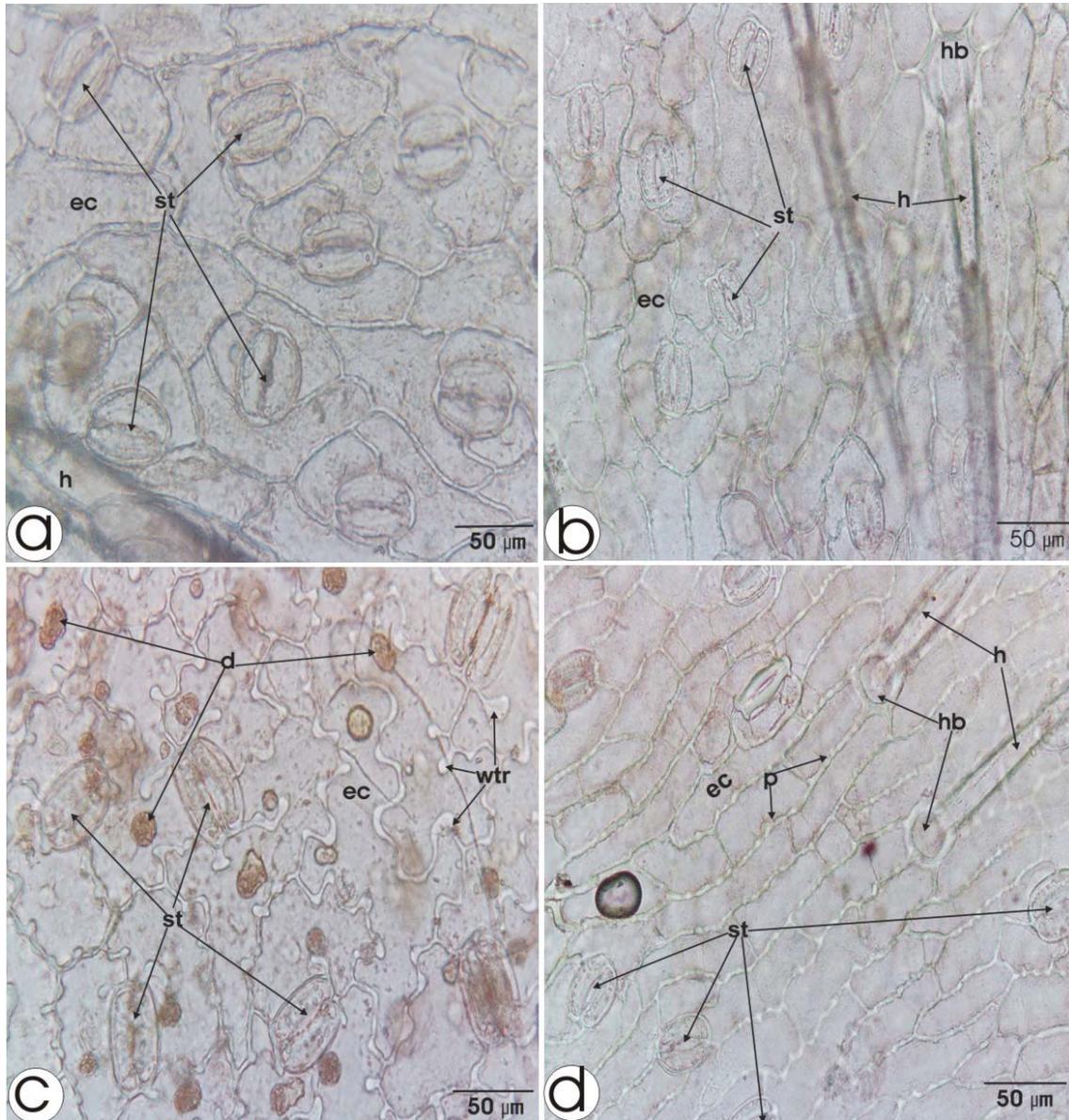


Fig. 3. Surface sections of the leaves. Lower leaf surface of *Moltkia coerulea* (a) and that of *M. aurea* (b); upper leaf surface of *M. coerulea* (c) and that of *M. aurea* (d). ec= epidermal cell; st= stoma; h= hair; hb=hair base; d=drus; wtr=wall thickness region; p=pith.

Table 3. Differential pollen and nutlet characters of *Moltkia* species in Turkey.

Characteristic/Species	<i>Moltkia coerulea</i>	<i>Moltkia aurea</i>
Pollen		
Shape	small sized, erect to suberect prolate-spheroidal to subprolate	small sized, semierect, oblate-spheroidal to prolate-spheroidal
The outline in equatorial view	pyriform	widely elliptic
The outline in polar view	circular to rounded triangular	widely elliptic
Polarity	heteropolar, one pole is about 3 x as broad as the other	isopolar, one pole is about as broad as the other
Aperture type and distribution	8-9-zonocolporate, rarely 10-zonocolporate	syncolpotae, 10-pantocolporate, rarely 12-pantocolporate
Tectum ornamentation	psilate	rugulate
Nutlet		
Colour	light-brown	pale-grey
Surface	verrucate	tuberculate

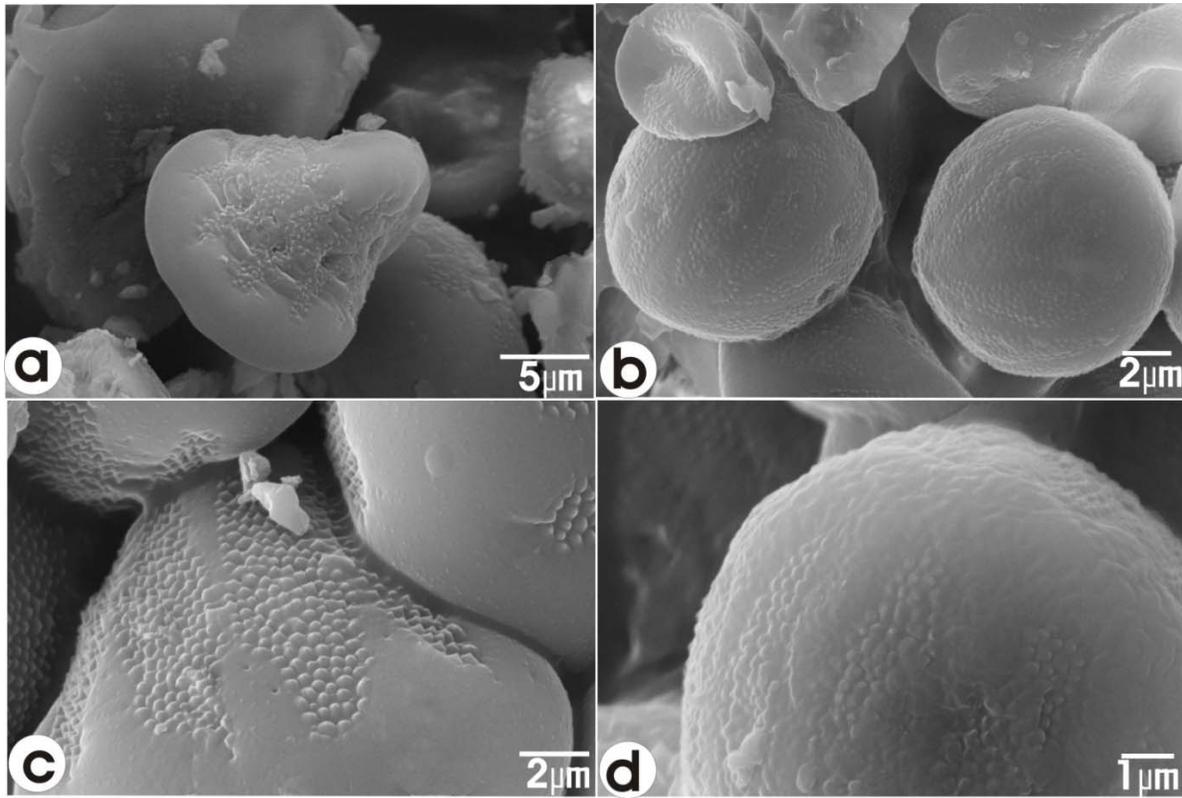


Fig. 4. General appearance (left) and surface details (right) of pollen grains of *Moltkia* species. (a & c) *Moltkia coerulea*, (b & d) *M. aurea*.

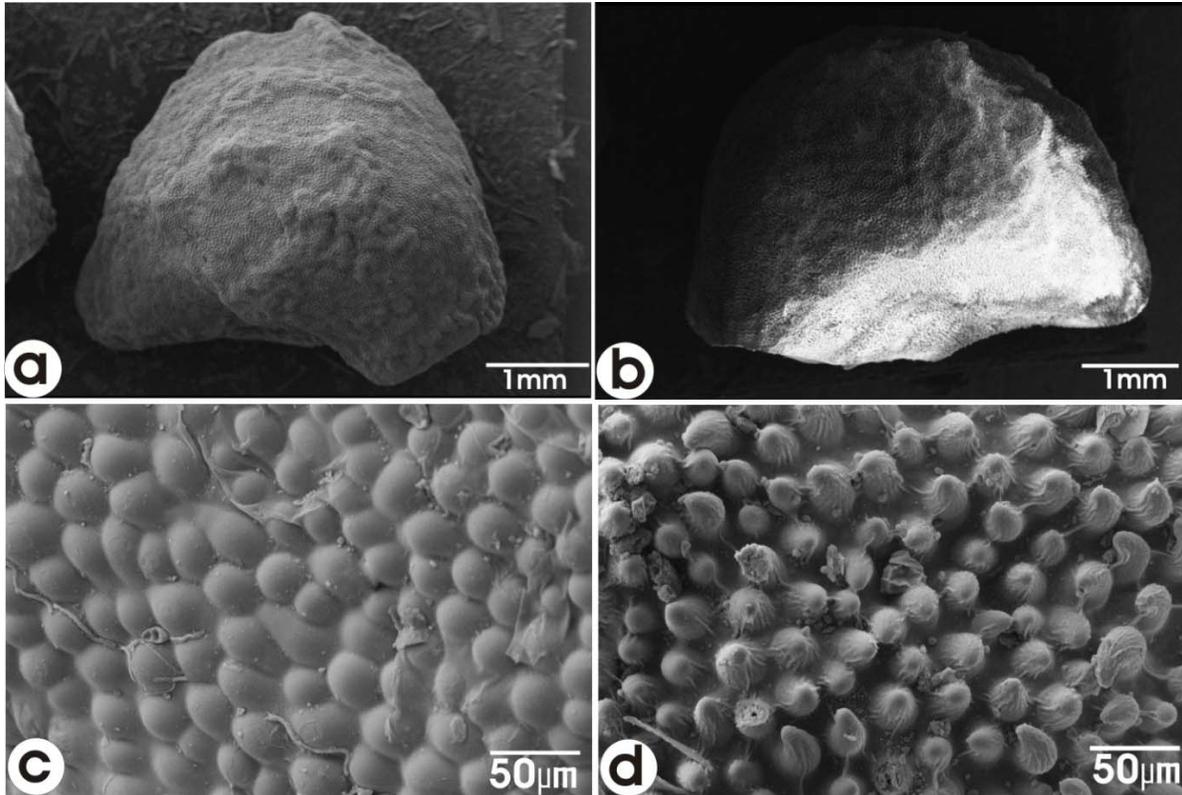


Fig. 5. General appearance (left) and surface details (right) of nutlets of *Moltkia* species. (a & c) *Moltkia coerulea*, (b & d) *M. aurea*.

Discussion

Various scientists have used anatomical characters to elucidate taxonomy among various species (Fukuhara & Shinwari, 1994; Gilaini *et al.*, 2002; Yousuf *et al.*, 2008). Anatomical examinations show that there are clear differentiations between *Moltkia* species in Turkey. The stem cortex is narrow and composed of 2-4-layered flattened quadrangular and ovoidal cells with thin walls in *M. coerulea*, but it is wide and composed of 7-9 layered more or less orbicular cells with thick walls in *M. aurea*. Other differentiations of the stem anatomy are met in vascular cambium and endodermis. While the vascular cambium is indistinguishable and the endodermis is composed of 1-3-layered quadrangular cells conspicuously differentiated from cortex in the stem of *M. coerulea*, the former is distinguishable and composed of radially prolonged cells and the latter is less distinguishable and composed of 1-layered cells similar to cortex ones in that of *M. aurea*.

Anatomical differences in the leaf are present in the vascular bundle and on the both surfaces. In *M. aurea*, the walls of xylem parenchyma cells are intricated, the vascular bundle sheath is less distinct and composed of orbicular, ovoidal, quadrangular and rectangular cells below, irregular shaped with intricated walls above. Whereas, in *M. coerulea*, the walls of xylem parenchyma cells are not intricated, the vascular bundle sheath is more distinct and composed of more or less orbicular and rectangular cells. Both leaf surfaces display some differences between the species in terms of stomatal density and the size of epidermal and guard cells. However the most remarkable differences are present in the lower surfaces. In *Moltkia coerulea*, the lower epidermal cells have heterogeneously thickened anticlinal walls and the guard cells belonging to the lower stomata are 57-70µm long and 18-24µm wide, but the formers have thick anticlinal walls and the latters are 34-43µm long and 13-17µm wide in *M. aurea*.

According to the results, *Moltkia* species growing in Turkey display important anatomical, palynological and nutlet micromorphological differences. Whereas, it was stated that the diagnostic characters between the two species are only seen corolla colour and nutlet shape in the flora of Turkey (Riedl, 1978). According to Riedl (1978), the nutlets of *Moltkia coerulea* are beaked, but those of *Moltkia aurea* are not beaked. However, in this study, it is seen that the nutlets in both species are more or less beaked. In addition, while the nutlet surface is punctate to tuberculate and ±rugose in *M. coerulea*, and it is punctate-rugose in *Moltkia aurea* according to the flora of Turkey, the present study shows that the nutlet surface is verrucate in *Moltkia coerulea*, and tuberculate in *M. aurea*. Nutlet surface ornamentation is reticulate, ruminant, rugose, pustulate or irregular in *Onosma* species growing in Turkey (Akçin, 2007b; Binzet & Akçin, 2009b). With the conspicuous verrucate and tuberculate nutlet surface ornamentation, the genus *Moltkia* is separated from the related genus *Onosma*.

Previous palynotaxonomic studies in the *Boraginaceae* were shown to be valuable in delimiting some genera and also in the study of the evolutionary

trends within the whole family (Bigazzi & Selvi, 1998; Clarke, 1979). *Anchusella*, *Borago*, *Brunnera*, *Elizaldia*, *Lithodora*, *Symphytum* and *Trachystemon* genera as currently circumscribed, are matched by palynological data. However, *Boraginaceae* is one of the more eurypalynous families and palynological data do not support a broad concept of some genera (Bigazzi & Selvi, 1998). In addition, not only is there a wide range of morphological types in some genera, but also there is a large proportion of species which can be recognized from their pollen grains (Clarke, 1977). In the present study, the 2 *Moltkia* species can be recognized from their pollen grains as well because the species are readily distinguished in tectum ornamentation, polarity, shape of grains and aperture type, number and distribution.

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