

# Morphological, Anatomical and Palynological Studies on Endemic *Matthiola anchoniifolia* Hub. -Mor. (*Brassicaceae*)

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## Abstract

In this paper, anatomical, palynological and seed micromorphological properties of an endemic plant *Matthiola anchoniifolia* Hub.-Mor. are recorded for the first time. A description and descriptive illustrations of the species are given based on the collected specimens for morphological study. Seed surface of *M. anchoniifolia* is examined by scanning electron microscope. The seed of *M. anchoniifolia* was compressed, brownish in colour and the cells of testa were nearly 60-80 µm in diameter and ranged from isodiametric, tetragonal or pentagonal. The anticlinal walls were straight or weakly curved while the outer periclinal walls were concave to flat with smooth surface. In anatomical study, cross sections of root, stem and stem leaf are examined. The root had secondary structure. Periderm consists of 5-8 layers of cells for phellem. Cortex consists of 9-12 layered parenchymatic tissue under the periderm. Secondary phloem ring-shaped, 6-9 layered and consists of companion cells and grouped sieve tubes. Stem had primary structure when analyzed. It is circular with a few irregular ribs in cross section. Cortex is 8-12 layered and parenchymatous. Stoma cells are present on both epidermis. Leaf is isobilateral. There are unicellular and ramified hairs on both surface. Palisade parenchyma cells are 1-2 layered and spongy parenchyma cells are 5-12 layered. *M. anchoniifolia* has tricolpate pollen type, prolate pollen shape and reticulate exine ornamentation.

**Keywords:** anatomy, endemic, *Matthiola anchoniifolia*, morphology, palynology, seed, SEM

## Introduction

*Brassicaceae* has been represented by 365 genus and 3250 species in the world and it is indicated as a huge family (Simpson, 2006). The major centres of distribution of the family are in the Mediterranean, Irano-Turanian and Saharo-Sindian regions (Hedge, 1976). In terms of the *Brassicaceae* family, Turkey is one of the richest country with 61 genera and 653 native species (Al-Shehbaz *et al.*, 2007). The genus of *Matthiola* R.Br. (*Brassicaceae*) is distributing in Africa, Europe and Asia. This genus represented approximately 50 species in the world and with 10 species in Turkey (Cullen, 1965; Dirmenci *et al.*, 2006; Heywood, 1993). Among the endemic taxa identified for Turkey is *Matthiola anchoniifolia* Hub.-Mor., *M. longipetala* (Vent.) DC. subsp. *pumilio* (Sibht. & Smith) P.W. Ball, *M. montana* Boiss. and *M. trojana* T. Dirmenci, F. Satıl & G. Tümen (Dirmenci *et al.*, 2006; Ekim *et al.*, 2000). *M. anchoniifolia* has been distributed in Sivas province in Turkey (Cullen, 1965). According to Red Data Book of Turkish Plants, treat category of *M. anchoniifolia* Hub.-Mor. is lower risk/conservation dependent (LR / cd) (Ekim *et al.*, 2000). There is no report on the anatomy, palynology and seed micromorphology of *M. anchoniifolia* which is an en-

demic species in Turkey. In this study, morphological, anatomical and palynological properties of *M. anchoniifolia* were given in detail for the first time.

## Material and methods

The specimens which were examined in this study had collected in Sivas (Turkey) from Ulaş and Kangal town in Sivas. The localities were given as follows:

B6 Sivas: Ulaş town, Ziyarettepe, 1406 m, 39° 33' 08,9" N, 37° 01' 12,1" E, 12.05.2012, M. Tekin 1189; *ibid.* 12.06.2012, M. Tekin 1248.

B6 Sivas: Kangal-Gürün road 16. km, 1530 m, 39° 07' 52,2" N, 37° 14' 33,4" E, 21.06.2012, M. Tekin 1251.

Voucher specimens were deposited in the Cumhuriyet University Herbarium, Faculty of Science and Department of Biology (CUFH). Morphological features and taxonomic descriptions of the plant were carried out according to Cullen (1965). For anatomical studies, the root, stem and stem leaf of the some specimens were fixed and conserved in 70% ethyl alcohol. Hand sections from fixed material were taken with a razor blade and sections were stained with Alcian blue (Sigma) for pectic substances, Safranin (Sigma) for lignin in the rate of 3/2 (Yılmaz, 2009).

Sections were waited in blend waited in blend dye about 5 minutes for staining. The stained sections were mounted in glycerin-gelatin to obtain permanent preparations (Jensen, 1962). Sections were examined using an Olympus light microscope BX51. Images were taken with an Olympus DP 70. For palynological investigations the pollens supplied from herbarium specimens and were prepared by Wodehouse method (Wodehouse, 1965). For measurements of polar axis, equatorial diameter, exine thickness and lumina size of pollen grains were used 100 $\times$  objective of Olympus light microscope CX21. Measurements were based on 50 pollen grains. For SEM studies, pollen grains coated with gold in a sputter coater. Morphological observations were made and micrographs were taken with a LEO 440 SEM at magnification 5000 $\times$ ; 8500 $\times$ ; 30000 $\times$ . For morphological descriptions of pollen, followed the terminology of Punt *et al.* (1994). For seed micromorphology, seeds were mounted on aluminum stubs. Specimen stubs were fixed to the specimen holder of Scanning Electron Microscope (LEO Supra 35 VP FEG SEM) and maintained at accelerating potential voltage of 1.5 kV and photomicrographs were taken at 150 $\times$  and 500 $\times$  magnifications in Sabanci University.

## Results and discussion

Morphological properties [*Matthiola anchoniifolia* Hub.-Mor. in Bauhinia (1963)] (Fig. 1, 2, 3)

Plant 15-45 cm and perennial. Stem herbaceous, woody at the base. Basal leaf 15-38  $\times$  3-7 mm, sessile, linear-oblong to linear-oblongate, entire, canescent with dendroid unicellular eglandular hairs. Leaf apex subacute to oblong. Stem leaf 25-56  $\times$  3-10 mm, sessile, linear to narrowly oblanceolate, canescent with unicellular branched hairs, entire and subacute at apex. Inflorescence raceme, 4-8 flowered. Flowers hermaphrodite, hypogynous with two planes of symmetry. Bracts absent. Pedicel 2-6 mm at

anthesis. Calyx 8.8-10.1 mm. Calyx tube length 8-9 mm and diameter 2.5-3 mm. Calyx teeth 0.8-1.1 mm. Corolla cruciform; petals 4, 22-25  $\times$  4-6 mm and brown colored. (Fig. 1, 2); (Tab. 1).

In the present study morphology, anatomy, palynology and seed micromorphology of *M. anchoniifolia*, a narrow deployed endemic species, was examined in detail. Some additions are required for morphological features (Cullen, 1965). Biometric measures belonging to the species is presented in Tab. 1. Cullen (1965) did not report any information about the length of plant, stem leaf, pedicel length at flowering time, inflorescence, calyx, corolla, stamen and pistil features.

In our study the length of plant was about 15-45 cm., stem leaf as 25-56  $\times$  3-10 mm., inflorescence as raceme and 4-8 flowered. Pedicel length was 2-6 mm at flowering time. Bract absent, calyx tubular, sepal oblong and 8.8-10.1 mm, calyx tube 8-9  $\times$  2.5-3 mm; calyx teeth 0.8-1.1 mm. Petal 22-25  $\times$  4-6 mm, approximately oblanceolate and brown colored. Stamen tetradynamous, outer 2 short stamens 6.6-7 mm; inner 4 long stamens 9.2-10 mm. Pistil 3-3.2  $\times$  0.7-0.8 mm, lanceolate, canescent and stigma green colored. Although Cullen (1965) reported in Flora of Turkey the basal leaf of *M. anchoniifolia* to be oblong-linear, we determined it to linear-oblong to narrowly linear-oblongate and the basal leaf 15-38  $\times$  3-7 mm in size. Similarly, although Cullen (1965) reported that pedicel length was 7-10 mm at fruiting time, siliques 8-12  $\times$  0.35-0.45 cm and seed 5  $\times$  3 mm, we measured pedicel length at fruiting time as 5-12 mm, siliques 5-15  $\times$  0.3-0.45 cm and seed 3-4.2  $\times$  2-3 mm in size.



Fig. 1. *Matthiola anchoniifolia* in natural habitat: a General view in flowering time; b General view in fruiting time

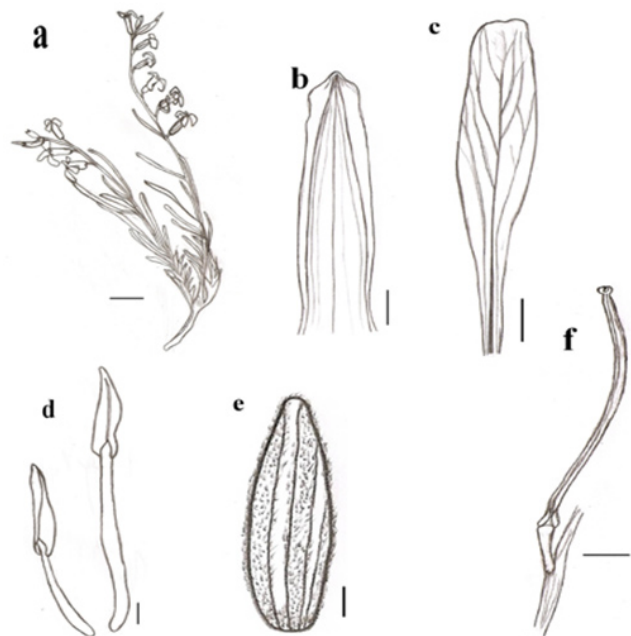


Fig. 2. Drawings of *Matthiola anchoniifolia*: a General view (Scale bar: 2 cm); b Sepal (Scale bar: 1mm); c Petal (Scale bar: 3 mm); d Stamens (Scale bar: 0.7 mm); e Pistil (Scale bar: 0.4 mm); f Fruit (Scale bar: 1 cm)

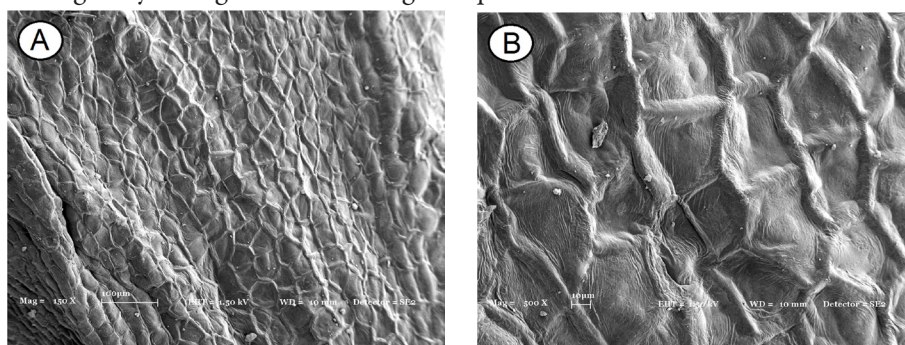
Tab. 1. The comparison with literature knowledges of the morphological characters obtained from *Matthiola anchoniifolia*

	M. Tekin, G. Yılmaz, E. Martin (present study)	Cullen (1965)
Plant length	15-45 cm	Not recorded
Basal leaf	15-38 × 3-7 mm sessile, linear oblong to narrowly linear-ob lanceolat	oblong-linear
Stem leaf	25-56 × 3-10 mm sessile, linear to narrowly oblanceolate, canescent, entire and subacute at apex.	Not recorded
Bract	Absent	Not recorded
Inflorescence	raceme, 4-8 flowered	Not recorded
Pedicle length at anthesis period	2-6 mm	Not recorded
Pedicle length at fruiting period	5-12 mm	7-10 mm
Sepal	8.8-10.1 mm	Not recorded
Calyx tube	8-9 × 2.5-3 mm, tubular	Not recorded
Calyx teeth	0.8-1.1 mm	Not recorded
Petal	22-25 × 4-6 mm, brown colored, approximately oblanceolate	Not recorded
Stamen	Tetradynamous Outer 2 short stamens 6.6-7 mm; inner 4 long stamens 9.2-10 mm	Not recorded
Pistil	3-3.2 × 0.7-0.8 mm, lanceolate, canescent, stigma green colored	Not recorded
Fruit	Siliquae, 5-15 × 0.3-0.45 cm, tomentose and striate	8-12 × 0.35-0.45 cm, tomentose and striate
Seed	3-4.2 × 2-3 mm in size, 0.3-0.5 mm in thickness, oblong or occasionally oblong-suborbicular	5 × 3 mm

The shapes of the cells of testa ranged from isodiametric, tetragonal or pentagonal 60-80  $\mu\text{m}$  in diameter in *M. anchoniifolia*. The anticlinal walls were observed to be straight or slightly curved while the outer periclinal walls were observed to be concave to flat with a smooth surface (secondary sculpture) and a slightly fibrous pattern in *M. anchoniifolia* (Fig. 3).

In previous studies, Tantawy *et al.* (2004) determined seed morphological characters of *M. longipetala* subsp. *bicornis* (Sibth) Ball., *M. longipetala* (Vent) DC. subsp. *bitra* (Conti) and *M. longipetala* subsp. *incana* R.Br. In *M. longipetala* subsp. *bicornis* the seed surface sculpture was reticulate with more or less broad and raised anticlinal walls. The periclinal walls were more or less raised with smooth striated lateral sides and central knobs. However in *M. longipetala* subsp. *bitra* seeds the seed surface pattern was domate with cracks. The anticlinal walls were shallow raised with smooth irregularly arranged folds running in

different directions. The periclinal walls were very narrow and deeply depressed. In addition, in the other examined taxa *M. longipetala* subsp. *incana* the seed surface pattern was reticulate. The anticlinal walls were more or less broad, raised with smooth surfaces. The periclinal walls were shallow depressed with smooth-papillate surface in certain areas and smooth-striated in others (Tantawy *et al.*, 2004). Kasem *et al.* (2011) investigated seed exomorphic characters of 32 taxa of *Brassicaceae* by LM and SEM. In their study seed morphological characters of *M. longipetala* (Vent) DC. subsp. *incana* were determined again and they pointed out that seeds of this taxa were globose and yellowish in colour with glabrous texture. The seed size was 1.5 × 0.2 mm (Kasem *et al.*, 2011). In this study seed of *M. anchoniifolia* was compressed, brownish in colour and the cells of testa were nearly 60-80  $\mu\text{m}$  in diameter. The anticlinal walls were straight or weakly curved while the outer periclinal walls were concave to flat with smooth surface

Fig. 3. Seed coat surface of *M. anchoniifolia*: a General view (Mag. 150x); b Testa surface (Mag. 500x)



in *M. anchoniifolia*. It was similar to *M. longipetala* subsp. *incana* with this feature.

#### Anatomical properties

The root had secondary structure when the plants collected. Periderm consists of three different tissues, phellem (5-8 layers of cells are brown due to suberised cell wall), phellogen (1 layer) and phelloderm (1 layer), from exterior to interior. Cortex consists of 9-12 layered parenchymatic tissue under the periderm (Fig. 4).

Parenchyma cells are  $40-85 \times 45-125 \mu\text{m}$  in size. Cambium is not distinguishable. Secondary phloem ring-shaped, 6-9 layered and consists of companion cells and grouped sieve tubes. Secondary xylem consist of trachea and tracheid. In the secondary xylem parenchyma cells could be observed between xylem vessels (Fig. 4).

Stem had primary structure when analyzed. Stem is circular with a few irregular ribs in cross section. Epidermis cells are  $12-30 \times 15-40 \mu\text{m}$  in size, ovoid to ovoid-oblong in shape. There are ramified unicellular eglandular hairs on epidermis. Cortex is 8-12 layered and parenchymatous. Cells of cortex are  $20-70 \times 25-100 \mu\text{m}$  in size and ovoidal. There are sclerenchyma fibers between xylem bundles. The pith is large and consists of parenchymatous cells. Pith cells are  $35-115 \times 45-140 \mu\text{m}$  in size and circular to circular-ovoid in shape (Fig. 5).

There is a single layered epidermis on the upper and lower surface of the leaf. Thickness of cuticle is  $5-7 \mu\text{m}$ . The shape of epidermal cell is oblong-ovoid. There are unicellular and ramified hairs on both surface. Upper epidermis cells are  $18-35 \times 10-45 \mu\text{m}$  and lower epidermis cells are  $15-30 \times 8-40 \mu\text{m}$ . Stoma cells are present on both epidermis. Leaf is isobilateral. Palisade parenchyma cells are 1-2 layered,  $12-27 \times 35-70 \mu\text{m}$  and generally cylindrical shaped. Spongy parenchyma cells are 5-12 layered,  $10-42 \times 18-55 \mu\text{m}$  and circular, ovoid or rarely irregular shaped (Fig. 6).

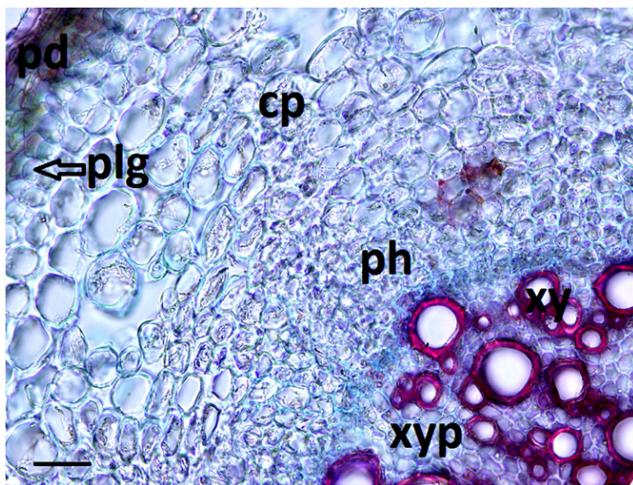


Fig. 4. Cross section of root of *Matthiola anchoniifolia*: (cp: cortex parenchyma; pd: periderm; ph: phloem; plg: root phellogen; xy- xylem; xyp- xylem parenchyma (Scale bar=100  $\mu\text{m}$ )

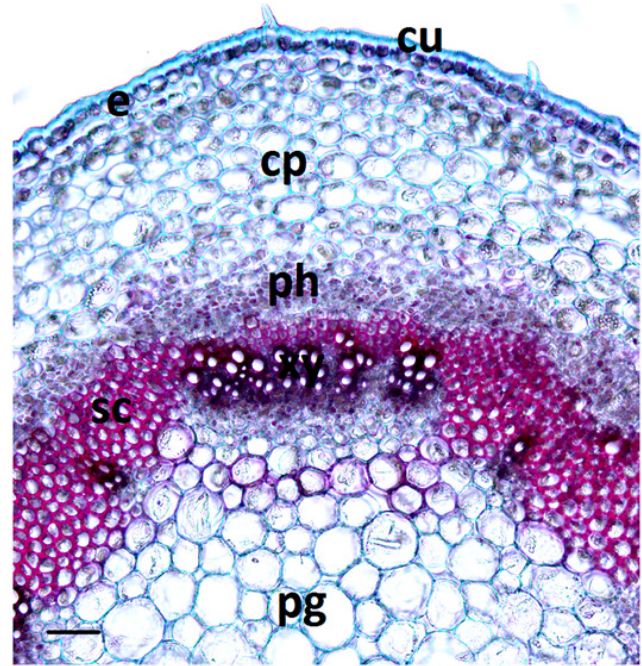


Fig. 5. Cross section of stem of *Matthiola anchoniifolia* : (cp- cortex parenchyma; cu- cuticle; e- epidermis; pg- pith region; sc- sclerenchyma (Scale bar: 100  $\mu\text{m}$ )

#### Palynological properties

The shape of pollen grains is prolate. Exine surface ornamentation is coarsely reticulate. Exine thickness is  $2.1 - 2.6 \mu\text{m}$  in range and mean  $2.4 \mu\text{m}$  (Tab. 2, Fig. 7).

*Brassicaceae* is a stenopalynous family whose pollen grains are usually tricolpate and reticulate (Erdtman, 1972; Reile, 1992) but there are some differences between members of the same genus as in *Hesperis* L. in terms of some features, such as pollen shape (Pinar et al., 2009). Properties of pollen morphology of *M. anchoniifolia* were determined with our present palynological measurements and examinations. Our results were compared with those

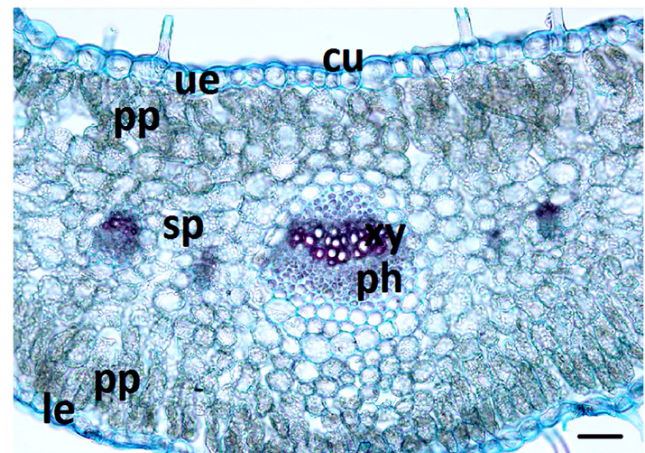


Fig. 6. Cross section of stem leaf of *Matthiola anchoniifolia*: (cu: cuticle; eh: epidermal hair; le: lower epidermis; ph: phloem; pp: palisade parenchyma; sp: spongy parenchyma; xy: xylem (Scale bar: 60  $\mu\text{m}$ )

Tab. 2. The comparison of the pollen morphological characters obtained from *Matthiola anchoniifolia* with other species (P: polar axis; E: equatorial diameter; P/E: the ratio between the polar axis and equatorial diameter; Pt: Pollen type; Ps: pollen shape; Lumina ca.: lumina size)

Taxon	<i>M. anchoniifolia</i> Present study	<i>M. arabica</i> (Abdel Khalik <i>et al.</i> )	<i>M. fruticulosa</i> (Abdel Khalik <i>et al.</i> )	<i>M. longipetale</i> subsp. <i>longipetale</i> (Abdel Khalik <i>et al.</i> )	<i>M. parviflora</i> (Abdel Khalik <i>et al.</i> )
Pollen feature					
Pollen type (Pt)	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate
Pollen shape (Ps)	Prolate	Prolate	Prolate	Prolate	Subprolate
Polar axis (P)( $\mu\text{m}$ )					
Range	(20-29)	(25-30)	(27-32)	(28-30)	(24-30)
Mean	25	29	30	29	28
Equatorial diameter (E)( $\mu\text{m}$ )					
Range	(16-20)	(17-20)	(18-21)	(20-22)	(20-30)
Mean	18	19	20	21	22
P/E ratio	1.39	1.53	1.5	1.38	1.27
Lumina ca. ( $\mu\text{m}$ )					
Range	(2.1-4.5)	(2.7-4.5)	(2.5-4.5)	(2.2-2.7)	(3.5-4.5)
Mean	3.1	3.4	3.5	2.4	3.9
Exine thickness ( $\mu\text{m}$ )					
Range	(2.1-2.6)	Not recorded	Not recorded	Not recorded	Not recorded
Mean	2.4	Not recorded	Not recorded	Not recorded	Not recorded
Ornamentation type	Coarsely reticulate	Coarsely reticulate	Coarsely reticulate	Coarsely reticulate	Coarsely reticulate

from related species of the same genus studied by Abdel Khalik *et al.* (2002).

Our results confirm the existence of minor differences in pollen size, shape, apertures and exine ornamentation in pollen grains of the other members of *Matthiola* genus. In *Brassicaceae*, pollen shapes vary more or less among genera within tribes but very rarely among species within the same genus (Abdel Khalik *et al.*, 2002). The pollen shape of *M.*

*anchoniifolia* is prolate. The palynological study of Abdel Khalik *et al.* (2002) on *Matthiola* also included *M. arabica* Boiss, *M. fruticulosa* (L.) Maire, *M. longipetale* (Vent.) DC subsp. *longipetale*. According to this study, all these species had the same shaped prolate pollen grains with the exception of *M. parviflora* (Schousb.) R. Br. with subprolate grains. When polar axis and equatorial diameter of *M. anchoniifolia* was compared with other *Matthiola* species,

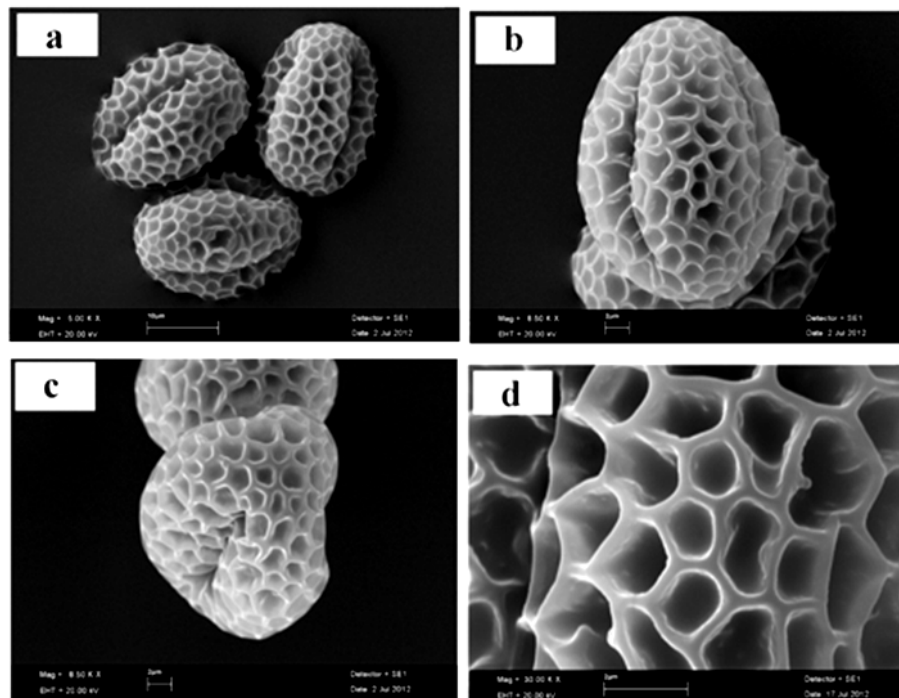


Fig. 7. SEM photographs of pollen of *Matthiola anchoniifolia*: a Multiple view of pollen (Scale: 10  $\mu\text{m}$ ) ; b Equatorial view and aperture (Scale: 2  $\mu\text{m}$ ); c Polar view and aperture (Scale: 2  $\mu\text{m}$ ) ; d Ornamentation (Scale: 2  $\mu\text{m}$ )



it appeared that pollen grains of *M. anchoniifolia* were the smallest (Tab. 2). Erdtman (1972), Rollins and Banerjee (1979), Lahham and Al-Eisawi (1987) reported slightly colpate or non aperturate pollen grains in a few *Matthiola* species. Abdel Khalik *et al.* (2002) reported especially in *M. arabica* that the colpi was less clear. However *M. fruticulosa*, *M. longipetale* subsp. *longipetale*, *M. parviflora* and in our study, the colpi of *M. anchoniifolia* was clear. *M. anchoniifolia* and all investigated *Matthiola* taxa in the study of Abdel Khalik *et al.* (2002) were tricolpate. Exine ornamentation type of *M. anchoniifolia* is coarsely reticulate. Abdel Khalik *et al.* (2002) reported that exine ornamentation of *M. arabica*, *M. fruticulosa*, *M. longipetale* subsp. *longipetale*, *M. parviflora* was the same with *M. anchoniifolia* and they were coarsely reticulate. Lumina size of *M. anchoniifolia*, *M. arabica*, *M. fruticulosa*, *M. parviflora* is more or less similar value apart *M. longipetale* subsp. *longipetale*, which have smaller value of the others. There is no report about exine thickness of *Matthiola* species in the study of Abdel Khalik *et al.* (2002) but in this study mean thickness of exine of *M. anchoniifolia* pollen grains was determined as 2.4  $\mu\text{m}$  (Tab. 2).

## Conclusion

Morphological, anatomical, palynological and seed micromorphological properties of *M. anchoniifolia* which was an endemic plant in Sivas province were reported in detail for the first time in this study.

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