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ANATOMICAL AND PALYNOLOGICAL CHARACTERISTICS OF ENDEMIC *ONOSMA POLYANTHA* DC. AND *ONOSMA MITIS* BOISS. & HELDR. FROM TURKEY

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Abstract

In this study, the anatomical and palynological characters of *Onosma polyantha* DC. and *Onosma mitis* Boiss. & Heldr. endemic to Turkey was investigated. To determine the anatomical characteristics, transverse sections of the root, stem and leaves, as well as adaxial and abaxial sections were taken from the leaves. It was determined that both species had a thick periderm tissue at the outermost part of the root. The endodermis was clearly observed and the pith was composed of tracheal elements. In the cross-section of the stem, only setose hairs were observed in *O. polyantha*, whereas in O. mitis, both setose hairs and rarely glandular hairs were present. The stomata were found to be at the same level as the epidermis in the *O. mitis*, while they were ± the upper level of the epidermis in *O. polyantha*. Two types of stomata (anomocytic and anisocytic) were seen on the lower and upper surfaces of the leaves, the density of the stomata was also higher on the lower surface of leaves in both species. In contrast, the stoma index was higher on the upper leaf surfaces than that of the lower side. The leaves were dorsiventral (=bifacial) in *O. mitis* and equifacial (=izobilateral) in *O. polyantha*. The pollen of both taxa were heteropolar, *O. polyantha* pollen grains were trisyncolporate at the distal pole, while in *O. mitis* pollen grains were trisyncolporate and tetrasyncolporate. Apocolpium was observed in the proximal poles of the trisyncolporate pollen, whereas it was not observed in the tetracolporate pollen of *O. mitis*. The pollen shape was spheroidal P/E=1.12 in *O. polyantha* and subprolata P/E=1.15 in *O. mitis*. In both species, the sculpture was scabrate (=granulate) and scabras were distributed on the surface of the pollen homogenously. The nutlet morphology of *O. polyantha* and *O. mitis* were also examined the nutlet ornamentations were reticulate, rugose, and reticulate-rugose, respectively; and epidermal cells were seen in different sizes. The aim of this study is to determine the anatomical and palynological characteristics of *O. mitis* and *O. polyantha* and to provide a more reliable diagnosis with the help of these characters.

Key words: Boraginaceae, *Onosma*, Anatomy, Palynology, Turkey.

Introduction

The Boraginaceae family comprises some 1600 species (Cecchi & Selvi, 2009; Kolarčik *et al.*, 2010; Mehrabian *et al.*, 2012; Luebert *et al.*, 2016, Chacon *et al.*, 2016), and are distributed throughout the tropical, subtropical and temperate regions of the world. About 44 genera and 375 species of the Boraginaceae family are distributed in Turkey (Binzet, 2012).

The *Onosma* L. Genus, belonging to the Boraginaceae family, contains more than 230 species, predominantly distributed in the Mediterranean region, southwest Asia and temperate Europe (Boissier, 1879; Riedl, 1967; Peruzzi & Passalacqua, 2008; Binzet *et al.*, 2010; Mehrabian *et al.*, 2011, 2014; Guner, 2012; Ranjbar & Almasi, 2014). The *Onosma* species are distributed in our country with 104 species and the endemism rate is approximately 52%. (Riedl 1978; Davis *et al.*,1988; Yıldırımlı, 2000; Riedl *et al.*, 2005; Binzet & Orcan, 2007; Kandemir & Türkmen, 2010; Aytaç & Türkmen 2011; Guner, 2012; Koyuncu *et al.*, 2013; Binzet, 2016a; Binzet, 2016b).

The *Onosma* species are grouped under three distinct sections in the flora of Turkey: *Protonosma*, *Podonosma* and *Onosma*. *Protonosma* and *Podonosma* are represented by one species, while the remaining species belong to *Onosma.* The latter section is divided into two subsections (subsect. *Asterotricha* with basal leaves covered by stellate trichomes and subsect. *Haplotricha* with basal leaves covered by simple setae) based on solely their indumentum types (Riedl, 1978).

Several *Onosma* taxa are used as dyes, herbs and in folk medicine. Fahad & Bano (2012) have recorded that, traditionally, *Onosma* specimens are used for the treatment of rheumatism, bladder pain, heart palpitations and kidney irritation. *O. argentatum* Hub.-Mor. *O. microcarpum* Steven ex DC. and *O. sericeum* Willd. are used for wound treatments in some rural areas of Turkey (Özgen *et al.*, 2003 and Özgen *et al.*, 2004). In addition, fresh flowers of some *Onosma* taxa are consumed as vegetables (Öztürk & Özçelik, 1991).

Anatomical and palynological characteristics of this genus are limited. There are several reports according to the morphology, anatomy and palynology of some *Onosma* species (Binzet & Orcan, 2003a; 2003b; 2009; Akçin & Binzet, 2011; Binzet & Akçin, 2009a; 2012; Binzet, 2011; Güven *et al.*, 2013; Akçin *et al.*, 2013; Binzet & Teke, 2014). Recently, *Onosma* species have also been subject to chemical studies at the same time (Özgen *et al.*, 2004; Çiftçi *et al.*, 2010; Morteza-Semnani *et al.*, 2006; Papageorgiou *et al.*, 1999; Hu *et al.*, 2006; Kundakovic *et al.*, 2006; Özgen *et al.*, 2006; El-Sahazly *et al.*, 2003; Kretsi *et al.*, 2003 and Mroczek *et al.*, 2004). There is no detailed study on the anatomical and palynological properties of *O. polyantha* and *O. mitis.* In this study, we presented the anatomical and palynological characteristics of endemic *O. polyantha* and *O. mitis*.

Materials and Method

The anatomical and palynological characters of endemic *O.polyantha* and *O. mitis* were investigated. Plant samples were collected from natural spreading areas. Some of these samples were placed in 70% ethyl alcohol for anatomical studies, some were placed in separate envelopes for palynological studies and others were dried as herbarium samples. Voucher samples were deposited in the herbarium of the Biology Department of the Faculty of Science and Arts at Mersin University, Turkey. *O. polyantha* and *O. mitis* species collected from two different localities, and the details are presented in Table 1.

Systematic descriptions of the species were made according to instructions in the Flora of Turkey (Riedl, 1978). Anatomical studies were performed using samples previously deposited in 70% ethyl alcohol. The roots, stems and leaves of each species were cross-sectioned with razor blades and covered with glycerin-gelatin (Vardar, 1987). The stomal index was calculated according to Meidner & Mansfield (1968), by calculating the number of stomata and epidermis cell counts in per square millimeters.

The Wodehouse preparation method was used for the palynological analysis (Wodehouse, 1935). Polar and equatorial axis, pollen shape, length of pores and colpus, the width of pores and colpus, intine thickness, exine thickness and length of polar triangular edge analysis were conducted with an Olympus CH20 light microscope (x10 ocu.; x100 obj.). An average of about 40 pollen grains was taken for determining the pollen size. Photomicrographs of anatomy and pollen were created by using the Olympus BX51 binocular light microscope.

For SEM analysis, pollen grains obtained from each specimen were transferred onto stubs and coated with platinum. The SEM micrographs were taken with ZEISS supra55 at Mersin University (MEİTAM). Pollen terminologies were used in accordance with Wodehouse (1935), Faegri & Iversen (1989) and Punt *et al.,* (1994).

Table 1. Locality of *O. polyantha* and *O. mitis*.

Taxa	Locality
O. polyantha	Sivas, Gürün, Şuğul valley, slopes, 16.06.2017, 1375m, 38°44'23"N 37°14'34"E, Binzet 201605.
O. mitis	Antalya, Korkuteli-Denizli 17 km, stony and rocky slopes, 08.06.2016, 1400m, 37°03'27"N 30°04'28"E, Binzet 201603

Results

Anatomical properties

***Onosma polyantha*:** It was observed in the cross-section that the root has a secondary structure (Fig. 1). There was a thick periderm tissue at the outermost part of the root. The Cortex tissue, consisting of 4-7 cells, was located under the periderm. The endodermis was clearly observed as a single layer. The vascular system covered a large area under the endodermis. There were 2 to 3 layers of cambium, placed between phloem and xylem. The xylem tissue covered a large area and the pith region consisted entirely of tracheal elements. Some regions within the xylem tissue contained sclerenchyma cell groups. The length of the trachea in the xylem tissue was 13.90–68.31x22.77–75.90µm (mean 35.42x42.04µm).

A transverse section was taken from the middle part of the stem and it was further observed (Fig. 1). In the cross-sections, the epidermis tissue consisted of an ordered cell layer of elliptical, rectangular and square shapes. There was a 7.5 µm cuticle layer on the epidermis cells. The lower and upper epidermis tissues were covered with setose trichomes and occasionally with glandular trichomes. The stomata rarely occurred ± above the epidermis level. Under the epidermis, there was a multi-layered parenchimatical cortex tissue. In the middle parts of the cortex tissue, there were 3 to 5 layers of collenchyma tissue. The endodermis was distinguishable and unilayer. Two to 4 layered cambium tissues were between the phloem and xylem. The xylem tissue formed a ring structure and was composed of many obscure vascular bundles towards the pith. The pith was composed of parenchyma cells and covered a relatively wide area.

The cross-sections and surface sections taken from the leaves were also observed (Fig. 1). The leaves had an amphistomatic type. The cross-sections taken from the leaves, rectangular, square and elliptical-shaped epidermis cells, were located on the upper and lower surfaces as a single layer. On the upper and lower epidermis, there was a cuticle layer of 7.5 to 20µm thickness. Setose trichomes and glandular trichomes were rarely observed on the adaxial and abaxial epidermis. Very typical cystoliths were detected at the base of setose trichomes. The stomata were located at the same level as the epidermis. The thickness of the mesophyll tissue varied from 460 to 620µm (mean=550 µm). Palisade parenchyma had three-layered cells in the upper surface and they were rectangular or cylindrical in shape, whereas in the lower surface the cells were two-layered. Spongy parenchyma with 4-6 layered cells was placed between the upper and lower palisade. In the lower surface of the main vein, between the epidermis and the main vein, there were 2 to 3 layers of collenchyma tissues. The vascular bundle in the main vein was in the shape of a semicircle. Other vascular bundles were surrounded by a parenchymal bundle sheath. Anomocytic and anisocytic types of stomata were observed in the lower and upper surfaces of the leaves, which were also surrounded by 3 to 4 epidermis cells. Stomata cells had a size of 31.87x38.45µm on the dorsal side and 10.95x30.36µm on the ventral side. Upper epidermis cells were 30x44.35µm, whereas lower epidermis cells were 31.19x11.13µm in size. The stomata index was 11.13 in the upper epidermis and 10.95 in the lower epidermis in *O. polyantha.* The stoma density was higher on the lower surface.

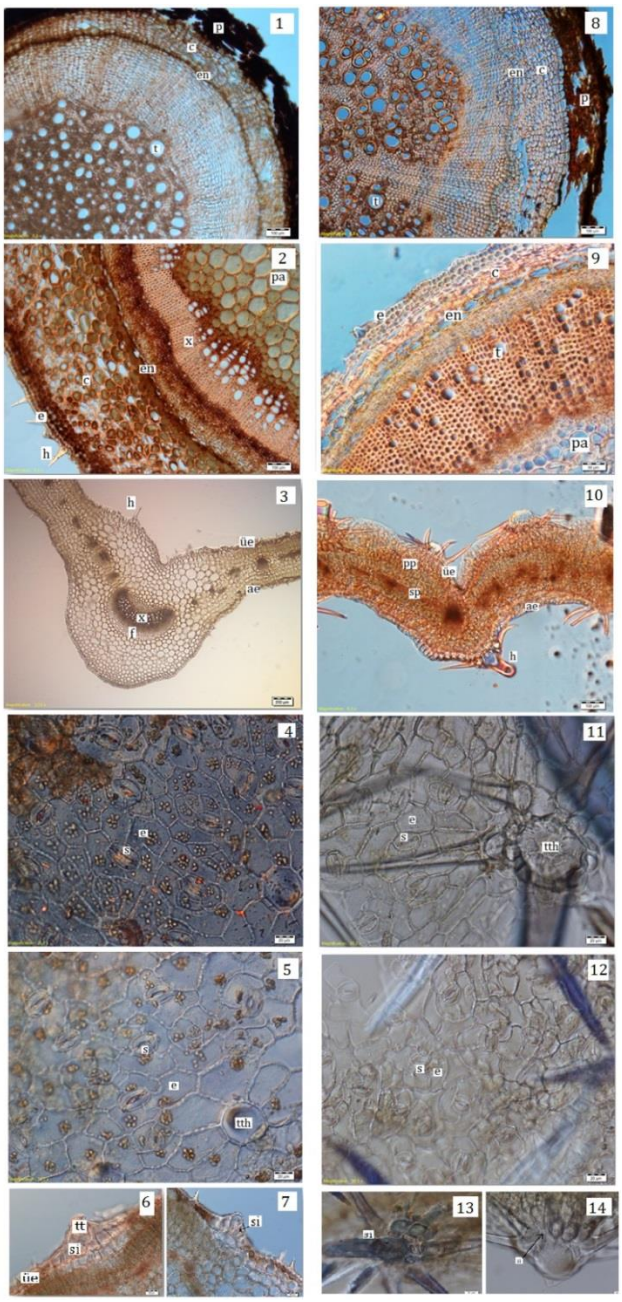

Fig. 1. *O. polyantha* (1: cross-section of the root, 2: cross-section of the stem, 3: transversal section of leaves, 4: the upper surface of the leaves, 5: the lower surface of the leaves; 6-7: cystolith. *O. mitis* (8: cross-section of the root, 9: cross-section of the stem, 10: cross-section of leaves, 11: the upper surface of the leaves, 12: the lower surface of the leaves; 13-14: cystolith).(c: cortex, p: periderm, en: endodermis, pa: parenchymatic pith cell, x: xylem, f: phloem, e: epidermis, h: hair, t: trache, üe: upper epidermis, ae: lower epidermis, sp: spongy parenchyma, pp: palisade parenchyma, s: stomata, tth: hair base cell, tt: hair base, si: cystolith).

***Onosma mitis*:** When the cross-section taken from the root was examined, the following tissues were observed (Fig. 1). A secondary structure was observed. When the cross-section of the root was examined, there was a thick periderm tissue on the outermost side. Under the periderm, 12-18 ordered cortex tissues were observed. The Cortex tissue consisting of 12-18 cells during tissue was located under the periderm. The endodermis was distinguishable as one-layered. In the middle region of the cortex tissue, there were indistinctly 2-4 layered collenchyma tissues to be seen. The xylem tissue covers a large area, and sclerenchyma cells were located in some regions. The pith consisted of tracheal elements and sclerenchyma cells. The size of the trachea in the xylem tissue was 25.30-75.90x25.30-63.25µm (mean 50.60x38.73µm).

A transverse section taken from the stem was observed as following (Fig. 1). Mostly outside the epidermis of the stem consisted of uniseriate, with different cell sizes. Setose trichomes with cystoliths at the base and glandular trichomes were rarely seen on the epidermis. The stomata were located at the same level of the epidermis. Under the epidermis, there were 8-10 ordered collenchyma tissues, which were usually unclear in the cell walls. The endodermis was clearly observed as 2-3 layered under the collenchyma tissue. There was a cambium tissue formed by 1-3 layers of flattened cells, which were not distinguishable between phloem and xylem. The xylem tissue formed an annular structure. The pith consisted of parenchymatic cells and covered a wide area.

When the cross-sections and surface sections taken from the leaves were examined, it was seen that the leaves were dorsiventral and amphistomatic (Fig. 1). In the cross-section of the leaf, there was a cuticle layer on the cells of the single-layered epidermis. The epidermis tissue, formed by rectangle, square, elliptical-shaped cells, was single-layered on the upper and lower surfaces. Setose trichomes with cystoliths were distinguishable at the base, stellate hairs and glandular trichomes rarely were seen on the adaxial and abaxial epidermis. The stomata were located at the same level of the epidermis cells. The thickness of the mesophyll tissue varies from 130 to 177µm (mean=160µm). The palisade parenchyma consisted of rectangular and cylindrical three-layered cells on the upper surface and two-layered cells on the lower surface. The spongy parenchyma was placed between the upper and lower palisade parenchyma with 4-6 layered cells. The cuticle thickness was between 3.79-3.06µm on the upper epidermis and between 4.55–5.56µm on the lower epidermis. The stomata were anamocytic and anisocytic on both sides. The stomata cell sizes were measured at 23.19x7.96µm on the dorsal side and 26.76x8.72µm on the ventral side. Upper epidermis cells were 48-64x22-13µm, lower epidermis cells were 46-80x23-11µm. Stomata index was 14.28 of the upper epidermis and 13.40 of the lower epidermis in *O. mitis.* The epidermis cell walls appeared to be more undulated than the upper surface. On the lower surface of the main vein, between the epidermis and the main vein, there were 2-3 rows of collenchyma tissues. The vascular bundle in the main vein was in the shape of a semicircle. The other circular vascular bundles were surrounded by a parenchymal bundle sheath.

Palynological characters: The palynological characteristics of both *Onosma* species examined are summarized in Table 2 and are shown in Figs. 2-3. The general palynological properties, based on LM and SEM studies are shown below:

Pollen grains of *O. polyantha* were heteropolar and spheroidal in shape (P/E: 1.12). The Amb shape was three angular-circular. Sculpture scabrate (=granulate), and scabras were homogeneously dispersed on the pollen surface. 8-10 skabra number per 1 μm^2 in mesocolpium, colpi and pori margins distinguishable. Structure tectatae, ect/end≅2/3. Intrastructure.

Pollen grains of *O. mitis* were heteropolar, the pollen type was trisyncolporate, tetrasyncolporate and subprolata in shape (P/E: 1.15). The pollen was usually trisyncolporate and a small number of pollen was tetrasyncolporate. The amb shape was tri-angular in trisyncolporate pollen and square in tetrasyncolporate pollen grains. Sculpture scabrate (=granulate), and scabras were homogeneously dispersed on the pollen surface. 10-15 scabra number per 1µm^2 in mesocolpium, colpi margins distinguishable. Structure tectatae, ect/end≅3/5. Intrastructure.

The sizes of the examined nutlets showed some variations. Nutlets of both *Onosma* species varied in the ranges of 3-4x2-3.5mm. Nutlet sizes were 4x3-3.5mm in *O. polyantha*, 3x2mm in *O. mitis*. Nutlet shapes are presented in Figure 4. In particular, the ventral keel was observed in the nutlet of the examined species. The ornamentation reticulate and epidermal cells were observed in different sizes in *O. polyantha* and rugose, rugose-reticulate, the boundaries of epidermal cells were not distinguishable in *O. mitis* (Fig. 4).

Discussion

The anatomical characteristics of the Boraginaceae family were explained by Metcalfe & Chalk (1979), Watson & Dallwitz (1991). The anatomical characteristics of the examined species suit with those of the Boraginaceae family (Metcalfe & Chalk, 1979). To our best knowledge, no anatomical and palynological characteristics of *O. polyantha* and *O. mitis* were available in the literature, except their general taxonomic properties. In this study, the anatomical and palynological characteristics of *O. polyantha* and *O. mitis* were investigated. Endemic *O. polyantha* belongs to the subsection of *Haplotricha*, whereas *O. mitis* belongs to the subsection of *Asterotricha*.

In anatomical studies, it was determined that both species have a typical dicotyledonous root and stem structure. Both examined species had a secondary root structure and a distinguishable one layered endodermis. The xylem tissue contains sclerenchyma cell groups in both species. The pith region of the root consisted of tracheal elements. In *Onosma* species as *O. giganteum* Lam. (Binzet & Orcan, 2003b), *O. bracteosum* Hausskn. & Bornm. (Akçin & Engin, 2005), *O. sieheanum* Hayek (Binzet & Akçin, 2009a), *O. mersinana* Riedl, Binzet & Orcan (Binzet & Orcan, 2009), *O. argentata* Hub.-Mor. (Özkan *et al.*, 2016). *O. polyantha* had primary xylem elements in the pith region. The pith region of *O. mitis* was composed of primary xylem and sclerenchyma cells. The length of the trachea in the xylem tissue was 13.90–68.31x22.77–75.90µm (mean 35.42x42.04µm) in the root of *O. polyantha* and the length of the trachea in the xylem tissue was 25.3-75.9x25.3-63.25µm (mean 50.6x38.73µm) in the root of *O. mitis*.

In both species, setose trichomes and glandular trichomes were rarely present on the stem epidermis. In addition, cystoliths were located at the base of the setose trichomes in both species. In the middle parts of the cortex tissue, in some regions, there were 3-5 layered collenchyma tissues in *O. polyantha* and collenchyma, which were usually squashed and the cell walls were not distinguishable with 8-10 layers, which were located under the epidermis in *O. mitis*. In both species, the endodermis was distinguishable and unilayer in *O. polyantha* and 2-3 layered in *O. mitis*. Cambium is distinguishable in *O. polyantha* as 2-4 layer, while it was undistinguishable in *O. mitis* as 1-3 layered. The pith was composed of parenchymatical cells and covered a wide area in both species.

The leaf anatomy, especially the leaf epidermis, provides important taxonomic data for *Onosma* genus (Dasti *et al.*, 2003). In *O. polyantha*, there were short and tall setose trichomes, short simple trichomes, and few glandular trichomes on the upper and lower epidermises of leaves. In *O. mitis*, short simple trichomes, short and long porrect-stellate trichomes and glandular trichomes were rarely seen on both the upper and lower epidermises of the leaves. Setose trichomes contained cystolith in the bases. Pignatti (1982) used trichomes (setae=porrect stellate) in leaves as taxonomic characters for determining *Onosma* species distributed from Italy.

As the thickness of the mesophyll tissue varied from 460-620µm (mean=550µm) in *O. polyantha*, the thickness of the mesophyll tissue varied from 130 to 177µm (mean=160µm) in *O. mitis*. Palisade parenchyma consisted of three-layered cells on the upper surface and two-layered cells on the lower surface and the spongy parenchyma with 4-6 layered cells lied between the upper and lower palisades in both species.

Table 2. Palynological characteristics of the *Onosma* taxa.

	O. polyantha	***O. mitis***
Pollen shape(P/E)	Sphaeroidal P/E=1.12	Subprolata P/E=1.15
Structure	Tectatae, ect/end ≅2/3(W)	Tectatae, ect/end ≅3/5(W)
P	15.02 ± 0.83	16.33 ± 0.75
E	13.41 ± 0.66	14.19 ± 0.48
plg	3.35 µm ± 0.40	3.20 µm ± 0.30
plt	3.65 µm ± 0.70	3.70 µm ± 0.65
clg	11.83 µm ± 0.75	11.70 µm ± 0.87
clt	3.60 µm ± 0.30	3.57 µm ± 0.25
ex	0.60 µm	0.60 µm
i	0.47 µm	0.53 µm
t	6.70 µm	6.90 µm

P: polar axis length, E: equatorial axis length, plg: porus length, plt: porus width, clg: colpus length, clt: colpus width, ex: exine thickness, i: intine thickness, t: length of one side of triangular polar area (apocolpium)

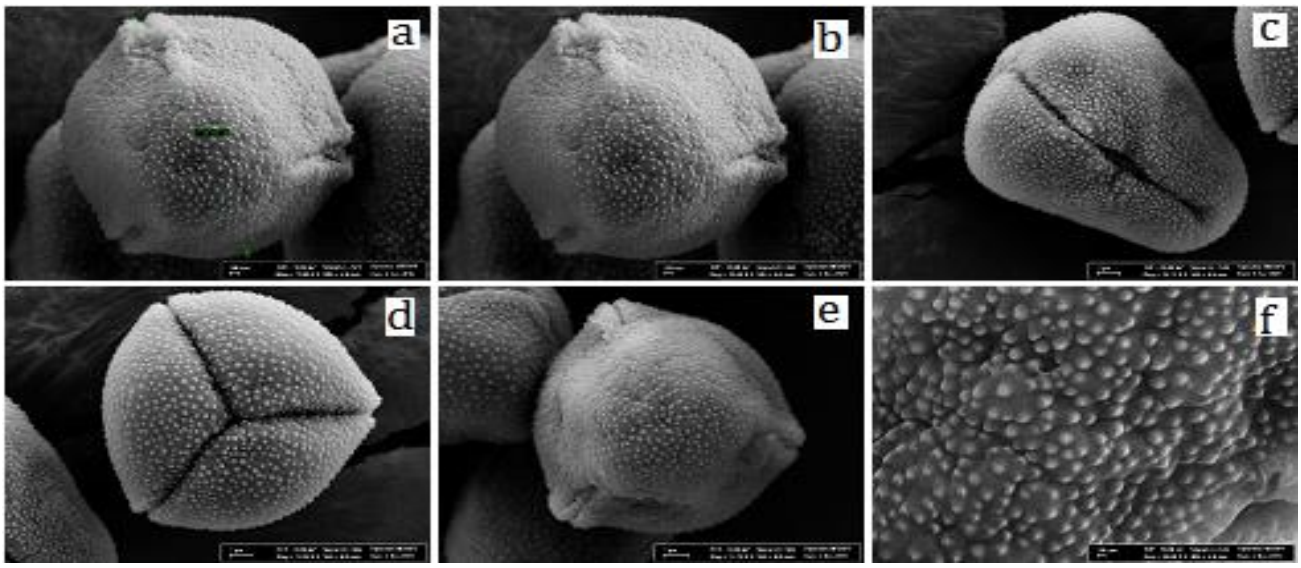

Fig. 2. SEM photographs of the pollen grains of *O. polyantha* a, b, e: proximal view, c: polar view, d: distal view, f: ornamentation.

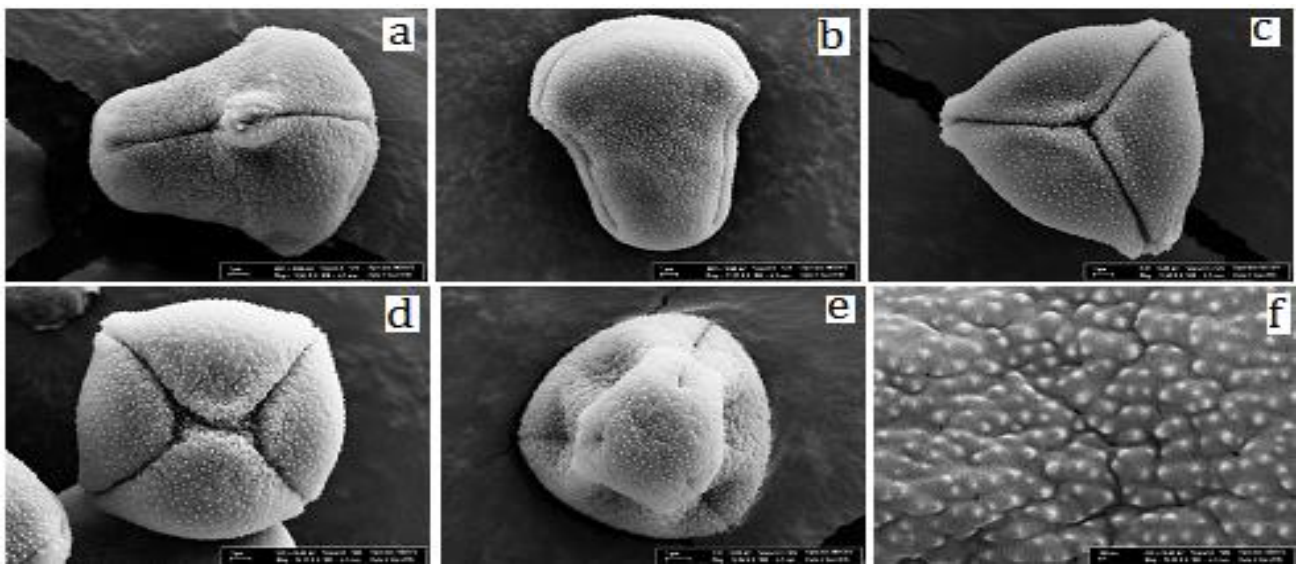

Fig. 3. SEM photographs of the pollen grains of *O. mitis* a,b: polar view, c,d: distal view, e; proximal view, f: ornamentation.

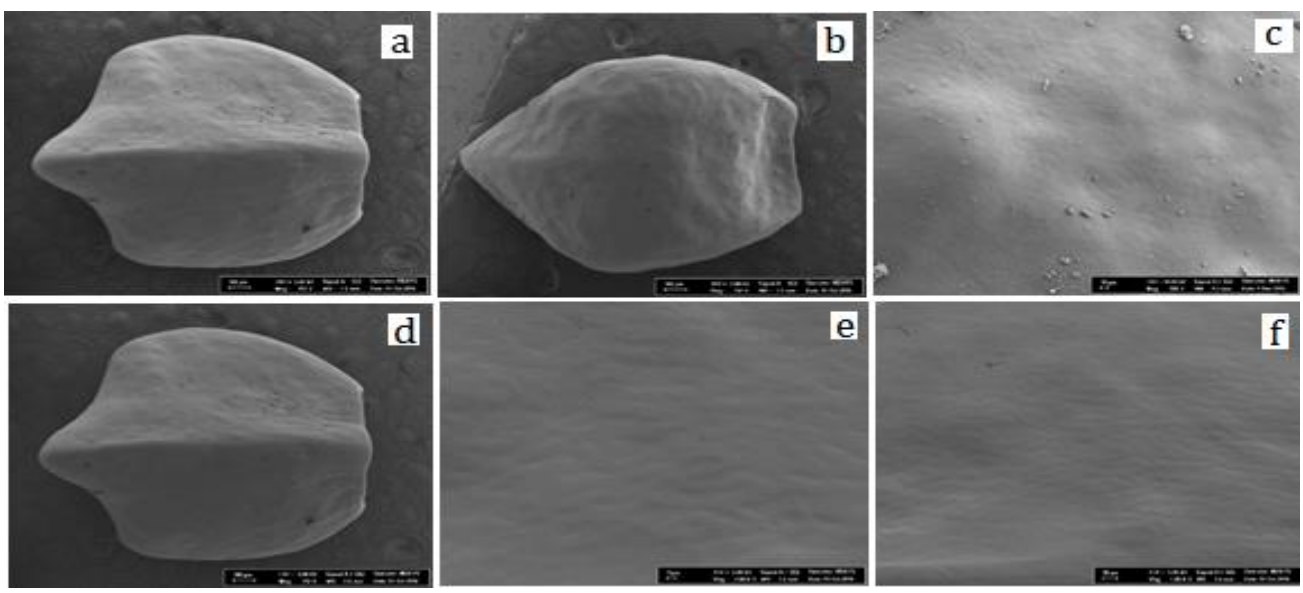

Fig. 4. SEM photographs of the nutlet surfaces of examined *Onosma* species. a-c: *O. polyantha*, d-f: *O. mitis* (SEM).

The epidermis was single-layered and its cells were rectangle, square, elliptical-shaped on the abaxial and adaxial surfaces in both examined species. Boraginaceae leaves are isobilateral and of bifacial type (Metcalfe and Chalk 1979). Azizian *et al*., (2000) reported that *Onosma* had two different leaf anatomies: the leaf is dorsiventral in sections of *Protonosma* and *Podonosma*, and isobilateral in sect. of *Onosma*.

In both species, the leaves are isobilateral (=equifacial). It has also been confirmed by previous studies that the leaves of *Onosma* species are isobilateral (Akçin & Engin, 2001; 2005; Binzet & Orcan, 2003a; 2003b; Akçin, 2004; Binzet & Orcan, 2009; Binzet & Akçin, 2009a). Stomata were present on both the lower and upper surfaces of all leaves in *Onosma* genus. Anomocytic and anisocytic stomata were seen in the Boraginaceae family (Metcalfe & Chalk, 1979). The leaf anatomies and indumentum of 14 different *Onosma* species were examined by Azizian *et al.,* (2000), and it was determined that the stomata were generally anomocytic in that study. The types of stomata were determined as anomocytic and anisocytic in *O. armena* DC., *O. intertexta* Hub.-Mor., *O. sieheana* Hayek, *O. frutescens* Lam. and *O. inexspectata* Teppner (Akçin, 2007a; Binzet & Akçin, 2009a; 2012). In another study presented by Zarinkamar (2007), the stomata were also identified as dominant anamocytic and anisocytic types in *O. microcarpa* DC. and *O. dichroantha* Boiss. Dasti *et al.,* (2003) reported that although the anomocytic type was dominant, hemiparacytic, helicocytic, staurocytic and brachyparatetracytic types of stomata were also seen in *O. stephonia.* Our results were similar with Metcalfe & Chalk (1979), Akçin (2007a), Binzet & Akçin (2009b; 2012). In our study, the stomata type was determined as anisocytic and anomocytic in both species. In the examined leaves of the *Onosma* species, the stomatal size was 31.87x38.45µm on the dorsal side and 10.95x30.36µm on the ventral side in *O. polyantha*, while the stomatal size was 23.19x7.96µm on the dorsal side and 26.76x8.72µm on the ventral side in *O. mitis*. The Stomata index was 11.13 of the upper epidermis and 10.95 of the lower epidermis in *O. polyantha*, 14.28 of the upper epidermis and 13.40 of the lower epidermis in *O. mitis*.

The main pollen characters of examined the *Onosma* species were summarized in Table 2 and illustrated in Figs. 2-3. According to LM and SEM observations, the pollen grains were heteropolar and trisyncolporate and sphaeroidal in shape (P/E: 1.12) in *O. polyantha* and the pollen grains were heteropolar and trisyncolporate, tetrasyncolporate and subprolata in shape (P/E: 1.15) in *O. mitis.* While the Amb shape was three angular-circular in *O. polyantha*, the Amb shape was tri-angular in the trisyncolporate pollen grains and square in the tetrasyncolporate pollen in *O. mitis*. In both species, the sculpture was scabrate (=granulate) and scabras were distributed on the surface of the pollen homogenously. 8-10 scabra per 1 μm^2 in mesocolpium in *O. polyanyha* and 10-15 skabra number per 1 μm^2 in mesocolpium in *O. mitis*. The palynological characteristics of both species were suitable with those of former studies (Maggi *et al.,* 2008; Binzet *et al.,* 2014).

Riedl (1978) reported that the external nutlet sizes, shapes, characters, colors and ornamentations were of limited taxonomic value. However, the sculpturing of the nutlet surface patterns, as observed by SEM, showed specific variations. The results of this study are in agreement with the findings of Akçin (2007b) and Binzet & Akçin (2009b). Nutlet sizes showed some variations where the nutlets of the studied *Onosma* species varied in the ranges of 3 - 4x2-3.5mm, they were 4x3-3.5mm in *O. polyantha* and 3x2mm in *O. mitis*. In particular, a ventral keel was observed in the nutlet of the examined species. The ornamentation reticulate in *O. polyantha* and rugose, rugose-reticulate, in *O. mitis* were seen.

Acknowledgements

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