Pak. J. Bot., 49(2): 579-588, 2017.

ANATOMICAL, MORPHOLOGICAL AND PALYNOLOGICAL STUDIES OF SOME *ONOSMA* L. (BORAGINACEAE) TAXA ENDEMIC TO ANATOLIA

HALİL İBRAHİM TEKE[1] AND RIZA BİNZET[2*]

[1]*Department of Biology, Faculty of Sciences and Arts, Adıyaman Üniversity, Adıyaman, Turkey*
[2]*Department of Biology, Faculty of Sciences and Arts, Mersin Üniversity, 33343 Mersin, Turkey*
[*]*Corresponding Author: rbinzet@gmail.com, rbinzet@mersin.edu.tr*

Abstract

In this study, the morphological, anatomical and palynological characters of *Onosma discedens* Hausskn. ex. Bornm., *O. nana* DC. and *O. sorgerae* var. *subglabriflora* Teppner were investigated. All the examined species are endemic to Anatolia, Turkey. The root cortex of examined species is multilayered. The raphide crystals are observed in epidermal cells and basal hair cells on both side of epidermis in *O. sorgerae* var. *subglabriflora*. Leaves are isobilateral (=equifacial) in *O. nana* and *O. sorgerae* var. *subglabriflora*, whereas leaves are dorsiventral(=bifacial) in *O. discedens*. Stomatas are observed on both surfaces (=amfistomatic) in all examined species. Stomata index is 9.25 of upper epidermis and 10.71 of lower epidermis in *O. discedens*, 9.61 of upper epidermis and 10 of lower epidermis in *O. nana* and 9.67 of upper epidermis and 13.63 of lower epidermis in *O. sorgerae* var. *subglabriflora*. The pollen grains are heteropolar, trisyncolporatae and sphaeroidal shape. In *O. discedens*, sphaeroidal (P/E: 1.13 W) and subprolate in Acetolysis and in *O. nana*, subprolate in shape and in *O. sorgerae* var. *subglabriflora.* Amb is three angular in all examined *Onosma* species. Sculpture is scabrate-rugulate in all species. Nutlet ornamentation is rugose.

Key words: Endemic, Morphology, *Onosma*, Systematics, Anatolia, Turkey.

Introduction

The Boraginaceae fa

mily consists of c. 131 genera and ± 2500 species divided in five subfamilies and seven tribes (Retief & Vanwyk, 1997). The Boraginaceae are distributed throughout the tropical, subtropical and temperate regions of the world. The centers of highest diversity in the North Temperate Zone are the Irano-Turanian and Mediterranean regions, and in the tropics are Central America and northern and central South America. The Boraginaceae are poorly developed in the colder regions. In contrast, Turkey has about 300 species whereas Iran, Afghanistan and Pakistan have about 380 species (Al-Shehbaz, 1991).

Onosma L. genus belongs to the family Boraginaceae, order Lamiales, consisting of over 150 species growing in typical xeric habitats, are mostly perennial, usually suffruticose or biennial herbs. The genus has its greatest species diversity in southeast Europe and Asia Minor and is known to include several serpentine endemics, especially in the southern Balkans (Stevanovic *et al*., 2003). However, more detailed studies showed that the number of species was underestimated. According to nine Floras, more than 230 *Onosma* taxa are recognized (Riedl, 1978; Boissier, 1879; Dinsmor, 1932; Tutin *et al*., 1972; Hayek & Markgraf, 1970; Meikle, 1985; Teppner, 1991; Ge-Ling *et al*., 1995). Anatolia is an important centre of origin for *Onosma* comprising about 103 species (108 taxa), 51 of which and 1 variety are endemic to Turkey (Riedl, 1978; Davis *et al*., 1988; Yildirimli, 2000; Riedl *et al*., 2005; Binzet & Orcan, 2007; Kandemir & Türkmen, 2010; Aytaç & Türkmen, 2011, Güner, 2012; Koyuncu *et al*., 2013; Binzet, 2016a, Binzet, 2016b).

Onosma species have specific indumentums formed by a single large bristle (seta) growing out of the top of a tubercle, and (present in some groups only) several rays of smaller setae attached circularly to the base of the tubercle and directed radially (called asterosetules or stellate setae; Javorka, 1906; Ball, 1972).

Based on morphological characteristics, mainly the indumentum type, three groups have been recognized within the genus, originally described as sections: *Onosma* sect. *Asterotricha* Boiss., sect. *Haplotricha* Boiss., and sect. *Heterotricha* Boiss., (Boissier, 1879). Section *Asterotricha* is characterized by stellate setae, Haplotricha lack stellate hair, and *Heterotricha* possess an intermediate indumentum type. These three Boissier sections are currently treated as informal groups (Peruzzi & Passalacqua, 2008). Division into three infrageneric groups seems to be supported also by karyological data. Representatives of the section Heterotricha group are characterized by bimodal chromosome sets 2n=12L+14S and 2n=12L +8S (L and S denote large and small chromosomes, respectively; Teppner 1971) and probably originated via hybridization between taxa of the Haplotricha (2n=12L) and Asterotricha (2n=14S) groups (Teppner, 1971, 1972; Vouillamoz, 2000; Martonfi *et al*., 2008).

Taxonomic treatments within these three groups are highly controversial. Many species were described based on minor morphological differences, and consequently they have often been confused. Riedl (1978) pointed out that the classification appeared to be partly artificial and in need of re-investigation and new data (karyological and palynological) may provide useful reference points in the future classification of the genus.

Several *Onosma* species are used as folk medicine, herbs and dyes. In folk medicine, these plants are employed for burns, wounds and ailments (Khajuria & Jain, 1993; Ozgen *et al*., 2003). *O. microcarpum* Steven ex DC., *O. sericeum* Willd. and *O. argentatum* Hub.-Mor. are used for the treatment of wounds in rural areas in Turkey (Özgen *et al*., 2003 and Özgen *et al*., 2004). The flowers of some species are consumed as vegetables (Öztürk & Özçelik, 1991).

Studies on the anatomy of *Onosma* genus are limited. Metcalfe & Chalk (1979) and Watson & Dallwitz (1991) studied the anatomical characters of some species of family Boraginaceae. Akçin & Engin (2001; 2005) and Akçin (2004; 2007) Binzet & Akçin (2009) studied the anatomical and ecological properties of some *Onosma* species. Binzet & Orcan (2003a; 2003b; 2009), Binzet & Akçin (2009), Binzet (2011), Binzet & Akçin (2012) also investigated the anatomical features and palynological characteristics of *Onosma* species.

Materıal and Methods

In this study, the morphological, anatomical and palynological characters of *O. discedens*, *O. nana*, and *O. sorgerae* var. *subglabriflora* is investigated. Plant samples were collected from natural populations and some of them were used for morphological, anatomical and palynological studies while others were dried as herbarium samples. Voucher samples were deposited in the herbarium of Science and Art Faculty of Mersin University. The samples were collected from the following locations (Table 1).

Table 1. Locality information of the examined *Onosma* taxa.

Taxa	Locality
O. discedens	B7 Erzincan: Kemaliye-İliç 15 km., rocky slopes and steppe, 1453 m., 39º 22′ 13″ N 38º 27′ 89″ E, 08.06.2010, Binzet 201030; Erzincan: Kemaliye-İliç 10 km., rocky slopes and steppe, 1430 m., 39º 20′ 23″ N 38º 28′ 41″ E, 31.05.2011, Binzet 201111.
O. nana	C5 Niğde; Southern of Demirkazık village, Yarpuz valley, rocky areas, 2170-2250 m, 37º 48′ 42″ N 35º 07′ 02″E, 10.06.2011, Binzet 201120; 07.07.2011, Binzet 201145.
O. sorgerae var. *subglabriflora*	C7: Adıyaman, Nemrut Mountain, steppe and stony areas, 1950 m, 37º 58′ 25″ N 038º 43′ 85″E, 11.05.2010, Binzet 20108; 01.06.2011, Binzet 201116.

Taxonomical descriptions of the specimens were made according to Riedl (1978). Morphological measurements were based on 10-15 specimens. Anatomical studies were carried out on the samples kept in alcohol 70 %. Cross and surface sections of root, stem and leaves were excised by hand and they were covered with glycerin-gelatin (Vardar 1987). Microphotographs were taken with Olympus BX51 microscope. Stomatal index was calculated according to Meidner & Mansfield (1968).

Slides of the pollen grains obtained from herbarium and fresh materials and slides were prepared using the methods described by Wodehouse (1935) and Acetolysis (Erdtman, 1952) respectively for light microscope study. LM observation (polar and equatorial axis, pollen shape, Length of pores (pori) and colpus (colpi), width of pores (pori) and colpus (colpi), exine thickness, intine thickness and length of polar triangular edge) were conducted with a Olympus BX 40 research microscope (x10; x100). Pollen dimensions All of species were measured in such amounts that the resulting data followed Guassian curves.

For SEM studies, pollen grains were first treated with 70 % alcohol, then dried before mounting on stubs with gold and examined under JMS-6400 SEM at the Osmangazi University (ESOGUSTEM). The terminologies for polen morphology were used in accordance with Erdtman (1952), Faegri & Iversen (1964) and Punt *et al*. (1994).

Morphological properties

Onosma discedens Hausskn. ex Bornm. Mag. Bot. Lap. 30: 78 (1931).

Perennial, stem erect and numerous, 18–30 cm, dense greyish hairy. Clothed at base with remains of leaves. Basal and lower cauline leaves 50-130x5-10 mm, linear–lanceolate, margins revulate, acute, silvery beneath with almost silky dense adpressed hairs, green above, hairs sparse. Upper cauline leaves 30–50x5–10 mm, sessile shorter and broader, lanceolate, apex acute, dense adpressed setae. otherwise similar. Inflorescence dense cymes grouped into a terminal head, elongating after flowering. Bracts , -35x-15 mm, broadly lanceolate, upper narrowly linear, Pedicels -6 mm in flower, -10 mm in fruit. Calyx 12–15 mm in flower, -20 mm in fruit, densely patent yellowish setose. Corolla -16 mm, whitish to cream, scarcely longer than calyx, annulus glabrous, narrowly cylindrical, villous, lobes 1.2-1.5x1 mm. Anthers c. 6 mm, Filaments c. 3 mm, Stilus 15-17 mm. Nutlets 4x3 mm, ovoid-triquetrous, brownish, apex cuspidate. Endemic, Ir-Tur. element. Flowering Time and Habitat: June, stoney and rocky slopes and steppe.

Onosma nana DC., Prodr. 10: 65 (1846). Syn: *O. microsperma* auct. non Steven ex Boiss. (1875).

Perennial. Stems simple, erect 8–20 cm, patent stose, setae arising from small tubercles, shortly hairy. Clothed at base with remains of leaves. Basal leaves 20–55x2-3mm, narrowly oblanceolate, apex acute petiolate, ± patent setae, setae 1.5-2 mm, short patent hairy. Cauline10–24x2-3 mm, linnear, sessile, apex acute, patent setae, setae – 3 mm, short hairy. Inflorescence of 1-2 terminal usually few flowered cymes. Bracts 10–12x1-2 mm, ovate-lanceolate, patent setae, acute. Pedicels 1-2(-3) mm. Calyx 10-20 mm, lobes linear, apex subacute, not or slightly accrescent after flowering, lobes setose on both surfaces. Corolla white to pale blue at first, becoming pink or sky blue or pale blue-violet, 20–25 mm, glabrous or minutely papillate, cylindrical-campanulate, annulus glabrous, lobes 2.5-2.8 x 1mm, sparsely hairy. Anthers c. 6 mm, Filaments c. 7 mm, Stilus -28 mm. Nutlets 4x2.7 mm with dorsal keel and short beak, pale grey. Endemic, E. Medit. Element. Flowering Time and Habitat: May-July, rocky areas and stoney slopes.

Onosma sorgerae Teppner in Phyton 20: 139 (1980, sphalm. 'sorgerae'). var. *subglabriflora* Teppner in Phyton 20: 141(1980).

Perennial. Stem erect, strongly branched, 10-20 cm, sende sterile rosette, sparsely patent setae and dense ± retrorse hairy. Rosette leaves narrowly obovate or narrowly oblanceolate, gradually attenuate into petiole, 20-70x2-6 mm, setae arising from shortly stellate hairy tubercles. Flowering stems erect, 2-3 branched, Basal leaves 20–70x2-9 mm, spatulate-narrow oblanceolate, setae arising from shortly stellate hairy tubercles. Cauline leaves 16–25x3–5 mm, narrowly obovate or narrowly oblanceolate, apex acute, sessile, setae 1.5-2 mm, stellate rays 0.3-0.6 mm. Inflorescence capitat at first, later slightly elongating. Bracts 14-16x3-9 mm in flower, -23 mm in fruit, lanceolate. Pedicels 1-2 mm in flower, -4 mm in fruit, patent setae. Calix 12-18 mm in flower,, 18-23(-25) mm in fruit, pilose to base within. Corolla 19-24 mm clavate, cream-pale pink at first, later blue-violet, glabrous, lobes c. 1 x 2.5 mm, pilose. Anthers 8 mm, Filaments 4 mm, Stilus 25-28 mm. Nutlets 3.5-5.5x2.5-3.5 mm, apex acute, smooth and shinning grey-brownish. Endemic, Ir.-Tur. element. Flowering Time and Habitat: May-June, stoney and rocky areas.

Anatomical properties

Onosma discedens

In transverse sections of the root, a secondary structure was observed. Outermost periderm tissue is composed of multilayers. Cortex tissue were seen bellow the periderm as multilayered and covers a wide area. Endodermis is indistinguishable. Cambium with 2-4 layers were located between phloem and xylem. Xylem parenchima cells were seen in xylem tissue. The pith consist of tracheal cells.

In transverse section taken from the middle part of the stem following characters were observed (Fig. 1). The epidermis consisted of uniseriate, oval and rectangular cells. Setose, glandular and eglandular trichomes were seen on the epidermis. Stomata were located at the level of the epidermis. Collenhyma with 2-3 layers is located under the epidermis. Cortex tissue consisted of 10-16 layers. Cambium is distinguishable between phloem and xylem. Xylem tissue is composed of many obscure vascular bundle towards the pith. The pith consisted of cylindrical parenchymatic cells and covered wide area.

In the transverse and surface sections taken from the leaf following characters were observed. Epidermis formed by various shaped cells is single layered on the adaxial and abaxial surface. Cells of upper epidermis are 17-55 µm x 12-38 µm, lower epidermis cells 17-50 µm x 7-38 µm. Both epidermises are covered with dense setose trichomes. Stomatas are observed on both surfaces (=amfistomatic). Stomatas occur on the lower level with the adaxial and abaxial epidermis. Stomata on the lower surface outnumber those on the upper surface. . Stomata index is 9.25 for the upper epidermis and 10.71 for the lower epidermis. Stomata are anamocytic and anisocytic. Stomata are surrounded by 3-5 cells on both side leaves and measured 7.5x30 µm on the dorsal and ventral side. Leaves are dorsiventral(=bifacial) in type. Palisade parenchyma has three layered cells on upper sides and they are rectangular, cylindirical shape and measured as 38-56 x 7-15 µm with spongy parenchyma with 4-6 layered cells on lower sides. Vascular tissue is surounded by a parenchymal bundle sheath. (Fig. 1)

Onosma nana

A transverse section taken from the root was observed as followed (Fig. 1). A secondary structure is observed. Outermost, periderm tissue is composed of 8-12 layers. Cortex consist of 10-12- flattened parenchimatical cells. Endodermis is distinguishable. Cambium of 3 layers lies between phloem and xylem. The pith consist of primer xylem elements.

A transverse section taken from the middle part of the stem was obsorved as follow (Fig. 1). The most outside; the epidermis of stem consists of uniseriate, oval and rectangular cells. Setose, glandular and eglandular trichomes were seen on the epidermis. Stomata is located at the upper level of the epidermis. Collenhyma with 3-4 layered were located under the epidermis. 3-4 layered squashed cells are take places the dorsal part of the cortex tissue. Cambium is distinguishable. Xylem tissue is composed of many vascular bundle towards the pith. The pith consist of cylindrical parenchymatic cells and cover wide area.

The transverse and surface sections taken from the leaf were observed as follows (Fig. 1). Epidermis tissue formed by various shaped cells is single layered on the adaxial and abaxial surface. Upper epidermis cells 20-63 µm x 10-33 µm, lower epidermis cells 15-55 µm x 7-20 µm. Epidermis membrane in bellow surfaces are more undulate than the upper surfaces. Setose and glandular trichomes present on the adaxial and abaxial epidermis. Stomatas occur on the both surfaces and upper level with epidermis cells. Stomata index is 9.61 for the upper epidermis and 10 for the lower epidermis. Stomatas are observed on both surfaces (=amfistomatic). Stomata are anamocytic and anisocytic. Stomata are surrounded by 3-5 cells on both side leaves and measured 7.5x27 µm on the dorsal and ventral side. Leaves are isolateral(=equifacial) in type. Palisade parenchyma has two layered and three layered cells rarely and they are rectangular, cylindirical shape and measured as 27-38 x 8-15 µm on the upper surface and single layered on the lower surface. Spongy parenchyma with 2-3 layered cells lies between the upper and lower palisade. Vascular tissue is surounded by a parenchymal bundle sheath.

Onosma sorgerae var. *subglabriflora*

A secondary structure is observed in root. Peridermis is multilayered at the outermost of the cross-section (Fig. 1). Cortex tissue is composed of 15-20 layered oval and circular parechymatical cells. Endodermis is distinguishable as one layered. Cambium of 3-5 layers lies between phloem and xylem. Sclerenchyma cell and sclerenchyma groups were located in xylem tissue. The pith consist of tracheal cells.

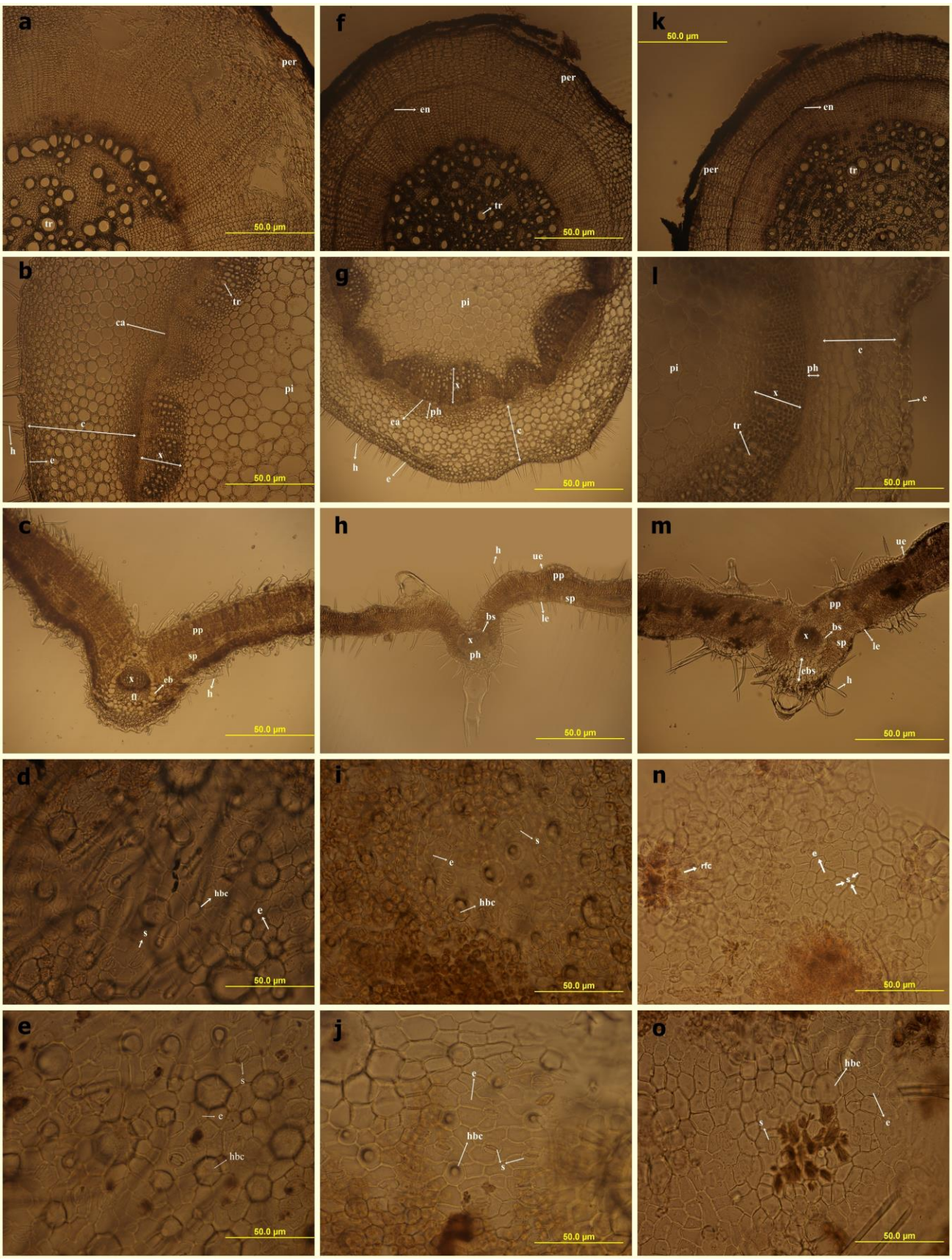

Fig. 1. *Onosma discedens*(a: cross section of root, b: cross section of stem, c: transversal section of leaves, d: lower surface of the leaves, e: upper surface of the leaves; *Onosma nana* (f: cross section of root, g: cross section of stem, h:transversal section of leaves, i: lower surface of the leaves, j: upper surface of the leaves) l-p; *Onosma sorgerae*(k: cross section of root l: cross section of stem m: transversal section of leaves n: lower surface of the leaves o: upper surface of the leaves). bs: bundle sheat, ca: cambium, c: cortex, e: epidermis, ebs: extaention bundle sheat, en: endodermis, h: hair, hbc: hair base cell, le: lower epidermis, pp: palisade parenchyma, per: periderm, ph: phloem, pi: pitht, rfc: raphide cyrstal, s: stomata, sp: spongy parenchyma, tr: trache, ue: upper epidermis, x: xylem

A transverse section taken from the middle part of the stem has the following characteristics (Fig. 1). The epidermis consists of uniseriate, square, oval and rectangular cells. Setose, glandular and eglandular trichomes are seen on the epidermis. Stomata is located at the level of the epidermis. Raphide crystals are observed in epidermis cells, 2-3 layered of cortex which is under the epidermis and in the base cell hairs. 3-4 layered squashed cells are present in the central part of the cortical tissue. Cambium is distinguishable and 1-3 layered. Xylem tissue is composed of many vascular bundle towards the pith. The pith consist of cylindrical parenchymatical cells of different size and cover wide area.

The transverse and surface sections taken from the leaf are observed as follows (Fig. 1). The adaxial and abaxial epidermises of the leaf consist of uniseriate, rectangular, pentagonal, hexagonal and heptagonal cells in transverse section upper epidermis are 18-53x15-30 µm and lower epidermis cells are 15-43x10-25 µm. Epidermis membrane in bellow surfaces are more undulate than the upper surfaces. The raphide crystals are observed in epidermis cells and basal cells of hairs on both side of epidermis. Setose and glandular trichomes are present on the adaxial and abaxial epidermis. Stomatas occur on the both surfaces and the same level with epidermis cells. Stomata on lower side outnumber those on lower side. Stomatas are observed on both surfaces (=amfistomatic). Stomata are anamocytic and anisocytic. Stomata are surrounded by 3-6 cells on upper side and surrounded by 4-5 epidermis cells on bellow side and measured 105x20 µm on the dorsal and ventral side. Leaves are isolateral(=equifacial) in type. Stomata index is 9.67 for the upper epidermis and 13.63 for the lower epidermis. Palisade parenchyma has two layered and they are rectangular, cylindirical shape and measured as 38-48 x 8-11 µm on the upper surface and single layered on the lower surface. Spongy parenchyma with two layered cells lies between the upper and lower palisade. Vascular tissue is surounded by a parenchymal bundle sheath. (Fig. 1).

Palynological characters: The results of LM data on the pollen grains of *Onosma* species examined are summarised in the Table 2 and they are shown in Figures 2-4. The general pollen characteristics, based on LM and SEM studies, is given below:

Pollen grains of *O. discedens* is heteropolar and sphaeroidal in shape (P/E: 1.08 W; P/E: 1.12 E). Amb shape is three angular. Sculpture scabrate-rugulate, scabrae outnumber on equatorial region and distal pole with 15 scabrae per 1 μm^2 in mesocolpium, colpi and pori margins ± distinguishable (Fig. 2).

Pollen grains of *O. nana* is heteropolar, pollen type is trisyncolporatae and sphaeroidal in shape in Wodehouse (P/E: 1.13 W) and subprolate in Acetolysis (P/E: 1.15 E). Amb shape is triangular. Sculpture scabrate-rugulate and scabrae are distributed on the surface of pollen homogenouslly with 14 scabrae number per 1 μm^2 in mesocolpium, colpi margins ± distinguishable (Fig. 3).

Pollen grains of *O. sorgerae* var. *subglabriflora* is heteropolar, polen type is trisyncolporatae and subprolata in shape (P/E: 1.13 W; P/E: 1.15 E). Amb shape is triangular. Sculpture scabrate-rugulate and scabrae outnumber on equatorial region with 18 scabrae per 1 μm^2 in mesocolpium, colpi margins are distinguishable (Fig. 4).

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

	O. discedens	***O. nana***	***O. sorgerae* var. *subglabriflora***
Pollen shape(P/E)	Sphaeroidea P/E:1.08 (W) Sphaeroidea P/E:1.12 (E)	Sphaeroidea P/E:1.13 (W) Subprolata P/E:1.15(E)	Subprolata P/E:1.14(W) Subprolata P/E:1.20(E)
Structure	Tectatae, ect/end ≅ 2/3(W);1/3 (E)	Tectatae, ect/end ≅ 1/2 (W,E)	Tectatae, ect/end ≅ 1/2 (W,E)
P	13.91 ± 0.76 (W) 14.16 ± 0.73(E)	14.76 ± 0.74 (W) 14.40 ± 0.75 (E)	15.13 ± 0.48 (W) 15.43 ± 0.73 (E)
E	12.77 ± 0.67(W) 12.54 ± 0.59(E)	12.8 ± 0.57 (W) 12.43 ± 0.92 (E)	13.17 ± 0.34 (W) 12.85 ± 0.75 (E)
plg	2.85 ± 0.26(W) 4.12 ± 0.18(E)	2.60 ± 0.23 (W) 4.02 ± 0.11 (E)	3.4 ± 0.25 (W) 3.98 ±0.18 (E)
plt	3.18 ± 0.20(W) 4.15 ± 0.13(E)	2.9 ± 0.22 (W) 4.2± 0.14 (E)	3.57 ± 0.29 (W) 4.11 ± 0.23 (E)
clg	11.78 ± 0.62(W) 11.41 ± 0.56(E)	12.22 ± 0.48 (W) 11.92 ± 0.41 (E)	13.26 ± 0.66 (W) 12.76 ± 0.76 (E)
clt	4.25 ± 0.28(W) 4.02 ± 0.11(E)	4.41 ± 0.18 (W) 3.98 ± 0.12 (E)	3.83 ± 0.23 (W) 3.97 ± 0.20 (E)
t	5.75 (W) 6.9 (E)	6.76 (W) 7.04 (E)	6.19 (W) 6.22 (E)
ex	0.48 (W) 0.81 (E)	0.49 (W) 0.91 (E)	0.7 (W) 0.77 (E)
i	0.71 (W)	0.69 (W)	0.7 (W)

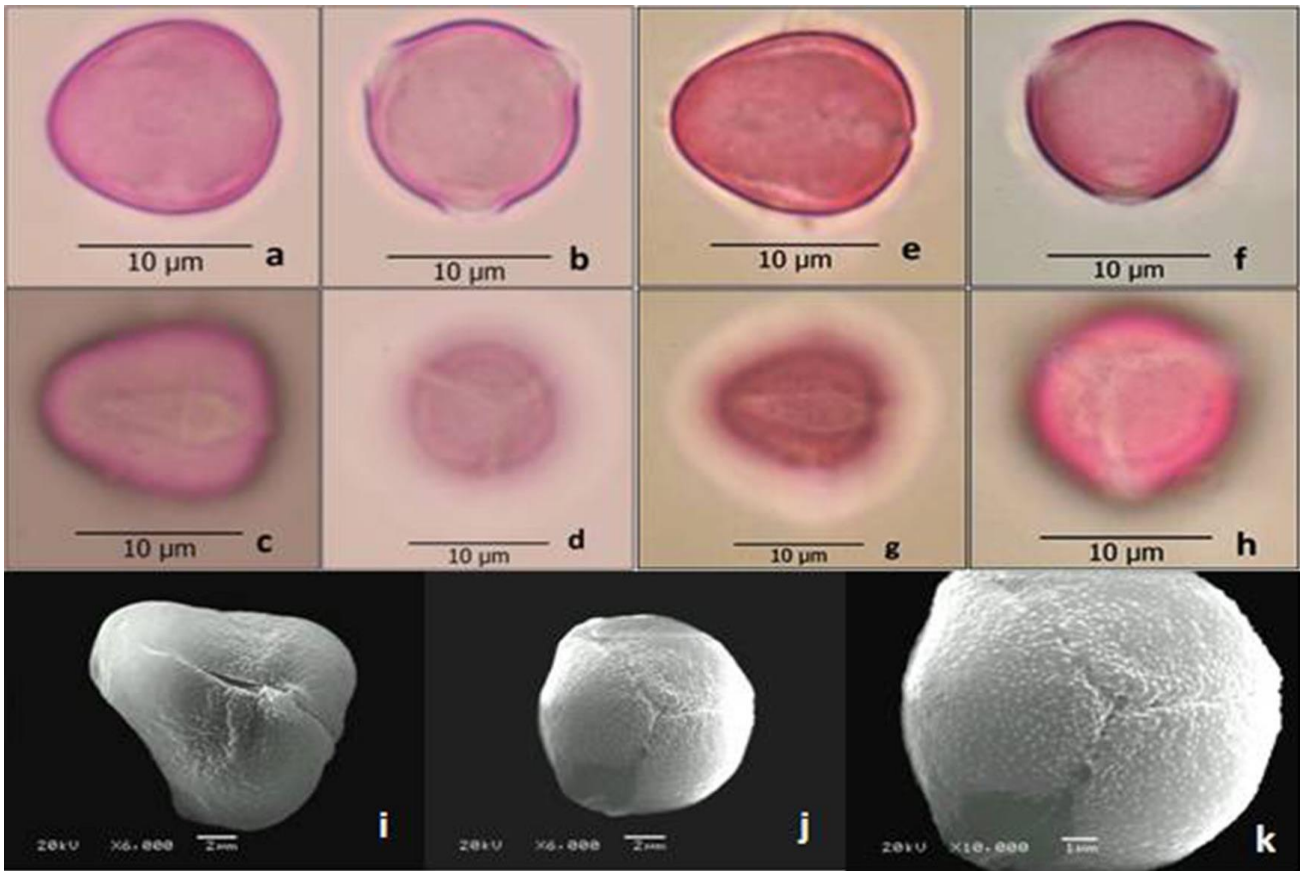

Fig. 2. *O. discedens* a,c: equatorial view (E); b, d: polar view (E); e, g: equatorial view (W); f, h: polar view (W), i-k: SEM photographs of the pollen grains.

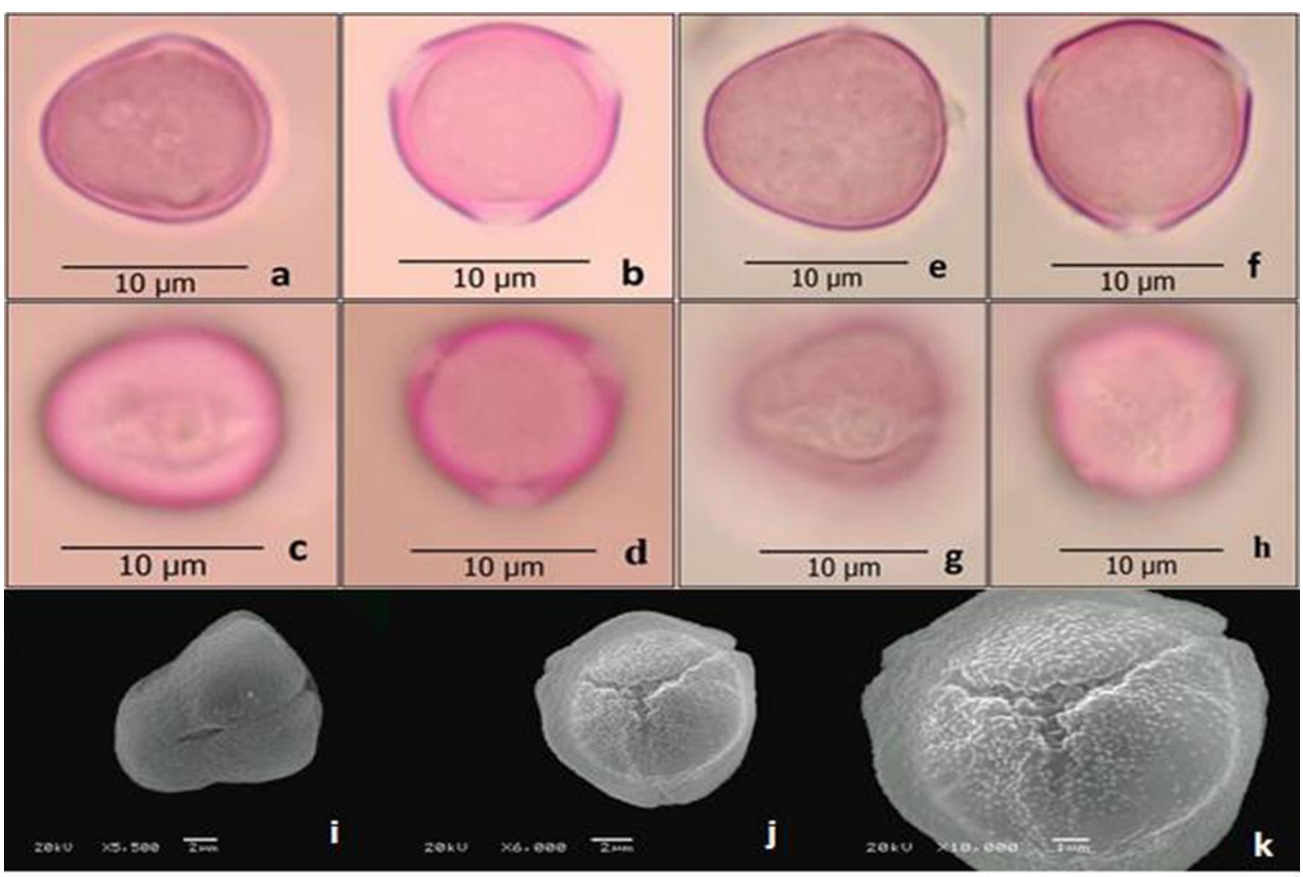

Fig. 3. *O. nana* a,c: equatorial view (E); b,d: polar view (E); e,g: equatorial view (W); f,h: pola view (W), i-k: SEM photographs of the pollen grains.

Fig. 4. *O. sorgerae* var. *subglabriflora*, a,c: equatorial view (E); b,d: polar view (E); e,g: equatorial view (W); f,h: polar view (W). i-k: SEM photographs of the pollen grains.

Studied nutlet size show some variations which ranges 3.5-5.5x2.7-3.5 mm. *O. nana* has the smallest nutlets (4x2.7 mm). *O. sorgerea* var. *subglabriflora* has the largest nutlets (3.5-5.5x2.5-3.5mm). Nutlet shape is also variable (Fig 4). Especially in nutlet of examined species sharp ventral keel were seen. Nutlet ornamentation is rugose type in all the three species. This type is characterised by the epidermal cells of the nutlet surfaces having small or fine wrinkles (Fig. 5).

Dıscussıon

In this study, the morphological, anatomical and palynological characteristics of *Onosma discedens*, *O. nana* and *O. sorgerae* var. *subglabriflora* were investigated. *Onosma discedens* and *O. nana* belong to the subsection *Haplotricha* whereas *O. sorgerae* var. *subglabriflora* belong to the subsection *Asterotricha*. All the examined species are perennial and endemic. Although our morphological results generally agree with the description in Flora of Turkey, some differences have been also identified. Basal and cauline leaves of *O. discedens* are 60-70x5-7 mm, and pedicel is 6-8 mm in nuks in Flora of Turkey. While in this study base and cauline leaves of *O. discedens* are 50-130x5-10 mm, and pedicel is -10 mm in nuks.

Some morphological characteristics of the examined species are also reported here for the first time. In *O. discedens*; upper cauline leaves 30-50x5-10 mm, lanceolate, acute, with ± adpressed setae intensely, cymes elongated after flowering, bracts -35x-15 mm, pedicels -6 mm, corolla -16 mm, lobes 1.2-1.5x1 mm, anther 6 mm, filaments 3 mm and stylus 15-17 mm. In *O. nana*; bracts10-12x1-2 mm, corolla lobes 2.5-2.8x1 mm, nutlets 4x2.7 mm. In *O. sorgerae* var. *subglabriflora* stem 10-20 cm, cauline leaves 16-25x3-5mm, setae 1.5-2 mm, stellate hairs 0.3-0.6 mm, bracts 14-16x3-9 mm in anthesis, -23 mm in nuks, pedisel 1-2 mm in anthesis, -4 mm in nuks, calyx 12-18 mm in anthesis, corolla lobes 1 x 2.5 mm, anthers 8 mm, stylus 25-28 mm.

Metcalfe & Chalk (1979), Watson & Dallwitz (1991) studied the anatomical characters of the family Boraginaceae. The anatomical features of the examined species are similar to the other members of the family Boraginaceae (Metcalfe & Chalk, 1979). The cross section of root showed that the root had a secondary structure. Parenchyma cells groups were seen in xylem tissue in roots of *O. discedens*, Sclerenchyma cell and sclerenchyma cell groups were located in xylem tissue in roots of *O. sorgerae* var. *subglabriflora*. While, the pith region of root generally consisted of primary xylem elements in some *Onosma* species such as *O. giganteum* Lam.(Binzet & Orcan, 2003b), *O. bracteosa* Hausskn. & Bornm. (Akçin & Engin, 2005), *O. sieheana* Hayek (Binzet & Akçin, 2009), *O. mersinana* Riedl, Binzet & Orcan (Binzet & Orcan, 2009). The pith region of root consist of parenchymatic cells in the other *Onosma* species such as *O. intertexta* Hub.-Mor. (Binzet & Akçin, 2009), *O. frutescens* Lam. and *O. inexspectata* Teppner (Binzet & Akçin, 2012). The pith region of all examined species are composed of primary xylem elements.

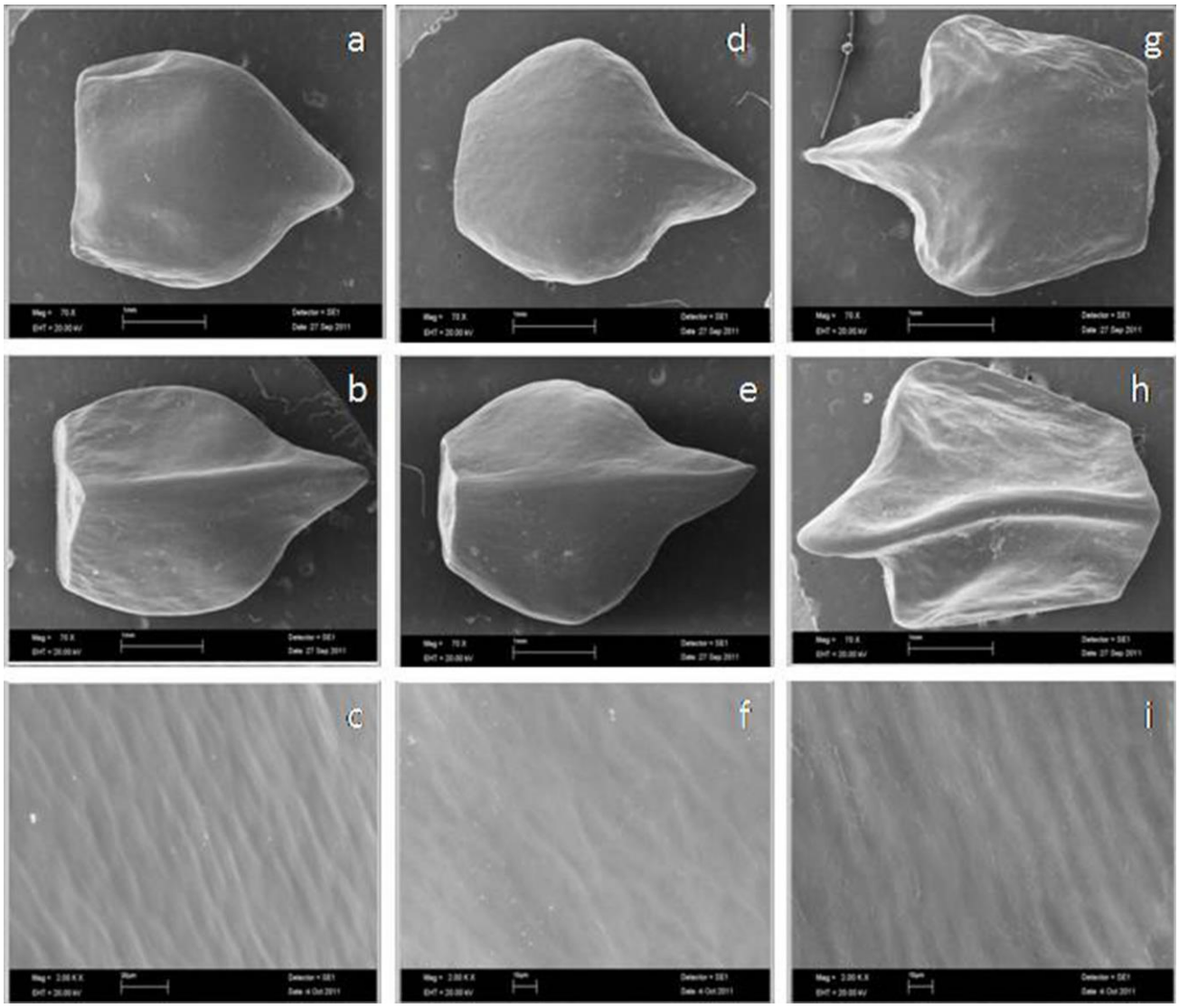

Fig. 5. Nutlet surfaces of examined *Onosma* species**.** a-c: *O. discedens*, d-f: *O. nana*, g-i: *O. sorgerae* var. *subglabriflora*

As stomata is located at the upper level of the epidermis of stem in *O. nana*, stomata is located at the level of the epidermis of stem in *O. discedens* and *O. sorgerae* var. *subglabriflora*. Collenchyma with 2-3 layered were seen under the epidermis of stem in *O. discedens* and collenchyma with 3-4 layered were located under the epidermis of stem in *O. nana*. Crystals were seen in pith cells in some *Onosma* species such as *O. mutabile* (Binzet & Orcan, 2003a), *O. intertexta* and *O. sieheana* Hayek (Binzet & Akçin, 2009). The pith of stems were composed of parenchymatical cells and cover wide are in all examined species.

Upper epidermis cells are 17-55 x 12-38 μm and lower epidermis cells are 17-50 x 7-38 μm in *O. discedens*, upper epidermis cells are 20-63 x 10-33 μm and lower epidermis cells are 15-55 x 7-20 μm in *O. halophila* and upper epidermis cells are 18-53 x 15-30 μm and lower epidermis cells are 15-43 x 10-25 μm in *O. sorgerae* var. *subglabriflora*. Epidermis membrane in lower surfaces are more undulate than the upper surfaces in both of *O. nana* and *O. sorgerae* var. *subglabriflora*. The presence of crystals in Boraginaceae family has important characters (Metcalfe & Chalk, 1979). The raphide crystals are observed in epidermis cells and basal hair cells on both side of epidermis in *O. sorgerae* var. *subglabriflora*.

Metcalfe & Chalk (1979) reported that the Boraginaceae family has both isobilateral and bifacial leaves. According to Azizian *et al*. (2000), two distinct leaf anatomical characteristics are present within the *Onosma* genus: in sections *Protonosma* and *Podonosma*, leaf is dorsi-ventral and in section *Onosma* leaf is isobilateral. Species of *Onosma* genus generally have isobilateral leaf (Akçin & Engin 2001, 2005; Binzet and Orcan 2003a,b; Akçin 2004, Binzet and Orcan 2009; Binzet and Akçin 2009; Binzet & Akçin, 2012). In this study (in section *Onosma*), while leaves are isobilateral (=equifacial) in both of *O. nana* and *O. sorgerae* var. *subglabriflora*, leaves are dorsiventral (=bifacial) in *O. discedens*. Stomatas were observed on both surfaces (=amfistomatic) in all examined species.

Metcalfe & Chalk (1979) reported that there are both anomocytic and anisocytic stomoata in Boraginaceae family. The leaf anatomies and trichome features of fourteen *Onosma* species were investigated by Azizian *et al*. (2000), who observed that the stomata

are mainly anomocytic type. Akçin (2007) and Binzet & Akçin (2009; 2012) reported that stomata are anisocytic and anomocytic type respectively, in *O. armena* DC., *O. intertexta*, *O. sieheana*, *O. frutescens* and *O. inexspectata*. According to Zarinkamar (2007), dominant stomatal type is anomocytic, with anisocytic cells present as a subordinate type in some species such as *O. microcarpa* DC. and *O. dichroantha* Boiss. Dasti *et al*. (2003) explained that although anomocytic type was dominant type helicocytic, hemiparacytic, staurocytic and brachyparatetracytic stomata were seen in *O. stephonia*. Our findings are similar with Metcalfe & Chalk (1979), Akçin (2007), Binzet & Akçin (2009; 2012). In our study stomata type was anomocytic and anisocytic in all species. Stomata index is 9.25 of upper epidermis and 10.71 of lower epidermis in *O. discedens*, 9.61 of upper epidermis and 10 of lower epidermis in *O. nana* and 9.67 of upper epidermis and 13.63 of lower epidermis in *O. sorgerae* var. *subglabriflora*. Vascular bundles are surrounded by a parenchymatic sheat in all examined species.

According to LM and SEM investigations, the pollen grains are heteropolar and trisyncolporatae. sphaeroidal in shape (P/E: 1.08 W; P/E: 1.12 E) in *O. discedens*, sphaeroidal in shape in Wodehouse (P/E: 1.13 W) and subprolata in Acetolysis (P/E: 1.15 E) in *O. nana*, subprolata in shape (P/E: 1.13 W; P/E: 1.15 E) in *O. sorgerae* var. *subglabriflora*. Amb shape is three angular in all examined *Onosma* species. Sculpture scabrat-rugulat, scabras outnumber on equatorial region and distal pole and 15 scabra number per 1 μm^2 in mesocolpium in *O. discedens*, sculpture scabrat-rugulat and scabras are distributed on the surface of pollen as homogenouslly. 14 skabra number per 1 μm^2 in mesocolpium in *O. nana* and sculpture scabrat-rugulat and scabras outnumber on equatorial region. 18 skabra number per 1 μm^2 in mesocolpium in *O. sorgerae* var. *subglabriflora.*

Binzet & Akçin (2009) reported that, the nutlet colour of the studied 14 *Onosma* taxa appeared to be brown or gray; however it is difficult to distinguish taxa on the basis of fruit colour. Therefore the colour of nutlet was not used as diagnostic character to distinguish *Onosma* species.

There is an opinion that external nutlet size, shape, characters, colour and ornamentation are of limited taxonomik value (Riedl, 1978). However, the sculpturing of the nutlet surface patterns, as seen by SEM, shows specific variations.

In this study, nutlets of studied *Onosma* species vary in the range 3.5-5.5x2.7-3.5 mm. *O. nana* has the smallest nutlets (4x2.7 mm). *O. sorgerea* var. *subglabriflora* has the largest nutlets (3.5-5.5x2.5-3.5 mm). Nutlet shape shows some variations. Especially in nutlet of examined species sharp ventral keel were seen. Nutlet size, shape and surface ornamentation of 14 *Onosma* species were studied by Binzet & Akçin (2009) and they reported that rugose ornamentation seen in *O. angustissima* and *O. gigantea* nutlet surfaces. In this study, nutlet ornemantation is rugose type in all of species. This type is characterised by the epidermal cells of the nutlet surfaces having small or fine wrinkles (Fig. 5).

Acknowledgments

This study is Ms Thesis by Halil İbrahim TEKE and was supported by Adıyaman University Research Found (Project No: ADYÜ BAPFEF-YL-2011/0014).

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(Received for publication 26 October 2015)