

Full Length Research Paper

The anatomical properties of two *Onosma* L. (Boraginaceae) species from Turkey

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In this study, the morphological and anatomical characteristics of *Onosma frutescens* Lam. and *Onosma inexpectata* Teppner were investigated. *O. inexpectata* is an endemic species. Both examined species have secondary root structure, and cortex is multilayered and composed of parenchymatous cells. While, xylem is composed of sclerenchymatous cells and tracheary elements in roots of *O. frutescens*, xylem is composed of tracheal elements in roots of *O. inexpectata*. Glandular and eglandular trichomes are present on the epidermis of stem of both species. Parenchyma cells are $28.12 \pm 7.55 \times 24.90 \pm 5.42 \mu$ in stem of *O. frutescens*. Parenchyma cells are $37.35 \pm 8.66 \times 17.09 \pm 3.44 \mu$ in stem of *O. inexpectata*. Stomata are anisocytic and anomocytic. Vascular bundles are surrounded by a parenchymatous sheath in both species.

Key words: Boraginaceae, *Onosma*, morphology, anatomy, Turkey.

INTRODUCTION

Onosma L. species belong to the family Boraginaceae, order Lamiales and subclass Dicotyledoneae. It has been reported that there are 150 species of the genus *Onosma* L. (Boraginaceae) on Earth (Al-Shehbaz, 1991; El-Shazly et al., 2003; Naz et al., 2006). However, recent studies and revisions increased the number of species in the genus *Onosma* to over 230 species (Riedl, 1978; Boissier, 1897; Dinsmor, 1932; Tutin et al., 1972; Hayek and Markgraf, 1970; Shishkin, 1974; Meikle, 1985; Teppner, 1991; Ge-ling et al., 1995).

The genus *Onosma* (Boraginaceae) is represented by about 104 taxa (99 species) in Turkey and the rate of endemism among native species is 50%. *Onosma* has about 50 endemic species and 1 endemic variety (Riedl, 1978; Davis et al., 1988; Yıldırım, 2000; Riedl et al., 2005; Binzet and Orcan, 2007; Kandemir and Türkmen, 2010; Aytaç and Türkmen, 2011). After the publication of the Flora of Turkey and East Aegean Islands (Riedl, 1978), some species have been described for Turkey. These include *Onosma propontica* Aznavour, *Onosma kaheirei* Teppner, *Onosma taurica* Palas var. *viridis*

Borbos, *Onosma mirabilis* A. P. Khokhrjakov, *Onosma nydeggeri* Hub.-Mor., *Onosma mersinana* Riedl, Binzet and Orcan, *Onosma riedliana* Binzet and Orcan, *Onosma beyazoglui* Kandemir and Türkmen and *Onosma aksoyii* (Aytaç and Türkmen, 2011). The genus has been divided into three sections: *Protonosma*, *Podonosma* and *Onosma*. *Protonosma* and *Podonosma* sections are represented by one species; the other *Onosma* species belong to *Onosma* sections. This section is separated into two subsections according to solely indumentum type: *Asterotricha* (Boiss.) Gürke. and *Haplotricha* (Boiss.) Gürke. Riedl (1978) pointed out that the classification appears to be partly artificial and in need of re-investigation and new data (caryological and palynological) may provide useful reference points in the future classification of the genus.

The members of this genus are used as folk medicine, herbs and dyes. *Onosma sericeum* Willd., and *Onosma microcarpum* Steven ex DC. are used for the treatment of wounds in rural areas in Turkey (Ozgen et al., 2003). The flowers of some species are consumed as vegetables (Öztürk and Özçelik, 1991).

Studies on the anatomy of *Onosma* genus are limited. Metcalfe and Chalk (1979) and Watson and Dallwitz (1991) explained the characteristics properties of family Boraginaceae. Akçin and Engin (2001, 2005), Akçin

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

Taxa	Locality	Collection date	Herbarium no.
<i>O. frutescens</i>	C3: Antalya: Gazipaşa, Northern of Sugözü village, 36° 25' N 032° 27' E, 800 m	19-05-2005	Binzet 30
	C4: Mersin: Anamur, Yukarı Kükür village, 36° 14' N 032° 43' E, 20-09-2005, 600 m	20-09-2005	Binzet 31
<i>O. inexpectata</i>	C6: Osmaniye: Hasanbeyli-Fevzipaşa, 37° 06' N 036° 36' E, 1150 m	25-05-2004	Binzet 3

Table 2. Anatomical measurements of *Onosma frutescens* and *O. inexpectata*.

Plant part		<i>O. frutescens</i>		<i>O. inexpectata</i>	
		Breadth (μm) Mean \pm SE	Length (μm) Mean \pm SE	Breadth (μm) Mean \pm SE	Length (μm) Mean \pm SE
Root	Periderm cells	34.79 \pm 6.27	10.28 \pm 2.04	20.18 \pm 4.82	5.74 \pm 1.14
	Parenchyma cells	18.18 \pm 4.12	10.74 \pm 2.27	12.42 \pm 2.29	9.28 \pm 2.24
	Diameter of trachea	40.78 \pm 10.21	-	29.88 \pm 6.46	-
Stem	Epidermis cells	21.17 \pm 3.64	15.79 \pm 3.32	10.55 \pm 2.35	10.90 \pm 3.53
	Collenchyma cells	26.92 \pm 8.14	20.28 \pm 5.53	13.54 \pm 4.03	13.08 \pm 3.32
	Parenchyma cells	28.12 \pm 7.55	24.90 \pm 5.42	37.35 \pm 8.66	17.09 \pm 3.44
	Diameter of trachea	24.81 \pm 7.28	-	17.70 \pm 3.99	-
Leaf	Diameter of pith ray cells	56.92 \pm 13.52	-	33.31 \pm 10.14	-
	Upper epidermis cells	14.89 \pm 4.98	14.07 \pm 4.79	23.13 \pm 6.17	13.22 \pm 3.28
	Lower epidermis cells	20.35 \pm 6.15	23.48 \pm 3.68	16.31 \pm 3.39	13.93 \pm 4.20
	Palisade parenchyma cells	10.79 \pm 9.27	32.68 \pm 4.58	10.90 \pm 2.07	6.50 \pm 1.35
	Spongy parenchyma cells	19.25 \pm 4.58	10.15 \pm 2.22	11.50 \pm 2.32	6.60 \pm 2.22

(2004, 2007a, b), and Binzet and Akcin (2009) studied the anatomical and ecological properties of some *Onosma* species. Binzet and Orcan (2003a, b, 2009) investigated the anatomical structure and palynological characteristics of five *Onosma* species.

MATERIALS AND METHODS

Plant samples were collected from natural populations of southern Turkey, between 2004 and 2005 (Table 1). Voucher specimens are kept at the Herbarium of the Faculty of Art and Science of Mersin University. Taxonomical descriptions of the specimens were made according to Riedl (1978). Samples for anatomical studies were fixed in 70% alcohol. Cross and surface sections of root, stem and leaves were excised by hand and were covered with glycerin-gelatin (Vardar, 1987). Microphotographs were taken with Nikon FDX-35 and Olympos BX51 microscopes. All measurements and observations were made using imaging software (Table 2). For Scanning Electron Microscopy, dried leaves were mounted on stubs using double-sided adhesive tape. Samples were coated with 12.5 to 15 nm of gold. Coated leaves were examined and photographed with JMS-6400 Scanning Electron Microscope.

RESULTS

Onosma frutescens Lam.

A transverse section taken from the root was observed as shown in Figure 1. Periderm is multilayered. Phellem cells are brown and light brown. Phellogen is distinguishable. Cortex is multilayered and parenchymatic. Parenchymatic cells are $18.18 \pm 4.12 \times 10.74 \pm 2.27 \mu$. Phloem is definite. Cambium cells are 1 to 2 layered, flat and distinguishable. Xylem is composed of sclerenchymatic cells and tracheary elements. Concentric rings are definite in the xylem. The pith consists of parenchymatic cells (Figure 1).

Cuticle layer is thick on the stem. Glandular and eglandular trichomes are present on the epidermis. Some eglandular trichomes have multilayered base. Collenchyma is multilayered. Parenchyma cells with thick walled are $28.12 \pm 7.55 \times 24.90 \pm 5.42 \mu$ in size. Squashed cells can occur in the cortex. Endodermis is

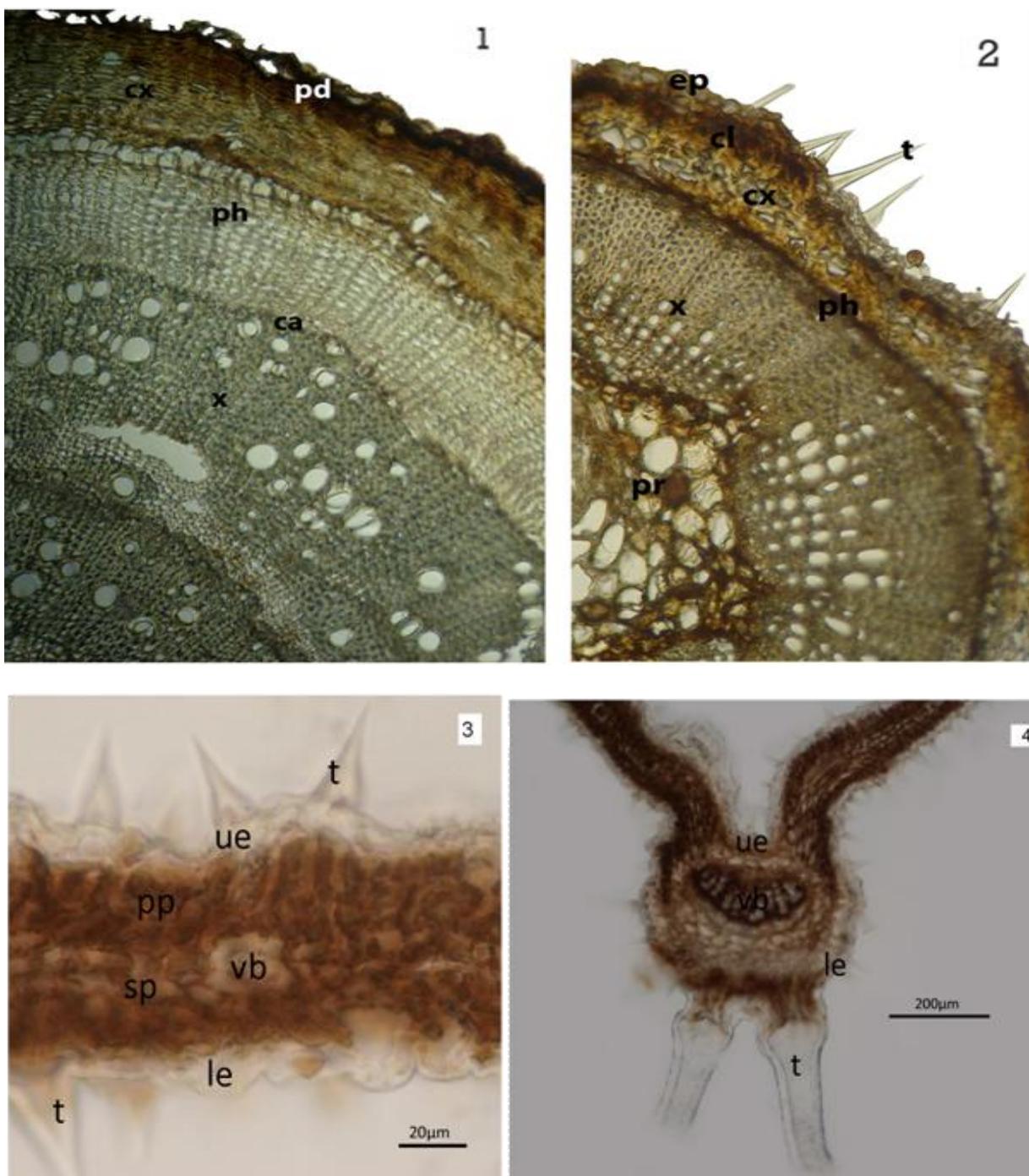


Figure 1. *O. Frutescens*. 1 = Cross-section of root. 2 = Cross-section of stem. 3 and 4 = Cross-section of leaf. pd: peridermis, cx: cortex, ph: phloem, ca: cambium, ep: epiderma, cl: collenchyma, cx: cortex, ph: phloem, x: xylem, pr: parenchyma t: hair üe: upper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, ae: lower epidermis, id: vascular tissue.

distinguishable and unilayered. Xylem and phloem elements are clear. Cambium is undistinguishable. The diameter of trachea cells are $24.81 \pm 7.28 \mu$. Tracheids cells are dense in xylem tissue. Pith cells are large and cylindrical (Table 2, Figure 1).

A transverse and surface section of the lamina and both epidermises was studied. Epidermal cells of both surfaces are arranged in a single layer. There are short simple trichomes, short and tall patent-setose trichomes and glandular trichomes on both epidermises. Shortly,

trichomes with smooth walls are dense on both surfaces. Patent-setoses have ornamental walls. Both epidermises have thick cuticle and undulate cell walls. Leaf is equifacial. Mesophyll consists of 2 to 3 layers of palisade parenchyma cells on the upper epidermises and 2 layers of palisade parenchyma cells on the lower epidermises. Spongy parenchymatic cells with large intercellular cavities are 2 to 3 layered. Vascular bundles are surrounded by a parenchymatic sheath.

Stomata are anomocytic and anisocytic. Stomata occur on the both surfaces and same level with epidermis cells (Figures 1 and 3).

***Onosma inexpectata* Teppner**

A transverse section taken from the root was observed as shown in Figure 2. Phellem is multilayered and dark brown. Phellogen is uniseriate and distinguishable. Cortex is multilayered and composed of parenchymatic cells and phloem. Cambium cells are 1 to 2 layered and distinguishable. Xylem is composed of tracheal elements and covers a large area in root. Trachea cells are 15 to 30 μ and organized scattered. Tracheids with thick walls are dense in xylem tissue. Parenchymatic cells are present in the pith region (Figure 2).

The epidermis of stem consists of uniseriate, rectangular or orbicular cells. Glandular and eglandular trichomes are present on the epidermis. The eglandular trichomes include simple trichomes and porrect-stellate trichomes with ornamental cuticle. The glandular trichomes are capitate types. Collenchyma is generally 1 to 2 layered. Parenchyma cells are $37.35 \pm 8.66 \times 17.09 \pm 3.44 \mu$ in size. Endodermis is distinguishable and unilayered. Xylem and phloem elements are clear. Cambium is distinguishable. Tracheids with thick walls are dense in xylem tissue. Pith cells are large and cylindrical.

The adaxial and abaxial epidermises of the leaf consist of uniseriate, oval or rectangular cells in transverse section. Both epidermises are covered with short simple trichomes, short and long porrect-stellate trichomes and glandular trichomes. Eglandular trichomes have ornamental cuticle and contain crystals in the bases. Leaf is isobilateral. Palisade parenchyma cells are 2 to 3 layered on the upper surface and 1 to 2 layered on the lower surface. Spongy parenchyma cells are 3 layered. Vascular bundles are collateral and surrounded by a bundle sheath. Stomata are anisocytic and anomocytic on both epidermises. Anisocytic stomata are denser than ones on the upper epidermis (Table 2, Figures 2 and 3).

DISCUSSION

In this study, anatomical properties of *Onosma frutescens* belonging to the subsection Haplotricha, and *Onosma*

inexpectata an endemic plant belonging to the subsection Asterotricha, were examined. Metcalfe and Chalk (1979), and Watson and Dallwitz (1991) explained the anatomical characteristic of the family Boraginaceae. The anatomical properties of the both species are suitable with the family Boraginaceae (Metcalfe and Chalk, 1979). The cross section of root showed that the root had secondary structure. Xylem is composed of sclerenchymatic cells and tracheary elements in roots of *O. frutescens*, and xylem is composed of tracheal elements in roots of *O. inexpectata*. Parenchyma cells are $18.18 \pm 4.12 \times 10.74 \pm 2.27 \mu$ in stem of *O. frutescens*. Parenchyma cells are $12.42 \pm 2.29 \times 9.28 \pm 2.24 \mu$ in stem of *O. inexpectata*. While, the pith region of root, generally consist of primary xylem elements in some *Onosma* species such as *Onosma bracteosum* (Akçin and Engin, 2005) and *Onosma giganteum* Lam. (Binzet and Orcan, 2003), *Onosma sieheanum* (Binzet and Akçin, 2009), and *Onosma mersinana* (Binzet and Orcan, 2009). The pith region of root consist of parenchymatic cells in the other *Onosma* species such as *Onosma intertextum*. The pith region of *O. frutescens* and *O. inexpectata* is composed of parenchymatic cells.

In both species, glandular and eglandular trichomes are present on the epidermis of stem. As collenchyma is multilayered in *O. frutescens*, collenchyma is generally 1 to 2 layered in *O. inexpectata*. In all two species, endodermis is distinguishable and unilayered in stem. Parenchyma cells are $28.12 \pm 7.55 \times 24.90 \pm 5.42 \mu$ in stem of *O. frutescens*. Parenchyma cells are $37.35 \pm 8.66 \times 17.09 \pm 3.44 \mu$ in stem of *O. inexpectata*. Cambium is distinguishable in *O. inexpectata*, while it is undistigunishable in *O. frutescens*. The pith is composed of parenchymatic cells in both species. The presence of crystals in Boraginaceae family has important characters (Metcalfe and Chalk, 1979). Crystals were seen in pith cells in some *Onosma* species such as *Onosma mutabile* (Binzet and Orcan, 2003), *O. siehanum* and *O. intertextum* (Binzet and Akçin, 2009), but it is were not seen in both species. *Onosma* section was separated into two subsection according to indumentum type: *Asterotricha* (Boiss.) Gürke and *Haplotricha* (Boiss.) Gürke. In *O. frutescens*, short simple trichomes, short and tall patent-setose trichomes and glandular trichomes on both epidermises of leaves and shortly trichomes with smooth walls are dense on both surfaces. Patent-setoses have ornamental walls. In *O. inexpectata*, short simple trichomes, short and long porrect-stellate trichomes and glandular trichomes are seen on the both upper and lower epidermis of leaves. Eglandular trichomes have ornamental cuticle and contain crystals in the bases.

Pignatti (1982) used setae (porrect-stellate) in leaf as taxonomic characters for determining *Onosma* species in Italy. Metcalfe and Chalk (1979) pointed out that family Boraginaceae has both bifacial and isobilateral leaves. According to Azizian et al. (2000), two distinct leaf anatomical structures are present within the genus

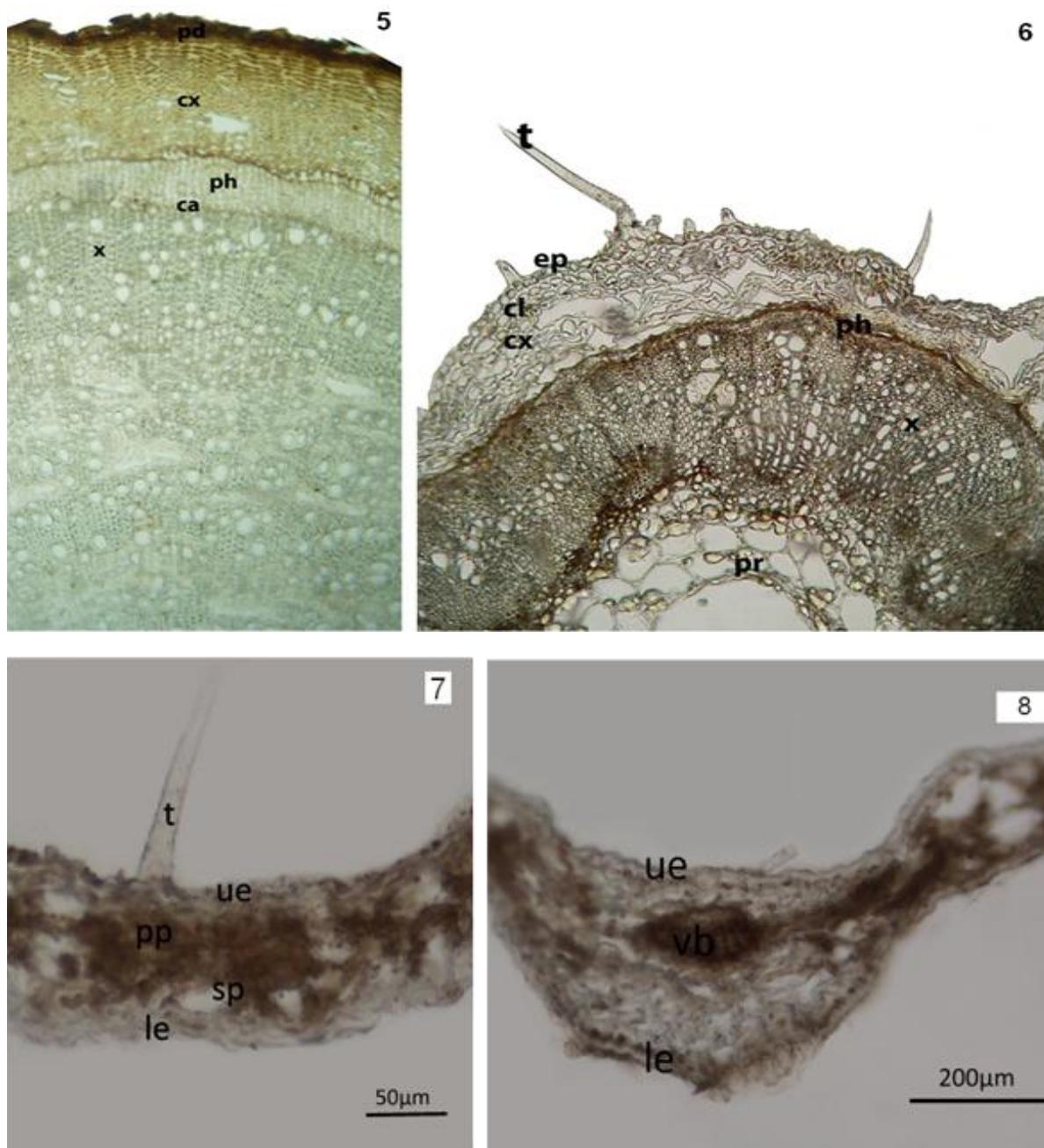


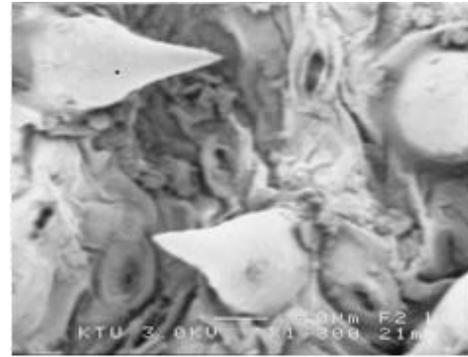
Figure 2. *O. inexpectata*. 5 = Cross-section of root. 6 = Cross-section of stem. 7 and 8 = Cross-section of leaf. pd: peridermis, cx: cortex, ph: phloem, ca: cambium, ep: epiderma, cl: collenchyma, cx: cortex, x: xylem, pr: parenchyma t: hair ue: upper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, ae: lower epidermis, id: vascular tissue.

Onosma: in sections *Protonosma* and *Podonosma*, leaf is dorsi-ventral and in sect. *Onosma*, leaf type is isobilateral. In our study (in sect. *Onosma*), leaves are isobilateral. *Onosma* species generally have isobilateral leaf (Akçin and Engin, 2001, 2005; Binzet and Orcan, 2003 a,b; Akçin, 2004; Binzet and Orcan, 2009; Binzet and Akçin, 2009). Metcalfe and Chalk (1979) reported that there are both anomocytic and anisocytic stomata in

Boraginaceae. 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. Akçin (2007b) and Binzet and Akçin (2009) reported that stomata are anisocytic and anomocytic, respectively, in *O. armenum*, *O. intertextum* and *O. sieheanum*. According to Zarinkamar (2007), dominant stomatal type is anomocytic, with anisocytic cells present



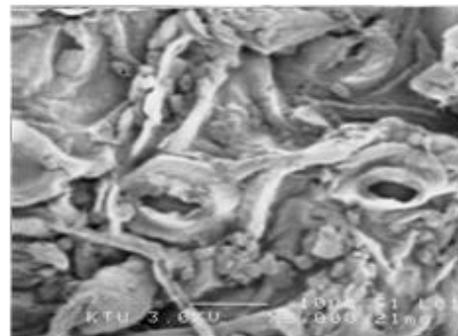
O. frutescens Lower surface of leaf



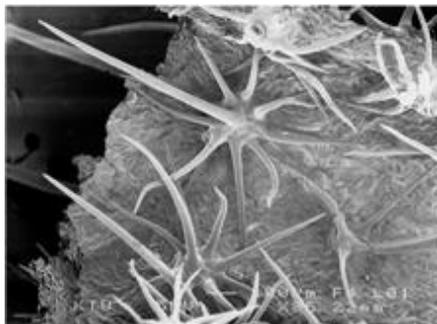
O. frutescens Lower surface of leaf



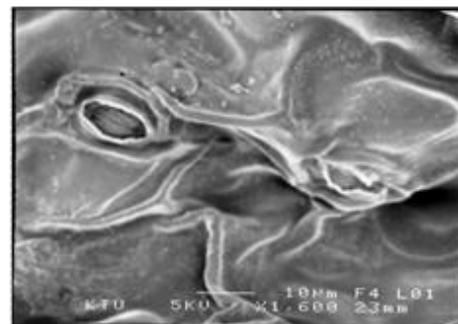
O. frutescens Upper surface of leaf



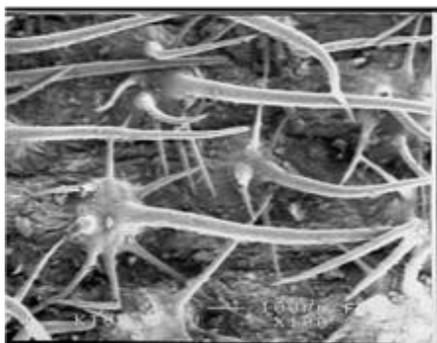
O. frutescens Upper surface of leaf



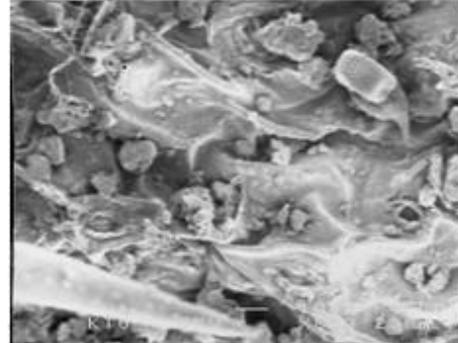
O. inexpectata Lower surface of leaf



O. inexpectata Lower surface of leaf



O. inexpectata Upper surface of leaf



O. inexpectata Upper surface of leaf

Figure 3. Scanning electron micrographs of surfaces of *O. frutescens* and *O. inexpectata* leaves. Indumentum view on the left (Bars = 100 µm), stomatas on the upper and lower surface of leaves on the right (Bars = 10 µm).

as a subordinate type in some species such as *O. microcarpum* DC. and *O. dichroanthum* Boiss. Our findings are similar with Metcalfe and Chalk (1979), Akçin (2007b) and Binzet and Akçin (2009). Stomata type was anisocytic and anomocytic in two species. Vascular bundles are surrounded by a parenchymatic sheath in both species.

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