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MICROMORPHOLOGICAL AND ANATOMICAL INVESTIGATION ON SIX SPECIES OF *ONOSMA* L. (BORAGINACEAE) FROM TURKEY

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Abstract

Micromorphology and anatomy of six *Onosma* L. species, viz. *O. argentata* Hub.-Mor., *O. neglecta* Riedl, *O. proballanthera* Rech. f., *O. rechingeri* Riedl, *O. sericea* Willd. and *O. stenoloba* Hausskn. *ex* Riedl from Turky were investigated. Stem anatomy revealed that cuticle layer ranged from 0.6 µm in *O. argentata* to 1.7 µm in *O. proballanthera*. Parenchymatous cells of *O. neglecta* and *O. stenoloba* possessed more intense starch than the other species studied. In leaf anatomy, the longest palisade parenchyma was found in *O. neglecta*, while the smallest was noted in *O. argentata*. Mesophyll structure of *O. argentata*, *O. sericea* and *O. rechingeri* was equifacial (isobilateral), while *O. neglecta, O. proballanthera* and *O. stenoloba* presented bifacial (dorsiventral) structure. Rugose nutlet ornamentation was observed in *O. argentata, O. neglecta* and *O. sericea*, whereas reticulate type was found in *O. proballanthera, O. rechingeri* and *O. stenoloba. Onosma stenoloba* could easily be distinguished from other species by its aesterotrichous indumentum, and in contrary, other species possessed haplotrichous type of indumentum. Micromorphological features of nutlet surface, anatomical features of epidermal surface (trichomes and stomata), and lamina mesophyll structure (dorsiventral and isobilateral) could be useful in solving taxonomic problem of the genus.

Introduction

Onosma L. belonging to the family Boraginaceae consists of about 150 species, distributed mainly in West and Central Asia and in the Mediterranean area, and grows in dry, sunny, rocky, sandy, and steppe habitats (Cecchi and Selvi, 2009; Binzet *et al*., 2010, Kolarcik *et al*., 2010). *Onosma* are biennial or perennial herbs, characterized by scorpioid cymes, linear or linear-lanceolate calyx lobes that are parted to base, corolla without ribs or deep furrows, unappendaged corolla throat, sagittate anthers coherent at base, capitate stigma and ovate to triangular nutlets. Cronquist (1981) included the family Boraginaceae in the Order Lamiales of the sub-class Asteridae of Magnoliopsida. Güner *et al*. (2012) states that the Boraginaceae stands the ninth position among the families in Turkey in term of number of species, and is represented by 44 genera and 375 taxa in the flora of Turkey. One hundred and three endemic taxa represent approximately 50% of the *Onosma* taxa in Turkish flora (Güner *et al*., 2012; Binzet, 2016). The indumentum of leaves and stem of *Onosma* taxa consists of three separate components: setae (rarely hairs) often slightly raised, or pancake-shaped with multicellular tubercles; setules, sometimes shortened to tiny spinules or produced as hairs, stellately arranged around the base of

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the seta; and tiny hairs forming a pubescent, puberulous or tomentose surface covering between the setae. The setae provide the plant its typically hispid indumentum and they are brittle and easily detached, penetrating the skin and causing an irritant rash (Riedl, 1978). The members of *Onosma* are medicinally important and considered as cardiotonic, purgative, anthelmintic, and used for treatment of diabetes, leucoderma, dyspepsia, abdominal pain, and bronchitis (Hayta *et al.,* 2014; Özgen *et al*., 2004). In addition, they are economically important for beekeeping because of their attractive flowers, and rich in nectar (Dukas and Dafni, 1990).

Taxonomic significance of micromorphology and anatomy in delimitation of taxa and establishment of interspecific relationships are well documented (Tschan and Denk, 2012; Meng and Mao, 2013; Begum *et al*., 2014). Metcalfe and Chalk (1950) studied the anatomy of the family Boraginaceae and found setae (hairs), epidermal surface and cystoliths as diagnostic characters. Several attempts have been made on micromorphology and anatomy of different Turkish species of *Onosma* (Akçin and Engin, 2001, 2005; Akçin, 2004, 2007; Binzet and Akçin, 2009; Akçin and Binzet, 2010). Micromorphological studies of epidermal and nutlet surface of some *Onosma* taxa highlighted the importance of these features (Akçin, 2009; Binzet and Akçin, 2009; Akçin *et al.,* 2013; Mehrabian *et al.,* 2014).

In the present study, the micromorphological and anatomical structure of six species of *Onosma*, namely *O. argentata, O. neglecta, O. proballanthera, O. rechingeri, O. sericea* and *O. stenoloba* from Bingöl and its environs of Turkey were investigated for the first time. Morphological characters are controlled by the genes of a species as well as influenced by phenotypic plasticity, and because of these phenomena proper identification of species sometimes become problematic. In this regard, micromorphology and anatomical studies provide powerful tools for species delimitation and interspecific relationship. The main objectives of the present study are two folds: i) to explore the anatomical features of stem, leaf and epidermal surface of six Turkish *Onosma* species, and ii) to investigate stem, leaf and nutlet micromorphology by scanning electorn microscopy of those species which could contribute to the taxonomy of *Onosma*.

Materials and Methods

Plant materials

Six *Onosma* species were collected from natural habitats of Bingöl, Turkey from 2016 to 2017. The taxonomic identity of the species were confirmed following Riedl (1967, 1978). The list of the species employed in this study along with their Turkish names, localities and vouchers are presented in Table 1. The voucher specimens have been deposited at the Munzur University Herbarium.

Anatomical studies

Anatomical studies were carried out on specimens kept in 70% ethanol. Cross-sections of stem and leaves were stained with Phloroglucinol–HCl, toluidin, safranine and lugol's solution and the chlorophyll pigments in leaves were removed with chloral hydrate (Yakar-Tan, 1982; Selvi *et al*., 2014). After dyeing, the sections were prepared as permanent slides and examined under the light microscope Olympus BX53 with photograph attachment, and the photographs were taken and digitized. Stomatal density on abaxial and adaxial surfaces of the leaves were counted under a light microscope. Stomatal index were calculated following Meidner and Mansfield (1968).

Micromorphological studies

Epidermal surface (stem and leaves) and nutlet surface of six *Onosma* species were studied by Tabletop Scanning Electron Microscopy (SEM). For SEM, small pieces of leaves and stem with nutlet were fixed on aluminum stubs using double-sided adhesive, and coated with gold palladium

to a thickness of 40−50 nm. The SEM micrographs were taken in a NeoScope JCM-5000 at an accelerating voltage of 10 kV (Selvi *et al.*, 2013). SEM studies took place in the Basic Sciences Research and Applied Center of Balıkesir University, Turkey.

Table 1. List of *Onosma* L. species along with their locality and voucher specimens.

Species	Turkish name	Locality	Vouchers
**O. argentata* Hub.-Mor.	Gümüşemcek	Turkey, B8 Bingöl: between Bingöl and Elazığ, limestone slopes, 39°34'48.05"N, 39°58'1.07"E, 1505 m, 06.07.2017.	UC-2012
**O. neglecta* Riedl	Bahaemziği	Turkey, B8 Bingöl: between Kuruca and Karakoçan, roadsides, 38°55'21.30"N, 40°19'57.28"E, 1540 m, 09.07.2016.	UC-2013
**O. proballanthera* Rech. f.	Yaylaemziği	Turkey, B8 Bingöl: from Bingöl to Solhan roadsides, rocky slopes, 38°49'32.90"N, 40°51'33.85"E, 1898 m, 11.07.2016.	UC-2016
**O. rechingeri* Riedl	Geçmıjmıjok	Turkey, B8 Bingöl: Genç town, towards from Genç to Bingöl, limestone rocky, 38°49'38.12"N, 40°32'38.40"E, 1070 m, 11.07.2016.	UC-2015
O. sericea Willd.	Kâğıtemcek	Turkey, B8 Bingöl: between Bingöl and Elazığ, rocky slopes, 38°56'22.30"N, 40°10'24.28"E, 1460 m, 10.07.2016.	UC-2014
**O. stenoloba* Hausskn. *ex* Riedl	Tosyaemceği	Turkey, B8 Bingöl: towards from Bingöl to Ilıcalar village, roadsides, 39°1'12.57"N, 40°44'18.84"E, 1385 m, 12.07.2016.	UC-2017

*Denotes species endemic to Turkey.

Results and Discussion

Anatomical studies

Anatomical investigation reveals variation in stem, lamina and midvein structure among the *Onosma* species investigated. A comparative account of biometric measurement of stem anatomy of the investigated species is provided in Table 2. At the outermost level, the cuticle layer ranges from 0.6 µm in *O. argentata* to 1.7 µm in *O. proballanthera* and *O. sericea*. The epidermal layer is lowest in *O. sericea*, while it is highest in *O. neglecta*. The maximum pith/stem ratio has been observed in *O. neglecta* (0.69) followed by *O. proballanthera* (0.68), whereas the minimum ratio (0.59) has been noticed in *O. sericea* (Table 2). Though no significant differences in stem cells in the investigated species have been observed, however, these features are found useful to some extent for distinguishing some species.

Single layered epidermal tissue consisting of oval, cubic or rectangular cells are found below the cuticle. In the epidermis, a few number of eglandular and rarely glandular trichomes are seen. Eglandular trichomes comprises 1−3 cells, upright or slightly curled, while glandular trichomes consist of 1−2 capitate stem cells with a round head. Cortex layer consists of collenchyma, parenchyma and endodermis, and at the top of the cortex layer 3-6-rows of collenchyma layers are found which is followed by 2−5 layered parenchyma cells. The parenchymatous cells of *O. neglecta* and *O. stenoloba* contain more intense starch than the remaining species. In the inner part of the cortex, there are 1−2 layered endodermis, and 3-layered phloem are observed below the endodermis. The cambium between phloem and xylem is often indistinguishable. Xylem in the

Table 2. Stem anatomical structure of six species of *Onosma* L.

Parameters (μm)	Species																	
	O. argentata			*O. neglecta*			*O. proballanthera*			*O. rechingeri*			*O. sericea*			*O. stenoloba*		
	Min.	Max.	Std.	Min.	Max.	Std.	Min.	Max.	Std.	Min.	Max.	Std.	Min.	Max.	Std.	Min.	Max.	Std.
Cuticle	0.6	1.4	0.5	0.7	1.2	0.3	0.9	1.7	0.5	0.8	1.3	0.2	0.7	1.7	0.8	0.8	1.6	0.4
Epidermis layer	13	21	2.5	16	27	2.4	13	24	3.1	14	22	2.6	12	22	2.7	15	26	3.3
Cortex layer	162	245	26.1	142	245	27.4	170	272	41.2	164	258	36.4	142	248	28.4	178	244	26.4
Collenchyma layer	41	107	21.3	25	101	17.4	32	89	15.4	39	105	22.3	42	110	27.5	41	106	21.3
Parenchyma layer	25	32	3.8	21	35	3.5	27	40	4.1	25	32	3.8	16	32	3.9	18	33	3.2
Endodermis width	14	26	3.6	12	24	2.7	14	24	3.6	14	26	3.6	11	23	2.4	14	25	2.1
Phloem layer width	21	33	3.4	23	34	2.7	21	32	3.3	21	33	3.4	18	35	4.1	17	34	4.9
Xylem layer	162	421	65.8	145	390	43.5	152	408	56.5	162	421	65.8	164	440	72.5	180	434	56.9
Trachea (width)	13	29	4.8	14	47	6.5	11	34	5.2	13	29	4.8	14	40	5.2	12	41	3.3
Pith region	38	105	21.6	49	110	19.6	27	96	3.7	38	105	21.6	19	89	7.5	32	118	23.1
Pith/stem ratio		0.65			0.69			0.68			0.65			0.59			0.67	

form of bundles towards pith, along with some regions with phloem is interrupted by the annulus to the pith. The pith region consists of round or polygonal parenchymatous cells with thin walls and fragmented (Fig. 1). In the cross section of leaf, the cuticle layer varies from 0.8 µm in *O. neglecta* to 2.7 µm in *O. rechingeri*. The longest palisade parenchymatous cell has been found in *O. neglecta* followed by *O. stenoloba*. In contrast, the smallest palisade parenchyma is noted in *O. argentata*. The length of palisade parenchyma remains the same in *O. proballanthera* and *O. sericea* (Table 3).

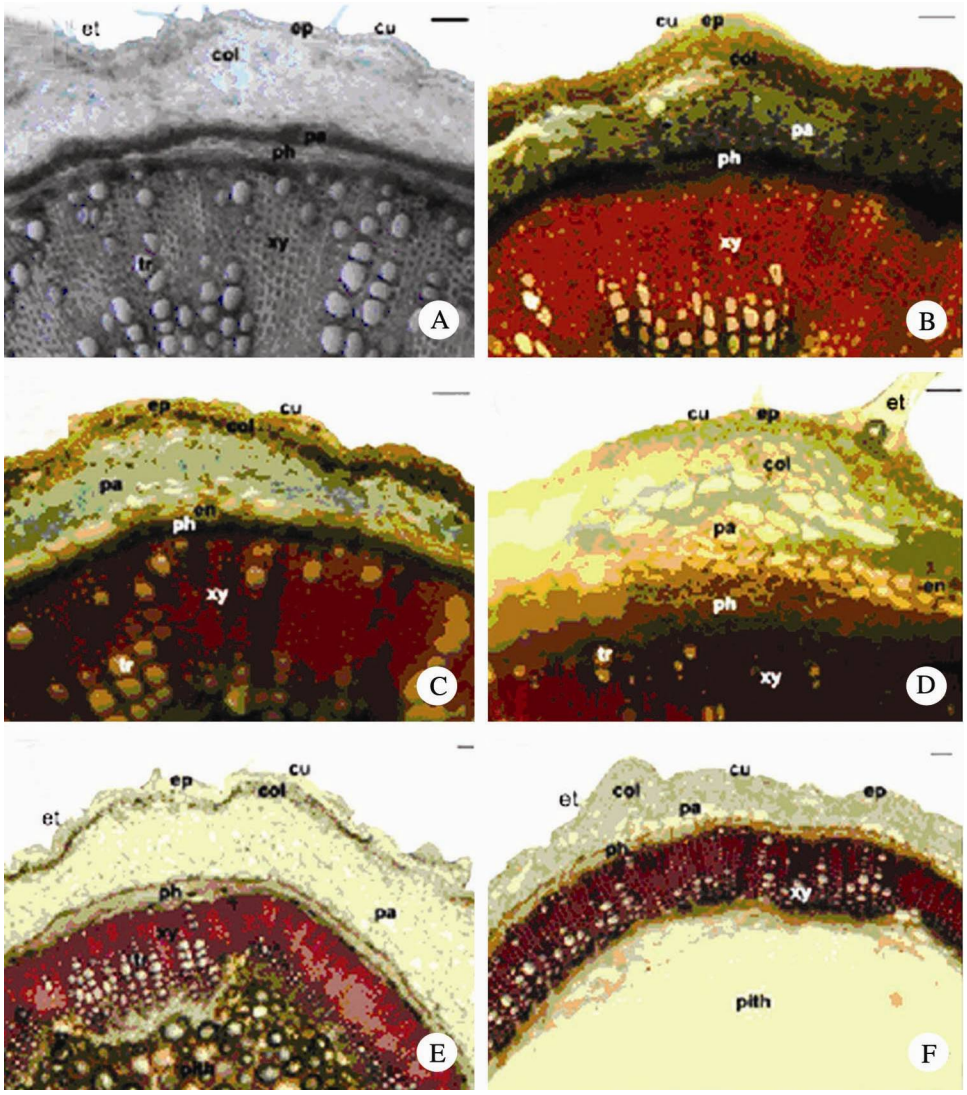

Fig. 1. Cross section of stem of six *Onosma* species: A. *O. argentata*; B. *O. neglecta*; C. *O. proballanthera*; D. *O. rechingeri*; E. *O. sericea*; F. *O. stenoloba.* cu: cuticle, ep: epidermis, et: eglandular trichome, col: collenchyma, pa: parenchyma, ph: phloem, xy: xylem, tr: trachea (Bar = 50 µm).

Table 3. Leaf anatomical structure of six species of *Onosma* L.

Parameters (μm)	Species																	
	O. argentata			*O. neglecta*			*O. proballanthera*			*O. rechingeri*			*O. sericea*			*O. stenoloba*		
	Min.	Max.	Std.	Min.	Max.	Std.	Min.	Max.	Std.	Min.	Max.	Std.	Min.	Max.	Std.	Min.	Max.	Std.
Lamina width	178	220	121	250	319	27.3	244	372	38.6	189	230	14.1	244	372	38.6	298	347	18.2
Cuticle	1.1	2.4	1.2	0.8	1.8	0.3	0.9	2.1	0.4	1.2	2.7	1.1	0.9	2.1	0.4	1.3	2.2	0.9
Upper epidermis width	13	28	5.1	14	24	3.1	20.5	29	2.9	15	30	6.1	20.5	29	2.9	19	27	3.6
Palisade cell length	24	35	2.1	36.7	58.3	5.8	33	46.3	3.7	27	39	3.1	33	46.3	3.7	30	57	4.1
Palisade cell width	5.3	11.4	1.2	14.3	21.7	2.5	13.8	27.1	3.6	6.3	10.4	1.3	13.8	27.1	3.6	18	34	4.7
Spongy cell width	12.6	20.5	2.2	22	47	8.1	17.1	40	6.8	12.5	21.5	2.5	17.1	40	6.8	23	41	3.9
Mesophyll layer	137	168	8.1	199	271	25.5	202	304	39.5	145	170	9.1	202	304	39.5	197	342	21.6
Lower epidermis width	11	18	2.5	12	19	2.7	16	26	3.7	13	20	2.1	16	26	3.7	15	24	4.2
Collenchyma cell	7	22	3.9	10	19.3	2.9	6.4	19.6	5.2	10	21	11.3	6.4	19.6	5.2	8	21	4.9
Middle vein Trachea diameter	6	19	3.9	10	19.3	2.9	6.4	19.6	5.2	8.8	19.2	2.1	6.4	19.6	5.2	5	20	2.9
Phloem cell	3.6	8.2	1.2	6	11	1.9	7.6	10.8	0.9	2.6	6.4	3.1	7.6	10.8	0.9	4.6	9.2	1.1

The upper and lower epidermis of leaves are covered with a thin cuticule in all the species studied. The upper epidermal cells are more significant than the lower ones. Epidermal cells consist of a single-row, tightly lined, rectangular, square and oval-shaped cells. The epidermis is densely covered with eglandular trichomes, and rarely with glandular trichomes. The eglandular trichomes are tubercled, 1−3 celled, straight or slightly curved shape, whereas the glandular trichomes are rare and capitate. The study also reveals variation in mesophyll structure among the species. *O. argentata*, *O. sericea* and *O. rechingeri* exhibit equifacial (isobilateral) type of leaves, while *O. neglecta*, *O. proballanthera* and *O. stenoloba* have bifacial (dorsiventral) leaves (Fig. 2).

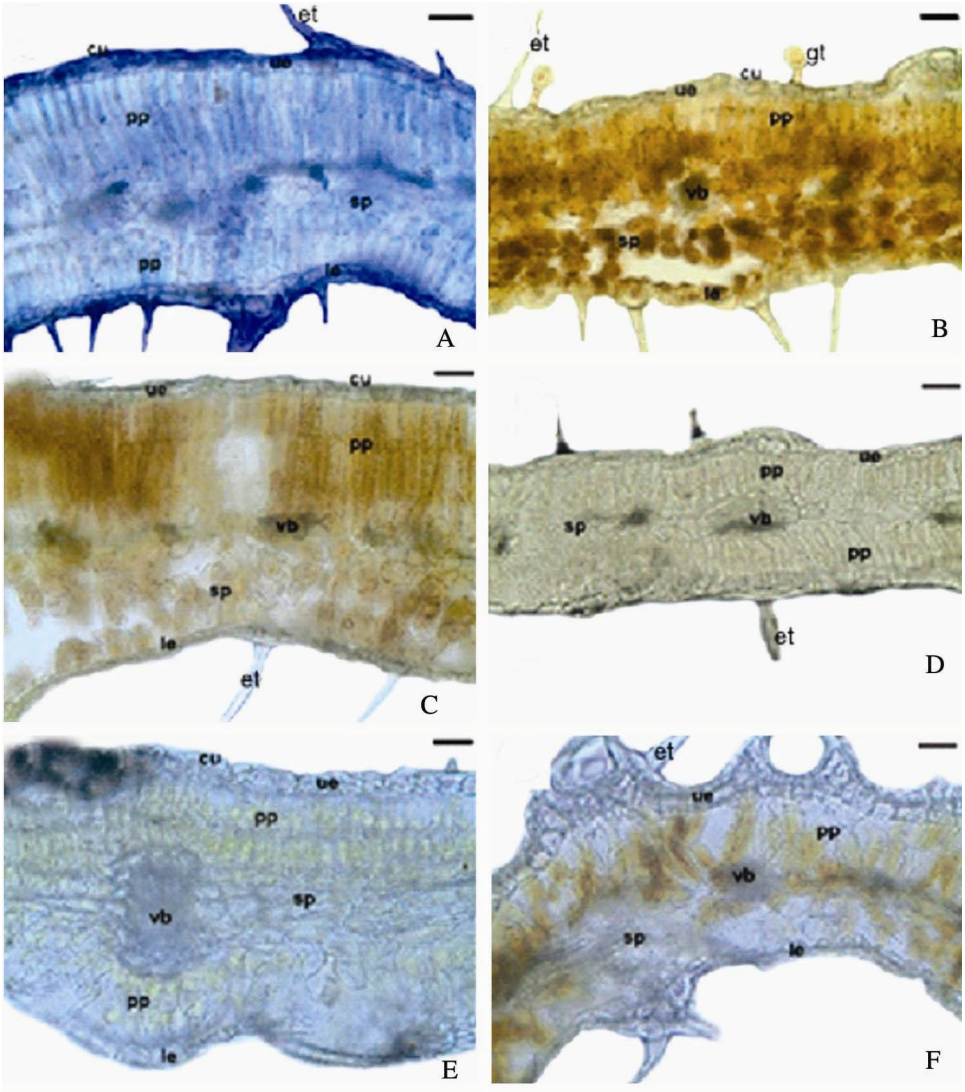

Fig. 2. Lamina mesophyll layer of six *Onosma* species: A. *O. argentata*; B. *O. neglecta*; C. *O. proballanthera*; D. *O. rechingeri*; E. *O. sericea*; F. *O. stenoloba*. cu: cuticle, ue: upper epidermis, et: eglandular trichome, gt: glandular trichome, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle, le: lower epidermis (Bar = 50 µm).

This feature has been found important for distinguishing the species of *Onosma*. In the middle vein region, collateral type of vascular bundle (closed collateral) has been found in all the species. There are several subsequent collenchyma layers at the top of the xylem and below the phloem (Fig. 3).

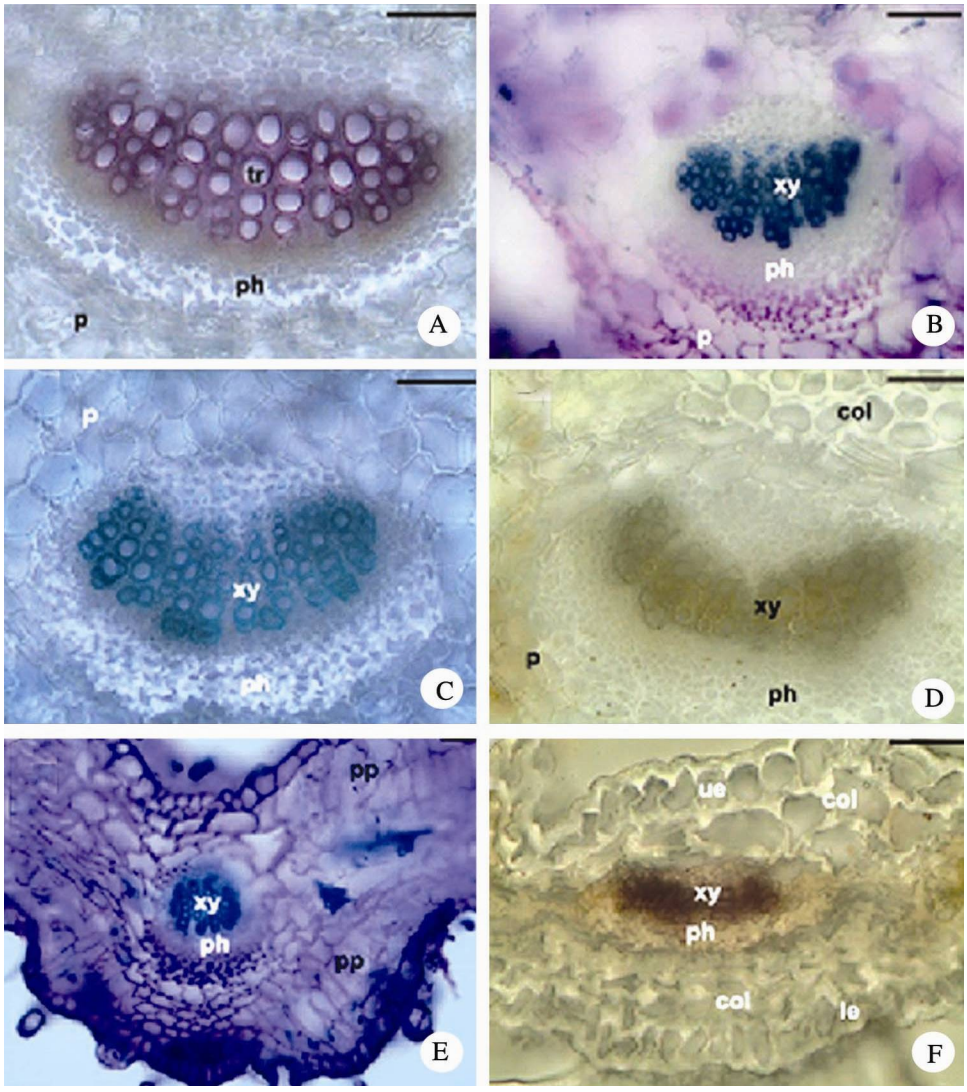

Fig. 3. Lamina middle veins of six *Onosma* species: A. *O. argentata*; B. *O. neglecta*; C. *O. proballanthera;* D. *O. rechingeri*; E. *O. sericea*; F. *O. stenoloba.* col: collenchyma, p: parenchyma, ph: phloem, xy: xylem, tr: trachea (Bar = 50 µm).

The palisade parenchyma consist of two layers with plentiful chloroplasts which are cylindrical and tight. The spongy parenchyma is oval or round with 2−4 rows, and are loosely arranged. In the middle vein region, there is a wide closed collateral type of vascular bundle. Underneath of the xylem there are 2−4 rows of phloem. Above of middle vein 1−2 rows of

collenchyma layers are noticed, and 2−4 rows are observed below the middle vein. Stomata is found both in upper and lower surface of leaf (Amphistomatic). Anomocytic and anisocytic types of stomata are found in the investigated species (Fig. 4). Tissues and cells in stem and lamina of all investigated species have been found to contain calcium oxalate crystals, either in solitary or in clustered form. Cystoliths are frequent in the basal parts of hairs.

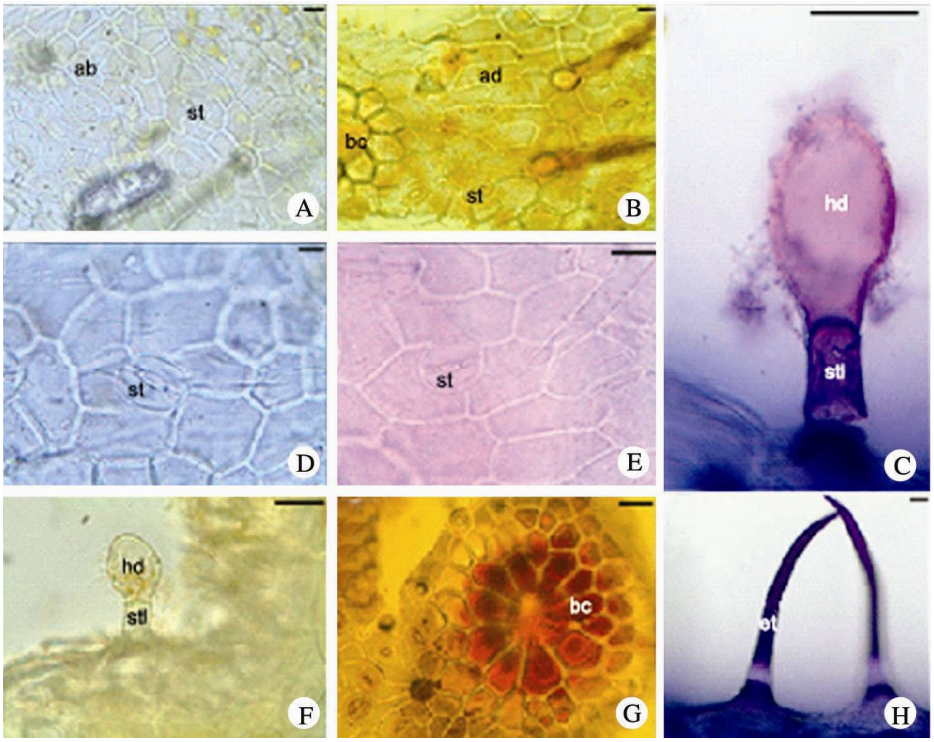

Fig. 4. Epidermal surface of six *Onosma* species: A. Lamina-abaxial surface (*O. proballenthera*); B. Lamina-adaxial surface (*O. sericea*); C. Capitate trichome (*O. neglecta*); D. Anomocytic stomata (*O. proballenthera*); E. Anisocytic stomata (*O. neglecta*); F. Capitate trichome on lamina (*O. rechingeri*); G. Base cells of seta (*O. argentata*); H. Eglandular trichomes (setae) on lamina (*O. neglecta*). st: stomata, ad: adaxial surface, ab: abaxial surface, hd: head cell, stl: stalk, et: eglandular trichome; bc: trichome base cell (Bar = 20 µm).

Micromorphological studies

Epidermal surface of stem and leaves, and nutlet surface of six *Onosma* species have been investigated by SEM. Micromorphological studies have shown that both stem and leaves contain dense eglandular and sparsely glandular trichomes. Eglandular trichomes are usually 1−2 celled, straight or slightly curled. The glandular trichomes are less common, consisting of a 1−2 stem cells with a round head (Fig. 4). Trichomes play an important role in identifying the members of the family Boraginaceae. Metcalfe and Chalk (1950) have shown that distribution and forms of trichomes are useful to differentiate between the different genera and taxa of the family Boraginaceae.

The stem and leaf epidermal surfaces of the *Onosma* species in our study are heavily covered with feathers (setae) and rarely glandular trichomes. Tubercled long trichomes on leaves contain

beams at the base of *O. stenoloba*. Unlike other species, on the leaf surfaces of *O. rechingeri*, there are simple hairs with pointed or lying ends. At the bottom of some setae, large cells containing cystolith are observed (Fig. 4). The indumentum structure of the stem and leaves differ according to the tubercles of the setae. If the tubercles are glabrous they are haplotrichous, and when tubercles stellately at base are asterotrichous. Based on indumentum structure, *O. stenoloba* can easily be distinguished from all other species as it is asterotrichous, whereas, the remaining *Onosma* species have haplotrichous type of indumentum (Fig. 4). The most excessive glandular trichomes have been found in the leaves of the *O. rechingeri* and *O. neglecta*. The trichome is made up of a pear-shaped head cell and a single or two-celled stem cell. Stomata have been found intensely and usually are of anomocytic in all species, however, anisocytic stomata are found seldomly in *O. neglecta* and *O. stenoloba*. Akçin *et al.* (2013) examined the stomata of *O. sericea* and *O. stenoloba* and found anomocytic and anisocytic types of stomata in these species. In our study, anisocytic stomata have been found in both of these two species, however, the anomocytic stomata has been observed only in *O. stenoloba*.

The nutlet micromophology of six *Onosma* species are presented in Table 4. Nutlets of the studied *Onosma* species vary in size and shape. The smallest nutlets (2.5−3.6 × 2.5−3.5 mm) are found in *O. stenoloba*, while the largest nutlets (3.9−4.3 × 3.2−3.7 mm) are observed in *O. rechingeri*. Different shapes of nutlets are found in the examined species, viz. oblong-ovoid (*O. rechingeri, O. stenoloba*), ovoid (*O. argentata, O. neglecta, O. sericea*), and orbicular to ovoid (*O. proballanthera*). In the nutlets of *O. neglecta, O. rechingeri* and *O. stenoloba* sharp ventral keels are seen. Two types of ornamentation of nutlets have been determined - the rugose type and the reticulate type. Rugose type is characterised by the epidermal cells of the nutlet surface having small or fine wrinkles, and this type of ornamentation has been observed in *O. argentata, O. neglecta* and *O. sericea*. The reticulate type is characterised by the epidermal cells of the nutlet surface which are formed in a reticulate ornamentation with varied sizes and shapes of mesh, and this type of ornamentation has been found in *O. proballanthera, O. rechingeri* and *O. stenoloba* (Fig. 5 and Table 4). Binzet and Akçin (2009) found some variations in nutlet surfaces of 14 *Onosma* species and determined four main types of surface ornamentation, viz. ruminate, rugose, reticulate and pusticulate. Among those four types we found only rugose and reticulate types of ornamentation in our study.

Table 4. Nutlet characters of six species of *Onosma* L.

Species	Nutlet size (mm)	Shape	Color	Epidermal cell
O. argentata	3.3-3.8 × 2.7-3.0	Ovoid, acute; dorsal side convex, ventral side roof-like	Brownish	Rugose
O. neglecta	3.5-4.4 × 2.75-3.4	Ovoid, with prominent beak; dorsal side convex, ventral side keeled	Chestnut-brown	Rugose
O. proballanthera	3.7-4.5 × 2.8-3.6	Orbicular to ovoid, with prominent beak; dorsal side convex, ventral side weakly keeled	Light brown	Reticulate
O. rechingeri	3.9-4.3 × 3.2-3.7	Oblong- ovoid, acute; dorsal side convex, ventral side keeled	Brownish	Reticulate
O. sericea	3.6-4.5 × 2.3-3.5	Ovoid, acute; dorsal side convex, ventral side roof-like	Brownish	Rugose
O. stenoloba	2.5-3.6 × 2.5-3.5	Oblong-ovoid, acute; dorsal side convex, ventral side keeled	Brownish	Reticulate

Micromorphological characters of epidermal surface of stem and leaf as revealed from scanning electron microscopy are shown in Fig. 6. Eglandular and glandular trichomes have been observed in the stem and leaves of all species investigated. Glandular trichomes were less frequent than eglandular trichomes. Eglandular trichomes are rigid, unicelluar and patent-setose in all

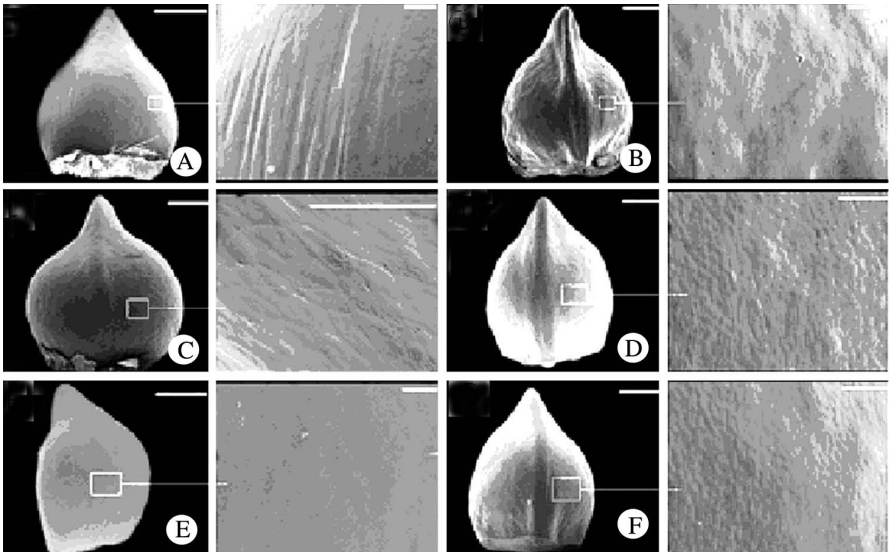

Fig. 5. Seed micromorphology of six *Onosma* species: A. *O. argentata*; B. *O. neglecta*; C. *O. proballanthera;* D. *O. rechinger;* E. *O. sericea*; F. *O. stenoloba* (Bar = 1 mm (general view), 100 µm (surface ornamentation).

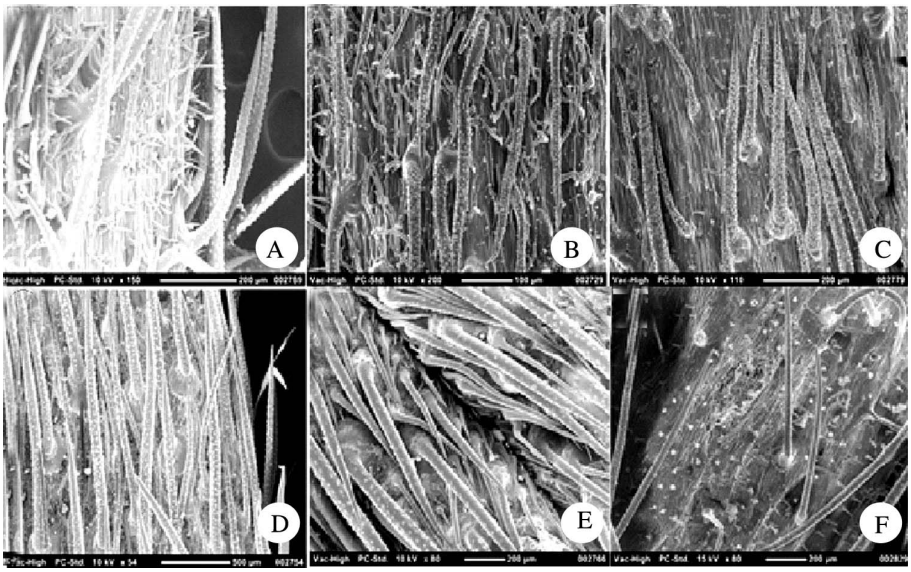

Fig. 6. Micromorphology of epidermal surface (stem and leaves) of *Onosma* species by SEM: Stem (A,B,C), Leaves (D,E,F): A. *O. argentata*; B. *O. rechingeri*; C. *O. stenoloba*; D. *O. neglecta*; E. O. *sericea*; F. *O. proballanthera.* (Bar = 200 µm).

species. Glabrous tubercules have been found at the base of stem and leaf of all species except *O. stenoloba.* The setae surface of those species cover dense or sparse papilae, where setae of *O. stenoloba* consist of tubercles with pilies at the base. The findings based on anatomical and micromorphological studies were found somewhat consistent with those of Akçin and Engin (2001), and Akçin and Binzet (2010). However, our results differs from those earlier studies in terms of number of layers of cells and tissues, the density of trichomes and the types of leaf mesophyll (Fig. 6). The present study based on micromorphological and anatomical investigation employing six *Onosma* species, of which five are endemic to Turkey, is the first of its nature. Though there are similarities in micromorphology and anatomy of stem, leaf and nutlets among the species studied, however, some of these characters have been found useful for delimation of the species. More particularly, micromorphological features of nutlet surface of *Onosma*, anatomical features of epidermal surface (trichomes and stomata), and types of lamina mesophyll (dorsiventral and isobilateral) could be useful in species delimitation and solving taxonomic problem of the genus.

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