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Leaf epidermis morphology of some *Onosma* (Boraginaceae) species from Turkey

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Abstract: The leaf epidermis of 14 *Onosma* L. species was examined by light and scanning electron microscopy (SEM). The leaf characters studied showed some variations. Morphologic features of the epidermal cells such as shape, structure of the anticlinal walls, stomatal size, outer stomatal rims, peristomatal rims, and apertures were summarised in the examined taxa. It was found that these characters are variable in the genus but usually they are stable within a species. Epidermal cells of the investigated taxa are polygonal, elongated, or irregular. Anticlinal walls of the epidermal cells are generally straight to curved and repand. Apertures are long, ellipsoidal, and narrow. SEM studies in particular provided some useful information concerning the systematics of *Onosma* taxa.

Key words: Epidermis, *Onosma*, micromorphology, SEM

1. Introduction

Onosma L. is one of the largest genera, with 98 species in the family in Turkey. The endemism rate for the genus in Turkey is 50% (Yıldırımlı, 2000; Riedl et al., 2004; Binzet & Orcan, 2007; Kandemir & Türkmen, 2010; Aytaç & Türkmen, 2011). Metcalfe and Chalk (1979) explained the general characteristic properties of the family Boraginaceae, including some details of the genus *Onosma*. Karyological (Teppner, 1981, 1988), chemical (Mellidis & Papageorgiou, 1987; Khajuria & Jain, 1993; El-Shazly et al., 2003; Özgen et al., 2004), and micromorphological (Akçin, 2007a) studies have been conducted on *Onosma* species. There are a number of studies on the anatomy of the genus. The leaf anatomy of some of the taxa studied in this paper was observed by light microscope (Akçin & Engin, 2001, 2005; Binzet & Orcan, 2003; Akçin, 2004, 2007b). Nevertheless, none of these studies had the structure of the epidermal cells as their primary focus. Some species of *Onosma* are difficult systematically. Riedl (1978) pointed out that the classification appears to be partly artificial and in need of re-investigation and new data may provide useful reference points in a future classification. Some *Onosma* species were examined to determine nutlet surface patterns using scanning electron microscopy (SEM) (Akçin, 2007a; Binzet & Akçin, 2009; Akçin & Binzet, 2011). The importance of leaf and epidermis morphology for classification has been recognised and studied by many authors (Baranova, 1972; Metcalfe & Chalk, 1979; Baranova, 1992; Ma et al., 2004; Yang & Lin, 2005; Mavi et al., 2011; Szymura & Wolski, 2011). These characteristics, which probably are taxonomically valuable, have not yet been investigated in *Onosma* taxa.

The micromorphological and morphological characters of the epidermis and stomata of *Onosma* species were studied to determine their taxonomic significance and implications.

2. Materials and methods

The plant specimens used in the present study were collected from different localities in Turkey. The plant materials were identified and deposited at Ordu University Herbarium. Collection data for the studied species are given in Table 1. Taxonomic descriptions of the specimens were performed according to Riedl (1978). Anatomic materials were fixed in 70% alcohol. Surface sections were cut freehand and they were covered with glycerine–gelatin (Vardar, 1987). The permanent slides were photographed with a Nikon FDX-35 microscope including software (NIS - Elements, Version 3.00 SP5) (Figures 1, 2). All measurements and observations were performed on the figures. Stomata were measured from 15 mature leaves. The terminology mainly follows Baranova (1972), Metcalfe and Chalk (1979), and Yang and Lin (2005).

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Table 1. Collection data for the *Onosma* species.

Taxa	Locality
O. ambigens	A7 Gümüşhane: Around Bayburt, 1200 m, *Akçin* 992.
O. albo-roseum	A5 Amasya: Around castle, 450 m, *Akçin* 1038.
O. araraticum	A5 Amasya: Ak Dağ, 1600 m, *Akçin* 1037.
O. armenum	A4 Ankara: Around Çubuk 2 Dam, 1150 m, *Barbaros* 1007.
O. bornmuelleri	A5 Kastamonu: Ilgaz, 890 m, *Akçin* 1031.
O. bourgaei	A4 Çankırı: Ilgaz, 2000 m, *Akçin* 996.
O. bracteosum	A6 Samsun: Ladik, 950 m, *Akçin* 1016.
O. intertextum	A6 Tokat: Around Niksar, 350 m, *Akçin* 1047.
O. isauricum	A5 Amasya: Akdag, 1150 m, *Akçin* 1015.
O. mutabile	A6 Ordu: Mesudiye, 1500 m, *Akçin* 1035.
O. roussaei	A6 Samsun: Çakallı, 350 m, *Akçin* 1049.
O. sericeum	A4 Ankara: Around Çubuk 2 Dam, 110 m, *Barbaros* 1008.
O. stenolobum	A5 Amasya: Vermiş village, 1200 m, *Akçin* 1010.
O. tauricum var. *tauricum*	A5 Kastamonu: Tosya, 900 m, *Akçin* 1048.

For scanning electron microscopy, dried mature leaves were mounted on stubs using double-sided adhesive tape. Samples were coated with 12.5–15 nm of gold. Coated leaves were examined and photographed with a JMS-6060 LV scanning electron microscope (Figures 3, 4).

For statistical analyses, one-way analysis of variance (ANOVA) and the general linear model were applied using SPSS 15.0 (Table 2; Figure 5).

3. Results

Morphological characteristics of leaf epidermal cells such as shape, structure of the anticlinal walls, stomatal sizes, outer stomatal rims, peristomatal rims, and apertures are summarised in Tables 2 and 3. It was found that these characters vary among the examined taxa.

3.1. Epidermal cells

The epidermal cells on both the adaxial and abaxial surfaces of *Onosma* are polygonal, elongated, or irregular. The pattern of anticlinal walls of cells may vary among the examined taxa. The anticlinal walls of the epidermis cells are generally straight to curved and repand. Straight to curved anticlinal wall is the dominant type in the adaxial epidermis and repand is the dominant type in the abaxial epidermis in the examined taxa. There are sinuous types in 2 taxa (Table 2; Figures 1, 2).

3.2. Stoma features

Stomata are present on both adaxial and abaxial sides in the studied species (Figures 3, 4). SEM observations showed that the stomata are sunken (Figures 3, 4) or slightly sunken (Figures 3, 4) among the examined taxa.

Stoma types for the examined taxa are staurocytic, anisocytic, and anomocytic. Anomocytic stoma is the dominant type in both the adaxial and abaxial epidermis in some of the examined taxa. Anizocytic stomata occur more densely on the abaxial and adaxial surfaces of the rest of the investigated taxa.

Stomatal sizes vary from species to species. All detailed measurements are presented in Table 2. It is seen that the outer stomatal rims are generally raised in the examined taxa. Although some investigated species have hardly visible raised rims, a few taxa have double outer stomatal rims (Figures 3, 4).

Peristomatal rims are raised, overlapping, stout, or smooth in the examined taxa. In some species, peristomatal rims are barely perceptible (Figures 3, 4).

The outer stomatal ledge aperture may vary in different species. Long, ellipsoidal, and narrow apertures are present in the studied species. Long apertures are generally present in *Onosma* taxa but ellipsoidal and narrow apertures are also seen (Figures 3, 4).

The cuticular membrane is striate in all the examined taxa. Wax granules, flakelets, and thick layers wax are observed in some species.

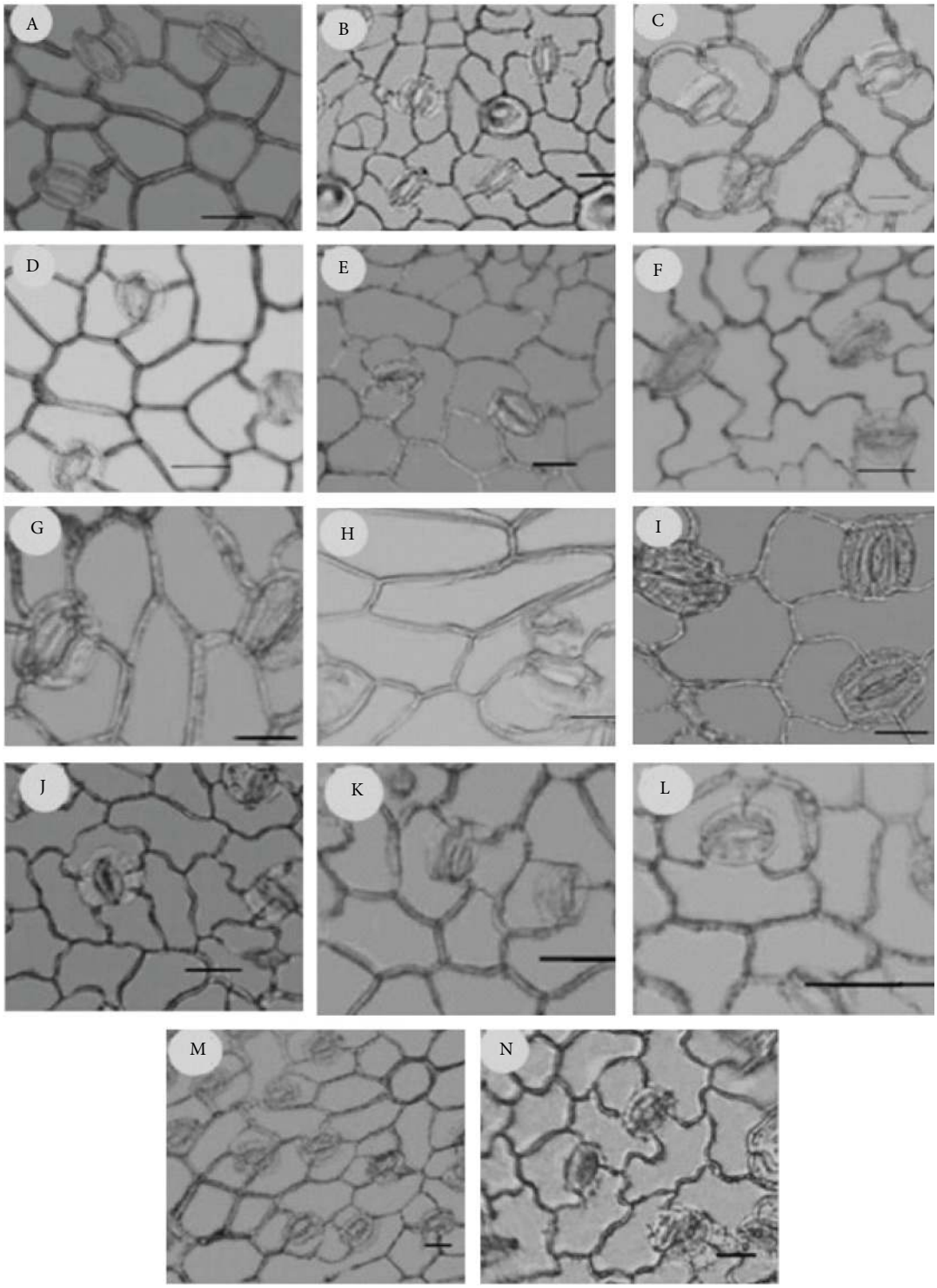

Figure 1. Surface sections of the examined taxa (LM): A, B- *Onosma ambigens,* C, D- *O. albo-roseum,* E, F- *O. araraticum,* G, H- *O. armenum,* I, J- *O. bornmuelleri,* K, L- *O. bourgaei,* M, N- *O. bracteosum.* (A, C, E, G, I, K, M: abaxial surface; B, D, F, H, J, L, N: adaxial surface). Scale bar: 20 μm.

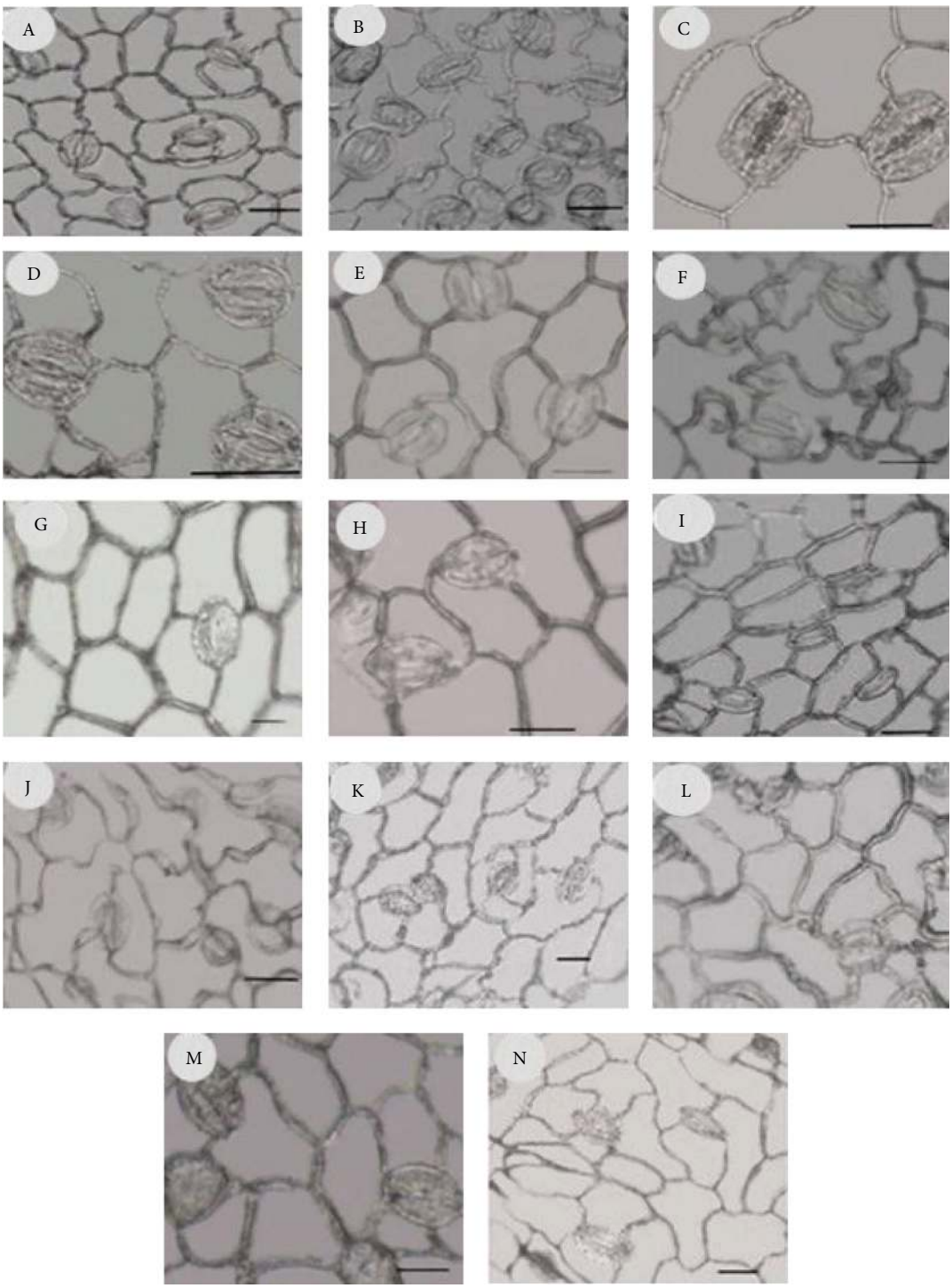

Figure 2. Surface sections of the examined taxa (LM): A, B- *Onosma intertextum,* C, D- *O. isauricum,* E, F- *O. mutabile,* G, H- *O. roussaei,* I, J- *O. sericeum,* K, L- *O. stenolobum,* M, N- *O. tauricum* var. *tauricum.* (A, C, E, G, I, K, M: abaxial surface; B, D, F, H, J, L, N: adaxial surface). Scale bar: 20 μm.

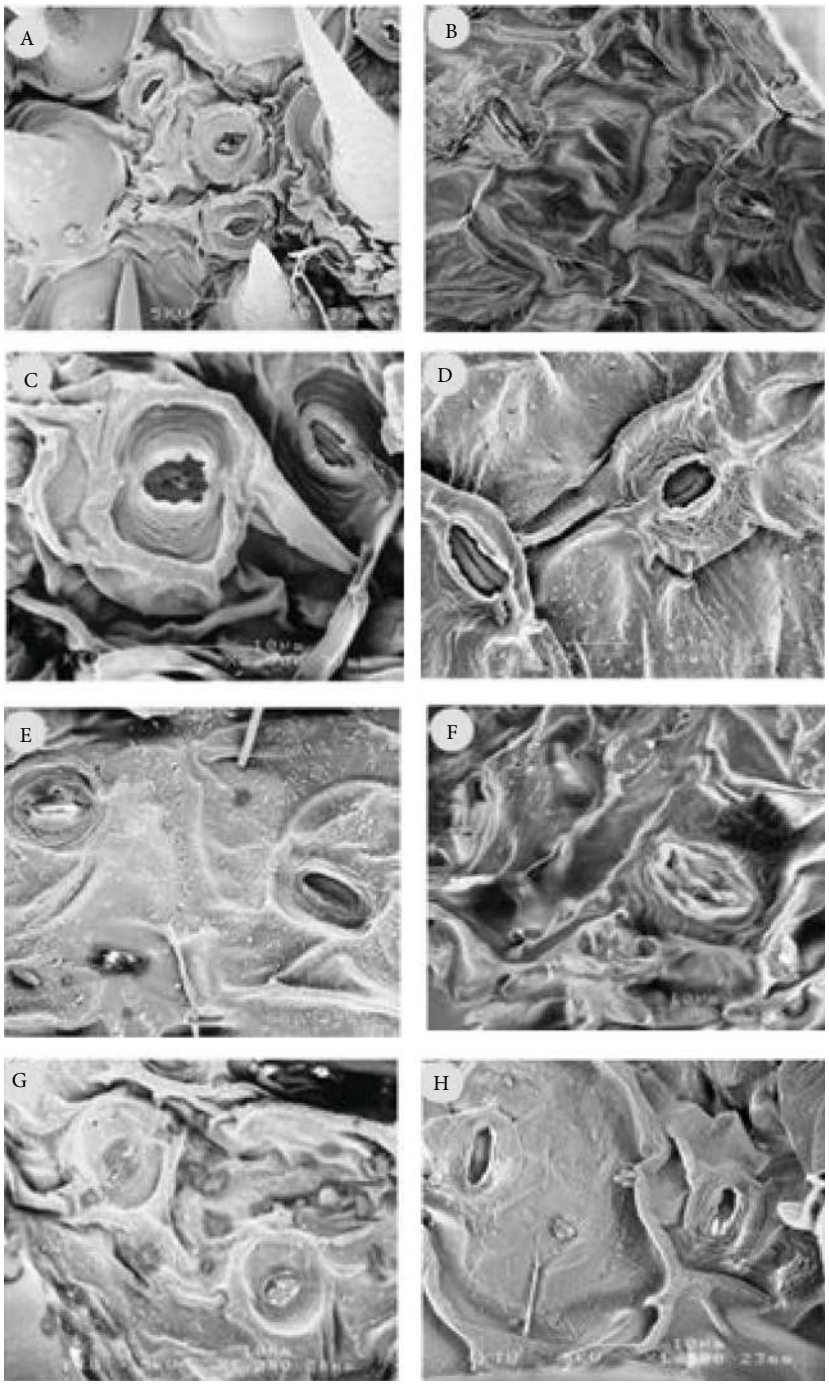

Figure 3. SEM images of stomata: A- *Onosma ambigens,* B- *O. albo-roseum,* C- *O. araraticum,* D- *O. armenum,* E- *O. bornmuelleri,* F- *O. bourgaei,* G- *O. bracteosum,* H- *O. intertextum.*

4. Discussion

This paper presents a comparative anatomical study of the leaf epidermis of 14 *Onosma* species, carried out using light microscopy and SEM. The results of the study show that the micromorphological characters used vary among the investigated taxa. Szymura and Wolski (2011) determined that micromorphological characters of the leaves of *Solidago* L. species are taxonomically useful. We found that some micromorphological characters such as structure of the anticlinal walls, outer stomatal rims, peristomatal rims, and apertures are important characters.

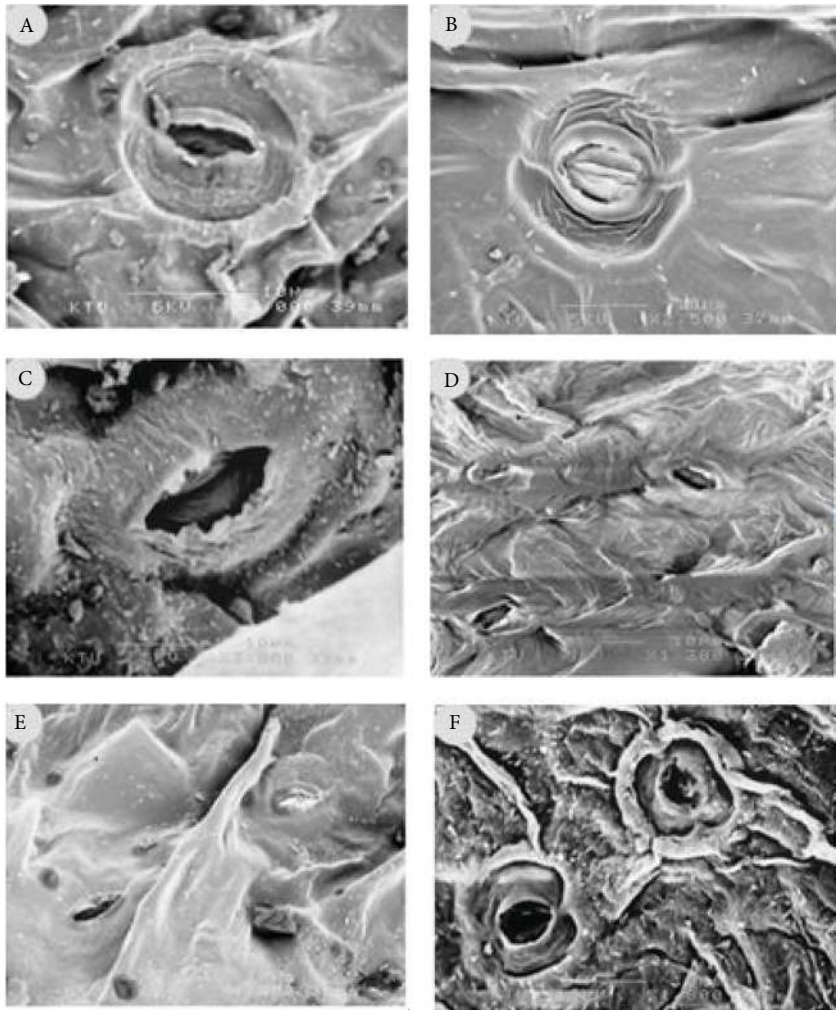

Figure 4. SEM images of stomata: A- *Onosma isauricum,* B- *O. mutabile,* C- *O. roussaei,* D- *O. sericeum,* E- *O. stenolobum,* F- *O. tauricum* var. *tauricum.*

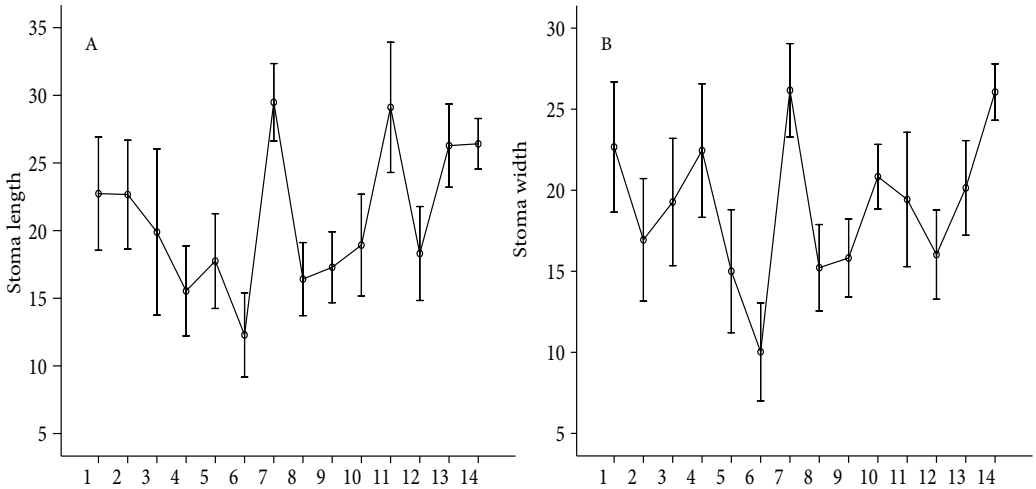

Figure 5. A- Error bar of the stoma length in examined taxa; B- Error bar of the width in examined taxa. 1: *Onosma ambigens;* 2: *O. albo-roseum;* 3: *O. araraticum;* 4: *O. armenum;* 5: *O. bornmuelleri;* 6: *O. bourgae;* 7: *O. bracteosum;* 8: *O. intertextum;* 9: *O. isauricum;* 10: *O. mutabile;* 11: *O. roussaei;* 12: *O. sericeum;* 13: *O. stenolobum;* 14: *O. tauricum* var. *tauricum.*

Table 2. Leaf epidermal properties of studied *Onosma* taxa with LM.

	Adaxial epidemis		Adaxial epidemis		Size of stomata (Mean ± SE)					
	P.A.V.	Stoma type	P.A.V.	Stoma type	Length (µm)	S.E.M.	Sig.	Length (µm)	S.E.M.	Sig.
O. ambigens	Str-cur	Ano, Sta, Ani	Repand	Ano, Sta, Ani	23.05c ± 2.77	4.39	***	19.20b ± 5.84	4.03	***
O. albo-roseum	Str-cur	Ani, Sta	Str-cur	Ani, Sta	23.06c ± 0.18	4.03	***	16.46e ± 0.20	3.57	***
O. araraticum	Repand	Sta, Ano, Ani	Repand	Sta, Ani, Ano	21.41d ± 0.4	9.42	***	20.33d ± 0,17	3.84	***
O. armenum	Str-cur	Ano, Ani	Str-cur	Ano, Ani	15.47g ± 0.22	2.77	***	22.35b ± 3.23	4.24	***
O. bornmuelleri	Str-cur	Ano, Ani, Sta	Repand	Ani, Ano	19.32f ± 0.52	3.06	***	16.42f ± 3.41	3.59	***
O. bourgaei	Str-cur	Ani-Ano	Str-cur	Ani, Ano, Sta	12.39h ± 0.1	2.41	***	10.32g ± 2.9	2.28	***
O. bracteosum	Str-cur	Sta, Ani, Ano	Repand	Ano, Ani	28.94a ± 0.36	2.05	***	26.76a ± 1.17	2.07	***
O. intertextum	Str-cur	Ani-Ano	Sinuous	Ano, Ani	17.11g ± 0.28	1.82	***	15.15f ± 0.13	1.77	***
O. isauricum	Str-cur	Ano, Sta, Ani	Repand	Sta, Ano, Ani	17.23f ± 3.18	1.71	***	15.32f ± 2.21	1.44	***
O. mutabile	Str-cur	Sta, Ani	Sinuous	Ano, Ani	20.72e ± 1.83	3.53	***	19.61c ± 9.61	0.98	***
O. roussaei	Str-cur	Ani, Sta	Str-cur	Ani, Ano	28.31a ± 31	5.79	***	19.33d ± 2.91	4.28	***
O. sericeum	Str-cur	Ano, Ani	Str-cur	Ano, Ani	17.83d ± 1.4	3.01	***	16.37e ± 3.24	1.89	***
O. stenolobum	Str-cur	Ano, Ani	Repand	Ano-Ani	26.14b ± 0.38	2.36	***	20.59c ± 0.31	2.13	***
O. tauricum var. *tauricum*	Str-cur	Ano, Ani	Repand	Sta, Ano, Ani	26.11b ± 0.57	0.87	***	25.85a ± 0.59	0.75	***

***P < 0.001, Abbreviations: S.E.M.: Standard error of means, Sig.: Significance level, abcdefgh: Letters in the same column are different. P.A.W.: Pattern of anticlinal walls, Str-cur: straight to curved, Ano: Anomocytic, Ani: Anisocytic, Sta: Staurocytic

Table 3. Leaf epidermal properties of studied *Onosma* taxa with SEM.

Taxa	Outer stomatal rim	Peristomatal rim	Aperture	Membrane and wax ornamentation
O. ambigens	raised	overlapping	long and wide	striated and granular
O. albo-roseum	double and raised	barely perceptible	long and narrow	densely striated
O. araraticum	raised	raised	ellipsoidal	striated and granular
O. armenum	double	stout and raised	long	striated
O.bornmuelleri	raised	overlapping and raised	long	striated, thick layered, and granular
O. bourgaei	double	overlapping	long	striated
O. bracteosum	raised	stout and raised	ellipsoidal	hardly visible, striated
O. intertextum	raised	smooth	long and narrow	striated
O. isauricum	raised	raised	long	striated, flaked, and granular
O. mutabile	raised	raised	ellipsoidal	striated
O. roussaei	overlapping	stout	long	striated, flaked, and granular
O. sericeum	hardly visible raised	raised	long	striated, flaked
O. stenolobum	hardly visible raised	barely perceptible	long	striated, flaked, and granular
O. tauricum var. *tauricum*	raised	Surrounded by curved ridges of cuticle on the subsidiary cells	ellipsoidal	striated and granular

The leaf epidermis and leaf anatomy provide comprehensive taxonomic data (Dasti et al., 2003). In the present study, the pattern of anticlinal walls of cells varies among the examined species. The anticlinal walls of the epidermis cells are generally straight to curved and repand. Sinuous types are observed only in *O. intertextum* Hub.-Mor. and *O. mutabile* Boiss. (Figures 3, 4). Yang and Lin (2005) reported that anticlinal walls of cells show some differences in *Schisandra* Michaux. They found that characters of the leaf epidermis are constant in the genus. In the present study, we found that outer stomatal rims, peristomatal rims, outer stomatal ledge aperture, cuticular membrane, and wax ornamentation are useful characters for delimiting the examined taxa. Outer stomatal rims are generally raised in the examined taxa. *O. albo-roseum* Fisch. & C.A.Mey., *O. armenum* DC., and *O. bourgaei* Boiss. have double outer stomatal rims. Single rims prevail in the other species. The outer stomatal rims were double in evergreen groups of *Schisandra*, in contrast to single rims seen in deciduous groups of this genus (Yang & Lin, 2005). The term peristomatal rim was proposed for low ridges of the cuticle. The shape of peristomatal rims can show differences in plants (Wilkinson, 1979). The examined *Onosma* species have raised, overlapping, stout, or smooth peristomatal rims. Peristomatal rims are surrounded by curved ridges of cuticle on the subsidiary cells in *O. tauricum* var. *tauricum* Pallas ex Willd. (Figure 4). Stout peristomatal rims are present in *O. armenum*, *O. bracteosum* Hausskn. & Bornm., and *O. roussaei* DC. (Figures 3, 4). Sunken stomata depressed below the leaf surface may be due to the thickness of the cuticle, the height of the epidermal cells, or the presence of a hypodermis that may be up to several layers thick (Wilkinson, 1979). It was found that *O. isauricum* Boiss. & Heldr., *O. sericeum* Willd., *O. tauricum* var. *tauricum*, *O. araraticum* H.Riedl, and *O. bracteosum* have sunken stomata (Figures 3, 4). Slightly sunken stomata were seen in *O. bornmuelleri* Hausskn. and *O. mutabile* (Figures 3, 4).

Some authors consider that stomatal size is too variable to be of diagnostic value (Davis & Heywood, 1963; Abdulrahaman et al., 2011). Most authors agree that stomatal size is usually sufficiently stable to be used

as a diagnostic character (Pant & Gupta, 1966; Wilkinson, 1979; Dasti et al., 2003). In the examined *Onosma* species, stomatal size is 12.39 ± 0.1–28.94 ± 0.36 × 10.32 ± 2.9–26.76 ± 1.17 µm. According to the results, stomatal size is longest in *O. roussaei* and *O. bracteosum*. *O. bourgaei* has the shortest long and short axes. Statistically, there are significant differences between species in terms of stomatal size. Stomatal size is found to be a significant character in the studied *Onosma* taxa (Figure 5). Stomata are located on both adaxial and abaxial surfaces in all the leaves. Stoma types are anomocytic, staurocytic, and anisocytic. Metcalfe and Chalk (1979) pointed out that there were both anomocytic and anisocytic stomata in this family. The same results were determined by Akçin and Engin (2001) and Akçin (2004), who performed some anatomical studies on Turkish *Onosma* taxa. Dasti et al. (2003) studied the foliar stomata of 32 species belonging to 3 tribes of the family Boraginaceae. They indicated that the prevalent type of stoma was anomocytic. Other types such as paracytic, diacytic, helicocytic, actinocytic, and cyclocytic were rare. They also explained that, although anomocytic was a dominant type, helicocytic, hemiparacytic, staurocytic, and brachyparatetracytic stomata were seen in the examined *Onosma* taxa.

We also found that the cuticular membrane of the leaf epidermis of all the taxa has striae. Wax granules, flakelets, and thick layers wax were observed in some species. *O. albo-roseum*, *O. bourgaei*, *O. intertextum*, and *O. mutabile* do not have wax structures.

In this study, we report that micromorphological characters such as structure of the anticlinal walls, outer stomatal rims, peristomatal rims, apertures, and wax ornamentation are important characters. They are taxonomically useful for discriminating *Onosma* taxa.

References

Abdulrahaman AA, Oyedotun RA & Oladele FA (2011). Diagnostic significance of leaf epidermal features in the family Cucurbitaceae. *Insight Botany* 1: 22–27.

Akçin ÖE (2004). An investigation on the morphology, anatomy and ecology of endemic *Onosma bornmuelleri* Hausskn. *Ecology* 13: 13–19.

Akçin ÖE (2007a). Nutlets micromorphology of some Onosma L. (Boraginaceae) species from Turkey. *Biologia* 62: 684–689.

Akçin ÖE (2007b). The morphological and anatomical properties of endemic *Onosma armenum* DC. (*Boraginaceae*) species. *International Journal of Natural and Engineering Sciences* 1: 37–43.

Akçin ÖE & Engin A (2001). Comparative morphological and anatomical study on species of *Onosma isauricum* Boiss. & Heldr. and *O. stenolobum* Hausskn. ex H.Riedl. *The Herb Journal of Systematic Botany* 8: 75–95.

Akçin ÖE & Engin A (2005). The morphological, anatomical and ecological properties of endemic *Onosma bracteosum* Hausskn. & Bornm. (Boraginaceae) species. *Turkish Journal of Botany* 29: 317–325.

Akçin ÖE & Binzet R (2011). Micromorphological studies on nutlets of some *Onosma* L. (Boraginaceae) species from Turkey. *Pakistan Journal of Botany* 43: 743–752.

Aytaç Z & Türkmen Z (2011). A new *Onosma* (Boraginaceae) species from southern Anatolia, Turkey. *Turkish Journal of Botany* 35: 269–274.

Baranova M (1972). Systematic anatomy of the leaf epidermis in the Magnoliaceae and some related families. *Taxon* 21: 447–469.

Baranova M (1992). Principles of comparative stomatographic studies of flowering plants. *Botanical Review* 58: 1–99.

Binzet R & Orcan N (2003). Morphological and palynological studies on *Onosma roussaei* DC. and *Onosma giganteum* Lam. (*Boraginaceae*). *The Herb Journal of Systematic Botany* 10: 57–76.

Binzet R & Orcan N (2007). A new species of *Onosma* L. (Boraginaceae) from Southern Turkey. *Novon* 17: 8–10.

Binzet R & Akçin ÖE. (2009). Nutlet size, shape and surface ornamentation in 14 *Onosma* L. (Boraginaceae) species. *Acta Botanica Croatica* 68: 117–126.

Dasti AA, Bokharı TZ, Malik AS & Akhtar R (2003). Epidermal morphology in some members of family Boraginaceae in Baluchistan. *Asian Journal of Plant Sciences* 2: 42–47.

Davis PH & Heywood VH (1963). *Principles of Angiosperm Taxonomy*. pp. 210–230. Edinburgh: Oliver and Boyd.

El-Shazly A, Abdel-Ghani A & Wink M (2003). Pyrrolizidine alkoloids from *Onosma arenaria* (Boraginaceae). *Biochemical Systematics and Ecology* 31: 477–485.

Kandemir A & Türkmen Z (2010). A new species of *Onosma* (Boraginaceae) from eastern Turkey. *Turkish Journal of Botany* 34: 277–282.

Khajuria RK & Jain SM (1993). Two new naphthoquinones from the roots of *Onosma hispidum*. *Indian Journal of Chemistry* 32: 390–391.

Ma QW, Li CS, Li FL & Vickulin SV (2004). Epidermal structures and stomatal parameters of Chinese endemic *Glyptostrobus pensilis* (Taxodiaceae). *Botanical Journal of the Linnean Society* 146: 153–162.

Mavi DÖ, Doğan M & Cabi E (2011). Comparative leaf anatomy of the genus *Hordeum* L. (Poaceae). *Turkish Journal of Botany* 35: 357–368.

Mellidis AS & Papageorgiou VP (1987). Lipids from roots of *Onosma heterophylla*. *Phytochemistry* 26: 842–843.

Metcalfe CR & Chalk L (1979). *Anatomy of Dicotyledons I*. London: Oxford University Press.

Özgen U, Çoşkun M, Kazaz C & Seçen H (2004). Naphthoquinones from the roots of *Onosma argentatum* Hub.-Mor. (Boraginaceae). *Turkish Journal of Chemistry* 28: 451–454.

Pant DD & Gupta KL (1966). Development of stomata and foliar structure of some Magnoliaceae. *Botanical Journal of the Linnean Society* 59: 265–277.

Riedl H (1978). *Onosma* L. In: Davis PH (ed.) *Flora of Turkey and the East Aegean Islands,* Vol. 6, pp. 326–376. Edinburgh: Edinburgh University Press.

Riedl H, Binzet R & Orcan N (2004). A new species of *Onosma* (Boraginaceae- Lithospermeae) from southern Turkey. *Edinburgh Journal of Botany* 61: 127–130.

Szymura M & Wolski K (2011). Leaf epidermis traits as tools to identify *Solidago* L. taxa in Poland. *Acta Biologica Cracoviensia Series Botanica* 53: 38–46.

Teppner H (1981). Karyosystematik von *Onosma stellulatum, O. pygmaeum* und *O. leptanthum* (Boraginaceae). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 102: 297–306.

Teppner H (1988). *Onosma kaheirei* spec. nova und *O. erectum* (Boraginaceae) aus Griechenland. *Phyton (Austria)* 28: 115–131.

Vardar Y (1987). *Preparation Techniques in Botany*. İzmir: Ege University Press.

Wilkinson HP (1979). The plant surface (mainly leaf), In: Metcalfe CR & Chalk L. (ed.) *Anatomy of the Dicotyledons,* pp. 97–165. Oxford: Clarendon Press.

Yang ZR & Lin Q (2005). Comparative morphology of the leaf epidermis in *Schisandra* (Schisandraceae). *Botanical Journal of the Linnean Society* 148: 39–56.

Yıldırımlı Ş (2000). The chorology of the Turkish species of Boraginaceae family. *The Herb Journal of Systematic Botany* 7: 257–272.