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MICROMORPHOLOGICAL STUDIES ON NUTLETS OF SOME *ONOSMA* L. (BORAGINACEAE) SPECIES FROM TURKEY

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Abstract

In this study, the nutlet micromorphology of 9 taxa of Turkish *Onosma* species viz., *O. sericeum* Willd., *O. cassium* Boiss., *O. rutilum* Hub.-Mor., *O. sieheanum* Hayek, *O. albo-roseum* Fisch. & Mey. *ssp. albo-roseum* var. *albo-roseum, O. inexspectatum* Teppner, *O. auriculatum* Aucher ex DC., *O. heterophylum* Griseb and *O. thracicum* Velen. was examined using both light and scanning electron microscopy. According to surface ornemantations, three main types, reticulate, rugose and elongated, and 2 subtypes (rugose and rugose-reticulate) were defined and illustrated. Nutlet morphology of the examined specimens exhibits some variation in size and shape. Nutlet size ranges between 2-7 x 1.5-5 mm. Nutlets are ovoid, broadly ovoid oblong-ovoid and bipyrimidate.

Introduction

The Boraginaceae family are distributed throughout the tropical, subtropical and temparate regions of the world (Al-Shehbaz, 1991). *Onosma* L., is a genus with ca. 150 species occuring in dry, cliffy and sunny habitats, and distributed mainly in Eurasia and Mediterranean area. *Onosma* is the largest genus in the family Boraginaceae with about 102 taxa (97 species) in Turkey and the rate of endemism among native species is about 50% (Riedl, 1978; Davis *et al*., 1988; Yıldırımlı, 2000; Riedl *et al.,* 2005; Binzet & Orcan, 2007). The classification of *Onosma* species appears to be partly artificial and there is need for re-investigation and that new data may provide useful reference points in a future classification (Riedl, 1978). Since the genus was revised for the *Flora of Turkey* (Riedl, 1978), some new anatomical, karyological, palynological and chemical studies have been reported. These include anatomical studies (Akçin & Engin, 2001, 2005; Akçin, 2004, 2007a; Binzet & Orcan, 2003); karyological studies (Teppner, 1980, 1981, 1988, 1991, 1996a, 1996b); palynological studies (Binzet & Orcan, 2003; Maggi *et al*., 2008); chemical studies (Mellidis & Papageorgiou, 1987; Khajuria & Jain, 1993; El-Shazly *et al*., 2003; Özgen *et al*., 2004; Çadirci *et a*l., 2007) of *Onosma* species.

Micromorphological features on nutlet surface of *Onosma* species could be useful in solving some taxonomic problems (Akçin, 2007b). In this study we have carried out a detailed analysis of nutlet micromorphology of 9 species of the genus *Onosma* in Turkey.

Material and Methods

Mature nutlets from 9 *Onosma* species were freshly collected at flowering stage during 2003-2007 from different locations in Turkey. *O. sericeum* Willd., *O. cassium* Boiss. *O. rutilum* Hub.-Mor., belong to *Haplotricha* subsect. and *O. sieheanum* Hayek *O. albo-roseum* Fisch. & Mey. ssp. *albo-roseum* var. *albo-roseum, O. inexspectatum* Teppner, *O. auriculatum* Aucher ex DC., *O. heterophylum* Griseb. and *O. thracicum* Velen belong to *Asterotricha* subsect. Sample specimens are stored at the Herbarium of the Department of Biology at the University of Mersin. Collection data of the studied specimens are given in Table 1.

Table 1. The locality of the investigated species.

Taxa	Locality
O. sericeum Willd.	Kilis: Kilis-Gaziantep 20 km, roadside, 700 m, 25.05.2004, Binzet 67.
O. cassium Boiss.	Hatay: İskenderun, Arsuz, Işıklı village-Kale village, rocky slopes and open forest, 150 m, 29.06.2004, Binzet 11.
O. rutilum Hub.-Mor.	Mersin: Silifke-Gülnar, Balandız plateau, under forest and open forest, stony and rocky slopes, 820 m, 14.05.2005, Binzet 13.
O. sieheanum Hayek	Mersin: Kırobası-Mut 8 km, roadside, 1430 m, 06.05.2005, Binzet 34.
O. albo-roseum Fisch. & Mey. ssp. *albo-roseum* var. *albo-roseum*	Kayseri: Sarız-Göksun 5 km, road side and rocky slopes, 1750 m, 27.05.2004, Binzet 79.
O. inexspectatum Teppner	Osmaniye: Hasanbeyli-Fevzipaşa 2 km, slopes and open forest, 1150 m, 25.05.2004, Binzet 3.
O. auriculatum Aucher ex DC.	Mersin: Mut–Silifke 25 km, slopes, 130 m, 08.06.2003, Binzet 15.
O. heterophylum Griseb	Bartın: Bartın entrance, roadside and slopes, 30m, 09.06.2007, Binzet 129
O. thracicum Velen	Eskişehir: Mihalıççık, Kartal kule around, 1648m17.05.2007, Binzet 130

In order to determine the average nutlet sizes, 10 nutlets from each species were measured. For scanning electron microscopy, dried mature nutlets were mounted on stubs using double-sided adhesive tape. Samples were coated with 12.5-15 nm of gold. Coated nutlets were examined and photographed with JMS-6400 Scanning Electron Microscope. Observations were made on the surface patterns of nutlets as in Stearn (1973).

Results and Discussion

Nutlet size of studied species shows some variations. Nutlet size ranges between 2-7x1,5-5mm. *O. alboroseum* ssp. *alboroseum* var. *alboroseum* from the *Asterotricha* subsection, has the largest nutlets (6-7x4-5mm); *O. thracicum* from the *Asterotricha* subsection, has the smallest nutlets (2-2.5x1.5-2mm) (Table 2).

Colour of studied nutlets shows some variations. Nutlet colour is pale grey-brown in *O. sericeum, O. cassium* and *O. rutilum*; pale brown in *O. sieheanum,* and *O. thracicum*; light brown-brown and usually brown dotted in *O. alboroseum* ssp. *alboroseum* var *alboroseum*; grey-brown in *O. inexspectatum*; brown in *O. auriculatum*; light brown in *O. heterophyllum.*

Nutlet shape is ovoid in *O. rutilum, O. alboroseum, O. auriculatum, O. heterophyllum* and *O. thracicum*; broadly ovoid in *O. sericeum* and *O. inexspectatum*; oblong-ovoid in *O. cassium*; bipyrimidate in *O. sieheanum*. There are distinct ventral keels in nutlets of *O. rutilum, O. alboroseum* and *O. thracicum*.

Based on the surface ornamentation of the nutlet in *Onosma* species, three main types can be determined: Type I (reticulate-type, *O. cassium*), Type II [It has been divided into two subtypes: respectively rugose (*O. alboroseum, O. rutilum, O. sieheanum*) and rugose-reticulate (*O. heterophyllum, O. auriculatum*)] and Type III (*O. sericeum, O. inexspectatum, O. thracicum)*.

Type I (Figs a-b)

Epidermal cells of nutlet surface are formed in a reticulate ornamentation with varied sizes and shapes of the meshes. The reticulate patterns consist of large rounded-polygonal cells with prominent walls. Cells are clear. This type includes only *O. cassium*.

Type II : This type may be divided into 2 subtypes.
Subtype 1 (Figs c-h)

Rugose is characterised by the epidermal cells of nutlet surface that have small or fine wrinkles. This subtype is recognised on the nutlet of 3 studied species, i.e. *O. alboroseum, O. rutilum* and *O. sieheanum.* In *O. alboroseum,* cells walls are thick and straight. The epidermis cells are not clear. In *O. rutilum,* the surface epidermis cells and cells walls are not clear. In *O. sieheanum*, the surface epidermis cells are elongated rectangular and narrow-large. Cells walls are usually clear and straight or wavy.

Subtype 2 (Figs ı-l)

Surface subtype is rugose-reticulate. The nutlet surface has a prominent undulate-ridged pattern formed by elongated rectangular and narrow-large cells. Cell walls are clear, thick, elevated and wavy. This subtype is recognised on the nutlet of 2 studied species, i.e., *O. heterophyllum, O. auriculatum.* In *O. heterophylum*, the cells are ±clear. Boundaries of cells are elevated and thick. In *O. auriculatum*, the cells are elongated rectangular with elevated, wavy boundaries.

Table 2. A comparison of characters studied for *Onosma* taxa nutlets.

Taxa	Nutlet size	Shape	Colour	Nutlet surface	Subsection	Phytogeographic region
O. sericeum Willd.	4,5-5x3,6-4mm	Broadly-ovoid, acute, with ventrally and dorsally keeled	Pale graey-brown	Type III	*Haplotricha*	Ir.-Tur. element
O. cassium Boiss.	3-5x3-5mm	Oblong-ovoid, subacute, with ventral and dorsal keel, short beaked	Pale brown-brown	Type I	*Haplotricha*	E. Medit. element
O. rutilum Hub.-Mor.	3-4x2-2.5mm	Ovoid, acute, with distinct ventral and indistinct dorsal keel, short beaked	Pale graey-brown	Type II	*Haplotricha*	Ir.-Tur. element
O. sieheanum Hayek	2.8-3x2.5-3mm	Acute, bipyramidate with prominent ventral keel	Pale brown	Type II	*Asterotricha*	Ir.-Tur. element
O. albo-roseum Fisch. & Mey. ssp. *albo-roseum* var. *albo-roseum*	6-7x4-5 mm	Ovoid, subacute, with distinct ventral keel and indistinct dorsal keel, short beaked	Light brown-brown and usually brown dotted	Type II	*Asterotricha*	Ir.-Tur. element
O. inexspectatum Teppner	3-3.3x2-2.7mm	Broadly ovoid, acute, with prominent ventral keel	Gray-brown	Type III	*Asterotricha*	E. Medit. element.
O. auriculatum Aucher ex DC.	3.5-4x3-3,5mm	Ovoid, acute, indistinct dorsal keel and prominent ventral keel	Brown	Type II	*Asterotricha*	Ir.-Tur. element
O. heterophyllum Griseb	3-3.5x2-2.5mm	Ovoid, beak acute, incurved, with prominent ventral keel and lateral ridges	Light brown	Type II	*Asterotricha*	Euro-Sib. element
O. thracicum Velen.	2-2.5x1.5-2mm	Ovoid, incurved, with distinct ventral and indistinct dorsal keel, short beaked	Pale-brown	Type III	*Asterotricha*	Euro-Sib. element

Ir.-Tur.: Irano- Turanien, Medit: Mediterranean, Euro-Sib.: Euro-Siberian

Type III (Figs m-s)

The epidermal cells of nutlet surface formed in elongated type with varied sizes and shapes. Three species observed belong to the elongated type, i.e. *O. sericeum, O. inexspectatum, O. thracicum.* In *O. sericeum* the surface epidermis cells are elongated usually rectangular and large-narrow. Cell walls are clear, thin and straight. In *O. inexspectatum,* epidermis cells are ±elongated rectangular-hexagonal and large. Cell walls are clear, thin and straight. In *O. thracicum*, the surfaces epidermis cells are elongated and large-narrow. Cell walls are ± clear and straight-wavy.

Morphological and microphological features of nutlet of nine *Onosma* species were determined. The examined species belong to section *Onosma*. Three species, *O. sericeum, O. cassium* and *O. rutilum* belong to subsection *Haplotricha.* The other species belong to subsection *Asterotricha. O. rutilum, O. sieheanum* and *O. inexspectatum* are endemic for Turkey. According to Willis (1973), the center of distribution and maxium concentration of genus *Onosma* is Iran. But *Onosma* is represented by about 102 taxa (97 species) in Turkey and the rate of endemism among native species is about 50% (Riedl, 1978; Davis *et al*., 1988; Yıldırımlı, 2000; Riedl *et al.,* 2005; Binzet & Orcan, 2007). According to Flora of Iran, *Onosma* is represented by 73 species in Iran, 17 of which are endemic (Khatamsaz, 2002). Consequently Turkey has more species than Iran, Iran can not center of distribution of genus *Onosma*.

The members of *Onosma* genus are very similar and this similarity often causes to identification problems. The species are distinguished on the basis of indumentum composed of specific trichomes leading in the past to several mistakes in taxonomy (Ball, 1972; Maggi, 2008). Riedl (1978) needs to include new characteristics that may provide useful reference points in a future classification. Boraginaceae fruits are characterized by one-seeded nutlets with a sclerified exocarp protecting the seeds (Diana *et al*., 2002).

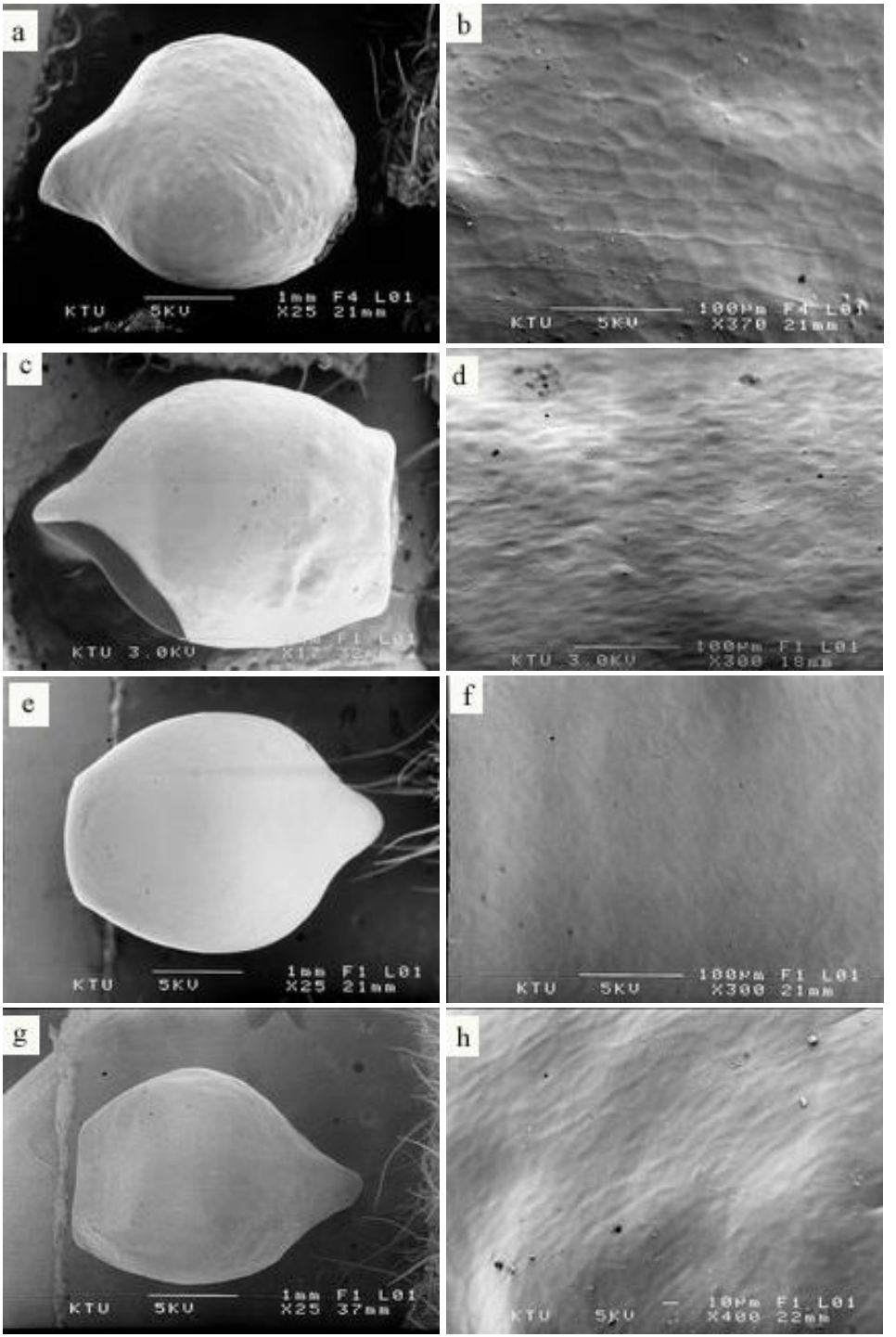
Morphological characters of seeds provide valuable information on the evolutionary classification of flowering plants (Corner, 1976; Takhtajan, 1991). Fruit surface and seed coat morphologies are beneficial for systematic (Juan *et al*., 2000; Özcan, 2002; Akçin, 2007b, 2008).

External nutlet characters, size, shape and colour are of limited taxonomic value according to the *Flora of Turkey* (Riedl, 1978) and our observations. However, the micromorphological characters of the nutlet surface patterns as seen by scanning electron microscope show some variations.

Examined nutlet sizes of species show some variations. Nutlet size is not very useful for distinguishing of species. However, nutlet size helps to identify some taxa such as *O. alboroseum* ssp. *alboroseum* var. *alboroseum* and *O. thracicum*. *O. alboroseum* has the largest nutlets (6-7x4-5mm) whereas *O. thracicum* has the smallest nutlets (2-2.5x1.5-2mm).

Colour of studied nutlets shows some variations. Nutlet colours are pale gray-brown, pale brown, light brown-brown, gray-brown and brown. A lot of species have the same fruit colour. It is difficult to distinguish between taxa depending on the colour of the fruit. Therefore the colour of nutlet was not used as diagnostic character to distinguish *Onosma* species in this study.

Nutlet shape is also a variable character, ranging from ovoid, broadly ovoid to bipyrimidate. There are distinct ventral keel and indistinct dorsal keel in nutlet of *O. rutilum, O. alboroseum* and *O. thracicum. O. sericeum* and *O. cassium* have dorsal and ventral keel. Nutlet shape of *O. sieheanum* are bipyrimidate.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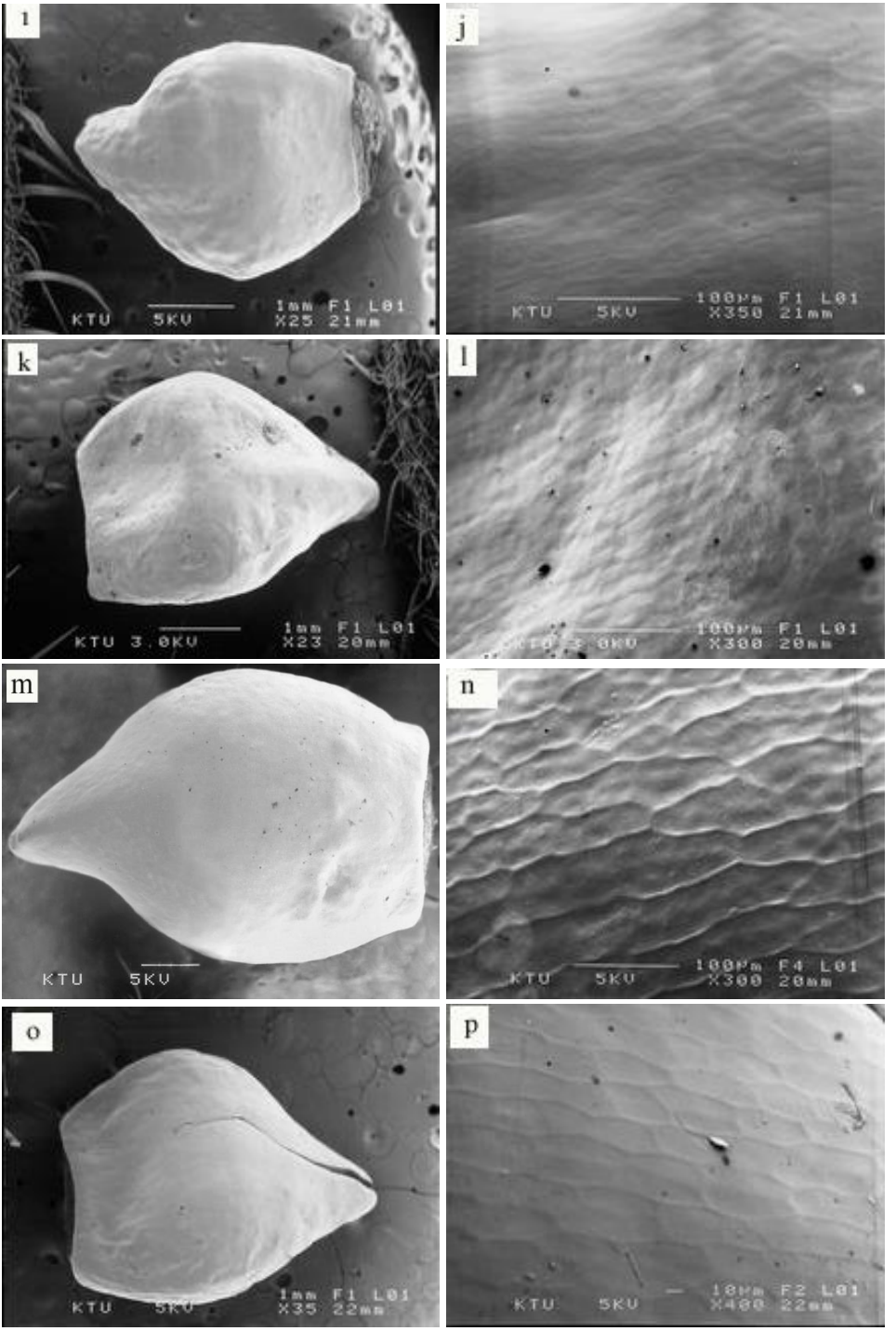
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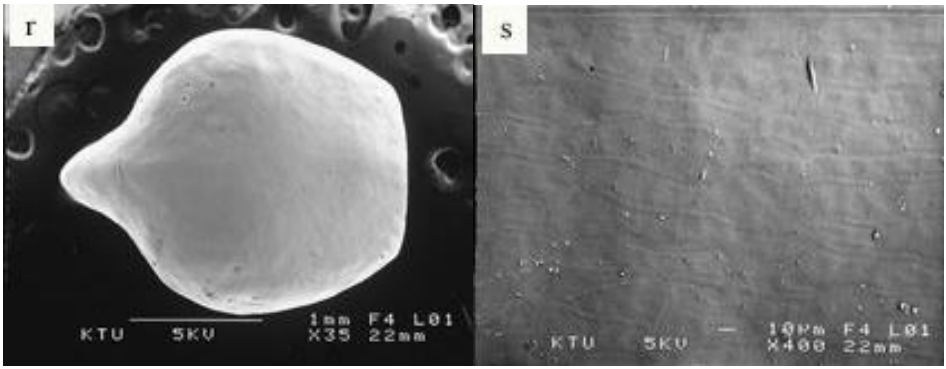

Figs. a-s. SEM micrographs of nutlet surface: Type I: a,b: *O. cassium* (bars= 1mm; 100µm), Type 2: subtype 1: c,d: *O. alboroseum* ssp. *alboroseum* var. *alboroseum* (bars= 1mm; 100µm); e,f: *O. rutilum* (bars= 1mm; 100µm); g,h: *O. sieheanum* (bars= 1mm; 10µm); subtype 2: ı,j: *O. heterophyllum* (bars= 1mm; 100µm); k,l: *O. auriculatum* (bars= 1mm; 100µm); Type 3: m,n: *O. sericeum* (bar = 1mm; 100µm); o, p : *O. inexspectatum* (bars= 1mm; 10µm); r,s: *O. thracicum* (bars= 1mm; 10µm).

Three main types in *Onosma* species can be determined based on surface ornamentation: Type I (reticulate-type), Type II (rugose and rugose-reticulate) and Type III. Most species of *Onosma* have nutlet surface type II (Rugose and Rugose-reticulate), such as *O. alboroseum, O. rutilum, O. sieheanum, O. heterophyllum* and *O. auriculatum.* The other species determined belong to type I and III. Type I, which includes *O. cassium*, is clearly separate from the other two established types because epidermal cells of nutlet surface are formed in a reticulate ornamentation. In this study, the unknown nutlet features of *O. sieheanum* are given for the first time here in detailed.

O. sericeum, O. cassium and *O. rutilum* belong to *Haplotricha* subsection (Riedl, 1978). These species have a different nutlet size, shape and surface types (Table 2). Except for *O. cassium*, same surface type was seen in both species which belong to subsection *Asterotricha* and species belong to subsection Haplotricha. Therefore, it is difficult to use nutlet surface features at subsection level.

This study indicated that nutlet morphology is useful in suggesting relationship at species level. Akçin (2007b) stated that micromorphological features on nutlet surfaces of *Onosma* could be useful in solving taxonomic problems. Aytaş Akçin & Ulu (2008) reported that mericarp characters are very useful for the identification of *Anchusa* L. (Boraginaceae) species.

Nutlet characters within the genus *Onosma* are of taxonomic significance. It is clear that external nutlet characters, especially surface ornamentation, could help us to classify the species of the complex genus *Onosma* in the future.

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References

Akçin, O.E. 2004. Endemik *Onosma bornmuelleri* Hausskn.'nın morfolojisi anatomisi ve ekolojisi üzerine bir araştırma (An investigation on the morphology, anatomy and ecology of endemic *Onosma bornmuelleri* Hausskn.). *Ecology,* 13: 13-19.

Akçin, O.E. 2007a. The morphological and anatomical properties of endemic *Onosma armenum* DC. (*Boraginaceae*) species. *International Journal of Natural and Engineering Sciences,* 1(2): 37-43.

Akçin, O.E. 2007b. Nutlets micromorphology of some *Onosma* L. (Boraginaceae) species from Turkey. *Biologia, Bratislava,* 62(6): 684-689.

Akçin, O.E. 2008. Seed coat and fruit surface micromorphology of some *Cynoglossum* L. (Boraginaceae) species. *Bangladesh Journal of Botany,* 37: 115-119.

Akçin, O.E. and A. Engin. 2001. *Onosma isauricum* Boiss. & Heldr. ve *O. stenolobum* Hausskn. Ex H. Riedl türleri üzerinde karşılaştırmalı morfolojik ve anatomik bir araştırma (Comparative morphological and anatomical study on species of *Onosma isauricum* Boiss. & Heldr. and *O. stenolobum* Hausskn. Ex H. Riedl). *Herb Journal of Systematic Botany,* 8: 75-95.

Akçin, O.E. and A. Engin. 2005. The morphological and anatomical and ecological properties of endemic *Onosma bracteosum* Hausskn. & Bornm. (Boraginaceae) species. *Turk. J. Bot*., 29: 317-325.

Al-Shehbaz, I.A. 1991. The Genera of Boraginaceae in the Southeastern United States: *Jour. Arnold. Arb.* Suppl. Ser. 1: 1-169.

Aytaş Akçin, T. and S. Ulu. 2008. Micromorphological Characters of Fruit of Some *Anchusa* L. (Boraginaceae) Species from Turkey. *International Journal of Natural and Engineering Sciences,* 2: 63-67.

Ball, P.W. 1972. *Onosma* L., pp. 89-94. In: *Flora Europea*. (Eds.): T.G. Tutin *et al*., Vol. 3, Univ. Press, Cambridge.

Binzet, R. and N. Orcan. 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. and N. Orcan. 2007. A new species of *Onosma* L. (Boraginaceae) From Southern Turkey. *A Journal for Botanical Nomenclature,* 17: 8-10.

Cadirci, E., H. Süleyman, H. Aksoy, Z. Halici, U. Ozgen, A. Koç and N. Ozturk. 2007. Effects of *Onosma armeniacum* root extract on ethanol-induced oxidative stress in stomach tissue of rats. *Chemico-Biological Interactions,* 170: 40-48.

Corner, E.J. 1976. *The seeds of the dicotyledons*. Vol. 2, Chambridge, Chambridge University press.

Davis, P.H., R.R. Mill and K. Tan. 1988. *Flora of Turkey and the East Aegean Islands*, 10, Edinburgh University press, Edinburgh, pp.184-185.

Diana, N., H. Förther and H. H. Hilger. 2002. A Systematic analysis of Heliotropium, Tourneforti, and allied taxa of the Heliotropiaceae (Boraginales) based on ITSI sequences and morphological data. *Amer. J. Bot*., 89: 287-295.

El-Shazly, A., A. Abdel-Ghani and M. Wink. 2003. Pyrrolizidine alkoloids from *Onosma arenaria* (Boraginaceae). *Biochem. System. Ecol.,* 31: 477-485.

Juan, R., J. Pastor and I. Fernandez. 2000. SEM and light microscope observations on fruit and seeds in Scrophulariaceae from southwest Spain and their systematic significance. *Annals of Botany,* 86: 323-338.

Khajuria, R.K. and S.M. Jain. 1993. Two new naphthoquinones from the roots of *Onosma hispidum. Indian J. Chem*., 32: 390-391.

Khatamsaz, M. 2002. Boraginaceae, In: *Flora of Iran*. (Eds.): Assadi M. Et. Al. Research Institute of Forests and Rangelands, Tehran, pp. 1-504.

Maggi, F., V. Kolarcik and P. Martonfi. 2008. Palynological analysis of five selected *Onosma* taxa. *Biologia,* 63(2): 183-186.

Mellidis, A.S. and V.P. Papageorgiou. 1987. Lipids from roots of *Onosma heterophylla*. *Phytochem*., 26: 451-454.

Özcan, T. 2002. SEM observations on petals and fruits of some Turkish endemic *Bupleurum* L. (Umbelliferae) species. *Botanical Journal of the Linnean Society,* 138: 441-449.

Özgen, U., C. Maksut, C. Kazaz and H. Secen. 2004. Naphthoquinones from the roots of *Onosma argentatum* Hub.-Mor. (Boraginaceae). *Turk. J. Chem*., 28: 451-454.

Riedl, H. 1978. "*Boraginaceae*" *Flora of Turkey and the East Aegean Islands*, 6, (Ed.): P.H. Davis. Edinburgh University press, Edinburgh, pp. 237-437.

Riedl, H., R. Binzet and N. Orcan. 2005. A New Species of *Onosma* (Boraginaceae-Lithospermeae) From Southern Turkey. *Edinb. J. Bot*., 61(2&3): 127-130.

Stearn, W. T. 1973. *Botanical Latin*. David & Charles, Newton, Abbot, London, pp. 566.

Takhtajan, A. 1991. *Evolutionary Trends in Flowering Plants*. New York, Colombia University Press.

Teppner, H. 1980. Die *Onosma alboroseum*-Gruppe (Boraginaceae). *Phyton (Austria)* 20: 135-157.

Teppner, H. 1981. Karyosystematick von *Onosma stellulatum, O. pygmaeum* und *O. leptanthum* (Boraginaceae). *Bot. Jahrb. Syst.,* 102: 297-306.

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

Teppner, H. 1991. Karyology of some Greek *Onosma* species (Boraginaceae). *Bot. Chron*., 10: 271-292.

Teppner, H. 1996a. Remarks to the *Onosma* species *O. bourgaei, O. spruneri* and *O. stellulata* (Boraginaceae) offered. *Samentauschverzeichnis* (*Bot. Garten Inst*. *Bot. Univ. Graz*), pp. 33-39.

Teppner, H. 1996b. Die *Onosma*-Arten (Boraginaceae-Lithospermeae) Rumaniens. *Stapfia,* 45: 47-54.

Willis, J.C. 1973. *A dictionary of the flowering plants and ferns.* Univ. Press, Cambridge.

Yıldırımlı, Ş. 2000. The chorology of the Turkish species of Boraginaceae family, *The Herb J. System. Bot*., 7(2): 257-272.

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