

## Nutlet size, shape and surface ornamentation in 14 *Onosma* species (Boraginaceae)

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Nutlets of 14 species of *Onosma* L. in Turkey were examined with scanning electron microscope. Nutlet (mericarp) morphology of the examined specimens exhibits some variation in shape and size. Nutlets of species vary in the range  $2.5\text{--}6 \times 2\text{--}4.5$  mm. The studied nutlet surfaces show some variations. According to surface ornamentations 5 types were defined and illustrated: Type 1 (Ruminate type), Type 2, Type 3 (Rugose-type), Type 4 (Reticulate-type) and Type 5 (Pusticulate-type). The reticulate is the most common type in the examined species. Six of the species observed belong to the reticulate type, i.e. *O. papillosum*, *O. lycaonicum*, *O. caerulescens*, *O. rascheyanum*, *O. mersinana* and *O. riedliana*. In addition, the nutlet features of *O. papillosum* H. Riedl and *O. lycaonicum* Hub.-Mor., previously unpublished, are given for the first time here in detail. It is clear that external nutlet characteristics could especially help in the classification of the species of the complex genus *Onosma* in the future.

**Keywords:** *Onosma*, Boraginaceae, nutlet, mericarp, morphology, ornamentation, SEM

### Introduction

The family Boraginaceae comprises about 131 genera and 2500 species, mainly annual, bi-annual or perennial herbs and shrubs, some trees and a few lianes, distributed throughout the temperate and subtropical regions of the world. The family is mainly distributed in dry, cliffy and sunny habitats of Eurasia, the Mediterranean region and western North America (RETIEF and VAN WYK 1997), with a maximum concentration in Iran (WILLIS 1973). This family has about 34 genera and 350 taxa in Turkey (YILDIRIMLI 2000). The genus *Onosma* L. is the largest genus with about 97 species in Turkey and the rate of endemism among the native species is about 50 % (RIEDL 1978, DAVIS et al. 1988, YILDIRIMLI 2000, RIEDL et al. 2005, BINZET and ORCAN 2007). RIEDL (1978) reported that the *Onosma* genus has been divided into three sections: *Protonosma*, *Podonosma* and *Onosma*. *Protonosma* and *Podonosma* sections are represented by 1 species, the other *Onosma* species belong to *Onosma* sections. This section is separated into two subsections according to indumentum type: *Asterotricha* (Boiss.) Gürke and *Haplotricha* (Boiss.) Gürke. RIEDL (1978) reported that the

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classification of *Onosma* species appears to be partly artificial and in need of re-investigation and that new data may provide useful reference points in a future classification. There have been karyological studies (TEPPNER 1980, 1981, 1988, 1991, 1996a, 1996b); anatomical studies (AKÇIN and ENGIN 2001, BINZET and ORCAN 2003, AKÇIN 2004, AKÇIN and ENGIN 2005, 2007a); palynological studies (BINZET and ORCAN 2003); chemical studies (MELLIDIS and PAPAGEORGIOU 1987, KHAJURIA & JAIN 1993, EL-SHAZLY et al. 2003, ÖZGEN et al. 2004) of *Onosma* species. The first study of the ultramorphology of Boraginaceae including *Onosma* was done by KHATOON et al. (1994). The first study on nutlet micromorphology of Turkish *Onosma* has confirmed micromorphology as a useful tool in the *Onosma* taxonomy (AKÇIN 2007b).

### Material and methods

Nutlets of 14 species from two subsections of *Onosma* were collected in 1998–2006. *O. sintenisii* Hasskn. ex Bornm, *O. papillosum* H. Riedl and *O. bulbotrichum* DC belong to *Haplotricha* subsect. and *O. lycanicum* Hub.-Mor., *O. intertextum* Hub.-Mor., *O. briquetii*

**Tab. 1.** The localities of the examined species.

Taxa	Locality
<i>O. sintenisii</i> Hasskn. ex Bornm	Sivas, Divrigi-Kemaliye 44 km, slopes, 26.V.2006, 1060 m, Binzet 99.
<i>O. papillosum</i> H. Riedl	Adana: Yesilkent-Tufanbeyli 7 km, Steppe, 1560 m, 16.06.2005, Binzet 22.
<i>O. bulbotrichum</i> DC	Adiyaman, Karahöyük village, slopes, 24.V.2006, 650 m, Binzet 85.
<i>O. lycanicum</i> Hub.-Mor.	Mersin: Mut-Karaman, Sertavul around, steppe, 1670 m, 19.06.2004, Binzet 89.
<i>O. intertextum</i> Hub.-Mor.	Gümüşhane, Around Karaca cave, 1300m, Akçin 1049.
<i>O. briquetii</i> Czeck.	Amasya, Akdag, Çalardi plateau, 28.V. 2006, Binzet 128.
<i>O. bourgaei</i> Boiss.	Çankiri: Ilgaz, roadside, 2000 m, Akçin 996.
<i>O. rascheyanum</i> Boiss.	Kahramanmaraş: Çağlayancerit-Pazarcik 15 km, stony and rocky slopes, 970 m, 26.05.2004, Binzet 87.
<i>O. caerulescens</i> Boiss.	Kahramanmaraş: Pazarcik-Gölbasi 20 km, roadside, 900 m, 27.05.2004, Binzet 2.
<i>O. angustissimum</i> Hasskn. et Bornm.	Adana: Gülek, gülekkale around, stony and rocky slopes, 1550 m, 26.06.2004, Binzet 61.
<i>O. ambigens</i> Lacaita	Çorum: Iskilip to Tosya, 450 m, Ergen 1008.
<i>O. giganteum</i> Lam.	Osmaniye: Yarpuz-Hasanbeyli 15 km, roadside, 750 m, 30.06.2004, Binzet 52
<i>O. mersinana</i> Riedl, Binzet et Orcan	Mersin: Müglü Deresi village, rocky slopes, 1100 m, 07.05.2003, Binzet 18.
<i>O. riedliana</i> Binzet et Orcan	Mersin: Gülnar-Ermenek 42 km, roadside open field, 1300 m, 05.06.2004, Binzet 24.

Czecz., *O. bourgaei* Boiss., *O. rascheyanum* Boiss., *O. caerulescens* Boiss., *O. angustissimum* Hasskn. et Bornm., *O. ambigens* Lacaita, *O. giganteum* Lam., *O. mersinana* Riedl, Binzet et Orcan and *O. riedliana* Binzet et Orcan belong to *Asterotricha* subsect. Sample specimens are stored at the Herbarium of the Department of Biology at the University of Mersin and University of Ordu. Collection data of the studied specimens are given in table 1.

In order to determine the average nutlet sizes, 15 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 (STEARN 1973).

## Results

### Nutlet size

Studied nutlet size shows some variations. Nutlets of studied *Onosma* species vary in the range 2.5–6×2–4.5 mm. *O. riedliana* has the smallest nutlets (2.5×2 mm) and *O. bulbotrichum* has the largest (6×4.5 mm).

### Nutlet colour

Colour of studied nutlets shows some variations. The colour of the nutlets is brown in *O. sintenisii*, *O. intertextum* and *O. bourgaei*; gray-pale brown in *O. papillosum*, *O. mersinana*, *O. briquetii* and *O. riedliana*; pale brownish in *O. ambigens* and *O. rascheyanum*; pale gray in *O. bulbotrichum*; gray in *O. lycaonicum*; reddish brown in *O. caerulescens*; gray brown in *O. angustissimum* and *O. giganteum*.

### Nutlet shape

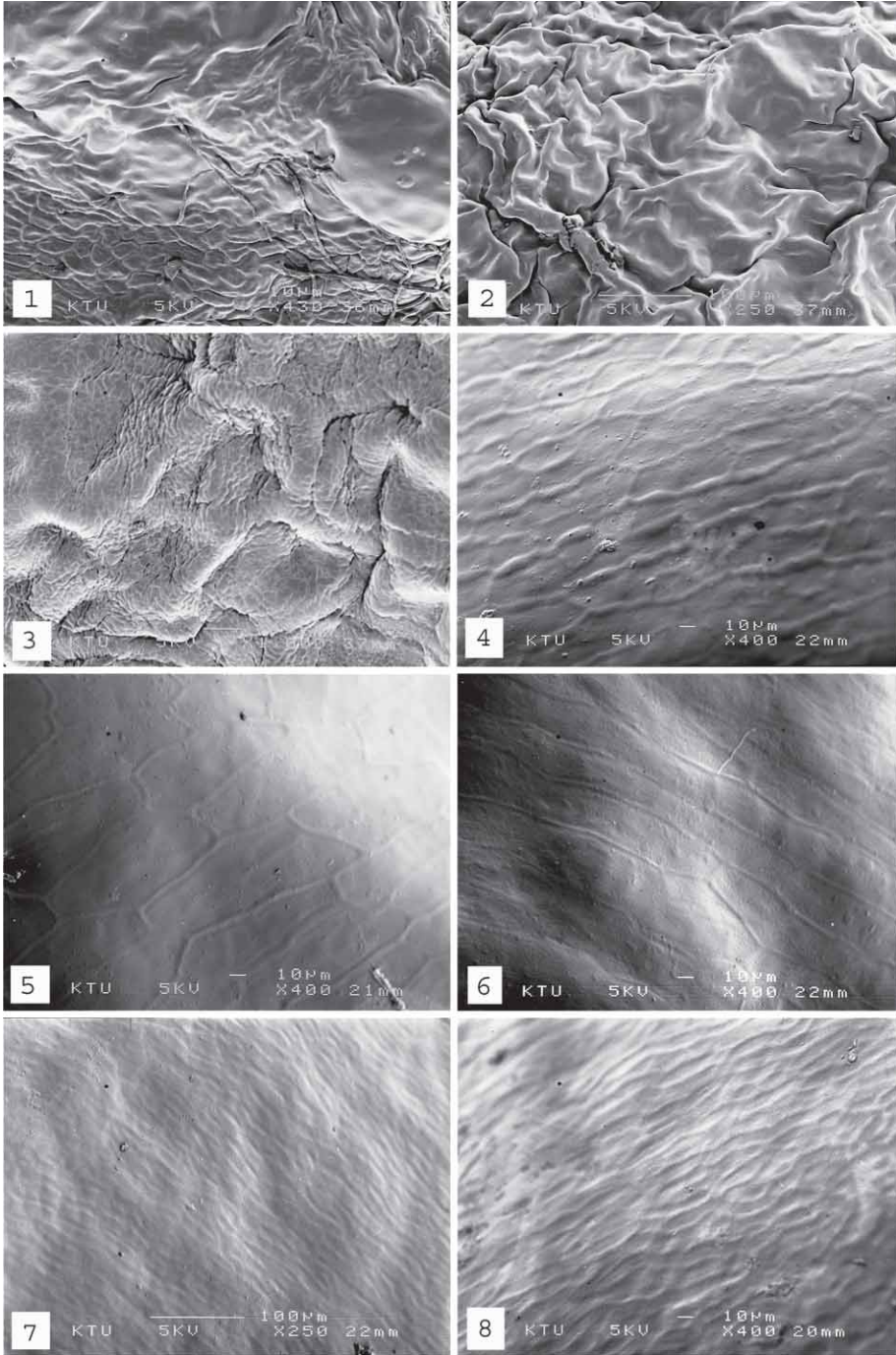
Nutlets of 14 taxa were examined in detail in this study. Nutlet shape shows some variations. Nutlet shape is oblong-ovoid in *O. sintenisii*; ovoid in *O. bulbotrichum*, *O. lycaonicum*, *O. bourgaei*, *O. rascheyanum*, *O. caerulescens*, *O. angustissimum*, *O. giganteum*; ovoid-globose in *O. intertextum*; oblong in *O. briquetii* and *O. mersinana*; oblong globose in *O. riedliana*. In the nutlets of *O. bulbotrichum* and *O. caerulescens* in particular sharp ventral and dorsal keels were seen.

### Nutlet ornamentation

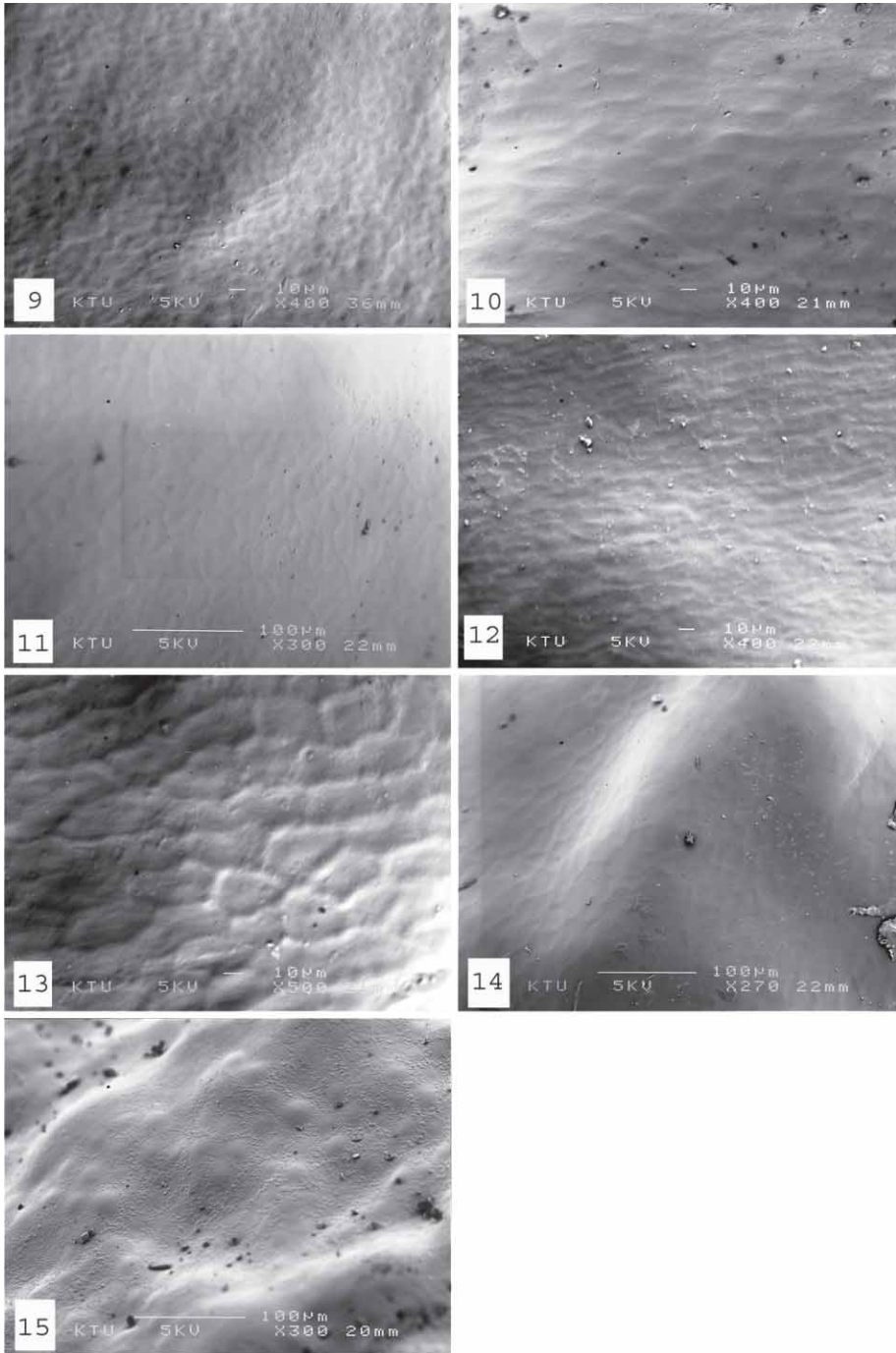
Some variations in nutlet surfaces were determined. Five main types in *Onosma* species can be distinguished according to surface ornamentation: Type 1 (Ruminant type), Type 2, Type 3 (Rugose type), Type 4 (Reticulate type) and Type 5 (Pusticulate type); within these some types, variants can be recognised.

Type 1 (Figs. 1–3) is characterised by a surface pushed into a series of large ridges superimposed on the pattern formed by the cell walls. Two species observed belong to this type, i.e. *O. ambigens* and *O. bourgaei*.

Type 2 (Figs. 4–6) has epidermal cells of nutlet surface formed in an elongated type with varied sizes and shapes. Three species observed belong to the elongated type, i.e. *O.*



**Plate 1.** SEM micrographs of nutlet surfaces. Type 1: 1 – *O. ambigens*, 2,3 – *O. bourgaei*; type 2: 4 – *O. briquetii*, 5 – *O. intertextum*, 6 – *O. sintenisii*; type 3: 7 – *O. angustisimum*, 8 – *O. giganteum*;



**Plate 1. – continued.** Type 4: 9 – *O. papillosum*, 10 – *O. lycaonicum*, 11 – *O. caerulescens*, 12 – *O. rascheyanum*, 13 – *O. mersinana*, 14 – *O. riedliana*; type 5: 15 – *O. bulbotrichum*.



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

Taxa	Nutlet size	Shape	Colour	Nutlet surface	Subsection	Phytogeographic region
<i>O. sintenisii</i> Hasskn. ex Bormn	3.5×3mm	Oblong-ovoid, acute, with ventral and dorsal keel, short beaked	Brown	Type II	Haplotricha	Ir.-Tur. Element
<i>O. papillosum</i> H. Riedl	4×2.5mm	Oblong-ovoid, acuminate, with ventral and dorsal keel, short beaked	Gray-pale brown	Type IV	Haplotricha	Ir.-Tur. Element
<i>O. bulbotrichum</i> DC	6×4.5mm	Ovoid, obtuse, with sharp ventral and indistinct dorsal keel, beak strongly incurved	Pale gray	Type V	Haplotricha	Ir.-Tur. Element
<i>O. lycaonicum</i> Hub.-Mor.	3×2mm	Ovoid, acute-acuminate, with ventral and dorsal keel, short beaked	Gray	Type IV	Asterotricha	Ir.-Tur. Element
<i>O. intertextum</i> Hub.-Mor.	4×3 mm	Ovoid-globose, acuminate	Brown	Type II	Asterotricha	Ir.-Tur. element?
<i>O. briquetii</i> Czece.	3×2.5	Oblong-ovoid, acuminate	Gray-pale brown	Type II	Asterotricha	Ir.-Tur. Element
<i>O. bourgaei</i> Boiss.	3×2.5mm	Ovoid, acute beak, indistinct dorsal keel	Brown	Type I	Asterotricha	Ir.-Tur. Element
<i>O. rascheyanum</i> Boiss.	5×3mm	Ovoid, beak acute, incurved, with prominent ventral keel and lateral ridges	Pale brownish	Type IV	Asterotricha	Ir.-Tur. Element
<i>O. caeruleascens</i> Boiss	4×3mm	Ovoid, acuminate, with sharp ventral and less prominent dorsal keel, short beaked	Reddish-brown	Type IV	Asterotricha	Ir.-Tur. Element
<i>O. angustissimum</i> Hasskn. et Bormn.	3.5×3 mm	Ovoid, acuminate-acute, with ventral and dorsal keel, short beaked	Gray-brown	Type III	Asterotricha	Ir.-Tur. Element
<i>O. ambigens</i> Lacaite	3×2.5	Dorsally compressed, beak acute	Pale brownish	Type I	Asterotricha	Ir.-Tur. Element
<i>O. giganteum</i> Lam.	3.5×2mm	Ovoid, acute, with ventral and dorsal keel, very short beaked	Gray-brown	Type III	Asterotricha	Medit. Element
stright <i>O. mersinana</i> Riedl, Binzet et Orcan	3×2mm	Oblong, obtuse-acuminate with dorsal keel	Gray-pale brown	Type IV	Asterotricha	Medit. Element
<i>O. riedliana</i> Binze et Orcan	2.5×2 mm	Oblong-globose, acuminate, with dorsal keel and short beak	Gray-pale brown	Type IV	Asterotricha	Medit. Element

*briquetii*, *O. intertextum* and *O. sintenisii*. In *O. briquetii*, the surface epidermis cells are elongated rectangular and narrow-large. Cell walls are clear and wavy. *O. intertextum* has elongated rectangular and large epidermis cells. Cell walls are clear and straight. In the nutlet of *O. sintenisii*, the surface epidermis cells are elongated-rectangular and large-narrow. Cell walls are clear and straight.

Type 3 (Figs. 7–8) is the rugose type, characterised by the epidermal cells of the nutlet surface having small or fine wrinkles. This type is recognised on the nutlet of two studied species, i.e. *O. angustissimum* and *O. giganteum*.

Type 4 (Figs. 9–14) is the reticulate surface type. The epidermal cells of the nutlet surface are formed in a reticulate ornamentation with varied sizes and shapes of mesh. Most species of *Onosma* have the type 4 nutlet surface, such as *O. papillosum*, *O. lycanicum*, *O. caerulescens*, *O. rascheyanum*, *O. mersinana* and *O. riedliana*. In these species, *O. mersinana* is especially distinct from the others. In *O. mersinana*, the cells are clear. Boundaries of cells are elevated and thick. *O. caerulescens* and *O. riedliana* are similar. In these species, the cells are small and isodiametric. Cell walls are  $\pm$ clear. The other similarity is seen in *O. papillosum* and *O. rascheyanum*. The surface type is reticulate. Cells are  $\pm$ clear. Boundaries of cells are elevated. In *O. lycanicum*, the surface type is reticulate. Cells are  $\pm$ clear, cell walls are elevated.

Type 5 (Fig. 15) is characterised by the pustulate pattern and by the epidermal cells of the nutlet surface having compressed tubercles. Boundaries and cells are not clear. This type is recognised on the nutlet of *O. bulbotrichum*.

## Discussion

The examined 14 species belong to the section *Onosma*. Three species, *O. sintenisii*, *O. papillosum* and *O. bulbotrichum* belong to the subsection *Haplotricha*. The other species belong to the subsection *Asterotricha*. Nine species are endemic for Turkey. RIEDL (1978) needs to include new characteristics that may provide useful reference points in a future classification. The species of *Onosma* members are very similar and this similarity often causes identification problems. Boraginaceae fruits are characterized by one-seeded nutlets with a sclerified exocarp protecting the seeds (DIANA et al. 2002).

In the present study, the nutlet colour of the studied 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 in this study.

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

Nutlet size and shape show specific characteristics. *Onosma riedliana* has the smallest nutlets (2.5×2 mm), whereas *O. bulbotrichum* has the largest nutlets (6×4.5 mm). Nutlet shape in *O. bulbotrichum* and *O. caerulescens* is characterized by sharp ventral and dorsal keels.

In this study, five types of fruit surface were examined. Most species of *Onosma* have nutlet coat type 4, such as *O. papillosum*, *O. lycanicum*, *O. caerulescens*, *O. rascheyanum*, *O. mersinana* and *O. riedliana*. The other species determined belong to types 1, 2, 3

and 5. *O. ambigens* and *O. bourgaei* are morphologically similar and have type 1, and this type is very distinct. *O. briquetii*, *O. intertextum* and *O. sintenisii* are type 2. *O. angustisimum* and *O. giganteum* have type 3 nutlet coat sculpturing. *O. bulbotrichum* has type 5. *O. sintenisii*, *O. papillosum* and *O. bulbotrichum* belong to the subsection Haplotricha. These species have a different surface type. Except for *O. bulbotrichum*, the same surface type was seen in both species belonging to the subsection Asterotricha and species belonging to the subsection Haplotricha. Accordingly it is difficult to use nutlet surface features at subsection level.

In this study, the nutlet features of *Onosma papillosum* H. Riedl and *O. lycaonicum* Hub.-Mor. are given for the first time, and in the morphology is described as a useful tool for species identification, as indicated previously (AKÇIN 2007b). Mericarp characters are useful for the identification of *Anchusa* L. species (AYTAŞ AKÇIN and ULU 2008). Fruit surface and seed coat morphologies are considered useful characters for species identification of *Cynoglossum* (AKÇIN 2008). In these studies, the micromorphology of several plant seeds and fruits has been examined with SEM and their importance in plant taxonomy has been emphasized (OLGUN 1997, ÇOŞKUNÇELEBI et al. 2000, KHALIK et al 2008). The external nutlet characters, especially surface ornamentation, could help in classification species of the complex genus *Onosma* in the future.

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