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Nutlet surface micromorphology of Turkish *Satureja* (Lamiaceae)

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Abstract: There are some taxonomic uncertainties within the Turkish members of *Satureja*. It is extremely difficult to distinguish some of *Satureja* species because of their great morphological similarity. They are represented by fifteen species in Turkey. In this study, the nutlet surface features of species were examined using both stereoscopic and scanning electron microscopy (SEM). The investigated species can be divided into two main types, the more or less smooth and the sculptured, and four subtypes, undulate-reticulate, reticulate, reticulate-protuberculate and papillate-tuberculate. This work offers the description, comparison and illustration of all of these types and subtypes. In addition, the unknown nutlet features of eight *Satureja* species are described in detail for the first time. It is clear that in future studies, external nutlet characters, especially surface texture, could be useful in species classification of the complex *Satureja* genus.

Key words: *Satureja*; Lamiaceae; micromorphology; nutlet; SEM

Indroduction

Satureja L. (Lamiaceae) includes about 200 species of herbs and shrubs, often aromatic, widely diffused in the Mediterranean area, Asia and boreal America (Rustaiyan et al. 2004), and is represented by 15 species in Turkey (Davis 1982; Tumen et al. 2000). Five of these species are endemic. *Satureja*, for the most part, grows in the south and west Anatolia regions of Turkey.

Satureja species are low shrubs, suffruticose herbs or annuals. Many members of the genus have aromatic and medicinal characteristics. The aerial parts of these species have distinctive tastes and can be used to season stuffing, meat pies, and sausages (Eminagaoglu et al. 2007). These species are widely used as herbal tea and spices in Turkey due to the pleasant aroma. Members of this genus are called "kekik" in Turkey, and some of *Satureja* species are exported as thyme (Satıl et al. 2002). In folk medicine, some species, especially *S. cuneifolia* Ten., *S. thymbra* L. and *S. hortensis* L. are traditionally used as digestive aids and diuretics in various regions of Turkey (Baytop 1999). As a medicinal plant, *S. hortensis* has been traditionally used as a stimulant, stomachic, carminative, expectorant, antidiarrheic, and aphrodisiac (Sefidkon & Jamzad 2005).

In previous systematic revisions of any laminaceous group of taxa, the micromorphological characteristics of nutlet surfaces have been either totally ignored or only seldom mentioned in spite of their stability. Nutlet morphology in Lamiaceae has proved useful to varying degrees at different levels of the taxonomic hierarchy. In recent times, the importance of the SEM in the study of nutlet surfaces has been demonstrated for various genera of Lamiaceae (Husain et al. 1990; Demissew & Harley 1992; Marin et al. 1996; Budantsev & Lobova 1997; Jamzad et al. 2000).

There are some taxonomic uncertainties within the species of *Satureja*. In this paper, we report a comparative study on nutlet surface morphology for 15 species of the genus in order to improve present knowledge and to provide morphological separation of similar species.

Material and methods

The plant material was collected in different regions of Turkey (Table 1). Voucher specimens were deposited in the Herbarium of the Science and Arts Faculty of Balıkesir University, Turkey. Measurements and optical observations of structural features and colours of the nutlets were carried out with a Wild M5 stereomicroscope.

For scanning electron microscopy (SEM), dry, mature nutlets were mounted directly on stubs, using single-side adhesive tape, coated with gold, and the photographs were taken with EVO-50. The terminology for describing nutlet surface sculpturing mainly follows Stearn (1992).

Results

Nutlets of 15 species were examined in detail for this study. Nutlets of *Satureja* are pale to dark brown or

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Table 1. Collection data of *Satureja* species studied.

Taxa	Collection data	Herbarium No.
S. thymbra L.	B1 Izmir: Kiraz-Sarıgöl 15. km, 20.06.2001	FS1046
	C4 Antalya: Gazipaşa to Anamur, 22.06.2001	FS1048
S. cuneifolia Ten.	C2 Denizli: Babadağ, Taşdelen plateau, 1600 m, 22.08.2001	FS1043
	B1 Izmir: Kiraz, 17.09.2001	FS1042
**S. cilicica* P.H.Davis	C6 Kahramanmaraş: Andırın-Geben plateau, 1400 m, 27.08.2000	FS1180
**S. amani* P.H.Davis	C5/6 Hatay: Hassa, Amanos mountain	TD1221
S. icarica P.H.Davis	A1 Çanakkale: Gökçeada, Keklik hill, 250 m, 22.09.2002	FS1024
**S.wiedemanniana* (Lalem.) Velen.	A5 Amasya: Kral grave vicinity, 400–500 m, 24.07.2002	TD2033
**S. parnassica* Heldr. & Sart. ex Boiss. subsp. *sipylea* P.H. Davis	B1 Manisa: Spil Mountain, 1500m, 17.08.2001	FS1382
	A1 Balıkesir: Marmara Island, 400 m, 11.08.2001	FS1383
S. spinosa L.	C2 Muğla: Fethiye, Babadağ 1750 m, 10.08.2001	FS1381
S. coerulea Janka	A1 Kırklareli: Dereköy 23. km. 600 m, 29.10.2001	TD1625
S. spicigera. (C. Koch) Boiss.	A7 Ordu: Akkuş-Aybastı plateau, 11.08.2001	TD1403
	A7 Trabzon: Beşikdüzü, Yeşilköy, 850 m, 14.10.2005	FS1421
S. boissieri Hausskn. ex Boiss.	B7 Adıyaman: Yazıbaşı village, 2000 m, 30.09.2001	FS1027
S. macrantha C.A.Mayer	A9 Erzurum: Şenkaya-Akşar 5. km, 08.08.2002	FS1040
**S. aintabensis* P.H. Davis	C6 Gaziantep: Dülükbaba, 900 m, 14.07.2001	FS1012
	C7 Urfa: Akabe place, 700 m, 19.07.2001	FS1004
S. pilosa Velen.	B1 Balıkesir: Edremit, Kazdağ-Kapıkule, 1400 m, 15.09.2004	FS1385
S. hortensis L.	A9 Erzurum: Şenkaya-Akşar 5. km, 08.08.2002	FS1042
	C6 Kahramanmaraş: Andırın plateau, 1500 m, 26.07.2001	FS1016

FS – Dr. Fatih Satıl, Balikesir University, TD – Dr. Tuncay Dirmenci, Balikesir University, * – Endemic

black in colour. Their size is 0.8–2 × 0.4–1.1 mm, oblong, obovate-oblong to broadly oblong-ovoid, trigonous and apically truncate, obtuse, acute-obtuse or rounded, with a more or less clear bilobed areole. They are hairless or apically haired.

Nutlets of *S. aintabensis* P.H. Davis, *S boissieri* Hausskn. ex Boiss., *S. hortensis*, *S. macrantha* C.A. Mayer, *S. spicigera* (C. Koch) Boiss. and *S. thymbra* are glabrous. Among the other species examined, two kinds of trichomes can be distinguished: (i) large sessile oil glands found at the nutlet apex (only in *S. cuneifolia*); and (ii) tiny, stalked glandular and eglandular hairs found mainly at the nutlet apex and on the median edge in the remaining species (*S. cilicica*, *S. coerulea, S. icarica, S. parnassiaca* subsp. *sipylea, S. pilosa, S. spinosa, S. thymbra, and S.wiedemanniana)*. Both types show a wide range of surface sculpturing patterns on the nutlet epicarps. The nutlet epicarps have a very characteristic multicellular-protuberance pattern with circular or hexagonal outlines of variable thickness. As a result of our observations with both the stereoscopic microscope and SEM, two main types of nutlet surface ornamentation, viz., more or less smooth and sculptured, can be distinguished in *Satureja* species. Several variants can be recognized within these types.

The more or less smooth nutlets may be divided into three subtypes:

Undulate-reticulate: The nutlet surface has a prominent undulate-reticulate pattern, which is formed by hexagonal cells. Taxa with this type of nutlets are: *S. aintabensis*, *S. amani* P.H.Davis, *S. cilicica* P.H.Davis, *S. cuneifolia S. parnassica* Heldr. & Sart. ex Boiss. subsp. *sipylea* P.H. Davis and *S. wiedemanniana* (Lalem.) Velen., (Figs 2, 4, 5, 9, 13, 21, 32). The surface of *S. boissieri* Hausskn. ex Boiss. is undulate-reticulate and also has an irregular reticulate-granular pattern (Fig. 7).

Reticulate: The surface sculpturing is reticulate with deep spherical-ovale pits and high prominent ridges in *S. icarica* P.H. Davis (Fig. 17). The nutlet surface is composed of polygonal cells**;** densely reticulate in *S. pilosa* Velen. (Fig. 23).

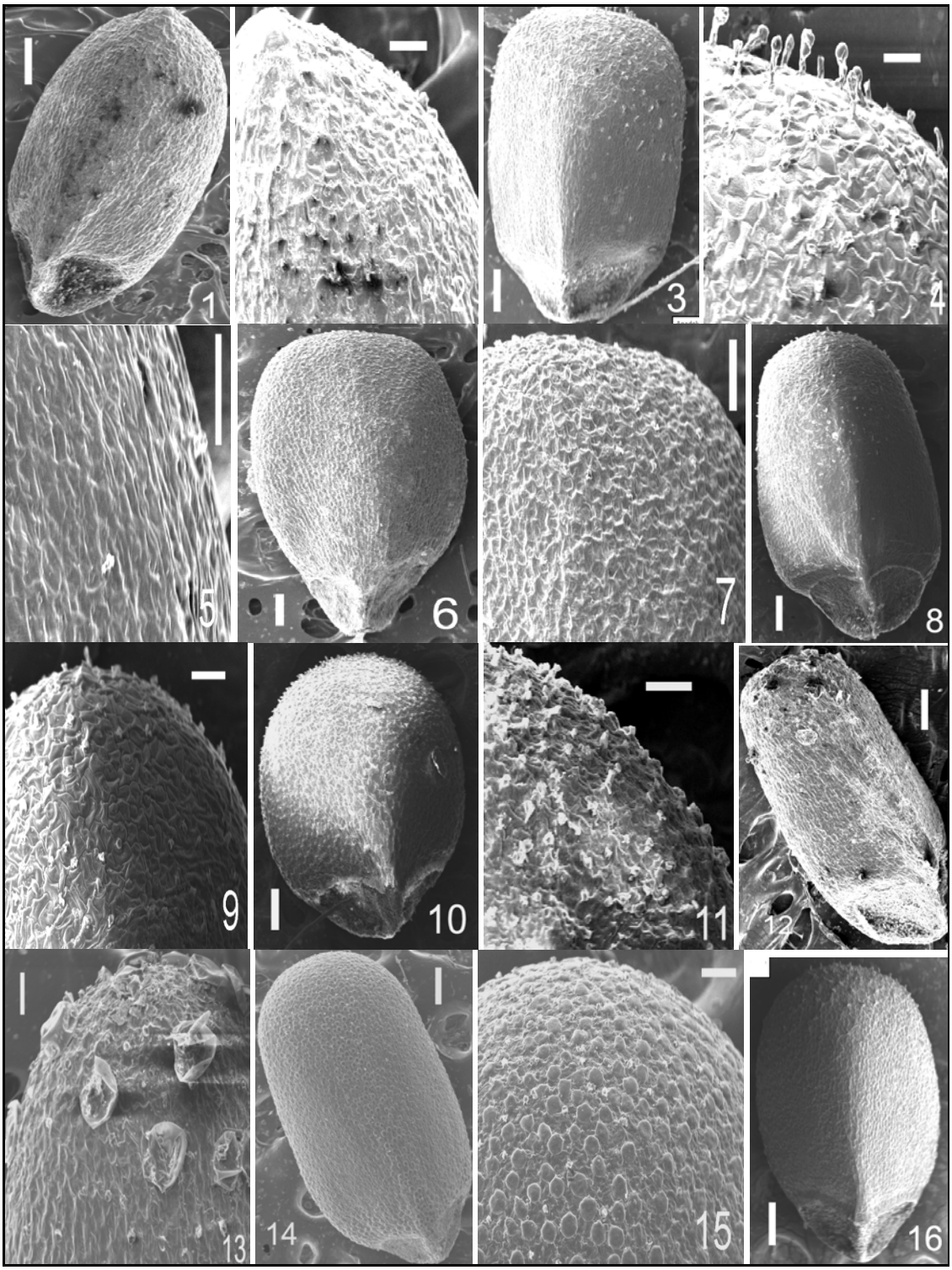
Reticulate-protuberculate: The nutlet surface has a prominent reticulate-protuberculate pattern in *S. coerulea* Janka (Fig. 11). However, the surface sculpturing shows protuberances with a reticulate-undulate cellular pattern in *S. macrantha* (Fig. 19) and *S. spinosa* L. (Fig. 27).

The sculptured nutlets are characterized by papillate to tuberculate surfaces. The surface of *S. hortensis* (Fig. 15) is regularly papillate with rounded cells. The surface sculpturing is reticulate-small tuberculate, with shallow polygonal pits in *S. spicigera* (Fig. 25). The nutlet surface of *S. tymbra* has a granular to papillate pattern (Fig. 29).

A summary of the distribution of the nutlet surface characters (nutlet size, shape, colour, surface pattern) and values cited by Davis (1982) are given in Table 2. The table shows that nutlet characters have a wide range of variation.

Discussion

The variability in nutlet colour, size, shape and areole are of limited taxonomic value, whereas the sculpturing of the nutlet surface pattern shows a wide range of variation, not only among the different genera among Lamiaceae, but also at the infra-sectional and infra-specific

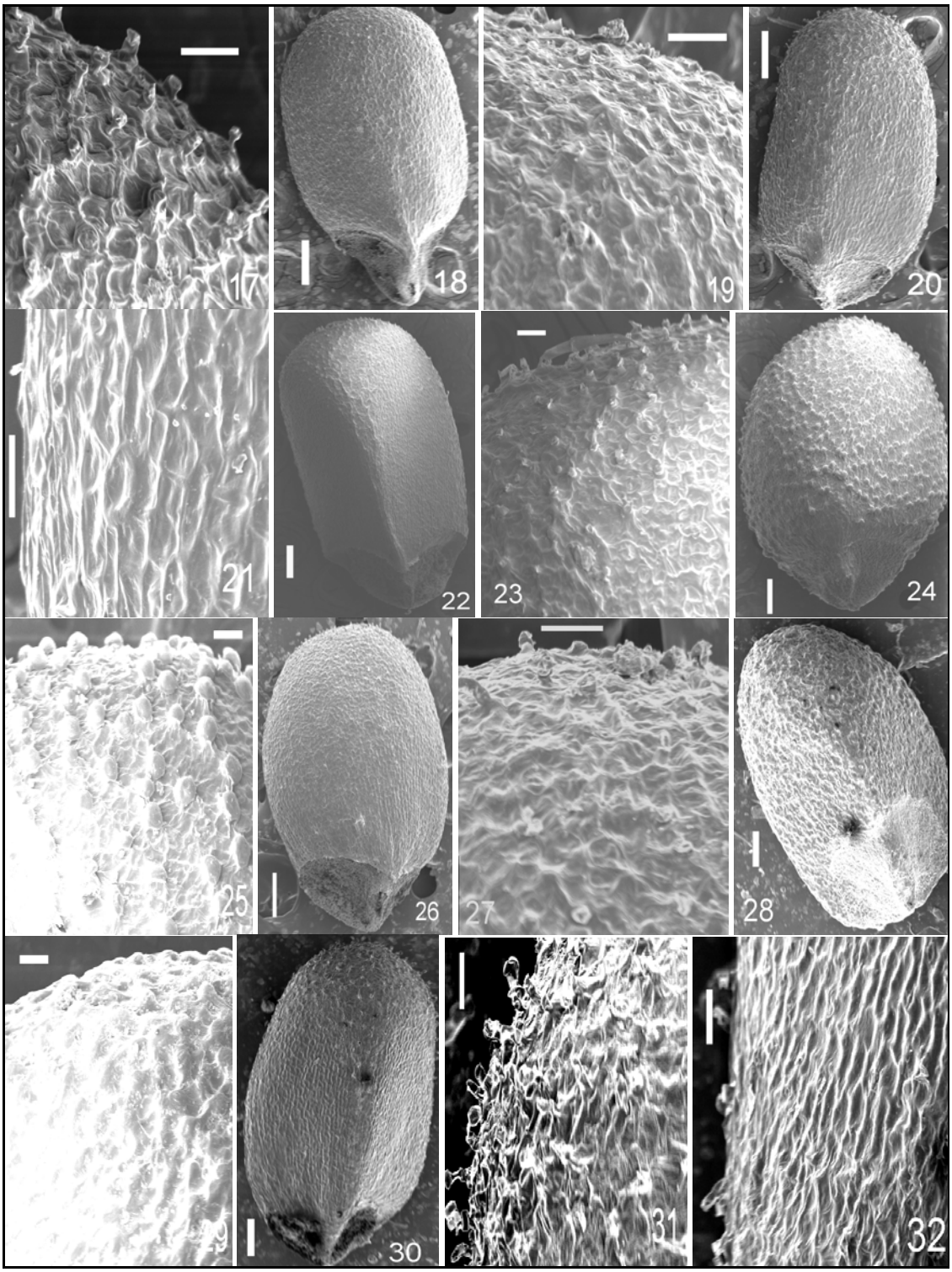


Figs 1–16. *Satureja* nutlets and their coat surfaces in SEM. *S. aintabensis* (1–2), *S. amani* (3–5), 6–7 *S. boissieri,* 8–9 *S. cilicica,* 10–11 *S. coerulea* 12–13 *S. cuneifolia* 14–15 *S. hortensis*, 16 *S. icarica.* Scale bars: 1, 3, 6, 8, 10, 12, 14, 16 = 200 μm; 2, 4, 5, 9, 11, 13, 15 = 60 μm; 7 = 100 μm; 17 = 40 μm.

level (Husain et al. 1990). *Satureja* is a polymorphic genus, and it is greatly in need of a monographic treatment as suggested by Davis (1982).

We tend to believe that an overall study of nutlet microcharacters of *Satureja* species could be of great importance for infrageneric classification and a better understanding of the phylogeny and evolution of this very interesting genus.

The taxonomic significance of nutlet characters in the genus *Satureja* has already been pointed out by Husain et al. (1990). They investigated five species belonging to Eusaturejeae and divided them into two well-defined groups. Group I consists of *S. cuneifolia, S. subspicata* and *S. montana* and nutlets of this group have characteristic protuberances, with discoid or hexagonal outlines of variable thickness, without trichomes and oil



Figs 17–32. *Satureja* nutlets and their coat surfaces in SEM. 17 *S. icarica*, 18–19 *S. macrantha*, 20–21 *S. parnassica* subsp. *sipylea*, 22–23 *S. pilosa*, 24–25 *S. spicigera,* 26–27 *S. spinosa,* 28–29 *S. thymbra*, 30–32 *S. wiedemanniana.* Scale bars: 18, 20, 22, 24, 26, 28, 30 = 200 μm; 29 = 60 μm; 17, 19, 21, 23, 25, 27, 31, 32 = 40 μm.

glands. Group II includes *S. pilosa* and *S. horvatii*, and the nutlets are without protuberances but have papillate 4-celled trichomes and large sessile oil glands. In our investigations, the nutlet surface of *S. cuneifolia* has a prominent undulate-reticulate pattern, which is formed by hexagonal cells and large sessile oil glands in the apex. In *S. pilosa*, the nutlet surface is composed of polygonal cells and is densely reticulate, without large sessile oil glands but with tiny stalked glands and rare trichomes. Our findings generally do not agree with those reported earlier (Husain et al. 1990). Therefore, the taxonomic identification of *S. cuneifolia* and *S. pilosa* may be doubtful.

Two main types of nutlet surface can be distinguished in *Satureja* species based on their exocarp characters, viz., hairless and hairy in the apical part of the

Table 2. A comparision of characters studied for *Satureja* nutlets.

Taxa	Nutlet size/shape	Colour	Nutlet coat surface	Flora of Turkey
S. aintabensis	1.2–1.7 × 0.5– 0.8mm broadly oblong	brown	undulate-reticulate	unknown
S. amani	1.1–1.3 × 0.8–0.9 mm obovate-oblong	dark brown	undulate-reticulate	unknown
S. boissieri	1.2–1.5 × 0.7–1 mm broadly oblong, ovoid	brown	undulate-reticulate	unknown
S. cilicica	1.2–1.4 × 0.7–1 mm broadly oblong	brown, dark brown	undulate-reticulate	unknown
S. coerulea	1–1.2 × 0.8–1.1 mm broadly oblong, ovoid	brown, dark brown	reticulate-protuberculate	1.5 mm
S. cuneifolia	1.2–1.4 × 0.5–0.6 mm oblong	pale brown	undulate-reticulate	1–1.4 mm obovate-oblong obtuse
S. hortensis	1.3–1.5 × 0.9–1mm, ovoid-oblong	black	regular papillate	1–1.2 mm broadly oblong
S. icarica	1–1.2 × 0.6–0.9 mm oblong, obovate	brown	reticulate	unknown
S. macrantha	1.4–1.5 × 0.6–0.8 mm obovate-oblong	brown	reticulate-protuberculate	1.5 × 0.75 mm
S. parnassiaca subsp. *sipylea*	0.8–1.1 × 0.4–0.5 mm broadly oblong	brown	undulate-reticulate	unknown
S. pilosa	1.2–1.5 × 0.8–1 mm oblong	brown	reticulate	unknown
S. spicigera	1.1–1.4 × 0.7–1 mm broadly oblong, ovoid	dark brown	small tuberculate	1.2 mm broadly oblong, ovoid
S. spinosa	0.8–1.2 × 0.5–0.8 mm obovate- oblong	pale brown	reticulate-protuberculate	1 mm obovate-oblong,obtuse
S. thymbra	1.5–2 × 1.1mm broadly oblong	dark brown	granular-papillate	2 mm, oblong, obtuse and glandular hairy
S. wiedemanniana	1–1.3 × 1–0.5–0.7 mm broadly oblong	pale brown	undulate-reticulate	unknown

nutlet. The first type is characterized by the presence of large sessile oil glands which occur only in *S. cuneifolia* collected from two different localities, and make it easily distinguishable from the other species. The second type is typified by tiny stalked glandular hairs which occur in *S. amani, S. cilicica, S. courelea, S. icarica, S. parnassica* subsp. *sipyle, S. pilosa, S. sipinosa* and *S. wiedemanniana.* The remaining species are glabrous.

According to our results, the nutlets of *S. parnassica* subsp. *sipyle* are the smallest (0.8–1.1 × 0.4–0.5 mm), while those of *S. thymbra* are the largest (1.5–2 × 1–1.1 mm). Nutlet sizes of seven species were recorded by Davis (1982). Nutlet lengths reported here are usually similar to those of five species reported by Davis (1982), with the exception of two species. The nutlet length for *S. courelea* in our study is smaller than that reported by Davis (1982), while it is longer for *S. hortensis.*

Nutlets are usually oblong or obovate-oblong in shape, while only three species (*S. boissieri, S. courelea* and *S. spicigera*) are of an oblong-ovoid shape. Although the apex of *Satureja* nutlets is generally obtuse, truncate or rounded, it is acute or acute-obtuse only in *S. aintabensis.*

Of the many types of nutlet epicarp patterns observed, the most common by far is the reticulate pattern according to Husain et al. (1990). Two patterns can be distinguished; viz., the more or less smooth and the sculptured. In some *Satureja* species, the nutlet surface is more or less smooth and has a reticulate pattern or one of its variants. However, the nutlet surfaces of *S. hortensis, S. sipicigera* and *S. thymbra* are sculptured, and they have a pattern quite different from those of the other species analysed. Nutlets of *S. hortensis* are papillate. Papillae are mainly very small with rounded cells. Nutlets of *S. sipicigera* could be described as reticulate, small-tuberculate, and those of *S. thymbra* show a granular to papillate pattern.

S. cuneifolia is morphologically very close to *S. amani* and *S. cilicica* according to Davis (1982). Furthermore, *S. cuneifolia* and *S. wiedemanniana* show great morphological similarity (Davis 1982). Our results clearly indicate that *S. cuneifolia* is different from all the other species because of the large sessile oil glands. *S. aintabensis* is apparently nearest in relation to *S. macrantha* and shows some resemblance to *S. hortensis* according to Davis (1982). While *S. aintabensis* can be separated from *S. macrantha* on the basis of an acute or acute-obtuse apex, it is also easily distinguished from *S. hortensis* by its sculpturing surface pattern. *S. wiedemanniana* is also allied to S. *parnassica* subsp. *sipylea* according to Davis (1982). However, nutlet morphology in S. *parnassica* subsp. *sipylea* and *S. wiedemanniana* displays a great similarity. Furthermore, some *Satureja* species have been investigated with RAPD-PCR (Random Amplified Polymorphic DNA by the Polymerase

Chain Reaction) and *S. parnassica* subsp. *sipylea* and *S. wiedemanniana* were found very similar in molecular structure. This study also supports our findings (Oz Aydın 2004).

In this work, the nutlet features of *S. aintabensis, S. amani, S. boissieri, S. cilicica, S. icarica, S. parnassica* subsp*. sipylea, S. pilosa* and *S. wiedemanniana* are reported in detail for the first time.

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