



Nutlet morphology of six *Clinopodium* taxa, previously treated as *Acinos*, in Turkey

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Abstract

A group of Lamiaceae that has caused confusion over its generic boundaries are those species belonging to the complex surrounding the genera *Satureja*, *Calamintha*, *Micromeria*, *Clinopodium* and *Acinos*. In the current study, nutlet morphology of six *Clinopodium* taxa, previously treated as *Acinos* in Flora of Turkey, is studied for the first time using stereoscopic and scanning electron microscopy (SEM), and detailed descriptions and illustrations of nutlets are described and illustrated here. According to our results, the nutlets are trigonous and oblong or obovoid shaped. The size of nutlets ranges from 1.2-2.2 mm in length and 0.6-1.5 mm in width. They are brown or dark brown in colour, and the surface patterns are papillate, cellular, ridged, reticulate-ridged or reticulate-areolate. Because nutlet surface shows variations from population to population, *Clinopodium alpinum* (syn: *Acinos alpinus*) and *C. graveolens* subsp. *rotundifolium* (syn: *A. rotundifolius*) should be described as species complexes. Our results show that the nutlet surface can be useful as a taxonomic character.

Key words: *Acinos*, *Clinopodium*, Lamiaceae, nutlet, morphology

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Öncesinde *Acinos* olarak bilinen Türkiye'deki altı *Clinopodium* taksonunun nutlet morfolojisi

Özet

Lamiaceae'nin *Satureja*, *Calamintha*, *Micromeria*, *Clinopodium* ve *Acinos* cinslerine ait türlerin bir grubu genel sınırları içinde karışıklığa neden olmaktadır. Bu çalışmada, öncesinde *Acinos* olarak bilinen Türkiye Florası'ndaki altı *Clinopodium* taksonunun nutlet morfolojisi stereo ve taramalı electron mikroskobu (SEM) kullanılarak ilk kez çalışılmış ve nutletlerin ayrıntılı tanımları ve resimleri burada verilmiştir. Sonuçlarımıza göre, nutletler üçgenimsi, oblong ya da obovoid şekillidir. Nutletler 1.2-2.2 mm uzunluğunda ve 0.6-1.5 mm genişliğindedir. Kahverengi ya da koyu kahverengi renginde ve yüzeyleri papilli, hücreli, damarlı, ağsı-damarlı ya da ağsı-adacıklıdır. Nutlet yüzeyleri populasyondan, populasyona farklılık gösterdiği için *Clinopodium alpinum* (syn: *Acinos alpinus*) ve *C. graveolens* subsp. *rotundifolium* (syn: *A. rotundifolius*) kompleks türler olarak tanımlanmıştır. Çalışma sonuçlarımız nutlet yüzeylerinin taksonomik bir karakter olarak faydalı olabileceğini göstermektedir.

Anahtar kelimeler: *Acinos*, *Clinopodium*, Lamiaceae, morfoloji, tohum

1. Introduction

Species of the genus *Acinos* Mill. have been placed in various genera in previous treatments of the genus; they were placed in the genus *Calamintha* Mill. by Boissier (1879), Rechinger (1943) and Webb (1966) or in the genus *Satureja* L. by Briquet (1895–1897). The name *Acinos* has been included in the synonymy of *Clinopodium* by Harley et al. (2004), and recently all species of the genus *Acinos* have been transferred into the genus *Clinopodium* L., and with another insertions, this concept of a broad *Clinopodium* was accepted by the authors (Govaerts, 1999; Bräuchler et al., 2006; Dirmenci et al., 2010a,b).

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Taking the above references into account, the status of taxa is complex and they have many synonyms some taxonomic problems remain. For example; *Clinopodium alpinum* (L.) Kuntze (1891: 515) (syn: *Acinos alpinus* (L.) Moench (1794: 407)) and *C. graveolens* (M.Bieb.) Kuntze subsp. *rotundifolium* (Pers.) Govaerts (1999: 17) (syn: *A. rotundifolius* Persoon (1806: 131)) are morphologically variable between populations of the same species and should therefore be treated as species-complexes (Davis and Leblebici, 1982; Strid and Tan, 1991; Kaya et al., 1999a,b; Kaya, 2002), which were divided at subspecific levels in earlier treatments (e.g. Tutin et al., 1972; Silic, 1979).

The genus *Acinos* are widespread and are represented in Turkey by five species comprising six taxa (Davis and Leblebici, 1982; Davis et al., 1988). After the last taxonomic rearrangement into *Clinopodium*, the accepted names in Turkey are as follows: *Clinopodium acinos* (L.) Kuntze (1891: 513) (syn: *Acinos arvensis* (Lam.) Dandy in Clapham (1946: 326)), *C. alpinum* (syn: *A. alpinus*), *C. graveolens* subsp. *rotundifolium* (syn: *A. rotundifolius*), *C. suaveolens* (Sm.) Kuntze (1891: 516) (syn: *A. suaveolens* (Sm.) G. Don ex Loudon (1830: 239), *C. troodi* (Post) Govaerts subsp. *grandiflorum* (Hartvig & Å.Strid) Govaerts (1999: 19) (syn: *A. troodi* (Post) Leblebici subsp. *grandiflorus* Hartvig & Strid (1987: 331)) and *C. troodi* (Post) Govaerts subsp. *vardaranum* (Leblebici) Govaerts (1999: 19) (syn: *A. troodi* (Post) Leblebici subsp. *vardaranus* Leblebici (1974: 406)). The two subspecies of *C. troodi* are endemic to Turkey.

Morphological and anatomical studies on the fruit and seed structure are played an important role in systematics. Nutlets contain good characters for species recognition (Budantsev and Lobova, 1997) in *Clinopodium*. The importance of scanning electron microscopy (SEM) in the study of nutlet surface and the taxonomic value of nutlet characters has been frequently demonstrated for various genera (Husain et al., 1990; Budantsev and Lobova, 1997; Xifreda and Mallo, 2004; Mosquero et al., 2002, 2005; Kaya and Dirmenci, 2008; Dinç et al., 2013; Kaya et al., 2009; Kaya and Dirmenci, 2012; Satil et al., 2012). In most Lamiaceae the four ovulary lobes each develop into a dry, trigonous, one-seeded mericarp that is obovoid to oblong in shape (Davis and Leblebici, 1982).

In the present work, stereoscopic microscopy and SEM were used to determine the morphology of the nutlet surface, both to improve the present knowledge of the species and to evaluate the usefulness of these features for systematic purposes.

2. Materials and methods

Plant materials were collected from several populations in Turkey (Table 1). Voucher specimens are deposited at the Herbarium of the faculty of Pharmacy of Anadolu University (ESSE). At least 15–20 dry mature nutlets of each of the six taxa were studied morphologically. Measurements and optical observations of nutlet colours were carried out under a stereomicroscope (Wild M5). For SEM, dry mature nutlets were mounted directly on stubs, using single-side adhesive tape and were then coated with gold using ion-sputtering apparatus. SEM photographs were taken with EVO-50. The terminology of nutlet coat surface sculpturing mainly follows Husain et al. (1990) and Stearn (1992).

Table 1. Collection data of taxa studied

Taxa	Collection data	Herbarium No
<i>C. acinos</i>	A1 (E) KIRKLARELİ: Kadıköy	ESSE 10534
<i>C. alpinum</i>	A2 (A) BURSA: Uludağ	ESSE 10511
	B1 MANİSA: Spil Mountain	ESSE 10527
	B1 BALIKESİR: Kaz Mountain	ESSE 10550
<i>C. graveolens</i> subsp. <i>rotundifolium</i>	B3 ESKİŞEHİR: Sivrihisar	ESSE 10515
	B3 ESKİŞEHİR: University campus	ESSE 10541
	B1 KÜTAHYA: Domaniç	ESSE 10520
<i>C. suaveolens</i>	B1 BALIKESİR: Edremit	ESSE 10510
<i>C. troodi</i> subsp. <i>grandiflorum</i>	C2 DENİZLİ: Acıpayam	ESSE 10501
<i>C. troodi</i> subsp. <i>vardaranum</i>	C2 MUĞLA: Köyceğiz	ESSE 10503

3. Results

The shape of the studied *Clinopodium* (*Acinos s.s.*) nutlets is trigonous and oblong or obovoid. Their size ranges from 1.2–2.2 mm long and 0.6–1.5 mm wide. They are brown or dark brown in colour. The apices are obtuse to obtuse-rotundate and the bases are acute. Areoles are bilobed, V-shaped, and white in colour. Minute papillae are sparsely distributed on nutlet surfaces. A summary of nutlet characters (nutlet size, shape, colour, surface pattern, epidermal cell shape) is provided in Table 2.

Clinopodium acinos (Figures 1, 2): Nutlets are 1.3–1.6 × 0.6–0.8 mm, oblong, narrowly obovoid and dark brown. The surface sculpture is slightly papillate. The seed coat consists of irregular rounded or ovoid cells with ridges.

Clinopodium alpinum (Figures 3–8): Nutlets are 1.2–2.0 × 0.6–1.2 mm, oblong to broadly obovoid and brown. The surface sculpture is prominently ridged in the Bursa specimens. The seed coat is composed of shallow, discoid cells with radiating ridges (Figures 3, 4). The surface sculpture is reticulate-areolate in the Manisa samples. In those the seed

coat consists of regular 4–6-polygonal cells with radiating ridges in the centre and prominent reticulum walls (Figures 5, 6). In the Balıkesir specimens, the seed coat is reticulate-ridged and composed of more or less prominent discoid cells with undulating walls, pitted and sunken in cells and interspaces (Figures. 7, 8).

Clinopodium graveolens subsp. *rotundifolium* (Figures 9–14): Nutlets are $1.7\text{--}2.2 \times 0.8\text{--}1.0$ mm, narrowly obovoid to obovoid-oblong and brown or dark brown. In the Sivrihisar specimens, the surface sculpture is reticulate-areolate. The seed coat consists of 5–6-polygonal cells with a spot in the centre and broken, more or less prominent reticulum walls (Figures 9, 10). In samples of the Eskişehir locality, the surface sculpture is irregularly cellular. The seed coat consists of irregular rounded cells with undulating radiate and broken walls (Figures 11, 12). The surface sculpture is reticulate-areolate in the Kütahya locality. The seed coat consists of regular 5–6-polygonal cells striated in the centre and with prominent reticulum walls (Figures 13, 14).

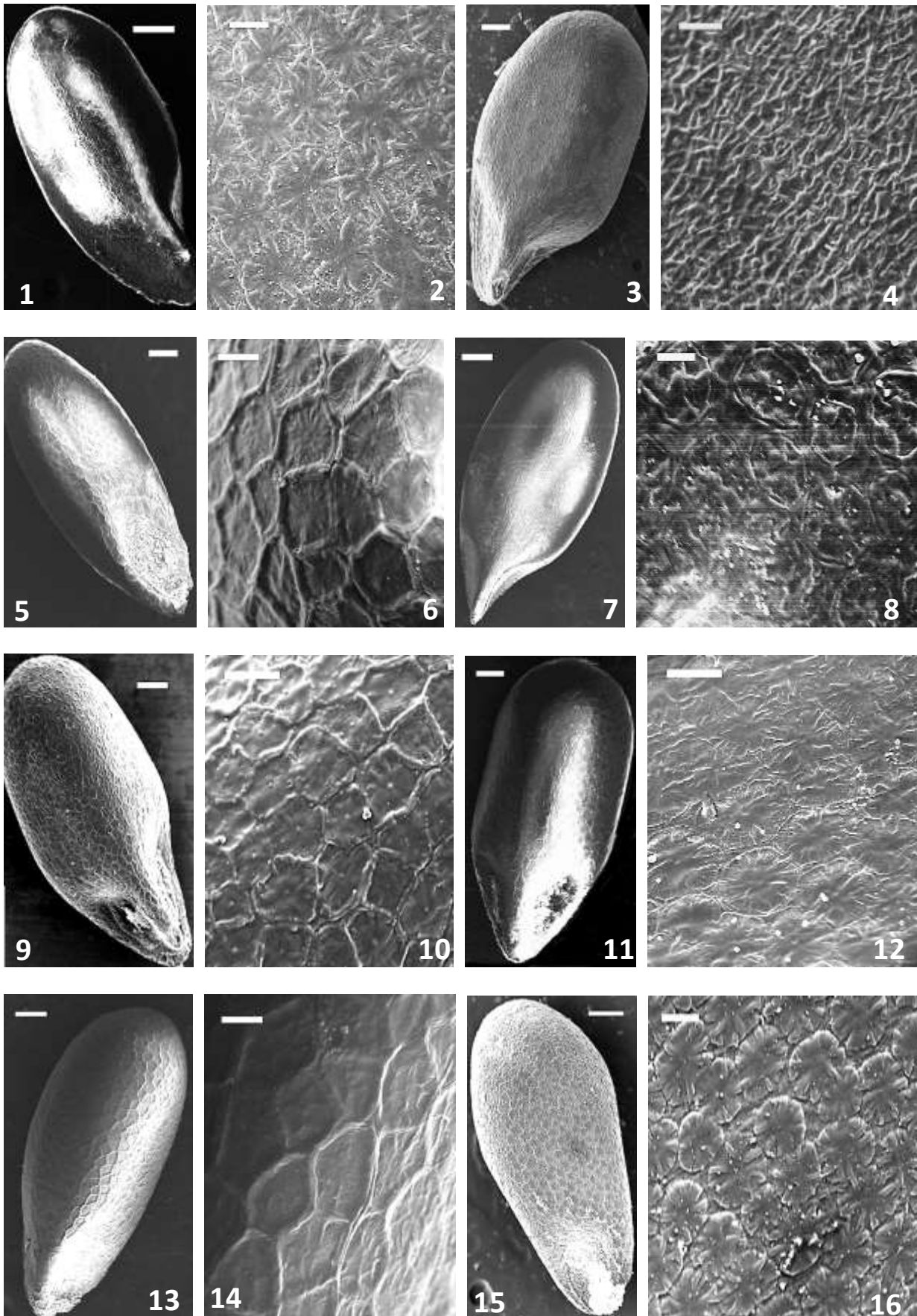
Clinopodium suaveolens (Figures 15, 16): Nutlets are $1.3\text{--}1.8 \times 0.8\text{--}1.0$ mm, obovoid-oblong and dark brown. The surface sculpturing is slightly papillate and the seed coat is composed of more or less rounded cells with ribbed and sunken interspaces.

Clinopodium troodi subsp. *grandiflorum* (Figures 17, 18): Nutlets are $1.8\text{--}2.0 \times 0.9\text{--}1.3$ mm, obovoid-oblong and dark brown. The surface sculpturing is more or less cellular. The seed coat is composed of square or rarely ovoid cells, minutely pitted and straight in the middle of the interspace.

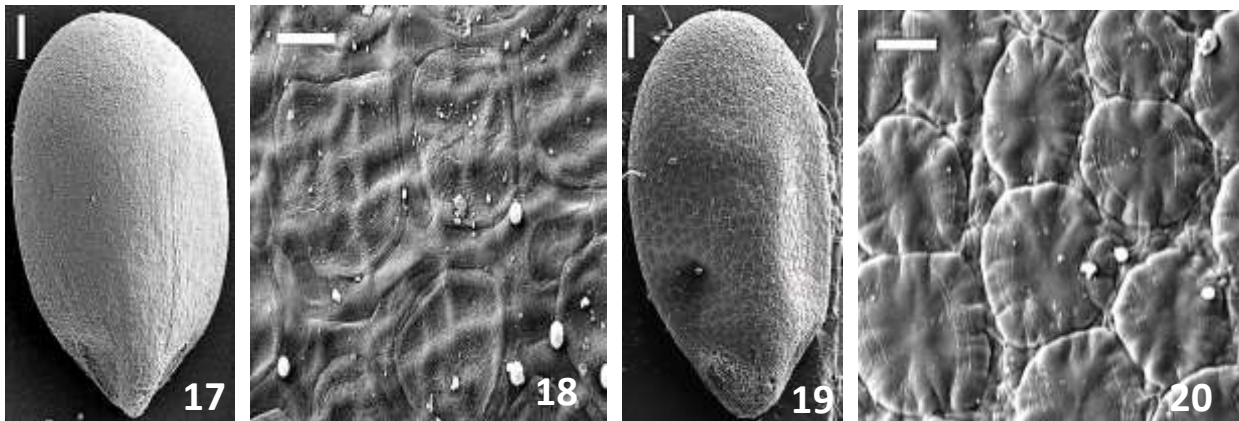
Clinopodium troodi subsp. *vardaranum* (Figures 19, 20): Nutlets are $1.8\text{--}2.2 \times 0.9\text{--}1.5$ mm, obovoid-oblong and brown. The surface sculpturing is papillate. The seed coat is composed of rounded or ovoid cells that are thinly striate and have an undulate margin and are rugose in the middle of interspaces.

Table 2. A comparison of morphological traits in studied nutlets

Taxa	Nutlet size / shape	Colour	Nutlet coat surface	Epidermal cell shape
<i>C. acinos</i>	$1.3\text{--}1.6 \times 0.6\text{--}0.8$ mm oblong, narrowly obovoid	dark brown	slightly papillate	irregular rounded or ovoid cells
<i>C. alpinum</i>				
Bursa	$1.3\text{--}1.8 \times 0.6\text{--}1.0$ mm oblong, obovoid	brown	prominent ridges	shallow discoid cells
Manisa	$1.2\text{--}1.5 \times 0.8\text{--}1.0$ mm broadly obovoid	brown	reticulate-areolate	regular 4–6-polygonal cells
Balıkesir	$1.7\text{--}2.0 \times 0.8\text{--}1.2$ mm oblong, obovoid	brown	reticulate-ridges	more or less prominent discoid cells
<i>C. graveolens</i> subsp. <i>rotundifolium</i>				
Sivrihisar	$1.7\text{--}2.0 \times 0.8\text{--}1.0$ mm narrowly obovoid	brown	reticulate-areolate	5–6-polygonal cells
Eskişehir	$1.7\text{--}2.0 \times 0.8\text{--}1.0$ mm narrowly obovoid	brown	irregular cellular	irregular rounded cells
Kütahya	$1.8\text{--}2.2 \times 0.8\text{--}1.0$ mm obovoid-oblong	dark brown	reticulate-areolate	regular 5–6-polygonal cells
<i>C. suaveolens</i>	$1.3\text{--}1.8 \times 0.8\text{--}1.0$ mm obovoid-oblong	dark brown	slightly papillate	more or less rounded cells
<i>C. troodi</i> subsp. <i>grandiflorum</i>	$1.8\text{--}2.0 \times 0.9\text{--}1.3$ mm obovoid-oblong	dark brown	more or less cellular	square or rarely ovoid cells
<i>C. troodi</i> subsp. <i>vardaranum</i>	$1.8\text{--}2.2 \times 0.9\text{--}1.5$ mm obovoid-oblong	brown	papillate	rounded or ovoid cells



Figures 1–20. Nutlets and their coat surfaces in SEM. 1–2 *C. acinos*, 3–8 *C. alpinum*; 3–4 Bursa locality, 5–6 Manisa locality, 7–8 Balıkesir locality, 9–14 *C. graveolens* subsp. *rotundifolium*; 9–10 Sivrihisar locality, 11–12 Eskişehir locality, 13–14 Kütahya locality, 15–16 *C. suaveolens*,



Figures 17–18 *C. troodi* subsp. *grandiflorum*, 19–20 *C. troodi* subsp. *vardaranum*, Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 =200 μm ; 18, 20 =20 μm ; 2, 4, 6, 8, 16 =40 μm ; 10, 12, 14 =60 μm .

4. Conclusions

Despite their stability as characters, the micromorphological characters of nutlet surfaces have either been totally ignored. In addition, the micromorphological characters of nutlets are largely unknown in the Turkish species, apart from nutlet shape, colour and size. Nutlets of *Clinopodium* taxa treated as *Acinos* in the Flora of Turkey are obovoid-oblong and glabrous (Davis and Leblebici, 1982). There are some studies about nutlet morphologies of some *Clinopodium s.l.* taxa (Husain et al., 1990), but nothing else has been known about the *Clinopodium s.l.* taxa growing in Turkey. Here, the nutlet features of six *Clinopodium s.l.* taxa are described for the first time in detail.

According to our results, nutlet dimensions vary among the examined taxa, the smallest and the narrowest nutlets measure 1.2–1.6 \times 0.6–1.0 mm in *C. acinos* and *C. alpinum* (Manisa locality), and the longest and the largest nutlets, 1.8–2.2 \times 0.9–1.5 mm, in *C. troodi* subsp. *vardaranum* (Table 2). The nutlets are usually obovoid-oblong or oblong in shape, however, narrowly or broadly obovoid nutlets are also found. The variability in nutlet size, shape and colour are of limited taxonomic value. As a result of the observations carried out using SEM, some micromorphological nutlet types can be distinguished: papillate or slightly papillate in *C. acinos*, *C. suaveolens* and *C. troodi* subsp. *vardaranum*, prominent ridges, reticulate-areolate and reticulate-ridges in *C. alpinum*, reticulate-areolate and irregular-cellular in *C. graveolens* subsp. *rotundifolium* and more or less cellular in *C. troodi* subsp. *grandiflorum* (Figures 1–20). Nutlet surfaces are a significant character to separate the taxa. The nutlets of the some taxa are easily distinguished from the others with regard to epidermal cell shape. Various epidermal cell shapes are observed in different localities of *C. alpinum* and *C. graveolens* subsp. *rotundifolium* while the epidermal cells shapes in *C. suaveolens* and *C. acinos* are almost similar to each other. The round or ovoid cell shapes with undulate margin are observed in epidermal cells of *C. troodi* subsp. *vardaranum* while the square or rarely ovoid cell shapes are observed in epidermal cells of *C. troodi* subsp. *grandiflorum* (Figures 1–20). The epidermal cell shapes can also be of considerable diagnostic and systematic value.

According to Husain et al. (1990), the variability in nutlet colour, size and shape are of limited taxonomic value, whereas the sculpturing of the nutlet surface, as seen by SEM, show a wide range of variation, not only between the different genera of Lamiaceae, but also at the infrasectional and infraspecific levels. In Lamiaceae tribe Saturejeae, the nutlet morphology has already been studied Husain et al. (1990), in *Acinos*, *Calamintha*, *Clinopodium*, *Hyssopus* L., *Melissa* L., *Micromeria* Benth., *Origanum* L. and *Satureja*. The study of Husain shows that nutlet morphology has taxonomic value at different levels of the taxonomic hierarchy in the tribe. Husain et al. (1990) reported that the nutlets of *Clinopodium alpinum* subsp. *hungaricum* (Simonk.) Govaerts (1999: 16, syn.: *Acinos hungaricus* (Simonk.) Šilic) were irregular, shallow and had patterned ridges. A pattern with prominent ridges was also observed in our specimens of *Clinopodium alpinus* from the Bursa locality. Our findings are generally in agreement with those reported earlier (Husain et al., 1990).

Mosquero et al. (2005) investigated nutlets of *Clinopodium alpinum* subsp. *alpinum* (syn: *A. alpinus* subsp. *meridionalis*) and *Micromeria graeca* (L.) Benth. ex Reichenb. subsp. *graeca* from Spain. In their studies, nutlet surface of *Acinos alpinus* subsp. *meridionalis* is recognized as polygonal cellular with parallel radials and undulated in margins. Furthermore, they observed abundant minute papillae on the nutlet surface as also observed in our taxa examined. The reticulate and areolate surface types are determined in some species of the genus *Glechon* Spreng. (Xifreda and Mallo, 2004). The results obtained by Mosquero et al. and Xifreda and Mallo are generally similar to ours.

Nutlet surface morphology of Turkish *Satureja s.s.* has been investigated previously by Kaya et al. (2009). The genus *Satureja s.s.* is similar to the genus *Clinopodium s.s.* and, it can be separated on the basis shape calyx morphologically. Kaya et al. determined two types in *Satureja* nutlet surfaces: the more or less smooth and the sculptured, and four subtypes, undulate-reticulate, reticulate, reticulate-protuberculate and papillate-tuberculate. The reticulate and papillate types of nutlet surfaces match our samples. The nutlets of Turkish *Ziziphora* taxa have been

examined by Kaya and Dirmenci (2012) and they have been reported slightly papillate surface pattern and similar minute papillae on nutlet surface. Our results are in accordance with their study.

In an earlier study (Kaya, 2002), some morphological variations of *C. alpinum* and *C. graveolens* subsp. *rotundifolium* had been reported and ultimately, these taxa were described as complex and variable species. The compositions of the essential oils of *C. alpinum* (Kaya et al., 1999a) and *C. graveolens* subsp. *rotundifolium* (Kaya et al. 1999b) were of a complex nature. In this study, we investigated the nutlets of *C. alpinum* and *C. graveolens* subsp. *rotundifolium* from different localities. We observed different results as prominent ridges in the Bursa samples, reticulate-areolate in the Manisa samples and reticulate-ridges in the Balıkesir samples of *C. alpinum*, reticulate-areolate in the Sivrihisar and Kütahya samples and irregular-cellular in the Eskişehir samples of *C. graveolens* subsp. *rotundifolium*. In the light of the data obtained from the present study, these taxa can be viewed as counterparts of a complex taxon with variations from population to population.

Many environmental factors change due to changes in elevation. For instance, daily temperature differences, cloudiness, humidity and effects of wind, evaporation and mean temperature decrease with elevation increase. All these changes play an important role on the morphological and anatomical characteristics of the plant (Gönüz and Özörgücü, 1999). In western Anatolia *Clinopodium alpinum* is grown in various habitat types at elevations between 900 and 2300 m. *Clinopodium graveolens* subsp. *rotundifolium* is the most widespread taxon in Turkey (rarer in the East) and grows in various habitat types at elevations between sea level and 2200 m, and it is the most tolerant ecologically..

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