

NUTLET MORPHOLOGY AND ITS TAXONOMIC SIGNIFICANCE IN THE GENUS *MENTHA* L. (LAMIACEAE) FROM TURKEY

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Abstract

The nutlet morphology of 11 taxa of *Mentha* L. (*M. pulegium*, *M. aquatica*, *M. × piperita*, *M. × dumetorum*, *M. spicata* subsp. *spicata*, *M. spicata* subsp. *tomentosa*, *M. × villosa-nervata*, *M. longifolia* subsp. *longifolia*, *M. longifolia* subsp. *typhoides*, *M. × rotundifolia* and *M. suaveolens*) distributed throughout Turkey was investigated by scanning electron microscopy (SEM). The shape of all studied nutlets was broadly oblong or ovoid. Nutlet size ranged from 0.54 to 0.97 mm in length and from 0.37 to 0.66 mm in width. The smallest and biggest nutlets were found in *M. × villosa-nervata* and *M. aquatica*, respectively. The *Mentha* taxa studied can be divided into three groups, based on nut sculpturing type such as distinctly boreticulate, inconspicuously boreticulate and reticulate. This study has shown that some nutlet morphological characteristics can be utilised as additional diagnostic characteristics in delimitations of *Mentha* at the species and infraspecific levels.

Introduction

Mentha L., one of the most important genera of the family Lamiaceae, has worldwide distribution and it consists of perennial aromatic herbs. Some *Mentha* species, such as *M. pulegium* L., *M. longifolia* (L.) Huds., *M. spicata* L., *M. × piperita* L. and *M. × villosa-nervata* Opiz, are traditionally used in folk medicine (Baytop, 1999). Mint oil and their constituents obtained from different species of *Mentha* are also used in perfumery, cosmetics and food industries (Kokkini, 1994).

Mentha is a taxonomically difficult genus because of extensive hybridization, vegetative propagation, polyploidisation and cultivation (Harley, 1972; Harley and Brighton, 1977; Tucker *et al.*, 1980). The genus comprises 18 species and 11 hybrids placed into four sections, namely *Pulegium*, *Tubulosae*, *Eriodontes* and *Mentha* according to the latest taxonomic treatment (Tucker and Naczi, 2007). Harley (1982) recognized 11 *Mentha* taxa belonging to two sections (*Pulegium* and *Mentha*) from Turkey and then two hybrids have been added to Flora of Turkey (Tarimcilar and Kaynak, 1997a, b). In this study, the treatment of Harley (1982) has been followed for the nomenclature of *Mentha*.

There are some studies about monophyly of *Mentha* and phylogenetic relationships within the genus (Gobert *et al.*, 2002; Bunsawat *et al.*, 2004; Shasany *et al.*, 2005). Saric-Kundelic *et al.* (2009) investigated the utility of morphological, anatomical and phytochemical characters for the identification of *Mentha* species, hybrids, varieties and cultivars in Bosnia-Herzegovina and Slovakia. In various genera of family Lamiaceae, the nutlet morphology, anatomy, pericarp structure and their taxonomic significance have been reported by some studies (Husain *et al.*, 1990; Marin *et al.*, 1994; Ryding, 2010). However, accounts on the mericarp morphology of some

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taxa of *Mentha* examined in this study are rather limited (Duletic-Lausevic and Marin, 1999; Moon *et al.*, 2009). We aim in this study, with the aid of scanning electron microscope (SEM), to provide detailed data on nutlet morphology of 11 *Mentha* taxa found in Turkey and to determine which characteristics of their nutlets may be used for taxonomic purposes.

Materials and Methods

Plant materials:

Nutlets of 11 taxa of *Mentha* collected from different parts of Turkey were investigated. The materials used in this study were composed mainly of herbarium specimens, which were deposited in the herbarium of Uludag University (BULU). The specimens used for SEM micrographs were presented in Table 1.

Nutlet size and SEM analyses:

For nutlet length and width, 50 nutlets were measured per taxon. However, at least 10 nutlets were measured for hybrids. In order to ensure that the nutlets were of normal size and maturity, they were examined using a stereomicroscope. For SEM, nutlets of taxa were transferred directly to a double-sided tape-affixed stub and were coated with gold-palladium, using a BAL-TEC SCD 005 sputter. The micrographs were obtained from a CARL ZEISS Evo 40 SEM using a voltage of 20 kV at the Microscopy Laboratory of Science and Art Faculty of Uludag University. The micrographs were used to describe surface sculpturing type of nutlets. The terminology for nutlet shape and surface sculpturing mainly follows that of Barthlott (1981) and Stearn (1983).

Table 1. List of taxa used for SEM micrograph (GT- Gül Tarmcılar).

No.	Taxon	Collection data	Vouchers
1	<i>M. aquatica</i> L.	A2 Bursa: Fadilli village, 9 m, 3.9.2004	GT 30514
2	<i>M. × dumetorum</i> Schult.	A1Kirklareli: Babaeski, 60 m, 23.8.2003	GT30448
3	<i>M. pulegium</i> L.	A2 Istanbul: Cavusbasi, 16.8.2005	GT 30533
4	<i>M. longifolia</i> (L.) Huds. subsp. <i>longifolia</i>	A2 Bursa: Gemlik, Hayriye village, 10 m, 8.9.2006	GT 30592
5	<i>M. longifolia</i> (L.) Huds. subsp. <i>typhoides</i> (Briq.) Harley	A2 Istanbul: Sile, 15.8.2005	GT30530
6	<i>M. × piperita</i> L.	A2 Istanbul: Cavusbasi, Kavaklık, 16.8.2005	GT 30535
7	<i>M. × rotundifolia</i> (L.) Huds.	B1 Balikesir: Bandırma to Erdek, 130 m, 27.8.2004	GT 30508
8	<i>M. spicata</i> L. subsp. <i>spicata</i>	A1 Tekirdag: 1 km to Hayrabolu, 70 m, 23.8.2003	GT 30452
9	<i>M. spicata</i> L. subsp. <i>tomentosa</i> (Briq.) Harley	A2 Bilecik: Pazaryeri, Bahcesultan, 1050 m, 6.9.2006	GT 30562
10	<i>M. suaveolens</i> Ehrth.	A2 Yalova: Sultaniye, 25 m, 7.6.2006	GT 30570
11	<i>M. × villosa-nervata</i> Opiz.	B1Canakkale: Saros, Kocacesme village, 35 m, 25.8.2004	GT 30470

Results and Discussion

The characteristics of nutlet (i.e. size, colour, presence or absence of trichomes and surface sculpturing) are summarized in Table 2. Micrographs of nutlets belonging to all studied taxa are presented in Figures 1-4. We found that the shape of all studied nutlets was broadly oblong or ovoid and that nutlet colour varied from pale to dark brown. The nutlets of *M. pulegium*, *M. aquatica* and *M. dumetorum* were pale brown, while those of *M. × piperita*, *M. × villosa-nervata*

and *M. × rotundifolia* were dark brown. However, the colour of the nutlets in *M. spicata* subsp. *spicata*, *M. spicata* subsp. *tomentosa*, *M. longifolia* subsp. *longifolia*, *M. longifolia* subsp. *typhoides* and *M. suaveolens* varied from chestnut brown to dark brown. Moreover, short or long trichomes were observed on the surface of nutlets of *M. aquatica*, *M. × dumetorum*, *M. spicata* subsp. *tomentosa* and *M. longifolia* subsp. *longifolia*. Nutlet size ranged from 0.54 to 0.97 mm in length and from 0.37 to 0.66 mm in width. The smallest nutlet was found in *M. × villosa-nervata* and the biggest nutlet was found in *M. aquatica* (Table 2).

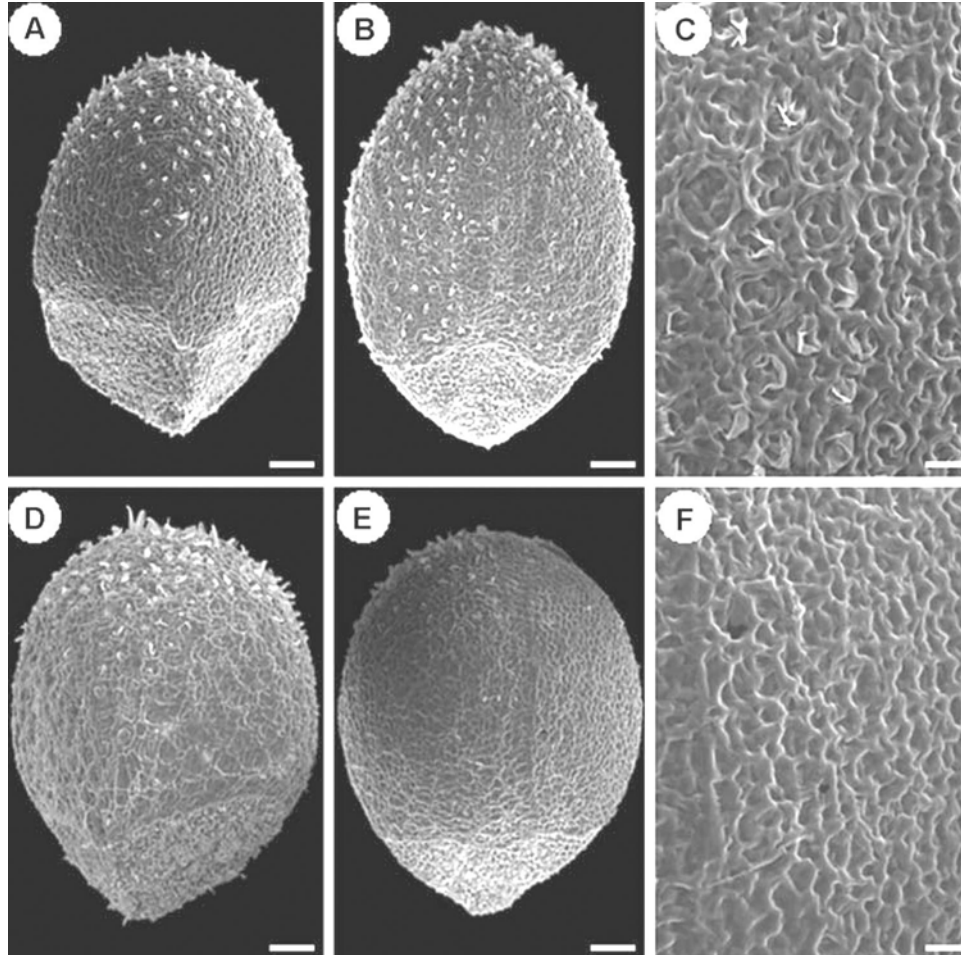


Fig. 1. SEM micrographs of nutlets of *Mentha aquatica* (A-C); *M. × dumetorum* (D-F); Ventral view (A, D); dorsal view (B, E); surface sculpturing (C, F). Scale bars: A, B, D, E = 100 μ m; C, F = 20 μ m.

Under SEM, three types were observed in the *Mentha* taxa based on surface sculpturing pattern:

Type I. Distinctly boreticulate: a surface with penta- or hexagonal-shaped small cells, and the walls of these cells are high, irregular and having depressions. This sculpturing pattern was seen in *M. aquatica* and *M. × dumetorum* (Fig. 1C, F).

Table 2. Nutlet characteristics of the studied taxa of *Mentha* L.

Taxon	Length (mm)	Width (mm)	Sculpture	Presence/ absence of trichomes	Colour	Figures
	Mean± SD	Mean± SD				
<i>M. aquatica</i>	0.9± 0.07	0.6± 0.05	TYPE I	short hair	pale brown	Fig. 1A-C
<i>M. × dumetorum</i>	0.8± 0.15	0.6± 0.06	TYPE I	short hair	pale brown	Fig. 1D-F
<i>M. pulegium</i>	0.7± 0.01	0.5± 0.04	TYPE II	absent	pale brown	Fig. 2A-C
<i>M. × piperita</i>	0.7± 0.04	0.5± 0.04	TYPE II	absent	dark brown	Fig. 2D-F
<i>M. spicata</i> subsp. <i>spicata</i>	0.8± 0.02	0.6± 0.01	TYPE II	absent	chestnut to dark brown	Fig. 2G-I
<i>M. spicata</i> subsp. <i>tomentosa</i>	0.7± 0.01	0.5± 0.01	TYPE III	scarcely hair	chestnut to dark brown	Fig. 3A-C
<i>M. longifolia</i> subsp. <i>longifolia</i>	0.6± 0.06	0.5± 0.07	TYPE III	long hair	chestnut to dark brown	Fig. 3D-F
<i>M. longifolia</i> subsp. <i>typhoides</i>	0.7± 0.01	0.5± 0.03	TYPE III	absent	chestnut to dark brown	Fig. 3G-I
<i>M. × villosa-nervata</i>	0.6± 0.02	0.4± 0.03	TYPE III	absent	dark brown	Fig. 4A-C
<i>M. suaveolens</i>	0.6± 0.02	0.5± 0.02	TYPE III	absent	chestnut to dark brown	Fig. 4D-F
<i>M. × rotundifolia</i>	0.6± 0.02	0.4± 0.01	TYPE III	absent	dark brown	Fig. 4G-I

Type II. Inconspicuously biretulate: a surface covers inconspicuously penta- or hexagonal-shaped small cells, and these cells having various walls. *M. pulegium*, *M. × piperita* and *M. spicata* subsp. *spicata* exhibited this type of sculpturing. Only in *M. pulegium*, the nutlets with cells having rigid cell boundary and having star-shaped extensions at their centres (Fig. 2C). The nutlets of *M. × piperita* and *M. spicata* subsp. *spicata* with cells having wrinkled or often unclear walls (Fig. 2F, I).

Type III. Reticulate: a surface with penta- or hexagonal-shaped cells having large lumen and smooth, regular walls and forming a net-like appearance on their surface. The nutlets of *M. spicata* subsp. *tomentosa*, *M. longifolia* subsp. *longifolia*, *M. longifolia* subsp. *typhoides*, *M. × villosa-nervata*, *M. suaveolens* and *M. × rotundifolia* exhibited this type (Figs 3C, F, I; 4C, F, I).

When the nutlet characteristics of the investigated *Mentha* taxa were compared with previous literature (Ball, 1972; Borisova, 1977; Tarimcilar and Kaynak, 2002), our results are more or less similar to their findings. The shape of nutlets examined in this study was broadly oblong or ovoid. Borisova (1977), Harley (1982) and Tarimcilar and Kaynak (2002) have reported that the nutlet shape of the genus *Mentha* varies from globose to ovoid or obovoid.

Duletic-Lausevic and Marin (1999) found nutlet dimensions 0.7×0.5 mm in *M. pulegium* and *M. longifolia*, 0.8×0.6 mm in *M. aquatica*, 0.6×0.4 mm in *M. spicata* and *M. × rotundifolia*, and 0.6×0.5 mm in *M. suaveolens*. Moon *et al.* (2009) examined nutlet characteristics (i.e. size, colour, shape and surface sculpturing) of *Mentha aquatica*, *M. longifolia*, and *M. suaveolens* and reported the length and width measurements (mm) as $1 \pm 0.05 \times 0.7 \pm 0.02$, $0.6 \pm 0.03 \times 0.5 \pm 0.02$ and $0.6 \pm 0.03 \times 0.4 \pm 0.02$, respectively. Nutlet shape of these taxa is widely elliptic, surface sculpturing type is reticulate, and colour varies from yellowish brown to reddish dark brown (Moon *et al.*, 2009).

According to our results, the nutlets of *M. aquatica*, *M. × dumetorum*, *M. spicata* subsp. *tomentosa* and *M. longifolia* subsp. *longifolia* have trichomes. The presence or absence of trichomes on nutlet is an important character to discriminate *M. longifolia* subsp. *longifolia* and subsp. *typhoides* which have the similar nutlet size, sculpturing and colour (Table 2). On the other

hand, Duletic-Lausevic and Marin (1999) stated that the nutlets of *M. spicata*, *M. rotundifolia* and *M. suaveolens* lack trichomes and that *M. aquatica* and *M. longifolia* exhibit nutlets with or without trichomes.

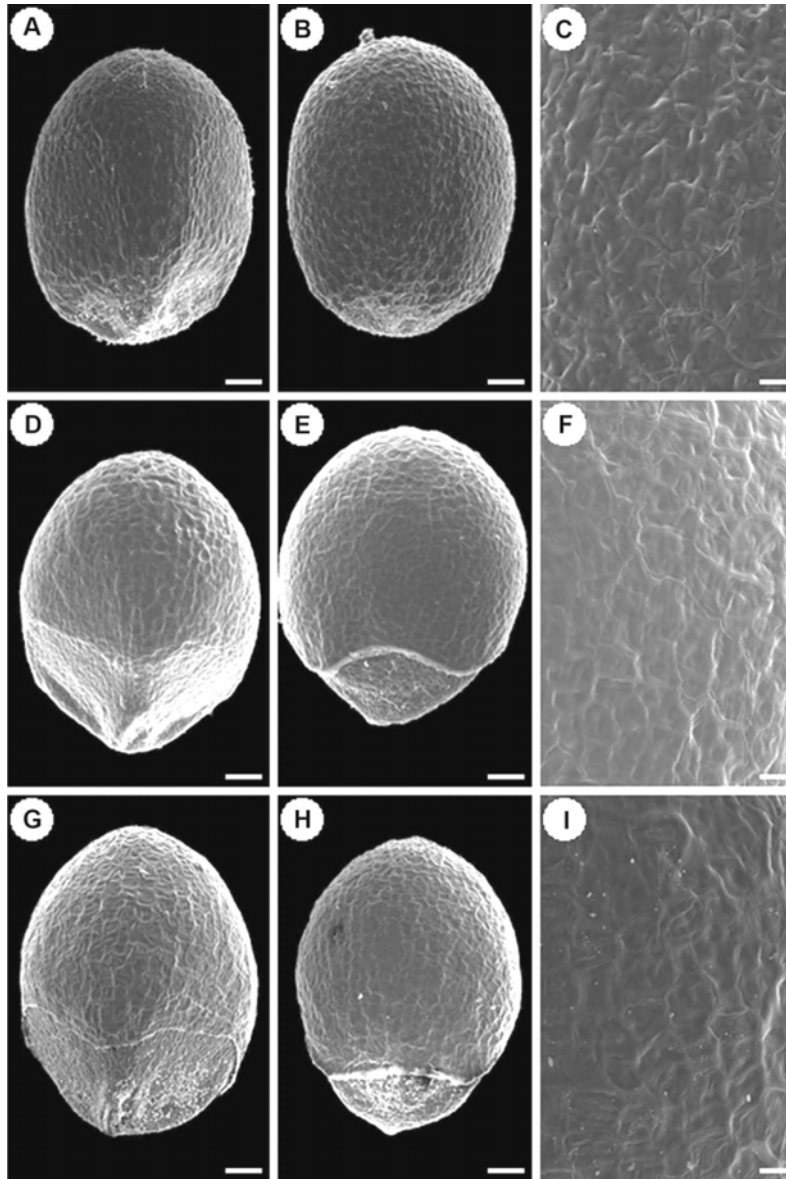


Fig. 2. SEM micrographs of nutlets of *M. pulegium* (A-C); *M. × piperita* (D-F); *M. spicata* subsp. *spicata* (G-I). Ventral view (A, D, G); dorsal view (B, E, H); surface sculpturing (C, F, I). Scale bars: A, B, D, E, G, H = 100 μ m; C, F, I = 20 μ m.

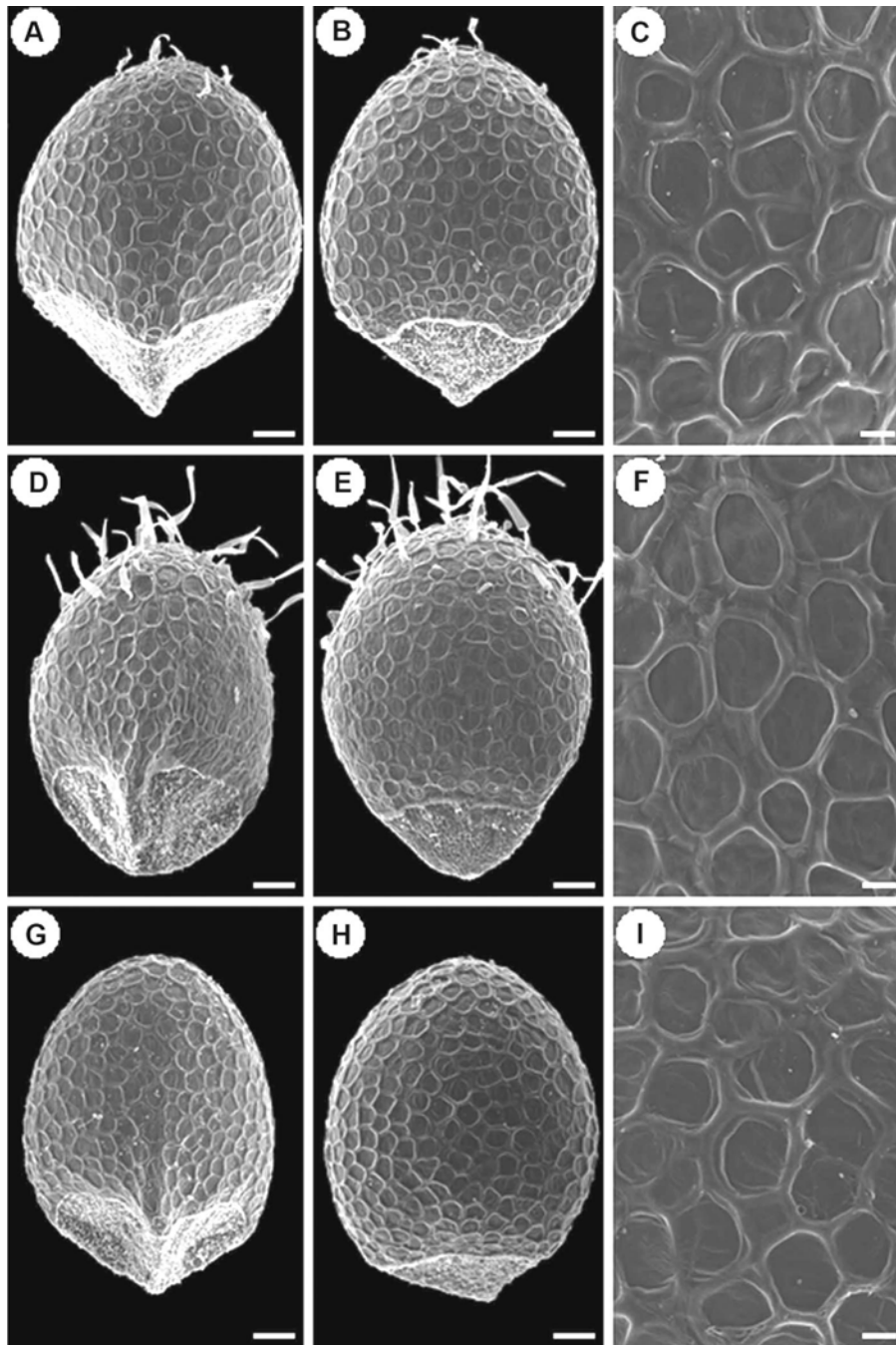


Fig. 3. SEM micrographs of nutlets of *M. spicata* subsp. *tomentosa* (A-C); *M. longifolia* subsp. *longifolia* (D-F); *M. longifolia* subsp. *typhoides* (G-I). Ventral view (A, D, G); dorsal view (B, E, H); surface sculpturing (C, F, I). Scale bars: A, B, D, E, G, H = 100 μ m; C, F, I = 20 μ m.

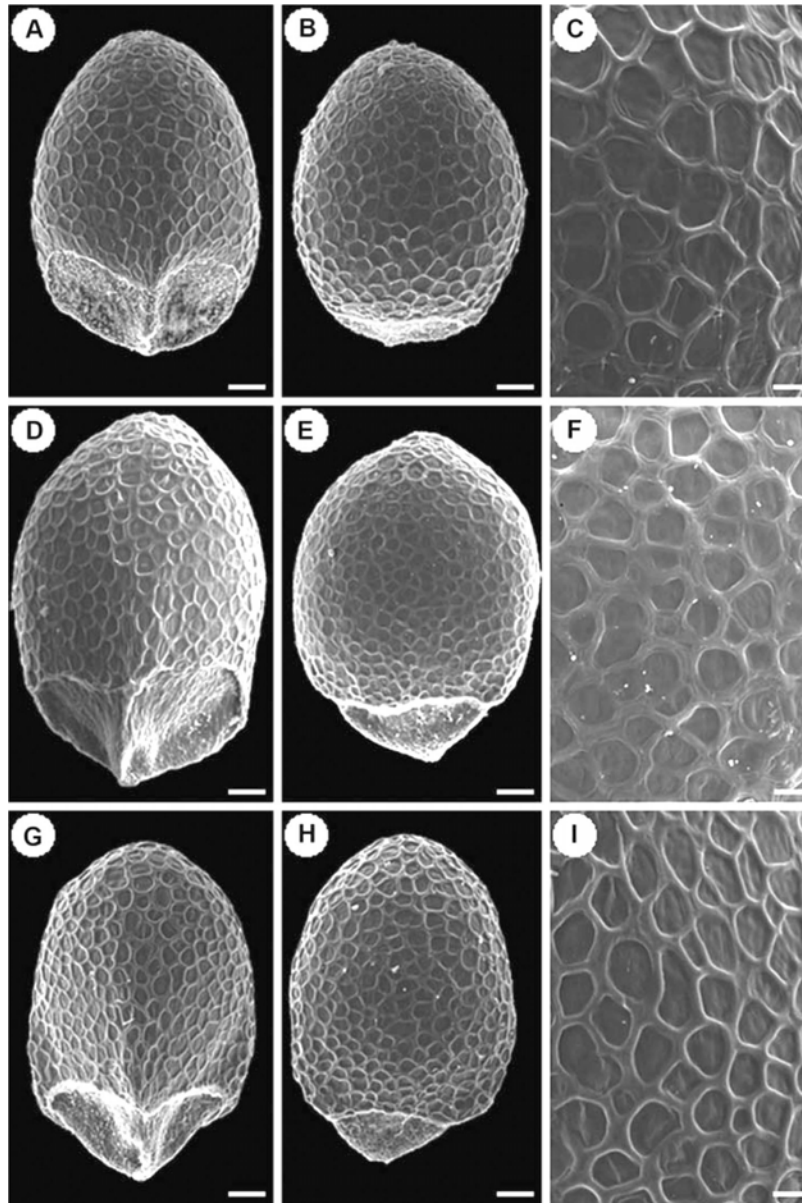


Fig. 4. SEM micrographs of nutlets of *M. × villosa-nervata* (A-C); *M. suaveolens* (D-F); *M. × rotundifolia* (G-I). Ventral view (A, D, G); dorsal view (B, E, H); surface sculpturing (C, F, I). Scale bars: A, B, D, E, G, H = 100 μ m; C, F, I = 20 μ m.

Mentha taxa employed in this study can be divided into three informal groups, with regard to nutlet characteristics basically sculpturing patterns. Group I includes *M. aquatica*, *M. × dumetorum* (*M. aquatica* \times *M. longifolia*) and they are similar to each other both in terms of the morphological features and the nutlet characteristics. However, *M. × dumetorum* differs from *M. aquatica* in its more oblong spikes and narrower leaves (Tarimcilar and Kaynak, 1997a, 2002).

Group II consists of *M. pulegium*, *M. spicata* subsp. *spicata* and *M. × piperita* (*M. aquatica* × *M. spicata*). Of the studied *Mentha* taxa, only *M. pulegium* is located in sect. *Pulegium*, whereas the others are included in sect. *Mentha*. Sect. *Pulegium* is distinguished from sect. *Mentha* by its bracts similar to leaves, tubular calyx, weakly 2-lipped, with distinctly unequal calyx teeth, hairy within calyx throat, gibbous corolla tube. Sect. *Mentha* have variable bracts, calyx tubular or campanulate, with more or less equal calyx teeth, glabrous calyx throat and straight corolla tube (Harley, 1982). Moreover, the inflorescence of *M. × piperita* is morphologically similar to *M. spicata* in that it forms a terminal spike, but it differs from *M. aquatica* in its more lanceolate leaves that have shorter petioles (3-9 mm or rarely more).

Group III includes *M. spicata* subsp. *tomentosa*, *M. longifolia* subsp. *longifolia*, *M. longifolia* subsp. *typhoides*, *M. × villosa-nervata*, *M. suaveolens* and *M. × rotundifolia*. The nutlet surfaces of this group are covered with penta- or hexagonal-shaped cells that form a particularly net-like appearance. *M. × villosa-nervata* (*M. spicata* × *M. longifolia*) is morphologically different from the parents in its narrower spikes and smaller leaves and calyx (Tarimcilar and Kaynak, 1997b, 2002). *M. × rotundifolia* (*M. suaveolens* × *M. longifolia*) resembles *M. suaveolens* in its pale green and strongly rugose leaves, but it differs in that its leaves are more oblong and have an acute apex (Harley, 1982; Tarimcilar and Kaynak, 2002).

Hybrids can be distinguished from their parental species in terms of some nutlet features. As seen in Table 2, *M. × dumetorum* mainly differs from *M. aquatica* and *M. longifolia* with its smaller and distinctly biretulate sculpturing nutlet. The nutlets of *M. × piperita* are smaller than those of *M. spicata* subsp. *spicata*, but they are more similar to *M. spicata* than *M. aquatica* in terms of nutlet characteristics. They are easily distinguishable from *M. aquatica* due to its inconspicuously biretulate, glabrous and dark brown nutlet. *M. × villosa-nervata* differs from *M. longifolia* subsp. *longifolia* and *M. spicata* subsp. *spicata* by its glabrous, dark brown and reticulate nutlet, respectively. The nutlet characteristics of *M. × rotundifolia* and *M. suaveolens* display a great similarity with each other.

A key can be established based on nutlet characteristics for Turkish *Mentha* taxa:

1	Nutlet sculpturing biretulate	2
-	Nutlet sculpturing reticulate	6
2	Nutlet sculpturing distinctly biretulate	3
-	Nutlet sculpturing inconspicuously biretulate	4
3	Nutlets at least 0.83 mm long	<i>M. aquatica</i>
-	Nutlets at least 0.65 mm long	<i>M. × dumetorum</i>
4	Nutlets 0.78-0.82 mm long	<i>M. spicata</i> subsp. <i>spicata</i>
-	Nutlets shorter than 0.78 mm	5
5	Nutlet cells with star-shaped extensions at their centres	<i>M. pulegium</i>
-	Nutlet cells without star-shaped extensions at their centres	<i>M. × piperita</i>
6	Nutlets without hair	7
-	Nutlets with hair	10
7	Nutlets 0.47-0.53 mm wide	8
-	Nutlets 0.37-0.43 mm wide	9
8	Nutlets 0.69-0.71 mm long	<i>M. longifolia</i> subsp. <i>typhoides</i>
-	Nutlets 0.58-0.62 mm long	<i>M. suaveolens</i>

- | | | |
|----|-------------------------------|---|
| 9 | Nutlets at least 0.39 mm wide | <i>M. × rotundifolia</i> |
| - | Nutlets at least 0.37 mm wide | <i>M. × villosa-nervata</i> |
| 10 | Nutlets 0.69-0.71 mm long | <i>M. spicata</i> subsp. <i>tomentosa</i> |
| - | Nutlets 0.54-0.66 mm long | <i>M. longifolia</i> subsp. <i>longifolia</i> |

The utility of nutlet characters, i.e. shape, size, presence or absence of hairs, nature of indumentum, surface sculpturing, exocarp cellular morphology and anatomy of the nutlet has been shown at various taxonomic levels in different genera of Lamiaceae (Husain *et al.*, 1990; Marin *et al.*, 1994; Duletic-Lausevic and Marin, 1999; Moon and Hong, 2006). Our findings also showed that the nutlet size, presence/absence of trichomes, surface sculpturing pattern are valuable diagnostic characteristics for separating closely related taxa of *Mentha*. In conclusion, we can say that nutlet morphological characteristics combined with other morphological characters can be used for delimitation of taxa at the species and infraspecific levels in the genus *Mentha*. Furthermore, this study provides the detailed data on the nutlet features of Turkish *Mentha* taxa.

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