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Research Paper

Anatomical and Micromorphological Investigations on Turkish Endemic *Marrubium cephalanthum* subsp. *montanum* (Lamiaceae)

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Abstract: *M. cephalanthum* subsp. *montanum* is a endemic subspecies which grows on steppe and rock slopes of Northern Anatolia. In this study, anatomical and micromorphological characters of *Marrubium cephalanthum* Boiss. & Noe subsp. *montanum* Akgül & Ketenoğlu were observed. In anatomical studies, cross-sections of the root, stem, leaf and the surface sections of leaves were determined. It was observed that the root of the investigated taxon had a secondary thickening. Collenchyma layer in stem was prominent. The leaf is dorsiventral and amphistomatic. For the micromorphological studies, the dried stem, leaf, calyx and nutlet samples were examined using scanning electron microscope (SEM). Eglandular trichomes were mostly observed on the stem, leaf and calyx. Glandular trichomes are present more sparsely on these organs of investigated taxon. In addition, the nutlet surface is reticulate.

Keywords: *Anatomy, Marrubium, SEM, trichome, endemic*

Introduction

The genus *Marrubium* L. (Lamiaceae) contains herbaceous plant distributed in the Irano-Turanian and Mediterranean phytogeographic regions and rarely in America and Australia (Hedge, 1992). The genus has more than 40 species in the world (Akgül *et al*., 2008). In Turkey, the genus has 21 taxa of which 12 are endemic (Davis *et al*., 1988; Ekim *et al.*, 2000; Aytaç *et al*., 2012). High endemism level (% 57) shows that Turkey is an important gene centre of the genus (Fırat, 2016). The genus *Marrubium* is characterized by densely hairy which cover all plant parts, flowers arranged densely on the stems, having tubular calyx and corolla (Ahvazi *et al*., 2016).

Some of the *Marrubium* species are used due to their medicinal value and most of them have great importance (Meyre- Silva and Cechinel-Filho, 2010). In additionally, mentholated leaves of the plant are used as spice and tea (Estilai and Hatemi, 1990). Some *Marrubium* species such as *M. vulgare* L. are cultivated as ornemantal plants (Büyükkartal *et al*., 2016).

In Lamiaceae, the systematic importance of trichome types was demonstrated by many researchers (Abu-Assab and Cantino, 1987; Marin *et al*., 1994; Navorro and El-Qualidi, 2000). Glandular trichomes are widespread on leaves and flowers of the Lamiaceae members and the taxonomical importance of the structure of these trichomes is well known in the Lamiaceae (Ascensao *et al*., 1999; Kaya *et al*., 2007; Kahraman *et al.*, 2010). Stellate and branched hairs are rather common in many genera of Lamiaceae such as *Marrubium, Ballota* L. and *Stachys* L. (Jamzad, 1999; Upson and Andrew, 2004). It was also reported that some species of *Marrubium* was characterized by having stellate trichomes in all their parts (Ahvazi *et al*., 2016). Nutlet micromorphology has been also considered useful in systematics of the Lamiaceae (Dinç and Doğan, 2006; Kaya and Dirmenci, 2008). Especially recent studies performed on trichome and nutlet surface micromorphology emphasize the great value of micromorphological characters in Lamiaceae taxonomy (Hedge, 1992; Kahraman *et al*., 2011; Satıl *et al*., 2012).

Up to now some anatomical studies of the family Lamiaceae have been conducted (Kaya *et al.*, 2000; Uysal, 2003; Erken, 2005; Büyükkartal *et al.*, 2016), but studies on anatomy and micromorphology of *M. cephalanthum* subsp. *montanum* are rather limited. Therefore, the present study aims to examine anatomical and micromorphological features of Turkish endemic *M. cephalanthum* subsp. *montanum* to determine the various type of trichomes and their distribution on the stem, leaf, calyx, nutlet.

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Materials and Methods

Plant samples were collected during the flowering and fruiting time from natural populations in Çorum province (Turkey) in the in the year 2014. The specimens were dried according to herbarium techniques and they were deposited at the Ondokuz Mayıs University, Faculty of Art and Science Herbarium (OMUB). Cullen (1982) was followed for taxonomic identification of species. Anatomical studies were performed on average 30 fresh specimens kept in 70 % alcohol. In these samples, root, stem and leaves transverse sections and surface sections of leaves were studied. The photographs of sections were taken with Nikon Coolpix 5200 digital camera. Image- J program was used for measurements of all anatomical characters. Stomata index was calculated according to the method described by Meidner and Mansfield (1968). For scanning electron microscopy, dried stem, leaf, calyx and nutlet samples were mounted on aluminium stubs and covered by gold after which they were studied by JEOL-JSM 7001 S scanning electron microscope (SEM) and photographed. The general trichome terminology follows Cantino (1990) and Koul *et al*. (2000).

Results and Discusssions

Anatomical Properties

Root anatomy

In the transverse section of root, the cells of periderm layer on the outermost surface are squashed (Fig.1A). There is a multilayered secondary cortex (11-12 layers) under the periderm. Cambium cells are distinguishable. Xylem is composed of tracheary elements. Phloem is consisted of squashed cells and less distinguishable. Pith rays with 1-2 layers are present(Fig. 1A). In the center of root, there is a pith consists of primary xylem elements. The mean diameter of vessel members is 15.82±3.68 µm (Table 1).

Table 1. Root, stem and leaf anatomical characteristics of *M. cephalanthum* subsp. *montanum*.

		Width (µm) (mean±SD)	Length (µm) (mean±SD)
Root	Thickness of periderm	30.54±3.90	-
	Parenchyma cells	20.54±6.89	16.01±5.91
	Thickness of phloem	61.92±7.10	-
	Thickness of xylem	290.96±15.89	-
	Diameter of vessels	15.82±3.68	-
Stem	Thickness of epidermis	21.58±4.12	-
	Parenchyma cells	15.99±4.68	42.03±8.73
	Thickness of cortex	101.33±23.58	-
	Thickness of collenchyma	154.66±53.29	-
	Thickness of phloem	48.67±11.23	-
	Thickness of xylem	140.46±33.14	-
	Diameter of vessels	23.81±4.07	-
	Pith cells	23.32±7.32	48.29±26.58
Leaf	Upper epidermis cells	13.05±3.34	12.08±1.89
	Thickness of palisade parenchyma	33.78±2.47	-
	Thickness of spongy parenchyma	36.25±4.33	-
	Lower epidermis cells	9.54±2.41	9.81±2.25
	Adaxial stomata	18.20±1.17	21.74±1.77
	Abaxial stomata	18.91±1.61	22.27±1.87

SD: Standard deviation

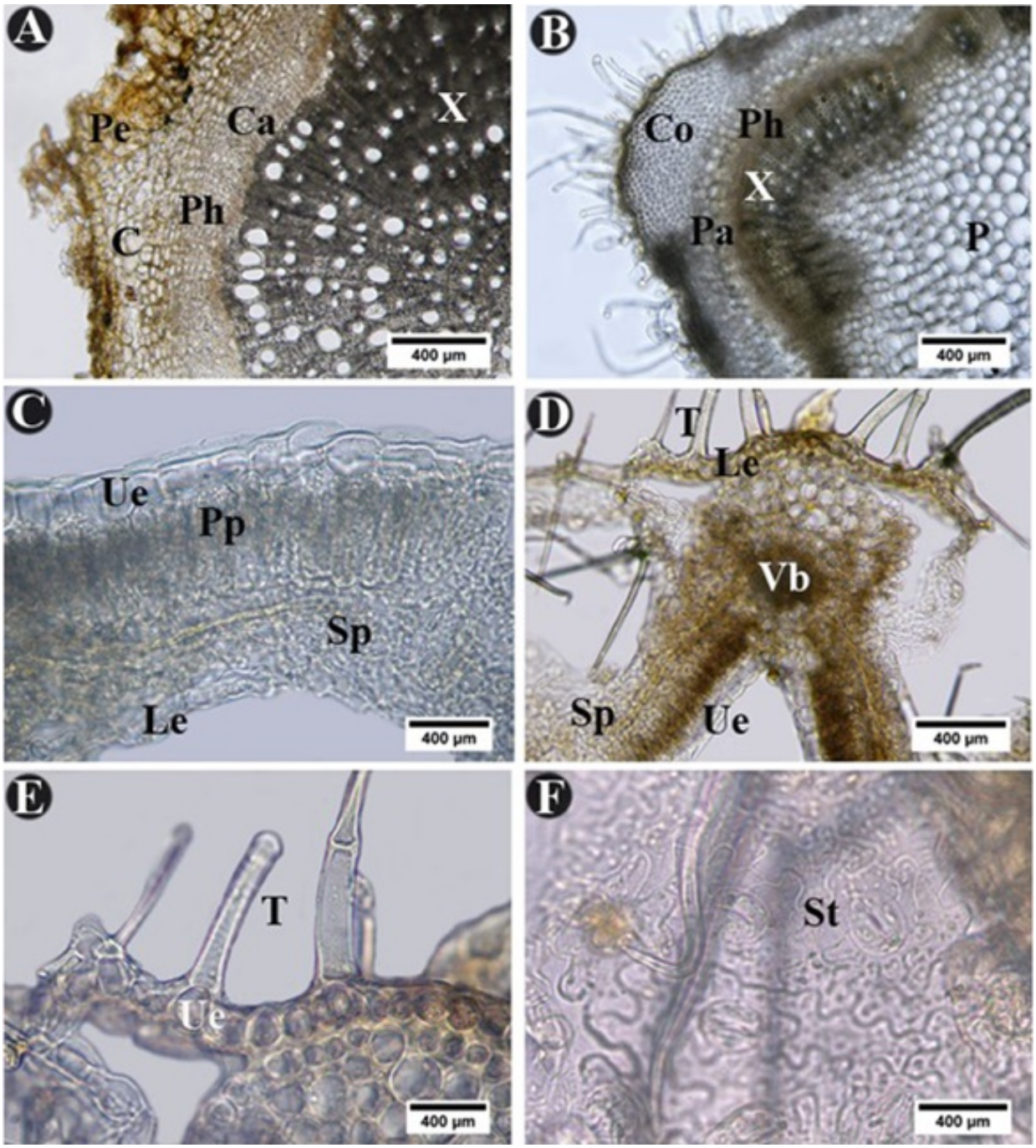

Figure 1. Root, stem and leaf anatomy of *M. cephalanthum* subsp. *montanum* A- Cross section of root, B- Cross section of stem, C- D- Cross section of leaf, E- Light micrograph of trichomes of *M. cephalanthum* leaf, F- Abaxial epidermis surface of leaf. (C-cortex, Ca-cambium, Co-collenchyma, Le- lower epidermis, P-pith, Pa-parenchyma, Pe- periderm, Ph-phloem, Pp-palisade parenchyma, Sp-spongy parenchyma, St- stomata, T-trichome, Ue-upper epidermis, Vb- vascular bundle, X-xylem).

Stem anatomy

Epidermis consist of orbicular or rectangular cells. It is covered by a thick cuticle (Fig. 1B). The non-glandular and glandular trichomes on the epidermis are present. Collenchyma is 10-12 layered and cortex parenchyma cels are 15.99±4.68 x 42.03±8.73 µm (Table 1).Collenchyma layer covers a large area at the corners. The cambium between the xylem and phloem is not distinguishable. The mean diameter of vessel elements is 23.81±4.07 µm (Table 1). In the centre of the stem, there is a large pith region. The pith consist of large and cyclindrical parenchymatic cells and become smaller towards the central part of the stem (Figure 1B).

Leaf anatomy

The transverse section of the lamina revealed that the upper and lower epidermis cells are uniseriate, quadrangular and oval (Fig. 1C). However, upper epidermal cells are larger than the lower ones (Table 1). There are mostly eglandular and rarely glandular trichomes on the both epidermis (Figs. 1D,E). The leaf is of the bifacial (dorsiventral) type. The mesophyll is composed of palisade parenchyma cells and isodiametric with large intercellular space spongy parenchyma cells (Fig. 1C). Vascular bundles are collateral type and surrounded by a parenchymatic bundle sheath (Fig. 1D). The stomata type is anomocytic and they are mostly occur on the lower surface of leaf (Fig. 1F). Stomatal index is 19.16±3.98 for the lower epidermis and is 17.08±3.52 for the upper epidermis (Table 2).

Table 2. Stomata index of *M. cephalanthum*.

Stomata index	Mean±SD
Upper leaf surface	17.08±3.52
Lower leaf surface	19.16±3.98

SD: Standard deviation

Micromorphological Properties

Two different trichome types on the stem, leaf and calyx of *M. cephalanthum* were observed: eglandular and glandular trichomes. Glandular trichomes can be distinguished by unicellular to multicellular stalk cell and a large head and these types of trichomes were hardly seen due to the presence stellate trichomes on the stem, calyx and leaf. Whereas, non-glandular trichomes are present in large numbers on all surfaces of these organs (Figs. 2A, C, D). Upper leaf surface of *M. cephalanthum* subsp. *montanum* is covered by mostly eglandular trichomes and these trichomes are especially more abundant in basal leaves. A few branched stellate trichomes were present on the lower surface of leaf. These trichomes especially are more abundant on the leaf midrib region. Strongly sinous walls in upper and lower leaf epidermis are observed in this species (Fig. 2B). SEM observation showed that the calyx of *M. cephalanthum* subsp. *montanum* has mostly eglandular trichomes (Figures. 2 C,D). We also observed abundant eglandular trichomes on calyx teeth and calyx throat of *M. cephalanthum* subsp. *montanum* (Fig. 2C). The mature nutlets of *M. cephalanthum* generally ovate in outline (average 2.8 mm long, 1.34 mm wide) and brown in colour (Figure 2E). The ornemantation is reticulate (Figure 2F).

In the present study, the observations and measurements of some anatomical and micromorphological characters of *M. cephalanthum* subsp. *montanum* are presented for the first time. Anatomical structures of roots of the investigated taxa have the same general anatomical characters of the family Lamiaceae. The root anatomy showed that the species has woody roots with secondary growth, 1-2 rows of ray cells. The center of root was filled with xylem elements. Büyükkartal *et al*. (2016) also determined that *M. bourgaei* Boiss. and *M. heterodon* (Benth.) Boiss & Bal. had woody roots with secondary growth and the root center of both taxa consisted of tracheids. Our results are consistent with those of Metcalfe and Chalk (1972) and those of some other studies on Lamiaceae family and *Marrubium* genus (Baran and Özdemir, 2009; Kahraman *et al.*, 2010; Büyükkartal *et al.* 2016).

Stem anatomical examinations showed that the stem has well- defined collenchyma with 10-11 layers in the corners. Metcalfe and Chalk (1972) and Watson and Dalwitz (1978) reported that many Lamiaceae species have multilayered collenchyma as a characteristic feature. Also some researcher reported a well developed collenchyma tissue in the stem corners (Baran and Özdemir, 2006; Kahraman *et al*., 2010; Tüylü *et al*., 2017). In additionally, Büyükkartal *et al.* (2016) were observed several layers of lamellar collenchyma beneath the epidermis in stem of *M. heterodon* (Benth.) Boiss & Bal. In the stem cross- sections, the vascular bundles were collateral type and xylem was positioned towards the center. Tüylü *et al.* (2017) also reported that the vascular bundles are collateral type and the xylem is positioned towards the center in stem of *M. lutescens* Boiss. and *M. cephalanthum* subsp. *akdaghicum.* Our results support those of Büyükkartal *et al.* (2016) and Tüylü *et al.* (2017).

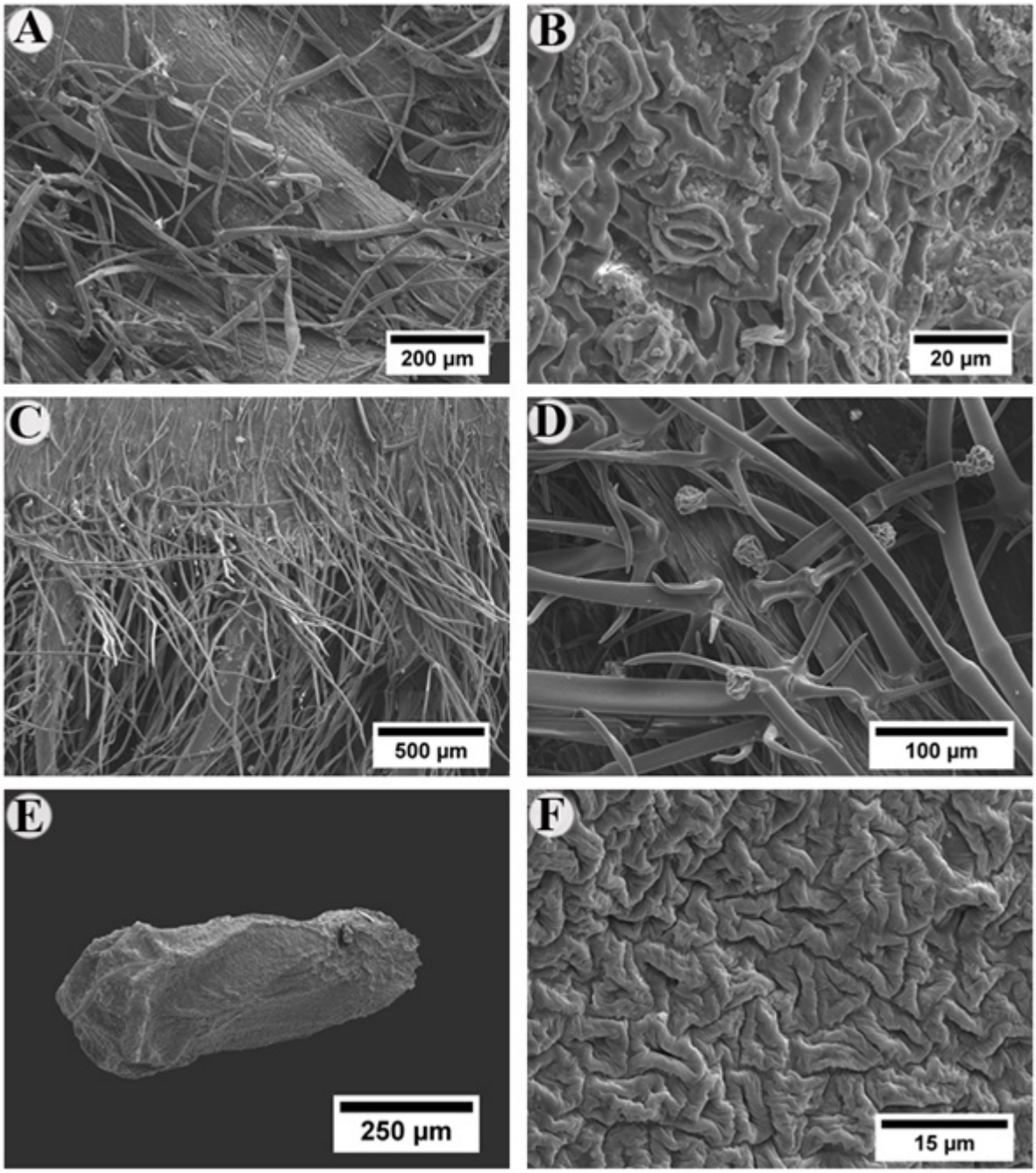

Figure 2. SEM micrographs of *M. cephalanthum* subsp. *montanum*; A- Eglandular trichomes on stem, B- Abaxial epidermis surface of leaf, C- Trichomes on calyx teeth and throat, D- Trichomes on calyx, E- Nutlet, F- Nutlet surface.

We also showed that *M. cephalanthum* subsp. *montanum* has bifacial leaf. Mesophyll consisted of palisade and spongy parenchyma cells with large intercellular space. The same leaf character has been also observed in the leaves of other *Marrubium* taxa (Akgül *et al.,* 2008; Hatamneia *et al.*, 2008; Büyükkartal *et al*., 2016). Our observation showed that *M. cephalanthum* subsp. *montanum* has amphistomatic leaves and the number of stomata on abaxial surface was higher than adaxial surface. Tüylü *et al.* (2017) reported that the number of stomata per mm^2 in lower surface was higher than the upper surface in *M. heterodon*. Our results are aggrement with previous study. Cantino (1990) also mentioned that both amphistomatic and hypostomatic leaves are found in the members of the Lamiaceae family. Akgül (2004) also reported that the leaves of *M. cephalanthum* are amphistomatic. Similar results were also reported in the other *Marrubium* taxa (Büyükkartal *et al.,* 2016; Tüylü *et al*., 2017).

Trichome types and micromorphology of the indumentum have taxonomic importance in Lamiaceae (Metcalfe and Chalk, 1972; Cantino, 1990; Navorro and El Oualidi, 2000). Both eglandular and glandular trichomes are recorded in the members of the family (Kahraman *et al.*, 2010; Celep *et al*., 2011; Mousavi *et al*., 2014; Çalı, 2017). The stem, leaf and calyx of *M. cephalanthum* subsp. *montanum* covered by densely stellate trichomes. Akgül (2004) also reported that the stem and leaves of *M. cephalanthum* has covered by characteristic stellate trichomes. Glandular trichomes have taxonomic value at specific and subspesific level in the Lamiaceae family (Husain *et al.*, 1990). According to the results of present study, glandular trichomes were capitate and generally more sparsely on the stem, calyx and both leaf surfaces. These glandular trichomes were reported in some Turkish *Marrubium* species (Bisio *et al.*, 1999; Akgül, 2004; Salmaki *et al.*, 2009). Çalı (2017) determined that peltate trichomes and subtype IA capitate trichomes (with globose unicellular or bicellular head and a stalk of one or two cells) were seen abundantly on all organs of *M. cephalanthum* subsp. *montanum*. Our results obtained from SEM are consistent with those of Çalı (2017). The type of nutlet sculpturing has been considered as an important taxonomic character in Lamiaceae (Salmaki *et al*., 2009; Kahraman *et al*., 2011; Satıl *et al*., 2012). According to our study, nutlet ornamentation type of *M. cephalanthum* subsp. *montanum* has been determined as reticulate. These findings are consistent with the observations of Akgül *et al.* (2008).

Conclusion

The results of this study exhibited that Turkish endemic *M. cephalanthum* subsp. *montanum* has some characteristic anatomical and micromorphological features. According to obtained results from the micromorphological features, the stem, leaf and calyx of *M. cephalanthum* subsp. *montanum* was densely covered by characteristic stellate trichomes. As a consequence, our results can be provide useful data in the future studies about this genus.

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