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ریزریختشناسی فندقچه سرده فراسیون و خویشاوندان و اهمیت ساز گانشناختی آن (تیره نعنائیان: طایفه فراسیونیها)

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چکیده. فندقچههای ۲۰ آرایه از طایفه فراسیونیها توسط میکروسکوپ الکترونی نگاره مورد بررسی قرار گرفتند و توضیحات مفصلی از ویژگیهای ریزریخت-شناسی آنها برای تمامی گونههای مورد مطالعه ارائه شده است. فندقچههای مطالعه شده در اندازه، شکل، رنگ و تزئینات سطحی تنوع خوبی نشان میدهند. شکل فندقچه در اغلب گونههای مورد بررسی تخممرغی است، همچنین اشکال گرد، تخم مرغی پهن، بیضوی، سرنیزهای، مثلثی و مستطیلی را در تعداد کمی از گونهها می-توان یافت. شش نوع اصلی الگوی تزئینات سطحی برای فندقجهها قابل تشخیص است که شامل: شبکهای، لانکدار، نردبانی، جویده، تاولدار و یشتهیشتهای-ریزدانهای است. رایج ترین نوع تزئینات سطحی فندقچه در بین گونههای مورد مطالعه نوع شبکهای است، اما تنوع در اندازه و شکل سلولهای تشکیل دهنده آنها صفات تشخیصی مفیدی را نیز فراهم می کند. تحقیقات ما نشان می دهد که نوع تزئینات در تحدید و تعریف آرایهها در سطح گونهای و در بین گونههای Marrubium و خو بشاوندان آن مفيد است.

واژههای کلیدی. آرایه شناسی، تزئینات سطحی، صفات تشخصی، لیدیسیان، میکروسکوپ الکترونی نگاره

Nutlet micromorphology of the genus Marrubium L. and allies and its systematic implication (Lamiaceae: tribe Marrubieae)

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Abstract. Nutlets of 20 taxa of the tribe Marrubieae were examined by scanning electron microscopy (SEM) and detailed descriptions of nutlet micromorphological features for all examined taxa are provided. The nutlets exhibited variation in size, shape, color and surface sculpturing. The nutlets shape of most species studied is ovate, but rounded, broad ovate, elliptic, lanceolate, triangular and oblong can also be found in a few species. Six basic types of the sculpturing pattern of nutlet surface can be distinguished: reticulate, foveolate, scalariform, ruminate, pusticulate and colliculate-granulate. The most common type of nutlet sculpturing among the studied species is reticulate, but the variation in size and shape of their composing cells provided useful diagnostic characters. Our investigation revealed that the type of sculpturing was more useful in taxon delimitation among Marrubium species and allies at the species rank.

Keywords. diagnostic characters, Labiatae, surface sculpturing, scanning electron microscopy, taxonomy

*دكتر شهريار سعيدي سال گذشته به ديار باقي شتافت. يادش را گرامي ميداريم.

INTRODUCTION

Marrubieae Vis., with five genera and ca. 91 species, is one of the 10 Lamioideae tribes distributed mainly in southern Europe and North Africa (Siadati *et al.*, 2018). They are mostly nonaromatic herbs or subshrubs with campanulate to rotate calyx and often with secondary calyx lobes, zygomorphic and 2-lipped corolla and stamens included or shortly exerted from the corolla (Siadati *et al.*, 2018; Harley *et al.*, 2004).

According to one of the most recent phylogenetic studies, the monophyletic Marrubieae contains four major clades: the first one including member of Acanthoprasium (Benth.) Spach and Moluccella L., the second one comprising species of Ballota L., the third clade containing Pseudodictamnus Fabr. and related species and the fourth one including Marrubium L. species (Siadati et al., 2018). Acanthoprasium, with two species, is a European genus with a woody habit, spiny bracteoles and upper lip of corolla totally hairy. According to Bendiksby et al. (2011), the annual or short-lived perennials Moluccella, contains eight species in southwestern Asia and the Mediterranean regions, are characterized by a zygomorphic calyx more or less expanded at the mouth, internally glabrous, and usually lobed with both primary and secondary indentations. The genus Ballota are subshrubs to perennial herbs with herbaceous bracteoles and shortly exerted stamens from the corolla and extend from Macaronesia, Europe to Mediterranean and Western Asia (Siadati et al., 2018). According to Bentham (1832-1836), the genus Ballota had traditionally been divided into three sections based on two main morphological characters, woody against herbaceous habit and the type of bracteoles (spinose against herbaceous): (1) sect. Acanthoprasium Benth., which is raised to generic rank by Bendiksby et al. (2011), (2) sect. Beringeria (Neck.) Benth., which is recently raised to generic rank by Siadati et al. (2018) and (3) sect. Ballota Benth. (Bentham 1832-1836). Based on recent phylogenetic studies, the genus Ballota re-circumscribed to encompass the members of sect. Ballota with about three species. Moreover, members of sect. Beringeria recently raised to generic rank as Pseudodictamnus, which accommodates perennial herbs with herbaceous bracteoles, expanded calvx limb with 6-20 teeth and widely distributed from Eurasia to North Africa (Siadati et al., 2018). The genus Marrubium with about 50 species have usually toothed and petiolate leaves, 5-15 (-30) lobed calvx covered by dense stellate trichomes, corolla tube shorter than calyx and stamens included in the corolla tube (Harley et al., 2004).

Studies on nutlet micromorphology within Lamiaceae showed that nutlets features *e.g.*, shape, morphology of the abscission scar and surface

sculpturing, were potentially useful at different taxonomic levels (e.g. Demissew & Harley, 1992; Marin et al., 1994; Ryding, 1995; Oran, 1996; Ryding, 1998; Salmaki et al., 2008; Moon et al., 2009; Satil et al., 2012; Krawczyk & Głowacka, 2015; Eyvaz?zadeh Khosroshahi & Salmaki, 2018). Among different nutlet characters, type of sculpturing has been considered to be taxonomically most important (Oran, 1996; Kahraman et al., 2011), however, color, size and shape of nutlets were considered unimportant, either because they did not vary or the variation was random or too great (Oran, 1996). Abscission scars were invariable, but the variation at higher levels may be significant (Guerin, 2005). There are no reports on the taxonomic significance of the nutlets micromorphology among different genera of Marrubieae, except that by Mosquero et al. (2007), who provided a description of the morphology and anatomy structure in Marrubium vulgare L. Akgül et al. (2008) illustrated the range of variability in seed characters in Marrubium species found in Turkey and Hassan & Al-Thobaiti (2015) provided a detailed description of morphological nutlet characteristics Marrubium vulgare in Saudi Arabia. Thus, the main goal of this study was to provide a detailed description of nutlet micromorphology of the genus Marrubium and allies.

MATERIALS AND METHODS

Nutlets of 20 species representing all five genera of tribe Marrubieae were selected to investigate the value of seed characters in the classification of genera and species. Nutlets were collected from herbarium specimens, deposited in the herbaria M (Botanische Staatssammmlung München), MSB (Münich Systematic Botany), and TUH (Central Herbarium of Tehran University). A list of voucher specimens is presented in Table 1. A total of two species of Acanthoprasium, two species of Moluccella, four taxa representing three species of Ballota, two species of Pseudodictamnus and 10 species of Marrubium were analyzed. Nutlets were observed in advance, using a stereomicroscope to ensure that they were of normal size and maturity. Nutlets observations were made using scanning electron microscopy.

For SEM observations, dried nutlets were mounted on aluminum stubs using double-sided adhesive and sputter-coated with a thin layer (ca. 30 nm) of gold and examined by means of a Hitachi SU3500 (Japan) scanning electron microscopy at an accelerating voltage of 5-30 kV. This paper follows the terminology of Bojnanský & Fargašová (2007) and Stearn (1983) for the seed shape and surface ornamentation.

Table. 1. Taxa, voucher specimens and collection data of selected species of *Marrubium* and its allied genera deposited in the herbaria M (Botanische Staatssammmlung München), MSB (Münich Systematic Botany), and TUH (Central Herbarium of Tehran University).

Species	Collection data					
Acanthoprasium frutescens (L.) Spenn.	France: Schlucht von Aiglum, 30 km westlich und nördlich Nice, O. Angerer s.n. (M)					
Acanthoprasium integrifolium (Benth.)	Cyprus: – 1880 (M)					
Ryding						
Ballota nigra subsp. anatolica P. H. Davis, Cult.	Turkey: Nigde, near Aydinkent, Yayikli, P. Rasmont 55330 (M)					
Ballota nigra subsp. ruderalis (SW.) Briq.	Jugoslawien: Makedonija, 13 km ONO von Skocivir an der Straße zum Kajmakchalan (Grenzgebirge), D. Podlech & Lippert 26027 (M)					
Ballota platyloma Rech.f.	Iran: Mazandaran, Zentral- Elburs, Im Einzugsgeblet des oberen Tedschen-Flusses, unterhalb Kom-rud bala, lochere Gebuschvegetation, Felsschutt; 60 km ostlich von Firuzkuh, E. Behboudi & P. Aellen s.n. (M)					
Ballota saxatilis Sieber ex C.Presl	Libanon/Syrien: -, s.n. (M)					
Pseudodictamnus hispanicus (L.) Salmaki & Siadati	Spain: Valencia Segunto, inruinis, 508 (M)					
Pseudodictamnus aucheri (Boiss.) Salmaki &	Iran: Chahar-Mahal-e Bakhtiari, Kuh-e Rig from Dehno, Zarre					
Siadati	17941 (TUH)					
Marrubium alyssoides Pomel,	Marokko: d' Oujda, 5 km W E1 Aioun; Felder, Eucalyptushainen N der Straβe nach Taza (P 1), W. Lippert 21821 (M)					
Marrubium anisodon K. Koch	Afghanistan: Logar, Weshang des Tera-Passes bei Nyazi, D. Podlech 18498 (M)					
Marrubium astracanicum Jacq.	Caucasus: Krasnoselskoie, montes Areguni, in vicinitate pagi Tokludza, V. Vašák 257082 (M)					
Marrubium catariifolium Desr.	Caucasica: M. Senser 257078 (M)					
Marrubium cuneatum Banks & Sol.	Iran: Kurdistan, In grraminosis siccis jugi prope Salavatabad 25 km E Sanandaj, K.H. Rechinger 42784 (M)					
Marrubium leonuroides Desr.	Caucasus: Kislovodsk, in abruptis, I. Akinfiev 7382 (MSB)					
Marrubium litardierei Marmey	Marokko: d'Er-Rachidia, Hoher Atlas, Tizi n'Talrent an der Straβe von Midelt nach Straβenrand, D. Podlech 47337 (MSB)					
Marrubium peregrinum L.	Austeria: Nordburgenland, Parndorfer Platte, trockene, grasige Stellen am E-Fuβ des Heidl bei Nickelsdorf, ca. 0.45 km SSE des Bahnübergangs am NE-Fuβ des Heidl (Kote 133), T. Barta 2004-351 (M)					
Marrubium persicum C.A. Mey.	Armenia: Ararat, Ararat valley 12 km NNW of Ararat town, 7 km NW of Vedi, Erah range, Fayvush & al. XI/1040 (M)					
<i>Marrubium propinquum</i> Fisch. & C.A. Mey.	Caucasus: Krasnoselskoie, montes Areguni, in vicinitate pagi Tokludza, V. Vašák s.n. (M)					
Moluccella aucheri (Boiss.) Scheen,	Iran: Baluchistan, 20 km a Zahedan meridiem versus, K.H. Rechinger 14562 (M)					
Moluccella laevis L.	Iraq: Erbil (Kurdistan). In agris demssis inter Mirza Punstam et					

RESULTS AND DISCUSSION

Several main features of the investigated nutlets *i.e.* shape, length, width, as well as sculpturing patterns, projections of outer periclinal walls and anticlinal walls are summarized in Table 2. Selected SEM micrographs of nutlets studied were presented in Figures 1-3. In general, the color of nutlets in all studied species was dark brown to black. The nutlets were oblong (*e.g.*, *M. catariifolium* Desr., Fig. 2G), triangular (*e.g.*, *Mo. laevis* L., Fig. 3H), broad ovate (*e.g.*, *B. nigra* subsp. *ruderalis* (Sw.) Briq., Fig. 1F)

to rounded (e.g., P. aucheri (Boiss.) Salmaki & Siadati, Fig. 1L) and elliptic (e.g., A. frutescens (L.) Spenn) to lanceolate (e.g., B. nigra subsp. anatolica P.H. Davis, Fig. 1D) in shape, but the most common type was ovate (e.g., B. platyloma Rech. f., Fig. 1H; M. cuneatum Banks & Sol., Fig. 2I and M. persicum C.A.Mey., Fig. 3B). The size of nutlet ranged from 1.34×1.08 mm (in M. leonuroides Desr.) to 4.04×1.45 mm (in Mo. aucheri (Boiss.) Scheen). Nutlets of investigated taxa exhibited six types of surface sculpturing patterns. The most common type

Pania, ca 500 m. 11230 (M)

Table 2. Details of nutlet characteristics of the studied taxa of Marrubium and its allied genera.

Table 2. Details of nutlet ch	Nutlet Shape	Nutlet length (mm)	Nutlet width (mm)	Surface Sculpturing	Apex	Outer	Anticlinal Wall	Figures
Acanthoprasium frutescens	Elliptic	2.75	1.38	Reticulate	+	Deep Concave	Riased	Fig. 1 A
Acanthoprasium integrifolium	Triangular	2.75	1.39	Scalariform	+	Shallow Concave	Riased	Fig. 1 B, C
Ballota nigra subsp. anatolica	Lanceolate	2.09	0.9	Foveolate	_	Concave	Riased	Fig. 1 D, E
Ballota nigra subsp. ruderalis	Broad ovate	1.85	1.16	Reticulate	_	Flat	_	Fig. 1 F, G
Ballota platyloma	Ovate	1.85	1.05	Reticulate	_	Flat	Riased	Fig. 1 H, I
Ballota saxatilis*	Ovate	2.3	1.29	Reticulate	_	Concave	Riased	Fig. 1 J, K
Pseudodictamnus aucheri	Rounded	1.78	1.32	Reticulate	_	Concave	Riased	Fig. 1 L, M
Pseudodictamnus hispanicus	Ovate	2.22	1.27	Reticulate	_	Concave	Riased	Fig. 1 N, O
Marrubium alyssoides	Ovate	1.86	1.01	Ruminate	_	Concave	Riased	Fig. 2 A, B
Marrubium anisodon	Oblong	1.78	0.99	Reticulate	_	Shallow Concave	Riased	Fig. 2 C, D
Marrubium astracanicum	Ovate	1.79	1.07	Pusticulate	_	Convex	Riased	Fig. 2 E, F
Marrubium catariifolium	Oblong	2.28	1.22	Reticulate	_	Deep Concave	Riased	Fig. 2 G, H
Marrubium cuneatum	Ovate	2.21	1.21	Reticulate	_	Flat	Riased	Fig. 2 I, J
Marrubium leonuroides	Ovate- lanceolate	1.34	1.08	Reticulate	_	Concave	Riased	Fig. 2 K, L
Marrubium litardierei	Ovate	2.09	1.1	Reticulate	_	Shallow Concave	Riased	Fig. 2 M, N
Marrubium peregrinum	Oblong	1.65	0.96	Reticulate	+	Concave	Riased	Fig. 3 O, A
Marrubium persicum	Ovate	1.79	1	Foveolate	_	Concave	Riased	Fig. 3 B, C
Marrubium propinquum	Oblong	1.81	1.03	Reticulate	_	Concave	Raised and Wavy	Fig. 3 D, E
Moluccella aucheri	Triangular	4.04	1.45	Reticulate	_	Concave	Riased	Fig. 3 F, G
Moluccella laevis	Triangular	3.04	2.13	Colliculate- Granulate	_	Convex	Represented by channels	_

*Due to lack of sufficient evidence, *Ballota saxatilis* has not yet been assigned to *Pseudodictamnus*, but it shares several morphological features with the members of this genus.

of sculpturing pattern was reticulate (e.g., A. frutescens, Fig. 1A), however, other types of sculpturing patterns, like scalariform (A. integrifolium (Benth.) Ryding, Fig. 1C), foveolate (M. persicum, Fig. 3C), ruminate (M. alyssoides Pomel, Fig. 2B), pusticulate (M. astracanicum Jacq., Fig. 2F) and colliculate-granulate (Mo. laevis, Fig. 3I), were observed.

Our study represents the first investigation on nutlet micromorphology on the genus *Marrubium* and its allied genera. Variation in shape, size, presence or absence of trichomes at the apex of

nutlets and particularly surface sculpturing appeared to have taxonomic value in some groups of Lamiaceae (Husain *et al.*, 1990; Oran, 1996; Navarro & El-Oualidi, 2000; Moon & Hong, 2006; Moon *et al.*, 2009; Kahraman *et al.*, 2011). Özkan *et al.* (2009) found that variation of shape, size, surface sculpturing and color were useful in distinguishing groups, species and subspecies among 12 examined species of *Salvia*. In *Stachys* (Salmaki *et al.*, 2008) nutlet micromorphology provided valuable data in separating the related species within sections, although these characters were not useful in

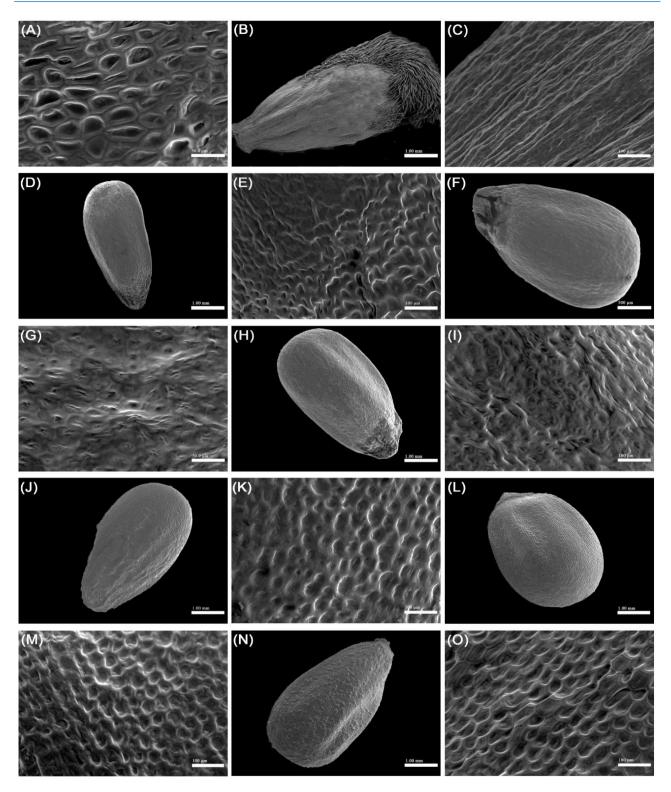


Fig. 1. Scanning electron micrographs of *Marrubium* and its allied genera. **A.** *Acanthoprasium frutescens*. **B, C.** *A. integrifolium*. **D, E.** *Ballota nigra* subsp. *anatolica*. **F, G.** *B. nigra* subsp. *ruderalis*. **H, I.** *B. platyloma*. **J, K.** *B. saxatilis*. **L, M.** *Pseudodictamnus aucheri*. **N, O.** *P. hispanicus*.

separating large natural groups. Variations in size and the type of sculpturing as well as the shape of its composing cells had been considered to provide the most valuable characters at species level (*e.g.*, Kahraman *et al.*, 2011; Tarimcilar *et al.*, 2013; Salmaki *et al.*, 2008; Eyvazadeh Khosroshahi & Salmaki, 2018). For instance, the polygonal cells in

M. peregrinum L. (Fig. 3A) were much smaller than those in M. leonuroides (Fig. 2L). Moreover, M. anisodon K.Koch (Fig. 2D) and M. litardierei (Fig. 2N) were different in the depth of pits on surface. Due to the particular value of microsculpturing, a comprehensive discussion is given below, indicating the importance of sculpturing patterns and its

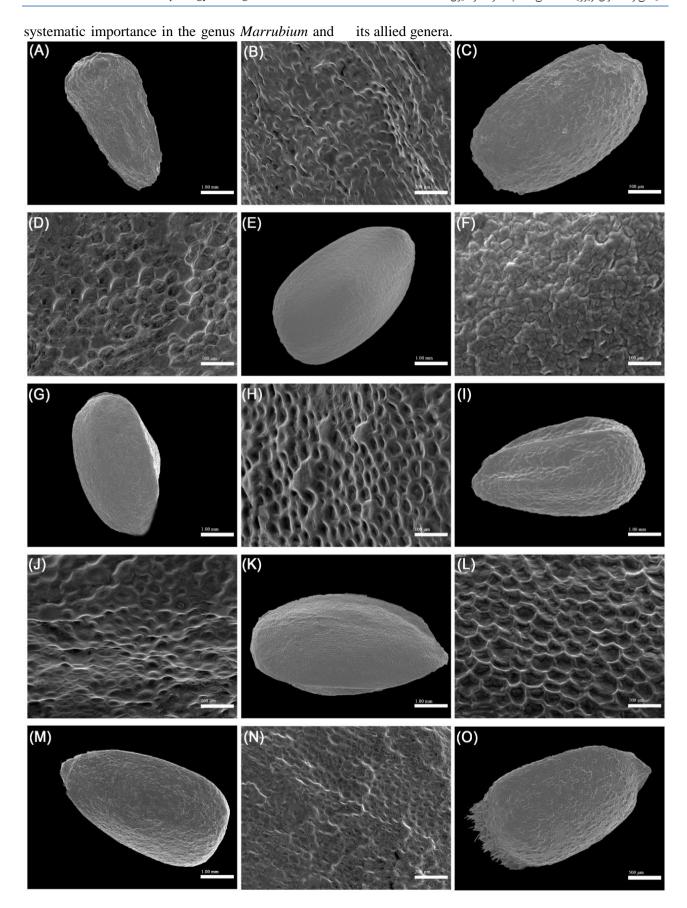


Fig. 2. Scanning electron micrographs of *Marrubium* and its allied genera. **A, B.** *Marrubium alyssoides*; C, D: *M. anisodon*; **E, F.** *M. astracanicum*; **G, H.** *M. catariifolium*; I, J: *M. cuneatum*; **K, L.** *M. leonuroides*; **M, N.** *M. litardierei*, **O.** *M. peregrinum*.

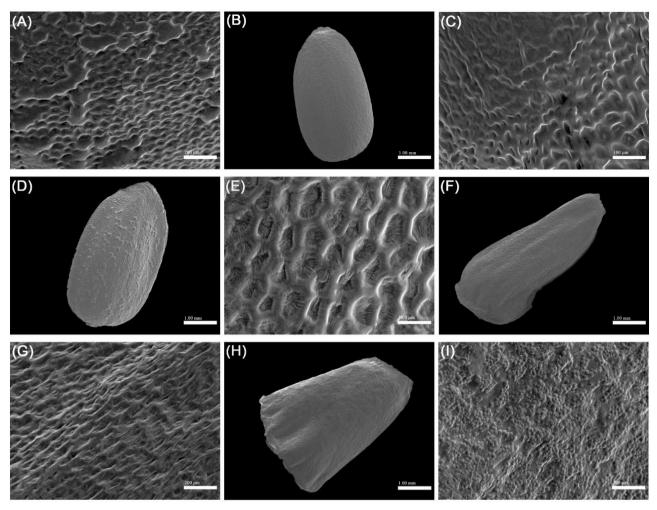


Fig. 3. Scanning electron micrographs of *Marrubium* and its allied genera. **A.** *Marrubium* peregrinum; **B, C.** *M.* persicum; **D, E.** *M.* propinquum; **F, G.** Moluccella aucheri; **H, I.** Mo. laevis.

Acanthoprasium - This genus includes two species based on the latest phylogenetic study (Bendiksby et al., 2011), characterized by simple hairs and broadly campanulate calyx with spiny lobes. Both species of Acanthoprasium are well distinguished by having bearded nutlets. The present study showed that the presence of trichomes at the apex of nutlets could be used as a taxonomic marker in the delimitation of Acanthoprasium nutlets from the rest of Marrubieae. However, these two species were different in shape and sculpturing pattern. While, A. frutescens is characterized by elliptic nutlets as well as reticulat pattern. While, A. frutescens is characterized by elliptic nutlets as well as reticulate pattern of nutlet sculpturing (Fig. 1A), A. integrifolium distinguished by triangular nutlets and scalariform sculpturing (Fig. 1B–C).

Moluccella— The genus Moluccella, characterized by glabrescent stems and leaves as well as an expanded calyx, is distributed from southern Europe to central Asia, Pakistan and Kashmir (Scheen et al., 2010; Bendiksby et al., 2011). In the present study, two out of eight species of this genus were examined. Although both of these species were

similar in shape and possession of the largest nutlets (Fig. 3F-H), they were different in the pattern of sculpturing. Moluccella nutlet aucheri. distinguished by reticulate pattern of nutlet sculpturing, differs from Mo. laevis with colliculategranulate pattern of nutlet sculpturing. In addition, the type of anticlinal walls was different between these two species. While Mo. aucheri possesses raised anticlinal walls, Mo. laevis is characterized by anticlinal walls represented by channels. Due to the limited taxon sampling on the genus Moluccella, a more comprehensive study needs to be done for indicating the taxonomic importance of nutlet characters.

Ballota— Recently, a narrower circumscription of the genus *Ballota* was proposed by Siadati *et al.* (2018) to encompass the members of sect. *Ballota* (sensu Patzak, 1958) with about three species. These three species are characterized by the following morphological features: five main calyx teeth, rarely with a few additional minute teeth as well as simple and glandular indumentum (Siadati *et al.*, 2018). Although nutlet morphology was not useful in the recognition of the genus *Ballota* from the other

genera of the tribe Marrubieae, it was helpful in the delimitation of taxa at species level. *Ballota nigra* subsp. *anatolica* with lanceolate nutlets was characterized by foveolate pattern of nutlet sculpturing (Fig. 1D–E). On the other hand, *B. nigra* subsp. *ruderalis* (Fig. 1F–G) and *B. platyloma* (Fig. 1H–I) have broadly ovate and ovate nutlets, respectively, and possess reticulate pattern of nutlet sculpturing.

Pseudodictamnus- The genus Pseudodictamnus, with about 28 species, comprises perennial herbs with herbaceous bracteoles, expanded calyx limb with 6-20 teeth and corolla tube shorter than or equaling the calyx with branched and simple hairs (Siadati et al., 2018). This genus, which is phylogenetically known to be the closest relative of Ballota and Marrubium, shares similar nutlet features with them. For instance, the pattern of nutlet sculpturing among all three species of the genus Pseudodictamnus was reticulate, which is a common type among the species of Ballota and Marrubium. Moreover, these three species were similar in the following characters: reticulate pattern of nutlet sculpturing with concave projection in outer periclinal wall and raised anticlinal walls. However, they were different in the shape of nutlets (Fig. 1 J, L, N).

Marrubium— *Marrubium*, with ca. 50 species, is morphologically well characterized by the following features: usually toothed and petiolate leaves, thyrsoid inflorescence; calyx 5-15(-30)-lobed, corolla tube shorter than calyx; stamens included in corolla-tube, posterior corolla lip 2-lobed (Harley *et al.*, 2004). In addition, several characters, such as rounded or subtruncate nutlets at apex and glabrous or with sessile glands at apex, have been mentioned in previous studies (Harley *et al.*, 2004).

It is not surprising that *Marrubium*, the largest genus of the tribe Marrubieae, shows considerable diversity in nutlet characters such as size, shape, and sculpturing pattern. *Marrubium leonuroides* and *M. catariifolium* possessed the smallest and biggest nutlets among the examined species, respectively. The common nutlet shapes among the *Marrubium* species were ovate and oblong. The common sculpturing pattern of nutlet in *Marrubium* species was reticulate, however, they showed some variation in shape and size of the composing cells.

Based on morphological characters the genus *Marrubium* has been divided into six sections by Seybold (1978). *Marrubium* sect. *Microdonta*, which was represented here by *M. astracanicum*, *M. catariifolium*, *M. leonuroides* and *M. propinquum*, morphologically characterized by five straight or curved calyx teeth and purple corolla. Although all the species attributed to this section were different

in shape, they showed similar type of sculpturing, except in M. astracanicum, which possessed pusticulate sculpturing. While Marrubium alyssoides belonging and M. persicum, Marrubium sect. Stellata, were similar in shape of nutlets but differed in the type of sculpturing, both studied species of Marrubium sect. Marrubium (M. anisodon and M. cuneatum) differed in the shape of nutlets but possessed a similar reticulate type of sculpturing. Marrubium peregrinum, belonging to sect. Ramosa, possessed the smallest oblong nutlets (1.65×0.96 mm) among the studied species and simple hairs at apex.

This section is morphologically well characterized by widely branched stems and few-flowered verticillasters with white corolla (Davis, 1982). Nutlet sculpturing feature was helpful in the separation of species, however, it appeared uninformative at the level of section.

CONCLUSION

Nutlet micromorphology provided valuable data in the separation of the related species within genera of the tribe. Our findings revealed that the nutlet shape, sculpturing pattern and the absence/presence of simple hairs at apex of nutlet were the most significant features in the separation of taxa at species level. However, these characters were not useful in the separation of sections within genera. Our results, in accordance with previous studies of Lamiaceae (e.g., Oran, 1996), showed that the shape of the nutlets were insignificant in assessing the relationships among Marrubium species and its relatives in the tribe Marrubieae. It seemed also that, contrary to other genera of Lamiaceae (e.g., Guerin, 2005), nutlet characters were of low phylogenetic value in Marrubieae.

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