

## ریزریخت‌شناسی فندقچه سرده فراسیون و خویشاوندان و اهمیت سازگان‌شناختی آن (تیره نعنائیان: طایفه فراسیونی‌ها)

سوده سیادتی<sup>۱</sup>، شهریار سعیدی مهرورز<sup>۱\*</sup> و یاسمن سلمکی<sup>۲</sup>

<sup>۱</sup> گروه زیست‌شناسی، دانشکده علوم، دانشگاه گیلان، رشت، ایران؛ <sup>۲</sup> قطب تبارزایی موجودات زنده و بخش علوم گیاهی، دانشکده زیست‌شناسی، پردیس علوم، دانشگاه تهران، تهران، ایران

مسئول مکاتبات: سوده سیادتی، [ssiadati@phd.guilan.ac.ir](mailto:ssiadati@phd.guilan.ac.ir)؛ یاسمن سلمکی، [ysalmaki@ut.ac.ir](mailto:ysalmaki@ut.ac.ir)

**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، تخم‌مرغی پهن، بیضوی، سرنیزه‌ای، مثلثی و مستطیلی را در تعداد کمی از گونه‌ها می‌توان یافت. شش نوع اصلی الگوی تزئینات سطحی برای فندقچه‌ها قابل تشخیص است که شامل: شبکه‌ای، لانه‌دار، نردبانی، جویده، تاول‌دار و پشته‌پشته‌ای-ریزدانه‌ای است. رایج‌ترین 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 می‌کند. تحقیقات ما نشان می‌دهد که نوع تزئینات در تحدید و تعریف آرایه‌ها در سطح گونه‌ای در بین گونه‌های *Marrubium* و خویشاوندان آن مفید است.

**واژه‌های کلیدی.** آرایه‌شناسی، تزئینات سطحی، صفات تشخیصی، لبدیسیان، میکروسکوپ الکترونی نگاره

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

Soudeh Siadati<sup>1</sup>, Shahryar Saeidi Mehrvarz<sup>1</sup> & Yasaman Salmaki<sup>2</sup>

<sup>1</sup>Department of Biology, Faculty of Science, University of Guilan, P.O. Box, 41335-1914 Rasht, Iran; <sup>2</sup>Center of Excellence in Phylogeny of Living Organisms and Department of Plant Science, School of Biology, College of Science, University of Tehran, P.O. Box 14155-6455, Tehran, Iran.

Correspondent author: Soudeh Siadati, [ssiadati@phd.guilan.ac.ir](mailto:ssiadati@phd.guilan.ac.ir); Yasaman Salmaki, [ysalmaki@ut.ac.ir](mailto:ysalmaki@ut.ac.ir)

**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, ruminant, 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

\*دکتر شهریار سعیدی سال گذشته به دیار باقی شتافت. یادش را گرامی میداریم.

دریافت: ۱۳۹۷/۱۰/۱۶؛ اصلاح: ۱۳۹۷/۱۲/۲۰؛ پذیرش: ۱۳۹۸/۰۱/۲۰؛ انتشار: ۱۳۹۸/۰۷/۲۸؛ Received 06.01.2019/ Revised 10.03.2019/ Accepted 09.04.2019/ Published 20.10.2019

## 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 calyx 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 calyx 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 the morphological nutlet characteristics of *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 Staatssammlung 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 Staatssammlung München), MSB (Münich Systematic Botany), and TUH (Central Herbarium of Tehran University).

| Species   | Collection data  |
|---|--|
| <i>Acanthoprasium frutescens</i> (L.) Spenn.                    | France: Schlucht von Aiglum, 30 km westlich und nördlich Nice, O. Angerer s.n. (M)   |
| <i>Acanthoprasium integrifolium</i> (Benth.) Ryding             | Cyprus: – 1880 (M)   |
| <i>Ballota nigra</i> subsp. <i>anatolica</i> P. H. Davis, Cult. | Turkey: Nigde, near Aydıncık, Yayıklı, P. Rasmont 55330 (M)  |
| <i>Ballota nigra</i> subsp. <i>ruderalis</i> (Sw.) Briq.        | Jugoslawien: Makedonija, 13 km ONO von Skocivir an der Straße zum Kajmakchalan (Grenzgebirge), D. Podlech & Lippert 26027 (M)  |
| <i>Ballota platyloma</i> Rech.f.                                | Iran: Mazandaran, Zentral- Elburs, Im Einzugsgebiet des oberen Tedschen-Flusses, unterhalb Kom-rud bala, lochere Gebuschvegetation, Felsschutt; 60 km ostlich von Firuzkuh, E. Behboudi & P. Aellen s.n. (M) |
| <i>Ballota saxatilis</i> Sieber ex C.Presl                      | Libanon/Syrien: –, s.n. (M)  |
| <i>Pseudodictamnus hispanicus</i> (L.) Salmaki & Siadati        | Spain: Valencia Segunto, inruinis, 508 (M)   |
| <i>Pseudodictamnus aucheri</i> (Boiss.) Salmaki & Siadati       | Iran: Chahar-Mahal-e Bakhtiari, Kuh-e Rig from Dehno, Zarre 17941 (TUH)  |
| <i>Marrubium alyssoides</i> Pomel,                              | Marokko: d' Oujda, 5 km W El Aioun; Felder, Eucalyptushainen N der Straße nach Taza (P 1), W. Lippert 21821 (M)  |
| <i>Marrubium anisodon</i> K. Koch                               | Afghanistan: Logar, Weshang des Tera-Passes bei Nyazi, D. Podlech 18498 (M)  |
| <i>Marrubium astracanicum</i> Jacq.                             | Caucasus: Krasnoselskoie, montes Areguni, in vicinitate pagi Tokludza, V. Vašák 257082 (M)   |
| <i>Marrubium catariifolium</i> Desr.                            | Caucasica: M. Senser 257078 (M)  |
| <i>Marrubium cuneatum</i> Banks & Sol.                          | Iran: Kurdistan, In grraminosis siccis jugi prope Salavatbad 25 km E Sanandaj, K.H. Rechinger 42784 (M)  |
| <i>Marrubium leonuroides</i> Desr.                              | Caucasus: Kislovodsk, in abruptis, I. Akinfiyev 7382 (MSB)   |
| <i>Marrubium litardierei</i> Marmey                             | Marokko: d'Er-Rachidia, Hoher Atlas, Tizi n'Talrent an der Straße von Midelt nach Straßenrand, D. Podlech 47337 (MSB)  |
| <i>Marrubium peregrinum</i> L.                                  | Austria: Nordburgenland, Parndorfer Platte, trockene, grasse Stellen am E-Fuß des Heidl bei Nickelsdorf, ca. 0.45 km SSE des Bahnübergangs am NE-Fuß des Heidl (Kote 133), T. Barta 2004-351 (M)             |
| <i>Marrubium persicum</i> 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)   |
| <i>Moluccella aucheri</i> (Boiss.) Scheen,                      | Iran: Baluchistan, 20 km a Zahedan meridiem versus, K.H. Rechinger 14562 (M)   |
| <i>Moluccella laevis</i> L.                                     | Iraq: Erbil (Kurdistan). In agris demssis inter Mirza Punstam et Pania, ca 500 m. 11230 (M)  |

## 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

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

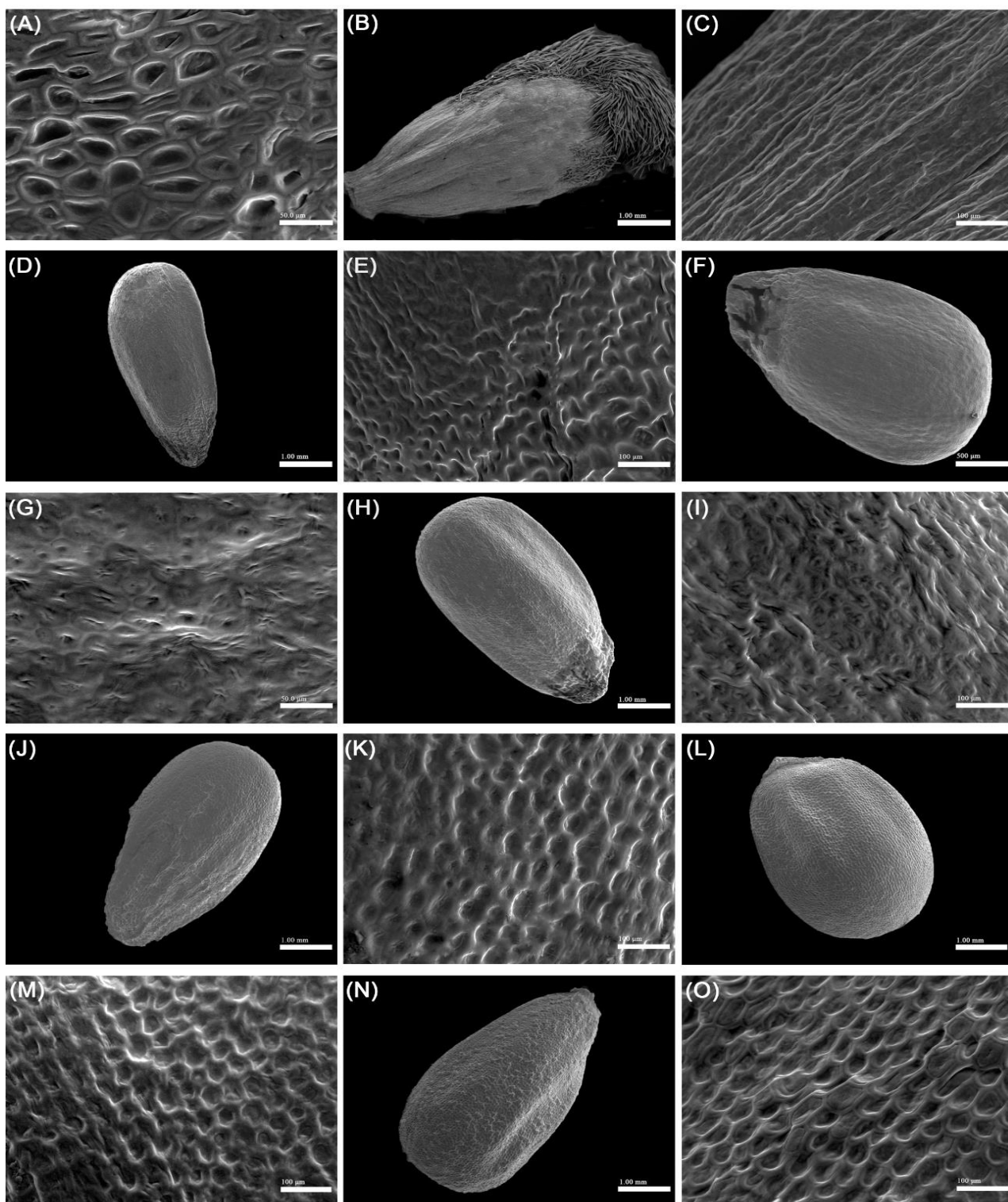
| Taxon  | Nutlet Shape     | Nutlet length (mm) | Nutlet width (mm) | Surface Sculpturing   | Apex Bearded | Outer Periclinal Wall | Anticlinal Wall         | Figures     |
|--|------------------|--------------------|-------------------|-----------------------|--------------|-----------------------|-------------------------|-------------|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

\*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

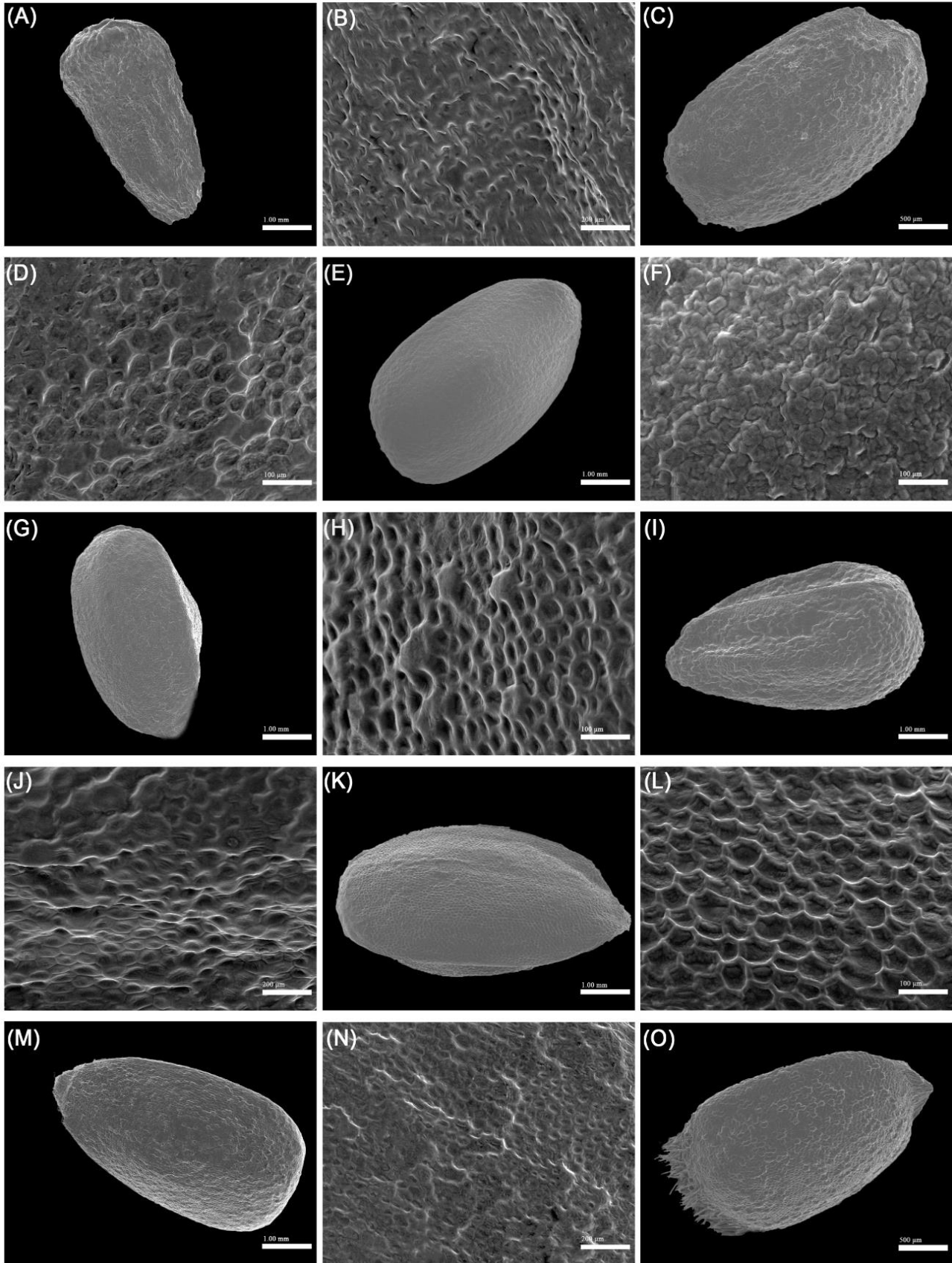


**Fig. 1.** Scanning electron micrographs of *Marrubium* and its allied genera. **A.** *Acanthoprasium frutescens*. **B, C.** *A. integrifolium*. **D, E.** *Ballota nigra* subsp. *anatolica*. **F, G.** *B. nigra* subsp. *ruderalis*. **H, I.** *B. platyloma*. **J, K.** *B. saxatilis*. **L, M.** *Pseudodictamnus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

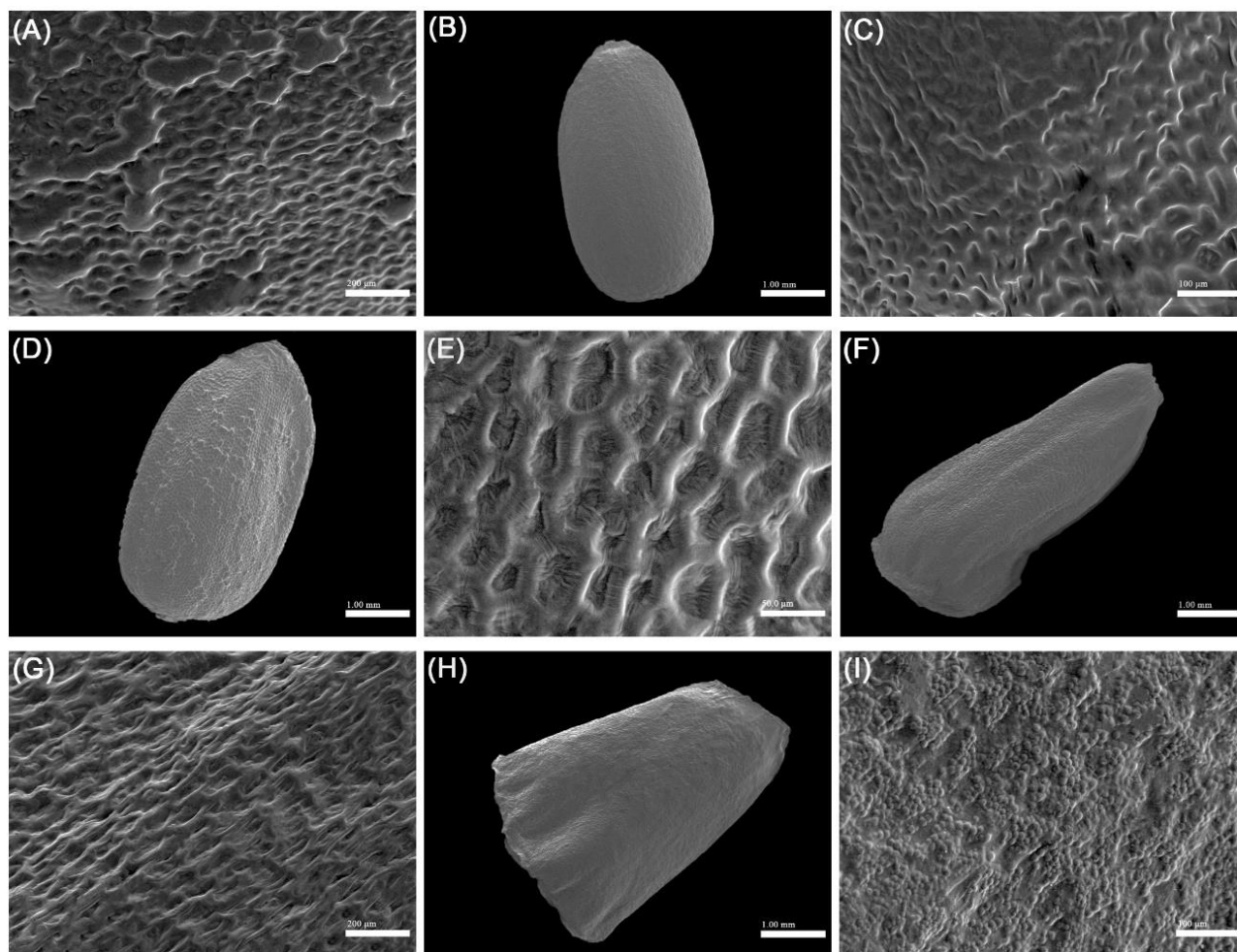
separating large natural groups. Variations in size and the type of sculpturing as well as the shape of its composing cells had been considered to provide the most valuable characters at species level (e.g., Kahraman *et al.*, 2011; Tarimcilar *et al.*, 2013; Salmaki *et al.*, 2008; Eyvazadeh Khosroshahi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

systematic importance in the genus *Marrubium* and its allied genera.



**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 reticulate pattern. While, *A. frutescens* is characterized by elliptic nutlets as well as reticulate pattern of nutlet sculpturing (Fig. 1A), *A. integrifolium* is 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 nutlet sculpturing. *Moluccella aucheri*, distinguished by reticulate pattern of nutlet sculpturing, differs from *Mo. laevis* with colliculate-granulate 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* and *M. persicum*, belonging to *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.

## ACKNOWLEDGEMENT

We are grateful to “Alexander von Humboldt Stiftung” for partial financial support, and the Research Council of the University of Tehran for a grant to Yasaman Salmaki. This research project was also supported by the University of Guilan. The authors would like to thank Mr. Javadi (Shahid Beheshti University) for his assistance in providing the scanning electron micrographs.

## REFERENCES

- Akgül, G., Ketenoglu, O., Pinar, N. M. and Kurt, L. 2008. Pollen and seed morphology of the genus *Marrubium* (Lamiaceae) in Turkey. – *Ann. Bot. Fennici* 45: 1–10.
- Bendiksby, M. Thorbek, L., Scheen, A.-C., Lindqvist, C. and Ryding, O. 2011. An updated phylogeny and



- classification of Lamiaceae subfamily Lamioideae. – Taxon 60: 471-484.
- Bentham, G.** 1832-1836. Labiatarum genera et species. – James Ridgway & Sons, London, 582 pp.
- Bojnanský, V. and Fargašová, A.** 2007. Atlas of seeds and fruits of central and east European flora: the Carpathian Mountains region. – Springer Science & Business Media, Netherlands, 1046 pp.
- Davis, P.H.** 1982. Flora of Turkey and east Aegean islands 7. Edinburgh University Press, Edinburgh, 964 pp.
- Demissew, S. and Harley, M.M.** 1992. Trichome, seed surface and pollen characters in *Stachys*, Lamioideae (Labiatae) in tropical Africa. In: Harley RM, Reynolds T (eds) advances in Labiatae science. – Royal Botanic Gardens, Kew. 149-166.
- Eyvazadeh Khosroshahi, E. and Salmaki, Y.** 2018. Nutlet micromorphology and its systematic implications in *Phlomooides* Moench. – Nova Biol. Rep. 5: 82-94.
- Guerin, G. R.** 2005. Nutlet morphology in *Hemigenia* R. BR. *Microcorys* R. BR. (Lamiaceae). – Pl. Syst. Evol. 254: 49-68.
- Harley, R.M., Atkins, S., Budantsev, A.L., Cantino, P.D., Conn, B.J., Grayer, R., Harley, M.M., De Kok, R., Krestovskaya, T.V., Morales, R., Paton, A.J., Ryding, O. and Upson, T.** 2004. Labiatae. – in: Kubitzki, K. & Kadereit, J.W. (eds.), The families and genera of vascular plants, 7: 167-275 – Berlin, Heidelberg: Springer.
- Hassan, S. and Al-Thobaiti, A.** 2015. Morphological nutlet characteristics of some Lamiaceae taxa in Saudi Arabia and their taxonomic significance. – Pak. J. Bot. 47: 1969-1977.
- Husain, S.Z., Marin, P.D., Šilic, C., Qaiser, M. and Petković, B.** 1990. A micromorphological study of some representative genera in the tribe Saturejeae (Lamiaceae). – Bot. J. Linn. Soc. 103: 59-80.
- Kahraman, A., Celep, F., Doğan, M., Guerin, G.R. and Bagherpour, S.** 2011. Mericarp morphology and its systematic implications for the genus *Salvia* L. section *Hymenosphace* Benth. (Lamiaceae) in Turkey. – Plant Syst. Evol. 292: 33-39.
- Krawczyk, K. and Glowacka, K.** 2015. Nutlet micromorphology and its taxonomic utility in *Lamium* L. (Lamiaceae). – Plant Syst. Evol. 301: 1863-1874.
- Marin, P.D., Petković, B. and Duletić, S.** 1994. Nutlet sculpturing of selected *Teucrium* species (Lamiaceae): a character of taxonomic significance. – Plant Syst. Evol. 192: 199-214.
- Moon, H. K. and Hong, S.P.** 2006. Nutlet morphology and anatomy of the genus *Lycopus* (Lamiaceae. Mentheae). – Pl. Res. J. 119: 633-644.
- Moon, H. K. Hong, S.P. Smets, E. and Huysmans, S.** 2009. Micromorphology and character evolution of Nutlets in tribe Mentheae (Nepetoideae, Lamiaceae). – Sys. Bot. 34: 760-776.
- Mosquero, A.M. and J. Pastor, R.J.Y.** 2007. Morfología y anatomía de núculas de *Marrubium* (Lamiaceae) en el suroeste de España. – Lagasalia 27: 23-29.
- Navarro, T. and El-Oualidi, J.** 2000. Trichomes morphology in *Teucrium* L. (Labiatae), A taxonomic review. – An. Jard. Bot. Madrid 57: 277-297.
- Oran, S.A.** 1996. Ultrastructure of nutlet surface of the genus *Salvia* L. in Jordan and the neighbouring countries. – Dirasat. Nat. Eng. Sci. 23: 393-408.
- Özkan, M., Aktaş, K., Özdemir, C. and Guerin, G.** 2009. Nutlet morphology and its taxonomic utility in *Salvia* (Lamiaceae: Mentheae) from Turkey. – Acta Bot. Croat. 68: 105-115.
- Patzak, V.A.** 1958. Revision der Gattung *Ballota* Section *Ballota*. – Ann. Naturhist. Mus. Wien. 62: 57-86.
- Ryding, O.** 1995. Pericarp structure and phylogeny of Lamiaceae- Verbenaceae complex. – Plant Syst. Evol. 198: 101-141.
- Ryding, O.** 1998. Phylogeny of the *Leucas* Group (Lamiaceae). – Syst. Bot. 23: 235-237.
- Salmaki, Y., Zarre, S. and Jamzad, Z.** 2008. Nutlet morphology of *Stachys* (Lamiaceae) in Iran and its systematic implication. – Feddes Repert. 119: 631-645.
- Satıl, F. Kaya, A, Akçiçek, E. and Dirmenci, T.** 2012. Nutlet micromorphology of Turkish *Stachys* sect. *Eriostomum* (Lamiaceae) and its systematic implications. – Nordic J. Bot. 30: 352-364.
- Scheen, A.-C Bendiksbj, M., Ryding, O., Mathiesen, C., Albert, V.A. and Lindqvist, C.** 2010. Molecular phylogenetics, character evolution and suprageneric classification of Lamioideae (Lamiaceae). – Ann. Missouri Bot. Gard. 97: 191-219.
- Seybold, S.** 1978. Revision der Persischen *Marrubium*-Arten (Labiatae) Vorarbeiten zur Flora Iranica. – Stuttgarter Beitr. zur Naturkunde Ser. A (Biol.) 310: 1-31.
- Siadati, S. Salmaki, Y., Saeidi Mehrvarz, Sh., Heubl, G. and Weigend, M.** 2018. Untangling the generic boundaries in tribe Marrubieae (Lamiaceae: Lamioideae) using nuclear and plastid DNA sequences. – Taxon 67: 770-783.
- Stearn, W.T.** 1983. Botanical Latin, Chapter XXV. – David & Charles, Newton Abbot, London and North Pomfret, Vermont, 153 pp.
- Tarimcilar, G. Yilmaz, Ö., Daşkin, R. and Kaynak, G.** 2013. Nutlet morphology and its taxonomic significance in the genus *Mentha* L. (Lamiaceae) from Turkey. – Bangladesh J. Plant Taxon. 20: 9-18.

\*\*\*\*\*

**How to cite this article:**

**Siadati, S., Saeidi Mehrvarz, S. and Salmaki, Y.** 2019. Nutlet micromorphology of the genus *Marrubium* L. and allies and its systematic implication (Lamiaceae: tribe Marrubieae). – Nova Biol. Reperta 6: 338-346.