

Research article

Nutlet micromorphology and its taxonomic significance in *Lamium* sect. *Amplexicaule* (Lamiaceae)

Çiğdem CAN ÖZKER^{1,*}, Zeynep ATALAY^{2,*}, Ferhat CELEP^{1,*}

¹Kırıkkale University, Department of Biology, Kırıkkale, Turkey

²TÜBİTAK, Kavaklıdere, Ankara, Turkey

*Corresponding authors e-mail: cigdemcank@gmail.com, ferhat_celep@hotmail.com

Abstract: In *Lamium*, species-level identification is usually very difficult, particularly in closely related species. Here, we investigated nutlet micromorphological characteristics of five taxa belonging Lamium sect. *Amplexicaule* (*L. amplexicaule*, *L. aleppicum*, *L. eriocephalum* subsp. *eriocephalum*, *L. eriocephalum* subsp. *glandulosidens*, *L. macrodon*) and additional data taken from literature for *L. persepolitanum* by stereo-binocular microscopy and scanning electron microscopy to show their taxonomic value. The size of nutlets varies from 2.12 mm (*L. amplexicaule*) to 4.27 mm (*L. eriocephalum* subsp. *glandulosidens*) in length, 1.13 mm (*L. amplexicaule*) to 2.22 mm (*L. eriocephalum* subsp. *glandulosidens*) in width. The basic shape of nutlets is oblong, oblong-ovate, obovate and triangular. Five nutlet sculpturing patterns are present namely reticulate, penta-hegzagonal reticulate, penta-hegzagonal colliculate, verrucate, verrucate with reticulate pattern. The results shown that the variation of the nutlet surface sculpturing pattern, nutlet shape, size, and macromorphological characters provide useful diagnostic properties at species level.

Keywords: Lamium, Lamiaceae, nutlet, taxonomy, Scanning Electron Microscopy.

Citation: Can Özker, Ç., Atalay, Z., & Celep, F. (2023). Nutlet micromorphology and its taxonomic significance in *Lamium* sect. *Amplexicaule* (Lamiaceae). *Acta Biologica Turcica*, 36(1), F4:1-7.

Introduction

Lamium L. is the type genus of Lamiaceae (mint family). Within Lamiaceae, *Lamium* is a member of the subfamily Lamioideae, and distributed in Eurasia (especially Türkiye), North Africa (including Macaronesia), and Central and East Asia (Harley et al. 2004). Europe and Türkiye together encompass about 90% of the diversity within the genus (Mennema 1989). *Lamium* typically occur in forest understories, rocky mountain slopes and screes, and cultivated field habitats (Mennema 1989).

Delimitation of *Lamium* from its related genera has been a significant challenge in the taxonomic history of the genus. Due to the absence of recognizable morphological characters, circumscription of the genus has undergone many changes through time. Several East

Asian labiates with uncertain generic position have been placed in *Lamium*, which served as a repository genus (Mennema 1989, Atalay 2016a, 2016b). According to the recent morphologic and phylogenetic studies (Ryding 2003, Scheen et al. 2010, Bendiksby et al. 2011, Celep et al. 2011, Atasagun et al. 2015, Salmaki et al. 2015, Krawczyk et al. 2014), the number of species is constantly changing. According to the Plant List (Govaerts et al. 2010), 24 species (42 taxa; including species, subspecies and varieties) of *Lamium* have been accepted. However, 275 taxa have been treated in the genus as either synonyms, subspecies, varieties or unresolved names, which clearly illustrates the taxonomic and nomenclatural problems of the genus *Lamium* has.

Türkiye is the main biodiversity center of the genus with 47 taxa, ca. 52% of which are endemic (Mill 1982, Duman 2000). However, in his monograph, Mennema (1989) treated many of the earlier species, distributed both in Türkiye and in the World, as subspecies, varieties or synonyms and reduced the species number to 16 in the world. On the basis of molecular phylogenetic studies, three previously synonymized species (*Lamium aleppicum* Boiss. & Hausskn. ex Boiss., *L. paczoskianum* Vorosch., and *L. armenum* Boiss.) have been resurrected (Bendiksby et al. 2011, Krawczyk et al. 2014). In addition, Celep (2017) described *L. bilgii* Celep and *L. cappadocicum* Celep & Karaer (Celep et al. 2022a), which are closely related to the *L. garganicum* complex, and resurrected *L. ponticum* along with a new subspecies from Türkiye (Celep, 2021).

In the latest monograph of the genus, Mennema (1989) accepted three subgenera, subg. *Orvala*, subg. *Galeobdolon*, and subg. *Lamium*. Within subg. *Lamium*, Mennema (1989) distinguished three sections, sect. *Amplexicaule* Mennema, sect. *Lamium* and sect. *Lamiotypus* Dumortier. In sect. *Amplexicaule*, Mennema (1989) recognized 3 species, *L. amplexicaule*, *L. eriocephalum* and *L. macrodon*, which the species have annual or perennial life form, amplexicaule floral leaves (bracts) and straight corolla tube. Additionally, Mennema (1989) recognized five varieties under *L. amplexicaule* (*L. amplexicaule* var. *amplexicaule*, *L. amplexicaule* var. *bornmuelleri*, *L. amplexicaule* var. *aleppicum*, *L. amplexicaule* var. *orientale*, and *L. amplexicaule* var. *incisum*). *Lamium eriocephalum* subsp. *glandulosidens*, endemic to Türkiye, was treated as a synonym under *L. eriocephalum* by Mennema (1989).

Bendiksby et al. (2011) produced the first comprehensive phylogeny of the genus. In contrast to Mennema (1989), the resurrection of *L. aleppicum* (Syn. *L. amplexicaule* var. *aleppicum*) was suggested by Bendiksby et al. (2011). Additionally, Jamzad (2012) transferred *Stachys persepolitana* Boiss. to the genus *Lamium* as *Lamium persepolitanum* (Boiss.) Jamzad. Subsequently, the taxonomic treatment of Jamzad (2012) was supported by the phylogenetic study of Salmaki et al. (2015), and they suggested that *L. persepolitanum* is closely related to *L. amplexicaule* and the species should be placed in the sect. *Amplexicaule*. Therefore, the sect. *Amplexicaule* now includes five species as *L. amplexicaule*, *L. aleppicum*, *L. eriocephalum*, *L. macrodon* and *L. persepolitanum*.

Nutlet morphology has been shown to be taxonomically significant in the Lamiaceae (Salmaki et al. 2008, Moon et al. 2010; Ryding 1994, 2010, Kahraman et al. 2011, Celep et al. 2014, 2022b). In particular, nutlet sculpturing patterns have been regarded as a very valuable character (Celep et al. 2022b). However, all species examined in *Lamium* have a reticulate sculpturing pattern that is of low diagnostic value (Krawczyk and Glowacka 2015). Cell wall thickenings are of taxonomic importance and mostly distinguish taxa at the level of genus and subfamily (Barthlott 1981). This finding was supported by Krawczyk and Glowacka (2015), as the cell wall thickening observed in the nutlets of *Lamium* divided the genus into two groups. *Lamium amplexicaule* and *L. macrodon* were placed in the same species group. This finding largely corresponds to the phylogenetic lineages recognized in the genus *Lamium* (Krawczyk and Sawicki 2013).

This study aims to give a detailed account of comparative morphological and nutlet micromorphological characteristics of all members of the sect. *Amplexicaule*; *L. amplexicaule*, *L. macrodon*, *L. aleppicum*, *L. eriocephalum* (including the subspecies, *L. eriocephalum* subsp. *eriocephalum* and *L. eriocephalum* subsp. *glandulosidens*), and *L. persepolitanum* based on the new data and literatures.

Material and Methods

Plant material

Since 2012, as a part of the taxonomic revision of the genus *Lamium* in Türkiye, we have carried out extensive field studies and collected a large number of specimens within Türkiye. Additionally, we have examined hundreds of herbarium specimens at ANK, AEF, BM, E, GAZI, HUB, K, KNYA, LE and Z herbaria. Moreover, population sizes and phenological and ecological characteristics were observed in the field. Plant specimens were collected during anthesis from natural populations. Voucher specimens were deposited in Kırıkkale University, and a list of specimens examined is provided in Table 1.

Nutlet Micromorphology

Nutlets of five *Lamium* taxa were examined under a stereomicroscope to ensure that they were mature and of normal size. Morphometric measurements of nutlets were made under the stereomicroscope Leica S8AP0 coupled with a Leica DFC 295 digital camera. For scanning electron microscope (SEM) analyses, selected nutlets were

directly mounted on aluminum stubs with double adhesive tape and coated with gold prior to examination with a JEOL-6060 scanning electron microscope at TPAO (Turkish Petroleum Anonymn Cooperation, Ankara). Length and width of 30 mature nutlets from each taxa were

measured using Carnoy 2.0 (Schols et al. 2002). Minimum-maximum range, mean, and standard deviations of nutlet length and width were calculated. The nutlet terminology follows Stearn (2004).

Table 1. Collection data of *Lamium* specimens examined for nutlet characters. Taxa endemic to Türkiye are indicated by an asterix (*)

Taxon	Locality
<i>L. eriocephalum</i> subsp. <i>eriocephalum</i> Benth. *	Türkiye, Niğde, Aladağlar, 08.07.2013, BB 3913
<i>L. eriocephalum</i> subsp. <i>glandulosidens</i> (Hub.-Mor.) R. R. Mill *	Türkiye, Antalya, Alanya, Gökbel, 13.07.2014, F.Celep 3558
<i>L. amplexicaule</i> L.	Türkiye, Muğla, Fethiye, Babadağ, 18.04.2013, F.Celep 1794
<i>L. aleppicum</i> Boiss. & Hausskn. ex Boiss.	Türkiye, Kahramanmaraş Ahırdağı, 24.04.2013, F.Celep 1836
<i>L. macrodon</i> Boiss. & A. Huet	Türkiye, Antalya, Alanya, Gökbel, 20.04.2013, F.Celep 1811

Results

Morphology

Literature surveys indicated that there are differences between the morphologic terminology used by Mennema (1989) and Mill (1982). According to Mennema (1989), members of the sect. *Amplexicaule* have amplexicaul floral leaves, bracts absent and corolla tube straight. However, Mill (1982) used term ‘bracts’ and ‘bracteol’ instead of “amplexicaule floral leaves” and “bracts”, respectively. In our results and discussion, we followed Mill’s (1982) terminology.

According to our herbarium and field observations as well as previous research (Mill 1982, Mennema 1989), *L. eriocephalum* clearly differs from the other species in the section *Amplexicaule* by its perennial habit, densely tomentose to lanate hairy inflorescence with slightly to deeply palmatifid amplexicaul floral leaves and larger flowers (30-40 mm) (not 9-24 mm in *L. amplexicaule*, *L. macrodon* and *L. aleppicum*, 24-28 mm in *L. persepolitanum*). *Lamium aleppicum* differs from remaining three species (*L. amplexicaule*, *L. macrodon* and *L. persepolitanum*) by its amplexicaule ovate-oblong to oblong floral leaves (Mill 1982). *Lamium macrodon* differs from *L. amplexicaule* and *L. aleppicum* by its setose (not triangular) and 4-10 mm long (not 1.5-4.5 mm) calyx teeth. *Lamium persepolitanum*, which has recently been placed in the section *Amplexicaule* (Salmaki et al. 2015), differs from remaining species in the section by its non-amplexicaule floral leaves, striate corolla tube (no striation on the corolla tube of the other species) and deeply bifid upper lip of corolla (not flat or indistinctly bifid). Therefore, the morphological features of *L.*

persepolitanum does not fully support its inclusion in the sect. *Amplexicaule*.

Nutlet Micromorphology

Mature nutlets of *L. eriocephalum* subsp. *eriocephalum* are 4.07-4.53 mm long (4.31 ± 0.2) and 1.62-1.89 mm wide (1.77 ± 0.05). The nutlet shape is oblong. Surface sculpturing pattern of the mature nutlets is reticulate (Figures a-b). Mature nutlets of *Lamium eriocephalum* subsp. *glandulosidens* are 4.38-4.78 mm long (4.58 ± 0.12) and 2.18-2.34 mm wide (2.24 ± 0.05). The nutlet shape is oblong. Surface sculpturing pattern of the mature nutlets is penta-hegzagonal reticulate (Figures c-d). Mature nutlets of *L. amplexicaule* are 2.13-2.28 mm long (2.18 ± 0.1) and 1.00-1.11 mm wide (1.07 ± 0.03). The nutlet shape is mostly oblong-obovate. The nutlet surface is glabrous and brown with raised white reticulate marbling spots. Surface sculpturing pattern of the mature nutlets is pentangular-hexangular colliculate (Figures e-f). Mature nutlets of *L. macrodon* are 2.18-2.51 mm long (2.30 ± 0.15) and 1.10-1.19 mm (1.15 ± 0.12) wide. The nutlet shape is mostly obovate. The nutlet surface is glabrous and brown with raised white reticulate marbling spots. Surface sculpturing pattern of the mature nutlets is irregularly verrucate (Figures g-h). Mature nutlets of *L. aleppicum* are 2.70-3.16 mm long (2.84 ± 0.12) and 1.15-1.56 mm wide (1.40 ± 0.11). The nutlet shape is mostly obovate. The nutlet surface is glabrous and brown with raised white reticulate marbling spots. Surface sculpturing pattern of the mature nutlets is pentangular-hexangular colliculate (Figures i-j).

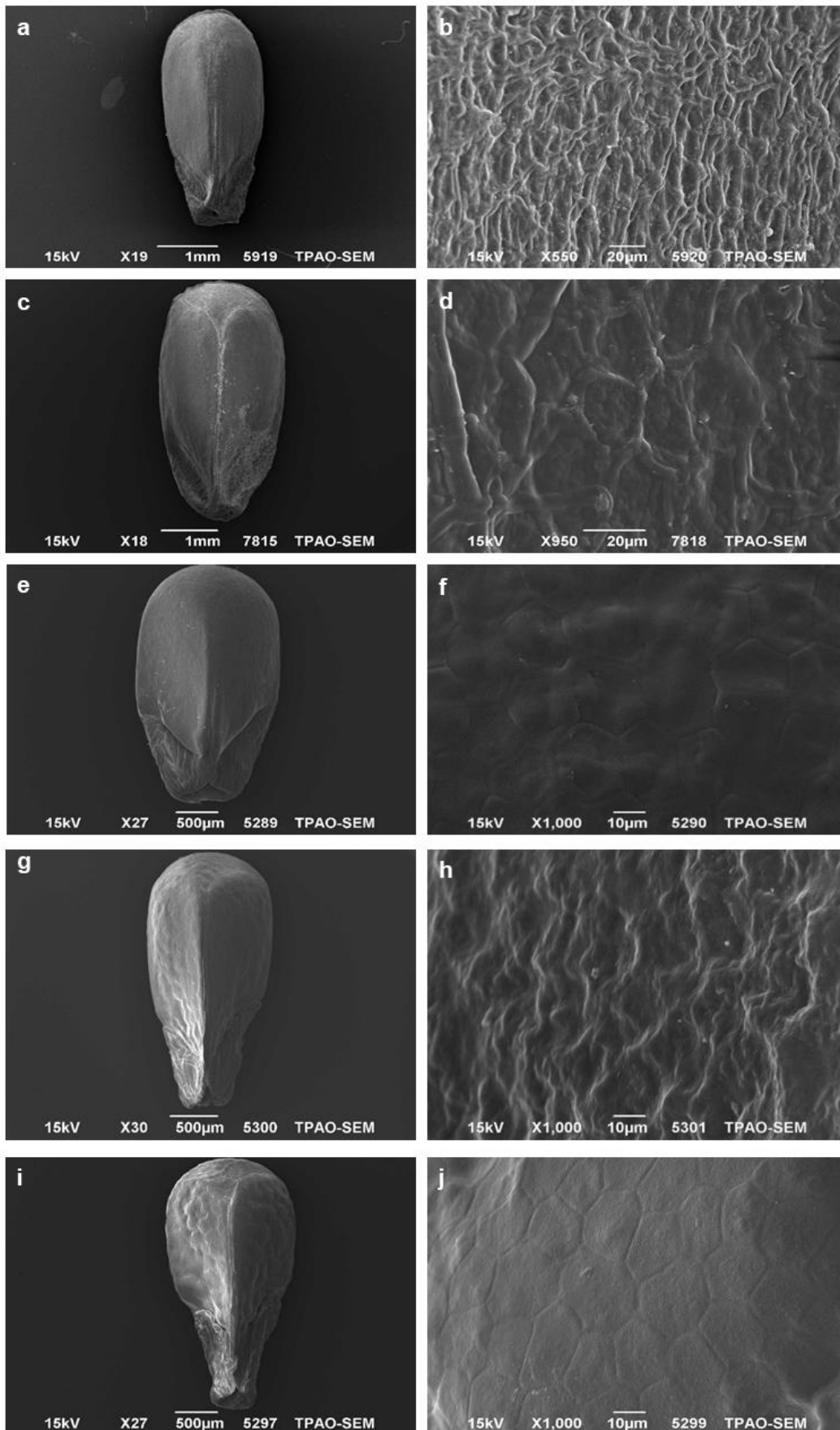


Figure. Nutlet micromorphology of a-b) *L. eriocephalum* subsp. *eriocephalum*, c-d) *L. eriocephalum* subsp. *glandulosidens*, e-f) *L. amplexicaule*, g-h) *L. macrodon*, i-j) *L. aleppicum*

Nutlet characteristics of *L. persepolitanum* was reported by Salmaki et al. (2008). According to their report (Salmaki et al. 2008), mature nutlets of *L. persepolitanum* are 2.50 mm long and 2.12 mm wide. The

nutlet shape is triangular. Surface sculpturing pattern of the mature nutlets is verrucate with reticulate pattern (Table 2).

Table 2. Nutlet characteristics of the examined *Lamium* taxa.

Taxon	Mericarp Shape	Mericarp length (mm) Mericarp width (mm)	Exocarp cell size on the nutlet surface (µm)	Spot	Surface ornamentation
<i>L. eriocephalum</i> subsp. <i>eriocephalum</i>	Oblong	4.00 ± 0.18 (3.80-4.60)	20.41±4.13(10.95-30.12)	absent	reticulate or penta-hegzagonal reticulate
<i>L. eriocephalum</i> subsp. <i>glandulosidens</i>		1.63 ± 0.30(1.32-2.22)			
<i>L. amplexicaule</i>	Oblong- obovate	4.27 ± 0.18 (3.95-4.63)	19.23±5.93(9.96-29.01)	absent	penta-hegzagonal reticulate
<i>L. aleppicum</i>		1.68 ± 0.30(1.32-2.22)			
<i>L. macrodon</i>	Obovate	2.39 ± 0.14(2.12-2.55)	21.94±5.21(15.20-30.86)	present	pentangular-hexangular colliculate
<i>L. persepolitanum</i>		1.16 ± 0.05(1.13-1.24)			
<i>L. persepolitanum</i>	Triangular	2.87 ± 0.14(2.71-3.13)	22.31±6.77(8.17-36.25)	present	pentangular-hexangular colliculate on protuberance and reticulate among the protuberance verrucate with some
<i>L. persepolitanum</i>		1.35 ± 0.15(1.21-1.73)			
<i>L. persepolitanum</i>	Triangular	2.86 ± 0.22(2.51-3.13)	10.95±2.35(8.58-14.95)	present	protuberances in small magnification
<i>L. persepolitanum</i>		1.25 ± 0.10 (1.18-1.38)			
<i>L. persepolitanum</i>	Triangular	2.50 (Salmaki et al. 2008)	-	unknown	verrucate with reticulate pattern
<i>L. persepolitanum</i>		2.12 (Salmaki et al. 2008)	-		

Discussion

The present study aimed to provide information on the morphology and nutlet micromorphology of five *Lamium* taxa (*L. amplexicaule*, *L. macrodon*, *L. aleppicum* and *L. eriocephalum* subsp. *eriocephalum* and *L. eriocephalum* subsp. *glandulosidens*) belonging to the sect. *Amplexicaule*. Moreover, the taxonomic placement of *L. persepolitanum* is discussed based on available morphological and nutlet micromorphological data (Salmaki et al., 2008, 2015).

Bendiksby et al. (2011) pointed out that the infrageneric classification of Mennema (1989) was not corroborated by their molecular phylogenetic results. Our field, herbarium and literature surveys strongly support the suggestion of Bendiksby et al. (2011). In addition, Bendiksby et al. (2011) suggested that “*L. amplexicaule* is polyphyletic” and “*L. aleppicum* should be resurrected”. Though Mennema (1989) and Bendiksby (2011) reported that *L. aleppicum* has narrower leaves, and Mill (1982) reported that *L. aleppicum* has oblong to oblong-ovate bracts (floral leaves), our field studies and population observations pointed out that *L. aleppicum* is morphologically hardly distinguishable from *L. amplexicaule* in field and herbarium specimens.

Our field and herbarium studies indicate that *L. eriocephalum*, *L. amplexicaule*, *L. macrodon* and *L.*

persepolitanum clearly differs morphologically from one another. Particularly, *L. eriocephalum* differs from the remaining sect. *Amplexicaule* members by its perennial habit, densely tomentose to lanate hairy inflorescence with slightly to deeply palmatifid amplexicaul floral leaves and larger flowers (30-40 mm) (not 9-24 mm in *L. amplexicaule*, *L. macrodon* and *L. aleppicum*, 24-28 mm in *L. persepolitanum*). Additionally, *L. eriocephalum* subsp. *glandulosidens* differs morphologically from the type subspecies by its bracts (floral leaves) ovate and palmate divisions 1.5-3.5 mm (not 4-10 mm, very rarely 2.5-3.5), calyx 12-13 mm and teeth subequal with the calyx tube.

Lamium persepolitanum is an endemic Iranian species. The taxonomy and nomenclature of *L. persepolitanum* are quite complicated. The species was first described by Boissier in 1846 as *Stachys persepoliatana* Boiss. from *Persia australis*, Kuh-e Ayup (Ajub) prope Persepolis. Bunge later pointed out that the species should be transferred to the genus *Lamium*. However, Boissier (1879) and Briquet (1897) conserved the name *Stachys perseopolitana*. Nevertheless, Boissier (1879) added a note under *Stachys persepolitanum* as “*an suadente cl. a Bunge potius Lamii species?*”. Independently, two herbarium specimens on the one herbarium sheet in the Kew herbarium were labelled as *Lamium persepolitanum*

Bunge, which were collected by Stapf from S. Persia, Shiraz, wood at foot of Kuh-i-Bil, 19-20 May 1885. Without any doubt, these specimens were previously described species *Stachys persepolitana*, however we could not find any literature describing whether the species was transferred from *Stachys* to *Lamium* or the species author was Bunge. It means that *L. persepolitana* Bunge is a *nomen nudum*. In addition to this, Parsa (1948) did not accept the name *L. persepolitana* Bunge and described the new species as *Lamium iranicum* using the same Stapf's specimens in 1948.

In the *Lamium* account of Flora of Iran, Jamzad (2012) transferred the *Stachys persopolitana* Boiss. to *Lamium* as *Lamium persepolitana* (Boiss.) Jamzad and Salmaki et al. (2012) made a lectotypification. Most recently, Salmaki et al. (2015) confirmed that the species should be placed in the genus *Lamium* based on the molecular phylogenetic and morphological data, and suggested that it should be placed in the section *Amplexicaule*. The overall taxonomic history of the species and the nomenclatural priority indicate that the correct species name should be *Lamium persepolitana* (Boiss.) Jamzad and *L. iranicum* is a synonym of it.

According to previous research (Boissier 1846, 1879, Briquet, Jamzad 2012, Salmaki et al. 2015) and herbarium studies, corolla morphology of *L. persepolitana* is more similar to perennial *L. garganicum* complex as opposed to the annual *L. amplexicaule* (sect. *Amplexicaule*) due to its deeply bifid upper lip of corolla, striate corolla tube and non-amplexicaule floral leaves. On the other hand, *L. persepolitana* differs from perennial *L. garganicum* complex by its annual habit and non-bracteolate verticillasters.

According to nutlet size, *L. eriocephalum* subsp. *eriocephalum* and *L. eriocephalum* subsp. *glandulosidens* are clearly larger than the other taxa in the sect. *Amplexicaule*. The other species (*L. amplexicaule*, *L. macrodon*, *L. aleppicum*) have more or less similar nutlet size. In addition, *L. eriocephalum* has not got spot on the nutlet, however the other species have spot on the nutlet surface. According to nutlet shape, *L. eriocephalum* has oblong, *L. amplexicaule* has oblong-obovate, *L. aleppicum* and *L. macrodon* have obovate and *L. persepolitana* has triangular nutlet shape. Though, nutlet features are not separate *L. macrodon* and *L. aleppicum*, they are easily separated based on calyx teeth shape and size, and bract shape as given in the results.

To sum up, some nutlet characters (i.e. size and surface sculpturing pattern) combined with the analysis of macromorphological features are important in distinguishing species in the sect. *Amplexicaule*.

Acknowledgments

This paper is a part of the master thesis of Çiğdem Can Özker (Kırıkkale University). We thank the curators of following herbaria ANK, AEF, GAZI, HUB, E, K, BM, G, and L for allowing us to study their *Lamium* collections. We thank the editor and two anonymous reviewers for their valuable comments.

Ethical Approval

No need to ethical approval for this study.

Funding Statement

We wish to thank the Scientific and Technical Research Council of Türkiye (Project Number: 112 T 131, TÜBİTAK) for their financial assistance.

Conflict of Interest

The authors declare that they have no conflict of interest.

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