Nutlet micromorphology and its taxonomic utility in *Lamium* L. (Lamiaceae)

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Abstract This paper presents results of examination of fruit surface in the genus *Lamium* L. (Lamiaceae) using scanning electron microscopy (SEM). The purpose of this study was to define new features that differentiate taxa difficult to identify on the basis of macromorphological features, such as species of hybrid origin and subspecies within *Lamium*. The results of our research show that the SEM analysis of fruit surface allowed defining new diagnostic features for almost all studied representatives of the genus *Lamium* except for *L. confertum* from *L. coutinhoi* and *L. album* subsp. *album* from *L. album* subsp. *barbatum*. The most useful feature in distinguishing the examined species was cuticle striation. In turn, the type of secondary sculpturing of the cell walls divided the species studied into two main groups. One of them consisted of four taxa (*L. album*, *L. flexuosum*, *L. garganicum*, *L. orvala*) with thickenings of the cell walls in the form of irregular ridges and tubercles. The other one comprised all the remaining species, with cell wall thickenings forming regular papillae. The only species which could not be qualified to any of these groups was *L. moschatum*. The obtained results demonstrated also high similarity of *L. galeobdolon* to the remaining species of *Lamium*, especially *L. flexuosum* and

L. orvala. This observation provides an argument for the inclusion of *L. galeobdolon* to the genus *Lamium*.

Keywords *Lamium* · Taxonomy · Nutlet · SEM · Micromorphology · Lamiaceae

Introduction

Dead-nettle *Lamium* L. (Lamiaceae) is a genus comprising 25 species of herbaceous plants (Govaerts et al. 2010). Morphological diversity within this genus is relatively low. Representatives of particular species differ in details of flower structure, leaf shape or their arrangement on the stem. Part of the taxa distinguished in *Lamium* are difficult to identify because of the very subtle morphological features that differentiate them. This particularly applies to *L. incisum* and *L. confertum*, which are polyploidal species of hybrid origin (Jørgensen 1927; Taylor 1991; Dvořáková 2000). *L. incisum* is easy to confuse with *L. purpureum*, while *L. confertum* shows a very high morphological similarity to *L. amplexicaule* (Szafer et al. 1986; Dvořáková 2000). There are also difficulties with the delimitation of some subspecies distinguished within *Lamium*. For instance, one of the controversial taxa is *L. garganicum* subsp. *laevigatum*, distinguished on the basis of pubescence and the shape of the upper lip of the corolla (Arcangeli 1882; Ball 1972), but combined into one taxon with *L. garganicum* subsp. *garganicum* by some researchers (Mennema 1989; Govaerts et al. 2010). Also the systematic order within the genus *Lamium* is a disputable issue. On the basis of morphological features, the genus is divided into three subgenera: *Lamium*, *Orvala* and *Galeobdolon* (Mennema 1989). In the biggest one—subgenus *Lamium*, there are three sections: *Amplexicaule*, *Lamiotypus* and

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Lamium. The division into subgenera has been set taking into account: pubescence of anthers, shape and pubescence of calyx, shape of corolla tube and locations of floral leaves. Division of subgenus *Lamium* into three sections also relies on the shape of corolla tube and the presence or absence of floral leaves. Recently, this classification, determined solely on the basis of morphological features, has been undermined by phylogenetic studies (Bendiksby et al. 2011), however a new division has not yet been proposed.

Since macromorphological features are often an insufficient criterion for identification of some representatives of *Lamium*, and they fail to fully reflect phylogenetic relationships within the genus, we decided to search micromorphological features characteristic for the representatives of *Lamium*. According to the study conducted on a group of about 5,000 species of plants, a very valuable taxonomic criterion at the level of species, order, and family is the sculpturing of the seed surface (Barthlott and Voigt 1979; Barthlott 1981). The usefulness of mericarp morphology, anatomy and pericarp structure in taxonomy was also proved by numerous studies carried out in Lamiaceae at various taxonomical levels (Husain et al. 1990; Demissew and Harley 1992; Ryding 1992, 1994, 1995; Marin et al. 1994, 1996; Oran 1996; Budantsev and Lobova 1997; Jamzad et al. 2000; Guerin 2005; Moon and Hong 2006; Salmaki et al. 2008; Kahraman et al. 2011; Satil et al. 2012). For instance, Marin et al. (1994) and Ryding (1995) concluded that nutlet characters were potentially useful within Lamiaceae at the level of section, genus and species. Studies on *Stachys* L. showed that nutlet micromorphological characters appear to be useful in distinguishing infrageneric groups (Demissew and Harley 1992) and in the species-level taxonomy (Satil et al. 2012). In *Salvia* L., one of the largest genera of Lamiaceae, it has been shown that gross morphology of nutlets and their sculpturing pattern are variable and taxonomically useful at a species level (Oran 1996; Kahraman et al. 2011). In all these studies, the analysis based on scanning electron microscopy (SEM) has been successfully applied.

The purpose of this study was to provide a detailed description of fruit micromorphology in *Lamium* by using SEM and to evaluate the systematic significance of such characteristics in the light of the variation between taxa of the genus. The observed variability was also analyzed in relation to the evolutionary history of this genus.

The subject of our research was fruit characteristic for the genus *Lamium*, i.e., schizocarp splitting apart into four mericarps (nutlets). Each of them contains one seed covered with a dry, nonsplitting, leathery pericarp. The mericarps are ovoid, flat at the apex, triangular. They possess elaiosomes, the presence of which indicates adaptation to seed dispersal by myrmecochory (Dvořáková 2000).

Morphological variation of fruits is observed between species with respect to fruit size, color and the presence or absence of convex spots on their surface. Micromorphological variability of fruits in the genus *Lamium* has not been widely studied until now. To date this variability has been described only for the subspecies of *L. galeobdolon* (L.) L. (Czarna and Bednorz 2011), which by some taxonomists (e.g., Dvořáková 2000; Mirek et al. 2002; Rothmaler 2007) is excluded to the separate genus as *Galeobdolon luteum* Huds. Identification of *L. galeobdolon* is based on three main morphological features distinguishing it from other *Lamium* species: color of the corolla, the number and shape of lobes in the corolla's lower lip (Sychowa 1967; Ball 1972; Szafer et al. 1986; Krawczyk et al. 2013). It is debatable whether those key features are sufficient to place this taxon in separate genera. Opinions are divided, and taxonomists take different approaches to the problem. The comparison of nutlet micromorphology of *L. galeobdolon* with other *Lamium* species will enable determining whether the fruits of this species stand out against the tested genus.

Materials and methods

The study included 19 taxa of the genus *Lamium*, belonging to 14 species (Table 1). In the case of *L. album* subsp. *album*, *L. amplexicaule* and *L. purpureum*, the research material was collected in the course of our own field study. Other fruits were obtained from herbarium specimens, kindly provided by domestic and foreign herbaria: BR, COA, GJO, H, L, TUR. Three to four fully developed, mature and dry nutlets were examined for each taxon after a number of specimens had been compared under a light microscope for similarity. Nutlet length and width (Table 2) were measured in 20 samples per specimen of each taxon. The terminology of nutlet micromorphology was adopted after Barthlott (1981) and Salmaki et al. (2008).

Observations were done under Nikon SMZ1000 light microscope equipped with Nikon Digital Sight camera. Then, 3–4 *Lamium* fruits of each taxa were mounted on copper tables, and sputter-coated with gold in argon atmosphere (Fine coater JFC-1200, JEOL). Mericarp texture was imaged by a scanning electron microscope (JSM 5310LV, JEOL) at 15 kV and analyzed by NSS Version 3.0 (EDS Noran System 7.0, Thermo Scientific). Light and electron microscopy allowed observing the structure of fruit surface but it was not possible to determine the origin of the protuberances present in mericarps of some species, macroscopically observed as whitish spots. Suspecting that these are thickenings of the cuticular layer, we performed cuticle and cutin staining according to the methodology of

Table 1 Information about specimens used in the study; country of origin, year of collection, voucher information

Species	Country	Year of collection	Barcode number
<i>L. album</i> subsp. <i>album</i> L.	Poland	2010	–
<i>L. album</i> subsp. <i>barbatum</i> (Siebold and Zucc) Mennema	Japan	1968	L 0790173
<i>L. amplexicaule</i> L.	Poland	2009	–
<i>L. bifidum</i> subsp. <i>balcanicum</i> Velen	Greece	1980	BR 549820
<i>L. bifidum</i> subsp. <i>bifidum</i> Cirillo	France	1990	BR 5499274
<i>L. confertum</i> Fr.	Finland	1988	H 675201
<i>L. coutinhoi</i> J.G.Garcia	Portugal	2004	COA 33918
<i>L. flexuosum</i> Ten.	Italy	1968	TUR 170353
<i>L. garganicum</i> subsp. <i>corsicum</i> (Gren. and Godr.) Mennema	Italy	1978	L 0790158
<i>L. garganicum</i> subsp. <i>garganicum</i> L.	Austria	2008	GJO 49925
<i>L. garganicum</i> subsp. <i>laevigatum</i> (Ces., Pass. and Gibelli) Arcang.	France	1973	H 1134555
<i>L. garganicum</i> subsp. <i>striatum</i> (Sm.) Hayek	Serbia	1968	TUR 265431
<i>L. gevorense</i> (Gómez Hern.) Gómez Hern. and A.Pujadas	Spain	2005	COA 33931
<i>L. incisum</i> Willd.	Finland	2009	TUR 394510
<i>L. macrodon</i> Boiss. and A.Huet	Turkey	1983	H 1490027
<i>L. maculatum</i> (L.) L.	Romania	1988	TUR 297792
<i>L. moschatum</i> Mill.	Greece	1997	H 1695935
<i>L. orvala</i> L.	Slovenia	1970	TUR 246641
<i>L. purpureum</i> L.	Poland	2010	–

Wędzony (1996), after Heslop-Harrison (1977). Dried seeds of *Lamium* were placed for 20 min in auramine O solution for cuticle localization (lit). The characteristic fluorescence (excitation 405 nm, emission 470–520 nm) of cuticle was analyzed using a laser scanning microscope (LSCM, SP5, Leica), with LA AF Version: 2.4.1 build 6384. We examined fruits of *L. amplexicaule* with numerous spots and *L. flexuosum*, where spots were not observed.

Results

Morphological variation of the pericarp

Evaluation of *Lamium* mericarps under a light microscope revealed that their surface, colored in various shades of brown, was usually spotted with whitish, convex, shiny spots. Depending on the species, spots occurred singly or in large numbers, covering almost the entire surface of the fruit. Total absence of these spots was found only in the case of two species: *L. flexuosum* and *L. orvala*. Analysis of the smooth and spotted surface of fruits, carried out using confocal microscopy and appropriate staining, revealed cutin saturated cell walls and cuticular thickening present on their surface (Fig. 1). It was found that the protrusions seen as spots emitted fluorescent light, similarly to

thickened parts of the cell wall in the area of smooth surfaces. It allows concluding that the observed structures were probably cuticular platelets.

Observation of fruit surface using SEM showed that the outer layer of the pericarp of species studied was formed by approximately isodiametric, penta- to octagonal cells, forming reticulate surface sculpturi. *L. moschatum* was the only species where elongated cells were observed towards the axis of the mericarp (Fig. 2). Cell boundaries were generally straight or slightly undulated (due to the thickenings on the margins of cell walls). Anticlinal cell walls in most species were slightly channelled, although in some species (e.g., *L. album*, *L. garganicum*), an outline of the cells was difficult to observe because of the rich ornamentation of the surface of the fruit. Anticlinal walls of *L. flexuosum* and *L. orvala* were the only ones that were raised. The surfaces of the outer periclinal cell walls were usually concave, and cells with a flat surface were also observed in several taxa (Table 2). The margins of the periclinal walls were raised or flat. In some species, thickenings of the cell walls were present. They were regular (e.g., *L. garganicum* subsp. *garganicum*), ribbed (e.g. *L. coutinhoi*) or band-like, forming a ring on the periphery of the wall (e.g., *L. maculatum*).

The studied species can be divided into two main groups based on the secondary sculpturing of the cell walls observed. The first group consisted of species which cell wall

Table 2 Characteristic of the pericarp surface of the examined *Lamium* species

Species	Nutlet size (mm)	Cuticular platelets	Cell size (µm)	Cell boundaries	Anticlinal cell walls	Curvature of the outer periclinal cell wall	Margins of the outer periclinal cell wall	Relief of the outer periclinal cell wall	Striation
<i>L. album</i> subsp. <i>album</i>	3.2–3.6 × 1.4–1.6	Few	20–30	Straight, hardly visible	Channelled	Strongly concave	Raised, strongly thickened	Irregular ridges present in selected group of cells	Delicate, arranged radially, on the margins of cell wall
<i>L. album</i> subsp. <i>barbatum</i>	3.1–3.3 × 1.3–1.8	Few	15–30	Straight, hardly visible	Channelled	Strongly concave	Raised, strongly thickened	Irregular ridges present in selected group of cells	Delicate, arranged radially, on the margins of a wall or parallel in its central part
<i>L. amplexicaule</i>	2.1–2.3 × 1.0–1.2	Numerous	15–25	Straight or slightly undulated	Channelled	Concave	Raised	Papillae, numerous especially on the periphery of the wall, sometimes merging with each other	Lack
<i>L. bifidum</i> subsp. <i>balkanicum</i>	2.0–2.2 × 1.2–1.3	Numerous	20–35	Straight	Channelled	Slightly concave or flat	Slightly raised, sometimes bend-like thickened	Very numerous papillae, merging into strands or larger tubercles	Lack
<i>L. bifidum</i> subsp. <i>bifidum</i>	2.1–2.2 × 1.1–1.3	Numerous	20–35	Straight	Channelled	Slightly concave or flat	Flat or slightly raised	Numerous, short papillae, merging into larger tubercles	Lack
<i>L. confertum</i>	2.5–2.6 × 1.4–1.5	Numerous	20–45	Straight or slightly undulated	Channelled	Slightly concave or flat	Flat or slightly raised	Numerous, short papillae, often merging into ribs	Lack
<i>L. coutinhoi</i>	1.3–1.4 × 2.6–2.7	Numerous	20–25	Undulated	Channelled	Strongly concave	Strongly raised, thickenings forming ribs	Numerous, short papillae, often merging into ribs	Lack
<i>L. flexuosum</i>	2.7–3.3 × 1.4–1.7	Lack	20–40	Straight	Raised	Strongly concave	Strongly raised, with band-like thickenings forming a ring	Irregular tubercles in a central part of cell wall	Delicate, arranged mostly in the axial direction
<i>L. garganicum</i> subsp. <i>garganicum</i>	2.4–3.0 × 1.0–1.2	Few	20–30	Straight	Channelled	Strongly concave	Strongly raised, thickened	Thickenings in the form of irregular ridges often occupy the entire surface of the wall	Clearly visible, running radially or in different directions
<i>L. garganicum</i> subsp. <i>striatum</i>	2.4–2.5 × 1.1–1.2	Few	15–25	Straight	Channelled	Concave	Slightly raising, thickened	Part of cells, almost completely filled with margin thickenings	Clearly visible both in concave parts of cell wall and in the raised edges
<i>L. garganicum</i> subsp. <i>corsicum</i>	2.2–2.3 × 1.1–1.3	Few	15–35	Straight or slightly undulated, hardly visible	Channelled	Concave or flat	Slightly raising, thickened	Abundant, irregular protuberances covering up to the entire cell surface	Lack

Table 2 continued

Species	Nutlet size (mm)	Cuticular platelets	Cell size (µm)	Cell boundaries	Anticlinal cell walls	Curvature of the outer periclinal cell wall	Margins of the outer periclinal cell wall	Relief of the outer periclinal cell wall	Striation
<i>L. garganicum</i> subsp. <i>laevigatum</i>	2.8–3.1 × 1.2–1.5	Few	20–25	Straight	Channelled	Concave	Sometimes with band-like thickenings	Thickenings in the form of ridges and merging tubercles, covering up to the entire wall	Lack
<i>L. gevorense</i>	2.5–2.7 × 1.2–1.3	Numerous, sometimes merging	20–30	Straight	Channelled	Concave	Slightly raising	Small papillae of equal size, scattered on the whole surface of the walls	Lack
<i>L. incisum</i>	2.7–2.9 × 1.2–1.3	Numerous, sometimes merging	20–30	Straight or slightly undulated	Channelled	Concave or flat	Slightly raising	numerous papillae of various sizes; often merging with each other, especially on the raised cell margins	Lack
<i>L. macrodon</i>	1.9–2.0 × 0.9–1.0	Very numerous, often merging	15–25	Straight	Channelled	Flat or concave	Flat or slightly raising	Numerous small papillae, mainly in the central part of cell wall	Lack
<i>L. maculatum</i>	3.0–3.6 × 1.6–1.7	Numerous, small	25–30	Straight or slightly undulated	Channelled	Slightly concave	Slightly raising, with band-like thickenings	Numerous, merging papillae, sometimes protuberances covering the entire cell surface	Lack
<i>L. moschatum</i>	2.4–2.8 × 1.2–1.4	Few	10–15 wide 20–35 long	Straight	Channelled	Slightly concave or flat	Slightly raising, with band-like thickenings	A thick, even layer of the cuticle present on the whole cell surface with small depressions in its central part	Lack
<i>L. orvala</i>	3.9–4.5 × 1.6–1.9	Lack	20–30	Slightly undulated	Raised	Strongly concave	Raised	Some cells filled with irregular protrusions in the form of folds	Irregular, clearly visible
<i>L. purpureum</i>	1.5–1.9 × 0.8–0.9	Numerous	10–35	Straight	Channelled	Slightly concave or flat	Flat or slightly raised	Numerous, regular, big papillae	Lack

Fig. 1 Fruit surface of *Lamium amplexicaule* (a, b, c) and *L. flexuosum* (d, e, f) observed in light microscope (a, d) and laser scanning microscope (b, c, e, f). Wax platelets visible in b and c are

marked with orange circles. Parts b and e present cuticle fluorescence observed for different values of Z axis than figures c and f

thickenings formed regular papillae (*L. amplexicaule*, *L. bifidum*, *L. confertum*, *L. coutinhoi*, *L. gevorense*, *L. incisum*, *L. macrodon*, *L. maculatum*, *L. purpureum*). The other group comprised taxa with thickenings of the cell walls in the form of irregular ridges and tubercles (*L. album*, *L. flexuosum*, *L. garganicum*, *L. orvala*). *L. moschatum* did not fit into any of these groups, because the surface of the mericarps was formed by cell walls covered with a thick, even layer of the cuticle, with some slight depressions present in the central part of the cell wall. Taxa of the second group, excluding *L. garganicum* subsp. *garganicum corsicum* and *L. garganicum* subsp. *laevigatum*, had additional cuticle striations. Depending on the taxon, they were located on the margin of a cell wall or in a central portion and extended radially, parallelly or irregularly to the axis of mericarp (Table 2).

Diagnostic features of subspecies

A careful analysis of sculpturing on the surface of fruits enabled defining new diagnostic features that facilitate correct identification of similar taxa. Subspecies of *L. garganicum* had an easily observable feature, i.e., the

presence or absence of striation. In addition, individual taxa differed in the shape of the periclinal wall and the structure of thickenings present on this wall. The difference in ornamentation of the fruits was also observed in two subspecies of *L. bifidum*. Subspecies *balcanicum* had groups of cells with band-like thickened edges and low thickenings in the form of papillae as well as areas of cells densely covered with thickenings in the form of ridges (Fig. 3). In subspecies *bifidum*, there were no band-like thickenings on the margins of the walls. Surface of periclinal walls was covered with tubercles to a various extent, but they did not form ridges (Fig. 3). There were no micromorphological features observed in *L. album* that would allow identification of the distinguished subspecies.

Diagnostic features of species of hybrid origin

Comparison of sculpturing of nutlets of *L. incisum* and *L. purpureum* enabled defining traits that distinguish these two species. Papillae of different sizes were found on the surface of the pericarp cells in *L. incisum* that sometimes merged into larger tubercles. They were located mainly on the periphery of the external periclinal cell walls (Fig. 4).

Fig. 2 SEM micrographs of mericarp surfaces in some taxa of *Lamium*: *L. maculatum* (a, b, c), *L. moschatum* (d, e, f), *L. orvala* (g, h, i), *L. purpureum* (j, k, l)

In *L. purpureum*, the papillae were large and evenly distributed over the entire surface of the cell wall (Fig. 2), which differentiated this species from *L. incisum*.

Features that distinguish *L. confertum* from *L. amplexicaule* were also identified. In these species they were also related to the protuberances on the surface of cells. Papillae of various sizes were present in *L. confertum*, merging on the edges of cells into ribbed structures (Fig. 5). *L. amplexicaule* had specific small papillae of

equal size, distributed fairly evenly but they were slightly more numerous on the margins of the cell walls (Fig. 3).

When discussing micromorphological features specific for mericarps of *L. confertum*, one should pay attention to their very strong resemblance to the fruits of *L. coutinhoi*. The structures observed on the surface of mericarps of both species were similar to each other to such a degree that the distinction of species based on these traits was, in our opinion, impossible (Fig. 5).

Fig. 3 SEM micrographs of mericarp surfaces in some taxa of *Lamium*: *L. album* subsp. *album* (a, b, c), *L. album* subsp. *barbatum* (d, e, f), *L. amplexicaule* (g, h, i), *L. bifidum* subsp. *balcanicum* (j, k, l), *L. bifidum* subsp. *bifidum* (m, n, o)

Fig. 4 SEM micrographs of mericarp surfaces in some taxa of *Lamium*: *L. garganicum* subsp. *laevigatum* (a, b, c), *L. garganicum* subsp. *striatum* (d, e, f), *L. gevorense* (g, h, i), *L. incisum* (j, k, l), *L. macrodon* (m, n, o)

Fig. 5 SEM micrographs of mericarp surfaces in some taxa of *Lamium*: *L. confertum* (a, b, c), *L. coutinhoi* (d, e, f), *L. flexuosum* (g, h, i), *L. garganicum* subsp. *corsicum* (j, k, l), *L. garganicum* subsp. *garganicum* (m, n, o)

Discussion

Our results showed that *L. flexuosum* and *L. orvala* differed to a large extent from other *Lamium* species. At the same time, a comparison of the characteristics of nutlets of *L. flexuosum* and *L. orvala* to the description of fruits of *L. galeobdolon* (Czarna and Bednorz 2011), indicated a high similarity of these three species. Their common features included a lack of cuticular plates, a lack of cell wall thickening in the form of papillae, the presence of striations and convex anticlinal cell walls. Furthermore, the analysis of pericarp morphology revealed no significant differences between *L. galeobdolon* and the remaining species from the *Lamium* genus. Quite the contrary, there were many common features indicating the similarity of mericarps of *L. galeobdolon* and the other dead-nettles, in particular *L. flexuosum* and *L. orvala*. The observed similarity can be explained by the evolutionary history of the species studied. The results of phylogenetic studies showed that *L. galeobdolon* did not form a separate phylogenetic line in relation to the genus *Lamium* and thereby confirmed that the presence of *L. galeobdolon* in *Lamium* was justified (Krawczyk et al. 2013; Krawczyk and Sawicki 2013). Molecular studies confirmed a close relationship of *L. flexuosum*, *L. galeobdolon* and *L. orvala* (Krawczyk et al. 2014). The infrageneric classification based on macromorphological features also emphasizes the similarity of *L. galeobdolon* and *L. flexuosum* which constitute the subgenus *Galeobdolon*, while *L. orvala* belongs to the monospecific subgenus *Orvala* (Mennema 1989).

The results of molecular studies were congruent with the observed morphological variability of pericarp also with respect to the status of *L. garganicum* subsp. *laevigatum*. The difference noticed in sculpturing of the fruits of this taxon compared to second subspecies of *L. garganicum* is an argument to consider *L. garganicum* subsp. *laevigatum* a separate taxon. This result is consistent with the analysis of variation of barcoding sequences in the genus *Lamium*, which also indicated the distinctiveness of this subspecies (Krawczyk et al. 2014; Krawczyk and Sawicki 2013).

Our findings showed that the analysis of fruit surface with the use of SEM allowed defining new diagnostic features for almost all studied representatives of the genus *Lamium*. Many of the variable nutlet characters have been shown to be diagnostic and of systematic value in other groups of Lamiaceae (Husain et al. 1990; Oran 1996; Salmaki et al. 2008). However particular features were of different taxonomic values depending on the studied group of plants. Among different nutlet characters studied, the type of sculpturing has been considered to provide the most valuable ones (Oran 1996). In *Lamium*, the general nutlet surface patterns described by Salmaki et al. (2008) showed rather low diagnostic value, since all the species studied

within the genus were characterized by more or less reticulate sculpture. Only the thorough analysis of details of pericarp surface sculpturing provided valuable data for delimitation of dead-nettle species.

As noted by Barthlott (1981), cuticle striation is usually a valuable diagnostic feature. However the importance of this character in the identification of taxa higher than species seems to be limited. In turn, cell wall thickenings are both of diagnostic and taxonomic importance. According to Barthlott (1981), these structures most often distinguish taxa at the level of the genus and subfamily. Our results confirmed Barthlott's observations. The type of cell wall thickening observed in the pericarp of *Lamium* divided the species studied into groups, which largely coincided with phylogenetic lines distinguished in the genus studied (Krawczyk and Sawicki 2013). Sculpturing of the cell walls in the form of papillae arose independently in two phylogenetic lines. One of them consists of: *L. amplexicaule*, *L. bifidum*, *L. gevorensense* and *L. incisum*, while the second one comprises: *L. confertum*, *L. coutinhoi*, *L. maculatum* and *L. purpureum*. The papillae also evolved in the species *L. macrodon*, closely related to *L. moschatum*. The lack of visible cell wall thickening in *L. moschatum* may be the result of speciation. However, it cannot be ruled out that the cell wall thickenings are present in this species, but they are invisible due to the thick layer of cuticle on the surface of the fruit.

Conclusion

Nutlet micromorphology provides diagnostic features for almost all representatives of *Lamium* included in this study. One of the most valuable features, useful in distinguishing examined species, is cuticle striation, while cell wall thickenings are both of diagnostic and taxonomic importance. Although the analysis of fruit surfaces does not resolve phylogeny of the genus completely, it confirms to a large extent the picture of phylogenetic relationships derived from molecular studies. The obtained results combined with the analysis of macromorphological features and the results of phylogenetic studies provide a sound basis for creating a new taxonomical classification within the genus *Lamium*. However, in our opinion, a thorough reanalysis of macromorphological features is necessary to establish such a classification.

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References

- Arcangeli G (1882) Compendio della flora italiana; ossia, Manuale per la determinazione delle piante che trovansi selvatiche od inselvatichite nell'Italia e nelle isole adiacenti, Torino. doi:[10.5962/bhl.title.9995](https://doi.org/10.5962/bhl.title.9995)
- Ball PW (1972) *Lamium* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds) Flora Europaea, vol 3. Cambridge, pp 147–148
- Barthlott W (1981) Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nordic J Bot* 1:345–355. doi:[10.1111/j.1756-1051.1981.tb00704.x](https://doi.org/10.1111/j.1756-1051.1981.tb00704.x)
- Barthlott W, Voit G (1979) Mikromorphologie der Samenschalen und Taxonomie der Cactaceae: ein raster-elektronen-mikroskopischer Überblick. *Pl Syst Evol* 132:205–229
- Bendiksby M, Brysting AK, Thorbek L, Gussarova G, Ryding O (2011) Molecular phylogeny and taxonomy of the genus *Lamium* L. (Lamiaceae): disentangling origins of presumed allotetraploids. *Taxon* 60:986–1000
- Budantsev AL, Lobova TA (1997) Fruit morphology, anatomy and taxonomy of tribe Nepeteae (Labiatae). *Edinburgh J Bot* 54:183–216. doi:[10.1017/S0960428600004029](https://doi.org/10.1017/S0960428600004029)
- Czarna A, Bednorz L (2011) New diagnostic features of taxa within *Lamium* subg. *Galeobdolon* (Lamiaceae). *Roczn Akad Roln Poznaniu* 390. *Bot Stec* 15:49–51
- Demissew S, Harley MM (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, pp 149–166. doi:[10.1016/j.flora.2008.11.001](https://doi.org/10.1016/j.flora.2008.11.001)
- Dvořáková M (2000) *Lamium* L.–hluchavka. In: Slavík B (ed) *Květena České Republiky*, vol. 6. Praha, pp 596–603
- Govaerts R, Paton A, Harvey Y, Navarro T (2010) World checklist of Lamiaceae and Verbenaceae. Available at: <http://www.kew.org/wcsp/lamiaceae>. Accessed 21 November 2013
- Guerin GR (2005) Nutlet morphology in *Hemigenia* R.Br. and *Microcorys* R.Br. (Lamiaceae). *Pl Syst Evol* 254:49–68. doi:[10.1007/s00606-005-0311-y](https://doi.org/10.1007/s00606-005-0311-y)
- Heslop-Harrison Y (1977) The pollen–stigma interaction: pollen-tube penetration in *Crocus*. *Ann Bot (Oxford)* 41:913–922
- Husain SZ, Marin PD, Šilic C, Qaiser M, Petković B (1990) A micromorphological study of some representative genera in the tribe *Saturejeae* (Lamiaceae). *Bot J Linn Soc* 103:59–80. doi:[10.1111/j.1095-8339.1990.tb00174.x](https://doi.org/10.1111/j.1095-8339.1990.tb00174.x)
- Jamzad Z, Harley MM, Ingrouille M, Simmonds MSJ, Jalili A (2000) Pollen exine and nutlet surface morphology of the annual species of *Nepeta* L. (Lamiaceae) in Iran. In: Harley MM, Morton CM, Blackmore S (eds) *Pollen and spores: morphology and biology*. Kew, pp 385–397
- Jørgensen CA (1927) Cytological and experimental studies on the genus *Lamium*. *Hereditas* 9:126–136. doi:[10.1111/j.1601-5223.1927.tb03514.x](https://doi.org/10.1111/j.1601-5223.1927.tb03514.x)
- Kahraman A, Celep F, Doğan M, Guerin GR, Bagherpour S (2011) Mericarp morphology and its systematic implications for the genus *Salvia* L. section *Hymenosphace* Benth. (Lamiaceae) in Turkey. *Pl Syst Evol* 292:33–39. doi:[10.1007/s00606-010-0394-y](https://doi.org/10.1007/s00606-010-0394-y)
- Krawczyk K, Sawicki J (2013) The uneven rate of the molecular evolution of gene sequences of DNA-Dependent RNA polymerase I of the genus *Lamium* L. *Int J Molec Sci* 14(6):11376–11391. doi:[10.3390/ijms140611376](https://doi.org/10.3390/ijms140611376)
- Krawczyk K, Korniak T, Sawicki J (2013) Taxonomic status of *Galeobdolon luteum* Huds. (Lamiaceae) from classical taxonomy and phylogenetics perspectives. *Acta Biol Cracov Ser Bot* 55(2):18–28. doi:[10.2478/abcsb-2013-0016](https://doi.org/10.2478/abcsb-2013-0016)
- Krawczyk K, Szczecińska M, Sawicki J (2014) Evaluation of 11 single-locus and seven multi-locus DNA barcodes in *Lamium* L. (Lamiaceae). *Mol. Ecol Res* 14(2):272–285. doi:[10.1111/1755-0998.12175](https://doi.org/10.1111/1755-0998.12175)
- Marin PD, Petković BP, Duletić S (1994) Nutlet sculpturing of selected *Teucrium* species (Lamiaceae): a character of taxonomic significance. *Pl Syst Evol* 192:199–214. doi:[10.1007/BF00986252](https://doi.org/10.1007/BF00986252)
- Marin PD, Duletić S, Petković B (1996) Nutlet ornamentation in selected *Salvia* L. species (Lamiaceae). *Fl Medit* 6:203–211
- Mennema J (1989) A taxonomic revision of *Lamium*. PhD Thesis. Leiden University, Leiden
- Mirek Z, Piękoś Mirkowa H, Zajac A, Zajac M (eds) (2002) Flowering plants and pteridophytes of Poland—a checklist. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków
- Moon HK, Hong SP (2006) Nutlet morphology and anatomy of the genus *Lycopus* (Lamiaceae: Menthae). *J Pl Res* 119:63–644. doi:[10.1007/s10265-006-0023-6](https://doi.org/10.1007/s10265-006-0023-6)
- Oran SA (1996) Ultrastructure of nutlet surface of the genus *Salvia* L. in Jordan and the neighbouring countries. *Dirasat Nat Engin Sci* 23:393–408
- Rothmaler W (2007) *Exkursionsflora von Deutschland*. Elsevier, München
- Ryding O (1992) Pericarp structure and phylogeny within Lamiaceae subfamily Nepetoideae tribe Ocimeae. *Nordic J Bot* 12:273–298. doi:[10.1111/j.1756-1051.1992.tb01304.x](https://doi.org/10.1111/j.1756-1051.1992.tb01304.x)
- Ryding O (1994) Pericarp structure and phylogeny of Lamiaceae subfamily Pogostemonoideae. *Nordic J Bot* 14:59–63. doi:[10.1111/j.1756-1051.1994.tb00572.x](https://doi.org/10.1111/j.1756-1051.1994.tb00572.x)
- Ryding O (1995) Pericarp structure and phylogeny of Lamiaceae-Verbenaceae complex. *Pl Syst Evol* 198:101–141. doi:[10.1007/BF00985109](https://doi.org/10.1007/BF00985109)
- Salmaki Y, Zarre S, Jamzad Z (2008) Nutlet micromorphology and its systematic implication in *Stachys* L. (Lamiaceae) in Iran. *Feddes Rept* 119:607–621. doi:[10.1002/fedr.200811187](https://doi.org/10.1002/fedr.200811187)
- Satıl F, Kaya A, Akçiçek E, Dirmenci T (2012) Nutlet micromorphology of Turkish *Stachys* sect. *Eriostomum* (Lamiaceae) and its systematic implications. *Nordic J Bot* 30:352–364. doi:[10.1111/j.1756-1051.2011.01306.x](https://doi.org/10.1111/j.1756-1051.2011.01306.x)
- Sychowa M (1967) *Galeobdolon* Adans. In: Pawłowski B (ed) *Flora Polska, Rośliny Naczyniowe Polski i Ziemi Ościennych*. Instytut Botaniki Polskiej Akademii Nauk, Warszawa-Kraków
- Szafer W, Kulczyński S, Pawłowski B (1986) *Rośliny Polskie*. Wydawnictwo Naukowe PWN, Warszawa
- Taylor R (1991) The origin of *Lamium hybridum*, a case study in the search for the parents of hybrid species. *Northw Sci* 65:116–124
- Wędzony M (1996) *Mikroskopia fluorescencyjna dla botaników*. PAN Zakład Fizjologii Roślin im. Franciszka Górskiego, Kraków