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Taxonomic and ecological significance of seed morphology and ultrastructure in species of *Lotus* formerly classified in *Dorycnium* (Leguminosae – *Loteae*)

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Summary. Seed morphology and ultrastructure of *Lotus* species from sections *Dorycnium* (*L. dorycnium* and *L. graecus*), *Bonjeanea* (*L. rectus*, *L. hirsutus* and *L. strictus*) and *Lotus* (*L. corniculatus*) have been studied using light and scanning electron microscopy. The seeds of studied species have a conservative structure typical for most *Papilionoideae*. Seed size, shape and color only partially allow distinguishing species in the studied group. The most morphologically distinguishable seeds are those of members of the polyphyletic section *Bonjeanea*, with *L. rectus* having the smallest and *L. strictus* the largest seeds. Seed size ranges in the species of the section *Dorycnium* overlap both between the members of the section and with seeds of *L. hirsutus* (section *Bonjeanea*). Seed surface ultrastructure around the hilum is very stable among studied species, whereas that on the lateral seed side is more variable. The types of surface sculpture on the lateral seed side do not strongly correlate with taxonomic position of species, but some correlations with eco-geographical patterns can be traced. The type of seed surface micromorphology with inconspicuous primary sculpture and thick secondary cuticular deposits was revealed in taxa, which distribution is connected with the Mediterranean region (*L. hirsutus*, *L. dorycnium* subsp. *gracilis* and partially *L. rectus*). Foveolate-papillose primary sculpture with thin secondary cuticular deposits is typical for species occurring in milder climate (*L. corniculatus*, *L. strictus*, *L. graecus*). Studied species can be subdivided into two groups by endosperm thickness. We believe that thin endosperm revealed in *L. strictus* and *L. corniculatus* may be a trait connected with the acceleration of development when spreading from the Mediterranean to the temperate climatic zone.

Таксономическая и экологическая значимость признаков морфологии и ультраструктуры семян видов *Lotus*, включавшихся ранее в *Dorycnium* (Leguminosae – *Loteae*)

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Ключевые слова: микроморфология, семя, сканирующая электронная микроскопия, *Lotus corniculatus*, *Lotus graecus*, *Lotus dorycnium*, *Lotus hirsutus*, *Lotus rectus*, *Lotus strictus*.

Аннотация. С применением бинокулярного микроскопа и СЭМ изучены морфология и ультраструктура семян видов *Lotus* из секций *Dorycnium* (*L. dorycnium* и *L. graecus*), *Bonjeanea* (*L. rectus*, *L. hirsutus* и *L. strictus*)

и *Lotus* (*L. corniculatus*). Семена изученных видов имеют консервативную структуру, характерную для большинства *Papilionoideae*. Размер, форма и окраска семян лишь частично позволяют разделять виды в исследуемой группе. Наиболее морфологически различимы семена видов из секции *Bonjeanea*, при этом *L. rectus* имеет самые мелкие, а *L. strictus* – самые крупные семена. Размеры семян у видов секции *Dorycnium* перекрываются как между членами секции, так и с семенами *L. hirsutus* (секция *Bonjeanea*). Ультраструктура поверхности семян вокруг рубчика очень стабильна среди изученных видов, тогда как на боковой стороне семян она более изменчива. Типы скульптуры поверхности на боковой стороне семян не сильно коррелируют с таксономическим положением вида, а больше соответствуют эколого-географическим условиям. Так, тип ультраскульптуры с малозаметной первичной скульптурой и толстыми вторичными кутикулярными отложениями выявлен у видов, распространение которых связано со Средиземноморьем (например, *L. hirsutus*, *L. dorycnium* subsp. *gracilis* и частично *L. rectus*), а ямчато-бугорчатая первичная скульптура с тонкими вторичными кутикулярными отложениями характерна для видов, обитающих в более умеренном климате (*L. corniculatus*, *L. strictus*, *L. graecus*). По толщине эндосперма изученные виды можно разделить на две группы. Мы полагаем, что тонкий эндосперм, обнаруженный у *L. strictus* и *L. corniculatus*, может быть признаком, связанным с ускорением развития при распространении из Средиземноморья в умеренную климатическую зону.

Introduction

Seeds of *Papilionoideae* are usually formed from campylotropous ovules. The seed coat (testa) is formed from the outer integument. Tegmen (inner integument) is not specialized and is completely resorbed by the time the seed ripens. Seeds of *Papilionoideae* are exotestal (Corner, 1976). Typical papilionoid seed coat pattern includes epidermis (Malpighian layer or exotesta), hypodermis (sclereid layer) and parenchyma (Corner, 1976; Gunn, 1981).

The epidermis is formed by one layer of palisade or Malpighian cells, which are radially elongated thick-walled macrosclereids. Malpighian cells have special caps, and below the caps is the light line (Barton, 1965). The light line, according to different points of view, is formed as a result of bending of the outer thickening of epidermal cells (Corner, 1976) or reflecting the high density of cellulose microfibrils (Boesewinkel, Bowman, 1990). Outside, the exotesta is covered with a cuticle, which is thinner in the *Papilionoideae* subfamily than in the *Mimosoideae* and *Caesalpinioideae* and usually sculptured (Ponomarenko, 1985). The variety of cuticle sculpture types in *Papilionoideae* exceeds that in *Caesalpinioideae* and *Mimosoideae* (Manning, Staden, 1987). Features of the cuticle sculpture are used for diagnostic and systematic purposes at different taxonomic levels. Under the cuticle, there is a subcuticular layer developed to varying degrees in different representatives of the subfamily (Barton, 1965; Ponomarenko, 1985, 1996).

Hypodermis located under the epidermis is represented by single-layer hourglass-shaped osteosclereids (Manning, Staden, 1987). In *Papilionoideae*, this layer is thicker than in the other Leguminosae subfamilies and is clearly separated

from the underlying parenchyma (Ponomarenko, 1985; Manning, Staden, 1987). The cells of the hypodermis are separated by the intercellular spaces and probably participate in carrying water to the asteroid tissue and the tracheid block, which finally evaporates through the fissure in the hilum, when the seed ripens.

The parenchyma consists of a small number of layers of usually flattened cells; in comparison with *Mimosoideae* and *Caesalpinioideae* in the *Papilionoideae* subfamily, its thickness is usually the smallest (Ponomarenko, 1985).

In the area of the hilum, so-called hilum complex is formed. When seed is separated from the end of the funicle, one layer of funicle cells often remains attached to the palisade tissue, forming an outer layer (counter palisades) in the hilum region (Manning, Staden, 1987). The hilum is dissected by a median hilum fissure, which functions as a hygroscopic valve that regulates the evaporation of water by the seed (Hyde, 1954). The hypodermis in the hilum region is usually interrupted, and the parenchyma thickens and is replaced by aerenchyma, or asteroid tissue involved in the dehydration of the seed. Directly under the hilum fissure is the so-called “tracheid island” or tracheid block, which is a characteristic feature of the seed coat of the *Papilionoideae* subfamily (Hyde, 1954; Barton, 1965). The remains of the funicle can form an epihilum or rim-aril; the latter is noted for representatives of the genus *Lotus* (Arambarri, 1999), although Ponomarenko (1985) believed that only epihilum was a characteristic of *Papilionoideae*. Around the hilum, in *Papilionoideae* seeds a hilar rim is usually present, that is raised and colored differently (Gunn, 1981). It is formed by the asteroid tissue located under the epidermis.

The micropyle is a small fissure or hole; in the *Papilionoideae* subfamily it is smaller than a hilum

(Manning, Staden, 1987). It is located opposite the radicular lobe (Yakovlev, 1991), on the other side of the hilum, than the lens (Gunn, 1981). Three main forms of micropyle are distinguished for *Papilionoideae*: bifurcated (or Y-shaped), deltoid (or triangular) and punctate (Manning, Staden, 1987; Arambarri, 1999).

Raphe is a continuation of the funicle that runs along the seed from the hilum to the chalaza (Boesewinkel, Bowman, 1990; Yakovlev, 1991). Raphe and chalaza carry a vascular bundle. The opposite section of the seed (from the chalaza to the hilum) is called anti-raphe. Asymmetric seeds of *Papilionoideae* have a raphe shorter than anti-raphe (Corner, 1976). Often in the area of the raphe the testa has a different color than in other areas.

The lens is a zone in the seed coat, the special structure of which makes it permeable (Yakovlev, 1991). The lens of the *Papilionoideae* seeds is located below the hilum and is usually a slight projection often colored differently than the rest of the seed coat.

Most representatives of the *Papilionoideae* subfamily have an endosperm developed to varying degrees (Ponomarenko, 1985).

Seeds of *Papilionoideae* possess a large embryo, in the cotyledons of which a large amount of reserve nutrients accumulates. Ponomarenko (1985) made the conclusion that the embryo in legumes is a fairly stable structure that does not carry such an important set of representative features. She believed that in the structure of the embryo it is impossible to distinguish the levels of structural organization, unlike the seed coat and the hilum. Arambarri (1999) considered some characteristics of the embryo to be important for the taxonomy of the genus *Lotus*.

Manning and Staden (1987) demonstrated the important taxonomic significance for the differentiation of tribes and genera of the *Papilionoideae* subfamily of such features of the ultrastructure and anatomy of seeds as the shape of the hilum and micropyle, the nature of cells and thickness of the epidermis and hypodermis, the nature of the hilum aerenchyma, the location and shape of the pits on tracheoids (in the tracheid block), as well as sculpture of the exotesta surface.

Arambarri (1999) studied seeds of about 45 species of *Lotus* (*Papilionoideae*: *Loteae*) and 22 species of related genera. She concluded that seed ultrastructure revealed using scanning electron microscope (SEM) (the shape and position of the micropyle, the shape of the rim-aril and testa sculpture), as well as seed vasculature, are most

useful for diagnosing species and groups of closely related species of the genus *Lotus*. Kramina (2014) studied seed micromorphology using SEM in 39 species of *Lotus* belonging to sections *Lotus*, *Tetragonolobus*, *Lotea*, *Pedrosia*, *Heinekenia*, and *Chamaelotus* and two monospecific genera of the same tribe, *Kebirita* Kramina et D.D. Sokoloff and *Antopetitia* A. Rich. Using terminology of Arambarri (1999) to describe seed ultrastructure, Kramina applied the approach of Barthlott (1981) to describe testa sculpture. The study by Kramina (2014) did not reveal strict correspondence between seed ultrastructure and sectional subdivision of *Lotus* and demonstrated that similar sculpture types can be observed in different sections.

The nature of exotesta sculpture revealed using SEM was often used for taxonomic and diagnostic purposes in various tribes and genera of *Papilionoideae* (Lersten, 1981), in particular *Vigna* (Kumar, Rangaswami, 1984; Ojeda et al., 2013), genera of the tribe *Genisteae* (Pandey, Jha, 1988), *Melilotus* and *Vicia* (Voronchikhin, 1992, 1993), *Trifolium* (Rodriguez, 1995), *Onobrychis* (Özkan et al., 2015), and others.

Manning and Staden (1987) showed that in a number of genera from advanced tribes of *Papilionoideae*, in particular in *Medicago* (Trifolieae), *Lessertia* and *Sutherlandia* (*Galegeae*), as a result of changes in the wall chemistry the outer ends of epidermal cells are separated from each other, which leads to the appearance of a foveolate-papillose character of the testa. A similar phenomenon is observed in representatives of the genus *Lotus* (Arambarri, 1999; Kramina, 2014).

In *Papilionoideae*, the cuticle on the testa is usually sculptured, only occasionally smooth. The predominant types of sculpture in the subfamily are rugose and reticulate, less often foveolate and papillose. Often different types of sculpture are combined together or transitional types are formed (Manning, Staden, 1987). Manning and Staden (1987) also noted that similar types of cuticle sculpture are presented in different, sometimes unrelated, tribes, which may indicate their independent origin.

The morphology and ultrastructure of seeds in *Lotus* sections *Dorycnium* and *Bonjeanea* formerly classified within the genus *Dorycnium* are insufficiently studied. Seed morphology in four species from Turkey (*L. hirsutus*, *L. graecus*, *L. dorycnium*, and *L. axilliflorus*) was investigated by Çelebioğlu (1977), but seed ultrastructure was not studied.

The goal of our work was to study seed morphology and ultrastructure in representatives of the genus *Lotus* belonging to sections *Dorycnium* and *Bonjeanea* formerly classified within *Dorycnium* Mill. (Sokoloff, 2003). We intended to elucidate the relationship between the seed structure and the taxonomic and phylogenetic position of species and their ecological and geographical features.

Material and methods

Morphology and ultrastructure of seeds were studied in six species and four subspecies of the genus *Lotus* belonging to the sections *Dorycnium*, *Bonjeanea*, and *Lotus*. The seeds were collected in the wild or taken from herbarium specimens stored in Herbaria GAZI, LE, MA, MHA, MW, and P.

The origin of the material examined:

Lotus section *Dorycnium*

Lotus dorycnium L. subsp. *herbaceus* (Vill.) Kramina et D. D. Sokoloff: Crimea, Gurzuf, 23 VII 2020. T. E. Kramina, S. V. Polevova s. n. (MW).

Lotus dorycnium L. subsp. *herbaceus* (Vill.) Kramina et D. D. Sokoloff: Crimea, Malyy Mayak. 23 VII 2020. T. E. Kramina, S. V. Polevova s. n. (MW).

Lotus dorycnium L. subsp. *germanicus* (Gremli) Kramina et D. D. Sokoloff: Germany, Bayern. 17 VIII 2001. J. Müller s. n. (MW 0798254).

Lotus dorycnium L. subsp. *gracilis* (Jord.) Kramina et D. D. Sokoloff: Spain, Valencia, Saler. 14 VIII 1965. S. A. Renvoize 340 (LE).

Lotus dorycnium L. subsp. *dorycnium*: Austria superior, in graminosis ad Steyr. Zimmeter 417 (MW 0780771).

Lotus graecus L.: Crimea, near Vinogradnoye village, Mount Castel. 23 VII 2020. T. E. Kramina, S. V. Polevova s. n. (MW).

Lotus graecus L.: Bulgaria, 45 km SE of Burgas, 2 km SW of Tsarevo. 16 VIII 2005. Seregin, Bocharnikov E-918 (MW 0780775).

Lotus graecus L.: Bulgaria, Strandzha. 06 VII 1957. Unknown collector s. n. (MW 0780774).

Lotus section *Bonjeanea*

Lotus hirsutus L.: Portugal, Algarve, Vila do Bispo. 06 VI 2001. L. Medina, S. Nisa, M. Pardo de Santayana (MA 690879).

Lotus hirsutus L.: Spain, Valencia, the seeds obtained from The Botanical Garden of the University of Valencia. 2019. ES-0-VAL-476-97.

Lotus hirsutus L.: Spain, Girona, Lloret de Mar. 18 VI 1994. D. D. Sokoloff s. n. (MW 0780792, MW 0780795, MW 0799541, MW 0799542).

Lotus hirsutus L.: France, Corse. 22 VI and 03 VIII 1878. Reverchon s. n. (LE).

Lotus rectus L.: Crete, Rethymno. 15 VIII 2012. D. D. Sokoloff s. n. (MW).

Lotus rectus L.: Italy, Sardinia, Capoterra. III 1828. P. Thomas s. n. (LE).

Lotus rectus L.: France, Montpellier. Ledebour s. n. (LE).

Lotus rectus L.: Greece, Melissa prope Naupliam. 1860. Berger s. n. (LE).

Lotus strictus Fisch. et C. A. Mey.: Bulgaria, Haskovo. VIII 1906. Adamović s. n. (LE).

Lotus strictus Fisch. et C. A. Mey.: Turkey, C4 Konya, Aslim Bataklıği. 26 IX 1996. M. Vural, H. Duman, N. Adıgüzel 7731 (GAZI).

Lotus section *Lotus*

Lotus corniculatus L.: Russia, Moscow Region, Pushchino. 06 IX 1986. T. E. Kramina s. n. (MW).

The seeds were photographed with a stereomicroscope Nikon SMZ1500 equipped with an DS-Fi2 camera. Some stacked micrographs using several optical sections were composed using the software package HeliconFocus 7.7.5.

For scanning electron microscopy (SEM) seeds samples of specimens were collected and dried on the air. Dry specimens were attached to a SEM stub by carbon conductive tabs, then were coated with gold and palladium using EIKO IB-3 Ion Coater. Gold film deposition in Ar gas at 0.1 Torr pressure. Average film thickness 15–20 nm. The specimens were observed under Camscan-S2. Accelerating voltage 20 kV. SEI mode. MicroCapture software and JEOL, JSM-6380LA. Accelerating voltage 20 kV. SEI mode. SEM Control User Interface Version 7.11 (JEOL) software.

Morphometry was carried out for 105 seeds (from 2 to 22 seeds for each taxon), studies using SEM for two to three samples for each species and subspecies.

When describing the morphology and ultrastructure of seeds, we used the terminology of Arambarri (1999). When describing the types of seed surface sculpture, we used the approach of Barthlott (1981) with the isolation of primary, secondary, and tertiary sculpture. The primary sculpture is determined by the shape of exotesta cells and the degree of curvature of their outer periclinal wall. The secondary sculpture is a fine relief of cell walls defined by cuticular deposits or specific types of thickenings. The tertiary sculpture is defined by epicuticular secretions. The thickness of the endosperm and cotyledons was measured on longitudinal sections of seeds taken in the middle part, and then their thick-

ness was calculated as a percentage relative to the total thickness of the seed.

Results

Descriptions of seed morphology and ultrastructure (characters are summarized in Tables 1–3)

Lotus dorycnium subsp. *herbaceus* (Figs. 1A, 2A–C, 4A–C): seeds 1.7–2.2 × 1.3–1.6 mm, ovate or globose; smooth; glossy; brown or greenish-brown; mottled. Radicular lobe discernible or inconspicuous. Hilum ovate. Rim-aril thin. Hilar

rim rounded and discoloured (yellowish). Raphe deltoid; testa above raphe darker than the rest of the seed coat. Lens is a small protuberance. Endosperm thick. Epidermis layer rather thin (27–40 μm). Micropyle bifurcate. Surface micromorphology on the hilum side: primary sculpture foveolate (a depression in the center of a group of exotesta cells), secondary sculpture rugulate (folds are directed to the center of a group of exotesta cells) and reticulate. Surface micromorphology on lateral sides: primary sculpture inconspicuous, secondary sculpture roughened or wrinkled.

Table 1

Morphometric characters of seeds of *Lotus* species of sections *Dorycnium*, *Bonjeanea*, and *Lotus*. For each character, the average value is indicated, as well as the minimum and maximum (in brackets).

Section	Species	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Epidermis thickness (mkm)	Hypodermis thickness (mkm)	Endosperm thickness (%)	Cotyledon thickness (%)
<i>Dorycnium</i>	<i>L. dorycnium</i> subsp. <i>herbaceus</i>	1.94 (1.69–2.17)	1.42 (1.26–1.57)	1.28 (1.10–1.45)	34.2 (26.9–40.5)	4.5 (3.5–5.4)	31.2 (30.1–32.4)	60.9 (59–62.7)
	<i>L. d.</i> subsp. <i>gracilis</i>	1.51 (1.45–1.55)	1.19 (1.11–1.27)	1.09 (1.03–1.14)	51.6 (47.8–53.7)	14.6 (12–16.7)	28.2	57.9
	<i>L. d.</i> subsp. <i>germanicus</i>	1.81 (1.76–1.86)	1.57 (1.52–1.65)	1.41 (1.33–1.45)	39 (38.4–39.6)	10.8 (9.6–12)	29.3 (25.2–31.7)	62.6 (56.9–67.6)
	<i>L. graecus</i>	1.79 (1.34–2.38)	1.33 (1.01–1.47)	1.22 (0.46–1.48)	47.3 (41.2–54.3)	11 (5.2–15.7)	32.8 (29.6–36)	60.3 (55.5–65.1)
<i>Bonjeanea</i>	<i>L. rectus</i>	1.19 (0.96–1.41)	1.09 (0.88–1.25)	0.89 (0.59–1.14)	38.7 (32.1–48.8)	8.4 (2.7–18.1)	27.4 (24.5–31.7)	64.8 (60.4–68)
	<i>L. hirsutus</i>	1.93 (1.48–2.5)	1.57 (1.34–1.87)	1.22 (0.73–1.73)	45.8 (37.1–51.6)	5.4 (3.5–7.7)	36.3 (32–40.6)	55.6 (51.7–59.4)
	<i>L. strictus</i>	2.36 (2.28–2.4)	1.75 (1.65–1.84)	1.5 (1.36–1.7)	51.1 (45–57.7)	8.2 (6.4–10.9)	8.2 (6.6–11.1)	81.5 (80.6–82.3)
<i>Lotus</i>	<i>L. corniculatus</i>	1.47 (1.34–1.69)	1.22 (1.08–1.39)	0.99 (0.88–1.09)	33.8 (31.2–39.6)	4.5 (2.1–10.8)	14.75 (14.7–14.8)	77.3 (77.1–77.4)

Lotus dorycnium subsp. *gracilis* (Figs. 2D–G, 4D–F): seeds like in the previous subspecies but smaller in size: 1.4–1.5 × 1.1–1.3 mm. Rim-aril thicker. Epidermis also thicker (48–54 μm).

Lotus dorycnium subsp. *germanicus* (Figs. 2H–I, 4G–H): seeds like in subsp. *herbaceus* but wider: 1.8–1.9 × 1.5–1.7 mm, and darker (dark-brown).

Lotus graecus (Figs. 1B, 2J–L, 4I–K): seeds 1.3–2.4 × 1–1.5 mm, ovate, oblong or irregularly

globose; smooth; semiglossy or dull; light-brown or greenish-light-brown; monochrome or purple pointed or mottled. Radicular lobe inconspicuous or slightly discernible. Hilum suborbicular. Rim-aril thick. Hilar rim rounded and often discoloured (yellow or brown). Raphe deltoid; testa above raphe darker than the rest of the seed coat. Lens is a small protuberance. Endosperm thick. Epidermis layer rather thick (41–54 μm). Micropyle deltoid-

bifurcate. Surface micromorphology on the hilum side: primary sculpture foveolate (a depression in the center of a group of exotesta cells), secondary sculpture rugulate (folds are directed to the center of a group of cells) and reticulate. Surface micromorphology on lateral sides: primary structure foveolate-papillose (papillae correspond to the tops of epidermal cells, and a depression in the center of a group of cells), secondary structure smooth or rugulate.

Lotus rectus (Figs. 1D, 3A–C, 5A–D): seeds 1–1.4 × 0.9–1.3 mm, globose; smooth; semiglossy; dark-brown, rarely light-brown, sometimes mottled. Radicular lobe discernible. Hilum suborbicular. Rim-aril thick. Hilar rim rounded and often discoloured. Raphe deltoid. Lens is a small protuberance or inconspicuous. Endosperm thick. Epidermis layer moderately thick (32–49 µm). Micropyle deltoid. Surface micromorphology on the hilum side: primary sculpture foveolate (a depression in the center of a group of exotesta cells), secondary sculpture rugulate (folds are directed to the center of a group of cells). Surface micromorphology on lateral sides: primary sculpture foveolate-papillose (papillae correspond to the tops of epidermal cells, and a depression in the center of a group of cells), secondary sculpture smooth or rugulate, sometimes also reticulate.

Lotus hirsutus (Figs. 1C, 3D–F, 5E–G): seeds 1.5–2.5 × 1.3–1.9 mm, irregularly ovate or triangular-ovate; smooth; semiglossy or dull; brown,

light-brown or greenish; mottled. Radicular lobe discernible. Hilum recessed, ovate. Rim-aril thick. Hilar rim rounded and often discoloured. Raphe deltoid; testa above raphe is coloured darker than the rest of the seed coat. Lens is a small protuberance. Endosperm the thickest among studied species. Epidermis layer rather thick (37–52 µm). Micropyle bifurcate. Surface micromorphology on hilum side: primary sculpture foveolate (a depression in the center of a group of exotesta cells), secondary sculpture rugulate (folds are directed to the center of a group of cells) and reticulate. Surface micromorphology on lateral side: primary sculpture inconspicuous, secondary sculpture roughened.

Lotus strictus (Figs. 1F, 3G–H, 5H–J): seeds 2.3–2.4 × 1.6–1.8 mm, rounded-oblong; smooth; semiglossy; yellowish-brown or brown; mottled. Radicular lobe discernible. Hilum ovate. Rim-aril thin. Hilar rim rounded, discoloured. Raphe deltoid; testa above raphe coloured darker than the rest of the seed coat. Lens is a small protuberance. Endosperm thin. Epidermis layer thick (45–58 µm). Micropyle bifurcate. Surface micromorphology on hilum side: primary sculpture foveolate (a depression in the center of a group of exotesta cells), secondary sculpture rugulate (folds are directed to the center of a group of cells). Surface micromorphology on lateral side: primary sculpture foveolate-papillose (papillae correspond to the tops of epidermal cells, and a depression in the center of a group of cells), secondary sculpture smooth or rugulate.

Table 2

The types of seed surface sculpture in *Lotus* species of sections *Dorycnium*, *Bonjeanea*, and *Lotus*

Section	Species	Seed surface micromorphology on the hilum side		Seed surface micromorphology on lateral sides	
		Primary sculpture	Secondary sculpture	Primary sculpture	Secondary sculpture
<i>Dorycnium</i>	<i>L. dorycnium</i> subsp. <i>herbaceus</i>	foveolate	rugulate-reticulate	inconspicuous	roughened or wrinkled
	<i>L. d.</i> subsp. <i>gracilis</i>	foveolate	rugulate-reticulate	inconspicuous	roughened or wrinkled
	<i>L. d.</i> subsp. <i>germanicus</i>	foveolate	rugulate-reticulate	inconspicuous	roughened
	<i>L. graecus</i>	foveolate	rugulate-reticulate	foveolate-papillose	smooth or rugulate
<i>Bonjeanea</i>	<i>L. rectus</i>	foveolate	rugulate	foveolate-papillose	smooth or rugulate, sometimes reticulate
	<i>L. hirsutus</i>	foveolate	rugulate-reticulate	inconspicuous	roughened
	<i>L. strictus</i>	foveolate	rugulate	foveolate-papillose	smooth or rugulate
<i>Lotus</i>	<i>L. corniculatus</i>	foveolate	rugulate-reticulate	foveolate-papillose	smooth or rugulate

Table 3
Morphological seed characters in *Lotus* species of sections *Dorycnium*, *Bonjeanea*, and *Lotus*

Section	Species	Outlines	Sheen	Colour	Radicular lobe	Hilum	Rim-aril	Micropyle
<i>Dorycnium</i>	<i>L. dorycnium</i> subsp. <i>herbaceus</i>	ovate or globose	glossy	brown or greenish-brown; mottled	discernible or inconspicuous	ovate	thin	bifurcate
	<i>L. d.</i> subsp. <i>gracilis</i>	ovate or globose	glossy	brown or greenish-brown; mottled	discernible or inconspicuous	ovate	thick	bifurcate
	<i>L. d.</i> subsp. <i>germanicus</i>	ovate or globose	glossy	dark-brown; mottled	discernible or inconspicuous	ovate	thin	deltoid-bifurcate
	<i>L. graecus</i>	ovate, oblong or irregularly globose	semiglossy or dull	light-brown or greenish-light-brown; monochrome or mottled	inconspicuous or slightly discernible	suborbicular	thick	deltoid-bifurcate
<i>Bonjeanea</i>	<i>L. rectus</i>	globose	semiglossy	dark-brown, rarely light-brown; monochrome or mottled	discernible	suborbicular	thick	deltoid
	<i>L. hirsutus</i>	irregularly ovate or triangular-ovate	semiglossy or dull	brown, light-brown or greenish; mottled	discernible	recessed, ovate	thin	bifurcate
	<i>L. strictus</i>	rounded-oblong	semiglossy	yellowish-brown or brown; mottled	discernible	ovate	thin	bifurcate
<i>Lotus</i>	<i>L. corniculatus</i>	globose, rounded-oblong, ovate or triangular-ovate	dull or semiglossy	yellowish-brown, dark or light-brown; monochrome or mottled	discernible	suborbicular	thick	deltoid or bifurcate

Lotus corniculatus (Figs. 1E, 3I–J, 5K–M): seeds 1.3–1.7 × 1.3–1.4 mm, globose, rounded-oblong, ovate or triangular-ovate; smooth; dull or semiglossy; yellowish-brown, dark or light-brown; monochrome or mottled. Radicular lobe discernible. Hilum suborbicular. Rim-aril thick. Hilar rim rounded, discoloured. Raphe deltoid; testa above raphe coloured darker than the rest of the seed coat. Lens inconspicuous. Endosperm thin. Epidermis layer thin (31–40 µm). Micropyle deltoid or bifurcate. Surface micromorphology on the hilum side: primary sculpture foveolate (a depression in the center of a group of exotesta cells), secondary sculpture

rugulate (folds are directed to the center of a group of cells) and reticulate. Surface micromorphology on lateral sides: primary sculpture foveolate-papillose (papillae correspond to the tops of epidermal cells, and a depression in the center of a group of cells), secondary sculpture smooth or rugulate.

Seed size comparison

It is impossible to distinguish members of the *L. dorycnium* complex and *L. graecus* (section *Dorycnium*) based on seed size alone (Fig. 6A). Both seed length and width overlap between the two species. Among subspecies of *L. dorycnium*, there is

some differentiation in seed size, e. g. *L. dorycnium* subsp. *gracilis* has the smallest seeds, and the seeds of *L. dorycnium* subsp. *germanicus* are slightly wider than those of other subspecies, however clear separation of subspecies was not observed.

On the contrary, *Lotus* species of the section *Bonjeanea* are rather well differentiated from each other by seed size (Fig. 6B). *Lotus rectus* has the smallest seeds, *L. hirsutus* has middle-sized seeds, and the seeds of *L. strictus* are the largest. The seeds of *L. corniculatus* (section *Lotus*) taken for comparison are also small in size but slightly longer than those of *L. rectus*.

It is important to note that the seeds of *L. hirsutus* of the section *Bonjeanea* are of the same size class as those of *L. dorycnium* and *L. graecus* of the section

Dorycnium. The seeds of these three species cannot be distinguished from each other by size.

Endosperm and seed coat thickness

Seeds of *L. strictus* and *L. corniculatus* are characterized by the thinnest endosperm (8–15 % of the overall seed thickness). In all other studied species, endosperm ranged within 27–36 % of the overall seed thickness. This character negatively correlated with the relative thickness of cotyledons: 77–81 % of the overall seed thickness in *L. strictus* and *L. corniculatus* and 55–65 % in other species.

Relative seed coat thickness was similar among the studied species (7.4–8.3 % on both sides), except for *L. dorycnium* subsp. *gracilis*, which had the thickest seed coat (13.9 %).

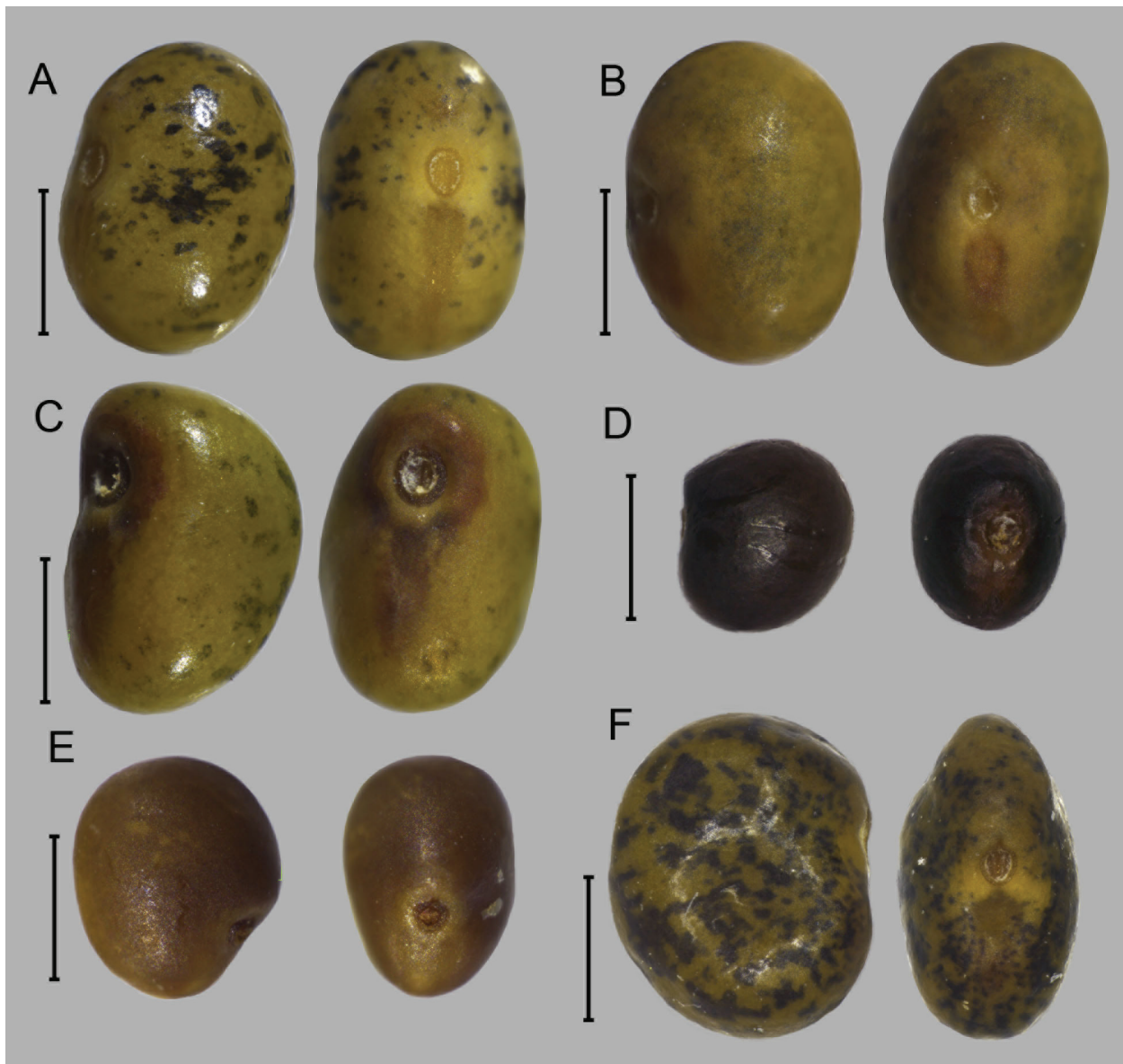


Fig. 1. Morphology of the seeds of *Lotus* species. Lateral and hilar view: A – *L. dorycnium* subsp. *herbaceus*; B – *L. graecus*; C – *L. hirsutus*; D – *L. rectus*; E – *L. corniculatus*; F – *L. strictus*. Scale bars = 1 mm.

Discussion

All studied species of *Lotus* are characterized by a conservative seed structure, which is typical for *Papilionoideae* seeds (Gunn, 1981).

Seed size, shape and color only partially allow distinguishing species in the studied group. The most pronounced difference was observed among the species of *Lotus* section *Bonjeanea*. This correlates with high degree of genetic isolation among species of this section revealed in phylogenetic analyses

conducted by both nuclear ribosomal ITS1-2 and a set of plastid DNA markers (Kramina et al., 2021). At the same time, the separation of species within the section *Dorycnium* is not possible, moreover, seed characters do not allow distinguishing *L. hirsutus* from species of *Lotus* section *Dorycnium*. This is in agreement with recently obtained results of molecular phylogenetic study, which demonstrated the absence of genetic isolation between *L. hirsutus* and *L. dorycnium* by plastid DNA markers (Kramina et al., 2022).

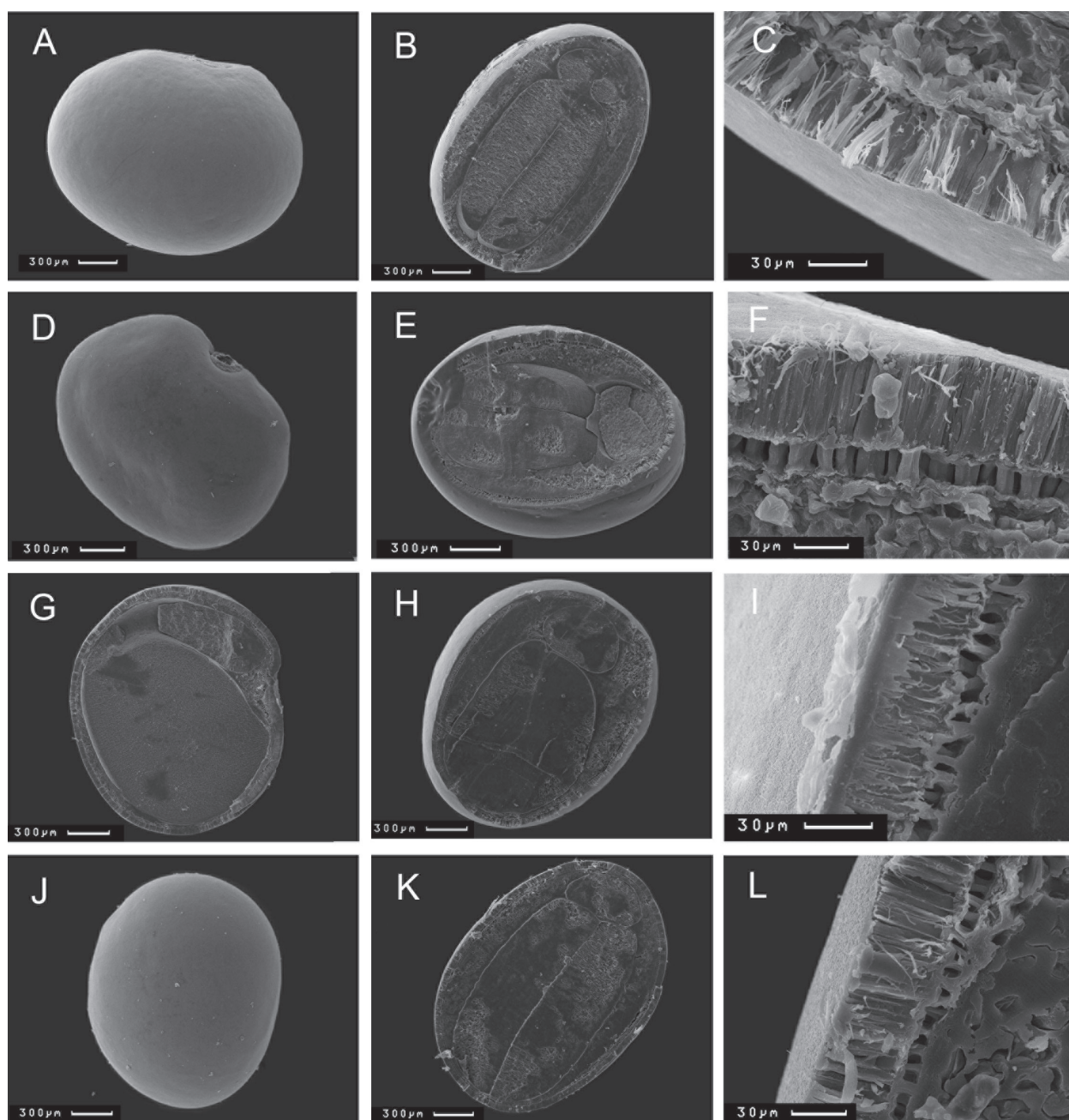


Fig. 2. Seed lateral view (A, D, J), seed longitudinal section (B, E, G, H, K), and seed coat section (C, F, I, L) of *Lotus* species: A–C – *L. dorycnium* subsp. *herbaceus*; D–G – *L. dorycnium* subsp. *gracilis*; H–I – *L. dorycnium* subsp. *germanicus*; J–L – *L. graecus*.

The analysis of seed ultrastructure revealed that the surface sculpture around the hilum is very conservative among studied species, whereas the surface ultrastructure on the lateral seed side is more variable. Our study demonstrated that the types of surface ultrastructure on the lateral seed side do not correlate with taxonomic position of the species. Similar types of surface ultrastructure are developed in species that are not closely related to each other. For example, foveolate-papillose primary sculpture with smooth or rugulate secondary sculpture is typical for *L. graecus* (section *Dorycnium*), *L. strictus* (section

Bonjeanea), and *L. corniculatus* (section *Lotus*). The same structure was revealed in the majority of species of *Lotus* section *Lotus* and in some other sections (e. g., sections *Heinekenia* and *Lotea*) (Kramina, 2014). *Lotus dorycnium* s. l. (section *Dorycnium*) and *L. hirsutus* (section *Bonjeanea*) were characterized by inconspicuous primary sculpture and roughened or wrinkled secondary sculpture. We consider that the types of surface ultrastructure on the lateral seed size more corresponds to eco-geographical conditions than to taxonomic position. Thus, the type

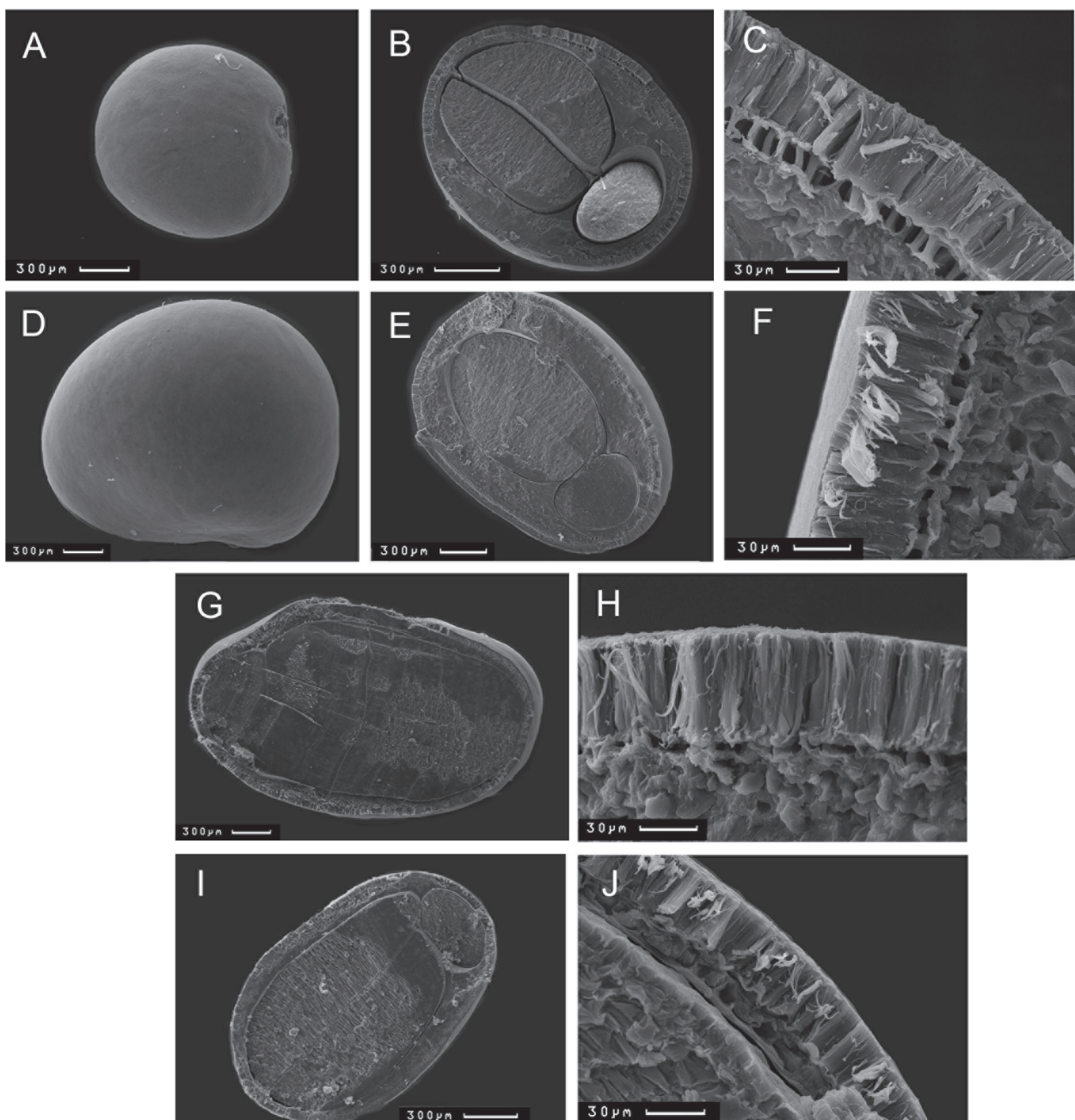


Fig. 3. Seed lateral view (A, D), seed longitudinal section (B, E, G, I), and seed coat section (C, F, H, J) of *Lotus* species: A–C – *L. rectus*; D–F – *L. hirsutus*; G–H – *L. strictus*; I–J – *L. corniculatus*.

of ultrastructure with inconspicuous primary sculpture and thick cuticular secondary sculpture was revealed in taxa with distribution connected with the Mediterranean region, e. g. *L. hirsutus*, *L. dorycnium* subsp. *gracilis* and partially *L. rectus*. Thick cuticular deposits hide the primary sculpture of the seed coat and contribute to the adaptation to the dry and hot summer period typical of the Mediterranean type of climate. Species common in milder climates (*L. corniculatus*, *L. strictus*, *L. graecus*) are characterized by less

thick cuticle deposits that do not hide the primary sculpture of the seed coat surface.

Lersten (1981) pointed out that the testa surface patterns are significant within and between adjacent tribes, e. g. papillose sculpture characteristic for the tribe *Vicieae*. However, Manning and Staden (1987) revealed the similar types of sculpture in unrelated tribes, e. g. the papillose sculpturing present in *Abrus* (*Abreae*), *Medicago* (*Trifolieae*), and *Erythrina* (*Phaseoleae*). Sometimes, the same type of seed surface ultrastructure may differ among taxa by some

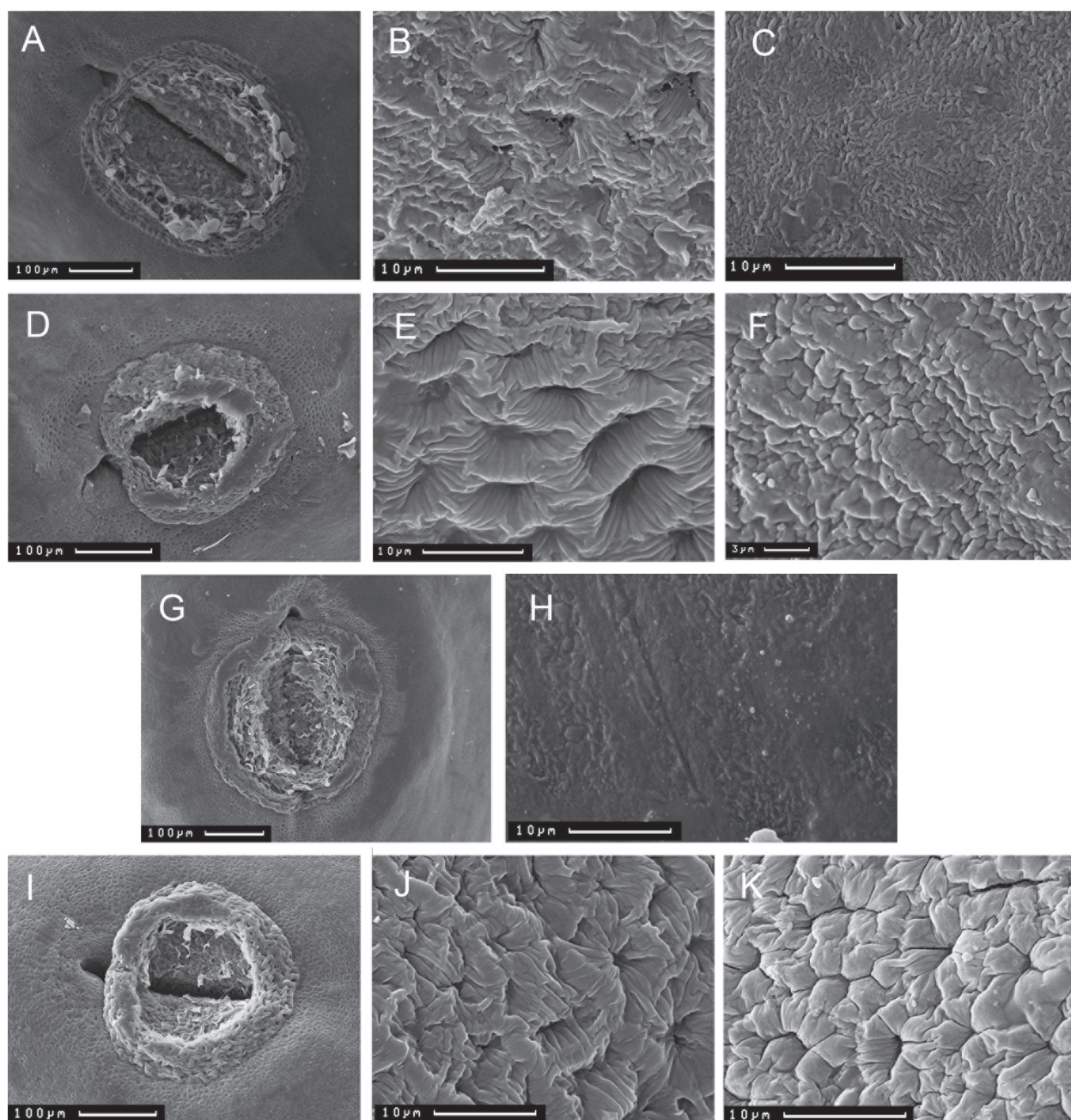


Fig. 4. Hilum (A, D, G, I), sculpture on the hilum side (B, E, J) and on the lateral side (C, F, H, K) of the seed in *Lotus* species: A–C – *L. dorycnium* subsp. *herbaceus*; D–F – *L. dorycnium* subsp. *gracilis*; G–H – *L. dorycnium* subsp. *germanicus*; I–K – *L. graecus*.

quantitative parameters, e. g. cell size or number of epidermis cells in a group (Voronchikhin, 1992). To date, there is still insufficient information to assess the taxonomic significance of seed coat sculpture in many groups of *Papilionoideae*, including the tribe *Loteae*.

Our study revealed the difference in the degree of endosperm development between studied species, which were subdivided into groups with thin (*L. strictus*, *L. corniculatus*) and rather thick endosperm (remaining species). The degree of endosperm development was studied

by Ponomarenko (1985) within the whole Leguminosae family. She identified three levels of seed organization. At the first level, which includes seeds of the genera *Brachystegia* and *Bauhinia* (*Caesalpinioideae*), the endosperm is absent or is represented by remains. At the second level, which includes many representatives of *Caesalpinioideae* and *Mimosoideae*, the endosperm is most developed and its surface is ruminated, only some taxa do not have it (*Acacia*, some *Albizzia*). At the third level represented in the *Papilionoideae* subfamily, almost all representatives have an endosperm, but

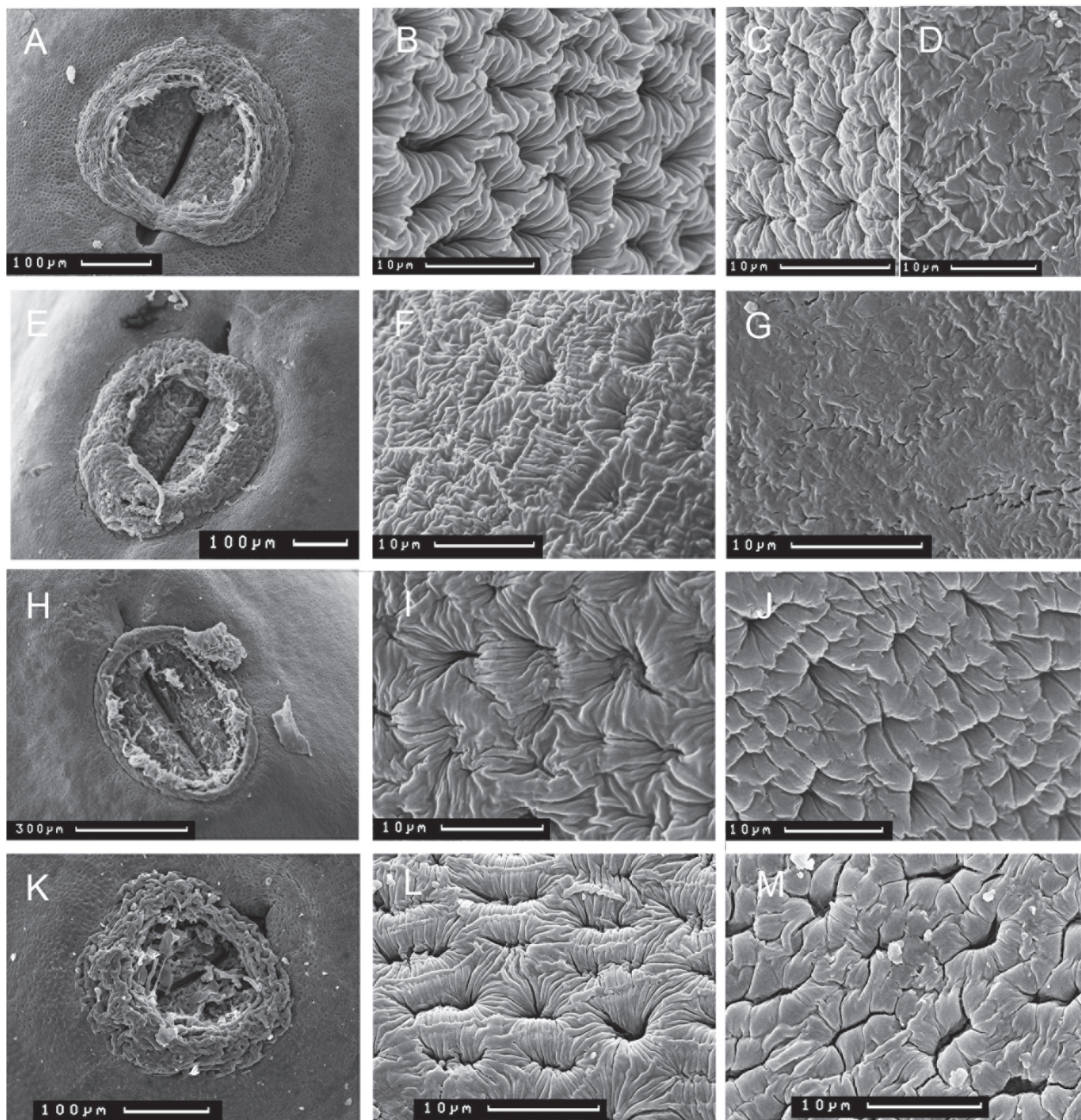


Fig. 5. Hilum (A, E, H, K), sculpture on the hilum side (B, F, I, L) and on the lateral side (C, D, G, J, M) of the seed in *Lotus* species: A–D – *L. rectus*; E–G – *L. hirsutus*; H–J – *L. strictus*; K–M – *L. corniculatus*.

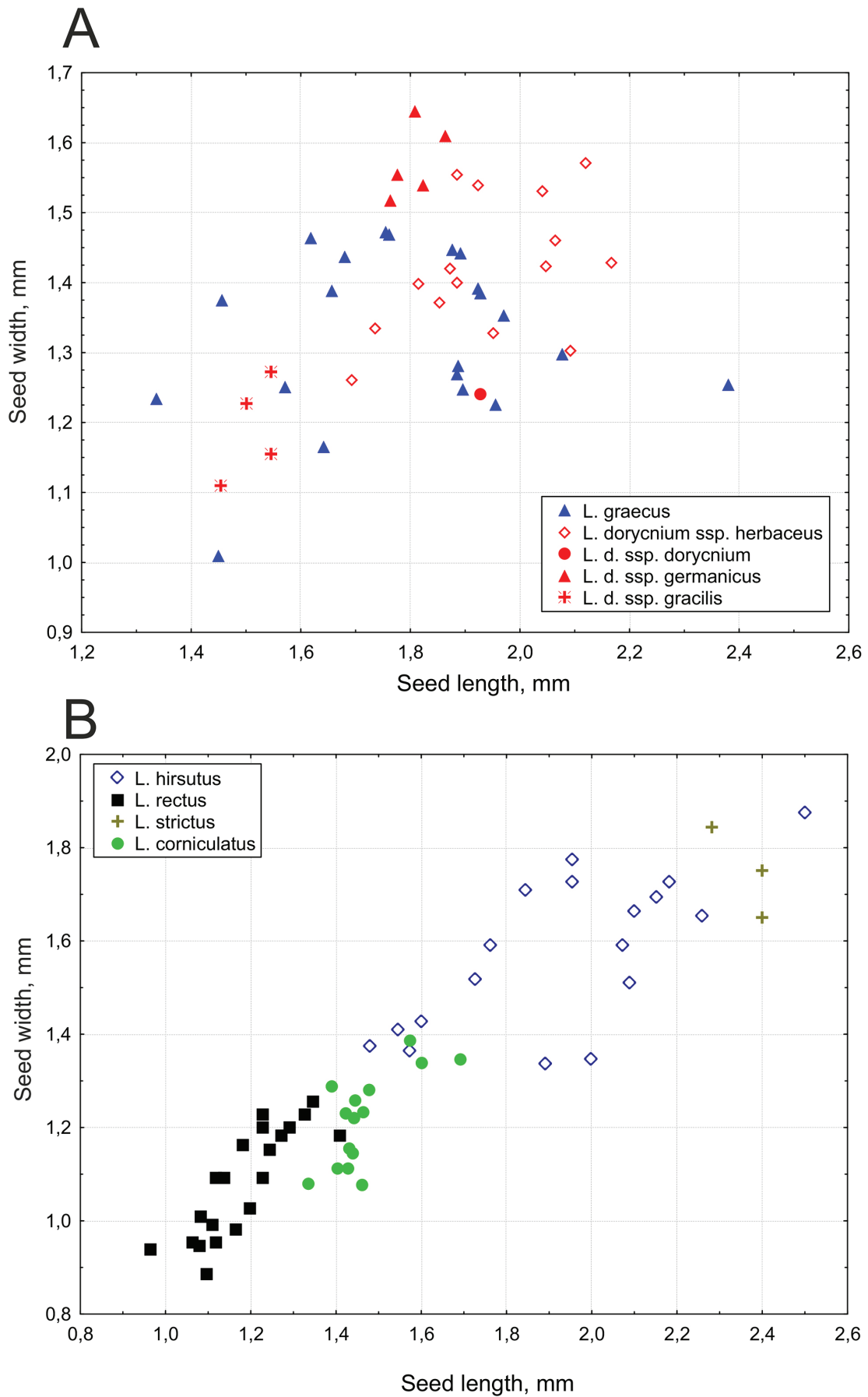


Fig. 6. Seed size in *Lotus* species of sections *Dorycnium* (A), *Bonjeanea*, and *Lotus* (B).

it is thinner than at the second level. Ponomarenko (1985) considered the absence of endosperm to be a primitive state of the legume trait, but noted that in the evolution of legumes from trees to herbal life forms, different tendencies can be observed, leading to an increase or decrease in the degree of endosperm development. We do not consider the thin endosperm observed in *L. strictus* and *L. corniculatus* to be a primitive state of the trait, but we believe that this trait rather indicates the acceleration of development when spreading from the subtropical to the temperate climatic zone. The thin seed coat and endosperm can also contribute to faster water uptake, as demonstrated by Pascualides and Planchuelo (2007) for *Crotalaria juncea* L. (Leguminosae).

Conclusion

The seeds of studied species of *Lotus* sections *Dorycnium* and *Bonjeanea* have a conservative structure, which is typical for most *Papilionoideae*. The seed size, shape and color only partially allow distinguishing species in the studied group. The most distinguishable seeds are from the section *Bonjeanea*, with *L. rectus* having the smallest and *L. strictus* the largest seeds. The seed sizes in the species of the section *Dorycnium* (*L. graecus* and *L. dorycnium*) overlap both between the members of the section and with the seeds of *L. hirsutus* (section *Bonjeanea*). The seed surface ultrastructure around the hilum is very conservative among studied

species, whereas that on the lateral seed side is more variable. The types of surface ultrastructure on the lateral seed side do not strongly correlate with taxonomic position of the species but more corresponds to eco-geographical conditions. Thus, the type of ultrastructure with inconspicuous primary sculpture and thick secondary cuticular deposits was revealed in the species, which distribution is connected with the Mediterranean region, e. g. *L. hirsutus*, *L. dorycnium* subsp. *gracilis* and partially *L. rectus*, and foveolate-papillose primary sculpture with thin secondary cuticular deposits is typical for species occurring in milder climates (*L. corniculatus*, *L. strictus*, *L. graecus*). Studied species can be subdivided into two groups by endosperm thickness. We believe that thin endosperm discovered in *L. strictus* and *L. corniculatus* may be a trait connected with the acceleration of development when spreading from the Mediterranean to the temperate climatic zone.

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