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RESEARCH PAPER

Pericarp structure in some species in the tribe *Sileneae* DC. (Caryophyllaceae, *Viscaria* group)

Tatyana I. Kravtsova[1]* & Yana V. Bolotova[2]

Tatyana I. Kravtsova[1]*
e-mail: TKravtsova@binran.ru

Yana V. Bolotova[2]
e-mail: yabolotova@mail.ru

[1] Komarov Botanical Institute RAS, Saint Petersburg, Russia

[2] Amur Branch of the Botanical Garden-Institute FEB RAS, Blagoveshchensk, Russia

* corresponding author

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ABSTRACT

The pericarp structure was studied in the representatives of genera *Atocion*, *Minjaevia*, and *Viscaria*, forming a separate *Viscaria* clade on the phylogenetic tree of the tribe *Sileneae*, and in *Ixoca*, previously a member of this group. It was found that, despite of differences in the capsule morphology and dehiscence in *A. armeria* and *M. rupestris* on the one hand, and *V. vulgaris* on the other, there are many similar features in their pericarp anatomy and micromorphology that supports their close relationships. The highest similarity in the pericarp structure, as well as in the capsule morphology, is between *A. armeria* and *M. rupestris*. Pericarp structure in *V. vulgaris* is less derived compared to them and differs by the absence of silicified palisade layer in the exocarp and indistinct differentiation of pericarp sclerenchyma into different (morphologically and in orientation) cell layers. Pericarp anatomy confirms the separation of genus *Ixoca* from the *Viscaria* group.

Keywords: Caryophyllaceae, *Sileneae*, *Atocion*, *Ixoca*, *Minjaevia*, *Viscaria*, fruit, molecular phylogeny, pericarp micromorphology and anatomy, taxonomy

РЕЗЮМЕ

Кравцова Т.И., Болотова Я.В. Строение перикарпия у некоторых видов трибы *Sileneae* DC. (Caryophyllaceae, *Viscaria* group). Строение перикарпия изучено у представителей родов *Atocion*, *Minjaevia* и *Viscaria*, составляющих на молекулярно-филогенетическом дереве трибы *Sileneae* кладу *Viscaria*, а также у *Ixoca*, ранее входившего в эту кладу. Выяснено, что, несмотря на различия в морфологии и способе вскрывания коробочек у *A. armeria* и *M. rupestris* с одной стороны, и *V. vulgaris* с другой, в анатомическом строении и микроморфологии их перикарпия имеется много общих черт, что подтверждает близкое родство этих таксонов. Наибольшее сходство в строении перикарпия, как и в морфологии коробочек, обнаружено между *A. armeria* и *M. rupestris*. Менее продвинутая по сравнению с ними структура перикарпия у *V. vulgaris* отличается отсутствием палисадного окремневшего экзокарпия и неотчетливой дифференциацией склеренхимы на слои, различающиеся по морфологии и ориентации клеток. Анатомия перикарпия подтверждает обособленность рода *Ixoca* от родов группы *Viscaria*.

Ключевые слова: Caryophyllaceae, *Sileneae*, *Atocion*, *Ixoca*, *Minjaevia*, *Viscaria*, микроморфология и анатомия перикарпия, молекулярная филогения, плод, таксономия

The systematics of the tribe *Sileneae* (Caryophyllaceae) is currently insufficiently developed; the taxonomic structure and the phylogenetic relationships of the genus *Silene* L., the largest in the Caryophyllaceae, numbering about 700 species, are debatable issues. It is considered sometimes to be very wide and includes almost all of other genera of the tribe, except *Agrostemma* L. (Greuter 1995), then divides into large numbers of small genera, the largest number in Tzvelev (2001) classification. The taxonomic revisions of the tribe also vary significantly in the opinion of authors from our country (Tzvelev 2001, Lazkov 2003, 2006). Molecular phylogenetic studies of the *Sileneae* with different molecular markers of nuclear and chloroplast origin (Oxelman & Lidén 1995, Oxelman et al. 1997), allowed elucidation of genera relationships in the tribe. These investigations revealed that separate clades on the molecular phylogenetic trees are as follows: basal clades *Agrostemma*, *Eudianthe*, *Petrocoptis*, the *Viscaria* group, the *Lychnis* group, and the terminal one, the largest *Silene* group, including two subclades. The *Viscaria* group initially included four morphologically distinct small genera: *Atocion* Adans., *Ixoca* Raf. (=*Heliosperma* (Rchb.) Rchb.), *Minjaevia* Tzvel. and *Viscaria* Bernh., the close relationships of which is very doubtful in the opinion of Tzvelev (2001). The close relationships between *Atocion* and *Viscaria* is one of the unexpected results obtained by Oxelman & Lidén (1995), which was confirmed by all subsequent molecular studies (Fraiman, Heidari & Oxelman 2009). In the previous classifications of the tribe, on the base of traditional methods, the *Atocion* was related to *Silene* s.str., and *Viscaria* to *Lychnis* (for example, Chowdhuri 1957). The contradictions with the traditional systematics of the tribe *Sileneae* are associated with the possible reticular character of their evolution (Erixon & Oxelman 2008, Fraiman, Heidari & Oxelman 2009, Mikhailova 2016b).

According to recent data (Fraiman & Oxelman 2007, Fraiman, Eggens & Oxelman 2009), the position of *Ixoca* as a sister group, in relation to *Viscaria vulgaris*, is erroneous; its real position within *Sileneae* remains unclear. A study of this genus with the involvement of low-copy nuclear genes indicates its hybrid origin. At least two lineages have been involved in the origin of *Ixoca*: an ancestor of *Viscaria* and *Atocion* and the other of *Eudianthe* Rchb. and/or *Petrocoptis* A. Braun ex Endl. (Fraiman, Eggens & Oxelman 2009). Recent results (Mikhailova 2016a, b) have shown that genus *Ixoca* forms a separate basal clade on the molecular phylogenetic tree.

The data of molecular phylogenetic analysis resulted in the revision of the tribe *Sileneae* classification, in which 8 genera were recognized (Oxelman et al. 2001). Monotypic genus *Minjaevia* (*Minjaevia rupestris* (L.) Tzvel.) was included in *Atocion*, as *Atocion rupestre* (L.) Oxelman (Oxelman et al. 2001, Fraiman, Heidari & Oxelman 2009, Fraiman et al. 2013).

The close relationships between *Atocion* and *Viscaria* can be considered as an example of glaring contradictions between morphology and molecular phylogeny, which are not uncommon in modern botany. There are significant variations within the *Viscaria* group in several carpological characters used in *Sileneae* taxonomy to distinguish genera: carpel and capsule teeth number; septa presence or absence; septicidal or loculicidal dehiscence. Capsules of *A. armeria* and *M. rupestris* have some characteristic features of *Silene* s.str.: three styles, a loculicidal-septicidal capsule with incomplete septa, dehiscent via 6 teeth. These genera are included sometimes into *Silene* s.l., in the sections *Compactae* (Boiss.) Schischk. and *Nanosilene* Otth (or *Rupifraga* Otth), respectively (Schishkin 1936, Chowdhuri 1957).

Viscaria and *Ixoca* capsules have another structure. In *V. vulgaris*, there are five styles, capsules are five-locular with incomplete septa, with unique loculicidal dehiscence via five teeth. In *Ixoca* species, there are three styles, capsules are one-locular, without septa, loculicidal-septicidal, dehiscent via six teeth.

The anatomical structure of fruits in the species, constituting the *Viscaria* group, as well as in the whole tribe *Sileneae*, is insufficiently studied. The pericarp structure was investigated in capsule teeth region in *Viscaria vulgaris*, *Ixoca carpatica* (=*Heliosperma quadrifidum*), and several *Lychnis*, *Melandrium* and *Silene* species (Weberbauer 1898, Guttenberg 1971). Exocarp sclereids in teeth region were found to be specialized cells – large, thick-walled, with strongly thickened U-shaped secondary wall, they take part in capsule dehiscence along septa and/or mid ribs. Their cell walls have transverse (in relation to capsule longitudinal axis) strands-like thickenings. Various thicknesses of outer and inner sclereid walls in the exo- and mesocarp, and the presence of unlignified inner layers contribute to the curvature of the pericarp in the teeth at drying. Between *Viscaria*, *Silene*, *Lychnis*, *Melandrium* on the one hand, and *Heliosperma*, *Petrocoptis*, certain *Alsineae* on the other, the differences are detected in teeth structure – in the number of mechanically active layers of sclereids, the form of sclereids, the form of their wall thickenings.

In some of the species, different anatomical types of the dehiscence were previously identified (Devyatov 1991): in *Ixoca arcana* (*Heliosperma alpestre*) at the site of cracking cells of the exocarp are smaller (lower) than neighboring cells, in *Viscaria vulgaris*, the cells of subepidermal layer are smaller. This character, however, varies in the second species. In a number of works, there are data on exocarp micromorphology for several species (Melikian & Devyatov 2000, Kravtsova & Romanova 2015, Kravtsova 2017): it was found to be colliculate in *Atocion*, *Minjaevia*, *Viscaria*, and a different type in *Ixoca*.

The aim of this study was to characterize the pericarp, its micromorphology and anatomical structure, in species combined in the smolkova clade (*Viscaria* group) and to consider the importance of these characteristics for systematics of the group. A comparative analysis also includes two species of the genus *Ixoca*, previously attributed to this group.

MATERIAL AND METHODS

The study is based on mature fruits (capsules) of five species (15 specimens) from Herbaria LE and KW, carpological collection (Lec) of Botanical Museum of Komarov Botanical Institute, and those collected in nature. The accepted taxonomy of the group corresponds to the classification of Tzvelev (2001). The following specimens were investigated:

Atocion armeria (L.) Raf.: Park of Komarov Botanial Institute, 4 IX 2004, N.N. Tzvelev 26 (LE); Gomelskaya Province, Dobrush station, 25 VII 1926, Ju. Krechetovich 24 (LE); St.-Petersburg, lawn in S. Kowalewskaya street, IX 2014, G.Ju. Konechnaya; Far East, Blagoveshchensk, Amur Branch of the Botanical Garden-Institute FEB RAS, IX 2016, Ya.V. Bolotova.

Ixoca arcana (Zapal.) Ikonn.: West Ukraine, Stanislavskaya (Ivanovo-Frankowskaya) oblast, 21 VII 1940, A.I. Pojarkova et al. (LE); Botanical Museum of Komarov Botanical Institute, LEc № 9833.

Ixoca carpatica (Zapal.) Ikonn.: Herb. Mus. Univ. Leopoldinensis N 104699, Carpathians, Charnohora, VIII. 1927, J. Mądalski (LE); Ivanovo-Frankowskaya oblast, 28 VI 1963, I.V. Artemchuk (LE);

Minjaevia rupestris (L.) Tzvelev. Karelia, the bank of Lake Onega, cape Cherest, 26 VII 2013, G.Ju. Konechnaya (LE); Prov of Nuland, between stations Oggelbu and Frederixberg, 15.07.1911, N.V. Shipchinsky 1138 (LE); Finland, Lojo, V. Rotert, 31VII (LE); Finland, Lojo, V. Rotert 2563 (LE);

Viscaria vulgaris Bernh. Ukraine, Velikolukskiy district, 22 VI 1921, Bulavnina et al. (KW); Pskov region., Oparino village, 14 VIII 1915, Kuznetsov, LEc № 9832; Moscow, Main Botanical Garden, A.N. Shvetsov.

The investigation was carried out using equipment of Core Centre "Cell and Molecular Technology in the Plant Science" at the Komarov Botanical Institute (St. Petersburg). The pericarp surface was studied using SEM Jeol JSM-6390LA (JEOL, Japan) in two regions: beneath the teeth and in the middle. The classification and terminology by Barthlott (1981), Murley (1951), and Botanical Latin (Stern 1973) were used for its description. The anatomical structure of the pericarp was studied using light and scanning electron microscopes along the entire length of the capsule. Longitudinal sections of the capsule walls were

made in the middle of the carpel, cross sections – in two regions, beneath the teeth and in the capsule middle. For anatomical study, fruits were first softened with heating in a mixture of water, 96 % ethanol and glycerol in equal proportions. Sections of the pericarp 12 and 24 μm thick were prepared using freezing microtome. The sections were stained with phloroglucinol with sulfuric acid, gentian-violet, by toluidine blue, Sudan IV for the detection of lignin and cutin in the pericarp tissues. To determine the locality of silica in the cell walls, the fragments of capsules were kept for 48 hours in hydrofluoric acid. Observations were made, and photomicrographs were taken using light microscope AxioImager A1 (Carl Zeiss) equipped with digital imaging AxioCam MRc5 and software AxioVision (Carl Zeiss).

RESULTS

The observations show that mature capsules of the species studied are not large: for *A. armeria* and *V. vulgaris*, they are 5–10 мм in length, with long carpophore, lesser and with short carpophore in *Ixoca* species (5–7 mm) and *M. rupestris* (3–4 mm). They are thin-walled, cylindrical (*A. armeria*), cylindrical and elongated-ovate (*I. arcana*, *I. carpatica*), ovate (*V. vulgaris*, *M. rupestris*). The peculiarity of *Ixoca* is longer than in other species, not limited to the teeth, the area of capsule cracking. *Ixoca* capsules, perhaps, represent the transition to the capsules, opening via septa; these capsules are also thin and fragile, easily destroyed.

Several transversal pericarp regions (belts) may be distinguished along the fruit: a teeth region, a region of large-celled exocarp beneath the teeth (sometimes is not developed), a middle small-celled region, a base region of thick-walled cells, somewhat lesser than above.

In the capsule teeth the pericarp is, more or less, thickened (Figs 1–4). Its sclerenchymatous part is mainly two-layered; exocarp sclereids in most genera are radially elongated, with the strongly U-shaped thickened wall. The thickenings of radial walls have the form of transversal (in relation to fruit) strands. Mesocarp sclereids are, more or less, large and longitudinally elongated, radially elongated at the ends of teeth (Fig. 3C), constitute of 1 or 1–2 layers, in the outer layer they have strongly thickened outer walls.

Beneath the teeth, the pericarp is thinner, includes two sclerenchyma layers, both at the top of a capsule and in the middle, where the number of layers reduced to one in *Ixoca* species (Fig. 4G, H). Structural differences between

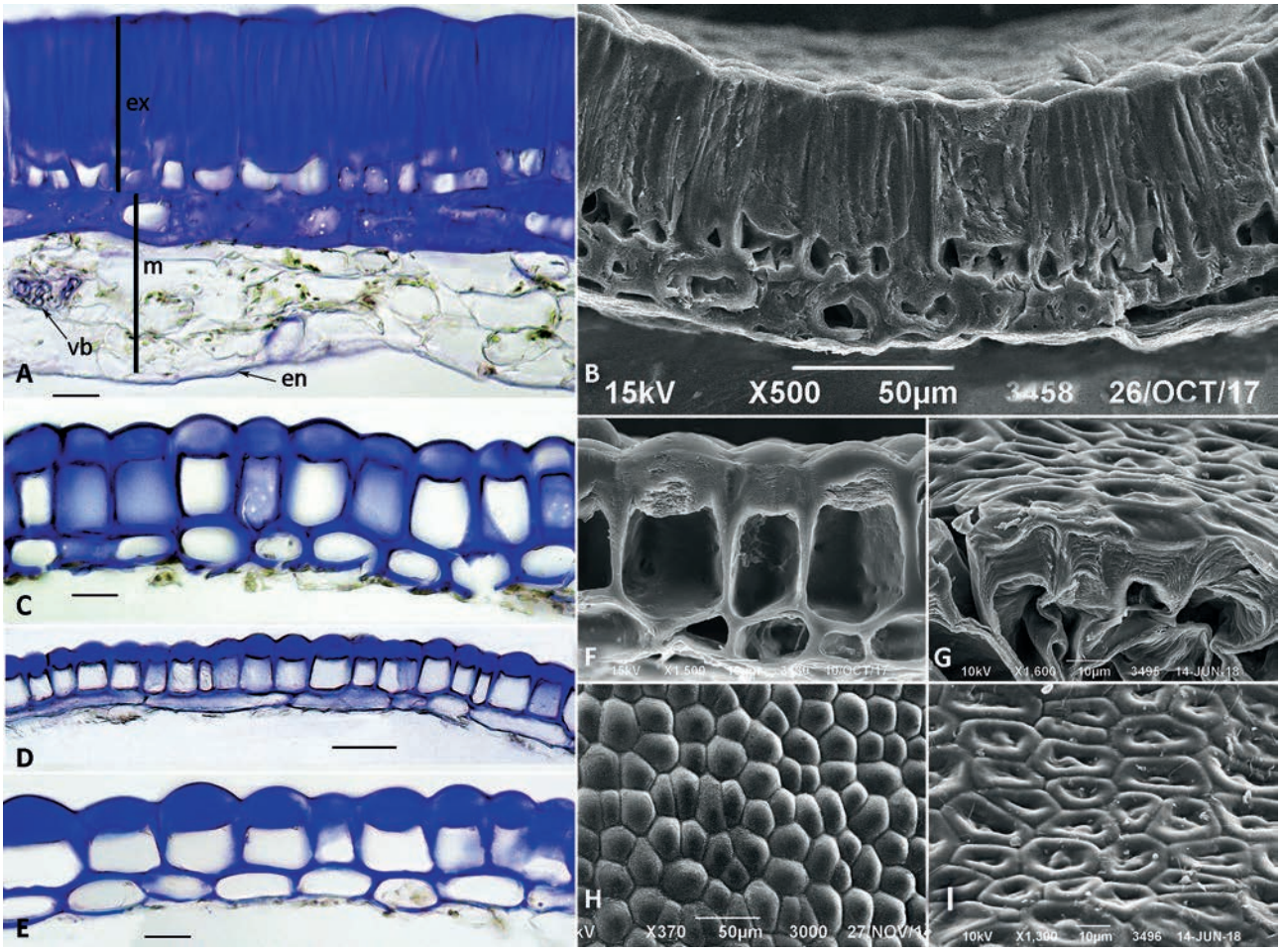

Figure 1 Pericarp structure in *Atocion armeria* (L.) Raf. A, B – longitudinal sections of pericarp in the teeth region; C, E – cross sections of the sclerified part of pericarp: beneath teeth (C), in capsule middle (E); D – longitudinal section of the sclerenchymatous part of the pericarp; F, G – cross section of exocarp before and after treatment with hydrofluoric acid, respectively; H, I – exocarp surface before and after treatment with hydrofluoric acid, respectively; A, C–E – LM, B, F–I – SEM. Structural elements: en – endocarp, ex – exocarp, m – mesocarp, vb – vascular bundle. Scale bars: A, C, E – 20 μm, B, D, H – 50 μm, F, G, I – 10 μm

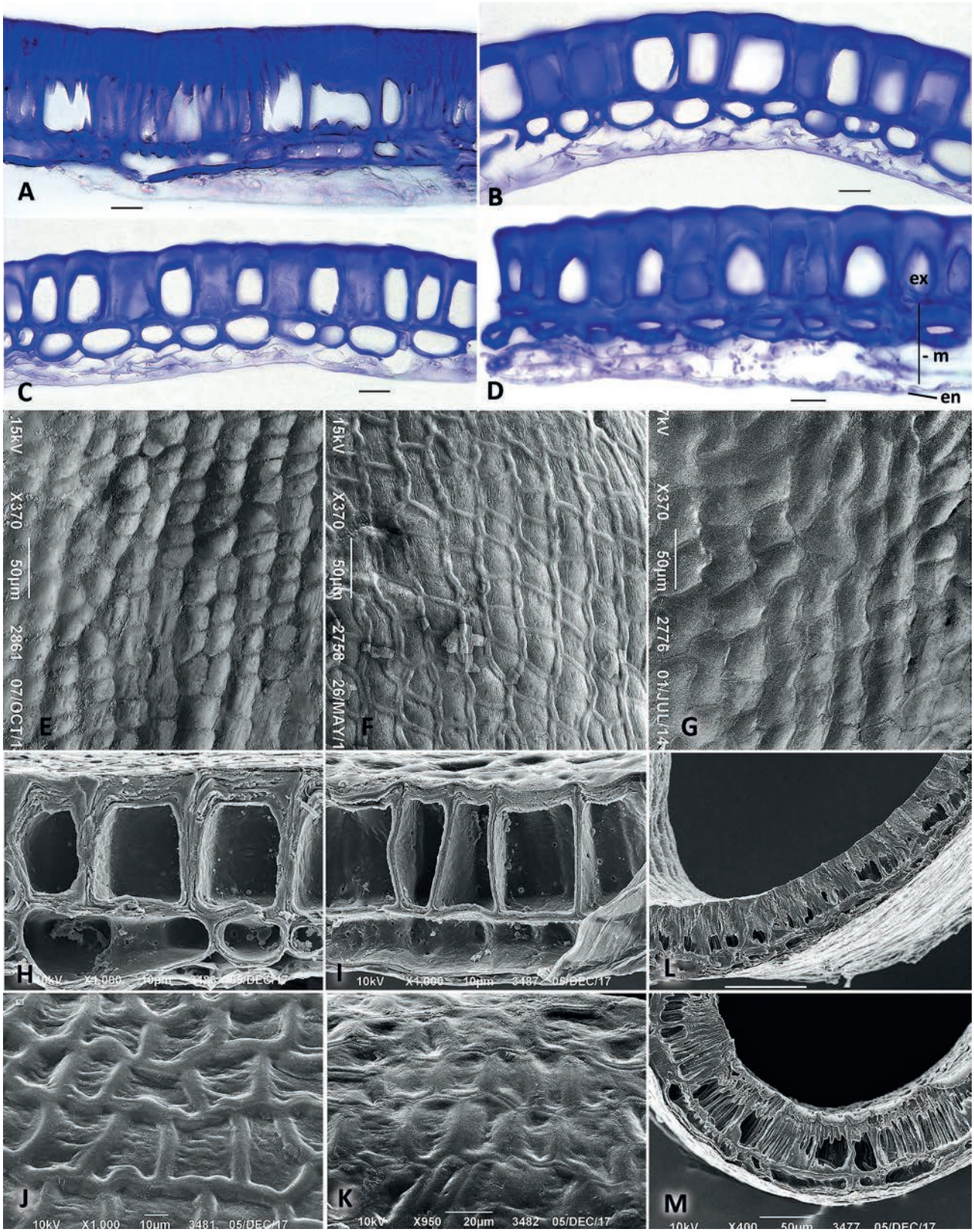

Figure 2 Pericarp structure in *Minjaevia rupestris* (L.) Tzvelev. A longitudinal section of the pericarp in the tooth region; B–D – cross sections of the pericarp (B – beneath the tooth, C, D – in the capsule middle for different specimens); E–G – the exocarp surface in different specimens; H, I – longitudinal sections of pericarp before and after treatment with hydrofluoric acid, respectively; J, K – different patches of exocarp surface after treatment with hydrofluoric acid; L, M – longitudinal sections of a capsule tooth before and after treatment with hydrofluoric acid, respectively. A–C, G, H–M – leg. Schipchinsky 1138; D – leg. Konechnaya, F – leg. Rotert. A–D – LM, E–M – SEM. Scale bars: A–D, K – 20 μm, E–F, M – 50 μm, H, J – 10 μm, L – 100 μm

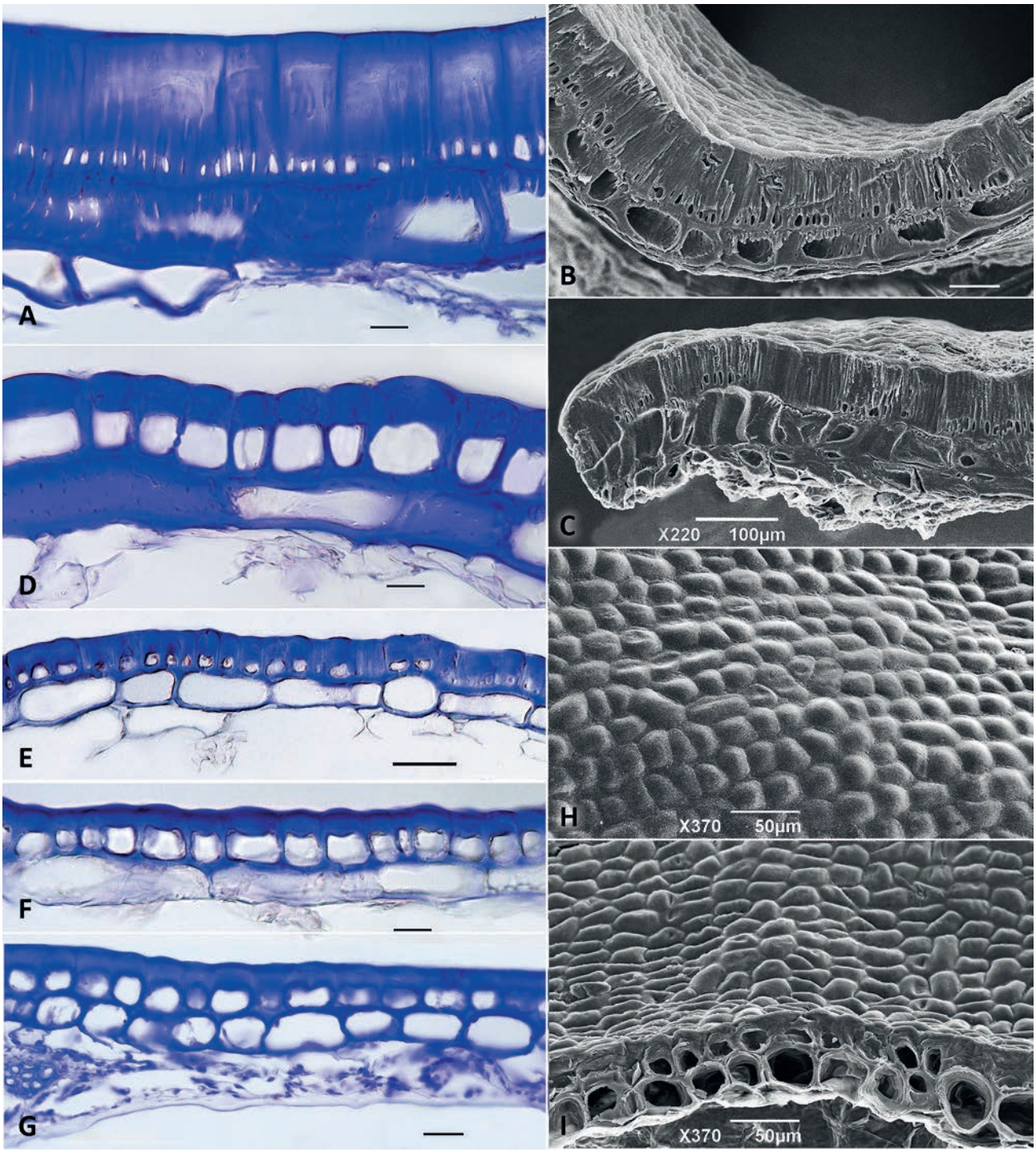

Figure 3 Pericarp structure in *Viscaria vulgaris* Bernh. A, B – longitudinal sections of pericarp in the tooth region. The arrow points at the inner fragmentary sclerified layer; C – tip of a tooth; D – longitudinal section of the pericarp sclerenchyma beneath the tooth; E–G – longitudinal (E, F) and cross (G) sections of the pericarp in the capsule middle; H – exocarp surface; I – cross section of the pericarp and its surface after treatment with hydrofluoric acid; A–E, I – Pskov region, leg. Kuznetsov; F–H – Velikolukskiy district, leg. Bulavnina. A, D–G – LM, B, C, H, I – SEM. Scale bars: A, D, F, G – 20 µm, B, E, H, I – 50 µm, C – 100 µm

different parts of the fruit are usually small; they concern cell's altitude and thickness of cell walls. In the capsule middle, the sclerified zone of the mesocarp is represented by lignified parenchyma. The pericarp has a general plan of the structure and is differentiated into exo-, meso-, and endocarp (Fig. 1A, 2D). Hard exocarp, developed from the outer epidermis of the ovary, consists of sclereids having different shapes and orientation relative to the longitudinal axis of the fruit, and differently thickened wall. The mesocarp, developing from the mesophyll, includes the outer sclerified zone of elongated along the fruit, often fiber-like cells (sclereids under the teeth, parenchyma cells below) and the inner parenchymatous zone beneath. Indistinctly visible membranous, usually structureless endocarp consists of compressed cells of inner epidermis with slightly thickened outer tangential walls. Vascular bundles of middle ribs and

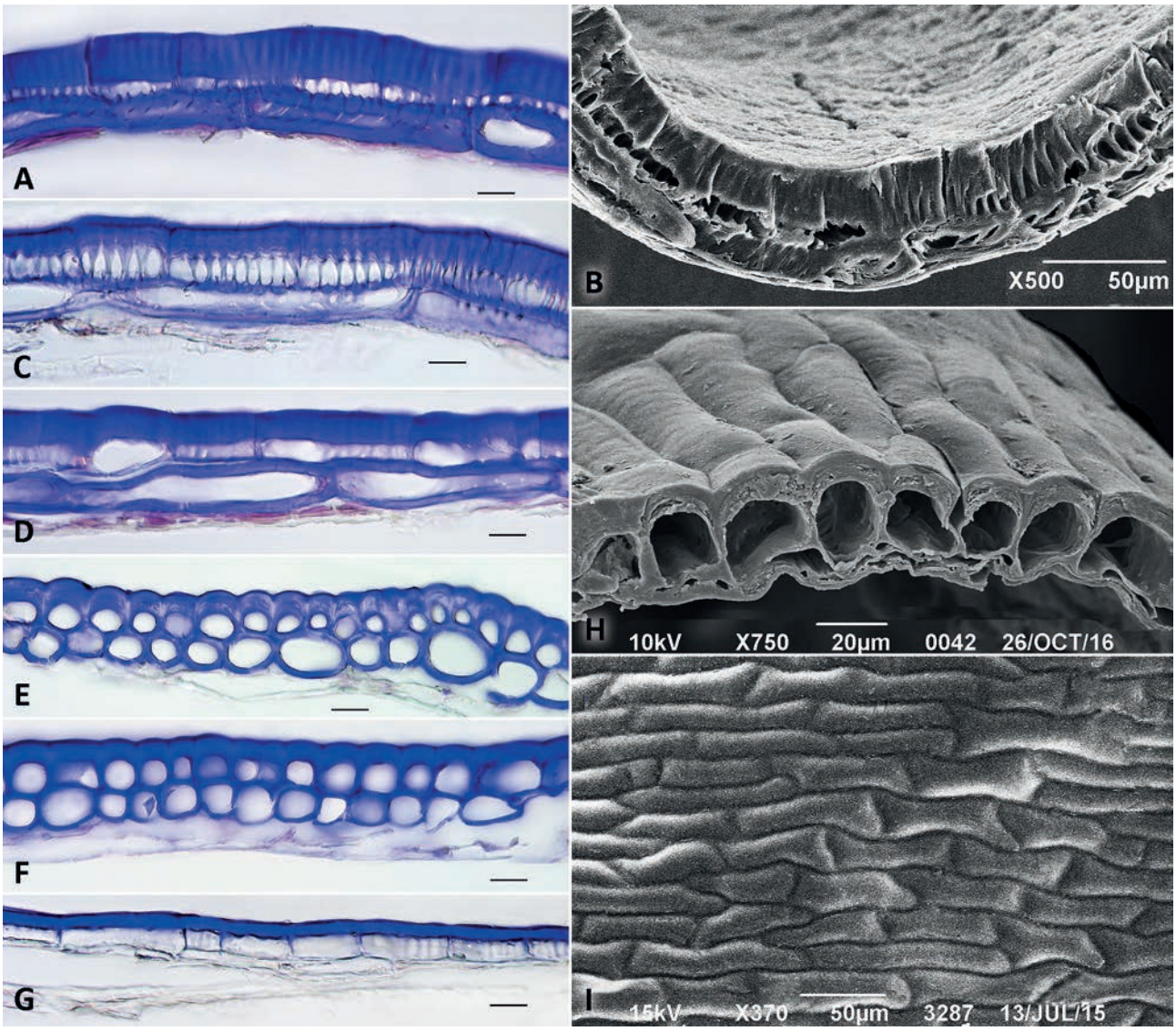

Figure 4 Pericarp structure in *Ixoca arcana* (Zapal.) Ikonn. and *I. carpatica* (Zapal.) Ikonn. A–C – longitudinal sections of the pericarp or sclerenchyma layers only in the tooth region (A, C – different fragments of different thickness); D – longitudinal section of the pericarp sclerenchyma layers beneath the tooth; E, F, H – cross sections, and G – longitudinal section of pericarp or sclerenchyma layers only in the capsule middle; I – exocarp surface. A, B, D, E, H – *I. carpatica* C, F, G, I – *I. arcana*. A, C–G – LM, B, H, I – SEM. Scale bars: A, C–H – 20 µm, B, I – 50 µm

their branches are situated in the parenchyma of the mesocarp, under the sclereids. Exo- and endocarp are covered with thin cuticle. In the region of middle ribs and septa between locula, exocarp sclereids are low and narrow, smaller than neighboring cells, and mesocarp sclereids in these places are usually smaller and more thick-walled, in *A. armeria* and *V. vulgaris* they are situated in two layers. In *V. vulgaris*, this special pericarp structure is characteristic only for the midrib regions, the septa region is notable only for more layers (two) of mesocarp sclereids.

Atocion armeria. In the capsule teeth, the pericarp is 80–100 µm thick, with two-layered sclerenchyma (Fig. 1A, B), sometimes three-layered in some places. Exocarp sclereids are radially elongated 60–80 µm thick, with a small cavity. Mesocarp sclereids are much lower, mostly compressed, somewhere rounded, wall thickenings are porous with round pits, in places are similar with those in the exocarp.

Beneath the teeth, there is a large-celled region of the exocarp about half the teeth length (Fig. 1C, F). The pericarp is 70-90 µm thick, with two-layered sclerenchyma (in septa and midrib it is three-layered), with crenate outer borderline; exo- and mesocarp sclereids are smaller in comparison with teeth. In the exocarp, they are low columnar, mushroom-shaped, 50–60 µm in height, with irregularly thickened walls; their outer tangential wall is strongly thickened, lenticular to round in section. Mesocarp sclereids are smaller, oval, rarely rounded in cross-section, of different diameters. In the capsule middle the pericarp is thinner (40–70 µm thick), consisting of more thin-walled and less tall cells (Fig. 1 D, E). Exocarp sclereids are 30–50 µm thick, radially elongated on the longitudinal section, infrequently cuboid on the transverse section, because the cells are often slightly elongated across the fruit (Fig. 1H). The mesocarp consists of compressed lignified parenchyma cells.

Exocarp surface has a colliculate primary sculpture (Fig. 1H). A treatment of the pericarp with hydrofluoric acid resulted in thinning of the sclereid walls in the exocarp, which indicates the presence of silica in them. Dissolution of silica, positioned by layers, caused the outer tangential wall caved into the cell cavity (Fig. 1G, J). Due to this, the sculpture of the exocarp surface has become annulate (Fig. 1J): the cell boundaries are still marked by a groove, the peripheral part of the outer cell wall is convex and has the form of a round roller; its central part is concave with protruding tubercle centered.

Minjaevia rupestris. In the capsule teeth, the pericarp is 75–105 μm thick, with two-layered sclerenchyma (Fig. 2A, L, M). The thick-walled sclereids of the exocarp, radially elongated or cuboid, have a rather large, irregularly-rectangular cell lumen. Mesocarp sclereids are much smaller, compressed and elongated along the fruit, with round pits in the wall. Somewhere wall thickenings are similar to those that exist in the exocarp.

Beneath the teeth, there is a large-celled region in the exocarp approximately the same length as teeth. The pericarp is 60–90 μm thick, with smooth outer borderline (Fig. 2B). In the capsule middle it is slightly thinner due to thinner-walled and less tall sclerenchyma cells (Fig. 2C). Exocarp sclereids are mostly radially elongated, 35–55 μm in height, fenestrate in section, with a large cavity and scarcely convex outer tangential walls. The form of thickenings of their walls is different between specimens (Fig. 2B–D): in sclereids with less thickened walls the thickenings of their outer walls are poorly expressed lenticular or falcate, and the large lumen is irregularly rounded-rectangular (Fig. 2B, C). In other specimens the sclereids have thicker walls with another wall thickening and an ovate or rounded-triangular cell lumen in the cross section. Mesocarp sclereids are oval, unequal-oval, or round in the cross section.

Exocarp micromorphology, as well as its anatomy, varies between different specimens. The outer wall of exocarp cells can be slightly convex, smooth or slightly concave. Therefore, in addition to the usual colliculate primary exocarp sculpture with slightly convex outer tangential cell walls (Fig. 2E), there is a reticulate-colliculate (Fig. 2F), shallow-reticulate, smooth with reticulate pattern, as well as transitional type with weakly expressed reticulation and cells of irregular form (Fig. 2G).

After the treatment with hydrofluoric acid the cell walls in sclerenchyma layers of the pericarp become thinner (Fig. 2H, I), especially the outer tangential wall of the exocarp sclereids, which turned out to be concave into the cell cavity. The sculpture of the treated exocarp surface also varies in specimens. Basically, it is reticulate (Fig. 2J), somewhere foveate-reticulate (Fig. 2K), and cell boundaries being not marked by a groove, sometimes is similar to that in *A. armeria* – feebly marked annulate. After silica dissolution, teeth sclereids changed also: their cavity increased in both sclerenchyma layers, and the pits, separating in the exocarp vertical strands of the wall thickenings, became wider, making the strands more clearly visible (Fig. 2L, M).

Viscaria vulgaris. In the capsule teeth the pericarp is 100–150 μm thick, with two-three layered sclerenchyma (Fig. 3A–C); the inner sclerified layer is weakly developed, discontinuous (shown with the arrow in Fig. 3A). It is represented by compressed lignified parenchyma cells. Exocarp sclereids are radially elongated, 60–80 μm in height, with strongly thickened cell wall, and a small lumen. The sclereids of outer mesocarp layer are large, only slightly smaller in height of the exocarp sclereids, slightly longitudinally elongated, radially elongated at the tooth end (Fig. 3C). They are thick-walled; wall thickenings are porous with linear pits, in places similar to those in the exocarp.

Beneath the teeth the large-celled region of the exocarp is short. The pericarp is 70–85 μm thick, with a smooth outer borderline (Fig. 3D). The sclereids are large in both layers, almost equal in height. They are radially elongated or cuboid in the exocarp, and longitudinally elongated in the underlying layer. In the capsule middle (Fig. 3E–G) the pericarp is 40–60 μm thick, sclerenchyma cells of both layers are much smaller and sometimes are more thin-walled than above. Exocarp sclereids are from low columnar (Fig. 3E) to slightly tangentially elongated (Fig. 3F, G, H), with a poorly pronounced lenticular thickening of the outer tangential wall, arciform or horseshoe-shaped in section; one or the other type prevails, depending on the specimen. The mesocarp consists of large, longitudinally elongated lignified parenchyma cells, both prosenchymatous and widely oval. The primary sculpture of the exocarp is colliculate (Fig. 3H). It is virtually unchanged or slightly changed after the treatment with hydrofluoric acid (Fig. 3I); most of the exocarp sclereids retain the convex outer wall.

Ixoca. The pericarp of *Ixoca carpatica* and *I. arcana* has similar structure, there are slight differences regarding cell shape from above (see later) and their altitude in teeth: higher (up to 40 μm) in *I. carpatica*, instead of approximately 30 μm in *I. arcana*.

In the capsule teeth, the pericarp is 50–60 μm thick, with two-layered sclerenchyma (Fig. 4A–C). Exocarp sclereids are longitudinally to transversely elongated on the longitudinal section, 30–40 μm thick, thickenings of their radial walls are weaker in comparison with other species, have the form of thin strands at the cell base. Mesocarp sclereids are large, thick-walled, longitudinally and obliquely elongated, the same height as exocarp sclereids, their radial walls are with linear pits.

Beneath the teeth (Fig. 4D) the large-celled region of the exocarp is almost absent, consists of few cells. In the capsule middle, the pericarp is 35–50 μm thick, two-layered (Fig. 4E, F), or 15–30 μm thick, single-layered in less sclerified capsules; the cell structure remained only in the exocarp (Fig. 4G, H). Exocarp sclereids are small, 15–30 μm in height, does not exceed the sclereids of underlying layer in height, horseshoe-shaped, radially elongated or square in cross section, with arciform outer tangential wall, cross-hatched from above (Fig. 4H); longitudinal radial walls are provided with scalariform thickenings, which change to reticulate and porous thickenings with round pits (Fig. 4D, G). Mesocarp cells are longitudinally elongated.

Exocarp surface has striate or reticulate-striate primary sculpture (Fig. 4J) in different capsule regions, in *I. arcana* mostly reticulate-striate. Exocarp sclereids in *I. carpatica* are

60–225 μm long, linear, fiber- and bone-like (osteosclereids), with convex outer walls. In *I. arcana*, they are shorter (30–130 μm), oblong or elongated-polygonal to linear. After the treatment with hydrofluoric acid the outer tangential wall of the exocarp sclereids is slightly concave, convex or smooth, has wrinkled secondary sculpture; with longer exposure to the acid, the exocarp surface becomes shallow-reticulate.

DISCUSSION

The results of comparative anatomical study of fruit in the representatives of the tribe Sileneae revealed several taxonomically important pericarp characters: a number of pericarp layers in upper and low capsule parts, heights and shapes of the exocarp sclereids and the form of their wall thickenings. Earlier, Weberbauer (1898) displayed a high value of the capsule teeth structure. Our results allow supplementation of the diagnostic features with the character of the ratio of sclereid's height in exo- and mesocarp and silica absence or presence in the sclereid walls.

Changes in the thickness of the exocarp sclereids walls, which we observed in *A. armeria*, *M. rupestris* and *I. arcana* after pericarp treatment with hydrofluoric acid, indicate a significant amount of silica in them, located in layers, especially a lot in the thick outer wall, and also in the pores area in the teeth. In the treated pericarps of these species, the sculpture of the exocarp surface has changed (and in different way in *A. armeria* and *M. rupestris*) in comparison with an intact one due to silica dissolution in the outer tangential cell walls and their concavity. In *V. vulgaris,* such changes are absent or weakly expressed, which indicates a minor role of silica (if any) in the construction of the pericarp of this species.

For the species studied, three main types of the exocarp sclereids may be distinguished beneath teeth:

1) large, 30–60 μm in height, forming a palisade layer, exceeding the height of mesocarp sclereids considerably, columnar or cuboid, mushroom-shaped or fenestrate in section, with a more or less convex outer tangential walls, impregnated with silica; they correspond to colliculate, reticulate or transitional form (*Minjaevia*) of primary sculpture of the exocarp surface in *Atocion* and *Minjaevia*;

2) small, 20–40 μm in height, not forming a palisade layer, less than or equal to the height of mesocarp sclereids, for the most part low columnar or cuboid, horseshoe or arcuate in section, with poorly convex outer tangential wall, not impregnated with silica (?or weakly impregnated); they correspond to the colliculate sculpture of the exocarp surface in *Viscaria*;

3) low, 15–30 μm in height, longitudinally elongated, from above polygonal to linear, fiber- or bone-like, horseshoe in section, less than or equal to the height of mesocarp sclereids, correspond to the striate or reticulate-striate sculpture in *Ixoca*.

Depending on the form of the cell wall's thickenings, two variants of sclereids can be distinguished within the type (1): mushroom-shaped sclereids in *A. armeria* with convex outer wall, lenticular to round in section; the sclereids with poorly convex outer tangential wall in *M. rupestris* , fenestrate in section, either with irregularly rounded-rectangular cell lumen and slight lenticular thickening of the outer cell wall or with ovate cell lumen and the outer cell wall mainly without the lenticular thickening.

The mesocarp sclereids are either mostly prosenchymatous, longitudinally elongated (*Atocion*, *Minjaevia*, *Ixoca*), or broader, sometimes broadly oval, the same height as in the exocarp (*Viscaria*).

The results obtained confirm the lack of close relationships between *Ixoca* and the genera of the *Viscaria* group. The genus is peculiar in the striate and reticulate-striate primary sculpture of the exocarp surface, and the structure of exocarp sclereids – longitudinally elongated, often osteosclereids and fiber-like sclereids having secondary thickenings of longitudinal radial walls. It differs from other species by a similar form of the sclereids in the both sclerenchyma layers and having the same orientation in relation to the fruit. The capsule teeth also have a special structure: though the sclerenchyma is two-layered (a common feature for other genera of the *Viscaria* group), the exocarp sclereids are tangentially (not radially) elongated, provided with weaker wall thickenings. Such teeth structure, as Weberbauer (1898) noted, is also available in *Petrocoptis* and members of certain genera (*Arenaria*, *Moehringia*) of the tribe *Alsineae*. *Petrocoptis* is also indicated as one of the possible ancestors of the genus *Ixoca* based on molecular studies (Fraiman, Eggens & Oxelman 2009).

The analysis of the pericarp structure in the *Viscaria* group showed that despite the differences in the capsule morphology and pattern of its opening in *A. armeria* and *M. rupestris* on the one hand, and *V. vulgaris* on the other, there are many similar features in their pericarp anatomy and micromorphology that supports a close relationship of these taxa. The primary sculpture of exocarp surface is colliculate, the pericarp is thin, the sclerenchyma is two layered both on top of the capsule and in the middle. Structural differences between different parts of the fruit are small and relate mainly to cell height and thickness of cell walls. In the middle of the fruit the mesocarp is represented by lignified parenchyma. The exocarp sclereids are low columnar or cuboid, with convex (in different degrees in the species) outer tangential wall. The capsule teeth are formed mainly by two sclereid layers as well, what distinguishes this group from most of Sileneae, having multicellular teeth, composed of three layers of sclereids.

The greatest similarity in the pericarp structure, as in the capsule morphology, is between *Atocion armeria* and *Minjaevia rupestris*: both have a large-celled region in the exocarp beneath the teeth; moreover, the exocarp sclereids exceed considerably the mesocarp sclereids in height and have a large cavity. The mushroom-shaped exocarp sclereids turned out to be a distinctive character of Atocion armeria. Silica locality in the outer tangential cell walls is different between the two species as follows from different sculpture of treated exocarp surface.

Several structural and chemical differences of the pericarp mark out *Viscaria vulgaris* in the group studied. The lack of palisade layer, not impregnated with silica cell walls of the pericarp sclereids, and less pronounced differentiation of pericarp sclerenchyma into two different (morphologically and in cell orientation) cell layers

determine less derived pericarp structure in *Viscaria* in comparison with *Atocion* and *Minjaevia*. The distinctive features also include a big contrast between the large-cellular pericarp in teeth and small-cellular one in the rest part of a capsule, the absence of the large-celled region in the exocarp beneath the teeth. The presence of the third, discontinuous inner lignified layer in the teeth can be a sign of their transitional structure to *Silene*.

It should be noted that for *Minjaevia rupestris*, unlike other species studied, a prominent infraspecific variation of both exocarp micromorphology and anatomical structure is characteristic. The exocarp surface sculpture may be a colliculate, reticulate-colliculate, reticulate or intermediate type; the exocarp sclereids differ in the form of wall thickenings between specimens.

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