Spore morphology of *Selaginella borealis*, *S. sanguinolenta* and *S. helvetica* 
(Selaginellaceae, Lycopodiophyta)

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Summary. For the first time we have conducted a study of three species of the genus *Selaginella* P. Beauv. from Russia: *S. sanguinolenta* (L.) Spring, *S. helvetica* (L.) Link and *S. borealis* (Kaulf.) Rupr. using the method of scanning electron microscopy (SEM). In a comparative analysis of the morphology of micro- and megaspores of *S. sanguinolenta*, *S. helvetica* and *S. borealis*, we first compiled an information on representatives of *Selaginella* from the territories of China and Russia. A distinctive feature of *S. borealis* and *S. sanguinolenta* based on the megaspore morphology is the different nature of the exosporium surface: *S. borealis* has the exosporium with rounded-polygonal tubercles, and *S. sanguinolenta* has the exosporium with roller-like folds rising above the sporoderma. *S. sanguinolenta* is characterized by following species-specific features of microspores: the presence of radially arranged convolutions on the proximal surface of the spore, outside the laesura, and convoluted folds on the distal surface of the microspores. *S. helvetica* is characterized by the following microspore morphology: the hemispherical distal side is in the equatorial position, and flat proximal side; the surface of the exosporium is granular, completely covered with rounded-polygonal tubercles. In the research we confirmed the presence of *S. borealis* in China (Yunnan). It has been established that the morphology of *S. sanguinolenta* microspores from the Khabarovsk Territory, the Republics of Buryatia, Tuva and the Irkutsk Region is identical to the microspores of representatives of *S. sanguinolenta* from China (Yunnan); and the morphology of *S. helvetica* microspores from the territory of the Chita Region, Trans-Baikal and Primorye Territories is identical to the morphology of the species samples from Liaoning Province (China).
Genus *Selaginella* P. Beauv. (Selaginellaceae) is the largest family in the Lycopsidophyta (Zhou, Zhang, 2015). Representatives of Selaginellaceae are distributed almost worldwide, mainly in the tropics (Tryon, Lugardon, 1991, Zhou et al., 2015b). Representatives of the genus prefer not only the main habitats of the temperate, subtropical and tropical zones, including the desert, but also enter arctic areas and climb the mountains in the alpine belt (Jermy, 1990; Zhang et al., 2013; Zhou, Zhang, 2015).

In the territory of Russia, the genus is represented by 8 species (Tzvelev, 2004), of which the complex *Selaginella borealis–S. sanguinolenta* is most difficult. Many researchers recognize both these species as independent (Tzvelev, 2004), and some consider them as one polymorphic species (Zhang et al., 2013).

The spore morphology of *Selaginella* representatives is important in the systematics of the genus. Using the spore morphology researchers differentiate some taxonomic and complex species (Zhou et al., 2015a). Such conclusions were made by Zhou’s research team based on 70 *Selaginella* species from China. Using the morphological characteristics of megaspores and/or microspores, the researchers divided the spores of the Chinese representatives of the genus into 15 types, and three more types were divided into different subtypes. Wherein some types reflect the species composition of the clades and subclades identified in further molecular genetic studies of Zhou et al. (2015b).

According to Japan samples the megaspore morphology of *S. helvetica* refers to seventh spore type – “The *Selaginella pallidissima* type” (species composition of the type: *S. pallidissima, S. denticulata, S. helvetica, S. jugorum, S. laxistrobila, S. nipponica, S. pallidissima, S. prostrata, S. pseudonipponica, S. rubella, S. tama-montana*) (Zhou et al., 2015a). Megaspores of this type are characterized by a rounded or almost rounded shape, the presence of a wide laesura, occupying a length of 1/2–3/4 the size of the equatorial diameter on the proximal side. The proximal surface of the megaspore is warty, and the distal surface is covered with furrows with a curved ornament.

Similar characteristics on the *S. helvetica* megaspores are given by researchers on samples from Europe and North Asia (without specifying the herbarium samples from the Herbarium “S” of the Swedish Museum of Natural History). At the same time, there is an additional clarification in terms of the sizes of *S. helvetica* megaspores – 350–400 μm in the morphological description (Korall, Taylor, 2006).

*S. helvetica* spores on samples from China (Liaoning province) are characterized by the presence of a hemispherical microspore. The microspore laesura occupies 3/4 the size of the equatorial diameter on the proximal side. The proximal and distal surfaces of microspores are warty, sometimes with ridges (Zhou et al., 2015a).
Zhou et al. (2015a) used *S. sanguinolenta* specimens (including specimens of *S. borealis*, without a species separation of these two taxa) from Yunnan Province to study the morphology of spores. According to this study the megaspore morphology of *S. sanguinolenta* is related to the first spore type – “The *Selaginella sanguinolenta* type” (species composition of the type: *S. nummularifolia*, *S. rossii*, *S. sanguinolenta*). Megaspores of this type have a tetrahedral form; a laesura occupies a length of 3/4 the size of an equatorial diameter. The proximal and distal surfaces of megaspores are warty. The microspore form of *S. sanguinolenta* is also tetrahedral, as megaspore one. Laesuras are clearly defined and reach the equator in length. The proximal and distal microspore surfaces are wrinkled (Zhou et al., 2015a).

We should note, that Zhou et al. have proposed generalized morphological spore types features which characterize the group of species studied. This creates difficulties in identifying certain species using the characters of the spore morphology, that is especially important in species differentiation in such complex taxa as *S. borealis* and *S. sanguinolenta*. In this study, the task was to identify the species-specific features of the megaspore morphology of *S. borealis* and *S. sanguinolenta* representatives, as well as to clarify the information on the morphology of *S. sanguinolenta* and *S. helvetica* microspores.

**Material and methods**

A material for the study was the spore samples from the herbarium material of the Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences (NS), G. G. Popov Herbarium of the Central Siberian Botanical Garden of the SB RAS (NSK), Irkutsk State University (IRKU), Siberian Institute of Plant Physiology and Biochemistry SB RAS (IRK). In total, we selected 10 samples for the study on 3 species of the genus *Selaginella*, from the territory of Republics of Tuva and Buryatia, Irkutsk and Chita Regions, Khabarovsk and Primorye Territories. The spore studies were conducted in the laboratory of the Institute for Water and Environmental Problems (IHEP SB RAS, Barnaul), using a Hitachi S 3400 N electronic scanning microscope from Hitachi High-Technologies Corp. Spores were applied on double sided tape, fastened on metal object tables with a diameter of 10 mm. The spore surface was treated with a gold-palladium mixture in an Emitech SC 7620/QT S vacuum evaporation unit for about 6 minutes.

All spore samples were examined under high vacuum. The studied samples were scanned with an increase from ×400 (general view of the spores) to ×14 000 (study of the surface of exospore).

The analysis of spores was carried out according to the following morphological features, μm: 1 – equatorial diameter; 2 – the polar axis; 3 – laesura length; 4 – laesura width; 5 – diameter of the tubercles on the proximal side of the spore; 6 – diameter of the tubercles on the dorsal side of the spore; 7 – diameter of the tubercles on the proximal side of the spore near the laesuras; 8 – the width of the curved folds on the distal side; 9 – the width of the convolutions on the proximal side. The measurements were carried out in a 20-fold repetition.

**Results and discussion**

**Morphological descriptions of micro- and megaspores of the *Selaginella* species.**

*S. sanguinolenta* (L.) Spring

SEM description (Fig. 1.). The spores in the proximal-polar and distal-polar positions are rounded-triangular, not lobed. The equatorial diameter is 46.76 (from 32.8 to 64.3) μm. The polar axis is 26.3 (from 25.4 to 27.3) μm. In the equatorial position, the distal side is hemispherical, proximal – conical. Laesura arms are straight, 15.72 (from 13.4 to 17.0) μm long, 2.27 (from 1.8 to 2.9) μm wide. The surface on the proximal side of the spores out of the laesuras is flat, or with the presence of convolutions radially located from the edges of the laesuras, 1.3 (0.8 to 2.2) μm wide. There are convoluted folds, 3.74 (from 1.8 to 7.1) μm wide, across the distal surface of the spore. The surface of the exospore is rough, without outgrowths.

Specimens examined: “Khabarovskiy territory, Ayano-Mayskiy district, vicinity of village Aym, left rocky bank of river Maya, *Pinus sylvestris* forest, almost continuous cover on the rocks, abundant. Leg. S. Kharkevich, T. Buch. Det. S. Kharkevich. 21 VI 1978” (IRK); “Buryatia, Eravninsky District, state farm “Tuldunsky”, rocky slope. 23 VII 1989. No. 0172. Coll. T. Ivantsova. Det. A. Zarubin” (IRKU); “Irkutsk Region, Slyudyansky District, st. Maritui, 121 km. On the rock at the foothill. 27 VI 1989. No. 0160. Kacherova” (IRKU); “Tuvinskaya ASSR, Tandinsky District, East Tannu-Ola, near Lake Chagytai. 51°00’N, 94°50’E. Starched steppe. 29 VII 1983. No. 28. I. M. Krasnoborov. NS0004571” (NS); “Tuvinskaya ASSR, Kaa-Khem District, near collective farm Derziz, 15 km from the mouth of the Derziz river, H = 800 m. Granite overlooks on a
Fig. 1. SEM-photographs of microspores of *Selaginella sanguinolenta*: A – the distal side of the spore; B – proximal side of the spore; C – the spore in equatorial position; D – the spore in equatorial-proximal position; E – distal spore surface; F – proximal spore surface with laesura. The scale bar is 30 μm (A), 20 μm (B–D), 10 μm (E) and 5 μm (F).
Fig. 2. SEM photographs of microspores of *Selaginella helvetica*: A – the distal side of the spore; B – the proximal side of the spore; C – the spore in equatorial position; D – the spore in equatorial-proximal position; E – the distal spore surface; F – proximal spore surface with a laesura. Scale bar of 20 μm (B, D), 10 μm (A, C) and 5 μm (E, F).
steep eastern slope. 22 VII 1974, No. 333. I. Krasnоборов, V. Khanminchun. NS0003983” (NS).

**Selaginella helvetica** (L.) Link

SEM description (Fig. 2.). Spores in the proximal-polar position are rounded-triangular, not lobed; round in the distal-polar position. The equatorial diameter is 29.78 (from 27.5 to 32.0) μm. The polar axis is 23.43 (from 22.1 to 24.5) μm. In the equatorial position, the distal side is hemispherical, proximal – flat. The laesura arms are straight, 15.97 (from 15.0 to 17.1) μm long, 1.33 (from 1.0 to 1.6) μm wide, rising above the sporoderm surface, clearly expressed or immersed in it. Exosporium torulose. Tubercles are rounded-polygonal, 0.9 (from 0.6 to 1.2) μm in diameter, up to 0.2–0.4 μm in diameter near laesuras. The surface of exosporium is granular.

Specimens examined: “Trans-Baikal Territory, Mogochinsky District, r. Shilka, left bank, Chasovinka village, H = 345 m. Point 352. 53°29′21″N, 120°02′51″E. The forested (larch, birch) rocky slope of the north-eastern exposure. Field number: 1627. 12 VII 2011. No. 19520. Coll. S. G. Kazanovsky, O. D. Chernova. Det. S. G. Kazanovsky” (IRK); “Primorskiy territory, Krasnoarmeyskiy district, vicinity of village Roschyno, dry plumb rocks along road, continuous cover. Leg. S. Kharkevich, I. Vyshin. Det. S. Kharkevich. 25 VII 1984” (IRK); “Eastern Transbaikalia, r. Shilka, rocks to the valley of the Kurengi river, near the town of Sretensk, northern slope. 14 VIII 1963. G. Peshkova” (NSK); “Chita Region, vicinity of city of Nерchinsk, r. Nерча at the confluence of the river Shilka. Rocky slope to r. Nерча, raw, semi-enriched. 12 VII 1989. No. 1559. Coll. A. Bardunov. Det. A. Kiseleva” (IRK).

**Selaginella sanguinolenta** (L.) Spring

SEM description (Fig. 3.). The spores in proximal-polar and distal-polar positions are rounded. The equatorial diameter is 303.0 (from 297 to 312) μm. The exosporium is covered with roller-like folds, rising above the sporoderm, reaching a width of 8.3 (from 5.8 to 12.2) μm. The distance between adjacent roller-like folds reaches the width of the folds themselves. The surface of the exosporium is rough.

Specimen examined: “Tuvinskaya ASSR, Tandinsky District, East Tannu-Ola, in env. Lake Chagytai. 51°00′N, 94°50′E. Starched steppe. 29 VII 1983. No. 28. I. M. Krasnоборов. NS0004571” (NS).

**Selaginella borealis** (Kaulf.) Rupr.

SEM description (Fig. 4.). The spores in the proximal-polar and distal-polar positions are tetrahedral to rounded. Equatorial diameter is 296.2 (from 274 to 309) μm. The polar axis is 243.7 (from 237 to 253) μm. In the equatorial position, the distal side of the spore is hemispherical, proximal – conical. The laesura arms are straight, 123.0 (from 112 to 130.0) μm long, 13.24 (from 9.2 to 18.00) μm wide, rising above the sporoderm. Exosporium is nodular. The tubercles are rounded-polygonal, 4.9

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Fig. 3. SEM photographs of megaspores of *Selaginella sanguinolenta*: A – distal side of the spore; B – distal spore surface. Scale bar 200 μm (A) and 50 μm (B).
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Fig. 4. SEM photographs of megaspores of Selaginella borealis: A – distal side of the spore; B – proximal side of the spore; C – the spore in equatorial position; D – the spore in equatorial-proximal position; E – distal spore surface; F – proximal spore surface with a laesura. Scale bar 200 µm (B, D), 100 µm (A, C), 50 µm (F) and 30 (E).
The main distinctive feature of *S. borealis* and *S. sanguinolenta* based on the characteristics of the megaspore morphology is the nature of the exospore surface. Representatives of *S. borealis* have the exospore with rounded-polygonal tubercles of 3.8–4.9 µm in diameter, unlike the *S. sanguinolenta* exospore covered with roller-like folds rising above sporoderm and reaching a width of 8.3 µm. The *S. borealis* exospore surface is granular and *S. sanguinolenta* one is rough. The form of the megaspore of *S. borealis* is rounded to tetrahedral, whereas *S. sanguinolenta* has the megaspore form only rounded. The size of the equatorial diameter differs in *S. borealis* and *S. sanguinolenta* slightly – 296.2 µm and 303 µm, respectively.

In a comparative analysis of the megaspore samples of our study with the samples presented in the work of Zhou et al. (2015a), images B, C and D of fig. 1 of *S. sanguinolenta* megaspores do belong to *S. sanguinolenta*, and picture A of fig. 1 – to *S. borealis* with characteristic tubercles on the exospore surface and the tetrahedral form of megaspore peculiar only to it. Thus, our study has confirmed the presence of *S. borealis* in the territory of Yunnan (China).

We have also established that the SEM data and the description of the microspore morphology of representatives of *S. sanguinolenta* from the Khabarovsk Territory, the Republics of Buryatia, Tuva and the Irkutsk Region are identical to the microspore morphology of *S. sanguinolenta* from China (Yunnan). Species-specific features of microspores characterizing *S. sanguinolenta* are the presence of radially arranged convolutions on the proximal surface of the spore, outside the laesura, and convoluted folds on the distal surface of the microspore.

As a result of our study, the microspore morphology and images of *S. helvetica* representatives from the Chita Region, Trans-Baikal and Primorye Territories turned out to be identical to the morphology of the studied specimens from Liaoning province (China) in the work of Zhou and co-authors. Species-specific features of microspores characterizing *S. helvetica* are hemispherical distal side in the equatorial position and flat proximal side; the surface of the exospore granular, completely covered with rounded-polygonal tubercles.

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