Arctoa (2023) **32**: *151–157*
doi: 10.15298/arctoa.32.12

NEW SPECIES OF *SOLENOSTOMA* (SOLENOSTOMATACEAE, MARCHANTIOPHYTA) FROM ALASKA

НОВЫЙ ВИД *SOLENOSTOMA* (SOLENOSTOMATACEAE, MARCHANTIOPHYTA) С АЛЯСКИ

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Abstract

A morphological and molecular phylogenetic study of a peculiar *Solenostoma* from Alaska with emarginate to lobed leaves and long purple rhizoids revealed an unknown species. The species is described as *Solenostoma alaskanum* sp. nov. It is characterized by medium-sized plants, numerous, very long, rigid and sporadically dense, dark purple to dark red-brown rhizoids, mostly short two-lobed or emarginated, rounded-quadrate to oblong leaves, distinct hyalodermis, large granulate oil-bodies and *trn*L-F and *trn*G-intron sequences other than in closely related species. The differences from morphologically similar species with bilobed or emarginate leaves and purple rhizoids occurring in Western North America, are discussed.

Резюме

Морфологическое и молекулярно-филогенетическое изучение своеобразной *Solenostoma* с выемчатыми до лопастных листьями и пурпурными ризоидами с Аляски выявило новый вид. Вид характеризуется средними размерами, многочисленными очень длинными и густыми пурпурными до темно-красно-коричневых ризоидами, преобладанием двулопастных или выемчатых листьев, наличием гиалодермиса, крупными зернистыми масляными тельцами и отличными от других видов последовательностями локусов *trn*L-F и интрона *trn*G. Рассматриваются отличия от других морфологически похожих видов рода с двулопастными и выемчатыми листьями и пурпурными ризоидами, встречающимися на Западе Северной Америки.

KEYWORDS: liverworts, *Solenostoma alaskanum* sp. nov., description, morphology, differentiation, phylogeny, *trn*L-F and *trn*G-intron, distribution, ecology

INTRODUCTION

During the expedition organized by D. Horton in 1992 to Alaska (Konstantinova, 2021) some specimens were collected, which caused great difficulties with identification. One such specimen contained a very peculiar plant with rounded, oblong, mostly shallowly two-lobed or emarginate leaves and dark purple rhizoids. In the collected specimen there were several loose mats without admixture of other liverworts. Since the plants in the specimen were sterile, there were neither perianths nor androecia, and it was impossible to determine it accurately even to genus. After sequencing the specimen and conducting phylogenetic analysis, it became obvious that the plant belongs to the genus *Solenostoma*, but it is not identical to any of the previously described species of the genus and most likely is a species new to science. A thorough morphological examination of the specimen also showed no similarity with the previously described species. All this convinced us that this is a species new for science, the description of which is given below.

MATERIAL AND METHODS

Collections and morphological study

The morphological study is based on one specimen collected by Konstantinova in Kenai Peninsula, Alaska, during the field work organized by Dr. Diana Horton in 1992 (Konstantinova, 2021). The specimen is preserved in the herbarium of the Polar-Alpine Botanical Garden Institute, Kirovsk, Russia (KPABG) and a duplicate is stored in the herbarium of the Main Botanical Garden, Moscow (MHA). The plants were studied using stereomicroscope (Nikon SMZ 8007) and compound light microscope Nikon Eclipse SOi with digital camera DS Fi1. The isolated shoots of *Solenostoma alaskanum* were photographed using an Olympus MVX-10 stereomicroscope equipped with a digital camera Lumenera Infinity 3-6, other photomicrographs were obtained using light

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microscopes Nikon Eclipse 50i equipped with a Nikon DS-Fi1 digital camera and Leitz Wetzlar Orthoplan equipped with a digital camera Nikon D700. In order to better illustrate the three-dimensional objects, photomicrographs were combined from several optical sections using the stacking software Helicon Focus 8 (Kozub *et al*., 2008).

Sampling for molecular analyses. Since there were no gametangia in the specimen from Alaska and it was difficult to identify its genus, the *trn*L-F and *trn*G-intron cpDNA were sequenced for this specimen and BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was done to determine its sequence similarity. The species of the genus *Solenostoma* appeared to be the most similar by both DNA markers (up to 97%). A molecular phylogenetic study was subsequently implemented to reveal the affinity of the Alaskan specimen. The ingroup comprises 32 species of Solenostomataceae published in Bakalin *et al.* (2014) and 31 species published in Shaw *et al.* (2015); additionally 10 samples from three species were included as newly sequenced. The outgroup was represented by *Marsupella anastrophylloides* Bakalin, Vilnet et Maltseva from the allied family Gymnomitriaceae. Table 1 includes the list of specimens newly sequenced for this study with voucher details and GenBank accession numbers. A list of all specimens included in the phylogenetic analyses is provided in Appendix 1.

DNA isolation, PCR amplification and DNA sequencing. The DNeasy Plant Mini Kit (Qiagen, Germany) was used for DNA extraction from dried liverwort specimens according with the manufacturer's protocol. The *trn*L-F and *trnG*-intron regions were amplified and sequenced with primers provided in Taberlet *et al.* (1991) and Shaw *et al.* (2005). PCR was carried out in 20 µl volumes with the following amplification cycles: 3 min at 94°C, 30 cycles (30 s 94°C, 40 s 56°C (*trn*L-F) or 64°C (*trnG*-intron), 60 s 72°C) and 2 min. of final extension time at 72°C. The amplified fragments were visualized on 1% agarose TAE gels by EthBr staining, purified using the Cleanup Mini Kit (Evrogen, Russia), and then used as a template in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) following the standard protocol provided for 3100 Avant Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic analysis. The obtained sequence data were combined in two alignments (*trn*L-F and *trn*G-intron) with automatic ClustalW option incorporated into the program BioEdit 7.0.1 (Hall, 1999). The preliminary phylogenetic test revealed congruent topologies for both datasets and they were combined for subsequent estimations, absent data were coded as missing.

The maximum likelihood analysis (ML) was performed with IQ-TREE (Nguyen *et al*., 2015), the Bayesian analysis (BA) with MrBayes v. 3.2.1 (Ronquist *et al*., 2012). The ModelFinder (Kalyaanamoorthy *et al*., 2017) resolved the K3Pu+F+I model as the best fit evolutionary model of nucleotide substitutions. The ultrafast bootstrapping procedure (Hoang *et al*., 2018) with 200 replicates and four rate categories of gamma distributions was used. The obtained ML tree topologies were redrawn in NJplot (Perričre & Gouy, 1996). For the Bayesian analysis each part of the combined dataset was assigned the GTR+I+G model as recommended by the program's creators; gamma distributions were approximated with four rate categories. Two independent runs of the Metropolis-coupled MCMC were used to sample parameter values in proportion to their posterior probability. Each run included three heated chains and one unheated chain, and the two starting trees were chosen randomly. The number of generations was five millions. Trees were saved every 100th generation. The average standard deviation of split frequencies between two runs was 0.005085. The first 12500 (25%) trees were discarded in each run, and 75000 trees from both runs were sampled after burn-in. Bayesian posterior probabilities were calculated from trees sampled after burn-in. The FigTree v.1.3.1 was used to visualize Bayesian phylogenetic tree (http://tree.bio.ed.ac.uk/software/figtree/).

The level of sequence divergence between species was estimated as the average pairwise *p*-distances for *trn*L-F and *trn*G-intron in Mega 11 (Tamura *et al*., 2021) using the pairwise deletion option for counting gaps.

RESULTS

Newly generated sequence data of *trn*L-F and *trn*G-intron were obtained for 11 specimens and deposited into GenBank. The combined *trn*L-F+*trn*G-intron alignment comprises 75 specimens and has a total length of 1326 positions, among them 553 positions belong to *trn*L-F and 773 – to *trn*G-intron.

The ML calculation of the *trn*L-F+*trn*G-intron dataset resulted in a single tree with an arithmetic mean of Log likelihood -9082.52, the means of Log likelihood in both runs sampled in the BA analysis were -9139.89 and -9136.88, respectively. The tree topologies reconstructed by both methods were congruent. The topology from the ML analysis is provided on Fig. 1 with indication of bootstrap support (BS) values from ML calculation and Bayesian posterior probabilities (PP) from BA.

The analysis of the taxa combined here from two earlier studies (Bakalin *et al.,* 2014, Shaw *et al.,* 2015) resulted in the same main clades corresponding to genera or subgenera of Solenostomataceae, but the backbone topology was not resolved due to polytomy. We follow Bakalin's treatment here and label clades following the narrow generic concept: *Solenostoma, Plectocolea, Metasolenostoma* Bakalin et Vilnet and *Protosolenostoma* (Amakawa) Bakalin et Vilnet. The specimen from Alaska was found in the base of the clade composed by Russian and Asian multiply sampled accessions of *S. obovatum*, *S. obscurum*, as well as *S. rotundatum*, *S. flagellatum*, *S. emarginatum* and only a single North

Fig. 1. Phylogram obtained by maximum likelihood for the family Solenostomataceae based on *trn*L-F+*trn*G-intron dataset. Bootstrap supports from maximum likelihood and Bayesian posterior probabilities more than 50% (0.50) are indicated. The branches from nodes with supports 100/1.00 are in bold. The geographical regions and GenBank accession numbers are provided, accessions obtained in this study are marked with asterisk. The level of nucleotide sequence divergence of *Solenostoma alaskanum* from other species of the genus are shown (*trn*L-F/*trn*G-intron, %).

Table 1. The list of specimens sequenced in this study with voucher details and GenBank accession numbers.

Taxon	Specimen voucher	GenBank accession number	
		*trn*L-F cpDNA	*trn*G-intron cpDNA
Solenostoma alaskanum sp. nov.	USA: Alaska, *Konstantinova K32-92* (KPABG)	OR865669	OR865680
Solenostoma obovatum	Russia: Buryatia Rep., *Mamontov 439-1-1* (KPABG)	OR865672	OR865683
Solenostoma obovatum	Russia: Buryatia Rep., *Mamontov 436-1-3* (KPABG)	OR865673	OR865684
Solenostoma obscurum	Russia: Buryatia Rep., *Mamontov 436-1-6* (KPABG)	OR865674	OR865685
Solenostoma obscurum	Russia: Buryatia Rep., *Mamontov 616-3-1* (KPABG)	OR865675	OR865686
Solenostoma obscurum	Russia: Trans-Baikal Terr., *Mamontov 491-1-9069* (KPABG)	OR865676	OR865687
Solenostoma obscurum	Russia: Trans-Baikal Terr., *Mamontov 179-1* (KPABG)	OR865677	OR865688
Solenostoma obscurum	Russia: Trans-Baikal Terr., *Mamontov 180-21-1* (KPABG)	OR865678	OR865689
Solenostoma obscurum	Russia: Trans-Baikal Terr., *Mamontov 356-3-1* (KPABG)	OR865679	OR865690
Solenostoma pseudopyriflorum	Russia: Primorsky Terr., *Mamontov 47-1-10* (LE)	OR865671	OR865682
Solenostoma pseudopyriflorum	Russia: Buryatia Rep., *Mamontov 618-1-2* (MHA, KPABG)	OR865670	OR865681

American specimen, *S.* cf. *schusterianum*. The relation of the tested specimen is robustly supported by 98% BS in ML and 1.00 PP in BA. The nucleotide sequence divergence of the Alaskan specimen from the selected *Solenostoma* species varied from 2.5–8.1% in *trn*L-F and 2.7–7.3% in *trn*G-intron which agrees the with the level of species differentiation suggested in Bakalin et Vilnet (2014). These data, along with the peculiar morphological features of the sample described below, convinced us that it is a new species.

TAXONOMY

Solenostoma alaskanum Konstant., Vilnet & Mamontov sp. nov.

Diagnosis. The species is characterized by medium-sized plants, numerous/ very long and sporadically dense, dark purple to dark red-brown rhizoids, stems with distinct hyalodermis, mostly two-lobed or emarginated, rounded-quadrate to oblong leaves, large granulate oil-bodies and differentiation in *trn*L-F and *trn*G-intron nucleotide sequences from other species of the genus.

Holotype: U.S.A., **Alaska**, Kenai Borough, 4.5 km South of Ingram Creek crossing on Hwy 1 south of Portage, along Ingram Creek, 60°50'N, 149°05'W. In pure mats on debris on moist cliff on bank of creek Coll. N. Konstantinova with D. Horton, R.M. Schuster, 29.06.1992. Holotype KPABG 126087, isotype MHA.

Etymology. The name refers to the U.S.A. state where the species was collected.

Description. Plants 2–2.5 mm wide and 2.2–3 cm long, with light green linear apices to dark green and almost black-green below in nearest part of shoots, with numerous ventral intercalary branches and stolons. Cells of dorsal stem surface very long, ca. (13)20–25×(30)50–100 (150) μm. Stems in cross section 15–17 cells thick, round to widely elliptical, 225–280×300–350 μm, with distinct hyalodermis of large cells with slightly thickened lateral walls, 20–25×25–33 (35) μm, cortical cells in one or two rows distinctly smaller than outer cells, ca. (12)15–17×17–20 μm, thick-walled. Cells of medulla isodiametric, 17–25 μm, moderately thick-walled. Rhizoids numerous, very long and dense, dark red-purple-violet to red-brown, over the whole ventral stem surface up to the apices. Leaves slightly obliquely inserted and just slightly decurrent on straight line dorsally and arched ventrally, leaf free dorsal and ventral zone absent. Leaves variable on one shoot, alternating several large and small, rounded-quadrate to oblong, distinctly elongate, varying on one shoot from 0.5×0.6 mm to 0,7–0.75×0.9 mm and 1.35×1.30 mm, with a rounded extended ventral base and decurrent dorsal side, many leaves emarginate up to 0.8–0.1 of their length with slightly uneven, rounded-obtuse lobes, but in some small leaves with one "lobe" blunt-pointed. Mid-leaf cells 17–22×(20)24–28 μm, with moderate trigones, at margins just slightly smaller, (15)17–20(25) μm, elongated along margin or perpendicular to margin, cells at base of leaves distinctly elongated, 22–24×(30)32–40(50) μm, cuticula striolate papillose. Oil-bodies 2–4 per mid-leaf cell, dark grey, spherical to ellipsoidal, finely granulate, 9–12×12–15 (17) μm immediately after soaking, preserved 30 years after collecting but rapidly disappearing when soaked. Cetera ignota.

DISCUSSION

When identifying collections from Alaska the plants of a peculiar liverwort with abundant dark purple rhizoids and partly lobed or emarginate leaves immediately attracted our attention. However, since there were no gametangia, it was difficult to decide to which genus this specimen should be attributed. Molecular genetic analysis made it possible to clarify this and showed that the collected plants belong to *Solenostoma.* In the genus *Solenostoma*, as far as we know, there are two species with bilobed or emarginate leaves and purple rhizoids, i.e., *Plectocolea biloba* S. Hatt. ex Amakawa (=*Solenostoma bilobum* (S. Hatt. ex Amakawa) Potemkin & Nyushko) and *Plectocolea emarginata* Amakawa (=*Solenostoma emarginatum* (Amakawa) Váňa, Hentschel et Heinrichs). However, both these species differ from *S. alaskanum* in 1) smaller size of plants, not exceeding 1–1.7 mm in width and 7–10 mm in length vs. 2–2.5 mm wide and 2.2–3 cm long in *S. alaskanum*; 2) shape of leaves which are subquadrate in both species vs. mostly oblong and distinctly elongated in *S. alaskanum*; 3) cells with indistinct trigones vs. distinct, moderate in size to

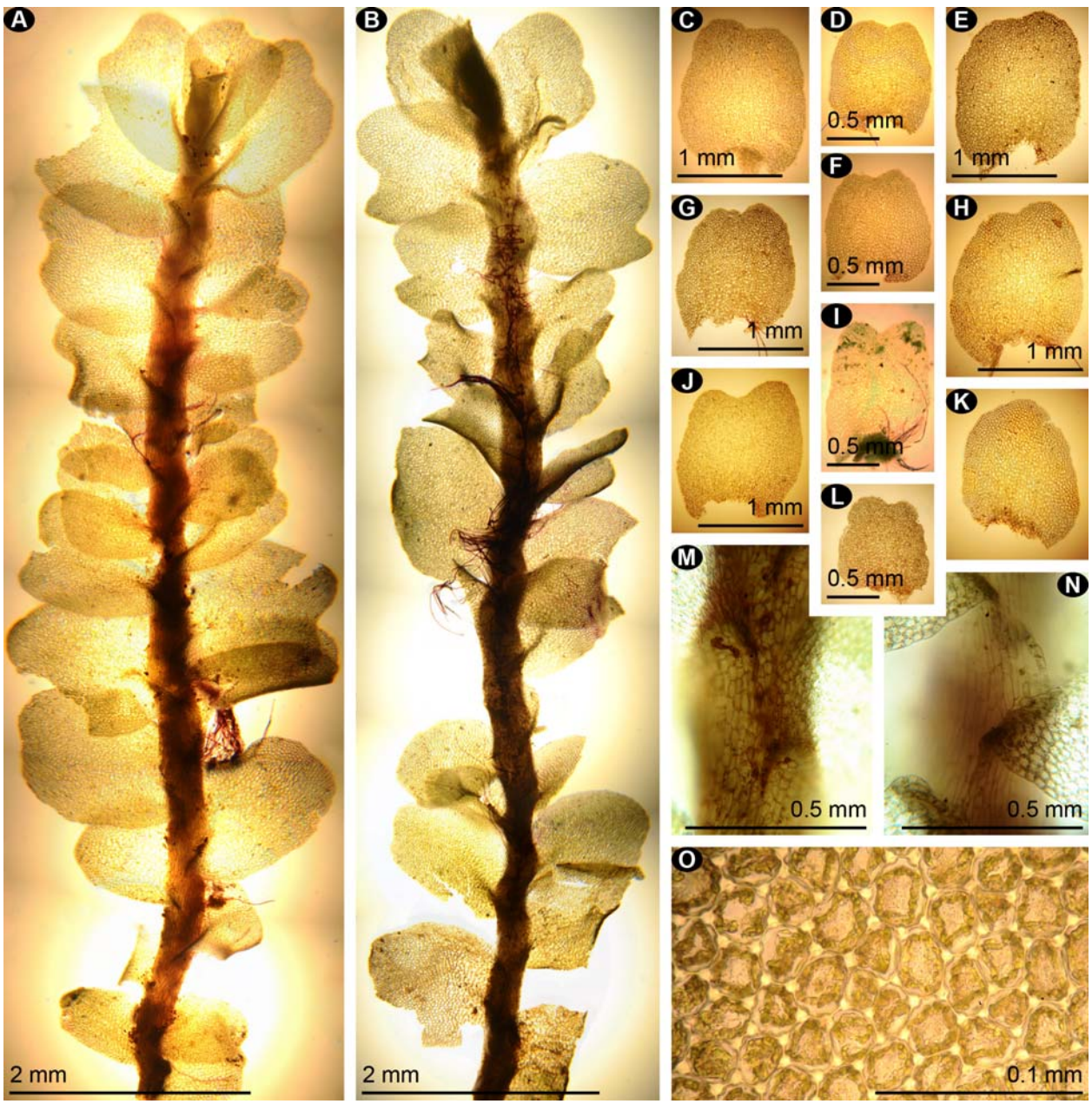

Fig. 2. *Solenostoma alaskanum* (all from holotype): A: plant habit (dorsal view); B: plant habit (ventral view); C–L: leaves; M: bases of leaves (ventral aspect); N: bases of leaves (dorsal aspect); O: median leaf cells.

large trigones in *S. alaskanum*; 4). Smooth, nearly homogeneous cuticle vs. distinctly striolate in *S. alaskanum*. In addition, S. *bilobum* differs in having angular lobes with apiculate apices, often ending in two superposed cells, smaller, just to 7–9×3–5 µm, nearly homogeneous oil-bodies vs. large, to 12×17 µm in *S. alaskanum.* Additionally, *Solenostoma emarginatum* and *S. alaskanum* clearly differ by both sequenced DNA loci (*p*-distances 4.4/2.7%)

One more species phylogenetically allied and morphologically similar to the described species is *Solenostoma schusterianum* (J.D. Godfrey et G. Godfrey) Váňa, Hentschel et Heinrichs. But the latter species differs from *S. alaskanum* in 1) larger size of plants, being 2.1–4.8 mm wide and 2–6 cm long; 2) purple colored ventral bases of leaves, whereas we have not found any trace of red or purple color apart of rhizoids in *S. alaskanum*; 3) shape of leaves, including rounded to acuminate or retuse apex vs. mostly emarginate or bilobed apex in *S. alaskanum*; 4) much larger mid-leaf cells, which are 35–50 (–58)×33–43 µm, with large and bulging trigones vs. (17)22×(20)24–28 µm in *S. alaskanum*.

It is difficult to say anything about its distribution and ecology since *S. alaskanum* is only known from one specimen from one location. One can only assume that the species is quite rare, like three other closely related and / or morphologically somewhat similar species *Solenostoma bilobum* and *S. emarginatum,* of which the lat-

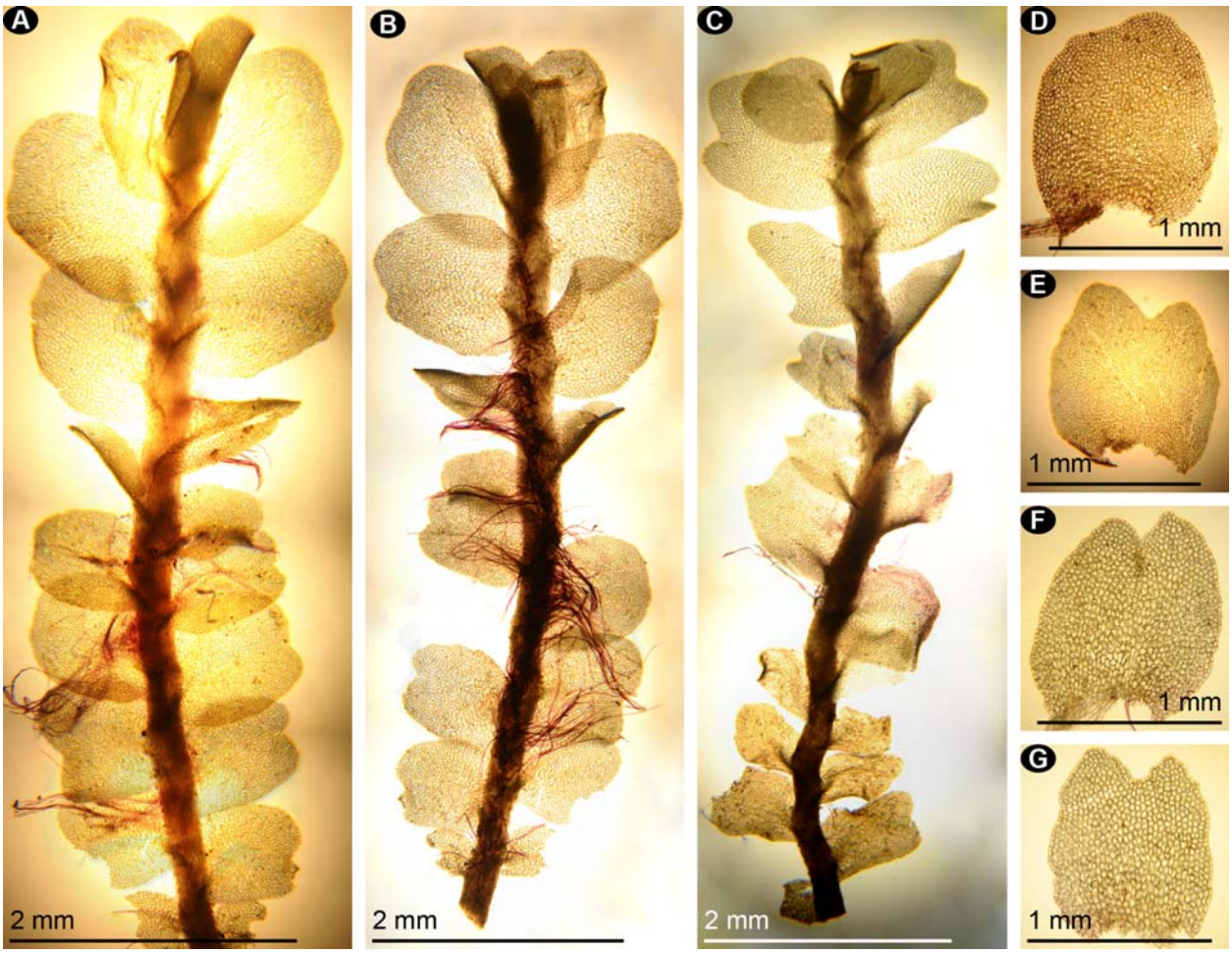

Fig. 3. *Solenostoma alaskanum* (all from holotype): A, C: plant habit (dorsal view); B: plant habit (ventral view); D–G: leaves.

ter is an endemic of Japan, whereas the former is a Japanese-Korean endemic with several known localities in the Kuril Islands (Bakalin, 2014) as well. The third species, *Solenostoma schusterianum,* is a western-North American (from southern Alaska mainland and the Aleutians to Washington) boreo-montane species (l.c.). Based on the only known locality and habitat, we tentatively classify *S. alaskanum* as a montane suboceanic species.

It should be noted as well that the floras of the liverworts of Alaska, British Columbia, and Far East of Russia are far from being fully studied. These large regions with very diverse nature conditions and, to a greater extent, with an ancient relict flora, will bring many more surprises during future studies. It can be fairly confidently assumed that *Solenostoma alaskanum* will be found in the coastal mountains of Alaska and British Columbia and probably in the Kuril Islands, like *Solenostoma bilobum*.

ACKNOWLEDGEMENTS

We sincerely thank V. Bakalin for a fruitful discussion and useful comments. A. Hagborg is gratefully acknowledged for valuble comments and linguistic corrections. The study was carried out within institutional research project of the Avrorin Polar-Alpine Botanical Garden-Institute, RAS NN №122091900008-4, and using large scale research facilities "Herbarium of the Polar-Alpine Botanical Garden-Institute (KPABG)", reg. No. 499397. The Ministry of Higher Education and Science of Russia is thanked for grant 075-15-2021-678 supporting Center of Common Use «Herbarium MBG RAS».

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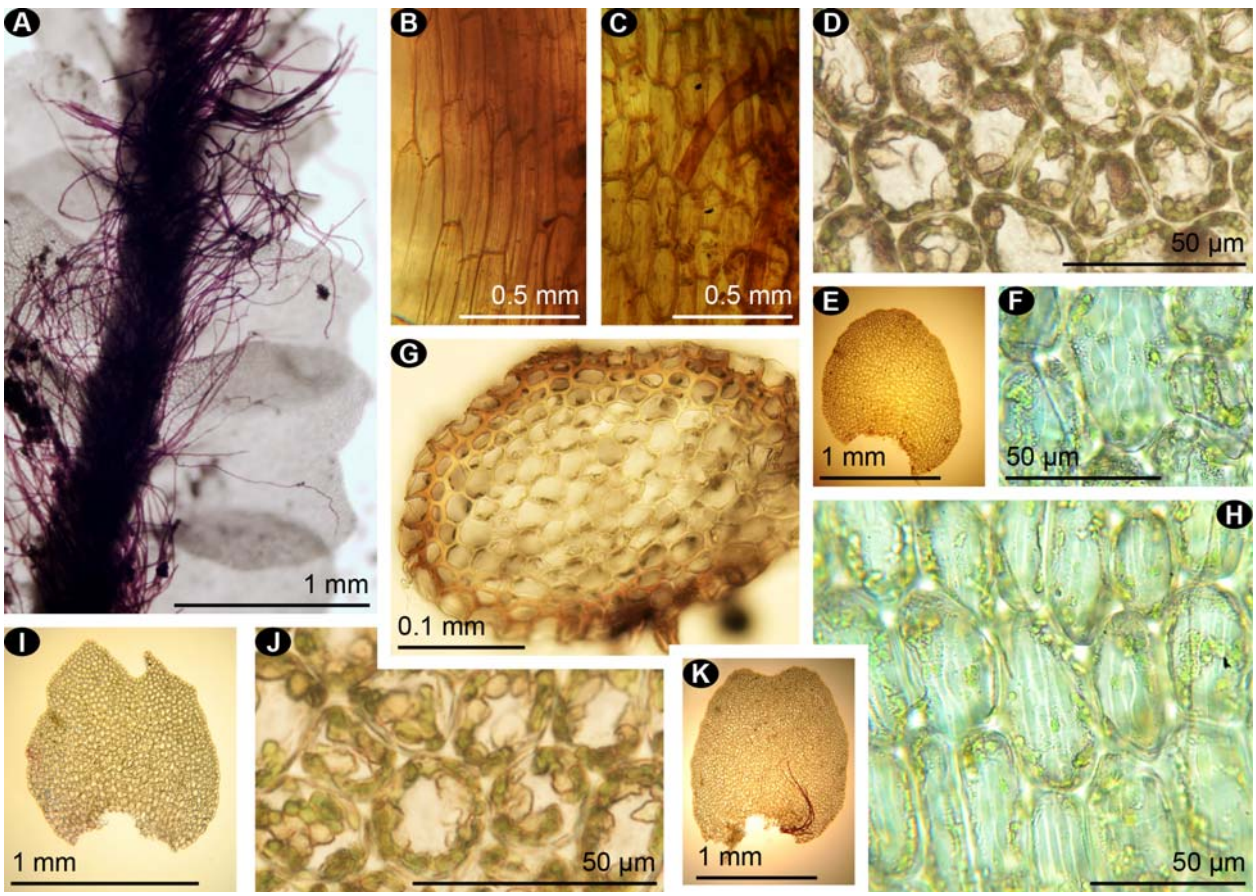

Fig. 4. *Solenostoma alaskanum* (all from holotype): A: shoot fragment (ventral view); B: cortical stem cells (dorsal view); C: cortical stem cells (ventral aspect); D, J: leaf cells showing persistent oil-bodies; E, I, K: leaves; F, H: basal leaf cells showing cuticular papillae; G: stem cross section.

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Received 13 November 2023 Accepted 5 December2023