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# A FURTHER RANGE EXTENSION OF THE GENUS *ARVERNELLA* (BRYOPHYTA)

# ДАЛЬНЕЙШЕЕ РАСШИРЕНИЕ АРЕАЛА РОДА *ARVERNELLA* (BRYOPHYTA)

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Abstract

A molecular phylogenetic analysis of tiny Amblystegiaceae plants from mountains of southern Siberia revealed an undescribed species of *Arvernella*. This genus was recently described from France and subsequently found in Sakhalin in the Russian Far East. Siberian plants represent the third species of the genus. Morphologically they are somewhat more similar to European ones, whereas the latter differ more strongly from both Asiatic species genetically. The newly described species is one of the smallest moss species in Russia with leaves 0.17–0.20 mm long and 0.06–0.07 mm wide only. It is currently known from four localities, the most distant being 530 km apart.

Резюме

Изучение с помощью молекулярно-генетических методов очень мелких растений из семейства Amblystegiaceae, собранных на юге Сибири, выявило еще один не описанный вид из рода *Arvernella*. Этот род был недавно описан по образцам из Франции и впоследствии найден на о. Сахалин на российском Дальнем Востоке, откуда был описан второй вид. Сибирские растения представляют третий вид рода *Arvernella*. По некоторым морфологическим признакам этот вид более сходен с европейским, который, однако, сильнее отличается от азиатских видов по последовательностям ДНК. Сибирский вид *Arvernella* – это одно из самых мелких растений во флоре мхов России, его листья всего 0.17–0.20 мм длиной и 0.06–0.07 мм шириной. *Arvernella sibirica* в настоящее время известна из четырех местонахождений, расстояние между самыми дальними из них около 530 км.

KEYWORDS: mosses, ITS, new species, South Siberia

## Introduction

The recent biodiversity studies in Russia increased the number of species in the local floras of many regions, and especially in Siberia – huge and quite incompletely known territory. Advances in the studies of Anabar Plateau in the southern Taimyr were summarized by Fedosov *et al*. (2011); Yakutia exploration in two recent decades was recently overviewed by Ignatov *et al*. (2022). Most of the new species described from these areas were supported by molecular phylogenetic data. Recent studies in Baikal area by a large group of bryologists with subsequent broad application of DNA barcoding demonstrated the power of such 'integrative floristics method' that revealed tens of unexpected records for species known from so faraway localities that their findings in Baikal surroundings were thought to be impossible (Fedosov *et al.*, 2022).

A similarly unexpected discovery was recently recognized for the genus *Arvernella* in the Russian Far East (Ignatov *et al*., 2021). This genus has been described only recently for one species from France (Hugonnot & Hedenäs, 2015). The plant is very small, so it remained undescribed up to the 21st century in a relatively well-known region – Western Europe. DNA sequences confirmed that the Far Eastern plants belong to the same genus, but is another species.

Subsequent attempts to find more representatives of *Arvernella* were undertaken. We looked through herbarium collections for the smallest pleurocarpous mosses, and sequenced the most promising candidates.

## Material and methods

### *Specimen search*

We looked for potential *Arvernella* specimens in collections in MHA, MW, NSK, and LE, searching among *Serpoleskea confervoides* and *Pseudoamblystegium subtile* that are also small plants, and could be thus misidentified instead of *Arvernella*; moreover, the latter genus was described and illustrated only in the last years. Most promising specimens from NSK herbarium were selected for sequencing.

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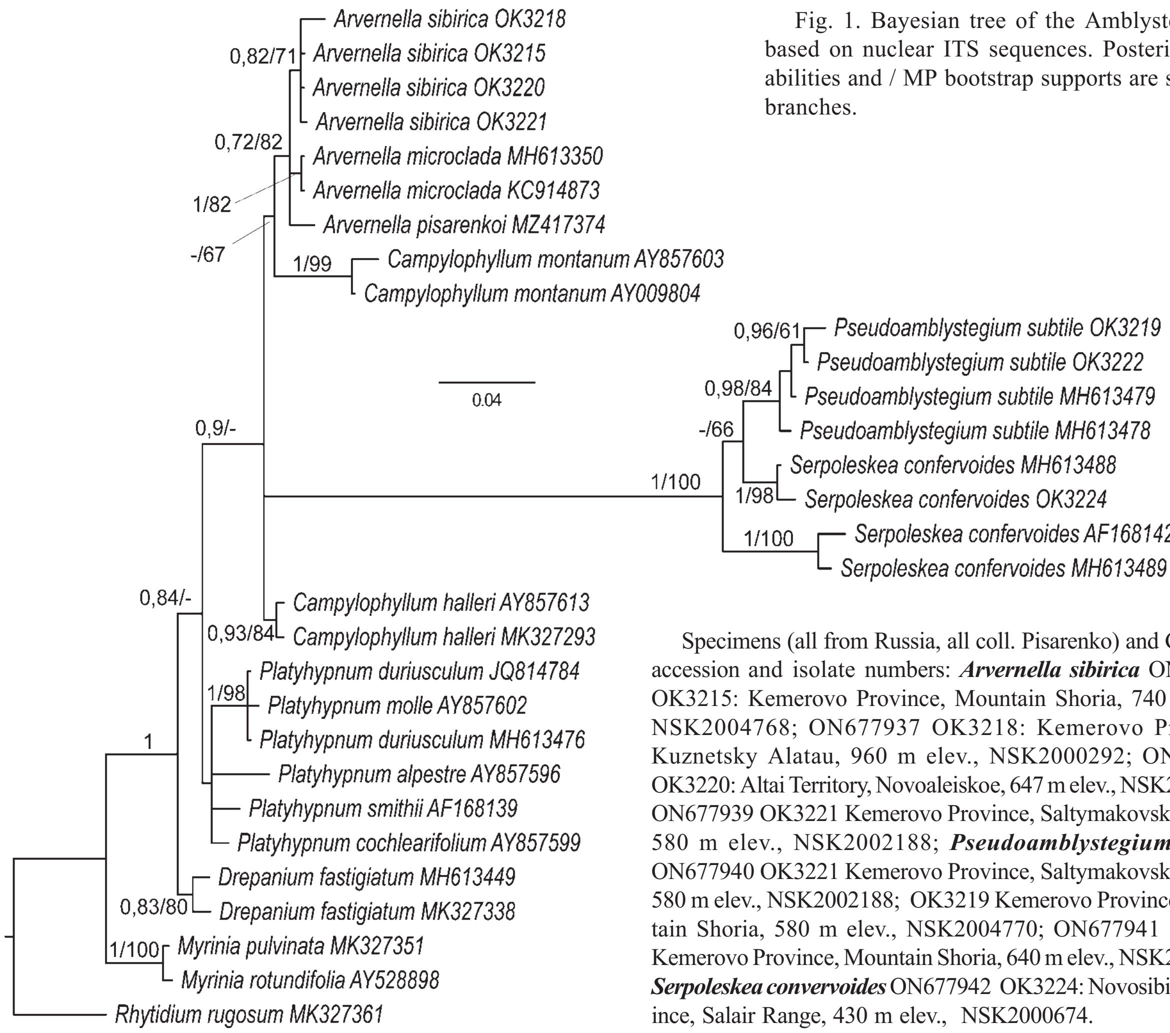


Fig. 1. Bayesian tree of the Amblystegiaceae based on nuclear ITS sequences. Posterior probabilities and / MP bootstrap supports are shown at branches.

Specimens (all from Russia, all coll. Pisarenko) and GenBank accession and isolate numbers: ***Arvernella sibirica*** ON677936 OK3215: Kemerovo Province, Mountain Shoria, 740 m elev., NSK2004768; ON677937 OK3218: Kemerovo Province, Kuznetsky Alatau, 960 m elev., NSK2000292; ON677938 OK3220: Altai Territory, Novoaleiskoe, 647 m elev., NSK2000685; ON677939 OK3221 Kemerovo Province, Saltymakovsky Range, 580 m elev., NSK2002188; ***Pseudoamblystegium subtile*** ON677940 OK3221 Kemerovo Province, Saltymakovsky Range, 580 m elev., NSK2002188; OK3219 Kemerovo Province, Mountain Shoria, 580 m elev., NSK2004770; ON677941 OK3222 Kemerovo Province, Mountain Shoria, 640 m elev., NSK2004634; ***Serpoleskea convervoides*** ON677942 OK3224: Novosibirsk Province, Salair Range, 430 m elev., NSK2000674.

***Sequence acquisition***

The laboratory protocol and sequencing were essentially the same as in our previous moss studies, described in detail by, e.g., Gardiner *et al.* (2005). Nuclear ITS sequences were obtained for Siberian plants; however, the *atp*B-*rbc*L region was not amplified with the same primers and same protocol which were used for successful amplification of the Far Eastern *Arvernella*.

***Molecular analysis***

ITS sequences from Siberian plants were checked by BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi, on December 2021), and since their position appeared to be the closest to *Arvernella*, they were added to the alignment compiled so to include also taxa close to *Arvernella* by BLAST, essentially the same used for the analysis in the previous paper (Ignatov *et al*., 2021), but with the exclusion of many only remotely related taxa composing the terminal clade of the family. The genera taken for analysis usually form a basal grade to the Amblystegiaceae, including *Myrinia*, *Platyhypnum*, *Drepanium,* and *Campylophyllum*. *Serpoleskea confervoides* and *Pseudoamblystegium subtile* were included as superficially most similar plants. Sequences were aligned using Bioedit (Hall, 1999).

Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist *et al.*, 2012), with 10 000 000 generations, and the chain temperature 0.02 in all analyses. Convergence of each analysis was evaluated using Tracer1.4.1 (Rambaut & Drummond, 2007). Consensus trees were calculated after omitting the first 25% trees as burn-in.

Maximum parsimony analysis was performed in Nona (Goloboff, 1994) in the Winclada shell (Nixon, 1999), with bootstrap calculations for 2000 replications (N searches 100, starting trees per rep 100, max trees 100, do max).

## RESULTS

***DNA analysis***

Molecular phylogenetic tree (Fig. 1) resolves Siberian plants assigned to *Arvernella* by BLAST in a clade with other species of *Arvernella*, although the combined *Arvernella* clade (PP=0.72, BS=82) and the clade of Siberian plants of *Arvernella* (PP=0.82, BS=71) are rather weakly to moderately supported. However, the clade of two specimens of the European *A. microclada* is well supported (PP=1.0, BS=82).

***Morphological analysis***

A comparison with the Far Eastern *A. pisarenkoi* and with the descriptions of *A. microclada* revealed

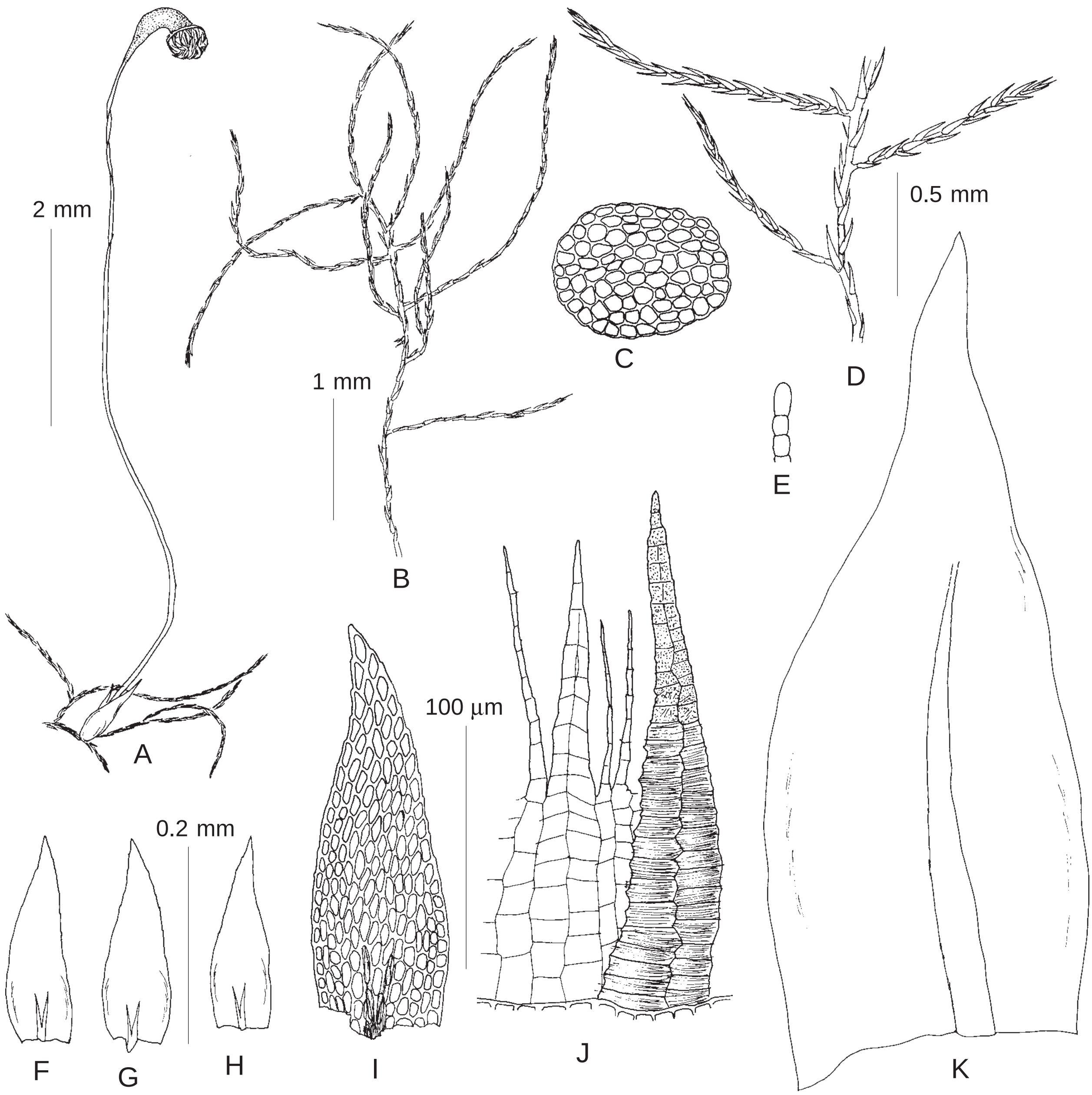


Fig. 2. *Arvernella sibirica* Ignatov & Ignatova (from holotype). A: plant with sporophyte, dry; B: sterile plant, dry; C: stem transverse section; D: stem and branches, wet; E: axillary hair; F–H: stem leaves; I: leaf cells; J: part of peristome; K: perichaetial leaf. Scale bars: 2 mm for A; 1 mm for B; 0.5 mm for D; 0.2 mm for F–H, K; 100 µm for C, I–J.

that the Siberian specimens possess all important features of the genus (Fig. 2), e.g., the small plant size; stem transverse section formed by homogeneous cells; ovate-lanceolate leaves; short, double or forked costae in stem leaves; entire leaf margins; short laminal cells; strongly enlarged perichaetial leaves with long costae; and autoicous sexual condition. The differences of Siberian plants from two other species of the genus are quantitative; they are discussed below, in the taxonomy section.

## Discussion

Despite the low support of the clade of Siberian plants, their morphology clearly indicate that they are conspecific, and the same is clear also from the same area of occurrence and the lack of differentiation within the clade (Fig. 1), excepting a small difference in branch length, which in fact is caused by a somewhat incomplete sequence data.

Siberian plants differ from Far Eastern plants by morphology, being somewhat more similar to European plants. The latter, however, have more prominent genet-

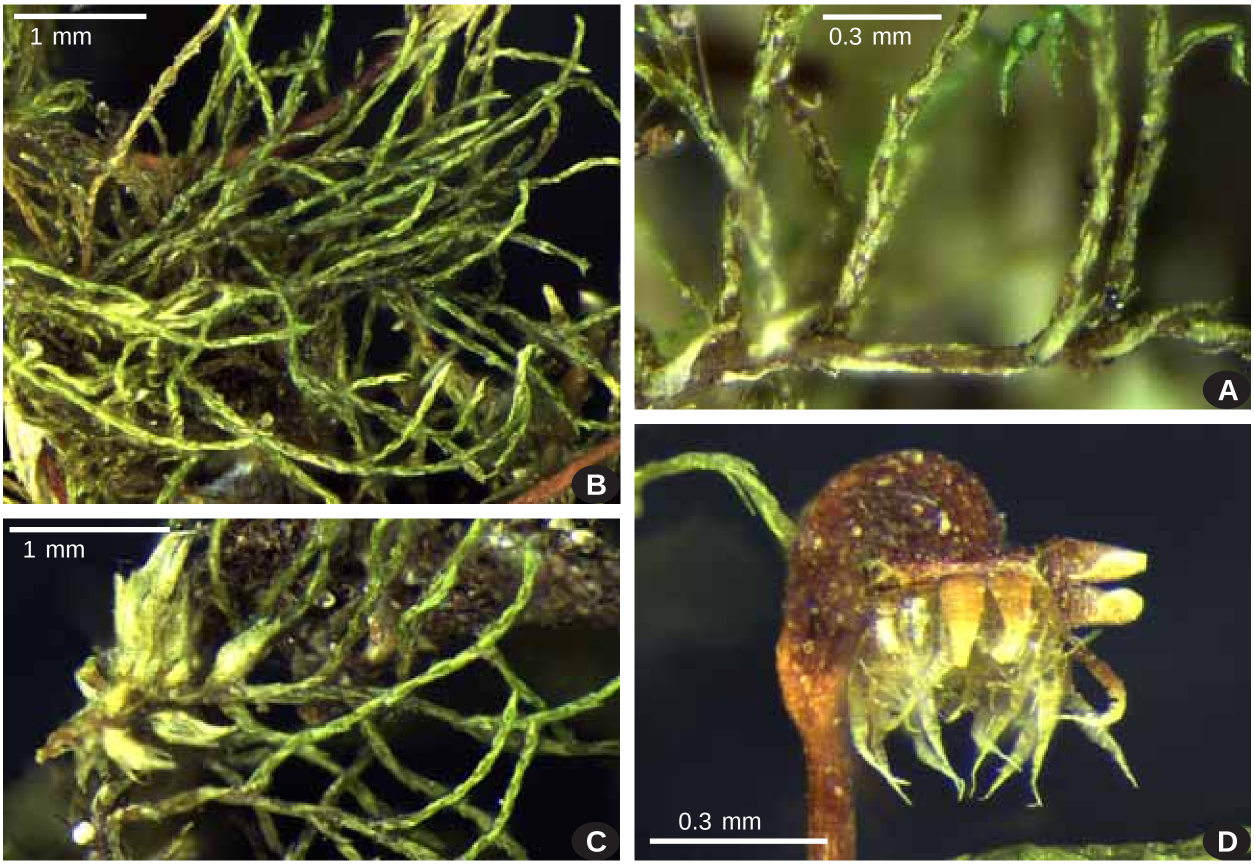


Fig. 3. *Arvernella sibirica* (from holotype). A–B: habit; C: plant with perichaetia; D: capsule.

ic differences, resulted in high support for *A. microclada* clade. Therefore, we describe Siberian plants as a new species.

## Taxonomy

**Arvernella sibirica** Ignatov & Ignatova, species nova. Figs. 2–3.

**Type**: Kemerovo Province, Tashtagol District. Gornaya Shoriya; watershed of Taenza and Mrassu Rivers; small stand of old-growth tall-herbaceous fir forest among young secondary aspen and birch communities (Gs10-18), 53.03750°N, 88.35981°E;740 m alt., 4 Aug 2010 Coll. Pisarenko O.Yu. NSK2004768 (Holotype MHA, Isotypes NSK, DNA isolate OK3221).

**Diagnosis**: *Arvernella sibirica* is similar to *A. pisarenkoi* in a very small plant size, shape of leaves, shape and size of leaf cells, but differs in having smaller leaves, 0.17–0.20×0.06–0.07 mm vs. 0.27–0.40×0.10–0.16 mm in *A. pisarenkoi*; not differentiated alar groups vs. clearly differentiated, consisting of subquadrate cells; shorter costae, to 0.2 the leaf length vs. to 0.2–0.4 the leaf length; and smaller spores, 8–10 µm vs. 10–13 µm. *Arvernella sibirica* differs from *A. microclada* in smooth vs. prorate leaf cells and smaller spores, 8–10 µm vs. 10–15 µm.

**Description**: *Plants* minute, in lax, tiny, delicate mats, green to dark green. *Stems* creeping, to 4 mm long, in transverse sections composed of homogeneous, firm-walled cells, without central strand and hyalodermis, terete foliate, irregularly branched, branches diverging at about right angle, ca. 1 mm long, terete foliate; rhizoids inserted below leaf insertion; axillary hairs 4–5-celled, upper cell ca. 16×10 µm; paraphyllia absent; proximal branch leaves linear to lanceolate. *Stem leaves* appressed when dry, erect when moist, 0.17–0.20×0.06–0.07 mm, lanceolate, gradually narrowed to apex, slightly narrowed to base, not decurrent; *costa* forked, to ca. 0.2 the leaf length, distinctly delimited; margins plane, finely serrulate throughout; upper and median *laminal cells* rhomboidal or elongate-rhomboidal, 10–15(–20)×4–5 µm, with length to width ratio 1.5–2.0(–2.5):1, firm-walled, smooth; cells at leaf margins in lower 1/2 the leaf length in 2–3 rows subquadrate to short-rectangular, 5–11×5–6 µm, alar cells not differentiated. *Branch leaves* similar to stem leaves. *Autoicous*. *Perigonia* bud-like, perigonial leaves ovate, strongly concave. *Perichaetial* leaves straight, 0.7–0.8×0.3 mm, triangular-lanceolate, acuminate, eplicate, with thick, gradually tapered, indistinctly delimited costa to 0.6 the leaf length. *Setae* to 7 mm, erect to somewhat flexuose, smooth, brownish to reddish-brown. *Capsules* inclined to somewhat pendent, urn 0.8–1.0×0.2–0.3 mm, elongate-ovoid, strongly contracted below mouth when dry and empty. *Annuli* deciduous. *Opercula* low conic and shortly and broadly rostrate. *Exostome* teeth 250–260 µm long, cross-striolate below, papillose above; *endostome* with basal membrane ca. 1/2 of its length, segments narrow, scarcely perforated, slightly shorter than exostome teeth, cilia 1–2, slightly shorter than segments, nodose. *Spores* 8–10 µm.

***Differentiation***: *Arvernella sibirica* shares some morphological characters with both other species of *Arvernella*. In leaf size it is closer to *A. microclada*: 0.17–0.20×0.06–0.07 vs. 0.14–0.35×0.055–0.080 mm. Plants of both *A. sibirica* and *A. microclada* appear extremely tiny due to closely appressed leaves (contrary to *A. pisarenkoi* with erect leaves). Mid-leaf cells of these species are also similar: 10–15(–20)×4–5 µm in *A. sibirica* and 17–22 ×3–10 µm in *A. microclada*; however, in the latter species cells are strongly prorate dorsally but in *A. sibirica* they are smooth. In addition, spores of *A. sibirica* are smaller: 8–10 µm vs. 10–15 µm in *A. microclada*. *Arvernella pisarenkoi* differs from two other species in slightly larger size of plants, larger leaves (0.27–0.40 ×0.10–0.16 mm) and longer costae. Both *A. pisarenkoi* and *A. sibirica* have leaves with distinct, forked costae, whereas leaves of *A. microclada* are often ecostate or with indistinct costae.

*Arvernella sibirica* is also similar to *Serpoleskea confervoides* – another tiny plant with curved capsules and leaves with short double costae; the latter species differs from both species of *Arvernella* in having stems with differentiated sclerodermis (cells are uniform in stem cross sections of *Arvernella*) and ecostate inner perichaetial leaves (with strong costae extending to mid-leaf in *Arvernella*).

The differences between three species of the genus can be also described in the key to identification:

1. Costae absent or very short and indistinct; median leaf cells abaxially strongly prorate . *A. microclada*
— Costae distinct, to 0.2–0.4 the leaf length; median leaf cells smooth or abaxially weakly prorate ....... 2
2. Leaves 0.27–0.40×0.10–0.16 mm; alar groups clearly differentiated; spores 10–13 ìm ......... *A. pisarenkoi*
— Leaves 0.17–0.20×0.06–0.07 mm; alar groups not differentiated; spores 8–10 ìm ............... *A. sibirica*

**Distribution and ecology**: The samples of *Arvernella sibirica* were collected at four points of the northwestern periphery of the Altai-Sayan mountain region (Fig. 4).

The localities where the species was collected are largely similar. All of them are situated in hyper humid regions surrounded by areas with sharply continental climate. An average annual temperature is about 0°C; the sum of temperatures above 10°C is not more than 1500°C. Annual precipitation everywhere is more than 800 mm, that is at least twice higher than in neighboring plains, and more than half of the amount falls in the warm period. In winter snow depth exceeds 80 cm and due to wind-caused redistribution can reach to 170–190 cm (Pilnikova, 1993). So thick snow layer keeps soil non-freezing during the winter period, with temperatures on a soil surface being about 0°C (Lashchinsky & Sedelnikov, 1991).

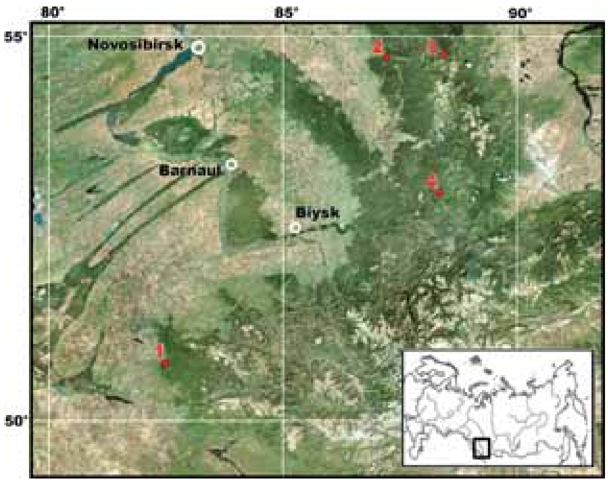


Fig. 4. Known localities of *Arvernella sibirica*: 1) Foothills of the western Altai Mts., upper reaches of the Aley River, 650 m alt. [Altai Territory, Tretyakov District]; 2) Saltymakov Range, which is the northern part of specific geological structures, a so-called "Melafir horseshoe", the basalt mold of Permian age, 580 m alt. [Kemerovo Province]; 3) Central part of Kuznetskiy Alatau Range, northern slope of the Malaya Tserkovnaya Mt., 960 m alt. [Kemerovo Province]; 4) Mountain Shoria, watershed of Taensa and Mrassu Rivers, 740 m alt. [Kemerovo Province].

The dominant tree species in all areas is *Abies sibirica.* Tall-herbaceous communities are the characteristic feature of the vegetation cover of these regions; they cover vast areas and form a mosaic with fir parcels. In most cases tall-herbaceous communities are polydominant; the herb layer is closed, tall, and multi-tiered. The average height of herbage is 1–1.5 m, but generative sprouts of some species often exceed 3 m. *Anthriscus sylvestris, Heracleum dissectum, Crepis sibirica, Bupleurum aureum, Aconitum septentrionale, Cirsium helenioides, Saussurea latifolia,* and *Euphorbia lutescens* are the most constant and abundant.

Coarse herbage acts as a significant environment-forming factor: it forms its own phytoclimate, which affects not only the illumination but also the regime of humidity and temperature. So, under the herbage canopy, diurnal temperature fluctuations are smoothed out, and the air humidity during the daytime is 15–30% higher than above it (Lashchinsky & Sedelnikov, 1991). In winter, under a thick snow cover, the processes of decomposition of plant residues continue to go on. As a result, despite the enormous productivity of the communities, there is almost no litter on soil and nothing besides of low light prevents the growth of mosses. Small stones of several centimeter, laying on soil under this canopy are thus in specific condition. Small species of Amblystegiaceae occur on such rocks and three collections from such environment appeared to be *Arvernella sibirica*. The fourth collection of this species was gathered from the base of *Padus avium* trunk in fir forest with tall herbs.

Other mosses abundant under tall-herb canopy are: *Oxyrrhynchium hians, Sciuro-hypnum reflexum, Mnium*




Fig. 5. Tall-herb vegetation with scattered or dense *Abies sibirica* stands. A–B: Saltymakov Range (B is close-up of A); C: Mountain Shoria.

*spinosum, Rhodobryum roseum, Fissidens bryoides, F. taxifolius,* occasionally *Eurhynchium angustirete* and *Thamnobryum neckeroides*, the two latter species being mixed with *Arvernella sibirica* in the holotype collection.

The specificity of the habitat conditions of this type of vegetation is emphasized by one more interesting species: *Rhynchostegium rotundifolium.* It is known in all Siberia only from two localities, 2 and 4 in Fig. 4 (Pisarenko, 2014), and in collection in loc. 2 it was mixed with *Arvernella sibirica*.

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## Literature cited

FEDOSOV V.E., E.A.IGNATOVA, M.S. IGNATOV & A.I. MAKSIMOV. 2011. Rare species and preliminary list of mosses of the Anabar Plateau (Subarctic Siberia). – *Arctoa* **20**: *153–174.*

FEDOSOV, V.E., O.M. AFONINA, M.S. IGNATOV, E.A. IGNATOVA, S.G. KAZANOVSKY, O.I. KUZNETSOVA, Y.S. MAMONTOV, N.A. KONSTANTINOVA, D.E. KOLTYSHEVA, S.KUBEŠOVÁG, P.M. LAMKOWSKI, A.MANUKJANOVÁ, N.S. GAMOVA, A.V. FEDOROVA, S.V. DUDOV, A.V. VERKHOZINA & J. KUČERA. 2022. Integrative floristics: a modern approach to biodiversity surveys in the molecular era, as applied to an expedition to the Khamar-Daban range, southern Siberia, Russia. – *Journal of Bryology*. doi 10.1080/03736687.2022.2078767

GARDINER, A., M. IGNATOV, S. HUTTUNEN & A. TROITSKY. 2005. On resurrection of the families Pseudoleskeaceae Schimp. and Pylaisiaceae Schimp. (Musci, Hypnales). – *Taxon* **54**: *651–663*.

GOLOBOFF, P.A. 1994. NONA: A Tree Searching Program. – *Tucumán, Argentina: Program and documentation, published by the author*.

HUGONNOT, V. & L. HEDENÄS. 2015. *Arvernella microclada* Hugonnot & Hedenäs (Amblystegiaceae), a new minute species from France, requiring a separate genus. – *Journal of Bryology* **37**(3): *184–191*.

IGNATOV, M.S., E.A. IGNATOVA & O.I. KUZNETSOVA. 2021. A rare European endemic moss genus *Arvernella* is discovered in Sakhalin, Russian Far East, where it is also rare. – *Arctoa* **30**: *1–7.* doi: 10.15298/arctoa.30.01

IGNATOV, M.S., E.A. IGNATOVA, E.I. IVANOVA, V.G. ISAKOVA, O.V. IVANOV & A.P. SEREGIN. 2022. MHA Herbarium: Collections of mosses from Yana-Indigirka Region, Yakutia, Russia. – Biodiversity Data Journal 10: e77341. https://doi.org/10.3897/BDJ.10.e77341

NIXON, K.C. 1999. Winclada (BETA) ver. 0.9.9. Available from: http://www.cladistics.com/about_winc.html

LASHCHINSKY, N. N. & V. P. SEDELNIKOV (ed.)] ЛАЩИНСКИЙ, Н. Н., В. П. СЕДЕЛЬНИКОВ. 1991. Экология сообществ черневых лесов Салаира. – [Ecology of chernevaia taiga communities on Salair] *Новосибирск, Наука* [*Novosibirsk, Nauka*]: *73* pp.

[PILNIKOVA, Z.N. (ed.)] ПИЛЬНИКОВА З.Н. (ред.) 1993. Научно-прикладной Справочник по климату СССР. Серия 3. Многолетние данные. Томская, Новосибирская, Кемеровская области, Алтайский край. – [Scientific and Applied Climate Reference Book of the USSR. Series 3. Long-term data. Tomsk, Novosibirsk, Kemerovo Provinces and Altai Territory] *СПб, Гидрометеоиздат* [*St.-Petersburg, Gidrometeoizdat*] **20**(1–6): *717* pp.

PISARENKO, O.Yu. 2014. Mosses of Salair-Kuznetsk region (Altai-Sayan mountain country) and adjacent plains of west Siberia – *Arctoa* **23**: *33–62*.

RAMBAUT, A. & A.J. DRUMMOND. 2007. Tracer v1.4. Available from http://beast.bio.ed.ac.uk/Tracer.

RONQUIST, F, M. TESLENKO, P. MARK, Van der, D.L. AYRES, A. DARLING, S. HÖHNA, B. LARGET, L. LIU, M.A. SUCHARD & J.P. HUELSENBECK. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. – *Systematic Biology* **61:** *539–542.*

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