

BRYOPHYTE MOLECULAR BARCODING RECORDS. 13

БРИОЛОГИЧЕСКИЕ НАХОДКИ ПО РЕЗУЛЬТАТАМ ДНК-МАРКИРОВАНИЯ. 13

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Abstract

DNA-barcoding revealed/confirmed the range extension of the following bryophytes: *Arvernella pisarenkoi* (Sakhalin Province, Kuril Islands, Russia), *Protoaongstroemia sachalinensis* (Kemerovo Province, Russia), *Brachythecium glareosum* (Leningrad Province, Russia), *Oligotrichum hercynicum* (Leningrad Province, Russia), *Gymnomitrium commutatum* (Murmansk Province, Russia), *Lophozia svalbardensis* (Norway, Svalbard), and *Calliergon giganteum* subsp. *sibiricum* (Norway, Svalbard), and *Sasaokaia aomoriensis* (Primorsky Territory, Russia).

Резюме

С помощью ДНК-баркодинга выявлены или подтверждены находки за пределами основного ареала следующих видов мохообразных: *Arvernella pisarenkoi* (Сахалинская область, Курильские острова, Россия), *Protoaongstroemia sachalinensis* (Кемеровская область, Россия), *Brachythecium glareosum* (Ленинградская область, Россия), *Oligotrichum hercynicum* (Ленинградская область, Россия), *Gymnomitrium commutatum* (Мурманская область, Россия), *Lophozia svalbardensis* (Норвегия, Шпицберген) и *Calliergon giganteum* subsp. *sibiricum* (Норвегия, Шпицберген) и *Sasaokaia aomoriensis* (Приморский край, Россия).

KEYWORDS: mosses, liverwort, new records, molecular markers, nrITS, Russia, Norway

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INTRODUCTION

This paper continues the series of brief reports of new findings proved in the course of the bryophyte DNA barcoding studies. It presents various finding where the sequencing either confirms species identities, which are ambiguous by various reasons, or disclose their affinities, or support generic placements of certain taxa that have never been investigated for molecular markers earlier, or have never been barcoded previously, or have been barcoded from different parts of the world. Being obtained in the course of screening rather than special projects of a particular group, such data may remain unsubmitted to DNA databases and stay neglected and not searchable among published materials.

1. *Arvernella pisarenkoi* Ignatov & Ignatova

Contributors: A.S. Kartasheva, M.S. Ignatov, O.I. Kuznetsova

Specimen: RUSSIA: Kuril Islands, Urup Island, Tokotan Lake, 45.85303°N, 149.789208°E, *Alnus* stand, on soil. 18.VIII.2021 A.S. Kartasheva s.n. (MHA9132205).

DNA: Isolate OK4298. GenBank accession numbers PX705562 (nrITS); PX715252 (*trnL-F*); PX715253 (*atpB*).

Sequences of all three markers were identical to that of respective sequences from the holotype.

This species was described by a single specimen from Sakhalin (Ignatov *et al.*, 2021). Before that, the genus was considered to be endemic of France (Hugonnot & Hedenäs, 2015), where it was discovered as a new species that belongs to an undescribed genus, which was extremely surprising for a relatively well studied Central Europe. Subsequently, a European species, *A. microclada*, was found in other regions of France (Tinguy *et al.*, 2019) and in Germany (Hugonnot & Pépin, 2025). In Russia, Pisarenko *et al.* (2022) discovered this genus in several localities in the mountains of South Siberia, but those plants were not fully identical to either European, or Far Eastern populations in DNA sequences and in morphology as well, and therefore they were described as *A. sibirica*. So, *Arvernella pisarenkoi* remained up to now the only species known solely by the holotype specimen. The new record is the first for Kuril Islands, extending its known distribution area southwards. The plant was found as an admixture to *Myuroclada longiramea* in *Alnus fruticosa* stand.

2. *Protoaongstroemia sachalinensis* Fedosov, Ignatova & Jan Kučera

Contributors: A.E. Nozhinkov, E.A. Ignatova. V.E. Fedosov, A.V. Fedorova

Specimen: Kemerovo Province, Krapivensky Municipal District, right bank of Tom River, 54.61418°N, 87.47111°E, 155 m a.s.l., on river bank on claiish alluvium. 1.VIII.2024 (MHA9132208).

DNA: Isolate OK4289. GenBank accession number PX712823 (*trnS-F*).

Like *Arvernella*, this taxon was described as new species from the new genus based on a single specimen,

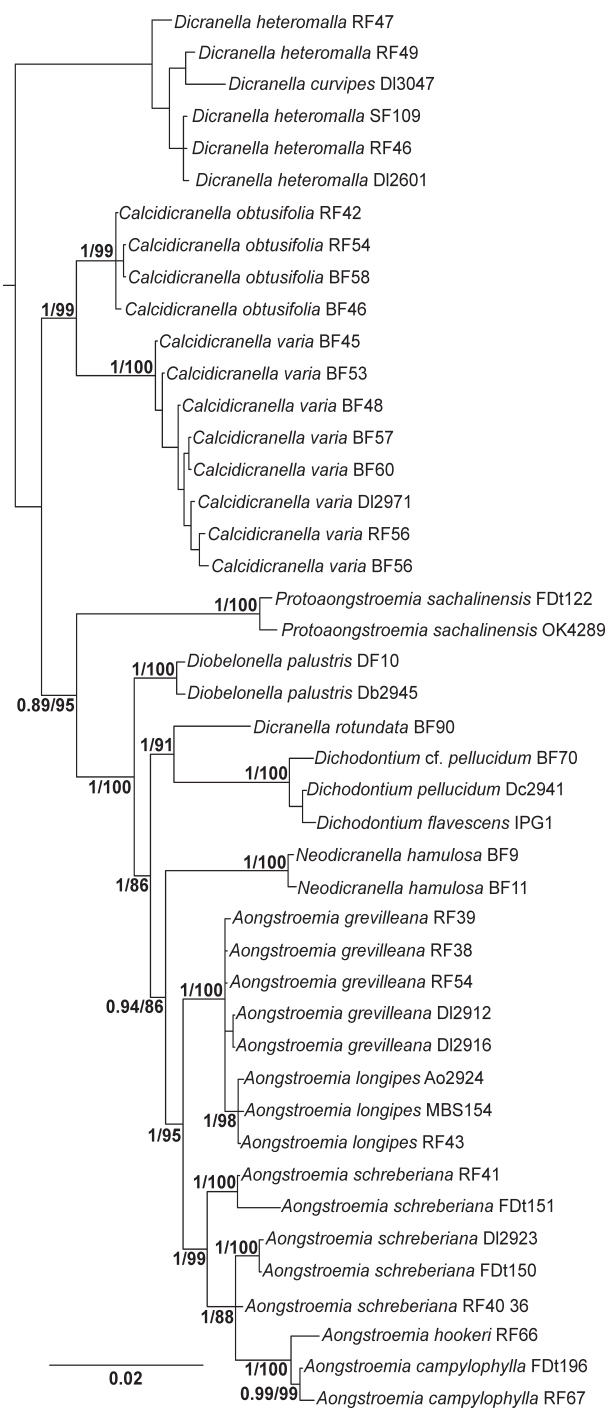


Fig. 1. Bayesian phylogenetic tree of the family Aongstroemiaceae based on the combined plastid *trnF-trnS* and mt *nad5* sequences.

since in molecular phylogenetic reconstruction it did not group with any genus of the family (Fedosov *et al.*, 2023). However, unlike *Arvernella*, this genus has fair morphological justification and can be easily recognized among other small *Aongstroemia*-like pioneer mosses due to two-to three-stratose lamina in upper leaf portion. Although the newly obtained sequence possesses few differences from the holotype (Sakhalin Island, Tym' River bank), it shares molecular synapomorphies of the latter and groups

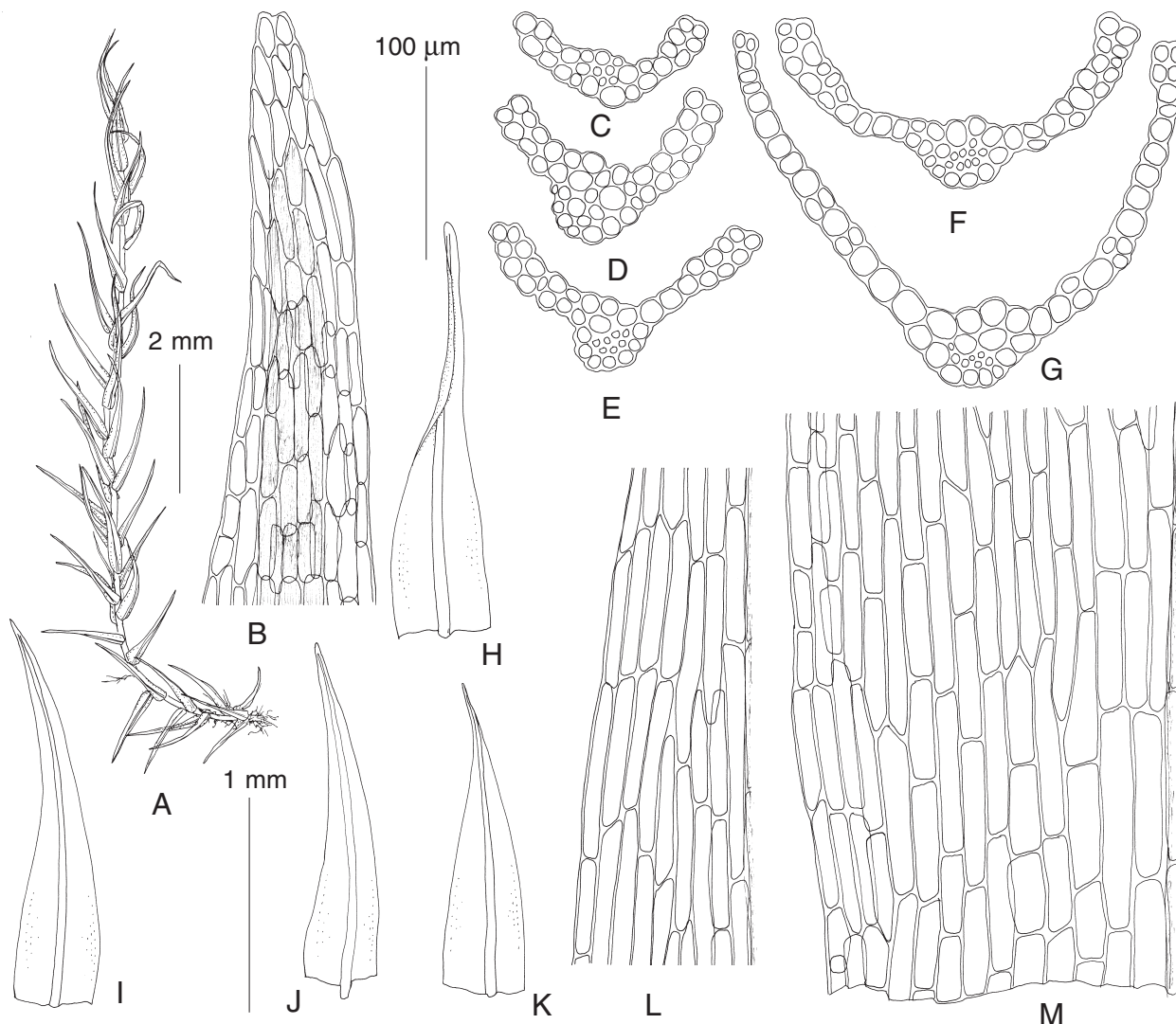


Fig. 2. *Protoaongstroemia sachalinensis* (from: Kemerovo Province, 1.08.2024, Nozhnikov s.n., MHA). A: habit, dry; B: upper leaf cells; C–G: leaf transverse sections; H–K: leaves; L: mid-leaf cells; M: basal leaf cells.

with it (Fig. 1). Distribution of this species remains poorly known since both known localities originate from North Asia, huge area, weakly sampled for bryophytes, but disjunction in its distribution between Sakhalin Island and humid mountain areas of South Siberia again reminds that among two Asian species of *Arvernella* (see Pisarenko *et al.*, 2022).

3. *Brachythecium glareosum* (Bruch ex Spruce) Bruch, Schimp. & W. Gümbel

Contributors: E.V. Smirnova, E.V. Kushnevskaya, M.S. Ignatov

Specimens: RUSSIA: Leningrad Province, Lomonosovsky District, 1.3 km west of Lomakha village, 59.66688°N, 29.00825°E, right bank of Lamoshka River, on shaded dry limestone (~10 m above the valley floor), 22.VIII.2023, coll. E.V. Smirnova & E.V. Kushnevskaya, MHA9132206.

DNA: Isolate OK4306: GenBank accession number PX705354 (nrITS).

Brachythecium glareosum has a more western distribution, reaching in Leningrad Province its eastern boundary. It was repeatedly reported from the province, but some records lack voucher specimens in herbaria (Borszczow, 1857; Elenkin & Beketov, 1919; Botch & Vasilevich, 1992), and some were re-identified as *B. salebrosum* (e.g., Schmalhausen, 27.VII.1871 LECB). The only collection confirmed is from Boksitogorsky District, Ragusha River (coll. M. Botch, 19.VIII.1982, det. G. Vyunova, LE). Our locality is the second for the Province and first proved by DNA.

4. *Oligotrichum hercynicum* (Hedw.) Lam. & DC

Contributors: E.V. Smirnova, A.V. Fedorova, M.S. Goldstein, E.V. Kushnevskaya & V.E. Fedosov

Specimens: RUSSIA: Leningrad Province, Karelian Isthmus, Vyborgsky District, 60.288135°N, 29.959094°E, the dirt road, on a roadside paved with fine gravel, 11.IX.2022, coll. M.S. Goldstein & E.V. Kushnevskaya, MW9118991.

DNA: Isolate: BF88; GenBank accession number PX724617 (nrITS).

The arctomontane species *Oligotrichum hercynicum* spreads southwards, as noted in the neighboring territories of Finland (Enroth, 2002) and Republic of Karelia (Maksimov & Maksimova, 2007). This species was first recorded in Leningrad Province in 2022 in its typical habitat, on a roadside of the dirt road. The plants are not well-developed, with narrow leaves (<0.55 mm wide, with width:length ratio 1:4), few and low ventral lamellae (<10 lamellae per leaf, <8 cells high), weak dorsal lamellae (1–2 per leaf or absent), upper lamina cells 12.5 µm, and short rectangular basal lamina cells (width:length ratio 1:2). However, molecular data confirm the species identification: obtained ITS sequence is identical to those of *O. hercynicum* from Chukotka Peninsula (DQ333436) and Kuznetsky Alatau (DQ529252), studied by Ivanova et al. (2005). The species is thought to have been spread by means of mining and logging equipment.

5. *Gymnomitrium commutatum* (Limpr.) Schiffn.

Contributors: N.A. Konstantinova, E.A. Borovichev & A.A. Vilnet

Specimen: Russia, Murmansk Region, Lapland State Nature Biosphere Reserve, Monchegorsk District, Volchya Tundra Mts, unnamed peak between Volchya Tundra and Mochesnyunchorr Mountains, 68°04'18"N – 32°23'17"E, 681 m alt., rock outcrops, exposed rock ledges, on fine soil, mixed with *Diplophyllum taxifolium* (Wahlenb.) Dumort. and *Lophozia* sp., 18.VIII.2012 Borovichev # BE243-1-12, det. Konstantinova (KPABG (hepatics) 127612).

DNA: GenBank accession number: PX533057 (nrITS1).

There were *Isopaches decolorans*, *Anthelia juratzkana*, and *Marsupella sprucei* in the envelop KPABG 21382 along with a small bryophyte mat (ca. 2.5×1.5 cm) consisted of *Gymnomitrium commutatum* which shoots creeping along the ground with an admixture of single shoots of *Diplophyllum taxifolium* (Wahlenb.) Dumort. and *Lophozia* sp. This mat was moved to a separate envelop with herbarium number KPABG (H) 127612.

It is the first finding of *G. commutatum* in Murmansk Region, European Russia, and northern Europe. Previously, it was known in Europe only from the mountains of central Europe and Iceland. It also occurs sporadically in South Greenland (Schuster, 1988), western North America, Japan (Damsholt, 2002), and China (Pippo, 1990), Korea, Nepal, Bhutan (Long & Grolle, 1990). In Russia, the species is sporadically found in the mountainous regions near and east of Baikal Lake (Mamontov, 2024). It's important to note that it has not been found yet in relatively well-studied Scandinavia. Molecular genetic study confirms previous records of *Gymnomitrium commutatum* from Alaska, Austria, Siberia, and Russian Far East as a single genetic unit with low level of infraspecific variability (Mamontov et al.,

2018). The obtained ITS1 nrDNA of specimen from Murmansk Region support the morphological identification of plants as *Gymnomitrium commutatum*, with level of molecular variability 0.5–1.1% to previously studied specimens from Central Europe, Asia and America.

Gymnomitrium commutatum is the Red-listed species that is assessed as VU according IUCN criteria in Red data Book of Russia (Mamontov, 2024).

6. *Lophozia svalbardensis* Konstant., Vilnet & Mamontov

Contributors: N.A. Konstantinova & A.A. Vilnet

Specimen: Norway, Svalbard, Nordaustlandet, Prince Oscar's Land, gentle slope with a puddle between large rocks surrounded by protruding spots covered with lichens. In cracks in the rocks, 80°12'16"N – 22°32'07"E, 156 m alt., 6.VIII.2006 Konstantinova & Savchenko # K112-1b-06 (KPABG(H)111804), det. Konstantinova.

DNA: GenBank accession number PX508779 (trnL-F).

This is the second finding in the Svalbard archipelago.

During the revision of doubtful specimens identified as *Isopaches bicrenatus* from the Arctic regions, one specimen identified as this species was redefined as *Lophozia* cf. *svalbardensis*. There were no perianthia or androecia in the specimen, but the plants had numerous, light purple and violet gemmae. To confirm the identification, the trnL-F cpDNA was sequenced and compared with published accession of the type specimen of *Lophozia svalbardensis* (MW297149). Obtained sequence supports the identity of specimen # K112-1b-06 as *Lophozia svalbardensis*. This species was described by us from Svalbard, Nordaustlandet, Gustav V Land, Murchisonfjorden (Konstantinova et al., 2020), and, until now, it was known only from this locality in the archipelago. Later *Lophozia excisa* var. *succulenta* (*Lophozia excisa* var. *succulenta*) was synonymized with *Lophozia svalbardensis* (Potemkin & Vilnet, 2021). As a result, the species is currently known in West Greenland, Yamal Peninsula, Gydan'skiy Peninsula, Arctic Alaska (l.c.), and on Sverdrup Island in Kara Sea (Kotkova et al., 2024). The new locality on Svalbard indicates a much wider distribution both in the archipelago and in the Arctic as a whole. The nucleotide sequence data confirm absence of trnL-F infraspecific variability in *Lophozia svalbardensis* through area.

7. *Calliergon giganteum* ssp. *sibiricum* Ignatova & Chernjad.

Contributors: O.A. Belkina & A.A. Vilnet

Specimens / DNA: 1) Norway, Svalbard, Billefjorden, west of the Pyramiden village, a south-eastern slope of Odinfjellet Mt., Planteryggen, 78°39'36"N; 16°5'53"E, elevation 220 m, wet hummocky dwarf shrub-moss-dominated tundra (place with seepage), on wet soil near base of hummock, 26.VII.2008, O.A. Belkina (B81-1-08), KPABG(m) 117445. DNA: Isolate M13a; GenBank ac-

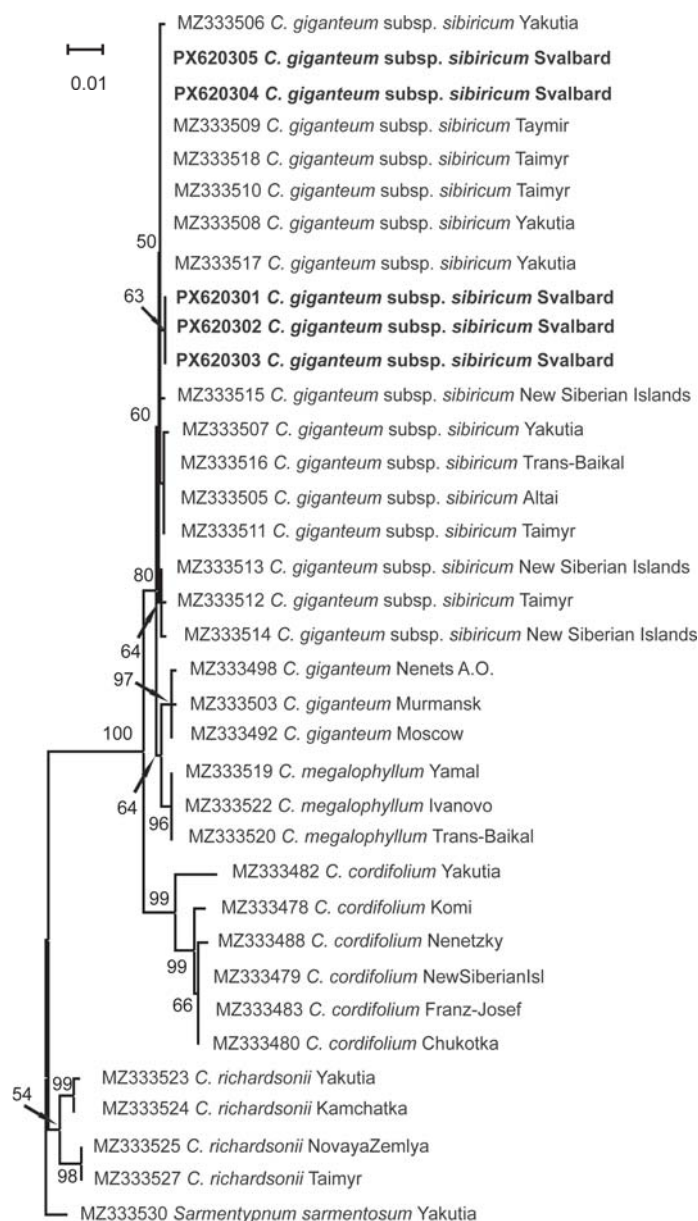


Fig. 3. The Neighbor-joining phylogenetic tree for the genus *Calliergon*, bootstrap support values more than 50% are indicated, GenBank accession numbers are marked, the newly sequenced specimens from Svalbard are in bold.

cession number PX620301 (nrITS). 2) same area, 78°39'42"N; 16°6'39", elevation 264 m, gentle part of east-south-eastern slope, banks and branched bed of shallow stream covered with mosses, on bottom, with *Scorpidium cossonii* (Schimp.) Hedenäs and *Sarmentypnum sarmentosum* (Wahlenb.) Tuom. & T.J. Kop., 30.VII.2008, O.A. Belkina (B140-96-08), KPABG(m) 117579. DNA: Isolate M13c; GenBank accession number PX620302 (nrITS). 3) Norway, Svalbard, Billefjorden, west of the Pyramiden village, Mimerdalen Valley near foot of south-eastern slope of Odinfjellet Mt., 78°39'22"N; 16°10'27", elev. 90 m, hummocky wet tundra with predominance of *Dryas octopetala* L., *Cassio-*



Fig. 4. Stem leaves of *Calliergon giganteum* subsp. *sibiricum* Svalbard. Scale bars 1 mm.

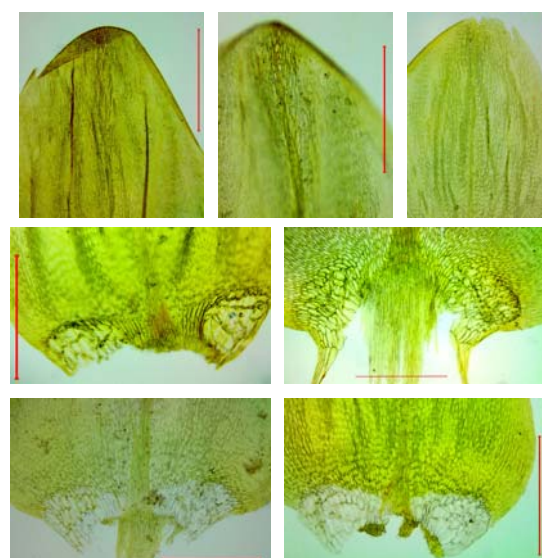


Fig. 5. Leaf distal parts and bases of *Calliergon giganteum* subsp. *sibiricum* Svalbard; scale bars 0.5 mm.

pe tetragona (L.) D. Don and mosses and with water between hummocks, on wet soil in depression, 3.VIII.2008, O.A. Belkina (B190-5-08), KPABG(m) 119295. DNA: Isolate M13d; GenBank accession number PX620304 (nrITS). 4) same, the foot of the south-eastern slope of Odinfjellet Mt., Estheriahaugen, 78°39'37"N; 16°10'7"E, elev. 100 m, small ornithogenic bog, on wet soil, with *Scorpidium* spp., *Drepanocladus turgescens* (T. Jensen) Broth., 3.VIII.2008, O.A. Belkina (B194-1-08), KPABG(m) 119316. DNA: Isolate M13e; GenBank accession number PX620305 (nrITS). 5) same, north of the Pyramiden village, north-western coast of Petuniabukta, marine terrace on eastern

Table 1. The nrITS *p*-distances in the genus *Calliergon*, %
Taxon Intraspecific Interspecific

		1	2	3	4
1	<i>C. giganteum</i> subsp. <i>sibiricum</i>	0.2			
2	<i>C. giganteum</i> subsp. <i>giganteum</i>	0.2	0.8		
3	<i>C. megalophyllum</i>	0.0	0.7	0.7	
4	<i>C. cordifolium</i>	0.9	2.5	2.7	2.9
5	<i>C. richardsonii</i>	0.8	4.6	4.7	4.7 5.5

foot of Svenbrehøgda Mt.; 78°43'19"N; 16°26'10"E, elev. 27 m, depression with wide shallow stream with many hummocks, on wet hummock, with *Scorpidium cossonii* 29.VII.2008, O.A. Belkina (B131/1a-08), KPABG(m) 118354. DNA: Isolate M13b; GenBank accession number PX620303 (nrITS).

Calliergon giganteum (Schimp.) Kindb. was not previously mentioned in Svalbard (Frisvoll & Elvebakk, 1996; Lönnell *et al.*, 2025). The subspecies *sibiricum* was segregated by Ignatova *et al.* (2021) for plants with narrow costa that are common in Siberia. The specimens from Svalbard also have a large group of alar cells that are sharply defined and mostly extended to the costa, they are colorless or brownish in older leaves. The costa, quite wide at the base, quickly narrows, reaching the cucullate apex of the leaf (Fig. 4, 5). The leaves are relatively small, many ones are shorter and wider compared to those of *C. giganteum* ssp. *giganteum*; but sometimes narrow leaves occur.

To confirm morphological identification of Svalbard's specimens the ITS1-2 nrDNA sequences were generated according with protocols described in Belkina & Vilnet (2021) and incorporated in dataset with previously published accessions for the genus *Calliergon* (Ignatova *et al.*, 2021). The phylogenetic estimation was provided by Neighbor-joining method released in Mega 11 (Tamura *et al.*, 2021) to avoid misleading relation based on low variability of obtained dataset. The *p*-distance model, accounting of transitions and transversions, pairwise deletion option for gap treatment and 1000 bootstrap replicates were used in calculation. The sequence variability was estimated as the average pairwise *p*-distances using the pairwise deletion option for counting gaps. Obtained topology (Fig. 3) illustrates that all five tested specimens from Svalbard form the clade with specimens of *C. giganteum* ssp. *sibiricum* from Yakutia, Trans-Baikal Territory, Altai, Taimyr and New Siberian Islands. The *C. giganteum* ssp. *sibiricum* clade related to clades of *C. giganteum* and *C. megalophyllum*. The sequence divergence among Svalbard's specimen consists only in single substitution in ITS1 of specimens B81-1-08, B140-96-08, B131/1a-08, that differ them from all other tested accessions. The *p*-distance variability in *C. giganteum* ssp. *sibiricum* consists 0.2% that are corresponding with variability in phylogenetically allied and morphologically similar *C. giganteum* (0.2%) (Table 1). All three taxa are slightly diverged from each other by ITS1-2 sequence data (0.7 and 0.8%) compared with *C. cordifolium* (2.5–2.9%) or *C. richardsonii* (4.6–5.5%).

The subspecies *sibiricum* has been recorded in the Asian part of Russia, including the continental Arctic and Arctic Ocean islands (New Siberian, Severnaya Zemlya, Vrangeli Island), as well as European Franz Josef Land (Czernyadjeva, Ignatova, 2022). Now this taxon was firstly found on the Svalbard archipelago. It is rather widespread along the lower part of the south-eastern slope of Odinfjellet Mt. and at its foot. We have also collected specimens of this subspecies in other points: at elevation 90 m (KPABG(m) 119281), 100 m (KPABG(m) 119329, KPABG(m) 119331), 200 m (KPABG(m) 117443), and 230 m (KPABG(m) 117453).

8. *Sasaokaea aomoriensis* (Paris) Kanda

Contributors: V.A. Kuptsova, E.A. Chubar, N.G. Razzhigaeva, M.S. Ignatov & O.I. Kuznetsova

Specimens: RUSSIA: Primorsky Territory, Khasan Distr., Lotos Lake, 42°27.4741'N, 130°38.1970'E north-western shore, swampy meadow with hummocks of *Carex appendiculata*. 10.VII.2024 Coll. E.A. Chubar #14 (MHA9132207).

DNA: Isolate OK4305; GenBank accession number PX705355 (nrITS).

The species was reported from Russia by Gorobets (2004) from island in the Peter the Great Bay of the Sea of Japan, but unfortunately later the specimen was destroyed. It was also reported from Khabarovsk Territory (Kuptsova *et al.*, 2018), based on identification of Ignatov, but later the molecular studies proved that in fact this specimen belongs to the floating form of *Elodium paludosum* (Ignatov, 2022).

The present collection from Primorsky Territory was made in somewhat peculiar habitat, which is described here in details.

The Lotos Lake, 2.9×1.8 km in size and no more than 2 m deep, is situated 9 km apart from the coast of Sea of Japan, 2.5 km apart from Tumannaya River, from which it is separated since the late Holocene (Mikishin *et al.*, 2008). Some ridges up to 90 m elev. surround the lake (Fig. 6). The western shore of the lake is swampy, and there is a succession of several plant communities from hill slope with springs to open water: (1) *Phragmites*+*Carex*, with large-tussocks of *Carex appendiculata* (10–25 m wide); (2) *Thelypteris palustris* +*On-*

Fig. 6. The Lotos Lake.



oclea sensibilis+*Calamagrostis angustifolia* (10–15 m wide); (3) *Carex* spp.+*Calamagrostis* +*Phragmites* alternating with *Phragmites*+*Carex lasiocarpa* (ca. 45 m wide); (4) pure *Phragmites* in shallow water up to 70 cm deep (few to 100 m wide); (5) scattered mire areas without *Phragmites* and *Carex lasiocarpa* are delimited by *Carex cryptocarpa* C. A. Mey. (*Carex lyngbyei* Hornem.), and have *Menyanthes trifoliata*, *Iris laevigata*, *Equisetum fluviatile*, *Carex pseudocuraica*, and *Zizania latifolia*; (6) open water with *Nelumbo nucifera*, *Nymphoides coreana*, *Trapa* spp., *Salvinia natans*, *Ceratophyllum demersum*, *Utricularia minor*, *U. macrorhiza*, *Myriophyllum* spp., *Potamogeton* spp., at places with *Schoenoplectus tabernaemontani*. Mosses cover 35–40% of the surface in 1, 2, and 3 of these vegetational types, and include *Plagiomnium ellipticum*, *Climacium dendroides*, *Thuidium subglaucinum*, and *Sasaokaia aomoriensis*. *Sasaokaia* was observed in between *Carex appendiculata* hummocks and along small brooks (42°27.4741'N, 130°38.1970'E).

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