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BRYOPHYTE MOLECULAR BARCODING RECORDS. 14

БРИОЛОГИЧЕСКИЕ НАХОДКИ ПО РЕЗУЛЬТАТАМ ДНК-МАРКИРОВАНИЯ. 14

Oxana I. Kuznetsova (ed.)[1], Alina V. Fedorova[1], Vladimir E. Fedosov[2,3], Michael S. Ignatov[1,2], Elena A. Ignatova[2], Yury S. Ishchenko[1], Valeria O. Khairetdinova[4], Lyubov E. Kurbatova[5], Elena D. Lapshina[6], Ilya V. Filippov[6], Ekaterina M. Ryzhova[4], Anna V. Shkurko[1] & Anna A. Vilnet[7]

Оксана И. Кузнецова (ред.)[1], Алина В. Федорова[1], Владимир Э. Федосов[2,3], Михаил С. Игнатов[1,2], Елена А. Игнатова[2], Юрий С. Ищенко[1], Валерия О.Хайретдинова[4], Любовь Е. Курбатова[5], Елена Д. Лапшина[6], Илья В. Филиппов[6], Екатерина М. Рыжова[4], Анна В. Шкурко[1], Анна А. Вильнет[7]

Abstract

DNA-barcoding revealed/confirmed the range extension of the following bryophytes: *Schistidium elegantulum* (Leningrad Province, Russia), *S. pruinosum* (Leningrad Province, Russia), *Didymodon validus* (Moscow, Russia), and *Sphagnum olafii* (Buryatia, Yakutia, and Khabarovsk Territory, Russia).

Резюме

С помощью ДНК-баркодинга выявлены или подтверждены находки за пределами основного ареала следующих видов мохообразных: *Schistidium elegantulum* (Ленинградская область, Россия), *S. pruinosum* (Ленинградская область, Россия), *Didymodon validus* (г. Москва, Россия), and *Sphagnum olafii* (Бурятия, Якутия и Хабаровский край, Россия).

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

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. ***Schistidium elegantulum*** H.H. Blom

Contributors: E.M. Ryzhova, L.E. Kurbatova, V.O. Khairetdinova, A.A. Vilnet

Specimen: RUSSIA: Leningrad Province, Vyborg District, Polyanskoye rural settlement, outskirts of the village of Pesochnoe, Fort Ino, 60.16901°N, 29.38391°E, southwest side of the concrete pillbox wall, 13.VIII.2025, E.M. Ryzhova & V.O. Khairetdinova (LE B-0049045).

DNA: Isolate M18. GenBank accession number PZ252753 (nrITS).

The morphological identification of *Schistidium* specimen gathered on pillbox wall of fortification building of the First World War was hampered by the absence of sporophytes. The BLAST search (https://blast.ncbi.nlm.nih.gov)

[1] – Tsitsin Main Botanical Garden, Russian Academy of Sciences, Botanicheskaya Str., 4, Moscow 127276 Russia. E-mails: oikuznets@gmail.com; alina_77777@mail.ru; misha_ignatov@list.ru; shen-ku@bk.ru. ORCID: (OK) 0000-0002-5513-1329; (AF) 0000-0001-7362-2124; (MI) 0000-0001-6096-6315; (YuI) 0000-0001-8473-6813; (AS): 0000-0001-7682-932.

[2] – Lomonosov Moscow State University, Faculty of Biology, Plant Ecology and Geography Dept., Leninskie Gory Str. 1–12, Moscow 119234 Russia. E-mails: fedosov_v@mail.ru; arctoa@list.ru. ORCID: (VF) 0000-0002-5331-6346, (EI) 0000-0001-6287-5660.

[3] – Botanical Garden-Institute, Far Eastern Branch of the Russian Academy of Sciences, Makovskogo Street, 142, Vladivostok, 690024, Russia

[4] – St Petersburg University (SPbU), Universitetskaya Emb., 7/9, St Petersburg 199034 Russia. E-mails: katrin.ryzhova.2002@mail.ru; st037699@student.spbu.ru. ORCID: (ER) 0009-0003-4013-1945; (VKh): 0009-0006-4847-4004.

[5] – Komarov Botanical Institute of the Russian Academy of Sciences, Prof. Popov Str., 2, St. Petersburg, Russia, 197376. E-mail: kurbatovale@binran.ru. 0000-0003-4695-5331.

[6] – Yugra State University, 16 Chekhov St., Khanty-Mansiysk, 628012 Russia E-mail: e_lapshina@ugrasu.ruб filip83pov@yandex.ru. ORCID (EL): 0000-0001-5571-7787, (IF) 0000-0002-3639-553X

[7] – Polar-Alpine Botanical Garden and Institute of the Kola Science Center of the Russian Academy of Sciences, Apatity, Russia, 184209. E-mail: anya_v@list.ru. ORCID: (AV) 0000-0001-7779-2593.

Fig 1. The maximum likelihood phylogenetic tree for *Schistidium elegantulum* and related species. GenBank accession numbers and voucher details are provided. The newly sequenced specimen from Leningrad Province is in bold.

of its nrITS1-2 reveals the 95-98% similarity with accessions of *Schistidium elegantulum*. To prove the species identity and evaluate its molecular variability, the nrITS1-2 dataset was created including *S. elegantulum* accessions from several European countries, as well as the single available Russian accession, accession from Krasnodar Territory of Russia (DQ822022/HM031071).

Five closely related according to Blom *et al.* (2025) taxa were added to phylogenetic reconstruction by maximum likelihood approach with IQ-Tree (Nguyen *et al*., 2015). Tree topology (Fig.1) shows the position of the specimen from Leningrad Province in a clade with numerous specimens of *Schistidium elegantulum.* The *p*-distance estimation in Mega 11 (Tamura *et al.*, 2021) reveals the variability till 1.3% in nrITS1-2 of selected specimens of *S. elegantulum*, with the highest similarity of the Leningrad's specimen to the accession from Krasnodar Territory.

In Russia, *Schistidium elegantulum* is known from the Caucasus, Eastern Siberia, and Primorsky Territory. In European Russia, this species was recorded from Moscow, Lipetsk, Ryazan, Rostov, and Voronezh Regions (Ivanov *et al*., 2017). One of the geographically close record of this species is from Pskov Region (*Zolotov & Zolotova s.n*. 13.IX.1999, MHA) and Karelia (Ignatovf et al., 2006). Several finds are provided for Baltic states and southern Finland (Hyvärinen *et al*., 2019). Our record supported by nucleotide sequence data is the first for the Leningrad Province.

2. *Schistidium pruinosum* (Wilson) G. Roth

Contributors: E.M. Ryzhova, L.E. Kurbatova, A.A. Vilnet

Specimens: RUSSIA: Leningrad Province, Lomonosovsky district, Lebyazhenskoye urban settlement, Fort Krasnaya Gorka, 59.97623°N, 29.33280°E, north-eastern descent, inner part of the concrete pillbox wall, 13.X.2024, E.M. Ryzhova & L.E. Kurbatova (LE B-0049044).

DNA: Isolate M17. GenBank accession number PZ252752 (nrITS), PZ263162 (*trn*L-F).

The *Schistidium*-like specimen with poorly defined papillae on the leaf surface gathered in the abandoned fortification could not be reliably identified based on morphological features. Hofbauer *et al.* (2016) proposed the nrITS2 as a barcode for identification of poorly developed *Schistidium* plants colonizing building surfaces, allowing to discover a hidden diversity in this genus.

We were able to obtain nrITS1-2 and *trn*L-F sequenc-

Fig 2. The maximum likelihood phylogenetic tree for *Schistidium pruinosum* and related species. GenBank accession numbers and voucher details are provided. The newly sequenced specimen from Leningrad Province is in bold.

es for the tested specimen. BLAST search (https://blast.ncbi.nlm.nih.gov/) of nrITS1-2 provided 91–96% similarity with two accessions of *Schistidium pruinosum* from the Russian part of Caucasus (HM053933, Kabardino-Balkarian Republic, 27.VII.2004 *Ignatov & Ignatova s.n.,* MW and HM053932, Republic of Adygeya, 26.VI.2003 *Akatova s.n*., MW); these two specimens were found to be distantly related by Milyutina *et al.* (2010). The *trn*L-F sequence suggests 99% similarity with the corresponding part of the whole chloroplast genome of *Schistidium strictum* (Turner) Loeske ex Mãrtensson (OZ350023). Due to a limited number of accessions for *trn*L-F region of *Schistidium* species, an estimation of the phylogenetic affinity and species identity of the tested specimen was based on nrITS1-2 sequences only. The dataset with 16 accessions of the species closely related to *Schistidium pruinosum* (according to the topologies provided by Milyutina *et al.*, 2010, and Hofbauer *et al.*, 2016) was analyzed by maximum likelihood method in the program IQ-Tree (Nguyen *et al*., 2015). In the obtained tree (Fig. 2), the specimen from Leningrad Region was found in the robustly supported clade composed of *Schistidium pruinosum* from Republic of Adygeya and two unidentified specimens from Germany and Italy. The specimen of *Schistidium pruinosum* from Kabardino-Balkarian Republic revealed the sister affinity to this clade. The *p*-distances of tested specimen calculated in Mega 11 (Tamura *et al*., 2021) are 0.2% to both specimens from European countries and 0.6% to the specimen from Adygeya. The Kabardino-Balkarian specimen is diverged from the listed *Schistidium pruinosum* accessions in 4.3-5.5%, which is similar to the distances between pairs of species *Schistidium apocarpum – S. strictum* or *S. lancifolium – S. andreaeopsis.* According to the phylogenetic position and sequence similarity with European accessions, the Leningrad specimen should be treated as *Schistidium pruinosum*. This is the first record of this species for the region.

In Russia *Schistidium pruinosum* is known from Republic of Bashkortostan (*Zolotov 07-42*, MHA9116800), several regions of the Caucasus (Ignatova & Blom, 2017), Khabarovsk Territory (*Ignatov & Ignatova 13-514*, MHA9116799), and Murmansk Region (Kozhin *et al*., 2019).

3. ***Didymodon validus*** Limpr.

Contributors: M.S. Ignatov, E.A. Ignatova, Y.S. Ishchenko, A.V. Fedorova

Specimen: RUSSIA: Moscow, 55.841932°N, 37.617073°E, ca. 140 m elev., Tsitsin Main Botanical Garden, alpinarium of the Caucasian plants, on vertical surface of the limestone wall faced to the lawn, 29.X.2024, *Ignatov & Ishchenko 24-2064*, MHA.

DNA: Isolate OK4187. GenBank accession number PZ252753 (nrITS), PZ715504 (*trn*G).

This is a common species in Asian Russia, Caucasus and some montaneous regions of Europe. However, in Cental European Russia it was known as a rare species from more southern Kursk, Lipetzk, Voronezh Provinces and recently discovered in Ryazan Province (Kuznetsova *et al*., 2025). The new collection was done in the Botanical Garden, on an artificially constructed hill for Caucasian plants. This place was studied earlier (Ignatov & Ignatova, 1990), and there was found only *D. fallax*, the most common species of the genus *Didymodon* s.l. in Moscow Province. Therefore, we presume spreading of *D. validus* in recent years. BLAST seach found ITS of this specimen similar for >99.5% with 18 specimens of *D. validus* and noone else species; BLAST of *trn*G found 100% similarity only with six accessions of *D. validus*. This specimens has been published using identification by morphology (Ignatov et al., 2025), but considering numerous misidentifications in this genus we provide here a molecular barcoding confirmation.

4. ***Sphagnum olafii*** Flatberg

Contributors: A.V. Shkurko, O.I. Kuznetsova, E.D. Lapshina & V.E. Fedosov

Specimens: RUSSIA: (1) Buryatia, Tunkinskiy Distr., vicinity of Mondy, Khullugaima Mt. cirque, 51.769°N, 100.891°E, damp alpine tundra, 17.VII.2023 coll. *Fedosov V.E. #319* (MW). (2) Yakutia, Kytalyk National Park, to the west of the Chokurdakh settlement, 70.796°N, 145.853°E, polygonal bog, on permafrost ridge dividing polygons and occupied by *Salix pulchra, Carex aquatilis, Sphagnum squarrosum, Aulacomnium* spp., *etc*., 21.VII.2023 coll. *Fillipov I.V. #125F-4* (s124) (MHA), 05366 (YSU). (3) Khabarovsk Territory, Amur District, Sanboli surroundings, Alga River valley, 49.336°N, 135.121°E, *Betula ovalifolia, Chamaedaphne calyculata*, *Carex lasiocarpa* & *Sphagnum* spp. (*S. imbricatum, S. obtusum, S. orientale*) dominated mire, 1.VIII.2025 *Lapshina E.D. #38E/1-1a* (MHA), 07299 (YSU). (4) Khabarovsk Territory, Amur District, Selgon surroundings, 49.579°N, 135.417°E, dwarf shrub (*Chamaedaphne calyculata*)–horsetail (*Equisetum fluviatile*)–*Sphagnum* spp. (*S. imbricatum, S. obtusum, S. orientale, S. subsecundum*) dominated mire, 2.VIII.2025 coll. *Filippov I.V. #42F-2a* (MHA), 07388 (YSU).

DNA: Isolates/GenBank accession numbers (1) OK3765 / PZ569218; (2) OK852 / PZ569217; (3) OK4432 / PZ569216; (4) OK4508 / PZ569215. (RAPD-A).

Sphagnum olafii (sect. *Acutifolia*) was described from Svalbard (Flatberg, 1993). The species has circumpolar distribution at high latitudes, where it occupies predominantly arctic and alpine tundra communities and mires (Flatberg, 1993; Flatberg *et al.*, 2016). In Russia this species occurs from Yamalo-Nenets Autonomous District to Chukotka (Flatberg *et al.*, 2016; Beldiman *et al*., 2020; Lapshina & Filippov, 2025), while the southernmost localities was reported from alpine tundra in Zabaikalsky Territory (Flatberg *et al*., 2016) and South Buryatia (Fedosov *et al*., 2025). Recently, the plants morphologically similar to *S. olafii* were collected from Khabarovsk Ter-

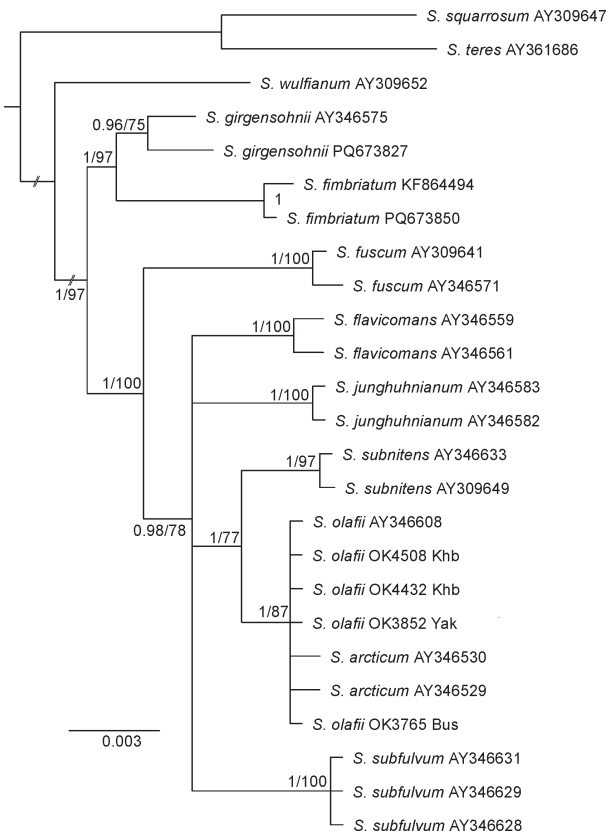

Fig. 3. Bayesian phylogenetic tree of *Sphagnum* subg. *Acutifolia* species based on the nuclear RAPD-A sequences and focused on the species of *S. subnitens* complex. Posterior probabilities >0.7 and bootstrap supports >70 are shown at branches.

ritory where they grew in minerotrophic *Betula ovalifolia*-sedge (*Carex lasiocarpa*)-*Sphagnum* mires with *Chamaedaphne calyculata* and *Equisetum fluviatile* in the Middle Amur Lowland in the southern taiga subzone, which is rather unusual for this Arctic-alpine species. To check the identity of these plants we used molecular barcoding approach to compare with the available GenBank accessions and other *S. olafii* specimens collected from arctic and alpine tundra communities, including the specimen from East Sayan (Buryatia).

For DNA barcoding we used anonymous nuclear region RAPD-A; protocols of DNA extraction follows Fedosov *et al.*, 2016, amplification and sequencing follow Shaw *et al*. (2003). To evaluate affinities of the studied specimens, we added our dataset with 23 accessions of RAPD-A region representing *S. olafii* from Svalbard and some other species of *S.* subg. *Acutifolia*: *S. arcticum* Flatberg & Frisvoll, *S. fimbriatum* Wilson, *S. flavicomans* (Cardot.)Warnst., *S. fuscum* (Schimp.) H. Klinggr., *S. girgensohnii* Russow, *S. junghuhnianum* Dozy & Molk., *S. squarrosum* Crome, *S. subfulvum* Sjörs, *S. subnitens* Russow & Warnst., *S. teres* (Schimp.) Ångstr. and *S. wulfianum* Girg. Sequences were aligned using MAFFT v.7487 for Windows (Katoh *et al.*, 2002) and then alignments were edited manually in BioEdit (Hall, 1999). Topologies inferred from RAPD-A were considered with the indel data scored using the simple indel coding (SIC) approach (Simmons & Ochoterena, 2000) in SeqState 1.4.1. (Müller, 2005) for Bayesian (BI) reconstructions and in FastGap 1.2 (Borchsenius, 2009) for Maximum likelihood (ML). Settings of phylogenetic analysis follow Shkurko *et al*. (2026) in present volume. DNA barcoding confirmed the morphology-based identification of the species (Fig. 3) and its range extensions southward in mountains of south Siberia and Southern part of Russian Far East.

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