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NEW SPECIES OF LIVERWORT FROM YAKUTIA (RUSSIAN ARCTIC): WHEN STRIKING MORPHOLOGICAL DIFFERENCES ARE NOT SUPPORTED FROM DNA

НОВЫЙ ВИД ПЕЧЕНОЧНИКА ИЗ ЯКУТИИ (РОССИЙСКАЯ АРКТИКА): КОГДА ЯРКИЕ МОРФОЛОГИЧЕСКИЕ РАЗЛИЧИЯ НЕ ПОДДЕРЖИВАЮТСЯ ДНК МАРКЕРАМИ

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Abstract

A new liverwort species *Rudolgaea aenigmatica* is described from Yakutia based on a combination of striking morphological features, particularly very narrow, abaxially concave, undulate leaves with unevenly and irregularly curved margins, deep sinuses, lobes sharply tapering into a long, more or less narrow, sometimes incurved apex, margins often with long teeth, teeth-like lobes or cilia near the base that strikingly differs the species from all known liverworts. Molecular phylogenetic study resolved it in one clade with *Rudolgaea borealis.* The nucleotide sequences of two nuclear and five chloroplast DNA markers reveal the identity between *R. borealis* and the newly described species. ISSR analysis provides common bands composition for both species from 11 primers. At present, both species do not obtain molecular differentiation. The described species occupies similar habitats and is found in the same plant communities as *R. borealis*. Such obvious morphological differences in the absence of clear molecular and ecological differentiation have been described for liverworts for the first time, and at this stage of knowledge we cannot find an explanation for this.

Резюме

Новый вид печеночников *Rudolgaea aenigmatica* описан из Якутии на основании сочетания крайне своеобразных морфологических особенностей, в частности, волнистых, выпуклых на спинную сторону, очень узких и с глубокой вырезкой дву-трех-лопастных листьев с неровными, неравномерно отогнутыми краями с зубцами или длинными 2-3-х клеточными в основании ресничками и с удлиненными узкими лопастями, переходящими в часто узкую, крючковидно загнутую верхушку. Молекулярно-генетическое исследование трех образцов этого вида выявило их положение в одной кладе с *Rudolgaea borealis.* Нуклеотидные последовательности двух ядерных и пяти хлоропластных маркеров являются идентичными для *R. borealis* и нового вида. ISSR анализ выявил общий состав бэндов для обоих видов, полученных с использованием 11 праймеров. Оба вида не имеют четкой молекулярной дифференциации. Описанный вид обитает в сходных местообитаниях и встречается в тех же растительных сообществах, что и *R. borealis*. Столь явные морфологические различия при отсутствии четкой молекулярной и экологической дифференциации описаны для печеночников впервые и на данном этапе знаний объяснения этому не найдены.

KEYWORDS: *Rudolgaea aenigmatica*, morphology, molecular phylogeny, *rpb*2, *rpl*16, ISSR, plant communities, polygonal mires, floating fens.

INTRODUCTION

A peculiar liverwort, which we could not attribute to any of the known species, was discovered during the identification of specimens collected in the northeast of the Republic of Sakha (Yakutia) in the Berelekh River basin, a left tributary of the Indigirka River when studying mire habitats (Lapshina & Filippov, 2025). The liverwort was found as isolated shoots in mats and turfs of mosses. Its distinctive appearance, unlike any other known liverwort, and the absence of perianthia, androecia, and sporophytes did not allow these plants to be classified as belonging to any known species. To determine the phylogenetic relationship of this mysterious plant, the sequencing of widely used markers for liverworts barcoding (ITS1-2 nrDNA and *trn*L-F cpDNA) were implemented. Unexpectedly, the identity of the tested loci was shown with *Rudolgaea borealis*. The latter species was described by Frisvoll & Moen (1980) as *Lophozia borealis* subgen. *Leiocolea* but later was transferred in the genus *Gymnocolea* by Schuster (1986). Recently, based on

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a molecular phylogenetic study, it was shown that this species, along with *Gymnocolea fascinifera* Potemkin are not related to the genus *Gymnocolea,* and based on the integrative approach the new genus *Rudolgaea* was described (Potemkin & Vilnet, 2021). The morphological differences between the genus *Rudolgaea* and the genus *Gymnocolea* or *Lophozia* subgen *Leiocolea, i.e. Mesoptychia* in the absence of perianthia are expressed less strongly than the phylogenetic ones, which explains the placement of *R. borealis* in mentioned above genera (Schuster, 1986; Frisvoll & Moen,1980). The specimens we are considering here, on the contrary, are morphologically completely unlike any species of the mentioned above genera (*Rudolgaea, Gymnocolea,* and *Mesoptychia*). Because the Yakutian plants were morphologically very different from *R. borealis,* we sequenced the additional six DNA markers for available specimen of *R. borealis* and the newly collected plants. The Inter Simple Sequence Repeat (ISSR) method (Bornet & Branchard, 2001) for genome-wide screening of a high number of loci was additionally used to search genetic differences among specimens. As we know, the ISSR has worked poorly for assessment of infraspecific liverwort's diversity due to presence of fungal endophytes that could bias the results. Nevertheless, infraspecific structure of *Mannia fragrans* (Hock *et al.*, 2008) and some *Calypogeia* species (Buczkowska & Dabert, 2011) were successfully shown by ISSR approach.

The aim of this study is to describe the species based on morphological features, identify its phylogenetic position and provide results of integrative study showing presumably genetic identity of morphologically different plants as an example of a taxonomical puzzle.

MATERIAL AND METHODS

Morphological study

The plant morphology was studied using traditional microscopy techniques. The micrographs were taken using stereomicroscope Nikon SMZ 8007 and compound light microscope Nikon Eclipse SOi equipped with digital camera DS Fi1. Five specimens (see studied specimens) were studied in detail by the first author, the rest (mostly single shoots in mats of mosses) were identified and noted in the specimens by Elena Lapshina.

The specimens are deposited in the Herbarium of Polar-Alpine Botanical Garden-Institute of the Kola Science Center, Russian Academy of Science (KPABG(H)) and in the Biological collection of Yugra State University (YSU), Khanty-Mansiysk (herbarium numbers in square brackets after the original numbers). The specimen that contains the most abundant shoots of the species was chosen as the type.

Sampling for molecular analyses

Three specimens from Republic of Sakha (Yakutia) #44F-5-23(KPABG(H) 127871, YSU 05590), #47F-1-23 (KPABG(H) 127875, YSU 05599), #85E-4-23 (KPABG(H) 127874, YSU 05571) were selected to be tested by molecular genetic methods. Firstly, routine for liverworts barcoding loci ITS1-2 nrDNA and *trn*L-F cpDNA were obtained and BLAST search (https://blast.ncbi.nlm.nih.gov/) resolved them as near identical with *Rudolgaea borealis* sequenced previously from Gydan (*Troeva #G1-138*) and Taimyr (*Lapshina #4354,* KPABG-125157) Peninsulas (Potemkin & Vilnet, 2021; Kuznetsova *et al*., 2022).To solve this problem additional DNA markers were used: *rpb*2 nrDNA, *rbc*L, *rpl*16, *rps*4, *trn*T-F (incl. *trn*L-F), *psb*A-*trn*H cpDNA.

Following the available molecular data for family Anastrophyllaceae, phylogenetic estimation was provided based on ITS1-2, *trn*L-F and *rbc*L sequences. Other sequenced loci were used only for *p*-distance estimation. The ITS1-2+*trn*L-F dataset includes 42 previously published accessions from 18 genera and 35 species, the *rbc*L dataset includes 19 accessions from 12 genera and 13 species. *Obtusifolium obtusum* from the phylogenetical-

Table 1. Primers for the polymerase chain reaction and cycle sequencing reactions.

DNA marker	Primer sequence (5'-3')	Annealing temperature in PCR, °C	Reference
ITS1-2 nrDNA	Forward ACCTGCGGAAGGATCATTG	56	Friedl, 1996
ITS1-2	Reverse GATATGCTTAAACTCAGCGG	56	Milyutina *et al*., 2010
*rpb*2 nrDNA	Forward TGGGGAATGATGTGTCCTGC	50	Liu et al., 1999
*rpb*2	Reverse CCCATGGCTTGCTTCCCCAT	50	Liu et al., 1999
*trn*L-F cpDNA	Forward CGAAATCGGTAGACGCTACG	54	Taberlet *et al*., 1991
*trn*L-F	Reverse ATTTGAACTGGTGACACGAG	54	Taberlet *et al*., 1991
*trn*T-F	Forward CCATAATGAAAACTGAATTTTTG	52	Hernández-Maqueda *et al.*, 2008
*trn*T-F	Reverse CATYGAGTCTCTGCACCT	52	Quandt *et al*., 2004
*rbc*L	Forward ATGTCACCACAAACAGAGACT AAA GC	50	Kress & Erickson, 2007
*rbc*L	Reverse GTATCTATTGTTTCATATTC	50	Bakalin *et al*., 2024
*psb*A-*trn*H	Forward GTTATGCATGAACGTAATGCTC	52	Newmaster & Subramanyam, 2009
*psb*A-*trn*H	Reverse CGCGCATGGTGGATTCACAATCC	52	Newmaster & Subramanyam, 2009
*rpl*16	Forward GCTATGCTTAGTGTGTGACTCGTT	52	Jordan *et al.*, 1996
*rpl*16	Reverse GTAATCCAAGCTGGTTCAAGTGC	52	Olsson *et al.*, 2009
*rps*4	Forward ATGTCCCGTTATCGAGGACCT	50	Nadot *et al.*, 1994
*rps*4	Reverse TACCGAGGGTTCGAATG	50	Souza-Chies *et al.*, 1997

Table 2. ISSR primers used for PCR

ISSR primer	Primer sequence (5'-3')
M5	ATA TAT ATA TAT ATA T
M14	GAC AGA CAG ACA GAC A
UBC 840	GAG AGA GAG AGA GAG AAY T
UBC 844B	CTC TCT CTC TCT CTC TGC
UBC 868	GAA GAA GAA GAA GAA GAA
UBC 881	GGG TGG GGT GGG GTG
HB8	GAG AGA GAG AGA GG
17898A	CAC ACA CAC ACA AC
17898B	CAC ACA CAC ACA GT
17899A	CAC ACA CAC ACA AG
17899B	CAC ACA CAC ACA GG

ly allied family Obtusifoliaceae (Bakalin *et al*., 2021) was chosen as an outgroup. The list of specimens for phylogenetic estimations is in Appendix 1.

DNA extraction, amplification and sequencing

For DNA extraction the HiPure SF Plant DNA Kit (Magen, China) was used following the procedure described in the manufacturer protocol. 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 at appropriate annealing temperature (Table 1), 60 s 72°C) and 2 min. of final extension time at 72°C. The pairs of primers for the polymerase chain reaction are listed in Table 1. The amplicons were visualized on 1% agarose TAE gels by EthBr staining, cleaned using the Cleanup Mini Kit (Evrogen, Russia), and used as a template in sequencing reactions with the ABI Prism BigDye Terminator v. 3.1 Kit (Applied Biosystems, USA) following the standard protocol provided for 3730 Avant Genetic Analyzer (Applied Biosystems, USA).

ISSR analysis

Initially, several species from Anastrophyllaceae were sampled to check the usefulness of ISSR primers taken from published studies (Hock *et al*., 2008; Buczkowska & Dabert, 2011) to expose genetic differences in the target family. Follow the results of ITS1-2 and *rpb*2 amplification, the presence of fungal endophyte was determined in *Anastrophyllum astorgae* #YuSM-928-3-7385, *Sphenolobus minutus* #K10-2a-23, *Schljakovianthus quadrilobus* #K 52-1-18, *Tetralophozia filiformis* #C-73-44-18, *Isopaches bicrenatus* #K129-1-19, and *Gymnocolea inflata* #41089, thus these specimens could not be included in ISSR analysis. The tested specimens from Yakutia, *Rudolgaea borealis* KPABG-125157 and *Vietnamiella epiphytica* #V-9-7-17 had no endophytes. Preliminary analysis of *Vietnamiella epiphytica* and *Rudolgaea borealis* with several ISSR markers provided different bands for both species, suggested possibility of method to distinguished genetic diversity in Anastrophyllaceae. The experiment with 11 different primers (Table 2) complemented to a target microsatellite were implemented only for three tested Yakutian specimens and *Rudolgaea borealis* KPABG-125157. 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 50°C, 60 s 72°C) and 2 min. of final extension time at 72°C. The amplicons were visualized on 1.7% agarose TAE gels by EthBr staining and registered by photo. Images were analysed manually.

Table 3. The arithmetic means of Log likelihood obtained in molecular phylogenetic estimation

Dataset	ML	BA 1st run	BA 2nd run
ITS1-2+*trn*L-F	-7345.117	-7258.01	-7257.97
*rbc*L	-3055.761	-3078.07	-3077.78

Phylogenetic analysis

The nucleotide sequence data and alignments for estimations were produced in BioEdit 7.0.1 (Hall, 1999). The ITS1-2+*trn*L-F dataset includes 1392 nucleotide positions, the *rbc*L dataset –1374 positions. Follow repeatedly published results of Anastrophyllaceae phylogeny the ITS1-2 and *trn*L-F were tested as combined dataset, *rbc*L dataset was analysed separately due to limited and differed taxa sampling in ingroup. The phylogenetic estimation based on other sequenced DNA markers was impossible due to absence of sequenced accessions to produce dataset for the whole family.

The ITS1-2+*trn*L-F and *rbc*L datasets were separate-

Table 4. The value of *p*-distances for selected specimens, "-" – non calculated value due to absence of sequence data

Specimen	*p*-distances, ITS1-2/*rpb*2/*trn*T-F (incl. *trn*L-F)/*rbc*L/*rpl*16/*rps*4/*psb*A-*trn*H, %					
	1	2	3	4	5	6
1 *Vietnamiella epiphytica* KPABG-122594						
2 *Gymnocolea inflata* KPABG-125590	9.6/3.3/8.6/-/ 8.1/5.8/7.9					
3 *R. borealis* Gydan LE	10.4/-/4.3/2.5/-/-/-	6.3/-/7.0/-/-/-/-				
4 *R. borealis* Taimyr KPABG-125157	10.9/2.3/4.3/2.6/7. 3/4.8/8.5	8.1/4.1/7.1	6.6/2.1/7.0/-/ -/-/-	0.0/0.0/0.2/-/		
5 *R. aenigmatica* ELOn44-5	10.7/2.1/4.0/2.5/ 7.3/4.8/8.5	6.6/2.0/6.9/-/ 8.1/4.1/7.1	0.0/0.0/0.3 /-/-/-/-	0.0/0.0/0.1/ 0.0/0.0/0.0/0.0		
6 *R. aenigmatica* ELOn47	10.7/2.1/4.4/2. 5/7.3/4.8/8.5	6.6/2.0/6.9/-/ 8.1/4.1/7.1	0.0/0.0/0.5/ -/-/-/-	0.0/0.0/0.1/0.0/ 0.0/0.0/0.0	0.0/0.0/0.0/0.0 /0.0/0.0/0.0	
7 *R. aenigmatica* ELOn85-4	10.7/2.1/4.1/2.5 /7.3/4.8/8.5	6.6/2.0/6.9/-/ 8.1/4.1/7.1	0.0/0.0/0.2 /-/-/-/-	0.0/0.0/0.1/0.0/ 0.0/0.0/0.0	0.0/0.0/0.0/0.0 /0.0/0.0/0.0	0.0/0.0/0.0/ 0.0/0.0/0.0/ 0.0

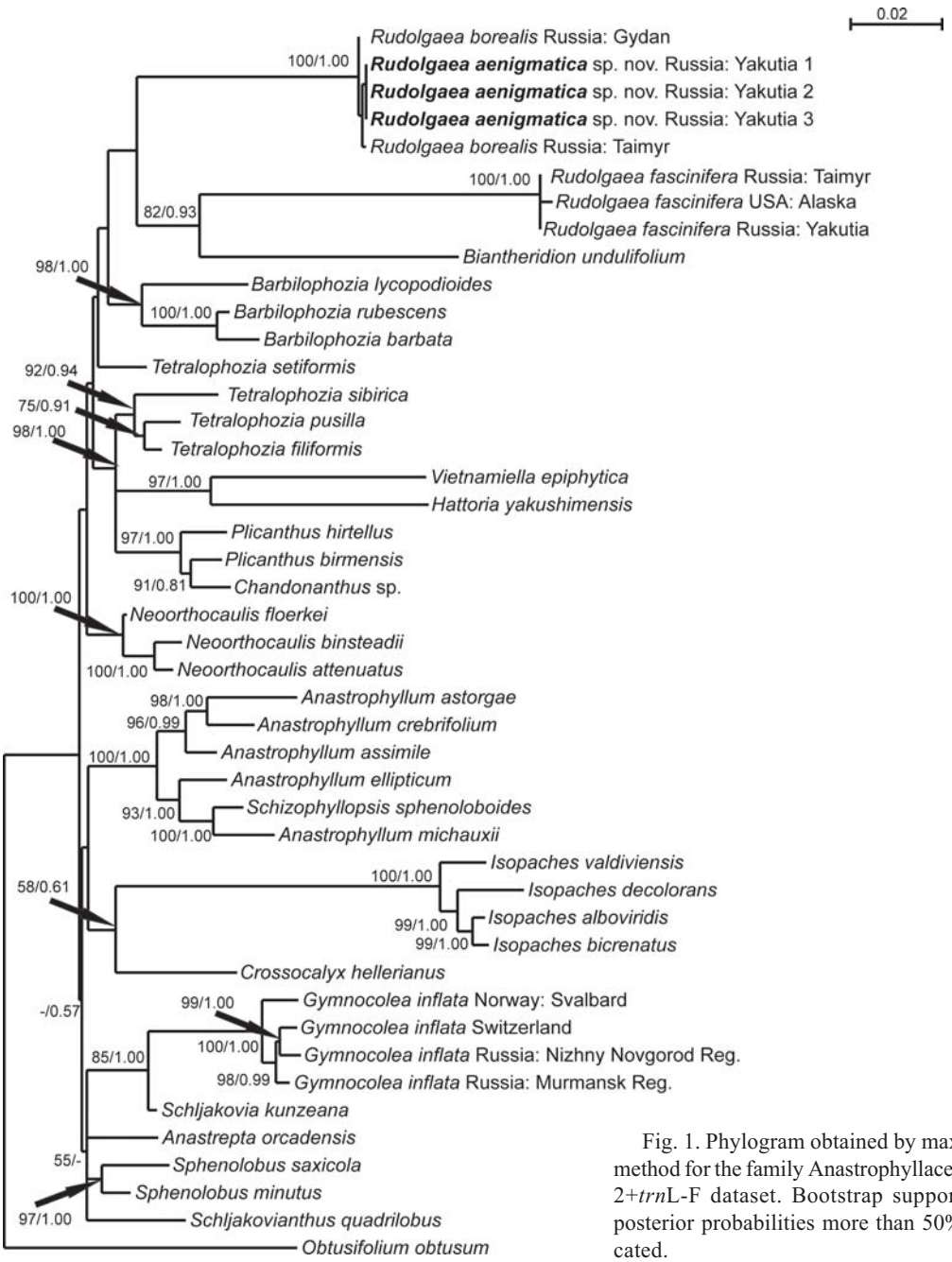

Fig. 1. Phylogram obtained by maximum likelihood method for the family Anastrophyllaceae based on ITS1-2+*trn*L-F dataset. Bootstrap supports and Bayesian posterior probabilities more than 50% (0.50) are indicated.

ly assigned with the maximum likelihood (ML) method in the program IQ-TREE (Trifinopoulos *et al*., 2016) and the Bayesian approach (BA) – in MrBayes v. 3.2.1 (Ronquist *et al*., 2012). The program ModelFinder (Kalyaanamoorthy *et al*., 2017) selected the best fit evolutionary model of nucleotide substitutions for each dataset: the TNe+I+G4 model was suggested for ITS1-2+*trn*L-F, the TN+F+R2 – for *rbc*L. These models and the ultrafast bootstrapping procedure (Hoang *et al*., 2018) with 1000 replicates were used for ML estimations. For the Bayesian analysis the GTR+I+G model was implemented for each dataset; 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 for both datasets was one million. Trees were saved every 100th generation. The average standard deviation of split frequencies between two runs for ITS1-2+*trn*L-F dataset was 0.009000, for *rbc*L dataset – 0.006846. The first 2500 (25%) trees were discarded in each run and 15000 trees from both runs were sampled after burn-in for each dataset. Bayesian posterior probabilities were calculated from trees sampled after burn-in.

The sequence variability was estimated as the average pairwise *p*-distances for ITS1-2, *rpb*2, *rbc*L, *rpl*16,

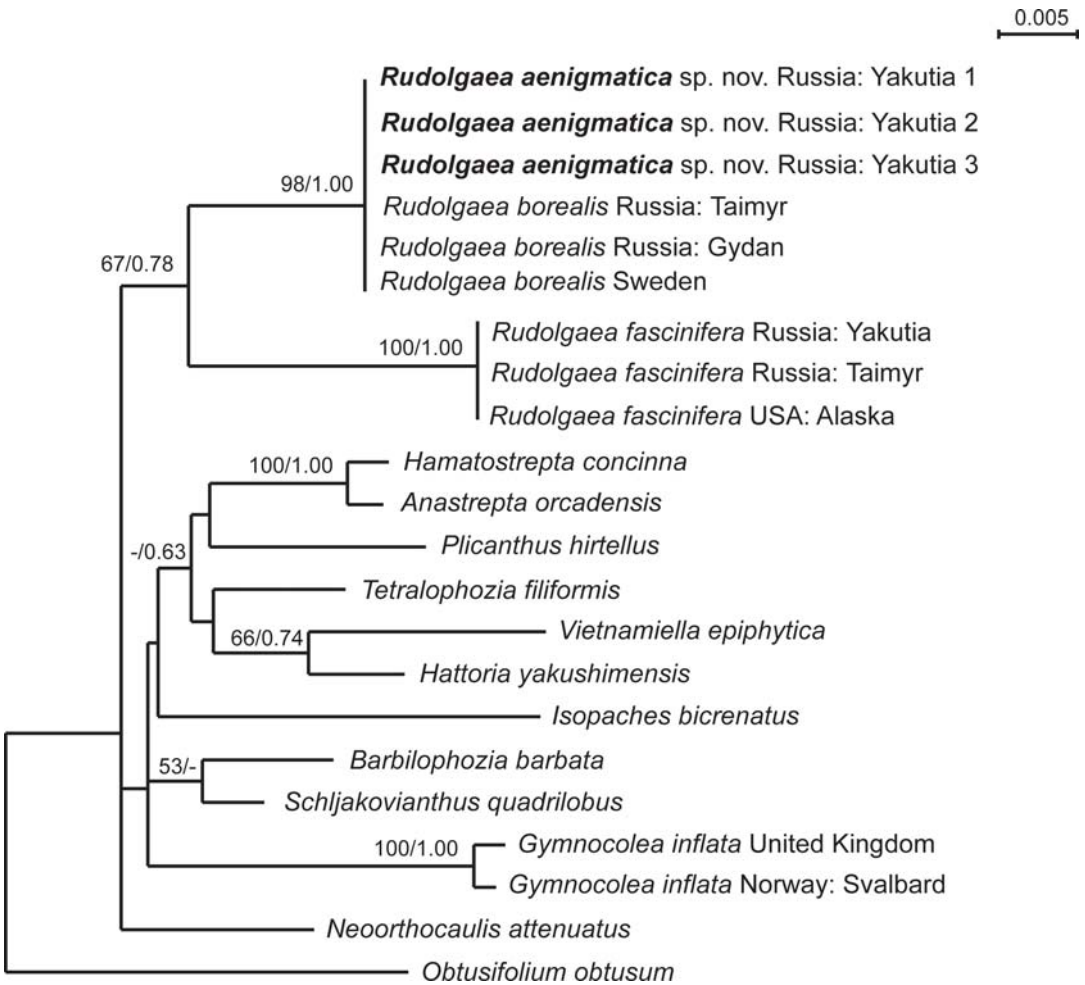

Fig. 2. Phylogram obtained by maximum likelihood method for the family Anastrophyllaceae based on *rbc*L dataset. Bootstrap supports and Bayesian posterior probabilities more than 50% (0.50) are indicated.

*rps*4, *trn*T-F (incl. *trn*L-F), and *psb*A-*trn*H cpDNA in Mega 11 (Tamura *et al*., 2021) using the pairwise deletion option for counting gaps.

Results

Molecular phylogenetic study

In total 38 accessions were newly generated in this study and deposited into GenBank (Appendix 2). Among them 21 accessions for three specimens of mysterious plants from Yakutia, six for *Rudolgaea borealis* KPABG-125157, four for *Vietnamiella epiphytica* KPABG-122594, two for *Obtusifolium obtusum* KPABG-14526. The arithmetic means of Log likelihood obtained in ML and BA analyses of ITS1-2+*trn*L-F and *rbc*L datasets are shown in Table 3.

For each dataset congruent tree topologies were reconstructed after phylogenetic estimation by two methods. The Fig. 1 illustrated the ML topology achieved for ITS1-2+*trn*L-F with indication of bootstrap support (BS) values from ML calculation and Bayesian posterior probabilities (PP) from BA. The results of *rbc*L calculations are presented in Fig. 2.

Three tested specimens from Yakutia compose a clade with two *Rudolgaea borealis* specimens from Gydan and Taimyr Peninsulas (Fig. 1, 2). The ITS1-2+*trn*L-F tree demonstrates a minute variability within this clade throughout short branch lengths, the *rbc*L tree has not provide branches to specimens suggested their identity. The supports of a clade of *R. borealis* plus Yakutian specimens are BS=100% and PP=1.00 (100/1.00) in the ITS1-2+*trn*L-F, and 98/1.00 -in the *rbc*L. Increasing specimens sampling for current analyses for the genus *Rudolgaea* and other genera of Anastrophyllaceae do not sufficiently increase supports of monophyly for the genus *Rudolgaea.* Opposite, the sister relation of *Rudolgaea borealis* and *Rudolgaea fascinifera* did not obtain supports in the ITS1-2+*trn*L-F compared with PP=0.86 in Potemkin & Vilnet (2021). The decrease of PP support value from 0.96 (Potemkin & Vilnet, 2021) to 0.78 is found in *rbc*L estimation. Moreover, *Biantheridion undulifolium* was placed in a sister relation to *R. fascinifera*-subclade with 82/0.93 supports in ITS1-2+*trn*L-F topology (Fig. 1). The genus *Rudolgaea* is characterized by interspecific *p*-distances compared with intergeneric one among other taxa of Anastrophyllaceae (Potemkin

& Vilnet, 2021), thus spreading of sampling for analysis could support phylogenetic divergence of *Rudolgaea* and its artificial inclusion into one taxonomical unit. Similar statement may be true for the genus *Tetralophozia* with unsupported separation of *Tetralophozia setiformis* from the clade with other three species of *Tetralophozia* (Fig.1). The monophyly of *Tetralophozia* was not sufficiently supported in special generic study (Bakalin *et al*., 2022). The quite low values of internal nodes in topology of Anastrophyllaceae were repeatedly received as in previous studies (Vilnet *et al*., 2010; Bakalin *et al*., 2020; Potemkin & Vilnet, 2021). It once again raises a question of the need for a special research of the family phylogeny with modern genomic methods.

The *p*-distance was calculated for three mysterious specimens from Yakutia, *Rudolgaea borealis* KPABG-125157, *R. borealis* Gydan LE, *Vietnamiella epiphytica* KPABG-122594 and *Gymnocolea inflata* KPABG-125590 (Table 4). All DNA markers are robustly differentiated *Rudolgaea borealis* from *Vietnamiella epiphytica* and *Gymnocolea inflata* from 2 till 11% by distinct markers. The values of *p*-distances between *R. borealis* and *R. fascinifera* are 5.3% in ITS1-2, 5.5% in *trn*L-F and 3.1% in *rbc*L (Potemkin & Vilnet, 2021, Table 2) that highly distinguished both species. The variability among *Rudolgaea borealis* specimens were found only in *trn*L-F region, it counts 0.1–0.5% that corresponds to 1-2 substitutions in the 3'-ends of tRNALeu and tRNAPhe genes and could be a problem of sequencing procedures. Three new tested Yakutia specimens occurred identical to each other and, with exclusion of minor differences in *trn*L-F, with specimens of *Rudolgaea borealis* from Gydan and Taimyr.

The search of specific ISSR bands to distinguish *Rudolgaea borealis* KPABG-125157 and three Yakutia' specimens failed. Images of fingerprints obtained after PCR from eleven different primers became fully identical.

TAXONOMY

Rudolgaea aenigmatica Konstant., Vilnet et Lapshina sp. nov. Figs. 3–4.

Diagnosis. The species is characterized by very narrow leaves (the length/wide ratio reaches 1.7–2), sinuses depth to 0.75 the leaf length, lobes of leaves mostly with long narrow often crescent apex, margin unevenly curved on ventral side, often with teeth 2–6 cells wide at base or with cilia near or slightly above the base on the dorsal, and sometimes on both dorsal and ventral sides of leaves.

TYPE: Russia, Republic of Sakha (Yakutia), to the west of the Chokurdakh settlement, Kytalyk National Park, valley of Berelekh River, 70.852018°N, 146.219206°E #044F-5-23 Filippov I.V. 13.VII.2023 [Holotype KPABG(H) 127871; isotype YSU 05590], sedge-*Hypnum* community within extensive khasyrey (bottom of deflated thermokarst lakes), scattered, mixed with *Meesia triquetra, Aneura pinguis, Scorpidium revolvens, Scorpidium scorpioides*. Isolate A189a, GenBank accession numbers nrDNA: PZ280164 (nrITS), PZ281231 (*rpb*2); cpDNA PZ281202 (*trn*T-F), PZ281207 (*rbc*L), PZ281211 (*rpl*16), PZ281219 (*rps*4), PZ281225 (*psb*A-*trn*H).

Etymology. The name refers to the extremely unusual, surprising for the genus shape of leaves.

Description

Plants (1)1.5–2.5(3) mm wide and to 25–30 mm long, with curly leaves, almost black in lower part of shoots, higher up gradually becoming lighter – brown or chocolate brown, in upper parts of the shoot sometimes brownish-golden to light green, sometimes pellucid. **Branches** malleable, mostly terminal *Frullania* type or lateral intercalary near apex and in the middle of shoot, rarer ventral intercalary. **Rhizoids** are relatively abundant in some areas of the stem, but in large sections, especially near the apex of the shoot absent, colorless or brownish, ca. (10)12–15(20) μm, never arising from the ventral leaf base. **Stem** near apex almost colorless, below becomes dark brown to almost black, in cross-section 9–11 cells wide, elliptical, with some cells mycorrhizal, 180–230 × 250–300 μm, cortical cells dark brown, thick-walled, much smaller than inner cells, ca. (14)15–20 μm, inner cells ca. 22–25(35) × 25–35(40) μm, thin-walled, with small trigones (Fig. 4: H). Cells on ventral side of stem slightly thick–walled, 15–18 × 30–50 μm, on dorsal side slightly thicker-walled and larger, 18–20 × 50–80(100) μm. **Leaves** distant, obliquely inserted, from decurrent to long decurrent on dorsal side, undulate, dorsally convex, with margins unevenly and irregularly more or less widely curved toward the ventral side; large leaves from 1.35 mm wide and 1.8 mm long in lower parts of shoot to smaller, ca 0.35 mm wide and 0.6 mm long, in upper part of shoot ca. (1.33)1.4–1.9 (2) as long as wide, 2–3(4)-lobed; some leaves partly two or several layers thick at base (Fig. 4: D, H). **Sinus** reaches 0.5–0.75 (0.9) of the leaf length, often reflexed, mostly narrow rounded at the base and U- or V -shaped or sometimes slit-shaped. **Lobes** lanceolate, distinctly unequal, dorsal lobe much smaller and narrower, often cilia-like, just 2–5 cells at base. Lobes mostly more or less sharply tapering into a long, more or less narrow, often incurved apex ending in two or several superposed cells, upper of which almost isodiametric, ca. 15-18 μm. **Margins** of leaves often widely curved toward the ventral side (Fig. 3: K–L). **Cells** with small to moderate trigones. **Marginal cells** partly elongated in lobes, ca. 16–20(25) × (25)30–35 μm (Fig. 4: E, G, I), below almost isodiametric, ca. 20–25 × 25–28 μm, midleaf cells (20)23–28(35) × 30–37(45) μm (Fig. 4: B), near base of leaves slightly larger, (22)25–30(35) × (25)30–50(65) μm (Fig. 4: C, D); walls between cells usually deeper pigmented than walls of open cell surface, yellow to yellow-brown and light and dark red-brown in pigmented parts, cuticle coarsely striate. **Underleaves** irregular, variable, absent on some sectors of stem, narrow lanceolate when present, sometimes from

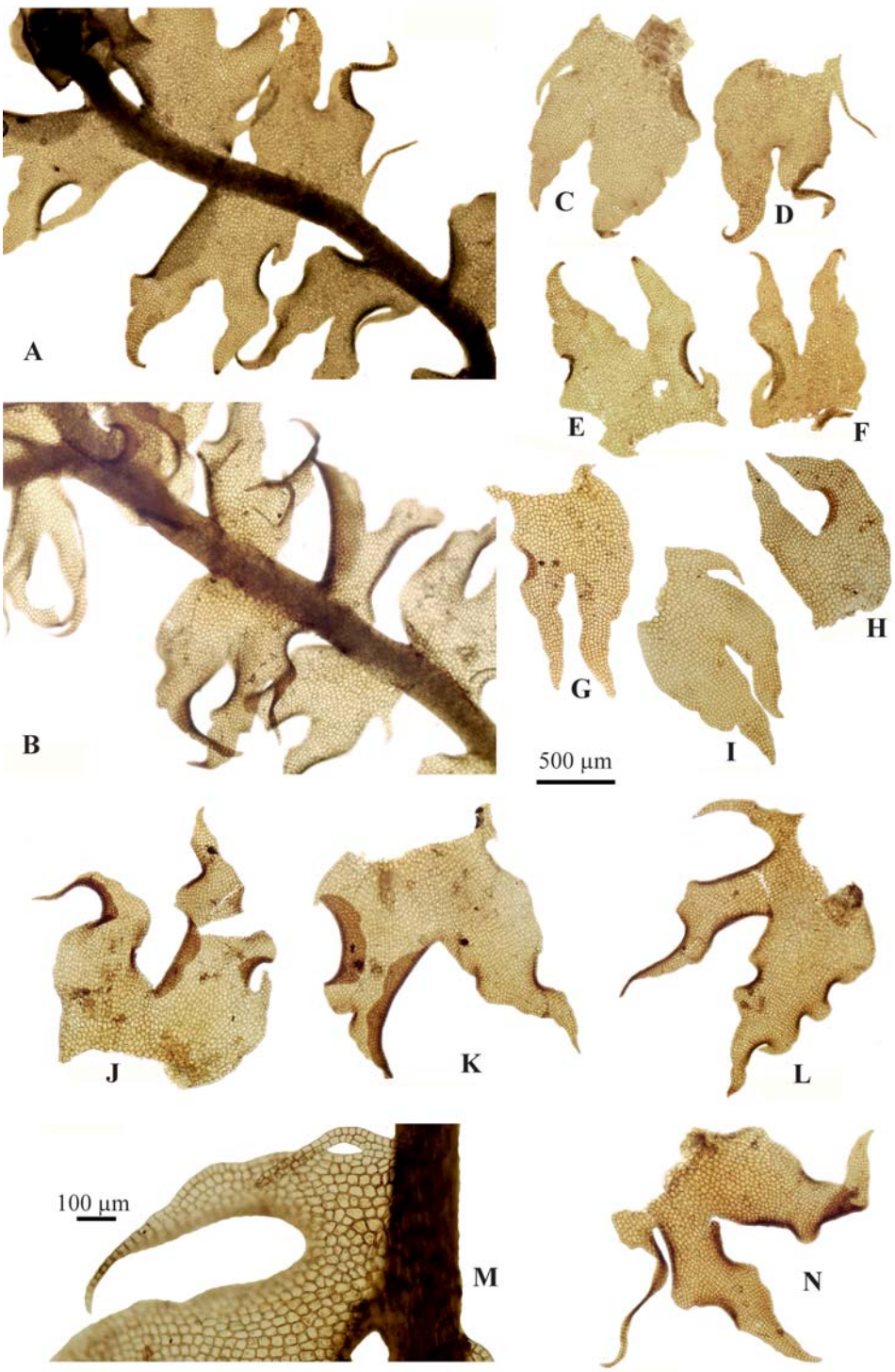

Fig. 3. *Rudolgaea aenigmatica* Konstant., Vilnet, Lapshina. A, B: parts of sterile shoots; C–N: leaves (A, C, D, E, F – from E. Lapshina #83-1-2, YSU # 05559; B, G, H, I, J, K, L, M, N – from I. Filippov #044F-5-23, YSU # 05590).

3–4 cells wide and several cells long base turning into a long ending, one cell wide or two cells wide and many cells long (Fig. 4: J). Generative structures were not found.

Additional specimens examined: RUSSIA: **Republic of Sakha (Yakutia)**, north-eastern part to the west of the Chokurdakh settlement, Kytalyk National Park, valley of Berelekh River, 70.85909°N, 146.22586°E #91E-9 [KPABG(H) 127872; YSU 05587], Lapshina E. D. 13.VII.2023, *Comarum palustre*-sedge (*Carex chordorrhiza, C. aquatilis* subsp. *stans*)–*Hypnum* hollow, single shoots in mats dominated by *Mesoptychia rutheana* with admixture of some shoots of *Scorpidium revolvens, Meesia triquetra, Aneura pinguis, Bryum neodamense*, *Cephaloziella* sp.; ibid 70.85175°N, 146.21793°E #83E/1-2-23 [KPABG(H) 127873**;** YSU 05559], Lapshina E.D. 13.VII.2023, sedge (*Carex chordorrhiza*)-*Hypnum* community in khasyrey, several shoots in mats dominated by *Sphagnum orientale, Bryum* cf. *pseudotriquetrum* and admixture of *Cinclidium* sp.*, Aneura pinguis, Scor-*

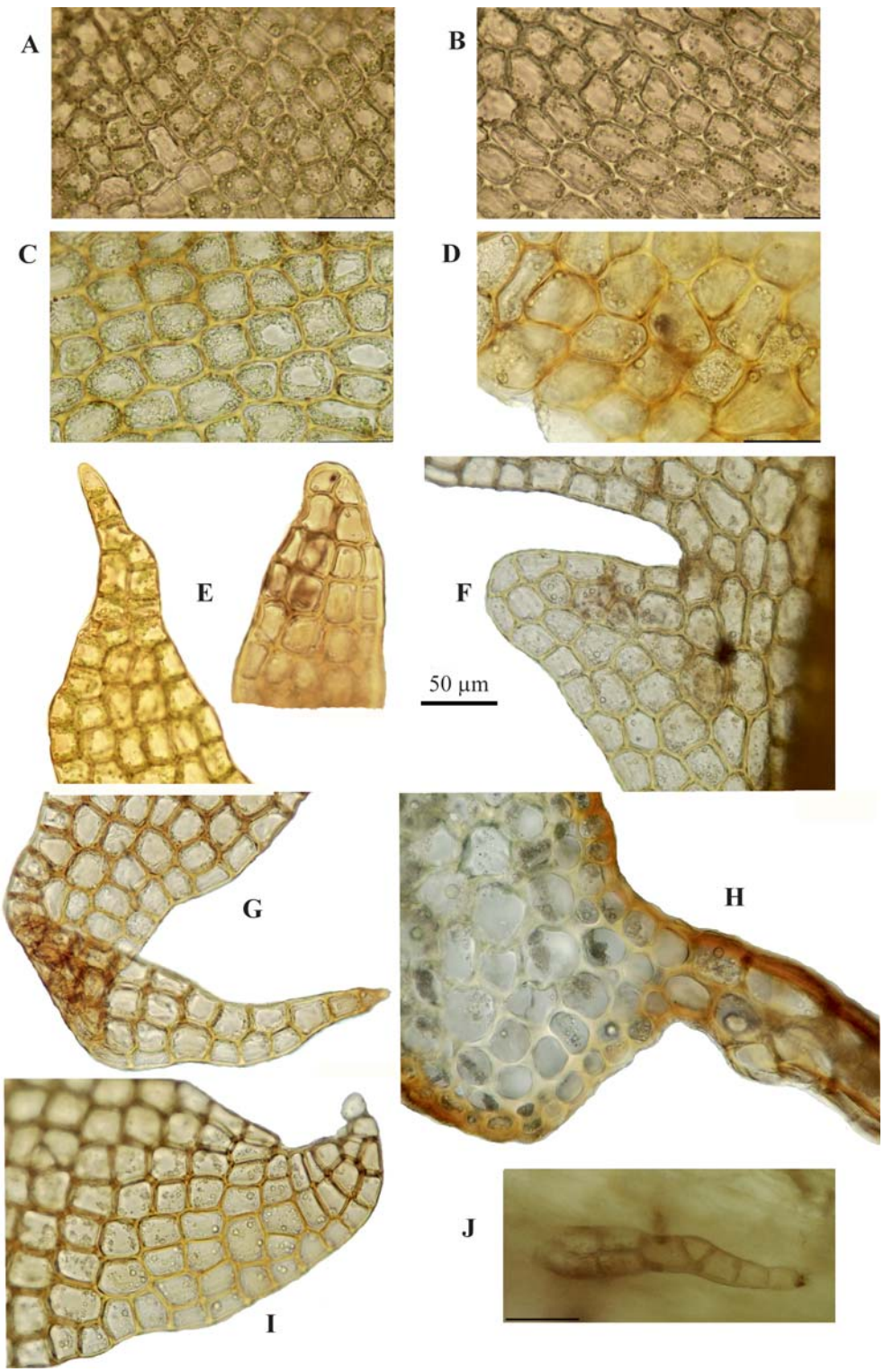

Fig. 4. *Rudolgaea aenigmatica* Konstant., Vilnet, Lapshina. A: cells in leaf lobe; B: midleaf cells; C: cells near base; L: cells at leaf base; E, G, I: apices of lobes; F: teeth-like lobe; H: cross section of stem; J: underleaf. A, B, E from E. Lapshina #83E/1-2 (YSU 05559); C, D, F, G, H, I, J from I. Filippov #044F-5-23 (YSU 05590).

pidium revolvens, Polytrichum jensenii; ibid 70.85305°N, 146.21829°E #85E-4-23 [KPABG(H) 127874; YSU 05571], Lapshina E.D. 13.VII.2023, sedge (*Carex chordorrhiza*)-*Hypnum* community in khasyrey, many shoots mixed with *Calliergon richardsonii, Aneura pinguis, Cinclidium subrotundum, Bryum neodamense, Meesia triquetra;* ibid. 70.857605°N, 146.226456°E #47F-1 [KPABG(H) 127875; YSU 05599] Filippov I.V 13.VII.2023, single shoots in mats dominated by *Calliergon richardsonii* and *Scorpidium revolvens* and admixture of *Pseudocalliergon brevifolium, Aneura pinguis, Mesoptychia ru-*

Fig. 5. A: Characteristic habitat of *Rudolgaea aenigmatica* (waterlogged sedge-*Hypnum* phytocenoses in polygonal mire complex near lake); B: Continuous *Hypnum* moss cover of *Scorpidium revolvens* under dense sedge layer formed by *Carex aquatilis* subsp. *stans* and *C. chordorrhiza.*

theana, Rudolgaea borealis, Cephaloziella sp.

Ecology. *Rudolgaea aenigmatica* occupies habitats similar to *R. borealis* and, like this species, is found as individual shoots in mats of mosses of rich fens. Scattered shoots of *Rudolgaea aenigmatica* were found among mosses in eleven specimens from eight plant communities. These communities occurred in three mire complexes located several tens kilometers from each other (Table 5).

Differentiation. The differences of the described species are immediately obvious and it is impossible to confuse it with other species both of the same genus or other species of the same habitats. *Rudolgaea aenigmatica* differs from all liverworts occurring in somewhat similar habitats, particularly from species of the genus *Rudolgaea, Gymnocolea inflata*, and *Odontoschisma fluitans* by its very narrow, undulate, dorsally convex leaves, with margins unevenly and irregularly, more or less widely curved toward the ventral side, deeper sinuses, 0.5–0.75 (0.9) of the leaf length, rather sharply tapering into a long, more or less narrow, often incurved apices, presence of long cilia or cilia-like lobes near base of ventral or dorsal side or both sides. *Rudolgaea aenigmatica* differs from *Gymnocolea inflata* and *Odontoschisma fluitans* in tendency to form a third and sometimes fourth shortened lobe and undulate, abaxially concave leaves vs. flat or slightly concave leaves of the mentioned species. Such unusual combination of features immediately excludes all the listed species. It should be noted that some tendencies that are only emerging in other species of the genus are very clearly expressed in *Rudolgaea aenigmatica*. It concerns, first of all, the ability to form cilia, teeth, outgrowths at the base of the leaves or even a third lobe. All species of the genus tend to form a third and sometimes even a fourth lobe. For *R. borealis* it was shown by Frisvoll & Moen (1980: 139, Fig. 2). Potemkin (1993: 77) described *R. fascinifera* as having entire margins "without any teeth or slime papillae"; however, later Konstantinova *et al*. (2024: 169) found that in *R. fascinifera* "along with two-lobed leaves, there are also three-lobed leaves and leaves with more or less outlined third lobe in the form of a protrusion at the base of the leaf". However, in *R. fascinifera* (cf. Konstantinova *et al*., 2024: 167) or *R. borealis* (Damsholt, 2002) the teeth or outgrowths at the base of the leaf are very small, just several cells, whereas *R. aenigmatica* has very long, often only slightly shorter than the lobes, two- or multi-celled at the base, teeth-like lobes ending in a long, one cell wide apex or a broad, short, multicellular third lobe often shorter than the other lobes (Fig. 3). The emerging convexity of the leaves and somewhat sinuose margins of leaves noted by Damsholt (2002) for *R. borealis* are much more clearly expressed in *R. aenigmatica.* Very narrow leaves with width/length ratio up to ca. 1:0.7–2.0 are described and illustrated for *R. fascinifera* by Potemkin (1993) and Potemkin & Vilnet (2021) but this species has leaves with short, acute to apiculate or obtuse and rotund lobes and margins entire, occasionally more or less crooked, without any teeth, cilia or slime papillae.

KEY TO IDENTIFICATION OF SPECIES OF *RUDOLGAEA*

1 Leaves undulate, distinctly convex dorsally, narrow, (1.3)1.4–2 (2.5) as long as wide, divided to 0.5–0.75 of the leaf length into 2–3(4) unequal, sometimes cilia-like lobes that are sharply tapering into a long, more or less narrow, often incurved apex ending in two or several superposed cells. Margins of leaves often widely curved toward the ventral side, leaves at base two layers thick in two or three rows .... *Rudolgaea aenigmatica*

— Leaves not undulate, flat or only slightly convex, as wide as long or longer than wide, but usually the ratio of width to length does not exceed 1:1.35, divided to 0.2–0.3(0.45) of the leaf length into 2 blunt or short, cuspidate lobes, apex not curved, margins entire or with single slime papillae or outgrowth . 2

2. Leaves flat, very malleable in shape, from 1.1 to 2 times as long as wide, often narrowed to the base and somewhat back-trapezoidal, lobes often clearly uneven with wider and longer ventral part; plants not glistening; restricted to acidic or neutral sites .. .. *R. fascinifera*

— Leaves slightly convex dorsally and their lower halves channelled, about 1.1(–1.3) as long as wide, symmetrical or slightly asymmetrical, not narrowed to the base; plants green to golden brown, glistening; restricted to rich fens ..... *R. borealis*

DISCUSSION

The performed phylogenetic analysis showed the position of the studied plants in the genus *Rudolgaea* of the Anastrophyllaceae family and the almost complete identity of the studied loci with *Rudolgaea borealis*. The genus *Rudolgaea* Potemkin & Vilnet was recently established based on reevaluation of morphological and molecular features of species attended to the genus *Gymnocolea*; it includes the type species *Rudolgaea fascinifera* and the second one, *R. borealis* (Potemkin & Vilnet, 2021). Despite the lack of generative organs and differences in the studied genetic markers, we decided to describe a new species, based on significant morphological differences from *R. borealis* and occurrence in different (albeit similar) plant communities at three distant sites (Table 5). The diversity of the family Anastrophyllaceae from integrative evidence increased in the last decade due to the description of several new species, particularly *Anastrophyllum astorgae* (Mamontov & Vilnet, 2023), *Isopaches valdiviensis* (Konstantinova *et al*., 2025), *Tetralophozia sibirica* (Bakalin *et al*., 2022), and even the new genus *Vietnamiella* (Bakalin *et al*., 2020). All recently described species of the family are distinguished morphologically that correlates with sufficient genetic divergence from known taxa. The case of *Rudolgaea borealis* and *R. aenigmatica* is completely different: their significant morphological differences are not supported genetically.

Several traditionally used barcoding regions and phylogenetic markers for plants (plastid *rbc*L, *psb*A-*trn*H, *rps*4, *trn*L-*trn*F and the nuclear ITS2) successfully tested in delimitation of moss and liverwort species (Kress & Erickson, 2007; Liu *et al*., 2010; Hassel *et al*., 2013) do not provide differences between *R. borealis* and *R. aenigmatica*.

The modern idea of super-barcoding based on comparative analysis of chloroplast or mitochondrial genomes suggests new appropriate loci to distinguish the species, which are hardly differentiated by traditional barcode markers. These could be, for example, the gene cluster *rpo*A–*rps*11–*sec*X–*inf*A–*rps*8–*rpl*14–*rpl*16–*rps*3–*rpl*22–*rps*19–*rpl*2–*rpl*23 (Dong *et al*., 2021) or protein-coding regions *ycf*1, *ycf*2, *ycf*66 (Ślipiko *et al*., 2020). Having failed to find molecular divergence between morphologically dissimilar specimens of *Rudolgaea borealis* and *R. aenigmatica* with traditionally used liverwort markers, we sequenced part of *rpb*2 nrDNA gene and *rpl*16 cpDNA, but they also became identical. The lack of sufficient herbarium material of both *Rudolgaea borealis* and *R. aenigmatica* currently precludes genome sequencing to obtain a reliable genetic confirmation of species divergence. Furthermore, the results of nuclear ISSR analysis, commonly used to search for divergence between populations of the same species (Hassel & Gunnarsson, 2003), demonstrating the identical bands composition of *Rudolgaea borealis* and *R. aenigmatica* by eleven primers, call into question the prospect of genetic separation of these species.

We also failed to detect any differences in ecology of *Rudolgaea aenigmatica* and *R. borealis.* These two species occupy similar habitats and the same plant communities (Tables 5, 6), and twice recorded within the same relevé (Table 5). Moreover, one shoot of *Rudolgaea borealis* and several shoots of *R. aenigmatica* were found in the same mat within one specimen [KPABG(H) 127875]. An admixture of both *Rudolgaea aenigmatica* and *R. borealis* in one mat is also an evidence for recognizing them as different species (see, for example, Schuster, 1969: 286, interpreting the coexistence of *Lophozia hyperborea* and *L. quadriloba* as 'self-evident that two distinct species must exist'). Both species were collected in Yakutia in minerotrophic (moderately rich in mineral elements) fens: in sedge (*Carex chardorrhiza, C. aquatilis* subsp. *stans*)-*Hypnum* moss communities dominated by *Scorpidium revolvens, Meesia triquetra* on waterlogged polygons of rim-polygonal mires and lakeside floating mats across the bottoms of numerous drained thermokarst lake basins (khasyreis) and low river terraces (Fig. 5A). These communities are characterized by a continuous *Hypnum* moss cover of *Scorpidium revolvens*, and *Meesia triquetra* (Table 5, 6, Fig. 5B).

Rudolgaea aenigmatica and *R. borealis* are often accompanied, in greater or lesser abundance, with high consistency by *Aneura pinguis, Bryum neodamense, Campylium stellatum, Cinclidium subrotundum, Cinclidium latifolium, Mesoptychia rutheana, Pseudocalliergon brevifolium,* and *Scorpidium scorpioides*. (Table 5, 6). In Yakutia, a constant component of the moss cover of *Hypnum* mires is common in Eastern Siberia *Sphagnum orientale*. In all communities, specimens of *Rudolgaea borealis* and *R. aenigmatica* grow as scattered shoots among mosses. The total projective cover of the herbaceous layer in such communities varies widely (5–90%, usually 15–70%). The sparse upper sublayer (30 cm high) is formed by *Carex aquatilis* subsp. *stans*. The denser lower sublayer (15–20 cm high) is composed of *Carex chordorrhiza* and *Comarum palustre. Epilobium palustre, Eriophorum angustifolium, E. russeolum, Pedicularis sudetica, Rumex arcticus*, and *Saxifraga foliolosa* are found in small abundance (Table 6). Occasionally, low (15–20 cm high) bushes of *Salix fuscescens* form a no-

Table 5. Association ***Meesio triquetro–Caricetum chordorrhizae*** Lapshina et Filippov 2025 with described here *Rudolgaea aenigmatica* in the northeastern Yakutia

Plant cover, %											
dwarf shrubs	0	5	0	0	0	0	0	2	0	20	20
herbs	40	25	40	60	30	60	70	15	60	40	25
mosses & liverworts	100	90	90	100	50	70	70	90	75	100	100
Relevé nr. by author	044F23kt	050F23kt	047F23kt	091E23kt	127F23kt	085E23kt	083E23kt	166E23kt	077E23kt	130E23kt	128E23kt
Number of species	16	20	24	21	27	16	23	27	16	22	22
Relevé nr. in the table	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Mosses & liverworts											
Rudolgaea aenigmatica	+	+	+	+	+	+	1	.	.	.	.
Rudolgaea borealis	.	.	.	.	.	+	+	+	+	+	+
Meesia triquetra	3	50	20	45	5	10	30	30	60	20	20
Scorpidium revolvens	20	10	60	30	20	60	20	25	.	40	40
Sphagnum orientale	1	20	10	1	3	.	1	.	.	1	10
Aneura pinguis	1	1	1	1	1	1	1	1	5	5	1
Cinclidium subrotundum	1	1	1	1	3	2	15	.	1	.	.
Pseudocalliergon brevifolium	.	5	1	20	5	.	+	1	.	5	1
Polytrichum jensenii	3	1	.	1	1	.	2	.	.	1	1
Bryum neodamense	.	1	.	1	.	1	1	1	1	1	.
Mesoptychia rutheana	.	1	.	1	1	.	.	2	.	.	1
Scorpidium scorpioides	1	.	1	1	.	1	1	.	.	.	.
Bryum pseudotriquetrum	.	1	1	.	1	.	1	1	.	.	.
Cinclidium latifolium	.	1	1	.	1	.	.	15	.	.	.
Scapania paludicola	.	.	.	.	.	1	.	.	2	.	1
Campylium stellatum	.	.	.	.	5	.	1	20	.	.	.
Cephaloziella sp.	.	.	1	.	1	.	.	.	+	.	.
Calliergon richardsonii	.	.	1	.	1	1	.	.	.	.	.
Riccardia latifrons	.	.	.	.	1	.	.	.	+	.	.
Cephaloziella divaricata	.	.	.	.	.	1	+	.	.	.	.
Blepharostoma brevirete	.	.	.	.	1	.	.	.	.	.	+
Loeskypnum badium	1	.	.	.	.	.	.	.	.	30	30
Herbs & dwarf shrubs											
Carex chordorrhiza	25	15	25	40	1	50	60	2	50	30	20
Carex aquatilis subsp. *stans*	10	3	5	5	30	10	3	10	10	5	3
Comarum palustre	5	5	5	20	1	.	2	10	.	1	1
Eriophorum angustifolium	.	.	1	1	1	1	.	.	.	1	1
Epilobium palustre	1	.	1	1	.	1	1	.	1	.	.
Saxifraga foliolosa	1	.	1	1	.	.	.	1	1	1	.
Pedicularis sudetica	.	1	1	.	.	.	.	2	.	2	5
Eriophorum russeolum	.	.	1	.	.	1	1	1	.	.	.
Caltha arctica	1	.	1	.	.	.	1	.	.	.	.
Calamagrostis holmii	.	1	.	2	.	.	.	.	.	.	.
Bistorta vivipara	.	.	.	.	.	.	.	1	.	1	.
Luzula wahlenbergii	.	.	.	.	.	.	.	.	1	1	.
Saxifraga radiata	.	.	1	.	1	.	.	.	.	.	.
Rumex arcticus	.	1	1	.	.	.	.	.	.	.	.
Saxifraga cernua	.	.	.	1	.	.	.	.	1	.	.
Hierochloe pauciflora	.	.	.	.	.	.	.	.	.	1	1
Carex rotundata	.	.	.	.	.	.	1	.	.	+	.
Juncus triglumis	.	.	.	.	.	.	.	1	.	1	.
Calamagrostis neglecta	.	.	1	.	.	.	1	.	.	.	.
Aulacomnium turgidum	.	.	.	.	.	.	.	.	.	1	1
Salix saxatilis	1	5	.	1	.	.	.	1	.	20	20

Note. Species found in one relevé with abundance (indicated in brackets): *Andromeda polifolia* (2 1), *Betula exilis* (8 1), *Bryum longisetum* (9 1), *Carex marina* (8 3), *Cephalozia bicuspidata* (5 1), *Cephaloziella uncinata* (5 1), *Eriophorum medium* (11 1), *Fissidens osmundoides* (5 1), *Hamatocaulis vernicosus* (11 2), *Meesia uliginosa* (5 1), *Pedicularis pennellii* (8 1), *P. sceptrum-carolinum* (8 1), *Plagiochila arctica* (5 1), *Ranunculus pallasii* (9 1), *Salix phylicifolia* (8 1), *Saxifraga hirculus* (8 1), *Schljakovia kunzeana* (5 1), *Schljakovianthus quadrilobus* (11 1), *Sphagnum squarrosum* (7 2), *Utricularia vulgaris* (8 1), *Warnstorfia fluitans* (4 1), *W. sarmentosa* (8 1).

Dates and localities:

1 – 13.07.2023, 70.85202°N, 146.21921 °E;
2 – 13.07.2023, 70.85928°N, 146.22827°E;
3 – 13.07.2023, 70.85761°N, 146.22646°E;
4 – 13.07.2023, 70.85909°N, 146.22586°E;
5 – 21.07.2023, 70.79611°N, 147.84897°E;
6 – 13.07.2023, 70.85305°N, 146.21829°E;
7 – 13.07.2023, 70.85175°N, 146.21793°E;
8 – 19.07.2023, 70.75962°N, 147.23175°E;
9 – 12.07.2023, 70.88258°N, 145.9196°E;
10 – 16.07.2023, 70.77303°N, 146.72231°E;
11 – 16.07.2023, 70.77596°N, 146.72403°E.

Authors: E.D. Lapshina – rel. 4, 6–11; I.V. Filippov – rel. 1–3, 5.

Table 6. Arrangment of two species of *Rudolgaea* and their associates along the important environmental gradients (the range of each species is indicated by a color in the respective column).

	Poor-to-rich' fen gradient				Mud-bottom to hummock gradient			
	Poor fen	Inter-mediate fen	Modera-tely rich fen	Extre-mely rich fen	Mud-botom	Carpet	Law	Hummock
Mosses & liverworts								
Aneura pinguis								
Bryum neodamense								
B. pseudotriquetrum								
Calliergon richardsonii								
Campylium stellatum								
Cinclidium latifolium								
C. subrotundum								
Meesia triquetra								
Mesoptychia rutheana								
Polytrichum jensenii								
Pseudocalliergon brevifolium								
Rudolgaea borealis								
R. aenigmatica								
Scorpidium revolvens								
S. scorpioides								
Sphagnum orientale								
Herbs & shrubs								
Carex chordorrhiza								
Carex stans								
Comarum palustre								
Epilobium palustre								
Eriophorum angustifolium								
E. russeolum								
Pedicularis sudetica								
Salix saxatilis								
Saxifraga foliolosa								

ticeable admixture (3–30%). According to the ecological-floristic classification, this type of sedge-*Hypnum* communities belong to the ass. ***Meesio triquetro–Caricetum chordorrhizae*** Lapshina et Filippov 2025, recently described in the southern part of the tundra zone of Yakutia (Lapshina & Filippov, 2025). The number of species per sampling plot varies from 11 to 28 (average 19). A total of 75 species have been recorded in this association, of which 8 (11%) are highly constant. The communities of this association are widespread in the southern tundra subzone in Yakutia, where they occur in waterlogged polygons of rim-polygonal mires, hollows of flat-palsa-hollow mire complexes, and lakeside floating mats across numerous drained thermokarst lake basins (khasyreis). The surface is flat or gently undulating, formed by a continuous carpet of *Hypnum* mosses, often with areas of open water. The seasonal thaw depth of the permafrost is 30–40 cm (Lapshina & Filippov, 2025).

As far as we know, such obvious morphological differences in the absence of clear ecological and molecular differentiation have been described for liverworts for the first time, and at this stage of knowledge, no explanation for this has been found. Probably genetic differences between these two morphologically clearly distinct species will be found in future, but at present we were unable to detect them by available material and methods.

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Appendix 1. The list of specimens included in molecular phylogenetic estimation. The accessions started with PZ are obtained in this study.

Taxon	Specimen voucher	GenBank accession number		
		ITS1-2 nrDNA	*trn*L-F cpDNA	*rbc*L cpDNA
Anastrepta orcadensis (Hook.) Schiffn.	Russia: Republic of Buryatia, *Konstantinova 59-1-01* (KPABG-102486)	DQ875126 –	DQ875088 –	–
A. orcadensis	China: Yunnan Prov., *Long 34711* (E)	–	–	KF852268
Anastrophyllum assimile (Mitt.) Steph.	Russia: Irkutsk Reg., *Mamontov YuSM-468-1-5a* (KPABG-120896)	OP584689	OP573528	–
Anastrophyllum astorgae Mamontov & Vilnet	Chile, *Mamontov YuSM-928-3-7385* (KPABG-126098)	OR769965	OR762259	–
Anastrophyllum crebrifolium (Hook. f. & Taylor) Steph.	Chile, *Mamontov YuSM-929-3-7474* (KPABG-126097)	OR769963	OR762257	–
Anastrophyllum ellipticum Inoue	Russia: Altai Rep., *Mamontov 330/2* (KPABG)	KF836654 /KF836632	KF836664	–
Anastrophyllum michauxii (F.Weber) H. Buch	Russia: Komi Rep., *Dulin* (KPABG-109820)	KF836645	KF836655	–
Barbilophozia barbata (Schmidel ex Schreb.) Loeske	Netherlands, *Konstantinova 3b-5-99* (KPABG-102106)	EU791779	EU791676	–
B. barbata	Switzerland, *Cailliau et al.*	–	–	JX305536
Barbilophozia lycopodioides (Wallr.) Loeske	Russia: Republic of Karelia, *Bakalin* (KPABG-107299)	HQ897114	HQ897003	–
Barbilophozia rubescens (R.M.Schust. et Damsh.) Kartt. et L.Söderstr.	Russia: Magadan Reg., *Mochalova* (KPABG-106852)	EU791784	EU791678	–
Biantheridion undulifolium (Nees) Konstant. et Vilnet	Russia: Kemerovo Reg., *Konstantinova 56-1-00* (KPABG-101859)	EU791794	EU791671	–
Chandonanthus sp.	China, *He-Nygren 492*	–	AY463554	–
Crossocalyx hellerianus (Nees ex Lindenb.) Meyl.	Russia: Sakhalin Reg., *Bakalin 82* (KPABG)	KF836643	KF836666	–
Gymnocolea inflata (Huds.) Dumort.	Russia: Murmansk Reg., *Konstantinova 193-1-02* (KPABG-9512)	OR769957	OR762251	–
G. inflata	Russia: Nizhny Novgorod Reg., *Konstantinova 129-2a-03* (KPABG-106093)	GQ220783	GQ220785	–
G. inflata	Italy: Sued Tirol, *Schafer-Verwimp & Verwimp 41089* (KPABG-125590)	PX620329	PZ281205	–
G. inflata	Norway: Svalbard, *Konstantoniva 118-1-04* (KPABG-109976)	EU791787	EU791661	KF852306
G. inflata	United Kingdom, *Cailliau et al.*	–	–	JX305549
Hamatostrepta concinna Váňa et D.G.Long, Fieldiana	Myanmar, *Long 34854* (DUKE)	–	–	KF852407
Hattoria yakushimensis (Horik.) R.M.Schust.	Japan: Kagoshima, Yakushima Island, *Katagiri 428* (NICH)	LC744594	LC376049	LC376047
Isopaches alboviridis (R.M.Schust.) Schljakov	Russia: Perm Terr., *Konstantinova K303-1-04* (KPABG-108224)	PX620310	PX632760	–

Taxon	Specimen voucher	GenBank accession number		
		ITS1-2 nrDNA	*trn*L-F cpDNA	*rbc*L cpDNA
Isopaches bicrenatus (Schmidel ex Hoffm.) H.Buch	Russia: Murmansk Reg., *Konstantinova K129-1-19* (KPABG-124358)	OP584691	OP573530	–
Isopaches bicrenatus	USA: Vermont, *Shaw 6970* (DUKE)	–	–	KF852384
Isopaches decolorans (Limpr.) H.Buch	Russia: Murmansk Reg., *Borovichev BE243-1-12* (KPABG-21382)	PX620327	PX632779	–
Isopaches valdiviensis Mamontov, Vilnet & Konstant.	Chile: Los Ríos Reg., *Mamontov 754-3-9134* (MHA)	PX620328	PX632781	–
Neoorthocaulis attenuatus (Mart.) L.Söderstr., De Roo et Hedd.	Russia: Kamchatka Terr., *Bakalin 66-1-01-VB* (KPABG-103759)	OR769958	OR762252	–
Neoorthocaulis attenuatus	(H-4230531)	–	–	GU373417
Neoorthocaulis binsteadii (Kaal.) L.Söderstr., De Roo et Hedd.	Russia: Nenetzky Autonom. Area, *Lavrinenko s.n.* (KPABG-100333)	OR769959	OR762253	–
Neoorthocaulis floerkei (F.Weber et D.Mohr) L.Söderstr., De Roo et Hedd.	Russia: Sakhalin Reg., Paramushir I., *Bakalin K-96-10-04* (KPABG-107496)	OR769960	OR762254	–
Obtusifolium obtusum (Lindb.) S.W.Arnell	Russia: Murmansk Reg., *Konstantinova K401-2-06* (KPABG-14526)	PZ289441	PZ293230	–
O. obtusum	Russia: Kamchatka Terr., *Bakalin & Klimova K-102-2a-21* (VBGI)	–	–	OP205313
Plicanthus birmensis (Steph.) R.M.Schust.	Russia: Primorsky Terr., *Bakalin P-76-5-05* (KPABG-123309)	EU791791	EU791668	–
Plicanthus hirtellus (F.Weber) R.M.Schust.	Madagascar: Vakinankaratra, *Brinda* (MO-6969211)	LC886038	LC886014	LC885968
Rudolgaea aenigmatica	Russia: Republic of Sakha (Yakutia), *Filippov 44F-5-23* (KPABG-127871), 1	PZ280164	PZ281202	PZ281207
R. aenigmatica	Russia: Republic of Sakha (Yakutia), *Filippov 47F-1* (KPABG -127875), 2	PZ280165	PZ281203	PZ281208
R. aenigmatica	Russia: Republic of Sakha (Yakutia), *Lapshina 85E-4* (KPABG-127874), 3	PZ280166	PZ281204	PZ281209
Rudolgaea borealis (Frisvoll & Moen) Potemkin & Vilnet	Russia: Krasnoyarsk Terr.,Taimyr, *Lapshina 4354* (KPABG-125157)	ON175945	PZ281201	PZ281206
R. borealis	Russia: Gydansky Peninsula, *Troeva G1-138* (LE)	MZ343174	MZ353627	MZ032229
R. borealis	Sweden, *Cailliau & Hallingbaeck*	–	–	JX305563
Rudolgaea fascinifera (Potemkin) Potemkin & Vilnet	Russia: Republic of Sakha (Yakutia), *Lapshina 019E-6-23* (KPABG-126373)	PQ686974	PQ699322	PQ699389
R. fascinifera	USA: Alaska, *Potemkin 92-9701* (LE)	MZ297375	MZ298895	MZ298896
R. fascinifera	Russia: Krasnoyarsk Terr., Taimyr, *Lapshina 159E/3a-21* (KPABG-124490)	no data	no data	PQ738158
Schizophyllopsis sphenoloboides (R.M.Schust.) Váňa et L.Söderstr.	Russia: Republic of Sakha (Yakutia), *Bakalin* (KPABG-101592)	KF836653	KF836663	–
Schljakovia kunzeana (Huebener) Konstant. et Vilnet	Russia: Trans-Baikal Terr., *Bakalin 38-1-00* (KPABG-101737)	OR769961	OR762255	–
Schljakovianthus quadrilobus (Lindb.) Konstant. et Vilnet	Russia: Murmansk Reg., *Mamontov 412-3-1* (INEP-400334)	OR769962	OR762256	–
S. quadrilobus	USA: Alaska, *Shaw F982b/8* (DUKE)	–	–	KF852393
Sphenolobus minutus (Schreb. ex D.Crantz) Berggr.	Russia: Arkhangelsk Reg., *Savchenko CA-19-29* (KPABG-122709)	MT422255	MT431399	–
Sphenolobus saxicola (Schrad.) Steph.	Russia: Republic of Buryatia, *Konstantinova 123-3-02* (KPABG)	DQ875124	DQ875086	–
Tetralophozia filiformis (Steph.) Urmi	China: Sichuan Prov., *Bakalin & Klimova China-39-1-17* (VBGI-37281, KPABG-122599)	MZ231275	MZ229433	–
Tetralophozia filiformis	China: Yunnan Prov., *Shaw 5790* (DUKE)	–	–	KF852352
Taxon	Specimen voucher	GenBank accession number		
		ITS1-2 nrDNA	*trn*L-F cpDNA	*rbc*L cpDNA
Tetralophozia pusilla (Steph.) Bakalin & Vilnet	Republic of Korea: Gyeongsangnam-do, *Bakalin Kor-27-20-15* (VBGI, KPABG-120508)	MZ231277	MZ229435	–

Tetralophozia setiformis (Ehrh.) Schljakov	Russia: Murmansk Reg., *Konstantinova K201-1-07* (KPABG-18022)	MZ231280	MZ229440	–
Tetralophozia sibirica Vilnet & Bakalin	Russia: Trans-Baikal Terr., *Mamontov 411*(KPABG-121349)	MZ231279	MZ229437	–
Vietnamiella epiphytica Bakalin & Vilnet	Vietnam: Lao Cai Prov., *Bakalin V-9-7-17* (KPABG-122594)	MK277316	MK290984	MK290986

Appendix 2. GenBank accession numbers of additionally sequenced DNA markers for selected specimens (*rpl*16, *rps*4, *psb*A-*trn*H cpDNA, *rpb*2 nrDNA).

Species	Sample	*rpl*16	*rps*4	*psb*A-*trn*H	*rpb*2
Gymnocolea inflata	KPABG-125590	PZ281214	PZ281217	PZ281223	PZ281229.
Rudolgaea borealis	KPABG-125157	PZ281210	PZ281218	PZ281224	PZ281230.
Rudolgaea aenigmatica	KPABG-127871	PZ281211	PZ281219	PZ281225	PZ281231.
Rudolgaea aenigmatica	KPABG-127875	PZ281212	PZ281220	PZ281226	PZ281232.
Rudolgaea aenigmatica	KPABG-127874	PZ281213	PZ281221	PZ281227	PZ281233.
Vietnamiella epiphytica	KPABG-122594	PZ281215	PZ281216	PZ281222	PZ281228.