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LOPHOZIA SAVICZIAE SCHLJAKOV (LOPHOZIACEAE, MARCHANTIOPHYTA) – ONE MORE ALLOPOLYPLOID SPECIES OF LIVERWORTS

LOPHOZIA SAVICZIAE SCHLJAKOV (LOPHOZIACEAE, MARCHANTIOPHYTA) – ЕЩЕ ОДИН АЛЛОПОЛИПЛОИДНЫЙ ВИД ПЕЧЕНОЧНИКОВ

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Abstract

Lophozia savicziae is a poorly known species described in 1973 from Murmansk Region of Russia and also recorded from North Asia, Greenland, and West of North America including Alaska. The isotype specimen of *L. savicziae* (*Schljakov # 4-71* KPABG) was successfully sequenced. The obtained incongruence of topologies inferred from ITS1-2 nrDNA and *trn*L-F cpDNA data suggested a hybrid origin of *L. savicziae* from *L. silvicoloides* (paternal parent) and *Lophozia* sp. (maternal parent). *Lophozia savicziae* keeps both parental ITS1-2 copies that suggests a non-completeness of concerted evolution in this allopolyploid species. In the result of the integrative study all tested accessions of *L. savicziae* were found only in the Murmansk Region. The specimens identified as *L. savicziae* from Svalbard, several localities of the Murmansk Region, the Khanty-Mansi (Yugra) autonomous area, the Krasnoyarsk, the Kamchatka Territories, and the Chukotka Autonomous Area were referred to *L. silvicoloides* which apparently is not rare in tundra and high mountains. Three specimens were referred to *Lophozia* sp. Morphologically *L. savicziae* differs well from *Lophozia silvicoloides* in leaf and perianth shape. It is much more difficult to distinguish it from *Lophozia* sp., which we hesitate to describe due to the lack of appropriate data.

Резюме

Lophozia savicziae – малоизвестный вид, описанный в 1973 году из Мурманской области России, приводящийся также из Северной Азии, Гренландии и с запада Северной Америки, включая Аляску. Изотип *L. savicziae* (Шляков # 4-71 KPABG) был успешно секвенирован. Полученное несоответствие топологий, выявленное на основе данных ITS1-2 ядДНК и *trn*L-F хпДНК, позволило предположить гибридное происхождение *L. savicziae* от *L. silvicoloides* (родителя по отцовской линии) и *Lophozia* sp. (родителя по материнской линии). У *Lophozia savicziae* сохранились копии ITS1-2 обоих родительских видов, что свидетельствует о незавершенности концертной эволюции этого аллополиплоидного вида. В результате комплексного исследования образцы, соответствующие типу *L. savicziae,* были обнаружены только в Мурманской области. Образцы, определенные как *L. savicziae* со Шпицбергена, из нескольких районов Мурманской области, Ханты-Мансийского автономного округа (Югра), Красноярского и Камчатского краев и Чукотского автономного округа, были отнесены к *L. silvicoloides*, которая, по-видимому, не является редкостью в тундрах и высокогорьях. Три образца отнесены к *Lophozia* sp. Морфологически *L. savicziae* хорошо отличается от *Lophozia silvicoloides* формой листьев и периантия. Гораздо сложнее отличить ее от *Lophozia* sp., которую мы пока не описываем из-за недостатка данных.

KEYWORDS: Lophoziaceae, ITS1-2 nrDNA, *trn*L-F cpDNA, integrative taxonomy, hybridization, morphology, distribution, hybrid origin

Introduction

The genus *Lophozia* (Dumort.) Dumort. is one of the most complicated liverwort genera in the north of the Holarctic in terms of the interpretation of the described taxa. Eighteen binominals were listed in the World checklist of hornworts and liverworts (Söderström *et al*., 2016). Later two more species were described, particularly *Lophozia fuscovirens* (Bakalin, 2019) and *L. svalbardensis* (Konstantinova *et al*., 2020), and two species were transferred into *Lophozia* from other genera, including *L. koreana* from the genus *Tritomaria* Schiffn. ex Loeske (Bakalin *et al*., 2021) and *L. obscura* from the genus *Schistochilopsis* (N. Kitag.) Konstant. (Bakalin *et al*., 2020). Only five species are accepted more or less without a doubt, while the majority of species possess "knowledge problem" or even "serious doubts" (Söderström *et al*., 2016). One of the species marked as "knowledge problem" is *Lophozia savicziae* Schljakov. It means that "the taxon is not well known by the person evaluating it. It may be a newly described species or a species originally

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Полярно-альпийский ботанический сад
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Lophozia Savicziae Schljak.
Хибинский горный массив Северо-восточный склон Вудъяврчорра против морены Кукисвумчорра. Торфянистая дерновина на сыром скалистом выступе склона.
№ 4 Собр. 25/VII 19 71 г. Р. Н. Шляков
RS-4-71 Опред. VII 74 Шляков

Fig. 1. The label of *Lophozia savicziae* isotype preserved in KPABG.

not well described and not restudied recently". Clarifying the phylogenetic position and taxonomy of such species on the basis of an integrative approach with the inclusion of type specimens which are still suitable for DNA extraction, seems to us to be one of the most urgent and important tasks.

Lophozia savicziae was described from the Khibiny Mountains in the Murmansk Region (Schljakov, 1973). It is mainly known from specimens collected and identified by R.N. Schljakov and his students. The species has been recorded from the northern regions of Russia as well as from the mountains of South Siberia and Far East of Russia. Outside Russia, the species has only been recorded for isolated localities in Sweden and in Svalbard (Bakalin, 2005; Konstantinova *et al*., 2009). K. Damsholt (2002) considered the species questionable as *Lophozia wenzelii* (Nees) Steph. var. *lapponica* H.Buch et S.W.Arnell, but later attributed it to *Lophozia ventricosa* var. *grandiretis* (H.Buch et S.W.Arnell) R.M.Schust. et Damsh. (Damsholt, 2013). *Lophozia savicziae* has never been included into molecular phylogenetic estimations of *Lophozia.* Therefore, when last year the senior author collected a very good material of this species in the Khibiny Mountains (Murmansk Region), we decided to sequence plants from the collected specimen, as well as to try to sequence the plants of the isotype stored in the herbarium of the Polar-Alpine Botanical Garden-Institute of the Kola Science Centre of Russian Academy of Science (KPABG) (Fig. 1), and some specimens identified as *Lophozia savicziae* from different regions of Russia and Svalbard also preserved in KPABG. The aim of the current study is to verify the molecular genetic identity of specimens referred to *L. savicziae*, determine its phylogenetic position, and clarify morphological features that distinguish it from closely related species.

Material and methods

Morphological study

Specimens identified as *Lophozia savicziae* and morphologically somewhat similar *L. silvicoloides* stored in KPABG were revised. Morphology of thirteen more or less recently collected specimens identified as *Lophozia savicziae* and *L. silvicoloides* from remote areas of Eurasia and Svalbard and the isotype of *L. savicziae* (Murmansk Region, Khibiny Mountains, *Schljakov # 4-71*) involved in molecular study (see below) were studied more carefully using light microscopes equipped with digital cameras.

It should be clarified, however, that the specimen of Schljakov No. 4 (*Schljakov # 4-71,* KPABG(H)3752), collected by him on July 25, 1971 on the Mount Woodyavrchorr in Khibiny, is not indicated as an isotype in the protologue. The author of the description indicated specimen No. 6 as the type, which label is completely identical to the specimen No. 4 label, except for the date of identification, marked as July 1974 (Fig. 1). *Lophozia savicziae* was described in 1973 (Schljakov, 1973), based on the specimen identified initially as *L. murmanica* as is marked by the author (l.c.). In the specimen with the field number 4, Schljakov's field name is *Lophozia murmanica* as well (Fig. 1), it was exactly the same with No. 6, which was also named *L. murmanica* as written in the protologue (l.c.). All this convinces us that both specimens were collected simultaneously in one place, and in accordance with the explanation in the note 1 to Article 8.2: of the *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code)*(Turland *et al*., 2018), the only differences in field number are not an obstacle to recognizing the specimen as an isotype.

Sampling for molecular analyses

Thirteen specimens referred to *Lophozia savicziae* were molecularly tested for the first time. The specimens of *Lophoziopsis longidens* (*Konstantinova # K12-4-23*) and *Oleolophozia perssonii* (*Konstantinova & Savchenko # K3-10*) were newly sequenced in this study as well, and the latter species was used as an outgroup taxon according with modern phylogenetic treatment (Bakalin *et al*., 2024). Following our previous studies of the genera *Lophozia* and *Lophoziopsis* (Konstantinova *et al*., 2020) the ITS1-2 nrDNA and *trn*L-F cpDNA were selected as molecular genetic markers. Additionally datasets for molecular phylogenetic reconstructions were compiled by twenty nine specimens of both genera, including four specimens of *Lophozia silvicoloides* (Appendix).

Selected specimens studied including all specimens involved in the molecular investigation.

Lophozia savicziae Schljakov – **Russia**. Murmansk Region: Khibiny Mountains, Woodyavrchorr Mount (67.650801°N – 33.640917°E), 600 m a.s.l., 25.VII.1971 *Schljakov # 4-71* [KPABG(H)3752]; ibid. (67.60509°N – 33.60541°E), 500 m a.s.l., 20.VIII.2024 *Konstantinova # 25-3-24* [KPABG(H) 126740]; Panskie Tundra, Kamenik Mount, *Konstantinova # K203-2-07* [KPABG(H)18034]; Keivy Ridge, *Konstantinova # 65-2-97* [KPABG(H)6183]. ***Lophozia* sp.** – **Russia**. Kamchatka Terr.: Commander Isl., Bering Isl., *Bakalin # K-16-8-02-VB* [KPABG(H)103428 as *Lophozia savicziae*]; South Kamchatka (53°00'N – 158°25'E), 300 m a.s.l., 22.VIII.2001 *Bakalin # 73-2-01-VB* [KPABG(H)103798, as *Lophozia savicziae*]; Central Kamchatka (55°05'N – 159°00'E), 1000 m a.s.l, 2.IX.2002 *Bakalin # K-39-1-02-VB* [KPABG(H)104025 as *Lophozia savicziae*]. ***Lophozia silvicoloides*** N. Kitag. – **Russia**. Krasnoyarsk Terr., Sibiryakov Isl., 19.VII.1989 *Kuvaev* [KPABG(H)

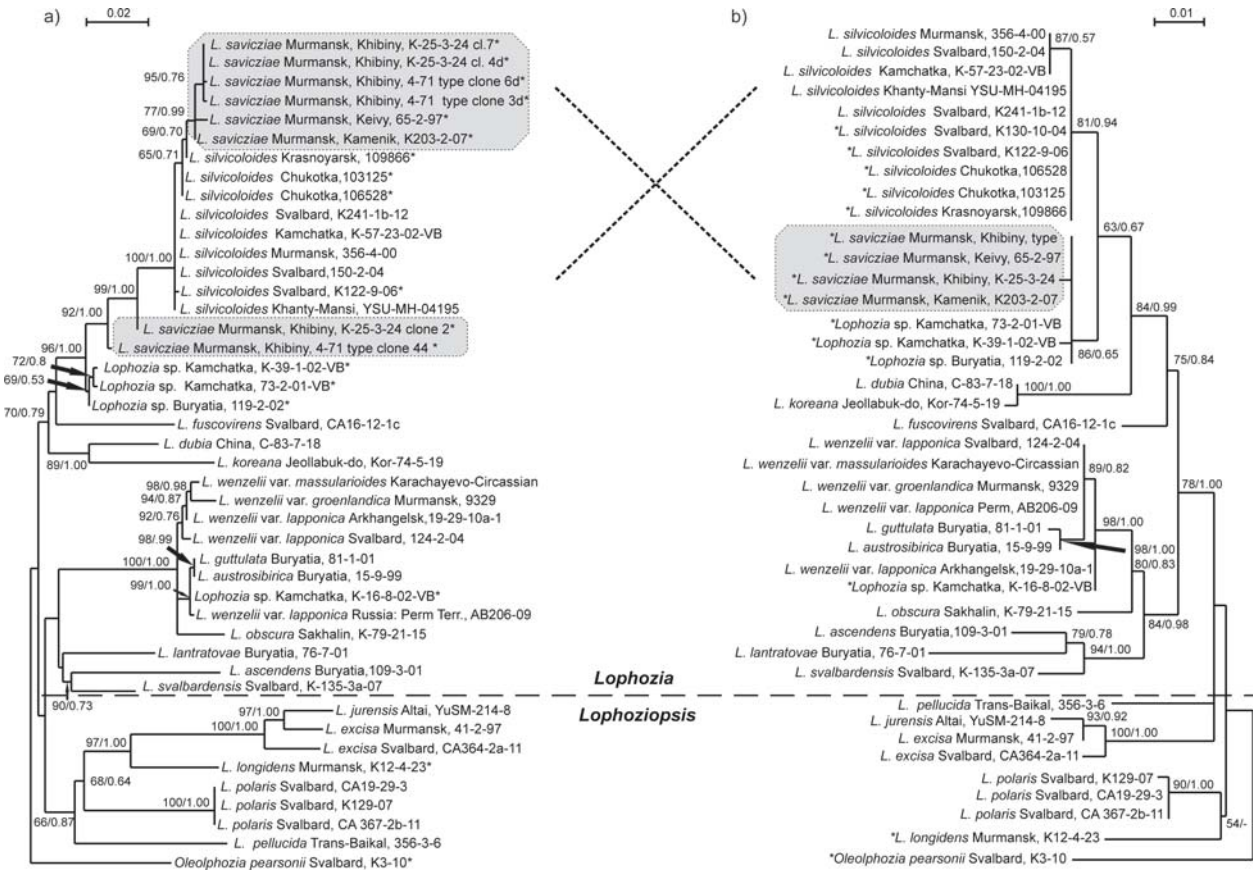

Fig.2. Phylogram obtained by maximum likelihood method for the genera *Lophozia* and *Lophoziopsis* based on a) ITS1-2 and b) *trn*L-F datasets. Bootstrap supports from maximum likelihood analysis and Bayesian posterior probabilities after Bayesian estimation more than 50% (0.50) are indicated. The voucher information are provided, specimens firstly tested in this study are marked with asterisk.

109866]; Buryatia Rep., *Konstantinova # 119-2-02* [KPABG(H) 126733, as *Lophozia savicziae*]; Chukotka Autonomous Area: 115 km of the Egvekinot-Iultin highway, 20.VIII.1970 *Afonina* [KPABG(H)103125, as *Lophozia savicziae*]; Arakamchechen Island, 19.VIII.1976 *Afonina* [KPABG(H)106528]; **Norway.** Svalbard: Nordaustlandet, Prins Oskar Land (78.002627°N – 15.850970°E), 85 m a.s.l., 31.VII.2006 *Konstantinova # K122-9-06* [KPABG(H) 111833, as *Lophozia savicziae*]; Western Spitsbergen Island, Nordenskiöld Land, Grøndalen (78°01'29"N – 14°21'02" E), 7 m a.s.l., 27.VII.2004 *Konstantinova* [KPABG(H)110030, as *Lophozia savicziae*].

DNA isolation, PCR amplification, DNA sequencing

DNA was extracted with the HiPure SF Plant DNA Kit (Magen, China) according with manufacturer's protocol. The ITS1-2 and *trn*L-F genetic regions were amplified and sequenced with pairs of primers provided by White *et al*. (1990) and Taberlet *et al*. (1991). PCR was carried out in 20 µl volumes with the following amplification cycles: 3 min at 94°C, 30 cycles (30 s 94°C, 40 s 56°C, 60 s 72°C) and 2 min. of final extension time at 72°C. The amplified fragments were visualized on 1% agarose TAE gels by EthBr staining, purified using the Cleanup Mini Kit (Evrogen, Russia), and used as a template in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) following the standard protocol provided for 3100 Avant Genetic Analyzer (Applied Biosystems, USA).

In a case of a heterogeneity of ITS1-2 sequences in specimens *Konstantinova # K-25-3-24* and *Schljakov # 4-71,* amplicons were cloned by protocol provided in Evrogen Joint Stock Company (Moscow, Russia). Three clones per each of two specimens were sequenced with primers for ITS1-2.

Phylogenetic analysis

The sequences for 15 specimens were assembled in the program BioEdit 7.0.1 (Hall, 1999). The ITS1-2 and *trn*L-F alignments were automatically produced with ClustalW (Thompson *et al*., 1994) and then manually corrected. The ITS1-2 and *trn*L-F datasets revealed incongruence in term of relation of *Lophozia savicziae* specimens in the preliminary estimation, thus phylogenetic analyses provided for each dataset separately. The maximum likelihood analysis (ML) was provided with the program IQ-TREE (Nguyen *et al*., 2015), the Bayesian analysis (BA) with MrBayes v. 3.2.1 (Ronquist *et al*., 2012). The best fit evolutionary models of nucleotide substitutions for ML calculations were selected in program ModelFinder (Kalyaanamoorthy *et al*., 2017): the

TN+F+G4 model was suggested for ITS1-2, the HKY+F+I+G4 for *trn*L-F. The ultrafast bootstrapping procedure (Hoang *et al*., 2018) with 1000 replicates was used. For the Bayesian analysis the GTR+I+G model as recommended by the program' creators was implemented for both ITS1-2 and *trn*L-F 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 was one million. Trees were saved every 100th generation. The average standard deviation of split frequencies between two runs for ITS1-2 dataset was 0.009441, for *trn*L-F dataset – 0.004992. 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 and *trn*L-F in Mega 11 (Tamura *et al*., 2021) using the pairwise deletion option for counting gaps.

Results

Molecular phylogenetic study

The 14 accessions of ITS1-2 and 15 accessions of *trn*L-F were firstly obtained and deposited into GenBank. The ITS1-2 dataset counts 895 positions, *trnL*-F – 515. The arithmetic mean of Log likelihood was -3579.465 for single tree obtained for ITS1-2 dataset in ML calculation, the means of Log likelihood in BA analysis were -3622.23 and -3620.34. The arithmetic means of Log likelihood were -1486.001 for single tree reconstructed from *trn*L-F dataset in ML analysis, and -1528.26 and -1527.56 in BA calculation.

Tree topologies reconstructed by both analyses for ITS1-2 dataset are similar, as well similarity in topology is found in two calculations of *trn*L-F dataset. Fig. 2 illustrates the ML topology with indication of bootstrap support (BS) values from ML calculation and Bayesian posterior probabilities (PP) from BA for ITS1-2 and *trn*L-F dataset consequently.

The genera *Lophozia* and *Lophoziopsis* take a relation on obtained phylogeny, the phylogenetic affinity among species are stable and correspond with the results of previous estimation (Konstantinova *et al*., 2020). Thirteen specimens named as *L. savicziae* did not form a single clade but were dispersed into four clades; moreover, some specimens were resolved in a different positions in trees inferred from ITS1-2 and *trn*L-F data. Specimens *Konstantinova # K122-9-06* and *# K241-1b-12* from Svalbard, *Afonina* KPABG(H)103125 and KPABG(H)106528 from Chukotka, and *Kuvaev* KPABG(H)109866 from Sibiryakov Island were resolved in a clade of *L. silvicoloides* in trees inferred from both genomic regions. The specimens *Bakalin # 73-2-01-VB* and *# K-39-1-02-VB* from Kamchatka Territory and *Konstantinova # 119-2-02* from Buryatia Republic composed a clade with sister relation to *L. silvicoloides*-clade in both trees; they are named here as *Lophozia* sp. We cannot be completely sure of the correctness of attributing the maternal species to this poorly known taxon. However, a number of morphological features (see below) allows us to make this assumption. Four specimens of *L. savicziae* from different localities in Murmansk Region, including isotype *Schljakov # 4-71,* were placed in *L. silvicoloides*-clade in ITS1-2 topology and in the clade with three specimens of *Lophozia* sp. in *trn*L-F topology. Evidently, this group of specimens presents a true *L. savicziae*, that originated through hybridization of paternal parent *L. silvicoloides* and maternal parent *Lophozia* sp. Among the clones obtained for *Schljakov # 4-71* and *Konstantinova # K-25-3-24,* one group (clones 7, 4d, 6d, 3d) is similar with the sequences of *L. silvicoloides* specimens, whereas the other group (clones 2, 44) are located in a separate phyla between *L. silvicoloides* and *Lophozia* sp. clades. Clone 2 from *Konstantinova # K-25-3-24* is characterized by ITS1 similar to *Lophozia* sp. clade, but ITS2 is similar to *L. silvicoloides*-clade. Clone 44 from *Schljakov # 4-71* has ITS1-2 resembling those of *Lophozia* sp.-clade, but with a number of substitutions. Two other specimens of *L. savicziae* from the Murmansk Region were not used for cloning but revealed heterogeneity in both spacers, and their dominant copy of ITS1-2 is similar to the *L. silvicoloides*-clade.

The *p*-distances were evaluated for *Lophozia silvicoloides*, true *L. savicziae* specimens including the obtained copies of both parental genomes, *Lophozia* sp. and three close relative species according phylogeny - *L. dubia, L. koreana* and *L. fuscovirens* (Table 1). True *L. savicziae, Lophozia silvicoloides* and *Lophozia* sp. are characterized by similar low level of infraspecific variability in both sequenced loci (0.2-0.3% in ITS1-2, 0.1-0.2% in *trn*L-F). The paternal ITS1-2 copies of *L. savicziae* differ from *L. silvicoloides* in 0.6%, which is within the frame of infraspecific variability in the genus *Lophozia* (Bakalin, Vilnet, 2019). The maternal ITS1-2 copy of *Konstantinova # K-25-3-24* differs in 1.7% from *Lophozia* sp., the maternal ITS1-2 copy of *Schljakov # 4-71* - 0.9% from *Lophozia* sp. The *trn*L-F dissimilarity of *L. savicziae* and *Lophozia* sp. is 0.1%. Both *Lophozia* sp. and *L. silvicoloides* are diverged by 2.6% in ITS1-2 and 1.4% in *trn*L-F. The level of differentiation between *Lophozia* sp. and *L. silvicoloides* is at least two times lower than the divergence with the phylogenetically allied species *L. koreana* (4.2-4.3% in ITS1-2, 2.5-3.3% in *trn*L-F), *L. dubia* (5.3-5.9% in ITS1-2, 2.5-3.6% in *trn*L-F) or *L. fuscovirens* (4.4-5.0% in ITS1-2, 2.5-3.0% in *trn*L-F).

Thus, *Lophozia silvicoloides* and *Lophozia* sp. present a complex of closely related species with a common origin, which in the course of evolution led to the creation of *L. savicziae* through hybridization.

Morphology

Lophozia savicziae shares with both parents numerous biconcentric oil-bodies,1–2-celled gemmae that are pellucid to greenish, and distinctly concave leaves. *Lophozia silvicoloides* differs from *Lophozia savicziae* in 1) the shape of perianth which is very large and long exserted from bracts, has laciniate-ciliate mouth with teeth 2–3 cells wide at base and 5–7 cells long vs. not long exserted and relatively small perianth with teeth 1–2(3) cells long in *L. savicziae*; 2) deeper (1/4–1/3) and mostly angulate sinuses of leaves vs. shallow (up to 0.25 of leaf length) crescentic sinuses of leaves in *L. savicziae*; 3) distinctly cuspidate lobes, often ending in 2(3) superposed cells and slightly elongated uppermost cell; 4) narrow, (0)2(3) cells wide postical free leaf zone vs. 3–7 cells wide in *L. savicziae*. *Lophozia* sp. differs from *L. savicziae* in 1) often antically decurrent leaves vs. not or slightly decurrent leaves in *L. savicziae*; 2) smaller size of midleaf cells, (18)20–22(25) µm, cells isodiametric, mixed with distinctly elongated cells 18–20 µm wide and 25–32 µm long vs. (20)22–40 (45) µm wide and (23)27–45 µm long in *L. savicziae* and *L. silvicoloides*; 3) smaller gemmae, that are pellucid to greenish but with admixture of few brownish, ca. (15)18–20(22) × 19–20(23) µm vs. (18)22–30 × 15–30(33) µm in *L. savicziae* and *Lophozia silvicoloides*; 4) narrower postical free leaf zone, which is 2(3) cells wide, vs 3–7 cells wide in *L. savicziae.* However, the size of cells and gemmae, the width of postical free leaf zone, as well as the color of the plants are quite variable in the studied specimens, which can clearly be seen from the range of sizes given above.

Distribution

At this stage we can only preliminary estimate distribution of the three species, mostly based on sequenced specimens, and our assumptions are as follows.

Lophozia silvicoloides is the most widespread. It has an almost circumpolar distribution, occurring from Svalbard in the west (Söderström *et al*., 2021), where it is probably not uncommon under suitable conditions, to the Magadan Region, Kamchatka, and the Khabarovsk Territory, the Kuril Islands and Japan in the east, the mountains of Primorsky Territory in the south (Bakalin, 2005). Between these extreme points, the species is known from the Urals, Sibiryakov Island, and it has been repeatedly recorded for the Murmansk Region. In North America, the species is so far known from Alaska (Bakalin, 2005).

Lophozia sp. is so far known from South and Central Kamchatka and South Siberia. Evidently, the species is more widespread and has the arctic-montane distribution.

Lophozia savicziae is only known for sure from the Murmansk Region.

Ecology

All three species grow on peat soil covering moist cliffs, on fine earth in crevices of rocky placers, and on peat soil on banks of streams; they occur from near sea level in Svalbard up to 1880 m a.s.l. in Khamar-Daban Mountains (South Siberia) and around 1000 m alt. on the Kamchatka Peninsula. Besides that, the widespread *Lophozia silvicoloides* occurs in Svalbard and Russian Arctic in moist moss (including *Sphagnum*) - low shrub tundras and in moss - *Pinus pumila* tundras. In Japan, from where *Lophozia silvicoloides* was described, it is found "on humus under shrubs of *Pinus pumila*, rarely descending to the upper edges of subalpine coniferous forest" (Kitagawa, 1965: 278).

DISCUSSION

The tree topologies obtained from nuclear and chloroplast markers reveal different phylogenetic relation of *L. savicziae* that suggests its hybrid origin from *Lophozia silvicoloides* and unknown species *Lophozia* sp. in a course of reticulate evolution. The allopolyploidy of *L. savicziae* is supported by the presence in its genome of two copies of ITS1-2 corresponding to both parental taxa – *Lophozia silvicoloides* and *Lophozia* sp. The parental copies of ITS1-2 were sequenced for the isotype specimen (*Schljakov # 4-71*) and for the specimen recently gathered at a distance of about 10 km from the type locality of *Lophozia savicziae* (*Konstantinova # K-25-3-24*). The ITS1-2 copies corresponded to *Lophozia silvicoloides* vary in a level of infraspecific variation. The ITS1-2 copies inherited from *Lophozia* sp. clearly differ from it due to the substitutions in *Schljakov # 4-71* or even a recombination event in *Konstantinova # K-25-3-24* in ITS2, whereas ITS1 remains similar to *Lophozia* sp. Apparently, the nuclear genome gradually loses one of

Table 1. The value of infra- and interspecific *p*-distances for the selected species of the genus *Lophozia*, n/c – non calculated value due to single specimen only, "-" - non calculated value due to absence nucleotide sequence data.

Taxon	Variability, ITS1-2/*trn*L-F, %							
	Infra-pecific	Interspecific						
		L savic.	*L. silvic.*	cl.2	4-71 cl.44	*L.* sp.	*L. fusc.*	*L. dubia*
L savicziae	0.3/0.1							
L. silvicoloides	0.2/0.2	0.6/1.3						
L. savicziae K-25-3-24 cl.2	n/c/-	1.4/-	1.1/-					
L. savicziae 4-71 cl.44 type	n/c/-	2.3/-	2.2/-	1.0/-				
Lophozia sp.	0.3/0.2	2.7/0.1	2.6/1.4	1.7/-	0.9/-			
L. fuscovirens	n/c/ n/c	5.0/2.9	4.8/2.5	4.7/-	4.3/-	4.4/3.0		
L. dubia	n/c/ n/c	5.9/2.5	5.9/3.6	6.2/-	5.7/-	5.3/2.6	7.2/3.8	
L. koreana	n/c/ n/c	4.2/2.5	4.3/3.3	4.7/-	4.5/-	4.3/2.6	6.3/3.2	5.1/0.3

two parental copies in the course of concerted evolution, and specimen collected close to the locus classicus 50 years later shows significant changes towards the predominance of only one copy of the nuclear genome. Liverworts plastid genome is inherited as uni-parental (Szweykowska-Kulinska *et al.*, 2002), and this parent is maternal (Natcheva & Cronberg, 2007). In case of *Lophozia savicziae,* the maternal parent is *Lophozia* sp., while the paternal parent is *L. silvicoloides. Lophozia savicziae* appears to be a genetically restricted taxon, due to its phylogenetic position and genetic divergence, based on nucleotide data obtained from the isotype specimen and additional three specimens from different localities in Murmansk Region of Russia.

Among leafy liverworts, a hybrid origin has been shown for *Porella baueri* (Boisselier-Dubayle *et al.*, 1998), *Porella platyphylloidea* (Heinrichs *et al*., 2011), *Calypogeia sphagnicola* (Buczkowska *et al*., 2012), *Plagiochila britannica* (Barbulescu *et al*., 2017), *Blepharostoma trichophyllum* (Bakalin *et al*., 2020), and *Lophoziopsis rubrigemma* (Konstantinova *et al*., 2023). The incompleteness of nrDNA concerted evolution was shown for *Barbilophozia rubescens,* which revealed the presence of two parental copies of ITS1-2 in its genome obtained in cloning procedure (Vilnet *et al*., 2012).

Morphologically, *L. savicziae* differs more or less well from *Lophozia silvicoloides* (see results) in leaf and perianth shape. However, the poor knowledge of the morphological variability of both taxa and misunderstanding of their boundaries resulted in many confusing identifications. Moreover, the same specimen could be identified by the same author in different ways in different years. An example is the specimen from the Grøndalen in Svalbard. It was identified as *Lophozia savicziae* and under this name is stored in the herbarium [KPABG(H) 110030], although on the draft label in envelope it is written by N. Konstantinova in brackets *L. silvicoloides,* and in the publications (Konstantinova, 2007; Konstantinova & Savchenko, 2008) this specimen is given as *L. silvicoloides*. It is so in many specimens of *L. silvicoloides* from Russia and Svalbard; these specimens often have red-brown color, which does not agree with the description of *L. silvicoloides* by Kitagawa (1965:276) who described its color as "pale green or pale brown". However, it should be emphasized that in some of the studied specimens with the colored plants almost pure green plants were also found. So, obviously, this feature is not essential for this species. In addition, it should be noted that there is no single specimen from Japan in our molecular sampling, and the species occurs in Japan "on humus under shrubs of *Pinus pumila*" (Kitagawa, 1965), whereas in Svalbard and Russia it occurs mostly on humus covered rocks and in moist moss – low shrub tundra. A comparative integrative study of the specimens from Japan with specimens from Russia and Svalbard is necessary to determine if all these plants belong to one species.

It is somewhat more difficult to characterize the morphological differences of *Lophozia* sp. which is very similar to *L. savicziae* in color and leaf shape. One of its distinguishing features is the size of the leaf cells, which are somewhat smaller in *Lophozia* sp. However, relatively small cells are also found in some specimens of *L. savicziae,* including the isotype. Another distinguishing feature is the width of the postical free leaf zone which is 1–2 cells wide in *Lophozia* sp. but 3–5 cells wide in *L. savicziae*. However, in small plants of *L. savicziae*, the postical free leaf zone may be quite narrow, just 1–2 cells wide. It should be added that none of the studied specimens of *Lophozia* sp. contain perianth and, accordingly, its characters are unknown. Considering all this, we hesitate to describe *Lophozia* sp. as new species based on these three specimens.

Thus, *L. savicziae* differs from both parents, although these differences are often difficult to detect because of the significant variability of almost all the considered characters, in particular, the color of plants, the size of leaf cells and gemmae, and the width of postical free leaf zone.

The difficulties in understanding of *Lophozia savicziae* are also evidenced by its synonymization by K. Damsholt with *Lophozia wenzelii* var. *lapponica* H.Buch et S.W. Arnell (Damsholt, 2002) or *Lophozia ventricosa* var. *grandiretis* (H.Buch & S.W.Arnell) R.M.Schust. (Damsholt, 2013). The obtained results show that *L. savicziae* is closely related neither to *L. ventricosa* nor *L. wenzelii* and cannot be considered as a variety of these species. However, it is important to emphasize that the interpretations of both *L. wenzelii* and *L. ventricosa* complexes are extremely contradictory and require careful study with expanded sampling based on an integrative approach.

The obtained results show that one of three species with biconcentric oil-bodies (*L. savicziae)* is an allopolyploid of the other two (*Lophozia silvicoloides* and *Lophozia* sp.). These three closely related taxa are clearly distinguished from other species of the genus *Lophozia.* Two other taxa of *Lophozia* characterized by biconcentric oil bodies (*L. schusteriana* and *L. silvicola*) were not considered. However, this should be a special study that requires significant efforts, which, however, can lead to interesting results and help in unwinding the tangle of problems associated with this complex.

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Appendix. The list of specimens included in this study with voucher details and GenBank accession numbers, newly obtained accessions are in bold. For some specimens the species names as stored in KPABG are provided in parentheses.

Taxon	*Voucher*	*GenBank accessions #*	
		nr ITS1-2	cp *trn*L-F
***Lophozia** ascendens* (Warnst.) R.M. Schust.	Russia: Buryatia Rep., *Konstantinova 109-3-01* (KPABG-104300)	DQ875089	DQ875054
L. austrosibirica Bakalin	Russia: Buryatia Rep., *Bakalin 15-9-99* (KPABG-109664)	DQ875105	DQ875069
L. dubia Schiffn.	China, *Bakalin C-83-7-18* (VBGI)	OR982393	OR995729
L. fuscovirens Bakalin & Vilnet	Norway: Svalbard, *Savchenko CA16-12-1c* (KPABG-121433)	MK774737	MK779914
L. guttulata (Lindb.) A. Evans	Russia: Buryatia Rep., *Konstantinova 81-1-01* (KPABG-104253)	DQ875108	DQ875072
L. koreana (Bakalin, S.S. Choi & B.Y. Sun) Maltseva, Vilnet & Bakalin	Republic of Korea: Jeollabuk-do, *Bakalin Kor-74-5-19* (VGBI-85004)	MW685441	MW654174
L. lantratovae Bakalin	Russia: Buryatia Rep., *Bakalin 76-7-01* (KPABG-102544)	DQ875090	DQ875055
L. obscura (Bakalin) A.V. Troitsky, Bakalin & Fedosov	Russia: Sakhalin Terr., Kuril Isl., Iturup Isl., *Bakalin K-79-21-15* (VBGI)	MT431697	MT381919
L. savicziae Schljakov	Russia: Murmansk Prov., *Schljakov 4-71* (KPABG-3752), type		
	clone3d	**PV480873**	
	clone6d	**PV480874**	
	clone44	**PV480875**	**PQ767091**
L. savicziae	Russia: Murmansk Prov., *Konstantinova K-25-3-24* (KPABG)		
	clone4d	**PV480876**	
	clone7	**PV480877**	
	clone2	**PV480878**	**PQ767092**
L. savicziae	Russia: Murmansk Prov., *Konstantinova K203-2-07* (KPABG-18034)	**PV485289**	**PV537237**
L. savicziae	Russia: Murmansk Prov., *Konstantinova 65-2-97* (KPABG-6183)	**PV485290**	**PV537238**
L. silvicoloides N. Kitag. (as *L.* cf. *savicziae*)	Norway: Svalbard, *Konstantinova K122-9-06* (KPABG-111833)	**PQ763426**	**PQ767090**
L. silvicoloides (as *L. savicziae*)	Norway: Svalbard, *Konstantinova K130-10-04* (KPABG-110030	No data	**PQ767089**
L. silvicoloides	Norway: Svalbard, *Konstantinova K241-1b-12* (KPABG)	MW298767	MW297148
L. silvicoloides	Norway: Svalbard, *Konstantinova 150-2-04* (KPABG)	DQ875100	DQ875065
L. silvicoloides (as *L. ventricosa* var. *ventricosa*)	Russia: Chukotka A.O., *Afonina 20.08.1970* (KPABG-103125)	**PV485291**	**PV537239**
L. silvicoloides (as *L. savicziae*)	Russia: Chukotka A.O., *Afonina 19.08.1976* (KPABG-106528)	**PV485292**	**PV537240**
L. silvicoloides	Russia: Kamchatka Terr., *Bakalin K-57-23-02-VB* (KPABG-104240)	DQ875098	DQ875063
L. silvicoloides	Russia: Khanty-Mansi Autonomous Area, *Filippov YSU-MH-04195* (YSU, LE)	OP811455	OP821982
L. silvicoloides (as *L. savicziae*)	Russia: Krasnoyarsk Terr., Sibiryakov Isl., *Kuvaev 19.07.1989* (KPABG-109866)	**PV485293**	**PV537241**
L. silvicoloides	Russia: Murmansk Prov., *Konstantinova 356-4-00* (KPABG-8088)	DQ875099	DQ875064
L. svalbardensis Konstant., Vilnet & Mamontov	Norway: Svalbard, *Konstantinova K-135-3a-07* (KPABG-123473)	MW298768	MW297149
L. wenzelii var. *groenlandica* (Nees) Bakalin	Russia: Murmansk Prov., *Bakalin 9329* (KPABG-9329)	DQ875109	DQ875073
L. wenzelii var. *lapponica* H. Buch & S.W. Arnell	Russia: Perm Terr., *Bezgodov AB206-09* (KPABG-122460)	MW298769	MW297150
L. wenzelii var. *lapponica*	Norway: Svalbard, *Konstantinova 124-2-04* (KPABG-123979)	DQ875112	DQ875076
L. wenzelii var. *lapponica*	Russia: Arkhangelsk Prov., Franz Josef Land, Ziegler Isl., *Savchenko*, CA19-29-10a-1 (KPABG)	MT422262	MT431406
L. wenzelii var. *massularioides* Bakalin	Russia: Karachayevo-Circassian Rep., *Onipchenko* 31.08.83 (MHA)	DQ875111	DQ875075
L. sp. (as *L. savicziae*)	Russia: Kamchatka Terr., Commander Isl., Bering Isl., *Bakalin K-16-8-02-VB (*KPABG-103428*)*	**PV485297**	**PV537245**
L. sp. (as *L. savicziae*)	Russia: Buryatia Rep., *Konstantinova 119-2-02* (KPABG-126733)	**PV485296**	**PV537244**
L. sp. (as *L. savicziae*)	Russia: Kamchatka Terr., *Bakalin 73-2-01-VB* (KPABG-103798)	**PV485294**	**PV537242**
L. sp. (as *L. savicziae*)	Russia: Kamchatka Terr., *Bakalin K-39-1-02-VB* (KPABG-104025)	**PV485295**	**PV537243**
***Lophoziopsis** excisa* (Dicks.) Konstant. & Vilnet	Norway: Svalbard, *Savchenko CA364-2a-11* (KPABG-119728)	MW298770	MW297151
L. excisa	Russia: Murmansk Prov., *Konstantinova 41-2-97* (KPABG)	DQ875092	DQ875057
L. jurensis (Meyl. ex Müll. Frib.) Mamontov & Vilnet	Russia: Altai Terr., *Mamontov YuSM-214-8* (KPABG)	MF803150	MF803152
L. longidens (Lindb.) Konstant. & Vilnet	Russia: Murmansk Prov., *Konstantinova K12-4-23* (KPABG-126020)	**PQ763429**	**PQ767093**
L. pellucida (R.M. Schust.) Konstant. & Vilnet	Russia: Trans-Baikal Terr., *Mamontov 356-3-6* (KPABG)	MW298773	MW297154
L. polaris (R.M. Schust.) Konstant. & Vilnet	Norway: Svalbard, *Savchenko CA19-29-3* (KPABG-122714)	MW298774	MW297155
L. polaris	Norway: Svalbard, *Konstantinova & Savchenko K129-07* (KPABG)	MT334459	MT338482
L. polaris	Norway: Svalbard, *Savchenko CA 367-2b-11* (KPABG)	MW298775	MW297156
***Oleolophozia** perssonii* (H. Buch & S.W. Arnell) L. Söderstr., De Roo & Hedd.	Norway: Svalbard, *Konstantinova & Savchenko K3-10* (KPABG-113981)	**PQ763430**	**PQ767094**