Arctoa (2025) **34**: *152–164*
doi: 10.15298/arctoa.34.12

MOLECULAR PHYLOGENETIC STUDY OF THE GENUS *ISOPACHES* WITH DESCRIPTION OF A NEW SPECIES FROM SOUTH AMERICA

МОЛЕКУЛЯРНО-ФИЛОГЕНЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ РОДА *ISOPACHES* С ОПИСАНИЕМ НОВОГО ВИДА ИЗ ЮЖНОЙ АМЕРИКИ

NADEZHDA A. KONSTANTINOVA[1], ANNA A. VILNET[1] & YURIY S. MAMONTOV[2]

НАДЕЖДА А. КОНСТАНТИНОВА[1], АННА А. ВИЛЬНЕТ[1], ЮРИЙ С. МАМОНТОВ[2]

Abstract

This is the first molecular phylogenetic study of the genus *Isopaches* based on a large set of specimens from numerous remote regions of Eurasia and North America. Along with three previously known species, it includes a new species from South America – *I. valdiviensis*. The latter is formally diagnosed, illustrated, and compared to similar taxa, including another southern hemisphere species, *I. pumicicola*. We have shown that the genus *Isopaches* is a group of closely related and slightly divergent taxa and revealed a possible hybrid relation between *I. bicrenatus* and *I. alboviridis*. The main morphological differences of the species including sex allocation, leaf width/length ratio, shape of gemmae etc., are discussed. Overlap in some of these characters in different species is shown. A key to all species of the genus is provided.

Резюме

Впервые проведено молекулярно-филогенетическое исследование рода *Isopaches*, основанное на большом наборе образцов из ряда регионов Евразии и Северной Америки. Наряду с тремя из ранее известных видов, в исследование включен образец, описанный нами как новый вид из Южной Америки – *I. valdiviensis*. Приводятся описание и иллюстрации вида, а также сравнение с другими видами рода, в том числе со вторым южнополушарным видом *I. pumicicola*. Установлено, что род *Isopaches* представляет собой группу близкородственных таксонов, выявлено возможное гибридогенное родство между *I. bicrenatus* и *I. alboviridis*. Обсуждаются основные морфологические различия видов, в том числе распределение полов, соотношение ширины листьев к длине, форма устья периантияи т.д. Показана значительная вариабельность некоторых из этих признаков у трех встречающихся в Голарктике видов. Приводится ключ для определения всех видов рода.

KEYWORDS: liverworts, molecular analysis, morphology, phylogeny, systematics, distribution, hybrid origin, ITS1-2 nrDNA, *trn*L-F, *rbc*L, *psb*A-*trn*H cpDNA

INTRODUCTION

Isopaches H.Buch is the oligotypic genus of leafy liverworts that according to modern treatments (Söderström *et al*., 2016; Hodgetts *et al*., 2020) includes four species, one of which *I. bicrenatus* (Schmidel ex Hoffm.) H.Buch is widespread in the Holarctic with isolated localities in the Southern Hemisphere, particularly in New Zealand and Brazil (Engel & Glenny, 2008). Among the other three species, *I. pumicicola* (Berggr.) Bakalin is an endemic of New Zealand, *I. decolorans* (Limpr.) H.Buch is extremely disjunct, represented on all continents except Australia and Antarctica, and *I. alboviridis* (R.M.Schust.) Schljakov is a poorly known disjunct Holarctic species. The latter was described from Greenland (Schuster, 1969) and recently found scattered in the north of Eurasia (Potemkin, 1990, 1993; Potemkin & Rozantseva 2015; Borovichev, 2008), mountains of South Siberia (Bakalin, 2004) and in Alaska (Potemkin, 1995). In 2019, one of us (YM) collected a specimen of *Cryptochila spegazziniana* (Spruce ex C.Massal.) Grolle, in Chile, where he discovered small plants with gemmae quite characteristic of the genus *Isopaches*, yet with a very different appearance from other species in the genus. Schuster (1995: 247), discussing the taxonomy of the genus (subgenus in his sense) in relation to the discovery of *Lophozia (Isopaches) decolorans* (Limpr.) Steph. in western North America, emphasizes that it "remains poorly known and that species limits in *Isopaches* have remained imprecisely". In terms of *I. alboviridis*, Schuster (1969) noted that it probably is "preponderantly dioecious Arctic subspecies" of *I. bicrenatus*.

Previously, some authors (Schuster, 1969, 1995, 2002; Paton, 1999; Damsholt, 2002; Engel & Glenny, 2008), considered the genus to be a subgenus of the genus *Lo-*

---

1 – Polar-Alpine Botanical Garden-Institute of the Kola Science Center of RAS, Kirovsk, Murmansk Region, 184256, Russia. E-mails: nadya50@list.ru, anya_v@list.ru. ORCID: (NK) 0000-0002-7600-0512; (AV) 0000-0001-7779-2593
2 – Tsitsin Main Botanical Garden of the Russian Academy of Sciences, Moscow, Russia, 127276. E-mail: yur-mamontov@yandex.ru. ORCID: 0000-0003-3851-0738

phozia. Firstly remote phylogenetic position of *I. bicrenatus* from *Lophozia* (Dumort.) Dumort. based on *trn*L-F cpDNA was shown by Yatsentyuk *et al.* (2004) and then again was supported from *trn*G-intron and *rps*4 cpDNA of *I. bicrenatus* and *I. decolorans* by De Roo *et al.* (2007). At present, the genus *Isopaches* is treated in the family Anastrophyllaceae (Söderström *et al.*, 2010, 2016). All former molecular studies (Yatsentyuk *et al*. 2004; De Roo *et al*. 2007; Vilnet *et al*., 2010) focused on phylogeny of suborder Cephaloziineae and included sequences from only a pair specimens of *I. bicrenatus* and *I. decolorans*, whereas *I. alboviridis* has never been tested molecularly and its relation within *Isopaches* stays unclear.

The main goal of this work, along with the description of a new species, was to evaluate morphological and molecular variability of *Isopaches* species based on an integrative study of a wide range of specimens from different geographically remote areas. Along with this, we tried to find out how well the poorly known *I. alboviridis* differs from the widespread *I. bicrenatus.*

MATERIAL AND METHODS

Collections and specimens studied

Specimens of *Isopaches* from KPABG, MHA, LE including previously collected specimen from South America were revised. All specimens stored in the herbarium KPABG that were identified as *I. decolorans* and *I. alboviridis* were studied in detail, and in the majority of the studied specimens of these species some plants were selected for molecular phylogenetic analysis with the exception of a few specimens that did not contain enough material. In addition, the most well-preserved, specimens of *I. bicrenatus* with gemmae, perianths, and sporophytes collected in various regions of Russia were involved in molecular phylogenetic studies. At that, we paid special attention to specimens from high-latitude regions and upper mountain belts, identified as *I. bicrenatus*, it is quite difficult to distinguish *I. bicrenatus* without gametangia from the poorly studied *I. alboviridis.* So, we selected plants for sequencing both from specimens collected in the most characteristic habitats of *I. bicrenatus* in the forest zone, and specimens from habitats near the northern and high-altitude limits of the species' distribution.

Morphological study

The plants of the newly described species were photographed using a Leitz Wetzlar Orthoplan light microscopes equipped with a digital camera, a Nikon D90. In order to better illustrate the three-dimensional objects, photomicrographs were combined using the stacking software HeliconFocus, and some of the obtained images were reconstructed into line drawings.

Sampling for molecular analyses

Twenty seven specimens of three known species of the genus *Isopaches* (*I. alboviridis, I. bicrenatus* and *I. decolorans*) were selected for molecular phylogenetic estimation. The specimen from Chile was tested to check its relation and identity. Follow published studies of Anastrophyllaceae (Vilnet *et al*., 2010; Bakalin *et al*., 2020; Potemkin & Vilnet, 2021) the ITS1-2 nrDNA, *trn*L-F and *rbc*L cpDNA were selected as molecular markers. For several specimens of *Isopaches* spp. the nucleotide sequences of *psb*A-*trn*H cpDNA were obtained. The single *rbc*L accession for *I. bicrenatus* from U.S.A. was downloaded from GenBank. The dataset for phylogenetic estimation additionally includes 15 species from 14 genera of family Anastrophyllaceae. *Obtusifolium obtusum* from the phylogenetically allied family Obtusifoliaceae (Bakalin *et al*., 2021) was selected as an outgroup. The list of specimens for phylogenetic estimation is in Appendix 1.

DNA isolation, PCR amplification and DNA sequencing

For DNA extraction the HiPure SF Plant DNA Kit (Magen, China) was used following the procedure described in the manufacturer's protocol. The pairs of primers for the polymerase chain reaction are listed in Table 1. 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 (ITS1-2), 54°C (*trn*L-F), 52°C (*rbc*L, *psb*A-*trn*H), 60 s 72°C) and 2 min. of final extension time at 72°C. 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).

Phylogenetic analysis

The newly sequenced loci for 32 specimens were assembled in the program BioEdit 7.0.1 (Hall, 1999). The alignments for phylogenetic reconstructions were automatically produced with ClustalW (Thompson *et al*., 1994) and then manually corrected. The ITS1-2 dataset includes

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

DNA marker	Primer sequence (5'-3')	Reference
ITS1-2 nrDNA	Forward ACCTGCGGAAGGATCATTG	Friedl, 1996
ITS1-2 nrDNA	Reverse GATATGCTTAAACTCAGCGG	Milyutina *et al*., 2010
*trn*L–F cpDNA	Forward CGAAATCGGTAGACGCTACG	Taberlet *et al*., 1991
*trn*L–F cpDNA	Reverse ATTTGAACTGGTGACACGAG	Taberlet *et al*., 1991
*rbc*L	Forward ATGTCACCACAAACAGAGACT AAA GC	Kress & Erickson, 2007
*rbc*L	Reverse GTATCTATTGTTTCATATTC	Bakalin *et al*., 2024
*psb*A-*trn*H	Forward GTTATGCATGAACGTAATGCTC	Newmaster & Subramanyam, 2009
*psb*A-*trn*H	Reverse CGCGCATGGTGGATTCACAATCC	Newmaster & Subramanyam, 2009

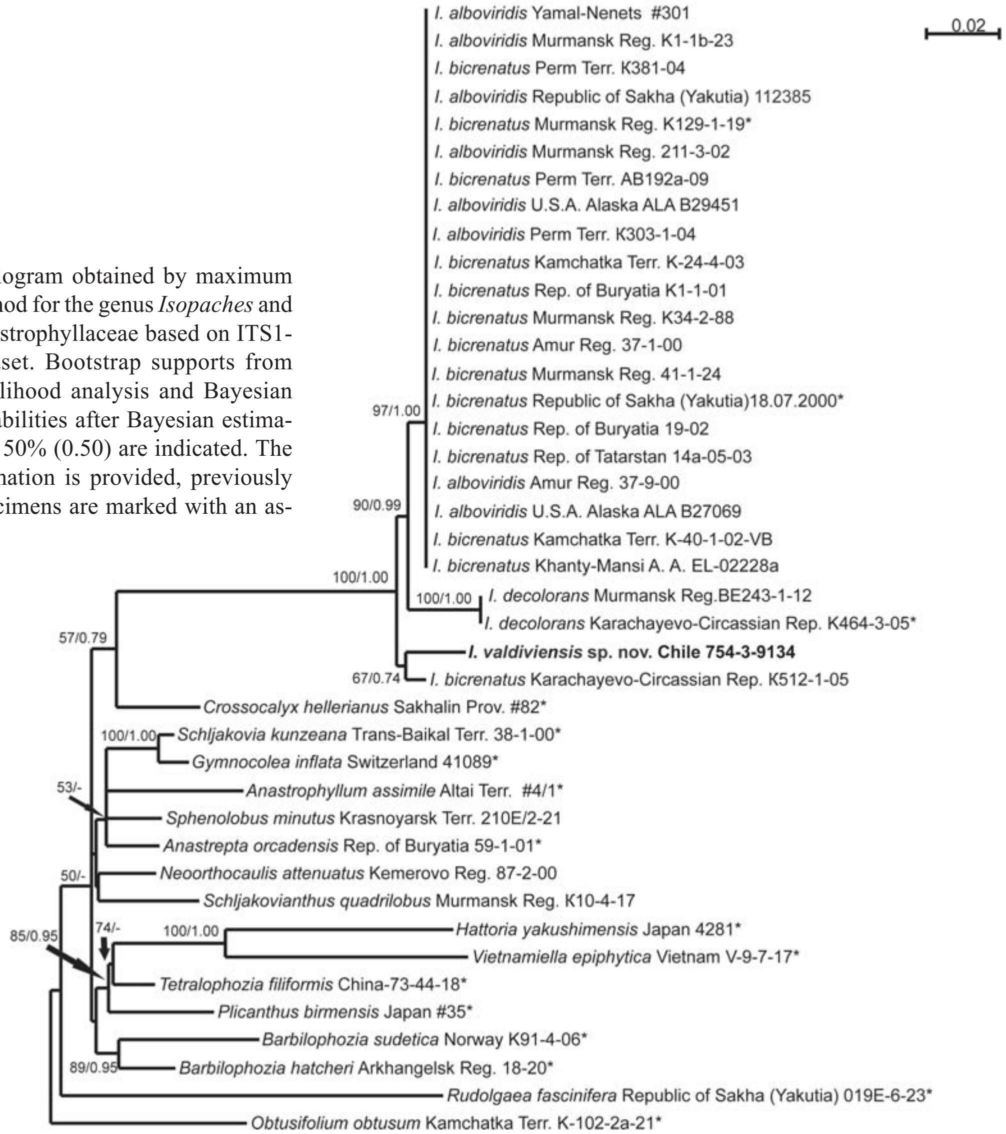


Fig. 1. Phylogram obtained by maximum likelihood method for the genus *Isopaches* and the family Anastrophyllaceae based on ITS1-2 nrDNA dataset. Bootstrap supports from maximum likelihood analysis and Bayesian posterior probabilities after Bayesian estimation more than 50% (0.50) are indicated. The voucher information is provided, previously sequenced specimens are marked with an asterisk.

41 accessions and 871 nucleotide positions, the *trn*L-F dataset – 44 accessions and 479 positions. A lot of accessions from GenBank were selected for the *rbc*L dataset and this alignment includes 22 sequences with 1330 positions. Due to differences in taxa sampling for the *trn*L-F and *rbc*L alignments it was not possible to combine them into single one. The *psb*A-*trn*H accessions for the ingroup are poorly presented in GenBank, thus dataset for this marker was produced only for estimation of sequence variability in *Isopaches*. The *psb*A-*trn*H dataset includes 11 accessions. The preliminary phylogenetic estimation reveals incongruence in ITS1-2 and *trn*L-F topologies, thus the nuclear and chloroplast markers were not able to unite in a single dataset for subsequent analyses.

Two analytical procedures were implemented for molecular phylogenetic study: the maximum likelihood (ML) with the program IQ-TREE (Nguyen *et al*., 2015) and the Bayesian approach (BA) – with MrBayes v. 3.2.1 (Ronquist *et al*., 2012). The program ModelFinder (Kalyaanamoorthy *et al*., 2017) was used to select the best fit evolutionary model of nucleotide substitutions for each dataset: the TN+F+G4 model was suggested for ITS1-2, the TPM3+F+G4 – for *trn*L-F, TN+F+I – for *rbc*L. The suggested model and the ultrafast bootstrapping procedure (Hoang *et al*., 2018) with 1000 replicates were used in ML estimations. For the Bayesian analysis the GTR+I+G model as recommended by the program's creators was implemented for each of three datasets; 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 0.005442, for *trn*L-F dataset – 0.007899, for *rbc*L dataset – 0.005740. The first 2500 (25%) trees were discarded in each run and 15000 trees from both runs were sampled after burn-in for each

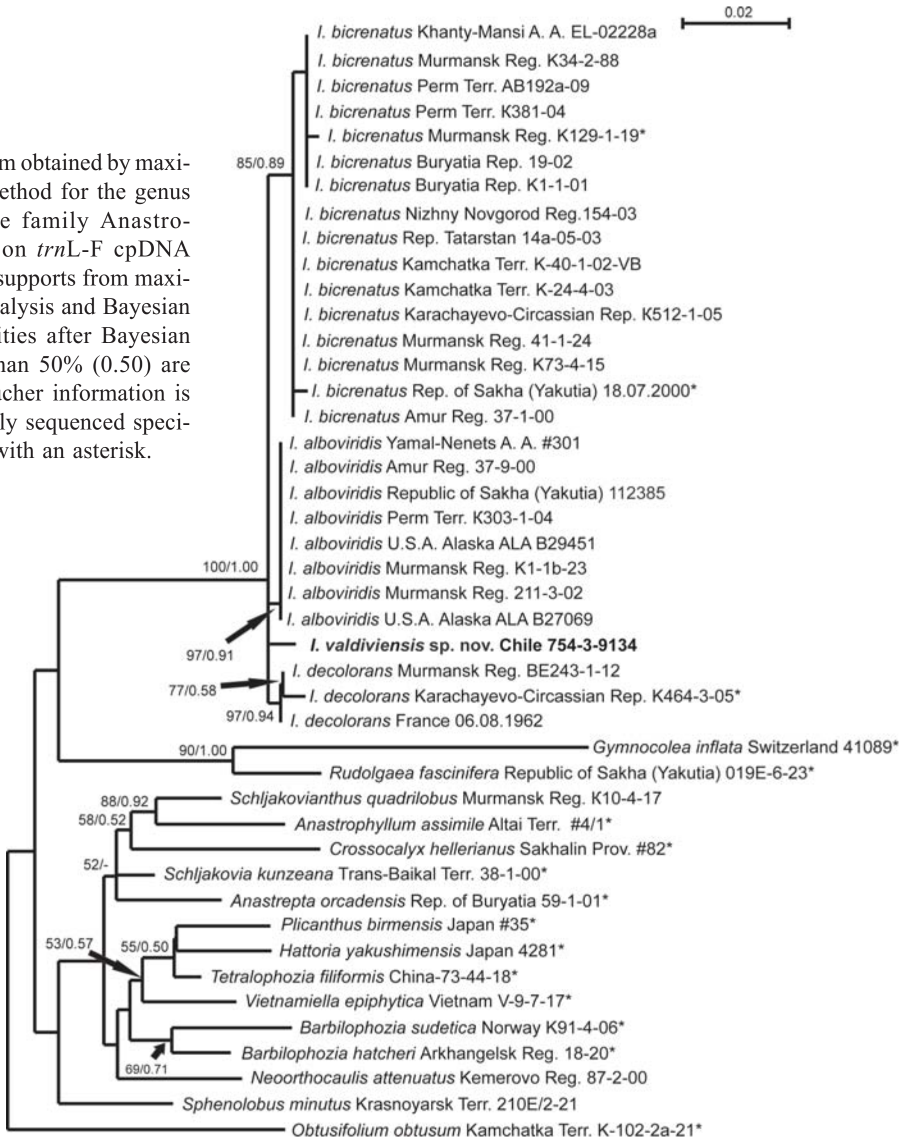


Fig. 2. Phylogram obtained by maximum likelihood method for the genus *Isopaches* and the family Anastrophyllaceae based on *trn*L-F cpDNA dataset. Bootstrap supports from maximum likelihood analysis and Bayesian posterior probabilities after Bayesian estimation more than 50% (0.50) are indicated. The voucher information is provided, previously sequenced specimens are marked with an asterisk.

dataset. Bayesian posterior probabilities were calculated from trees sampled after burn-in. The sequence variability was estimated as the average pairwise *p*-distances for all tested markers in Mega 11 (Tamura *et al*., 2021) using the pairwise deletion option for counting gaps.

RESULTS

Molecular phylogenetic study

The 26 accessions of ITS1-2, 29 – of *trn*L-F, 11 – of *psb*A-*trn*H, 9 - of *rbc*L were newly generated in this study and deposited into GenBank. 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 2.

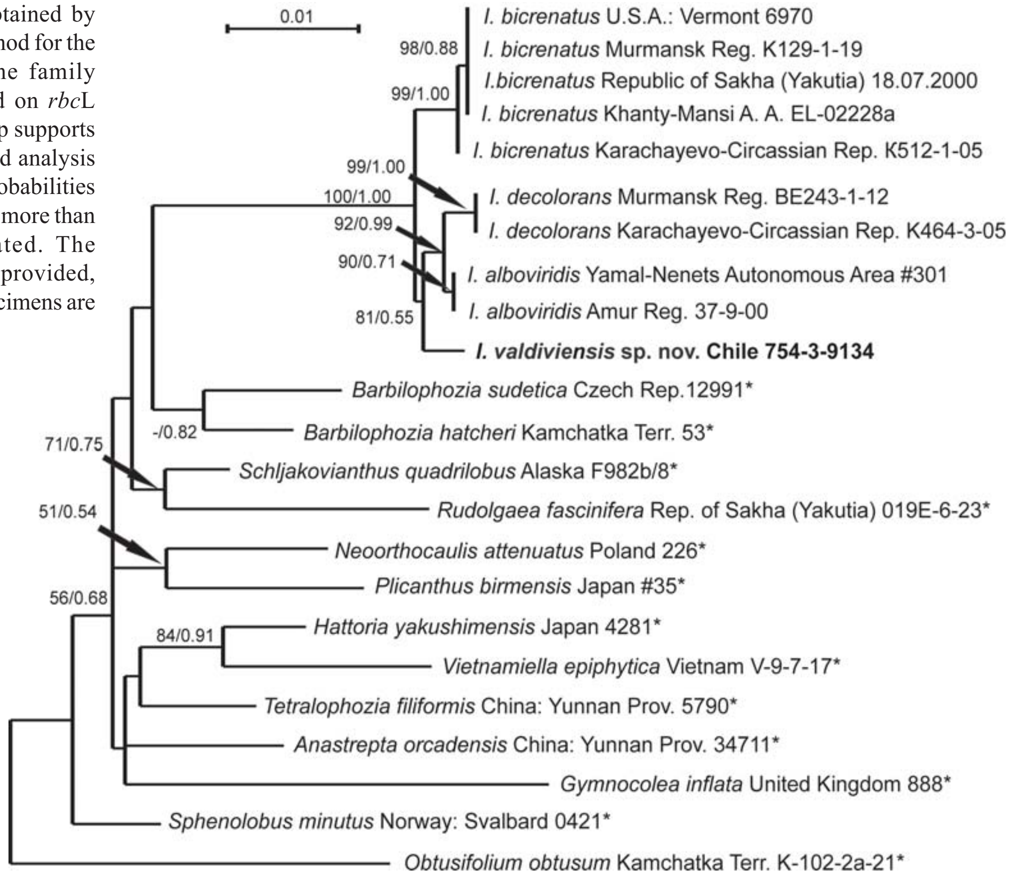
Topologies of trees constructed by different approaches for each of datasets became similar, thus on Figs. 1–3 the ML topologies achieved for ITS1-2, *trn*L-F and *rbc*L are presented with indication of bootstrap support (BS) values from ML calculation and Bayesian posterior probabilities (PP) from BA.

The specimens of the genus *Isopaches* composed a robustly supported (BS=100%, PP=1.00 or 100/1.00) terminal clade in all trees. Its affinity in Anastrophyllaceae as well as relation among other genera of family stays unclear, thus was repeatedly suggested in a number of previous studies (Vilnet *et al*., 2010; Bakalin *et al*., 2020; Potemkin & Vilnet, 2021). Eight specimen of *I. alboviridis* and 13 specimens of *I. bicrenatus* composed a grade in ITS1-2 (97/1.00) (Fig. 1). The subclade (100/1.00) of two *I. decolorans* specimens is sister related to this grade (90/0.99). The basal position in *Isopaches* belongs to the poorly supported subclade (67/0.74) composed by the specimen of *I. bicrenatus* from the Caucasus (*K512-1-05*) and the specimen of *Isopaches* from Chile (*Mamontov 754-3-9134*). From *trn*L-F data (Fig. 2), all three *Iso-*

Table 2. The arithmetic means of Log likelihood obtained in molecular phylogenetic estimation.

Dataset	ML	BA 1st run	BA 2nd run
ITS1-2	-3697.180	-3736.51	-3737.64
*trn*L-F	-1656.840	-1702.18	-1700.03
*rbc*L	-3187.370	-3202.27	-3204.24

Fig. 3. Phylogram obtained by maximum likelihood method for the genus *Isopaches* and the family Anastrophyllaceae based on *rbc*L cpDNA dataset. Bootstrap supports from maximum likelihood analysis and Bayesian posterior probabilities after Bayesian estimation more than 50% (0.50) are indicated. The voucher information is provided, previously sequenced specimens are marked with an asterisk.



paches species are located in the supported subclades – *I. bicrenatus* (85/0.89), *I. alboviridis* (97/0.91) and *I. decolorans* (97/0.94), but relation among them and the position of the specimen from Chile stay unclear due to polytomy. The Caucasian specimen of *I. bicrenatus* (*K512-1-05*) is located in the *I. bicrenatus*-subclade. The *rbc*L topology (Fig. 3) resolved sister relation (92/0.99) between *I. alboviridis* (90/0.71) and *I. decolorans* (99/1.00) subclades and poorly supported affinity (81/0.55) of Chile's specimen to them. The specimen of *I. bicrenatus K512-1-05* from Caucasus is placed in the base of *I. bicrenatus*-subclade with robust support (99/1.00).

Isopaches bicrenatus (with the exception of the specimen *K512-1-05*) and *I. alboviridis* are characterized by low values of infraspecific *p*-distances, which equal 0% in highly variable spacer *psb*A-*trn*H or in highly conservative gene *rbc*L (Table 3). The ITS1-2 and *trn*L-F *p*-distances vary 0–0.2% in both species. A significant similarity between *I. bicrenatus* and *I. alboviridis* was found in the studied nuclear marker, that is, 0.1% in ITS1-2. By contrast, the chloroplast markers are greatly different: the *p*-distance is 1.2% in *trn*L-F, 0.9% – in *psb*A-*trn*H, and 0.7% – in *rbc*L. The position of *I. bicrenatus* and *I. alboviridis* on the phylogenetic trees and the level of *p*-distances between them could suggest a hybrid relation, the phenomenon that is known for a number of liverwort species (Sawangproh & Cronberg, 2021). *Isopaches decolorans* is clearly distinct from *I. bicrenatus* and *I. alboviridis* by *p*-distances calculated for all tested DNA loci. The specimen of *I. bicrenatus* from the Caucasus (*K512-1-05*) and the specimen *I. decolorans* from France provided remarkable variability within their corresponding species. The first specimen is characterized by significant divergence in ITS1-2 to both *I. bicrenatus* and *I. alboviridis,* and the second – by high divergence in *psb*A-*trn*H to *I. decolorans*. The specimen from Chile also demonstrates significant values of *p*-distances to three *Isopaches* species in all molecular markers. Together with its distinct position on the obtained trees, it suggested that this specimen is a new species.

Morphological study

The specimen from Chile (*Mamontov 754-3-9134*) contains a few tiny plants scattered on sandy soil at the base or between large shoots of *Cryptochila spegazziniana*. At first glance, very small, thin and loosely foliated shoots do not look like compact plants of the genus *Isopaches*. However, the stem anatomy, the shape of the gemmae abundantly present at the tips of the shoots, as well as the characteristic thickening of the cell walls, indicated that the plants belong to the genus *Isopaches*. Despite the scarcity of the material and the absence of perianths we considered it appropriate to describe a new species based on the its phylogenetic position, the extremely distinctive appearance (particularly spreading leaves, which are not imbricate and long decurrent on the ventral side), and the geographical isolation.

TAXONOMY

Isopaches valdiviensis Mamontov, Vilnet & Konstant., species nova. Fig. 4–6.

TYPE: Chile, Los Ríos Region, Valdivia Province, Alerce Costero National Park, sclerophyllous shrubs with mosses along brook bank, 40°10'12.6"S, 73°27'16.7"W, 750 m a.s.l., on soil near road, 14.VI.2019, *Mamontov 754-3-9134* (KPABG127751, holotype).

Diagnosis: Differs from other species of the genus in the distant, antically secund and long decurrent on dorsal side leaves, and in ITS1-2 nrDNA, *trn*L–F, *psb*A-*trn*H and *rbc*L cpDNA sequences.

Etymology: the name corresponds to Valdivia Province, the part of the Los Ríos Region of Chile where the species has been found.

Description. Plants pale green, rather pellucid, upper parts of leaves sometimes light brown, very small, shoots 0.4–0.5 mm wide and up to 2 mm long. Stem 110–120 µm wide. Rhizoids scattered, colorless, 15 µm thick, rather long. Stem in cross section 7–8 cell thick, stem cells (in cross section) not differentiated, mostly isodiametric, 20–25 µm, or slightly elongated, 20 × 25 µm, mixed with single smaller cells just 15–18 µm, thin-walled, with minute trigones. Cells on the ventral side of shoots slightly elongated, 15–20 (–22) µm wide and (20–)25–37 µm long. Leaves distant, antically secund and long decurrent on dorsal side, asymmetrical (uneven?), trapezoidal-ovate, somewhat narrowed in the apex, with a width slightly exceeding the length, 300–325 µm wide and 250–300 µm long, widest near the base, divided up to 0.1–0.2 their length into two slightly uneven, obtuse to blunt-pointed lobes; sinus wide, obtuse, V- or U-shaped. Midleaf cells 20–25×22–27(–30) µm, mixed with single small isodiametric cells just 15×15 µm, slightly thick-walled, with very small trigones, cells of the base do not distinctly differ from the midleaf cells, cells of margins slightly smaller and mostly isodiametric, ca. 18–20(–22) µm. Cuticle mostly distinctly striolate. Gemmiparous leaves with cells elongated in upper part, shoots with prolonged gemmae formation, immature gemmae colorless, angular-ovoid, ripe gemmae red-brown, angulate- stellate, 2-celled, (15–)17–20 µm. Dioicous. Only one antheridium was found, antheridal stalk 1-seriate. Otherwise unknown.

Differentiation. *I. valdiviensis* differs from all other species of the genus in distant, antically secund and long decurrent on dorsal side leaves. By contrast, leaves are imbricate and more or less subtransversely inserted in all other species of the genus (except *I. pumicicola*). In *I. pumicicola*, another species found only in the Southern hemisphere, the leaves are strongly succubously inserted, but are longer than wide or as long as wide, strongly concave, "ovate, often rather broadly so" (Engel & Glenny, 2008). Moreover, this species is paroicous and lacks asexual reproduction (no gemmae have been found, although the species is known from several stations in New Zealand). By contrast, the leaves are wider than long in *I. valdiviensis* and more or less flat, while the species probably is dioicous (only antheridial plants have been found) and has asexual reproduction (several plants in the specimen bear abundant gemmae). In the key below we provide the most reliable taxonomic characters for distinguishing of all species of the genus *Isopaches*.

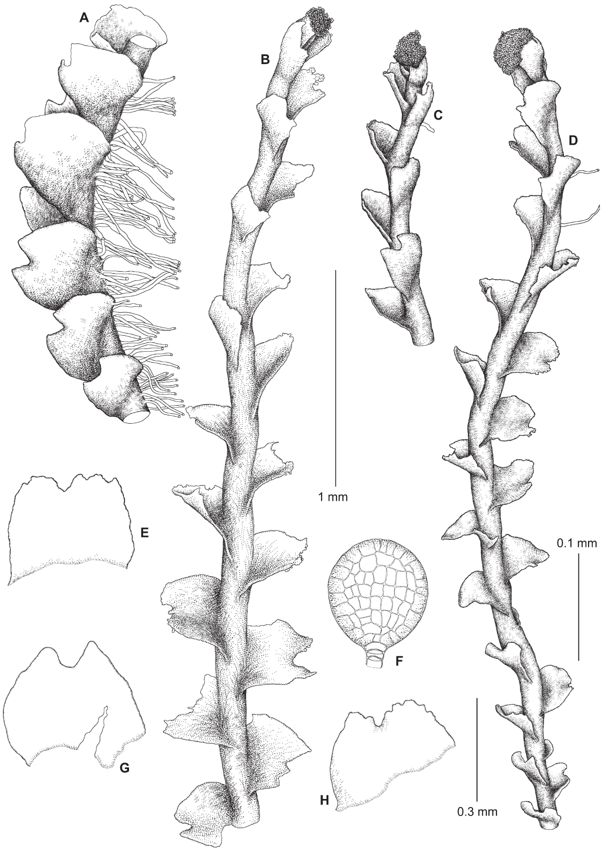


Fig. 4. *Isopaches valdiviensis* (from *Mamontov 754-3-9134*, KPABG): A – androecial shoot. B–D – sterile shoots. E, H – leaves. F – antheridium. G – androecial bract. Scale bars: 0.1 mm for F; 0.3 mm for E, G, H; 1 mm for A–D.

Table 3. The value of infra- and interspecific *p*-distances of ITS/*trn*L-F/*psb*A-*trn*H/*rbc*L, % for the genus *Isopaches*, n/c – non calculated (single specimen).

Taxon	Infraspecific variability	Interspecific variability			
		I. alboviridis	*I. bicrenatus*	*I. b.* K512-1-05	*I. decolorans*
I. alboviridis	0.2/0.1/0.0/0.0				
I. bicrenatus	0.1/0.2/0.0/0.0	0.1/1.2/0.9/0.7			
I. bicrenatus K512-1-05	n/c/ n/c/n/c/n/c	1.6/1.1/1.3/0.6	1.5/0.2/1.8/0.1		
I. decolorans	0.0/0.2/1.2/0.0	2.3/1.2/1.2/0.3	2.2/1.1/1.7/0.8	2.7/1.0/2.0/0.7	
I. valdiviensis	n/c/n/c/n/c/n/c	2.3/0.9/0.9/0.5	2.3/0.8/1.9/0.8	2.0/0.7/2.3/0.7	3.5/0.8/1.9/0.7

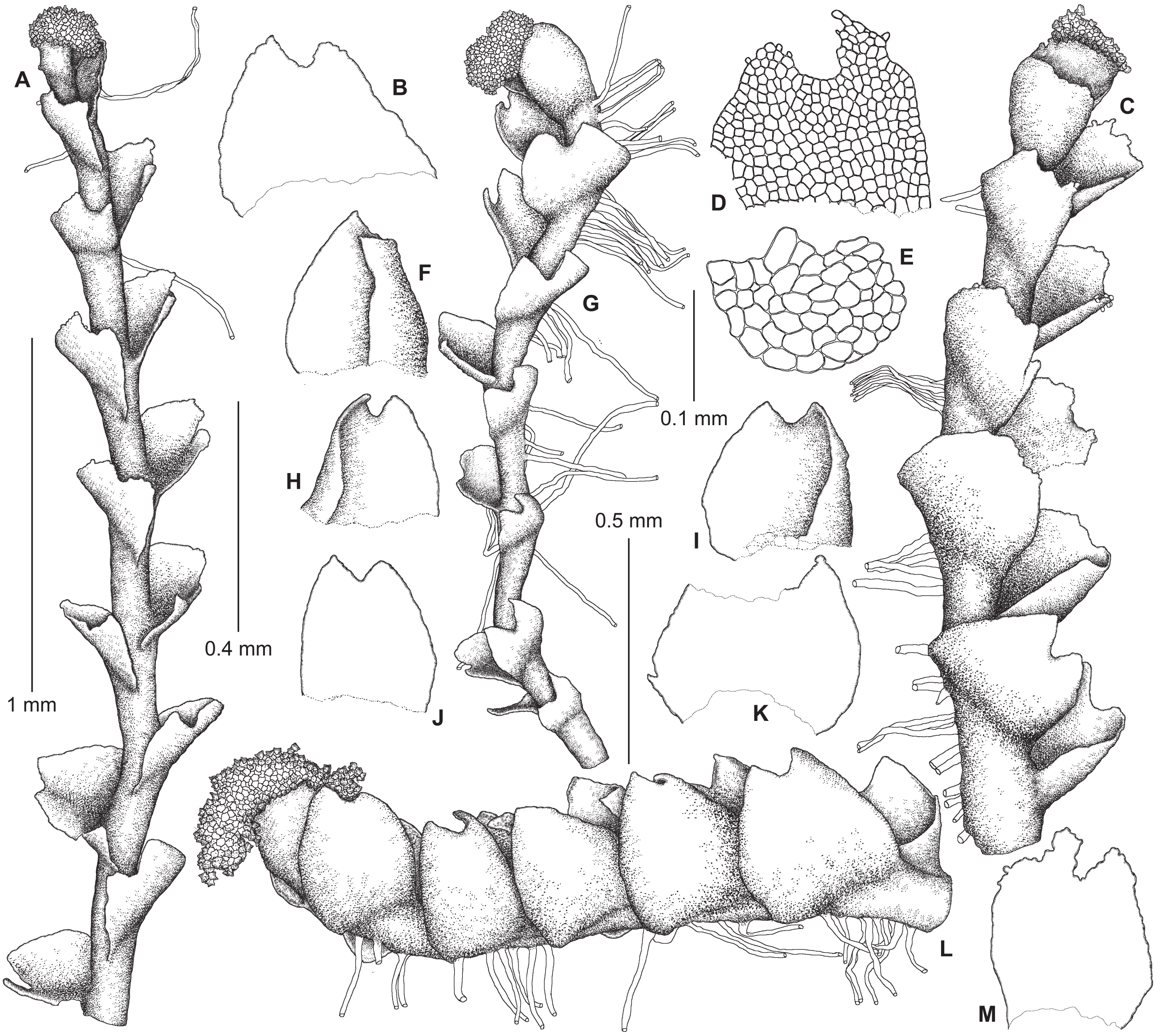


Fig. 5. *Isopaches valdiviensis* (A–M from *Mamontov 754-3-9134*, KPABG): A, G – sterile shoots. C, L – androecial shoots. B, D, J, K, M – leaves. E – stem cross section. F, H, I – androecial bracts. Scale bars: 0.1 mm for E. 0.4 mm for B, D, K, M. 0.5 mm for C, F, H–J, L. 1 mm for A, G.

Key to species of Isopaches

1. Plants without gemmae, leaves longer than wide, narrowed in the apex, lobes juxtaposed, sinus often sharply V-shaped, female bracts 2–3-lobed, lobes entire or with 1–2 teeth .... *I. pumicicola* (New Zealand)

— Plants always with more or less abundant gemmae, leaves wider than long or as wide as long, sinus crescentic, obtuse or rectangular 2

2. Dioicous, leaves distant, antically secund and long decurrent on dorsal side, asymmetrical (uneven?) trapezoidal .... *I. valdiviensis* (South America)

— Paroicous, heteroicous or without gametangia, mostly dense leaved, leaves more or less transversely inserted, concave, imbricate at least in upper part 3

3. Plants closely dense-leaved, leaves wider than long with width/length ratio to 1.25, very shallowly divided by crescentic sinus up to 0.2 leaf length, gemmae mostly bright red, 4–5-angled, irregularly polygonal to rectangular and triangular, rarely ellipsoidal or stellate and with not so strongly projected angles, mostly dioicous, but sometimes heteroicous or paroicous .... *I. decolorans*

— Plants with leaves as wide as long, slightly wider than long or slightly longer than wide, divided up to 0.25–0.4 leaf length by U- or V-shaped but not crescentic sinus; gemmae mostly distinctly stellate, sometimes with admixture of triangular, with strongly projecting angles 4

4. Plants paroicous, dioicous, or heteroicous, or without gametangia, leaf lobes mostly acute to apiculate, ending in (1)2, rarely 3–4 superposed cells with often (but not always) elongated apical cell, sinus usually angular; female bracts not dentate or with single teeth .... *I. alboviridis*

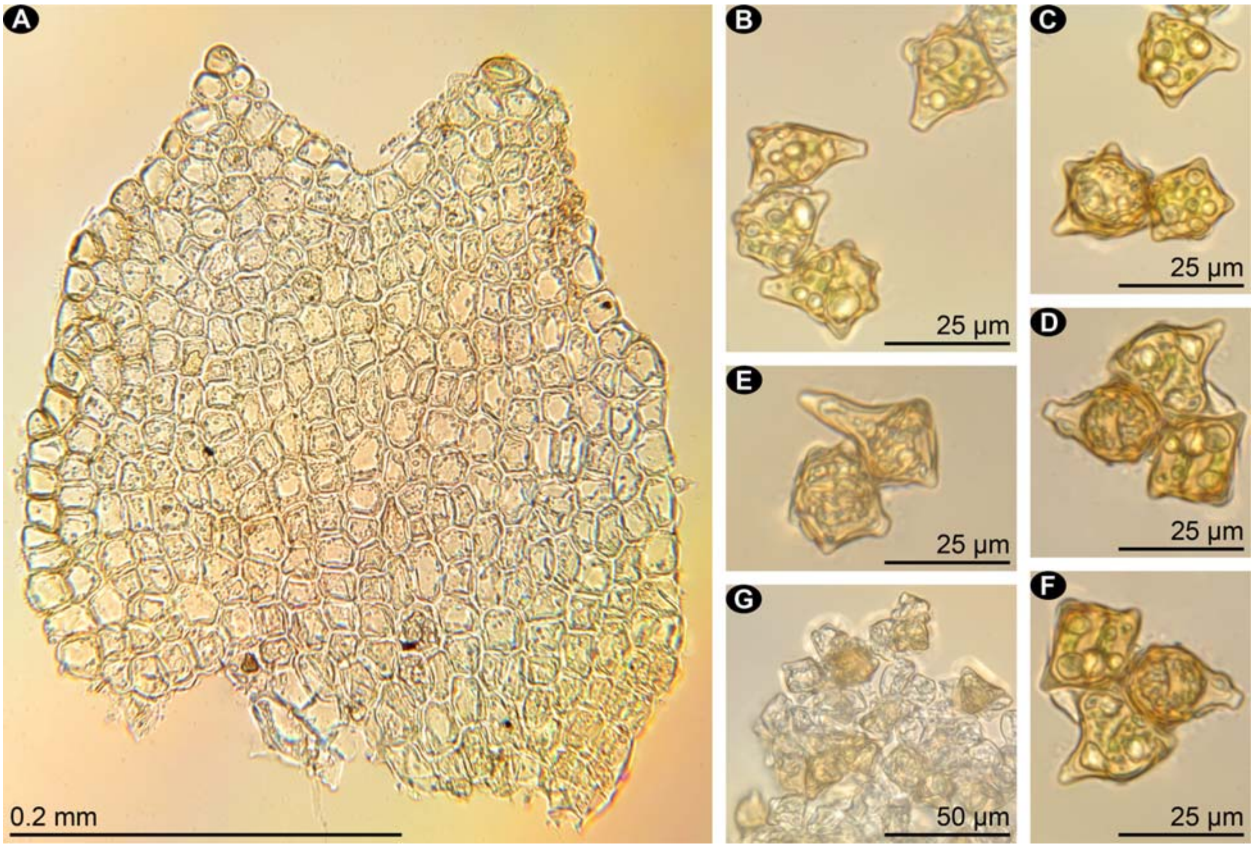


Fig. 6. *Isopaches valdiviensis* (A–G from *Mamontov 754-3-9134*, KPABG): A – leaf. B–G – gemmae.

— Plants mostly paroicous, almost always with perianths, leaf lobes mostly subacute or acute, ending in one-celled apex, apical cells not or slightly elongated, sinus obtuse or rectangular, perianth mouth usually lobulate-dentate with teeth of 2–3 distinctly elongated cells or 4-celled of less elongated cells; antheridial stalk 1–2(–3)-seriate, female bracts mostly coarsely dentate .... *I. bicrenatus*

DISCUSSION

Variation

One of the goals of our study was to clarify the differences between the species of the genus, and we will discuss the variability of the main morphological diagnostic features within the *Isopaches* below. The two southern hemisphere species were discussed above, and here we will focus primarily on the variability of the remaining three species. The values of the main morphological features are shown in Table 4. The table is based on our own observations, and some literature sources (Potemkin, 1993; Schuster, 1995).

We were able to study only three specimens of rare species *I. decolorans*: from Karachayevo-Circassian Republic, Murmansk Region, and the Alps (from where the species has been described). Phylogenetically, the tested specimens of *I. decolorans* are clearly separated from other species of the genus *Isopaches* by nuclear and chloroplast markers. Specimens from Russia possess similar nucleotide sequences of studied loci, but specimen from Alps is similar to them in *trn*L-F and differ in *psb*A-*trn*H (1.2%), that could suggest the haplotype diversity across the species area. Morphologically, the sequenced specimens of *I. decolorans* from the Karachayevo-Circassian Republic (Caucasus) and Murmansk Region matched the species description very well and differ more or less clearly from two other Holarctic species in the julaceous appearance, strongly concave to hemispherical, densely imbricate leaves with shallow crescentic sinus, red-colored and not stellate gemmae.

It is much more difficult to distinguish *I. alboviridis* and *I. bicrenatus.* Both species appear to be not clearly distinct phylogenetically due to the position of the tested specimens in the one grade on the ITS1-2 tree (Fig. 1) and a high similarity of nucleotide sequences, which corresponds with level of infraspecific variability in both species (Table 3). However, the divergence between these species in the chloroplast markers is higher and corresponds with level of interspecific differences in this genus, which was resolved in the separation of two clades on the trees (Figs. 2, 3). The obtained incongruence could suggest hybrid events in the evolution of the genus *Isopaches*. We did not registered the second parental copy of ITS1-2 in the chromatograms of both species and did not use the cloning procedure to detect copies as it was done for the phylogenetically allied genus *Barbilophozia* (Vilnet *et al*., 2012). The data of chromosome numbers for the genus *Isopaches* are unknown. We could assume two hypotheses to explain obtained relation of both species. The first one consists in their origin from one male parent through hybridization with different female parents taking into account maternal inheritance of cytoplasmic organelle in liverworts (Natcheva & Cronberg, 2007). The second hypothesis is in that one species as a male parent of other with completed concerted evolution of nuclear genome in descendant. In both hypotheses female parents stay unknown, possibly extinct, in spite of relatively young age of the genus – 28 Ma (Laenen *et al.*, 2014). Infraspecific variability registered in the specimens of *I. bicrenatus* from Karachayevo-Circassian Republic (*K512-1-05*) and *I. decolorans* from France points to the possible presence of significant molecular genetic divergence within the genus, which may be revealed in future studies. A possible parental species may be also found in such studies, as in the case of the poorly known subarctic species *Lophozia savicziae* Schljakov (Konstantinova & Vilnet, 2025).

The morphology of *I. bicrenatus* and *I. alboviridis* was repeatedly considered in detail by Potemkin (1990, 1992) and Schuster (1969, 1988, 1995). Both authors note that *I. alboviridis* is a very polymorphic species and discussed its variability in Greenland (Schuster 1988, 1995, Schuster & Damsholt, 1974) and North Asia (Potemkin, 1990, 1992). The study of the morphology of the specimens included in the molecular phylogenetic study revealed significant variability in diagnostic features and as result numerous misidentifications of species. Therefore, we discuss these differences in more detail below.

We found that at least a number of morphological features that were considered diagnostic for the *Isopaches* species in previous studies should be accepted with significant reservations, or do not work at all (see Table 4). Above all this applies to sex allocation. Damsholt (2002) reported *I. decolorans* as dioicous, though it was described as paroicous (Limpricht, 1879). Potemkin (1990, 1992) discovered paroicous plants of *I. decolorans* in Yamal Peninsula. *I. alboviridis* has been described as dioicous (Schuster, 1969), however, a few years later Schuster & Damsholt (1974: 85) noted that the species "may be sporadically paroecious". One of the specimens of *I. alboviridis* we studied and sequenced (*K1-1b-23*, Murmansk Region) turned out to be paroicous. There were no developed perianths, but some plants have gynoecia on the tops of shoots with androecia. The rest of the studied specimens of *I. alboviridis* were either sterile or dioicous. In addition, it should be noted that in the specimens of *I. bicrenatus*, along with paroicous plants with perianths there are antheridial plants with no traces of gynoecia. Gemmae are found only on these antheridial plants, while the perianthous plants usually do not bear gemmae.

One more character mentioned in the description of *I. alboviridis* (Schuster, 1969), the uneven lobes of leaves is very variable in all three discussed species. In all of them, the lobes are not equal to a greater or lesser extent, and the ventral side of leaf is larger and more convex than dorsal one. This is especially pronounced in bracts. In terms of the ratio of width to length of the leaf, *I. decolorans* differs well from the other two, since its leaves are very wide, whereas the other two species have leaves approximately equal in width to length or slightly more or slightly less (Table 4). Such a feature as the shape of apex of the leaves seems to be unreliable. Indeed, the leaf lobe apex in *I. alboviridis* was described as ending in (1–)2–3 (–4) superposed elongated cells in contrast to *I. bicrenatus* with acute and subacute lobes, or *I. decolorans* with lobes ending in one or infrequently two superposed not elongated cells. However, firstly, this feature is often impossible to see because the apex of the upper leaves is often discolored or destroyed by the gemma formation. Secondly, most of the leaves in the studied specimens of the *I. alboviridis* clade end in one or rarely two superposed cells.

Our data also do not confirm such a character as smaller cells of the leaf margins (compared to the midleaf cells) in *I. alboviridis*, in contrast to those that do not differ in size from the midleaf cells in *I. bicrenatus*. This character is mentioned both Potemkin (1993) and Schuster (1995). Study of the specimens from the clade of *I. bicrenatus* shows that marginal cells of leaves are mostly slightly smaller than midleaf cells. This is not always easy to detect due to the fact that the leaves in *I. bicrenatus* plants often are strongly concave and the leaf margin is difficult to place flat on the slide.

Another character by which the discussed species differ from each other is the perianth mouth. Schuster (1969) described the perianth mouth in *I. bicrenatus* as "lobulate-ciliate, the longer teeth 3-4 celled" in contrast to *I. alboviridis* in which the perianth mouth is feebly lobulate, sharply spinose-dentate to shortly ciliate-dentate, with teeth 1–2(–3) cells long. However, the perianth mouth in *I. bicrenatus* is not always strongly serrated, and some specimens have perianth mouths with scattered 1–2-celled teeth. Moreover, in the specimens from Central America, Bakalin (2008) describes plants with crenulate perianth mouth.

In general, the identification of species of the genus presents significant difficulties. This is especially true for *I. alboviridis* and *I. bicrenatus* whose hybrid relation have been discovered. Therefore, for the correct identification of the species it is necessary to consider a set of features in their interrelation, which was emphasized by the authors who studied the genus (Potemkin, 1993; Schuster, 1995).

Ecology

Schuster (1995) and Potemkin (1993) point out differences in the ecology of the *Isopaches* species, suggesting that the *I. bicrenatus* occurs on acidic substrates, while *I. alboviridis* is *Ca*-tolerant species. Later (Schuster,

Table 4. Distinctions between *I. bicrenatus, I. alboviridis*, and *I. decolorans*.

Character	*I. bicrenatus*	*I. alboviridis*	*I. decolorans*
Appearance	compact, leaves imbricate, more or less dense leaved especially in upper part of shoot, fresh plants odorous	leaves imbricate, more or less dense leaved specially in upper part of shoot, fresh plants not odorous	julaceous, closely densely leaved, looks like *Prasanthus* Lindb., fresh plants not odorous
Color of shoots	from almost clear green to dark or bright red-brown sometimes with vinaceous basal ventral part of leaves	whitish-green to golden-brown and dark light and dark brown and dark red brown	shiny golden yellow and grassy green
Sex allocation	Paroicous, mostly fertile, but often mixed with male gemmiparous plants	Dioicous, heteroicous, paroicous, often sterile or with androecia, proterandry often occurs	Dioicous or more rare paroicous
Leaf width/length ratio	0.95–1.2/1	ca. as wide as long	1.2–1.3/1
Leaf shape	concave	concave	concave to hemispherical
Leaf lobes	wider than long	longer or as wide as long	much wider than long
Apex of lobes	blunt (obtuse) to acute ending in one not or slightly elongated cells	cuspidate, ending in 1–2(–3) superposed cells with upper (or single) mostly (but not always) elongated cell, apical part often pellucid or destroyed	acute to apiculate
Sinus of leaves	obtuse to rectangular, 0.2–0.3 leaf length	V-U shaped, 0.25–0.4 leaf length	crescentic, 0.1–0.15(–0.2) leaf length
Marginal leaf cells	Usually slightly smaller than , inner, 17–20 µm measured along margin	in one-two rows along margin smaller than inner cells, ca. (11–)13–18(–20) µm measured along margin	
Gemmae	yellow to red brown, mostly stellate,	light brown, uniformly stellate with long but sometimes polygonal,	20–25 µm, two-celled, with thickened angles thickened angles, 18–25 ×
dark bright red-brown,	with thickened angles	(15–)17–20(–25) µm	4–5-angled, variable four-pentagonal, rarely ellipsoidal or stellate, with more or less thickened angles
Male bracts	in 2–4 pairs, distinctly unevenly bilobed, sometimes with an additional tooth	in 2–4(–7) pairs, larger than leaves, imbricate, concave, subequally bilobed or 3-lobed with small third lobes or teeth	larger than leaves, each with a conspicuous antical tooth
Female bracts	bilobed or with additional lobes coarsely dentate with teeth 1–3 cells long or with scattered 1-celled teeth or almost without teeth	2–3(–4)-lobed, coarsely dentate with variable teeth ending in 1–3(–4) uperposed cells, or with scattered teeth	2–3-lobed, much larger than leaves, with few or no teeth
Perianth mouth	numerous to scattered 1–3-celled teeth, the upper of which is cuspidate (a single 4-celled teeth occur)	shallowly lobulate, spinose-dentate with teeth to 1-2(3) cells long	lobulate with scattered teeth often 2–3-celled at base
Antheridial stalk	very short, 1–2(–3)-seriate	very short, 1-seriate	very short, 1-seriate

1988: 84) assumed that *I. alboviridis* "largely replaces *L. bicrenata* in non-acidic sites". This is indirectly confirmed by the records of the species in the Murmansk region, where it was found in areas with a predominance of basic rocks.

FUNDING

The work was carried out with the support of a grant from the Russian Science Foundation, No. 25-24-20015.

ACKNOWLEDGMENTS

We sincerely thank A. Hagborg for useful comments and linguistic corrections. We are also grateful to A.D. Potemkin, the curator of the Hepaticae part of the bryophyte division of LE herbarium for providing a number of specimens of *I. alboviridis* and *I. decolorans* for molecular phylogenetical study. The work with the collections was carried out within the framework of the State Project of the Polar-Alpine Botanical Garden and Institute using the large-scale research facilities at the Herbarium of the Polar-Alpine Botanical Garden and Institute (KPABG), reg. No. 499394. The study by Yu.S. Mamontov was performed within the State assignment (Nos. 122042500074-5 and 122042700002-6).

LITERATURE CITED

BAKALIN, V.A. 2004(2005). Hepatics of Stanovoye Nagor'e Uplands (Eastern Siberia). – *Arctoa* **13**: *229–240*.

BAKALIN, V., A. VILNET & V.S. NGUYEN. 2020. *Vietnamiella epiphytica* – a new genus and species of Anastrophyllaceae (Hepaticae). – *The Bryologist* **123**(1): *48–63*. https://doi.org/10.1639/0007-2745-123.1.048

BAKALIN, V.A. 2008. New data on distribution and taxonomy of some species in Lophoziaceae (Hepaticae). – *Arctoa* **17**: *161–164*. doi: 10.15298/arctoa.17.12

BAKALIN, V.A., V.E. FEDOSOV, A.V. FEDOROVA & W.-Z. MA. 2021. Obtusifoliaceae, a new family of leafy liverworts to accommodate *Konstantinovia*, newly described from the Hengduan Mts. (South China) and *Obtusifolium* (Cephaloziineae, Marchantiophyta). – *Plant Systematics and Evolution* **307**: 62. https://doi.org/10.1007/s00606-021-01779-8

BAKALIN, V.A., V.E. FEDOSOV, K.G. KLIMOVA, Y.D. ALTSEVA, A.V. FEDOROVA & S.S. CHOI. 2024. *Konstantinovia* is not monotypic, and a new attempt to determine relationships in Cephaloziellaceae-Scapaniaceae Superclade (Marchantiophyta). – *Plants* **13**: *15*. https://doi.org/10.3390/plants13010015

BOROVICHEV, E.A. 2008. New liverwort records from Murmansk Province. 1. – *Arctoa* **17**: *191*. https://doi.org/10.15298/arctoa.17.16.

DAMSHOLT, K. 2002. Illustrated Flora of Nordic Liverworts and Hornworts. – *Lund, Nordic Bryological Society, 840 pp.*

DE ROO, R.T., T.A. HEDDERSON & L. SÖDERSTRÖM. 2007. Molecular insights into the phylogeny of the leafy liverwort family Lophoziaceae Cavers. – *Taxon* **56**: *310-314*.

ENGEL, J.J. & D. GLENNY. 2008. A Flora of the Liverworts and Hornworts of New Zealand. Vol. 1. – *Missouri, Missouri Botanical Garden Press*, *897 pp*.

FRIEDL, T. 1996. Evolution of the polyphyletic genus *Pleurastrum* (Chlorophyta): inferences from nuclear-encoded ribosomal DNA sequences and motile cell ultrastructure. – *Phycologia* **35**: *456–469*. doi: 10.2216/i0031-8884-35-5-456.1

HOANG, D.T., O. CHERNOMOR, A. VON HAESELER, B.Q. MINH & L.S.VINH. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. – *Molecular Biology and Evolution* **35**: *518–522*. https://doi.org/10.1093/molbev/msx281

HODGETTS, N.G., L. SÖDERSTRÖM, T.L. BLOCKEEL, S. CASPARI, M.S. IGNATOV, N.A. KONSTANTINOVA, N. LOCKHART et al. 2020. An annotated checklist of bryophytes of Europe, Macaronesia and Cyprus. – *Journal of Bryology* **42**(1): *1–116*. https://doi.org/10.1080/03736687.2019.1694329

HALL, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – *Nucleic Acids Symposium Series* **41**: *95–98*.

KALYAANAMOORTHY, S., B.Q. MINH, T.K.F. WONG, A. VON HAESELER & L.S. JERMIIN. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. – *Nature. Methods* **14**: *587–589*. https://doi.org/10.1038/nmeth.4285

KONSTANTINOVA, N.A. & A.A. VILNET. 2025. *Lophozia savicziae* Schljakov (Lophoziaceae, Marchantiophyta) – one more allopolyploid species of liverworts. – *Arctoa* **34**: *44–51*. doi: 10.15298/arctoa.34.04

KRESS, W.J. & D.L. ERICKSON. 2007. A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding *trn*H-*psb*A spacer region. – *PLoS ONE* **2(6)**: *e508*. https://doi.org/10.1371/journal.pone.0000508

LAENEN, B., B. SHAW, H. SCHNEIDER, B. GOFFINET, E. PARADIS, A. DE SAMORE, J. HEINRICHS et al. 2014. Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts. – *Nature Communications* **5**: *6134*. https://doi.org/10.1038/ncomms6134

LIMPRICHT, K.G. 1879. Neue und kritische Lebermoose. – *Jahresbericht der Schlesischen Gesellschaft für Vaterländische Cultur* **57**: *311–317*.

LIU, Y.J., S. WHELEN & B.D. HALL. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. – *Molecular Biology and Evolution* **16**(12): *1799–1808*. https://doi.org/10.1093/oxfordjournals.molbev.a026092

MILYUTINA, I.A., D.V. GORYUNOV, M.S. IGNATOV, E.A. IGNATOVA & A.V. TROITSKY. 2010. The phylogeny of *Schistidium* (Bryophyta, Grimmiaceae) based on the primary and secondary structure of nuclear RDNA internal transcribed spacers. – *Molecular Biology* **44**: *883–897*. https://doi.org/10.1134/S0026893310060051

NATCHEVA, R. & N. CRONBERG. 2007. Maternal transmission of cytoplasmic DNA in interspecific hybrids of peat mosses, *Sphagnum* (Bryophyta). – *Journal of Evolutionary Biology* **20**: *1613–1616*. https://doi.org/10.1111/j.1420-9101.2007.01341.x

NEWMASTER, S.G. & R. SUBRAMANYAM. 2009. Testing plant barcoding in a sister species complex of pantropical *Acacia* (Mimosoideae, Fabaceae). – *Molecular Ecology Resources* **9**: *172–180*. https://doi.org/10.1111/j.1755-0998.2009.02642.x

NGUYEN, L.-T., H.A. SCHMIDT, A. VON HAESELER & B.Q. MINH. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. –*Molecular Biology and Evolution* **32**: *268–274*. https://doi.org/10.1093/molbev/msu300

PATON, J.A. 1999. The liverwort flora of the British Isles. – *Colchester, Harley Books*, *626 pp.*

POTEMKIN, A.D. & E.I. ROZANTSEVA. 2015. New and little-known for the Leningrad Region liverworts (Marchantiophyta) from Hogland Island (Gulf of Finland, Baltic Sea). – *Novosti Sistematiki Nizshikh Rastenii* **49**: *369–381*. https://doi.org/10.31111/nsnr/2015.49.369

POTEMKIN, A.D. & A.A. VILNET. 2021. Reappraisal of *Gymnocolea* and description of a new genus *Rudolgaea* (Anastrophyllaceae, Marchantiophyta). – *Arctoa* **30**: *138–148*. doi: 10.15298/arctoa.30.15.

[POTEMKIN, A.D.] ПОТЁМКИН А.Д. 1990. *Lophozia alboviridis* и *L decolorans* (Hepaticae) на Ямале. – [*Lophozia alboviridis* and *L. decolorans* (Hepaticae) on the Yamal Peninsula] *Ботанический Журнал* [*Botanicheskij Zhurnal*] **75**(8): *1086–1092*.

POTEMKIN, A.D. 1993. The Hepaticae of the Yamal Peninsula, West Siberian Arctic. – *Arctoa* **2**: *57–101*.

POTEMKIN, A.D. 1995. Contribution to the knowledge of the liverworts of North America. – *Fragmenta Floristica et Geobotanica* **40**: *323–338*.

RONQUIST, F., M. TESLENKO, P. VAN DER MARK, D.L. AYRES, A.

DARLING, S. HÖHNA, B. LARGET et al. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. – *Systematic Biology* **61**: *539–542*. https://doi.org/10.1093/sysbio/sys029

SAWANGPROH, W. & N. CRONBERG. 2021. Evidence for interspecific hybridization in bryophytes during pre-molecular and molecular eras. – *Bryophyte Diversity and Evolution* **043**(1): *180–205*.

SCHUSTER, R.M. 1969. The Hepaticae and Anthocerotae of North America. Vol. 2. – *New York and London, Columbia University Press, 1062 pp*.

SCHUSTER, R.M. 1988. The Hepaticae of South Greenland. – *Beihefte zur Nova Hedwigia* **92**: *1–255*.

SCHUSTER, R.M. 1995. Notes on Nearctic Hepaticae, XVII. *Lophozia decolorans*, new to North America and the subgenus Isopaches. – *The Bryologist* **98**(2): *246–250*.

SCHUSTER, R.M. 2002. Austral hepaticae. Part II. – *Beihefte zur Nova Hedwigia* **119**: *1–606*.

SCHUSTER, R.M. & K. DAMSHOLT. 1974. The Hepaticae of West Greenland from ca. 66° N to 72° N. – *Meddelelser om Grønland, udgivne af Kommissionen for Ledelsen af de Geologiske og Geografiske Undersřgelser i Grønland* **199**: *1–373*.

SÖDERSTRÖM, L., A. HAGBORG, M. VON KONRAD, S. BARTHOLOMEW-BEGAN, D. BELL, L. BRISCOE, E. BROWN et al. 2016. World checklist of hornworts and liverworts. – *PhytoKeys* **59**: *1–828*. doi: 10.3897/phytokeys.59.6261

SÖDERSTRÖM, L., R. DE ROO & T. HEDDERSON. 2010. Taxonomic novelties resulting from recent reclassification of the Lophoziaceae/Scapaniaceae clade. – *Phytotaxa* **3**: *47–53*.

TABERLET, P., L. GIELLY, G. PAUTOU & J. BOUVET. 1991. Universal primers for amplification of three non–coding regions of chloroplast DNA. – *Plant Molecular Biology* **17**: *1105–1109*. https://doi.org/10.1007/BF00037152

TAMURA, K., G. STECHER & S. KUMAR. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. – *Molecular Biology and Evolution* **38**: *3022–3027*. https://doi.org/10.1093/molbev/msab120

THOMPSON, J.D., D.G. HIGGINS & T.J. GIBSON. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. – *Nucleic Acids Research* **22**: *4673–4680*.

VILNET, A.A., N.A. KONSTANTINOVA & A.V. TROITSKy. 2010. Molecular insight on phylogeny and systematics of the Lophoziaceae, Scapaniaceae, Gymnomitriaceae and Jungermanniaceae. – *Arctoa* **19**: *31–50*. doi: 10.15298/arctoa.19.02

VILNET, A., N. KONSTANTINOVA & A. TROITSKY. 2012. Molecular phylogenetic data on reticulate evolution in the genus *Barbilophozia* Loeske (Anastrophyllaceae, Marchantiophyta) and evidence of non-concerted evolution of rDNA in *Barbilophozia rubescens* allopolyploid. – *Phytotaxa* **49**: *6–22*. https://doi.org/10.11646/phytotaxa.49.1.2

YATSENTYUK, S.P., N.A. KONSTANTINOVA, M.S. IGNATOV, J. HYVÖNEN & A.V. TROITSKY. 2004. On phylogeny of Lophoziaceae and related families (Hepaticae, Jungermanniales) based on *trn*L-*trn*F intron-spacer sequences of chloroplast DNA. – *In: Goffinet B., V. Hollowell & R. Magill (eds.) Molecular Systematics of Bryophytes. Monographs in systematic botany from the Missouri Botanical Garden*: **98**: *150–167*.

Received 28 October 2025 *Accepted 10 December 2025*

Appendix 1. The list of specimens for molecular phylogenetic estimation. The numbers start with PX are obtained in this study.

Taxon	Specimen voucher	GenBank accession number			
		nrITS	*trn*L-F	*rbc*L	*psb*A-*trn*H
Anastrepta orcadensis (Hook.) Schiffn.	Russia: Republic of Buryatia, *Konstantinova 59-1-01* (KPABG-102486)	DQ875126	DQ875088		
A. orcadensis	China: Yunnan Province, *Long 34711* (E)			KF852268	
Anastrophyllum assimile (Mitt.) Steph.	Russia: Altai Territory, *Ignatov #4/1* (KPABG)	KF836651	KF836661		
Barbilophozia hatcheri (A. Evans) Loeske	Russia: Arkhangelsk Region, Novaya Zemlya, *Sidorenko & Czernyadjeva 18-20* (LE-B-0028137)	OR536583	OR540408		
B. hatcheri	Russia: Kamchatka Territory, *Bakalin 53* (GOET)			KC184708	
B. sudetica (Nees ex Huebener) L. Söderstr., De Roo & Hedd.	Czech Republic, *Shaw 12991* (DUKE)			KF852392	
B. sudetica	Norway: Svalbard, *Konstantinova K91-4-06* (KPABG-111837)	EU791796	EU791679		
Crossocalyx hellerianus (Nees ex Lindenb.) Meyl.	Russia: Sakhalin Region, Sakhalin Island, *Bakalin Exsiccatae #82* (VLA, KPABG)	KF836643	KF836666		
Gymnocolea inflata (Huds.) Dumort.	Switzerland: Suedtirol, Oetztaler Alpen, *Schaefer-Verwimp & Verwimp 41089* (KPABG-125590)	PX620329	PX632782		
G. inflata	United Kingdom, *Cailliau et al. 888*			JX305549	
Hattoria yakushimensis (Horik.) R.M. Schust.	Japan: Kagoshima, Yakushima Island, *Katagiri 4281* (NICH)	LC744594	LC376049	LC376047	
Isopaches alboviridis (R.M. Schust.) Schljakov	Russia: Amur Region, *Bakalin 37-9-00* (KPABG-101728)	PX620307	PX632757	PX632786	PX632795
I. alboviridis	Russia: Murmansk Region, *Konstantinova 211-3-02* (KPABG-9563)	PX620308	PX632758		
I. alboviridis	Russia: Murmansk Region, *Konstantinova K1-1b-23* (KPABG-125996)	PX620309	PX632759		
I. alboviridis	Russia: Perm Territory, *Konstantinova K303-1-04* (KPABG-108224)	PX620310	PX632760		
I. alboviridis	Russia: Republic of Sakha (Yakutia), *Bakalin Hepatica Rossica Exsiccata #7* (KPABG-112385)	PX620311	PX632761		

Taxon	Specimen voucher	GenBank accession number			
		nrITS	*trn*L-F	*rbc*L	*psb*A-*trn*H
I. alboviridis	Russia: Yamal-Nenets Autonomous Area, *Bezgodov #301* (KPABG-118950)	PX620312	PX632762	PX632787	PX632796
I. alboviridis	U.S.A.: Alaska, *Potemkin ALA B29451* (LE-935801)	PX620313	PX632763		
I. alboviridis	U.S.A.: Alaska, *Potemkin ALA B27069* (LE)	PX620314	PX632764		PX632797
I. bicrenatus (Schmidel ex Hoffm.) H. Buch	Russia: Amur Region, *Bakalin 37-1-00* (KPABG-101720)	PX620315	PX632765		
I. bicrenatus	Russia: Republic of Buryatia, *Konstantinova K1-1-01* (KPABG)	PX620316	PX632766		
I. bicrenatus	Russia: Republic of Buryatia, *Konstantinova 19-02* (KPABG-104396)	PX620317	PX632767		
I. bicrenatus	Russia: Kamchatka Territory, *Bakalin K-40-1-02-VB* (KPABG-104037)	PX620318	PX632768		
I. bicrenatus	Russia: Kamchatka Territory, *Bakalin K-24-4-03* (KPABG-105425)	PX620319	PX632769		
I. bicrenatus	Russia: Khanty-Mansi Autonomous Area, *Lapshina EL-02228a* (KPABG)	PX620320	PX632770	PX632788	PX632798
I. bicrenatus	Russia: Murmansk Region, *Konstantinova K129-1-19* (KPABG-124358)	OP584691	OP573530	PX632789	PX632799
I. bicrenatus	Russia: Murmansk Region, *Konstantinova & Savchenko K73-4-15* (KPABG-120685)		PX632771		
I. bicrenatus	Russia: Murmansk Region, *Konstantinova K34-2-88* (KPABG)	PX620321	PX632772		
I. bicrenatus	Russia: Murmansk Region, *Konstantinova 41-1-24* (KPABG)	PX620322	PX632773		
I. bicrenatus	Russia: Nizhny Novgorod Region, *Konstantinova 154-03* (KPABG-106058)		PX632774		
I. bicrenatus	Russia: Perm Territory, *Konstantinova Ę381-04* (KPABG)	PX620323	PX632775		
I. bicrenatus	Russia: Perm Territory, *Bezgodov AB192a-09* (KPABG-117327)	PX620324	PX632776		
I. bicrenatus	Russia: Republic of Sakha (Yakutia), *Bakalin 18.07.2000* (KPABG)	EU791797	AY327788	PX632790	PX632800
I. bicrenatus	Russia: Republic of Tatarstan, *Ignatov & Ignatova 14a-05-03* (KPABG-109469)	PX620325	PX632777		
I. bicrenatus	Russia: Karachayevo-Circassian Republic, *Konstantinova K512-1-05* (KPABG-109798)	PX620326	PX632778	PX632791	PX632801
I. bicrenatus	USA: Vermont, *Shaw 6970* (DUKE)			KF852384	
I. decolorans (Limpr.) H. Buch	Russia: Karachayevo-Circassian Republic, *Konstantinova K464-3-05* (KPABG-109693)	EU791798	EU791680	PX632792	PX632802
I. decolorans	Russia: Murmansk Region, *Borovichev BE243-1-12* (KPABG-21382)	PX620327	PX632779	PX632793	PX632803
I. decolorans	France: Savoi, *Castelli 06.08.1962* (LE)		PX632780		PX632804
I. valdiviensis Mamontov, Vilnet & Konstant.	Chile: Los Ríos Region, *Mamontov 754-3-9134* (KPABG127751)	PX620328	PX632781	PX632794	PX632805
Neoorthocaulis attenuatus (Mart.) L. Söderstr., De Roo & Hedd.	Russia: Kemerovo Region, *Konstantinova 87-2-00* (KPABG-101941)	PX620332	PX632785		
N. attenuatus	Poland, *Strebel 226* (GOET)			KC184733	
Obtusifolium obtusum (Lindb.) S.W. Arnell	Russia: Kamchatka Territory, *Bakalin & Klimova K-102-2a-21* (VBGI)	OP082400	OP205298		
Plicanthus birmensis (Steph. ex Schiffn.) R.M. Schust.	Japan: Fukuoka, Mt. Houman, *Amamoto 35* (HIRO)	LC649032	LC649025	LC648981	
Rudolgaea fascinifera (Potemkin) Potemkin & Vilnet	Russia: Republic of Sakha (Yakutia), Kytalyk National Park, *Lapshina 019E-6-23* (KPABG-126373)	PQ686974	PQ699322	PQ699389	
Schljakovia kunzeana (Huebener) Konstant. & Vilnet	Russia: Trans-Baikal Territory, *Bakalin 38-1-00* (KPABG-101737)	OR769961	OR762255		
Schljakovianthus quadrilobus (Lindb.) Konstant. & Vilnet	Russia: Murmansk Region, *Konstantinova K10-4-17* (KPABG-121734)	PX620331	PX632784		
S. quadrilobus	U.S.A.: Alaska, *Shaw F982b/8* (DUKE)			KF852393	
Sphenolobus minutus (Schreb.) Berggr.	Russia: Krasnoyarsk Territory, Taimyr Peninsula, *Lapshina 210E/2-21* (KPABG-124536)	PX620330	PX632783		
S. minutus	Norway: Svalbard, *Hentschel Bryo 0421* (GOET)			DQ312475	
Tetralophozia filiformis (Steph.) Urmi	China: Yunnan Province, *Bakalin & Ma China-73-44-18* (VBGI)	MZ231276	MZ229434		
T. filiformis	China: Yunnan Province, *Shaw 5790* (DUKE)			KF852352	
Vietnamiella epiphytica Bakalin & Vilnet	Vietnam: Lao Cai Province, *Bakalin & Klimova V-9-7-17* (KPABG-122594)	MK277316	MK290984	MK290986	