

THE PHYLOGENETIC AFFINITY, DISTRIBUTION AND VARIABILITY OF  
*CRYPTOCOLEA IMBRICATA* R.M. SCHUST. (MARCHANTIOPHYTA)

ФИЛОГЕНЕТИЧЕСКОЕ ПОЛОЖЕНИЕ, ВАРИАБЕЛЬНОСТЬ И РАСПРОСТРАНЕНИЕ  
*CRYPTOCOLEA IMBRICATA* R.M. SCHUST. (MARCHANTIOPHYTA)

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Abstract

The phylogenetic relation of the poorly known Arctic genus *Cryptocolea* is clarified for the first time from an integrative approach. It is shown that the genus belongs to the family Southbyaceae, rather than Solenostomataceae, where it was placed earlier. Some morphological synapomorphies confirming the found relation are revealed including perianths totally hidden in the bracts, the character of margin of female bracts, and the surface of spores. A detailed description of the genus based on multiple specimens from Eurasia is given, the variability of morphological features and three DNA loci (ITS1-2 nrDNA, *trnL*-F and *rbcL* cpDNA) is discussed, and a new variety *C. imbricata* var. *minima* Konstant., Vilnet & Mamontov is described. Ecology and distribution of *C. imbricata* in Eurasia are clarified; the necessity of further studies of the genus is discussed, including molecular study of specimens from North America, as well as collecting specimens from bare soil in the High Arctic.

Резюме

Впервые на основании интегративного подхода пересмотрено филогенетическое положение малоизученного арктического рода *Cryptocolea*. Показано, что род относится к семейству Southbyaceae, а не к Solenostomataceae, куда его помещали ранее. Выявлен ряд морфологических синапоморфий, подтверждающих обнаруженное родство, в том числе полностью скрытый в покровных листьях периантий, характер края перихециальных листьев и скульптура поверхности спор. Выполнено подробное описание рода, рассмотрена вариабельность морфологических признаков и трех локусов ДНК (ITS1-2 ядДНК, *trnL*-F и *rbcL* хлДНК), описана новая разновидность *C. imbricata* var. *minima* Konstant., Vilnet & Mamontov. Уточнены экология и распространение *C. imbricata* в Евразии, показана необходимость дальнейшего изучения рода, в том числе включение в молекулярно-генетические исследования образцов из Америки и сбор и комплексное изучение образцов с пятен из пятнистых тундр Арктики.

KEYWORDS: liverworts, phylogeny, taxonomy, *Cryptocolea*, morphology, new variety, ITS1-2, *trnL*-F, *rbcL*, distribution, ecology

INTRODUCTION

*Cryptocolea* is a monotypic genus which was described by R.M. Schuster as “an isolated taxon of uncertain phylogenetic relationships” (Schuster, 1969: 889). He repeatedly (Schuster, 1953, 1969) considered the similarity of *Cryptocolea* with several families; in particular, he noted that the genus “shows superficial relationships to at least three families of Hepaticae: the Plagiochilaceae, the Jungermanniaceae (s. str., as in Schuster 1951), and the Southbyaceae” (Schuster, 1953: 415). At the same time, he placed the genus in Jungermanniaceae close to *Nardia* (Schuster, 1969). Later Crandall-Stotler et al. (2009) moved the genus to the Solenostomataceae Stotler et Crand.-Stotl. In the world checklist of liver-

worts (Söderström *et al.*, 2016), the genus keeps its position in Solenostomataceae; however, it is emphasized that this is done provisionally. As far as we know, the genus *Cryptocolea* had never been involved in the molecular phylogenetic studies. The aim of our study is primarily to clarify the phylogenetic position of the genus, and secondarily to characterize the morphological and genetic variability of species based on specimens from different regions of Eurasia.

MATERIAL AND METHODS

*Collections and morphological study*

The morphological investigation is based on specimens preserved in the herbarium of the Polar-Alpine Botanical Garden Institute, Kirovsk, Russia (КРАБГ)

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and the herbarium of the Main Botanical Garden, Moscow (MHA). Totally 20 specimens were studied using stereomicroscope (Nikon SMZ 8007) and compound light microscope Nikon Eclipse SOi with digital camera DS Fi1. The mats and isolated shoots of *Cryptocolea* were photographed using an Olympus MVX-10 stereomicroscope equipped with a digital camera Lumenera Infinity 3-6, other photomicrographs were obtained using light microscopes Nikon Eclipse 50i equipped with a Nikon DS-Fi1 digital camera and Leitz Wetzlar Orthoplan equipped with a digital camera Nikon D700. In order to better illustrate the three-dimensional objects, photomicrographs were combined from several optical sections using the stacking software Helicon Focus 8 (Kozub et al., 2008).

### Mapping

The locations on the map are placed according to the coordinates available from the specimen vouchers, or calculated from the description of the localities. All locations with coordinates from the information system L. (CRIS) were automatically plotted on the map since they were either checked by the authors, or their identification is beyond doubt, such as the specimens identified by V. Bakalin. The locations from the GBIF were critically evaluated for the reliability of the data source before being transferred to the map. Different kinds of data are marked on the map by different symbols.

### Sampling for molecular analyses

The specimens of *Cryptocolea imbricata* from Svalbard (Norway), Kamchatka, Khabarovsk, Trans-Baikal and Krasnoyarsk Territories of Russia were selected for molecular study. The ITS1-2 nrDNA and *trnL*-F cpDNA were chosen as appropriate molecular markers. BLAST search based on both sequenced loci did not provide high similarity with the members of family Solenostomataceae as was expected from the recently accepted taxonomic position of the genus (Söderström et al., 2016). Two accessions of the genus *Southbya* (KJ802083, KF942954) appeared to be the most similar to *Cryptocolea imbricata* with *trnL*-F sequence similarity up to 86–87%, and a number of accessions of the families Gymnomitriaceae and Jungermanniaceae with ITS1-2 sequence similarity up to 84–86%. The low level of similarity suggests the isolated phylogenetic position of *Cryptocolea*, and also insufficient sampling of related taxa and absence of appropriate DNA loci in GenBank, especially the ITS1-2 region for most genera of the Jungermanniineae. After BLAST estimation we sequenced a part of *rbcL* cpDNA gene for three *Cryptocolea* specimens to estimate the affinity of the genus, since this region was used in the fundamental study of macroevolutionary history of bryophytes (Laenen et al., 2014) and an expanded nucleotide dataset for liverworts was deposited in GenBank. All three nucleotide markers were also obtained for *Arnellia fenica*, *Southbya tophacea* and *Gongylanthus limbatus* to fill absent data for these taxa critical for current phylo-

genetic estimation. The ITS1-2 sequence data for 42 accessions, *trnL*-F for 91 accessions, *rbcL* for 68 accessions were downloaded from GenBank; they represent taxa from the suborder Jungermanniineae, a sister related Cephaloziineae and species from Myliineae placed in the outgroup. The list of specimens sequenced in this study with voucher details and GenBank accession numbers is shown in Table 1; the list of all specimens included in the phylogenetic study is provided in Appendix 1 (supplementary material).

### DNA isolation, PCR amplification and DNA sequencing

DNA from dried liverwort shoots was extracted with DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The ITS1-2, *trnL*-F and *rbcL* loci were amplified and sequenced with pairs of primers described in White et al. (1990), Taberlet et al. (1991) and Kress & Erickson (2007). 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, *trnL*-F) or 52°C (*rbcL*), 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).

### Phylogenetic analysis

The newly sequenced data were assembled and alignments were produced with the program BioEdit 7.0.1 (Hall, 1999). From the available sequence data two datasets were produced: ITS1-2+*trnL*-F and *rbcL*+*trnL*-F. The combination of the three DNA loci into a single alignment was inappropriate due the absence of specimens with all three sequenced loci, with exception of those obtained in this study. The species in ITS1-2+*trnL*-F and *rbcL*+*trnL*-F alignments are represented by different specimens that have an appropriate combination of DNA data from a single specimen. *Cryptocoleopsis imbricata*, *Endogemma caespiticia* and *Southbya tophacea* are represented by the same single *trnL*-F accession in the both alignments. The specimens of *Southbya nigrella* and *Geocalyx graveolens* were included in the combined ITS1-2+*trnL*-F with *trnL*-F only and in *rbcL*+*trnL*-F with both chloroplast loci. Seven specimens with only *trnL*-F sequences were placed in ITS1-2+*trnL*-F alignment, 18 specimens with *rbcL* only and eight specimens with *trnL*-F only data were included in *rbcL*+*trnL*-F alignment. Totally, the ITS1-2+*trnL*-F dataset has 58 specimens, *rbcL*+*trnL*-F – 82 specimens. All positions were taken into account and absent data and loci were coded as missing in both alignments.

The maximum likelihood analysis (ML) was performed with IQ-TREE (Nguyen et al., 2015) and the

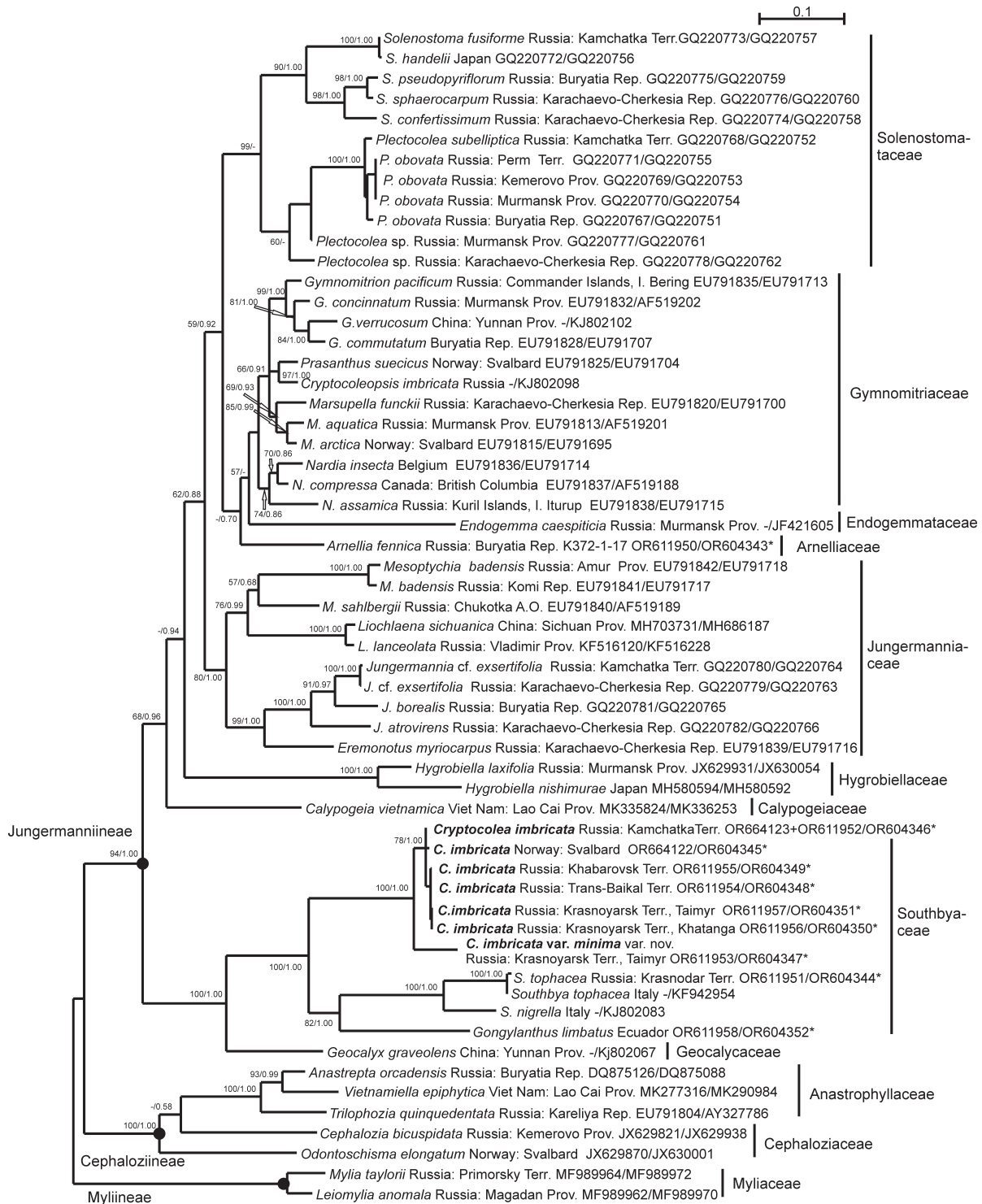


Fig. 1. Phylogram obtained by maximum likelihood for the suborder Jungermanniineae based on ITS1-2+trnL-F dataset. Bootstrap supports from maximum likelihood and Bayesian posterior probabilities more than 50% (0.50) are indicated. GenBank accession numbers are provided, accessions obtained in this study are marked with asterisk.

Bayesian analysis (BA) with MrBayes v. 3.2.1 (Ronquist *et al.*, 2012). The ModelFinder (Kalyaanamoorthy *et al.*, 2017) resolved the GTR+FO+G4 model as the best fit evolutionary model of nucleotide substitutions for both ITS1-2+trnL-F and *rbcL*+trnL-F datasets. The ultrafast

bootstrapping procedure (Hoang *et al.*, 2018) with 200 replicates was used. The obtained ML tree topologies were redrawn in NJplot (Perrière & Gouy, 1996). For the Bayesian analysis both datasets were assigned the GTR+I+G model as recommended by the program's creators; gam-

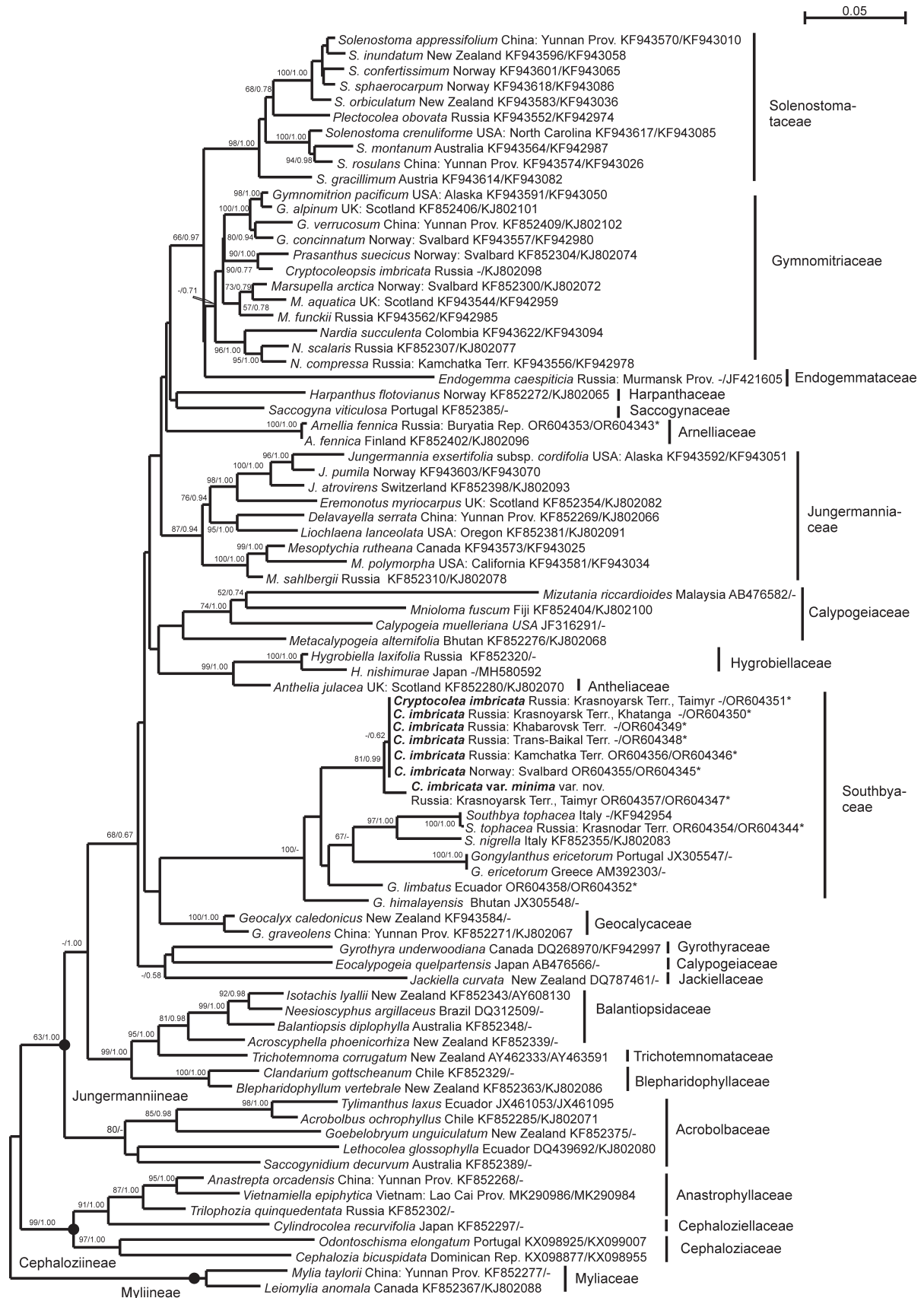


Fig. 2. Phylogram obtained by maximum likelihood for the suborder Jungermanniineae based on *rbcL*+*trnL*-F dataset. Bootstrap supports from maximum likelihood and Bayesian posterior probabilities more than 50% (0.50) are indicated. GenBank accession numbers are provided, accessions obtained in this study are marked with asterisk.

Table 1. The list of specimens sequenced in this study with voucher details and GenBank accession numbers.

Taxon	Specimen voucher	GenBank accession number		
		ITS1-2 nrDNA	<i>trnL</i> -F cpDNA	<i>rbcL</i> cpDNA
<i>Arnellia fennica</i>	Russia: Buryatia Rep., <i>Konstantinova &amp; Savchenko K372-1-17</i> (KPABG-122033)	OR611950	OR604343	OR604353
<i>Cryptocolea imbricata</i>	Norway: Svalbard, <i>Frisvoll 732314</i> (KPABG-122667)	OR664122	OR604345	OR604355
<i>Cryptocolea imbricata</i>	Russia: Kamchatka Terr., <i>Bakalin K-66-5-15</i> (KPABG-120389)	OR664123/ OR611952	OR604346	OR604356
<i>Cryptocolea imbricata</i>	Russia: Khabarovsk Terr., <i>Bakalin Kh-36-56-13</i> (KPABG-119106)	OR611955	OR604349	–
<i>Cryptocolea imbricata</i>	Russia: Krasnoyarsk Terr., <i>Khatanga Settl., Fedosov 13-3-0382</i> (KPABG-116993)	OR611956	OR604350	–
<i>Cryptocolea imbricata</i>	Russia: Krasnoyarsk Terr., <i>Taimyr, Fedosov G107969</i> (KPABG-107969)	OR611957	OR604351	–
<i>Cryptocolea imbricata</i>	Russia: Trans-Baikal Terr., <i>Mamontov YuSM-524-3-6</i> (KPABG-119564)	OR611954	OR604348	–
<i>Cryptocolea imbricata</i> var. <i>minima</i>	Russia: Krasnoyarsk Terr., <i>Taimyr, Lapshina 213E/4-3-21</i> (KPABG-124714)	OR611953	OR604347	OR604357
<i>Gongylanthus limbatus</i>	Ecuador, <i>Schaefer-Verwimp 24463</i> (MO)	OR611958	OR604352	OR604358
<i>Southbya tophacea</i>	Russia: Krasnodar Terr., <i>Konstantinova &amp; Savchenko K312-4-11</i> (KPABG-122707)	OR611951	OR604344	OR604354

ma 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 for the ITS1-2+*trnL*-F dataset and five millions for the *rbcL*+*trnL*-F dataset. Trees were saved every 100th generation. The average standard deviation of split frequencies between two runs in the ITS1-2+*trnL*-F analysis was 0.005607 and 0.006173 in *rbcL*+*trnL*-F. For the ITS1-2+*trnL*-F calculations the first 2500 (25%) trees were discarded in each run, and 15000 trees from both runs were sampled after burn-in, for *rbcL*+*trnL*-F 12500 (25%) trees were discarded in each run, and 75000 trees from both runs were sampled after burn-in. Bayesian posterior probabilities were calculated from trees sampled after burn-in. The FigTree v.1.3.1 was used to visualize Bayesian phylogenetic tree (<http://tree.bio.ed.ac.uk/software/figtree/>).

The sequence variability was estimated as the average pairwise *p*-distances for ITS1-2, *trnL*-F and *rbcL* loci of the genus *Cryptocolea* and related taxa in Mega 11 (Tamura *et al.*, 2021) using the pairwise deletion option for counting gaps.

## RESULTS

Newly generated sequence data were obtained for ten specimens tested in the current study, ten ITS1-2 and *trnL*-F and six *rbcL* accessions were deposited into GenBank. The ITS1-2+*trnL*-F alignment has a total length of 1620 positions, among them 1049 positions belong to ITS1-2 and 571 – to *trnL*-F. The total length of the *rbcL*+*trnL*-F alignment has 1954 positions, among them 1343 positions belong to *rbcL* and 611 – to *trnL*-F.

The single tree obtained in the ML calculation of the ITS1-2+*trnL* dataset has an arithmetic mean of Log like-

lihood -18009.90, both runs sampled in the BA analysis have the means of Log likelihood -17688.73 and -17686.93, respectively. The single ML tree calculated on *rbcL*+*trnL*-F dataset has an arithmetic mean of Log likelihood -20834.75, both runs sampled in the BA analysis have the means of Log likelihood -20556.77 and -20554.46. Tree topologies reconstructed for each dataset in both analytical procedures are congruent, thus Fig. 1 demonstrates the topology received in ML analysis of ITS1-2+*trnL* dataset, Fig. 2 – the topology from ML analysis of *rbcL*+*trnL*-F dataset, with indication of bootstrap support (BS) values from ML calculation and Bayesian posterior probabilities (PP) from BA.

Despite the different taxon sampling, the backbone topologies of the trees constructed from the combined nuclear and chloroplast loci and from two chloroplast loci are similar and agree with previously published topologies for Jungermanniales reconstructed from different combination of chloroplast markers (De Roo *et al.*, 2007; Gailliau *et al.*, 2013) and combined chloroplast, mitochondrial and nuclear markers (Shaw *et al.*, 2015). Seven specimens of *Cryptocolea* form a clade robustly supported in the analyses from ITS1-2+*trnL* dataset (BS=100%, PP=1.00), and less supported in the analyses from *rbcL*+*trnL*-F dataset (BS=81%, PP=0.99). In both trees the remote affinity of the *Cryptocolea*-clade to the terminal Solenostomataceae-clade, as well to the genera *Nardia* and *Arnellia*, is evident. The *Cryptocolea*-clade reveals a sister affinity (BS=100%, PP=1.00) to a clade composed by the genera *Southbya* and *Gongylanthus* (BS=82%, PP=1.00) in ITS1-2+*trnL* topology, and unsupported relation to these genera in *rbcL*+*trnL*-F topology. Molecular phylogenetic estimation suggests taxonomical changes in the Jungermanniineae by transferring the genus *Cryptocolea* from the family Solenostomataceae to the family Southbyaceae.

Table 2. The values of *p*-distances (%) for the family Southbyaceae, calculated from a) ITS1-2 and *trnL-F*, b) *rbcL* nucleotide sequence data.

Specimen	Kamchatka Terr. 120389	Taimyr 107969	Khabarovsk Terr. 119106	Trans-Baikal Terr. 119564	Khatanga 116993	Svalbard 122667	Taimyr 124714	Krasnodar 122707	Italy 35466	Italy 621	Ecuador 24463
<i>Cryptocolea imbricata</i>											
Kamchatka Terr. 120389											
<i>C. imbricata</i>	1.0/0.2										
Taimyr 107969											
<i>C. imbricata</i>	0.8/0.2	0.5/0.0									
Khabarovsk Terr. 119106											
<i>C. imbricata</i> 119564	1.0/0.2	0.6/0.0	0.4/0.0								
Trans-Baikal Terr. <i>C. imbricata</i>	0.8/0.2	0.2/0.0	0.2/0.0	0.4/0.0							
Khatanga 116993											
<i>C. imbricata</i>	1.1/0.0	1.9/0.2	1.5/0.2	1.1/0.2	1.1/0.2						
Svalbard 122667											
<i>C. imbricata</i>	7.6/2.0	7.4/2.1	7.4/2.1	7.6/2.1	7.4/2.1	7.1/1.9					
Taimyr 124714											
<i>Southbya tophacea</i>	122707	25.6/11.3	22.1/11.3	22.4/11.3	22.3/11.3	22.1/11.3	15.3/11.223.5/10.8				
Krasnodar											
<i>S. tophacea</i>	-/11.3	-/11.6	-/11.6	-/11.6	-/11.6	-/11.2	-/11.1	-/0.2			
Italy 35466											
<i>S. nigrella</i>	-/12.6	-/12.3	-/12.3	-/12.3	-/12.3	-/12.0	-/11.8	-/7.6	-/7.0		
Italy 621											
<i>Gongylanthus</i>	28.2/8.1	23.7/8.0	23.6/8.0	23.6/8.0	23.6/8.0	30.4/7.6	22.9/9.2	21.4/10.4	-/10.1	-/12.2	
<i>limbatus</i>											
Ecuador 24463											
Specimen	<i>C. imbricata</i> Kamchatka 120389	<i>C. imbricata</i> Svalbard 122667	<i>C. imbricata</i> Taimyr 124714	<i>Southbya tophacea</i> Krasnodar 122707	<i>S. nigrella</i> Italy 621	<i>Gongylanthus</i> <i>ericetorum</i> Portugal 36610	<i>G.ericetorum</i> Greece 6335	<i>G. limbatus</i> Ecuador 24463	<i>G. himalayensis</i> Bhutan 28706		
<i>Cryptocolea imbricata</i>											
Kamchatka 120389											
<i>C. imbricata</i>	0.2										
Svalbard 122667											
<i>C. imbricata</i>	0.7	0.9									
Taimyr 124714											
<i>Southbya tophacea</i>	5.4	5.2	5.6								
Krasnodar 122707											
<i>S. nigrella</i>	6.1	5.9	5.9	5.1							
Italy 621											
<i>Gongylanthus</i>	4.6	4.4	4.2	6.3	6.7						
<i>ericetorum</i> 36610											
Portugal											
<i>G.ericetorum</i>	4.7	4.5	4.4	6.3	6.5	0.0					
6335											
Greece											
<i>G. limbatus</i>	3.3	3.1	3.1	5.9	6.4	6.1	6.1				
Ecuador 24463											
<i>G. himalayensis</i>	3.6	3.4	3.4	6.9	6.7	6.1	6.1	2.4			
28706 Bhutan											

## MORPHOLOGY

In general, morphological variability of the specimens studied by us is quite low and is mainly in the intensity of color, plant size and leaf shape. Some of the most variable features are considered below.

**Color.** The species was described as “bright pellucid green with the older leaves becoming a light yellowish brown” (Schuster, 1969:890). In the material studied by us the color of plants varies greatly from bright green to

golden-brown, bronze and dark bronze. In some specimens plants are fully green, but more often the upper leaves are green or just with golden-brown or chestnut-brown colored margins, whereas lower leaves are mostly brown to dark brown. In some specimens all plants are bronze or even dark bronze-brown, but the inner parts of leaves covered by a lower leaf are always bright green or, in old material, pale green. Fig. 3



Fig. 3. *Cryptocolea imbricata* var. *minima* (A, C, D, G, I – from holotype). *Cryptocolea imbricata* var. *imbricata* (B, E, F, H, J – from Fedosov 13-3-0382): A, B: habit of plants in cushions; C–E, G: female shoots; F: sterile shoot; H–I: male shoots.

**Size** of plants vary greatly from just 0.4 mm wide and just 1.5–2 mm long on spots of bare soil in Taimyr Peninsula (KPABG-124714) to 1–1.3 mm wide and 12–15 mm long in specimens from the Kamchatka Peninsula. Fig.3

**Rhizoids** are described by Schuster (1969) as sporadic, rarely numerous, but in many studied specimens rhizoids were numerous. Moreover, the rhizoids are very dense and very long in the specimen KPABG-124714 from Taimyr.

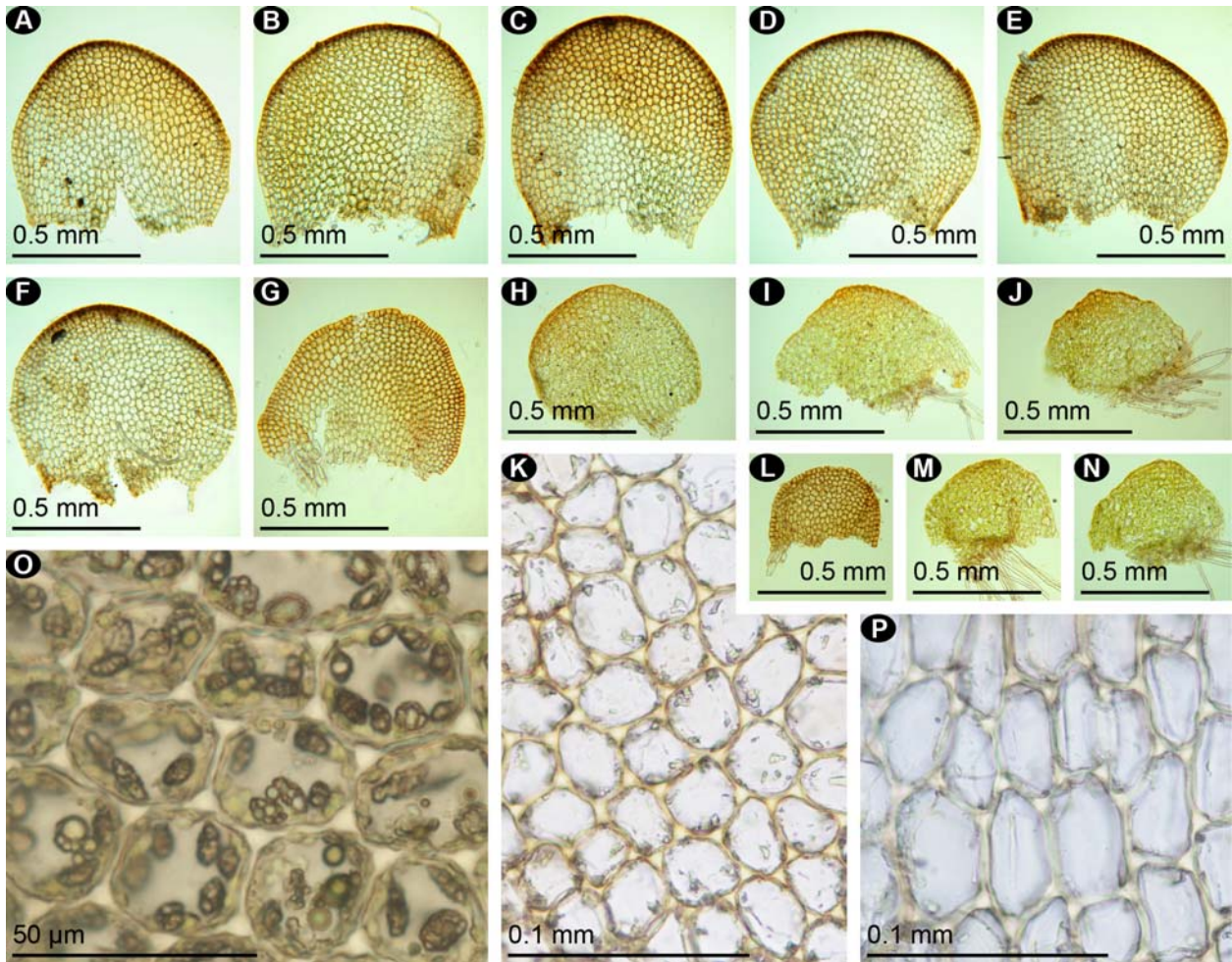


Fig. 4. *Cryptocolea imbricata* var. *minima* (G–J – from holotype). *Cryptocolea imbricata* var. *imbricata* (K, P – from *Bakalin Kh-36-56-13*; O – from *Mamontov 537-3-1*): A–J, L–N: leaves; K: median leaf cells; O: basal leaf cells with oil-bodies; P: basal leaf cells.

**Shape of leaves** varies from almost round to broadly ovate or transversely elliptical (KPABG 124714, 116993), but leaves are always broadly rounded at apex and mostly with margin folded inwards. Fig.4

**Leaf cells** in most specimens are smaller in the upper part of the leaf and along margins, gradually increasing in size towards the middle of leaf, and are the largest in the lower part of leaf. Cells are more or less elongate in the distal part of leaves and distinctly elongate, up to 2–2.5(–3) times as long as wide, at the base of leaves. In one specimen from Taimyr Peninsula (KPABG-124714), in which the leaves are very small, cells are large throughout the leaf, only slightly larger and more elongated at the base. Fig.4

**Sexuality.** The species is described as dioicous and in most specimens we found either antheridia or archegonia. But in Fedosov's specimen from Dickson (KPABG-107969), one antheridium was found on a plant with characteristically ventricose male bracts and with archegonia in terminal position on the same plant. This suggests that at least in some areas the species may have protandria.

**Papillae** clearly visible both on the outer and inner female bracts in some specimens, but in other specimens with young archegonia they may be completely undeveloped and appear only as separate larger marginal cells with colorless walls.

**Spores.** In the only specimen with sporophytes studied by us spores were somewhat larger (20–23 µm in diam.) than it was described by Schuster (1969), 14–16(17–19) µm. At the same time, the sculpture of the surface corresponds exactly to Schuster's description. Fig.5

#### TAXONOMICAL TREATMENT

The description of *Cryptocolea imbricata* provided below is based on specimens preserved in KPABG and MHA. Some of the characteristics described by Schuster (1969), but not found by us, are given in brackets.

*Cryptocolea imbricata* R.M. Schust., Amer. Midl. Nat. 49(2): 417, fig. 7:7, pl. 34, 1953.

**Plants** with a subtle pleasant smell of essential oil after soaking, (0.4)0.8–1.3 mm wide and 2–15 mm long, growing as isolated plants or in dense turfs or mats with admixture of other liverworts, not branched apart the innovation under gynoeceum or single short branch near



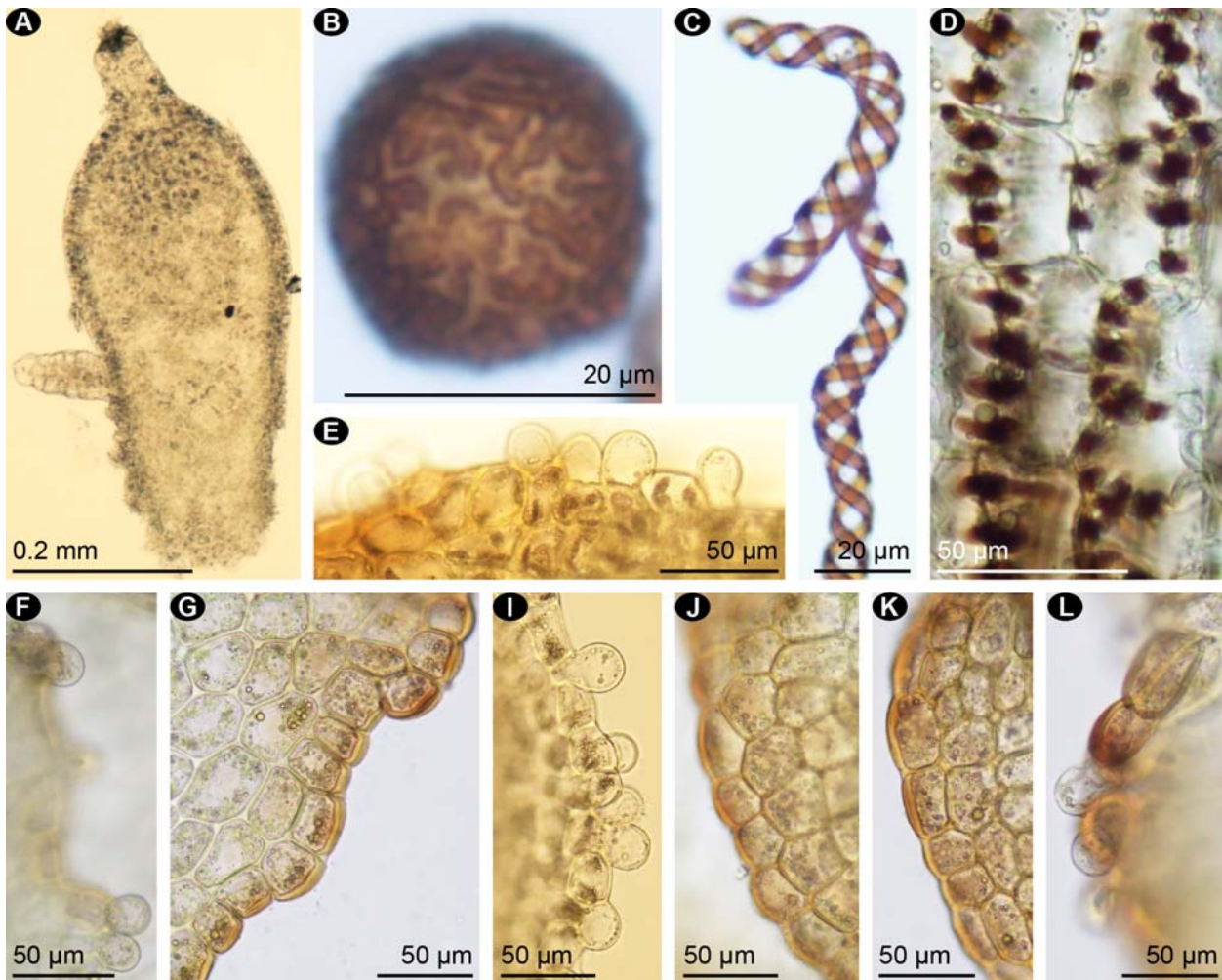


Fig. 5. *Cryptocolea imbricata* var. *minima* (F, G, J, K, L – from holotype). *Cryptocolea imbricata* var. *imbricata* (A–D from Bakalin K-48-4-15; E, I – from Bakalin K-66-5-15): A: calyptra; B: spore; C: two elaters; D: capsule wall; E, F, G, I, L: female bracts margins; J, K: male bracts margins.

apex; branches lateral, intercalary or of *Frullania* type. Plants green to golden and copper-bronze, or dark brown, often bright or glistening, deep brown along margins of leaves and gradually becoming green in the inner parts of leaves, or with copper-bright and deep brown color of the whole plant. **Stem** fleshy, cross section ellipsoidal, (175)200×250–300(325) µm, as wide as long, cells of medulla thin-walled, almost isodiametric or wide elliptical, 20–25×20–30 µm, with small trigones; cortical cells smaller, elongated along the margin, slightly thick-walled with brown walls, 13–17×17–20 µm, mycorrhizae not found. **Ventral stem sectors** not expressed at all or even the attachment lines of opposite leaves come one after the other (especially below) or near apex 1–2 cells wide; underleaves absent; cells on ventral side of stem 17–20×25–37 µm; cells on dorsal side larger, 25–30×50–75 µm. **Rhizoids** sporadic to numerous, very long, colorless or light brown, 10–13 µm in diameter. **Leaves** closely imbricate, slightly decurrent on densely leaved shoots to long decurrent on loosely leaved shoots, both on ventral and especially on dorsal side, where long, 1–2 cells wide

strips can reach a leaf below; leaves saucer-shaped, concave, broadly ovate or almost orbicular, from 400 (in var. *minima*) to 750–800 µm, to slightly elliptical and then 750–900×900–1100 µm, rarely transversely elliptical. **Cells** along the margins of leaves from almost isodiametric to slightly elongated, 20–25×(20)–25–30 µm in upper part, just slightly larger below and in the middle; smaller, isodiametric, 20–25×20–25 µm, mixed with elongated, (20)25–30×25–30–35(38) µm and more distinctly elongated towards the base, and at base (25)30–38(40)×35–50(65) µm, thin-walled, with straight to slightly wavy walls, with small, but distinct trigones. **Oil bodies** light to dark grey, (3)5–7(9) per cell, 5–9 µm of many, relatively large, uneven segments, spherical or wide ellipsoidal, to (3)4–8×7–10 (12) µm, more or less persistent. **Gemmae** absent.

Dioicous or heteroicous. **Male** plants in separate mats (in most collections only male plants are present), smaller than female plants, often 0.5 mm wide and just (1)2 mm long in the same mats as female, androecia compressed laterally, terminal, but then became intercalary,

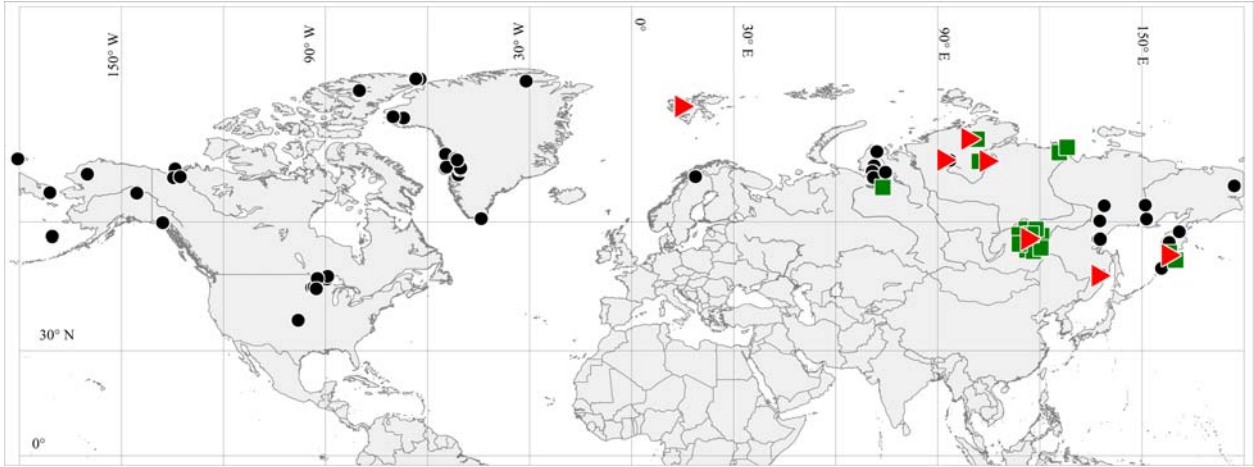


Fig. 6. Worldwide distribution of *Cryptocolea imbricata*. Squares - specimens revised by the authors; triangles - sequenced specimens; circles - literature and GBIF data

so there are often 2–3 androecia alternating with sterile leaves, each androecium consists of 3–4 pairs of bracts inflated at the base. Bracts distinctly ventricose, with marginal cell walls rounded, wave-like at the edges of margin. Bracts almost orbicular to broadly transversely elliptical, ca. 650–950  $\mu\text{m}$  wide, to 650–800  $\mu\text{m}$  long; antheridia 1–2 per bract, large, filling ventricose base of bract, 200  $\mu\text{m}$  wide and 250  $\mu\text{m}$  long, stalk biseriate.

**Female** plants with perigynium more or less low; perichaetial leaves at apex of shoot, very closely attached, nested one into the other in 3–6 (7) pairs, forming characteristic bilobate structure, outer bracts clearly ventricose, often reflexed in distal part, large, subquadrate or with length slightly exceeding the width or vice versa, ca. 700–900 (1300)  $\mu\text{m}$  wide and (700) 800–1350  $\mu\text{m}$  long, gradually decreasing in size, with young innermost colorless scale-like bracts, almost twice as small as outer bracts. Bracts along margins with slime papillae sometimes mixed with one-celled teeth or some kind of indentations formed on the site of destroyed papillae. Small bracteoles sometimes present. [Perianth rudimentary, totally hidden in bracts, with wide mouth, but orifice reduced by extensive plication, shallow plurilobulate, lobules blunt, with few slime papillae interspersed, developed after fertilization]; shoot calyptra with numerous sterile archegonia at the base was found in a single studied specimen with sporophyte (KPABG 120385). Capsule wall with numerous nodular thickenings in external cell layer, inner layer with more or less complete semi-annular bands. **Spores** brown, with a peculiar worm-like sculpture of the surface, 14–20 (23)  $\mu\text{m}$  in diam., elaters bispiral, (6) 7–10  $\mu\text{m}$  wide, with 2–3  $\mu\text{m}$  band. Fig. 5.

**Type.** Near Grand Portage, Minnesota, USA, R.M. Schuster 18592, 1950.

**Specimens examined.** RUSSIA: Far East, **Kamchatka Territory**, East Kamchatka: Pinachevsky Pass, 53°26'56"N, 158°38'17"E, 1280 m a.s.l. 18.VIII.2015 V. Bakalin [KPABG-

120389], moist cliffs, N-facing rocks, with *Frullania subarctica*; Ganalsky Range, 53°54'34"N, 158°03'39"E, 1330 m a.s.l. 06.VIII.2015 V. Bakalin [KPABG-120385, spor!], with *Trilophozia quinquedentata*; *ibid.*, 53°54'34"N, 158°03'35"E, 1450 m a.s.l. 07.VIII.2015 V. Bakalin [KPABG-121576, MHA-9091828]; **Khabarovsk Territory**, Tardoki-Yani Range, 48°53'N, 138°03'E, 2010 m a.s.l. 22.VIII.2013 V. Bakalin [KPABG 119106]; **Trans-Baikal Territory**, Stanovoye Nagor'ye Uplands, Kodar Range, 56°53'40"N, 117°19'21"E, 2000 m a.s.l. 10.VI.2015 Yu. Mamontov 524-3-6 [KPABG-119564], on base of moist rock, mixed with *Pseudotritomaria heterophylla*, *Gymnomitrium revolutum*, *Frullania subarctica*, *Herbertus aduncus*; *ibid.*, 56°44'54.9" N. 117°21'39.0" E, 1644 m a.s.l. 15.VI.2015 Yu. Mamontov 537-3-1 [KPABG-119573], on moist soil of a late snow bed, mixed with *Trilophozia quinquedentata*, *Scapania hyperborea* and *Fuscocephaloziaopsis pleniceps*; *ibid.*, 56°44'54.9" N. 117°21'39.0" E, 1644 m a.s.l. 15.VI.2015, Yu. Mamontov 537-3-6085 [MHA-9087205], on moist soil of a late snow bed, mixed with *Mesoptychia heterocolpos*; *ibid.*, 56°55'39.0" N. 117°42'32.8" E, 1755 m a.s.l., 03.VII.2013, Yu. Mamontov 351-3-2 [KPABG], on rock ledge moistened with dripping water, mixed with *Anastrophyllum assimile*, *Marsupella arctica*, *Odontoschisma macounii*, *Radula prolifera*; *ibid.*, 03.VII.2013, Yu. Mamontov 351-3-5 [MHA], on rock ledge moistened with dripping water, mixed with *Anthelia juratzkana*, *Fuscocephaloziaopsis pleniceps*, *Gymnomitrium revolutum*, *Odontoschisma macounii*; Kalar Range, 56°10'57.1" N, 117°20'03.9" E, 1756 m a.s.l. 20.VIII.2014 Yu. Mamontov 493-1-8981 [MHA], on soil-covered boulders, mixed with *Fuscocephaloziaopsis albescens*, *Marsupella emarginata*, *Radula prolifera*, *Schistochilopsis incisa*; *ibid.*, 20.VIII.2014 Yu. Mamontov 493-1-8983 [MHA-9088476], on soil-covered boulders, mixed with *Calycularia laxa*, *Scapania crassiretis*, *Trilophozia quinquedentata*; *ibid.*, 56°14'36.4" N, 117°26'23.8" E, 1516 m a.s.l. 26.VIII.2014 Yu. Mamontov 506-1-1 [MHA], on moist rock surface, mixed with *Cephalozia bicuspidata*, *Gymnomitrium concinatum*, *Sphenolobus saxicola*, *Tetralophozia setiformis*; Udokan Range, 56° 40' N, 118° 22' E, VIII.1987 A. Kozhevnikova & V. Filin [LE], on rocks, mixed with *Anastrophyllum assimile*, *Calycularia laxa*, *Marsupella emarginata*, *Sphenolobus minutus*. **Krasnoyarsk Territory**: Ereechka River,

71°02'00"N, 105°08'00"E, 22.VII.2013 V.E. Fedosov [KPABG 119106] with *Trilophozia quinquedentata*, *Aneura pinguis*, *Scapania crassiretis*, *Calycularia laxa*; Dikson district, "Ledyanaya bukhta", 74°28'50" N, 99°45'24" E, 20.VII.2004 V.E. Fedosov [KPABG 107969] with *Pseudotritomaria heterophylla*, *Blepharostoma brevirete*, *Mesoptychia heterocolpos*, *Trilophozia quinquedentata*; Dolgano-Nenets Autonomous Okrug, foothills of Byrranga Mountain Ridge, boulder fields of dolerite shales, on fine earth under rock, 74°27'59" N, 99°47'25"E, 30.VII.2004 V.E. Fedosov [KPABG 107960] with *Anthelia juratzkana*, *Blepharostoma brevirete*; *Pseudotritomaria heterophylla*; the slope in Kotuy River lower course, near Khatanga settlement, 71°12'00"N, 102°37'00"E, 24.VIII.2013 V.E. Fedosov [KPABG 117025] with *Blepharostoma brevirete*; **Republic of Sakha (Yakutia)**, Lena River Delta, near Bragino Village, left bank of Olenekskaya bayou, polygonal tundra-boggy complex, 20. VIII.1979 V.R. Filin [KPABG 124398, gyn.], with *Mesoptychia sahlbergii*, *Blepharostoma brevirete*, *Aneura pinguis*; ibidem, in cracks between polygons, 20. VIII.1979 V.R. Filin [KPABG 124397, andr.], **NORWAY: Svalbard, Dickson Land**, 79°2'16" N, 15°35'20"E, 04.VIII.1973 A.A. Frisvoll [KPABG 122667 duplicate from TRH 732314] mixed with *Blepharostoma brevirete*, *Mesoptychia collaris*.

**Variation.** The nucleotide sequence variability is quite low in all studied specimens of *Cryptocolea*. It does not exceed 1.9% in ITS1-2, 0.2% in *trnL-F* and 0.2% in *rbcL* (Table 2a, b). The only exception is the specimen from Taimyr (KPABG 124714), which differs from others in 7.1–7.6% in ITS1-2, 1.9–2.1% in *trnL-F* and 0.7–0.9% in *rbcL*, which is significantly lower than in the phylogenetically close genera *Southbya* and *Gongylanthus*. The species divergence within *Southbya* and *Gongylanthus* is more evident from both chloroplast loci, than the divergence of one Taimyr specimen (KPABG 124714) of *Cryptocolea imbricata* from the other six specimens gathered in remote localities. The rate of mutations varied in nuclear and chloroplast genome, the data concerning variability in the phylogenetically closed taxa are fragmentary, thus the taxonomical separation of the Taimyr specimen (KPABG 124714) from molecular evidence appears to be premature.

Morphologically, the specimen from Taimyr KPABG 124714 differs in very small size, just 0.4–0.5 mm wide and 1–1.5 (2) mm long; numerous very long and dense rhizoids throughout the length of plant; minute trigones; transversely elliptical, much smaller than in other studied specimens leaves which are 600–550 µm wide and 400–450 µm long; and almost not differentiated cells of leaves that are quite large from the margin to the middle, ca. (25) 32×(30) 35–37 µm, and just slightly larger at base where single cells can be 25–30×37–45 µm.

At this stage, we are reluctant to describe a new species based on one, quite poor specimen. Nevertheless, we consider it necessary to pay attention to such deviant forms by describing them as a separate variety based on both molecular and morphological differences.

***Cryptocolea imbricata* var. *minima*** Konstant., Vilnet et Mamontov, *var. nov.*

**Diagnosis.** *Cryptocolea imbricata* var. *minima* is characterized by very small size of plants, numerous very long and dense rhizoids, transversely elliptical leaves with cells of leaves almost not differentiated, female bracts not reflexed, and in differentiation in ITS1-2, *trnL-F* and *rbcL* nucleotide sequences.

**Holotype:** RUSSIA: **Krasnoyarsk Territory**, Taimyr Peninsula, Bataika river valley, spotty *Carex arctisibirica*-lichen moss tundra, 71°12'48"N, 92°41'26"E, 29 July 2021, with *Trilophozia scitula*, *Anthelia juratzkana*, E.D. Lapshina 213E/4-3-21 [KPABG 124714]. Other specimens examined: **Yamalo-Nenets Autonomous Okrug (Yugra)**, Nadymkiy District, dwarf shrub-lichen spotty tundra, on clay spot, 66°32'25"N, 73°52'9"E, 06.VIII.2019, E.D. Lapshina 385E/3-4-19 [KPABG 124450].

**Etymology.** The name reflects the very small size of plants.

The variety can be separated by the key below:

1. Plants medium-sized, shoots (0.7)0.8–1.3 mm wide and more than (4)5 mm long; leaves orbicular to broadly ovate, cells along margins and in upper part of leaf 20–25 µm, smaller than mid-leaf cells. ....  
..... var. *imbricata*
- Plants small, shoots 0.4–0.5 mm wide and 1–2 mm long; leaves, transversely elliptical up to 0.5–0.6 mm wide and just 0.4 mm long; leaf cells not differentiated, large, ca. 25–32×(30) 35–37 µm, including leaf margin..... var. *minima*

**Differentiation.** *Cryptocolea imbricata* is a quite distinct species. Female plants of the species differ from all other species with rounded, non-serrated leaves in having characteristic inflorescences, which form compact heads of closely imbricate, concave leaves, with the apical and marginal part of bracts often patent to reflexed. One of the most important features distinguishing this genus from the species of other genera with undivided leaves is the rudimentary perianth completely hidden in the bracts. Oil-bodies are quite characteristic, composed of many granules that never occur in any species of Sole-nostomataceae. It is more difficult to distinguish male plants; they are often differing, having quite specific bright golden brown to bright or dark bronze color, saucer-shaped, concave leaves, relatively large leaf cells, and pleasant smell of essential oil and relatively long persistent oil bodies composed of granules of various size. Fortunately, female plants occur in many specimens.

**Ecology.** The species occurs in wet calcareous sites both in lowland and mountain tundra belt. In the alpine belt of northern mountains the species grows on humus-covered or calcareous moist basic rocks, at base or in crevices of rocks where it occurs mostly mixed with other bryophytes, but can sometimes form almost pure mats, as e.g. in the Kamchatka Peninsula and the mountains of South Siberia. Its associates are other not common liverworts like *Gymnomitrium revolutum*, *Radula prolifera*, *Pseudotritomaria heterophylla*, *Frullania subarcti-*

ca, or in moist calcareous sites in northern regions widespread and more or less common *Calycularia laxa*, *Mesoptychia heterocolpos*, *M. sahlbergii*, *Scapania crassiretis*, *Trilophozia quinquedentata*, *Blepharostoma brevirete*, etc. In “difficult” habitats such as spots in spotted dwarf shrub-lichen or moss lichen tundra with low moisture the species occurs as the small dwarf variety *minima*. In such sites the described variety occurs as isolated scattered shoots among other bryophytes, e.g., *Tritomaria scitula*, *Anthelia juratzkana*, and *Jungermannia* sp.

**Distribution.** *Cryptocolea imbricata* was described by Schuster (1969) from an isolated location in Minnesota and then recorded by him from one station in Michigan and many localities in Ellesmere Island and Greenland (l. c.). The species was later found in Alaska (Steere and Inoue, 1978), Northwest Territories of Canada (Damscholt, 2007), a single locality in Europe (northern Sweden, Schuster & Mårtensson, 1978), one in Svalbard (Frisvoll & Elvebakk, 1996) and many localities in Asia, including its northern part (Konstantinova *et al.*, 2009, Schuster & Konstantinova, 1996; Fedosov *et al.*, 2015; Fedosov *et al.*, 2020; Konstantinova *et al.*, 2023), mountains of Yakutia (Sofronova, 2018), South Siberia (Mamontov, 2013), and Far East of Russia (Bakalin, 2010, 2015, Bakalin *et al.*, 2021) ( Fig. 6). The species occurs from almost sea level in the tundra zone, e.g. in the Yamal Peninsula (Potemkin, 1993) up to 2000 m alt in the Kodar Range and Tardoki-Yani Mountains (South Siberia, Bakalin, 2015) or Kamchatka Peninsula (see specimens examined).

Thus, *Cryptocolea imbricata* can be characterized as an arctomontane, almost circumpolar species.

#### DISCUSSION

*Cryptocolea imbricata* was described relatively recently, in the middle of the 20th century, and was for a long time known only from the north of North America, Greenland and Ellesmere Island (Schuster, 1969). Like a number of other liverworts described by Schuster from North America and Greenland (e.g. *Pseudotritomaria heterophylla* (R.M. Schust.) Konstant. et Vilnet, *Lophozopsis polaris* (R.M. Schust.) Konstant. et Vilnet, *Frullania stylifera* (R.M. Schust.) R.M. Schust., etc.), *Cryptocolea imbricata* turned out to be widespread in northern Asia what has been revealed in the course of intensive collecting liverworts in the last 40 years. The results obtained in this molecular study had shown the artificiality of placing the genus in the family Solenostomataceae and directed our attention to a number of morphological features that had been overlooked earlier. Meanwhile, these features are obviously important, since they are associated with the protective organs of archegonia, as well as with the sculpture of spores. Thus, in the Solenostomataceae to which *Cryptocolea* was previously assigned, the perianthium is always well developed and more or less protruding from bracts. In *Cryptocolea*, the perianthium

is rudimentary and totally hidden in the bracts. Schuster (1969: 895) stressed the “rather complex” development of gynoecia and noted that “no trace of any structure that can be certainly identified as the perianth is evident at the time of fertilization”. We did not find structures similar to perianths either in the only specimen with mature sporophyte we examined, or in the studied specimens with unfertilized archegonia. This is completely unlike the well-developed, more or less long perianths inserted from bracts in Solenostomataceae; however, it is quite similar to the perianths of Southbyaceae, which are poorly developed, mostly bilobate, shorter than bracts. Another feature never found in the Solenostomataceae, but characteristic of the Southbyaceae is the peculiar margin of female bracts. In *Cryptocolea* female bracts bear slime papilla and sometimes single one celled teeth along margins; this places it closer to *Southbya* with its dentate margin of female bracts. In our opinion a very important feature is the surface of spores, in particular, the combination of worm-like and granular surface both in *Southbya* (compare, Paton, 1999: 430, Fig.212) and *Cryptocolea*; it is quite different in Solenostomataceae with its minutely papillose spores. All these morphological features confirm the unnatural position of *Cryptocolea* in the Solenostomataceae and are consistent with the results of molecular phylogeny.

The main drawback of our study is that we have not been able to obtain fresh material of this species for sequencing from America, from where the species was described. Given the characteristic appearance of the species, its generally insignificant morphological variability, it can be neglected at this stage. However, it is necessary to keep this in mind and molecular study of specimens of *Cryptocolea* from America is necessary. Another, no less interesting area of future study is the purposeful integrative study of *Cryptocolea* specimens from bare soil in spotted tundra. Apart of type, one specimen from Nenets Autonomous Okrug [KPABG 124450] was referred by us to var. *minima* but on the basis of morphological features only. This specimen contains just several very small plants mixed with other species and very tightly pressed to the substrate, so we have not sequenced it. Apparently, the spots in the spotted tundra are quite a characteristic habitat for var. *minima*. And perhaps also, given the level of genetic divergence, the variety deserves to be separated into a distinct species. However, further research is needed to verify this idea. In general, the diversity of liverworts on spots in spotted tundra is greatly underestimated; it concerns species of the genera *Nardia*, *Solenostoma*, and *Marsupella*. In the specific conditions of such habitats, species often have an extremely uncharacteristic appearance. The question is how much it is fixed genetically. The problem lies in the complexity of studying such habitats, both due to the inaccessibility of these locations, and in huge diversity of such sites, as well as a significant variability of conditions even within

one area. It would seem that molecular genetic methods can solve at least some of these problems, however, the use of an integrative approach is very much limited by the lack of verified data of the most Arctic and Arctic-montane species.

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