

Article

New Updates on the Distribution of *Scapania umbrosa* (Schrad.) Dumort. (Scapaniaceae, Marchantiophyta) in Pacific Asia

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Abstract: The distribution of liverworts in the North Pacific is still poorly understood. *Scapania umbrosa*, which is rare in Asia, was first recorded during the study of the liverwort flora of the Commander Islands (the westernmost Aleutians). The results of the molecular genetic analysis not only confirmed that the specimen belonged to *Scapania umbrosa* but also demonstrated the genetic specificity of the discovered population in comparison with the European, Siberian, and Alaskan populations. The island population of *Scapania umbrosa* is likely a recently diverged isolate of the species. Previously reported data on the distribution of *Scapania umbrosa* in the Russian Far East from Kamchatka and Kunashir (VBI Herbarium), as well as from Sakhalin and Southern Primorye (reported in GBIF), are based on misidentifications. Morphological descriptions, distinguishing characters, line drawings, and photographs of *Scapania umbrosa* based on the collected material are provided.

Keywords: *Scapania umbrosa*; Scapaniaceae; taxonomy; liverworts; East Asia; Commander Islands; North Pacific



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1. Introduction

Many liverwort species are considered to have a circumpolar distribution. However, only a molecular genetic analysis can definitively show whether it is just one species or several. This may especially concern taxa with generally suboceanic distributions, where Pacific and Atlantic areas are separated by thousands of kilometers in Asia and/or North America. Molecular genetic studies in recent years have often supported the use of geographical approaches for describing taxa that are new for science. For example, this regularity was observed in *Frullania* [1], *Odontoschisma* [2], *Blepharostoma* [3], *Calypogeia* [4], and *Tetralophozia* [5]. One of the boreal subcircumpolar taxa is *Scapania umbrosa* [6], which is distributed quite sparsely. Heinrichs et al. [7] found it in the sister relationships with *Scapania apiculata* and placed into sect. *Apiculatae*, while Choi et al. [8], treated it in its own sect. *Umbrosae*. Indeed, two taxa are similar in the narrow and apiculate leaf lobes with rather prominently pachydermous leaf cell network, although there are several distinctions, including gemmae features and perianth armature. The ecology of two species is also looking somewhat similar. *Scapania apiculata* Spruce is an obligate epixylous taxon, while *S. umbrosa* (Schrad.) Dumort. only prefers these habitats, although occurring on other substrates too. The distribution of the species has some gaps, including between the Urals and East Siberia in Eurasia and between the west and east coasts of North America. This distribution feature may be simply caused by species requirements for high air humidity or may indicate genetic differentiation of the Pacific and Atlantic populations of the species. The distribution of *Scapania umbrosa* in Pacific Asia is uncertain. There are several reports (mostly summarized in [8]), and all of them are based on morphological studies, and there is no molecular evidence for the distribution of this species in this huge area after a strong gap in distribution from the Sub-Atlantic part of the area. When we found *Scapania umbrosa*

on the Commander Islands—the flora of which occupies an intermediate position between Eurasian and American floras in a phytogeographical sense [9–11]—we were inspired to verify the intraspecific differentiation of this taxon on the available material in comparison with available accessions from Europe and North America. The latter also resulted in the revision of the available herbarium material previously collected in the Russian Far East. The revision of the herbarium material revealed that at least a vast majority of the species reports (if not all) for the Far East are based on the mistakes in identifications. That makes especially valuable the comparison of newly obtained material from the Commanders also using molecular-genetic methods. Therefore, the main goal of the present account is to provide new and updated data on the distribution of *Scapania umbrosa* in the Pacific Asia and to discuss the possible genetic differentiations of the Commander Islands population from other genetically studied populations of the species.

2. Materials and Methods

2.1. Study Area and Specimen Collection

Specimens of *Scapania umbrosa* were collected by the senior author during field work on Bering Island in 2022. This was the third expedition (following the expeditions organized by Bakalin in 2002 and Bakalin and Klimova in 2021) devoted to the study of liverworts on the island. Bering Island is located in the northern part of the Pacific Ocean, ca. 180 km eastward from the Kamchatka Peninsula. The other island (Mednyi) of the Commander Archipelago is located 60 km east of Bering Island. Moreover, a durable gap of 340 km occurs between Mednyi Island and Attu Island, which belong to the Near Island group of the Aleutians eastward. Bering Island is devoid of forests; specifically, meadow and tundra landscapes with many watercourses, ponds, and mires dominate in this location. According to the Köppen–Geiger classification [12], most of Bering Island has a Dfc climate (subarctic climate), with some ‘spots’ of the ET climate (polar or tundra climate) on the Pacific Coast of the southern part of the island. The average annual temperature is positive (+2.1 °C), with the total amplitude of the annual temperature reaching only 15 °C; the average annual number of sunny days is approximately 40, and the annual precipitation is approximately 480 mm (due to data from the weather station of Nikol’skoe Settlement). A particular feature is the low intensity of the local rains associated with their significant duration. The average monthly relative humidity and annual average relative humidity are high; specifically, on average, the air humidity per year is more than 80%, and it changes little throughout the year [9,13].

Only one decaying log on which *Scapania umbrosa* was growing was found. Two specimens were collected from it. The collected material was transported to Vladivostok to the Laboratory of Cryptogamic Biota of the Botanical Garden Institute FEB RAS (herbarium international acronym VBGI [14]), where it was examined by using standard anatomic-morphological techniques. Photographs of living plants, plant parts, and oil bodies were obtained. After the identification process, the samples for DNA analysis were collected, while parts of the plants were rapidly dried in silica jelly.

To clarify the distribution of *Scapania umbrosa* in Pacific Asia, we checked records of the species in the Global Biodiversity Information Facility (GBIF) [15].

2.2. Molecular-Genetic Analysis

2.2.1. Taxon Sampling

To determine the phylogenetic position of the Bering Island population, the dataset for the molecular phylogenetic analysis was compiled. It includes one specimen of *Scapania umbrosa* from the Commander Islands (specimen voucher—Com-67-1-22) and three additional specimens of *S. umbrosa* that were collected from the Tuva Republic (Russia, South Siberia) (VB-99-6-1. . .33), Alaska State (USA) (NK28-1-92), and Saxony (Germany) (GLM-B-20091) (duplicates of all specimens are in the VBGI herbarium). The specimens from Siberia and Alaska were obtained from KPABG, courtesy of Prof. Konstantinova (VB-99-6-1. . .33, NK28-1-92, KPABG). Due to the fact that we succeeded in obtaining a

sequence for only one locus (*trnL–trnF*), the last specimen was not included in the resulting tree and in the throughout analysis, but it was uploaded to the NCBI database (GenBank).

Phylogenies were constructed for the *Scapania* sect. *Umbrosae* and *Scapania* specimens from several closely related sections following the general phylogeny of the genus published in [7,16]. Two specimens of *S. microdonta* were used as an outgroup for tree rooting. The specimen voucher details, including the GenBank accession numbers, are listed in Table 1.

Table 1. The list of voucher details and GenBank accession numbers for the specimens used in the phylogenetic analysis in the present paper. The newly obtained sequences are marked in bold.

GenBank Name	Accepted Name	Specimen Voucher	GenBank Accession Number	
			ITS1–ITS2 nrDNA	<i>trnL–trnF</i> cpDNA
<i>Scapania apiculata</i> Spruce	<i>Scapania apiculata</i> Spruce	Russia, Rep. Buryatiya, N.A. Konstantinova, 2 August 2022, HRE 49 (KPABG)	EU791741	EU791633
<i>Scapania umbrosa</i> (Schrad.) Dumort.	<i>Scapania umbrosa</i> (Schrad.) Dumort.	Germany, Eckstein 6509 (GOET)	JN631481	JN631615
<i>Scapania umbrosa</i> (Schrad.) Dumort.	<i>Scapania umbrosa</i> (Schrad.) Dumort.	Germany, Schroeder 3-9-1996 (JE)	JN631483	JN631617
<i>Scapania umbrosa</i> (Schrad.) Dumort.	<i>Scapania umbrosa</i> (Schrad.) Dumort.	Russia, Komi Rep., M. Dulin, MD 139-1-99 (KPABG)	EU791740	EU791632
<i>Scapania umbrosa</i> (Schrad.) Dumort.	<i>Scapania umbrosa</i> (Schrad.) Dumort.	Germany, Saxony, M. Reimann, 6 August 1997, GLM-B-20091	-	OR449400
<i>Scapania umbrosa</i> (Schrad.) Dumort.	<i>Scapania umbrosa</i> (Schrad.) Dumort.	Siberia, Tuva Republic, V.A. Bakalin, 8 June 1999, VB-99-6-1..33	OR437974	OR449401
<i>Scapania umbrosa</i> (Schrad.) Dumort.	<i>Scapania umbrosa</i> (Schrad.) Dumort.	USA, Alaska State, N.A. Konstantinova, 29 June 1992, NK28-1-92	OR437975	OR449402
<i>Scapania umbrosa</i> (Schrad.) Dumort.	<i>Scapania umbrosa</i> (Schrad.) Dumort.	Russia, Kamchatka Territory, Aleutsky District, Commander Islands, K.G. Klimova, 30 August 2022, Com-67-1-22 (VBGI)	OR437976	OR449403
<i>Scapania undulata</i> (L.) Dumort.	<i>Scapania undulata</i> (L.) Dumort.	Russia, Murmanskaya Province, N.A. Konstantinova, 208-2-02 (KPABG)	EU791751	EU791642
<i>Scapania undulata</i> (L.) Dumort.	<i>Scapania undulata</i> (L.) Dumort.	USA, Shevock et al. 29009 (GOET)	JN631489	JN631623
<i>Scapania undulata</i> (L.) Dumort.	<i>Scapania undulata</i> (L.) Dumort.	Russia, Sakhalin Province, Kuril Isl., Kunashir I., V.A. Bakalin, and K.G. Klimova, K-42-6-18, 57856 (VBGI), 122474 (KPABG)	MZ272024	MZ274278
<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	Russia, Rep. Buryatiya, N.A. Konstantinova, 136-4-01 (KPABG)	EU791749	EU791640
<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	Russia, Huneck 28-6-1990 (JE)	JN631473	JN631607
<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	Russia, N.A. Konstantinova, Hep. Ross. Exs. 50 (GOET)	JN631474	JN631608
<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	Russia, Murmansk Province, N.A. Konstantinova, 28 September 2019 (KPABG)	OP584680	OP573519

Table 1. Cont.

GenBank Name	Accepted Name	Specimen Voucher	GenBank Accession Number	
			ITS1–ITS2 nrDNA	<i>trnL–trnF</i> cpDNA
<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	<i>Scapania subalpina</i> (Nees ex Lindenb.) Dumort.	Russia, Murmansk Province, N.A. Konstantinova, 28 September 2019 (KPABG)	OP584680	OP573519
<i>Scapania obscura</i> (Arnell et C.E.O. Jensen) Schiffn.	<i>Scapania obscura</i> (Arnell et C.E.O. Jensen) Schiffn.	Russia, Magadan Province, V.A. Bakalin, Mag-22-6-10 (KPABG)	JX629927	JX630058
<i>Scapania uliginosa</i> (Lindenb.) Dumort.	<i>Scapania uliginosa</i> (Lindenb.) Dumort.	Russia, Murmanskaya Province, V.A. Bakalin, 25-7-01 (KPABG)	EU791739	EU791631
<i>Scapania uliginosa</i> (Lindenb.) Dumort.	<i>Scapania uliginosa</i> (Lindenb.) Dumort.	Austria, Schäfer-Verwimp and Verwimp 18181 (GOET)	JN631479	JN631613
<i>Scapania uliginosa</i> (Lindenb.) Dumort.	<i>Scapania uliginosa</i> (Lindenb.) Dumort.	Russia, Murmansk Province, N.A. Konstantinova, K26-3-88 (KPABG)	OP584677	OP573516
<i>Scapania paludosa</i> (Müll. Frib.) Müll. Frib.	<i>Scapania paludosa</i> (Müll. Frib.) Müll. Frib.	Russia, Kemerovskaya Province, N.A. Konstantinova, 4-3-00 (KPABG)	EU791747	EU791638
<i>Scapania paludosa</i> (Müll. Frib.) Müll. Frib.	<i>Scapania paludosa</i> (Müll. Frib.) Müll. Frib.	Russia, Permskiy Kray, N.A. Konstantinova, K316-2-04 (KPABG)	EU791748	EU791639
<i>Scapania rufidula</i> Warnst.	<i>Scapania rufidula</i> Warnst.	Russia, Rep. Yakutiya, V.A. Bakalin, 35-3-00 (KPABG)	EU791746	EU791637
<i>Scapania spitsbergensis</i> (Lindb.) Müll. Frib.	<i>Scapania spitsbergensis</i> (Lindb.) Müll. Frib.	Svalbard, Spitsbergen, N.A. Konstantinova, K 90-2-06 (KPABG)	EU791761	EU791652
<i>Scapania glaucocephala</i> (Taylor) Austin	<i>Scapania glaucocephala</i> (Taylor) Austin	Russia, Siberia, Buryatiya Rep., N.A. Konstantinova, 64-05-02 (KPABG)	EU791753	EU791644
<i>Scapania hyperborea</i> Jørg.	<i>Scapania hyperborea</i> Jørg.	Russia, Murmansk, N.A. Konstantinova, 509-3a-04 (KPABG)	EU791744	EU791635
<i>Scapania hyperborea</i> Jørg.	<i>Scapania hyperborea</i> Jørg.	Russia, Yakutia, V.A. Bakalin, 1-10-00 (KPABG)	EU791745	EU791636
<i>Scapania tundrae</i> (Arnell) H. Buch	<i>Scapania tundrae</i> (Arnell) H. Buch	Norway, Svalbard, N.A. Konstantinova, A.N. Savchenko, K42-1a-06 (KPABG)	MT334461	MT338486
<i>Scapania tundrae</i> (Arnell) H. Buch	<i>Scapania tundrae</i> (Arnell) H. Buch	Norway, Spitsbergen, N.A. Konstantinova, 140-1-04 (KPABG)	EU791725 and EU791742	EU791634
<i>Scapania paludicola</i> Loeske et Müll. Frib.	<i>Scapania paludicola</i> Loeske et Müll. Frib.	Russia, Karelia, V.A. Bakalin, 11 August 1997 (KPABG)	EU791743	AF519196
<i>Scapania paludicola</i> Loeske et Müll. Frib.	<i>Scapania paludicola</i> Loeske et Müll. Frib.	United Kingdom, Wales, Glamorgan, D.G. Long et al. 40,399 (E)	JN631459	JN631595
<i>Scapania paludicola</i> Loeske et Müll. Frib.	<i>Scapania tundrae</i> (Arnell) H. Buch	Norway, Svalbard, N.A. Konstantinova, K42-1a-06 (KPABG)	OP584679	OP573518
<i>Macrodiplophyllum microdontum</i> (Mitt.) Müll. Frib.	<i>Scapania microdonta</i> (Mitt.) Müll. Frib.	Russia, Primorye Territory, V.A. Bakalin, P-74-11-05 (GOET)	JN631445	JN631580
<i>Macrodiplophyllum microdontum</i> (Mitt.) Müll. Frib.	<i>Scapania microdonta</i> (Mitt.) Müll. Frib.	Russia, Siberia, Buryatiya Rep., N.A. Konstantinova, 146-12-01 (KPABG)	EU791769	AF519199

2.2.2. DNA Isolation, Amplification, and Sequencing

Two markers (the nuclear ITS1–ITS2 (nrDNA) region and chloroplast *trnL–trnF* (tRNA-Leu (*trnL*) gene, partial sequence + *trnL–trnF* intergenic spacer, complete sequence+ tRNA-Phe (*trnF*) gene, partial sequence) region) were used for the phylogenetic analysis. DNA was extracted from the dried liverwort tissues by using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The amplification of ITS1–ITS2 nrDNA and *trnL–trnF* cpDNA was performed by using an Encyclo Plus PCR kit (Evrogen, Moscow, Russia) with the primers listed in Table 2. To prevent cross-contamination, sterile mortars and pestles were used to isolate DNA from the specimen, and the samples were treated alternately, separately from each other. The PCR reaction was performed with positive and negative controls and jointly with samples from other families. First of all, the obtained chromatograms were tested using nucleotide BLAST (NCBI).

Table 2. Primers used in polymerase chain reaction (PCR) and cycle sequencing.

Locus	Sequence (5'-3')	Direction	Annealing Temperature (°C)	Reference
<i>trnL–trnF</i> cpDNA	CGAAATTGGTAGACGCTGCG	forward	62	[17]
<i>trnL–trnF</i> cpDNA	TGCCAGAAACCAGATTTGAAC	reverse	58	[17]
ITS1–ITS2 nrDNA	ACCTGCGGAAGGATCATTG	forward	58	[18]
ITS1–ITS2 nrDNA	GATATGCTTAAACTCAGCGG	reverse	58	[19]

The polymerase chain reaction was performed in a total volume of 20 µL, including 1 µL of template DNA, 0.4 µL of 50× Encyclo polymerase Mix (concentration in the final volume of the reaction mix is 1×), 4 µL of 5x Encyclo Red buffer (concentration in the final volume of the reaction mix is 1×), 0.4 µL of dNTP mixture (0.16 mM each), 13.4 µL (for *trnL–trnF* cpDNA)/12.4 µL (for ITS1–ITS2 nrDNA) of nuclease-free deionized water, 1 µL of dimethylsulfoxide/DMSO (5% in the final volume of the reaction mix) only for ITS1–ITS2 nrDNA, and 0.4 µL of each primer (forward and reverse, 0.2 µM in the final volume of the reaction mix). The concentration of Mg²⁺ in the final reaction was 3.5 mM. All reagents, excluding DMSO, are from the Encyclo Plus PCR Kit, Evrogen, Moscow, Russia. The DMSO reagent is from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India. Polymerase chain reactions were performed by using the protocols for amplification listed in Table 3.

Table 3. Protocols for PCRs.

Initial Denaturation	3 min—94 °C	
Denaturation	30 s—95 °C	
Annealing	20 s (<i>trnL–trnF</i> cpDNA), 30 s (ITS1–ITS2 nrDNA) at 58 °C (<i>trnL–trnF</i> cpDNA), 60 °C (ITS1–ITS2 nrDNA)	30 cycles
Elongation	30 s—72 °C	
Final elongation	3 min—72 °C	

Amplified fragments were visualized on 1% agarose TAE gels via EthBr (Ethidium bromide) staining and purified by using the Cleanup Mini Kit (Evrogen, Moscow, Russia). The DNA was sequenced by using the BigDye Terminator Cycle Sequencing Kit (ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit) v. 3.1 (Applied Biosystems, Waltham, MA, USA), with further analysis of the reaction products following the standard protocol on an automatic sequencer 3730 DNA Analyzer (Applied Biosystems, Waltham, MA, USA) in the Genome Center, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow.

2.2.3. Phylogenetic Analyses

Two datasets were compiled for the ITS1–ITS2 nrDNA and *trnL–trnF* cpDNA loci, aligned by using MAFFT [20–22], with standard settings, and then manually edited in BioEdit ver. 7.2.5 [23]. The topologies of the two trees based on the obtained alignments were similar; thus, we combined them into one alignment (ITS1–ITS2 plus *trnL–trnF*) and built a consensus tree. All of the positions of the final alignment were included in the phylogenetic analyses. Missing data at the ends of regions and gaps were treated as missing data.

Phylogenetic trees were reconstructed by using two approaches: maximum likelihood (ML) [24] with IQ-tree ver. 1.6.12 [25] and Bayesian inference (BI) [26] with MrBayes ver. 3.2.7 [27].

For the ML analysis, the best-fitting evolutionary model of nucleotide substitutions according to the Bayesian Information Criterion (BIC) value was TVMe+R3 (The TVMe model is transversion model, AG=CT, and equal base freq., R3, reflects the empirical base frequencies and heterogeneity rates across the sites), which was determined by ModelFinder (a model selection method implemented in IQ-tree) [28]. Consensus trees were constructed with 1000 bootstrap replicates.

BI analyses were performed by running two parallel analyses via the GTR+I+G model. The analysis consisted of four Markov chains. Chains were run for five million generations, and trees were sampled every 500th generation. The first 2500 trees in each run were discarded as burn-in; thereafter, 15,000 trees were sampled from both runs to produce the resulting tree. Bayesian posterior probabilities were calculated from the trees sampled after burn-in. The average standard deviation of the split frequencies between the two runs reached 0.001 before the analysis was stopped.

The sequence variability among the specimens of the genus *Scapania* sect. *Umbrosae*, sect. *Hyperborea*, and sect. *Apiculatae* was evaluated via the *p*-distances for each DNA locus in Mega XI [29] by using the pairwise deletion option for counting gaps.

To obtain a full picture of the relationships among the *Scapaniella*, *Umbrosae*, *Hyperborea*, and *Apiculatae* sections, we used Splits Tree and Neighbor Net methods that were implemented in SplitsTree v.4.14.2 [30] and TCS to visualize the molecular relationships within this complex. A haplotype network was constructed by using the TCS network inference method [31] via the PopART package (<http://popart.otago.ac.nz/>, accessed on 19 March 2024), which was accessed on 8 November 2019 [32]. The PopART program automatically removes positions with at least one N or a gap value from consideration.

3. Results

3.1. Molecular-Genetic Estimations

Seven accessions (three of ITS1–ITS2 nrDNA and four of *trnL–trnF* cpDNA) were produced and deposited into GenBank for the *Scapania umbrosa* specimens. They are very similar to sequences of *Scapania umbrosa* deposited in the GenBank.

The trees constructed for ITS1–ITS2 and *trnL–trnF* loci appeared highly congruent. The ML criterion recovered a bootstrap consensus tree (Figure 1) with a log-likelihood = −5940.817. The arithmetic means of the log-likelihoods in Bayesian analysis for each sampling run were −5964.64 and −5963.13.

The phylogenetic analysis revealed that the tested specimens of *Scapania umbrosa* collected on Bering Island should be placed in sect. *Umbrosae* together with specimens from Alaska, Tuva Rep., Germany, and Komi Rep. The divergence of *S. umbrosa* from other molecularly related species is listed in Table 4. It reached 7.02–8.84% for the ITS1–ITS2 locus and 2.09–3.6% for *trnL–trnF*. This divergence level is similar to the difference between *Scapania* species from neighboring clades; specifically, sect. *Apiculatae* and sect. *Hyperborea*. *Scapania umbrosa* from the Commander Islands was well distinguished both on the TCS network and on the Haplotype network (Figures 2 and 3).

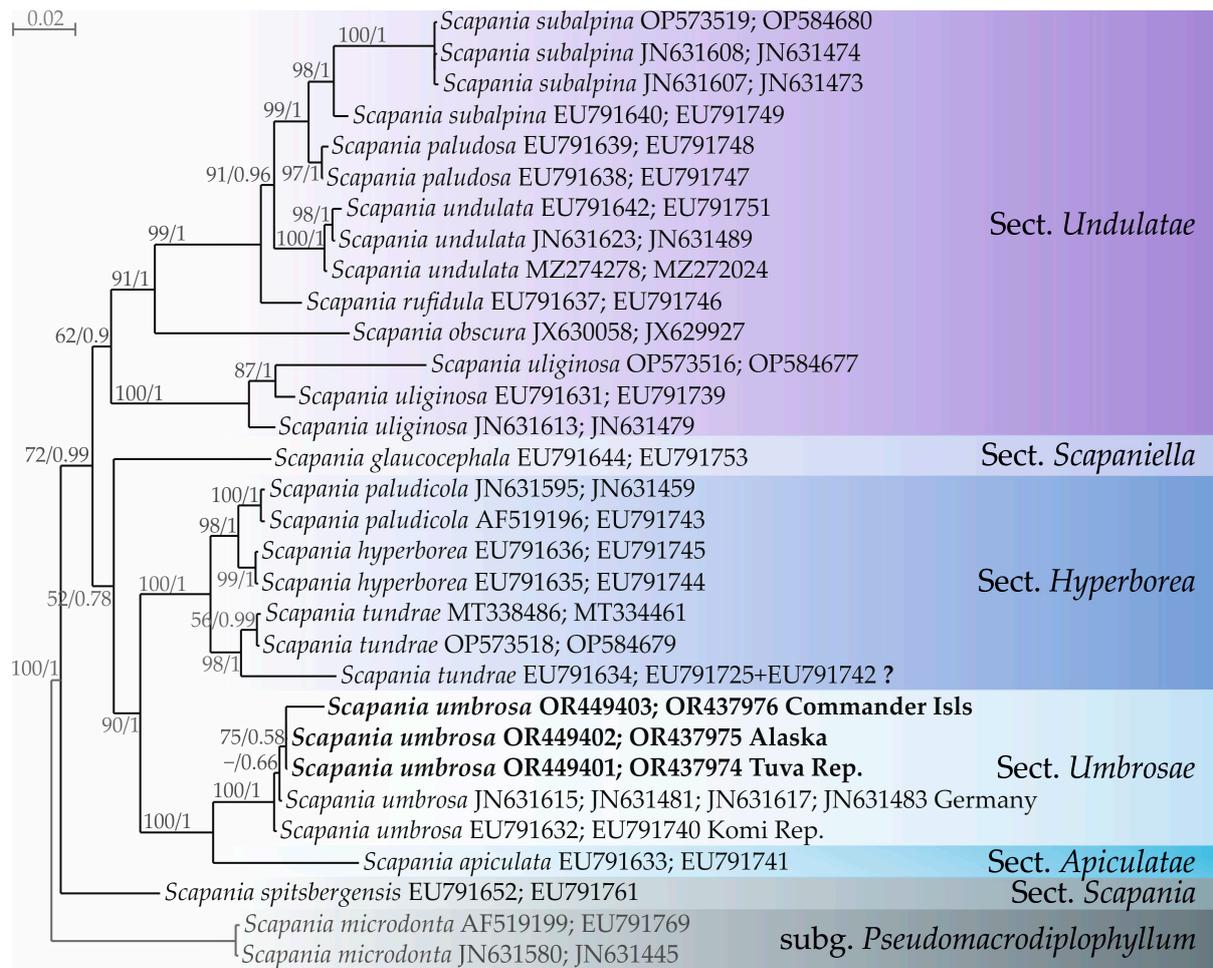


Figure 1. Phylogram obtained in a Bayesian (BI) and maximum likelihood (ML) analysis for the combined ITS1–ITS2 and *trnL–trnF* dataset for the *Scapania* species. GenBank accession numbers and voucher details are provided; bootstrap support values more than 50% (0.5 in BI) are indicated. Specimens obtained by the authors are marked in bold. Section subdivision follows [32]. Question mark is a specimen of *S. paludicola* from the NCBI database, which we renamed as described in Section 3.

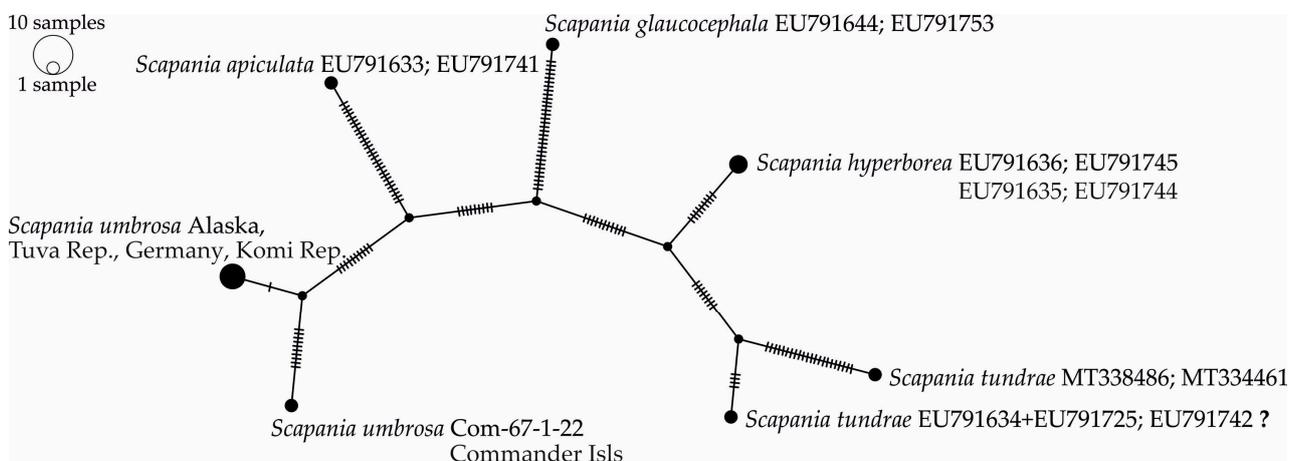


Figure 2. TCS haplotype network of ITS1–ITS2 + *trnL–trnF* sequences. Dashes indicate the number of nucleotide substitutions. Question mark is a specimen of *S. paludicola* from the NCBI database, which we renamed as described in Section 3.

Table 4. Intraspecific and interspecific *p*-distances, calculation based on ITS1–ITS2 (distances on lower left) and *trnL–trnF* (distances on upper right) nucleotide sequence data. The numbers of base differences per site from averaging over all sequence pairs within and between each group are shown. “*S. umbrosa* 1” group includes only the *S. umbrosa* specimen from the Commander Islands, “*S. umbrosa* 2” group includes all available *S. umbrosa* specimens at the moment of publishing, including a specimen from the Commander Islands, the “*S. umbrosa* 3” group includes all available *S. umbrosa* specimens at the moment of publishing excluding specimen from the Commander Islands. The justification for the allocation of the “*S. tundrae* 1” and “*S. tundrae* 2” groups is described in Section 3.

No.	Taxa	Intraspecific <i>p</i> -Distances, ITS1–ITS2/ <i>trnL–trnF</i> , %	Interspecific <i>p</i> -Distances, ITS1–ITS2/ <i>trnL–trnF</i> , %							
			1	2	3	4	5	6	7	8
1	<i>S. apiculata</i>	n/c/n/c		3.41	3.50	4.66	4.68	3.36	2.93	2.85
2	<i>S. hyperborea</i>	0/0	10.11		0	2.07	2.1	2.44	2.2	2.16
3	<i>S. paludicola</i>	0.13/0	10.1	1.99		1.78	1.8	2.3	2.12	2.09
4	<i>S. tundrae</i> 1	3.11/0.44	10.78	3.81	4.38		0.29	3.5	3.29	3.25
5	<i>S. tundrae</i> 2	0/0.43	10.14	3.02	3.44	1.56		3.6	3.38	3.34
6	<i>S. umbrosa</i> 1	n/c/n/c	8.48	8.23	8.66	8.84	8.5		0.24	0.29
7	<i>S. umbrosa</i> 2	0.73/0.14	7.31	7.62	8.16	8.31	7.71	1.45		0.1
8	<i>S. umbrosa</i> 3	0/0.09	7.02	7.47	8.03	8.18	7.52	1.81	0.36	

“n/c”—non calculated value due to single specimen.

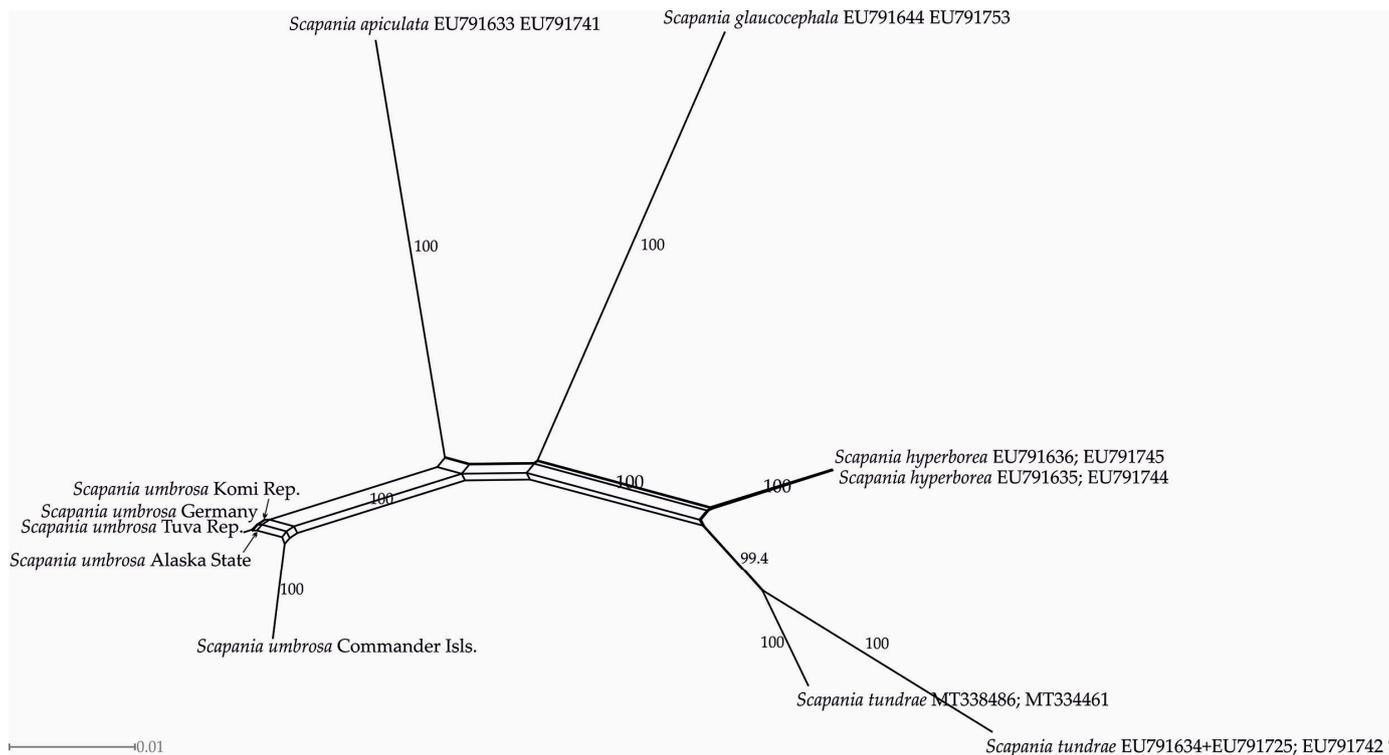


Figure 3. NeighborNet split network for specimens *Scapania* sect. *Umbrosae*, *Hyperborea*, and *Apiculatae*, based on the ITS1–ITS2 + *trnL–trnF* dataset. Bootstrap support values of 95 or higher are indicated. Question mark is a specimen of *S. paludicola* from the NCBI database, which we renamed as described in Section 3.

During the process of preparing the molecular-genetic portion of this study, we found that the sequences of *Scapania tundrae* and *Scapania paludicola* (ITS: MT334461, OP584679) that were loaded into the NCBI database were identical, and the *trnL–trnF* sequences

(MT338486, OP573518) also differed little from each other; thus, we renamed the *S. paludicola* in our tree and in Table 1 to *S. tundrae*. Due to the fact that the field number in GenBank was the same, we assumed that there was an error of unclear origin. Furthermore, the intraspecific *p*-distances between the sequences of *Scapania tundrae* were too high (ITS1–ITS2 nrDNA) (Table 4), which indicates that the sequence of *Scapania tundrae* (EU791725 + EU791742; EU791634) is likely another taxon. We marked this sequence by a question mark after the GenBank Accession Numbers in the consensus tree, TCS, and Haplotype networks (Figures 1–3), and we created 2 groups in Table 4 by attaching this sample to another *S. tundrae* and by using only those samples that were named “*S. tundrae*” in the GenBank database.

3.2. Herbarium Specimens and Morphology Implication

Specimens from Kamchatka (K-137-7-04, K-137-8a-04, KPABG, VBGI) and Kunashir Island (Kuril Islands) (K-53-16-06, VBGI) were revised and do not belong to *Scapania umbrosa*. Kamchatka plants are evidently juvenile and differ from *S. umbrosa* plants in the entire leaf margin; moreover, they do not clearly have conduplicate leaves and may represent unusual depauperate modifications of *S. obscura*. The plants in the Kunashir specimen were from the depressed form of *S. parvidens*, which is common on the island. It differs from *S. umbrosa* in its smaller cells, wider leaf lobes and 1(-2)-celled, shortly ellipsoidal gemmae. We attempted to clarify the species identity by using molecular genetic methods but were unable to obtain a reliable sequence.

We also checked the *Scapania umbrosa* records in Pacific Asia in the GBIF [15]. The locality of the indicated species for Magadan Province [33,34] is the result of a coordinate error and is referred to as Alaska according to the textual labels. One specimen that was previously identified as *Scapania umbrosa* and cited for Kamchatka was not located in the present study; therefore, we cannot confirm or reject the occurrence of that species on the Kamchatka Peninsula. There are two identical accessions of the same specimen that were simply placed in the GBIF twice [35,36]. The specimens from Sakhalin [37] and Southern Primorye (Kedrovaya Pad' Nature Reserve) [38] that appeared in the GBIF were stored at the Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences (TAA Herbarium); moreover, they were kindly revised by Dr. Nele Ingerpuu and were reidentified by her as *Douinia plicata* (Lindb.) Konstant. et Vilnet.

3.3. Taxonomy

Although the descriptions of *S. umbrosa* were published several times in the literature, the descriptions were based on European and American material [39–41], whereas the only description of *S. umbrosa* in [8], formally based on Asian material, seems to be founded on misidentified specimens. When considering the genetic differences among the studied populations on Bering Island, we provide a morphological description of *Scapania umbrosa* based on Com-67-1-22 (cf. specimens examined), which is the first description of *Scapania umbrosa* plants collected in Pacific Asia and confirmed via molecular genetic methods.

Description: Plants erect to ascending, green to yellowish brownish and greenish brownish, scattered or forming loose mats over decaying wood, 0.3–0.8(–1.1) mm wide and 3–7 mm long, dorsiventrally compressed. Rhizoids abundant in lower part of shoots, not in fascicles, erect to obliquely spreading, originating from the midline of the ventral side. Stem straight, not branched (branches not seen because the colony is quite young and probably originating from recent introduction by gemmae), cuticle striolate; stem cross-section transversely ellipsoidal, well-developed 110–130 × 130–160 μm, 10–12 cells high, thick-walled in 1–4 rows of cells, cells 10–20 μm in diameter, while lumen (due to the thick walls) is only 5–12 μm in diameter, inner cells in the not centered group, shifted to the dorsal side, nearly the same size as outer cells, but looks larger due to thinner cell walls. Leaves transversely inserted, distinctly conduplicate, obliquely spreading, dorsally shortly or barely decurrent (less 1/4 of stem width), ventrally decurrent for 1/2–2/3 of stem width; keel straight, at 30–40° with axis, 1–2-stratose; leaves when flattened in the

slide unequally bilobed, ventral lobe widely lanceolate $500\text{--}650 \times 200\text{--}440 \mu\text{m}$, dorsal lobe $460\text{--}570 \times 150\text{--}380 \mu\text{m}$; leaf margin in both lobes coarsely dentate in upper halves, with triangular teeth 1–2(–3) cells wide and 1–4 cells long, in lower third of leaf outer side the margin entire or with sparse unicellular teeth, rarely densely toothed. Cells along leaf margin with unevenly thickened walls (in leaf sinus margin walls commonly thinner than in leaf outer margin), with moderate in size concave to triangular trigones, cell size $10\text{--}15 \mu\text{m}$ along margin, cuticle obscurely verrucose; cells in lobe middle subsodiametric to oblong, $20\text{--}37 \times 12\text{--}22 \mu\text{m}$, with relatively thin walls, trigones moderate in size to large, cuticle distinctly papillose. Gemmae in sterile plants abundant, forming brownish clusters between upper leaves, in the slide gemmae color colorless to brownish and pinkish; bicellular, bacilliform, $15\text{--}23 \times 7\text{--}10 \mu\text{m}$, merely thin-walled or walls slightly thickened. Generative organs not observed.

Illustrations in present paper: Figures 4 and 5.

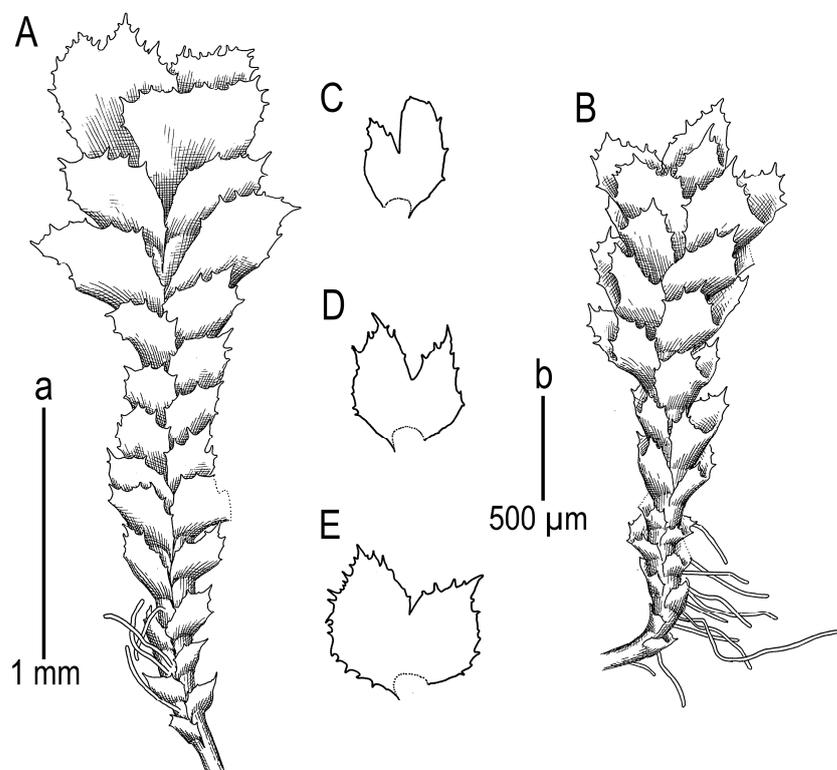


Figure 4. *Scapania umbrosa* (Schrad.) Dumort.: (A) plant habit, ventral view; (B) plant habit, dorsal view; (C–E) leaves. Scales: a—1 mm for (A,B); b—500 μm for (C–E). All from Com-67-1-22 (VBGI).

Specimens examined belonging to *S. umbrosa*: RUSSIA, the Russian Far East, Kamchatka Territory, Aleutsky District, Commander Islands, Bering Isl., southern part of the island, southern part of Mayatnik Bay ($54.81322 \text{ N } 166.64819 \text{ E}$), 10 m a.s.l., old driftwood in thickets of *Leymus mollis*, *Senecio cannabinifolius*, *Artemisia opulenta* on coastal meadow, rotten part of decaying log (driftwood), moist, in part shade, 30 August 2022, K.G. Klimova Com-67-1-22, Com-67-2-22 (VBGI); Siberia, Tuva Republic, Todzha Valley, Azas State Reserve, Azas Lake surroundings, swamp 5 km N of “Krasnyi Kamen” Station ($52.42639 \text{ N } 96.50222 \text{ E}$), decaying wood, 8 July 1999, V.A. Bakalin VB-99-6-1. .33 (KPABG, dupl. in VBGI); USA, Alaska State, Chugach Mtns., S of Portage ($60.83333 \text{ N } 148.91667 \text{ W}$), 60 m a.s.l., *Picea sitchensis* forest bordering intermediate fen, on rotting wood, 29 June 1992, N.A. Konstantinova NK28-1-92 (KPABG, dupl. in VBGI).

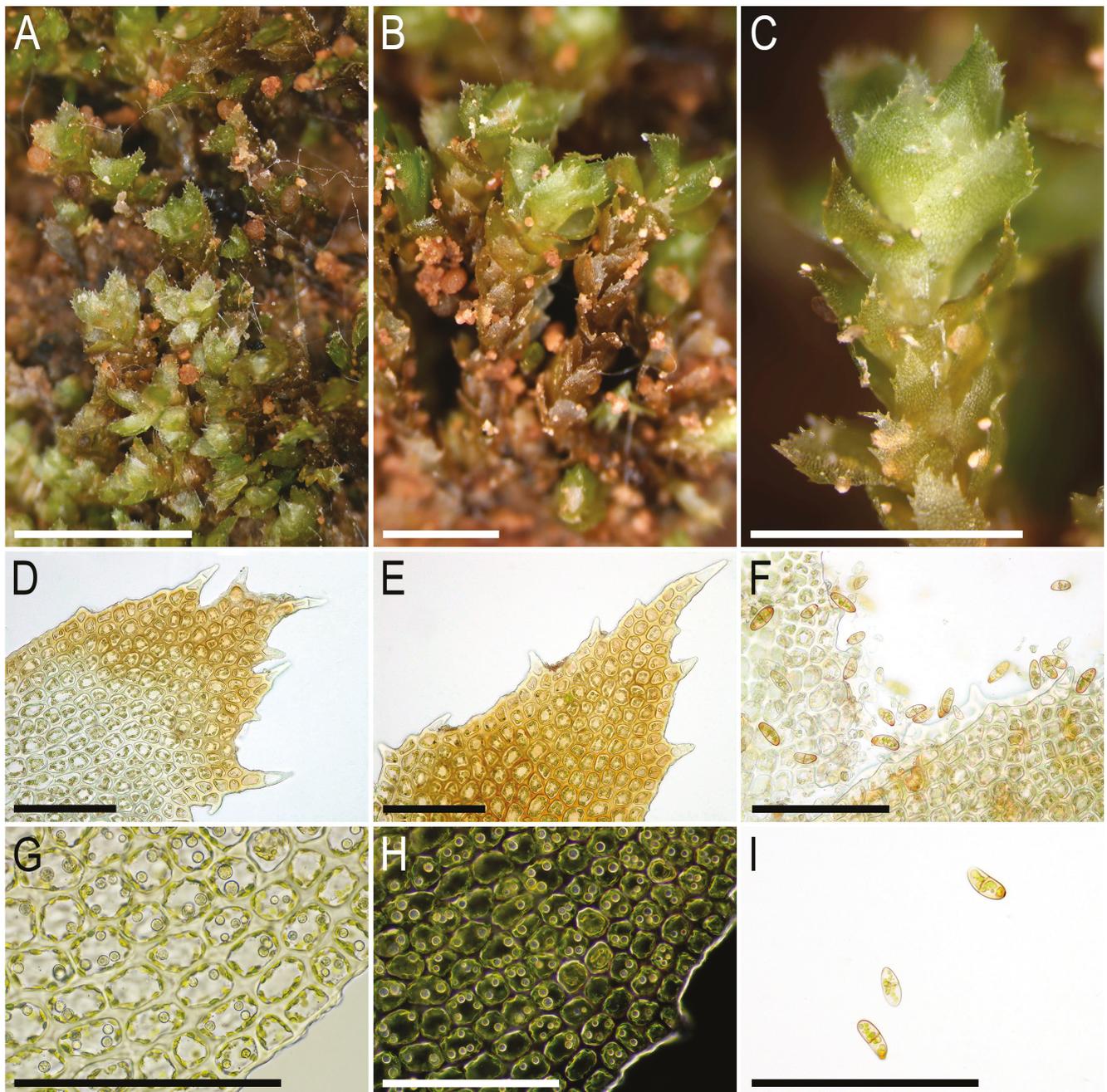


Figure 5. *Scapania umbrosa* (Schrad.) Dumort.: (A) mat, fragment; (B) shoots; (C) upper part of the shoot, dorsal view; (D) apex of ventral lobe; (E) apex of dorsal lobe; (F) gemmae on leaf lobes; (G) leaf margin cells with oil bodies; (H) leaf margin cells with oil bodies (photographed with dark field option); (I) gemmae. Scales: 2 mm for (A); 1 mm for (B,C); 100 µm for (D–I). All from Com-67-1-22 (VBGI).

Specimens examined not belonging to *S. umbrosa*, but being reported from the Pacific Asia under that name:

Scapania cf. *obscura* (Arnell et C.E.O. Jensen) Schiffn. (before reported as *S. umbrosa* in [32]) RUSSIA, Kamchatka Territory, Bystrinsky District, Nature Park Bystrinsky, Sredinnyi Range, the pass on watershed between Pravyy (Bolshoj) Kopkan River and Agapovskiy Stream (56.418333 N 159.215 E), 1400 m a.s.l., pit-run fines in snowbed habitat, 17 September 2004, V.A. Bakalin K-137-7-04, K-137-8a-04 (VBGI, KPABG) [42,43].

Scapania parvidens Steph. (before reported as *S. umbrosa* in [32]) RUSSIA, Sakhalin Province, Southern Kurils, Kunashir Island, middle part, the area of confluence of Kislaya and Lesnaya Rivers (44.005556 N 145.773056 E), 60 m a.s.l., on fain-grained soil of roadside in forest, 10 September 2007, V.A. Bakalin K-53-16-06 (VBGI, KPABG) [44].

Douinia plicata (Lindb.) Konstant. et Vilnet (before reported as *S. umbrosa* in [38]) RUSSIA, Primorye Territory, Khasansky District, Kedrovaya Pad' Nature Reserve (43.10331 N 131.49025 E), 25 July 1985, Kai Tönnsen (TAA5018484, TAA, the specimen was reidentified by N. Ingerpuu) [38].

Douinia plicata (Lindb.) Konstant. et Vilnet (before reported as *S. umbrosa* in [37]) RUSSIA, Sakhalin Province, Sakhalin Isl., Tymovsky Urban District, 15 km to the northwest from Lopatina Mt., bank of Dym River, spruce forest (50.92202 N 143.08144 E), 11 September 1975, Victor Masing (TAA5016210, TAA, the specimen was reidentified by N. Ingerpuu) [37].

4. Discussion

4.1. Molecular Divergence

The results of the phylogenetic analyses clearly show that the specimen from Bering Island belongs to *Scapania umbrosa*; conversely, they show that the insular species population is a recently diverged isolate of the species. Specifically, its haplotype differs by eleven nucleotide substitutions (Figure 2) from other haplotypes of specimens from Europe, Siberia, and Alaska, which were included in the analysis. In other words, plants in Alaskan specimens are genetically closer to plants in European and Siberian specimens than to plants from Commanders. Overall, the situation somewhat resembles what was recently described by Maltseva et al. [45] with *Scapania magadanica*. The latter was deeply nested within the clade of *S. kaurinii* but was clearly morphologically different from *S. kaurinii*. This problem was unsolvable by using the classical cladistic approach. However, the TCS haplotype network and NN split network showed five haplotypes for specimens of *S. magadanica*, forming a group that was “separated from the ‘*S. kaurinii*’ group by at least three nucleotide substitutions and from the closest of the revealed *S. kaurinii* haplotypes by five substitutions” [45] (p. 6). In the case of *Scapania umbrosa*, unexpectedly, we obtained only two haplotypes, namely *Scapania umbrosa* from Commanders and all of the remaining specimens from *S. umbrosa* (Germany; Komi and Tuva, Russia; and Alaska, USA). As previously mentioned, the revealed haplotypes are separated by eleven nucleotide substitutions, which may be rather common in infraspecific relationships. However, in contrast to the above-described case of the *S. kaurinii*–*S. magadanica* pair, we did not observe distinct morphological features to describe a new taxon. All of the differences are located within the infraspecific variation in *S. umbrosa*. Insular isolation has likely already led to molecular divergence but has not yet been reflected in the morphology of the population.

As noted above, we did not identify any reliable morphological differences between the population on the Commander Islands and other populations from other areas of distribution of this species worldwide. However, given the limited material available from the Commander Islands, it may be that we failed to identify some traits that could be considered environmentally induced and which could correlate with genetic differences. In addition, the lack of information on the distribution of *Scapania umbrosa* in the Aleutian Islands is not at all proof of the real absence of the species there, given the fragmentary data on the flora of Aleutian liverworts in general [46,47]. Thus, both of the following are equally probable: the distribution of the Alaskan and Commander haplotypes in the Aleutian Arch; and the distribution of only one (identified by us) haplotype, which may be connected by transitions with the haplotype known from Alaska, or may not be connected with it by the transitions.

4.2. Distribution

Scapania umbrosa occurs in almost all countries from the north to the south of Europe and generally has a sporadic distribution there [48]. In North America, the species range looks like two ‘stripes’ along the Atlantic and Pacific coasts of Canada and the USA, with

the ‘gap’ being present in the interior parts of the continent [39,49,50]. In the Russian Federation, *Scapania umbrosa* is also distributed sporadically and known from European Russia (North–West and Central Regions) [51–55] and from the Caucasus [56–58]. The first record of the species in Russian Asia (East Siberia, Todzha Valley) was published by Bakalin et al. [59]. For the Russian Far East, the species was first mentioned from the Stanovoy Uplands (Udokan Range, Amur Province) by Bakalin [60], and then from Southern Kurils [61] and from two locations in Kamchatka [62,63]. These specimens (K-137-7-04, K-137-8a-04, K-60-12-15, VBGI) were revised and confirmed by Choi et al. [8], except for one specimen (86-22-01, KPABG) from Kamchatka. We examined the morphology of previously confirmed specimens (K-137-7-04, K-137-8a-04, K-60-12-15, VBGI) and found that they did not belong to *Scapania umbrosa*, as discussed above.

Among adjacent countries, *Scapania umbrosa* is not known in Korea [64]. The species was mistakenly recorded in Japan from Shikoku [65] and was later referred to as *Scapania kamimurae* [66]. Afterwards, *Scapania kamimurae* was synonymized by Potemkin [67] under *Scapania integerrima*. Hence, according to published data, *Scapania umbrosa* was collected in Pacific Asia only in China and in Russia. This species was mentioned for Sichuan, Hunan, and Jiangxi [68]. There was no opportunity for us to study Chinese specimens, and we cannot confirm or disprove these identifications. If Chinese reports are not based on misidentification, then the question arises as to whether the plants from that country belong to already existing groups of haplotypes or form their own groups, when considering the serious geodetic gap between Chinese localities and other localities. However, we are inclined to suggest that reports of this species in China may be based on misidentification, for example, *S. griffithii* Schiffn., which is somewhat similar to *S. umbrosa* in bacilliform gemmae and dentate leaf lobes.

It is interesting that the species is not yet known in the Aleutians [46,47], although such findings of the species seem logical and expected; given the modern distribution of the species, it is logical to assume that it could migrate to Bering Island from Alaska through the Aleutian chain. Additionally, birds could be considered possible agents for reaching the island. Indeed, it is logical to expect that *Scapania umbrosa* may be introduced from other Aleutians, as we considered this way of migration for the Pacific North American *Gyrothyra underwoodiana*, which we unexpectedly discovered on Bering Island earlier [69]. However, this mechanism does not explain the absence of this species in the Aleutian Islands. Furthermore, in the case of *Scapania umbrosa*, the introduction by birds likely occurred long ago, given the genetic differences between the Bering and Alaskan populations.

4.3. Ecology

Scapania umbrosa has no strong confinement to a specific type of substrate. In addition to decaying wood in “an advanced stage of decay” (cf. [39] (p. 608)), it is also found on boulders and rocks with different levels of acidity (calcareous and noncalcareous sandstones, as well as granites) [39–41] and on soil along streams and roadsides [54]. Moreover, the species is confined to places with constantly high humidity.

On Bering Island, the species was collected from the rotten part of a decaying log (driftwood) in a coastal meadow dominated by *Leymus mollis* (Trin.) Pilg., *Senecio cannabifolius* Less., and *Artemisia* sp. Rotting wood is a rare type of substrate on the island due to the lack of forest vegetation. The main sources of wood include logs and trunks thrown up by the sea (driftwood). Obviously, a long process of gradual desalination is needed, and the process reaches the appropriate stage of decay before the driftwood becomes suitable for colonization by liverworts. All of these factors are responsible for the rarity of epixyloous taxa in the liverwort flora of the island and may be responsible for the rarity of *Scapania umbrosa*. However, there is no obvious reason as to why the species could not move within the island to other substrates it uses, such as rocks and soil. As noted in the Section 2, the climatic conditions of Bering Island are quite favorable for liverworts; specifically, high air humidity throughout the year and an extremely small number of sunny days make

the colonization of driftwood by liverworts possible even on open treeless marine terraces dominated by *Leymus mollis*.

4.4. Morphological Differentiation

The species is quite easily recognizable. The main morphological features that help to identify the taxon include quite narrow leaf lobes, distinctly decurrent ventral lobes, and narrow brownish bacilliform 2-celled gemmae. In general, the size of the species resembles the taxa of sect. *Curtae* but differs from the latter section members in the long decurrency of the ventral lobe; even narrower leaf lobes and pronounced dentation of the leaf lobes, a striking distinctive feature of the species.

The species may also be confused with small, underdeveloped forms of *Scapania undulata*, which differ in a lack of large trigones in leaf cells, evenly (versus unevenly) thickened cell walls along the leaf margin and rather short ellipsoidal 1-2-celled gemmae (versus bacilliform, 2-celled gemmae).

5. Conclusions

Currently, the only genetically confirmed locality of *Scapania umbrosa* in Pacific Asia is Commanders (Bering Island). In the discussion above, we consider the absence of this species from other islands of the Aleutian Arch. Indeed, it can be expected in other Aleutians as well. Moreover, taking the genetic difference, currently, the process of the formation of a new taxon may take place.

Admittedly, the bryoflora of the Commander Islands is very specific [9,10]; therefore, a finding such as *Scapania umbrosa* is not the only finding. For example, the abovementioned representative of the monotypic family, *Gyrothyra underwoodiana* [69] is located on Bering Island, which is the westernmost locality of the species worldwide. Similar examples are known among mosses, including North American *Claopodium bolanderi* Best, *Pseudoleskea baileyi* Best et Grout, *Brachythecium frigidum* (Müll. Hal.), amphioceanic *Rhytidiadelphus loreus* (Hedw.) Warnst., and *Ulota phyllantha* Brid. [11,70]; all have a distribution similar to that of *Scapania umbrosa* in general terms. It is fair to expect that, among the liverworts of the Commander Islands, there may be other interesting cases that have not yet been discovered.

In light of the abovementioned observations, a natural question arises as to how the Commander Islands should be considered in phytogeographical terms (either as part of Asia or as part of America).

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