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JUBULA HUTCHINSIAE SUBSP. *CAUCASICA* SUBSP. NOV. (JUBULACEAE, MARCHANTIOPHYTA) – A NEW TAXON FROM THE WESTERN CAUCASUS

JUBULA HUTCHINSIAE SUBSP. *CAUCASICA* SUBSP. NOV. (JUBULACEAE, MARCHANTIOPHYTA) – НОВЫЙ ТАКСОН С ЗАПАДНОГО КАВКАЗА

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Abstract

Jubula hutchinsiae (Hook.) Dumort. subsp. *caucasica* Konstant. & Vilnet is described based on ITS1-2 nrDNA, *trn*L-F and *trn*G cpDNA sequences and some morphological differences. Detailed description of the subspecies is provided, taxonomy, ecology and distribution of the taxon are discussed.

Резюме

На основе анализа сиквенсов ITS1-2 ядерной ДНК, *trn*L-F и *trn*G хлоропластной ДНК и небольших морфологических отличий описан новый подвид *Jubula hutchinsiae* (Hook.) Dumort. subsp. *caucasica* Konstant. & Vilnet. Приводится детальное описание таксона, обсуждаются таксономические особенности, экология и распространение подвида.

KEYWORDS: hepatics, Jubulaceae, *Jubula*, molecular phylogenetics, nrITS, *trn*L-F, *trn*G, Caucasus

INTRODUCTION

Jubula Dumort. is an oligotypic genus with temperate – tropical distribution. As it was noted by Zerov (1953), “there is a real mishmash in the treatment of species of this genus” (translation from the Ukrainian). After nearly 60 years this observation remains valid.

During a study of hepatics from the Caucasus the first author collected 32 specimens of *Jubula* in different regions of the western Caucasus (fig. 1). Following Guerke (1978) the specimens were identified as *Jubula hutchinsiae* (Hook.) Dumort. subsp. *javanica* (Steph.) Verd. but doubt about the correctness of this decision remained.

Jubula was first reported for the Caucasus from the territory of Georgia by Brotherus (1892) as *Frullania hutchinsiae* (Hook.) Nees var. *integrifolia* (Nees) Lindb. Later Verdoorn (1930) reported *Jubula hutchinsiae* (Hook.) Dumort. subsp. *javanica* (Steph.) Verd. for the Caucasus based on a collection of Voronov. Two species of *Jubula* were carefully described and illustrated in the treatment of the genus for the territory of the former USSR by Zerov (1953). *J. pennsylvanica* (Steph.) A. Evans was reported by Zerov (l.c.) for three localities in the Caucasus in Adzharia, Abkhazia, and the Krasnodar Territory in Russia) whereas *J. japonica* Steph. was reported from two localities in the Primorsky Territory based on specimens collected by Lazarenko (1936). Zerov (1953) referred all records from the Caucasus including his own ones to *J. pennsylvanica* because the Caucasian plants are characterized by almost totally entire margins of leaves and underleaves. The second important argument was temperate distribution vs. tropical for *Jubula hutchinsiae* subsp. *javanica.* In his monograph

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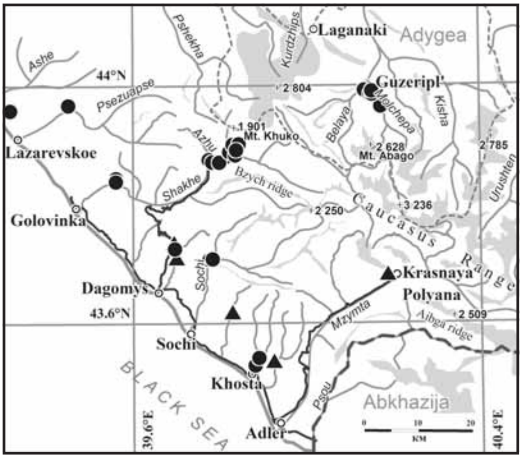


Fig. 1 Distribution of *Jubula hutchinsiae* subsp. *caucasica* Konstant. & Vilnet. Circles show specimes seen by the authors; triangles mark literature records (sub *J. pennsylvanica*).

of the genus *Jubula* Guerke (1978) stated that *J. pennsylvanica* is an American endemic and referred the specimens from the Caucasus that he had studied to *J. hutchinsiae* subsp. *javanica*. In the 1980s *J. pennsylvanica* was reported again by Duda (1982) from several localities on the slopes of the Caucasus facing the Black Sea. *J. hutchinsiae* subsp. *javanica* is recorded for the Caucasus in all recent check-lists and publications (Düll, 1983, Konstantinova et al., 1992, Schumacker & Váňa, 2005, Konstantinova & Bakalin, 2009, Konstantinova et al., 2009 etc.) based on Guerke's revision of *Jubula* (Guerke, 1978).

Several papers devoted to the study of *Jubula* have been published recently. Indian bryologists (Dey et al., 2011) reported *J. pennsylvanica* for Himalaya as the first Asian record of the species providing an identification key for Asian taxa of *Jubula*. Japanese bryologists (Katagiri et al., 2010) proposed new diagnostic features for differentiating the taxa of *Jubula* based on study of a vast material from Japan and Taiwan, including size and shape of male branches, degree of thickness of cell walls in cross section of stem, etc. As a result they treated *J. hutchinsiae* subsp. *javanica* as a separate species and provided the original key for distinguishing of the three discussed species: *J. hutchinsiae, J. javanica,* and *J. japonica.* Pätsch et al. (2010) suggested a great molecular diversification based on a molecular study of a large set of specimens of *Jubula,* but calculation of overlapping morphological features lead them to treat both *J. pennsylvanica* and *J. javanica* as subspecies of *J. hutchinsiae.*

Based on the treatment of Katagiri et al. (2010) Caucasian plants cannot be referred to *J. javanica* because they have relatively large male branches and lack thickened cell walls of the medula in the stem cross section. The Caucasian plants are morphologically very similar to *J. pennsylvanica,* which was Zerov's (1953) reason for treating them as *J. pennsylvanica.*

To determine better where our Caucasian samples should be referred, we sequenced the ITS1-2 nrDNA, *trn*L-F and *trn*G cpDNA loci and analyzed the results together with sequences obtained for *Jubula* by Pätsch et al. (2010). This brought us to an unexpected solution.

MATERIALS AND METHODS

Taxon sampling. The ITS1-2 nrDNA, *trn*L-F and *trn*G cpDNA loci of seven samples of *Jubula* gathered in the Russian part of the Caucasus, a single sample of *Jubula pennsylvanica* from the U.S.A., *J. javanica* from South Korea and *J. japonica* from the Russian Far East were sequenced as a part of this study. In addition we borrowed ITS1-2 and *trn*L-F nucleotide sequences of eighteen accessions belonging to five *Jubula* subspecies from the study of Pätsch et al. (2010) deposited in GenBank. We followed these authors using the phylogenetically closely allied *Nipponolejeunea pilifera* and *N. subalpina* as an outgroup. All analyzed samples are listed in Table 1, including GenBank accession numbers and voucher details.

DNA isolation, PCR amplification and DNA sequencing. DNA was extracted from dried liverwort tissue using the NucleoSpin Plant Kit (Macherey-Nagel, Germany). The amplification and sequencing were performed using primers suggested by Taberlet et al. (1991), Shaw et al. (2005), and White et al. (1990) for the *trn*L-F, *trn*G-intron, and ITS1-2 regions, respectively.

PCR were carried out in 20 µl volumes according to the following procedure: 3 min at 94°C, 30 cycles (30s 94°C, 40s 58°C, 60s 72°C) and 2 min of extension time at 72°C. Amplified fragments were visualized on 1% agarose TAE

Table 1. The list of subspecies of *Jubula hutchinsiae*, specimen vouchers and GenBank accession numbers for ITS and *trn*L-F/*trn*G. Accessions beginning with JN were produced for this study.

subspecies	**Specimen voucher**	**ITS**	***trn*L-F/*trn*G**
bogotensis	Costa Rica: Puntarenas, Gradstein 9449 (GOET)	FN396817	FN398012
bogotensis	Mexico: Veracruz, Gradstein s.n. (GOET)	FN396818	FN398013
caucasica	Russia: Caucaus 1, Cherkesskyi Pereval, Konstantinova & Savchenko K371-1-08 (KPABG)	JN836958	JN836968/ JN836978
caucasica	Russia: Caucaus 2, Sochi River, Konstantinova & Savchenko K446-7-08 (KPABG)	JN836959	JN836969/ JN836979
caucasica	Russia: Caucaus 3, Baranovka, Konstantinova & Savchenko K462-1-08 (KPABG)	JN836960	JN836970/ JN836980
caucasica	Russia: Caucaus 4, middle course of Shakhe River, Konstantinova & Savchenko K429-3-08 (KPABG)	JN836961	JN836971/ JN836981
caucasica	Russia: Caucaus 5, Belaya River, Konstantinova K 463-1-07 (KPABG)	JN836962	JN836972/ JN836982
caucasica	Russia: Caucaus 6, lower course of Shakhe River, Konstantinova & Savchenko K443-14-08 (KPABG)	JN836963	JN836973/ JN836983
caucasica	Russia: Caucaus 7, Guzeripl', Konstantinova, K456-5-07 (KPABG)	JN836964	JN836974/ JN836984
hutchinsiae	Portugal: Madeira 1, Schaefer-Verwimp & Verwimp 25675 (GOET)	FN396811	FN397099
hutchinsiae	Portugal: Madeira 2, Schaefer-Verwimp & Verwimp 25796 (GOET)	FN396812	FN398009
hutchinsiae	United Kingdom: Devon, Long 29077 (GOET)	FN396813	FN398010
hutchinsiae	United Kingdom: Wales, Long 35296 (GOET)	FN396814	FN398011
japonica	Japan: Miyazaki, Gradstein & Mizutani 2958 (GOET)	FN396810	FN397098
japonica	Russia: Primorsky Kray, Bakalin P-68-10-08 (KPABG)	JN836967	JN836977/ JN836987
javanica	South Korea, Bakalin Kor-12-6-08	JN836966	JN836976/ JN836986
javanica	Malaysia: Pahang, Schaefer-Verwimp & Verwimp 18870/A (GOET)	FN396802	FN397094
javanica	China: Yunnan, Long 34765 (GOET)	FN396805	FN397095
javanica	China: Fujian, Zhu 555 (HSNU)	FN396806	FN397096
javanica	Viet Nam: Vin-Phuc, Pocs & Tran Ninh 98103/A2 (GOET)	FN396808	FN397097
pennsylvanica	U.S.A.: Kentucky, Davison & Risk 2537 (UNAF)	FN396820	FN398014
pennsylvanica	U.S.A.: West Virginia, Davison 5201 (UNAF)	FN396821	FN398015
pennsylvanica	U.S.A.: Alabama 1, Davison 4707 (UNAF)	FN396822	FN398016
pennsylvanica	U.S.A.: Alabama 2, Davison 3775a (UNAF)	FN396823	FN398017
pennsylvanica	U.S.A.: Alabama 3, Davison 4690 (UNAF)	FN396824	FN398018
pennsylvanica	U.S.A.: North Carolina 1, Hyatt 8212 (UNAF)	FN396825	FN398019
pennsylvanica	U.S.A.: North Carolina 2, Davison s.n.(UNAF)	FN396826	FN398020
pennsylvanica	U.S.A.: Massachusetts, Konstantinova ACH-3-92 (KPABG)	JN836965	JN836975/ JN836985
Outgroup species			
Nipponolejeunea pilifera	Japan, Higuchi 41359	AY776307	AY776310
N. subalpina	Japan 1, Ohnishi 5611 (HIRO)	DQ987289	FJ380227
N. subalpina	Japan 2, Higuchi 41358	AY776306	AY776311

gels by EthBr staining, purified using the GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences, U.S.A.), and then used as a template in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, U.S.A.) following the standard protocol provided for 3100 Avant Genetic Analyzer (Applied Biosystems, U.S.A.).

Phylogenetic analyses. The nucleotide alignments for ITS1-2, *trn*L-F and *trn*G were obtained and manually corrected in BioEdit 7.0.1 (Hall, 1999). The *trn*G alignment for ten samples sequenced by us was only used to study sequence variability. The ITS1-2 and *trn*L-F alignments included 31 samples and were used for phylogenetic reconstructions. The variability of each

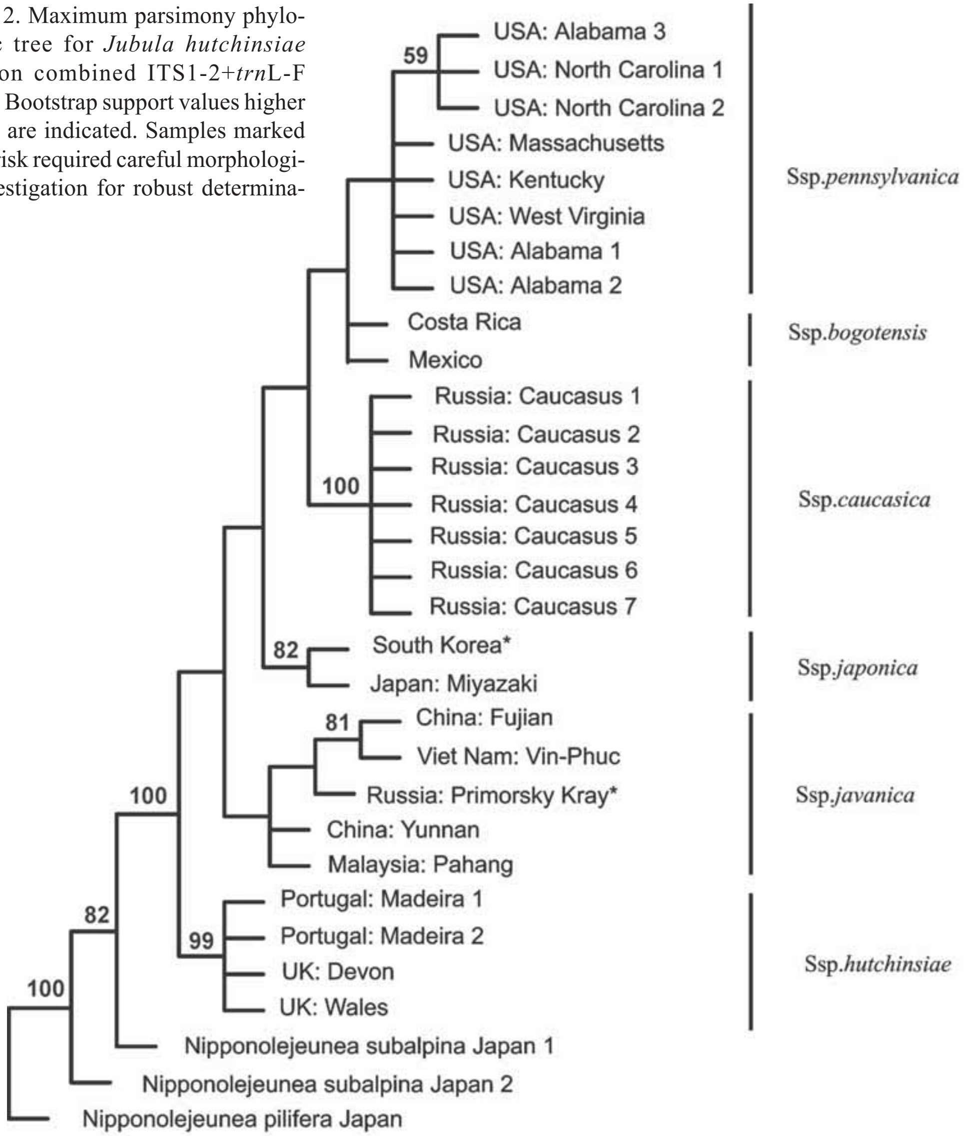


Fig. 2. Maximum parsimony phylogenetic tree for *Jubula hutchinsiae* based on combined ITS1-2+*trn*L-F dataset. Bootstrap support values higher than 50 are indicated. Samples marked by asterisk required careful morphological investigation for robust determination.

DNA locus within and between taxa was evaluated as means of *p*-distances calculated in Mega 3.0 (Kumar et al., 2004), using pairwise deletion option for counting gaps.

Reconnaissance phylogenetic analyses didn't reveal any incongruence between ITS1-2 and *trn*L-F datasets and both matrices were combined. With the exception of an ambiguously aligned P8 stem-loop region in the *trn*L-intron all positions of alignment were included in the phylogenetic analyses.

Two analytical procedures for phylogenetic calculations were implemented: the maximum parsimony method (MP) with TNT v. 1.1 (Goloboff et al., 2003) and the maximum likelihood method with PhyML (Guindon & Gascuel, 2003).

The parsimony analysis with TNT involved a New Technology Search with search minimal length tree by five reiterations and 1000 bootstrap resamplings, the default settings were used for the other parameters. Gaps in alignment were treated as missing data.

The best-fit evolutionary model of nucleotide substitutions, TN93, was determined using the ModelGenerator software (Keane et al., 2004). These models of nucleotide substitutions and four gamma rate categories were employed to estimate

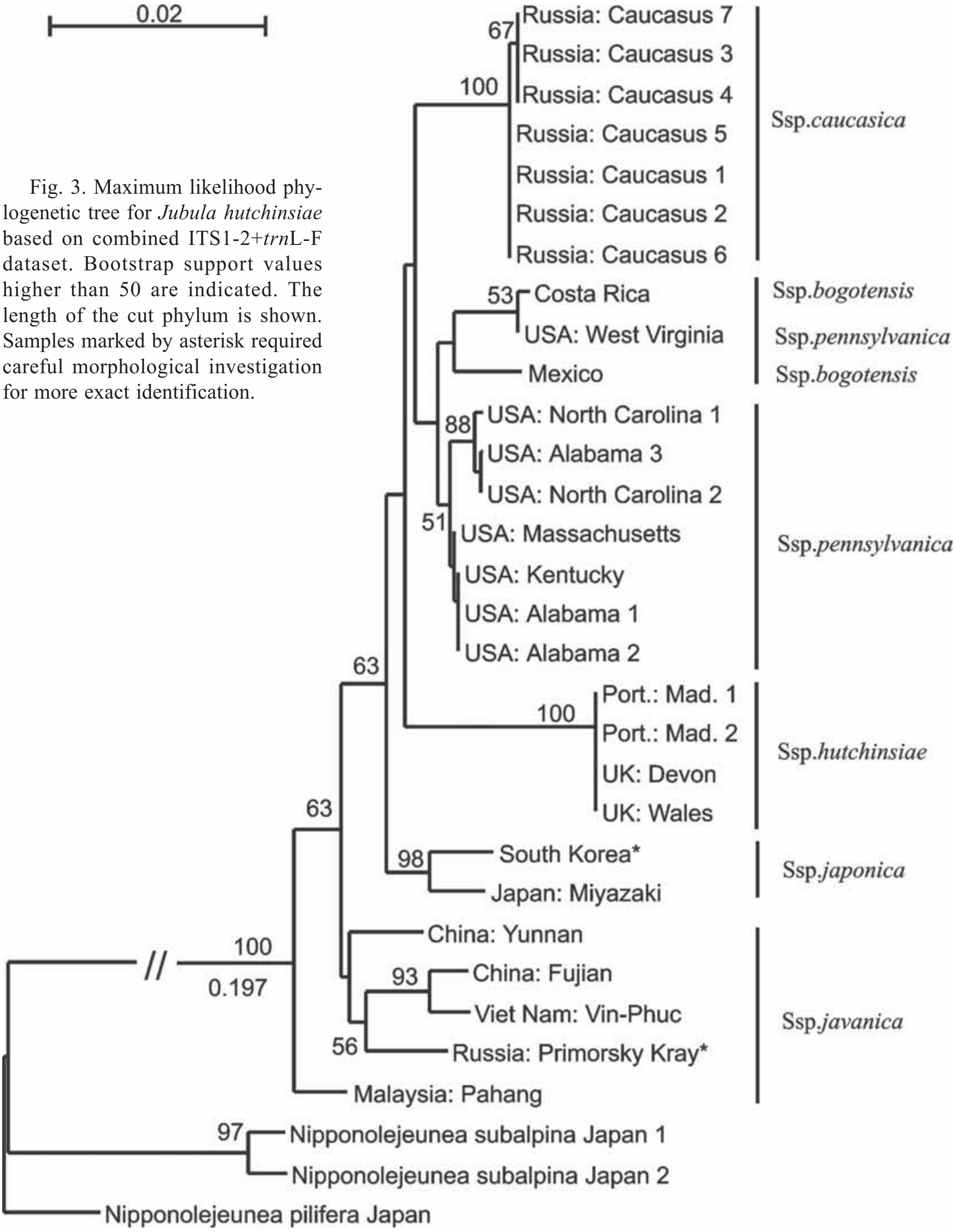


Fig. 3. Maximum likelihood phylogenetic tree for *Jubula hutchinsiae* based on combined ITS1-2+*trn*L-F dataset. Bootstrap support values higher than 50 are indicated. The length of the cut phylum is shown. Samples marked by asterisk required careful morphological investigation for more exact identification.

phylogeny by the ML method. Bootstrap supports (BS) for individual nodes were assessed using a resampling procedure with 500 replicates.

The haplotype diversity within the studied taxa of *Jubula* was estimated by TCS v.1.21 (Clement et al., 2000).

RESULTS

Phylogenetic reconstructions. The ITS1-2 alignment for 31 samples consists of 1039 sites, the *trn*L-F of 435 sites. The number of constant positions in ITS1-2/*trn*L-F alignments are 726 (69.87%)/394 (90.57%), variable positions are

274 (26.37%)/35 (8%), parsimony informative positions are 111 (10.68%)/32 (7.36%) respectively. The combined ITS1-2+*trn*L-F alignment among 1476 sites is characterized by 309 (21%) variable and 143 (9.7 %) parsimony informative positions. The *trn*G alignment for ten samples counted 703 sites, among them 680 (96.73%) positions are constant, 19 (2.7%), variable and 6 (0.85%) parsimony informative.

The MP analysis yielded 7 equally parsimonious trees with a length of 366 steps with CI = 0.920981 and RI = 0.923885 calculated in Mega 3. The MP tree with means of bootstrap support is shown in Fig. 2. The ML calculation resulted in a single tree (Fig. 3), the arithmetic means of Log likelihood was -3952.962446. All bootstrap supports with mean values higher than 50% are shown in Figs. 2-3.

The backbone topologies of both trees are different and only slightly supported. In contrast with Pätsch et al. (2010), the MP topology is characterized by unsupported polytomy and in ML topology some basal clades have support. On both MP and ML trees the clade of *J. hutchinsiae* subsp. *hutchinsiae* is supported (99% and 100% BS correspondingly). Caucasian samples of *Jubula* composed a clade with 100% BS support sister to the clade formed by *J. hutchinsiae* subsp. *pennsylvanica* + *J. hutchinsiae* subsp. *bogotensis* clade in both trees. Unfortunately we didn't achieve reliable support for the relation of the two latter subspecies as was the case in Pätsch et al. (2010). Support values higher than 50% were obtained for clades *J. hutchinsiae* subsp. *japonica* (82% MP, 98% ML), *J. hutchinsiae* subsp. *hutchinsiae* (99% MP, 100% ML) and *J. hutchinsiae* subsp. *pennsylvanica* (51% ML).

Molecular variability and haplotypes within Jubula subspecies. The subspecies of *Jubula* differ by nucleotide sequence length of the studied DNA loci (Table 2). The shortest ITS1-2 sequences belong to *J. hutchinsiae* subsp. *hutchinsiae,* 905 bp for both samples from Portugal and 911 bp for samples from the United Kingdom. The Caucasian samples of *Jubula* have 914 bp in the ITS1-2 sequences. The length of ITS1-2 sequences for the other four subspecies is larger ranging from 918 to 922 nucleotides. The absence of *trn*L-F length variability is found for *J. hutchinsiae* subsp. *japonica* (429 bp), *J. hutchinsiae* subsp. *pennsylvanica* (434 bp) and *J. hutchinsiae* subsp. *hutchinsiae* (435 bp). The length of *trn*L-F sequences of Caucasian samples of *Jubula* is 443 except for sample K371-1-08 (KPABG) which has a deletion of four nucleotides in the P8 stem-loop region of the *trn*L-intron.

The p-distances calculated for the ITS1-2 dataset suggest the low level of nucleotide substitutions for Caucasian samples of *Jubula* (0-0.1%) and *J. hutchinsiae* subsp. *hutchinsiae* (0%), and the high level for *J. hutchinsiae* subsp. *javanica* (0.4-2.5%). The diversity between species varies from 1.0 to 4.1% by ITS1-2 (Table 3). Nucleotide substitutions in the *trn*L-F sequences were found only in *J. hutchinsiae* subsp. *javanica* (p-distances 0.5-1.6%). In the whole the *trn*L sequence diversity between *Jubula* taxa is much lower and ranges from 0.2 to 1.4%.

Both substitutions and indels provide the haplotype diversity in *Jubula.* The highest haplotype diversity by both loci is found for *J. hutchinsiae* subsp. *javanica* (five haplotypes)*,* whereas North American *J. hutchinsiae* subsp. *pennsylvanica* has eight haplotypes for the ITS1-2 loci and a single *trn*L-F-haplotype. ITS1-2 haplotype differences are found between Portuguese and British populations of *J. hutchinsiae* subsp. *hutchinsiae* with a common *trn*L-F-haplotype. The ITS1-2 sequence variability of Caucasian samples of *Jubula* consists of a single substitution in the second spacer that produced two haplotypes. The deletion of four nucleotides in the P8 stem-loop region of the *trn*L-intron in a single Caucasian sample also revealed two *trn*L-F haplotypes, at the same time there are no mutations in the *trn*G sequences. It should be mentioned that each of three mutations was found in three different samples.

DISCUSSION

The overlapping values of DNA sequence variability within and between taxa of *Jubula* (Table 3) gives us an additional reason to agree with Pätsch et al. (2010) and accept *Jubula hutchinsiae* as a polymorphous species with geographically isolated subspecies. The samples of each subspecies composed clades on the obtained trees (except *J. hutchinsiae* subsp. *javanica* in the ML tree) but relations between them are unresolved

Table 2. The means of nucleotide sequence length and number of haplotypes for each subspecies *Jubula hutchinsiae*, calculated from the all studied loci (n: number of samples).

subspecies	n	ITS, bp	ITS haplotypes	*trn*L-F, bp	*trn*L-F haplotypes
bogotensis	2	918-919	2	435-437	2
caucasica	7	914	2	439-443	2
hutchinsiae	4	905-911	3	435	1
japonica	2	918-919	2	429	1
javanica	5	918-922	5	437-444	5
pennsylvanica	8	918-922	8	434	1

or poorly supported as also was stated in Pätsch et al. (2010). The 100% BS supported clade of Caucasian samples is sister to clade of *J. hutchinsiae* subsp. *pennsylvanica* +*J. hutchinsiae* subsp. *bogotensis* but without support on both trees. The low level of sequence variability in the Caucasian samples of *Jubula* revealed only two haplotypes in ITS1-2 and *trn*L-F loci compared to the more variable *J. hutchinsiae* subsp. *javanica* (five haplotypes in ITS1-2 and *trn*L-F) and *J. hutchinsiae* subsp. *pennsylvanica* (eight haplotypes in ITS1-2 and one in *trn*L-F). Thus Caucasian samples are strongly separated from the other *Jubula* subspecies.

Plants from the Caucasus differ from subsp. *javanica* in 1) totally entire margins of dorsal lobes vs. sometimes 1-2 spinose dentate in subsp. *javanica*; 2) large male inflorescences, usually longer and wider than the dorsal lobes vs. small male inflorescences, usually shorter and narrower than the dorsal lobes in subsp. *javanica*; 3) absence of gemmae vs. presence of discoid gemmae on the dorsal surface of the dorsal lobes in subsp. *javanica*; 4) thin-walled cells of the medulla in the cross-section of stem vs. thick-walled in subsp. *javanica.* Specimens from the Caucasus differ from *J. hutchinsiae* subsp. *hutchinsiae* in 1) often rounded to apiculate, entire-margined leaves; 2) the lack of a spur in the lobules; 3) entire-margined underleaves; 4) entire-margined bracts and bracteoles.

It is harder to distinguish Caucasian plants from *subsp. pennsylvanica.* The subspecies *caucasica* differs from subsp. *pennsylvanica* in: 1) generally slightly smaller lobes; 2) underleaves that are often only slightly elevated from the stem with lobes more or less flat or only slightly incurved at the base and shortly decurrent (less than the width of the stem) vs. strongly elevated underleaves with strongly incurved and longly decurrent lobes (more than the width of the stem) in subsp. *pennsylvanica*; 3) slightly reflexed ba-

Table 3. The means of *p*-distances, calculated from ITS1-2 and *trn*L-F, within and between subspecies of *Jubula hutchinsiae*.

ITS1-2	*caucasica*	*javanica*	*japonica*	*hutchinsiae*	*bogotensis*	*pennsylvanica*
caucasica	0-0.1					
javanica	2.0-3.4	0.4-2.5				
japonica	2.3-2.7	1.8-3.1	1.6			
hutchinsiae	1.6-3.3	1.1-4.1	1.1-3.6	0		
bogotensis	2.2-2.9	2.1-3.7	2.0-2.7	2.2-3.6	1.8	
pennsylvanica	1.3-1.9	2.2-4.8	2.1-3.2	0.8-3.8	1.3-3.2	0-1.3

*trn*L-F	*caucasica*	*javanica*	*japonica*	*hutchinsiae*	*bogotensis*	*pennsylvanica*
caucasica	0					
javanica	1.4-2.1	0.5-1.6				
japonica	1.2	0.5-1.4	0			
hutchinsiae	1.2-1.4	0.9-2.1	0.9	0		
bogotensis	0.93-1	0.2-1.2	0.2	0.9	0	
pennsylvanica	0.9-1	0.2-1.2	0.2	0.9	0	0

ses of sinus and non-reflexed margins of sinus of bracts and bracteoles vs. strongly reflexed bases and margins in bracts and bracteoles in subsp. *pennsylvanica*; 4) distinct small-celled zone between decurrent lobes of underleaves vs. indistinct or even absent in subsp. *pennsylvanica*. The subspecies *pennsylvanica* has ecology very similar to that of the Caucasian plants occuring in deep shaded wet ledges and walls of ravines, "on damp to dripping wet rocks" (Schuster, 1992).

The Caucasus is a strongly isolated mountain system with some sites that never were glaciated. As a result several centers of the tertial flora are preserved in the Caucasus (Pavlov, 1948). Some areas of Kolchida (also known as Colchis) with relict flora are restricted to the western Caucasus where many Caucasian endemic taxa of vascular plants occur. *Jubula hutchinsiae* subsp. *caucasica* was collected in just such areas. In particular it was found associated with *Rhododendron ponticum, Ruscus colchicus, Laurocerasus officinale* and another members of the flora of Colchis. It is reasonable to suppose a considerable and prolonged isolation of Caucasian populations of *Jubula* along with another Tertiary relic in the refugiums of the western Caucasus. The nearest localities of *J. hutchinsiae* subsp. *hutchinsiae* are mostly in coastal areas of southern Atlantic countries and on the Atlantic coast of Britain, Ireland and France. The nearest locality in Asia (as *J. pennsylvanica*) is in Himalaya (Dey et al., 2011). It is an amazing example of very slow evolution of liverwort species that evidently have diverged very little since Tertiary time.

DESCRIPTION

Jubula hutchinsiae subsp. **caucasica** Konstant. & Vilnet subsp. nov. Figs. 4-5.

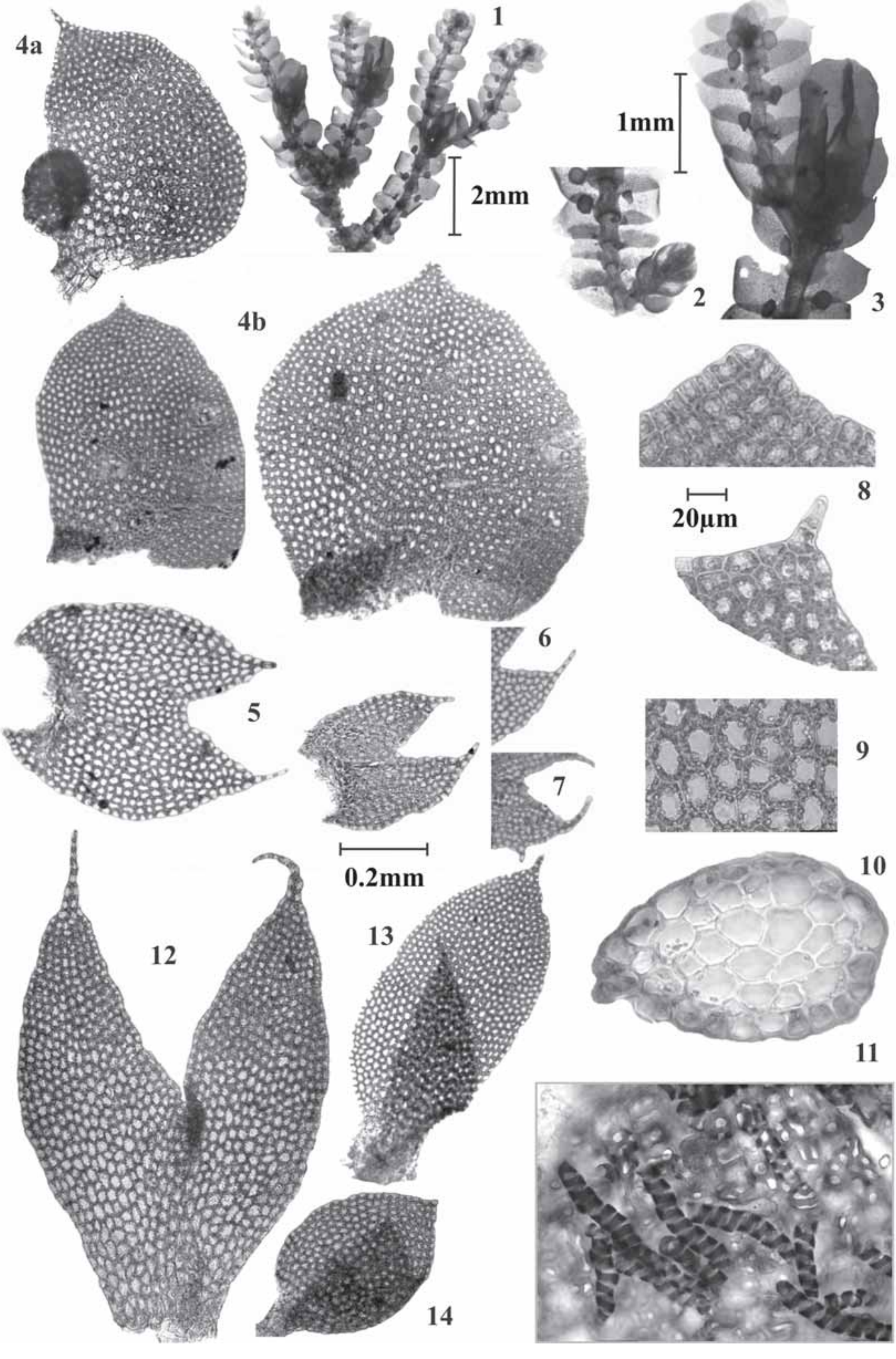
Subspecies haec Jubulae hutchinsiae subsp. pennsylvanicae marginibus loborum dorsalium amphigastriarumque integerrimis et statura androeciarum similis sed lobis amphigastriarum non reflexis et minus decurrentibus, zona inter lobos decurrentes amphigastriarum e cellulis minoribus formata et sequentiis DNA recedit.

Similar to *Jubula hutchinsiae* subsp. *pennsylvanica* in almost totally entire margins of dorsal lobes and underleaves as well as the size of male inflorescence but differs in non-reflexed and less decurrent lobes of underleaves, presence of distinct small-celled zone between decurrent lobes of underleaves and in DNA sequences.

Type: RUSSIA, Republic of Adygea, right bank of the Belaya River in surroundings of Guseripl' Town, cliffs on bank of small rivulet (43°59'30" N - 40°08'06" E), 682 m alt., the bottom of cliffs near water level, on rock and among mosses, 17.X.2007, Konstantinova # K463-1-07 (holotype KPABG, isotypes MW, MHA, VLA, LE).

Plants prostrate, light green in upper parts of shoots to dull or olive-green and dark green in the middle and at the base of shoots, some plants brown or blackish green especially in older parts, in the field mats often become dark green-blue because of presence of blue algae in lower parts of shoots, leafy shoots (1.0-)1.2-1.8 mm wide, 10-20 mm long, rhizoids from underleaf bases of few underleaves (bases of most underleaves without any trace of rhizoids), in clusters, light to dark brown, rarely colorless, ca. 5-7 μm wide, widened and branched at the end. Ventral cortical cells of stem rectangular, 19-22(-28) μm wide and (30-)36-44(-66) μm long, rarely nearly quadrate and then 25-28×25-28 μm, with light brownish, relatively thick walls, between decurrent lobes of underleaves with distinct small-celled zone consisting of thin-walled cells 8-9×11-16 μm. Stem in cross section (80-)110-150 μm high and 150-180(-230) μm wide, 5-7 cells high, cells of medulla isodiametric to slightly elongated (15-)24-28(-30)×(19-)22-30(-36) μm, central cells thin-walled, one layer near cortical cells slightly thick-walled and often with light brown walls of cells, cortical cells 15-20×20-22 μm, with thick brown walls. Branches of *Frullania*-type, with associated stem leaves without lobules, several small (sometimes hidden under leaves) sterile

Fig. 4. *Jubula hutchinsiae* subsp. *caucasica* Konstant. & Vilnet (from the holotype): 1 – Autoicous plant with ventral innovations; 2 – part of shoot with male branch; 3 – part of shoot with perianth; 4 – leaves: a: with galeate lobule; b: with explanate lobule; 5 – underleaves; 6-7 – distal part of underleaves with teeth on lateral margin; 8 – apices of dorsal lobes; 9 – median cells of lobe; 10 – stem transverse section; 11 – elaters over capsule wall; 12 – female bracteole; 13 – female bract; 14 – male bract.


4a
1
2mm
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14

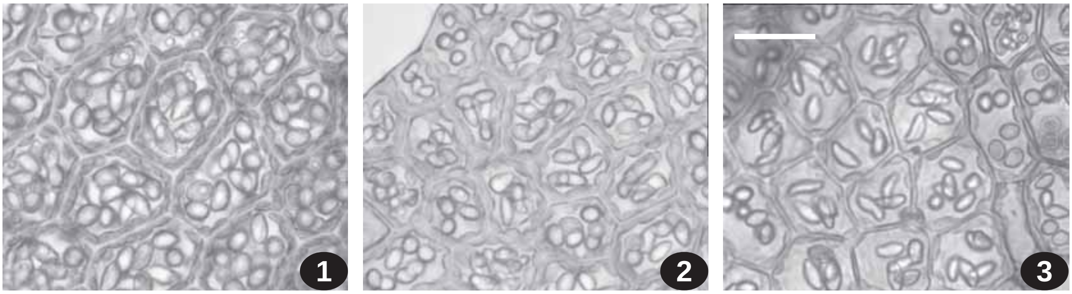

Fig. 5. *Jubula hutchinsiae* subsp. *caucasica* Konstant. & Vilnet (from #K364-11), oil-bodies: 1 – in the mid-leaf cells, 2 – in marginal cells, and 3 – in lobule cells. Scale bar 20 μm.

Lejeunea-type branches often present, the subfloral innovations are always of *Frullania*-type. Leaves imbricate, dorsal lobes flat to slightly convex, (350-)450-520(-600)×(570-)650-740(-800) μm, ovate to semicordate and with convex dorsal side, apices distinctly incurved, sometimes rounded or blunt, but more often acute or apiculate with uniseriate apex 1-3 (-4) cells long, often 2-celled at base. Cells of apical tooth usually isodiametric or slightly elongated, 11×14 μm, apical cells to 11×22 μm. Cells thin-walled, with or without minute trigones, median cells (14-)15-18(-22)×17-25(-27) μm, basal cells 17-20 ×(25)28-34 (-37) μm. Oil bodies (7-)8-10 per cell, colorless, sphaeric, fusiform to narrowly ellipsoidal, 4-5×7-10 μm, with admixture of smaller, 2.5-3×4-5 μm, widely ellipsoidal ones; in marginal cells oil bodies (2-)3-4(-5), small, sphaeric to widely ellipsoidal, ca. 3×3-5 μm. Lobules galeate and explanate, when galeate then obovate, narrowed to the mouth, narrow, ca 1.5-2.2 times long as wide, (91-)110-160(-175)×160-200(-220) μm or suborbicular and then 170×230 μm and orbicular, 170×170 μm, in one specimen one lobule had appendage. Underleaves distant, inserted on arcuate line, longly decurrent, however less than stem width, more or less apressed, with flat or slightly concave sides, usually longer than wide or with length equal to width, 340-600×400-700 μm, bilobed to 0.5-0.6 of their length, sinus U- or V- shaped, more or less reflexed, lobes mostly connivent or erect, acuminate-ciliate, with uniseriate apex of 2-4 elongated cells to 50-100 μm long, with cells 9-14×22-35(-40) μm, cells in undivided part of underleaves leptodermous polyhedral, without or with minute trigones somewhat elongated, 19-22×25-28 μm. Lateral margins of underleaf sometimes with spinose teeth.

Gemmae unknown.

Autoicous. Male branches of *Lejeunea*-type, often situated at the base of shoot quite distant from perianth (in some specimens difficult to find, because evidently destroyed), to 600 μm wide and 1400 μm long, sometimes as wide as long, definitely broader than leaves. Androecia spicate, compact, 1-2(-3) times as long as dorsal lobe, and distinctly wider, ventricose, male branch in cross-section 4 cells thick, 85-100×125 μm, with 16-17 peripheral cortical cells, bracts in (3-)5-7 pairs, subequally bilobed, with ventral lobe only slightly smaller than dorsal one. Dorsal lobe of male bracts ovate, 200-300 μm wide and 285-460 μm long, bracteole similar to underleaves but smaller and with obtuse lobes never ending in cilia, divided usually to 1/3 of their length, 285×220 μm, antheridia with uniseriate stalk, 2 per bract. Female inflorescences terminal. Female bracts in 1-2 pairs, unequally bilobed, keeled, with slightly reflexed margin in the bottom of sinus, but with non-reflexed margins of sides of sinus, dorsal lobe lanceolate, (260-)300-520×800 -1200 μm, acute to apiculate, ending in 2-celled apex, uppermost cell 11×25 μm, ventral lobe 230×520 μm, with 3-celled apices, uppermost cell 10-11×28 μm. Bracteole free from bracts, with 2 equal lanceolate lobes, 500×1140 μm long and 700 μm wide, usually with entire margins, sometimes with single teeth 4 cells long, with basal cell 14-15×28 μm and apical cell 12×28 μm, bilobed to the 0.9 of the length, keeled, with subequal lanceolate acuminate lobes ending in 4-celled apex, upper cell of which 11×28-30 μm.

Perianths were seen in most studied specimens but they were sometimes masked by leaves, often not fertilized, exerted to 0.3 of their length, smooth, 600-800 μm wide and 1200-1600 μm long, trigonous in transverse section, with one sharp postical keel and two lateral keels suddenly contracted to the distinct beak 60×60 μm wide and to 95 μm long, composed of (3-)4(-5) rows of cells, cells of 2-3 lower rows are nearly isodiametric, (8-)10-14 μm, whereas cells of 2 upper rows elongated, 8-11×17-20 μm, upper cells partly free, to 12×25-28 μm. Sporophytes in some collections present, but usually juvenille, mature capsules rare. Open capsule with 4 valves and attached elaters were found in two specimens. Capsule wall 2-layered, outer cells with radial banded thickenings and inner cells with reticulate thickenings. Elaters unispiral, 10-11 μm long, with red-brown spiral band, 6-7 μm wide (Fig. 4: 11). Spores not seen. Most collections were gathered in late September to middle October, so sporophytes likely ripen in the late October to November.

Variation. The morphological variability of *J. hutchinsiae* subsp. *caucasica* is very low in the Caucasus. The subspecies can be easily recognized in the field due to the characteristic dark green to dark bluish green color and apiculate apex incurved to ventral side. The size of plants, leaves, underleaves, etc. is slightly variable. Single teeth at the margin of underleaves can be seen in some specimens (Fig. 4: 7).

Ecology. The subspecies occurs from 50 to 1000 m alt., on shaded moist rocks and moist cliffs covered by fine earth, mainly in deep shaded canyons near running water: along streams and waterfalls, sometimes between exposed roots and on soil along trails in *Taxus* & *Buxus* woods. In pure mats or with *Metzgeria conjugata, Apometzgeria pubescens, Calypogeia fissa*, *Plagiochila porelloides,* and *Scapania verrucosa.*

Distribution. The range of the subspecies is restricted mainly to the western Caucasus (Fig. 1) where it has been reported from Russia, Abkhazia, and Georgia (Zerov, 1953, as *J. pennsylvanica*; Duda, 1982 as *J. pennsylvanica*; Konstantinova et al., 2002 and Konstantinova & Savchenko, 2011 as *J. hutchinsiae* subsp. *javanica*). The records from Turkey, Iran and Asia Minor (Guerke, 1978) probably should be referred to this taxon too.

The species is clearly restricted to areas with relict flora of Kolkhida.

Selected specimens examined. RUSSIA, **Republic of Adygea**: valley of Molchepa River right tributary of the Belay River in surroundings of Guseripl' Town, mouth of Filimonovskiy Creek, (43°59'45"N – 40°08'34"E), 665 m alt., on a rock covered by fine earth in the bed of a stream, 16.X. 2007, Konstantinova #K453-1-07 (KPABG); right bank of the Molchepa River, right tributary of the Belay River, in surroundings of Guseripl' Town (43°59'02"N – 40°08'53"E), 835 m alt., on rock near waterfall, 18.X.2007, Konstantinova #K471-2-07 (KPABG); valley of the Molchepa River, right tributary of the Belay River, in surroundings of Guseripl' Town, Filimonovskiy Creek, deep shaded canyon (43°59'36"N – 40°08'38"E), 693 m alt., on the bank of a stream, on rock at the bottom of rock outcrop, 16.X.2007, Konstantinova #K456-5-07 (KPABG); **Krasnodar Territory**: Right banks of the Vostochnyi Dagomys River near the Baranovka settlement (43°43'33"N – 39°41'45"E), 118 m alt., in a deep canyon with small waterfalls, on moist rocks, 13.X.2008, Konstantinova & Savchenko #K462-1-08 (KPABG); valley of the Shakhe River, cliffs on the right bank of the Belyi Stream (43°52' 15"N – 39°47'56"E), ca. 450 m alt., on a ledge of rock, among mosses 2.X.2008, Konstantinova & Savchenko # K429-3-08 (KPABG); lower course of Shakhe River, waterfalls on the Bolshoy Kichmay River (43°50'39"N – 39°33'39"E), 193 m alt., on loamy soil in *Buxus* stands, 10.X.2008, Konstantinova & Savchenko #K443-14-08 (KPABG); same place (43°50'20"N – 39°33'37"E), 68 m alt., on a cliff on the right bank near the 4-th waterfall, 10.X.2008, Konstantinova & Savchenko #K443-5-08 (KPABG); Shakhe River basin, north facing cliffs on the left bank of the Bushuika River (43°53'29"N – 39°50'18"E), 534 m alt., on rock, 28.IX.2008, Konstantinova & Savchenko #K402-1-08 (KPABG); Valley of the Sochi River, slope to the first Orekhovskiy waterfall (43°42'28"N – 39°46' 31"E), 154 m alt., on roots and rocks in a deep niche, 11.X.2008, Konstantinova & Savchenko #K446-7-08 (KPABG).

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