ISSN 1022-7954, Russian Journal of Genetics, 2007, Vol. 43, No. 11, pp. 1306–1313. © Pleiades Publishing, Inc., 2007.
Original Russian Text © A.A. Vilnet, I.A. Milyutina, N.A. Konstantinova, M.S. Ignatov, A.V. Troitsky, 2007, published in Genetika, 2007, Vol. 43, No. 11, pp. 1556–1564.

PLANT GENETICS

Phylogeny of the Genus *Lophozia* (Dumort.) Dumort. s. str. Inferred from Nuclear and Chloroplast Sequences ITS1-2 and TRNL-F

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Received October 6, 2006

Abstract—Maximum parsimony and maximum likelihood phylogenetic trees were constructed for 21 taxa of *Lophozia* s. str. and the related genera, *Schistochilopsis* (5 species), *Protolophozia elongata*, and *Obtusifolium obtusum* based on combined nuclear ITS1-2 and chloroplast *trnL-F* DNA sequences. The trees were characterized by similar topology. It was demonstrated that the genus *Lophozia* s. str. was monophyletic, excluding *L. sudetica*, which deserved isolation into a distinct cryptic genus. The species distribution among the clades disagreed with the sections distinguished based on anatomical and morphological data. The relationships within the genus *Schistochilopsis* were consistent with the sectioning of the genus, based on morphological characters. Analysis of molecular data provided more precise definition of the systematic position of a number of taxa. A low level of genetic divergence of geographically distant forms was demonstrated.

DOI: 10.1134/S1022795407110142

INTRODUCTION

Liverworts represent the most ancient group of land plants. Natural classification of this group is extremely complicated due to the low number of anatomical and morphological characters available for comparative analysis. *Lophozia* (Dumort.) Dumort. s. str. is one of most polymorphic and problematic for identification liverwort genus, especially in Arctic. The concept of some species and intraspecific taxa is controversial. Many taxa were described relatively recently. Furthermore, they are often known only from the type localities, or several more localities [1–4]. As a result, liverwort variation and phylogenetic relationships are scarcely studied. At present, a gradual transition towards more narrow concept of the genus *Lophozia* s. 1. is observed. Contrary to the ideas of Schuster on the genus size [1], many modern authors distinguish other distinct genera, *Leiocolea* (Muell. Frib.) H. Buch and *Barbilophozia* Loeske [5–8]. Most Russian authors share the ideas of Schljakov [3], who, following the narrow concept of the genera suggested by Scandinavian researchers [9, 10], in addition to the above genera, also recognizes *Isopaches* H. Buch, *Obtusifollium* (H. Buch) S. Arnell, and *Orthocaulis* H. Buch as isolated genera. Furthermore, this author raises the subgenera *Massula* K. Müll. emend R. M. Schust. and *Protolophozia* R. M. Schust. to the genus rank. Potemkin [11] supports the recognition of *Schistochilopsis* as a genus, but with inclusion of the *Obtusifolium* into it with the status of a section.

The arguments of the followers of either broad or narrow concept of the genera, based on anatomical and morphological characters, are briefly discussed by Bakalin [4]. The existing arguments, however, are not sufficiently conclusive to accept one or another concept of the genera in *Lophozia* s. 1. Earlier, based on the sequence data for the chloroplast DNA (cpDNA) *trnL-F* locus from the 47 species of the family Lophoziaceae, it was demonstrated that the "narrow" concept of the genera in this family was, probably, more correct [12]. In the present study, we tried to evaluate phylogenetic relationships within the *Lophozia* s. str. and in related taxa based on sequence analysis of the internal transcribed spacer of nuclear rDNA (ITS1-2) and the *trnL-F* locus of cpDNA of a higher number of taxa of *Lophozia* s. str., as well as its close relatives, *Schistochilopsis*, *Obtusifolium*, and *Protholophozia*.

MATERIALS AND METHODS

Taxon sampling. DNA was extracted from the plants selected from the herbarium specimens of Polar–Alpine Botanical Garden–Institute (KPABG) of Kola Research Center, Russian Academy of Sciences (table). The analysis included 21 out of 33 taxa of the genus *Lophozia* s. str., indicated for the world [4], as well as *Schistochiopsis* (5 species), *Protolophozia elongata*,

and *Obtusifolium obtusum*. The species as *Lophozia sudetica*, *L. silvicoides*, *L. excisa*, *Schistochilopsis incisa* and *S. opacifolia* were represented by several samples collected in different regions. *Anastrepta orcadensis*, *Anastrophyllum michauxii*, and *Sphenolobus saxicola* were used as an outgroup.

DNA isolation and sequencing. DNA was isolated using either CTAB-based method [13], or the kit for extraction of plant DNA, NucleoSpin Plant Kit (Macherey-Nagel, Germany).

Amplification of ITS1-2 and 5.8S rDNA was done using external and internal primers [14]. Amplification of the *trnL-F* region was performed using a pair of primers, which allowed production of the full-sized *trnL* intron and 3' exon sequences, as well as the sequence of the *trnL–trnF* intergenic spacer [15].

The amplification profile was 3 min at 94°C, followed by 30 cycles (94°C for 30 s, 58°C for 40 s, and 72°C for 60 s) with a final elongation at 72°C for 2 min. The produced DNA fragments were analyzed by electrophoresis in 1% agarose gel in 1 × TAE buffer with ethidium bromide. The samples were purified using the GFX(PCR DNA kit and Gel Band Purification Kit (Amersham Bioscience, United States).

DNA was sequenced using the method of cyclic sequencing with the ABI PRISM (BigDye (Terminator v. 3.1 reagent kit. The reactions were performed in the Genome Center of Joint Use (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow) with further analysis of the reaction products on the ABI PRISM 3100-Avant DNA automated sequencer. The sequences determined were submitted to the GenBank (table).

Phylogenetic analysis. Nucleotide sequences were aligned manually using the BioEdit software [16].

The trees were constructed using the methods of maximum parsimony (MP) and maximum likelihood (ML) as implemented in the TNT [17] and PHYML [18], respectively.

In the work with the TNT program the New Technology Search algorithms were used with the fivefold reiterated minimal tree search and 500 jackknife replications with the removal 36% of the positions. Other parameters were set as defaults. To calculate the Bremer support index, the trees that were 20 steps longer than the minimum tree were used.

To work with the PHYML program, an evolutionary model of the GTR + G + I nucleotide substitutions chosen by the ModelGenerator program [19] was selected. Eight gamma density categories and 100 bootstrap replications were used. (Our previous experience of treating the sequences of similar length showed that the bootstrap support values obtained at the increase of the replications number up to 1000, differed from those at 100 replications no more than the bootstrap index values obtained in case of the independent tree construction.)

RESULTS

The ITS1-2 and *trnL-F* sequences were determined in 36 samples. The *trnL-F* sequences for *Lophozia silvicola, L. sudetica, Schistochilopsis incisa*, and *Obtusifolium obtusum*, used in our previous investigations [12], were also included in the analysis.

For phylogenetic analysis, the ITS1-2 and *trnL-F* sequences were combined. The combined ITS1-2 and *trnL-F* sequences for *Lophozia polaris, L. silvicola, L. silvicoloides* (Spitsbergen), and *Obtusifolium obtusum* were constructed by uniting sequence data from different samples (table). Both *Schistochilopsis incisa* samples were represented only by the sequences of the *trnL-F* locus, while *Lophozia sudetica* was represented by the ITS1-2 sequence (K41-5-04).

The overall alignment for 39 samples consisted of 1416 nucleotide pairs (bp), among which 57% positions were conservative, 39% were variable, and 27.5% were parsimoniously informative. The *trnL-F* sequence contains larger number of conservative sites, compared to the ITS1-2 (69 and 51%, respectively), along with the low number of variable (28 versus 46%) and parsimony informative positions (20 versus 32%). Nucleotide composition is 24.2% (T), 24.5% (C), 23.8% (A), and 27.5% (G).

Among the *Schistochilopsis*, the shortest ITS1-2 sequence was found in *S. capitata* (787 bp), while *S. opacifolia* (832 bp) had the longest ITS1-2. Among the *Lophozia* s. str., the shortest ITS1-2 was in *L. wenzelii* var. *groelandica* (795 bp), and the longest ITS1-2 was in *L. sudetica* (June 4, 1998) (821 bp).Taken together, the length of the ITS1-2 fragment was longer in the representatives of the clade, formed by *L. excisa, L. propagulifera, L. polaris, L. polaris* var. *sphagnorum, L. longidens*, compared to other species of *Lophozia* s. str. (the Excisa clade, distinguished on phylogenetic trees, see below).

The length of the *trnL-F* sequence varied from 453 bp in *Schistochilopsis grandiretis* to 498 bp in *S. laxa* due to the insertion in the hairpin P8 of the *trnL* intron. An analogous situation was observed in the *Lophozia* s. str. species: two insertions within the hairpin P8 increased the length of the *trnL-F* sequence up to 497 bp in *Lophozia longidens, L. polaris*, and *L. polaris* var. *shagnorum*, compared to the length of this sequence in *L. austro-sibirica, L.* cf. *lacerata, L. ventricosa* var. *guttulata, L.* cf. *wenzelii* var. *groenlandica, L. wenzelii* var. *groenlandica, L. wenzelii* var. *litoralis,* and *L. wenzelii* var. *massularioides* (463 bp).

Thus, the species forming the Excisa clade differed from the other *Lophozia* s. str. by longer genomic regions examined, which were formed due to the insertions into ITS2 and P8.

A list of taxa used in the study with the indication of the herbarium vouchers and GenBank accession numbers

Taxon	Sampling locality, collector, KPABG herbarium number	GenBank accession number	
		trnL	ITS
Anastrepta orcadensis (Hook.) Schiffn.	Russia, Bury-atia, N. A. Konstantinova, 59-1-01	DQ875088	DQ875126
Anastrophyllum michauxii (F. Weber) H. Buch	Russia, Bury-atia, N. A. Konstantinova, 17-1-02	DQ875087	DQ875125
Lophozia ascendens (Warnst.) R. M. Schust.	Russia, Bury-atia, N. A. Konstantinova, 109-3-01	DQ875054	DQ875089
Lophozia austro-sibirica Bakalin	Russia, Buryatia, V. A. Bakalin, B 15-9-99	DQ875069	DQ875105
Lophozia excisa (Dicks.) Dumort	Spitsbergen, N. A. Konstantinova, 104-1-04	DQ875056	DQ875091
Lophozia excisa (Dicks.) Dumort.	Murmansk region, N. A. Konstantinova, 41-2-97	DQ875057	DQ875092
Lophozia excisa (Dicks.) Dumort.	Spitsbergen, N. A. Konstantinova, K-21-2-05	DQ875058	DQ875093
Lophozia heteromorpha R. M. Schust.	Russia, Kamchatka region, V. A. Bakalin, K-47-3-02	DQ875068	DQ875104
Lophozia cf. *lacerata* N. Kitag.	Russia, Commander Islands, V. A. Bakalin, K-3-2-02-VB	DQ875071	DQ875107
Lophozia lantratoviae Bakalin	Russia, Buryatia, V. A. Bakalin, 76-7-01	DQ875055	DQ875090
Lophozia longidens (Lindb.) Macoun	Russia, Murmansk region, N. A. Konstantinova, 360-2-00	DQ875059	DQ875094
Lophozia polaris (R. M. Schust.) R. M. Schust. et Damsh.	Russia, Kamchatka region, V. A. Bakalin, 30-01-02	DQ875060	No data
Lophozia polaris (R. M. Schust.) R. M. Schust. et Damsh.	Spitsbergen, N. A. Konstantinova, K-9-2-05	No data	DQ875095
Lophozia polaris (R. M. Schust.) R. M. Schust. et Damsh. var. *sphagnorum* (R. M. Schust.) R. M. Schust. et Damsh.	Russia, Yakutia, V. A. Bakalin, 23-11-00	DQ875061	DQ875096
Lophozia propagulifera (Gottsche) Steph.	Russia, Kamchatka region, V. A. Bakalin, K-53-6-02-VBD	Q875062	DQ875097
Lophozia schusteriana Schljakov	Russia, Murmansk region, V. A. Bakalin, G9331	DQ875067	DQ875103
Lophozia silvicola H. Buch	Russia, Karelia, V. A. Bakalin, August 2, 1998	AF519197 [12]	No data
Lophozia silvicola H. Buch	Russia, Nizhni Novgorod region, N. A. Konstantinova, 124-1-03	No data	DQ875102
Lophozia silvicoloides N. Kitag.	Russia, Murmansk region, N. A. Konstantinova, 356-4-00	DQ875064	DQ875099
Lophozia silvicoloides N. Kitag.	Russia, Kamchatka region, V. A. Bakalin, K-57-23-02-VB	DQ875063	DQ875098
Lophozia silvicoloides N. Kitag.	Spitsbergen, N. A. Konstantinova, 150-6-04	DQ875065	No data
Lophozia silvicoloides N. Kitag.	Spitsbergen, N. A. Konstantinova, 150-2-04	No data	DQ875100
Lophozia sudetica (Nees ex Huebener) Grolle	Russia, Murmansk region, V. A. Bakalin, June 4, 1998	AF519195 [12]	DQ875113
Lophozia sudetica (Nees ex Huebener) Grolle	Russia, Commander Islands, V. A. Bakalin, K-41-5-04	No data	DQ875115
Lophozia sudetica (Nees ex Huebener) Grolle	Russia, Kemerovo region, N. A. Konstantinova, 90-7-00	DQ875077	DQ875114
Lophozia cf. *wenzelii* (Nees) *Steph.* var. *groenlandica* (Nees) Bakalin	Russia, Kemerovo region, N. A. Konstantinova, 67-3-00	DQ875070	DQ875106
Lophozia ventricosa (Dicks.) Dumort. var. *guttulata* (Lindb. et S.W. Arnell) Bakalin	Russia, Buryatia, N. A. Konstantinova, 81-1-01	DQ875072	DQ875108

Table (Contd.)

Taxon	Sampling locality, collector, KPABG herbarium number	GenBank accession number	
		trnL	ITS
Lophozia ventricosa (Dicks.) Dumort. var. *longiflora* (Nees) Macoun	Russia, Chita region, N. A. Konstantinova, 11-5-00	DQ875066	DQ875101
Lophozia wenzelii (Nees) Steph. var. *groenlandica* (Nees) Bakalin	Russia, Murmansk region, N. A. Konstantinova, 9329	DQ875073	DQ875109
Lophozia wenzelii (Nees) Steph. var. *lapponica* H. Buch et S.W. Arnell	Spitsbergen, N. A. Konstantinova,124-2-04	DQ875076	DQ875112
Lophozia wenzelii (Nees) Steph. var. *litoralis* (S.W. Arnell) Bakalin	Russia, Murmansk region, V. A. Bakalin, 12-3-02	DQ875074	DQ875110
Lophozia wenzelii (Nees) Steph. var. *massularioides* Bakalin	Russia, the Caucasus, V. A. Onipchenko, August 31, 1983	DQ875075	DQ875111
Schistochilopsis capitata (Hook.) Macoun	Russia, Nizhni Novgorod region, N. A. Konstantinova, 132-03	DQ875080	DQ875119
Schistochilopsis incisa (Schrad.) Konstantinova	Russia, the Caucasus, K. O. Korotkov, August 23, 1999	AY327784 [12]	No data
Schistochilopsis incisa (Schrad.) Konstantinova	Russia, Murmansk region, N. A. Konstantinova,187-1-02	DQ875083	No data
Schistochilopsis grandiretis (Lindb. Ex Kaal.) Schiffn.	Russia, Kamchatka region, V. A. Bakalin, 99-5-01-VB	DQ875081	DQ875120
Schistochilopsis laxa (Lindb.) Grolle	Russia, Murmansk region, N. A. Konstantinova, 40-6-94	DQ875084	DQ875053 (5,8SpDNA, ITS2) DQ875122 (ITS1)
Schistochilopsis opacifolia (Meyl.) Konstantinova	Spitsbergen, N. A. Konstantinova, K-43-2-05	DQ875082	DQ875121
Schistochilopsis opacifolia (Meyl.) Konstantinova	Russia, Murmansk region, N. A. Konstantinova, 315-4-00	DQ875085	DQ875123
Sphenolobus saxicola (Schrad.) Steph.	Russia, Buryatia, N. A. Konstantinova, 123-3-02	DQ875086	DQ875124
Protolophozia elongata (Steph.) Schljakov	Russia, Murmansk region, V. A. Bakalin, 3-1-02	DQ875078	DQ875116
Obtusifolium obtusum (Lindb.) S.W. Arnell	Russia, Murmansk region, V. A. Bakalin, July 1, 2001	AY327769 [12]	No data
Obtusifolium obtusum (Lindb.) S.W. Arnell	Russia, Perm region, N. A. Konstantinova, K-315-1-04	No data	DQ875118

The ML phylogenetic tree is presented in the Fig. 1. In MP analysis, a single tree with the length of 1587 steps was found. It is presented in Fig. 2 with the indication of Bremer support index, jackknife support values, and the branch lengths. The ML and MP tree topologies were identical, except for relative positions of *Lophozia* cf. *wenzelii* var. *groenlandica*, and *L. wenzelii* var. *lapponica*, as well as of *Schistochilopsis incisa* (Murmansk region) within the clades.

DISCUSSION

Judging by the trees constructed, the genus *Lophozia* s. str. is monophyletic. At the same time, all samples of *Lophozia sudetica* formed a clade, which was separated from the other species belonging to *Lophozia* s. str. by the species of the genus *Schistochilopsis*, along with *Protolophozi elongata* and *Obtusifolium obtusum* (Figs. 1 and 2). Similar results were reported in the previous work of our laboratories on the analysis of the *trnL-F* region using another samples of taxa [12]. The ITS1-2 sequence variation among the *Lophozia sudetica* samples constituted 1 to 2%, and variation of the *trnL-F* in the same sample was 0.5%.

In phylogenetic reconstructions, *Obtusifolium obtusum* formed a clade, which was separated from the *Schistochilopsis* by the branch of *Protolophozia elongata*. A number of morphological characters (the leaf shape and insertion, the presence of amphigastria, the structure of the capsule wall, etc.), which differed *Obtusifo-*

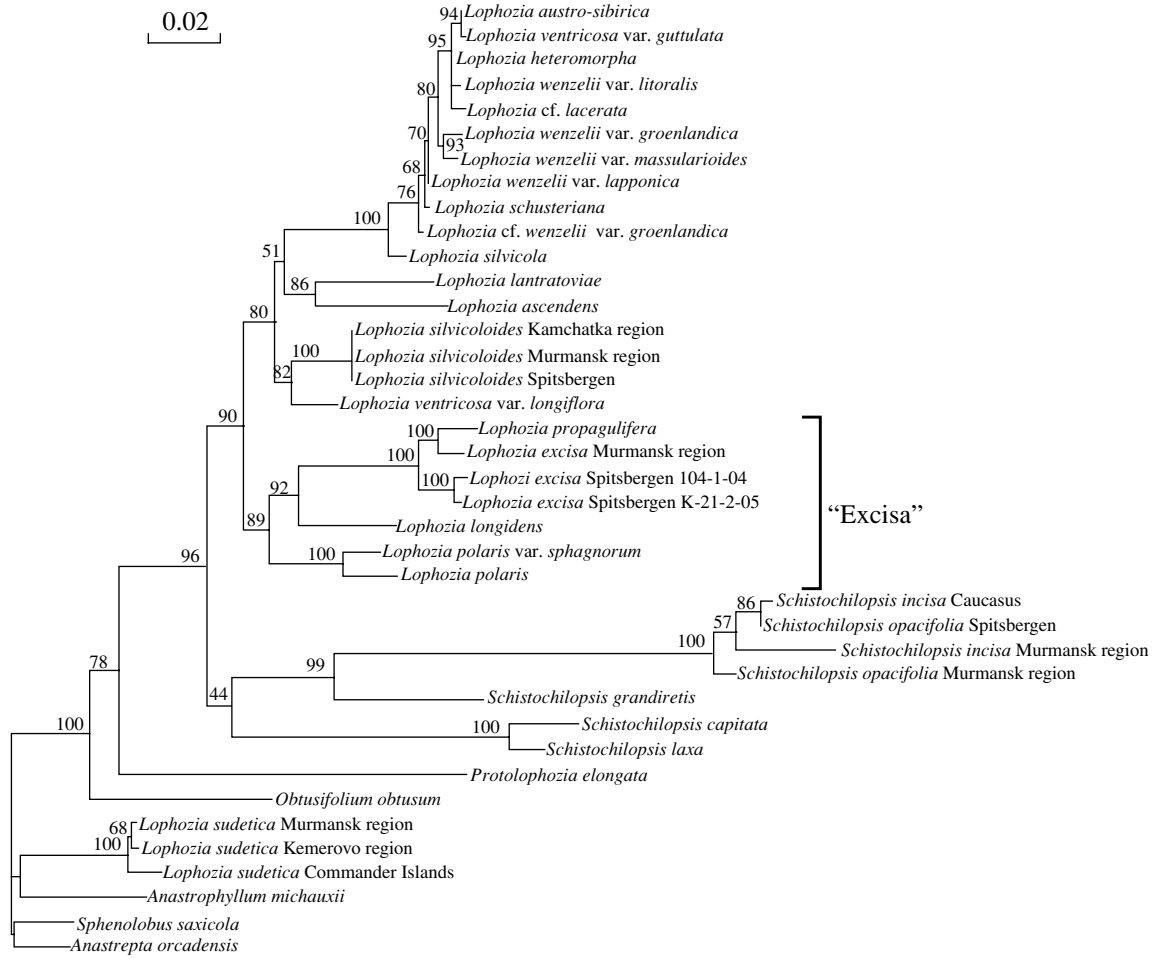

Fig. 1. Phylogenetic tree for the genus *Lophozia* s. str. and relative genera, based on combined nucleotide sequences of nuclear DNA ITS1-2 and cpDNA *trnL-F* locus by the method of maximum likelihood (log$l$ = –7761.38761). The bootstrap supports are indicated.

lium obtusum from all the other species of the genus *Schistochilopsis* were supported by the presence of a number of substitutions and indels, along with specific deletion within the P8 hairpin of the *trnL* intron. These data provided additional basis for treatment of the *Obtusifolium* rather as an individual genus [3, 4, 10, 20], than as the section of the genus *Schistochilopsis* [1, 11].

All the species of *Schistochilopsis* examined formed a clade, however, with weak support (Figs. 1 and 2). Tree positions of *Schistochilopsis laxa* and *S. capitata* were consistent with their grouping into the section *Heterogemma* (Joerg.) Potemkin (*Marshicae* R. M. Schust). Similarly, tree positions of *S. opacifolia*, *S. incisa*, and *S. grandiretis* were consistent with their grouping into the section *Incisae* (C.E.O. Jensen) Potemkin [8, 11, 21, 22].

Sequence differences between *Schistochilopsis opacifolia* and *S. incisa* were low (3 to 4%) and almost the same as between the two samples of *S. opacifolia* from Spitsbergen and Murmansk region. Morphologically studied samples of *S. opacifolia* and *S. incisa* from Murmansk region were very similar, differing only in the toothing patterns of the perianth mouth and leaves. These findings support of the viewpoint of Bisang [22] and Schuster [1], who recognized *Schistochilopsis opacifolia* as a subspecies of *S. incisa*.

Schistochilopsis grandiretis demonstrated the presence of substantial morphological differences from *S. opacifolia* and *S. incisa* (cell network pattern, stem coloration, and others), which characterized it as fairly distinct species. In the trees constructed (Figs. 1 and 2), it was a sister species to the *S. opacifolia* + *incisa*.

Comparison of the minimum numbers of nucleotide substitutions occurred during the evolution in accordance with the MP tree, suggests a relative molecular criterion of the taxa ranks within the genus *Schistochilopsis*. If we assume the number of 1 to 12 nucleotide substitutions as typical to the subspecies of *Schistochilopsis*, then the species status of *S. grandiretis, S. incisa* + *opacifolia*, as well as of *S. laxa* and *S. capitata* will be determined by 40 to 75 substitutions, while the sections of *Incisae* and *Heterogemma* will be determined by 128 to 170 substitutions.

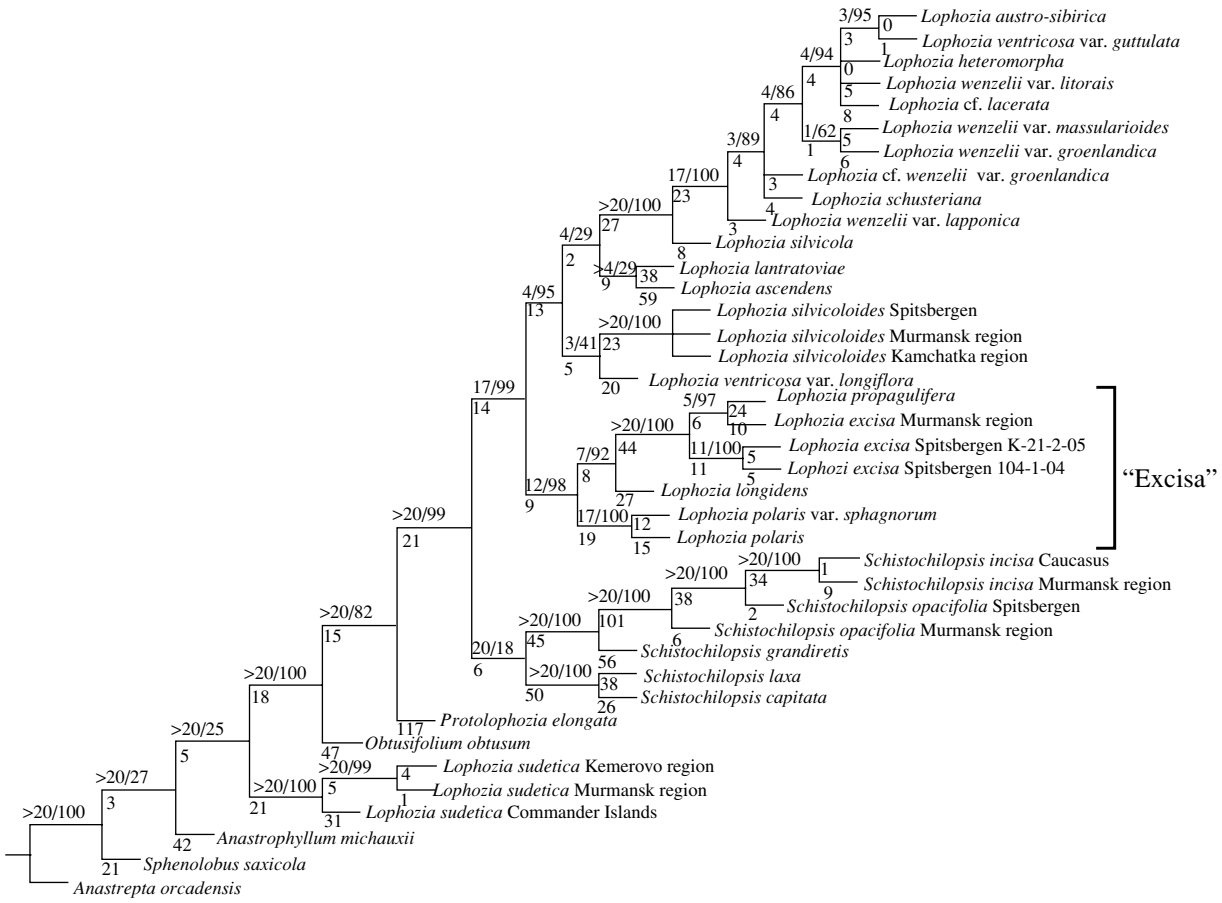

Fig. 2. Phylogenetic tree for the genus *Lophozia* s. str. and relative genera, based on combined nucleotide sequences of nuclear DNA ITS1-2 and cpDNA *trnL-F* locus by the method of maximum parsimony. The tree length is 1587. Numbers above branches indicate Bremer and jackknife support indices, and numbers below branches indicate branch lengths.

In all trees constructed, the species belonging to *Lophozia* s. str. (except for *Lophozia sudetica*, see above) were subdivided into two main clusters.

Except for the *Lophozia lantratoviae*, the species of the genus *Lophozia* s. str., having either reddish or brown-red gemma, formed a monophyletic group. This group was in general consistent with the volume of the section *Excisae*, according to Bakalin [4]. However, *Lophozia longidens* also falls into this section. Schuster [1] treated the latter species as an independent section, *Longidenatae* R. M. Schust. Schljakov [3] placed this species into the section *Guttulatae* Schljakov, while Bakalin [4] placed *L. longidens* into the section *Lophozia*. Thus, differently treated morphological characters (stem structure, growing pattern, leaf shape, bulbil coloration, and others) do not provide determination of the relationships of *Lophozia longidens*. Based on molecular data obtained, the species was placed into the section *Excisa*.

In modern classification systems, position of the little-known, predominantly Arctic species, *Lophozia polaris*, remains uncertain. In different works, the species was attributed to the sections *Lophozia* [1], *Heteromorphae* R. M. Schust. [3, 6], and finally, to *Excisa* [4]. Our data favor the latter viewpoint. Sequence differences between the two varieties of this species from two far distant regions (Spitsbergen and Yakutia) (26 substitutions in the MP tree) are comparable with those between the three forms of *Lophozia excisa* from the Spitsbergen and Murmansk region, as well as between *L. excisa* and *L. propagulifera* (32 to 46 substitutions).

Three forms of *Lophozia excisa* and *L. propagulifera* (*L. latifolia*) formed a separate subclade within the clade Excisa with the strongest support. *Lophozia excisa* is one of widely distributed and polymorphic species of the genus. This situation is reflected in the variation of the obtained genome loci in the species samples from different regions and ecological conditions (table). The difference between the two large-cell forms of *L. excisa* from Spitsbergen and Murmansk region (K21-2-05 and 41-2-97, respectively) is close to that between the small-cell form of *L. excisa* (104-1-04) and *L. propagulifera* (32 and 46 substitutions, respectively). Sequence differences between the large-cell forms of *L. excisa* from Murmansk region and Spitsbergen are comparable to those revealed upon comparison

of large- and small-cell specimens from Spitsbergen. Morphological differences between the specimens mentioned were also rather high (leaf thickness at the basement, leaf shape, plant size, and others). Further investigations will show whether these characters are stable, and whether the forms described deserve awarding a certain taxonomic rank.

In the group of *Lophozia* with green gemma, a clade with strong support is formed by *Lophozia silvicoloides* and *L. ventricosa* var. *longiflora*. Furthermore, the sequences of the *Lophozia silvicoloides* specimens from the Commander Islands, Murmansk region, and Spitsbergen are identical and remarkably different form the examined sequences of *L. ventricosa* var. *longiflora* from Chita region (by 5% at the ITS1-2 and by 2% at the *trnL*). Substantial morphological differences of these species (perianth mouth structure, oil bodies' pattern, and others), along with molecular data confirm the species status of *Lophozia silvicoloides* and demonstrate the absence of close relationships with *L. silvicola* (Figs. 1 and 2), which were earlier suggested by Schuster [1].

Lophozia ascendens and *L. lantratovie* group into one clade, albeit their ITS1-2 and *trnL-F* sequences in the MP tree are separated by 97 steps. *L. ascendens* is placed in the sections: *Longidentatae* R. M. Schust. [1], *Guttulatae* Schljakov. [3], and *Lophozia* [4]. However, according to Bakalin [4], the last section includes the two former ones. Concerning *L. lantratoviae*, it should be noted that this recently described species is also included by its author into the section *Lophozia*. The two species discussed are substantially different relative to their morphology, and they occupy different ecological niches. Specifically, *L. ascendens* is obligate epixylous, while *L. lantratoviae* is found on the fine soil at the banks of the mountain rivers.

Lophozia ventricosa–Lophozia wenzelii represent the most complicated and intricate complex. Many varieties, subspecies, and species were included into these taxa as different ranks and variants. As shown in the Figs. 1 and 2, genetic differences between them are low. Nevertheless, in the trees constructed, the samples belonging to different taxa studied and attributed to *L. wenzelii*, were united into two groups. One group consisted of *L. wenzelii* var. *groenlandica* and *L. wenzelii* var. *massularioides*. *L. wenzelii* var. *lapponica* appeared to be close to them. However, *L. wenzelii* var. *litoralis* fell into another clade. In any case, attribution of *L. wenzelii* and *L. schusteriana* to the section *Sudeticae*, suggested by Schljakov [3], and then by Bakalin [4], was not confirmed. The trees constructed in the present and previous studies [12] showed that *Lophozia sudetica* formed an independent clade, which was far distant from the other species of *Lophozia* s. str.

The tree position of recently described *Lophozia austro-sibirica*, which is known only from the type locality in Southern Siberia and from one site in Western Siberia [4], should be discussed in more detail. This species formed an isolated clade together with *L. ventricosa* var. *guttulata* with very strong support. In these taxa, the genomic sequences examined differed only by one substitution in the *trnF* gene. Morphological differences were very small, and mostly constituted in the fact that *L. austro-sibirica* was monoecious, while *L. ventricosa* var. *guttulata* was dioecious. It seems likely that *L. austro-sibirica* and *L. ventricosa* var. *guttulata* represent one species (the presence of monoecious and dioecious individuals was described in some Holarctic species of *Lophozia*, *Isopaches*, and some others).

We would like to note the low genetic divergence between the geographically distant populations of some taxa shown for the loci examined in this study.

Thus, based on sequence analysis of nuclear DNA ITS1-2 and cpDNA *trnL-F* loci in a great number of *Lophozia* s. str. species, including the poorly studied and recently described taxa, it was demonstrated that the genus of interest was monophyletic, except for *Lophozia sudetica*, which deserved isolation into a genus. None of the existing sectioning of *Lophozia* s. str. was supported by the tree topology. However, one out of two clearly distinguished groups was consistent with volume of the section *Excisae*, according to Bakalin [4]. This group contained all the species with red or brownish gemma examined, excluding *Lophozia lantratoviae*. Among the species with either colorless or green gemma the clade *Lophozia silvicoloides* + *L. ventricosa* var. *longiflora* was distinguished. The relationships within the complex *Lophozia ventricosa–Lophozia wenzelii* remain unclear. The recently described species, *L. austro-sibirica*, practically does not differ from *L. ventricosa* var. *guttulata* in the sequences of the genomic regions examined, and probably can not be treated as a distinct species. Species rank of *L. lantratoviae*, described in 2003, is confirmed by substantial genetic differences from the other species of the genus.

The tree relationships within the *Schistochilopsis* were consistent with the sectioning of the genus based on morphological characters. The remarkable genetic differences between *S. laxa* and *S. capitata* confirmed their species status. The idea on the inclusion of *Obtusifolium obtusum* into *Schistochilopsis* was not supported.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (grant nos. 06-04-48225 and 06-04-49493).

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