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ANIMAL GENETICS

Phylogeny of Charrs of the Genus *Salvelinus* Based on Mitochondrial DNA Data

A. G. Oleinik[a], L. A. Skurikhina[a], and Vl. A. Brykov[a, b]

[a] *Zhirmunsky Institute of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, 690041 Russia*
e-mail: alla_oleinik@mail.ru

[b] *Far Eastern Federal University, School of Natural Sciences, Vladivostok, 690600 Russia*

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Abstract—Charrs of the genus *Salvelinus* (including *Salvethymus*) represent a monophyletic group of salmonid fishes that diverged from the common ancestor without subdivision into subgenera. The phylogenesis of the genus is characterized by four cycles of mitochondrial genome divergence. The first one, belonging to the Late Miocene—the border between Miocene and Pliocene (6 to 4 million years ago)—was associated with the consecutive divergence of the *S. fontinalis*, *S. namaycush*, *S. levanidovi*, and *S. leucomaenis* basal branches. Two divergence events, including separation of the ancestral lineage of Western Pacific group of *S. m. krascheninnikovi* and the following segregation of the common ancestor into two mitochondrial phyla, happened within the period of 3 to 2 million years ago. The next cycle is attributed to the time interval of about 1 million years ago and includes the divergence of both phyla. In one phylum, a relatively quick isolation of Arctic and Eastern Pacific phylogroups, along with the divergence of the latter phylogroup into *S. confluentus* and *S. m. lordi* lineages, took place. At the same time, the second phylum diverged into the *S. m. malma* and *S. alpinus* phylogenetic groups. At the final stage (Middle to Late Pleistocene), differentiation of the taxa within the phylogenetic groups took place.

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INTRODUCTION

Charrs of the genus *Salvelinus* (Salmoniformes: Salmonidae) represent an important component of the arctic and subarctic freshwater ecosystems of Europe, Eastern Siberia, Northeast Asia, and North America belonging to the Pacific Ocean, Atlantic Ocean, and Arctic Ocean basins. Moreover, the degree of morphoecological variability in charrs appeared to be much higher compared to other representatives of fish fauna of northern arctic communities. Most of the researchers denote that charrs are one of the most interesting, yet taxonomically complex group among the salmonids. Therefore, despite good morphological research [1–4 and others], many fundamental issues of the genus phylogeny remain unresolved. In this regard, molecular genetic studies of charrs are of great interest, since they enable determination of the genetic differences between the populations at different levels of phenotypic divergence. Moreover, these studies make it possible to determine the hierarchy of the relationships among the taxa, their mutual isolation, the time of occurrence, and the degree of divergence from the common ancestor.

The prospects for resolving the phylogenetic issues in young closely related groups, which include the charrs of the genus *Salvelinus*, are associated with the use of mitochondrial DNA. In this case, use of the more slowly evolving nuclear markers often faces the problem of the limited number of phylogenetically informative characters (synapomorphies), which leads to the construction of polytomous dendrograms with a great number of weakly supported nodes. However, despite the large number of publications [5–17 and others], no generalized phylogeny of the mitochondrial groups, which would agree with morphological phylogeny of the genus *Salvelinus*, has previously been constructed. Our studies of the genetic differentiation and divergence in charrs of the genus *Salvelinus* with PCR-RFLP analysis of mtDNA fragments [11–13] provided insight into the evolution of the main phylogenetic groups of charrs. However, the proposed hypothesis was not free of the limitations associated with the composition of the examined taxa and the characters. For these reasons, the obtained reconstruction, as well as the phylogenetic hypothesis formulated on its basis [11], was initially considered as preliminary, requiring confirmation for the enlarged taxa composition. These findings stimulated further phylogenetic studies of charrs.

MATERIALS AND METHODS

The study is based on original material from the collection of the authors, which was formed from 1995 through 2005. The examined charrs of the genus *Salvelinus* included white charr *S. albus*, Arctic charr *S. alpinus alpinus* and *S. a. oquassa*, Chukchi charr

S. andriashevi, bull trout *S. confluentus*, Krogius charr *S. krogiusae*, longhead charr *S. kronocius*, white-spotted charr *S. leucomaenis*, Levanidov charr *S. levanidovi*, northern Dolly Varden *S. malma malma*, southern Asian Dolly Varden *S. m. krascheninnikovi*, southern American Dolly Varden *S. m. lordi*, Schmidt's charr *S. schmidti*, Taranetz charr *S. taranetzi*, *Salvelinus* sp. 4 from Nachikinskoe Lake [according to 18], and *Salvelinus* sp. (Table 1). Atlantic salmon *Salmo salar* was used as outgroup. The detailed laboratory protocol of mtDNA analysis was described earlier [11–13]. In accordance with the objectives of the study, different sets of 20 restriction endonucleases (MBI Fermentas, Lithuania; SibEnzyme, Russia) were used, including *Asu*I, *Ava*I, *Ava*II, *Bst*NI, *Bst*UI, *Dde*I, *Eco*RV, *Hha*I, *Hin*fI, *Mbo*I, *Mbo*II, *Msp*I, *Rsa*I, *Sty*I, *Bsu*RI, *Vsp*I, *Bcl*I, *Ssp*I, *Bme*1390I, and *Taq*I. The data on the sizes of the restriction fragments and the composition of combined haplotypes can be obtained from the authors upon request. Statistical treatment of the data was performed using the MRBAYES version 3.2 [19], PAUP version 4.0b10 [20], PHYLIP 3.67 [21], and REAP [22] software packages.

Formation of the taxon-character matrices for phylogenetic analysis was performed using restriction sites as characters and combined mtDNA haplotypes or isolated taxonomic groups, as operation taxonomic units (OTUs). Statistical analysis included phenetic analysis of genetic similarity over the whole set of characters, phylogenetic analysis of the relationships, cladistic analysis of phylogenetically informative characters for determination of the divergence times, testing of the relationships stability upon varying quantity and quality of differentiating characters, and testing of the terminal group composition with the use of distance (UPGMA and NJ) and character-based (MP, ML, BI) methods.

Dendrograms based on a clustering algorithm were constructed using the nucleotide divergence estimates as the genetic distances between mtDNA haplotypes. Calculations were made using original formulas designed for the restriction sites [23–25], as well as the modified formula (K2P) [26]. If the taxa played the roles of terminal groups, nucleotide divergence was estimated based on the evolutionary model of Nei and Tajima [27, 28] (the DA program in the REAP software package). Then, the distance matrix was transferred to the PHYLIP 3.67 for clustering and testing of the dendrogram topologies. Phenetic analysis in the PAUP 4.0b10 and PHYLIP 3.67 programs (the RESTDIST, SEQBOOT, NEIGHBOR, CONSENSE steps) was conducted sequentially for each data matrix. At this stage, testing was also performed for relationship stability with respect to the quantity and quality of differentiating characters and the nucleotide substitution model chosen for calculating pairwise distances.

Phylogenetic analysis using maximum parsimony (MP) for each character set was performed for a number of substitution models with two searching strategies (heuristic search, branch-and-bound). A heuristic search for the best-fit tree (the TBR and MulTrees options) was carried out in 100 replicates, limiting the maximum possible tree number to 1000. If best tree identification was impossible, the differences between alternative topologies were estimated using Templeton [29] and Kishino-Hasegawa [30] tests. The search for the best-fit MP tree was also performed with the branch-and-bound algorithm by PENNY among the 1000 pseudoreplicates obtained from the initial matrix in the SEQBOOT software program.

Phylogenetic analysis with maximum likelihood (ML, BI) was performed based on the nucleotide substitution model F84 [30], which was most appropriate for the restriction sites and was chosen a priori by the MRBAYES 3.2 program. A heuristic search for the best-fit ML tree was performed in 30 to 100 replications (TBR option) with a random pattern of the OUT inclusion. Analysis with a Bayesian maximum likelihood approach (Bayesian Inference, BI) was carried out with the following preset parameters of the Monte Carlo algorithm (MCMC): simultaneous launching of ten Markov chains (nine hot and one cold), 5100000 cycles, with selection of each 500th and discarding of the first 5001 of the generated trees (burnin = 5001). The cladogram reconstruction was performed with the maximum parsimony approach based on maximum number of phylogenetically informative (synapomorphic) characters with a heuristic search in the PAUP 4.0b10 program and branch-and-bound algorithm by PENNY. The analysis parameters described above were maintained in all variants.

The statistical significance of all topologies was examined with bootstrap [31] with 1000 random permutations for each data set. Robustness of the branching notes was evaluated by bootstrap support (*BS*), as well as by posterior probabilities (*PP*) for the BI trees. Graphic representation of the dendrograms was obtained in the TreeView program (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). The divergence time was calculated based on the genetic divergence between the taxa [27, 28] for the three mtDNA divergence time estimates: 0.92% [32], 1% [33], 1.6% [34] nucleotide substitutions per million years per two lineages.

RESULTS AND DISCUSSION

Phylogenetic Relationships among the mtDNA Haplotypes in Charrs of the Genus Salvelinus

Phylogenetic analysis combined two sequential stages of reconstructions based on the PCR-RFLP analysis of three (*ND1/ND2*, *Cytb/D-loop*, *ND5/ND6*) (185 haplotypes) and six (*ND1/ND2*, *Cytb/D-loop*, *ND5/ND6*, *ND3/ND4L/ND4*, *A8/A6/COIII*, *COI/COII*)

Table 1. Data on the examined *Salvelinus* taxa

Taxon	Location (symbol)	Sample size	
		N_1	N_2
S. leucomaenis	Izmeny Bay (**LEIZ**)*, Kamchatka River (**LEKA**)	13	10
S. levanidovi	Yama River (**LVYA**)*	13	13
S. taranetzi	Achchen Lake (**TRAC**)*, Pekulineiskoe Lake (**TRMP**)	27	15
S. krogiusae	Dal'nee Lake (**KGDA**)*	5	5
S. andriashevi	Estikhed Lake (**ANES**)*	2	2
Salvelinus sp. 4	Nachikinskoe Lake (**SPNA**)*	17	17
S. alpinus alpinus	Lama Lake, Arylakh Lake, Aily Lake (**ARGA.002-014**)* Fjellfrosvatnet Lake (**ARGA.015-017**)*, Sitasjaure Lake (**ARLS**), Muckross Lake (**AMUC**)	49	9
S. alpinus oquassa	Floods Pond (**ARMA**)*	22	
S. albus	Raduga River (**ALKA**)*, Kronotskoe Lake (**ALKR**)	58	10
S. kronocius	Kronotskoe Lake (**KRKR**)*	36	36
S. schmidti	Kronotskoe Lake (**SHKR**)*	44	44
S. confluentus	Nakina River, Mystic Creek, Murray River, Chowika River (**BTNA**)*	5	5
S. malma krascheninnikovi	Akur River (**MSAK**)*, Val River (**MSVL**), Aniva Creek (**MSAN**), Teplyi Creek (**MSTE**), Bezymyannyi Creek (**MSRU**), Lesnaya River (**MSLE**), Smolnyi Creek (**MSSM**), Tumnin River (**MSTU**), Shamora River (**MSSC**), Tatarka River (**MSTT**), Gryaznaya River (**MSGR**), Yuvishi River (**MSYU**), Tashiusu River (**MSTA**)	238	10
S. malma malma	Achchen Lake (**MNAC**), Pekulineiskoe Lake (**MNMP**), Anadyr River (**MNAN**), Apuka River (**MNAP**), Kamchatka River (**MNKA**)*, Kronozkaya River (**MNKR**)*, Krokur Lake (**MNKK**), Grishkin Creek (**MNNA**)*, Paratunka River (**MNPA**)*, Yama River (**MNYA**), Kholodnyi Creek (**MNHL**), Kukhtui River (**MNKU**), Kongakut River (**DKON**), Saviukviak River (**DSAV**), Kivalina River (**DKIV**), Kelly River (**DKEL**), Cobblestone River (**DCOB**), Sinuk River (**DSIN**), Solomon River (**DSOL**), Kanektok River (**DKAN**), Kashaiak River (**DKR**), Becharof Lake (**DBEL**), Frosty Creek (**DFRO**), Russel Creek (**DRUS**), Quartz Creek (**DQUA**), Snow Creek (**DSNO**), Auke Bay (**DAUK**)	436	30
S. malma lordi	Auke Bay (**DSAU**)*, Zeballos River (**DSZE**)*, Mill Creek (**DSMI**)*, Toba River (**DSTO**)*, Nooksack River (**DSNO**)*, Dungeness River (**DSDA**)*	14	14
Salvelinus sp.	Grand Lake (**MNGR**)	30	

Sample size: N_1, *ND1/ND2*, *Cytb/D-loop*, *ND5/ND6* fragments; N_2, *ND1/ND2*, *Cytb/D-loop*, *ND5/ND6*, *ND3/ND4L/ND4*, *A8/A6/COIII*, *COI/COII* fragments. The samples examined at six mtDNA fragments are designated by asterisks.

(93 haplotypes) mtDNA fragments. The lack of the data for the *12S/16S* fragment cannot lead to any significant shift in the final results. This is because comparative analysis of 67 completely sequenced mitochondrial genomes of the six salmonid species [35] showed that ribosomal genes in this group of fishes were highly conserved. Regardless of the character set used, no principal changes, either in the haplotype grouping or in the branching order within the clusters uniting relative charr taxa, were observed. Because of this, here we present only one dendrogram topology with the maximum number of terminal groups, identified in 1012 charrs (Fig. 1). A number of haplotypes groups differentiate such taxa as *S. levanidovi*, (haplotypes LV), $BS > 98\%$; *S. leucomaensis* (LE), $BS > 98\%$; *S. m. krascheninnikovi* (MS), $BS > 98\%$; *S. taranetzi* (TR), $BS > 50\%$; *Salvelinus* sp. 4 (SP), $BS > 85\%$: *S. alpinus* (AL), $BS > 98\%$; *S. m. lordi* (LO), $BS > 98\%$;

S. confluentus (BT), *BS* > 98%. Statistically significant topological elements were represented by the mtDNA haplotype macroclusters as follows: (1) ((*S. m. malma*, *S. albus*, *S. kronocius*, *S. schmidti*) *S. alpinus*) (*BS* > 94%); (2) ((*S. taranetzi*, *S. andriashevi*, *S. krogiusae*) *Salvelinus* sp. 4) (*BS* > 96%); (3) (*S. m. lordi*, *S. confluentus*) (*BS* > 50%). An increase in the character number first leads to polytomy resolution at the tree base and determines the divergence of the three basal branches of *S. leucomaensis*, *S. levanidovi*, and *S. m. krascheninnikovi*. Second, it makes it possible to determine the divergence order in the haplotype group (*S. taranetzi*, *S. krogiusae*, *S. andriashevi*). At the same time, an increase in the character number had no influence on the structure of the haplotype group cluster (*S. m. malma*, *S. albus*, *S. kronocius*, *S. schmidti*), where no stable structural elements associated with the presumptive taxonomic differentiation were observed.

A characteristic feature of the tree topology of the genus *Salvelunus* phylogenetic group is the presence of two clusters in which the polyphyletic haplotype composition is supported by high statistical estimates. It is suggested that the reason for the poor branch resolution of the phylogenetic group (*S. taranetzi*, *Salvelinus* sp. 4, *S. krogiuse*, *S. andriashevi*) within the gene trees lies in true radiation-associated polytomy. In this case, radiation is the preferable explanation, because it correlates with the dispersal in the new adaptive zone. In addition to unresolved topology, which is mostly caused by the preservation of ancestral polymorphism, this scenario implies similar divergence estimates between the main clades. A monophyletic group (*S. taranetzi*, *Salvelinus* sp. 4, *S. krogiuse*, *S. andriashevi*) demonstrates the congruence with the description parameters indicated. There are no doubts that the suggested hypothesis requires confirmation and, in light of the specific features of the mtDNA inheritance, at the level of nuclear genes.

At the same time, the second phyletic group (*S. m. malma*, *S. albus*, *S. schmidti*, *S. kronocius*) forms steady mtDNA haplotype clusters within all trees, but without the haplotype clustering support in accordance with taxonomic affiliation. Recent genealogical analysis of mtDNA haplotypes showed that the observed pattern most likely was the consequence of a complex combination of several factors in the background of ancestral polymorphism preservation [12, 13]. However, at the present stage, it is impossible to see the difference between the influence of incomplete lineage segregation and historical introgression, as well as to estimate the gene flow in the young taxa preserving ancestral polymorphism [36].

Phylogenetic Relationships of Charrs of the Genus Salvelinus Based on the Data of mtDNA Analysis

Based on the data of cladistics analysis, as well as phenetic and phylogenetic analysis of the mtDNA haplotypes, a phylogram representing the divergence of maternal phylogenetic lineages of charrs of the genus *Salvelinus* was reconstructed (Fig. 2). From the phylogram it follows that evolution of the group contained four divergence cycles. The first two cycles included sequential divergence of the basal branches of *S. levanidovi*, *S. leucomaenis*, and *S. m. krascheninnikovi* and later segregation of the phylum from the common ancestor. The next cycle led to the formation of the *S. alpinus* and *S. m. malma* phylogenetic groups within one phyletic branch. Simultaneously with this event, the second phylum diverged into the common ancestor of *S. confluentus* and *S. m. lordi* and the common ancestor of the *S. taranetzi* phylogenetic group. At the stage of evolution, divergence of the taxa within phylogenetic groups took place.

According to the phylogram, monophyletic groups of charrs, which are marked by the corresponding phylogenetic groups of mtDNA haplotypes, are confirmed (*S. m. lordi*, *S. confluentus*); ((*S. taranetzi*, *S. andriashevi*, *S. krogiusae*) *Salvelinus* sp. 4); ((*S. confluentus*, *S. m. lordi*) (*Salvelinus* sp. 4, *S. krogiusae*, *S. taranetzi*, *S. andriashevi*)); (*S. m. malma, S. albus, S. kronocius, S. schmidti*); ((*S. m. malma, S. albus, S. kronocius, S. schmidti*) *S. alpinus*). Comparison of the dendrogram topologies and the best-fit trees, which were generated for different terminal groups, showed that the used complex of characters contained a phylogenetic signal providing certain information on phylogenesis.

Phylogenesis of Charrs of the Genus Salvelinus: Presumptive Evolutionary Model

Comparative analysis of the dendrograms obtained based on the genetic data [5–11, 13, 14, 16, 17, 37–41] provided highly reliable confirmation of the divergence order and the evolutionary relationships between charrs of the genus *Salvelinus*. The presence of phylogenetic signal was also tested with data on the karyotype evolution [1, 42, 43 and other]. According to molecular genetic studies, the genus *Salvelinus* is statistically significantly monophyletic. Brook trout *S. fontianlis* is thought to be the basal phylum, which is closest to the common ancestor of the genus. *S. namaycush*, *S. levanidovi*, *S. leucomaenis*, and *S. m. krascheninnikovi* also belong to basal taxa. In this group, a coincidence of the rates and the direction of evolution for different genetic markers was shown, since these taxa represent two karyological lineages (*S. fontinalis*, *S. namaycush*, *S. levanidovi*) and (*S. leucomaenis*, *S. m. krascheninnikovi*). The first of these lineages groups the species with the most primitive karyotypes among the charrs [43]. It is noteworthy that *S. m. krasheninnikovi* represents the latest branch that diverged from the common generic ancestor, and it is a sister group relative to all other charr taxa.

The next stage of phylogenesis is associated with the segregation of the ancestral stem into two phyla, the divergence of which resulted in the formation of

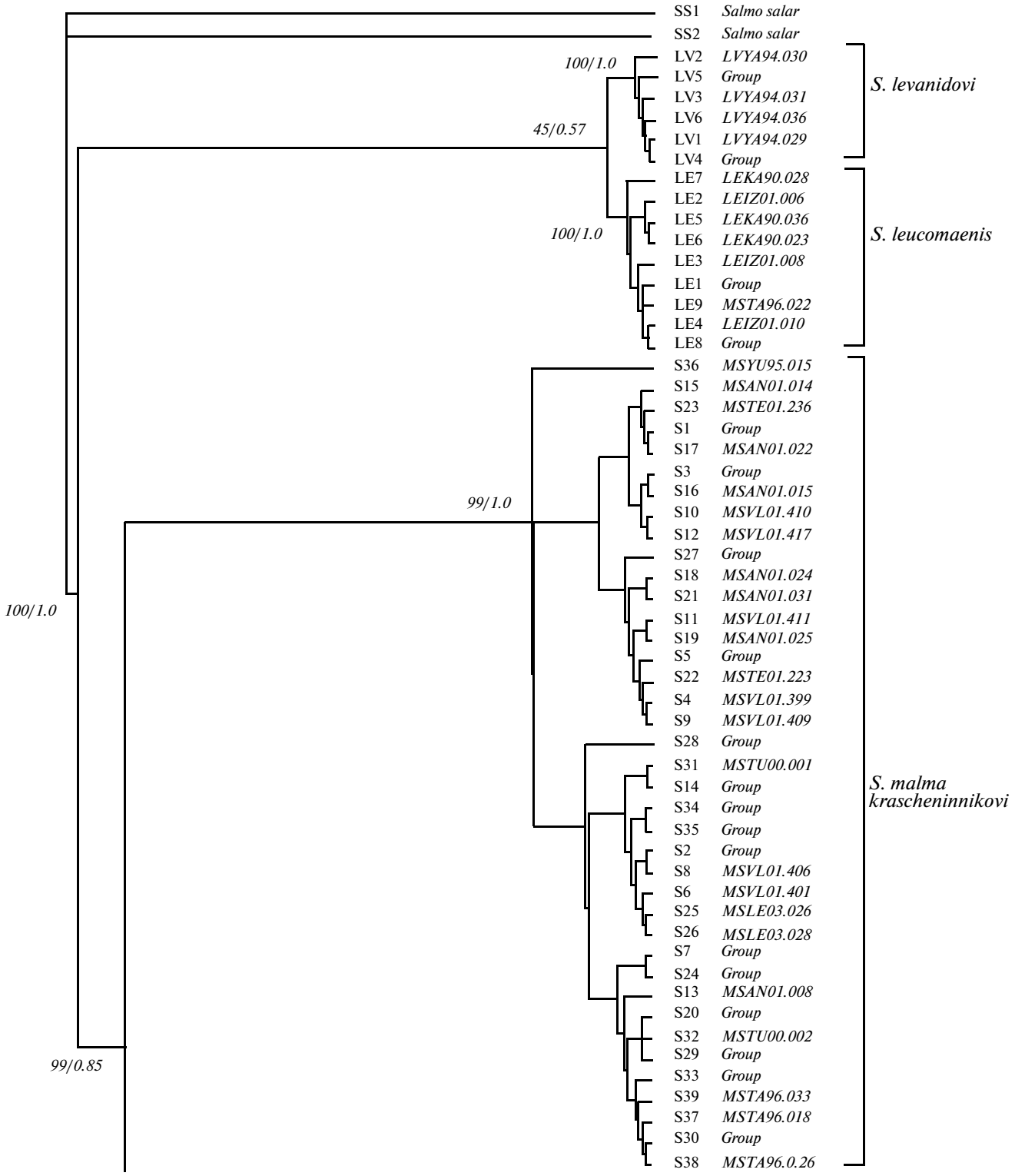


Fig. 1. Phylogenetic relationships among charrs of the genus *Salvelinus* based on the analysis of the mtDNA *ND1/ND2*, *Cytb/D-loop*, and *ND5/ND6* fragments. Consensus topology of the dendrograms of 185 mtDNA haplotypes, generated using MP (*heuristic search*, *l*, 609; CI, 0486; HI, 0.514; RI, 0.923; RC, 0.449) and BI (lnL = –3281.85) algorithms. The figures designate statistical support of the nodes, *BS* (>50%), for MP and *PP*, for BI trees, respectively. Designations of the individuals carrying certain haplotypes correspond to the locality symbols (Table 1). Groups unite the individuals from different localities with appropriate haplotypes.

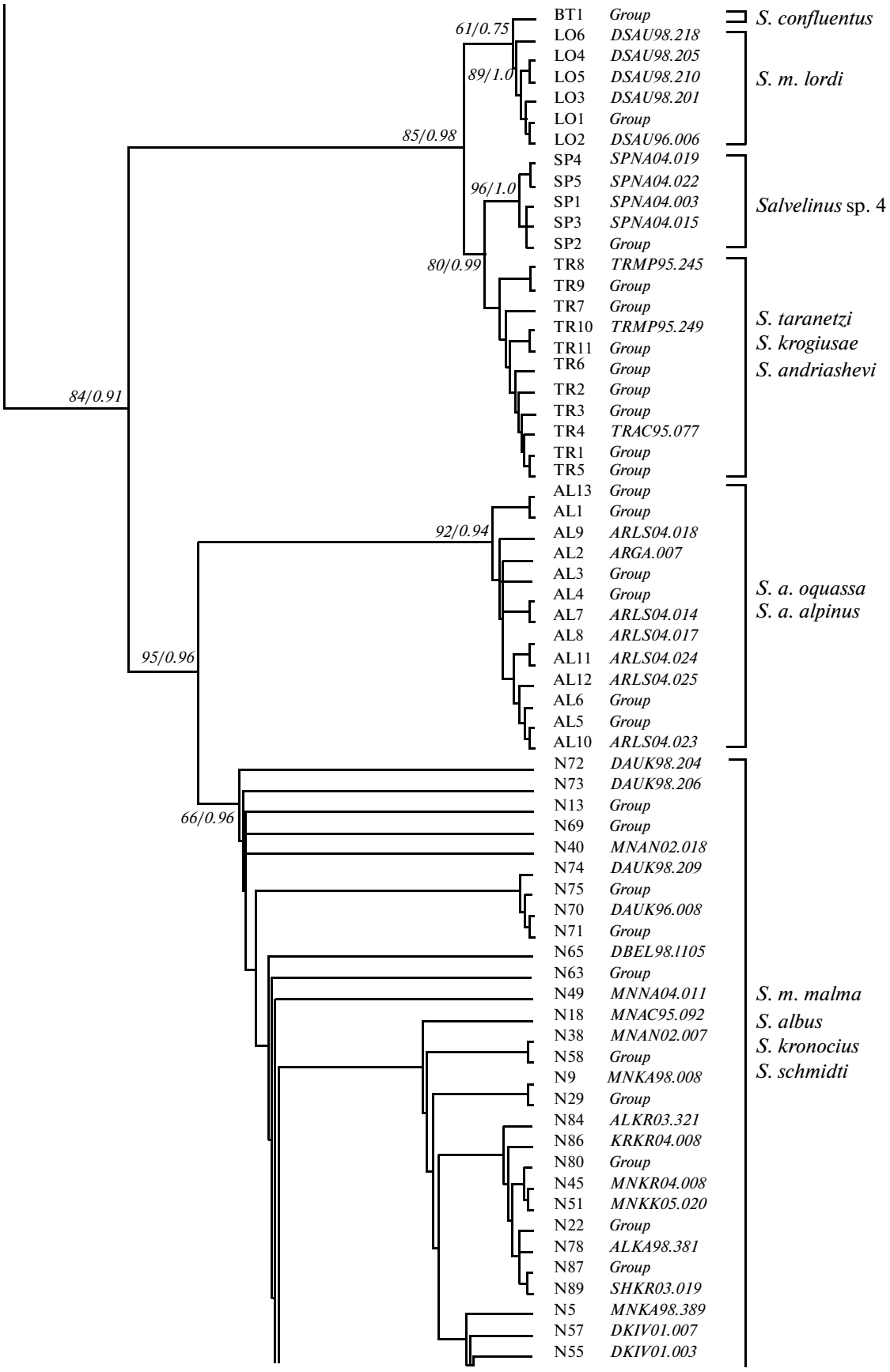
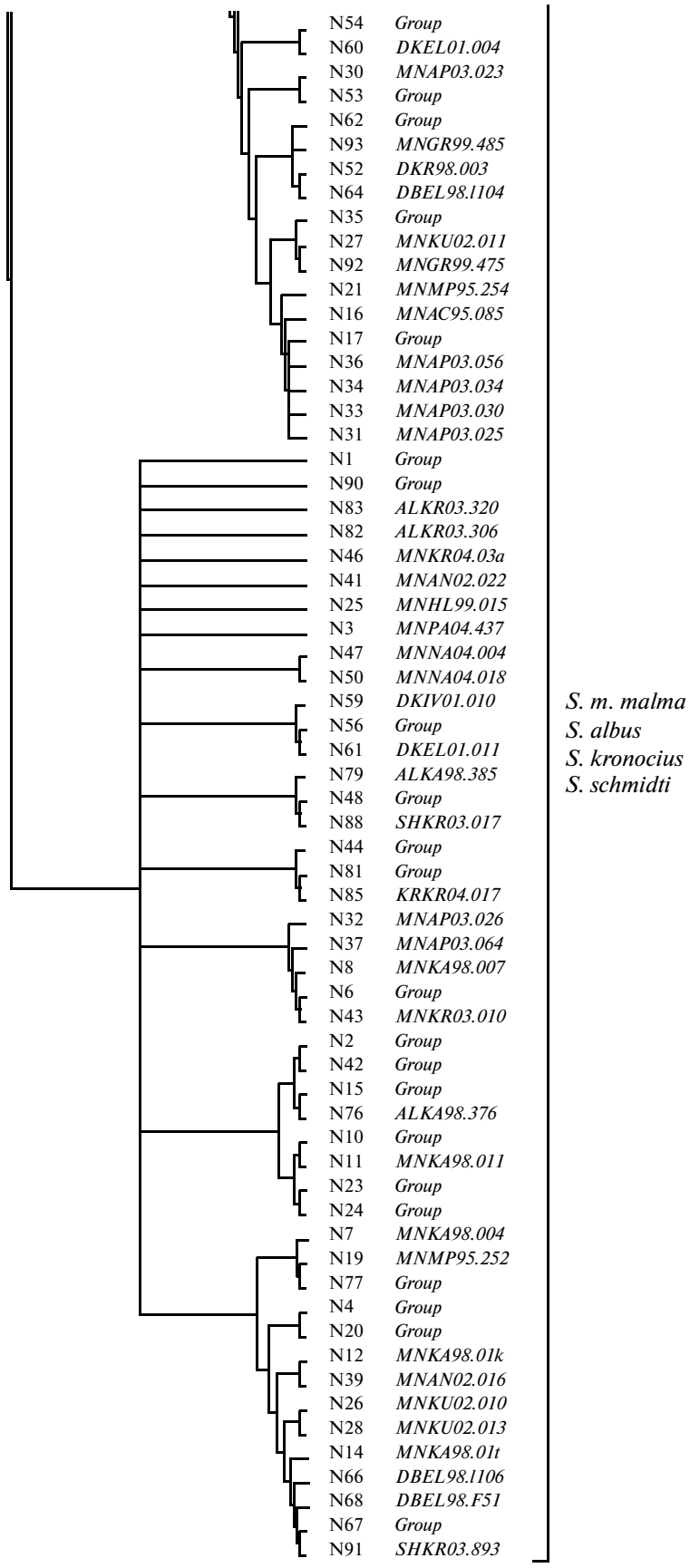

61/0.75
89/1.0
85/0.98
96/1.0
80/0.99
84/0.91
92/0.94
95/0.96
66/0.96
BT1 Group
LO6 DSAU98.218
LO4 DSAU98.205
LO5 DSAU98.210
LO3 DSAU98.201
LO1 Group
LO2 DSAU96.006
SP4 SPNA04.019
SP5 SPNA04.022
SP1 SPNA04.003
SP3 SPNA04.015
SP2 Group
TR8 TRMP95.245
TR9 Group
TR7 Group
TR10 TRMP95.249
TR11 Group
TR6 Group
TR2 Group
TR3 Group
TR4 TRAC95.077
TR1 Group
TR5 Group
AL13 Group
AL1 Group
AL9 ARLS04.018
AL2 ARGA.007
AL3 Group
AL4 Group
AL7 ARLS04.014
AL8 ARLS04.017
AL11 ARLS04.024
AL12 ARLS04.025
AL6 Group
AL5 Group
AL10 ARLS04.023
N72 DAUK98.204
N73 DAUK98.206
N13 Group
N69 Group
N40 MNAN02.018
N74 DAUK98.209
N75 Group
N70 DAUK96.008
N71 Group
N65 DBEL98.1105
N63 Group
N49 MNNA04.011
N18 MNAC95.092
N38 MNAN02.007
N58 Group
N9 MNKA98.008
N29 Group
N84 ALKR03.321
N86 KRKR04.008
N80 Group
N45 MNKR04.008
N51 MNKK05.020
N22 Group
N78 ALKA98.381
N87 Group
N89 SHKR03.019
N5 MNKA98.389
N57 DKIV01.007
N55 DKIV01.003
S. confluentus
S. m. lordi
Salvelinus sp. 4
S. taranetzi
S. krogiusae
S. andriashevi
S. a. oquassa
S. a. alpinus
S. m. malma
S. albus
S. kronocius
S. schmidti

Fig. 1. (Contd.)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N46 MNKR04.03a
N41 MNAN02.022
N25 MNHL99.015
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N47 MNNA04.004
N50 MNNA04.018
N59 DKIV01.010
N56 Group
N61 DKEL01.011
N79 ALKA98.385
N48 Group
N88 SHKR03.017
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N81 Group
N85 KRKR04.017
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N37 MNAP03.064
N8 MNKA98.007
N6 Group
N43 MNKR03.010
N2 Group
N42 Group
N15 Group
N76 ALKA98.376
N10 Group
N11 MNKA98.011
N23 Group
N24 Group
N7 MNKA98.004
N19 MNMP95.252
N77 Group
N4 Group
N20 Group
N12 MNKA98.01k
N39 MNAN02.016
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N28 MNKU02.013
N14 MNKA98.01t
N66 DBEL98.1106
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N91 SHKR03.893
S. m. malma
S. albus
S. kronocius
S. schmidti

Fig. 1. (Contd.)

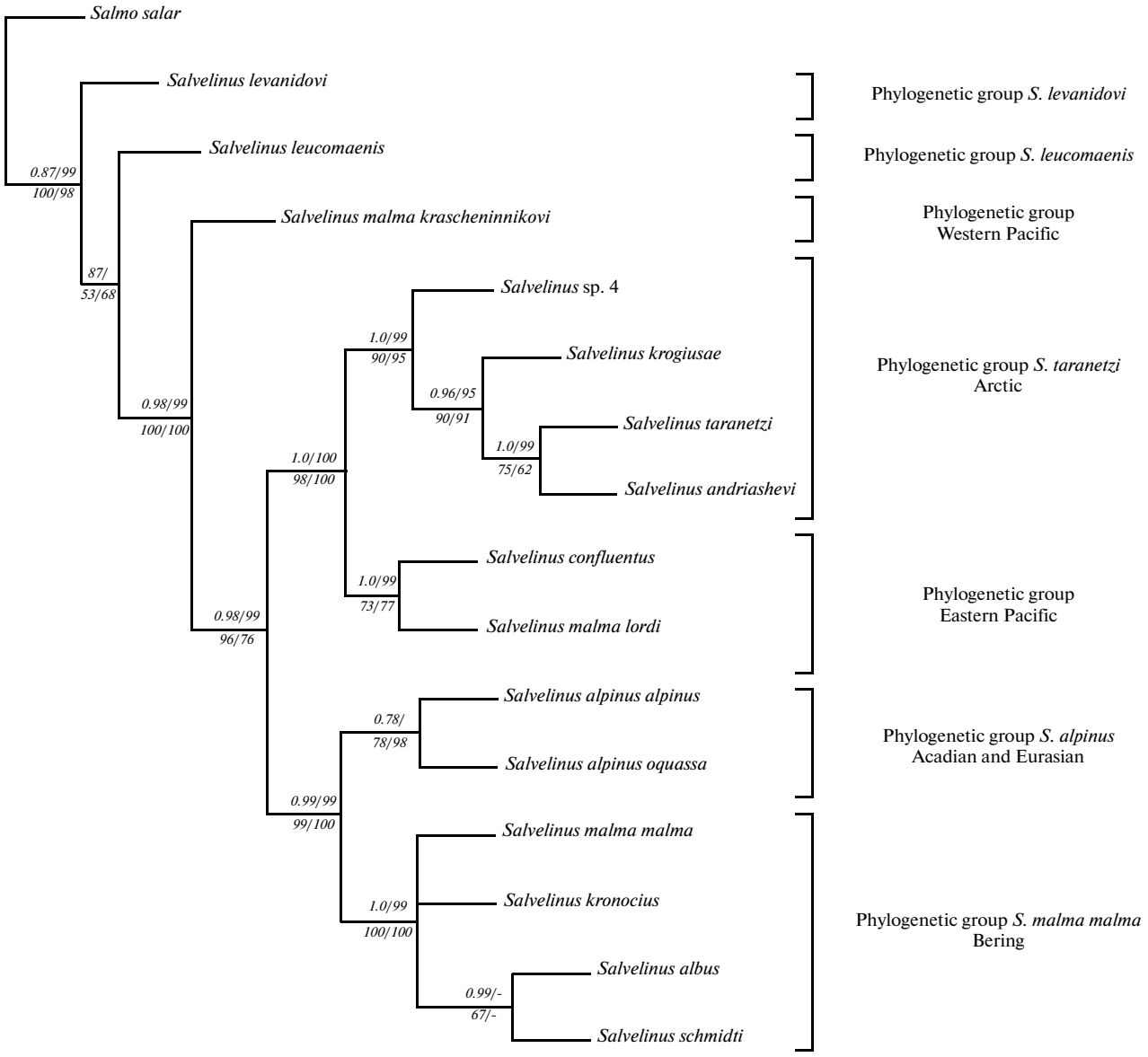

Fig. 2. Phylogenetic relationships among charrs of the genus *Salvelinus* based on the analysis of the mtDNA *ND1/ND2*, *Cytb/D-loop*, *ND5/ND6*, *ND3/ND4L/ND4*, *A8/A6/COIII*, and *COI/COII* fragments (912 characters, 297 phylogenetically informative), consensus phylogram. *Salmo salar* was used as the outgroup. The figures at the nodes designate the *PP* and *BS* (>50%) statistical support values for the BI and ML trees (above the axis), *BS*, for the NJ and MP trees (below the axis).

four monophyletic groups. Despite some topological discrepancies, the genetic data are in good agreement with the suggested phylogenetic hypothesis. After combination of the data on phylogeography of the mtDNA haplotypes [7–9, 15, 16, 39–41, 44–50] and nuclear allozyme loci [51–54], an extended taxonomic composition of phylogenetic charr groups can be presented.

The phylogenetic group of *Salvelinus taranetzi* (Arctic) includes Asian populations of Taranetz charr *S. taranetzi*, *Salvelinus* sp. 4 from Nachikinskoe Lake, Krogius charr *S. krogiusae* (Dal'nee Lake), Chukchi charr *S. andriashevi* (Estikhed Lake), Boganida charr *S. boganidae*, small-mouth charr *S. elgyticus* (Elgygytgyn Lake), and Kolyma–Chukotka group of lake charrs *Salvelunus* sp. from Naivak Lake, Chukotka; Maxi Lake, Juliet Lake, Kolyma River basin. This phylogenetic group also includes *S. a. erythrinus* (initially, *S. a. stagnalis*) from the Northwest Territories of North America and from isolated lake populations of Arctic Alaska, and probably, *Salvethymus svetovidovi*.

The Eastern Pacific phylogenetic group unites *S. m. lordi* and the formally unnamed "*coastal*" and "*interior*" forms of *S. confluentus*.

The phylogenetic group of Arctic charr *Salvelinus alpinus* includes lake charrs *S. a. salvelinus* from Cen-

Table 2. Degree of mtDNA sequence divergence (*ND1/ND2*, *Cytb/D-loop*, *ND5/ND6*, *ND3/ND4L/ND4*, *A8/A6/COIII*, *COI/COII*) in charrs of the genus *Salvelinus* (as percentage of nucleotide substitutions per site) [27, 28]

No.	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Salmo salar*														
2	*Salvelinus leucomaenis*	13.63													
3	*S. levanidovi*	14.56	8.53												
4	*S. taranetzi*	12.82	6.74	7.00											
5	*S. andriashevi*	12.80	6.78	7.11	0.055										
6	*S. krogiusae*	12.78	6.76	7.02	0.130	0.075									
7	*Salvelinus* sp. 4	12.56	6.94	7.17	0.282	0.226	0.250								
8	*S. malma lordi*	12.35	6.69	7.00	0.882	0.843	0.919	1.02							
9	*S. alpinus*	12.05	6.74	7.02	2.04	2.03	2.06	2.11	2.11						
10	*S. malma krascheninnikovi*	13.03	6.54	6.39	1.98	2.06	2.03	2.18	2.15	2.67					
11	*S. malma malma*	12.55	7.36	6.85	2.33	2.38	2.35	2.41	2.56	1.25	3.16				
12	*S. albus*	12.53	7.24	6.81	2.25	2.30	2.27	2.35	2.49	1.24	3.09	0.015			
13	*S. kronocius*	12.58	7.35	6.89	2.29	2.34	2.31	2.39	2.52	1.23	3.15	0.038	0.018		
14	*S. schmidti*	12.53	7.24	6.72	2.21	2.25	2.22	2.30	2.43	1.24	3.04	0.035	0.019	0.022	
15	*S. confluentus*	12.58	6.87	7.04	0.971	0.912	0.937	1.09	0.89	2.57	2.49	2.92	2.84	2.88	2.78

tral Europe, European populations of *S. a. alpinus* from the Atlantic Ocean and Arctic Ocean basins, eastern Siberian populations of *S. a. alpinus*, and *S. a. oquassa* from the eastern coast of North America. All of the examined populations, species (or forms) of charrs from Transbaikalia and Taimyr, including Dryagin charr *Salvelinus drjagini* from Lama Lake, Boganida charr *S. boganidae* from Lama Lake, *S. a. alpinus* (Pucheglazka) from Lama Lake and Sobach'e Lake, *S. a. alpinus* (Putoranchic) from Ayan Lake, and *S. a. erythrinus* (Davatchan) from Frolikha Lake, Gol'tsovoe Lake, and Leprindo Lake, also belong to this group. According to consistent data, the phylogenetic group of Arctic charr unites Acadian, Atlantic, and Siberian (according to [7]), or Acadian and Eurasian (according to [16]), phylogroups of *S. alpinus*.

The phylogenetic group of *Salvelinus malma malma* (Bering) unites northern Dolly Varden *S. m. malma* from Asia and North America, white charr *S. albus*, and endemic charrs of the Kronotskoe Lake, longhead charr *S. kronocius* and Schmidt's charr *S. schmidti*. This group also includes stone charr from the Kamchatka River basin, lake charrs *Salvelinus* sp. from the water bodies belonging to the Okhotsk Sea basin (Elekchanskie Lakes, Mak-Mak Lake, Khaddy Lake, Grand Lake), and possibly neiva *S. neiva* from Ueginskoe Lake.

Based on phylogenetic data and the genetic divergence estimates (Table 2), molecular dating of the main mtDNA divergence stages in charrs of the genus *Salvelinus* was performed. Despite the broad confidence interval of absolute time estimates of the stages of charr divergence, the data presented are comparable with the time estimates obtained using other genetic data, based on sequence analysis of certain nuclear and mitochondrial genes [32, 55] and on the generalized data for the five mitochondrial genes [32]. It should be noted that the calculations in the works cited were made with several calibration points, along with more exact statistical methods allowing nonstrict constancy of the molecular-evolution rates in different lineages.

According to the hypothesis, the basal group of taxa (*S. fontinalis*, *S. namaycush*, *S. levanidovi*, *S. leucomaenis*) diverged within the Late Miocene, the border between Miocene and Pliocene (6 to 4 million years ago). Two divergence events, including separation of the ancestral lineage of Western Pacific group and the following segregation of the hypothetical ancestor into two mitochondrial phyla, grouping independently evolving charr taxa, happened within the period of 3 to 2 million years ago. The third cycle is attributed to the time interval of about 1 million years ago (Early Pleistocene) and includes the divergence of both phyla. In one phylum, a relatively quick isolation of Arctic and Eastern Pacific phylogroups, along with the divergence of the latter phylogroup into *S. confluentus* and *S. m. lordi* lineages, took place. At the same

time, another phylum diverged into the *S. m. malma* and *S. alpinus* phylogenetic groups. Diversification within the phylogenetic groups took place in Middle to Late Pleistocene (0.781 to 0.0117 million years ago).

Based on comparative analysis of different data on mtDNA divergence in a number of families of marine, anadromous, and semi-anadromous fishes (Salmonidae, Osmeridae, Cyprinidae) occupying a common historical range, two periods of divergent evolution in the history of the species in Northern Pacific, which coincided with global paleoclimtic and paleogeological rearrangements, were previously identified [34]. The defining value of biogeographical factors in cladogenesis of different fish groups was repeatedly emphasized elsewhere [56, 57]. Time estimates of the first two divergence stages in the main mitochondrial lineages leading modern charrs coincide with the periods of radiation of salmonid fishes of the genus *Oncorhynchus* calculated for both nuclear allozyme and mitochondrial genes [34, 58]. However, it appeared that charrs experienced two more periods of radiation in the Pleistocene, during which the differentiation of most of the taxa took place. It is possible that the processes of speciation in this group were mostly determined by climatic and geographical changes in Late Cenozoic.

The problem of the isolation of subgeneric charr taxa deserves special interest. Based on morphological and karyological data, a consensus opinion was formed that charrs are taxonomically subdivided into the *Baione*, *Cristivomer*, and *Salvelinus* subgenera [59, 60]. This interpretation implies that *Salvelinus fontinalis* (*Baione*) and *Salvelinus namaycush* (*Cristivomer*) are independent phyletic lineages that diverged from the common ancestor of the genus. Reconstruction of phylogenetic relationships among charrs based on molecular genetic markers provided no unambiguous resolution of the basal branches, since *Salvelinus fontinalis* and *Salvelinus namaycush* either formed sister groups [6, 10, 40] or clustered together with the taxa belonging to the subgenus *Salvelinus* in many dendrograms [7, 10, 15, 17, 40, 61]. A number of taxonomic solutions were suggested. According to one of these, the genus *Salvelinus* is subdivided into three subgenera, including (1) *Salvethymus* with a single species *Salvethymus svetovidovi*; (2) *Baione* with the two species, *Salvelinus fontinalis* and *Salvelinus namaycush*; (3) *Salvelinus* [62]. At the same time, the opposite opinions are advocated, according to which (1) *S. fontinalis* and *S. namaycush* should be considered as members of the genus *Salvelinus* [40]; (2) there are no reasons to isolate any superspecific taxa of charrs [3].

Note that two related aspects under discussion can be distinguished in the context of the problem raised. One of these aspects concerns the establishment of conformity between the level of divergence of independently evolving phylogenetic lines of charrs and their possible taxonomic status. The second aspect is associated with the analysis of possible reasons for the basal branch clustering on the dendrograms obtained for qualitatively different characters. It should be noted that researchers obtaining statistically supported nodes in most cases do not usually focus on the second topic. However, if the topology serves as a basis for further discussion on the group's phylogeny, this aspect is the defining for further taxonomic conclusions. For instance, formation of the common cluster of *S. fontinalis* and *S. namaycush* is most often associated with hybridization [6, 10, 37, 40, 61]. However, conclusions on the stable phylogenetic relationships and the composition of sister pairs in charrs are usually supported by data on single genes. Since a monophyletic group (*S. namaycush*, *S. fontinalis*) is formed on the denodrograms inferred from the data, including the sequences of the internal transcribed spacer ITS2 of rDNA [6, 10, 61], the presence of a phylogenetic signal can be associated with the specific features of gene evolution. Sequence analysis of other genes or the genetic marker combinations leads to other dendrogram topologies, including those in which *S. fontinalis* and *S. namaycush* do not occupy the positions of basal branches [10, 17, 40].

It should be noted that a speculative hypothesis on the possible separation of charrs of the genus *Salvelinus* into two groups, one of which unites *S. fontinalis*, *S. namaycush*, and *S. leucomaenis*, was suggested by K.A. Savvaitova based on comparative analysis of meristic and morphological characters [63]. It is now known that the four species (*S. fontinalis*, *S. namaycush*, *S. levanidovi*, *S. leucomaenis*) are the most diverged taxa of the genus [5, 7, 11, 37, 39]. Analysis of the best-fit gene trees of charrs points to noticeable differences between branches uniting closely related taxa and branches leading to distant basal species, which often form common clusters [7, 15, 17]. According to our data and that from the literature [39], the number of nucleotide substitutions per site in *S. levanidovi* and *S. leucomaenis* (from 0.08531 to 0.06393) is several times higher than that among other taxa (from 0.03163 to 0.01230). Similar results were obtained for the whole mitochondrial genome [5] and the *ND3* and *Cytb* individual genes in *S. fontinalis*, *S. namaycush*, and *S. leucomaenis* [6, 37], and the ITS2 rDNA in *S. fontinalis* [6]. It seems likely that specificity of the genus *Salvelinus* consists in the unification of taxa with phylogenies of different complexity. The first group includes taxa with a high degree of divergence, which correspond to the long branches of phylogenetic tree (*S. fontinalis*, *S. namaycush*, *S. levanidovi*, *S. leucomaenis*). The second group includes the remaining taxa with low divergence that form short internal branches of the phylogenetic tree. For such taxonomic compositions, the conflict between gene tree topologies is determined by the high divergence level and unequal branch lengths [64, 65]. As a result, to obtain a stable topology of genetically distant taxa, it is necessary to overcome the long-branch attraction effect [66], which is manifested in trees inferred from the mtDNA sequences, as well as

from nucleotide and protein sequences of nuclear genes [67, 68].

It is evident that the available genetic data are insufficient to discuss the phylogenesis of the basal group. There are no studies in which all of these species were analyzed in the framework of a single taxon-character matrix. This profoundly complicates identification of the phylogenetic signal on the divergence of the phyletic lineages at the phylogram bases of the genus *Salvelinus*. Conflict phylogenetic signals associated with the evolution patterns of certain genes, as well as nonphylogenetic signals from the long-branch attraction effect in this group of taxa, can appear in each tree. Because of this, the only way to get a stable, well-supported phylogenetic hypothesis is to add the new data to the existing ones,with subsequent reassessment of the results. However, since the group of taxa under discussion, in addition to *S. fontinalis* and *S. namaycush*, also includes *S. levanidovi* and *S. leucomaenis*, it is more reasonable to consider all of these species representing the genus *Salvelinus* in equal taxonomic rank. It should be noted that M.K. Glubokovsky previously spoke against the isolation at the subgenus level of the two groups representing archaic (*Salvelinus svetovidovi*, *S. fontinalis*, and *S. namaycush*) and advanced charr species [3]. As the main argument, he noted that grouping of archaic species led to the formation of paraphyletic, from the cladistic position, taxa.

In this context, special attention should be paid to the long-finned charr *Salvethymus svetovidovi*, which represent a separate endemic genus of the family Salmonidae [69]. Based on the level of morphological and ecological differentiation, it was suggested that *Sv. svetovidovi* was one of the most ancient taxa among the salmonid fishes and was phylogenetically close to the common ancestor of charrs of the genus *Salvelinus* [4, 69]. Karyological analysis generally confirmed this suggestion. This is because *Sv. svetovidovi* has a unique karyotype, which evolved owing to Robertsonian translocations independently from the species of the genus *Salvelinus*, albeit preserving some characters in common with charrs [43]. More recently, a mosaic combination of morphological and karyological characters typical of both phylogenetically advanced and archaic salmonid taxa, suggesting deep specialization, was described in the long-finned charr [4]. In addition, other alternative hypotheses were suggested, according to which *Sv. svetovidovi* could be either an advanced species of the genus *Salvelinus* or the earliest diverged branch among charrs [70].

Molecular genetic study of the relationships of *Sv. svetovidovi* is limited to the analysis of the mtDNA CR region [7, 9, 15, 17, 41, 49, 50], *Cytb* and *COI* genes [17], and RAPD-PCR markers [40]. Moreover, in all mtDNA studies, the same data, obtained from two specimens of long-finned char and represented in the GenBank database, were used. According to the obtained data, *Sv. svetovidovi* formed a separate phyletic branch, which was almost equally diverged from the six phylogroups, uniting the haplotypes of most of the examined charr taxa (*S. alpinus*, *S. m. malma*, *S. taranetzi*, *S. confluentus*, *S. m. lordi*, *S. boganidae*, *S. elgyticus*, *S. albus*). However, in different gene trees, the phylum of *Sv. svetovidovi* does not occupy basal position relative to the monophyletic group of the genus *Salvelinus*. On the contrary, it is located within the dendrogram, diverging next to *S. fontinalis*, *S. namaycush*, *S. leucomaenis* [7], or next to *S. fontinalis*, *S. namaycush*, *S. leucomaenis* and *S. m. krascheninnikovi* [9, 15, 41, 50]. Nevertheless, the data available unambiguously points to the independent taxonomic status of *Sv. svetovidovi* within the genus *Salvelinus*, disapproving the phylogenetic hypotheses postulating the early divergence of the *Salvelinus* branch from the charr common ancestor [3, 70]. Even closer relationships between long-finned charr and other charr species have been observed at the RAPD-PCR markers [40], according to which two synapomorphies with *S. neiva* were identified in *Sv. svetovidovi*.

Thus, complex analysis of the personal and literature data indicates that charrs of the genus *Salvelinus* (including *Salvethymus svetovidovi*) are a monophyletic group that diverged from the common ancestor without subdivision into subgeneric taxa. It was demonstrated that a specific feature of the genus *Salvelinus* is the grouping of taxa with phylogenetic problems of different complexity. The first group includes taxa with a high divergence level and long branches (*S. fontinalis*, *S. namaycush*, *S. levanidovi*, *S. leucomaenis*), while the second group contains the remaining taxa with low divergence levels and short internal tree branches. Because of this, the general dendrogram topology of the genus *Salvelins* should be characterized as complex, with long external and short internal branches, and susceptible to the long-branch attraction effect upon phylogenetic reconstructions. As a result, clustering of the basal branches most likely represents a nonphylogenetic signal and thus cannot be interpreted as relationships of monophyletic groups.

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