Article

Morphological and Genetic Variability in *Radix auricularia* (Mollusca: Gastropoda: Lymnaeidae) of Lake Baikal, Siberia: The Story of an Unfinished Invasion into the Ancient Deepest Lake

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Abstract: This article aims to reconstruct the invasion of the ear-shaped pond snail, *Radix auricularia* (Linnaeus, 1758), to Lake Baikal, East Siberia. This species is widely distributed in the Palaearctic and Northern America, and since the early 20th century has formed abundant and sustained populations in Lake Baikal. The data provided on the morphological and genetic variability of *R. auricularia* help to better describe and delineate the species. With an integrative approach involving morphological and molecular data, we improved the knowledge of the intraspecific variability of *R. auricularia* in the most important characteristics used for its determination. Molecular sequences of nuclear spacer fragment ITS-2 and mitochondrial gene fragment *cyt–b* were obtained from 32 individuals of *Radix* (including seven outgroup *Radix* species) collected from various parts of Lake Baikal and adjacent waterbodies and compared with sequences of 32 individuals of *R. auricularia* from different regions of the Palaearctic as well as with individuals determined as *R. intercisa* from Lake Baikal, *R. iturupica* from the Kurile Islands, *R. ussuriensis* from the Khabarovsk region, *R. narzykulovi* from Tajikistan, and *R. schubinae* from the Amur region. Molecular genetic analyses revealed that all specimens collected from Lake Baikal belong to *R. auricularia*. There are no genetically distinct groups of snails that would correspond to two morphospecies previously recorded in Lake Baikal (e.g., *R. auricularia* s. str. and *R. intercisa*). Variability of the characteristics that are commonly used for species identification (shell morphology, mantle pigmentation, shape and position of the bursa copulatrix, length and position of the bursa duct, length ratio of preputium to penial sheath) were found in individuals analysed with molecular genetics to be broader than recognised in the current literature. Some shells of *R. auricularia* collected from Lake Baikal resemble shells of another lymnaeid species, *R. balthica*, and without molecular assessment can be confused with the latter. Geometric morphometric analysis of more than 250 shells revealed no observed hiatus between Baikalian and non-Baikalian *R. auricularia*. The probable stages and pathways of *R. auricularia* invasion to Lake Baikal's ecosystem are outlined and discussed. Factors such as global climate warming and human activity stimulated and facilitated the ongoing dispersal of ear pond snails within Lake Baikal.

Keywords: Lake Baikal; biological invasion; pond snails; shell morphology; molecular taxonomy; geometric morphometry

1. Introduction

The uniqueness of Lake Baikal, located in southeast Siberia, and its biota has repeatedly been reviewed in the scientific literature (e.g., [1–5]). This waterbody can be viewed as a kind of 'natural laboratory', where numerous 'experiments' in evolutionary divergence and ecological adaptation have been conducted, and numerous taxa of animals of various rank (species, genera, even families) endemic to Lake Baikal have evolved as the result of these 'experiments' [1,4,6–8]. Among these taxa, snails (Mollusca: Gastropoda) represent one of the most interesting cases. In total, around 150 species of gastropods belonging to 8 families are known in the fauna of Lake Baikal; nearly 70% of them are endemic [9–11]. It is an ancient malacofauna whose roots can be traced back to the Paleogene, if not the late Mesozoic [12–14], and, during its long history, some snail families formed flocks of endemic species and genera. Such flocks of taxa are among the gastropod families Acroloxidae, Baicaliidae, Benedictiidae, Planorbidae, and Valvatidae [1,9,14–19].

On the other hand, mollusc families such as Bythiniidae, Lymnaeidae, and Physidae are represented in the lake almost exclusively by species known from elsewhere, i.e., from other regions of Northern and Central Asia and even Europe [11]. Most representatives of these families do not occur in the so-called 'open Baikal', and instead are restricted to waterbodies situated on the shores of Lake Baikal and sometimes connected to the main part of the lake. Some of these waterbodies are the former bays of Lake Baikal. The totality of such waterbodies forms a specific system of habitats, which is called "sorovaya zona" (the sor zone) in Russian literature (or the "Littoral–sor zone"; [1] p. 170). "Sor" is the local Russian term for permanent waterbodies, sometimes considerably large ones, which can be connected to Lake Baikal by permanent or non-permanent channels. Some bays of Lake Baikal have small inlets where hydrological and ecological conditions are similar to those of the 'sors'. For example, such inlets may harbour aquatic vegetation virtually identical to the vegetation of East Siberian lakes and ponds and completely lack macrophyte species endemic to Lake Baikal. The majority of animal species endemic to Lake Baikal are absent from the 'sor zone' as well [1]. The "sor zone" is, thus, an intermediate habitat between the open Lake Baikal and the extra-Baikalian habitats and may be viewed as a 'waiting area' for the Palearctic taxa of animals extending their ranges to occupy the open Baikal. In other words, it acts as a 'reservoir' of taxa potentially able to colonize Lake Baikal. The lake is now facing drastic changes in its environment, including to the structures of animal and plant communities (e.g., [20–24] and references therein), and suffers from the ever-increasing impacts of human activity, which creates conditions favourable for alien animals of Palearctic origin and facilitates their colonization of Lake Baikal.

The case of *Radix auricularia* (Linnaeus, 1758), the ear pond snail, a freshwater gastropod of the family Lymnaeidae Rafinesque, 1815, which has formed abundant and sustainable populations in the open Lake Baikal, is one such recent colonisation (or invasion, to use a more expressive term), which may potentially contribute to further alteration of Lake Baikal's aboriginal ecosystem structure and function. This snail species is among the most widely distributed and ubiquitous species of Gastropoda in Eurasian freshwaters; its range in the Old World extends throughout Europe, Northern and Central Asia, Northern Africa, and eastward to the Far East [25–30]; non-native populations of the ear-shaped pond snail from North America are also known [31].

Maack [32], the natural history teacher at the Irkutsk Gymnasium, was the first author to record *R. auricularia* from Lake Baikal, namely from the Kultuk settlement located on the southwest shore of the lake. Unfortunately, his record is very brief and does not contain any details on the exact place where the species was found, whether it was in the open littoral or in the 'sor zone', which is vastly developed near Kultuk. Later on, Lindholm [33] and Kozhov [15], in their formative monographs on Lake Baikal Mollusca, gave an overview of the taxonomy and distribution of *Radix* in the lake. According to these authors, the snails are limited in their distribution to the 'sor zone' as well as to the most sheltered habitats of Lake Baikal, such as small inlets and bights, the mouths of rivers emptying into Lake Baikal, and similar niches. Kozhov [15] noted that *Radix* occurs at low depths, not exceeding 25 m,

with most individuals concentrated between 0.5 and 5 m depth. Both authors described and illustrated the conchological variability of this species, which appears to be very high. This led the malacologists to delineate a series of intraspecific varieties and morphs of the ear pond snail in Lake Baikal. Lindholm [33] described two new varieties—*Limnaea auricularia* var. *intercisa* and *L. a.* var. *lapidaria*; B. Dybowski [34] proposed to classify the snails living in Posolsk sor (=Posolsk Bay) as a variety *L. intercisa* var. *sorica*; later Kozhov [15] added more new taxonomic entities to this list. The differences between these taxa were relatively slight; the new varieties and morphs were based on distinctions in shell size and proportions, and the authors themselves acknowledged that it was sometimes difficult to draw a sharp boundary between these infraspecific entities. We will avoid further examination of taxonomies proposed by Lindholm [33], B. Dybowski [34], and Kozhov [15].

In 1967, Starobogatov and Streletzkaja [35] proposed to unite all intraspecific morphs of the Baikalian *Radix* under a subspecific name *Lymnaea auricularia torquilla* (Westerlund, 1877). In the subsequent Russian literature, this subspecies was elevated to full species rank and was usually referred to as *Lymnaea (Radix) intercisa* (Lindholm, 1909), whereas the name *L. torquilla* was reserved for some extra-Baikalian populations. It was thought that even *Lymnaea intercisa* was not endemic to Lake Baikal, but widely distributed in eastern and western Siberia as well as in eastern Kazakhstan [11,27,36–38]. Sitnikova et al. [11] recognized two species of *Radix* in the lake, *R. auricularia* s. str. and *R. intercisa*.

However, in 2004, Stift et al. [39] and de Boer et al. [40] returned to a more traditional taxonomic approach, classifying the Baikalian *Radix* as belonging to the Palearctic *Lymnaea (Radix) auricularia* and not delineating any subspecies or varieties within it. The authors used multivariate analysis of linear shell measurements of *Radix* of Lake Baikal to assess the degree morphological variability and compare it to specimens of populations from other regions. Stift et al. [39] provided distribution information which showed that, since the mid-20th century, *R. auricularia* expanded its presence in Lake Baikal and entered its steep rocky littoral, thus becoming "a significant novelty in the Baikal malacofauna" ([39] p. 105). It only took about half a century for this species to colonize areas that were previously considered to maintain Lake Baikal endemics only. One can say that we are observing an ongoing invasion of a widely distributed Palearctic species to a new environment, which may potentially have great implications for conservation. Many species of invasive freshwater snails and bivalves are known to be aggressive and successful competitors able to effectively replace the aboriginal species of molluscs (e.g., [41–43]). Thus, there is a need for a deepened understanding of the case of *Radix auricularia* gaining a foothold in a dominion of unique malacofauna.

The main goals of this study were: (1) To assess both genetic and morphological variability of *Radix auricularia* from the 'sor zone' and the open Lake Baikal; (2) To trace the origins and possible ways of intralacustrine dispersal of this snail in Lake Baikal using all available information; (3) To reveal the phylogenetic relationships of Baikalian *R. auricularia* and its conspecifics from other regions of the Palearctic.

2. Material and Methods

2.1. Material Sampling

The snails used in this study were collected from 35 sites located at various parts of Lake Baikal and adjacent waterbodies during the field seasons of 2011 and 2016–2019. Molluscs were collected, depending on their depth, by hand, sieve, dredging from on-board a research vessel, or by scuba diving. The sampling localities of *Radix* were scattered throughout the lake, from the northernmost to the southernmost parts (Figure 1). Supplementary field observations on the occurrence and abundance of *R. auricularia* in the open Baikal and the "sor zone" were made by TYS and MVV in June 2021 during a cruise on board R/V Papanin.
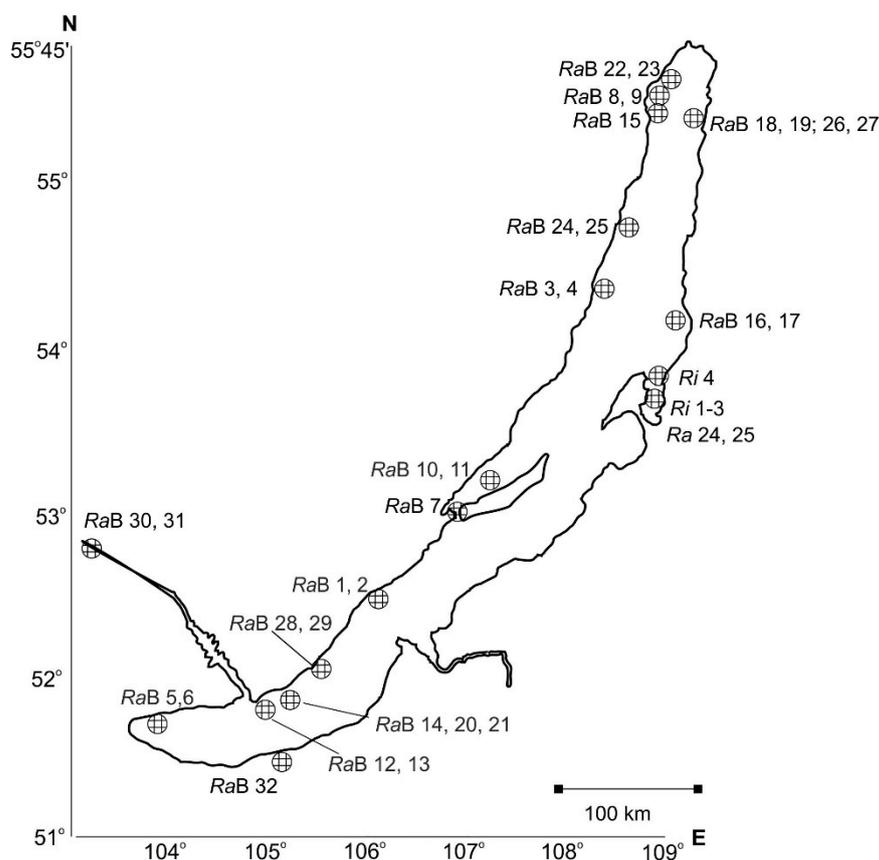
Figure 1. A map of Lake Baikal indicating sampling sites of molluscs used in this study. Numbers correspond to the numbers of *Radix* samples in Supplementary Table S1. RaB—*R. auricularia* from Lake Baikal; Ri—*Radix intercisa*; Ra—*R. auricularia*.

Both living animals and their empty shells (for the purpose of identification and variability study) were collected; the former were put directly into 70–80% ethanol or isopropyl alcohol in-field. Morphological and molecular genetic examination was carried out in the laboratories.

We studied the type series of *Limnaea* (*Gulnaria*) *auricularia* var. *intercisa* Lindholm, 1909 kept in the malacological collection of the Zoological Institute, Russian Academy of Sciences (ZIN) in St. Petersburg (see [10] for details) as well as numerous samples of *Radix auricularia* of Siberia and adjacent countries (Kazakhstan, Mongolia), which are kept in ZIN and in the Laboratory of Macroecology and Biogeography of Invertebrates, St. Petersburg State University (LMBI). In total, more than 250 specimens of *Radix* and other lymnaeid species (see Supplementary Table S1) were studied in this research.

2.2. Morphological Treatment and Taxonomic Identification of Radix Specimens

The primary taxonomic identification of collected snails was carried out by comparison with museum specimens and descriptions and illustrations of different *Radix* species available from the literature [10,15,27–29,33]. Both shell and internal anatomy characteristics were used for identification. In some cases, we were able to compare our individuals with the type specimens kept at ZIN.

All specimens used for molecular genetic studies are listed in Supplementary Table S1. These specimens are stored either in the mollusc collection of the Senckenberg Natural History Collections, Dresden (SNSD), if used for morphological analyses, and/or samples of tissues stored in the tissue collection of the Molecular Laboratory (SGN-SNSD-Mol-Lab). Some voucher specimens taken from the samples used for molecular analyses and shell

morphology are stored in LMBI and in the Limnological Institute, Siberian Branch of the Russian Academy of Sciences, Irkutsk.

Shell morphology, mantle pigmentation and anatomy were documented from the specimens studied. Genital organs were dissected and measured using stereo microscope (Nikon SMZ18; Nikon GmbH, Düsseldorf, Germany). Photographs were taken with a digital camera (Nikon DS-Fi2 and Canon 80D; Nikon Inc. and Canon Inc., Tokyo, Japan).

Samples of tissues taken from the foot were fixed in absolute ethanol or 98% isopropyl alcohol for analysis. They were registered in the tissue collection of the SGN-SNSD-Mol-Lab by assigning a tissue voucher number and a corresponding collection number in the mollusc collection of SNSD, and are stored at −80 °C.

Characteristics examined for morphological analyses were: shell morphology, mantle pigmentation, shape and position of the bursa copulatrix, length and position of the bursa duct, and length ratio of preputium to penial sheath.

The absolute shell height (SH) was measured with accuracy to the nearest 0.1 mm along the line connecting the shell apex and the basal part of shell, and was used as a proxy for snail body size. The measurements were made using digital callipers.

The geometric morphometric approach allowing one to decompose the shell form into shape and size was applied to study shell shape alterations between populations of *Radix auricularia* from different parts (or localities) within Lake Baikal as well as between populations of Baikal and some other waterbodies situated in Siberia and adjacent areas (Table 1). Shell photographs were taken with a Canon EOS 1100D (Canon Inc., Japan) digital camera equipped with a Canon MP-E 65 mm macro lens. In total, 278 images of shells originating from 26 localities were analysed: 23 localities represented the Baikalian populations of *R. auricularia*, whereas the remaining three were situated in Siberia and Western Mongolia (Table 1). The latter were included in the analysis to reveal probable differences in shell shape between Baikalian and extra-Baikalian populations of the studied species.

A configuration of 14 landmarks was used to characterize shell shape (in frontal view). The positions of landmarks (Figure 2) are described as follows: LM1—shell apex; LM2–5 are placed on the opposite sutural points of the penultimate whorl(s). LM6 is the most external point of the body whorl contour above the aperture. LM7 lies on the border of the parietal and palatal margins of the aperture. LM8 corresponds to the uppermost point of the aperture. LM9 is the most external point of the body whorl contour on its left side. LM10 is the most external position on the external part of the palatal margin of the aperture. LM11 is placed on the border of the parietal and columellar margins of the aperture. LM12 is the most external point of the columellar lip. LM13 is the lowest point of the body whorl at its confluence with the aperture margin. LM14 corresponds to the lowest point of the aperture at its basal margin.
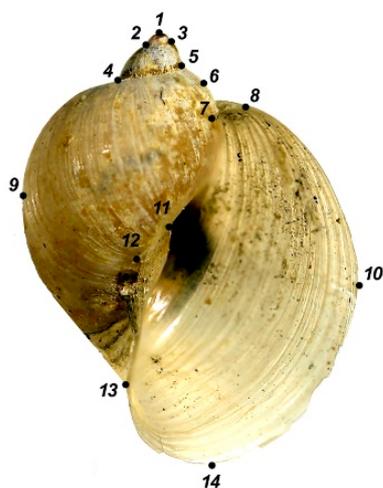
Figure 2. Configuration of landmarks (black dots) used for morphometric analysis of conchological variation in *Radix auricularia*.

Table 1. List of samples of *Radix auricularia* from Lake Baikal and other regions used in the geometric morphometric study, including snail numbers used in sequence analyses (*—"sor zone").

Region	Locality, Coordinates (Snail Numbers Correspond to Figure 1)	N
Baikal Lake		
Northwestern Baikal	Bolshaya Kosa Bay, N 54°45′; E 108°50′ (RaB 24, 25)	5
	Ayaya Bay, N 55°27′, E 109°52′ (RaB 18, 19, 26, 27)	15
	Near Zarechnoye settlement, N 55°27′, E 109°52′ (RaB 18, 19, 26, 27)	7
	Near Cape Slyudyanskiy N 55°30′; E 109°12′ (RaB 15)	9
	Muzhinay Cape, N 54°51′, E 108°54″	6
	Near Valukan Cape, N 54°18′; E 109°26′ (RaB 16, 17)	5
	Near Elokhin Cape, N 54°33′; E 108°39′ (RaB 3, 4)	10
	Near Solontsovyi Cape, N 54°10′, E 108°22′	2
	Onokochanskaya Bay, N 55°32′, E 109°11′	2
	Senogda Bay, N 55°34′; E 109°13′ (RaB 8, 9)	5
Maloye More Strait (=Small Sea)	Zagli-nuur Bay, N 53°02′; E 106°75′ *	2
	Kharin-Irgi Bay, N 53°03′, E 106°55′ (RaB 7) *	2
	Shibeteyskaya Bay, 53°08.08′; E 107°05.53′ (RaB 10, 11)	5
Southwestern Baikal	Babushka Bay, N 52°15′; E 105°42′ (RaB 28, 29)	2
	Peschanaya Bay, N 52°15′41″, E 105°42′9″	3
	Near Bolshiye Koty settlement (Zhilishche Ravine), N 51°53.96′; E 105°03.84′ (RaB 14, 20, 21)	12
	Near Buguldeyka settlement, N 52°32′; E 106°05′ (RaB 1, 2)	8
	Near Listvennichnoye settlement, N 51°51′; E 104°50′ (RaB 12, 13)	13
	Near Cape Zub (Khabartuy), N 51°43′; E 103°52′ (RaB 5, 6)	2
Chivyrkuy Bay and eastern Baikal	Near Cape Fertik, N 53°48′, E 109°03′ (Ri 4) *	23
	Near Katun' village, N 53°41′ E 109°17′ (Ri 1, 2, 3) *	24
	Kotovo Bay, N 53° 39′ E 108° 58′ (Ra 24, 25) *	6
	Zmeinaya Bight, N 53°46′ E 109°0′ *	20
Total:		188
Other territories		
Western Mongolia	Khar-Nuur Lake, N 48°22′ E 95°37′ (near Ra 23, 26)	32
Eastern Siberia	Irkutsk Region, an oxbow of the Kirenga River, N 54°42′ E 105°47′	22
Western Siberia	Omsk Region, Krivoye Lake, N 56°46′ E 74°36′ (near Ra 3)	36
Total:		90

Landmarks were set on digital shell images using tpsDIG v2.26 software [44]. Analysis of landmark data was performed using PAlaeontological STatistics (PAST) v2.0 [45] and tpsRelw v1.67 software [46].

Generalized Procrustes analysis was used to superimpose 2D-landmark configurations, and we used the projected Procrustes coordinates as raw data in subsequent statistical

analyses. Shape variation was studied using principal components of aligned landmark coordinates (relative warp analysis) implemented in tpsRelw. Thin-plate spline deformation grids were produced to visualize morphological variation along axes of the reconstructed morphospace. We avoided the use of centroid size (CS) as a proxy for body size since values of CS appeared to be tightly correlated with SH (Spearman's rS = 0.75; $p < 0.0001$).

2.3. Molecular Techniques and Sequence Analyses

　　For molecular analyses, we obtained sequence data of the nuclear ITS-2 spacer (partial, with 28S ribosomal RNA gene, partial sequence), which is 344 bp long in *Radix labiata* (Rossmässler, 1835) and up to 410 bp in *R. auricularia* (the length of the ITS-2 spacer varies within genera and species), and a 329 bp fragment of the *cyt–b* gene as mitochondrial marker.

　　We used new sequences and already published sequences from earlier publications [47–55]. For primers and protocols for DNA extraction, polymerase chain reaction (PCR), purification of PCR products, and DNA sequencing see [47]. For the new sequences for this publication, total genomic DNA was extracted using the innuPREP DNA Mini Kit (Analytik Jena AG, Jena, Germany).

　　PCR was performed using 1–5 μL of DNA in a 20 μL volume containing 1 unit of Taq polymerase (Bioron DFS-Taq, Bioron GmbH, Ludwigshafen, Germany), 2 μL PCR buffer 10× incl. MgCl2 (25 mM), 10 pmol of each primer, 0.25 mM of each dNTP (Carl Roth GmbH, Karlsruhe, Germany), and ultrapure H_2O. From the *cyt–b* gene, a region of about 370 bp was amplified with the primers UCytb151F and UCytb270R [56] and a temperature profile of 94 °C 4 min (94 °C 40 s, 54 °C 40 s, 72 °C 1 min) × 40, 72 °C 6 min. The primers used for ITS-2 were LT1 [57] and ITS2-Rixo [58]. The temperature profile used was 94 °C 4 min (94 °C 30 s, 50 °C 30 s, 72 °C 1 min) × 40, 72 °C 7 min. PCR products were visualised on 1% agarose gel. PCR products were cleaned using ExoSAP-IT enzymatic clean-up (Applied Biosystems, Foster City, CA, USA; 1:20 dilution, modified protocol: 30 min at 37 °C, 15 min at 80 °C).

　　The primers used for cycle sequencing were UCyt*b*151F for *cyt–b* and LT1 for ITS-2. Samples were sequenced in both directions if necessary. The total reaction volume of 10 μL contained 2.25 μL sequencing buffer, 0.5 μL premix, 5 pmol of the respective primer, 0.5–6 μL DNA template, and ultrapure H_2O. Using the ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), 25 cycles were performed at 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Reaction products were purified using Sephadex^{TM} G-50 fine (Cytiva, Freiburg, Germany). Sequencing was performed on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA).

　　All DNA-sequences have been placed in the European Nucleotide Archive (ENA, see http://www.ebi.ac.uk/ena/) (accessed on 20 January 2022) and are also available from GenBank (Table 1).

　　For outgroup comparison, we used the species *Austropeplea viridis* (Quoy and Gaimard, 1833) from the same family Lymnaeidae. We included sequences of *Radix auricularia*, *R. natalensis* (Krauss, 1848), *R. rubiginosa* (Michelin, 1831), *R. dolgini* (Gundrizer and Starobogatov, 1979), *R. labiata*, *R. balthica* (Linnaeus, 1758), *R. lagotis* (Schrank, 1803), *R. schubinae* (Kruglov, Starobogatov and Zatravkin, 1989), *R. iturupica* Kruglov and Starobogatov, 1989), *R. narzykulovi* Izzatulaev, Kruglov and Starobogatov, 1983), *R. ussuriensis* (Kruglov and Starobogatov, 1989), and *R. intercisa* (Milaschewitsch in Lindholm, 1909) in the ingroup for inter- and intraspecific comparisons. The specimens of *R. auricularia* used for analysis were collected from different areas of Russia and from Mongolia, China, Kyrgyzstan, Tajikistan, Tunisia, Bulgaria, Turkey, Italy, Denmark, Germany, Switzerland, and Hungary (see Supplementary Table S1).

　　For the sake of consistency with our previous articles [47–53], we follow here the taxonomy and nomenclature of Lymnaeidae used in the cited works (but see [30,59] for an alternative view on the system of these snails).

　　Alignment was performed using the sequence alignment editor BioEdit [60]. ITS-2 alignment was obtained using the Clustal algorithm of MEGA4 [61] and improved by eye. For post-alignment editing, see Schniebs et al. [50]. MEGA 4 was also used to check the

mitochondrial sequences for stop codons. Thus, we adopted an analytical approach under the maximum parsimony (MP) criterion that can utilize the indel information [62]. Losing this information by analysing using distance or maximum likelihood would mean losing the greatest part of the phylogenetic signal. Phylogenetic analyses of the ITS-2 spacer and *cyt–b* fragment were carried out using PAUP (v4.0b10; [63]; settings: gapmode = NewState, addseq = closest, maxtree = 10,000; number of bootstrap replicates = 10,000). For presentation of the MP results for ITS-2 and *cyt–b*, one of the 10,000 best trees, respectively, was chosen to illustrate branch lengths (one showing the same overall topology as the majority rule consensus tree was chosen).

For maximum-likelihood analyses including bootstrap support, we used RAxML (raxmlGUI 0.9 beta 2, [64,65]). Although often misleading, the suited nucleotide models were determined using jModelTest v0.1.1 [66], since the parameters of the GTR model were estimated in RAxML, and this also included all the simpler models. The best-fit model for *cyt–b* selected by Akaike information criteria (AIC) was GTR + G. The best-fit model for IST-2 was HKY+G. Accordingly, GTRGAMMA was selected for maximum-likelihood analyses of *cyt–b*, and GTRGAMMAI was selected for ITS-2. The settings were ML + thorough bootstrap with 100 (replicate) runs and 1000 (bootstrap) repetitions.

In order to visualise tight, non-dichotomous branching, a haplotype network (median joining; [67]; no loops) was computed from 61 *cyt–b* sequences with the program "Network" (https://www.fluxus-engineering.com/) (accessed on 4 October 2021). Haplotype balloons were coloured according to geographic origin.

3. Results

### 3.1. Molecular Phylogeny and Phylogeography

In the hypothesis of the phylogenetic relationships of the analysed *Radix* specimens based on one of the 100,000 best maximum-parsimony (MP) trees of the mitochondrial *cyt–b* marker (tree length = 415, CI = 0.5398, RI = 0.9068) illustrated in Figure 3, most of the basal branches have less than 95% bootstrap support. The clades of the species themselves have full or very high (96%) support, except for *R. ampla* (85%). The specimens preliminarily determined as *R. schubinae*, *R. iturupica*, *R. narzykulovi*, *R. ussuriensis*, *R. intercisa*, *R. auricularia* as well as the *Radix* specimens collected from Lake Baikal form a distinct clade, with 100% bootstrap support, that is separate from *R. rubiginosa*, with 53% bootstrap support. Together with *R. natalensis*, these *Radix* species form a large sister clade to all other *Radix* species analysed, with 95% bootstrap support. The RAxML tree of the *cyt–b* fragment (see Figure 3B) shows nearly the same result; however, the bootstrap supports are lower: the distinct clade formed by the specimens of *R. schubinae*, *R. iturupica*, *R. narzykulovi*, *R. ussuriensis*, *R. intercisa*, *R. auricularia*, and the *Radix* specimens collected from Lake Baikal has only 91% bootstrap support. Together with *R. natalensis* and *R. rubiginosa*, these *Radix* specimens are separated from all other *Radix* species analysed, with only 92% bootstrap support. All other branches of the RAxML tree separating species have bootstrap supports <67%. The clades of the species themselves have full (*A. viridis*, *R. rubiginosa*, *R. natalensis*), high (*R. baltica*, *R. dolgini*, *R. labiata*), or low support (*R. lagotis* 80%, *R. ampla* 63%).

The MP tree of the nuclear marker ITS-2 based on one of the 10,000 best MP trees (tree length = 618, CI = 0.7330, RI = 0.9634) is shown in Figure 4A. Most of the basal branches are fully or very highly supported. The clades of the species themselves received full or nearly full support in most cases, except for the clade formed by the specimens of *R. auricularia*, *R. schubinae*, *R. iturupica*, *R. narzykulovi*, *R. ussuriensis*, and *R. intercisa* (54%), as well as for *R. lagotis*, *R. ampla*, and *R. balthica* (<50%).
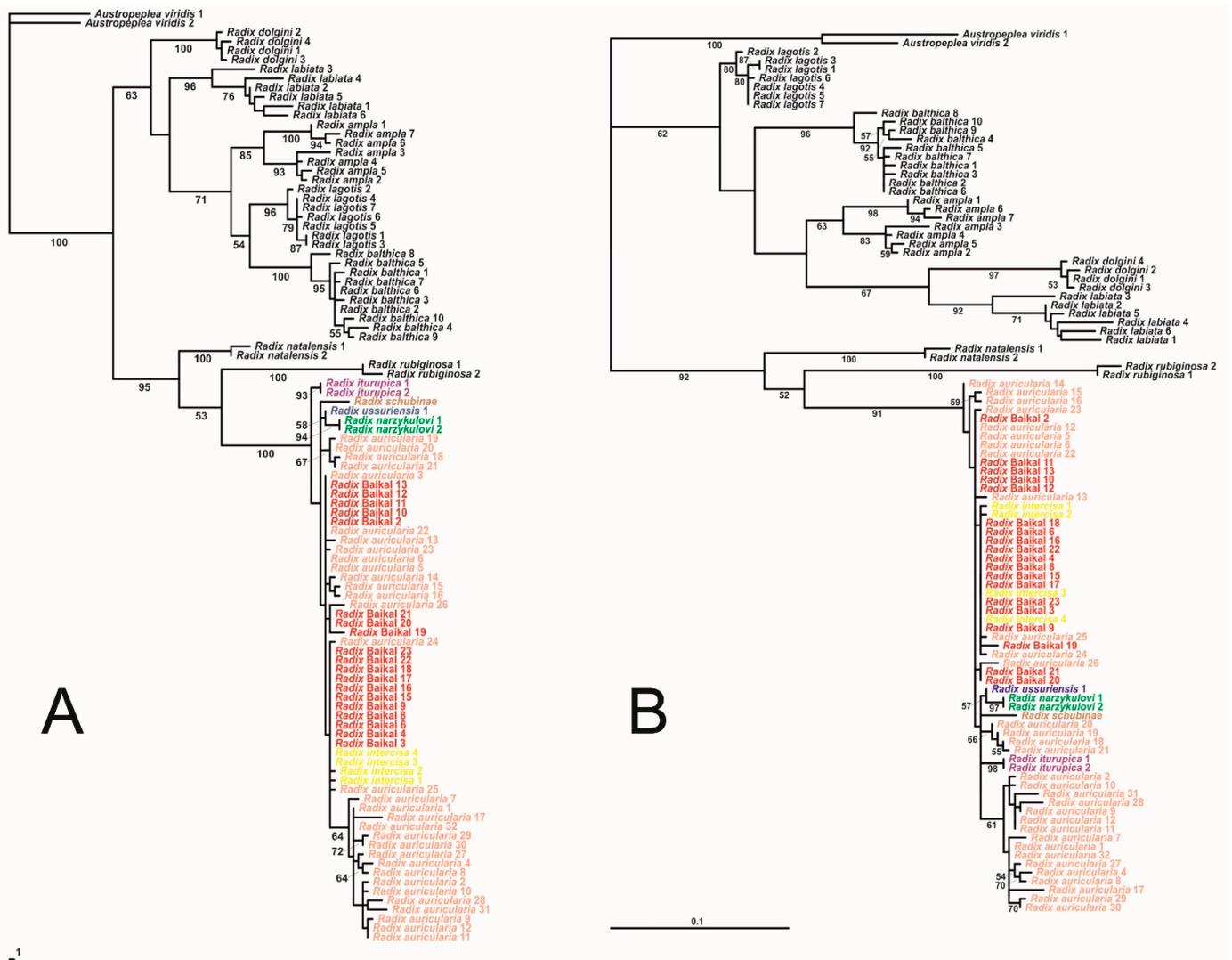
Figure 3. Hypotheses of the phylogenetic relationships of analysed *Radix auricularia* specimens based on the sequenced fragment (329 bp) of the mitochondrial *cyt–b* marker using different algorithms for tree building: (**A**) based on one of the 100,000 best maximum-parsimony (MP) trees (tree length = 415, CI = 0.5398, RI = 0.9068) and (**B**) based on RAxML. Branch lengths are proportional to the number of substitutions, and the overall topology corresponds to that of the strict consensus tree. Bootstrap support values above 50% are reported below nodes.

The RAxML tree of the nuclear marker ITS-2 (see Figure 4B) has a slightly different topography of the branches than the MP tree. The main difference between the MP and the RAxML tree of the ITS-2 spacer is that in the MP tree *R. dolgini* and *R. labiata* form well supported (92%) sister groups, whereas in the RAxML tree *R. dolgini* forms the sister group (85% support) of a larger clade in which *R. labiata* is the fully supported sister group to a cluster of *R. lagotis*, *R. ampla*, and *R. balthica* with 96% bootstrap support. In the latter, only *R. ampla* forms a distinct species clade with 69% bootstrap support. The clade formed by the specimens of *R. auricularia*, *R. schubinae*, *R. iturupica*, *R. narzykulovi*, *R. ussuriensis*, and *R. intercisa* is supported with only 51% while *R. labiata*, *R. natalensis*, and *R. rubiginosa* have fully supported species cluster each.
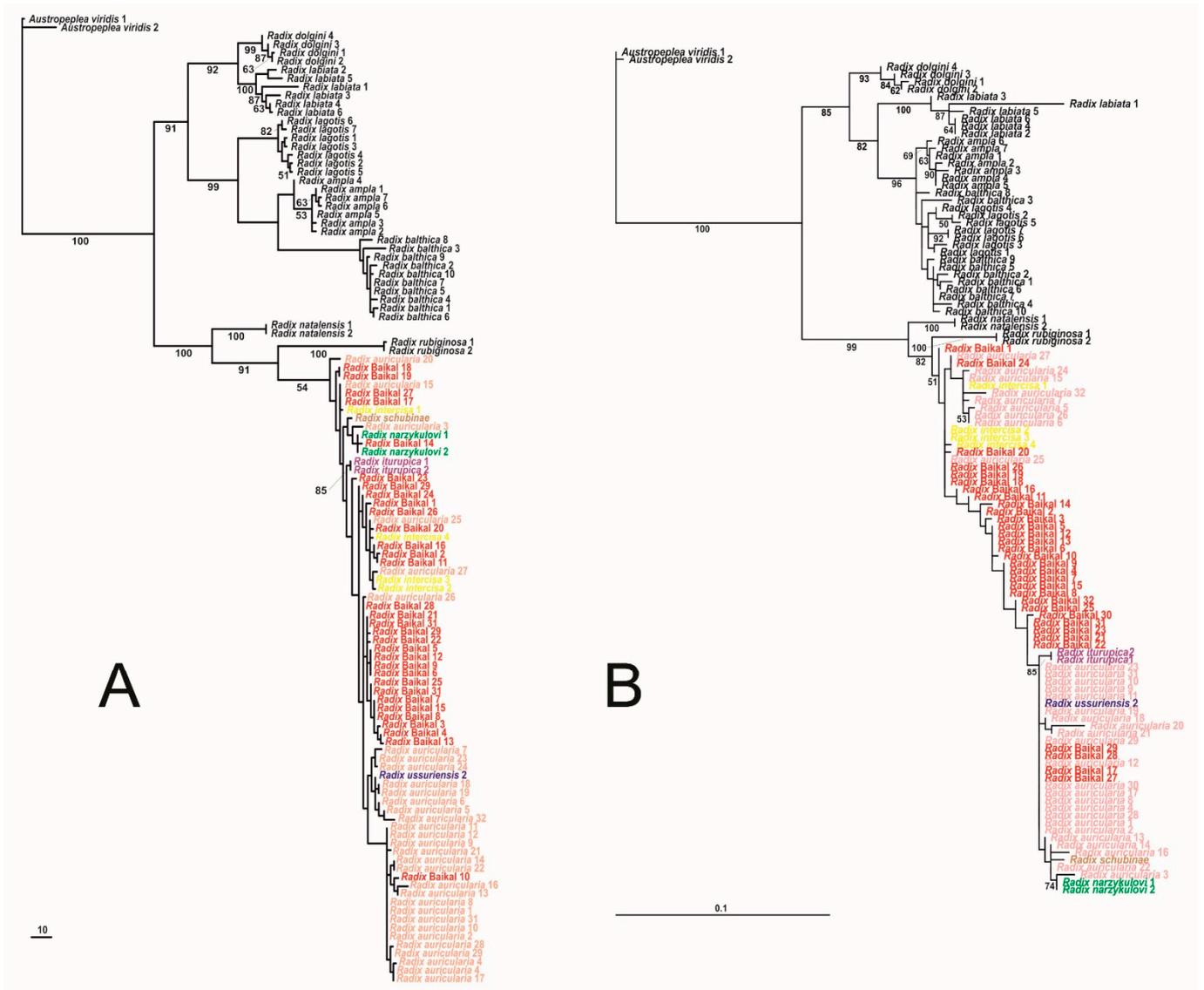
Figure 4. Hypotheses of the phylogenetic relationships of analysed *Radix auricularia* specimens based on the nuclear ITS-2 marker using different algorithms of tree building, (**A**) based on one of the 10,000 best MP trees (tree length = 618, CI = 0.7330, RI = 0.9634) and (**B**) based on RAxML. Branch lengths are proportional to the number of substitutions and the overall topology corresponds to that of the strict consensus tree. Bootstrap support values above 50% are reported below nodes.

The network (see Figure 5) reveals two identical haplotypes in Eastern and Central Europe (*Radix auricularia* 32 and *Radix auricularia* 1), Southern Europe (*Radix auricularia* 11 and *Radix auricularia* 12), Central Asia (*Radix narzykulovi* 1 and *Radix narzykulovi* 2), the Far East (*Radix iturupica* 1 and *Radix iturupica* 2), and Lake Baikal (*Radix* Baikal 20 and 21), as well as a second group of 13 identical haplotypes within Lake Baikal (*Radix intercisa* 3 and 4, *Radix* Baikal 3, 4, 6, 8, 9, 15–18, 22, and 23). Furthermore, the haplotype that is shared between Lake Baikal, Siberia, and the Far East is found in 9 individuals (*Radix* Baikal 2, *Radix* Baikal 10–13, *Radix auricularia* 3, 5, 6, and 22).
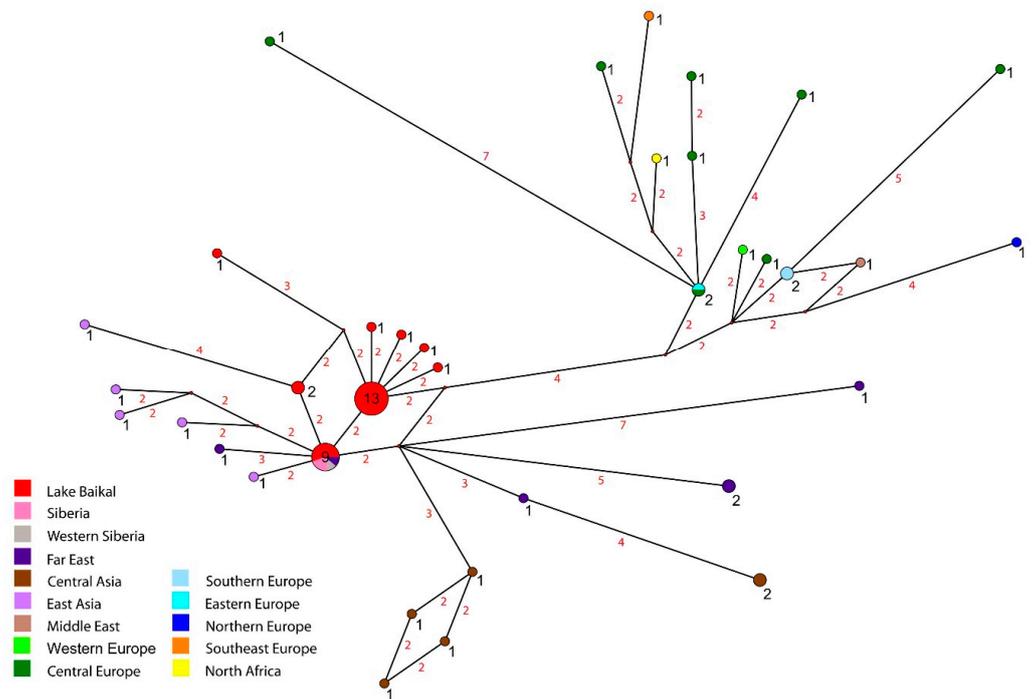
Figure 5. Haplotype network of *cyt–b* sequences of 61 *Radix auricularia* specimens from different locations in Asia, Europe, and North Africa. The size of the balloons is proportional to the number of individuals having each haplotype, which corresponds to the black number inside or next to the balloon. The small red dots represent internal haplotypes not represented by the dataset. The length of the connecting lines is proportional to the number of substitutions between haplotypes, corresponding to the red number on the line.

*3.2. Morphology of the Baikalian Representatives of Radix*

Shell. The shells of *R. auricularia* from Lake Baikal, including the 32 specimens examined by molecular genetic methods, demonstrate a very high degree of phenotypical disparity. They vary from individuals that show only slight growth in the width of the first two whorls, a concave lateral line from the apex to the upper border of the body whorl, and a large ear shaped aperture (e.g., Figure 6C,J,T), to others with fast growing whorls, a convex lateral line, and an ovate (Figure 6B,W) or elongated ovate (Figure 6E,H,V) aperture. The height of shells of specimens used in molecular analyses ranged from 9.4 to 20.5 mm, their width from 5.3 to 15.8 mm, their aperture height from 6.2 to 15.8 mm, their aperture width from 4.1 to 12.1 mm, and the number of whorls from between 3 and 4. The columellar fold varied from strong (e.g., Figure 6C,E), overly weak (e.g., Figure 6G,H) to almost straight (Figure 6N,X). Some shells of *R. auricularia* collected from Baikal resemble shells of another lymnaeid species, *Radix balthica* (e.g., Figure 6N,U), and without a molecular assessment can be misidentified as the latter. The type series of *Limnaea* (*Gulnaria*) *auricularia* var. *intercisa* contains specimens of very different shell shapes (see Figure 6A).
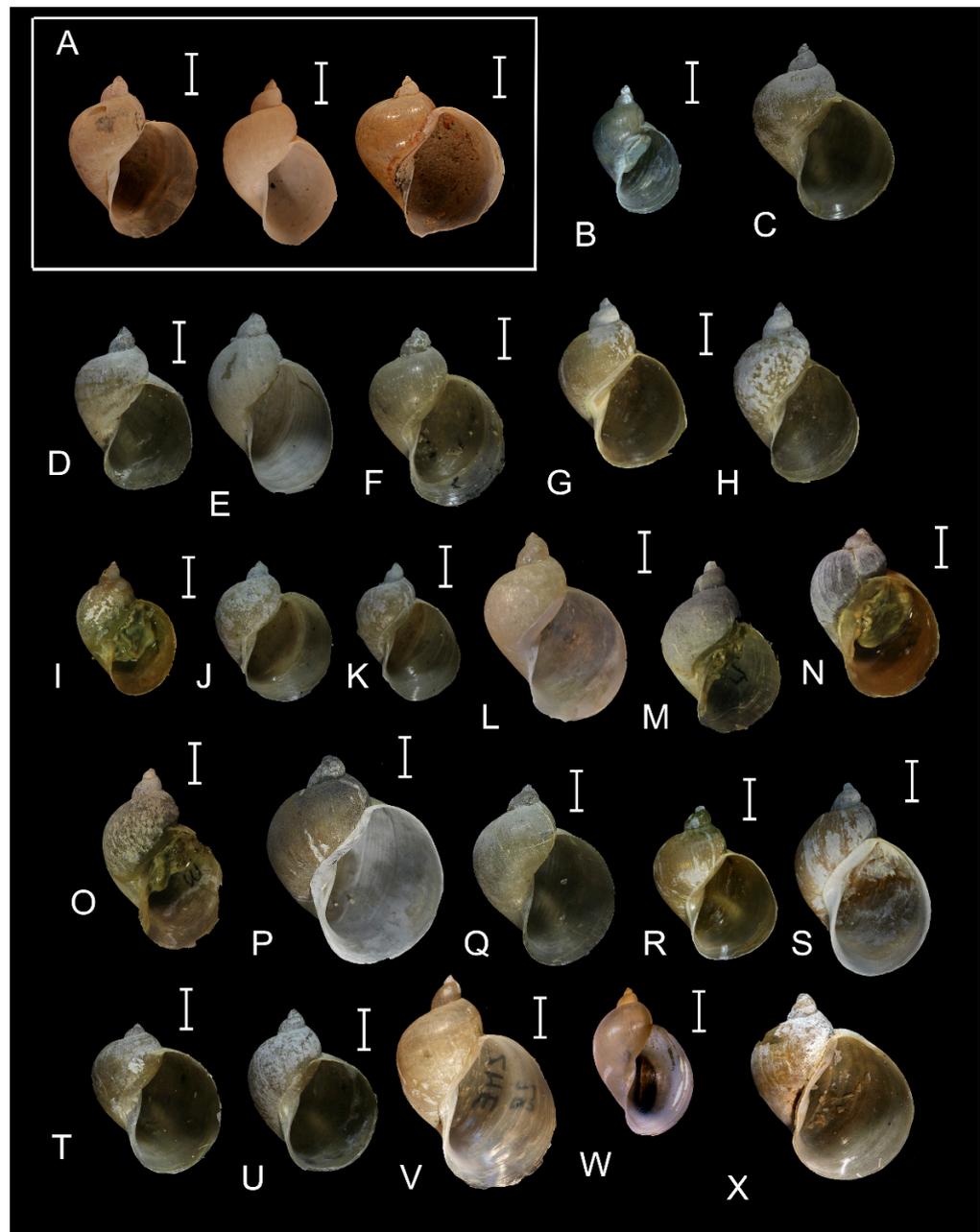
Figure 6. (**A**) The lectotype (left shell) and two paralectotypes of *Limnaea auricularia* var. *intercisa* Lindholm (ZIN). (**B**) Bol'shie Koty (SW), groundwater 'puddle', 3–5 m from shoreline. (**C**) Elokhin Cape (NW Baikal), depth 4–10 m. (**D,E**) Bol'shie Koty, near Zhilische, depth 2.5–4.2 m. (**F**) Listvenich-noye, 1–9 m depth. (**G,H**) Peshanaya Bay. (**I**) Cape Zub (Khabartuy), depth 3–10 m. (**J,K**) North of Buguldeyka, 2–3 m depth. (**L**) Maloye More, Kharin–Irgin Bay, 1.2 m depth. (**M**) Shibeteyskaya Bay. (**N**) Valukan Cape, 0.1–0.15 m depth. (**O**) Bolshaya Kosa Bay, depth 30 m. (**P**) Senogda Bay, depth 0.5 m. (**Q**) Slyudyanskiy Cape (NW), depth 2.8–3.2 m. (**R,S**) Baikal near Zarechnoe, 0.5 m depth. (**T,U**) Ayaya Bay (NE), depth 0.5 m. (**V–X**) Chivyrkuy Bay, Zmeinaya Bight, 0.5 m depth. Scale bars = 5 mm.

Mantle pigmentation. The mantle pigmentation of the 32 specimens examined by molecular genetic methods shows a broad polymorphism (Figure 7). The primary colour of the mantle varies from black (e.g., Figure 7B,C,F,G), greyish black (e.g., Figure 7E), or bluish black (Figure 7H) to greyish brown (Figure 7J,K), and brown (Figure 7L). On top of this primary colour the mantle shows spots and/or dots of different size, colour, and

clarity. The mantle collar is from white (Figure 7G) to dark grey (Figure 7A), bluish grey (e.g., Figure 7F,H), or yellowish grey in different shades (e.g., Figure 7D,I,J), to brown (Figure 7L) in colour, and has numerous irregular patches of black, brown, or brownish black in different numbers and sizes. The colour of the mantle edge varies from white (Figure 7G) or grey (e.g., Figure 7J,L) to yellowish grey (Figure 7K), greenish grey (e.g., Figure 7C,I), and bluish grey (Figure 7H). Specimens sampled at the same places can show different mantle pigmentation (Figure 7A,B,H,I).
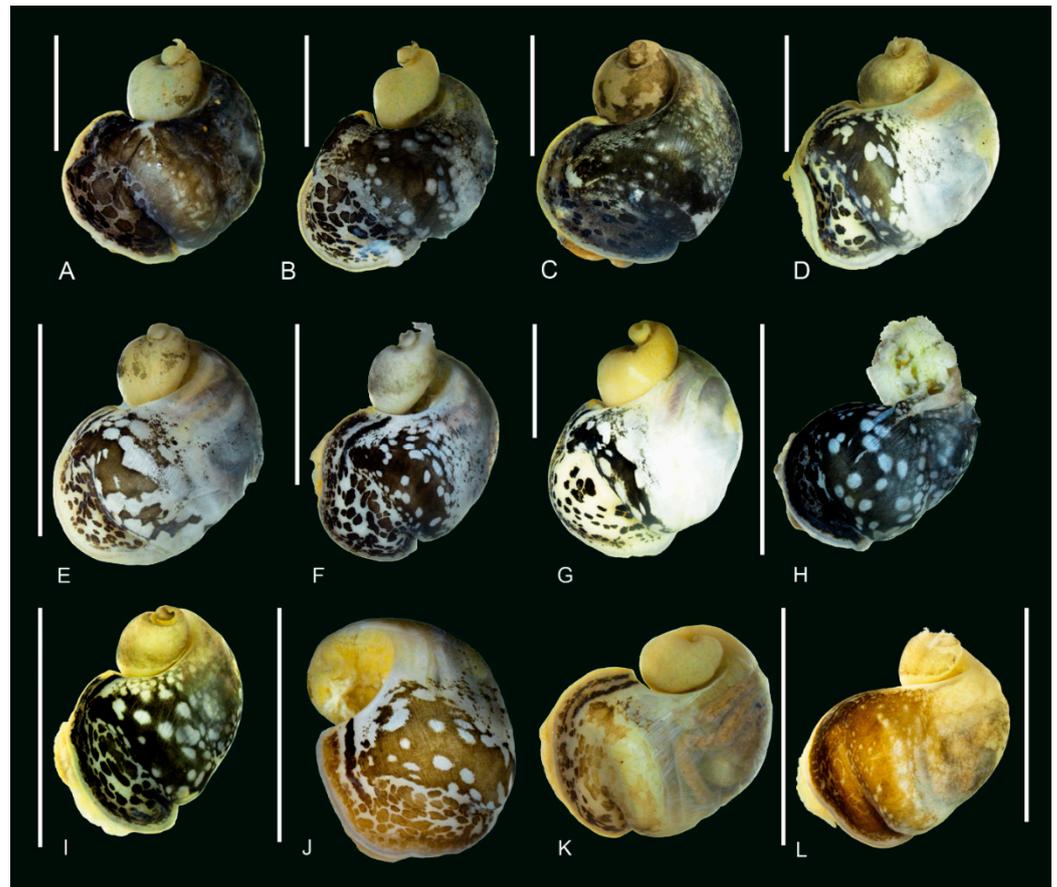
Figure 7. Variability in mantle pigmentation of *Radix auricularia* from Lake Baikal. (**A**) North of Buguldeyka, 2–3 m depth (*Radix* Baikal 2); (**B**) North of Buguldeyka, 2–3 m depth (*Radix* Baikal 1); (**C**) Listvenichnoe, 1–9 m depth (*Radix* Baikal 12); (**D**) North of Cape Elokhin, 4–10 m depth (*Radix* Baikal 3); (**E**) near Zarechnoe, 0.5 m depth (*Radix* Baikal 22); (**F**) Zhilische, 2.5–4.2 m depth (*Radix* Baikal 20); (**G**) North of Cape Elokhin, 4–10 m depth (*Radix* Baikal 4); (**H**) Angara River near Usol'e-Sibirskoe, 0–0.3 m depth (*Radix* Baikal 30); (**I**) Angara River near Usol'e-Sibirskoe, 0–0.3 m depth (*Radix* Baikal 31); (**J**) Maloye More, Kharin–Irgin Bay, 1.2 m depth (*Radix* Baikal 7); (**K**) Pereyemnaja River at its mouth into Lake Baikal (*Radix* Baikal 32); (**L**) Valukan Cape, 0.1–0.15 m depth (*Radix* Baikal 16). Scale bars = 10 mm.

Pigmentation on head and foot. The yellow or yellowish white body of the 32 *R. auricularia* specimens examined by molecular genetic methods clearly show visible blue–grey or brown–grey freckles (Figure 8A,B) or blurred blue–grey or brown–grey freckles on head and tentacles (Figure 8C–E) in most cases. Only two specimens lacked freckles (Figure 8F). The sole of the foot was coloured yellow, yellowish white, or blue–grey. No freckles could be found in any specimen on the sole of the foot.
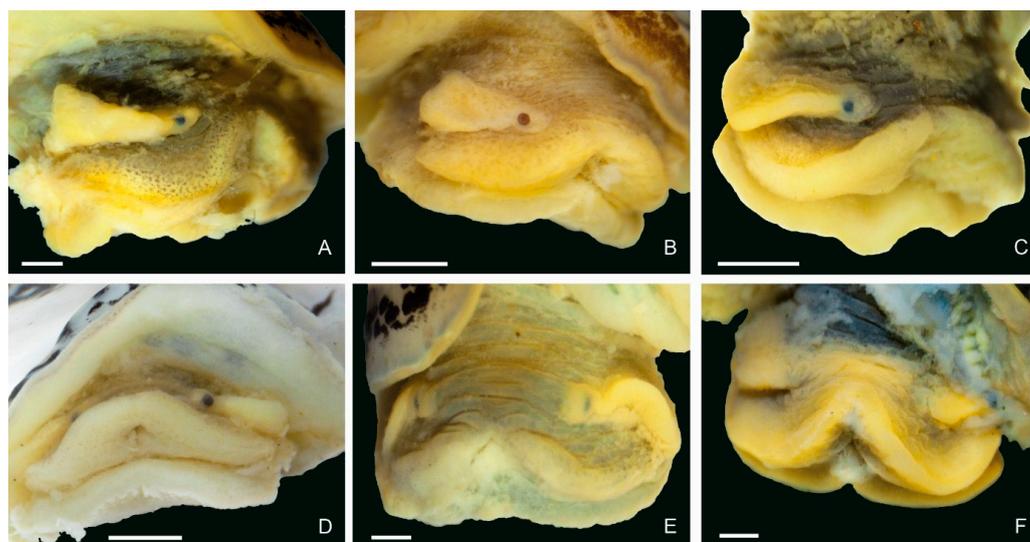
Figure 8. Variability in pigmentation on head and foot of *Radix auricularia* from Lake Baikal. Top row: view of right side, the dark point in the middle is the right eye. Bottom row: frontal view. (**A**) North of Cape Elokhin, 4–10 m depth (*Radix* Baikal 4); (**B**) Maloye More, Kharin–Irgin Bay, 1.2 m depth (*Radix* Baikal 7); (**C**) Pereyemnaja River at its mouth in to Lake Baikal (*Radix* Baikal 32); (**D**) near Zarechnoe, 0.5 m depth (*Radix* Baikal 22); (**E**) Maloye More, Shibetejskaya Bay, in a depth of 24–25 m (*Radix* Baikal 11); (**F**) North of Buguldeyka, 2–3 m depth (*Radix* Baikal 2). Scale bars = 1 mm.

Male genitalia. The ratio of the length of the preputium to that of the penial sheath varies from 0.75–1.51 (n = 8). The basic colour of the preputium is yellow (Figure 9A), yellowish grey (Figure 9B–D,H), yellowish green (Figure 9G), or bluish grey (Figure 9E,F). In most cases, additional pigmentation on the proximal part is grey (Figure 9A) or bluish grey (Figure 9C,D,G,H), reminding of freckles (n = 6). In one case, the bluish grey preputium has a dorsal pigmentation with yellow "freckles" (Figure 9E).

Figure 9. Variability in male genitalia of *Radix auricularia* from Lake Baikal: (**A**) Listvenichnoe, 1–9 m depth (*Radix* Baikal 13); (**B**) Listvenichnoe, 1–9 m depth (*Radix* Baikal 12); (**C**) Maloye More, Shibeteyskaya Bay, in a depth of 24–25 m (*Radix* Baikal 11); (**D**) North of Cape Elokhin, 4–10 m depth (*Radix* Baikal 3); (**E**) North of Buguldeyka, 2–3 m depth (*Radix* Baikal 1); (**F**) North of Buguldeyka, 2–3 m depth (*Radix* Baikal 2); (**G**) North of Cape Elokhin, 4–10 m depth (*Radix* Baikal 4); (**H**) Senogda Bay, 1.5 m depth (*Radix* Baikal 8). Scale bars = 1 mm.

The phallotheca was pure white (Figure 9D), yellowish (Figure 9H), yellowish with a very fine bluish grey pigmentation (Figure 9G), or whitish grey with a very fine bluish grey pigmentation (Figure 9E,F).

Bursa copulatrix. The shape of the bursa (n = 14) was very variable, from nearly spherical (Figure 10A), pyriform (Figure 10E), to elongated (Figure 10G,H). In one specimen, even a heart-shaped bursa (Figure 10B) was found.
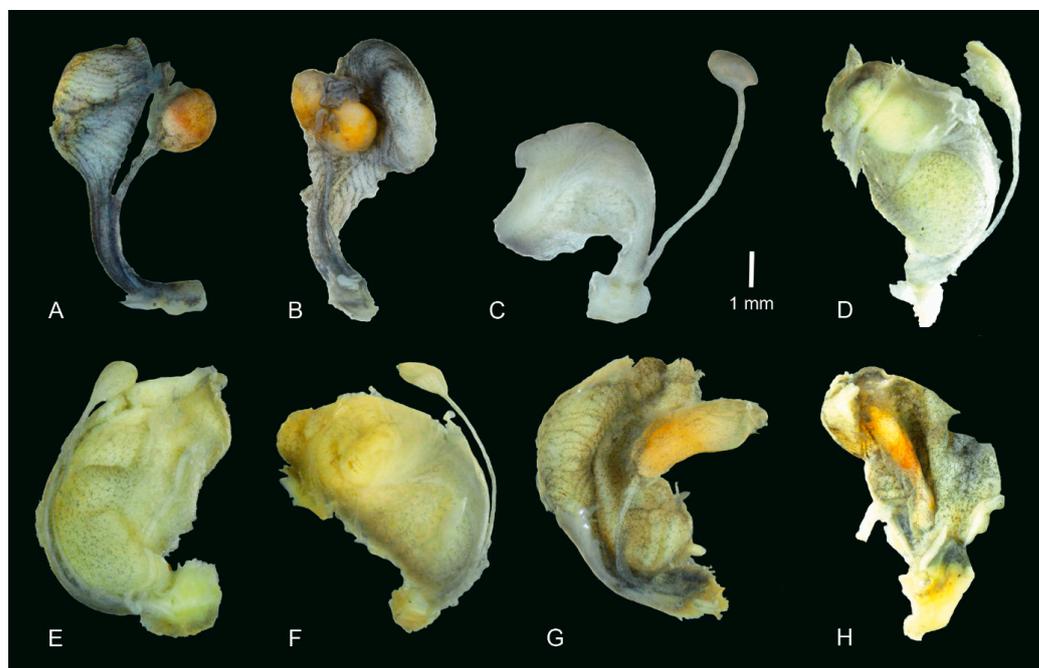

Figure 10. Variability of the form of the bursa copulatrix and the length of the bursa duct in *Radix auricularia* from Lake Baikal: (**A**) North of Buguldeyka, 2–3 m depth (*Radix* Baikal 1); (**B**) North of Buguldeyka, 2–3 m depth (*Radix* Baikal 2); (**C**) Ayaya Bay, 15 m depth (*Radix* Baikal 26); (**D**) North of Cape Elokhin, 4–10 m depth (*Radix* Baikal 3); (**E**) North of Cape Elokhin, 4–10 m depth (*Radix* Baikal 4); (**F**) Maloye More, Shibeteyskaya Bay, 24–25 m depth (*Radix* Baikal 11); (**G**) Listvenichnoe, 1–9 m depth (*Radix* Baikal 12); (**H**) Listvenichnoe, 1–9 m depth (*Radix* Baikal 13). Scale bar = 1 mm.

For six specimens, the length of the bursa duct as well as of the bursa could both be determined exactly. The ratios (length of bursa to length of bursa duct) varied between 5.53 and 1.17 in five specimens. In one specimen (Figure 10G), the bursa duct (2.99 mm) was nearly as long as the bursa (3.02 mm). For another specimen (Figure 10H), the sizes could not be determined exactly because the duct and bursa were filled and not exactly delimitable. However, it appears that the bursa duct is also nearly as long as the bursa in this specimen.

3.3. Morphological Comparison between Baikalian and Extra-Baikalian Populations of Radix auricularia

All shells included in the geometric morphometric analysis, independent of their geographic origin, fell into a single continuous morphospace, which cannot be subdivided into two or more internal groupings (Figure 11). The external phenotypical variation in *R. auricularia* extends from extremely ear-shaped specimens concentrated in the left part of the polygon to very elongated shells with high spires and slender body whorls that tend to occupy the right part of the morphospace (see Figure 11). A graded series of intermediate individuals creates a full connection between the two extremes. In Lake Baikal, both morphotypes (i.e., ear-shaped and slender) co-occur, and even a small sample may contain a mixture of the two (see Figure 6A). However, the slender morph appeared to be represented almost exclusively in Lake Baikal, while the populations of Siberia and Mongolia used in the analysis were represented by the (most-typical for the species) ear-shaped shells, with very short spires and greatly inflated body whorls.
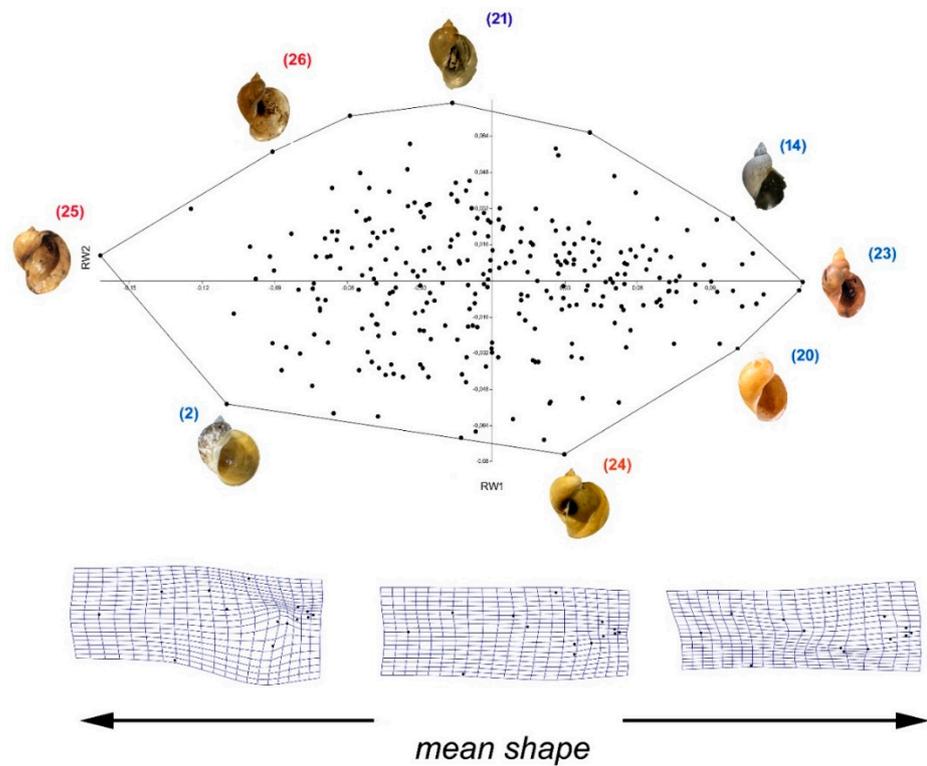
Figure 11. (**Above**)—continuous morphospace of *Radix auricularia* based on the results of geometric morphometric analysis. (**Below**)—thin-plate spline deformation grids illustrating phenotypical variation along axes of the reconstructed morphospace. Shell images (sizes not to scale) illustrate specimens located at the extreme points of the convex polygon containing all points. Numbers in brackets correspond to numbers of populations in Table 1. Red numbers designate non-Baikalian and blue numbers Baikalian individuals.

The extent of conchological variation in *R. auricularia* in Lake Baikal is approximately double that of *R. auricularia* from non-Baikalian localities, as exemplified by the distribution of the first relative warp (RW) values in the two samples (Figure 12B). The scatterplot (Figure 12A) illustrates the difference in shell morphology in the Baikalian *Radix* as compared with extra-Baikalian populations. There is no sharp boundary between the two samples, but the Baikalian snails are visibly shifted along the first RW axis towards the right end (see Figure 12A). In our opinion, this could indicate some alterations in phenotypic plasticity that have taken place in the Baikalian populations of *R. auricularia* as a direct consequence of their entering the lake ecosystem and the need to adopt to atypical life conditions (see Section 4).
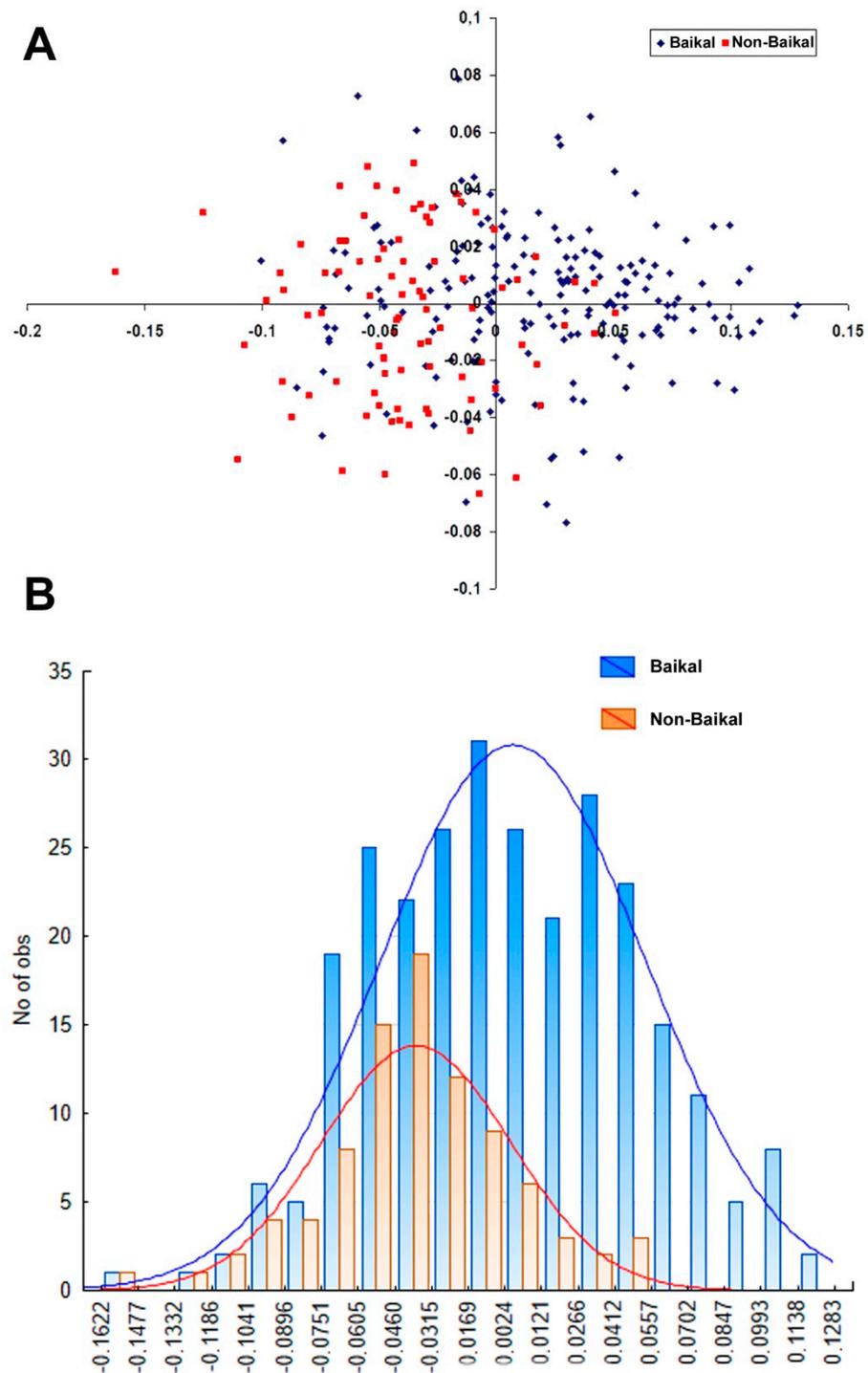
Figure 12. (**A**) Positions of the Baikalian and non-Baikalian specimens of *R. auricularia* in the space formed by the first two RWs. (**B**) Distribution of the first RW values in the Baikalian and non-Baikalian specimens of *R. auricularia*.

On the other hand, geometric morphometric analysis reveals no phenotypical differentiation among the Baikalian populations of this snail, whose shell morphology seems not to depend on either locality or ecology (see Supplementary Figure S1). The same lack of interpopulation morphological differentiation was observed in the non-Baikalian populations of *R. auricularia*, which cannot be distinguished based on geometric morphometric data (see Supplementary Figure S2).

4. Discussion

4.1. Molecular Phylogeny and Phylogeography

Our molecular genetic analyses of the mitochondrial marker *cyt–b* (Figure 3A,B) and the nuclear ITS-2 spacer (Figure 4A,B) show that the *Radix* specimens collected in different localities and in different water depths of Lake Baikal group together with the *R. auricularia* specimens from various regions and countries lying far outside the lake. We thus conclude that these specimens collected from Lake Baikal belong to *R. auricularia*. Though some authors [11,68] suggested earlier that there are two *Radix* morphospecies in Lake Baikal (i.e., *R. auricularia* s. str. and *R. intercisa*), our results do not support this assumption. There are even fewer reasons to consider the numerous varieties and morphs of *R. auricularia* described from Lake Baikal in the older works [15,33] as valid.

This is also confirmed by the network representation of mitochondrial sequences (Figure 5). The number of substitutions between the haplotypes of specimens from Lake Baikal is very small compared to the number of substitutions between other *R. auricularia* specimens used in the analysis. The group with 13 identical haplotypes includes two specimens that originally were determined as *R. intercisa*. Five specimens from three different sampling sites of Lake Baikal share the same haplotype with four specimens collected far from Lake Baikal in the Tomsk and Novosibirsk regions (Western Siberia), as well as in Yakutsk (Eastern Siberia).

In addition, the absence of reliable genetic distinction between the morphological species *R. schubinae, R. iturupica, R. narzykulovi,* and *R. ussuriensis* is evidence of their close molecular relationship with *R. auricularia*, which may suggest their synonymy with the latter. This result is shown here in a peer-reviewed publication for the first time, but Vinarski and Kantor [68] cited this fact based on (at that time still unpublished) molecular genetic results of K. Schniebs. Shirokaya et al. [69] mentioned possibly synonymy with *R. auricularia* for *R. narzykulovi*. Moreover, Sitnikova and Takhteev [70] already considered *R. intercisa* to be an intraspecific form of *R. auricularia*. However, since our research is geographically restricted to Lake Baikal, and since we did not study large samples of the abovementioned species (except for *R. intercisa*), the final solution to their taxonomic status must be presented elsewhere.

4.2. Morphology

Shell. The shells of *R. auricularia* from European populations are usually described as spherical–ovate with a large, ear-shaped aperture, a pointed short conical spire, and a strong columella fold (e.g., [27,29,71,72]). The shape of the line tangential to the whorls in adult shells is always described as concave (e.g., [27,29,47,71,72]). In most samples of *R. auricularia* from extra-Baikalian populations, which we have examined during this study, the majority of adult shells look more or less similar, and the degree of conchological disparity can be considered low or moderate. By contrast, *R. auricularia* from Lake Baikal seem to show a higher variability in shell morphology. The spire of the shell varies from pointed short conical to broader conical (see Figure 6). We found not only shells with a strong columella fold, but also individuals with a weak or straight one (see above). The shells show not only the described characteristic shell form but can have fast-growing whorls, a convex lateral line, and an ovate or elongated ovate aperture. The latter is characteristic of juvenile specimens of *R. auricularia* [29].

Shell heights of *R. auricularia* in the literature have the following values: about 20 mm high [29], 14–24 mm [28], 15–30 mm [70], up to 30 mm [26], and up to 34 mm [72,73]. The shells of the specimens from Lake Baikal analysed by genetic methods reached a maximum height of 20.55 mm and had a maximum of four whorls. In the literature, the number of whorls is reported as up to 4 [72], 4–4.5 [26], and up to 4.75 [73]. *R. auricularia* living in the deeper waters of the open Lake Baikal are considerably smaller. For instance, the mean shell height in a sample dredged from Peschanaya Bay on 21 June 2021 (see Supplementary Figures S3 and S4) was 13.2 ± 1.5 mm (min–max 10.6–17.1).

Geometric morphometric analysis revealed a clear shift in shell shape in the Baikalian populations of *R. auricularia* compared to their conspecifics from waterbodies of Siberia and adjacent countries. Though no hiatus between the two samples (i.e., Baikalian and non-Baikalian) may be observed, it is evident that introduction of the species in Lake Baikal's ecosystem resulted in alteration of their phenotype. As our results did not reveal any substantial genetic differences between Baikal and non-Baikal populations, one may consider this alteration a non-hereditary outcome of adaptation to living in deeper and colder waters of the lake. The precise understanding of what may induce such phenotypic changes would require a special study; here, we can propose only a tentative explanation (see below).

The absence of considerable differences in shell shape among Baikalian populations (see Supplementary Figure S1) indicates that shell alteration occurs independently in different locations, and these snails acquired their "Baikalian" phenotype simultaneously as a result of a parallel microevolution. A similar situation is observed in *R. auricularia* forming populations in geothermal waterbodies; in several habitats of such type separated by substantial geographic distances, phenotypically similar 'ecological races' of *R. auricularia* have formed (see [74,75]). The mechanism(s) of these parallel shifts in shell morphology requires special examination.

Mantle pigmentation. The typical mantle pigmentation reported for *R. auricularia* shows a few large white spots on a dark background (e.g., [26,28,29,47,71]). This pigmentation was observed only in a few of the specimens we analysed (Figure 7E,G). The other specimens show unexpected high variability of this characteristic. Some of the other specimens (Figure 7B,D,F,H–J) show mantle pigmentation described as typical for *R. balthica*: many medium-sized, light, distinct spots on a dark background (e.g., [28,47,71,76]); this has been found in *R. ampla*, too [52].

Pigmentation on head and foot. *R. auricularia* is known for having speckles reminding of freckles on the head, tentacles, and foot [47,72], or only on the foot [76,77]. This is the only *Radix* species with such pigmentation on the head and foot [29].

The specimens of *R. auricularia* from Lake Baikal analysed by molecular genetics show two variations of these freckles: clearly visible blue–grey or brown–grey freckles (Figure 8A,B) or blurred blue–grey or brown–grey freckles (Figure 8D,E). We found no freckles on the sole of the foot in any of the specimens analysed. In some individuals there were no freckles at all (Figure 8F). Thus, this characteristic shows higher variability than expected for *R. auricularia*.

Male genitalia. The ratio between lengths of preputium and penis sheath for *R. auricularia* is given as 1.0 (e.g., [26,28,29,71]) or 1.1 [27,72]. The individuals studied from Lake Baikal show ratios from 0.75–1.51. Additional pigmentation on the proximal part of the preputium resembling freckles as reported by Glöer and Pešić [77] was not observed in other *Radix* species analysed in more detail concerning this characteristic: *R. skutaris* Glöer and Pešić 2008 [77], *R. balthica* [47], *R. labiata* [48], and *R. ampla* [52]. However, this additional pigmentation was not observed in any of the individuals from Lake Baikal analysed by molecular genetics. Notably, similar pigmentation is frequent in some East Asian species of *Radix*, such as *R. rubiginosa* (Michelin, 1831) and *R. rufescens* (Grey, 1822) (M. Vinarski, personal observation).

Bursa copulatrix. Hitherto, the bursa duct in *R. auricularia* was known to be very long and thin (e.g., [26–29,47,71,72]) with the bursa positioned near the pericardium [47]. The shape of the bursa has been described as spherical (e.g., [27,72]), but Glöer ([29] p. 236) illustrated the female sex tract ([29] Figure 294: 4) of *R. auricularia* with an elongated, filled bursa. Most of the *R. auricularia* specimens from Lake Baikal had a very long and thin bursa duct the bursa positioned near the pericardium. However, this is the first time that a bursa duct nearly as long as the bursa positioned as the latter above prostate (see Figure 10G,H) was found in *R. auricularia*. Both spherical and elongated shapes of the bursa were observed within Baikalian *R. auricularia* (see Figure 10).

4.3. The Process of Radix auricularia Invasion to Lake Baikal Ecosystem

The data presented above as well as that available from published sources [1,39] allow us to propose a reconstruction of *Radix auricularia* invasion to Lake Baikal's ecosystem and its intralacustrine dispersal.

As stated in the introduction, this snail is a 'novelty' for the lake ecosystem, and up to the mid-20th century, the species was limited in its distribution to the littoral–sor zone and the shallowest nearshore parts of the lake, the conditions of which are similar to those in relatively small lakes and ponds. The lymnaeids could penetrate the littoral–sor zone along large rivers (Selenga, Barguzin, Kichera, Upper Angara) or with waterfowl (Anseriformes). It served as a 'waiting zone' for *R. auricularia*, which facilitated its entrance to the Baikal proper. This process started around 1950 and is still ongoing. Today, the snail is distributed throughout Lake Baikal (see Figure 1) and occupies depths from 0 to 30 m. Remarkably, the same depth range is reported for another lymnaeid species inhabiting an ancient lake—*Radix relicta* Poliński, 1929 in Lake Ohrid [78].

In bays of Lake Baikal and the rocky steep littoral, *R. auricularia* shares space and resources with a host of endemic species of the families Acroloxidae, Baicaliidae, Benedictiidae, Planorbidae, and Valvatidae. For example, in a bottom sample dredged on 22 June 2021 in Peschanaya Bay (southwestern Lake Baikal) for 6–7 m depth, we found very abundant individuals of *R. auricularia* living together with the endemic species *Choanomphalus* cf. *amauronius* (Bourguignat, 1861) (family Planorbidae), *Megalovalvata baicalensis* (Gerstfeldt, 1859), *M. demersa* (Lindholm, 1909) (family Valvatidae), *Korotnewia semenkevitschi* (Lindholm, 1909), *Parabaikalia elata* (W. Dybowski, 1875), *P. oviformis* (W. Dybowski, 1875), *P. florii* (W. Dybowski, 1975), and *Maackia bythiniopsis* (Lindholm, 1909) (family Baicaliidae).

In our opinion, there are a few factors that stimulated and facilitated the ongoing dispersal of the ear pond snail within Lake Baikal. One of them is global climate warming's affect on Lake Baikal [21]. According to Shimaraev and Domysheva [79], the temperature of the surface layer of water in the lake by the end of the last century increased by 0.9–1.5 °C in the southern and by 1.8–2.0 °C in the middle and northern basins; in recent years, the water temperature in some areas reached 18–20 °C. In the 0–300 m layer, the water temperature increased by 0.05–0.1 °C.

There was also a marked increase in water temperature in the open littoral zone. Voznesenskiy [80] reported the water temperature in Goloustnoye (during the warmest period on Lake Baikal, in August 1897–1898?) at depths of 2 and 5 m as 14.4 and 11.5 °C, respectively. A century later, Timoshkin and Zaitseva [81] showed that the bottom water temperature in Bolshiye Koty (37 km south of Goloustnoye) at a depth of 3.6 m increased in August 2006 to 15 °C; hence, at a depth of 2 m, the temperature could reach 16 °C, which is 2 degrees higher than at the end of the 19th century. In Listvenichny Bay near Cape Berezovoy, the bottom water temperature in August 2005 at a depth of 2.9 m reached 19 °C, and at a depth of 7.4 m—16 °C. For comparison: at the end of the 19th century, at depths of 10 and 25 m, the temperature was 9.2 and 5.5 °C, respectively; accordingly, from January to March, the temperature did not rise above 1 °C [80].

The water temperature in the 'sor' zone is considerably higher than in the open Lake Baikal. In the sor areas of the lake (Maloye More, Chivyrkuisky and Barguzinsky bays, Proval gulf) and coastal lakes associated with Lake Baikal, it rose in August 1957–1958 to 19–20 °C and in some areas to 25–27 °C [82,83]. Timoshkin and Zaitseva [81] found that the bottom water temperature in the water area bounded by the LIN pier in Zhilishche (near Bolshiye Koty settlement) in August 2007 reached 19 °C.

The ability of an invasive species to "thrive within a wide thermal window" ([84] p. 11) has been considered a key factor in the successful invasion of Lake Baikal. For example, the lower cold-tolerance of the widely distributed Holarctic amphipod *Gammarus lacustris* (Linnaeus, 1758), as compared with the cold-tolerance of the endemic Baikalian amphipods, was invoked to explain its inability to colonize the lake [84–86]. It may be noted that *R. auricularia* in the Palearctic is able to cope with a wide spectrum of temperatures; sustained populations of this species were recorded from Arctic waterbodies and cold highlands of

the Pamir Mountains and Tibetan Plateau, as well as from geothermal habitats with water temperatures lying between 30 and 40 °C [36,75,87–89]. Among lymnaeids, the ear pond snail is one of the most widely distributed and ecologically plastic species [25,27], which can be seen as an effective preadaptation to temperatures existing at different depths of Lake Baikal.

The next possible driver of the ongoing dispersal of the ear pond snail within Lake Baikal is human activity. Today, Lake Baikal is one of the most popular tourist sites in Siberia, attracting thousands of tourists per year. One of the most appealing activities for these people is boating. Numerous companies situated in Listvenichnoye, Turka, Olkhon, and other places on Lake Baikal offer boating excursions to different sightseeing places situated on the lake's shores. In the water surrounding the piers, conditions such as ambient temperature are created that closely match that of the 'sors' ('waiting zone'). Further, wave action is relaxed, and aquatic vegetation often develops, including thickets of *Elodea canadiens* (TYS, personal observation). It is no coincidence that *R. auricularia* was first found in the water surrounding the pier of Lake Baikal port at the source of the Angara River [1], and only later in the open littoral of the lake.

The boats, to which the snail may attach, serve as a vector for fast and relatively long-distance dispersal of *R. auricularia* from one bay to another. Overland transport of these boats can also facilitate mollusc dispersal, as recently demonstrated for *Dreissena polymorpha* [90,91] and other macroinvertebrates [92].

However, this activity characterizes the last 15–25 years only. It is, therefore, reasonable to think that in the mid-20th century, some natural factor(s) may have facilitated the snails' dispersal. The intralacustrine horizontal currents in Lake Baikal, which have, according to Krotova [93], a circular orientation in different depressions of the lake, could disseminate snails throughout Lake Baikal. For example, based on the similarity of the nucleotide sequences of *cyt–b*, we assume that from Shibiteiskaya Bay of Maloye More strait, snails dispersed south into the open littoral near Buguldeika and Listvyanka settlements. From the Chivyrkuisky Bay, snails were transported north from Valukan—Ayaya (northeastern open littoral)—Zarechnoye—Senogda—Elokhin (northwestern littoral). Perhaps the Angarsk Sor (the area of the Upper Angara and Kichera delta), Boguchanskaya Bay, as well as bays at the mouth of the Tyya River have also served as starting points for *R. auricularia* dispersal in Lake Baikal, but the genetic data on these populations remain unavailable. When they met, the snails spreading from different regions crossed, which is confirmed by the lack of supported structure due to low resolution between positions of most individuals on the *cyt–b* and ITS-2 trees. Moreover, the introgression was probably both nuclear and mitochondrial, but this assumption needs to be tested on more material in future studies.

The invasion of *R. auricularia* to Lake Baikal probably had numerous sources and, in our opinion, can hardly be explained by a single introduction event. This is proven by the absence of any genetic or morphological differentiation among populations of this species in the lake (see above). The question remains, what was the stimulus for the species to start entering Lake Baikal in relatively recent times? Why was the ear pond snail unable to enter this ecologically suitable waterbody earlier? Only hypothetically, we may link this process to increasing human impact on the lake ecosystem, which started to become prominent around the early 20th century. Before this, the Lake Baikal region was only loosely inhabited, with only a few large settlements and virtually no industrial sites. The last century has witnessed an enormous rise of industrial activity in the area, which has led to substantial alterations in the lake ecosystem.

Though in most cases *R. auricularia* and related lymnaeid species are limited to shallow waterbodies, where they inhabit the littoral zone within the first meters of depth, these snails are quite able to live much deeper. A classic example is the lymnaeids inhabiting the profundal zones of large Alpine lakes in Western Europe at considerable depths, 200–250 m and more. In the late 1860s, the Swiss physician and scientist François-Alphonse Forel, the "Father of limnology", discovered an unexpectedly rich fauna of bottom macroinvertebrates in the Geneva Lake, which he designated "Faune profonde" (*die Tiefenfauna* of German

authors) [94,95]. As Roszkowski [96] noted, the rich deep-water fauna of Lake Baikal was discovered at precisely the same time by Benedykt Dybowski, namely in 1869 (see W. Dybowski [97], who investigated samples made by his brother Benedykt, then a Polish exile in Siberia). Later on, analogous findings were made in other deep lakes of Switzerland and Germany. Molluscs, both snails and bivalves, were among this fauna, and some lymnaeid species were reported to live in depths from 25–280 m (see [96,98,99]). The object of this study, *R. auricularia*, was registered in the Alpine lakes at depths down to 80 m [100].

Brot [101], Clessin [100], and Piaget [98,102], who were among the first authors to study these deep-water lymnaeids, considered them separate species, based chiefly on their diminutive size and peculiar shell proportions. Later on, however, Roszkowski [96,103] brought anatomical evidence that these 'species' are nothing more than the 'profundal' ecological races of common species of Lymnaeidae that managed to colonize the lake's depths and adapted to their harsh conditions (low temperature, absence of sunlight, shortage of nutrients, etc.).

Some Alpine lymnaeid taxa described by Clessin and Piaget are morphologically similar to *Radix auricularia* of Lake Baikal living at comparable depths. For example, *Limnaea limosa* var. *roszkowskiana* Piaget, 1913 (a junior synonym of *R. auricularia*), described from the Geneva Lake in Switzerland from 20–30 m depth [102], very much resembles the slender morph of *R. auricularia* from Lake Baikal (see Supplementary Figure S3). A similar conchological morph evolved within deep-water populations of "*Limnaea limosa*" (most probably a synonym of *Radix balthica*) (see [97] for review). It gives us reason to link the formation of the slender morph of *R. auricularia* in Lake Baikal with a need to adapt to survive at depths exceeding 1–2 m, though we still lack any direct evidence for this assumption. It would be no surprise, although, to find parallel phenotypic changes in *R. auricularia* adapting to live in deep lakes located in separate and remote areas of Eurasia. Convergent shell evolution in aquatic pulmonates has many times been described in phylogenetically distant taxa belonging to this group (see, for instance, [104–106]).

The morphological variability among *R. auricularia* in Lake Baikal is unexpectedly very high and evidently exceeds what is typical for this snail in the waterbodies of Northern Eurasia. Some specimens analysed by molecular genetics show shell morphology, pigmentation of the mantle, head, and foot, ratio between lengths of preputium and penis sheath, length of the bursa duct, and position of the bursa that hitherto were not considered characteristic of this species.

One may hypothesize that we are observing a case of adaptive phenotypic plasticity, which often accompanies biological invasions by plant and animal species (see, for example, [107–109] and references therein). Following Lande [108], it would be reasonable to interpret this increased variability in Baikalian *R. auricularia* as "a rapid initial increase in plasticity which accelerates the evolution of a new optimal phenotype, followed by slow genetic assimilation of the new phenotype and reduction of plasticity". The predictions of this model correspond to the very young age of *R. auricularia* populations in the open Baikal, and we may expect the degree of phenotypic plasticity will decrease with time.

We must admit, though, that we lack information on if the plasticity found in our study is truly adaptive. As Stanley [110] once observed, the adaptive nature of shell shape in gastropods, whose shells are generally much less intimately associated with the substratum than those of bivalves, is not easy to determine. We are unable to fix the origin of the observed variations in shell habitus—we cannot answer whether they are genetic or ecophenotypic in nature.

The ecological observations made by one of the authors of this paper allow us to suggest that the open Baikal populations of *R. auricularia* possess some physiological and even behavioural adaptations for surviving in this specific environment. Snails of these populations live in cold water (summer temperatures +6 . . . +14 °C) and, being placed in water at room temperature, they die within 3–5 days. When the same snails were kept at a water temperature of +6 °C, they lived in aquaria for 6 months (T. Sitnikova, unpublished data). In shallow waterbodies and the littoral–sor zone, the ear pond snails lay egg masses

on coastal stones and leaves of aquatic vegetation. In the open littoral zone, they are unable to rise to the water's surface, impeded by waves during storms, and thus lay eggs on a stony substrate at depths of 3–5 m (T. Sitnikova, unpublished data).

These peculiarities find a parallel in considerable genetic distance separating snails taken from adjacent localities. For example, individuals of *R. auricularia* collected from a small pool in Bolshiye Koty settlement (population RaB 14 on the map in Figure 1) and from the open littoral off the settlement (Zhilishche; populations RaB 20-21) are separated by a distance of only 700 m. However, the genetic distance between them (by ITS-2 marker) exceeds that between RaB 14 from Baikal and *Radix narzykulovi* from the Pamir Mountains. Possibly, there is some physical barrier effectively preventing gene flow between snails in the shallow zone of Lake Baikal and the open littoral.

5. Conclusions

Our results cannot validate the delineation of *R. intercisa* as a distinct lymnaeid species of the genus *Radix*. One can hypothesize that, most probably, *Radix auricularia* have invaded Lake Baikal several times and from different water basins, and its population is possibly of polyphyletic origin. Taking into account the potential ability of radicine lymnaeid snails to colonize deep lakes, we may expect that the range of the ear pond snail in Lake Baikal will expand in the future, and the species will probably occupy deeper parts of the waterbody. This is of special interest since the introduction of such an ecologically plastic species of herbivorous snails may lead to the decline of the unique species of Baikalian snails or even to their complete competitive displacement.

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