

## On the genetic diversity and protoconch variability of snails of the genus *Caspiohydrobia* Starobogatov, 1970 (Caenogastropoda: Hydrobiidae)

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**ABSTRACT.** The genus *Caspiohydrobia* belongs to the less-known taxa of the family Hydrobiidae. According to a conchologically-based system, developed in the 1970-1980s by Yaroslav I. Starobogatov and his co-authors, the genus includes around 30 living species. However, the most recent data based on molecular evidence indicate that most (if not all) of these morpho-species should be considered junior synonyms of a widely distributed species, *Ecrobia grimmi*. However, the vast majority of nominal species described in the genus *Caspiohydrobia* have never been reassessed by molecular taxonomic methods. In this work, the genetic diversity of *Caspiohydrobia* spp. was studied based on a large sample taken in Lake Elanach in the Kurgan region (Trans-Urals, Russia). Conchological analysis made it possible to identify 7 nominal species of the genus *Caspiohydrobia* in the sample, whereas genetically all the studied individuals were the same, sharing a single COI haplotype. A phylogenetic tree of *Ecrobia* spp. has shown that all studied genetically specimens of *Caspiohydrobia* (including newly sequenced ones) forms a separate cluster on a cladogram, which can be identified as *Ecrobia grimmi* s. lato. However, a higher genetic diversity was revealed in a population of *Caspiohydrobia* spp., from a lake adjacent to the Caspian Sea (West Kazakhstan). Distinct morphotypes in the structure of the protoconch were found in samples of *Caspiohydrobia* spp. from the Caspian and Aral Seas. Although the characteristics of the protoconch (size, sculpture) do not give grounds to distinguish 'good' species within the group, the genetic and morphological diversity described in this work makes it possible to assume the ongoing microevolutionary processes within the discussed group and, possibly, the existence of young (incipient) species in Central Asia. Based on the study of the morphology of the protoconch, it has been suggested that the development of mollusks of the genus *Caspiohydrobia* (= *Ecrobia*, partim) can include the free-swimming larval stage; this hypothesis is, however, not supported by other evidence and needs to be carefully verified.

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О генетическом разнообразии и изменчивости протоконха моллюсков рода *Caspiohydrobia* Starobogatov, 1970 (Caenogastropoda: Hydrobiidae)

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**РЕЗЮМЕ.** Род *Caspiohydrobia* относится к числу слабоизученных таксонов семейства Hydrobiidae. Согласно конхологической системе, разработанной в 1970-1980-х годах Я.И. Старобогатовым и его соавторами, род включает около 30 ныне живущих видов. Однако новейшие данные, основанные на результатах молекулярных исследований, указывают на то, что подавляющее большинство этих (морфо-) видов следует считать младшими синонимами широко распространенного вида *Ecrobia grimmi*. Однако при этом большая часть номинальных видов, описанных в составе рода *Caspiohydrobia*, никогда не изучалась молекулярно-таксономическими методами. В этой работе рассматривается генетическое разнообразие *Caspiohydrobia* spp. на основе изучения большой выборки, взятой в озере Еланач в Курганской области (Зауралье, Россия). Конхологический анализ позволил идентифицировать в выборке 7 номинальных видов рода *Caspiohydrobia*, в то время как генетически все изученные особи оказались идентичными, имеющими один гаплотип COI. Филогенетическое древо *Ecrobia* spp. показывает, что все изученные генетически особи *Caspiohydrobia* (включая секвенированные в ходе данного исследования) образуют отдельный кластер на кладограмме, который может быть обозначен как *Ecrobia grimmi* s. lato. Однако высокое генетическое разнообразие было выявлено в популяции *Caspiohydrobia* spp., обитающей на территории Западного Казахстана в одном из озер, прилегающих к Каспийскому морю. Обособленные морфотипы в строении протоконха были обнаружены в выборках *Caspiohydrobia* spp. из Каспийского и Аральского морей. Хотя характеристики протоконха (размер, скульптура) не дают оснований выделять «хорошие» виды внутри данной группы, генетическое и морфологическое разнообразие, описанное в этой работе, позволяет предположить протекание микроэволюционных процессов внутри обсуждаемого рода и, возможно, существование молодых (зарождающихся) видов в Центральной Азии. На основании изучения морфологии протоконха было высказано предположение, что развитие моллюсков рода *Caspiohydrobia* (= *Ecrobia*, partim) может включать стадию свободноплавающей личинки; однако эта гипотеза пока не подтверждена другими доказательствами и нуждается в тщательной проверке.

variability of some representatives of the hydrobiid genus *Caspiohydrobia* Starobogatov, 1970, which is considered a relic of the Ponto-Caspian brackish-water malacofauna. The species of *Caspiohydrobia* are widely distributed in Central Asia, south to Iraq [found in the Sawa Lake; Haase *et al.*, 2010], and are members of the faunas of the Aral and Caspian seas. The available information on taxonomy, distribution, and ecology of extant species of *Caspiohydrobia* was recently summarized by Andreeva *et al.* [2022], who published an annotated and illustrated list of all living nominal species included in the genus. Since its description in 1970 [Starobogatov, 1970], both the volume and the species content of *Caspiohydrobia* were a topic of hot debates. There are two extreme points of view. According to the Soviet/Russian authors of Starobogatov's school, *Caspiohydrobia* is a 'good' genus, containing around 30 recent species [see reviews in Kantor, Sysoev, 2006; Vinarski, Kantor, 2016; Andreeva *et al.*, 2022]. The differences between these species are, however, rather minor, and they have usually been determined using a conchological (typological) approach [Logvinenko, Starobogatov, 1969; Starobogatov, Izzatullaev, 1974; Starobogatov, Andreeva, 1981; Frolova, 1984; Andreeva, Frolova, 1989; Andreeva *et al.*, 2020]. An opposite approach, based on an ample use of molecular genetic data, reveals that only one species, *Ecrobia grimmi* (Clessin, 1887), is distributed in Central Asia, and that most (if not all) nominal species described within *Caspiohydrobia* could be junior synonyms of the former. In this case, the very genus *Caspiohydrobia* should be synonymized with *Ecrobia* [Vandendorpe *et al.*, 2019; Wesselingh *et al.*, 2019]. The lack of substantial differences in the anatomical and radular characters among various species of *Caspiohydrobia* [Sitnikova *et al.*, 1992; Filippov, Riedel, 2009; Anistratenko, 2013; Andreeva *et al.*, 2022] additionally support this hypothesis.

On the other hand, most authors, discussing the taxonomy and species content of *Caspiohydrobia*, agreed that the available data, including the molecular one, are too scarce to judge decidedly on these topics [Wesselingh *et al.*, 2019; Andreeva *et al.*, 2022]. In particular, we still have no genetic (and sometimes anatomical) information on a few nominal species described from Tajikistan and assigned to this genus. The most recent publications, dealing with the molecular systematics of Central Asian hydrobiid snails [e.g. Vandendorpe *et al.*, 2019], follow a rather simple taxonomic approach, when all analyzed specimens from this region are identified as *Ecrobia grimmi*, without attempts to revise particular nominal species classified within *Caspiohydrobia*.

Here we report the results of our recent research on the conchological and genetical variability of selected species of the genus. Special attention was paid to the characteristics of the protoconchs, which have

## Introduction

The main goal of this communication is to provide new information on genetic and morphological

been recognized as a useful tool for species delimitation and identification in various groups of Caenogastropoda [Nützel, 2014], including hydrobiids [see, for example, Ponder *et al.*, 1999; Anistratenko, 2013; Vinarski *et al.*, 2014; Anistratenko *et al.*, 2021]. The variability of the protoconch microsculpture in the representatives of *Caspiohydrobia* (or *Ecrobia*) inhabiting inland waterbodies remains poorly investigated, except for *C. subconvexa* (Logvinenko et Starobogatov, 1969) from the Caspian Sea and *C. pavlovskii* Starobogatov et Izzatullaev, 1974 from the Aral Sea, which differed in the visibility of the boundary between the non-spiral part (= initial whorl) and smooth parts [Andreeva *et al.*, 2022].

We hope that the data provided in this publication will be helpful for future researchers that, possibly, may have a more extensive set of sequenced taxa and specimens at their disposal. One of the key points of this research is that we managed to obtain molecular information on some nominal species of *Caspiohydrobia*, identified morphologically by Svetlana Andreeva, the author who described (together with Ya.I. Starobogatov and E.S. Frolova) more than ten species in this genus [Starobogatov, Andreeva, 1981; Andreeva, Frolova, 1989]. A focus on a study of sympatric (and syntopic) samples of allegedly distinct species should provide a more reliable inference on taxonomy within the genus *Caspiohydrobia*.

## Material and methods

During this research, we studied samples of various *Caspiohydrobia* species kept in collections of the Zoological Institute of the Russian Academy of Sciences, St.-Petersburg (ZIN); the Laboratory of Macroecology & Biogeography of Invertebrates of the St.-Petersburg State University (LMBI), and the Limnological Institute of the Siberian Branch of the Russian Academy of Science, Irkutsk (LIN). The newly obtained fixed specimens used in molecular genetic analysis were collected by E.S. Babushkin and M.V. Vinarski in July of 2022, in saline Lake Elanach, situated in Kurgan Region of Russia (South Transuralia; 55.179015 N, 67.348671 E). All specimens from the lake were collected from a small area of less than 10 m<sup>2</sup> by means of a sieve. The snails were found on submerged plants or dredged from the bottom substrate. A part of this sample was studied by S. Andreeva, who identified the specimens based on a conchological approach. Later, these specimens were given to LIN for a molecular study. Another part of the Elanach Lake samples was transferred to LIN without identification of particular specimens, which were labeled as *Caspiohydrobia* sp. (= *Ecrobia grimmi* s. lato). The rest of specimens (nearly 100 fixed individuals in total) are deposited in the LMBI collection.

Both identified and not identified snails were

used in the molecular analysis, which allowed us to compare the two approaches to species delimitation within *Caspiohydrobia*, i.e. these based on the ‘strict’ and the ‘broad’ species concepts.

DNA was extracted from the foot muscle tissue according to a protocol described by Sokolov [2000]. Gene fragments of the mitochondrial cytochrome c oxidase subunit I (COI) were amplified in PCR using the primers universal for invertebrates [Folmer *et al.*, 1994]. Amplification conditions were as follows: denaturation at 94°C for 5 min, 30 cycles at 94°C for 30 sec, 50°C for 45 sec, 72°C for 2 min, and a final elongation step at 72°C for 10 min. The amplicons were visualized on 1% agarose gel. The visible bands with amplicons of expected size were excised and cleaned up according to Maniatis *et al.* [1982]. The COI sequences were verified manually and aligned using BIOEDIT v.7.2.5 [Hall, 1999]. Sequencing was performed in Research and Production Company “SYNTOL”, Moscow, Russia.

Pairwise *p*-distances (%) between COI sequences were calculated using Mega 11 [Tamura *et al.*, 2021]. The haplotype network was constructed with TCS v. 1.2.1 [Clement *et al.*, 2000]. The threshold value of the statistical parsimony algorithm, defining the maximal number of mutational connections between pairs of haplotypes within the same network, was set to 0.95. Additionally, 13 COI sequences of *Ecrobia grimmi* were retrieved from GenBank for analysis (Table 1). The phylogenetic hypothesis of the probable relationships between *E. grimmi* s. lato (= *Caspiohydrobia* spp.) and other species of the genus *Ecrobia* was tested using a set of 44 sequences belonging to four species obtained from GenBank (see Table 1).

A Maximum Likelihood (ML) phylogenetic hypothesis was inferred using IQ-TREE v.1.6.8 [Nguyen *et al.*, 2015] and midpoint rooted. The most suitable model of molecular evolution was chosen using the Model Finder module within IQ-TREE [Kalyaanamoorthy *et al.*, 2017]. Branch support was assessed using bootstrap values [Schrempf *et al.*, 2019] and the Shimodaira–Hasegawa approximate likelihood ratio test (SHaLRT) [Anisimova *et al.*, 2011].

We examined samples of *Caspiohydrobia* spp. of three lakes of Central Asia and the southern part of Western Siberia taken from the collections of ZIN and LMBI: Mnogosopochnoye Lake (North Kazakhstan, Akmola Region, 52.867 N, 69.703 E); Salamatka Lake (Russia, Transuralia, Chelyabinsk Region, 55.221 T, 62.023 E.); Sargul’ Lake of the Chany Lake system (Baraba steppe, the southern part of Western Siberia; 54.595 N, 78.853 E). The studied shells originate from the Holocene deposits and belong to an extinct population [see Gusskov *et al.*, 2008 for details]. No living *Caspiohydrobia* are known from the Lake Chany area now.

Table 1. The sequences used for phylogenetic analyses, with their GenBank accession numbers and references.

Табл. 1. Перечень использованных для филогенетического анализа сиквенсов, с указанием соответствующих номеров Генбанка и ссылками на литературу.

Species name	Accession number	Locality	Reference
<i>Ecrobia grimmi</i>	MN167715, MN167716, MN167717, MN167718, MN167719	Kazakhstan: near Karakol Lake	Vandendorpe <i>et al.</i> , 2019
	GQ505913, GQ505912	Iraq: brackish Lake Sawa	Haase <i>et al.</i> , 2010
	MN167725	Russia: Daghestan, Sulak Bay of the Caspian Sea	Vandendorpe <i>et al.</i> , 2019
	MN167724, MN167723, MN167722, MN167721, MN167720	Russia: Chelyabinsk Region, Salamatka Lake	
<i>Ecrobia maritima</i>	MN167735, MN167734	Ukraine: Utlyukskiy Liman	Vandendorpe <i>et al.</i> , 2019
	MN167733	Ukraine: Odessa, Hydrological Station	
	MN167732	Ukraine: Molochnyi Liman	
	MN167731	Romania: Mangalia	
	MN167730	Italy: Po Estuary	
	MN167728	Greece: Kimi Aliveri	
	MN167726	Bulgaria: Rapotamo River Estuary	
	AY616139	Russia: Crimea, Sevastopol, Kruglaja Buchta	Kevrekidis <i>et al.</i> , 2005
	AY616140	Greece: Evros Delta, Monolimi Lagoon	
	KJ406199	Greece: Ahivadolimni at Milos Island, Cyclades	Szarowska, Falniowski, 2014
<i>Ecrobia truncata</i>	KF643431, KF643450, KF643453, KF643866, KF643941	Canada: Prince Edward Island, Malpeque	Layton <i>et al.</i> , 2014
	MN167741	USA: Damariscotta River	Vandendorpe <i>et al.</i> , 2019
	MN167740	USA: Menemsha Pond	
	MN167743	USA: Flax Pond	
	MN167742	USA: Duck Creek	
	AF449217	USA: New Jersey, Stone Harbor	Wilke, 2003
	AY616137	Tunisia: Tunis, Lac de Tunis	Wilke, 2016, unpublished
	KJ406201, KJ406202	Greece: brackish lake W of Kato Xirokhorion, S of Argos, W Peloponnisos, Ionian Sea	Szarowska, Falniowski, 2014
	AF118325	Mediterranean Sea, Orbetello Lagoon	Wilke, Davis, 2000
	AF118333	Great Britain: North Sea, Snetisham Lagoon	
	AF118341	Iceland: North Atlantic, Galgahraun, Alftanrs	
	AF118357, AF118358	Denmark: Kattegat, Odense Fjord	
	AF118369	Germany: Baltic Sea, Boiensdorfer Werder	
<i>Ecrobia ventrosa</i>	MN167748	Croatia, small bay of the sea	Vandendorpe <i>et al.</i> , 2019
	MN167749	France, Etang de Villepey	
	MN167750	France, canal near Etang de Gines	
	MN167751	Greece, spring east of Itea	
	MN167755	Italy, Saline Ettore Inversa	
	MN167756	Spain, near Punta Umbria	
	MN167757	Spain, mouth of Torrent de la Borges	
	MN167758	Spain, Sa Albufereta	
	MN167760	Spain, within the Port d'Alcúdia	
	MN167761	Tunisia, southern bank of Lac de Tunis	
MN167762	Tunisia, north of Temime		
<i>Ecrobia spatatiana</i>	MN167736	Croatia, Pontana Spring	Vandendorpe <i>et al.</i> , 2019
	MN167737	Croatia, Pirovac Spring	
	MN167738	Croatia, Krka River Estuary	
<i>Caspiohydrobia</i> spp.	PP190379–PP190388 (9 original sequences)	Russia, Kurgan Region, Lake Elanach	This study
<i>C. sidorovi</i>	PP190389–PP190392 (4 original sequences)		
<i>C. aralensis</i>	PP190393–PP190396 (4 original sequences)		
<i>C. kazakhstanica</i>	PP190398		
<i>C. borealis</i>	PP190399		
<i>C. johanseni</i>	PP190400–PP190401 (2 original sequences)		
<i>C. chrysopsis</i>	PP190402		

Specimens of various species of *Caspiohydrobia* from the Aral and Caspian seas were examined as well.

Scanning electron microscopy (SEM) photos of the protoconchs were taken at the Limnological Institute of RAS by means of a Quanta 200 microscope (Thermo Fischer Scientific Inc., USA). For visualization of the protoconchs young specimens with preserved shell apex were selected, since in adult shells, as a rule, the protoconch periostracum is partially or completely destroyed. The measurement of the protoconchs was made following the protocol of Filippov and Riedel [2009], with the use of Image-Pro Plus 6.0 for Windows. The macrophotographs of shells were taken at ZIN (Core Facilities Centre "Taxon") by means of a Nikon SMZ 25 stereomicroscope with a mounted Nikon DS-Ri2 digital camera.

Here, the approach of Hershler and Ponder [1998] and Nützel [2014], who distinguished two parts of a protoconch, designated as protoconch I and protoconch II, is followed. Protoconch I represents the embryonic shell built within the egg prior to hatching, whereas protoconch II is built during the larval phase of a gastropod species with planktotrophic larval development [Nützel, 2014]. An example of this is the planktotrophic species *Peringia ulvae* (Pennant, 1777), whose protoconch microsculpture was described in previous works [Bandel, 1975; Fish, Fish, 1977]. In all non-planktotrophic taxa of hydrobiids, the protoconch forms a single entity, which is homologous with the protoconch I of planktotrophic species [Hershler, Ponder, 1998].

For comparison, the protoconchs of two other hydrobiid species, the direct-developing *Ecrobia ventrosa* (Montagu, 1803) and *Peringia ulvae* with planktotrophic larvae, were studied. We use specimens of these species from ZIN collection. *E. ventrosa* – No. 5 in systematic catalogue; locality: Germany, Mecklenburg. 1914. Leg. Steusloff. *P. ulvae* – No. 14 in systematic catalogue; locality: Germany, North Sea, near the Norderney island. 1846. Leg. Philippi.

The taxonomy used in this paper generally follows that of Vinarski and Kantor [2016] and Andreeva *et al.* [2022].

## Results

In a sample of snails from Lake Elanach, specimens belonging to seven nominal species of *Caspiohydrobia* were identified morphologically. These are: *Caspiohydrobia aralensis* Starobogatov et Andreeva, 1981; *C. borealis* Andreeva et Frolova, 1989; *C. chrysoptis* (Kolesnikov, 1947); *C. johanseni* Frolova, 1984; *C. kazakhstanica* Starobogatov et Andreeva, 1981; *C. oviformis* (Logvinenko et Starobogatov, 1969), and *C. sidorovi* Starobogatov et Andreeva, 1981. Specimens of all of them, except

Table 2. A COI-based matrix of pairwise genetic distances (*p*-distances, %) among species of *Ecrobia* (including specimens from Lake Elanach identified as *Caspiohydrobia* spp.)

Табл. 2. Основная на последовательностях гена COI матрица парных генетических расстояний (*p*-расстояния, %) между видами *Ecrobia* (включая особей из оз. Еланач, обозначенных как *Caspiohydrobia* spp.)

	<i>E. grimmi</i>	<i>E. maritima</i>	<i>E. truncata</i>	<i>E. ventrosa</i>	<i>E. spalaitiana</i>	<i>Caspiohydrobia</i> sp.
<i>Ecrobia grimmi</i>	<b>0.691</b> <b>(0.157-0.942)</b>					
<i>E. maritima</i>	3.564	<b>1.469</b> <b>(0.157-2.198)</b>				
<i>E. truncata</i>	4.724	4.859	<b>0.546</b> <b>(0.157-1.413)</b>			
<i>E. ventrosa</i>	5.565	5.436	5.253	<b>0.943</b> <b>(0.314-2.198)</b>		
<i>E. spalaitiana</i>	6.000	6.274	6.035	3.440	<b>1.151</b> <b>(0.157-1.727)</b>	
<i>Caspiohydrobia</i> sp.	0.366	3.281	4.467	5.385	5.965	<b>0.000</b>

for *C. oviformis* (represented by empty shells), were sequenced during this study (Fig. 1). In total, 24 new COI sequences (637 base pairs length) were obtained (see Table 1).

Analysis of the matrix of pairwise genetic distances among species of *Ecrobia* (Table 2) showed that all obtained sequences belong to the species *E. grimmi* (genetic distance 0–0.366% substitutions). The 24 sequenced individuals of Lake Elanach shared the same haplotype, which is unique, i.e. not represented in samples from other localities, available from GenBank (Fig. 2). An analogous situation was

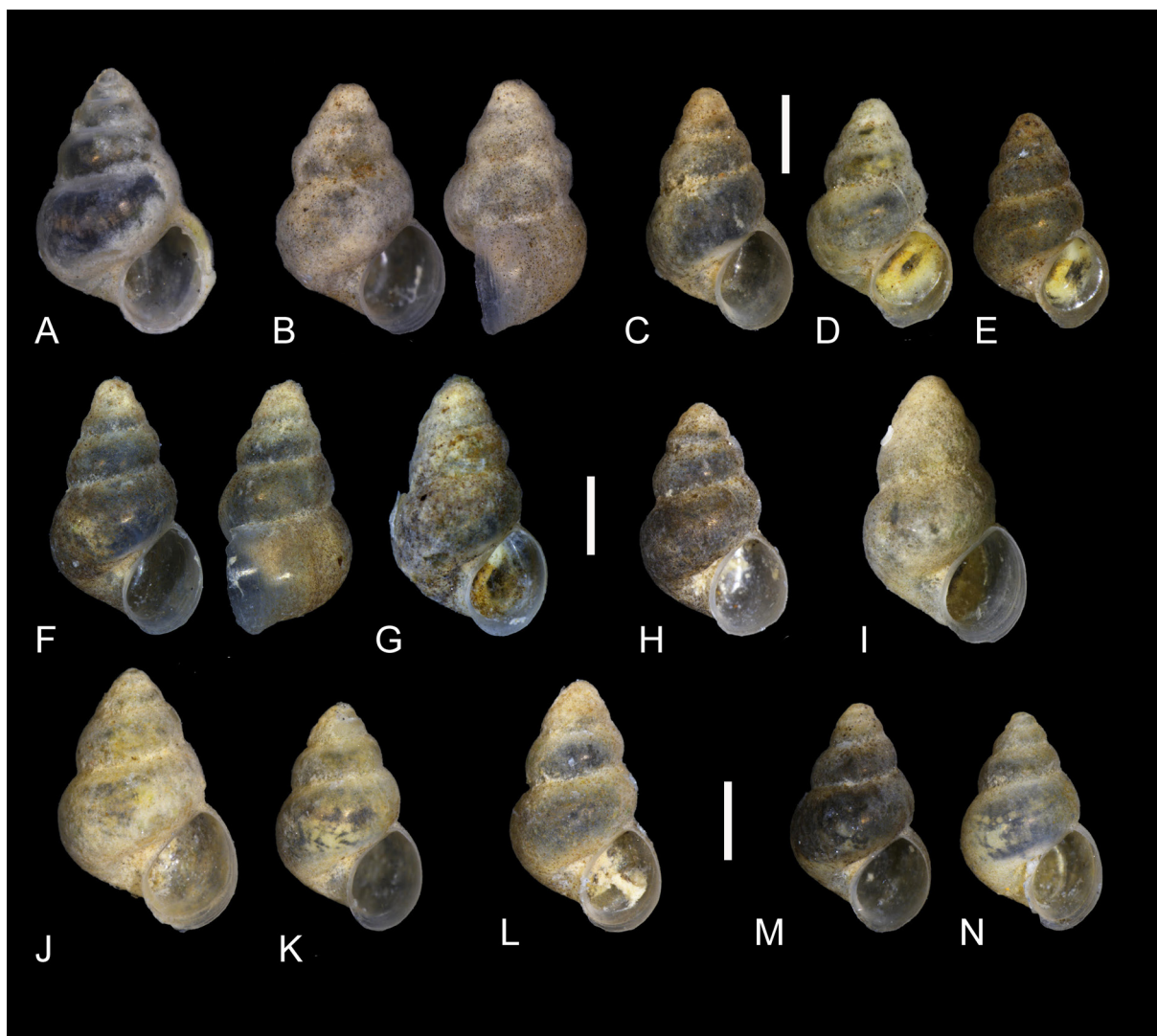
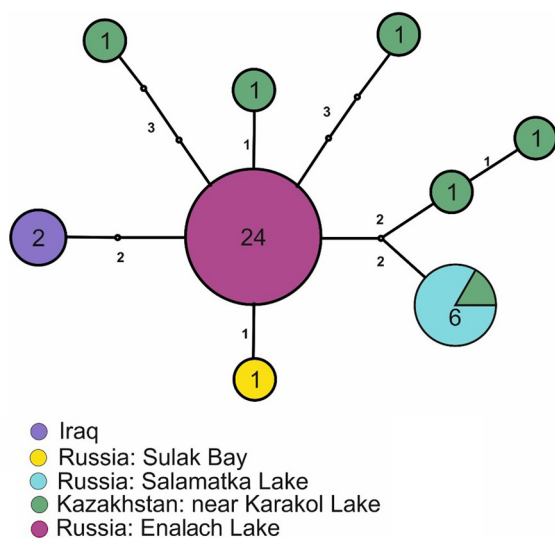


FIG 1. Shells of sequenced specimens of *Caspiohydrobia* spp. from Lake Elanach, Kurgan Region, Russia. Species identification made by S. Andreeva. A, B. *Caspiohydrobia kazakhstanica* (females). C-E. *C. aralensis*. F, G. *C. johanseni* (males). H, I. *C. cf. aralensis*. J, K. *C. borealis*. L. *C. chrysopsis*. M, N. *C. sidorovi* (females). Scale bars 1 mm.

РИС. 1. Раковины секвенированных экземпляров *Caspiohydrobia* spp. из озера Еланач, Курганская область, Россия. Идентификация видов выполнена С. Андреевой. А, В. *Caspiohydrobia kazakhstanica* (самки). С-Е. *C. aralensis*. F, G. *C. johanseni* (самцы). H, I. *C. cf. aralensis*. J, K. *C. borealis*. L. *C. chrysopsis*. M, N. *C. sidorovi* (самки). Масштабная линейка 1 мм.



revealed in most of other localities of *Caspiohydrobia* snails, where all specimens collected from the same habitat shared a common and locality-specific haplotype. This was true for *Caspiohydrobia* living in Lake Salamatka (Russia, Trans-Urals, Chelyabinsk Region), Sulak Lagoon (Russia, Dagestan), and Lake

FIG 2. A COI-based haplotype network of *Caspiohydrobia* spp. Numbers above/below the lines indicate the number of mutational steps between the nearest haplotypes. Numbers inside the circles denote the number of nucleotide sequences included in them.

РИС. 2. Основанная на последовательностях гена COI сеть гаплотипов *Caspiohydrobia* spp. Цифры над/под линиями указывают количество мутационных замен между ближайшими гаплотипами. Цифры внутри кружков обозначают количество включенных в них нуклеотидных последовательностей.

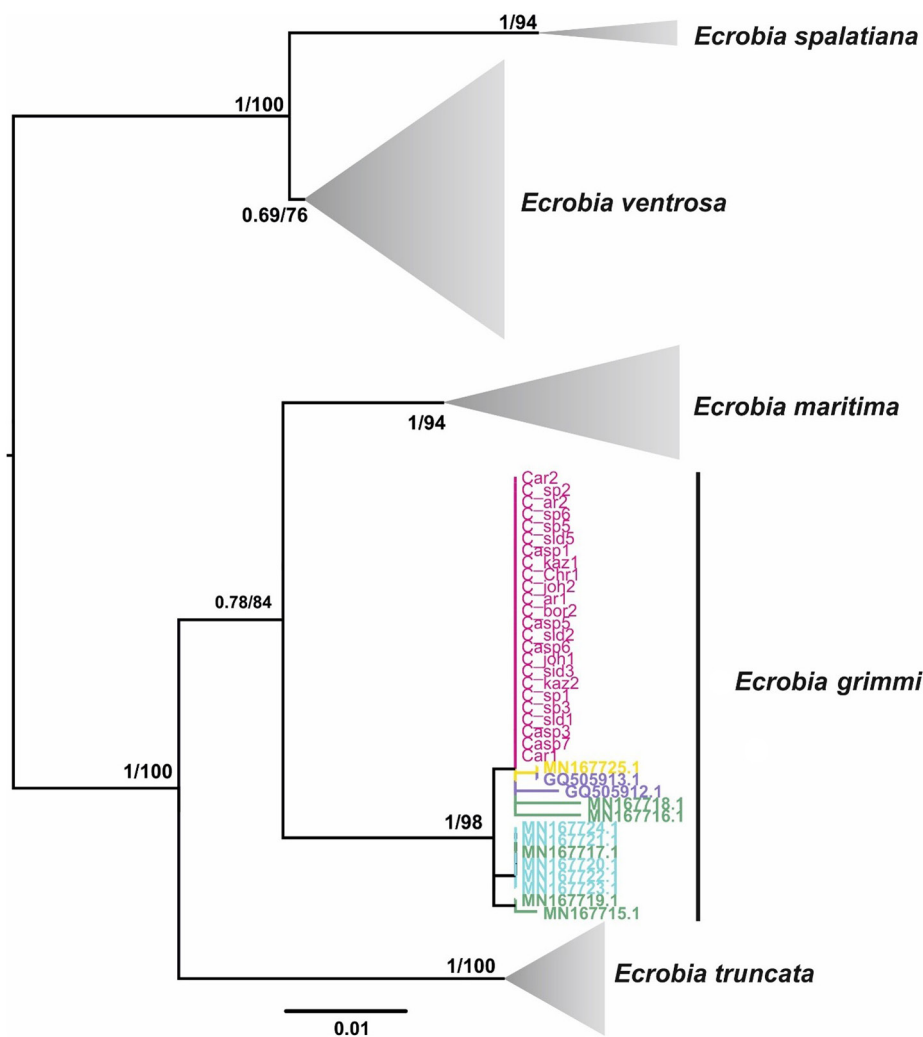


FIG 3. Collapsed *Ecrobia* ML phylogenetic tree based on COI nucleotide sequences with SH-aLRT/bootstrap supports. The colors were inherited from Fig. 2. Abbreviations: Casp – *Caspiohydrobia* sp.; C\_ar – *Caspiohydrobia aralensis*; C\_bor – *C. borealis*; C\_chr – *C. chrysopsis*; C\_joh – *C. johanseni*; C\_kaz – *C. kazakhstanica*; C\_sid – *C. sidorovi*.

РИС. 3. Свернутое филогенетическое дерево *Ecrobia*, полученное методом максимального правдоподобия (ML) с использованием нуклеотидных последовательностей COI с поддержками SH-aLRT/bootstrap. Цвета соответствуют цветам на рис. 2. Сокращения: Casp – *Caspiohydrobia* sp.; C\_ar – *Caspiohydrobia aralensis*; C\_bor – *C. borealis*; C\_chr – *C. chrysopsis*; C\_joh – *C. johanseni*; C\_kaz – *C. kazakhstanica*; C\_sid – *C. sidorovi*.

Sawa (Iraq). The highest genetic variability was recorded in a sample from Lake Karakol (Kazakhstan), where six distinct haplotypes were detected (five are unique; one is found also in Lake Salamatka). This result shows that the high conchological variability among snails collected from Lake Elanach does not correspond to a comparable genetic diversity. The six nominal species identified in this sample do not form separate clades in a cladogram; the specimens, not identified prior to sequencing and labelled as *Caspiohydrobia* sp., on the phylogenetic tree formed a mixed cluster with other specimens, whose species identity was determined (Fig. 3).

Table 3 summarizes the differences among shell protoconchs in species of brackish-water Hydrobiidae

studied by us. The protoconchs of snails from different – and geographically distant – localities are illustrated in Fig. 4.

Elanach Lake. We studied the protoconchs of the three nominal species identified in this sample – *C. borealis*, *C. chrysopsis*, and *C. kazakhstanica*, and found no differences between them (Fig. 4, A, Table 3).

Mnogosopchnoye Lake. Two shells (*C. aralensis*, *C. sidorovi*) were examined. Being of nearly the same whorl number (~ 6.0–6.5), they differ in size (shell size 3.8 and 2.8 mm, respectively), shape of whorls (convex in *C. aralensis*, oval flattened with thin suture edge in *C. sidorovi*), the presence of open umbilicus due to lowered last whorl (*aralensis*) or

Table 3. A comparison of protoconchal sculpture among the studied species / localities.

Табл. 3. Сравнение скульптуры протоконов у исследованных видов / из разных локалитетов.

Species / locality	The border between the non-spiral and smooth parts of the protoconch	Non-spiral part sculpture			Width of the non-spiral part, $\mu\text{m}$ (whorl number)	Protoconch I + protoconch II diameter, $\mu\text{m}$ (whorl number)
		Regular/Irregular	Size of the wrinkles and granules	Spiral lines or threads		
<i>Peringia ulvae</i>	Distinct, with 2-3 transverse folds	Irregular wrinkles and granules with a uniform net	Flattened small	A short longitudinal line (weak thickening)	150 (0.8)	277 (1.5)
<i>Ecrobia ventrosa</i>	Distinct, without thickenings and transverse folds	Regular	Of similar size	A single short line	180 (0.8)	275 (1.25)
<i>E. grimmeri</i> . The Caspian morphotype I	Distinct, with 2-3 transverse folds	Irregular	Of various size	1-2 long lines		
The Caspian morphotype II	Smoothed	Irregular	Of various size	Absent		
The Aral Sea morphotype I	Distinct, without folds	Very irregular, patchy	Of various size	Absent	172 (0.80)	232 (1.25)
The Aral Sea morphotype II	Unclear, smoothed	Irregular	Of similar size	1-2 of different length, one passing to smooth part	239 (0.8)	310 (1.25)
Lake Elanach ( <i>C. borealis</i> , <i>C. chrysopsis</i> , <i>C. kazakhstanica</i> )	Distinct, with 2-3 folds	Irregular, with smooth "spots"	Of various size	Short, in the form of threads and smooth strips	183-232 (0.8)	277-330 (1.25-1.50)
Lake Salamatka ( <i>C. oviformis</i> )	Distinct, with a few transverse thickenings	Very irregular, with "spots" of net	Of varying sizes, predominance of larger ones	Absent	191 (0.8)	283 (1.25)
Lake Mnogosopchnoye ( <i>C. aralensis</i> , <i>C. sidorovi</i> )	Distinct, without thickenings	Almost regular	Of similar size	Present	172, 180 (0.8)	260, 284 (1.25, 1.35)
Lake Sargul' ( <i>C. confiformis</i> )	Smoothed	Almost regular	Of similar size	Absent	Not measured	264, 275 (1.45)



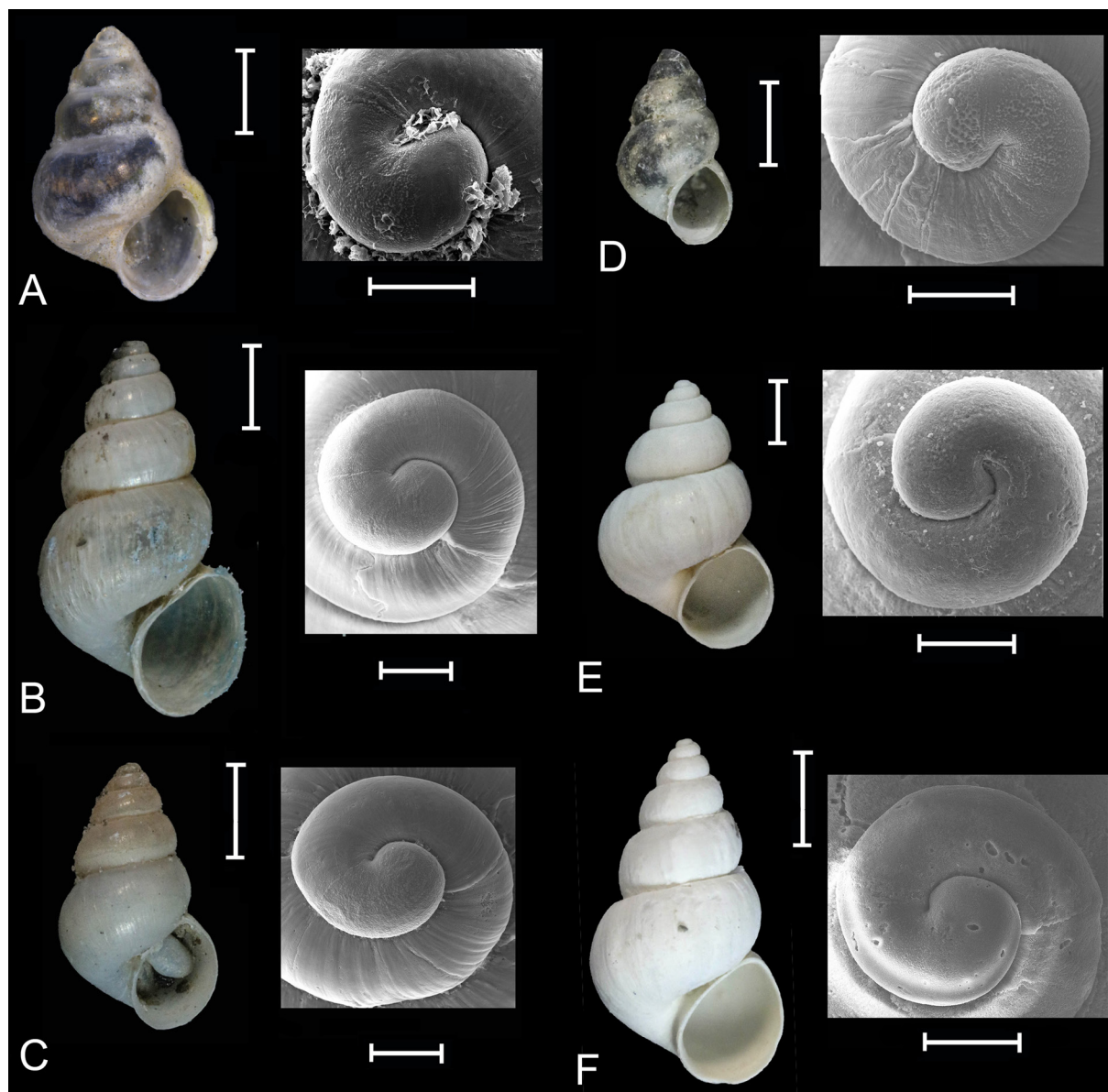


FIG 4. Shells and protoconchs of selected species of *Caspiohydrobia*. **A.** *C. kazakhstanica* (Lake Elanach). **B.** *C. aralensis* (Lake Mngosopochnoye, North Kazakhstan). **C.** *C. sidorovi* (Lake Mngosopochnoye). **D.** *C. oviformis* (Lake Salamatka). **E.** *C. coniformis* (South Siberia, Lake Sargul', the Chany Lake system, Holocene lacustrine deposits). **F.** *C. elongata* (from the same sample). Scale bars: 0.1 mm (protoconchs), 1.0 mm (shells).

РИС. 4. Раковины и протоконхи отдельных видов *Caspiohydrobia*. **A.** *C. kazakhstanica* (озеро Еланач). **B.** *C. aralensis* (озеро Многосопочное, Северный Казахстан). **C.** *C. sidorovi* (озеро Многосопочное). **D.** *C. oviformis* (озеро Саламатка). **E.** *C. coniformis* (Южная Сибирь, озеро Саргуль, Чановская озерная система, голоценовые озерные отложения). **F.** *C. elongata* (из той же пробы). Масштабные линейки: 0,1 мм (протоконхи), 1,0 мм (раковины).

without umbilicus (*sidorovi*). Their protoconchs, in turn, are identical (Fig. 4 B, C, Table 3).

Salamatka Lake. A single protoconch of a *C. oviformis* shell was studied (see Fig. 4 D), its teleoconch (5.5 whorls, shell height 2.4 mm) differs from shells of *Ecrobia grimmi* sensu lato by a curved outer lip of the aperture. The protoconch differs from other examined specimens in that the initial portion of the non-spiral part bears "spots" of large elevated wrinkles or granules with irregular edges. These "spots"

resemble net irregular sculpture. The initial smooth part has several transverse thickenings composed of small granules (of various shapes) and wrinkles (size in Table 3).

Sargul' Lake of the Chany Lake. The studied shells were of various size, shape, and whorl number; however, their protoconch morphology was virtually identical (Fig. 4 E, Table 3).

Aral Sea. We studied the protoconch morphology in six species. According to their characters,

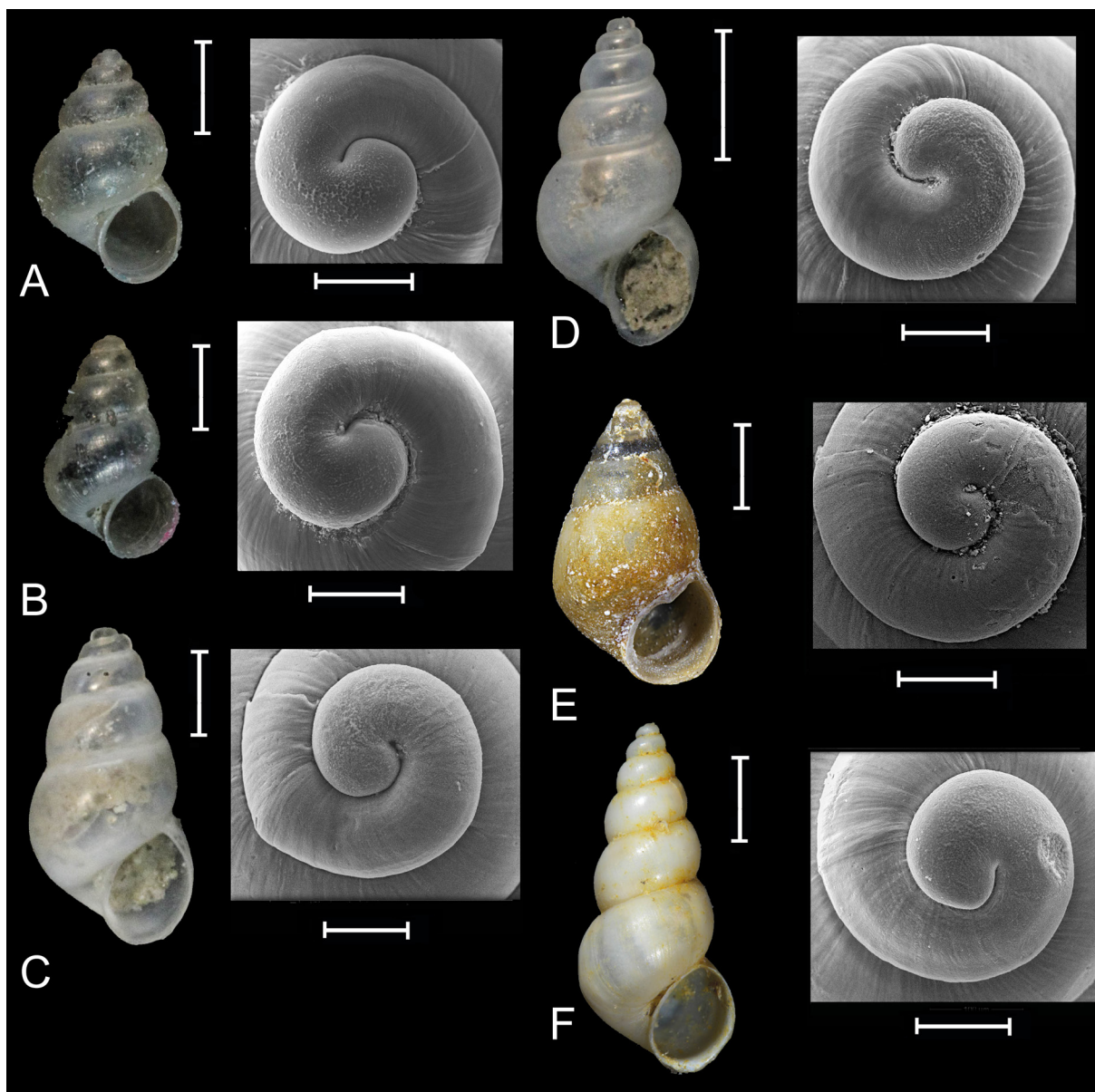


FIG 5. Shells and protoconchs of selected species of brackishwater hydrobiid snails. **A.** *Caspiohydrobia pavlovskii* (Aral Sea, Sary-Chaganak Bay). **B.** *C. sogdiana* (the same locality). **C.** *C. subconvexa* = Caspian morphotype 1. **D.** *C. conica* = Caspian morphotype 2. **E.** *Peringia ulvae* (North Sea, near the Norderney island). **F.** *Ecrobia ventrosa* (Germany, Mecklenburg). Scale bars: 0.1 mm (protoconchs), 1.0 mm (shells).

РИС. 5. Раковины и протоконхи отдельных видов солоноватоводных гидробиид. **A.** *Caspiohydrobia pavlovskii* (Аральское море, залив Сары-Чаганак). **B.** *C. sogdiana* (та же местность). **C.** *C. subconvexa* = Каспийский морфотип 1. **D.** *C. conica* = Каспийский морфотип 2. **E.** *Peringia ulvae* (Северное море, у острова Нордерней). **F.** *Ecrobia ventrosa* (Германия, Мекленбург). Масштабные линейки: 0,1 мм (протоконхи), 1,0 мм (раковины).

the studied species can be divided into two groups ("morphotypes") (Fig. 5, A, B). Morphotype I, represented by individuals identified as belonging to species *Caspiohydrobia pavlovskii* Starobogatov et Andreeva, 1981 and *C. parva* (Logvinenko et Starobogatov, 1969), is characterized by a distinct boundary between non-spiral and smooth parts. In the second group (*C. bergi* Starobogatov et Andreeva, 1981, *C. behningi* Starobogatov et Andreeva, 1981, *C. nikolskii* Starobogatov et Andreeva, 1981, and *C. sogdiana* Starobogatov et Izzatullaev, 1974), this

boundary is weakly or not traceable at all. Other characteristics see in Table 3.

Caspian Sea. In this waterbody, the presence of two more or less distinct protoconch morphotypes has been detected as well (see Fig. 5, C, D). The differences between them are given in Table 3. Morphotype I includes specimens identified as *C. grimmii* s. str., *C. subconvexa* (Logvinenko et Starobogatov, 1969), and *C. convexa* (Logvinenko et Starobogatov, 1966). The shells of the nominal species *C. turrita* (Logvinenko et Starobogatov, 1969), *C. cylindrica*

(Logvinenko et Starobogatov, 1969), and *C. conica* (Logvinenko et Starobogatov, 1969) belong to morphotype II.

The main difference between the protoconchs of *P. ulvae* and *E. ventrosa* is a visible border between protoconch I and II in *P. ulvae* (Fig. 5E) that is virtually absent in the latter species (Fig. 5 F, Table 3).

The sculpture of the non-spiral part of the examined protoconchs of different *Caspiohydrobia* varied significantly. However, the border between the non-spiral and smooth parts of the most protoconchs is similar to that in *E. ventrosa*, and the range of the width of the non-spiral part and the total protoconch diameter did not differ from that reported for *E. grimmi* [Filippov, Riedel, 2009].

## Discussion

The results presented above match the conclusions made by previous authors [Vandendorpe *et al.*, 2019; Wesselingh *et al.*, 2019; Andreeva *et al.*, 2022]. Neither genetic nor morphological data could reveal the existence of a species (or a group of species) in Central Asia, distinct from *Ecrobia grimmi* s. l. A molecular assessment of six nominal species of *Caspiohydrobia*, collected from Lake Elanach, has shown that they are genetically identical and share the same haplotype. The use of protoconch morphology for species discrimination was unsuccessful as well, which corresponds to the conclusions made earlier by Filippov and Riedel [2009].

Even though all *Caspiohydrobia* spp. sequences studied in our work are characterized by short genetic distances (haplotypes are separated by no more than three mutational steps), common haplotypes among snails collected in different geographic areas are nearly absent, indicating low gene flow and a high degree of isolation of individual populations. This type of haplotype connectivity in the network suggests the conservation of ancestral polymorphism with insignificant migration, as evidenced by the presence of geographically restricted unique haplotypes.

Unfortunately, the DNA sequences of almost all the representatives of this group from the Caspian Sea remain inaccessible to us. Similarly, the status of several nominal species of *Caspiohydrobia* described from the Aral Sea [Starobogatov, 1974; Starobogatov, Andreeva, 1981] and Tajikistan [Starobogatov, Izzatullaev, 1974] is also unclear. Possibly, these remote and well-isolated regions harbor endemic species other than *E. grimmi*.

Even the very limited data set analyzed in our work indicates the occurrence of microevolutionary processes in Central Asian populations of *E. grimmi*. The majority of the identified haplotypes are geographically specific, i.e. they are represented exclusively in a single ecosystem. The only exception is the haplotype from Lake Salamatka, located

in the Chelyabinsk region of Russia (coordinates 55.2111°N, 62.0253°E), which was also found in a sample from Lake Karakol in Kazakhstan. This break in the geographic specificity of the haplotype distribution may be due to long-distance dispersal by waterfowl, a scenario that has been used to explain the genetic diversity of *E. grimmi* [Haase *et al.*, 2010]. The distance between Salamatka and Karakol lakes is about 1460 km, which is about one and a half times the dispersal distance (800-900 km) reported by Haase *et al.* [2010]. Rather than a single long-distance dispersal event, we assume the presence of stepping stones in the form of brackish-water habitats between Salamatka and Karakol.

A special case is the snail population from the Lake Karakol area, represented in GenBank by six individuals, each of which has a specific haplotype. We did not have samples of mollusks from this lake at our disposal and did not study morphologically the individuals whose nucleotide sequences were used in the phylogenetic analysis. It should be noted, however, that this lake, located on the shore of the Caspian Sea in the Mangystau region of the Republic of Kazakhstan (coordinates 43.4677°N, 51.3106°E), was probably connected to the Caspian Sea in the recent past (it is possible that it is periodically connected to this day) and is inhabited, apparently, by Caspian malacofauna. The presence of two protoconch morphotypes in *Caspiohydrobia* from the Caspian Sea suggests it could harbor more than just a single species of *Caspiohydrobia* (= *Ecrobia*). The final solution of this question requires a morphological and genetic study of a freshly collected material from Lake Karakol and other lakes connecting with the Caspian Sea and application of a multilocus phylogeny approach.

Certain, though not very substantial, specific features in the protoconch structure found in some localities (e.g., in Lake Salamatka) may also indicate progressive differentiation of the populations inhabiting them. However, the genetic distances between individual populations of *E. grimmi* from Central Asia are markedly lower than the distances separating “good” species of this genus (Vandendorpe *et al.*, 2019). Thus, this single criterion does not allow us to confirm the species (or even subspecies) status of any of the studied populations.

The presence of the bipartite protoconchs in many of the studied species of *Caspiohydrobia* is a little problematic, since the planktotrophic development has not been reported for these taxa. When discussing the ecology of *Ecrobia maritima* (Milaschewitsch, 1916), Kevrekidis and Wilke [2005] suggested that this species is larviparous, based on an observation that its protoconch consists of a small initial whorl (protoconch I), ~175 µm in diameter, and a planktotrophic protoconch II comprising about 1.5 whorls. However, in the Aral Sea, *Caspiohydrobia*

sp. (or *Ecrobia grimmi* s. l.), characterized by direct development, also demonstrate a difference in the sculpture of the non-spiral part (= initial whorl) and the remaining, smooth surface of the protoconch, with high variability in the width of the non-spiral part and the total protoconch diameter [see also Filippov, Riedel, 2009]. The presence of a free-swimming planktonic larva in *Caspiohydrobia* would explain the current pattern of distribution of this group in Central Asia and neighboring regions. This territory was in the past covered by a large water basin, the remnants of which are the modern Caspian and Aral seas. Thus, a single basin, covering the territories of the present Aral and Caspian Sea, existed in the late Eocene – early Oligocene (the Turan Sea, 37–24 million years ago), as well as at the end of the Middle Miocene (about 13 million years ago) [Popov *et al.*, 2004]. In that time, the planktonic larvae of *Caspiohydrobia* could potentially spread over long distances and at a higher pace than today with the help of migrating birds. However, we are still lacking any evidence of the planktotrophic development in this group. Long-term monitoring of the plankton of the Aral Sea, carried out in the 1970s and 1980s using a standard methodology and on a permanent grid of sampling stations, did not record objects that could be identified as free-swimming *Caspiohydrobia* larvae (S. Andreeva, unpublished data). The existence of planktonic larvae in *Caspiohydrobia* is conceivable only in the form of a short-term stage, lasting no more than a few hours, or if the larvae do not rise into the water column, but are located just above the clutch, in which case they are not captured by the planktonic net and remain inaccessible to collectors. This question needs to be further investigated.

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