

Article

Keyhole into a Lost World: The First Purely Freshwater Species of the Ponto-Caspian Genus *Clathrocaspia* (Caenogastropoda: Hydrobiidae)

Vitaliy V. Anistratenko ¹ , Dmitry M. Palatov ^{2,3}, Elizaveta M. Chertoprud ⁴ , Tatyana Y. Sitnikova ^{3,5}, Olga Y. Anistratenko ^{1,6}, Catharina Clewing ⁷ and Maxim V. Vinarski ^{3,*} 

- ¹ Department of Invertebrate Fauna and Systematics, Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, B. Khmelnytsky Str. 15, 01054 Kiev, Ukraine; anistrat@izan.kiev.ua (V.V.A.); olga.anistrat@gmail.com (O.Y.A.)
- ² A.N. Severtzov Institute of Ecology and Evolution, 33, Leninsky Prospect, 117071 Moscow, Russia; triops@yandex.ru
- ³ Laboratory of Macroecology & Biogeography of Invertebrates, St. Petersburg State University, 7/9 Universitetskaya Emb., 199034 Saint Petersburg, Russia; sit@lin.irk.ru
- ⁴ Department of Hydrobiology, Biological Faculty, Lomonosov Moscow State University, 1, Leninskie Gory, 119991 Moscow, Russia; skytuna@yandex.ru
- ⁵ Limnological Institute, Siberian Branch of Russian Academy of Sciences, Ulan-Batorskaya Street 3, 664033 Irkutsk, Russia
- ⁶ Department of Cenozoic Deposits, Institute of Geological Sciences, National Academy of Sciences of Ukraine, O. Gontchar Str. 55b, 01054 Kiev, Ukraine
- ⁷ Department of Animal Ecology and Systematics, Justus Liebig University Giessen, Heinrich-Buff-Ring 26 (IFZ), 35392 Giessen, Germany; catharina.clewing@allzool.bio.uni-giessen.de
- * Correspondence: m.vinarski@spbu.ru



Citation: Anistratenko, V.V.; Palatov, D.M.; Chertoprud, E.M.; Sitnikova, T.Y.; Anistratenko, O.Y.; Clewing, C.; Vinarski, M.V. Keyhole into a Lost World: The First Purely Freshwater Species of the Ponto-Caspian Genus *Clathrocaspia* (Caenogastropoda: Hydrobiidae). *Diversity* **2022**, *14*, 232. <https://doi.org/10.3390/d14040232>

Academic Editors: Michael Wink and Alan Christian

Received: 23 February 2022

Accepted: 18 March 2022

Published: 22 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The species of the Ponto-Caspian gastropod genus *Clathrocaspia* Lindholm, 1930 have been recorded so far from the Caspian Sea Basin only from marine waters, whereas they inhabit the estuarine areas as well as the purely freshwater environments in the Azov–Black Sea Basin. This genus has recently been assessed as putatively extinct in the Caspian Sea. A new purely freshwater species *Clathrocaspia laevigata* sp. n. from the water-flows of the Samur River delta in Dagestan, Russia, is described. A morphological comparison of the new species with *C. brotzkajae* (Starobogatov in Anistratenko & Prisjajzhnjuk, 1992) from the Caspian Sea and *C. knipowitschii* (Makarov, 1938) inhabiting the Azov–Black Sea Basin shows their overall similarity. The major difference is that *C. laevigata* sp. n. almost completely lacks the reticulate teleoconch sculpture, whereas it is well-developed in all known *Clathrocaspia* species. The molecular data revealed probable sister relationships between the new species and *C. knipowitschii*. All Dagestan populations are ecologically and spatially isolated from the open sea and are very locally restricted. We suggest to consider newly described snail species as retained in a pure freshwater refuge located in the coastal area of the Caspian Sea. The discovery of such a refuge sheds more light on the origin, current state and the future of the unique Ponto-Caspian aquatic biota under global change and increasing anthropogenic impact.

Keywords: non-marine Hydrobiidae; morphology; taxonomy; Caspian Sea; Dagestan; refuge

1. Introduction

The highly endemic fauna of the Caspian Sea, which has evolved during the last several million years in the basin, is currently under a threat of immediate extinction (or, at least, profound degradation) caused by many factors, ranging from the global changes to various kinds of human activity (see [1–3] and references therein). This fauna includes many unique genera and species belonging to various animal groups, among which the Mollusca play a primary role. There are several dozens of endemic genera and species

of molluscs in the Caspian Sea (see [4–6] for review). The study of this malacofauna and its taxonomic diversity, ecology, distribution, and evolutionary origins is of crucial importance for the understanding of evolution and the prospects of the Caspian Sea biota. Unfortunately, a large portion of the gastropod species described from the Caspian Sea have never been studied alive, and some of them have not been discovered since their description, which raises a question about the actual diversity and species richness of the Caspian Sea malacofauna [6]. In the absence of fixed specimens, most snail species endemic to this waterbody were described based on empty shells only, without studying their anatomical and genetic traits, and the identity of the majority of these species has not been reassessed using anatomical and/or genetic information.

The known findings of living specimens of the Caspian Sea endemic gastropods are extremely rare and sporadic (except for *Theodoxus pallasii/major*, which is quite abundant, see [7,8]). This is characteristic of the entire, two-century-long history of exploration of the Caspian Sea malacofauna (e.g., [4,9–17]), and makes the taxonomy of this group highly uncertain and perplexing. A pronounced decline in the biodiversity of many groups of the Ponto-Caspian invertebrates, observed over the past century, has forced taxonomists to alert to a large-scale crisis of this unique biota both in the Caspian Sea and in the Azov–Black Sea Basin [3,6].

The striking absence of living specimens of many representatives of the Caspian Sea malacofauna available for research has even raised a question about the actual status of these species. It is possible that at least part of the endemic Caspian Sea gastropod species included in the faunal catalogues [4,6,18] does not belong to the recent biota and their shells are, in fact, subfossil ones, washed out from the Pleistocene or Early Holocene sediments [19]. In this situation, any finding of living individuals belonging to the Caspian Sea snail taxa gives a unique chance to study them both morphologically and genetically, thus providing a means for the elucidation of their taxonomic and phylogenetic position.

The Ponto-Caspian gastropod genus *Clathrocaspia* Lindholm, 1930 (Hydrobiidae: Caspiinae) gives a good example for the situation discussed above. There are five species of this genus in the Caspian Sea. Three of them (*C. brotzkajae*, *C. isseli*, and *C. pallasii*) have never been found alive, and all taxonomic information available for these species is based exclusively on their conchological traits [19]. As concerns the two other species (*C. gmelinii* and *C. gaillardi*), only a few living individuals of these were discovered in 1956, and since then, no further material has been studied. The genus *Clathrocaspia* has recently been assessed as putatively extinct in the Caspian Sea [19].

The known populations of the *Clathrocaspia* species in the Azov–Black Sea Basin are ecologically confined to the transitional brackish zone between freshwater and marine habitats. They inhabit the river mouth areas and estuaries (limans) of large rivers flowing into the Sea of Azov and the Black Sea [20–25]. During the last decade, alien populations of the Azov–Black Sea species *Clathrocaspia knipovitschii* (Makarov, 1938) have been discovered in different parts of the Danube River, up to 800 km upstream of its delta [26,27], which suggests that this species can live not only in brackish water but is also able to withstand freshwater conditions. On the contrary, all known records of living individuals and fresh empty shells of *Clathrocaspia* from the Caspian Sea were made exclusively in the open sea with local water salinity exceeding 12 ‰ [19].

Here, we report a rather surprising discovery of flourishing populations of a previously undescribed species of *Clathrocaspia* living in freshwater streams of the Samur Forest Reserve on the western coast of the Caspian Sea in Dagestan, Russia. The status of these populations as constituting an independent species has been confirmed both genetically and morphologically. We provide a detailed morphological account of the new species as well as the data on its ecology, distribution, and probable phylogenetic relationships with other species of the genus. This finding not only broadens our knowledge of the taxonomy, distribution, and ecology of *Clathrocaspia*. It also allows us to hypothesize the existence of a freshwater refuge located in the coastal area of the Caspian Sea, which may be important in the context of conservation of the endemic Caspian biota.

2. Material and Methods

2.1. Examined Material

More than 1000 living specimens of freshwater hydrobiid snails, provisionally identified as belonging to the genus *Clathrocaspia*, were collected by Dmitry Palatov between 3–12 April 2021 and 6–8 August 2021 from 14 springs, streams, and small rivers of the Samur River delta within the National Park “Samurskiy”, Magaramkent District of Dagestan, Russia (Figure 1, Table 1: localities Dag01–Dag67). The sampled area abounds in various types of lotic habitats; Figure 2 illustrates some of the localities where the snails have been found. Molluscs were collected with a sieve or by hand, sorted on-site from live material, and preserved in 80% ethanol. Table 1 provides a list of these samples, with characteristics of the localities, as well as information on museum samples inspected during this study. We examined several museum lots containing ethanol-fixed specimens of *Clathrocaspia* from the Caspian Sea collected by Boris M. Logvinenko in July to September 1956 off Derbent (Dagestan, Russia) and near the Mangyshlak Peninsula (Kazakhstan) (Figure 1, Table 1: localities 131/1956, 136/1956, 65/1956). These lots are deposited in the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, and, to the best of our knowledge, they constitute the only available samples of fixed *Clathrocaspia* from the Caspian Sea. These molluscs were dredged at a distance of 40–45 km from the coast and from depths 56–64 m. In the samples, among many thousands of empty shells, only a few individuals with soft bodies could be found. These snails, identified as *Clathrocaspia gmelinii* (Clessin & W. Dybowski in W. Dybowski, 1887) and *C. gaillardi* (Tadjallipour, 1977), were earlier studied by Sitnikova and Starobogatov [28], who published the first data on the female reproductive anatomy and the radula of *Clathrocaspia*.

For a study of conchological variation within *Clathrocaspia*, we used the type specimens of *C. brotzkajae* (Starobogatov in Anistratenko & Prisjzhnjuk, 1992) collected by B.M. Logvinenko in 1957 near the Mangyshlak Peninsula and by G.A. Alighadzhiev in 1960 near Derbent, Dagestan (see Figure 1, Table 1). As a material for comparison, we used two samples containing several tens of ethanol-fixed individuals of *C. knipowitchii* collected from two localities in the Lower Dnieper River near Kherson, Ukraine (the Black Sea Basin), in 2016 and 2018 (see Figure 1, Table 1).

In total, ca. 1200 specimens of various *Clathrocaspia* species represented by empty shells and fixed individuals were examined during this study.

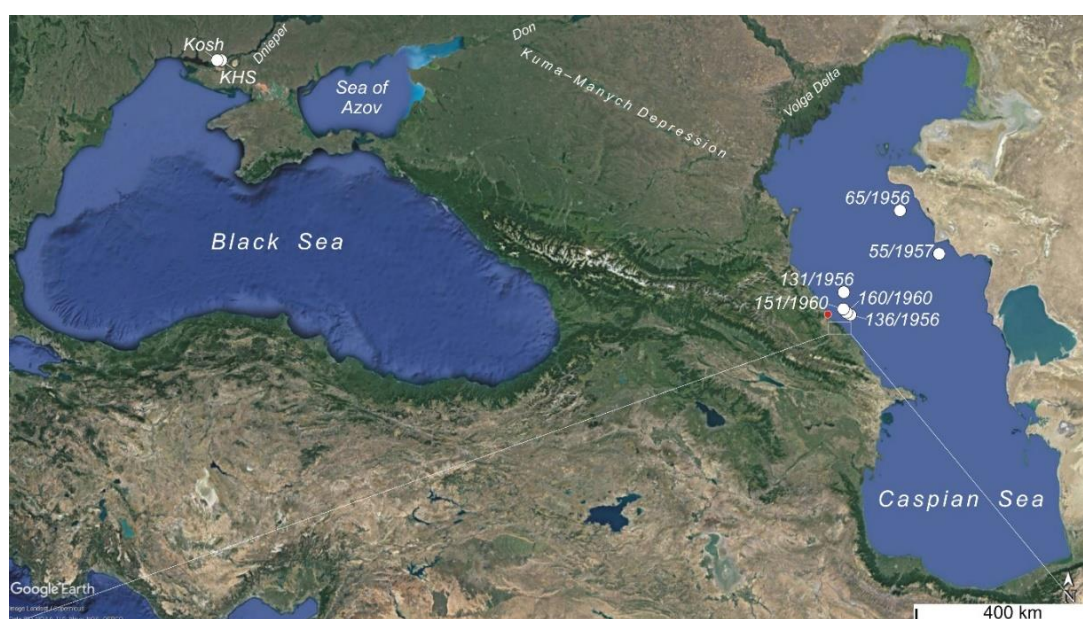


Figure 1. Cont.

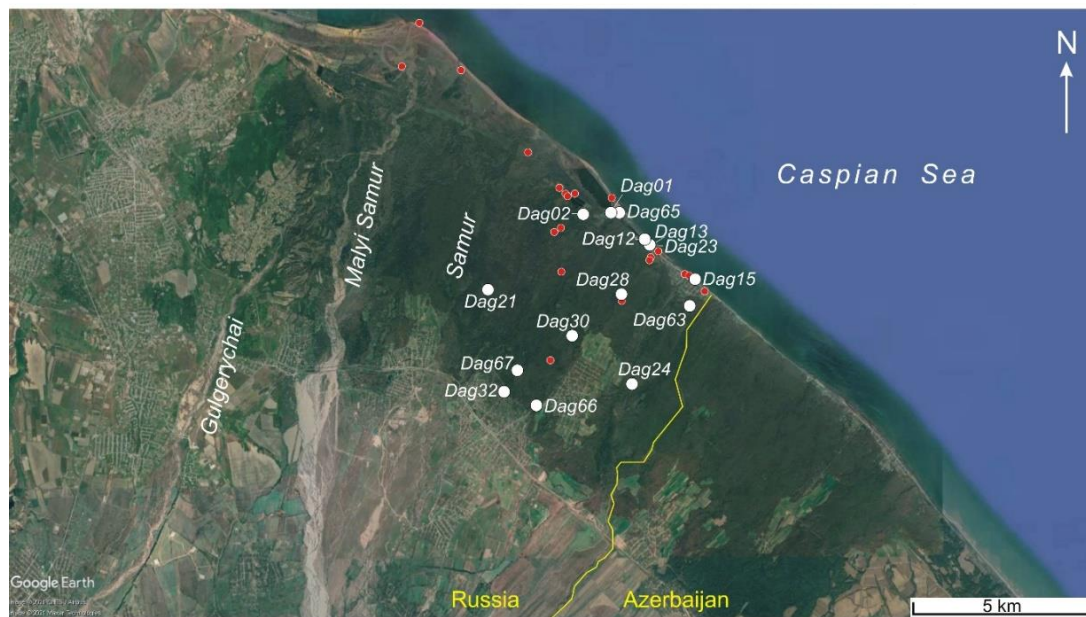


Figure 1. A map of the Ponto-Caspian region, with indication of study area and sampling points of *Clathrocaspia*. The numbers of localities correspond to those in Table 1. Small red dots show the sampling points where *Clathrocaspia* have not been found.

Table 1. The information of the studied material. The numbers of localities refer to those in the text and figures. Abbreviations: q.—quadrant, st.—station.

Locality	Species Name, Collection and Lot	Locality Description	m, a.s.l.	Image in This Study
			m, depthD	
Dag01	<i>C. laevigata</i> sp. n., ZIN 541-2021/3 ZMMU Lc-41241 (paratypes), Lc-41242 (paratypes)	3 April 2021. Samur delta area. One of the channels of the Karasu River near the reserve cordon. A river in a lowland forest, width 4–5 m, bottom with pebbles, stones, plant debris. 41°51′53.22″ N 48°33′17.40″ E. Coll. D.M. Palatov	–19 — —	2A
Dag02	<i>C. laevigata</i> sp. N., IZAN 607	3 April 2021. Samur delta area. The same channel of the Karasu River, 3 km upstream of the locality Dag01. A river in a lowland forest, width 4–5 m, bottom with pebbles, stones, alder roots, plant debris. Flow rate up to 0.5 m/s. 41°51′51.30″ N 48°32′43.86″ E. Coll. D.M. Palatov	–8 — —	
Dag12	<i>C. laevigata</i> sp. n., IZAN 608, JLU RM 21.10 UGSB 25914	6 April 2021. Samur delta area. A drain of a small fish pond at the northern end of the Primorskiy village, on the shore of the Caspian Sea. Width 3 m, bottom—stones with significant algal fouling. Flow rate 0.4–0.6 m/s. 41°51′29.04″ N 48°34′0.30″ E. Coll. D.M. Palatov	–25 — —	
Dag13	<i>C. laevigata</i> sp. n., private collection of D. Palatov	6 April 2021. Samur delta area. Another drain of a fish pond at the northern end of the Primorskiy village. Width 4–5 m, bottom—stones with significant algal fouling. Flow rate 0.3–0.6 m/s. 41°51′25.85″ N 48°34′3.03″ E. Coll. D.M. Palatov	–24 — —	2B

Table 1. Cont.

Locality	Species Name, Collection and Lot	Locality Description	m, a.s.l.	Image in This Study
			m, depthD	
Dag15	<i>C. laevigata</i> sp. n., ZIN 541-2021/5 IZAN 609, JLU RM 21.11 UGSB 25915	6 April 2021. Samur delta area. The Yalama River, 30 m above the mouth, on an open beach of the Caspian Sea in the Primorsky village. Width 3 m, bottom—stones, sand. Flow rate 0.3–0.6 m/s. 41°50′52.68″ N 48°35′0.90″ E. Coll. D.M. Palatov	–26 — —	2D
Dag21	<i>C. laevigata</i> sp. n., ZIN 541-2021/6	8 April 2021. Samur delta area. A small river in a lowland forest. Width 4–4.5 m. The bottom is silt, covered with silted driftwood. 41°50′42.72″ N 48°30′47.16″ E. Coll. D.M. Palatov	16 — —	2G
Dag23	<i>C. laevigata</i> sp. n., ZIN 541-2021/7 IZAN 610, JLU RM 21.12 UGSB 25916	10 April 2021. Samur delta area. A spring by a river on the Caspian coast in the same place as Dag13 locality, but separated from the main river. 41°51′25.85″ N 48°34′3.03″ E. Coll. D.M. Palatov	–24 — —	2C
Dag24	<i>C. laevigata</i> sp. n., ZIN 541-2021/8 IZAN 611, JLU RM 21.13 UGSB 25917	10.04.2021. Samur delta area. The Yalama River opposite Bilbil-Kazmalyar village. The river runs through a lowland forest. Width 4–5 m. The bottom is plant debris, driftwood, silt. Flow rate 0.2–0.6 m/s. 41°49′17.46″ N. 48°33′42.60″ E Coll. D.M. Palatov	20 — —	2E
Dag28	<i>C. laevigata</i> sp. n., ZIN 541-2021/1 (holotype) and 541-2021/2 (paratypes), IZAN 612, JLU RM 21.14 UGSB 25918	11 April 2021. Samur delta area. A stream in a lowland forest, 1.5 km W of Primorskiy village. Width 3–3.5 m. The bottom is pebbles, stones, some areas with silt, plant debris. Flow rate 0.3–0.6 m/s. 41°50′38.88″ N 48°33′31.14″ E. Coll. D.M. Palatov	2 — —	
Dag30	<i>C. laevigata</i> sp. n., private collection of D. Palatov	11 April 2021. Samur delta area. A stream in a lowland forest, north of Bilbil-Kazmalyar village. Width 3 m. The bottom is pebbles, stones, areas with silt, plant debris. Flow rate 0.3–0.6 m/s. 41°50′1.08″ N 48°32′29.70″ E. Coll. D.M. Palatov	17 — —	2F
Dag32	<i>C. laevigata</i> sp. n., ZIN 541-2021/9 IZAN 613, JLU RM 21.15 UGSB 25919	12 April 2021. Samur delta area. A stream in the bushes at the forest edge on the eastern outskirts of Tagirkent-Kazmalyar village. Width 1.5 m. The bottom is silt, driftwood, few rocky areas. Flow rate 0.3–0.5 m/s. 41°49′11.16″ N 48°31′8.28″ E. Coll. D.M. Palatov	35 — —	2H
Dag63	<i>C. laevigata</i> sp. n., ZIN 541-2021/10	6 August 2021. Samur delta area. The Yalama River, on the southern outskirts of Primorsky village. A river in a lowland forest, width 3–4 m. The bottom is driftwood, few rocky areas. Flow rate 0.3–0.5 m/s. 41°50′31.63″ N 48°34′53.82″ E Coll. D.M. Palatov	–3 — —	

Table 1. Cont.

Locality	Species Name, Collection and Lot	Locality Description	m, a.s.l.	Image in This Study
			m, depthD	
Dag65	<i>C. laevigata</i> sp. n., private collection of D. Palatov	7 August 2021. Samur delta area. One of the channels of the Karasu River near the reserve cordon, downstream of locality Dag01. Runs through a lowland forest, width 6–8 m, bottom with silt, pebbles, stones, plant debris. Flow rate 0.1–0.3 m/s. 41°51'53.34'' N 48°33'22.17'' E. Coll. D.M. Palatov	–21 –	
Dag66	<i>C. laevigata</i> sp. n., ZIN 541-2021/11	8 August 2021. Samur delta area. A stream in the bushes at the forest edge on the eastern outskirts of Tagirkent-Kazmalyar village. Width 3–4 m. The bottom is covered with heavily silted stones, driftwood. Water is muddy, noticeably polluted. Flow rate 0.3–0.5 m/s. 41°48'57.99'' N 48°31'45.16'' E Coll. D.M. Palatov	40 –	
Dag67	<i>C. laevigata</i> sp. n., ZIN 541-2021/12	8 August 2021. Samur delta area. A stream in the eastern outskirts of Tagirkent-Kazmalyar village. Width 3–4 m. The bottom is covered with stones with a touch of lime, driftwood. Water is muddy, noticeably polluted. Flow rate 0.3–0.5 m/s. 41°49'27.87'' N 48°31'21.59'' E Coll. D.M. Palatov	37 –	
65/1956	<i>C. gmelinii</i> , ZIN no number (still uncataloged)	31 July 1956. The eastern part of the Middle Caspian near Mangyshlak peninsula, q. 517/518, st. 65 N 50°30'00'' 43°42'30'' E Coll. B.M. Logvinenko	– 58	
131/1956	<i>C. gaillardi</i> , ZIN no number (still uncataloged)	5 September 1956. The western part of the Middle Caspian Sea near Dagestan, between Makhachkala and Derbent, q. 693B, st. 131. 42°22'30'' N 48°42'30'' E Coll. B.M. Logvinenko	– 56	
136/1956	<i>C. gmelinii</i> , ZIN no number (still uncataloged)	6 September 1956. The western part of the Middle Caspian Sea between Makhachkala and Derbent, q. 7436, st. 136. 42°7'30'' N 48°47'30'' E Coll. B.M. Logvinenko	– 64	
160/1960	<i>C. brotzkajae</i> , ZIN no number (still uncataloged)	Summer 1960. The Caspian Sea near Derbent, st. 160. 42°9'50'' N 48°42'50'' E Coll. A.G. Alighadzhiev	– 60	

Table 1. Cont.

Locality	Species Name, Collection and Lot	Locality Description	m, a.s.l.	Image in This Study
			m, depthD	
151/1960	<i>C. brotzkajae</i> , ZIN no number (still uncataloged)	Summer 1960. The Caspian Sea near Derbent, st. 151. 42°11'20'' N 48°39'40'' E Coll. A.G. Alighadzhiev	— 75	
55/1957	<i>C. brotzkajae</i> , ZIN no number (still uncataloged)	9 August 1957. The eastern part of the Middle Caspian Sea near Peschanyi Cape, q. 632в, st. 55. 42°52'30'' N 51°22'30'' E. Coll. B.M. Logvinenko	— 73	
KHS	<i>C. knipowitschii</i> , IZAN 390	18 May 2016. The Dnieper River near the Kherson Hydrobiological station. 46°35'54.55'' N 32°34'51.35'' E. Coll. V.V. Anistratenko	— 1.5–6.0	
Kosh	<i>C. knipowitschii</i> , IZAN 522	10 October 2018. The Dnieper River near Kherson City, Koshevaya branch. The bottom is covered with shell detritus and silted sand, with <i>Dreissena</i> druses; t° = 14 °C; transparency of water 3.0 m. 46°37'35.63'' N 32°34'10.31'' E. Coll. V.V. Anistratenko, I.V. Shevchenko	— 3.5	



Figure 2. Cont.



Figure 2. Images illustrating the diversity of biotopes inhabited by *Clathrocaspia* in the Samur Forest area. (A)—One of the channels of the Karasu River (locality Dag01); (B)—A fish pond drain at the northern end of Primorskiy village (locality Dag13); (A–C) spring by a river on the Caspian Sea coast (locality Dag23); (D)—The Yalama River, 30 m above the mouth, on an open beach of the Caspian Sea (locality Dag15); (E)—The Yalama River in a lowland forest near Bilbil-Kazmalyar village (locality Dag24); (F)—A forest stream, north of Bilbil-Kazmalyar village (locality Dag30); (A–G) small forest river (locality Dag21); (H)—A forest stream among the bushes on the eastern outskirts of Tagirkent-Kazmalyar village (locality Dag32).

2.2. Morphological Study

The adult shell characters and reproductive anatomy of *Clathrocaspia* were studied with Leica M 165 C stereomicroscope and photographed with digital camera ToupCam 9.0 MP attached to a light microscope Olympus CX21; stereomicroscope Micromed MC-5 ZOOM LED and Leica DM5000B microscope with digital camera Leica DFC425 equipped with normal light and differential interference contrast microscopy (DIC) optic. The anatomical data are mainly resulting from the dissections of *Clathrocaspia* sp. collected in localities Dag01, Dag15, Dag23, Dag28, Dag32, and Dag67, and of *C. knipowitschi* from the Lower Dnieper, Ukraine (localities KHS and Kosh; see Table 1). The female genitals and the male copulatory apparatuses were examined on temporary total preparations. The objects were stained with eosin alcohol solution, washed in 100% ethanol, and cleared in clove oil. In

addition, the structure of the female gonoduct was studied on a series of histological paraffin sections 5–7 μm thick, stained with hematoxylin, and encased in Canadian balsam [29]. The histological sections were prepared for one female collected at locality Dag28. The images were performed using a Nikon SMZ25 stereomicroscope and Axio Imager M1 light microscope with Axio Cm Mrc5 digital camera.

Seven standard shell dimensions (Figure 3) were taken from 135 *Clathrocasapia* spp. specimens. The morphological features of radula, operculum, teleoconch, and protoconch were additionally studied with the aid of a scanning electron microscope (SEM). Three parameters of the protoconch were measured: (1) the maximum diameter, (2) the number of whorls, and (3) the width of the initial cap-like part of the embryonic shell. Most of the SEM images were obtained at the Paleontological Institute of the Russian Academy of Sciences (Moscow) using a Vega3 Tescan microscope. Additional scanning electron micrographs were taken at the Institute of Geological Sciences, National Academy of Sciences of Ukraine (Kiev), with the aid of JSM-6490 SEM.

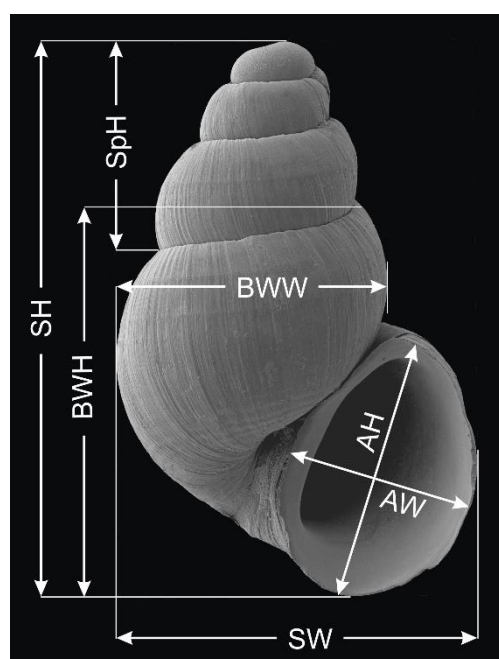


Figure 3. The standard measurements of the *Clathrocasapia* shell. Abbreviations: AH—aperture height; AW—aperture width; BWH—body whorl height; BWW—body whorl width; SH—shell height; SpH—spire height; SW—shell width.

2.3. Statistical Analysis

We used the principal component analysis (PCA) as the main tool for studying shell variability in *Clathrocasapia* spp.

The assessment of the significance of conchological differences between individuals of different sexes, as well as the establishment of a relationship between the SH covariate (shell height) and the variation in conchological differences among molluscs was carried out using the permutational multivariate analysis of variance (PERMANOVA) [30]. The PERMANOVA analysis was based on a Euclidean distance similarity matrix using normalized primary data on 7 conchological variables taken from 135 shells from the following samples: Dag01, Dag23, Dag28, Dag32, and Dag67 (see Table 1). The sexual dimorphism in shell traits was studied exclusively in Dag67 sample, from which 44 specimens (22 females and 22 males) were taken. The sex of these individuals was determined through dissection. The Type 3 sums of squares (partial) and permutation of residuals according to the reduced model were used. The number of permutations was set equal to 999, the significance level was $p < 0.05$. Statistical tests were carried out on the basis of PRIMER 7 with the PERMANOVA + add-on [31].

2.4. Molecular Genetic Methods

Genomic DNA was extracted from 18 specimens (representing 6 locations) using the CTAB (cetyltrimethylammoniumbromide) protocol described by Wilke et al. [32]. The mitochondrial barcoding gene COI (cytochrome *c* oxidase subunit I) was partially amplified using the universal primers LCO1490 and HCO2198 designed by Folmer et al. [33]. The following ramp-PCR conditions were used: initial denaturation of 94 °C for 4 min, followed first by 5 amplification cycles (denaturation of 94 °C for 1 min, annealing of 45 °C for 1 min, and elongation of 72 °C for 1 min) and followed by 35 cycles (denaturation of 94 °C for 45 s, annealing of 48 °C for 45 s, and elongation of 72 °C for 45 s); the reaction was terminated by a final elongation of 72 °C for 10 min. For visualization of forward and reverse sequences the LGC Sanger sequencing services (LGC Genomics GmbH, Berlin, Germany) was used. The alignment of the protein-coding COI fragment was performed by the eye.

The number of haplotypes was calculated in TCS [34]. For each unique haplotype, a nucleotide BLAST search (BLASTn suite: megablast with default settings) was conducted. Additionally, the mean, maximum, and minimum genetic *p*-distances within the undescribed species as well as to its closest relative (inferred from the BLAST search, the first five matches are used, see Table S1) were calculated in MEGA version 6.06 [35].

Abbreviations used in the paper: IZAN—Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine, Kiev, Ukraine; JLU—Justus Liebig University Giessen, Germany; ZMMU—Zoological Museum of Moscow State University, Moscow, Russia; ZIN—Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

3. Results

For nine specimens of *Clathrocaspia* sp. from the Samur River delta (sampled at five different localities, see Table S1), the barcoding gene fragment COI (with a length of about 658 bp) was successfully amplified. In total, four different haplotypes (C1a1–C1a4, see Table S1) could be identified. For all of them, the BLAST search yielded the same result that *C. knipowitschii* from the Dnieper River (Ukraine; GenBank accession no. MW385257) followed by *C. knipowitschii* from the Danube River (Hungary; MW385250) show the highest match (93.35–93.86% and 93.32–93.77% identity, respectively) to our newly sequenced specimens (for more details see Table S1). The mean genetic distance within *Clathrocaspia* sp. is 0.7% with a minimum of 0% and a maximum of 1.4%. The mean genetic distance between the Samur River delta snails and *C. knipowitschii* is 6.3% (minimum 6.1% and maximum 6.6%).

Alongside the results of an examination of shells, soft-body anatomy, and radular characters of the snails collected in the Samur River delta, these genetic data allow us to identify them as belonging to a previously unknown freshwater species of the genus *Clathrocaspia*. Its description, with taxonomic, ecological, and biogeographic remarks, is provided below. The ecological uniqueness (i.e., the ability to live under purely freshwater conditions) of these snails gives an additional evidence of their species independence among the Caspian Sea Basin representatives of *Clathrocaspia*.

3.1. Systematic Part

Class Gastropoda Cuvier, 1795

Subclass Caenogastropoda Cox in Moore, 1960

Order Littorinimorpha Golikov & Starobogatov, 1975

Superfamily Truncatelloidea Gray, 1840

Family Hydrobiidae Stimpson, 1865

Recently, it was suggested [36] to classify the Ponto-Caspian hydrobiids among three subfamilies: Caspiinae B. Dybowski, 1913; Hydrobiinae Stimpson, 1865; and Pyrgulinae Brusina, 1882 (see also [37]).

3.1.1. Subfamily Caspiinae B. Dybowski, 1913

The updated morphological diagnosis of this taxon has recently been provided by Anistratenko et al. [19]. The data on the radular morphology of the new species described below require a slight emendation of this diagnosis: the rachidian tooth of the radula with two **or three** pairs of basal cusps.

3.1.2. Genus *Clathrocaspia* Lindholm, 1930

Type species. *Caspia pallasii* Clessin & W. Dybowski in W. Dybowski, 1887; by original designation. Caspian Sea, Recent.

Seven recent species of *Clathrocaspia* are known in the Ponto-Caspian region; two of them inhabit only the Black Sea Basin, whereas the five remaining are endemic to the Caspian Sea [6,19]. The new species described below is the first and, so far, the only, known freshwater member of the genus *Clathrocaspia* in the Caspian Sea Basin. It is characterized by a sharply reduced teleoconch sculpture, and thus the diagnosis of the genus must be amended: The shells of *Clathrocaspia* are characterized by a well-developed **to almost absent** (=hardly visible) reticulate teleoconch sculpture.

3.1.3. *Clathrocaspia laevigata* V. Anistratenko, Palatov, Chertoprud & Vinarski sp. n.

Figure 4, Figure 5, Figure 6, Figure 7, Figure 8A–D, Figure 9A,B,D,E,G,K, Figures 10A and 11.
 urn:lsid:zoobank.org:act:B913C1C9-357D-4CD0-B31E-3AAB370BEBC5

Type material. Holotype ZIN 541-2021/1; 33 paratypes ZIN 541-2021/2, and 12 paratypes IZAN 612, all from the same locality. Specimens taken 11.04.2021 in a stream in a lowland forest, 1.5 km west of Primorskiy (locality Dag28 in Table 1). Other 13 paratypes in ZMMU: Lc-41241 (dried shells) and Lc-41242 (ethanol-fixed specimens), all collected from Dag01 locality.

Other material from different localities is stored in ZIN (629 specs., ZIN 541-2021/3–ZIN 541-2021/10; IZAN (110 specs., IZAN 607–IZAN 611, IZAN 613). Thirty-five specimens were transferred to JLU (RM 21.10 UGSB 25914–RM 21.15 UGSB 25919).

Type locality. Russia, Republic of Dagestan, Magaramkent District, the Samur River delta within the National Park “Samurskiy”; 41°50′38.88″ N 48°33′31.14″ E.

Etymology. The species epithet is derived from the Latin “laevigatus”—smooth, which denotes the extremely reduced sculpture of its shell.

Dimensions of the holotype shell, mm: SH = 1.91, SW = 1.24 (Figure 4A).

Description. The teleoconch is ovoid to broadly conical. The shell consists of 4.0–4.5 moderately inflated whorls with regularly increasing diameter; it is up to about 2.5 mm in height and up to 1.45 mm in width (for more information see Table 2). The whorls are separated by a deep suture and ornamented with fine axial growth lines, crossed by very weak spiral threads, resulting in an extremely delicate reticulate sculpture that is usually restricted to the last two whorls, sometimes even to the body whorl. The reticulate pattern is hardly visible under low magnification (Figure 4N,O), and the shell surface looks lacking any sculpture (smooth) compared with *C. knipowitschii* and *C. brotzkajae* in Figure 5. However, SEM micrographs demonstrate (Figure 6A–G and Figure 7A–C,G–I) that the teleoconch surface is covered by the typical for *Clathrocaspia* reticulate ornamentation, albeit strongly reduced. The aperture is wide, sometimes laterally expanded; the umbilicus is usually closed, sometimes looking like a narrow slit.

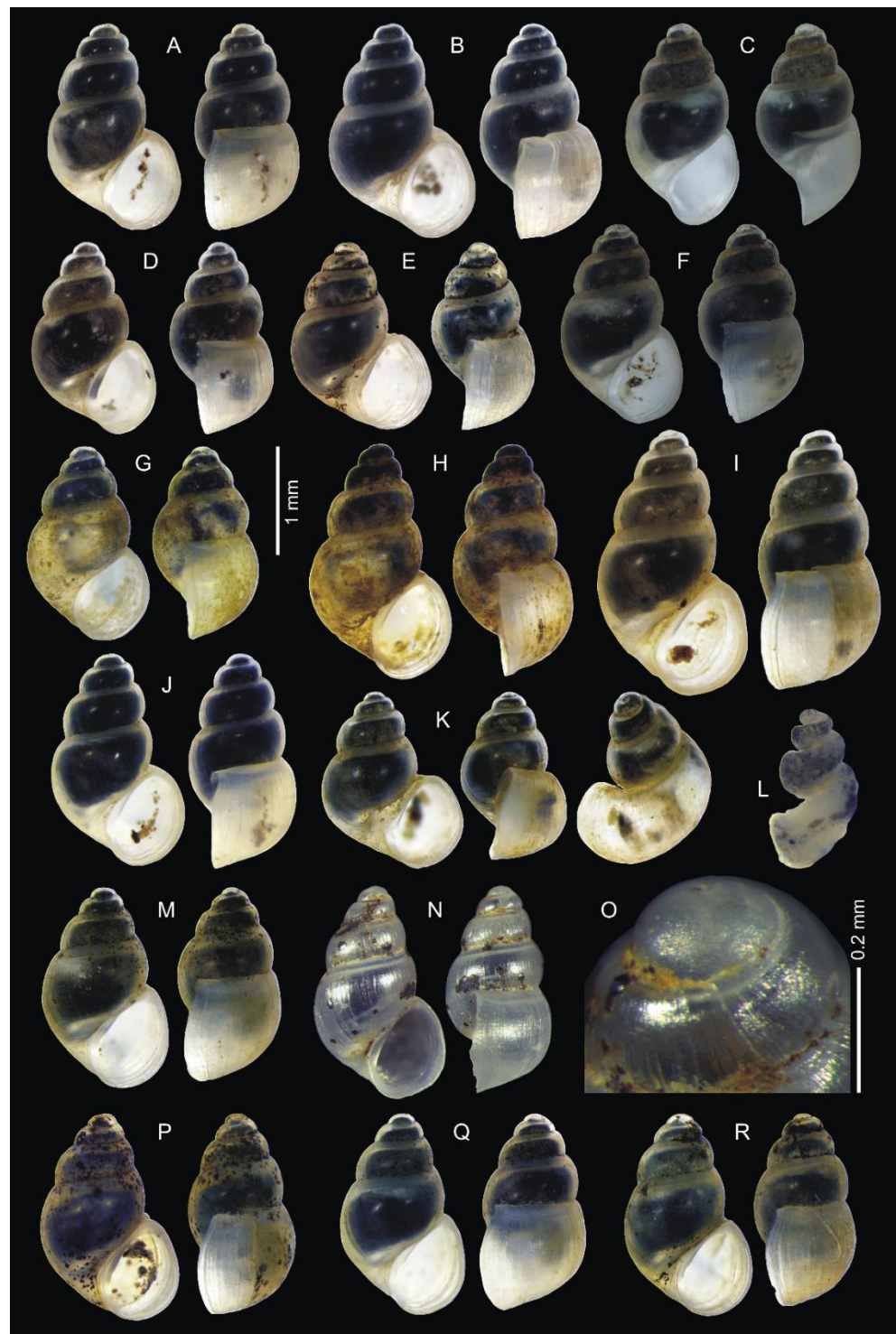


Figure 4. *Clathrocaspia laevigata* sp. n.: (A)—holotype, ZIN 541-2021/1; (B)—paratype, IZAN 612/a; (C)—paratype, IZAN 613/b; (D)—paratype, IZAN 612/c; (E)—paratype, IZAN 613/a; (F)—paratype, IZAN 613/c; (G)—specimen IZAN 610/2; (H)—specimen IZAN 610/3; (I)—paratype, IZAN 612/1 (the same individual as Figure 7A,D,G,I); (J)—specimen IZAN 612/4; (K)—specimen IZAN 612/2 (the same individual as Figure 9A,D,E); (L)—specimen IZAN 610/1, soft body; (M)—paratype, IZAN 613/3, loc. Dag32 (the same individual as Figure 7B,H,K,L); (N,O)—specimen IZAN 608/1 (N)—shell after treatment with bleach; (O)—the protoconch enlarged, a fine sculpture is visible in (O); (P)—specimen IZAN 613/2; (Q)—specimen IZAN 613/1 (the same individual as Figure 9B); (R)—specimen IZAN 613/4. (A,B,D,I–K)—from the locality Dag28; (C,E,F,M,P–R)—from the locality Dag32; (G,H,L)—from the locality Dag23; (N,O)—from the locality Dag12.

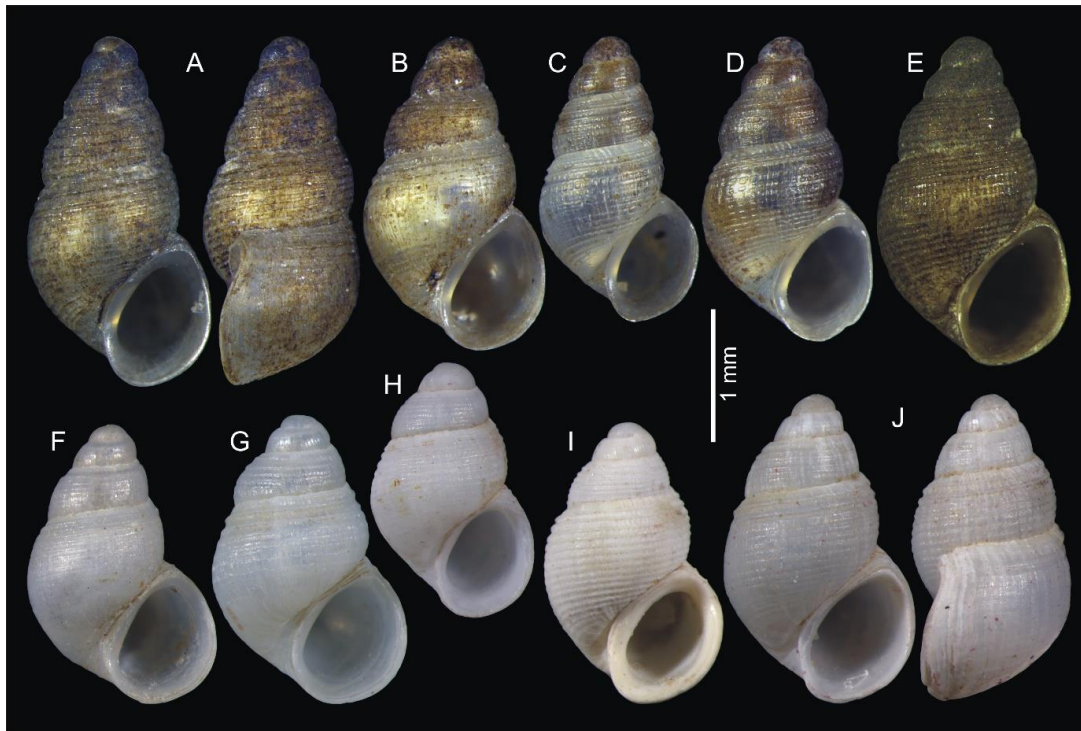


Figure 5. Shells of some other species of *Clathrocaspia*: (A–E)—shells of *C. knipowitschii*: (A)—specimen IZAN 522_8; (B)—specimen IZAN 522_6; (C)—specimen IZAN 390_8; (D)—specimen IZAN 390_3; (E)—specimen IZAN 522_9. (F–J)—shells of *C. brotzkajae*: (F)—holotype, ZIN; (G)—paratype, ZIN; (H)—paratype, ZIN; (I)—“paratype” of *C. “alighadzhievi”*, ZIN; (J)—the “holotype” of *C. “alighadzhievi”*, ZIN. (A,B,E)—from the locality Kosh; (C,D)—from the locality KHS; (F–H)—from the locality 151/1960; (I)—from the locality 160/1960; and (J)—from the locality 55/1957.

Table 2. The morphometric characteristics of shells of the *Clathrocaspia* species discussed in this study. Above lines—min–max values; below lines—mean value $\pm$ SD. Abbreviations according to Figure 3.

Species/Locality	n	Shell Dimension, in mm						
		SH	BWH	SW	BWW	SpH	AH	AW
<i>Clathrocaspia brotzkajae</i> (55/1957, 151/1960, 160/1960)	32	<u>2.08–2.42</u> 2.23 $\pm$ 0.09	<u>1.40–1.70</u> 1.53 $\pm$ 0.07	<u>1.23–1.58</u> 1.36 $\pm$ 0.08	<u>1.00–1.25</u> 1.13 $\pm$ 0.05	<u>0.65–0.88</u> 0.76 $\pm$ 0.06	<u>0.97–1.10</u> 1.02 $\pm$ 0.10	<u>0.73–0.85</u> 0.78 $\pm$ 0.03
<i>C. knipowitschii</i> (KHS)	11	<u>2.08–2.55</u> 2.27 $\pm$ 0.12	<u>1.38–1.68</u> 1.52 $\pm$ 0.08	<u>1.25–1.50</u> 1.38 $\pm$ 0.08	<u>0.90–1.17</u> 1.03 $\pm$ 0.07	<u>0.85–1.07</u> 0.93 $\pm$ 0.07	<u>0.92–1.05</u> 1.00 $\pm$ 0.04	<u>0.66–0.84</u> 0.75 $\pm$ 0.06
<i>C. knipowitschii</i> (Kosh)	19	<u>2.18–2.65</u> 2.41 $\pm$ 0.13	<u>1.50–1.75</u> 1.64 $\pm$ 0.08	<u>1.08–1.50</u> 1.37 $\pm$ 0.11	<u>1.00–1.21</u> 1.12 $\pm$ 0.06	<u>0.81–1.14</u> 0.97 $\pm$ 0.10	<u>0.90–1.20</u> 1.07 $\pm$ 0.08	<u>0.59–0.85</u> 0.75 $\pm$ 0.07
<i>C. laevigata</i> sp. n. (Dag01)	24	<u>1.53–1.95</u> 1.68 $\pm$ 0.10	<u>1.12–1.37</u> 1.21 $\pm$ 0.07	<u>0.93–1.14</u> 1.01 $\pm$ 0.06	<u>0.77–0.90</u> 0.84 $\pm$ 0.04	<u>0.36–0.66</u> 0.47 $\pm$ 0.08	<u>0.75–0.91</u> 0.81 $\pm$ 0.04	<u>0.51–0.65</u> 0.57 $\pm$ 0.04
<i>C. laevigata</i> sp. n. (Dag23)	11	<u>1.59–2.13</u> 1.87 $\pm$ 0.15	<u>1.20–1.47</u> 1.37 $\pm$ 0.08	<u>1.03–1.35</u> 1.21 $\pm$ 0.09	<u>0.86–1.05</u> 0.97 $\pm$ 0.05	<u>0.50–0.83</u> 0.67 $\pm$ 0.09	<u>0.80–0.99</u> 0.90 $\pm$ 0.07	<u>0.58–0.78</u> 0.68 $\pm$ 0.04
<i>C. laevigata</i> sp. n. (Dag28)	10	<u>1.65–2.42</u> 1.92 $\pm$ 0.21	<u>1.23–1.61</u> 1.35 $\pm$ 0.11	<u>1.13–1.45</u> 1.25 $\pm$ 0.09	<u>0.88–1.10</u> 0.95 $\pm$ 0.07	<u>0.56–0.94</u> 0.71 $\pm$ 0.11	<u>0.82–1.10</u> 0.90 $\pm$ 0.09	<u>0.65–0.80</u> 0.68 $\pm$ 0.05

Figure 6. *Clathrocaspia laevigata* sp. n. shell morphology: (A,J)—specimen ZIN 541-2021/3_01; ((A)—shell, (J)—protoconch); (B,H)—specimen ZIN 541-2021/3_02 ((B)—shell, (H)—protoconch); (C,I)—specimen ZIN 541-2021/3_03 ((C)—shell, (I)—protoconch); (D)—specimen IZAN 613/3; (E,L,Q)—specimen ZIN 541-2021/3_04 ((E)—shell, (L,Q)—protoconch); (F,M,T)—specimen ZIN 541-2021/3_05 ((F)—shell, (M,T)—protoconch); (G,R)—specimen IZAN 609/2 ((G)—shell, (R)—protoconch); (K,S)—protoconch of specimen ZIN 541-2021/3_06 ((K)—apical view, (S)—detailed view from side); (N)—operculum of specimen ZIN 541-2021/3_07 (from outside); (O)—operculum of specimen ZIN 541-2021/3_08 (from inside); (P)—protoconch of specimen ZIN 541-2021/3_09. (D)—from the locality Dag32; (G,R)—from the locality Dag15; all other specimens—from the locality Dag01.

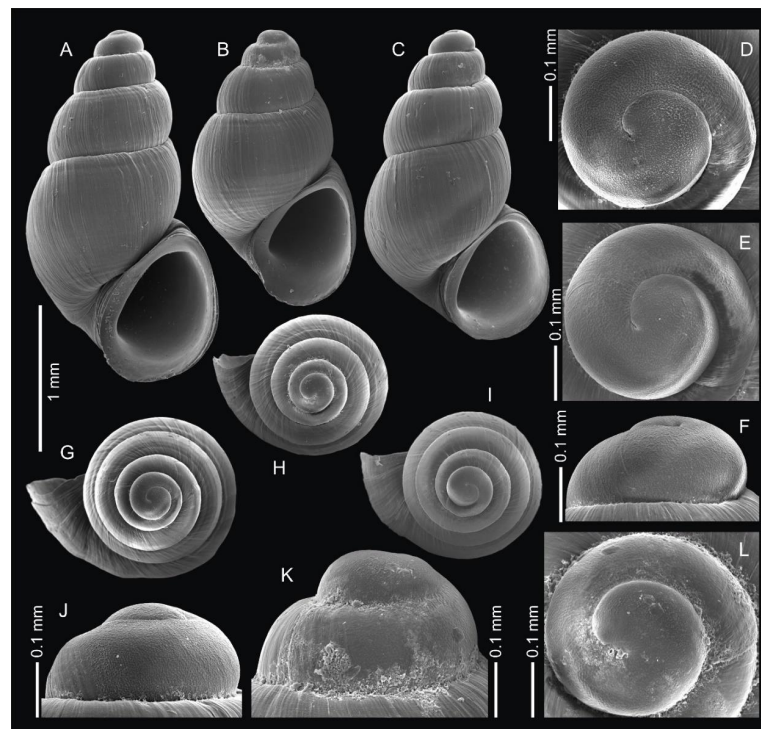


Figure 7. *Clathrocaspia laevigata* sp. n. shell morphology: (A,D,G,J)—paratype, IZAN 612/1, loc. Dag28 (the same individual as Figure 4I); (B,H,K,L)—paratype, IZAN 613/3, loc. Dag32 (the same individual as Figure 4M); (C,E,F,I)—specimen IZAN 612/3, loc. Dag28.

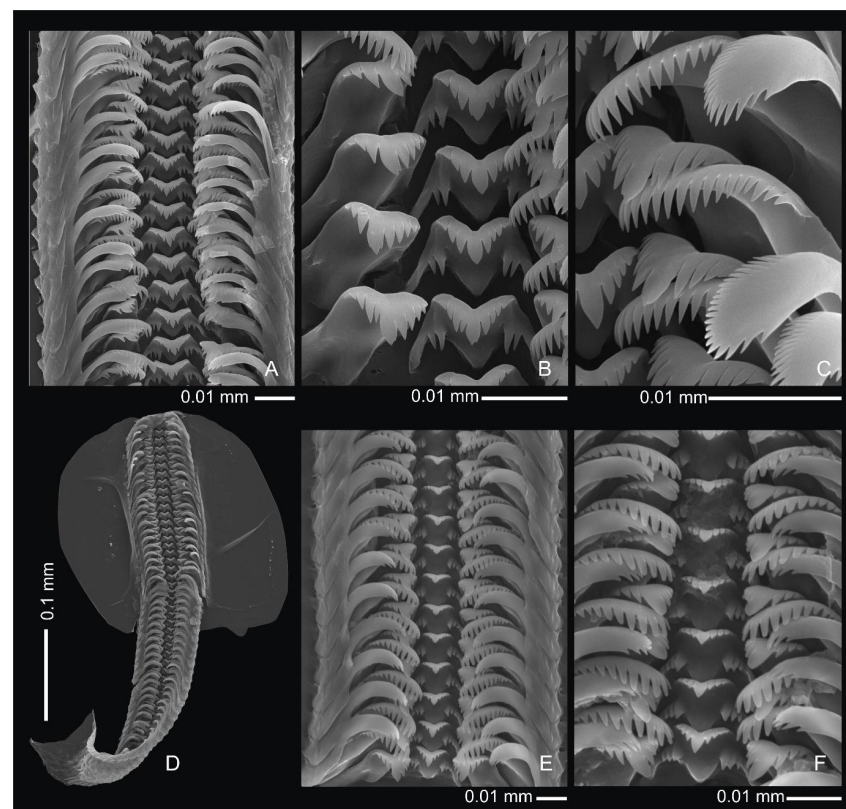


Figure 8. Details of the radula of *Clathrocaspia*: (A–D)—*Clathrocaspia laevigata* sp. n., from four different specimens from the locality Dag01; (E,F)—*C. knipowitschii*: (E)—specimen IZAN522_12; (F)—specimen IZAN522_13, both from the locality Kosh.

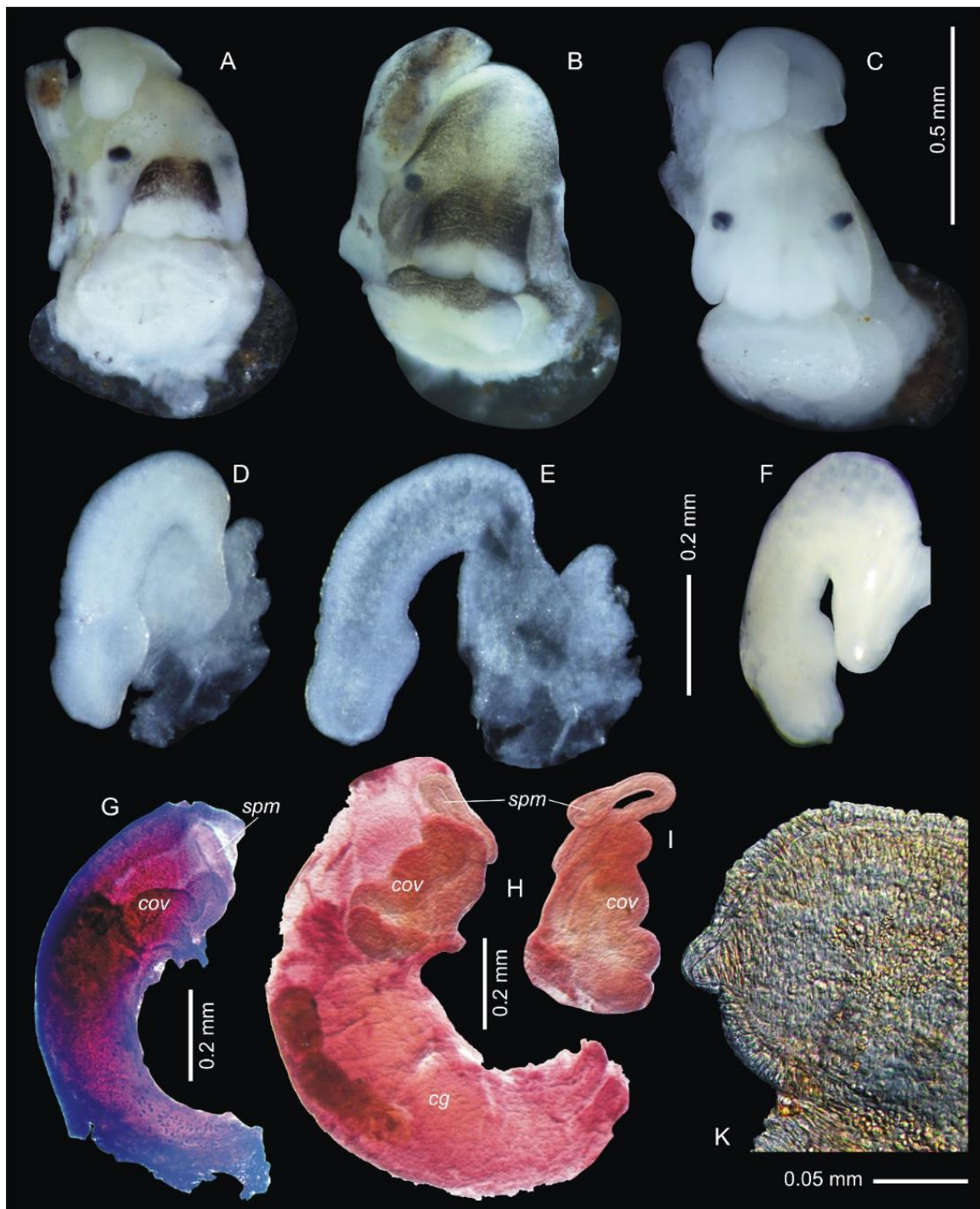


Figure 9. The soft body and details of the *Clathrocaspia* male and female reproductive system: (A,B,D,E,G,K)—*Clathrocaspia laevigata* sp. n.: (A,D,E)—male, specimen IZAN 612/2, loc. Dag28 (the same individual as Figure 4K) ((A)—front view, (D,E)—penis); (B)—female, specimen IZAN 613/1, loc. Dag32 (the same individual as Figure 4Q); (G)—ventral view of distal part of female genital system, specimen Dag28_TS, loc. Dag28; (K)—male, specimen Dag67_LCh, loc. Dag67 (pointed protrusion of the penis). (C,F,H,I)—*C. knipowitschii*: (C)—male, specimen IZAN 522_22, loc. Kosh (front view); (F)—male, specimen IZAN 522_13, loc. Kosh (penis); (H,I)—female, specimen IZAN 390_TS loc. KHS ((H)—ventral view and (I)—dorsal view of distal part of genital system). Abbreviations as seen in Figure 10.

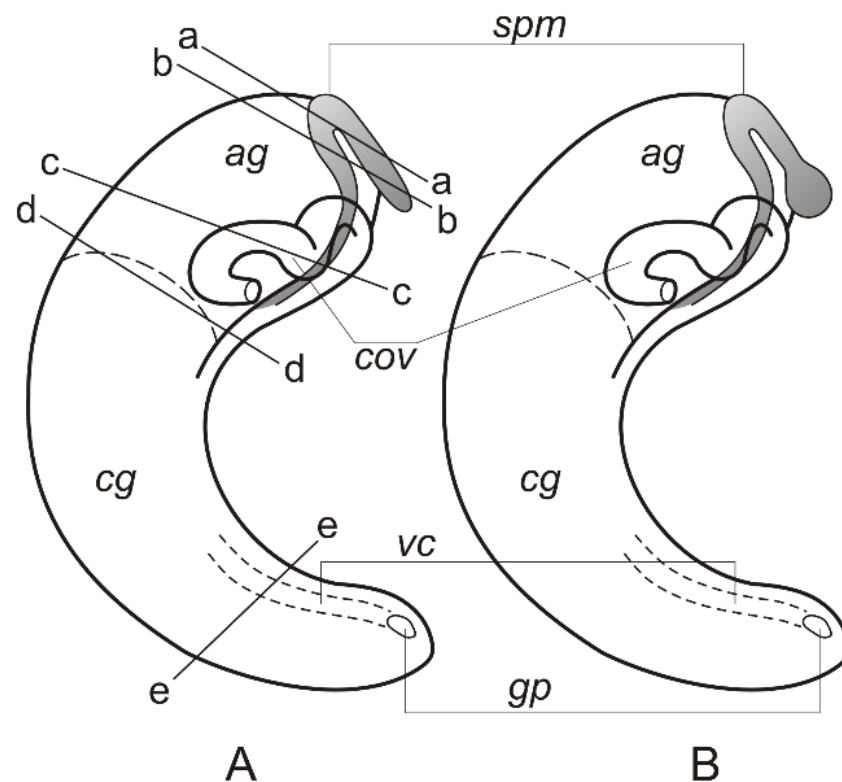


Figure 10. A scheme of the distal female genitalis of *C. laevigata* sp. n. (A) and *C. knipowitschii* (B) (ventral view of the oviduct and associated structures). Abbreviations: *ag*—albumen gland; *cg*—capsule gland; *cov*—coiled oviduct; *gp*—gonoporus; *spm*—spermatheca; *vc*—ventral channel of capsule gland. The histological sections of the *C. laevigata* sp. n. female genitals in positions a-a, b-b, c-c, d-d, and e-e are shown in Figure 11.

The protoconch (Figure 6H–M,P–T and Figure 7D–L) is relatively large, dome-like, consists of 1.35–1.45 whorls, and has the maximum diameter of 0.31–0.38 mm; the initial cap-like onset of the embryonic shell is 0.11–0.14 mm wide (Table 3). The protoconch surface is covered by a dense net of minute wrinkles and faint spiral threads placed at irregular interspaces (Figure 7D–L); the final 0.1 whorl of the protoconch is thickened (sometimes markedly), showing no or only traces of sculpture, except irregular growth lines. The transition to the teleoconch is abrupt, marked by a growth rim and the onset of growth lines (Figure 6H,J and Figure 7J,K).

Table 3. The morphometric characteristics of the protoconchs in three *Clathrocaspia* species. All measurements in mm. Above lines—min–max values; below lines—mean value $\pm$ SD. Abbreviations: NW—number of whorls; MD—maximum diameter of protoconch; WE—width of initial cap-like part of embryonic shell.

Species	Measurement		
	NW	MD	WE
<i>C. laevigata</i> (n = 16)	1.35–1.45	0.31–0.38	0.11–0.14
	1.41 $\pm$ 0.04	0.34 $\pm$ 0.02	0.12 $\pm$ 0.01
<i>C. knipowitschii</i> (n = 6)	1.15–1.30	0.30–0.38	0.13–0.15
	1.24 $\pm$ 0.05	0.34 $\pm$ 0.04	1.13 $\pm$ 0.02
<i>C. brotzkajae</i> (n = 2)	1.15–1.40	0.40–0.41	0.13–0.16

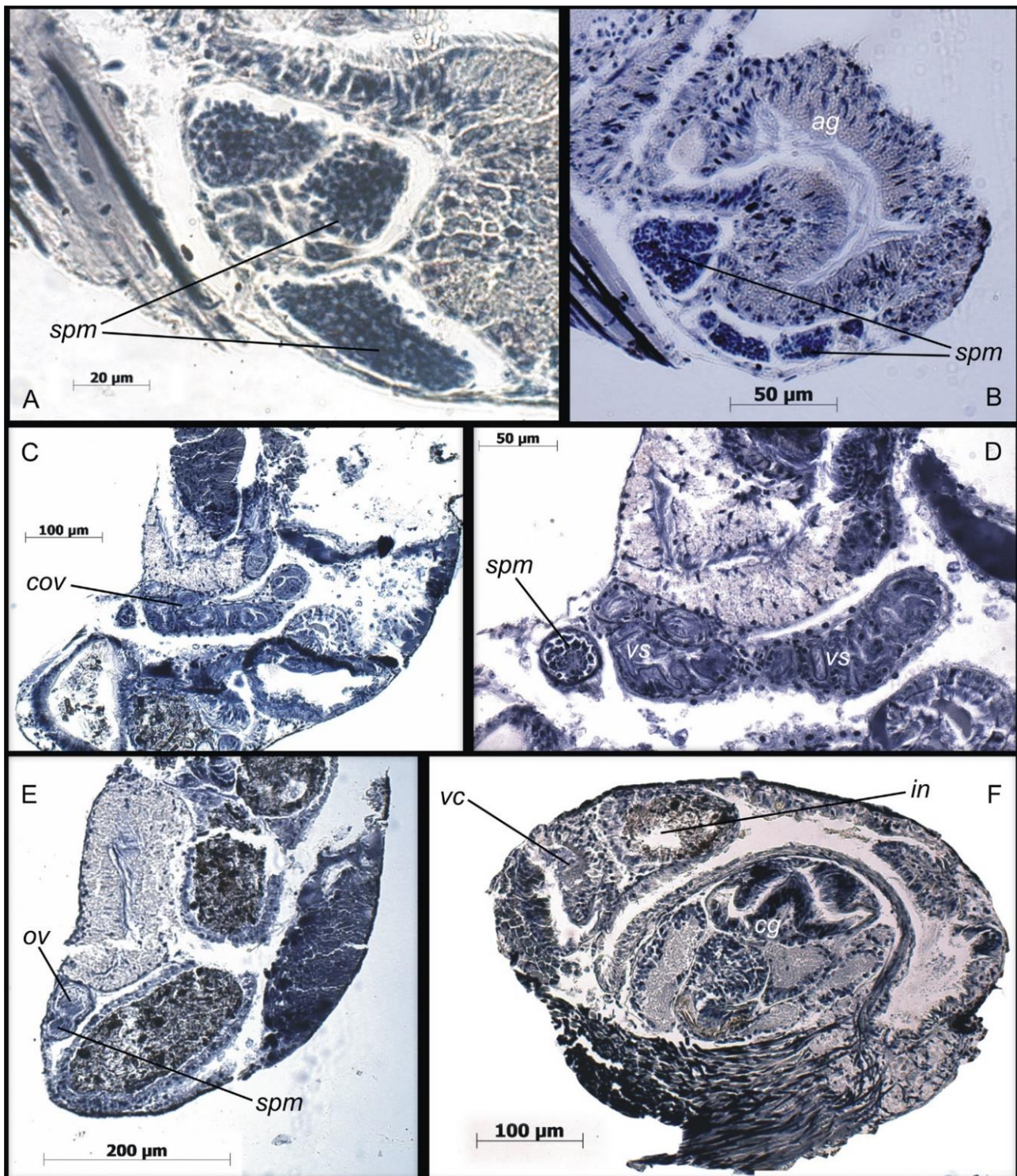


Figure 11. Histological sections of the female reproductive system of *C. laevigata* sp. n. (individual from the locality Dag28): (A,B)—sections a-a and b-b across two adjacent areas of spermatheca and albumen gland; (C,D)—section c-c across coiled renal loop and spermatheca; (E)—section d-d across oviduct and spermathecal duct; (F)—section e-e across capsule gland, ventral channel, and intestine. Abbreviations: *in*—intestine; *ov*—oviduct; *vs*—vesicles; other abbreviations as in Figure 10.

The operculum of the studied individuals (Figure 6N,O) is flat, thin, transparent, a little smaller than the aperture, ovate, and paucispiral with about 15 axial growth lines and a subcentral nucleus. The muscle attachment area edges are clearly differentiated.

There is no pronounced sexual dimorphism in the shell morphology of *Clathrocaspia laevigata* sp. n. According to non-parametric multivariate statistical analysis of shell variables, the differences between the sexes account for only 9.5% of all differences (PERMANOVA, $p < 0.05$). The shell size was the main factor in morphological differences ($p = 0.001$), in accordance with the PERMANOVA results; it is responsible for more than half of all interpopulation differences (54.9%). After excluding this effect (SH as a covariate), the differences between populations comprised only 9.9% of the total variability. Nevertheless, all populations are significantly different from each other, except for Dag 23 and Dag 32 (Tables 4 and 5). It can be assumed that the leading factor determining the differences between the studied samples is their possible age-related heterogeneity. The reliability of the differences between the samples, revealed after the size factor was removed, can be explained by various kinds of stochastic factors, which generate the observed pattern of intraspecific variability.

Table 4. Results of PERMANCOVA test with shell height (SH) and population identity (Population)) as covariates. p values are significant levels estimated by permutations of residuals ($p < 0.05$ are given in bold).

Source of Variation	df	SS	MS	Pseudo-F	p	Unique Perms	Variance Component (%)
SH	1	537.95	537.95	209.56	0.001	998	54.9
Population	4	82.144	20.536	7.9997	0.001	999	9.9
Residual variation	128	328.59	2.5671				35.3

Table 5. Results of pair-wise PERMANCOVA tests with shell height (SH) removed. P values are significant levels estimated by permutations of residuals ($p < 0.05$ are given in bold).

Samples	t	p	Unique Perms
Dag01 vs. Dag23	3.5008	0.001	999
Dag01 vs. Dag28	2.5745	0.001	999
Dag01 vs. Dag32	2.8936	0.001	999
Dag01 vs. Dag67	2.4135	0.004	999
Dag23 vs. Dag28	3.2519	0.001	999
Dag23 vs. Dag32	1.578	0.052	999
Dag23 vs. Dag67	3.1741	0.001	999
Dag28 vs. Dag32	3.0478	0.001	999
Dag28 vs. Dag67	2.733	0.001	999
Dag32 vs. Dag67	2.332	0.001	999

Radular morphology. The radula of *C. laevigata* sp. n. is typically taenioglossate, the length of the radular ribbon is around 0.45–0.50 mm, the width about 0.06–0.07 mm, and it bears 55–58 transverse rows (Figure 8A–D). The general shape of the rachidian (central) tooth is trapeziform with tapered latero-basal corners. The maximum width of the rachidian tooth is about 0.015–0.016 mm, and its height does not exceed 0.01 mm. On the cutting edge of the rachidian tooth, there are four lateral cusps on each side of the median cusp (4–1–4; Figure 8A–C), which is much larger than the lateral cusps and is triangular in shape. Two to three sub-basal cusps are well-developed on each side of the basal plate of the rachidian tooth. The lateral tooth formula is 3–1–3(4); the biggest cusp is long and massive (Figure 8B,C). On the inner marginal tooth, there are about 15–16 cusps, their length diminishing gradually from the long and slender distal cusps. The outer marginal tooth bears up to 23 long and slender cusps, their size gradually decreasing from the central part (Figure 8C, Table 6).

Table 6. Generalised data of the radular characteristics of *Clathrocaspia* species used in the present study.

Characteristics	<i>C. laevigata</i> sp. n.	<i>C. gmelinii</i> and <i>C. gaillardi</i> (after Sitnikova & Starobogatov 1998)	<i>C. knipowitchii</i>
number of transverse rows of the radula	55–58	no data	around 55
length of the radular ribbon, mm	0.45–0.50	no data	0.23–0.25
width of the radular ribbon, mm	0.06–0.07	no data	0.06–0.07
height of the rachidian (central) tooth, mm	0.0080–0.0100	0.0081–0.0105	0.0080–0.0108
width of the rachidian (central) tooth, mm	0.0150–0.0174	0.0129–0.0142	0.0150–0.0200
number of lateral cusps on the cutting edge of the rachidian tooth	4 (rarely 3)	4–5	3 (rarely 4)
number of sub-basal cusps on the cutting edge of the rachidian tooth	2–3	2–3	2–3
number of cusps on the lateral tooth	3–5	3	3–4
number of cusps on the inner marginal tooth	15–16	>10	12–14
number of cusps on the outer marginal tooth	20–23	no data	13–15

Soft body and the reproductive anatomy. The snout and the body are faintly brown or almost unpigmented (Figure 9A,B); the ctenidium is lying in the middle part of the mantle cavity; a large osphradium is located in the middle of the gill with 12–14 triangular leaflets ($n = 4$).

The penis is non-pigmented, colorless or light yellow, blunt, and broad; its base lies behind the left tentacle; in adults, it is bent towards the opening of the mantle cavity. The vas deferens runs approximately in the center of the penis or slightly closer to its base and opens at a pointed protrusion (Figure 9D,E,K).

The female reproductive system with an albumen gland, which is shorter in length than the capsule gland, coiled renal oviduct, and forming a large loop, covers slightly less than half of the surface of the albumen gland (Figures 9G and 10A). Inside the renal loop, small vesicles, possibly functioning as the bursa copulatrix, are seen (Figure 11C,D). The upper part of the albumen gland is covered by an expanded loop of spermatheca, which narrows towards the end. The spermatheca contains an assembly of “pouches” (or ampoules) (Figure 11A,B). The spermathecal duct narrows close to the junction with the oviduct (Figure 11E). The ventral channel runs along the lateral side of the capsule gland and is separated from the lumen of the gland with thickened folds (Figure 11F).

Distribution and ecology. *C. laevigata* sp. n. is known only from the Samur River delta, within the Samursky National Park, Dagestan (Russia). The available distributional data on *Clathrocaspia laevigata* sp. n. show that the range of the species covers the eastern part of the Dagestan shore (see Figure 1). Interestingly, the known locations of the new species are situated in the streams of the Samur River delta but not in the Samur River itself (see below). We expect to find other localities of this species in the similar waterbodies of the Samur Forest within the adjacent territory of Azerbaijan.

C. laevigata sp. n. is absent from lentic localities; several lakes in the vicinity of Makhachkala were examined, but no individuals of this species were found.

According to our data, these snails seem to prefer freshwater conditions (most streams have salinity up to 0.5‰), but they can withstand low salinity up to about 2‰. One population of *C. laevigata* sp. n. was discovered in a stream located directly on the open beach of the Caspian Sea (see Figure 1, locality Dag65). The upper course of this stream is freshwater, and apparently, the increased salinity at its mouth was caused by the influence of the salinated soils of its bed. The salinity tolerance of *C. laevigata* sp. n. is, hence, similar to that of *C. knipowitchii* in the lower courses of the Dnieper River, where this snail is reported from localities with salinity up to 1.5 ‰ [37]. Populations of *C. laevigata* sp. n. occur in lotic habitats of different type (streams, springs, and small rivers), the preferred substrate is rocky bottom. The water flow velocity in the studied streams varies between 0.2 and 0.6 m/s. The distribution of the population is somewhat patchy: usually these snails are found in some parts of streams but are absent in other parts of the same watercourse.

The known localities of *C. laevigata* sp. n. are situated in a relatively narrow zone near to the sea coast, with maximum distance from the sea about 6 km (see Figure 1). However, no specimens of this species were found in the sea proper, and it seems unlikely that the snails can resist the conditions of the adjacent parts of the Caspian Sea, with salinity of about 11‰.

One of the streams inhabited by the new species (Figure 1, locality Dag66) flows through cultivated fields and is getting highly polluted and muddy water and silted substrates. Almost no other living invertebrates have been found there, but *C. laevigata* sp. n., surprisingly, were present, forming a flourishing population with high density. This fact indicates the resistance of this species against anthropogenic pollution.

Remarks. Compared to other *Clathrocaspia* species studied during this work (*C. knipowitschii* and *C. brotzkajae*), *C. laevigata* sp. n. has significantly more thin shell walls, almost transparent teleoconch, and slightly longer embryonic shell (ca. 0.05–0.15 whorls) (Table 3). The reticulate sculpture of the teleoconch, which is well-developed in all previously described members of the genus, is absent in *C. laevigata* sp. n. (Figures 4–7). Possibly, both the thin-walled shell and the smooth teleoconch have evolved during the adaptation to living in freshwater habitats; however, the potential adaptive significance of these traits remains unexplained.

The mean shell height in *C. laevigata* sp. n. is less than in the other studied species of this genus (see Table 2). However, having a longer (but not larger!) protoconch and the cap-like onset almost identical in size to that of *C. knipowitschii* (Table 3), the Dagestan individuals of *Clathrocaspia* apparently grow more slowly than their congeners from the Dnieper River (see Section 4).

The three *Clathrocaspia* species studied conchometrically form almost separate, albeit partially overlapping, clouds of points in the plane formed by the two first principal components (Figure 12A). However, it should be noted that the differences between the *C. laevigata* sp. n. and the two other species revealed through this analysis lie chiefly in the differences in the first PC, which, in most cases, represent the ‘size variable’, being tightly correlated to the shell height. Thus, the observed separateness of the three clouds of points may be explained by the fact that the shells of the new species are relatively smaller (see above). Indeed, the scatter plot based on the second and third PCs shows the increased overlap between the three compared species in the morphospace (Figure 12B).

There are no significant differences in the quantitative parameters of the radula between *C. laevigata* sp. n. and *C. knipowitschii* (Figure 8, Table 6). However, some minor differences can be reported here. Namely, the rachidian tooth of the radula of snails from the Dnieper River bears usually three (rarely two) basal cusps, whereas, in the Dagestan individuals, teeth with two and three cusps occur equally often. Apart from this, the radula of each individual specimen of *C. laevigata* has a various number of basal cusps, while in all studied specimens of *C. knipowitschii* it has only either two or three cusps in one radular ribbon. Another constant difference revealed is that the inner marginal tooth of *C. laevigata* bears 15–16 cusps on the cutting edge, while in *C. knipowitschii*, there are 12–13 cusps. Judging from the data provided by Sitnikova & Starobogatov [28], the rachidian tooth of *C. gmelinii* and *C. gaillardi* is morphologically similar to that of *C. laevigata*.

The data on the reproductive anatomy of other *Clathrocaspia* species have been available for *C. gmelinii* and *C. gaillardi* inhabiting the Caspian Sea [28], and for *C. milae* (Boeters, Glöer & Georgiev in Boeters et al. 2015) of the Danube Basin [26]; the latter species has recently been synonymized with *C. knipowitschii* [27]. We additionally studied the soft body and copulatory apparatus as well as the female genitalia of *C. knipowitschii* from the native range of this species (Figure 9C,F,H,I). The copulatory apparatus morphology of *C. laevigata* sp. n. resembles that of *C. knipowitschii*; the general topography of the female reproductive organs of these species is also similar (Figures 9 and 10). However, the female anatomy of *C. laevigata* sp. n. differs from that of *C. knipowitschii* by the narrowed end of spermatheca—in *C. knipowitschii* it is ovate-broadened with a long duct (Figures 9 and 10). In *C. milae* (= *C. knipowitschii*), the second loop of the renal oviduct carries a large ovate receptaculum without a visible duct (see [26]: Figures 19–21). According to our data, no

significant differences in the female reproductive anatomy among *C. laevigata* sp. n., *C. gmelinii*, and *C. gaillardii* can be reported (see [28]: Figures 1, 12 and 13). The presence of not oriented sperm assembled in “pouches” (or ampoules) in the spermatheca of *C. laevigata* sp. n. suggests that the thickening of the penis is glandular, which allows males to produce spermatophores in the form of small ampoules.

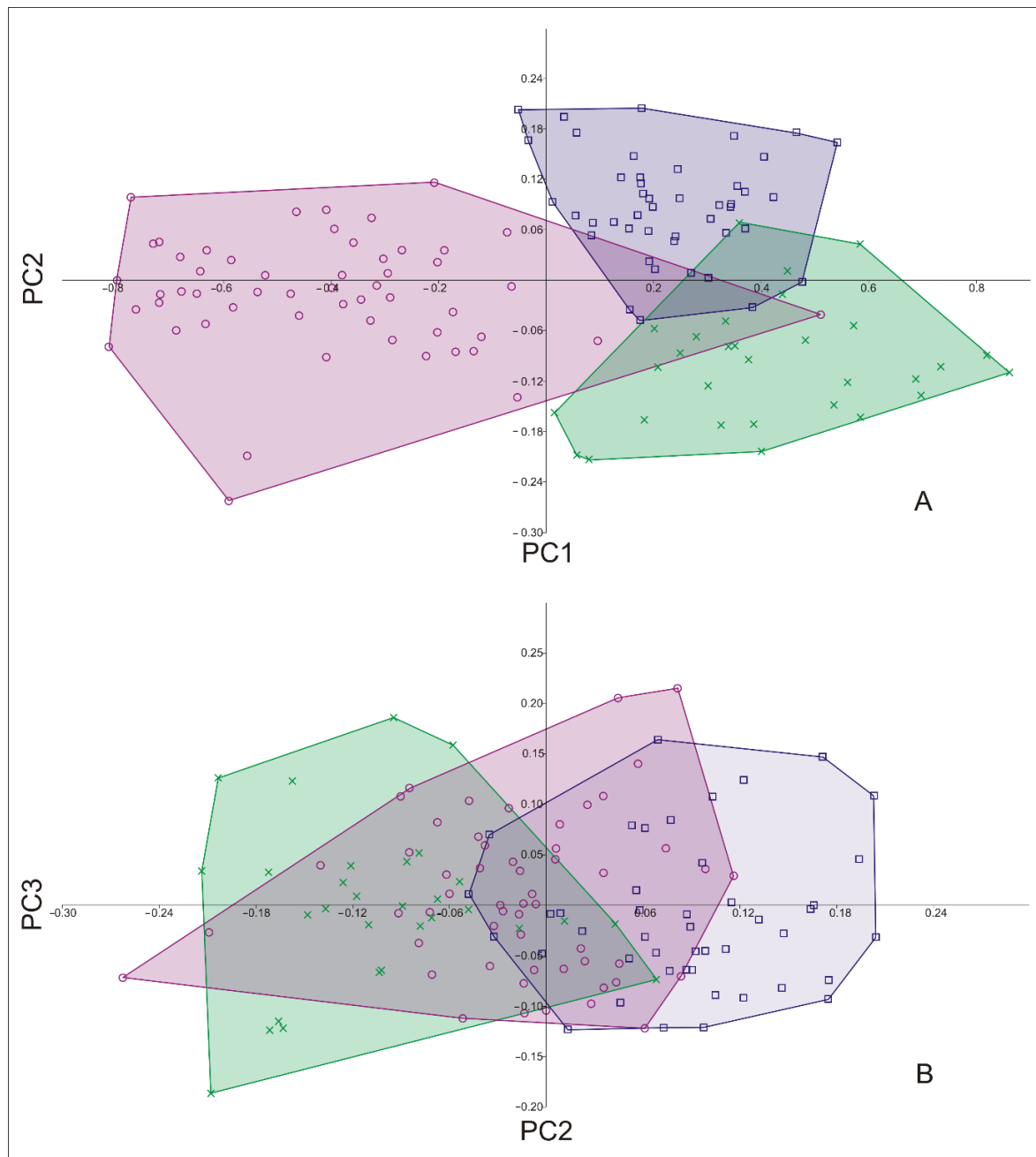


Figure 12. The results of the principal component analysis of shell measurements of three species of *Clathrocaspia*. The data of Table 2 were taken as the primary data. (A)—A scatter plot of individuals in the plane of the first two PCs. (B)—A scatter plot of individuals in the plane of the second and third PCs. The three first PCs explain 97.9% of the variation (PC1–90.0%; PC2–4.9%; PC3–3.0%). Purple color denotes *C. laevigata* sp. n., green denotes *C. brotzkajae*; and blue denotes *C. knipowitschii*.

4. Discussion

This surprising discovery of a new species of the genus *Clathrocaspia* in the streams of the Caspian Sea Basin poses some questions about its significance for the studies of ecology, biogeography, and conservation of the Ponto-Caspian endemic malacofauna. From the ecological point of view, the new species is remarkable as the first purely freshwater species of *Clathrocaspia* known to inhabit the Caspian Sea Basin.

Though the genetic distance between *Clathrocaspia* sp. and *C. knipowitschii* is considerably high for the COI gene, these two species remain the only members of the genus studied genetically. There is, therefore, a possibility that the snails found in Dagestan can belong to one of the already described nominal species of *Clathrocaspia*. However, as it was stated above, the chances to obtain fresh specimens of the Caspian Sea *Clathrocaspia* for a genetic study are rather low, and we are unable to check this possibility.

One of the curious biological features of Dagestan *Clathrocaspia laevigata* sp. n. is their ability to live and reproduce in the absence of the zebra mussel (*Dreissena polymorpha*). In our earlier publications, we documented the commensalistic interactions between *Dreissena* and *C. knipowitschii* populations in the Dnieper River delta, Black Sea Basin. *Clathrocaspia* snails are unable to reproduce without mussels, since their egg masses are deposited exclusively on the shell surface of *Dreissena* or among their byssus threads [19,38]. Remarkably enough, that almost all paleontological findings of *Caspia* s. lato from the Pliocene-Holocene sediments of the Ponto-Caspian Basin are made from deposits also containing shells of *Dreissena* or *Congeria*, the latter being the ancestral genus for some recent dreissenids [39]. Thus, the commensalistic relationships between these two groups of molluscs may be of substantial geological age.

As it was stated above, the adult shell of *C. laevigata* sp. n. is somewhat smaller on average than the shell of *C. knipowitschii*, whereas the maximum protoconch diameter in both species is practically identical (Tables 2 and 3). The same size of the hatchlings combined with a slightly smaller adult size implies a slower growth rate of the new species that can be determined by the colder waters in their habitats as compared to the rivers of the Black Sea region. The water temperature of the Dagestan streams was about 16–17 °C (measured on 6–8 August 2021) when the air temperature was around 40–43 °C (our data). The water temperature in the lower Dnieper near Kherson City in the same period can reach 26 °C, i.e., it is nearly 10 degrees higher than in the Dagestan streams (V. Anistratenko, unpublished data). As a rule, the growth rates in gastropods positively correlate with the ambient temperature. Similarly, the hatching can occur earlier under warmer conditions (for a discussion of these phenomena in relation to Caspiinae and the relevant references, see [19]). The scenario of the decreased growth rate in *C. laevigata* sp. n. as a function of the temperature fits well to the differences in the water temperature in the Samur River delta and the Dnieper River near Kherson.

The available faunal information reveals the macroinvertebrate fauna of the Samur River is rather rich and completely different from the fauna of streams in the Samur River delta. The reasons for the absence of *Clathrocaspia* in the Samur River are not clear yet. The Samur River is fed by glaciers and is in a state of flooding (often catastrophic) in summer, whereas, in winter, its level changes drastically due to rainfall. As a result, the extremely unstable hydrological regime causes unfavorable conditions of the river bottom substrates, which may explain the absence of *Clathrocaspia* snails from the Samur River. On the contrary, the hydrological regime of streams and springs in the Samur River delta is excellently stable. Probably for this reason, amphibiotic insects produce almost 100% of the biomass in the Samur River, whereas in the streams and springs of its delta, up to 80% of the total biomass is formed by molluscs and crustaceans [Dmitry Palatov, pers. observation]. Thus, three different macroinvertebrate faunas, namely, those of the Samur River (i), waterflows of its delta (ii), and the Caspian Sea (iii), having virtually no shared species, occur within a relatively restricted area.

The Samur River delta is protected legislatively in Russia as a part of a national park. The discovery of a new and presumably endemic gastropod species here increases the conservation significance of this territory. Moreover, the delta is the home for a series of other endemic aquatic invertebrates. In particular, four endemic crustacean species have recently been described from this area [40]. The list of aquatic molluscs of the Samur River delta includes 12 species of pulmonate snails such as *Lymnaea* spp. as well as *Valvata cristata* (O.F. Müller, 1774) and *Theodoxus fluviatilis* (Linnaeus, 1758) [D. Palatov, unpublished data]. Such a relatively high richness in macroinvertebrates is a little surprising since this area has periodically been flooded by the Caspian Sea's waters [41].

From the biogeographical point of view, the range of the genus *Clathrocaspia* lies within the Ponto-Caspian Region delineated by Starobogatov [42]. According to the available faunal and ecological information [4,13,27,40], the genus *Clathrocaspia* inhabits the Caspian Sea itself but does not occur in the Sea of Azov and the Black Sea. In the Azov–Black Sea Basin, *Clathrocaspia* predominantly live in the estuarine areas and limans of large rivers discharging into the Sea of Azov and the northern Black Sea [19–25,43,44]. The ranges of *Clathrocaspia* species are typically very limited, and, in some cases, these are restricted to a few locations: For instance, only two localities in the Don River delta are known for *C. logvinenkoi* (Golikov and Starobogatov, 1966) [24]. Recently, the non-native populations of *C. knipovitschii* have been found in different localities in the Danube River situated in Bulgaria, Hungary, Slovakia, Romania, and Serbia [27], which shows that *Clathrocaspia* can form sustainable populations in purely freshwater areas remote from the river estuarine areas. However, no such findings in the Caspian region have been reported. In terms of ecology, the molluscs discovered in Dagestan are strictly riverine and freshwater, withstanding a water salinity of up to about 2‰, like the Black Sea coast's *Clathrocaspia*.

The finding discussed in this study updates the biogeographic paradigm and assumes a new strategy for the further search for still undescribed species of this genus. The search should be directed not only in the Caspian Sea but aimed at small rivers on the western coast of the Caspian Sea. It can be expected, for example, the presence of freshwater *Clathrocaspia* in the lower reaches of the rivers in the Makhachkala region (Turali), as well as in the mouth of the Kura and Astara rivers. The focal habitats during these searches must not be large rivers but small streams/springs situated within their basins.

The localities of *Clathrocaspia* found in Dagestan cannot be assigned to any of the zoogeographical units in the regionalization scheme of the Caspian Sea proposed by Starobogatov [42]. The reason for this is that all the units delineated by Starobogatov are located within the aquatory of the lake proper and do not embrace the areas adjacent to the Caspian Sea. It may be necessary to establish a special zoogeographic unit for the habitats of *C. laevigata* sp. n. and similar habitats of the circum-Caspian freshwater *Clathrocaspia*, if found.

Another important open question regards the geological age of the discovered populations and possible mechanisms and sources of their formation. Unfortunately, the available molecular data about the genus *Clathrocaspia* are too scarce in order to discuss these issues here, even speculatively. Perhaps, a museomic study of the “historical DNA” extracted from the ethanol-fixed specimens of *Clathrocaspia* and related genera will help, in the future, to resolve these issues.

5. Conclusions

The recent discovery of a series of new species of freshwater invertebrates (molluscs, crustaceans) presumably endemic to the Samur River delta allows us to postulate the existence of a local microrefugium for the Ponto-Caspian species, which had survived here unfavorable periods, e.g., the changes in salinity or the Caspian Sea level's fluctuations. In addition to *Clathrocaspia*, the Ponto-Caspian amphipods (*Echinogammarus*) and mysids (*Limnomysis*), as well as ancient freshwater relic isopods (*Proasellus*) and amphipods (*Niphargus*, *Diasynurella*), inhabit small rivers, streams, and springs of this area [40]. Apparently, most of these crustacean species do not enter the Caspian Sea and live here only in freshwater environments.

The existence of a purely freshwater species of *Clathrocaspia* in the streams adjacent to the Caspian Sea opens an even more tempting perspective. Is it possible that at least some snail species, which are putatively listed among the Caspian Sea endemics but had never been found alive in this waterbody, live, in fact, in such freshwater refugia. If it is the case, then their empty shells, after having been moved to the sea with flowing water, might have been misinterpreted by the researchers as shells of the true Caspian Sea molluscs and described as such. In other words, the inflow of shells of purely freshwater species is, alongside the washing out the subfossil shells from the Pleistocene or Early Holocene sediments, a “source” of the presumably Caspian fauna. This hypothesis requires factual confirmation, but, if it appears true, it will cause a major revision of the current assessments of the Caspian Sea biodiversity and change the existing regionalization schemes based on the mollusc distribution.

The ecology and biogeography of *Clathrocaspia laevigata* sp. n. implies that further discoveries of endemic freshwater species of this group might be expected from waterbodies of a similar type located in the neighboring territories (e.g., Azerbaijan), as well as other areas of the circum-Caspian region such as Northern Iran. This consideration appeals to an urgent faunal investigation of such habitats, which may maintain a host of endemic species and even genera of aquatic molluscs and other macroinvertebrates. Given that the Ponto-Caspian region, and its unique biota suffer from a range of negative impacts, including extensive habitat degradation (see [1–3] and references therein), there is an urgent need for a taxonomic exploration for the circum-Caspian zone, which would help to determine the existing local hotspots of aquatic biodiversity and the potential microrefugia like that of the Samursky National Park.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14040232/s1>, Table S1. The data on specimens of *Clathrocaspia* spp. used in the molecular study.

Author Contributions: Conceptualization, V.V.A., O.Y.A. and M.V.V.; methodology, V.V.A., E.M.C. and M.V.V.; software, E.M.C. and C.C.; validation, V.V.A. and M.V.V.; molecular analysis, C.C.; statistical analysis, E.M.C. and M.V.V.; fieldwork, D.M.P.; morphological study, V.V.A., O.Y.A., E.M.C. and T.Y.S.; writing—original draft preparation, V.V.A.; writing—review and editing, all authors; visualization, V.V.A., O.Y.A., E.M.C., D.M.P., T.Y.S. and M.V.V.; supervision, V.V.A. and M.V.V.; project administration, V.V.A.; funding acquisition, M.V.V. All authors have read and agreed to the published version of the manuscript.

Funding: The basic financial support for this work was obtained from the Russian Scientific Fund (project No. 21-14-04401). Vitaliy Anistratenko and Catharina Clewing were supported by the German Research Foundation (DFG, grant no. WI1902/17). Olga Anistratenko was partially supported by the National Academy of Sciences of Ukraine, project no. 0122U001609.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The primary data and materials for this study are placed in some public repositories (zoological museums); the newly obtained DNA sequences were submitted to GenBank. See the text for more information.

Acknowledgments: The authors thank Pavel Kijaschko (ZIN, St. Petersburg) for his friendly help during our work with the collection of the Zoological Institute, Russian Academy of Sciences. The authors are grateful to Roman A. Rakitov (Paleontological Institute of Russian Academy of Sciences, Moscow) for the assistance provided in obtaining SEM micrographs. The authors also express their gratitude to Igor A. Kosevich (Lomonosov Moscow State University, Moscow) for his help in obtaining photographs using DIC microscopy. We are grateful to A. Vorobieva (Limnological Institute, Siberian Branch of Russian Academy of Sciences, Irkutsk) for making histological sections. The authors wish to thank three anonymous reviewers and the Academic Editor for their suggestions on how to improve the original text.

Conflicts of Interest: The authors declare no conflict of interests.

References

- Lattuada, M.; Albrecht, C.; Wilke, T. Differential impact of anthropogenic pressures on Caspian Sea ecoregions. *Mar. Pollut. Bull.* **2019**, *142*, 274–281. [CrossRef] [PubMed]
- Lattuada, M.; Albrecht, C.; Wesselingh, F.P.; Klinkenbuß, D.; Vinarski, M.V.; Kijashko, P.; Raes, N.; Wilke, T. Endemic Caspian Sea mollusks in hotspot and non-hotspot areas differentially affected by anthropogenic pressures. *J. Great Lakes Res.* **2020**, *46*, 1221–1226. [CrossRef]
- Gogaladze, A.; Son, M.O.; Lattuada, M.; Anistratenko, V.V.; Syomin, V.L.; Pavel, A.B.; Popa, O.P.; Popa, L.O.; ter Poorten, J.-J.; Biesmeijer, J.C.; et al. Decline of unique Pontocaspian biodiversity in the Black Sea Basin: A review. *Ecol. Evol.* **2021**, *11*, 12923–12947. [CrossRef] [PubMed]
- Logvinenko, B.M.; Starobogatov, Y.I. Mollusca. In *Atlas Bespozvonochnykh Kaspiyskogo Morya*. [The Atlas of Invertebrates of the Caspian Sea]; Birshtein, Y.A., Vinogradov, L.G., Kondakov, N.N., Kuhn, M.S., Astakhova, T.V., Romanova, N.N., Eds.; Pishchevaya Promyshlennost: Moscow, Russia, 1969; pp. 308–385. (In Russian)
- Kijashko, P. Chapter 5. Mollusca of the Caspian Sea. In *Opredelitel' Ryb i Bespozvonochnykh Kaspiyskogo Morya*. T. 1. Ryby i Mollyuski [Identification Keys for Fish and Invertebrates of the Caspian Sea. 1 Fish and Molluscs]; Bogutskaya, N.G., Kijashko, P.V., Naseka, A.M., Orlova, M.I., Eds.; KMK Scientific Press Ltd.: Moscow, Russia, 2013; pp. 292–392. (In Russian)
- Wesselingh, F.P.; Neubauer, T.A.; Anistratenko, V.V.; Vinarski, M.; Yanina, T.; Ter Poorten, J.J.; Kijashko, P.; Albrecht, C.; Anistratenko, O.; D'Hont, A.; et al. Mollusc species from the Pontocaspian region—An expert opinion list. *ZooKeys* **2019**, *827*, 31–124. [CrossRef] [PubMed]
- Anistratenko, V.V.; Zettler, M.L.; Anistratenko, O.Y. On the taxonomic relationship between *Theodoxus pallasi* and *T. astrachanicus* (Gastropoda: Neritidae) from the Ponto-Caspian region. *Arch. Molluskenkd.* **2017**, *146*, 213–226. [CrossRef]
- Sands, A.F.; Neubauer, T.A.; Nasibi, S.; Harandi, M.F.; Anistratenko, V.V.; Wilke, T.; Albrecht, C. Old lake versus young taxa: A comparative phylogeographic perspective on the evolution of Caspian Sea gastropods (Neritidae: *Theodoxus*). *R. Soc. Open Sci.* **2019**, *6*, 190965. [CrossRef] [PubMed]
- Eichwald, E. Faunae Caspii Maris primitiae. *Bull. Société Impériale Nat. Moscou* **1838**, *11*, 125–174.
- Eichwald, E. Zur Naturgeschichte des Kaspischen Meeres. *Nouv. Mémoires Société Impériale Nat. Moscou* **1855**, *10*, 283–323.
- Grimm, O.A. The Caspian Sea and its Fauna. Fascicle 1. *Tr. Aralo-Kaspiyskoy Ekspeditsii* **1876**, *2*, i–v + 1–168. (In Russian)
- Grimm, O.A. The Caspian Sea and its Fauna. Fascicle 2. *Tr. Aralo-Kaspiyskoy Ekspeditsii* **1877**, *2*, i–ii + 1–105. (In Russian)
- Dybowski, W. (1887–1888) Die Gasteropoden-Fauna des Kaspischen Meeres. Nach der Sammlung des Akademikers Dr. K.E. v. Baer. *Malakozoologische Blätter*, Neue Folge, *10*, 1–79. (1–3), 1–64 (Issue 1, 1887), 65–79 (Issue 2, 1888), pls. 1–3 (Issue 3, 1888). 10. Available online: <https://www.biodiversitylibrary.org/page/35483241#page/211/mode/1up> (accessed on 5 March 2022).
- Sowinsky, V.K. *An Introduction to the Study of the Fauna of the Ponto-Caspian-Aral Sea Basin, Viewed as a Separate Zoogeographical Province*; The St. Vladimir Imperial University Press: Kiev, Ukraine, 1902; 216p. (In Russian)
- Dybowski, B.; Grochmalicki, J. *Ueber Kaspische Schnecken aus Der Abteilung "Turricaspiinae" Subfam. Nova Zum Vergleich Mit Den Turribaikalia Nobis*; Zoological Museum of the Imperial Academy of Sciences: Petrograd, Russia, 1915; pp. 103–136, (a separatum of an unpublished article).
- Dybowski, B.; Grochmalicki, J. Studien über die turmförmigen Schnecken des Baikalsees und des Kaspimeeres (Turribaicaliinae-Turricaspiinae). *Abh. Der Kais.-Königlichen Zool.-Bot. Ges. Wien* **1917**, *9*, 1–55.
- Kolesnikov, V.P. A table for identification of the Caspian gastropods. *Byulleten' Mosk. Obs. Ispyt. Prir. Otd. Geol.* **1947**, *22*, 105–112. (In Russian)
- Vinarski, M.V.; Kantor, Y.I. *Analytical Catalogue of Fresh and Brackish Water Molluscs of Russia and Adjacent Countries*; A.N. Severtsov Institute of Ecology and Evolution of RAS: Moscow, Russia, 2016; 544p.
- Anistratenko, V.V.; Neubauer, T.A.; Anistratenko, O.Y.; Kijashko, P.V.; Wesselingh, F.P. A revision of the Pontocaspian gastropods of the subfamily Caspiinae (Caenogastropoda: Hydrobiidae). *Zootaxa* **2021**, *4933*, 151–197. [CrossRef] [PubMed]
- Makarov, A.K. Distribution of some crustaceans (Mysidacea, Cumacea) and liman mollusks in estuaries and open limans of the northern Black Sea region. *Zool. Zhurnal* **1938**, *17*, 1055–1062. (In Russian)
- Golikov, A.N.; Starobogatov, Y.I. Ponto-Caspian gastropods in the Azov-Black Sea Basin. *Zool. Zhurnal* **1966**, *45*, 352–362. (In Russian)
- Golikov, A.N.; Starobogatov, Y.I. Class Gastropoda Cuvier, 1797 [Mollusca-Gastropoda]. In *Opredelitel' Fauny Chernogo i Azovskogo Morey: Svobodnozhivushchiye Bespozvonochnyye*. T.3. Chlenistonogiye (Krome Rakoobraznykh), Mollyuski, Iglokozhiye, Shchetinkochelyustnyye, Khordovyye [Identification Key to the Fauna of the Black and Azov Seas, Free Living Invertebrates. Volume 3. Arthropoda (besides Crustacea), Mollusca, Echinodermata, Chaetognatha, Chordata]; Naukova Dumka: Kiev, Ukraine, 1972; pp. 65–166. (In Russian)
- Alexenko, T.L.; Starobogatov, Y.I. Species of *Caspia* and *Turricaspia* (Gastropoda, Pectinibranchia, Pyrgulidae) of the Azov-Black Sea Basin. *Vestn. Zool.* **1987**, *21*, 32–38.
- Anistratenko, V.V. Finding of the extremely rare hydrobiid *Caspia logvinenkoi* (Mollusca: Gastropoda) in the estuary of the River Don and its zoogeographical significance. *Mollusca* **2007**, *25*, 23–26.
- Anistratenko, V.V. On the taxonomic status of the highly endangered Ponto-Caspian gastropod genus *Caspia* (Gastropoda: Hydrobiidae: Caspiinae). *J. Nat. Hist.* **2013**, *47*, 51–64. [CrossRef]
- Boeters, H.D.; Glöer, P.; Georgiev, D.; Dedov, I. A new species of *Caspia* Clessin et W. Dybowski, 1887 (Gastropoda: Truncatelloidea: Hydrobiidae) in the Danube of Bulgaria. *Folia Malacol.* **2015**, *23*, 177–186. [CrossRef]

27. Szekeres, J.; Beermann, A.; Neubauer, T.A.; Ocadlik, M.; Paunovic, M.; Rakovic, M.; Csányi, B.; Varga, A.; Weigand, A.; Wilke, T.; et al. Rapid spread of a new alien and potentially invasive species, *Clathrocaspia knipowitschii* (Makarov, 1938) (Gastropoda: Hydrobiidae), in the Danube River. *Arch. Biol. Sci.* **2022**, *6*. [\[CrossRef\]](#)
28. Sitnikova, T.Y.; Starobogatov, Y.I. Reproductive system and radula of Caspian Pyrgulidae (Turricaspiinae and Caspiinae subfamilies—Gastropoda, Pectinibranchia). *Zool. Zhurnal* **1998**, *77*, 1357–1367. (In Russian with English summary)
29. Lillie, R.D. *Histopathologic Technic and Practical Histochemistry*; McGraw-Hill Book Company: New York, NY, USA, 1965.
30. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **2001**, *26*, 32–46. [\[CrossRef\]](#)
31. Anderson, M.J.; Gorley, R.N.; Clarke, K.R. *PERMANOVA + for PRIMER: Guide to Software and Statistical Methods*; PRIMER-E Ltd.: Plymouth, UK, 2008.
32. Wilke, T.; Davis, G.M.; Qiu, D.; Spear, R.C. Extreme mitochondrial sequence diversity in the intermediate schistosomiasis host *Oncomelania hupensis robertsoni*: Another case of ancestral polymorphism. *Malacologia* **2006**, *48*, 143–157.
33. Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **1994**, *3*, 294–299. [\[PubMed\]](#)
34. Clement, M.; Posada, D.; Crandall, K.A. TCS: A computer program to estimate gene genealogies. *Mol. Ecol.* **2000**, *9*, 1657–1659. [\[CrossRef\]](#)
35. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [\[CrossRef\]](#)
36. Anistratenko, V.; Anistratenko, O.; Kadolsky, D. Karl E. von Baer’s collection of Caspian Sea molluscs stored in the Zoological Museum of Lviv University, Ukraine. Part 2. Type materials of gastropod species described by Stephan Clessin and Władysław Dybowski in 1887–1888. *Arch. Für Molluskenkd.* **2019**, *148*, 35–62. [\[CrossRef\]](#)
37. Neubauer, T.A.; Van De Velde, S.; Yanina, T.; Wesselingh, F.P. A late Pleistocene gastropod fauna from the northern Caspian Sea with implications for Pontocaspian gastropod taxonomy. *ZooKeys* **2018**, *770*, 43–103. [\[CrossRef\]](#)
38. Alexenko, T.L.; Anistratenko, V.V. Peculiarities of reproduction of molluscs of two species of the genus *Caspia* (Gastropoda, Pectinibranchia, Pyrgulidae). *Vestn. Zool.* **1998**, *32*, 60–66. (In Russian)
39. Babak, E.V. The Pliocene and Quaternary dreissenids of the Euxine basin. *Tr. Paleontol. Inst. AN SSSR* **1983**, *204*, 1–104. (In Russian)
40. Palatov, D.M.; Marin, I.N. When males and females belong to different genera: An interesting case of *Synurella/Pontonyx* (Crustacea: Amphipoda: Crangonyctidae) co-occurrence. *Arthropoda Sel.* **2021**, *30*, 443–472. [\[CrossRef\]](#)
41. Novikova, N.M.; Polyanskaya, A.V. *The Samur Liana Forests: The Problem of Their Conservation under Conditions of the Developing Water Industry*; Institute of the Water Problems RAS: Moscow, Russia, 1994; 106p. (In Russian)
42. Starobogatov, Y.I. *Fauna of Mollusks and the Zoogeographic Regionalization of Fresh Waterbodies of the World*; Nauka: Leningrad, Russia, 1970; 372p. (In Russian)
43. Markovsky, Y.M. *Fauna of Invertebrates of the Lower River Courses of Ukraine, Life Conditions and Ways of Utilization. Part 1. The Basin of the Dniester Delta and Dniester Lagoon*; The Ukrainian Academy of Sciences Press: Kiev, Ukraine, 1953; 196p. (In Russian)
44. Markovsky, Y.M. *Fauna of Invertebrates of the Lower River Courses of Ukraine, Life Conditions and Ways of Utilization. Part 2. The Dnieper-Bug Lagoon*; The Ukrainian Academy of Sciences Press: Kiev, Ukraine, 1954; 207p. (In Russian)