

Article

Genetic and Morphological Characterization of the Invasive *Corbicula* Lineages in European Russia

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Abstract: Despite the full attention of malacologists to the study of *Corbicula* clams, stimulated mainly due to their high invasive potential and unique breeding system, studies based on an integrative taxonomic approach to identify various invasive lineages of this genus in European Russia are still limited. Our fieldwork was conducted in the Don, Volga, and Caspian Sea basins. In total, four distinct morphotypes belonging to the European forms R, Rlc, S, and “Int” of *Corbicula* clams were distinguished. According to our molecular genetic data, two *Corbicula* lineages, relevant to the nominal species *Corbicula fluminea* and *C. fluminalis*, can be defined. We observed a discrepancy between mtDNA haplotypes and morphological features for all individuals that were sampled from the Don and Kura Rivers. Identified mismatch may be due to the androgenetic reproduction of *Corbicula* that leads to cytonuclear inconsistencies. The 28S rRNA polymorphism in *C. fluminea* and *C. fluminalis* from studied localities was recorded. This occurrence is perhaps due to hybridization events between forms R, S, and Rlc. The biogeographic origins of *Corbicula* invasive lineages are discussed. *Corbicula fluminalis* (FW17, form S) has been detected for first time in the native range, namely in Azerbaijan, Tajikistan, Turkey, and Myanmar. We assume that the invasion of *C. fluminalis* into reservoirs and watercourses of the Caspian Sea basin (Republic of Dagestan and Stavropol Krai of Russia) was from Western Asia and Transcaucasia, and the introduction of *C. fluminea* to the Don and Volga River basins was from unspecified European countries.

Keywords: Cyrenidae; Asian clams; invasive species; Caspian Sea; Azov Sea; Volga River; hybridization; intragenomic variability



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1. Introduction

The *Corbicula* clams (Bivalvia: Cyrenidae) are native to Africa, Asia, Australia, and the Middle East [1]. However, these clams largely extended their range during the past century and are now widespread around the world [2]. In Europe, *Corbicula* was detected for the first time in the 1980s [3]. Currently, *Corbicula* clams are dispersed across all the main European countries, including Russia [4–8]. Invasive *Corbicula* clams were first discovered in European Russia in the Shura-Ozen River (Caspian Sea basin) in 2013 [9]. Later on, *Corbicula* clams were found in Agrakhan Bay, Kubyakinsky Bank, Lake Kuznechonok, Yuzbash-Sulak Collector, and Prislak Canal of the Caspian Sea from 2017–2021 [10,11]. In 2015, *Corbicula* was found in a cooler pond of the Kostromskaya Power Station, Volga River basin (Caspian Sea drainage) [12] and in heated waste water of the Arkhangelsk Thermal Power Plant, Northern Dvina River basin (White Sea basin) [7]. In 2017, several *Corbicula*

specimens were found in a heated channel of the Novocherkassk Hydroelectric Power Station (GRES) and in the Don River (Azov Sea drainage) [13–15].

The invasive lineages of *Corbicula* reproduce through androgenesis, which occurs after self-fertilization or outcrossing and herewith the oocyte is fertilized by an unreduced sperm [16–19]. The maternal nuclear DNA is then entirely extruded as two polar bodies and only the “male” pronucleus remains [16,18]. In addition, the sperm from one lineage is able to fertilize the eggs of another lineage (so-called “mitochondrial DNA capture”) and the nuclear genome of one lineage becomes associated with the mitochondria of another one, resulting in cytonuclear mismatches [16–18]. In some cases, “nuclear genome capture” may be observed, when part or the entire maternal nuclear DNA is not completely expelled from the egg, whereby the offspring inherits a hybrid genome [2,7,16,19]. Furthermore, such hybridization events could be a cause of genetic divergence between androgenetic species [2]. With respect to invasive *Corbicula* clams, the analysis of mitochondrial DNA alone may be biased. Therefore, an integrative taxonomic approach should be used, combining the analysis of nuclear and mitochondrial DNA sites, as well as the morphological and anatomical data [7,20–23].

Currently, five distinct invasive *Corbicula* lineages have been reported: (1) form A/R (COI mtDNA haplotype FW5), being widespread throughout East Asia, Europe, South and North America, and North Africa; (2) form B/Rlc (COI mtDNA haplotype FW1/FW4) that was found in East Asia, North America and Europe; (3) form C/S (COI mtDNA haplotype FW17) that was recorded in South America and Europe; and (4) a new form D that was described from North America [2,5,17,18,24].

Despite the close attention to the study of *Corbicula* clams, stimulated mainly due to their high invasive potential and unique breeding system [25,26], studies based on combining molecular genetics, conchological, and anatomical data for this genus in European Russia are still rare. Currently, only two populations have been covered by molecular genetic studies [7,27], and only COI gene fragments were analyzed for the population in the Volga River basin [27]. The objectives of the current study were: (1) to identify the invasive lineages of *Corbicula* in European Russia based on a comprehensive approach uniting molecular genetics, morphological, and anatomical data; (2) to evaluate the genetic diversity and possible vectors of introduction of *Corbicula* spp. to European Russia.

2. Materials and Methods

2.1. Study Area and Field Sampling

The field investigation was performed within several localities of European Russia: Don and Volga River basins, Shura-Ozen, Sulak, and Samur Rivers (Caspian Sea basin, Republic of Dagestan), and Kura River (Terek River basin, Stavropol Krai) in 2022 (Figures 1 and 2). *Corbicula* individuals were collected using a hand net and dredge and were fixed in 96% ethanol. A total of 439 specimens were gathered. The materials are stored in the collection of the Russian Museum of Biodiversity Hotspots, N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia.

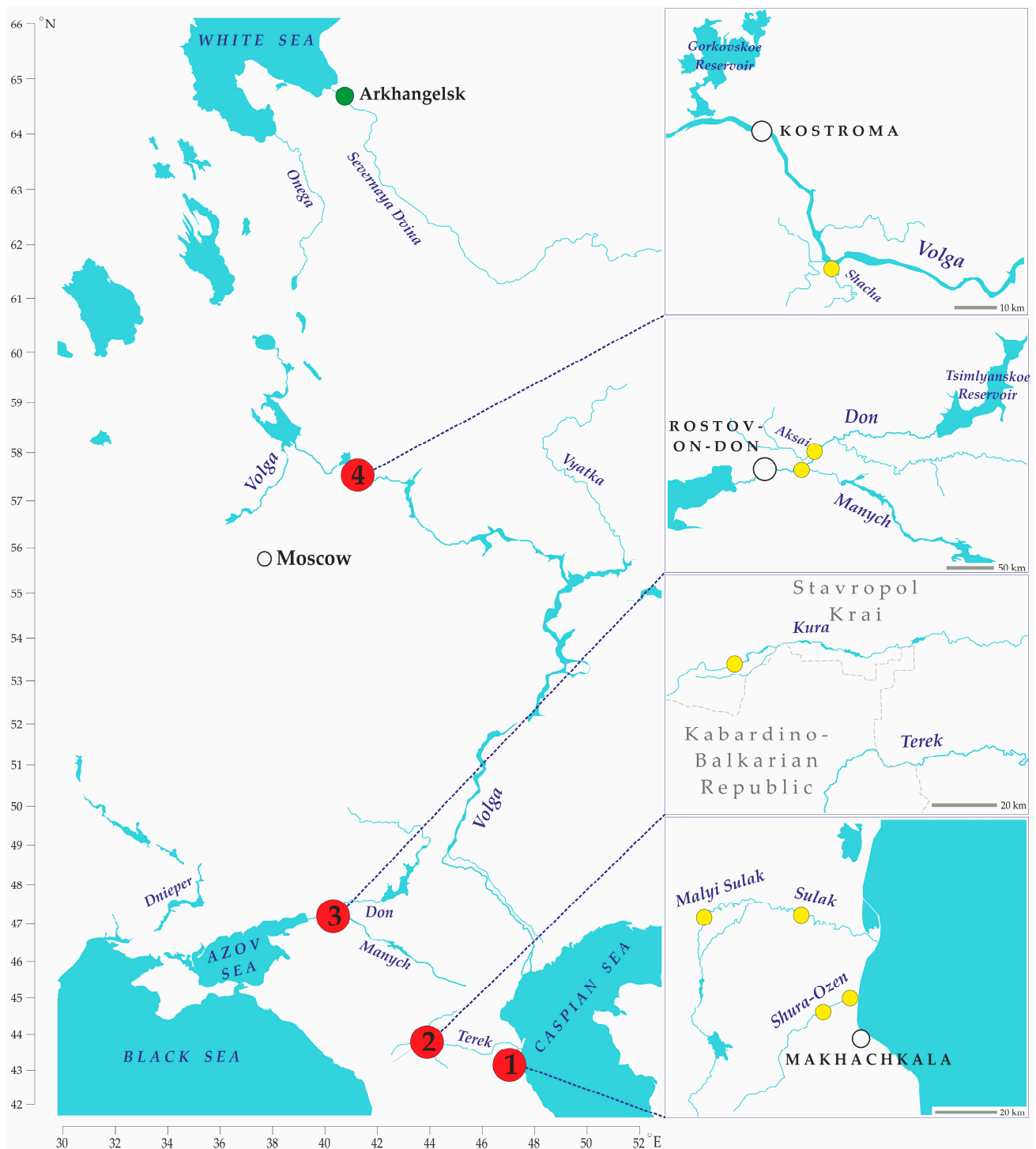


Figure 1. Map of records of Asian *Corbicula* clams in European Russia. (1) Caspian Sea basin, Republic of Dagestan; (2) Kura River, Terek River basin, Stavropol Krai; (3) Don River basin, Rostov Oblast; (4) Volga River basin, Kostroma Oblast. The green dot indicates the locality of the *Corbicula* according to Besspalaya et al. [7]. The red dots (sampling areas) and the yellow dots (sampling sites) indicate where *Corbicula* samples were collected in this study.



Figure 2. The habitat of *Corbicula fluminalis* in the Malyi Sulak River (Republic of Dagestan, Russia). Photos: O.V. Aksenova.

2.2. Morphological and Anatomical Studies

The *Corbicula* shells were measured using a caliper (with an accuracy of 0.1 mm): shell length (SL), height (SH), and width (SW) (Supplementary Table S1). A total of 439 specimens were measured in this study. The shell shape, sculpture, umbo position, shape of the hinge, and surface coloration of *Corbicula* clams were examined (see [7,8,20,21] for detail). Shell images were obtained using a Canon EOS 80D Digital Camera (Canon Inc., Tokyo, Japan). The ethanol-preserved individuals were dissected; their mantle, siphons, and gills were extracted for visual examination. The soft bodies and siphons were photographed using an Leica M165C stereomicroscope (Leica Microsystems, Wetzlar, Germany) with a FLEXACAM C1 digital camera (Leica Microsystems, Wetzlar, Germany). The morphotypes (A/R, B/Rlc, and C/S) were distinguished according to Pfenninger et al. [28], Korniushev [29], Marescaux et al. [30], Pigneur et al. [31], Bespalaya et al. [7], and Morhun et al. [32]. To assess the extent of conchological variation in *Corbicula* morphotypes, principal components analysis (PCA) was conducted, using the following measurements (SL, SH, and SW). In total, measurements of 196 adult clams were used. The statistical analysis was conducted using the software PAST v.3.0 [33]. The shell elongation (SEI) and shell inflation (SII) indices were calculated according to Bolotov et al. [34].

2.3. DNA Extraction, PCR Amplification, and DNA Sequencing

Here, we present original molecular data for 144 *Corbicula* specimens (Supplementary Table S2). Total DNA was extracted from 96% ethanol-preserved foot tissues using the NucleoSpin Tissue Kit (Macherey-Nagel GmbH & Co. KG, Dürren, Germany), following the manufacturer's protocol. The performed molecular genetic analysis included amplification and sequencing of mtDNA COI and nuclear 28S rRNA markers. The primer composites are presented comprehensively in our articles [35,36]. The PCR mix contained approximately

100 ng of total cell DNA, 10 pmol of each primer, 200 μ mol of each dNTP, 2.5 μ L of PCR buffer (with 20 mmol MgCl_2), 0.8 units Taq DNA polymerase (SibEnzyme Ltd., Novosibirsk, Russia), and H_2O was added for a final volume of 25 μ L. Temperature cycling was as follows: 95 $^\circ\text{C}$ (4 min), 36 cycles of 95 $^\circ\text{C}$ (50 s), 52 $^\circ\text{C}$ (50 s), 72 $^\circ\text{C}$ (50 s), and a final extension at 72 $^\circ\text{C}$ (5 min). The forward and reverse sequencing was performed on an automatic sequencer (ABI PRISM 3730, Applied Biosystems) using the ABI PRISM BigDye Terminator v.3.1 reagent kit. The resulting sequences were checked manually using the sequence alignment editor BioEdit v.7.2.5 [37].

2.4. Sequence Alignment and Phylogeographic Analyses

The sequences were aligned using the MUSCLE algorithm of MEGA7 [38]. Each new sequence was checked for similar sequences from GenBank through the nucleotide Basic Local Alignment Search Tool, BLASTn [39]. The phylogeographic analyses were executed based on a median joining network approach using Network v.4.6.1.3 software with default settings [40]. The dataset contains 318 COI sequences, including 202 sequences obtained from NCBI GenBank (Supplementary Tables S2 and S3). We followed [41] in using the term “lineage” for designation of the elementary taxonomic units within *Corbicula* spp. The 28S rRNA polymorphisms in selected *Corbicula* species were identified with the Toggle Converted Sites tool of MEGA11 at a 50% level [7,42].

3. Results

3.1. Shell Morphology and Anatomy of Clams

In total, four distinct morphotypes of *Corbicula* clams were distinguished: European forms R, Rlc, and S [28,30], as well as an “intermediate morphotype” (“Int”), which displayed an intermediate morphology between Rlc and S (Table 1). The average shell length $\pm$ SD of adult *Corbicula* relating to the R morphotype was 15.8 ± 4.5 mm (min–max 10.2–26.0 mm, $N = 22$), that of the S morphotype was 14.2 ± 2.8 (min–max 10.0–23.4 mm, $N = 118$), and that of the Rlc morphotype was 12.9 ± 3.9 (min–max 10.0–19.3 mm, $N = 17$).

Table 1. The morphological and molecular characteristics of invasive *Corbicula* clams.

Locality	Species (Lineages, Forms)	Morphotype	Shell Shape	External Shell Coloration	Internal Shell Coloration	Shell Sculpture	Umbo Position	Anatomy of Siphons	COI Haplo-type	Hybridizing Lineages **
Volga River basin	<i>Corbicula fluminea</i> (= <i>Corbicula</i> clade FW5; = <i>Corbicula</i> R form)	R	Round and broad, triangular	Dark brown	Matte, with purple marks	Distinctly pronounced deep concentric ridges	Slightly shifted	Light yellow color with black spots in the siphonal papilla and dark pigmentation around	FW5	R $\times$ Rlc
Don River basin	<i>Corbicula fluminea</i> (= <i>Corbicula</i> clade FW5)	Rlc	Oval triangular	Yellow or olive green	Yellow white, with small purple marks	Raised, regular concentric ribs	Centrally located	The papillae are long, have different lengths, and orange coloration with black spots and dark coloration around	FW5	R $\times$ Rlc
	<i>C. fluminea</i> (= R form) *	R	Oval triangular	Dark brown	White, with purple marks	Distinctly pronounced deep concentric ridges	Slightly shifted	N/A	N/A	N/A

Table 1. Cont.

Locality	Species (Lineages, Forms)	Morphotype	Shell Shape	External Shell Coloration	Internal Shell Coloration	Shell Sculpture	Umbo Position	Anatomy of Siphons	COI Haplo-type	Hybridizing Lineages **
Shura-Ozen Sulak, and Samur River basin (Republic of Dagestan)	<i>C. fluminalis</i> (= <i>Corbicula</i> clade FW17; <i>Corbicula</i> form S)	S	Narrow, saddle-like	Yellow or olive green	Deep purple	Tight spaced ridges	Significantly prominent	The bases of each siphon and tentacle have a dark pigmentation	FW17	R × S
Kura River (Stavropol Krai)	<i>C. fluminalis</i> (= <i>Corbicula</i> clade FW17; <i>Corbicula</i> form S)	“Intermediate form” Rlc + S	Oval triangular	Yellow or olive green	Deep purple	Raised, regular concentric ribs	Centrally located	Rather broad pigmented ring is not present. Siphonal papillae are pigmented	FW17	Rlc × R × S

Notes: * Only empty shells were found; ** based on analysis of 28S rRNA gene sequences (Table 2); N/A—not available.

Table 2. 28S rRNA polymorphism in selected *Corbicula* species *.

Species	432	433	434	438	446	485	533	549	550	570	655	726
<i>C. fluminea</i> (USA)	-	A	G	C	A	C	A	G	G	T	T	T
<i>C. fluminea</i> (Arkhangelsk Oblast) ** (N = 5)	-	A/C	A/G	A/C	.	.	A/C	.	.	C/T	.	.
<i>C. fluminea</i> (Kostroma Oblast) (N = 5)	-	.	.	.	.	.	.	.	.	.	.	.
<i>C. fluminea</i> (Kostroma Oblast) ** (N = 5)	-	A/C	A/G	A/C	.	.	A/C	.	.	C/T	.	.
<i>C. fluminea</i> (Rostov Oblast) (N = 5)	-	.	.	.	.	.	.	.	.	.	.	.
<i>C. fluminea</i> (Rostov Oblast) ** (N = 5)	-	A/C	A/G	A/C	.	.	A/C	.	.	C/T	.	.
<i>C. fluminalis</i> (Turkey) ** (N = 8)	-	C	A	A	.	.	C	A/G	G/T	C	G/T	.
<i>C. fluminalis</i> (Dagestan) ** (N = 4)	-	.	.	.	.	A	.	.	.	.	.	.
<i>C. fluminalis</i> (Dagestan) ** (N = 1)	-	C	A	A	.	.	C	.	.	C	.	.
<i>C. fluminalis</i> (Dagestan) *** (N = 5)	Var1	-	C	A	A	.	C	.	.	C	.	.
	Var2	G	C	A	A	.	C	A	T	C	G	.
<i>C. fluminalis</i> (Stavropol Krai) ** (N = 4)	-	.	.	.	.	A	.	.	.	.	.	.
<i>C. fluminalis</i> (Stavropol Krai) *** (N = 5)	Var1	-	C	A	A	.	C	.	.	C	.	.
	Var2	G	C	A	A	.	C	A	T	C	G	.
<i>C. fluminalis</i> (Stavropol Krai) *** (N = 1)	Var1	-	.	.	.	A	.	.	.	.	.	.
	Var2	G	C	A	A	.	C	A	T	C	G	.
<i>C. africana</i> (South Africa)	G	C	A	A	G	.	C	.	.	C	G	.
<i>C. africana</i> (South Africa)	G	C	A	A	.	.	C	.	.	C	.	C

Notes: *—Numbers in the top row refer to nucleotide positions within a condensed alignment of the sequences, letters are FASTA codes for nucleotides but with additional polymorphisms shown explicitly. Dots indicate a nucleotide matching the first sequence. Dashes indicate gaps after alignment of the sequences; **—Samples with two 28S rDNA variants of the same length; ***—Samples with two variants of 28S rRNA with different length.

The average shell length $\pm$ SD of adult specimens of the “intermediate morphotype” was 13.1 ± 1.5 mm (min–max 10.0–15.5 mm, $N = 39$). The current study presents the first report on records of *C. fluminalis* from the Samur and Kura Rivers. The main morphological characteristics of the *Corbicula* lineages are presented in Table S1 and Figure 3. The anatomical structures of siphons are described in Table 1.

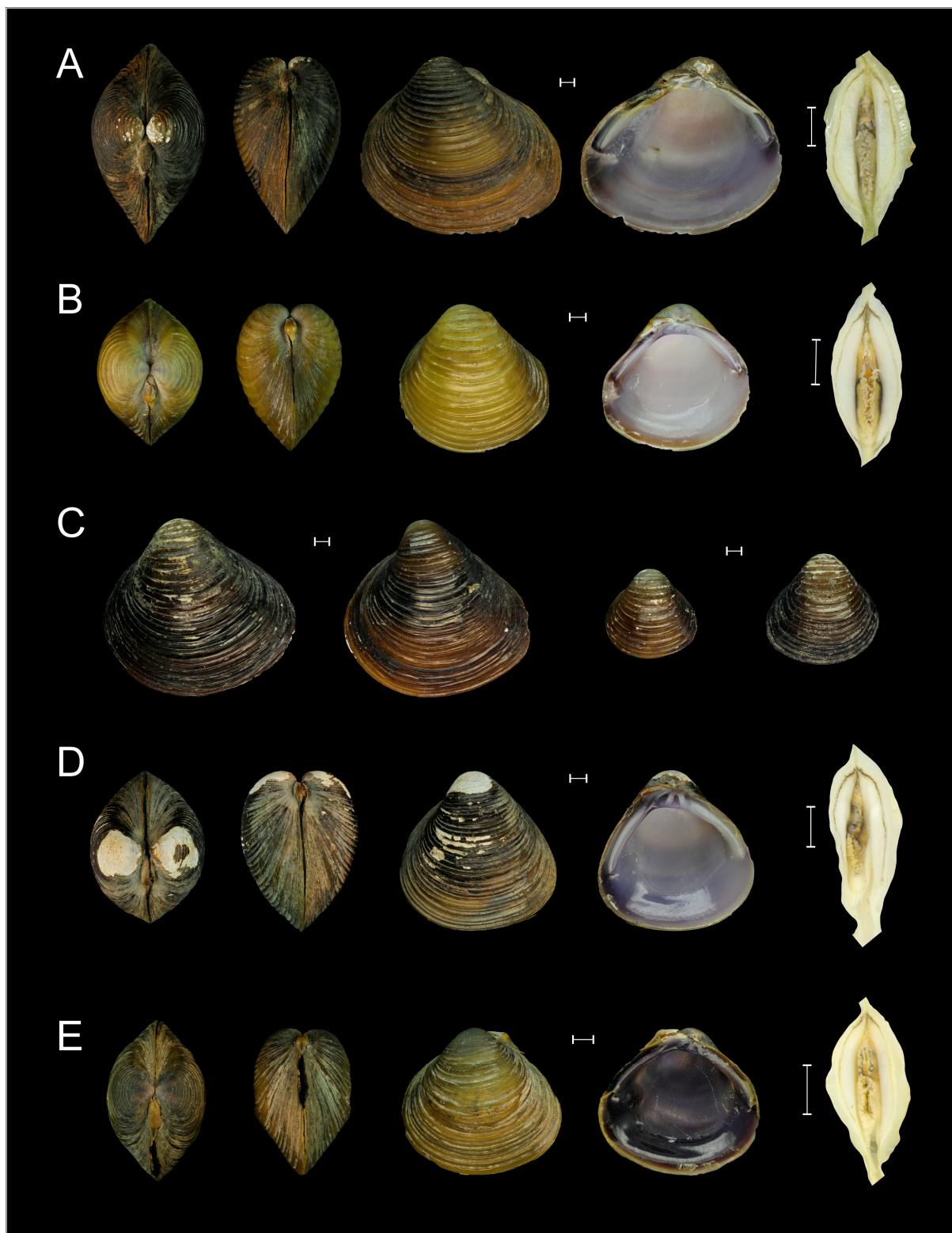


Figure 3. Morphology and anatomy of *Corbicula* clams from European Russia: (A) specimen MCorb-131.1, *C. fluminea* (=lineage FW5; =*Corbicula* R form), Volgorechensk TPP, Volga River basin, Kostroma Oblast; (B) specimen MCorb-133.1, *C. fluminea* (=Corbicula lineage FW5; =*Corbicula* Rlc form), Don River basin, Rostov Oblast; (C) *C. fluminea* (= *Corbicula* R form), Don River basin, Rostov Oblast; (D) specimen MCorb-136.2, *C. fluminea* (=Corbicula lineage FW17; =*Corbicula* form S; Caspian Sea basin, Republic of Dagestan); (E) specimen MCorb-141.1, *C. fluminea* (=Corbicula lineage FW17; =Int form; Kura River, Terek River basin, Stavropol Krai). Scale bars—2 mm. Photos: O.V. Aksenova.

Morphometrically, *Corbicula* lineages clearly differ from each other by the shell inflation index (Kruskal–Wallis test: $H = 93.6$, $df = 3$, $N = 196$, $p < 0.0001$; post hoc comparisons given in Supplementary Table S4) and the shell elongation index (Kruskal–Wallis test: $H = 60.73$, $df = 3$, $N = 196$, $p < 0.0001$; post hoc comparisons given in Supplementary Table S5).

At the same time, the PCA of shell variation (SL, SH, and SW) demonstrates that there is no difference between clams belonging to the R, Rlc, and S morphotypes of *Corbicula*, because all individuals form a single group in the multivariate space (Figure 4). The “intermediate morphotype” is morphologically different from the R, Rlc, and S morphotypes and forms a relatively separate group in the space of principal components PC1 and PC2 (Figure 4).

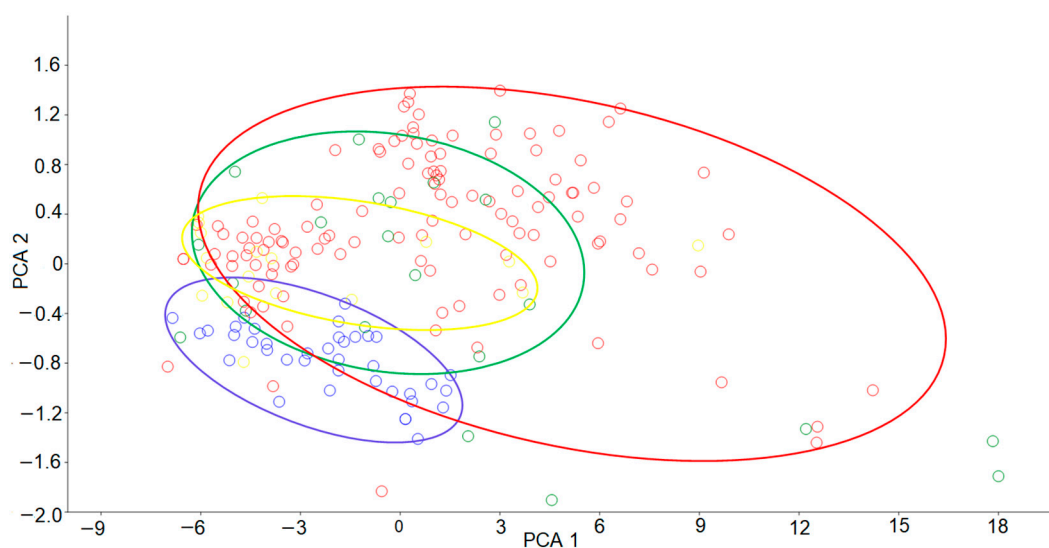


Figure 4. The principal components analysis (PCA) of the shell variation of R, Rlc, S, and “Int” forms of *Corbicula* clams from European Russia: R—green circles, Rlc—yellow circles, S—red circles, and “Int”—blue circles. Principal component PC1 explains 97.3% of the total variation, and PC2 explains 2.2% of it. Solid lines show 95% confidence ellipses.

3.2. Mitochondrial DNA Sequences

We obtained 144 new COI sequences from specimens sampled from the rivers of European Russia during this study (Supplementary Table S2). The COI haplotype analyses allowed us to define two distant genetic lineages (Figure 5). The first lineage apparently matches to *C. fluminea* (= *Corbicula* clade FW5; = *Corbicula* European form R [2,41]). It was found by us in the Don and Volga River basins. The second lineage genetically belongs to another clade, *C. fluminalis* (= *Corbicula* European form S; = *Corbicula* clade FW17 [2,41]); it was registered from the Shura-Ozen, Sulak, and Samur Rivers and Kura River drainage (Figure 5). In addition, the invasive *C. fluminalis* (FW17, form S) has been detected for the first time in its native range: Azerbaijan (Southwest Asia), Tajikistan (Central Asia), Turkey (Western Asia), and Myanmar (Southeast Asia) (Figure 5).

We discovered an incongruity between mtDNA haplotypes and morphotypes from the Don and Kura River basins (Table 1). The specimens that share the FW5 haplotype correspond to the morphotype Rlc, based on morphological and anatomical features. The specimens having the “intermediate morphotype” by the shell features show the FW17 haplotype of the mtDNA (Table 1).

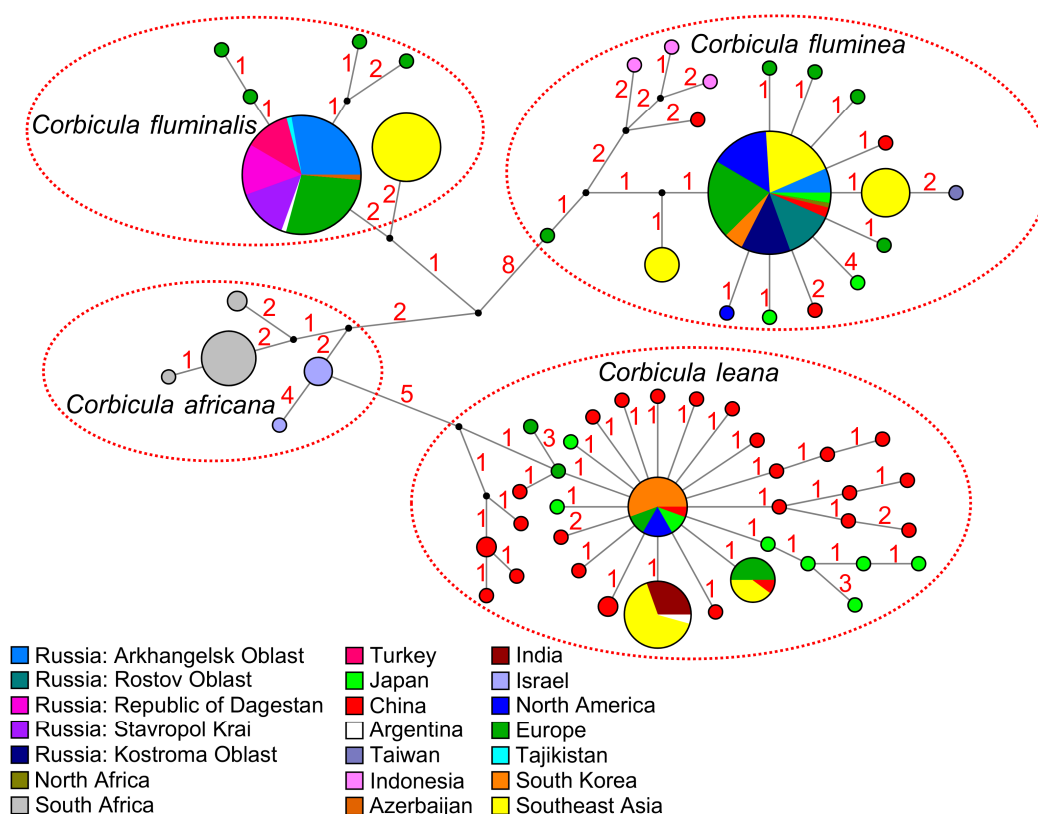


Figure 5. Median-joining haplotype network of 318 COI sequences (length = 604 bp; Supplementary Table S2) of *Corbicula* species. Circles represent different haplotypes, with the size reflecting their frequency (the smallest = 1 sequence). Red dotted contours indicate putative species-level haplogroups. Numbers near branches indicate numbers of nucleotide substitutions per site.

3.3. Nuclear DNA Sequence Polymorphism

We received 28S rRNA sequences from 56 individuals, including 28 individuals of *C. fluminea* (Müller, 1774) (= *Corbicula* clade FW5, European form R) and 28 individuals of *C. fluminalis* (Müller, 1774) (= *Corbicula* clade FW17; = *Corbicula* European form S) (Table 1). The 28S rRNA polymorphism of some individuals of *Corbicula* was detected: those from Shura-Ozen, Sulak, and Samur Rivers (Caspian Sea basin) at 50%, from Kura River (Terek River basin) at 60%, from Don and Volga River basins at 50% (Table 2). Thus, 18 genotyped specimens of *C. fluminea* and 19 genotyped specimens of *C. fluminalis* displayed polymorphic chromatograph profiles. Relying on the visual examination of sequences, we assume that specimens of *C. fluminalis* and *C. fluminea* from investigated sites could comprise elements of nuclear genomes occurring from several other *Corbicula* lineages (Table 2).

Then, we collated our 28S rRNA gene sequences with those received from GenBank according to Bepalaya et al. [7,20]. It is established that four individuals of *C. fluminalis* from Kura River have three variants of 28S rRNA: the first shows 99.76% similarity (with one nucleotide substitution) to that of *C. fluminea* from the USA [43] (GenBank accession No. KC429490); the second shows 99.61% similarity (with two nucleotide substitutions) to *C. fluminea* from South Korea [21] (GenBank accession No. MZ427291); the third shows 99.09% similarity (with six nucleotide substitutions) to *C. fluminea* from South Korea [21] (GenBank accession No. MZ427291; Table 2).

The 28S rRNA gene sequences from Shura-Ozen, Sulak, and Samur Rivers (Republic of Dagestan) (Figure 1, No. 2) were identical to that from Kura River (Stavropol Krai) (Figure 1, No. 1), but their combinations in each individual were different (Table 2).

In samples from the Don and Volga River basins, ten individuals of *C. fluminea* have 28S rRNA identical to that of *C. fluminea* from the USA [43] (GenBank accession No. KC429490); and ten individuals have 28S rRNA identical to *C. fluminea* from northern European Russia (Northern Dvina River basin) [7] (GenBank accession No. KX347247, KX347249, KX347250, MG757364, and MG757366, Table 2).

4. Discussion

4.1. Genetic and Morphological Analysis of *Corbicula* Lineages from European Russia

In general, two *Corbicula* lineages were discovered in the studied area: *C. fluminea* (= *Corbicula* clade FW5) was found in the Don and Volga River basins, and *C. fluminalis* (= *Corbicula* clade FW17) in the Caspian Sea basin (Shura-Ozen, Sulak, and Samur Rivers, Kura River basin) (Figure 4, Table 1). At the same time, we have recognized four distinct morphotypes of *Corbicula* clams based on morphological and anatomical data, i.e., R, Rlc, S, and “Int” [28,30] (Table 1). We recorded an incongruity between mtDNA haplotypes and morphological features for all the clams that were collected from the Don and Kura River basins (Stavropol Krai) (Table 1). Specimens from the Don River having the FW5 haplotype correspond to the morphotype Rlc based on morphological and anatomical features. Besides that, specimens from the Kura River have the “intermediate morphotype” (Rlc + S) by the shell features and show the FW17 haplotype of the mtDNA. Interestingly, the European Rlc form belongs to the same mitochondrial lineage as the American Form B, but the B morphotype has a deep purple inner shell surface in contrast to the Rlc morphotype [2,17,31,44]. Identified mismatches are possibly due to the specificity of *Corbicula* reproduction through androgenesis that may lead to cytonuclear inconsistencies [7,16,20,21]. According to a number of studies, cytonuclear mismatches usually have been detected in places in which different lineages are in coexistence [2,6,7,17,18,31,41,45]. In our research, we found lineages living in sympatry in the Don River basin, namely Rlc and R, and in the Caspian Sea basin (Republic of Dagestan), namely S, R, and Rlc [11]. In the Volga and Kura River basins, we found only individuals with R and “Int” morphotypes, respectively. It was established that the mixed “cytonuclear genotype” can rapidly spread and become fixed in a population [16]. Consequently, populations discovered by us could have been formed as a result of the invasion of androgenic hybrid *Corbicula* lineages. In addition, the possibility that we were unable to discover other lineages of *Corbicula* in the Volga and Kura River basins due to their low numbers cannot be excluded. For example, in a heated channel of the Arkhangelsk TPP, we found two lineages of *Corbicula*. Of these, the Rlc form was rare with a mean density of 0.6 ind./m² [7]. Moreover, Vastrade et al. [41] expressed a hypothesis that nuclear captures may also occur within a single lineage. Presumably, all points of view regarding the formation of populations in the Kura and Volga Rivers, described above, are not mutually exclusive, and in reality, several scenarios could take place.

4.2. The 28S rRNA Polymorphism

The 28S rRNA polymorphism in *C. fluminea* and *C. fluminalis* from studied localities is perhaps conditioned to hybridization events between forms A/R, C/S, and B/Rlc. In particular, the polymorphism discovered by us has probably happened from several hybridization actions followed by a “nuclear genome capture” between distinct *Corbicula* lineages. This phenomenon was described in detail in previous works [7,16,19–21]. A review of this body of literature shows that gene capture, considered initially as a rare event [19], is quite common in *Corbicula* populations [2,7,17,20,21,28,41]. For example, Vastrade et al. [41] revealed that most *Corbicula* individuals were heterozygous for 28S rRNA (84%). In our studies, the proportion of hybrid individuals varied from 50% to 60% depending on the population (Table 2). It is obvious that invasive *Corbicula* lineages originated as a result of androgenetic hybridization that is confirmed by the presence of a common 28S rRNA allele in invasive forms B, Rlc, and C/S and form A/R [6,16,41].

4.3. Dispersal Routes of the Invasive *Corbicula* Lineages to Aquatic Ecosystems of European Russia

Current molecular genetic studies indicate distinct geographic lineages for the invasive lineages of *Corbicula* [2,41]. In particular, the form A/R has originated in Japan, form B/Rlc is from the Asian mainland, and form C/S is from Africa [2,45]. Vastrade et al. [41] proposed an alternative hypothesis and suggested at least three distinct biogeographic sources of androgenetic lineages within the genus *Corbicula*, i.e., Southeast Asia as a source for the Vietnamese androgenetic *Corbicula* lineages, and Japan as a source for A/R, B, and Rlc forms. Furthermore, the authors suggested that the C/S form invaded South Africa and formed a population in this region. Hence, *C. africana* has probably originated from the C/S form rather than the opposite [41]. Until now, the FW17 (form C/S) invasive lineage has not been detected in the eastern part of the native range of *Corbicula* [2,5,41]. However, our COI haplotype network reveals that the COI sequences from Azerbaijan (Southwest Asia), Tajikistan (Central Asia), Turkey (Western Asia), and Myanmar (Southeast Asia) belong to *C. fluminalis* (=FW17; =form S). The sequences from Myanmar have four nucleotide substitutions from those from South America, Europe, Southwest Asia, and Central Asia (Figure 5). The sequences from Azerbaijan, Turkey, and Tajikistan were identical to those from South America and Europe or have only 1–2 nucleotide substitutions (Figure 5). Thus, the invasive lineage *C. fluminalis* (=FW17; =form S) has a wider distribution in Europe, South America, Southwest Asia, Southeast Asia, Western Asia, and Central Asia than it was thought previously. Taking into account that the COI sequences from the native range (Asia) analyzed by us were identical to samples from South America and Europe, we assume that invasion of this lineage has started from Asia, with a subsequent spread to Africa and South America, and then to Europe. This finding supports the hypothesis of Vastrade et al. [41] that the invasive lineage FW17 (form S) has probably expanded its range to South Africa. It cannot be excluded that the lineage FW17 may have also been introduced to the European subcontinent directly from the Asian populations. The COI sequences obtained by us from the Caspian Sea basin (Republic of Dagestan and Stavropol Krai of Russia) were identical to or have only 1–2 nucleotide substitutions compared to those from Europe, South America, northern European Russia, Azerbaijan, Tajikistan, and Turkey (Karasu River, Orontes River basin) (Figure 5). It should be noted that the type locality of *C. fluminalis* is situated in the Euphrates River, not far from the Orontes basin. Additionally, most individuals from the Caspian Sea basin share similar COI and 28S rRNA gene haplotypes, suggesting a possible common origin of these *Corbicula* lineages. Thus, we assume that the invasion of *C. fluminalis* (=form S; =*Corbicula* clade FW17) to reservoirs and watercourses of the Caspian Sea basin (Republic of Dagestan and Stavropol Krai) was from Western Asia and Transcaucasia. A similar hypothesis was expressed earlier by Nabozhenko M. and Nabozhenko S. [9] and by Voroshilova et al. [27]. All clams collected from the Don and Volga River basins share the single COI and 28S rRNA haplotypes that indicate a common origin of these *Corbicula* lineages. These specimens have an identical COI haplotype to sequences from South and North America, Europe, northern European Russia, China, and Japan. Consequently, we hypothesize that the introduction of *C. fluminea* (= *Corbicula* clade FW5) to the Don and Volga River basins was from unspecified European countries. Among the known dispersal mechanism of *Corbicula* clams, human activities such as transport of ballast water, construction of canals for various purposes, dams, irrigation and drainage systems, sand mining, and food resources, as well as natural passive transfer by migratory waterfowl and waterways, seem to be the most likely causes of global spreading of invasive lineages.

5. Conclusions

Here, we provide new and important insights into the invasive lineages of *Corbicula* clams in European Russia based on an integrative approach. According to our data, studied *Corbicula* populations comprise two androgenic invasive lineages, belonging to the biological species *C. fluminea* and *C. fluminalis*. We observed a discrepancy between mtDNA haplotypes and morphological features for all individuals that were sampled from the Don

and Kura Rivers, which may be due to the androgenetic reproduction of *Corbicula* that leads to cytonuclear inconsistencies. The 28S rRNA polymorphism in *C. fluminea* and *C. fluminalis* from studied localities is perhaps due to hybridization events between forms R, S, and Rlc. It is obvious that the frequency of this process in *Corbicula* clams is clearly underestimated and remains poorly understood. Future studies should focus on the genetic diversity and cytogenetic characteristics of *Corbicula* clams both in their native range and beyond.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w15183226/s1>, Table S1: Shell measurements of *Corbicula* clams from European Russia; Table S2: Information on newly sequenced specimens of *Corbicula* spp. from European Russia. Materials are from the collection of the RMBH, N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia); Table S3: Information on additional COI sequences of *Corbicula* clams obtained from GenBank, including taxon, accession number, region, and data source; Table S4: The shell inflation index across the four *Corbicula* morphotypes. Kruskal–Wallis test statistic/ p value: $H(\chi^2) = 93.6$; $p \leq 0.0001$. Significant pairwise comparisons following Bonferroni correction; Table S5: The shell elongation index across the four *Corbicula* morphotypes. Kruskal–Wallis test statistic/ p value: $H(\chi^2) = 60.73$; $p \leq 0.0001$. Significant pairwise comparisons following Bonferroni correction.

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Data Availability Statement: The specimens are deposited in the Russian Museum of the Biodiversity Hotspots (RMBH) of N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia). The new COI and 28S rRNA gene sequences obtained during this study are deposited in GenBank and presented in Supplementary Table S2.

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References

1. Araujo, R.; Moreno, D.; Ramos, M.A. The Asiatic clam *Corbicula fluminea* (Meuller, 1774) (Bivalvia: Corbiculidae) in Europe. *Am. Malacol. Bull.* **1993**, *10*, 39–49.
2. Pigneur, L.-M.; Etoundi, E.; Aldridge, D.C.; Marescaux, J.; Yasuda, N.; Van Doninck, K. Genetic uniformity and long-distance clonal dispersal in the invasive androgenetic *Corbicula* clams. *Mol. Ecol.* **2014**, *23*, 5102–5116. [\[CrossRef\]](#)
3. Mouthon, J. Sur la présence en France et au Portugal de *Corbicula* (Bivalvia, Corbiculidae) originaire d’Asie. *Basteria* **1981**, *45*, 109–116.
4. Crespo, D.; Dolbeth, M.; Leston, S.; Sousa, R.; Pardal, A. Distribution of *Corbicula fluminea* (Müller, 1774) in the invaded range: A geographic approach with notes on species traits variability. *Biol. Invasions* **2015**, *17*, 2087–2101. [\[CrossRef\]](#)
5. Gomes, C.; Sousa, R.; Mendes, T.; Borges, R.; Vilares, P.; Vasconcelos, V.; Guilhermino, L.; Antunes, A. Low genetic diversity and high invasion success of *Corbicula fluminea* (Bivalvia, Corbiculidae) (Müller, 1774) in Portugal. *PLoS ONE* **2016**, *11*, e0158108. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Penãrrubia, L.; Araguas, R.M.; Vidal, O.; Pla, C.; Vinãs, J.; Sanz, N. Genetic characterization of the Asian clam species complex (*Corbicula*) invasion in the Iberian Peninsula. *Hydrobiologia* **2017**, *784*, 349–365. [\[CrossRef\]](#)
7. Bespalaya, Y.V.; Bolotov, I.N.; Aksenova, O.V.; Kondakov, A.V.; Gofarov, M.Y.; Laenko, E.; Sokolova, S.E.; Shevchenko, A.R.; Travina, O.V. Aliens are moving to the Arctic frontiers: An integrative approach reveals selective expansion of androgenic hybrid *Corbicula* lineages towards the North of Russia. *Biol. Invasions* **2018**, *20*, 2227–2243. [\[CrossRef\]](#)

8. Bespalaya, Y.V.; Aksenova, O.V.; Kropotin, A.V.; Shevchenko, A.R.; Travina, O.V. Reproduction of the androgenetic population of the Asian *Corbicula* clam (Bivalvia: Cyrenidae) in the Northern Dvina River basin, Russia. *Diversity* **2021**, *13*, 316. [\[CrossRef\]](#)
9. Nabozhenko, M.V.; Nabozhenko, S.V. *Corbicula fluminalis* (O.F. Müller, 1774), A new clam species for the Russian Sector of the Caspian Basin. *Sci. South Russ.* **2016**, *12*, 61–64.
10. Khlopikova, M.V.; Guseynov, K.M.; Gasanova, A.S.; Barkhalov, R.M.; Zurkhaeva, U.D.; Guseynov, M.K. Russian first record of live clams of *Corbicula fluminalis* (Bivalvia: Corbiculidae) in the Dagestan Sector of the Caspian Sea Basin. *Russ. J. Biol. Invasions* **2019**, *10*, 79–82. [\[CrossRef\]](#)
11. Khlopikova, M.V.; Barkhalov, R.M.; Guseynov, K.M.; Gasanova, A.S.; Zurkhaeva, U.D. Invasions of new morphotypes of *Corbicula fluminalis* and *Corbicula fluminea* (Bivalvia: Corbiculidae) to the basin of the Dagestan Region of the Caspian Sea. *Russ. J. Biol. Invasions* **2023**, *14*, 251–261. [\[CrossRef\]](#)
12. Pryanichnikova, E.G.; Voroshilova, I.S.; Sabitova, R.Z. Introduction of *Corbicula fluminea* (Müller, 1774) (Mollusca: Bivalvia: Corbiculidae) in the Volga River Basin. *Inland Water Biol.* **2019**, *12*, 95–97. [\[CrossRef\]](#)
13. Zhivoglyadova, L.A.; Revkov, N.K. First records of *Corbicula fluminea* (O.F. Müller, 1774) (Mollusca: Bivalvia) from the Lower Don. *Ecol. Montenegrina* **2018**, *17*, 46–52. [\[CrossRef\]](#)
14. Zhivoglyadova, L.A.; Revkov, N.K. Invasion of the bivalve *Corbicula fluminea* (O.F. Müller, 1774) (Bivalvia: Cyrenidae) into the lower Don basin. *Aquat. Bioresour. Environ.* **2018**, *1*, 44–50. [\[CrossRef\]](#)
15. Zhivoglyadova, L.A.; Revkov, N.K.; Kovalev, E.A. Extension of the bivalve *Corbicula fluminea* (O. F. MÜLLER, 1774) areal in the lower Don River system. *Mar. Biol. J.* **2018**, *3*, 73–75.
16. Pigneur, L.M.; Hedtkke, S.M.; Etoundi, E.; Van Doninck, K. Androgenesis: A review through the study of the selfish shellfish *Corbicula* spp. *Heredity* **2012**, *108*, 581–591. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Lee, T.; Siripattawan, S.; Ituarte, C.F.; Foighil, D.O. Invasion of the clonal clams: *Corbicula* lineages in the New World. *Am. Malacol. Bull.* **2005**, *20*, 113–122.
18. Hedtkke, S.M.; Stanger-Hall, K.; Baker, R.J.; Hillis, D.M. All male asexuality: Origin and maintenance of androgenesis in the Asian clam *Corbicula*. *Evolution* **2008**, *62*, 1119–1136. [\[CrossRef\]](#)
19. Hedtkke, S.M.; Glaubrecht, M.; Hillis, D.M. Rare gene capture in predominantly androgenetic species. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 9520–9524. [\[CrossRef\]](#)
20. Bespalaya, Y.V.; Aksenova, O.V.; Gofarov, M.Y.; Kondakov, A.V.; Kropotin, A.V.; Kononov, O.D.; Bolotov, I.N. Who inhabits the world's deepest crater lake? A taxonomic review of *Corbicula* (Bivalvia: Cyrenidae) clams from Lake Toba, North Sumatra, Indonesia. *J. Zool. Syst. Evol. Res.* **2021**, *59*, 400–410. [\[CrossRef\]](#)
21. Bespalaya, Y.V.; Kropotin, A.V.; Kondakov, A.V.; Aksenova, O.V.; Gofarov, M.Y.; Kim, S.K.; Lee, J.H.; Travina, O.V.; Vikhrev, I.V.; Vinarski, M.V.; et al. A taxonomic reassessment of native and invasive species of *Corbicula* clams (Bivalvia: Cyrenidae) from the Russian Far East and Korea. *Zool. J. Linn. Soc.* **2023**, *197*, 104–126. [\[CrossRef\]](#)
22. Sheehan, R.; Etoundi, E.; Minchin, D.; Doninck, K.V.; Lucy, F. Identification of the invasive form of *Corbicula* clams in Ireland. *Water* **2019**, *11*, 1652. [\[CrossRef\]](#)
23. Modesto, V.; Ilarri, M.; Labecka, A.M.; Rodríguez, N.F.; Coughlan, N.E.; Liu, X.; Sousa, R. What we know and do not know about the invasive Asian clam *Corbicula fluminea*. *Hydrobiologia* **2023**. [\[CrossRef\]](#)
24. Tiemann, J.S.; Haponski, A.E.; Douglass, S.A.; Lee, T.; Cummings, K.S.; Davis, M.A.; Ó Foighil, D. First record of a putative novel invasive *Corbicula* lineage discovered in the Illinois River, Illinois, USA. *BiolInvasions Rec.* **2017**, *6*, 159–166. [\[CrossRef\]](#)
25. Korniushev, A.V.; Glaubrecht, M. Novel reproductive modes in freshwater clams: Brooding and larval morphology in Southeast Asian taxa of *Corbicula* (Mollusca, Bivalvia, Corbiculidae). *Acta Zool.* **2003**, *84*, 293–315. [\[CrossRef\]](#)
26. Kropotin, A.V.; Bespalaya, Y.V.; Aksenova, O.V.; Bolotov, I.N. Reproductive Mode of *Corbicula tobae* (Martens, 1900): Brooding and larval morphology in lake Toba (Indonesia). *Diversity* **2022**, *14*, 700. [\[CrossRef\]](#)
27. Voroshilova, I.S.; Pryanichnikova, E.G.; Prokin, A.A.; Sabitova, R.Z.; Karabanow, D.P.; Pavlov, D.D.; Kurina, E.M. Morphological and genetic traits of the first invasive population of the Asiatic clam *Corbicula fluminea* (O.F. Müller, 1774) naturalized in the Volga River basin. *Russ. J. Biol. Invasions* **2021**, *12*, 36–43. [\[CrossRef\]](#)
28. Pfenninger, M.; Reinhardt, F.; Streit, B. Evidence for cryptic hybridization between different evolutionary lineages of the invasive clam genus *Corbicula* (Veneroida, Bivalvia). *J. Evol. Biol.* **2002**, *15*, 818–829. [\[CrossRef\]](#)
29. Korniushev, A.V. A revision of some Asian and African freshwater clams assigned to *Corbicula fluminalis* (Müller, 1774) (Mollusca: Bivalvia: Corbiculidae), with a review of anatomical characters and reproductive features based on museum collections. *Hydrobiologia* **2004**, *529*, 251–270. [\[CrossRef\]](#)
30. Marescaux, J.; Pigneur, L.-M.; Van Doninck, K. New records of *Corbicula* clams in French rivers. *Aquat. Invasions* **2010**, *5*, 35–39. [\[CrossRef\]](#)
31. Pigneur, L.M.; Marescaux, J.; Roland, K.; Etoundi, E.; Descy, J.P.; Van Doninck, K. Phylogeny and androgenesis in the invasive *Corbicula* clams (Bivalvia, Corbiculidae) in Western-Europe. *BMC Evol. Biol.* **2011**, *11*, 147. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Morhun, H.; Vinarski, M.V.; Labecka, A.M.; Velde, G.; van der Son, M.O. Differentiation of European invasive clams of the genus *Corbicula* (Cyrenidae) using shell shape analysis. *J. Molluscan Stud.* **2022**, *88*, eyab045. [\[CrossRef\]](#)
33. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 9.

34. Bolotov, I.N.; Makhrov, A.A.; Gofarov, M.Y.; Aksenova, O.V.; Aspholm, P.E.; Bespalaya, Y.V.; Kabakov, M.B.; Kolosova, Y.S.; Kondakov, A.V.; Ofenböck, T.; et al. Climate warming as a possible trigger of keystone mussel population decline in oligotrophic rivers at the continental scale. *Sci. Rep.* **2018**, *8*, 35. [[CrossRef](#)] [[PubMed](#)]
35. 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. Biotech.* **1994**, *3*, 294–299.
36. Mollaret, I.; Jamieson, B.G.; Adlard, R.D.; Hugall, A.; Lecointre, G.; Chombard, C.; Justine, J.L. Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *Mol. Biochem. Parasitol.* **1997**, *90*, 433–438. [[CrossRef](#)] [[PubMed](#)]
37. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95–98.
38. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [[CrossRef](#)]
39. Johnson, M.; Zaretskaya, I.; Raytselis, Y.; Merezuk, Y.; McGinnis, S.; Madden, T.L. NCBI BLAST: A better web interface. *Nucleic Acids Res.* **2008**, *36*, W5–W9. [[CrossRef](#)]
40. Bandelt, H.J.; Forster, P.; Röhl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **1999**, *16*, 37–48. [[CrossRef](#)]
41. Vastrade, M.; Etoundi, E.; Bournonville, T.; Colinet, M.; Debortoli, N.; Hedtke, S.M.; Nicolas, E.; Pigneur, L.-M.; Virgo, J.; Flot, J.-F.; et al. Substantial genetic mixing among sexual and androgenetic lineages within the clam genus *Corbicula*. *Peer Community J. Evol. Biol.* **2022**, *2*, e73. [[CrossRef](#)]
42. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [[CrossRef](#)] [[PubMed](#)]
43. Sharma, P.P.; Zardus, J.D.; Boyle, E.E.; Gonzalez, V.L.; Jennings, R.M.; McIntyre, E.; Wheeler, W.C.; Etter, R.J.; Giribet, G. Into the deep: A phylogenetic approach to the bivalve subclass Protobranchia. *Mol. Phylogenet. Evol.* **2013**, *69*, 188–204. [[CrossRef](#)] [[PubMed](#)]
44. Siripattawan, S.; Park, J.K.; Foighil, D.O. Two lineages of the introduced Asian freshwater clam *Corbicula* occur in North America. *J. Molluscan Stud.* **2000**, *66*, 423–429. [[CrossRef](#)]
45. Haponski, A.E.; Ó Foighil, D. Phylogenomic analyses confirm a novel invasive North American *Corbicula* (Bivalvia: Cyrenidae) lineage. *PeerJ* **2019**, *7*, e7484. [[CrossRef](#)]

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