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Phylogeography and Genetic Diversity of Duck Mussel *Anodonta anatina* (Bivalvia: Unionidae) in Eurasia

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Abstract: The duck mussel *Anodonta anatina* is widely distributed throughout the freshwater basins of Northern, Central, and Western Eurasia, and it has a comprehensive genetic structure. This study was devoted to the analysis of lineages, which are defined based on COI gene sequences. Our new dataset was expanded by samples from freshwater basins of Northern and Central Eurasia. It allowed us to reveal a high level of genetic diversity for the widely distributed trans-Eurasian lineage of *A. anatina* for the first time. As for results, representative samples from the Russian Plain, Southern Siberia, and the Ural region showed the presence of multiple interactions between duck mussel populations, indicating the existence of connections between freshwater basins in this region during the Late Quaternary. The genetic group from the freshwater basins of Northern Eurasia may be divided into two sub-lineages, which have differences in genetic structure and distribution patterns. It was revealed that there was a postglacial expansion of duck mussels in the freshwater basins of Northern Eurasia after deglaciations of these territories and that the wide distribution of this species in this region was shaped via ancient connections between periglacial waterbodies. The lineage of *A. anatina* from the Ponto-Caspian region is a genetically rich and diverged group, which is present in the riverine basins of West-Central Asia related to the Caspian Sea.

Keywords: *Anodonta anatina*; freshwater ecosystems; widely distributed species; phylogeography; population genetics; reconstruction; quaternary

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1. Introduction

Anodonta anatina was studied intensively for decades as a widely distributed species in several climatic zones of Eurasia [1–11]. These studies were focused on the assessment of the species diversity of duck mussels due to the variability of shell shape for this species in the various waterbodies it inhabits. Applying molecular methods for the identification of duck mussel specimens allowed to reveal a broad phenotypical plasticity within one species. It established the existence of deeply separated genetic lineages, which are distributed in certain freshwater basins in Eurasia [4,10,11].

Froufe et al. [4] suggested dividing duck mussel populations in Europe into three genetic groups based on the analysis of COI gene sequences. The three groups assumed isolated populations in the following regions: (1) Western Iberia; (2) the Apennines and the Ebro River basin; and (3) the European non-Iberian and non-Italian haplotypes from the Central and North European populations. The authors estimated pairwise distances

between genetic groups listed above to be from 1.9 ± 0.6 % between Iberian and European samples to 3.1 ± 0.9 % between Italian and Iberian samples. These results showed the presence of significant differences in molecular variance among the studied regions and within the studied populations with the highest variability of the parameter among regions. Additionally, the diversification of the groups was assessed using such parameters as F_{ST} between samples (populations). It allowed us to determine the highest values of genetic differences between the Ebro River basin and riverine basins belonging to the Atlantic Ocean basin, which supported the conclusion on the existence of a separate population of the duck mussel there that is genetically close to Italian samples.

Gomes-dos-Santos et al. [12] provided data on the indices of genetic diversity estimated from the COI sequencing data for *A. anatina* populations from several regions of the Iberian Peninsula, belonging to the Iberian genetic group. They revealed significant negative values of both Fu's Fs and Tajima's D neutrality tests for the duck mussel populations from Southwestern and Northwestern Iberia, which belong to this group. This suggested an ability to expand their distribution area and supported previously received results on genetic patterns for the Iberian population of duck mussels [4].

Tomilova et al. [10] added one more COI genetic group of *A. anatina* to this list. They associated an occurrence of the separate genetic lineage of the freshwater mussels with comprehensive geological, paleotectonic, and palaeohydrological environments during the Late Quaternary, which has coincidence with the separation and following diversification of several groups of freshwater animals, including mussels, fish, and some arthropods. Additionally, they provided demographic and population genetic studies for existing genetic groups, along with newly added ones. This led the authors to determine the divergence time for the Azov refugia for freshwater animals in the Pliocene–Pleistocene period. The determined indices of genetic diversity supported conclusions on the presence of ancient refugia for freshwater mussels; additionally, it allowed us to make conclusions on the current state of the populations and on their possible future changes.

Bolotov et al. [9] revised the fauna of freshwater mussels in Russia. The duck mussel *A. anatina* is distributed there among three biogeographical provinces, from the Russian Plain to the Lena River basin in Eastern Siberia, where it had been divided previously into several ecophenotypes based on shells contours, but in fact, this species has shell shapes influenced by habitat parameters and climatic factors in a certain region [13].

There are populations of duck mussels in Eurasia separated by geographical barriers. Some of them are locally isolated in certain freshwater basins [11,14]. Additionally, Lopes-Lima et al. [11] made some remarks on the distribution of duck mussels in Western Asia, and the authors expanded the genetic groups of duck mussels in the haplotype network.

However, applying molecular methods for the assessment of the state of different freshwater species populations may reveal important patterns and develop actions for their future conservation, with special attention to certain regions, considered as distribution areas of endangered mussel populations [15,16]. Currently, duck mussel populations were studied only in local parts of their whole range in this way. Based on these considerations, the main goal of this paper was to investigate trans-Eurasian genetic diversity and the distribution patterns of *A. anatina* based on COI sequence data.

2. Materials and Methods

2.1. Data Sampling

New samples of *A anatina* were collected from the following riverine basins of Northern and Western Eurasia: Dnieper, Danube, Dniester, Volga, Garonne, Neva, Don, Neman, Ob, Dagomys, and, also, small separate rivers (Mamonovka River and Pregolya River) belonging to the Baltic Sea basin. In total, 79 specimens of duck mussels were sampled (Table S1). A tissue snip from each specimen was conserved in 96% ethanol directly after sampling. The samples of muscle tissue snips and shells were deposited in the Russian Museum of Biodiversity Hotspots [RMBH], Federal Center for Integrated Arctic Research, the Ural Branch of Russian Academy of Sciences, Arkhangelsk, Russia.

2.2. DNA Extraction, PCR, and Sequencing

Total genomic DNA was extracted from specimens using the NucleoSpin Tissue Kit (Macherey-Nagel GmbH and Co. KG, Düren, Germany), following the manufacturer's protocol. The COI sequences were amplified and sequenced using the combination of primers LCO1490 and HCO2198 [17], and LoboF1 and LoboR1 [18]. The PCR mix contained approximately 200 ng of total cellular DNA, 10 pmol of each primer, 200 µmol of each dNTP, 2.5 µL of PCR buffer (with 10 × 2 mmol $MgCl_2$), 0.8 units of Taq DNA polymerase (SibEnzyme Ltd., Novosibirsk, Russia), and H_2O, which was added up to a final volume of 25 µL. Thermocycling included one cycle at 95 °C (4 min), followed by 30–33 cycles at 95 °C (50 s), 48–50 °C (50 s), and 72 °C (50 s), and a final extension at 72 °C (5 min). Forward and reverse sequencing was performed using an automatic sequencer (ABI PRISM3730, Applied Biosystems) with the ABI PRISM BigDye Terminator v.3.1 Reagent Kit [19]. The resulting COI gene sequences were checked manually using BioEdit v. 7.2.5 [20].

2.3. Phylogeographic Analysis

The phylogeographic analysis was carried out using a median-joining network approach with Network v. 5.0.0.1 software with default settings [21]. We used the COI dataset that included 500 sequences for the analysis. These sequences were previously published in several works, and they were downloaded from the NCBI Genbank database [3–5,8–12,14,22–28]. Furthermore, we added 79 new sequences to the dataset (Table S1). Missing terminal sites were removed from this dataset, and all sequences were cut following the minimum sequence length (587 bp).

2.4. Population Genetic Analysis

We used the same COI dataset for the population genetic analysis as for the phylogeographic analysis. All specimens for the analysis were divided to five genetic groups (including four genetic lineages and two sub-lineages for one of them) before running the analysis, based on previous results of phylogenetic studies, as suggested by Tomilova et al. [10] and Lopes-Lima et al. [11]. Newly collected specimens were assigned to genetic groups based on the results of the phylogeographic analysis. The five groups of *A. anatina* sequences were considered the samples for population genetic analysis. Genetic diversity was estimated through haplotype diversity (H_d) and nucleotide diversity (π) calculations. Genetic differentiation between samples was estimated through the calculation of F_{ST} values by the method of Tajima and Nei, and inter- and intra-population genetic variability was estimated by AMOVA. We calculated Fu's Fs and Tajima's D neutrality tests to detect deviation from mutation–drift equilibrium in the studied samples. In the case of significance, in at least one neutrality test, we examined the frequency distributions of pairwise mismatch between sequences (MMD). The observed mismatch distribution was compared with that obtained under models of spatial expansion and population expansion for the evidence of model fit, by calculating the sum of squared deviations (SSD) of the observed data relative to the model and Harpending's raggedness statistic. Genetic diversity indices, F_{ST} distances, AMOVA, neutrality tests, and MMD were calculated using Arlequin v. 3.5.1.2 [29], all with 10,000 permutations. In the case where the observed MMD did not deviate significantly from the simulated one, the coalescent simulation of several demographic scenarios was carried out using DNASP v. 6.12.03 [30]. We checked four demographic scenarios, including growth and decline of population, bottleneck, and split/admixture scenarios. The most appropriate scenario was chosen based on the lowest value of probability for each of the three neutrality tests.

2.5. Molecular Dating Analysis

We used the equation $t = \tau/2\mu$, where τ is a moment estimator that represents a unit of mutational time, inferred from the mode of mismatch distribution, and μ is a mutation rate assessed in numbers of nucleotide substitutions per site per generation [31,32].

2.6. Statistical Analyses

Comparisons of *p*-distances between samples were carried out using Mann–Whitney U and Kruskal–Wallis H tests. Principal coordinate analysis and sample comparison were computed using PAST v 4.05 software [33].

3. Results

3.1. Phylogeography, Distributional Patterns, and Population Structure

We used the dataset of *A. anatina* COI sequences, which included 500 individuals, and they were collapsed to 119 unique haplotypes (Table S1). These sequences were divided to four genetic groups, which present samples from river basins of (1) the Iberian Peninsula (excluding the Ebro River basin) and freshwater basins in Morocco (IBER); (2) the North European Plain, Balkan Peninsula, Russian Plain, Asia Minor Peninsula, Western, Central, and Southern Siberia to the Lena River basin on the east and to southern parts of the Ob and Yenisey River basins (EUR); (3) the Italian Peninsula and the Ebro River Basin (ITAL); (4) the Azov-Prikubanskaya Lowland, the southern coast of the Caspian Sea, and the Kura River (located in Transcaucasia) (AZOV). Sample locations from three divergent genetic groups of *A. anatina* (EUR, ITAL, and AZOV) and samples from the Iberian lineage (excluding samples from Morocco) are shown in Figure 1.

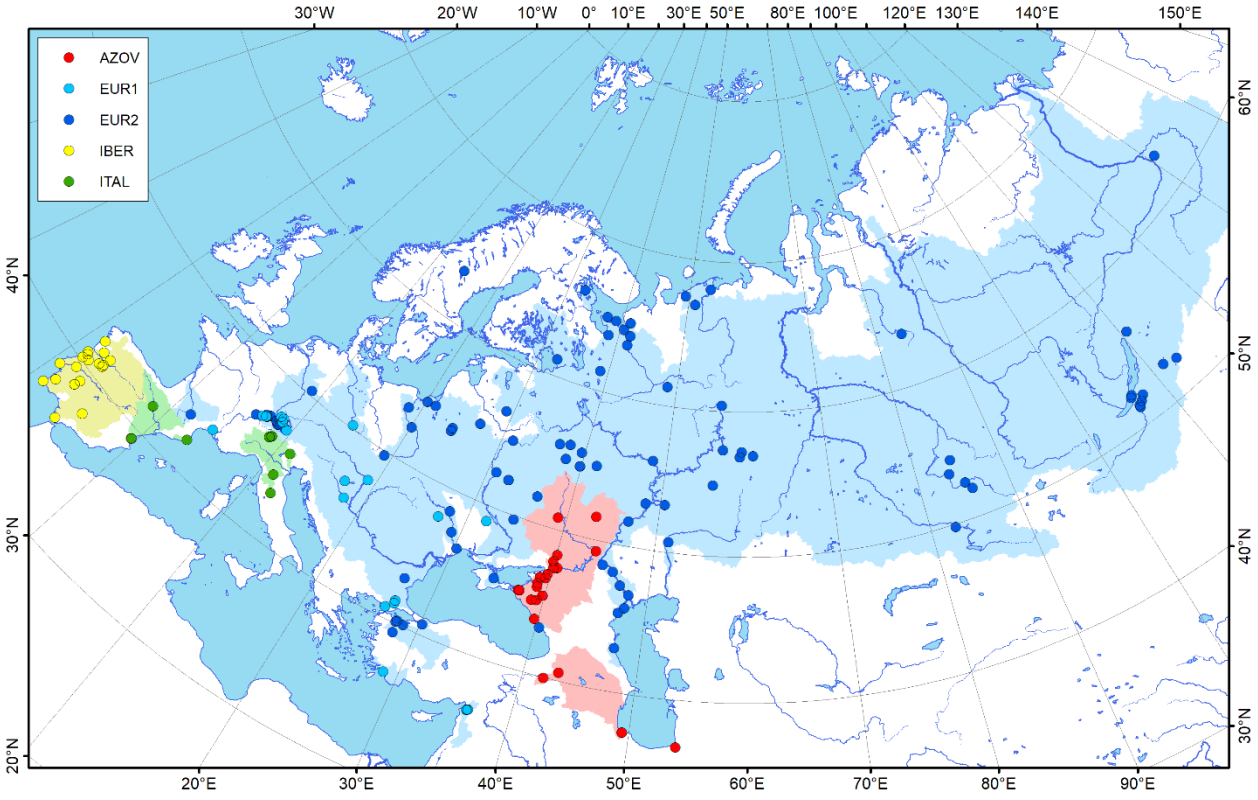

Figure 1. Sampling localities of *Anodonta anatina* in freshwater basins of Eurasia (our data and samples from NCBI GenBank). Point and freshwater basin colors represent the following lineages: IBER—Iberian lineage; EUR1 and EUR2—two Eurasian sub-lineages; AZOV—Azov lineage; and ITAL—Italian lineage (Table S1). The map was created using ESRI ArcGIS 10 software (https://www.esri.com/arcgis, accessed on 6 January 2023). The topographic base of the map was compiled with Natural Earth free vector and raster map data (http://www.naturalearthdata.com, accessed on 6 January 2023), GSHHG version 2.3.7 (http://www.soest.hawaii.edu/pwessel/gshhg, accessed on 6 January 2023) [34], and the HydroSHEDS database (http://www.hydrosheds.org, accessed on 6 January 2023) [35,36]. (Maps: Mikhail Yu. Gofarov).

The EUR genetic lineage was divided to two sub-lineages (EUR1 and EUR2) based on patterns observed in the haplotype network and were further checked by pairwise distances between the sub-lineages and by principal coordinate analysis of these sequence datasets. Sub-lineage EUR1 contains sequences from the western part of the duck mussel's distribution area in Europe, that is, the Danube, Southern Bug, Elbe, Rhine, Po, Vidourle, and Garonne River basins, as well as separate river basins of the Balkan Peninsula. Sub-lineage EUR2 contains sequences from river basins of the Asia Minor Peninsula, Black Sea basin, Arctic Ocean basin, and eastern part of the Baltic Sea basin, as well as from the Volga and the Elbe River basins. Statistically significant differences were observed between the two sub-lineages by principal coordinate 1 ($p < 0.001$). Additionally, the presence of statistically significant differences between samples of pairwise differences between the AZOV lineage, which is the closest to the EUR lineage, and the sub-lineages inside the EUR genetic group (EUR1 and EUR2), was revealed.

3.2. Genetic Diversity, Population Genetic Indices, and Tests

Four highly diverged genetic groups were identified in the haplotype network of *A. anatina* (Figure 2). The number of nucleotide substitutions between groups varies from seven (EUR vs. AZOV) to seventeen (IBER vs. ITAL). The Italian lineage presents eight unique haplotypes, and the two biggest of them are haplotypes from the Po and Ebro River basins. The Iberian lineage has several distributed haplotypes, which correspond to the Douro, Guadiana, Tejo, and Vouga River basins. Additionally, the lineage included samples from Moroccan riverine basins. The AZOV lineage included sequences from the Azov Sea river basins, and the most distributed haplotype is present in the Kuban, Don, Beisug, Chelbas, and Yeya River basins. Furthermore, this genetic group contains two haplotypes (Figure 2, haplotype A-B), which are distributed in the Kura River basin in Turkey and in separate rivers in the southern part of the Caspian Sea basin in Iran (the Sefid and Qarasu River basins). Samples from the Baranovskoe Lake (Figure 2, haplotype C) belonged to the AZOV genetic group, despite the sampling site being located in the Dagomys River basin (Black Sea basin). The largest numbers of sequences ($N = 305$) and unique haplotypes ($N = 62$) belonged to the EUR lineage, which covers a vast territory in Western, Northern, and Central Eurasia, in comparison to other *A. anatina* lineages, such as ITAL, IBER, and AZOV (Figure 1). The most distributed haplotypes in the EUR lineage form star-like structures, and they belong to duck mussel populations from the North European Plain, the Russian Plain, and the West Siberian Lowland.

Pairwise distances between *A. anatina* genetic groups varied from 1.39 to 3.68 % (Table 1). F_{ST} values were assessed to be from 0.561 to 0.924.

Table 1. Pairwise distances and F_{ST} values between genetic groups of *A. anatina* ($p < 0.00001$ for all F_{ST} values).

		***p*-Distance, %**				
		IBER	**EUR1**	**EUR2**	**ITAL**	**AZOV**
Population pairwise F_{ST}	IBER	X	2.25 ± 0.45	2.73 ± 0.55	3.34 ± 0.63	2.56 ± 0.51
	EUR1	0.633	X	1.39 ± 0.35	3.14 ± 0.65	2.29 ± 0.50
	EUR2	0.833	0.735	X	3.68 ± 0.74	2.44 ± 0.53
	ITAL	0.765	0.833	0.924	X	3.21 ± 0.64
	AZOV	0.561	0.592	0.766	0.725	X

Haplotype diversity varied from 0.706 ± 0.027 to 0.912 ± 0.017 among the studied populations. Nucleotide diversity was assessed to be from 0.002 ± 0.0015 to 0.011 ± 0.006. Significant values of neutrality tests were estimated only for the EUR group, and within this sample, significant negative values from both neutrality tests were revealed only for the EUR2 sub-lineage. The Tau parameter was estimated to be from 0.934 to 6.337 (Table 2).

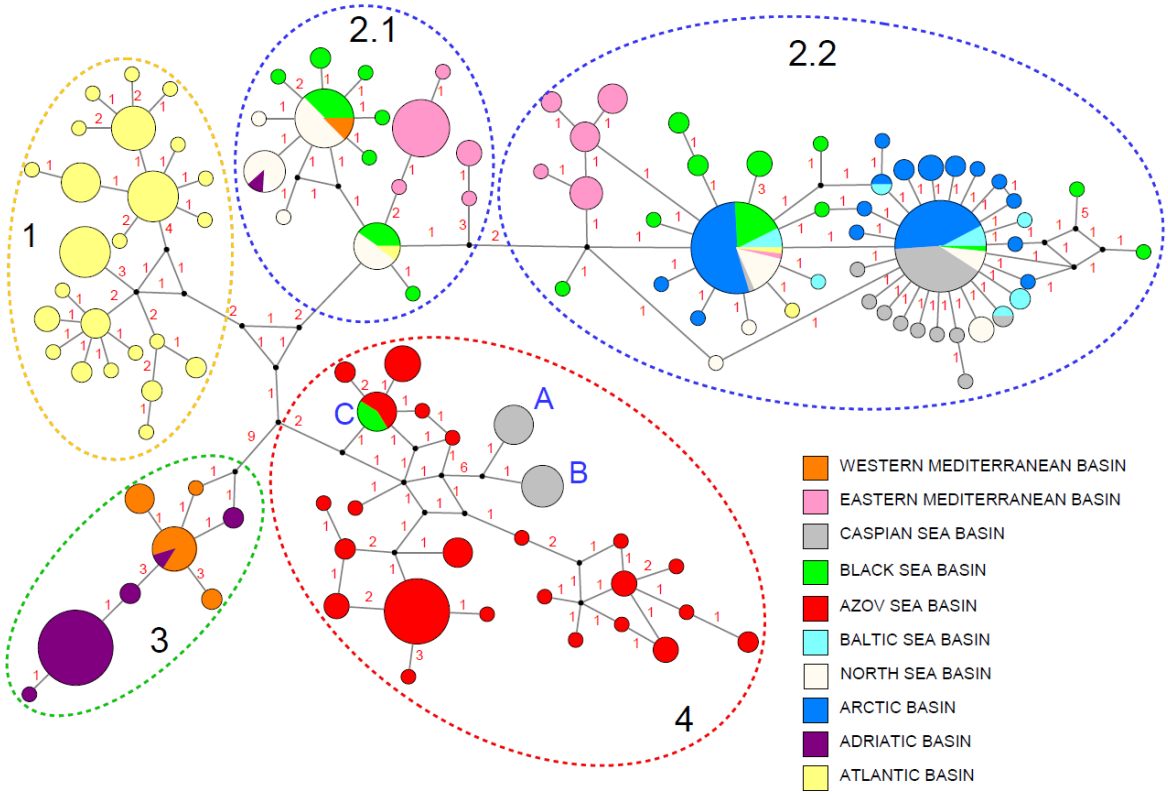

Figure 2. Median-joining haplotype network of the COI sequences of *A. anatina* dataset (*N* = 500; the list of sequences is presented in Table S1). The black numbers indicate genetic lineages, which were used as separate samples in population genetic analyses: 1. lineage IBER (*N* = 68 sequences); 2.1. sub-lineage EUR1 (*N* = 64 sequences); 2.2. sub-lineage EUR2 (*N* = 241 sequences); 3. lineage ITAL (*N* = 47 sequences); 4. lineage AZOV (*N* = 80 sequences). Blue letters indicate the haplotypes: A—Kura River basin, B—Southern Caspian basin, C—Baranovskoe Lake. The red numbers near branches indicate the number of nucleotide substitutions between haplotypes. The size of the circles corresponds to the number of available sequences for each haplotype (smallest circle = 1 sequence).

Table 2. Summary of genetic diversity indices estimated from the COI sequencing data for genetic lineages of *A.anatina*: sample size (*N*), number of haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π). The results of Fu's Fs and Tajima's D neutrality tests. Statistically significant values are followed by an asterisk ($p < 0.05$ for Tajima's D and $p < 0.02$ for Fu's Fs).

Lineage	*N*	h	H_d	π	Fu's Fs	Tajima's D	Mismatch Analysis (Spatial Expansion Model): Estimated τ
IBER	68	24	0.912 ± 0.017	0.010 ± 0.005	−5.389	−0.533	6.337
ITAL	47	8	0.658 ± 0.066	0.004 ± 0.003	0.307	0.041	4.110
AZOV	80	25	0.907 ± 0.021	0.011 ± 0.006	−4.064	0.017	5.939
EUR	305	62	0.810 ± 0.019	0.006 ± 0.003	−25.872 *	−1.939 *	5.452
incl.:							
EUR1	64	16	0.850 ± 0.024	0.005 ± 0.003	−3.660	−0.490	2.485
EUR2	241	46	0.706 ± 0.027	0.002 ± 0.0015	−28.793 *	−2.454 *	0.934

3.2.1. Mismatch Distribution Analysis

Mismatch distribution analysis showed values $p > 0.05$ for both the sum of squared deviation and for the Harpending's raggedness index for both sub-lineages from Western Europe (EUR1) and from Central Europe, Eastern Europe, and Asia (EUR2). Both distributions correspond to the spatial distribution model.

Mismatch analysis for the sample EUR1 revealed multimodal distribution (Figure 3). Additionally, the statistical significance ($p > 0.05$) for both tests of goodness-of-fit supported the previously defined hypotheses on the stable development of this population. We determined only one high pick and the unimodal distribution for the EUR2 population based on the results of the mismatch analysis (Figure 3). The results correspond to the postglacial population. The EUR2 sub-lineage had the lowest value of the Tau parameter, and it suggested the lowest numerical value of divergence time. The haplotype network showed this genetic structure as one widely distributed haplotype, with a large number of single haplotypes divided by one nucleotide substitution.

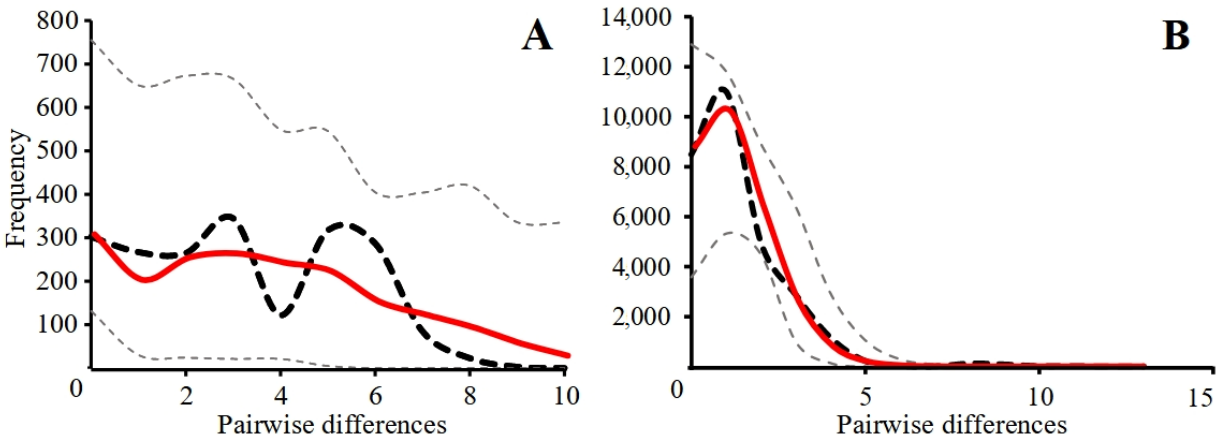

Figure 3. Mismatch distributions of *A. anatina* samples based on the mitochondrial COI gene: (**A**) mismatch distribution of the EUR1 sub-lineage; test of goodness-of-fit: sum of squared deviation = 0.015; *p* (Sim. SSD >= Obs. SSD) = 0.440; Harpending's raggedness index = 0.035; *p* (Sim. Rag. >= Obs. Rag.) = 0.620; (**B**) mismatch distribution of the EUR2 sub-lineage; test of goodness-of-fit: sum of squared deviation: 0.003; *p* (Sim. SSD >= Obs. SSD) = 0.150; Harpending's raggedness index = 0.061, *p* (Sim. Rag. >= Obs. Rag.) = 0.360. Bold dashed black lines indicate observed distribution, and solid red lines represent simulated distribution under a spatial expansion model. Dashed lines represent lower and upper confidence intervals ($p < 0.01$).

3.2.2. Coalescent Simulation

The simulation under the coalescent model revealed two scenarios with the highest values of significance in several neutrality tests, (including Fu's Fs, Tajima's D, and the Ramos-Onsins and Rozas R2 statistic) (Table 3), which were the "decline" and "bottleneck" scenarios. We rejected the decline scenario for the EUR2 population, according to data presented in the haplotype network (star-like shapes) and based on data on the number of specimens and unique haplotypes, which belonged to this sample. Therefore, the most likely scenario for sample EUR2 was established as the "bottleneck".

Table 3. The significance of Fu's, Tajima's, and Ramos-Onsins and Rozas's neutrality tests used in coalescent-based simulation: probabilities of the most appropriate scenario are in bold.

Neutrality Test	Criteria Value	Scenario			
		Growth	Decline	Bottleneck	Split/Admixture
Fu's Fs	−65.184	$p < 0.001$	$p < 0.001$	$\mathbf{p < 0.001}$	$p < 0.001$
Tajima's D	−2.480	$p < 0.001$	$p < 0.001$	$\mathbf{p < 0.001}$	$p < 0.001$
Ramos-Onsins and Rozas R2 statistic	0.014	$p < 0.05$	$p < 0.0005$	$\mathbf{p < 0.0005}$	$p < 0.05$

3.2.3. Divergence Time Estimation

The divergence time between the AZOV lineage and EUR1 sub-lineage was estimated as 799 ka BP (95% CI = 659–2496 ka BP), whereas the divergence time between EUR1 and EUR2 sub-lineages was estimated as 300 ka BP (95% CI = 211–731 ka BP), based on values of the Tau parameter received from the mismatch distribution analysis.

Two events of the distribution of the postglacial population correspond to interglacial periods on the geochronological scale, with data variation of oxygen isotopes in ice cores during the Quaternary. These two episodes are illustrated in the haplotype network by star-like shapes, and they included samples from the following freshwater basins: Neman, Bzyb, Oder, Vistula, Dniester, Dnieper, Danube, Rhine, Elbe, Pechora, Severnaya Dvina, Ob, Yenisey, Lena, Volga, and Ural River basins (Table S1).

The mean divergence of sub-lineage EUR2 from the AZOV lineage was higher than the mean divergence of sub-lineage EUR1 from the AZOV lineage (mean *p*-distances were 2.44 ± 0.53 % and 2.29 ± 0.50 %, respectively; Mann–Whitney test: $p < 0.0005$) (Table 1).

The PCoA revealed the presence of statistically significant differences between sub-lineages EUR1 and EUR2 using a comparison of these groups by principal coordinate 1 ($p < 0.0001$) (Figure 4).

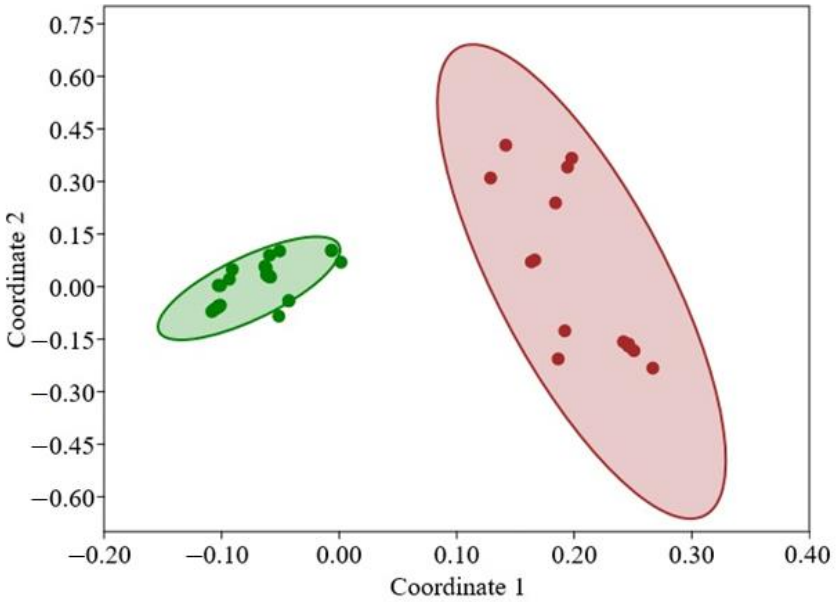

Figure 4. The results of the PCoA of the COI sequence datasets from sub-lineages EUR1 (green) and EUR2 (red).

The results of the test of genetic structure using AMOVA were significant (F_{ST} = 0.766, df1 = 4, df2 = 495, and $p < 0.0001$), and the highest variation was found among a group of populations (76.56 %), compared to the variation within populations (23.44 %).

4. Discussion

4.1. Distribution Patterns

4.1.1. Interglacial Environment as a Driver of *Anodonta anatina* Distribution during the Late Pleistocene in Northern Eurasia

The calculated ages of EUR2 sub-lineage divergence correspond to the Dnieper (Saale) glaciation with the subsequent transition to Mikulino (Eemian) interglaciation. This episode was characterized by the occurrence of giant interglacial waterbodies, which existed periodically in territories of the North European Plain and expanded to the east in Eurasia across the Russian Plain with the occurrence of warm climate episodes [37–39]. The haplotype network showed that the sub-lineage EUR2 has a star-like shape, corresponding to the origin of this group from several haplotypes, with their subsequent radiation in

suitable environmental niches. Multiple connections between periglacial waterbodies in Northeastern Europe was one of the main drivers of freshwater pearl mussel distribution after the Last Glacial Maximum in Northeastern Europe [40]. We can suggest a similar scenario for the wide distribution of *A. anatina*, but at significantly broader scales. The occurrence of the interglacial lakes allowed the spreading of freshwater mussels throughout the freshwater basins of Northern Eurasia (including Eastern Europe and Siberia). This scenario is supported by the received network's shapes for recent lineages, the central haplotype of which is presented there, and it is distributed in large river basins in a main part of the plain. Several haplotypes are separated from the central one by single nucleotide substitution, and these haplotypes are present in remote or locally separated freshwater basins (the Onega River basin, Neva River basin, and separate river basins of the Eastern Baltic Sea, Pechora River basin, the upper parts of the Yenisey River basin and Ural River basin, and the upper part of the Ob River basin).

Additionally, the distribution of duck mussels in the Late Quaternary among the freshwater basins of the southern part of the Russian Plain may be related to changes in Caspian Sea basin coastlines. Several authors provided data on changes in the sea level during the Khazarian and Khvalyn transgressions, which may have caused the connection between freshwater basins in the Russian Plain and may have led to the observed distribution of freshwater mussels [38,41–43].

4.1.2. Recent Distribution Episodes of the Duck Mussel Induced by Connection between Freshwater basins via Water Channels

The observed network shapes revealed the connection between two initially separated freshwater basins in Europe (the Rhine and Danube River basins). However, there are some pieces of evidence of the expansion of freshwater animals via the Rhine–Main–Danube Canal [44]. The haplotype network of *A. anatina* samples suggested the existence of similar distribution patterns for duck mussels, which may increase their dispersal between freshwater basins. We recorded haplotypes of the AZOV lineage out of the Azov Sea's riverine basins in an artificial waterbody of the Dagomys River basin (Baranovskoe Lake) that belongs to the Black Sea basin. The presence of duck mussel haplotypes from the AZOV lineage may be explained by the existence of a fish farm that engages in carp breeding. It is necessary to note that the largest river basins are currently connected by artificial channels in the Russian Plain (e.g., the Volga and Don Rivers) and that it may lead to the discovered distribution patterns and to changes in the genetic structure of a local mussel population, such as that of the duck mussel.

4.2. Genetic Diversity and Differentiation

We confirmed previous results that suggested a relatively low genetic diversity in the Italian lineage of duck mussels [4,10]. At the same time, new data on this group from the upper part of the Po River basin showed that the real level of genetic diversity in this group is compared with the one determined earlier. We should note here that this lineage includes several haplotypes, which are distributed among separate riverine basins (e.g., the Po, Tiber, Reno, and Ebro River basins). The shape of the haplotype network presented a population that existed in these basins for a long time, and it had a high level of divergence, both within the lineage and outside it, in relation to other samples.

The network structure for group IBER seemed similar to ITAL, but several separate haplotypes were revealed in a few large river basins (the Guadiana, Tejo, and Douro River basins). Additionally, we determined the highest mean value of the haplotype diversity for this group among all genetic groups. The Moroccan population of duck mussels also belongs to this genetic group, but its impact on the total diversity of this group is low. The previously defined AZOV lineage is also present in freshwater basins in Transcaucasia, including the relatively large Kura River basin and local populations in the Southern Caspian basin, according to recent studies [11]. Despite this, we confirmed here by network

shapes that the most distributed haplotypes from the lineage are present in the Kuban, Don, Beisug, Chelbas, Yeya, and Kirpili Rivers [10].

The EUR lineage is presented in the network by the largest number of unique haplotypes (Table 2) and it formed different structures there (Figure 2), including star-like shapes in the eastern part of the distribution area and several abundant haplotypes in the western part of Eurasia. These shapes for the western part of the duck mussel's range are quite similar to ones in the freshwater basins of the Iberian and Italian peninsulas. At the same time, we determined the presence of large numbers of singleton haplotypes towards to the eastern part of the range, which was influenced by several consequent glaciations over the Pleistocene epoch [37]. We observed the most likely differentiation between these two sub-lineages along the border between Europe and Asia, along the Bosfor Strait, Marmara, and Aegean Seas, and the sub-lineages are divided there by four nucleotide substitutions from each other.

The observed EUR1 sub-lineage did not deviate from a standard neutral model, according to negative and insignificant values of neutrality tests (Table 2), which supported the idea on some similarity of genetic structure of this sub-lineage to Iberian genetic group. Significant and negative values from both neutrality tests were observed for the EUR2 sub-lineage. Additionally, the lowest value of the Tau parameter indicated the lowest divergence time for this population. We determined that only one pick in the mismatch analysis for this sub-lineage corresponds to a star-like network shape. Previous studies of postglacial populations showed that such structure in a haplotype network indicates recent expansion [45]. The bottleneck scenario was selected as the most probable demographic scenario for the EUR2 sub-lineage after coalescent simulation. All this evidence may indicate the postglacial expansion of duck mussels in the freshwater basins of Northern Eurasia after the deglaciations of these territories, followed by a wide distribution of this species in this region via ancient connections between periglacial waterbodies [37]. This supports our conclusion on the distribution patterns of this species during periods of significant climatic variations in this region in the Late Quaternary.

4.3. Conservation Priorities

Some populations of this species distributed in arid and semi-arid regions may need conservation efforts [14]. Some of them exist in Western Asia in separate riverine basins, which belong to the Caspian Sea and to the Eastern Mediterranean [11,14]. Additionally, conservation efforts may be considered for isolated populations of duck mussels in Southern Europe, where they are distributed in separate riverine basins along the Mediterranean and Atlantic coasts [4].

5. Conclusions

The duck mussel has distribution patterns, which are related to regional environmental changes. Past climatic fluctuations and changes in the hydrological regime of certain territories had a significant influence on the current distribution and genetic diversity of populations. The occurrence of geographic barriers to *A. anatina* dispersal has determined the existence of separate populations or groups of populations. We determined relatively high values of genetic diversity for the duck mussel populations in Northern Eurasia.

We determined the presence of structures in the haplotype network with a widespread central haplotype and numerous singleton haplotypes. These divergent populations were found in conditions of spatial isolation. The observed pattern in the median-joining network showed a postglacial expansion of *A. anatina* from Southern and Western Europe towards the regions of Northern Eurasia. The postglacial patterns in the genetic structure were revealed for the recent group from these territories, and we determined the demographic scenario of a possible bottleneck in the Late Quaternary. *A. anatina* has been diversified in suitable environmental niches, which occurred after deglaciation. At the same time, the Azov genetic group is the closest group with high values of genetic diversity. These populations can be considered as key evolutionarily significant units for this model species,

compared to the stressed populations at the western edge of its range. Future studies should be aimed at the assessment of the diversity of stressed populations to develop conservation priorities for them.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15020260/s1, Table S1: List of *Anodonta anatina* (Linnaeus, 1758) COI sequences used in this study.

Author Contributions: A.A.L. and A.A.T. developed the concept of this study. A.A.L., A.A.T., A.V.K., E.S.K., I.V.V., M.Y.G., T.A.E., O.V.A., G.V.B., D.V.K., T.L.G., O.M., O.S.P., and I.N.B. collected samples. A.V.K. and A.A.T. designed and carried out molecular analyses. A.A.L. performed population genetic and statistical analyses. A.A.T. performed phylogenetic analysis. M.Y.G. created the map. A.A.L. wrote the paper, with input from I.N.B., A.A.T. and I.V.V. All authors have read and agreed to the published version of the manuscript.

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