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# Genetic diversity of zebra mussel (*Dreissena polymorpha* (Pallas, 1771)) as a reflection of successful invasion into the water bodies in Baltic Sea region

Aleksandra Morozova, Jeļena Oreha,* Nataļja Škute

Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Daugavpils, Latvia

## Abstract

*Dreissena polymorpha*, the zebra mussel, is one of the most widespread and ecologically disruptive invasive species in European freshwater systems. Despite its long presence in Latvia, genetic information on local populations has been lacking. This study provides the first comprehensive assessment of genetic diversity and population structure of *D. polymorpha* across seven Latvian waterbodies, representing lakes, a reservoir, and a river system. Individuals were genotyped using five polymorphic microsatellite loci. Genetic variation was analysed through the standard genetic parameters, namely, number of alleles and frequency, heterozygosity estimates and genetic differentiation was estimated using $F_{ST}$ statistics, Bayesian clustering, PCA, and Nei's genetic distance. All loci were polymorphic, and no evidence of null alleles or recent bottlenecks was detected. Populations exhibited high genetic diversity. Significant heterozygote deficits were found in most populations. Genetic differentiation among populations was moderate overall, though three geographically proximate lakes showed minimal differentiation. Bayesian clustering and PCA identified four distinct genetic groups, indicating that hydrological isolation and limited dispersal contribute to population structuring. These findings demonstrate that Latvian *D. polymorpha* populations maintain substantial genetic diversity and exhibit clear spatial genetic structuring. This genetic information provides an essential foundation for monitoring invasion dynamics and informing management strategies aimed at limiting further spread and ecological impact.

**Key words:** *Dreissena polymorpha*; invasion; microsatellites; population genetics; genetic variability; adaptability.

*Correspondence to: jelena.oreha@du.lv*

## Introduction

*Dreissena polymorpha*, commonly known as the zebra mussel, is a small freshwater bivalve native to the waterbodies of the Ponto-Caspian region and currently inhabits much of Europe and North America.

Zebra mussels (*Dreissena polymorpha*) prefer areas with weak currents, which facilitate natural dispersal. Their planktonic larvae (veligers) can swim freely in the water column, enabling long-distance downstream transport (Olson *et al*., 2018). Dispersal also occurs along river currents and through attachment to aquatic vegetation. Adult mussels use byssal threads to anchor themselves to hard surfaces such as boat hulls, ships, underwater equipment, and other substrates, contributing to anthropogenic spread (Johnson and Carlton, 1996). A novel phoretic interaction has recently been documented between zebra mussels and the carp minnow, the lake chub (*Couesius plumbeus*), suggesting that fish migration may further exacerbate mussel dispersal within aquatic systems (Ricciardi and Hill, 2023). Additionally, overland transport by anglers carrying bait from infested water bodies may further facilitate the spread of zebra mussels. The first invasion of the species *D. polymorpha* in Latvia was in the Gulf of Riga in the mid-1800s (Zając and Bonk, 2019). Today, this mollusc is widespread, and its numbers in Latvian waterbodies are increasing (Morozova and Shkute, 2023). Thus, *D. polymorpha* has become one of the most notorious invasive species in freshwater systems worldwide (Peñarrubia *et al*., 2016), leading to widespread ecological and economic impacts, including the disruption of local ecosystems and the clogging of water-intake pipes.

The relevance of this study stems from the continued expansion of *D. polymorpha* across European freshwater systems, including the Baltic region, where its ecological and economic impacts are intensifying (HELCOM, 2020). The species is listed among the "100 of the World's Worst Invasive Alien Species" due to its capacity to restructure communities, outcompete native molluscs, alter trophic interactions, and cause substantial economic losses through biofouling (Lowe *et al*., 2000; Birnbaum, 2011). Ongoing climate change and increasing anthropogenic pressures are expected to further facilitate its spread and exacerbate invasion-related impacts (Orlova and Panov, 2004).

Genetic analysis provides a powerful tool for evaluating the success of *D. polymorpha* invasions across diverse ecological contexts. For instance, the invasion of Lake Peipus (Estonia) has shown limited success, as evidenced by population-level genetic assessments indicating reduced expansion and low genetic diversity in this region (Travina et al., 2025). Such outcomes may be influenced by environmental instability, particularly in European freshwater systems that are increasingly vulnerable to climate change. Northern and eastern European countries, including Estonia, are

experiencing pronounced temperature fluctuations and hydrological shifts, which can disrupt the establishment and persistence of invasive aquatic species.

Microsatellite analysis is a well-established tool in population genetics, enabling the assessment of genetic diversity, population structure, and gene flow (Selkoe and Toonen, 2006). In invasive species research, this approach provides critical insights into colonization pathways, evolutionary history, and adaptive potential. For example, microsatellite markers have been used to trace the introduction routes of *D.polymorpha* across European and North American freshwater systems, revealing multiple independent invasion events and founder effects (Marshall and Stepien, 2021; Travina *et al*., 2025). Several studies have shown that invasive populations are genetically distinct from native populations, with limited admixture between different invasion fronts (Chen *et al*., 2021; Estoup *et al*., 2016).

As genetic data for *D. polymorpha* populations in Latvian waterbodies remain scarce, this study will provide the first comprehensive assessment of genetic diversity and connectivity across multiple aquatic systems in Latvia. These findings will establish a foundation for improved monitoring and management strategies aimed at mitigating the spread of *D. polymorpha* in the Baltic basin.

To investigate the invasion dynamics of *D. polymorpha* in Latvia, seven waterbodies representing different ecosystem types (river, reservoir, lakes) were selected. The study aimed to characterize the population genetic structure of the species using microsatellite markers.

We propose that the genetic variability and differentiation of zebra mussel populations in Latvian waterbodies reflects their adaptation to local habitat conditions and, potentially, limited gene flow between populations due to habitat characteristics and human activity.

## Methods

### Sample collection

Mussels were collected from 7 waterbodies, namely Pļaviņu Reservoir (Pļaviņu Res.) (an artificial lake created by a dam on Daugava River as part of the Hydroelectric Power Plant), the Lielupe River (Lielupe R.), Lake Laucesas (L. Laucesas), Lake Usmas (L. Usmas), Lake Sventes (L. Sventes), Lake Riču (L. Riču), Lake Drīdzis (L. Drīdzis) in Latvia (see location, surface area and depth of waterbodies in Tab. 1 and Fig. 1). The material was sampled in each waterbody in one locality at a depth of 2 m using a hand-held bottom scraper; in Pļaviņu Res. (middle course of the Daugava R.) before the dam and in lower course of Lielupe R. Approximately 50 mussels were taken from spatially separated patches within the colony (≥0.5-1 m apart) to avoid insert to analysis individual from the same shell cluster. Collected material was placed in 96% ethanol and stored at -80ºC to await DNA extraction.

### Microsatellite analysis

Genomic DNA was extracted from whole body tissue from each individual separately using Qiagen the DNeasy kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instructions. DNA was quantified and qualified spectrophotometrically by BioSpec-Nano (Shimadzu).

The extracted DNA was stored at -20°C. For subsequent molecular analysis, the DNA was diluted to a final concentration of 20 ng/µL. Extracted genomic DNA was used as a template for DNA amplification using the polymerase chain reaction (PCR). For genetic analysis 30 samples per each sampling site were chosen. The individuals were genotyped using five microsatellite loci: three of them were trinucleotide repeat loci (*Dpo101*, *Dpo221, Dpol A6*) (Naish and Boulding, 2001; Feldheim *et al*., 2011), two of them were tetranucleotide repeat loci (*Dpo260*, *Dpo272)* (Chapuis and Estoup, 2007). PCR was performed with fluorescently marked primers (TAMRA, HEX, FAM) in a volume of 10 µL using ABI 9700 thermocycler. PCR mixture components were: 100 ng of DNA sample, 10 mM Tris-HCl buffer with 50 mM KCl, 1.5 mM $MgCl_2$, 2 mM dNTP mix, 0.06 $10^6$ $U\ L^{-1}$ Taq DNA polymerase, 0.4 $mol\ L^{-1}$ of each primer. The PCR thermal cycling program had an initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 30 s, annealing at 50°C (for *Dpo260*, *Dpo272*), at 52°C (for *Dpo101*, *Dpo221*) and at 58°C (for *Dpol A6*) for 30 s, and extension at 72°C for 60 s, followed by a 7 min final extension at 72 ˚C and cooling at 4°C. Both positive and negative controls were used during PCR amplification. PCR products were examined on an ABI 310 automated analyzer using Genescan ROX500 size standard (Applied Biosystems, Thermo Fisher Scientific, Preston, Bedford, MA, USA), alleles were scored in GeneMapper 3.7 software (Applied Biosystem).

### Statistical analysis

The Micro-Checker 2.2.3 software was used to check the data for typographic errors in allele calls, to identify the null allele and genotyping errors in microsatellite data, short allele dominance (large allele dropout) and the scoring of the stutter peaks (Chapuis and Estoup, 2007; Van Oosterhout *et al*., 2004). The computer program Bottleneck 1.2.02. (Cornuet and Luikart, 1996) was used to detect the bottleneck effect on the studied population. The allele number in the locus, its frequency, alleles in the population, observed and expected heterozygosity, and the level in the polymorphic locus, genetic divergence was estimated by pair-wise $F_{ST}$ values

**Tab. 1.** The characteristics of Latvian waterbodies in which the *Dreissena polymorpha* was collected.

| Waterbodies | Position (coordinates) | Area (km²) | Mean depth (m) | Max depth (m) |
|---|---|---|---|---|
| Pļaviņu Reservoir (Daugava River) | 56°35′15″N 25°15′58″E | 35 | 14.6 | 47 |
| Lielupe River | 56°24′10″N 24°9′25″E | 17 600 | 5 | 15 |
| Lake Laucesas | 55º45′11″N 26º17′49″E | 1.86 | 5.4 | 15.5 |
| Lake Usmas | 57°12′N 22°10′E | 41.4 | 5.4 | 27 |
| Lake Sventes | 55°51′N 26°21′E | 7.348 | 7.8 | 38 |
| Lake Riču | 55°42′9″N 26°42′35″E | 12.83 | 9.7 | 51.9 |
| Lake Drīdzis | 55°58′N 27°16′E | 7.532 | 12.8 | 66.2 |

(Weir and Cockerham, 1984), using GeneAlex 6.41 software (Peakall and Smouse, 2006). The *p*-values for the pair - wise $F_{ST}$ values were corrected for multiple comparisons by Bonferroni corrections (BFCs) following Rice (1989). To estimate and visualize the genetic structure and differentiation of the studied populations and possible relatedness, the computer programs STRUCTURE 2.3 (Hubisz *et al*., 2009) and POPHELPER Structure WebApp v 1.0.10. (Francis, 2017) were used. A model assuming admixture and correlated allele frequencies between K populations (Burn-ins of 100,000 replications and 300,000 Markov chain Monte Carlo (MCMC) replicates) were used. Sampling locations were used as a priori information to assist the structuring (the LOCPRIOR model) as recommended for weak signals of structuring (Hubisz *et al*., 2009). Values of K between one and seven were tested, running STRUCTURE ten times for each K and using Evanno's ΔK method to determine the most suitable number of clusters (Evanno et al., 2005). The most likely (highest lnPr(X|K)) grouping was visualized using POPHELPER Structure Web App v 1.0.10 (Francis, 2017). The genetic relatedness of the populations was estimated with the help of Nei's (Nei *et al*., 1983) index of genetic distance (D) using the computer program Populations 1.2.32 (Langella, 2007). The dendrogram was created according to the UPGMA method using the computer program TREVIEW (Page, 1996).

## Results

The genetic variation of seven Latvian *Dreissena polymorpha* populations scoring five microsatellite loci was investigated. The data were verified in the software Micro-Checker 2.2.3., which identified no typographic errors or large allele dropout. According to Micro-Checker, putative null alleles were not detected. Allele frequencies followed an L-shaped distribution, i.e., no bias of allele frequencies toward mean values was observed in the population, as expected in a non-bottlenecked population at mutation-drift equilibrium. The level of allelic diversity and heterozygosity exhibited in invasive populations was characteristic of the source population and would not have been detected in the case of a severe bottleneck.

All five loci were polymorphic in all populations of *D. polymorpha* in all studied waterbodies. Allele size ranges were: 210-397 bp for *Dpol A6,* 112 - 288 bp for *Dpo260,* 112-288 bp for *Dpo272*, 168-339 bp for *Dpo101* and 66-287 bp for *Dpo221*. The number of alleles on each microsatellite locus was variable. The greatest number of alleles (38) was found in locus *Dpo101* and the minimum (18) in locus *Dpo272* (Tab. 2.)

The high polymorphism recorded is reflected in high expected heterozygosity ($H_E$) values (Tab. 3). Altogether values of observed heterozygosity ($H_O$) were lower than those expected for six popula-

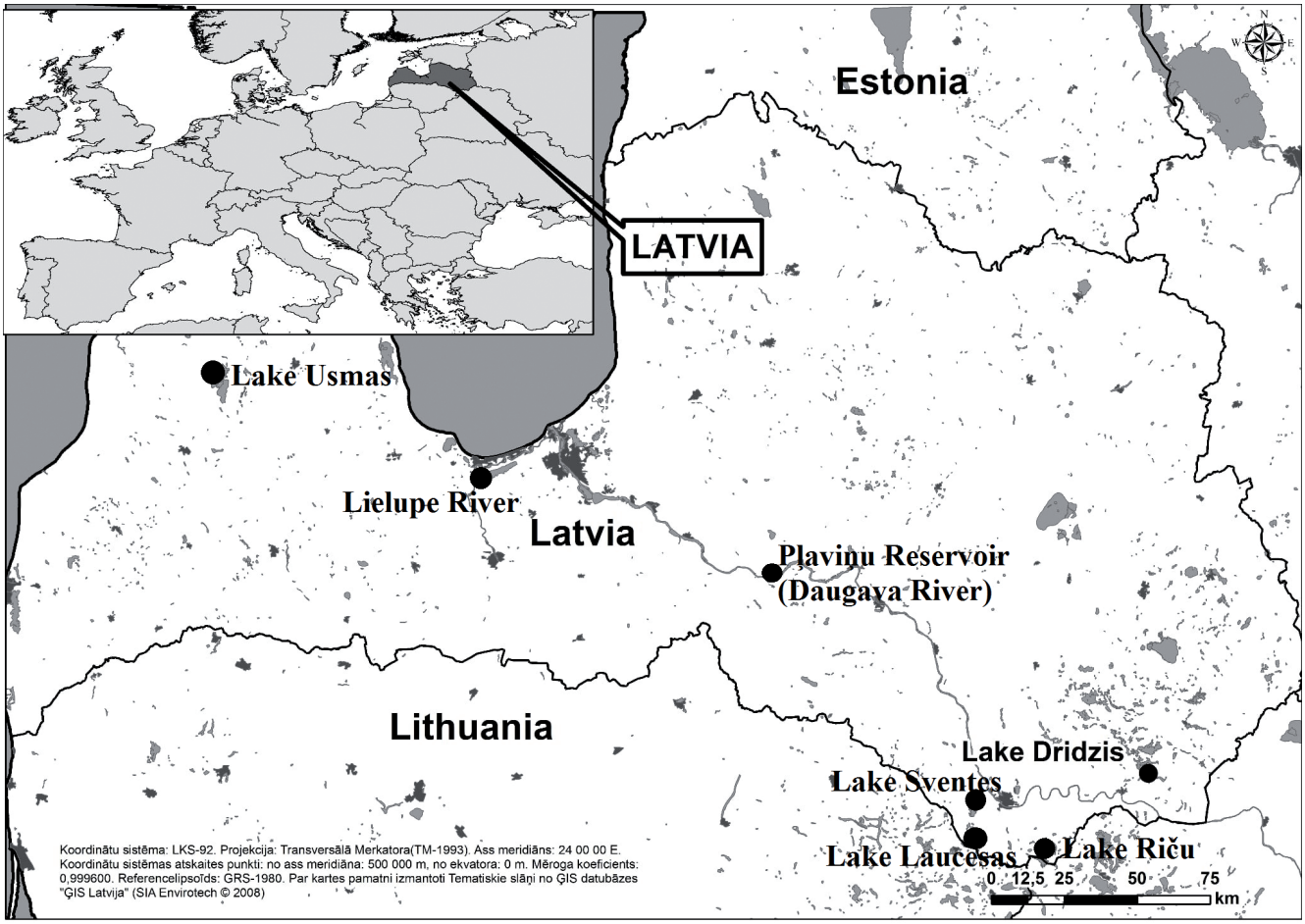


**Fig. 1.** *Dreissena polymorpha* sampling sites.

tions, besides Pļaviņu Reservoir population, where observed level of heterozygosity ($H_O$=0.81) was higher than expected value ($H_E$=0.77). The *L. Laucesas* population had the lowest observed heterozygosity level ($H_O$=0.43), but Pļaviņu Reservoir population had the highest observed heterozygosity level ($H_O$=0.80).

Deficiency of heterozygotes with high significance was shown also in each locus analysis (Tab. 4). Tab. 4 shows the significant and non-significant deviations of HWE in the individual locus tests (for each population) after sequential Bonferroni corrections (BFc). The individual locus tests displayed that 32 cases out of 35 had significant deviations of genotype frequencies from Hardy-Weinberg equilibrium (HWE) before and 30 cases out of 35 after BFc. No significant deviations from HWE were detected in Lake Riču and Lake Sventes (*Dpol260*) and in Pļaviņu Reservoir population in locus *Dpol272* before and after BFCs. Non-significant deviations of HWE in the individual locus tests after sequential BFc were revealed in Pļaviņu Reservoir population in locus *DpolA6* and in Lake Riču in locus *Dpol101*. A heterozygote deficit was detected in 26 out of 30 cases of significant HWE deviations after BFCs. Heterozygote excess was detected in four out of 30 cases of significant HWE deviations after BFCs (in locus *Dpol221* in Pļaviņu Reservoir population and in locus *Dpo101* in Lake Usmas, Lake Sventes and Lake Drīdzis).

The data of $F_{ST}$ and $R_{ST}$ statistics in total and in each studied microsatellite locus (the level of significance is given in brackets) are shown in Tab. 5. The estimation and comparison of both *F* and *R* statistics is relevant particularly when important variations in levels of differentiation are expected among sets of subpopulations.

All $F_{ST}$ and $R_{ST}$ values were significant besides $R_{ST}$ value in locus *Dpo272* (Tab. 5). It should be noted that the values of $F_{ST}$ and $R_{ST}$ were similar level only in *Dpo101* locus (it was moderate). Four loci (*Dpo272, Dpo260, Dpo101, Dpol A6*) had a moderate $F_{ST}$ differentiation, and one locus (*Dpo101*) had a moderate $R_{ST}$ differentiation. Only one of the used loci (*Dpo221*) had high $F_{ST}$ differentiation. However, using $R_{ST}$ statistics, two loci (*Dpo272, Dpo260*) had little genetic differentiation, and two loci (*Dpol A6, Dpo221*) had very high genetic differentiation.

**Tab. 2.** The characteristics of revealed alleles in the five microsatellite loci used for *Dressena polymorpha* population estimation.

| Locus | *Dpo272* | *Dpo101* | *DpolA6* | *Dpo260* | *Dpo221* |
|---|---|---|---|---|---|
| Avg. mean | 8.1 | 13.3 | 10.3 | 12.4 | 10.1 |
| Min | 5 | 7 | 8 | 8 | 5 |
| Max | 12 | 21 | 17 | 20 | 13 |
| NA | 18 | 38 | 35 | 34 | 35 |
| ad (bp) | 112-288 | 168-339 | 210-397 | 112-288 | 66-287 |

Avg. mean, average number of alleles per population; min, minimum number of alleles in population; max, maximum number of alleles in population; NA, total number of alleles in locus; ad (bp), range of allele size in base pairs.

**Tab. 3.** The level of heterozygosity in seven *Dreissena polymorpha* samples using five microsatellite loci.

| Population | $H_O$ | $H_E$ | $F_{IS}$ |
|---|---|---|---|
| Pļaviņu Reservoir (Daugava River) | 0.81 | 0.77 | -0.065 |
| Lielupe River | 0.53 | 0.82 | 0.363 |
| Lake Laucesas | 0.43 | 0.77 | 0.450 |
| Lake Usmas | 0.57 | 0.67 | 0.171 |
| Lake Sventes | 0.65 | 0.88 | 0.277 |
| Lake Riču | 0.60 | 0.86 | 0.310 |
| Lake Drīdzis | 0.62 | 0.85 | 0.285 |
| Mean | 0.60 | 0.80 | 0.256 |

$H_O$, observed heterozygosity; $H_E$, expected heterozygosity; $F_{IS}$, individual fixation index.

**Tab. 4.** Significance of departure from HWE after sequential Bonferroni corrections.

| | *Dpol272* | *Dpol260* | *Dpol101* | *DpolA6* | *Dpol221* |
|---|---|---|---|---|---|
| Pļaviņu Reservoir (Daugava River) | ns | *** | *** | ns | *** |
| Lielupe River | *** | *** | *** | *** | *** |
| Lake Laucesas | *** | *** | *** | *** | *** |
| Lake Usmas | *** | *** | *** | *** | *** |
| Lake Sventes | *** | ns | *** | *** | *** |
| Lake Riču | *** | ns | ns | *** | *** |
| Lake Drīdzis | *** | *** | *** | *** | *** |

ns, not significant; *$p$<0.05; **$p$<0.01; ***$p$<0.001.

The individual fixation index ($F_{IS}$) points to a reduction in heterozygosity due to non-random mating and is a measure of the deviation of genotypic frequencies from the HWE in subpopulations in terms of deficiency or excess of heterozygotes. When $F_{IS}>0$, there is a deficiency of heterozygous individuals (inbreeding); with $F_{IS}<0$ there is an excess of heterozygotes (unrelated mating), and $F_{IS}=0$ indicates random pairing (Astanei *et al*., 2005). The highest calculated value of the $F_{IS}$ coefficient was shown for the population of L. Laucesas with an average value for five loci (0.450) (Tab. 3). The lowest calculated value of the coefficient was shown in the Pļaviņu Reservoir population (-0.065).

The pair-wise $F_{ST}$ estimates of genetic differentiation between studied *Dreissena polymorpha* populations in Latvia are shown in Tab. 6. The values of genetic differentiation between L. Svente and L. Riču (0.041); L. Svente and Drīdzis (0.049); L. Riču and Drīdzis (0.053) are the smallest, which were revealed between zebra mussel populations in Latvia. The values of genetic differentiation between the Lielupe R. and L. Usma (0.207), L. Laucesas and L. Usmas (0.202), L. Drīdzis and L. Usmas (0.158) the greatest, which were revealed between studied zebra mussel populations in Latvia. Moderate genetic differentiation was shown in all other pairs of studied zebra mussels populations and it varied from 0.085 to 0.156 ($p\leq0.001$). The sequential BFCs did not change the significance level (*p*-value) from the pair-wise $F_{ST}$.

Bayesian clustering partitioned populations into four genetic groups (K=4; Fig. 2), placing lakes L. Svente, L. Riču and L. Drīdzis in one group; L. Laucesas and Pļaviņu reservoir in the second group; L. Usmas in the third group and Lielupe R. in the fourth group.

The Neighbour Joining tree (Fig. 3) shows genetic distances among seven zebra mussel (*Dreissena polymorpha*) populations from Latvian waterbodies. Pļaviņu Reservoir (Daugava R.) and Lake Laucesas are the most genetically distinct populations, indicating long-term isolation or unique evolutionary histories. Lakes Sventes, Riču, and Drīdzis form a closely related group, suggesting recent gene flow, shared ancestry, or geographic proximity. Lake Usmas and Lielupe River are genetically intermediate, with Usmas showing moderate differentiation and Lielupe forming a separate branch closer to the central group. These results highlight the existence of at least three genetically distinct clusters among the seven waterbodies studied, reflecting a combination of geographic isolation, environmental variation, and potential historical dispersal pathways. The observed genetic structuring has important implications for the conservation and management of zebra mussel populations in Latvia.

Fig. 4 presents a Principal Component Analysis (PCA) plot illustrating the genetic structuring among seven European *Dreissena polymorpha* populations. The PC1 (32.53%) and PC2 (54.72%) axes together explain a substantial proportion of the genetic variation among these populations. Lielupe River is located far to the left, indicating it is the most genetically distinct population. Lake Usmas is positioned far to the right, suggesting another genetically distinct group. Lake Riču, Lake Sventes, and Lake Drīdzis are clustered closely together, indicating a high level of genetic similarity. Lake Laucesas and Pļaviņu Reservoir are positioned relatively close, suggesting some level of shared genetic characteristics. Lake Laucesas and Pļaviņu Reservoir are positioned below the main cluster, suggesting some genetic differentiation but not as pronounced as Lielupe River or Lake Usmas, Lake Sventes, Lake Riču, and Lake Drīdzis form a central cluster, indicating they may have had more gene flow between them.

**Tab. 5.** Summary of $F_{ST}$ and $R_{ST}$ statistics in each investigated microsatellites locus and totally (the level of significance is given in brackets).

| Locus | $F_{ST}$ | *Nm* | $R_{ST}$ |
|---|---|---|---|
| *Dpo272* | 0.100 (0.001) | 2.108 | 0.006 (0.662) |
| *Dpo260* | 0.116 (0.001) | 1.876 | 0.044 (0.002) |
| *Dpo101* | 0.086 (0.001) | 2.584 | 0.151 (0.001) |
| *DpolA6* | 0.106 (0.001) | 2.030 | 0.426 (0.001) |
| *Dpo221* | 0.188 (0.001) | 1.129 | 0.316 (0.001) |
| Total | 0.120 (0.001) | 1.946 | 0.165 (0.001) |

A value lying in the range between 0 and 0.05 indicates little genetic differentiation; a value between 0.05 and indicates 0.15, moderate differentiation; a value between 0.15 and 0.25 high differentiation; and values above 0.25, very high genetic differentiation (Wright, 1978; Hartl and Clark, 1997).

**Tab. 6.** $F_{ST}$ values obtained during the pair comparison of *Dreissena polymorpha* samples from the studied samples.

| Pļaviņu Reservoir (Daugava River) | Lielupe River | Lake Laucesas | Lake Usmas | Lake Sventes | Lake Riču | Lake Drīdzis | Populations |
|---|---|---|---|---|---|---|---|
| | *** | *** | *** | *** | *** | *** | Pļaviņu Reservoir (Daugava River) |
| 0.156 | | *** | *** | *** | *** | *** | Lielupe River |
| 0.137 | 0.137 | | *** | *** | *** | *** | Lake Laucesas |
| 0.185 | **0.207** | **0.202** | | *** | *** | *** | Lake Usmas |
| 0.085 | 0.088 | 0.103 | 0.137 | | *** | *** | Lake Sventes |
| 0.112 | 0.103 | 0.119 | 0.129 | **0.041** | | *** | Lake Riču |
| 0.099 | 0.106 | 0.106 | **0.158** | **0.049** | **0.053** | | Lake Drīdzis |

The smallest and the highest $F_{ST}$ values are shown in bold; a value lying in the range between 0 and 0.05 indicates little genetic differentiation; a value between 0.05 and indicates 0.15. moderate differentiation; a value between 0.15 and 0.25. high differentiation; and values above 0.25. very high genetic differentiation (Johnson and Carlton, 1996; Olson *et al.*, 2018). ***$p<0.001$.

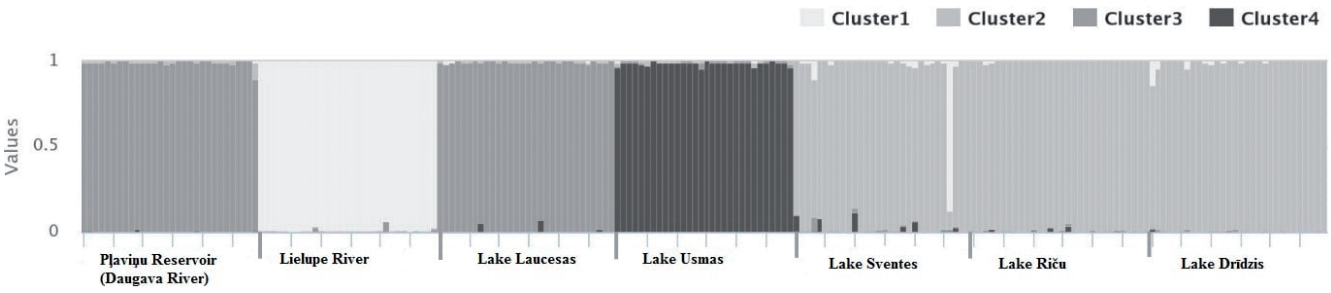


**Fig. 2.** Bayesian clustering of all individuals using STRUCTURE (Hubisz *et al*., 2009) assuming four genetic clusters of individuals (K=4). In the STRUCTURE analysis black lines separate individuals from different sampling sites and each individual is represented by a thin vertical line, which is partitioned into K-colored segments representing individual's estimated membership fractions in K clusters.

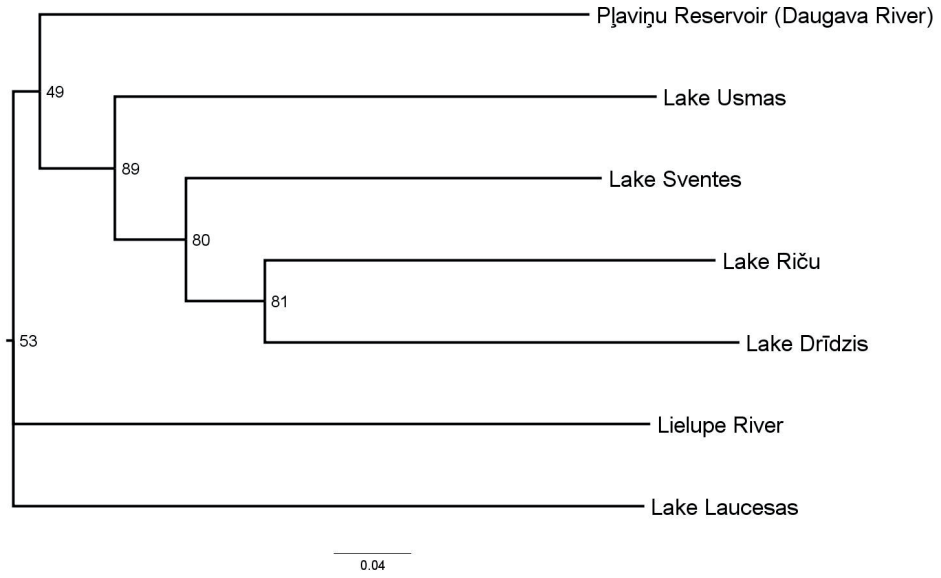


**Fig. 3.** Genetic differentiation among seven zebra mussel samples in Latvian waterbodies as revealed by a Neighbour Joining trees using Nei *et al*. (1983) genetic distance (Da).

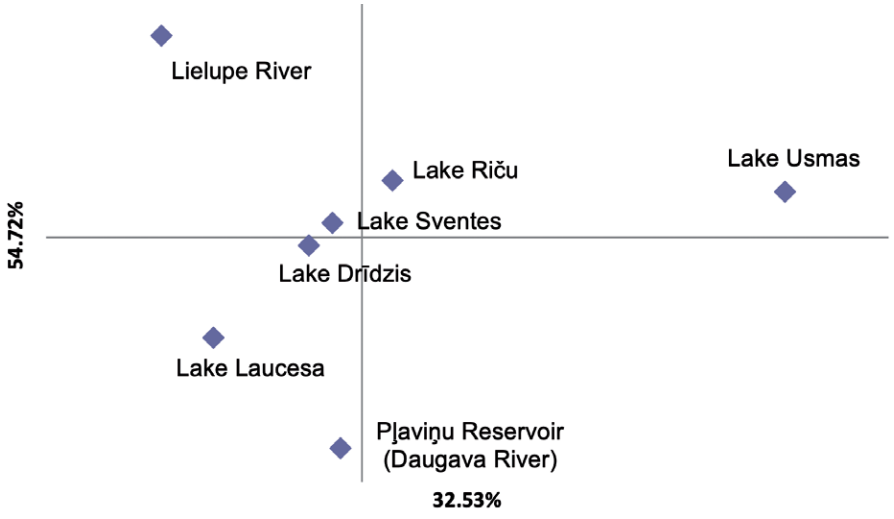


**Fig. 4.** Principal component analysis (PCA) plot of the genetic structuring among the seven Zebra mussel samples. PC1 and PC2 explain 32.53% and 54.72% of the total variation, respectively.

## Discussion

Genetic variation in *D. polymorpha* has been extensively studied to understand its invasion success and adaptability. Studies using microsatellite markers have revealed significant genetic diversity within and among populations. Native populations in the Ponto-Caspian region exhibit higher genetic diversity, reflecting their long evolutionary history and large effective population sizes compared to introduced populations. Invasive and introduced populations often show reduced genetic diversity due to founder effects and genetic bottlenecks associated with colonization events (Stepien *et al*., 2002; Gelembiuk *et al*., 2006; Travina *et al*., 2025). The present study shows high levels of variability in all invasive populations of zebra mussel in Latvia (mean observed heterozygosity: $H_O$ 0.60; mean allele number: A 10.8). Also, other researchers report about high levels of variability of *D. polymorpha* populations, for example, Great Lakes (US) population of *Dreissena polymorpha* (0.537-0.635; 15), Netherlands (0.577; 13.4), UK (0.504; 13.2) and Ireland (0.517-0.608; 10-12.6) and in the native Romanian population (0.653; 15.2) (Astanei *et al*., 2005). These results suggest that *D. polymorpha* even in invasive or geographically fragmented ranges, retains considerable genetic diversity, possibly due to multiple invasion or high reproductive output. values in Latvian populations are within the range observed for native and well-established alien populations although the mean numbers of alleles per locus are significantly lower (Tab. 5). Similar results were shown in the Iberian Peninsula`s zebra mussels population (Astanei *et al*., 2005). In the present study, a deficit of heterozygotes was recorded for the six zebra mussel populations (Lielupe R., L. Laucesas, L. Usmas, L. Sventes, L. Riču, L. Drīdzis populations). In only one population (Pļaviņu Reservoir) an excess of heterozygotes was recorded. Although decrease in the observed heterozygosity can induce a decrease in the average fitness of individuals and thus this measure has clear ecological consequences.

Various factors such as inbreeding, the Wahlund effect, selection, or the presence of null alleles may explain the observed heterozygote deficiency (Astanei *et al*., 2005) In that study, heterozygote deficiency in *D. polymorpha* populations was also detected by examining microsatellite markers and it was concluded that it was most likely caused by null alleles. However, other studies (Cornuet and Luikart, 1996; Naish and Boulding, 2001) did not report deviations from HWE. In our study the presence of null allele was not identified. Such possible causes of heterozygote deficiency are such factors as inbreeding, because in small populations individuals may mate with relatives more often than expected by chance and the Wahlund effect. When samples are taken from multiple genetically distinct subpopulations and analyzed together, the overall population appears to have fewer heterozygotes than expected. This is common in invasive species like *D. polymorpha*, which may colonize new areas in genetically distinct waves. Thus, heterozygote deficits are common in many mollusks, especially its been reported for dreissenid mussels (Astanei *et al*., 2005; Therriault *et al*., 2005; Gosling *et al*., 2008; Feldheim *et al*., 2011; Marescaux *et al*., 2016; Mallez and McCartney, 2018) and it can be attributed exactly to the Wahlund effect (Marshall and Stepien, 2021).

A decrease in allelic richness could lead to a reduction in the population's potential to adapt to future environmental changes since this diversity is the raw material for evolution by natural selection. Bottleneck effect and founder events are known to decrease the genetic diversity of a population and are often followed by a demographic expansion (Astanei *et al*., 2005; Brown and Stepien, 2009). Allelic richness is more sensitive than heterozygosity to founder events followed by expansions, because allelic richness does not consider the prevalence of alleles, only their presence. A rare allele that is lost in a founder event reduces allelic richness. However, there was no evidence that the Latvian zebra mussel populations had recently undergone a bottleneck effect in which a population can lose alleles. The allele size range in the studied populations from Latvia was similar to that in the study of 12 zebra populations from the Great Lakes (Naish and Boulding, 2001) and Lake Erie in North America and from Lake Mead in Southwestern United States (Astanei *et al*., 2005). However, in previous studies in Latvian lake Rāznas was shown quite high allele number per locus (Morozova and Shkute, 2023). This suggests that not all invasive populations suffer from low diversity, that is some of them maintain or restore diversity through multiple introductions or large founder populations.

Invasive populations in Western and Central Europe also exhibit significant genetic differentiation, with $F_{ST}$ values often exceeding 0.1 (Brown and Stepien, 2009). This differentiation is attributed to multiple introduction events from different source populations, as well as limited gene flow between isolated water bodies. For instance, populations in Rhine and Danube River basins in Europe show distinct genetic signatures, reflecting separate colonization histories and adaptation to local conditions (Astanei *et al*., 2005). Mainly, we have similar results in Latvian zebra mussel populations. The present study shows moderate differentiation between Latvian populations of zebra mussels. However, there are three samples (L. Riču, L. Drīdzis and L. Sventes) between which genetic differentiation is small (about 0.5). The possible reasons are, firstly, all three reservoirs are located quite near each other (Tab. 1 and Fig. 1) and belong to the same catchment area. Secondly, these lakes have recreation areas using water transport. Thus, the transfer of the zebra mussel into these lakes could have occurred via water transport. At the same time Latvian populations exhibit differentiation levels similar to those found in more isolated or hydrologically separated regions elsewhere in Europe.

Globally, the genetic structure of *D. polymorpha* populations reflects both natural and human-mediated dispersal. Native populations in the Ponto-Caspian region exhibit high genetic diversity and moderate differentiation, with $F_{ST}$ values typically ranging from 0.05 to 0.2 (Stepien *et al*., 2002). In contrast, invasive populations in North America and other regions often show reduced genetic diversity and higher differentiation, with $F_{ST}$ values sometimes exceeding 0.3 (Gelembiuk *et al*., 2006; Brown and Stepien, 2009). The studies of microsatellite markers identified distinct genetic clusters of *D. polymorpha* in waterbodies of North America, with Zebra mussel populations in the Great Lakes and Mississippi River basin showing significant differentiation ($F_{ST}$>0.2) due to separate invasion events from different European sources (Brown and Stepien, 2009). Similarly, populations in isolated water bodies often exhibit high $F_{ST}$ values due to limited gene flow and genetic drift (Astanei *et al*., 2005).

Latvian populations shown average *Nm* 1.95 (Tab. 5) suggests limited gene flow, which is sufficient to maintain some genetic exchange but not enough to homogenize populations. Similar *Nm* values (1.5-2.5) were reported by another author (Therriault *et al*., 2005) in Canadian populations, especially in newly invaded or fragmented habitats. Researchers (Müller *et al*., 2002) observed higher gene flow (*Nm*>3) in connected river systems, unlike the relatively more isolated lakes in Latvia. This suggests that Latvian populations are shaped by natural barriers and potentially limited

human-assisted dispersal compared to regions with greater navigational connectivity.

Bayesian clustering and Principal Component Analysis separated all investigated populations in four genetic groups. Lake Usmas is the most isolated, explaining its distinct genetic signature. Lielupe River is another outlier, suggesting genetic divergence from lake populations. Lakes Riču, Sventes, and Drīdzis are clustered, indicating high genetic similarity. These lakes are geographically close to each other and more isolated from others, leading to higher genetic similarity within lake clusters. Pļaviņu Reservoir and Lake Laucesas are separate but closer to the main lake cluster, suggesting partial differentiation. Pļaviņu Reservoir on the Daugava River is an artificial system, likely receiving genetic input from multiple sources, leading to intermediate differentiation. Dreissena population in Pļaviņu R. can receive genetic input from at least three major sources: upstream Daugava populations drifting or migrating into the reservoir, tributary populations entering from lateral inflows, and invasive individuals that colonize reservoir habitats. The altered hydrology and fragmentation caused by dams promote both mixing and partial isolation; may resulting in a genetically diverse population. According to other authors, intraspecific genetic diversity could be also the result from mutations, demographic history or selection (Doorenweerd *et al*., 2020; Travina *et al*., 2025).

Altogether the genetic characteristics of Latvian *D. polymorpha* populations observed in this study are consistent with the findings of Morozova and Shkute (2023), who reported high allelic richness and heterozygosity in Lake Rāzna. Their results support the interpretation that Latvian populations have not undergone severe bottlenecks and may have been established through multiple introduction events. The similarity in allele size ranges and level of heterozygosity between Rāzna and the present study suggests that Latvian waterbodies have a common invasion history, although local processes such as hydrological connectivity and human-mediated distribution have produced distinct genetic structures.

Future studies should focus on molecular analyses based on nuclear markers of *D. polymorpha* from native and invasive parts of its range.

## Conclusions

Studied populations of *Dreissena polymorpha* in Latvian waterbodies display high levels of genetic diversity. The observed genetic variability is comparable to that found in both native and long-established invasive populations elsewhere, indicating this invasive species successful establishment and potential adaptability. Given the ecological and economic impacts of *D. polymorpha*, these findings highlight the importance of continued genetic monitoring and research into environmental factors, migration barriers, and anthropogenic influences affecting these populations and detecting new introduction events. Continued research on these differences will be essential for managing the spread and impact of this aggressive and actively spreading invasive species and protecting native biodiversity.

## References


Astanei I, Gosling E, Wilson J, Powell E, 2005. Genetic variability and phylogeography of the invasive zebra mussel, *Dreissena polymorpha* (Pallas). Mol Ecol 14:1655-1666.

Birnbaum C, 2011. NOBANIS – Invasive Alien Species Fact Sheet – *Dreissena polymorpha*. Online Database of the European Network on Invasive Alien Species. Accessed: 25 October 2025. Available from: https://www.nonnativespecies.org/assets/Invasive_Alien_Species_Fact_Sheet_-_Dreissena_polymorpha_-_NOBANIS_-_downloaded_from_website_March_2014.pdf

Brown JE, Stepien CA, 2009. Invasion genetics of the Eurasian round goby in North America: tracing sources and spread patterns. Mol Ecol 18:64-79.

Chapuis MP, Estoup A, 2007. Microsatellite null alleles and estimation of population differentiation. Mol Bio. Evol 24:621-631.

Chen Y, Zhao L, Teng H, Shi C, Liu Q, Zhang J, Zhang Y, 2021. Population genomics reveal rapid genetic differentiation in a recently invasive population of *Rattus norvegicus*. Front Zool 18:6.

Cornuet JM, Luikart G, 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014.

Doorenweerd C, San Jose M, Barr N, Leblanc L, Rubinoff D, 2020. Highly variable COI haplotype diversity between three species of invasive pest fruit fly reflects remarkably incongruent demographic histories. Sci Rep 10:6887.

Estoup A, Ravigné V, Hufbauer R, Vitalis R, Gautier M, Facon B, 2016. Is there a genetic paradox of biological invasion? Annu Rev Ecol Evol Syst 47:51-72.

Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14:2611-2620.

Feldheim KA, Brown JE, Murphy DJ, Stepien CA, 2011. Microsatellite loci for dreissenid mussels (Mollusca: Bivalvia: Dreissenidae) and relatives: markers for assessing exotic and native populations. Mo. Ecol Resour 11:725-732.

Francis RM, 2017. Pophelper: an R package and web app to analyse and visualize population structure. Mol Ecol Resour 17:27-32.

Gelembiuk GW, May GE, Lee CE, 2006. Phylogeography and systematics of zebra mussels and related species. Mol Ecol 15:1033-1050.

Gosling E, Astanei I, Was A, 2008. Genetic variability in Irish populations of the invasive zebra mussel, *Dreissena polymorpha*: Discordant estimates of population differentiation from allozymes and microsatellites. Freshwater Biol 53:1303-1315.

Hartl DL, Clark AG, 1997. Principles of population genetics. Sinauer Associates, Sunderland.

HELCOM, 2020. Abundance and distribution of the Zebra mussel *(Dreissena polymorpha*). Baltic Sea Environment Fact Sheet. Accessed: 25 October 2025. Available from: https://helcom.fi/wp-content/uploads/2020/06/BSEFS-Abundance-and-distribution-of-the-Zebra-mussel.pdf

Hubisz MJ, Falush D, Stephens M, Pritchard AJ, 2009. Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9:1322-1332.

Johnson LE, Carlton JT, 1996. Biological invasions and cryptogenic species. Ecology 77:1653-1655.

Langella O, 2007. Populations 1.2.30: Population genetic software (individuals or populations distances, phylogenetic trees). Accessed: 25 October 2025. Available from: http://bioinformatics.org/~tryphon/populations/

Lowe S, Browne M, Boudjelas S, De Poorter M, 2000. 100 of the World's Worst Invasive Alien Species: A selection from the Global Invasive Species Database. IUCN/ISSG. Accessed: 25

October 2025. Available from: https://www.iucngisd.org/gisd/100_worst.php

Mallez S, McCartney M, 2018. Dispersal mechanisms for zebra mussels: Population genetics supports clustered invasions over spread from hub lakes in Minnesota. Biol Invasions 20:2461-2484.

Marescaux J, von Oheimb KCM, Etoundi E, von Oheimb PV, Albrecht C, Wilke T, Van Doninck K, 2016. Unravelling the invasion pathways of the quagga mussel (*Dreissena rostriformis*) into western Europe. Biol Invasions 18:245-264.

Marshall NT, Stepien CA, 2021. Genetic trajectories of zebra and quagga mussel invasions across three decades: Lake Erie versus Hudson River populations. Aquat Invasions 16: 147-166.

Morozova A, Shkute N, 2023. Genetic characteristics of the *Dreissena polymorpha* population in Latvia (Lake Rāzna) as part of the European population. Arch Biol Sci 75:133-139.

Müller JC, Hidde D, Seitz A, 2002. Canal construction destroys the barrier between major European invasion lineages of the zebra mussel. Proc Biol Sci 269:1139-1142.

Naish KA, Boulding EG, 2001. Trinucleotide microsatellite loci for the zebra mussel *Dreissena polymorpha*, an invasive species in Europe and North America. Mol Ecol Notes 1:286-298.

Nei M, Tajima F, Tateno Y, 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. J Mol Evol 19:153-170.

Olson JR, McCartney MA, Witt JDS, 2018. Dispersal potential of zebra mussel larvae in flowing water. Aquat. Invasions 13:311-322.

Orlova MI, Panov VE, 200). Establishment of the zebra mussel, *Dreissena polymorpha* (Pallas), in the Neva Estuary (Gulf of Finland, Baltic Sea): distribution, population structure and possible impact on local unionid bivalves. Hydrobiologia 514:207-217.

Page RD, 1996. TreeView: An application to display phylogenetic trees on personal computers. Bioinformatics 12:357-358.

Peakall R, Smouse PE, 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:228-295.

Peñarrubia L, Vidal O, Viñas J, Pla C, Sanz N, 2016. Genetic characterization of the invasive zebra mussel (*Dreissena polymorpha*) in the Iberian Peninsula. Hydrobiologia 779:227-242.

Ricciardi A, Hill JM, 202). Passive transport of a zebra mussel attached to a freshwater fish: A novel Dreissena dispersal mechanism? Biol Invasions 25:2057-2060.

Rice WR, 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Selkoe KA, Toonen RJ, 2006. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615-629.

Stepien CA, Taylor CD, Dabrowska KA, 2002. Genetic variability and phylogeographical patterns of a nonindigenous species invasion: A comparison of exotic vs. native zebra and quagga mussel populations. J Evol Biol 15:314-328.

Therriault T, Orlova M, Docker M, MacIsaac HJ, Heath DD, 2005. Invasion genetics of a freshwater mussel (*Dreissena rostriformis* bugensis) in eastern Europe: high gene flow and multiple introductions. Heredity 9516-23.

Travina OV, Bespalaya YV, Kondakov AV, Vikhrev IV, Aksenova OV, Aksenov AS, et al. 2025. Genetic diversity and expansion routes of the invasive zebra mussel *Dreissena polymorpha* in Eastern Europe. Hydrobiologia 852:1743-1758.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P, 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538.

Weir BS, Cockerham CC, 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Wright S, 1978. Evolution and the genetics of population. Variability within and among natural populations. University of Chicago Press, Chicago.

Zając K, Bonk M, 2019. New records of the invasive bivalve *Dreissena polymorpha* (Pallas, 1771) (Bivalvia: Dreissenidae) in the Carpathian Mountains. Folia Malacol 27:231-234.

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