

BIOLOGICAL INVASION SUCCESS OF *PONTOGAMMARUS ROBUSTOIDES* G. O. SARS, 1894 IN THE DAUGAVA RIVER AND ITS WATER RESERVOIRS (LATVIA)

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Abstract

We have used *Pontogammarus robustoides* for the first time in the Daugava River and its water reservoirs (Latvia) in order to find the genetic diversity of these populations using iPBS markers. During the research, we detected the most appropriate iPBS markers for future genetic studies of *Pontogammarus robustoides* populations of Daugava River and its water reservoirs. Based on these iPBS markers, we determined the genetic diversity of these populations. Research on the genetic structure of *Pontogammarus robustoides* populations in the lower waters of the Daugava River and its water reservoirs shows that the populations have grown up and are genetically stable and adaptable to changing environmental conditions, as evidenced by the relatively high population polymorphism level or plasticity, ranging from 65% in Riga to 82% in the Pļaviņas population. This means the higher level of polymorphism, the more viable the population is, the easier it is to adapt to changing environmental conditions. The biggest number of the amplified band was in Pļaviņas population – 78 amplified band and Ogresgals population - 75 amplified band, but the lowest number were in Riga and Tome population - 62 amplified band. The biggest number of polymorphic loci also were in Pļaviņas population (82 %) and in Ogresgals population (80 %), but the lowest number of polymorphic loci were in Riga (65 %) and Tome population (66 %). The estimated number of alleles in the loci ranges from 62 alleles (in the Riga and Tome populations) to 78 alleles (in the Pļaviņas population), the highest number of private alleles in the locus was found in the Pļaviņas population (5 private alleles), indicating a greater genetic difference in this population, while private alleles in the loci were not found in Veczeļķi, Riga, Ogresgals and Tome populations. The average level of heterozygosity ranged from 0.16 (Ogresgals and Tome populations) to 0.21 (Pļaviņas and Veczeļķi populations). Investigated populations are genetically similar or very related, genetic distance (D) or differentiation is very small from 0.04 to 0.11.

Keywords: Alien Ponto-Caspian amphipod, *Pontogammarus robustoides*, iPBS markers, Daugava River.

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INTRODUCTION

Crustaceans are one of the most important as a food chain link and the most diverse macro-invertebrate, and at the same time one of the most successful aliens in freshwater ecosystems. 53% of all alien species in European freshwater are directly crustaceans, mainly species from North America and the Ponto-Caspian region (Hänfling et al. 2011, Karatayev et al. 2009). Ponto-Caspian amphipods, including *Pontogammarus robustoides* are one of the most successful invaders in most European freshwaters (downstream of large rivers, estuaries, reservoirs and also lakes) and is affected by local macro-invertebrate communities (Berezina 2007, Jazdzewski et al. 2004, Grabowski et al. 2007, Grabowski et al. 2007a, Gumuliauskaitė & Arbačiauskas 2008). Such occurrence is related to species expansion in the Baltic Sea basin after deliberate introduction into the Kaunas Reservoir located on the Nemunas River and into Latvian waters, the nearest to Riga - the Lake Lielais Baltezers and the Lower Daugava River (the Ķegums Reservoir) in the 1960s as valuable fish food (Bodniece 1976, Kachalova & Lagzdin 1968). *Pontogammarus robustoides* is one of the most abundant and dominant amphipoda group in Latvian freshwater, especially in the lower reaches of the River Daugava and in water reservoirs with shallow, almost water-rich, diverse habitats and seasons (Grudule et al. 2007, Paidere & Brakovska 2023, Paidere et al. 2016, Paidere et al. 2019). At the same time, *Pontogammarus robustoides* successfully exceed or replace native amphipod species. The high invasiveness of species is due to its wide environmental tolerance, good adaptability, high fertility and behavior as an effective predator and omnivore (Arbačiauskas et al. 2013, Bacela & Konopacka 2005, Bacela-Spychalska

& Van der Velde 2013, Bacela-Spychalska 2016, Berezina 2016, Grabowski et al. 2007, Kobak et al. 2017, San Vicente 2018, Šidagytė & Arbačiauskas 2016). Studying population genetic variability of alien and indigenous species to be important for identifying the impact of the alien species on the native species community (Lawson et al. 2011, Lee 2002).

MATERIAL AND METHODS

Sampling sites, material collection and identification of *Pontogammarus robustoides* specimens

Samples of *Pontogammarus robustoides* from the Daugava River and its water reservoirs in Latvia (Fig. 1) were collected by qualitative sampling in the wadeable (up to 0.5 m) depths using a *Hydrobios* hand net with a mouth opening of 25x25 cm (500 µm mesh). The study sites' substratum mainly consisted of sand, silty sand, detritus, pebbles, some boulders, and macrophytes. Samples were sampled once a month, from April until September.

Identification was performed using specimen identification and length measurements were done with a *ZEISS Stemi 508doc* stereomicroscope fitted with an ocular micrometer (10:100). Identification of specimens was done following Eggers and Martens (2004), Eggers and Martens (2001), Karaman and Pinkster (1977), Jazdzewski 1975. The *Pontogammarus robustoides* sample material was stored at frozen after collecting and identification.

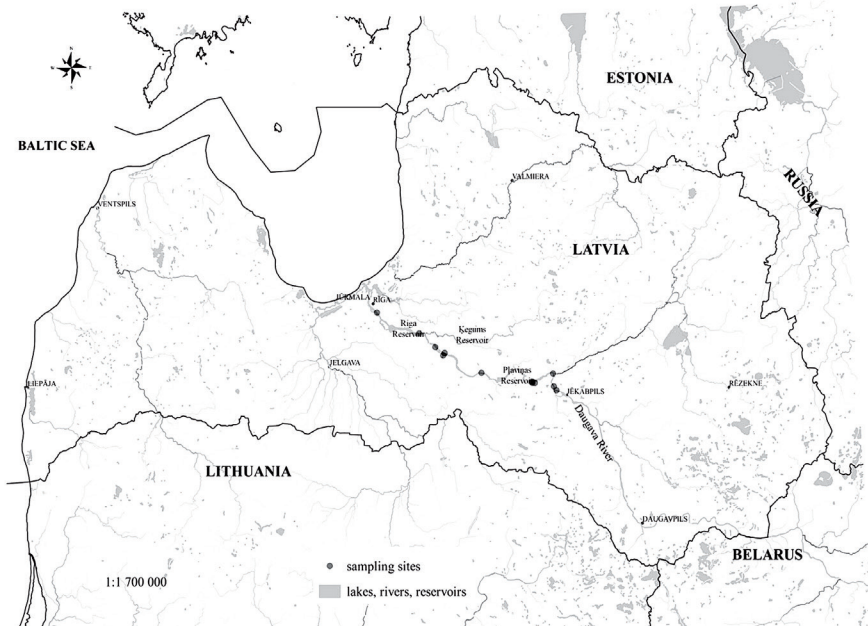


Figure 1. Localities of sampling sites in the Latvian reservoirs. Map courtesy J. Paidere.

Genetic analysis of *Pontogammarus robustoides*

DNA were extraction of *Pontogammarus robustoides* specimens by employing Invisorb® Spin 1 Tissue Mini Kit (STRATEC Molecular GmbH Berlin, Germany) (STRATEC, Molecular GmbH 2022, Brakovska & Paidere 2019).

The genetic variability among *Pontogammarus robustoides* population of the Latvian reservoirs was analyzed using nine iPBS markers - 2009, 2010, 2081, 2083, 2095, 2271, 2380, 2384, 2242 (Kalendar et al. 2010). So as mobile elements are frequently found in eukaryotic genomes, that is why retrotransposon-based markers are useful systems for analyses of genetic diversity of many organisms. iPBS technique is based on the long terminal repeat (LTR) retrotransposon possibility to integrate it self-copies into different places into the genome.

Statistical processing and analysis of the obtained data

The obtained data were processed and analyzed using the computer software GeneAlec 6.41 (Peakall & Smouse 2006). The number of alleles per locus, the private alleles in each population (Nei 1987) and the average heterozygosity level in polymorphic loci (Nei 1973) were measured, and their differences among *Pontogammarus robustoides* individuals from different sampling places were calculated. The genetic relatedness of *Pontogammarus robustoides* populations was estimated with genetic distance (D) (Nei 1978). Genetic differentiation among populations was estimated via principal component analysis (PCA) (Nei 1987). To estimate and visualize the genetic structure and differentiation of the studied *Pontogammarus robustoides* populations, STRUCTURE 2.3.4 (Hubisz et al. 2009) and STRUCTURE HARVESTER (Earl & vonHoldt 2012) were used.

RESULTS AND DISCUSSION

To evaluate the genetic structure of *Pontogammarus robustoides* in the Daugava and its reservoirs, according to parameters of gene diversity, nine universal iPBS markers were tested - 2009, 2010, 2081, 2083, 2095, 2271, 2380, 2384, 2242 (Kalendar et al. 2010), but further analysis used only three universal iPBS markers- 2081, 2083, 2242, which gave clear, interpretable results. For the studied populations of *Pontogammarus robustoides*, using iPBS markers 2081, 2083, 2242, 120 samples of *Pontogammarus robustoides* were analyzed and 94 interpretable loci were obtained, for which the number of alleles per locus, number of private alleles per locus, average heterozygosity,

number of polymorphic loci and genetic distance (D) (Nei 1978) were evaluated.

The estimated number of alleles in the locus of *Pontogammarus robustoides* individuals ranged from 62 (in the populations Riga and Tome) to 78 alleles (in the population Pļaviņas), but private alleles in the locus were found only in the population Pļaviņas (5 private alleles) and in the population Ķegums (1 private allele), it should be added that populations Veczeļķi, Riga, Ogresgals and Tome no private alleles were detected at all (Fig. 2). On the other hand, the average heterozygosity ranged from 0.16 (in the populations Ogresgals and Tome) to 0.21 (in the populations Pļaviņas and Veczeļķi), as can be seen, these differences are relatively small (Fig. 2).

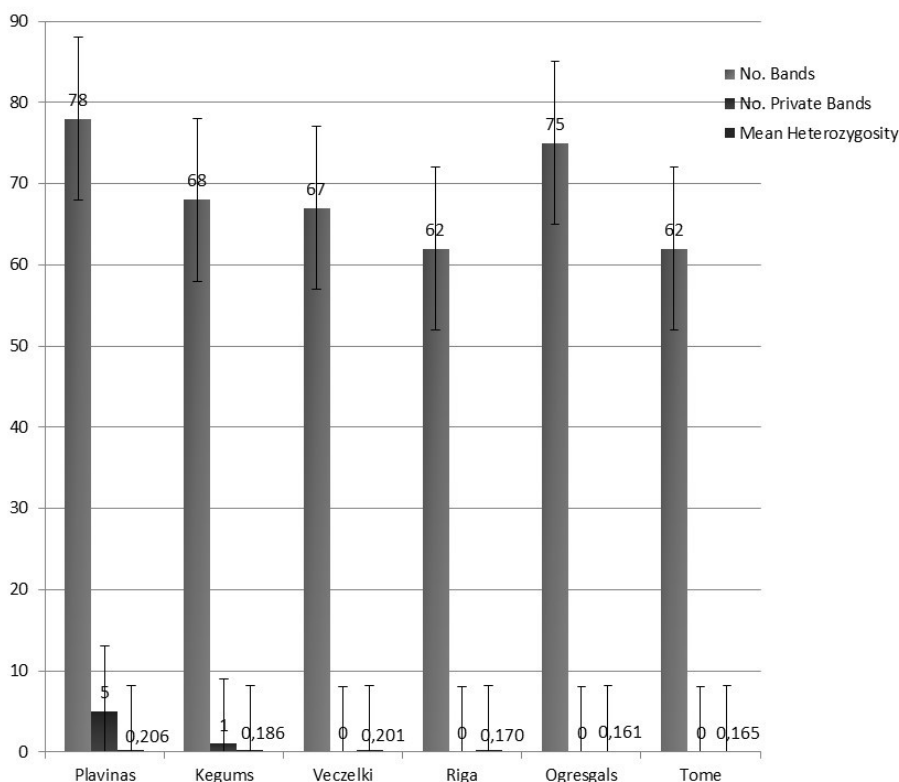


Figure 2. Number of alleles, private alleles per locus and average heterozygosity of the studied populations of *Pontogammarus robustoides*.

Heterozygosity is an important parameter of genetic diversity, as it reflects the number of individuals capable of transmitting the most gene variants to the next generation. In our case, it can be concluded that the studied populations can transmit a quite similar amount of gene variants to the next generations. The studies of other authors (Lowe et al. 2004) also emphasize that populations differ among themselves in the frequency of occurrence of gene alleles, which respectively reflect the population's variability potential and adaptability under the influence of changing environmental factors. Therefore, our obtained results confirm that the studied populations of *Pontogammarus robustoides* in the

Daugava and its reservoirs are stable, with high adaptation and survival/settlement capabilities. The number of polymorphic loci of *Pontogammarus robustoides* populations is very similar between the studied populations, i.e. from 65% (in the population Riga) to 82% (in the population Pļaviņas) (Fig. 3). The population of Pļaviņas (82%) and Ogresgals (80%) had the most polymorphic loci, while the population of Riga (65%) and Tome (66%) had the lowest number of polymorphic loci (Fig. 3). The higher the level of polymorphism, the more viable the population is, the easier it is to adapt to changing environmental conditions (Lowe et al. 2004).

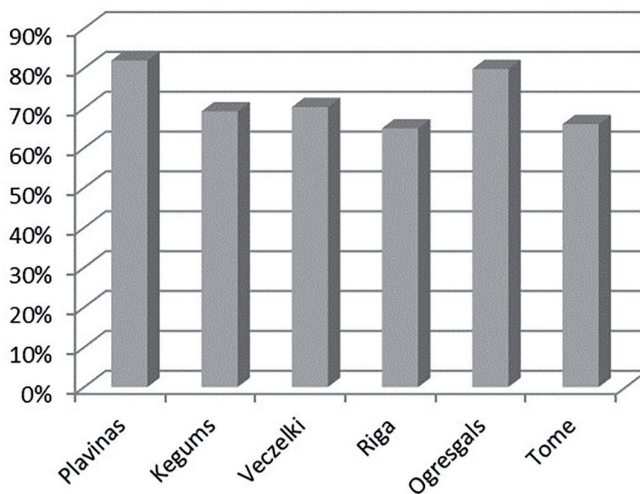


Figure 3. Percentage of polymorphic loci of *Pontogammarus robustoides* populations between sampling sites: Veczelķi, Riga, 3- Pļaviņas, Tome, Kegums, Ogresgals.

According to the genetic distance indices (D) (Nei 1978) among the studied populations of *Pontogammarus robustoides*, or the degree of genetic variation between populations (Lowe et al. 2004; Nei 1978; Slatkin & Barton 1989), the studied populations are genetically similar or very related, as the smallest genetic distance was found between populations Kegums and Veczelķi (0.02) and between Veczelķi and Riga (0.04), as well as Ogresgals and Tome (0.04)

(Tab. 1). The greatest genetic distance was found between populations Pļaviņas and Tome (0.11) as well as between populations Kegums and Ogresgals (0.11) (Tab. 1). Although the studied populations are genetically similar, after PCA analysis it can be seen (Fig. 4) that among the studied populations separate groups are formed by the populations of Kegums, Veczelķi, and by the populations of Pļaviņas and Veczelķi. Stable groups are also formed

by the populations of Ogresgals and Riga, as well as the populations of Ogresgals, Riga and Tome. The principal component analysis (PCA) plot represents PC 1 and PC 2 explain 31% and

22% of the total genetic diversity. The result could be explained by the spread of the species in time and space after its introduction.

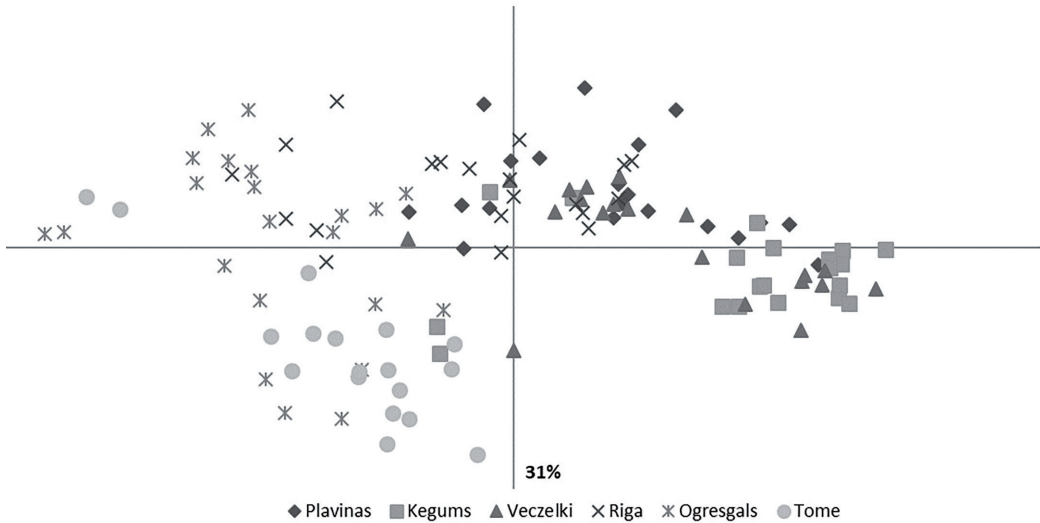


Figure 4. Genetic structure of *Pontogammarus robustoides* populations by genetic distance. Principal component analysis (PCA) plot to reach, PC 1 and PC 2 explain 31% and 22% of the total genetic diversity. The result could be explained by the spread of the species in time and space after its introduction.

Table 1. Genetic distance (D) (Nei 1978) among *Pontogammarus robustoides* populations between sampling sites: Veczeļķi, Riga, Pļaviņas, Tome, Ķegums, Ogresgals.

Sample sites	Pļaviņas	Ķegums	Veczeļķi	Riga	Ogresgals
Ķegums	0.062				
Veczeļķi	0.060	0.025			
Riga	0.066	0.072	0.042		
Ogresgals	0.091	0.114	0.088	0.051	
Tome	0.115	0.101	0.090	0.080	0.042

A similar result was obtained using Bayesian clustering analysis (STRUCTURE 2.3.4) (Hubisz et al., 2009) (Fig. 5) and number of

clusters of individuals using Evanno et al.'s (2005) clustering approach (Fig. 6).

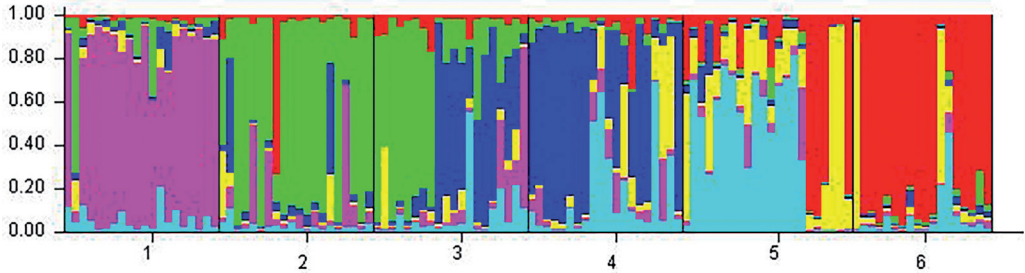


Figure 5. Bayesian clustering of individuals using STRUCTURE 2.3.4 (Hubisz et al., 2009). In the STRUCTURE analysis color lines separate individuals from different sampling sites and each individual is represented by a vertical line, which is partitioned into K-coloured segments representing an individual's estimated membership in K clusters (1- Veczeļķi, 2- Rīga, 3- Pļaviņas, 4- Tome, 5- Ķegums, 6- Ogresgals).

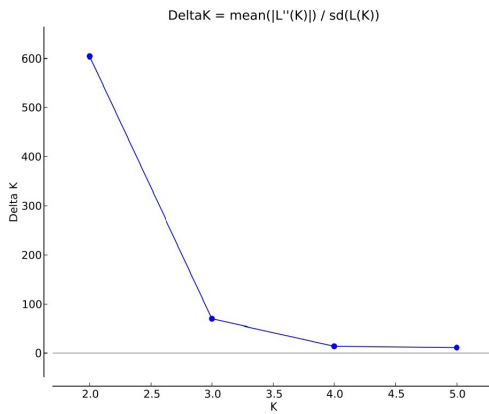


Figure 6. The number of clusters of individuals using Evano et al.'s (2005) clustering approach assuming two genetic clusters ($K=2$; $\Delta K=604$; $\text{Ln}P(K) \pm \text{SD} = -4331.10 \pm 0.26$).

CONCLUSIONS

Our of the genetic structure among *Pontogammarus robustoides* populations using non-specific PBS markers shows that the populations have settled in the Daugava waters, are genetically stable and able to adapt to changing environmental conditions. The populations genetic structure among *Pontogammarus robustoides* populations of the Rīga and

Pļaviņas shows high polymorphism, i.e. In addition, the existence of unique alleles of genes in the Pļaviņas population indicates a greater genetic difference of this population. Populations are genetically similar or very related (genetic distance or differentiation is very small from 0.04 to 0.11), however, they form different groups, which could be explained by the secondary distribution of the species in time and space after introduction.

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