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THE FIRST DATA ON THE GENETIC DIVERSITY OF RIVER LAMPREY *LAMPETRA FLUVIATILIS* (LINNAEUS, 1758) FROM THE VISTULA RIVER AND VISTULA LAGOON IN POLAND*

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Key words: conservation, environment, genetic diversity, microsatellite DNA, population.

Abstract

The genetic characteristic of seriously threatened river lamprey *Lampetra fluviatilis* L. from Vistula river and Vistula lagoon (Poland) was described in the present paper. The study, based on the nine microsatellite markers, was conducted to determine the genetic diversity and population structure of river lamprey in Vistula river and Vistula lagoon in Poland.

Observed heterozygosity ranged from 0.158 to 0.974 in lamprey from Vistula river, and 0.040 to 0.990 in lamprey from Vistula lagoon. The expected heterozygosity in river lamprey ranged 0.177–0.673 in specimens and 0.213–0.670 in population from Vistula lagoon. Eight loci appear to be diagnostic, due to occurrence of private alleles, for distinguishing the Vistula river and Vistula lagoon populations. The estimated effective population size (*Ne*) for the studied populations of river lamprey form Vistula river and Vistula lagoon equalled 72.4 (95% CI = 14.9–∞) and 25.8 (95% CI = 8.7–151.1), respectively. Constructed individual's tree based on DAS genetic distances and the Principal Coordinates Analysis (PCoA) exhibited three main genetic groupings within studied fish group.

The presented genetic characteristic of studied lamprey populations is important and necessary to develop and implement conservation actions of river lamprey in Poland.

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ZMIENNOŚĆ GENETYCZNA MINOGA RZECZNEGO Z RZEKI WISŁY I ZALEWU WIŚLANEGO – BADANIA WSTĘPNE

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Słowa kluczowe: minóg rzeczny, Wisła, Zalew Wiślany, zmienność genetyczna.

Abstrakt

Prezentowane badania dotyczą analizy genetycznej minoga rzecznego *(Lampetra fluviatilis)* z dwóch stanowisk w zlewni Wisły (z Wisły – okolice Czatkowy, woj. pomorskie oraz Zalewu Wiślanego). Analizę genetyczną prowadzono z zastosowaniem dziewięciu par starterów mikrosatelitarnego DNA.

Obserwowana heterozygotyczność (*Ho*) w badanych loci u minoga rzecznego z Wisły i Zalewu Wiślanego przyjmowała odpowiednio wartości 0,158–0,974 i 0,040–0,900. Oczekiwana heterozygotyczność (*He*) u minoga rzecznego z Wisły i Zalewu Wiślanego wynosiła odpowiednio 0,177–0,673 oraz 0,213–0,670. Osiem loci mikrosatelitarnego DNA określono jako diagnostyczne z uwagi na fakt występowania w nich alleli prywatnych. Efektywna liczebność populacji (*Ne*) minoga rzecznego z Wisły i Zalewu Wiślanego przyjmowała wartości odpowiednio 72,4 (95% CI = 14,9–∞) oraz 25,8 (95% CI = 8,7–151,1). Drzewo pokrewieństw filogenetycznych, skonstruowane na podstawie genetycznego dystansu DAS oraz analizy PCoA, wykazywało trzy podgrupy.

Prezentowane wyniki badań stanowią wstępne informacje na temat zmienności genetycznej minoga rzecznego zasiedlającego Wisłę i Zalew Wiślany, które mogą być wykorzystane w ochronie tego gatunku. Uzyskane wyniki zostaną wykorzystane w trakcie opracowywania metod przechowywania i kriokonserwacji nasienia minoga rzecznego w celu utworzenia banku nasienia.

Introduction

The European river lamprey *Lampetra fluviatilis* L. is a threatened species of anadromous ancient jawless vertebrates (agnathans), formerly widespread throughout western Europe (MAITLAND 1980, HARDISTY 1986). In Poland, the European river lampreys spend the adult phase of their life cycle in Baltic sea, where they feed parasitically on a wide variety of bony fish. Adults return to rivers for reproduction, where they become sexually mature, spawn and die. During last few decades this species have undergone a significant decline throughout Europe because of rivers and estuaries pollution, overexploitation, loss of spawning and larval habitat and by physical barriers to migration (LELEK 1987, OJUTKANGAS et al. 1995, NUNN et al. 2008, MAITLAND 2003). The drastic decline of the most river

lamprey populations initiated the development of conservation programs in Europe through the Bern Convention and the European Habitats Directive 92/43/EEC, indicating the river lamprey as a species requiring the designation of Special Areas of Conservation (SACs) (COUNCIL OF THE EUROPEAN COMMUNITIES 1992, MATEUS et al. 2012). Additionally, this species was listed in the IUCN Red List of Threatened Species 2013 (FREYHOF 2013). In Poland river lamprey was included in the Polish Red Data Book of Animals as an endangered species (WIESER 1992) and the populations of this species are very rare. In Poland, the river lamprey inhabit only a few rivers, for example the lower part of the Vistula river, and are under strict protection The adults of river lamprey were identified in ten Polish rivers (Wda, Drwęca, Wierzyca, Pasłęka, Łupawa, Radew, Wiepsza, Parsęta, lower parts of Oder and Vistula), Vistula and Szczecinski Lagoons (WITKOWSKI 2010). Accordingly, the obtaining of research samples is very difficult and requires many permits both national and local.

During the river lamprey conservation the year-round protection of this species, including a fishing ban, was enacted in Poland to counteract the steady decline of Polish river lamprey populations. This species is seriously threatened and effective protection together with supportive reproduction programs, should be designed and implemented in the most natural river lamprey populations. The existence of endangered populations is often dependent on supportive breeding (with use the innovative biotechnologies in aquaculture condition), rearing of juveniles under controlled conditions and releasing them into the natural environment. Furthermore, during the implementation of restoration plan of seriously endangered species/population the genetic screening, based on molecular analyses should be included as the key point in successful realization of conservation activity (FOPP-BAYAT 2008, FOPP-BAYAT and CIERESZKO 2012). Moreover the conservation of endangered river lamprey populations should involves the species or hybrid identification together with monitoring of important population genetic indices (e.g. gene diversity indicators, phylogeny, inbreeding coefficient, bottleneck effect). To date there is no information about genetic data of river lamprey from Vistula river estuarium in Poland. Therefore the main objective of the present study was the application the molecular analysis, based on microsatellite DNA markers, in highly endangered river lamprey from Vistula river and Vistula lagoon in Poland.

Material and Methods

This study was carried out in strict accordance with the recommendations in the Polish ACT of 21 January 2005 of Animal Experiments, Dz.U. z 2005 r. nr 33, poz. 289. The protocol was approved by the Local Ethical Committee for the Experiments on Animals in of the University of Warmia and Mazury in Olsztyn, Poland (Permit Number: 78/2012). The river lamprey were captured in fall (October) and spring (April) during spawning migration in the Vistula river near Czatkowa, Poland: N 54°8'4.18"; E 18°49'43.67" (49 specimens), and Vistula Lagoon, Poland: N 54°25'12.67"; E 19°44'8.99" (38 specimens) with the use of tunnel nets.

The fin clips were sampled and stored in 96% ethanol. Then the genomic DNA was extracted during the Chelex 100 procedure (WALSH et al. 1991). The nine microsatellite loci (*Lri-1*, *Lri-2*, *Lri-3*, *Lri-4*, *Lri-5*, *Lri-7*, *Lri-8*, *Lri-9* and *Lri-10*) were amplified during Polymerase Chain Reaction PCR (LUZIER et al. 2010). The PCR amplification was performed in 25-μL reaction volumes with approximately 40 ng genomic DNA, 1x PCR reaction Buffer (50 mM KCL, pH 8.5; Triton X-100), 3.3 mM $MgCl_2$, 0.25 mM of each deoxynucleotide triphosphate (dNTP), 0.4 μM of each primer, and 0.6 U Go *Taq* Flexi Polymerase (Promega, Madison, WI, USA). Re-distilled water was used to bring the reaction mixture to the desired final volume. PCR conditions were as follows: an initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, primer annealing at 50–62°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Amplification was conducted with a Mastercycler gradient thermocycler (Eppendorf, Germany). In order to enable genotyping of PCR products with an Applied Biosystem 3130 Genetic Analyser, forward primers were 5'–labeled with different fluorescent reporter dyes (Lri-1-VIC, Lri-2-FAM, Lri-3-NED, Lri-4-PET, Lri-5-FAM, Lri-7-VIC, Lri-8-NED, Lri-9-FAM and Lri-10-VIC). The lengths of the amplified DNA fragments were determined using the Applied Biosystems 3130 Genetic Analyser sequencer with application the GeneScan 600 [LIZ] size standard. Individual microsatellite fragments, amplified using primers with different attached fluorescent dyes, were arranged into sets and analyzed in multiplex mode. Fragment size and allele determination was performed using GeneMapper 4.0 genetic analyser software (Applied Biosystems), following the manufacturer's recommendations.

Data analysis

Before microsatellite DNA analysis, the received genotype raw data was checked for microsatellite null alleles, inconsistent values, scoring errors and large drop-out in samples using Micro-Checker software (version 2.2.3) (VAN OOSTERHOUT et al. 2004). For genetic characteristics of studied populations of river lamprey and genetic cluster recognition, a set of data analysis approaches were utilized. At the beginning, microsatellite allele frequencies, number of observed allele per locus (A_o), allelic range, number of effective alleles (A_e), allelic richness (A_r), number of private alleles (A_p), Shannon's index (*I*), the polymorphism information content (*PIC* value) and inbreeding coefficient (*Fis*) for each loci within tested population of river lamprey were calculated with PowerMarker (version 3.25), Fstat (version 2.9.3.2) and GenAlEx (version 6.5) (GOUDET 2002, LIU and MUSE 2005, PEAKALL and SMOUSE 2012). Additionally, the observed and (*Ho*) and expected heterozygosity (*He*) as well as Hardy-Weinberg (H-W) equilibrium for each locus were computed using Arlequin software (version 3.5) (EXCOFFIER and LISCHER 2010). The effective population size (*Ne*) was also assessed for examined fish by NeEstimator computer program (version 2.01) (DO et al. 2013). The linkage disequilibrium method was used for computing *Ne*, where the lowest allele frequency was 0.02. Calculated *Ne* values were subsequently corrected for underestimation from sampling errors with jackknifing 95% parametric confidence intervals (CIs). To delineate historical demography of the studied populations the test for the bottleneck assessment was conducted using the Bottleneck software (version 1.9), which tests for departure from mutation drift equilibrium based on heterozygosity excess or deficiency (Piry et al. 1999). For this purpose, an Infinite Allele Model (IAM), Stepwise Mutation Model (SMM) and two-phase model of mutation (TPM) were tested for sampled fish group. This method is based on the assumption that in non-bottlenecked broodstock (close to mutation drift equilibrium) the value of expected heterozygosity (*He*) is equal to Heq (heterozygosity expected in a mutation-drift equilibrium). The excess of *He* over Heq is the evidence of severe reduction in broodstock effective size that may occur because of a bottleneck event. Statistical tests were performed using the one-tailed Wilcoxon signed rank test. Additionally, the allele frequency distribution analysis was performed. In order to test genetic divergence between studied populations of river lamprey, the analysis of molecular variance (AMOVA), assessment of the allele sharing distances (*DAS*), Nei's standard genetic distance (*Ds*) (NEI 1972), genetic differentiation index (*Fst*) index, overall number of migrants as well as Bayesian clustering analysis

were utilized. The Arlequin software was used to perform the AMOVA test and to compute the overall genetic differentiation index (*Fst*) within tested populations. Computed individual pair-wise the allele sharing distances matrix was used to construct Neighbour-Joining tree of individuals with an application of Populations computer software (version 1.2.32) (LANGELLA 2002). The Principal Coordinates Analysis (PCoA) was performed on the basis of individual pair-wise matrices of Nei's standard genetic distance (*Ds*) using GenAlEx software (version 6.5). Overall number of migrants between studied populations of river lamprey was estimated by the Private allele method of SLATKIN (1985) and corrected for size using GenePop computer software (version 4.2.1) (ROUSSET 2008). Moreover, Bayesian clustering analysis implemented in computer software Structure (version 2.3.4) (PRITCHARD et al. 2000) was performed to estimate the most likely number of genetic clusters (*K*) in the studied populations. *K* was tested from one to 10 with 10 iterations. The admixture model was used with 30,000 burn-in periods and 1,000,000 Markov chain Monte Carlo (MCMC) replicates in each run. The most probable number of genetic clusters for analyzed microsatellite data set was estimated on the basis of obtained ln Pr(X | K) values and EVANNO at al. (2005) method (ΔK). For this purpose, the Structure Harvester online software (version 0.6.94) was used (EARL and VON HOLDT 2011). For accommodate the obtained genotypic data to the requirements of used computer software, every tetrasomic locus was examined as two disomic loci and as result the mean frequency was considered for estimation of genetic variation.

Results

Among the nine microsatellite DNA fragments applied in the present study, eight (*Lri-1*, *Lri-2*, *Lri-3*, *Lri-5*, *Lri-7*, *Lri-8*, *Lri-9*, *Lri-10*) were polymorphic, whereas one of them (*Lri-4*) was monomorphic. Moreover, all examined microsatellite loci were considered as disomic with the exception of *Lri-1*, which was considered as tetrasomic. Overall, 39 different alleles were found in the tested populations of river lamprey. A total length of identified alleles in the studied loci varied between 95 and 360 base pairs (bp).

Table 1 and Table 2 show all of evaluated genetic diversity parameters (*Ho*, *He*, A_r, A_o, A_e, *I* and PIC) for the studied populations of river lamprey from Poland. The average number of alleles per locus ranged from 3.8 (Vistula) to 3.4 (Vistula lagoon). The mean values of polymorphic information content (PIC) and the rate of Shannon's index (*I*) in examined populations of

river lamprey from Vistula and Vistula lagoon were 0.390, 0.796 and 0.366, 0.741, respectively (Table 1). Private alleles were identified in both analyzed populations, where Vistula population was characterized by the highest number of the private alleles (8) within the studied microsatellite loci (Table 1).

Table 1

Genetic diversity parameters of two investigated populations of river lamprey from Poland

Population	Locus	A_r	A_o	A_e	I	PIC	A_p
Vistula	*Lri-1*	4.000	5	3.044	1.189	0.612	–
	Lri-2	3.000	3	1.546	0.654	0.316	1
	Lri-3	6.000	6	2.034	1.016	0.470	1
	Lri-4	1.000	1	MONO	MONO	MONO	MONO
	Lri-5	5.000	5	1.512	0.698	0.316	2
	Lri-7	3.000	3	1.416	0.550	0.268	1
	Lri-8	4.000	4	1.206	0.381	0.163	2
	Lri-9	3.000	3	1.989	0.768	0.397	–
	Lri-10	4.000	4	2.826	1.111	0.577	1
	Mean	**3.667**	**3.8**	**1.946**	**0.796**	**0.390**	–
Vistula lagoon	*Lri-1*	4.380	6	3.032	1.225	0.612	1
	Lri-2	1.944	2	1.041	0.098	0.038	-
	Lri-3	4.944	5	1.981	1.011	0.4673	
	Lri-4	1.000	1	MONO	MONO	MONO	MONO
	Lri-5	3.759	4	1.571	0.683	0.329	–
	Lri-7	2.000	2	1.523	0.527	0.284	–
	Lri-8	2.000	2	1.268	0.367	0.189	–
	Lri-9	3.975	4	2.218	0.912	0.453	1
	Lri-10	4.520	5	2.709	1.108	0.559	2
	Mean	**3.169**	**3.4**	**1.918**	**0.741**	**0.366**	–

Explanations: A_r – allelic richness, A_o – observed alleles, A_e – expected alleles, I – Shannon's index, PIC – polymorphism information content, A_p – number of private alleles, MONO – monomorphic locus

The mean values of observed heterozygosity ranged from 0.441 (Vistula) to 0.436 (Vistula lagoon) and were close to the mean values expected under H-W equilibrium (*He*) – Table 2. Chi-Square test for Hardy-Weinberg Equilibrium (H-WE) showed that three (Vistula river) and four (Vistula lagoon) microsatellite loci deviated significantly from Hardy-Weinberg equilibrium (H-WE). In turn, the average values of *Fis* were negative in studied populations, ranging between -0.074 (Vistula) and -0.122 (Vistula lagoon). Moreover, the performed bottleneck test did not reveal any statistically significant *He* > Heq differences under all mutation models used in both of studied river lamprey populations (Table 2). The analysis of allele

frequency distribution revealed an L-shaped distribution. The estimated effective population size (*Ne*) for the studied populations of river lamprey form Vistula river and Vistula lagoon equalled 72.4 (95% CI = 14.9–∞) and 25.8 (95% CI = 8.7–151.1), respectively.

Table 2

Comparison of observed (*Ho*) and expected (*He*) heterozygosity, expected heterozygosity (Heq) in a Infinite Allele Model (*IAM*), Stepwise Mutation Model (*SMM*) and two-phase model of mutation (*TPM*) as well as fixation index (*Fis*) in examined populations of river lamprey

Population/ Locus	*Ho*	*He*	*P*	IAM		SMM		TPM		*Fis*
				Heq	*P*	Heq	*P*	Heq	*P*	
Vistula										
Lri-1	0.974	0.673	0.000	0.436	0.050	0.571	0.217	0.502	0.098	-0.463*
Lri-2	0.158	0.358	0.000	0.341	0.486	0.467	0.202	0.400	0.378	0.562*****
Lri-3	0.553	0.515	0.423	0.597	0.238	0.734	0.012	0.668	0.076	-0.074
Lri-4	MONO	–	–	–	–	–	–	–	–	–
Lri-5	0.342	0.343	0.421	0.534	0.140	0.672	0.003	0.607	0.042	0.003
Lri-7	0.289	0.298	0.065	0.342	0.425	0.466	0.144	0.397	0.289	0.029
Lri-8	0.158	0.173	0.029	0.434	0.075	0.595	0.003	0.524	0.022	0.090
Lri-9	0.368	0.504	0.099	0.337	0.244	0.465	0.494	0.395	0.327	0.271
Lri-10	0.684	0.655	0.902	0.445	0.103	0.597	0.339	0.517	0.154	-0.046
Mean	**0.441**	**0.440**	**–**	**0.433**	**–**	**0.571**	**–**	**0.501**	**–**	**-0.074**
Vistula lagoon										
Lri-1	0.990	0.670	0.000	0.453	0.094	0.596	0.234	0.516	0.182	-0.493*
Lri-2	0.040	0.040	1.000	0.194	0.299	0.225	0.196	0.218	0.239	-0.010
Lri-3	0.380	0.500	0.004	0.517	0.389	0.669	0.046	0.588	0.218	0.242*
Lri-4	MONO	–	–	–	–	–	–	–	–	–
Lri-5	0.360	0.367	0.145	0.417	0.391	0.580	0.054	0.507	0.185	0.019
Lri-7	0.320	0.347	0.680	0.183	0.225	0.231	0.337	0.212	0.284	0.078
Lri-8	0.200	0.213	0.528	0.184	0.356	0.224	0.480	0.201	0.412	0.063
Lri-9	0.560	0.555	0.234	0.434	0.287	0.586	0.311	0.505	0.452	-0.010
Lri-10	0.640	0.637	0.803	0.511	0.254	0.666	0.288	0.595	0.460	-0.004
Mean	**0.436**	**0.416**	**–**	**0.362**	**–**	**0.472**	**–**	**0.418**	**–**	**-0.122**

Explanations: *P* – level of significance; all statistically significant ($P < 0.05$) deviations Ho ≠ He and *He* > Heq were underlined, MONO – monomorphic locus

The estimated overall gene flow (*Nm*) between the populations was at the level 5.09 individuals per generation. Analysis of the genetic structure with AMOVA method showed that only 0.60% of the genetic variation was distributed among studied populations. Moreover, assessed genetic

differentiation between examined populations of river lamprey was at the level *Fst* = 0.006, being statistically insignificant ($P < 0.01$). Received results on individual multilocus genotype, based on structure analyses, did not find any signs of the further genetic clustering within the studied fish group (GIGHER et al. 2013, SCHEDINA et al. 2014, BRACKEN et al. 2015). Utilized method developed by EVANNO et al. (2005) for computation of ΔK did not reveal any specific value. The maximum value of ln Pr(X | K) was observed for K = 1, where the clear plateau of L'(K) values was present after K = 1. Contrastingly, constructed individual's tree based on DAS genetic distances and the Principal Coordinates Analysis (PCoA) exhibited three main genetic groupings within studied fish group (Figure 1 and Figure 2).

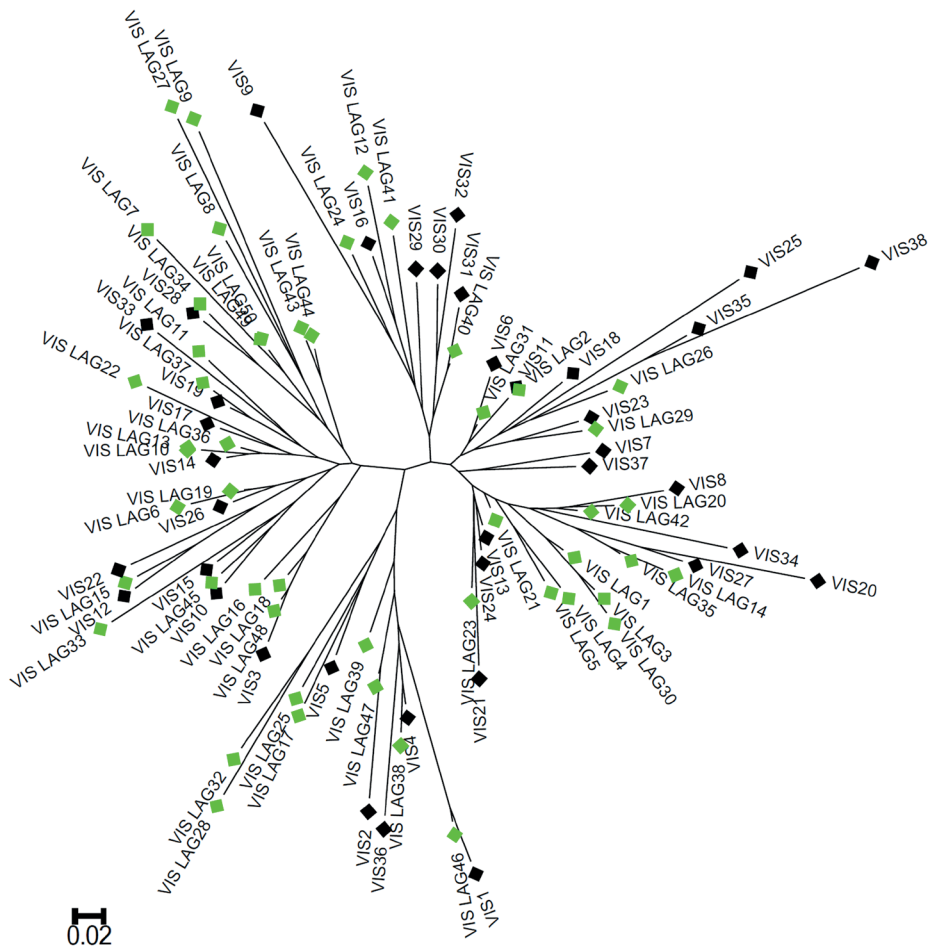

Fig. 1. The unrooted Neighbor-Joining tree of examined river lamprey (*Lampetra fluviatilis*) individuals based on allele sharing distances (*DAS*)

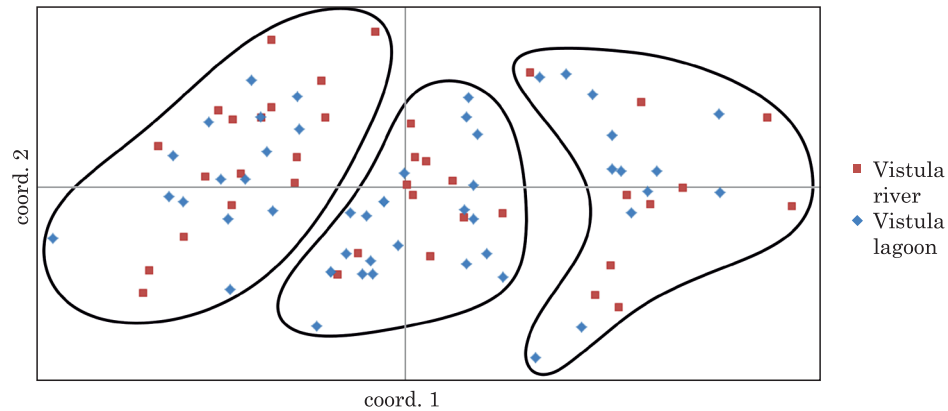

Fig. 2. Scatter plot of the Principal Coordinates Analysis (PCoA) based on an individual pair-wise Nei standard (*Ds*) genetic distance for examined specimens of river lamprey (*Lampetra fluviatilis*)

Discussion

The river lamprey populations in Poland inhabit an environment greatly affected by negative human impact. These pressures may have a strong, adverse impact on population viability need to be carefully assessed as well as managed at scales that are consistent with the population structure of river lamprey. However the decline of river lamprey is still significant in Vistula river and Vistula lagoon in Poland, but the established conservation plan (river lamprey is under partial protection: fishing of this species is forbidden) is realized without any information about genetic structure of this species. Indeed, scientific assessment of population structure, and implementation of conservation and management measures should be conducted based on genetic studies of conserved species/population.

In the present study the microsatellite DNA analysis was applied for identification of genetic diversity of two river lamprey populations in Poland. Such markers were sufficient and useful tools for delineating population structure of studied river lamprey populations. Application of microsatellite markers was helpful and allowed obtaining valuable data that will provide the starting point for the restoration programe of river lamprey in Vistula river estuarium.

To date, the applied genetic analyzes allowed the identification and characterization of the significant diversity of lampreys in the Iberian Peninsula area, a refugium region during the Pleistocene glaciations (MATEUS 2013). Moreover, this study enabled understanding the historical

processes of glacial colonization together with an assessment of the contemporary gene flow between species *Lampetra* sp. in Europe (MATEUS 2013). The application of sequencing analysis of entire genome has significantly contributed to the better understanding of the lampreys taxonomy and enabled the identification of differences between the common species of lampreys (MATEUS 2013). This is very important that the genetic analysis conducted on lamprey enabled to broaden the knowledge about their evolution providing the model to study the processes of speciation (GIGHER et al. 2013).

Population genetic studies of lampreys were frequently conducted on: i) comparison of distinct populations of one species, ii) migration analysis of lampreys, and iii) the estimation of relationship between separate taxa. Currently, the lampreys have become a model organisms in the studies of evolutionary biology associated with an anadromous and resident life styles (BRACKEN et al. 2015). The sea lamprey was the most frequently studied species because of its interesting evolutionary history and invasive character in Great Lakes. Based on genetic analysis, researchers tried to resolve the problems related with the species migration rate, dispersion abilities and its invasive impact on water ecosystems in order to derive genetically (DEROSIER et al. 2007, SPICE et al. 2012, TAYLOR et al. 2012). In turn, LUZIER et al. (2010) developed the microsatellite markers for brook lamprey (*Lampetra planeri*), and then applied them in study of the genetic population structure of other lamprey species. SPICE at al. (2011) conducted genetic studies on the Pacific lamprey (*Entosphenus tridentatus*), using 12 polymorphic microsatellite DNA that enabled indication the diagnostic loci distinguishing *Entosphenus* and *Lampetra* (SPICE et al. 2011). These markers were also used for the population analysis and making decisions in conservation of *Entosphenus tridentatus* (SPICE et al. 2011). Genetic research based on mitochondrial DNA fragment (*ATPase* subunit 6 and 8 and a part of *cytochrome b*) was carried out to identify the river lamprey and brook lamprey in Portugal (MATEUS et al. 2011). This analysis enabled the characteristics of haplotypes and phylogenetic analysis of studied lamprey species together with verification of its species (MATEUS 2011).

The above examples of analyzes, conducted in different species of lampreys, clearly indicate the importance of application of molecular markers in study of endangered organisms that are protected. It should be emphasized that in Poland, the genetic research on lampreys have not been conducted so far. Considering the lack of information about the genetic structure and biodiversity of lampreys in Poland, proposed in this paper, genetic research contribute to increased based information on the Polish populations of the river lamprey.

In the present study, examined fish group was characterized by similar levels of the genetic diversity compared to the lamprey populations across Europe and North America (McFarlane and Docker 2009, Gaigher et al. 2013, Schedina et al. 2014). However, observed genetic variability in this species seems to be explicitly lower than commonly described in teleost fish (Fopp-Bayat and Woznicki 2006, Fopp-Bayat et al. 2015, Kucinski et al. 2015). It is believed that hagfish and lampreys are an ancient group of vertebrates, being characterized by a ancient structure of their genomes. Recent evolutionary studies informed that a genome duplication event took place after the divergence between Cyclostomata (lampreys and hagfish) and gnathostomates (Holland et al. 1994, Thornton 2001). Therefore, it may explain lower variability of microsatellite DNA loci in lampreys with comparison to teleost fish species.

The applied, in the present research, sensitive genetic population tests showed a lack of significant genetic structure between the two examined populations of river lamprey. This result is consistent with described studies on anadromous lampreys from Japan, North America and Europe, where very little or no genetic structure were found (Goodman et al. 2008, Yamazaki et al. 2011, Bracken et al. 2015). The absence of population genetic structure seems to be a general rule in anadromous lamprey species, which is associated with the lack of natal homing (Waldman et al. 2008, Spice et al. 2012). Instead, it was proven that lampreys use pheromones released by stream inhabiting larvae to localize suitable spawning grounds (Fine et al. 2004). Both of the studied populations of river lamprey (Vistula river and Vistula lagoon populations) migrate annually upstream Vistula river for spawning sites. Despite the lack of clear signs for genetic structure within the studied populations, some evidences for asymmetric gene flow and limited panmixia were also found. Higher number of private alleles in population from Vistula river might suggest that the gene flow occurs unidirectional mainly from Vistula lagoon to Vistula river population. Additionally, the presence of private alleles in fish from both sites (Vistula river and Vistula lagoon) might be the evidence for the presence of few cryptic populations. Similarly, the obtained results on individual DAS genetic distances and the Principal Coordinates Analysis (PCoA) might suggest the existence of three mixed populations of river lamprey within studied region. Currently, this phenomenon can be connected to migratory behavior of lampreys. After the larval stage, lampreys metamorphose to young adults and then to sea phase. The sea migration of river lampreys is connected to host-fish on which lamprey parasitize. During this time, different populations of lampreys may mix because they can parasitize on one stock/population of sea fish (for example on cod).

After the sea phase, mature lampreys start spawning migration to rivers and during this time they could also mixed. Our results may indicate a situation where three populations of lampreys were mixed. Probably all three studied populations of river lamprey migrated to spawning sites, and some individuals did not complete their spawning migration.

In conclusion, the described data are very important in interpreting the phylogeographic, genetic and population context and they are the key point during development of management strategies and protection of this lamprey species in Poland. The primary genetic data will be valuable also in biodiversity monitoroing or supplementation plan of river lamprey during protection of endangered populations.

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