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Genetic variation and phylogenetic relationships among domesticated and wild paddlefish (*Polyodon spathula*) populations

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Aim. To determine the genetic variation and phylogenetic relationships among domesticated paddlefish populations from Ukraine and Poland with wild populations from the United States using microsatellite DNA markers. **Methods.** In total, 105 paddlefish individuals were analyzed using PCR technique, capillary electrophoresis, and statistical methods. **Results.** The evaluated mean number of alleles per locus (N_a) varied from 5.5 to 6.6 in the Polish and Ukrainian paddlefish stocks, respectively, whereas two-fold lower genetic polymorphism was demonstrated if compared to that found in wild population from the USA (11.1 alleles per locus). The mean observed (H_o) heterozygosity estimated in Ukrainian populations (0.709) was relatively close to those in the Polish (0.809) and wild (0.817) populations. Close genetic relationships were observed among the studied Ukrainian populations. The measured genetic similarity ranged from 0.908 (among populations from Kherson and Chernihiv regions) to 0.981 (among populations from Chernihiv and Vinnytsia regions). The Nei's genetic distances ranged from 0.019 (populations from Chernihiv and Vinnytsia regions) to 0.096 (populations from Kherson and Chernihiv regions). The constructed dendrogram demonstrated the genetic differences among hatchery-reared and natural paddlefish populations, which were separated into two single clusters according to their geographical origin. **Conclusion.** It has been revealed that Polish paddlefish population was more genetically distant from the studied Ukrainian populations of this species, which confirms the formation of Ukrainian and Polish populations from different parental individuals. The results obtained allow us to consider the investigated fish groups as a potential source of the exchange and enrichment of genetic resources of the paddlefish populations.

Keywords: paddlefish *Polyodon spathula*, DNA–markers, microsatellites, polymerase chain reaction, alleles, phylogenetic analysis

Introduction

The purposeful management of the genetic resources is becoming increasingly important in animal husbandry and aquaculture to preserve biodiversity in hatchery-reared stocks. Growing demand for fishery products requires the expansion of the production of highly valuable objects for the producing of marketable products in a relatively short time [1, 2].

Currently, American paddlefish (*Polyodon spathula*), as a representative of Acipenseriformes species, is of high commercial importance, and its cultivation in aquaculture allows an increase in the productivity of fishing enterprises by rearing in polyculture with other valuable fishes [3, 4].

Artificial paddlefish reproduction is widespread in the United States for restocking and conservation of natural genetic resources, producing marketable products, and for sport fishing of this species. A similar strategy has evolved since the creation of the Mississippi Interstate Cooperative Resource Association (MICRA) in 1991 providing the management of natural paddlefish populations through artificial reproduction and maintenance of its genetic diversity [5, 6].

In turn, the development of paddlefish aquaculture particularly in Europe, Asia and Cuba, is mainly focused on the production of commodity products as black caviar and delicatessen meat to meet the needs of the domestic market and export [7, 8]. Meanwhile, in Ukraine the paddlefish aquaculture is becoming increasingly popular as an optimal alternative to sturgeon species. However, the main challenge in the aquaculture sector is the risk of reduction of genetic variability in farmed

paddlefish populations due to small quantities of the genetic material imported from the USA and Russian Federation to establish its stocks. Likewise, in neighboring country Poland the broodstocks are relatively small and were formed from the progeny of a few spawners from Hungary [4, 12, 16]. Because of this, it is especially significant to evaluate genetic variation and identify genetic differences among paddlefish populations.

At present, the study of genetic processes occurring in paddlefish populations due to natural geographical isolation or under the influence of cultivation practice is at high of its development. The first main contribution proposed in the investigation of such genetic processes is the microsatellite DNA markers [9–14].

In population-genetic studies, microsatellites are widely used to determine genetic differentiation, carry out DNA identification and certification of species, and manage selection and breeding of valuable objects during their artificial reproduction [9, 15–18].

Based on the DNA analysis of paddlefish individuals, the controlled selection of spawner pairs can be performed in order to purposefully restore local populations and maintain genetic diversity of populations under artificial reproduction [11–14]. Additionally, the identification and assessment of genetic differences among the populations of natural reservoirs and farmed species will allow the establishment of phylogenetic relationships and determination of the origin of target objects for effective management of the genetic resources [13, 19–22].

The purpose of this study is to determine the genetic variation and phylogenetic relationships among the domesticated and wild paddlefish (*Polyodon spathula*) populations by STR-loci polymorphism.

Materials and Methods

Fish Samples

Samples (fin clips) were collected (105 in total) from paddlefish individuals of «Dnieper sturgeon fish hatchery named after acad. S.T. Artyushhyk» (n=32, Kherson region, 2016), «Chernihiv-rybhosp» (n = 35, Chernihiv region, 2017) and LTD Fish farm «Mercury» (n = 38, Vinnytsia region, 2017) [23]. The fin clips for the genetic investigation were stored in 97 % ethanol at -20 °C.

DNA Extraction

Genomic DNA was extracted («AmpliPrime» kit, Russian Federation) using silica-based method (SiO₂) [24] and subjected to spectrophotometric reading (BioSpec Nano Spectrophotometer, Shimadzu, Japan) to assess the DNA quality (DNA concentration varied between 48 and 65 ng/μl).

PCR Amplification

Multiplex PCR mix with a total volume of 20.0 μl contained 50.0 mM of Tris-HCl (pH 8.3), 1.5 mM of MgCl₂, 0.2 mM of each dNTP, 5 pM of forward and reverse primers and 1.5 U of Taq-DNA polymerase (Thermo Scientific™, Lithuania). PCR amplification (PCR) was performed on the Veriti 96 Well thermocycler (Applied Biosystems, USA) by standardized parameters: (a) initial denaturation – 5 min, 95 °C; (b) 30 cycles, including denaturation – 15 s, 95° C; primers hybridization – 25 s, 56 °C; elongation – 5 s, 72 °C and (c) final elongation – 5 min, 72 °C [25].

The selected polymorphic microsatellites, namely Psp21, Psp26 Psp28, which are presented in GenBank® (Table 1), were co-amplified in a single multiplex PCR reaction. One primer (forward or reverse) from each pair was end-labeled with a fluorescent dye.

Genotyping

Amplified products were denatured with formamide (Sigma, USA), then separated and detected via capillary electrophoresis (ABI Prism 3130x1 Genetic Analyzer, Applied Biosystems, USA). The DNA fragment size

Table 1. Specific information for the selected STR loci for individual identification and comparative analysis of paddlefish populations [10]

Locus	№ GenBank	Nucleotide sequence of primers (5' → 3')	Allele Size Range (Bases)
Psp12	AF406735.1	F:ATCTGATAACAATCTTCACAGTCC R: GAGTTCAGCTCGCTCTCC	218–228
Psp21	AF406736.1	F:TTCAGCAGGTAGTGAGACAGGCAG R:TCAAGTCCCATCCACTCT	142–170
Psp26	AF406737.1	F:TCGGTGTGTTGTGTGTGTATGC R:TGGTTCCAGTTTCGCTCATCC	130–160
Psp28	AF406738.1	F:CAAAGGCATCCCCTACCAC R:GCTGGACAAAAAGTATGGAGTGC	224–260

was analyzed with the GeneScan™ 500 LIZ size standard and GeneMapper analysis Version 3.7 Software (Applied Biosystems, USA).

Statistical analysis

Number of alleles per locus (Na), heterozygosity (observed (Ho) and expected (He)), Wright's fixation index (Fis) [26], probability value (P), Hardy-Weinberg equation (HWE) values were calculated using software MS Excel 2010, PowerStats V12 (Promega), Cervus v. 3.0.3, GENALEX 6.5, Statistica 8.0 (StatSoft, Tulsa, USA) [27–30].

Based on the allele frequencies, the genetic distances and coefficient of genetic identity were computed in agreement with Nei [31].

The Principal Coordinate Analysis (PCoA) was performed using GENALEX 6.5 program. The Neighbor-joining method (NJ) was used to construct the dendrogram of genetic distances between the studied fish groups using the POPTREEW Software [32].

Results

The comparative study were carried out between three cultured stock from Ukrainian regions (obtained as a result of own studies), two stocks from Poland studied by Kaczmarczyk D. (Kaczmarczyk, 2012): Pogorze, Silesian Voivodeship (n = 24) and Wasosze, Kujawsko-Pomorskie Voivodeship (n = 29) and wild populations from the United States investigated by Heist E. J. (Heist, 2002).

Table 2. Comparison of genetic diversity indices for Ukrainian and Polish (Kaczmarczyk, 2012) populations with wild populations from the USA (Heist, 2002)

Origin of the population	Na (mean ± SE)	Ho (mean ± SE)	He (mean ± SE)	Fis (mean ± SE)
Ukraine				
«Dnieper sturgeon fish hatchery named after acad. S.T. Artyushhyk» (Kherson reg.) n = 32	7.0±1.527	0.729±0.211	0.608±0.143	-0.159±0.129
«Chernigiv rybhos» (Chernihiv reg.), n = 35	5.7±1.330	0.724±0.142	0.650±0.050	-0.092±0.144
LTD Fishfarm «Mercury», (Vinnytsia reg.), n = 38	5.7±1.330	0.675±0.133	0.589±0.099	-0.121±0.186
Mean for populations	6.1±0.186	0.709±0.017	0.616±0.018	-0.124±0.019
Poland				
Wasosze, Kujawsko-Pomorskie (n = 29)	6.3±1.450	0.815±0.080	0.713±0.092	-0.153±0.047
Pogorze, Silesian Voivodeship (n = 24)	4.7±0.330	0.803±0.099	0.686±0.059	-0.062±0.116
Mean for populations	5.5±0.800	0.809±0.006	0.699±0.014	-0.108±0.046
USA				
Big Muddy and Mississippi rivers, Illinois, n = 28	10.0±1.730	0.821±0.074	0.809±0.056	-0.013±0.022
North Dakota, Missouri river, n = 39	12.3±2.400	0.845±0.043	0.841±0.041	0.004±0.009
Bayou Nezpique and Mermentau River, Louisiana, n = 45	11.0±2.080	0.785±0.015	0.789±0.034	0.002±0.044
Mean for populations	11.1±0.665	0.817±0.017	0.813±0.015	-0.002±0.005

Note: Departures from the Hardy-Weinberg equilibrium ($p \leq 0.05$) within investigated stocks.

For the comparative studies, the populations were chosen from different geographic regions, including Bayou Nezpique, Mermentau River, Louisiana (n = 45); Big Muddy and Mississippi, Illinois (n = 28); Missouri, North Dakota (n = 43); Red River near Natchitoches, Louisiana (n = 48); Red River below Lake Texoma, Oklahoma (n = 41); Grand Lake Neosho River, Oklahoma (n = 35).

Table 2 shows that the mean number of alleles per locus (N_a) varied from 5.5 to 6.6 in the Polish and Ukrainian paddlefish stocks, respectively, though it demonstrated a lower genetic polymorphism compared to that found in wild population from the USA (11.1 alleles per locus).

The mean observed heterozygosity (H_o) estimated in the Ukrainian stocks (0.709) was relatively close to those in the Polish stocks (0.809) and in wild populations (0.817). Meanwhile, the average observed heterozygosity was higher than expected for both Ukrainian ($H_o = 0.709$; $H_e = 0.616$) and Polish ($H_o = 0.809$; $H_e = 0.699$) paddlefish stocks. Similarly, the Wright's fixation index (F_{IS}) on average confirmed the predominance of heterozygous genotypes in domesticated populations from Ukraine (-0.124) and Poland (-0.108). The results for the populations from the USA showed that the average observed and expected heterozygosity values were at 0.817 and 0.813, respectively, indicating that the genotype frequencies in wild stocks were in Hardy-Weinberg equilibrium (HWE), as evidenced by the fixation index ($F_{IS} = -0.002$).

Based on the previously calculated frequencies for the identified allelic variants (Kurta, 2019), the Nei's genetic distances and genetic identity among Ukrainian paddlefish populations were revealed (Table 3).

Table 3. The pairwise Nei's genetic distance (below diagonal) and identity (above diagonal) matrix among Ukrainian paddlefish populations

Origin of the population	Kherson region	Chernihiv region	Vinnytsia region
Kherson region	-	0.908	0.937
Chernihiv region	0.096	-	0.981
Vinnytsia region	0.065	0.019	-

It has been established that local populations demonstrated a high level of genetic similarities, which signifies the common origin of these fish groups. The maximum level of the genetic similarity was determined between Chernihiv and Vinnytsia populations at 0.981, whereas the minimum level of the genetic similarity was observed between Kherson and Chernihiv populations at 0.908. The genetic similarity among investigated groups of paddlefish from Kherson and Vinnytsia regions was 0.937. According to the analysis of genetic distances for the studied populations, this index ranged from 0.019 (Chernihiv and Vinnytsia regions) to 0.096 (Kherson and Chernihiv regions), whereas among paddlefish populations from Kherson and Vinnytsia it was 0.065. The increased genetic identity of Ukrainian paddlefish populations is characterized by the presence of predominantly similar allelic variants and their frequency values at the studied loci.

The Principal Coordinate Analysis (PCoA) has been used to visualize the genetic relationships measured by Nei's genetic distance (Fig. 1).

The dissimilarity matrix used as input depicts on the plot shows that the population originated from Kherson region is more distant from those originated from the farms in Chernihiv and Vinnytsia regions.

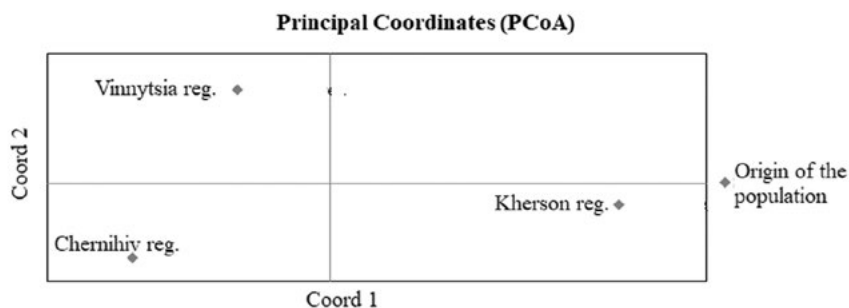


Fig. 1. Principal Coordinate Analysis (PCoA) based on Nei genetic distances for three paddlefish populations farmed in Ukraine

Phylogenetic analysis of Ukrainian populations using the neighbor-joining method (Fig. 2) showed that Chernihiv and Vinnitsia populations constitute a single cluster. In turn, the Kherson population formed a separate cluster.

Overall, the results of the research showed that the detected genetic distances are a consequence of the features of introduction of domesticated paddlefish populations within different geographic location in Ukraine. A high genetic identity among the three paddlefish populations confirms their common origin. The phylogenetic analysis showed that the investigated individuals of paddlefish populations from the Chernihiv and Vinnitsia regions were grouped into a single cluster. The data obtained indicate that these populations

originate from one stock and have common parental individuals. At the same time, the population from the Kherson region appeared more genetically distant, perhaps, due to the genetic material additionally imported to this region, which is confirmed by historical aspects of paddlefish introduction in Ukrainian fish farms [8, 32].

Meanwhile, the additional comparison allowed the establishing of the genetic relationships between paddlefish population from Ukraine and Poland and natural populations from the United States based on three microsatellite DNA-loci (Psp21, Psp26, Psp28).

The distribution of genetic heterogeneity among paddlefish populations from Ukraine, Poland and natural populations from the USA

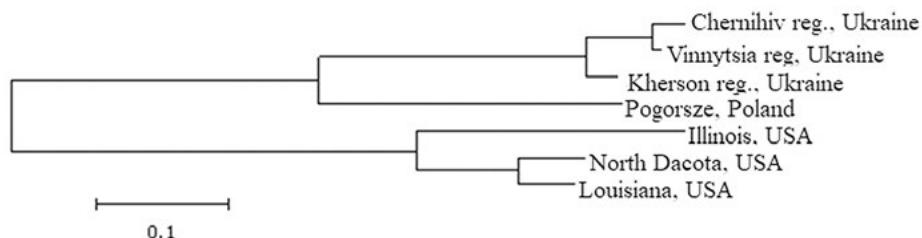


Fig. 2. Neighbor-joining dendrogram of the genetic distances among domesticated and wild paddlefish populations

Note: The stock from Wasosze (Poland) was excluded from phylogenetic analysis, since there is no data on allele frequencies.

showed that domesticated and natural populations fall into different clusters according to their population membership. At the same time, the Polish population was more genetically distant from the studied Ukrainian paddlefish populations. Such results are similar to those obtained in the D. Kaczmarczyk studies [14] and could be a consequence of the formation of Ukrainian and Polish brood populations from different parental individuals.

Analysis of the genetic relationships among paddlefish populations indicated the genetic delimitation of Ukrainian individuals from natural groups. Similarly, the genetic distances were found between Polish and natural populations. Such results are observed as a consequence of differences in the allele frequency spectrum identified in the gene pool of analyzed populations.

Discussion

The evaluated genetic variation in Ukrainian domesticated populations was compared with the respective values observed in Polish paddlefish populations and natural populations of this species from the USA. The mean number of alleles per locus was significantly lower in Ukrainian (6.6) and Polish (5.5) populations in comparison to the natural populations (11.1). These results indicate that almost a two-fold lower genetic variation was observed within domesticated populations from Ukraine and Poland than for the populations from natural reservoirs. The analysis of heterozygosity across the examined populations showed that the mean observed heterozygosity (H_o) estimated in Ukrainian populations (0.709) was relatively close to those in Polish (0.809) and wild populations (0.817). Moreover, the pro-

portion of H_o to H_e rates within domesticated stocks indicated the excess of heterozygous genotypes over the homozygous for both Ukrainian ($H_o = 0.709$; $H_e = 0.616$) and Polish ($H_o = 0.809$; $H_e = 0.699$) paddlefish stocks. These findings were confirmed by values of Wright's fixation index (F_{IS}) in Ukrainian (-0.124) and Polish (-0.108) populations. Despite the fact that the data obtained show high heterozygosity levels for the examined populations, keen attention should be paid to the genetic control of paddlefish breeding in order to prevent the loss of its genetic variability in the farmed populations.

The phylogenetic relationships of cultured paddlefish populations from Ukraine and Poland with natural populations from the United States were determined using three microsatellite DNA markers: Psp21, Psp26 and Psp28. The results demonstrate the close genetic relationships among Ukrainian paddlefish populations, confirming their common origin. Meanwhile, the genetic differences were found among Ukrainian and Polish populations, which allows considering the fish groups as a potential source for the exchange and enrichment of the paddlefish genetic resources.

The genetic distinctiveness between the paddlefish populations from Ukraine and Poland and natural populations from the United States indicate differences in the allele pool, in particular, low allelic diversity and allele frequency spectrum, due to cultivation of this species of fish in aquaculture.

Therefore, monitoring of the genetic processes in stocked paddlefish will allow an effective management of the genetic resources and preservation of the biodiversity that may be lost due to reproductive isolation and uncon-

trolled selection of spawning pairs for breeding. Particular attention should be given to selective and breeding work with the usage of genotyping of parental individuals for further formation of productive paddlefish brood stock. We recommend using the investigated microsatellites loci in the study of Ukrainian populations to analyze additional hatchery-reared brood populations of paddlefish from other geographic locations where the species was introduced.

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Генетична різноманітність та філогенетичні взаємозв'язки між domestикованими та дикими популяціями веслоноса (*Polyodon spathula*)

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Мета. Провести аналіз генетичного різноманіття та філогенетичних зв'язків domestикованих популяцій з України та Польщі із дикими популяціями зі США за мікросателітними ДНК-маркерами. **Методи.** Всього було проаналізовано 105 особин веслоноса, використовуючи методику ПЛР, капілярний електрофорез та статистичні методи досліджень. **Результати.** Визначено середню кількість алелів на локус (Na) для українських і польських популяцій, яка становила 6.1 та 5.5, відповідно, що вказувало на нижчий рівень поліморфізму порівняно з природними популяціями, для яких даний показник був майже вдвічі вищим і в середньому становив — 11.1 алеля. Значення спостережуваної гетерозиготності (Ho), визначене у штучно культивованих популяціях з України (0.709) та Польщі (0.809) були наближеними до рівня спостере-

жуваної генетризованості у диких популяціях (0.817). Встановлено, що для досліджуваних українських популяцій спостерігаються високі рівні генетичної подібності. Зокрема, розраховані значення генетичної схожості коливалися від 0.908 (між популяціями з Херсонської та Чернігівської областей) до 0.981 (між популяціями з Чернігівської та Вінницької областей). Показник генетичних дистанцій за Nei коливався від 0.019 (між популяціями з Чернігівської та Вінницької областей) до 0.096 (між популяціями з Херсонської та Чернігівської областей). Згідно побудованої дендрограми генетичних взаємозв'язків спостерігається розподіл доместикованих та природних популяцій на окремі кластери відповідно до їх географічного походження. **Висновки.** Було визначено, що польські популяції були генетично більш віддаленими, у порівнянні з українськими, що підтверджує особливості формування українських та польських популяцій від різних вихідних батьківських особин. Отримані результати дозволяють розглядати дані групи риб, як потенційне джерело для обміну та збагачення генетичними ресурсами популяцій веслоноса.

Ключові слова: веслоніс (*Polyodon spathula*), ДНК-маркери, мікросателіти, полімеразна ланцюгова реакція, алелі, філогенетичний аналіз

Генетическое разнообразие и филогенетические взаимосвязи между доместичированными и дикими популяциями веслоноса (*Polyodon spathula*)

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Цель. Провести анализ генетического разнообразия и филогенетических связей доместичированных популяций веслоноса из Украины и Польши с дикими популяциями из США по микросателитным ДНК-маркерам. **Методы.** Всего было проанализировано 105 особей веслоноса, используя методику ПЦР, капиллярный электрофорез и статистические методы

исследований. **Результаты.** Определено среднее количество аллелей на локус (Na) для украинских и польских популяций, которое составляло 6.1 и 5.5, соответственно, что указывало на более низкий уровень полиморфизма по сравнению с природными популяциями, для которых данный показатель был почти вдвое выше и в среднем составлял — 11.1 аллеля. Значения наблюдаемой гетерозиготности (Ho), определенные в искусственно культивируемых популяциях из Украины (0.709) и Польши (0.809), были приближенными к уровню наблюдаемой гетерозиготности в диких популяциях (0.817). Установлено, что для исследуемых украинских популяций наблюдаются высокие уровни генетического сходства. В частности, рассчитанные значения генетического сходства колебались от 0.908 (между популяциями из Херсонской и Черниговской областей) до 0.981 (между популяциями из Черниговской и Винницкой областей). Показатель генетических дистанций по Nei колебался от 0.019 (между популяциями из Черниговской и Винницкой областей) до 0.096 (между популяциями из Херсонской и Черниговской областей). Согласно построенной дендрограммы генетических взаимосвязей наблюдается распределение одомашненных и диких популяций на отдельные кластеры в соответствии с их географическим происхождением. **Выводы.** Было определено, что польские популяции были генетически более отдаленными, по сравнению с украинскими, что подтверждает особенности формирования украинских и польских популяций от различных исходных родительских особей. Полученные результаты позволяют рассматривать данные группы рыб, как потенциальный источник для обмена и обогащения генетическими ресурсами популяций веслоноса.

Ключевые слова: веслонос (*Polyodon spathula*), ДНК-маркеры, микросателлиты, полимеразная цепная реакция, аллели, филогенетический анализ

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