

ABSTRACT

For the dissertation for Doctor of Philosophy (PhD) degree
on the speciality “8D05104 – Genetics” of

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on the theme

Genetic analysis barbels of genus *Luciobarbus* Heckel, 1843 from Aral-Syrdarya basin based on the variability of nuclear and mitochondrial DNA markers

General description of the dissertation. The dissertation studies the genetic structure of barbels of the genus *Luciobarbus* Heckel, 1843 of the Aral-Syrdarya basin. Genetic analysis of barbels was conducted based on the variability of nuclear and mitochondrial DNA markers.

Relevance of the research topic. There are two species of barbels in the Aral-Syrdarya basin: aral *L. brachycephalus* and turkestan *L. conocephalus*. In the past, barbels were valuable commercial species of the Aral Sea and the Syrdarya River. The regulation of the Syrdarya and Amudarya River flows had a huge negative impact on the reproduction of the barbels population, which subsequently led to a catastrophic decline in numbers. Dams without fish passes blocked the barbel's path to traditional spawning grounds and reduced its numbers, so the barbels of the Aral-Syrdarya basin are listed in the Red Book of the Republic of Kazakhstan, and are also included in the IUCN Red List, where they have the conservation status of *Vulnerable*. In view of their status and in order to avoid potential damage to populations and maintain the ecological principle of "catch and release", samples were collected for genetic analysis. The species *L. brachycephalus* and *L. conocephalus* have largely overlapping habitats. Morphologically, these species are very close and they are difficult to identify; clarification of the species status and systematic position of barbels (proposals for research in the Red Book of the Republic of Kazakhstan) require molecular genetic studies. In addition, the issues of clarifying the species affiliation of the turkestan barbel *L. conocephalus* remain unstudied to this day. Probably, in the future, the barbels population will be maintained mainly through artificial reproduction with assessment of genetic diversity. Until now, specialized genetic studies on valuable, rare and endemic fish species have not been conducted.

The purpose of the research: To conduct a genetic analysis and assess the genetic diversity of the barbels of the genus *Luciobarbus* Heckel, 1843 from the Aral-Syrdarya basin based on nuclear and mtDNA markers.

Research objectives:

1. To map the habitats of barbels in the Aral-Syrdarya basin. To study the biological and morphological characteristics.
2. To assess the genetic diversity of barbels from the Aral-Syrdarya basin based on the use of microsatellite loci.
3. To study the genetic structure of the genus *Luciobarbus* Heckel, 1843 from the Aral-Syrdarya basin based on nuclear markers.

4. To assess the intrapopulation genetic diversity of the aral barbel based on the results of the *Cytb* gene of mtDNA.

5. To conduct a phylogenetic analysis of the genus *Luciobarbus* Heckel, 1843 using the *Cytb* gene of mtDNA.

6. To conduct a genetic passport of the aral barbel *L. brachycephalus* replacement broodstock kept in the State Enterprise "Kamyshlybash Fish Hatchery" based on nuclear and mtDNA markers.

The research objects: Barbels of the genus *Luciobarbus* Heckel, 1843 from the Aral-Syrdarya basin.

Research methods. During the work, cartographic, morphometric, molecular genetics and statistical methods were used.

The scientific novelty of the research. For the first time, the genetic diversity and structure of the genus *Luciobarbus* Heckel, 1843 from the Aral-Syrdarya basin was widely assessed in using 15 of 17 informative DNA microsatellite loci. Private alleles for the genus *Luciobarbus* Heckel, 1843 were found, allowing identification of the studied species. For the first time, the nucleotide sequence of the mtDNA marker *Cytb* of the Aral-Syrdarya basin barbels was determined, intrapopulation genetic diversity was studied and a haplotype network was constructed for the aral barbel, phylogenetic analysis of the genus *Luciobarbus* Heckel, 1843 was conducted, and based on the results, their differentiation was carried out and the species status of *L. conocephalus* was clarified. For the first time, a genetic passport of the aral barbel *L. brachycephalus* broodstock kept in the State Enterprise "Kamyshlybash Fish Hatchery" was conducted based on 4 out of 10 microsatellite loci and the *COI* gene of mtDNA.

Theoretical significance of the research. Since the study is at the intersection of genetics and ichthyology, the results obtained may have an impact on the development of these areas of science, both in fundamental and applied aspects. The results obtained contribute to the expansion of knowledge about the genetic structure of rare and endangered species of the ichthyofauna of Kazakhstan and can be used in evolutionary biology (microevolutionary processes), population genetics, fish taxonomy, comparative phylogenetics, issues of speciation and identification of ichthyogeographic patterns in the basins of Central Asia, where barbels are distributed.

Practical significance of the research. The results obtained in the course of this study can be used to develop effective, scientifically based plans for the conservation and restoration of barbels by nature conservation, fisheries and other organizations whose activities are aimed at the protection, reproduction and rational use of fish resources. The obtained data are especially important when planning the reintroduction of barbels, artificial breeding of the species, which requires taking into account the population-genetic structure, to prevent degradation of the gene pool of the species and critical extinction in the future. The conducted genetic passport of the replacement broodstock of the aral barbel *L. brachycephalus* is necessary for the targeted formation of producers and their further use in artificial reproduction for growing viable fish seeding material when stocking water bodies. Multifaceted complex molecular genetic studies are necessary for preserving the gene pool and

developing aquaculture and prove the need for their use in fish hatcheries within the Aral-Syrdarya basin.

The main provisions for the defence:

1. For the first time, four specimens of the turkestan barbel were caught in the studied catching points (near the city of Kyzylorda and rice checks), which suggests a wide distribution area. Barbels of the two species are close to each other in terms of a set of morpho-biological characteristics, most of which overlap.

2. Seventeen new specific microsatellite loci for the genus *Luciobarbus* Heckel, 1843 were tested, of which 15 were informative for assessing the genetic diversity.

3. The genetic structure based on nuclear markers was studied for representatives of the genus and they can also be useful for intrageneric genetic identification. It was revealed that within the range of some individuals of the aral barbel, nuclear genotypes of the turkestan barbel were found. Private alleles for the turkestan barbel were noted, such as: *M1447*, *M0244* and *M4215*, which demonstrate clear differentiation when dividing them into clusters.

4. When assessing the intrapopulation genetic diversity of the aral barbel, when calculating the indices of haplotype diversity, a total of 19 were identified. Phylogenetic analysis using Bayesian and maximum likelihood methods for the *Cytb* gene confirmed the presence of four main phylogroups among the analyzed individuals.

5. Based on the results of phylogenetic analysis of the genus *Luciobarbus* Heckel, 1843 based on the *Cytb* gene of mtDNA, their differentiation was carried out and the species status of the turkestan barbel, which is not a subspecies, but an independent species, was clarified.

6. For the first time, genetic passport of the aral barbel *L. brachycephalus* replacement broodstock kept in the State Enterprise "Kamyshlybash Fish Hatchery" was tested based on 4 of 10 nuclear markers and the *COI* gene of mtDNA.

Main research results and conclusions:

1. Based on the results of the barbel habitat mapping in the Aral-Syrdarya basin, the sampling area covered most of the known distribution area. The obtained values of the variation coefficient show that the characters in comparison have low variability among the two studied species. Most of the CV of meristic and plastic characters was less than 15%, which required additional molecular approaches.

2. Seventeen new specific microsatellite loci for the genus *Luciobarbus* Heckel, 1843 were tested, of which 15 were informative for assessing the genetic diversity of barbels and, according to the number of alleles for 15 loci, ranged from 2 to 12, with an average value of 6. The total number of alleles was 90. High values of total heterozygosity were found for loci with the largest number of alleles from 8 to 12: *M2044*, *M4215*, *M4474*, *M1287* and *M3444*, and two loci *M3318* and *M4455* were monomorphic and were excluded from subsequent analyses. For all genetic diversity indices, including mean allele number per locus, effective allele number and heterozygosity, *L. brachycephalus* from Basykara dam demonstrated higher values compared to all sampling points, and the point closest to the mouth of the Aral Sea had the lowest genetic diversity.

3. Nuclear marker-based genetic structure for representatives of the genus *Luciobarbus* Heckel, 1843 revealed 5 clusters ($K = 5$), Bayesian cluster analysis based on STRUCTURE v2.3.4 software. Individuals caught near the city of Kyzylorda and rice checks were identified as the aral barbel, but genetic structural analysis proved that they belong to another species - the turkestan barbel, which were noted for the first time in these places of the basin. A low level of introgression of the turkestan genotypes in the aral barbel in the Bayesian cluster was noted, and no genotypes of the aral barbel were found in the turkestan one, indicating limited genetic exchange between the two species. Private alleles for the turkestan barbel were noted, such as: *M1447*, *M0244* and *M4215*, which demonstrate clear differentiation when dividing them into clusters.

4. Based on the results of sequencing the complete mitochondrial gene *Cytb* (1141 bp), a haplotype network was constructed and, when calculating the indices of haplotype diversity, a total of 19 were identified. Phylogenetic analysis using Bayesian and maximum likelihood methods for the *Cytb* gene confirmed the presence of four main phylogroups among the analyzed individuals. Thus, it can be stated that *L. brachycephalus* has a high genetic intraspecific diversity.

5. Based on the mtDNA *Cytb* sequences, a phylogenetic tree of the aral barbel *L. brachycephalus*, turkestan barbel *L. conocephalus* and bulat-mai barbel *L. capito* was constructed using the NJ (Neighbor-Joining) method in comparison with obtained nucleotide sequences and samples from GenBank. Two large clades were formed on the tree, one of which is differentiated as *L. brachycephalus*, and the other is a clade with *L. capito* and *L. conocephalus*. The taxonomic status of the turkestan barbel *L. conocephalus* in Kazakhstan is controversial. The samples of Turkestan barbel from the Aral-Syrdarya basin, the Badam River, studied by the author, joined the Turkestan barbel from the Hari river (Iran). This confirms the species status of the turkestan barbel, which is not a subspecies (*Barbus capito conocephalus* or *Luciobarbus capito conocephalus*), but an independent species.

6. Based on nuclear and mitochondrial DNA markers, a preliminary testing of the genetic passport of the aral barbel *L. brachycephalus* replacement broodstock kept in the State Enterprise "Kamyshlybash Fish Hatchery" was carried out. For four (*Barb37*; *Barb79*; *BC8*; *BC37*) microsatellite loci, 14 alleles were identified, the average number of alleles per locus was 3.75). Using the mtDNA *COI* gene, the replacement broodstock kept in the State Enterprise "Kamyshlybash Fish Hatchery» was identified as belonging to the aral barbel with an accuracy of 99.86% nucleotide sequence coincidence.

Personal contribution of the author. The author of the dissertation has fully contributed to the selection of the research concept. The definition of the dissertation objective, setting research tasks, analysis of literature data, planning and implementation of methods, statistical processing of results, generalization and processing of the obtained data, and design were fully performed by the author of the dissertation. In addition, the author contributed to the publication of all scientific works: preparation of the manuscript, plotting of graphs and analysis of results, correspondence, and responses to reviewers.

Connection with the plan of basic scientific work. The dissertation work was completed by the candidate himself and funded within the framework of the scientific

and technical program BR10264236 "Scientific and technological support for the integrated development of aquaculture in Kazakhstan through the development and implementation of innovative technologies and new fish farming facilities" (2021-2023), where the author was responsible of the barbels genotyping section. The fundamental part of the dissertation and the main molecular genetic methods were carried out in the laboratory of molecular systematics, the Department of Biodiversity and Evolutionary Biology at the National Museum of Natural Sciences (Madrid, Spain) during the doctoral foreign internship.

Approbation of works. The research results and the main provisions of the dissertation were presented at international scientific conferences: International Scientific and Practical Conference "Actual Problems of Biodiversity and Biotechnology" (Astrakhan, Russian Federation, 2021); International Scientific and Practical Conference dedicated to the 70th anniversary of the Altai branch of FishRPC (Ust-Kamenogorsk, Kazakhstan, 2022); International Scientific Conference of Students and Young Scientists "Farabi Alemi" (Almaty, Kazakhstan, 2023); International Scientific and Practical Conference "Fisheries Research: Experience, Problems and Prospects" (Balkhash, Kazakhstan, 2023).

Publications. The main content of the dissertation is reflected in 9 printed works, including 2 articles in publications included in the first (Q1) and second (Q2) quartiles of the Web of Science and Scopus databases; 1 article in a journal included in the list of the CQAFSHE MSHE RK; 4 theses in the materials of international conferences and 2 methodological recommendations (Fisheries Committee of the Ministry of Ecology and Natural Resources of the RK).

Scope and structure of the dissertation. The dissertation work includes an introduction, literature review, materials and methods, results of own research, conclusion, list of references and appendices. The dissertation is presented on 139 pages of computer text, in compliance with the necessary standards, includes 13 tables, 25 figures. The list of references contains 306 sources.