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Development and Characterization of Fifteen Polymorphic Microsatellite Loci for Rare and Endangered Species within *Luciobarbus* Heckel, 1843 Genus in the Aral Basin and Their Conservation Application

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Abstract: Biodiversity conservation entails not only the preservation of specific taxa but also genetic diversity. Despite the crucial role of molecular data in freshwater fish conservation management, there is a scarcity of information regarding the genetic diversity of *Luciobarbus* Heckel, 1843 (Actinopterygii, Cyprinidae) populations in the Aral system. Therefore, the primary aim of this study was to provide genetic information on two native species of the *Luciobarbus* genus found in the Aral system: *L. conocephalus* (Kessler, 1872) and *L. brachycephalus* (Kessler, 1872). These species, like many others in the Aral system, confront the imminent threat of extinction due to system alterations. However, genetic studies on these species at the nuclear level are challenging because *Luciobarbus* is an allotetraploid genus. Consequently, genetic investigations thus far have focused mainly on sequencing mitochondrial genes due to their haploid nature. This study has successfully developed fifteen new polymorphic microsatellite loci, which can prove to be valuable for population genetics, conservation, and other pertinent research on these species.

Keywords: Aral; Syrdarya; microsatellite markers; allotetraploids; conservation; population genetics

Key Contribution: This study assessed the genetic diversity of rare and endangered species of the Aral Basin in Kazakhstan to address a knowledge gap in this area. An assessment of genetic diversity was not carried out before this work. This study provides valuable insights for further research aimed at investigating genetic structure.

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1. Introduction

The cyprinid family includes 152 genera and within freshwater fishes, this family stands out as the most diverse, comprising a remarkable 1695 species [1]. Within the cyprinid fishes, the genus *Luciobarbus* Heckel, 1843 includes 51 medium to large-sized species distributed along rivers draining into the Persian Gulf, the Mediterranean, the Black, Caspian, and Aral Seas and into the Atlantic Ocean, from the Iberian Peninsula in Europe to Central Asia and North Africa [2–4].

The Aral Sea was once the world's fourth-largest inland body of water in terms of surface area [5]. A lake basin, fed by two rivers, the Amu Darya and the Syrdarya, supports a diverse ecosystem and boasts a highly economically valuable fishery [6]. Currently, the Aral Sea is facing severe environmental degradation primarily attributable to a rapid reduction in its water level. The main consequence of this dwindling water level is an increase in

salinity, which reduces biodiversity [7]. It has been reported that the population of *Luciobarbus brachycephalus* (Kessler, 1872) has declined by 30% over the past 30 years and continues to decline due to the rising salinity of the Aral Sea and the construction of dams on its tributaries [8]. Two species of barbels of the genus *Luciobarbus* Heckel, 1843 inhabit water bodies within the Aral Basin: *Luciobarbus brachycephalus* (Kessler, 1872) and *Luciobarbus conocephalus* (Kessler, 1872). They are part of the Ponto-Caspian freshwater faunal complex [9,10], and their distribution ranges largely overlap [11–13]. *Luciobarbus brachycephalus* is a migratory species represented by two populations: one in the Caspian Sea Basin and the other in the Aral Basin. In contrast, *L. conocephalus* primarily inhabits freshwater environments and does not undergo extensive migration. *Luciobarbus conocephalus* is mainly found in the flat regions of the Amudarya, Syrdarya, Zeravshan, Kafirnigan, Kashkadarya, and Chu Rivers as well as in reservoirs on these rivers, in floodplain lakes, and in the main and waste channels of irrigation systems [14–16]. The Kazakhstan part of the range includes the Syrdarya Basin from the Shardara Reservoir to the lower reaches, including the basins of rivers flowing from the southwestern slopes of the Karatau Ridge (Arys, Bugun, Badam, and Keles Rivers) [17]. The taxonomic status of *L. conocephalus* has been a subject of debate [18], and it was initially classified as a subspecies [4] of *Luciobarbus capito* (Güldenstädt, 1773) and later considered to be a synonym of *L. capito* [19]. However, recent molecular and morphological studies [20] support the recognition of *L. conocephalus* as a distinct and valid species. In this study, we aligned with this more recent and supported taxonomy [20] and recognized the species as *L. conocephalus*.

Luciobarbus species display a high degree of morphological similarity, with only a few distinguishing traits. Previous studies on the systematic position and taxonomic classification of barbels in the Aral–Syrdarya Basin have predominantly relied on comparative morphological methods. However, these studies have yielded inconsistent results among different authors [21–24]. The morphological variation in the barbel traits of both species was limited, with significant overlap observed. An analysis of morphometric traits in juvenile individuals has revealed that the number of scales in the lateral line is the most visually significant characteristic [13]. The available literature highlights the necessity for further investigation and clarification of the genus's taxonomy [17], which requires molecular genetic analysis based on both mitochondrial and nuclear markers.

Many published works have focused on the evolution of the *Luciobarbus* genus by examining mitochondrial genes [25–30]. This genus represents an allopolyploid, specifically a tetraploid formed as a result of ancient hybridization, which renders the study of its nuclear genome particularly challenging. Regarding the evolution of ploidy in cyprinids, only general studies have been conducted, encompassing several species of the genus *Luciobarbus*, and only one study used a small number of nuclear genes to resolve relationships within the cyprinid family [31]. Mitochondrial and nuclear genes do not always correspond [32,33] because the mitochondrial genome is haploid and maternally inherited; therefore, its effective population size is four times smaller than the nuclear genome [34]. Therefore, intraspecific polymorphisms are lost more quickly than that in the nuclear genome since this rate is inversely proportional to the effective population size [32]. This is especially important for allopolyploid organisms such as the genus *Luciobarbus* [35]. Nonetheless, for a more precise interpretation of the genus's evolutionary history, additional exploration of the nuclear genome is imperative.

Among nuclear markers, microsatellites have been extensively employed due to their versatility in various applications across the domains of population genetics, conservation, and evolutionary biology. Their codominant characteristics, high levels of polymorphism, and reproducibility make them highly suitable tools for evaluating population structure and genetic diversity [36–41].

This study represents the first attempt to determine the genetic structure (variation), diversity (variability), and evolutionary history of populations of the genus *Luciobarbus* in the Aral Basin by investigating their nuclear genomes by employing microsatellites. These findings hold particular significance for the conservation and sustainable utilization of

resources in one of the ecosystems, the Aral Sea, which has been severely impacted by human activities.

2. Materials and Methods

Specimens of barbels were collected from the Aral Basin from May to September 2022. Along the Syrdarya River, specimens belonging to the genus *Luciobarbus* were found at five sampling points. The names and geographical coordinates of each locality are presented in Table 1.

Table 1. Sampling points and species of this study.

N°	Location	Species	Number of Sampled Individuals (*n* = 81)	Latitude	Longitude
1	Bairykum village	*L. brachycephalus*	7	42.0764	68.4753
2	Kyzylorda region	*L. brachycephalus* *L. conocephalus*	21 2	45.757381	62.322459
3	Basykara dam	*L. brachycephalus*	25	45.7583156	62.3254401
4	Rice check	*L. brachycephalus* *L. conocephalus*	22 2	45.21043	64.12054
5	Badam River	*L. conocephalus*	2	42.3088933	69.5388738

A map of the river basins (Figure 1) was constructed using QGIS 3.22.13 (QGIS Development Team 2021).

Eighty-one individuals were collected from the sampling points using nets. Fin clips were preserved in 96% ethanol. All the samples were collected using the appropriate permits from the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan. The specimens were released alive back into the river, with the exception of a small selection of 2 specimens from each population, and fin fragments are now deposited in the Collections and BioBank of the National Museum of Natural Sciences (MNCN-CSIC) in Madrid, Spain. For the first time, the developed microsatellite markers were tested in the MNCN-CSIC for the current populations analyzed. Total genomic DNA from the sampled tissues was obtained using Qiagen DNeasy 96 Blood and Tissue kits (QiagenTM, Venlo, Netherlands) following the manufacturer's recommendations.

A total of 17 primer pairs were designed for microsatellite loci (Table 2). These primers were grouped into 4 sets of multiplex (1: *M1287*, *M1417*, *M1182*; 2: *M2237*, *M2108*, *M2044*, *M2164*, *M2306*; 3: *M3230*, *M3264*, *M3318*, *M3444*; 4: *M4211*, *M4455*, *M4215*, *M4138*, *M4474*) PCRs according to the primer properties, forward and reverse sequences, expected amplicon size, repeat motif, tail of the primer, and type of fluorescent marker used.

PCRs were performed in a final volume of 12.5 µL containing 1 µL of DNA (10 ng/L), 6.25 µL of Type-it Microsatellite PCR Kit (Qiagen), 4 µL of PCR grade water, and 1.25 µL of the 10X primer mix. The optimal PCR protocol consisted of an initial denaturation step at 95 °C for 5 min: 30 cycles of 95 °C for 30 s, 57 °C for 90 s, 72 °C for 30 s; 8 cycles of 95 °C for 30 s, 53 °C for 90 s, 72 °C for 30 s; and a final extension step at 68 °C for 30 min. All the PCRs included a negative control to verify possible contamination. The tails of the oligonucleotides were attached to the 5′ ends of the primers for fluorescent labeling. The oligonucleotide tails used were the universal sequences *M13* (GGA AAC AGC TAT GAC CAT), CAG (CAG TCG GGC GTC ATC) and *T3* (AAT TAA CCC TCA CTA AAG GG). The oligonucleotide tails were labeled with PET, NED, and VIC dyes. The amplified PCR products were processed on an ABI Prism 3730 DNA Analyzer (250–500 LIZ size standards). Allele assignment was performed using GENEMAPPER 3.7 (Applied Biosystems, Foster City, CA, USA).

Table 2. Characterization of 17 nucleotide microsatellite loci for *Luciobarbus brachycephalus* (Kessler, 1872) and *Luciobarbus conocephalus* (Kessler, 1872).

Locus Name	Primer Sequence (5′–3′)	Amplicon Size	Repeat Motif	Tail of Primer	Fluorescent Marker
M1287	F:TAATTAGCAACAGGCCCGCA R: TGCGTTCCCGTGTTTGAATG	170	$(AG)_{10}$	T3	PET
M1417	F:CCAAGTCTCGCTATCCTCGG R:AAGAGGAGTGATGACAGCGC	114	$(CCG)_5$	CAG	NED
M1182	F:GCTCTCGTTCCAGTCCAGAC R: AGCATCTGGCCATGATGGAG	193	$(AATC)_7$	CAG	VIC
M2237	F:GAAGGTCACGTGGTTGTCCA R: AGGGAATTGGATGCAGCTCC	91	$(AG)_{12}$	CAG	PET
M2108	F:GCTGCGGATTGGTCAAGAAC R: GCTCTTCTCCTCTCATCCGC	91	$(AG)_{14}$	M13	NED
M2044	F:TATGCAGCTTCCACCCACTG R:GTTCACGCTGTTTGCTGGAG	103	$(AC)_{10}$	M13	NED
M2164	F:GGCGTTGTTGAGCCAATCAG R: TGACTTTGGCAGGACGTGTT	91	$(AGC)_5$	M13	VIC
M2306	F:CAGTCCCAGACTCTTCCAGC R:CCGGTGTGCGATCCAATCTA	302	$(ATC)_5$	CAG	NED
M3230	F:ATTGAGGATCCCGAGGCTCT R:CGATAAGCCCGTGAGACGTT	149	$(AGG)_5$	T3	PET
M3264	F:TGGTCATGCATGCGGTACAT R:AAGGTCACTGAAGTGCTGCA	159	$(ACAG)_6$	T3	NED
M3318	F:AGTGAAAGCATGTCCAGGCA R:GGAACTGGCCGTGAAATGG	217	$(AG)_{18}$	CAG	VIC
M3444	F:ATGACTCAGGTGAAGCAGGC R:CCGCTCCTGCTTGACTTCAT	223	$(AGC)_7$	CAG	VIC
M4211	F:CTAGACGAGCAGCACTGGAG R:CATTAGACAGCCGAGCCCTT	109	$(AC)_{11}$	CAG	PET
M4455	F:TGTATGACGCTGGTTGGAGC R:ATGATACGATCCCAGCGCTG	110	$(AC)_{11}$	M13	NED
M4215	F:CGAGCCGATCTCTGTCTGTG R:CCCAAACCCAAGAAAGTGCG	91	$(AATC)_{10}$	M13	PET
M4138	F:CTGGCTGTCAACCTGTGGAA R:CTCCAGAGTCCGTACCTGGA	153	$(AG)_{10}$	CAG	VIC
M4474	F:AACACTGACCATGTGACGCA R:CCAACTTCTGGTCCGGCATA	238	$(AC)_{11}$	T3	PET

The genetic diversity of each locus was estimated using the GenoDive v.3.0 program. [42], which allows the analysis of polyploid organisms. For this purpose, the following indices were estimated: the number of alleles per locus (N_A), the effective number of alleles per locus (A_E), the observed heterozygosity (H_o), the heterozygosity within populations (H_s), the total heterozygosity (H_t), the corrected total heterozygosity (H'_t), and the inbreeding coefficient (G_{is}). The genetic differentiation between populations was performed based on the *G'st* statistics [43]. The significance value for this parameter was estimated using 999 permutations. Previously, MICRO-CHECKER v2.23 [44] was used to explore the existence of null alleles and evaluate their impact on the estimation of genetic differentiation.

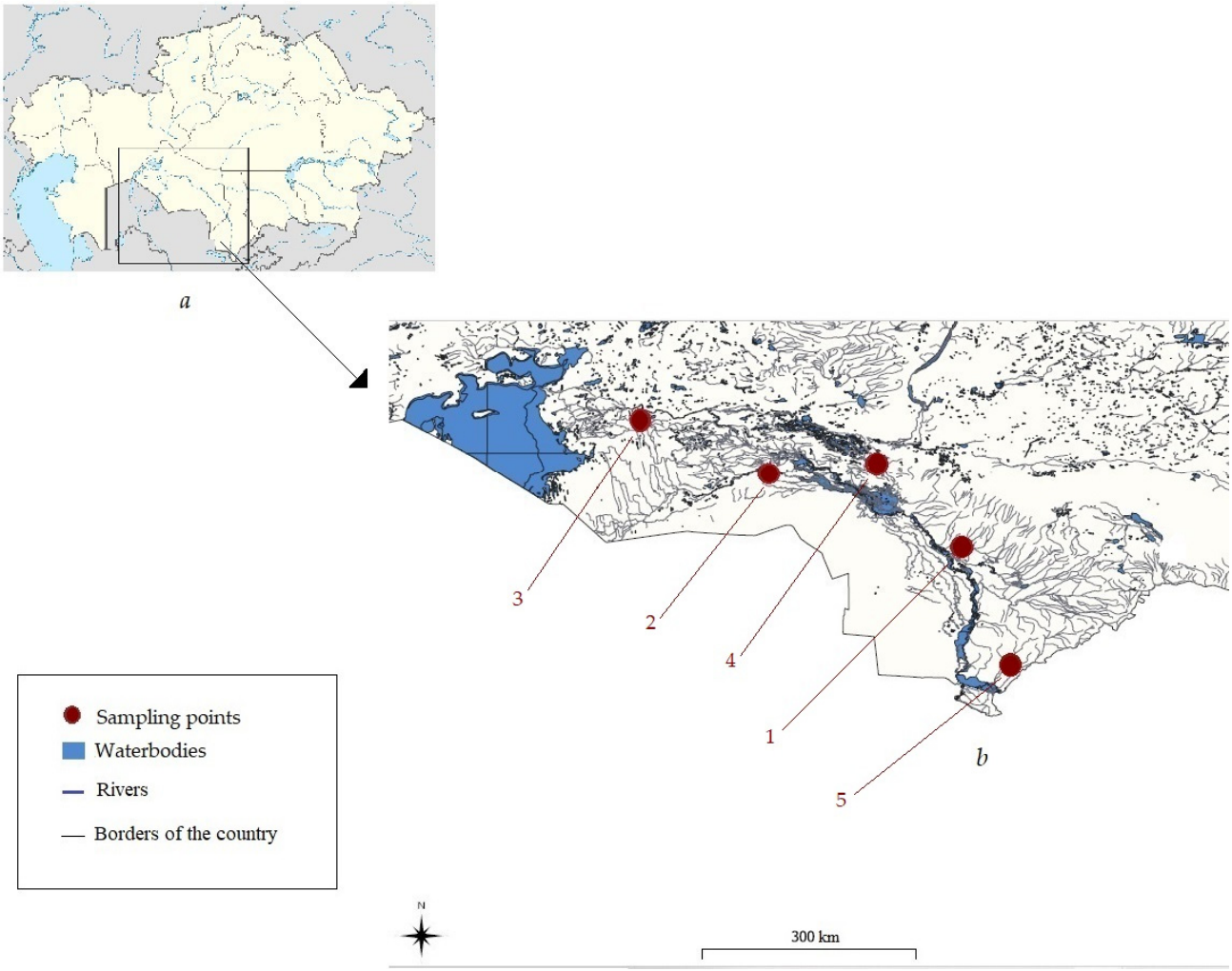


Figure 1. Sampling location of barbels; (**a**): Map of Republic of Kazakhstan; (**b**): Aral–Syrdarya Basin in Kazakhstan territory; 1–5: *L. brachycephalus* and *L. conocephalus*.

Polymorphism information content (PIC) is a way to evaluate the ability of genetic markers to detect polymorphisms among individuals. This parameter was calculated using the following formula [45]:

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

A model based on Bayesian clustering was used to study the population structure. The number of populations (K) with the highest posterior probability (mean lnProb [D]) was calculated using the program STRUCTURE 2.3.4 [46], assuming an admixed model and a uniform prior probability of the number of populations, K, and a ploidy of 4 (tetraploids). The MCMC simulations consisted of 1×10^6 burn-in iterations followed by 1×10^5 sampled iterations. Furthermore, the modal values of λ and ΔK [47] were also calculated to infer the best value of K in [48].

To determine the amount of genetic structuring among grouping levels, an analysis of molecular variance (AMOVA) [49] was carried out to estimate the percentage of variation in the allele frequency of the microsatellites among and within each population, grouping them by sampling location (population) for *L. brachycephalus*. The AMOVA was carried out in the GenoDive v.3.0 program [42].

3. Results

No evidence of shadow banding or genotyping errors was found, and the frequency of null alleles was low. Based on the allele frequencies observed in the analysis of 15 loci across 81 individuals, a total of 90 alleles were identified, accounting for a missing data percentage of 10.93%. The number of alleles per locus varied between 2 and 12, with a polymorphism information content (PIC > 0.5) of 0.774, indicating high polymorphism. Notably, one locus, *M2108*, exhibited a significant deviation from Hardy–Weinberg equilibrium ($p = 0.0001$).

The AMOVA revealed that the highest percentage of genetic variation for all the *L. brachycephalus* samples from four sampled populations was explained by the "within individuals" component (98.4%), while a low percentage of genetic variation explained the among individual and among population components at 0.3% and 1.3%, respectively.

For all measures of genetic diversity, including the average number of alleles per locus, the effective number of alleles, and heterozygosity, *L. brachycephalus* from the Basykara Dam consistently displayed higher values compared to all the other sampling locations. Within the examined populations of *L. brachycephalus*, the population closest to the Aral Sea estuary exhibited the lowest diversity.

The inbreeding coefficient values were statistically significant for all the studied populations, except for the population of *L. brachycephalus* from Rice Check, which is located in the headwaters of the Syrdarya River watershed (Table 3).

Table 3. Indices of genetic diversity for *L. brachycephalus* and *L. conocephalus* at each sampling point in the Aral–Syrdarya Basin.

Sampling Points	Species	N_a	N_e	H_o	H_e	G_{is}
Bairykum v.	*L. brachycephalus*	3.267	2.283	0.518	0.497	−0.042
Kyzylorda r.	*L. brachycephalus* / *L. conocephalus*	4.533	2.653	0.578	0.546	−0.058
Basykara d.	*L. brachycephalus*	5.067	2.707	0.540	0.539	−0.001
Rice check	*L. brachycephalus* / *L. conocephalus*	4.667	2.659	0.557	0.552	−0.008
Badam R.	*L. conocephalus*	1.867	1.812	0.433	0.428	−0.014

N_a: number of alleles; N_e: effective number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; G_{is}: inbreeding coefficient.

Among the seventeen tested microsatellites, fifteen loci amplified successfully (Table 4) and were polymorphic; two of the loci (*M3318* and *M4455*) were monomorphic and they were excluded from subsequent analyses. Among the fifteen amplified loci, *M2044*, *M4215*, and *M1287* showed the greatest degree of polymorphism (Table 4).

The number of alleles per locus ranged from 2 to 12 (mean 6). The observed heterozygosity and total heterozygosity ranged from 0.037 to 0.911 (average 0.525) and from 0.055 to 0.860, respectively. The highest effective number of alleles was 5.093 for the *M2044* locus and 3.030 for the *M4215* locus; the observed heterozygosity ranged from 0.911–0.715 for the *M2044*, *M1287*, *M1417*, *M1182*, *M3264*, *M3444*, and *M4138* loci. The heterozygosity within the population was noted for the loci *M2044*—0.842, *M4215*—0.721, and *M1287*–0.699, and high values of total heterozygosity were detected for the loci with the greatest number of alleles from 8 to 12: *M2044*—0.860, *M4215*—0.811, *M4474*—0.738, *M1287*—0.730, and *M3444*—0.676.

Structural analyses did not reveal a clear genetic structure between the four populations of *L. brachycephalus* (Figure 2, clusters 1–4), which were separated from sampling site 5, represented by *L. conocephalus* (Figure 2). On detecting the population structure for the analyzed barbels from different sampling points in the Aral–Syrdarya Basin, bar plots showing the division of runs by mode for the optimal (K = 5) number of clusters were generated (Figure 2), although no genetic structure was found. Only two clusters of both

species were separated: one with populations of the species *L. conocephalus* (sampling point 5) and the other with populations of *L. brachycephalus* (sampling points 1–4). However, some individuals belonging to *L. conocephalus* of the 2 and 4 at the sampling points collapsed into differentiated clusters (Figure 2).

Table 4. Indices of genetic diversity per locus.

Locus	N_a	N_e	H_o	H_s	H_t	H'_t	G_{is}
M1287	11.000	2.946	0.827	0.699	0.730	0.738	−0.184
M1417	3.000	2.640	0.879	0.650	0.646	0.645	−0.353
M1182	5.000	2.678	0.821	0.660	0.695	0.704	−0.243
M2237	5.000	1.409	0.186	0.320	0.321	0.321	0.420
M2108	3.000	1.057	0.037	0.057	0.055	0.055	0.341
M2044	12.000	5.093	0.911	0.842	0.860	0.864	−0.082
M2164	3.000	1.062	0.041	0.064	0.061	0.061	0.357
M2306	2.000	1.584	0.242	0.390	0.476	0.497	0.380
M3230	2.000	1.996	0.654	0.514	0.503	0.500	−0.273
M3264	6.000	2.403	0.758	0.602	0.591	0.588	−0.259
M3444	8.000	2.885	0.824	0.675	0.676	0.676	−0.221
M4211	4.000	1.514	0.255	0.357	0.374	0.378	0.288
M4215	9.000	3.030	0.237	0.721	0.811	0.833	0.672
M4138	5.000	2.145	0.715	0.550	0.542	0.540	−0.300
M4474	12.000	2.430	0.490	0.618	0.738	0.768	0.207
Mean	6.000	2.325	0.525	0.515	0.539	0.545	−0.020

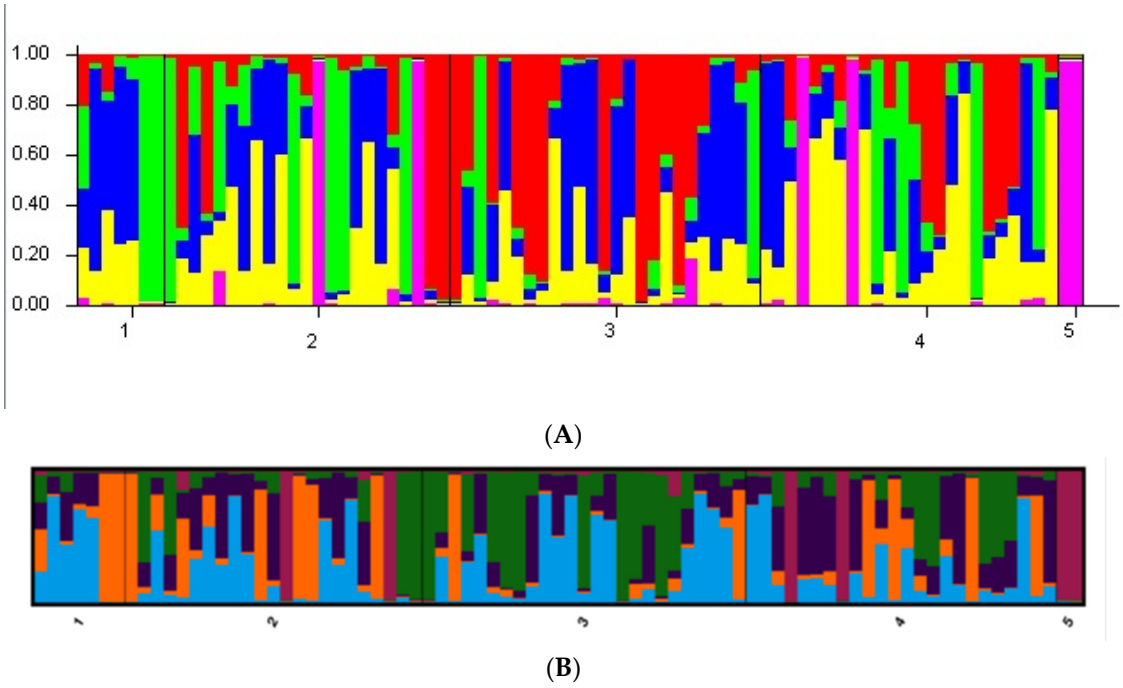
N_a: number of alleles; N_e: effective number of alleles; H_o: observed heterozygosity; H_s: heterozygosity within populations; H_t: total heterozygosity: H'_t; corrected total heterozygosity; G_{is}: inbreeding coefficient.

However, we assume that four specimens from sample points 2 and 4 (Kyzylorda region and Rice Check) are indeed *L. conocephalus* and not the result of introgression. Apparently, these four specimens were misidentified in the field. It is also worth noting that four specimens were caught in these places, although *L. brachycephalus* is traditionally found there.

Genetic introgression, also known as introgressive hybridization, is the transfer of genetic material from one species into the gene pool of another by repeated backcrossing of the interspecific hybrid to one of its parent species. Genetic introgression from native species is recognized as a detrimental impact resulting from biological invasions involving taxonomically similar invaders. The ecological consequences of genetic introgression from an invasive congener were tested using the endemic barbel populations from central Italy, where the invader was the European barbel *Barbus barbus*. The results indicate that the genetic introgression of an invasive congener from native species can result in substantial ecological consequences, including the potential for cascading effects [50].

However, our genetic analysis provided support for a low level of introgression of *L. conocephalus* into *L. brachycephalus* in the Bayesian cluster where *L. brachycephalus* was only present (one sampling point in Bairykum village), but not vice versa, indicating limited genetic exchange between the two species.

Bayesian structure analysis with microsatellites revealed differences between individuals of the two species; however, no evidence of intraspecific genetic structure was found within *L. brachycephalus*.



(**A**)

(**B**)

Figure 2. Bayesian cluster analysis based on STRUCTURE (**A**) and results analysis aligned in CLUMPAK (**B**). The colors represent the mean proportion of membership (Q) to each of the inferred groups K = 5. 1: Bairykum village (*L. brachycephalus*); 2: Kyzylorda region (*L. brachycephalus; L. conocephalus*); 3: Basykara Dam (*L. brachycephalus*); 4: Rice Check (*L. brachycephalus; L. conocephalus*); 5: Badam River (*L. conocephalus*).

The studied species exhibit significant morphological similarities, making it rather challenging to differentiate between them. In cases where their geographical ranges overlap, their separation primarily relies on ecological factors related to habitat and lifestyle (including migratory and resident forms). Our nuclear study effectively distinguished between these two species without any ambiguities. It is worth noting that previous research [17] suggested the possibility of interbreeding between them.

These microsatellite loci can also be used for intrageneric genetic identification, according to the results obtained, since individuals of *L. conocephalus* show clear differentiation when divided into clusters. For this purpose, we also noted private alleles for *L. conocephalus*, such as *M1447*, *M0244*, and *M4215* as well as *M2237*, which is only one locus that is not valid for individuals of this species.

4. Discussion

In this article, we present the first results of a study with nuclear microsatellite markers for two *Luciobarbus* species inhabiting the Aral Basin. The nuclear markers analyzed in this study represent the first microsatellites isolated from *Luciobarbus* and increase the available molecular resources for these tetraploid species, which makes it difficult to study the nuclear genome. These microsatellites have been suggested to be informative for the analysis of the genetic diversity and structure of two *Luciobarbus* species inhabiting the Aral Basin and they will provide significant insights into species genetic diversity and population structure in other species of the allotetraploid genus *Luciobarbus*, assisting in conservation and other relevant studies for its more vulnerable species.

Most of the habitats of rare and endangered barbels in the Aral Basin in Kazakhstan were surveyed, and the sampling points covered were Bairykum village, the Kyzylorda region, the Basykara Dam, Rice Check, and the Badam River. The rarity of the species *L. conocephalus* compared with *L. brachycephalus* has attracted attention because this population has a very limited distribution area in the geographical context of the Aral–Syrdarya Basin

within Kazakhstan. In addition to the drying of the Aral Sea and the erosion that this part of the basin has suffered, conservation measures should be implemented to prevent future actions that jeopardize the conservation of these two *Luciobarbus* species.

Of the 17 analyzed loci, 15 were polymorphic for all individuals; microsatellites were used to study the genetic structure and diversity of both *L. brachycephalus* and *L. conocephalus*. The number of alleles per locus is estimated to range from 2 to 12, namely *M2044* and *M4474*-12, *M1287*-11, *M4215*-9, and *M3444*-8, with an average value of 6, in comparison with the number of alleles that fluctuate from 2 to 7 in other species [51–54]. Additionally, at two points where *L. brachycephalus* is usually recorded, four individuals of *L. conocephalus* were also found, which was confirmed by our genetic results.

The contribution of this study, with fifteen newly developed microsatellites for *Luciobarbus*, is highly relevant given the precarious conservation status of the two species analyzed, especially *L. conocephalus*. The use of these microsatellites will allow us to gain a better understanding of the evolutionary history of the species within this genus and support the conservation of these rare and endangered species.

In this article, we embarked on a pioneering endeavor by isolating and employing microsatellite markers to unravel the enigmatic genetic landscape of the *Luciobarbus* genus within the confines of the Aral Basin. This landmark achievement significantly augments the molecular toolkit available for the study of *Luciobarbus,* a genus that has long confounded researchers due to its tetraploid nature. As such, the legacy of this research is poised to cast a long-lasting impact on the realms of ichthyology, genetics, and conservation biology.

5. Conclusions

In the conservation of biodiversity, the assessment of genetic diversity plays an important role. The conservation of a system of many genetically diverse local populations and their structure is necessary for the long-term survival of species and the functioning of ecosystems.

Therefore, the main goal of this study was to obtain genetic information about two native species of the genus *Luciobarbus* living in the Aral system: *L. conocephalus* (Kessler, 1872) and *L. brachycephalus* (Kessler, 1872). As a result of this research, nuclear markers for evaluating the genetic structure and diversity were successfully developed and applied. These microsatellite loci can also be used for intrageneric genetic identification. It should be noted that based on the level of genetic diversity, small, isolated populations are distinguished that show an average level of genetic diversity.

Despite the rare and endangered status of the barbels of the Aral Basin, in-depth studies to identify their population structure and assess their genetic diversity have not been carried out before this work. With the help of molecular genetic methods, it has been possible to largely solve this problem and make a significant contribution to the study of the genetic diversity of barbels, which has been unclear for more than 30 years. The results obtained from this study can be used to develop effective, science-based plans for the conservation of barbels and can be used by environmental, fishery, and other organizations whose activities are aimed at the protection, reproduction, and rational use of fish resources.

Reduced genetic diversity reduces the ability of a population to adapt to future environmental changes, such as biotic and abiotic changes, reducing the plasticity of population genomes. Further research is needed because these species, like many others in the Aral system, face an imminent threat of extinction due to changes in the drying water system. This emphasizes the need for continued investigation into these complexities and their implications.

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