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ARTICLE

Population genetic analysis of Carassius species in Mongolia based on mitochondrial and microsatellite markers

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Abstract: This study investigates the taxonomy and population genetic structure of the *Carassius* species distributed in Mongolia using mitochondrial cytochrome b (*Cytb*) and control region (*D-loop*) sequences, complemented by nuclear microsatellite markers. Specimens were collected from lake and rivers belonging to both the Arctic Ocean and Pacific Ocean basins of Mongolia. Phylogenetic analyses based on mitochondrial markers identified two distinct genetic lineages corresponding to *Carassius auratus* and *Carassius gibelio*. The phylogenetic tree strongly supported this division, with a high haplotype diversity (Hd = 0.938) indicating substantial haplotype diversity and a clear separation of the Onon river population (*C.auratus*) lineage from the other populations. Microsatellite markers also displayed high polymorphism, but showed only weak nuclear structure (PERMANOVA $R^2 = 0.031$), indicating substantial gene flow and polyploidy admixture that obscure the deep maternal divergence captured by mtDNA. Geographically, *C. gibelio* population was predominantly distributed in the Selenge river tributaries, Buir lake, and the Kherlen river, whereas *C. auratus* was mainly found in the Onon and Bulgan rivers. Overall, this integrative approach combining mitochondrial and nuclear markers provides the first comprehensive assessment of *Carassius* genetic diversity and distribution in Mongolia. The results contribute essential insights into the taxonomy, evolutionary divergence, and biogeographical history of these freshwater fish, offering a valuable foundation for their future conservation and management.

Keywords: *Carassius auratus*, *Carassius gibelio*, mitochondrial DNA, D-loop, Cytb, microsatellite markers, genetic diversity, freshwater fish, evolutionary divergence;

INTRODUCTION

The genus *Carassius* (Cyprinidae, Rafinesque, 1815) comprises of several morphologically and genetically similar freshwater fish species widely distributed across Eurasia. Among them, *Carassius auratus* (the crucian carp) and *Carassius gibelio* (the Prussian carp) are of particular ecological and economic importance due to their broad distribution, reproductive plasticity, and adaptability [1].

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Species of the genus *Carassius* exhibit remarkable reproductive flexibility, capable of both sexual and gynogenetic reproduction [2]. Gynogenesis, a form of asexual reproduction in which sperm activates egg development without male genetic contribution, is widespread in *C. gibelio* and contributes to its rapid colonization and genetic complexity [3]. Populations may include diploid, triploid, and tetraploid individuals, with triploids often reproducing clonally, while maintaining high heterozygosity, thereby enhancing ecological adaptability and reproductive success [4].

These species also display morphological plasticity, adapting readily to environmental variations, such as temperature, oxygen concentration, and food availability, which has facilitated their wide distribution across Eurasia, including diverse river and lake systems in Mongolia [1]. Hybridization and introgression between *C. auratus* and *C. gibelio* are common, often blurring species boundaries and complicating taxonomic identification [1].

Previous studies on Mongolian *Carassius* were limited mainly to morphological descriptions of local populations [5]. Later genetic analyses by Kalous et al. (2012) [6] revealed that Mongolian specimens fall into two distinct mitochondrial lineages, indicating hidden diversity within what was historically treated as *C. gibelio*. One of these lineages was reported as originating from “Uvs Lake,” but these records appear mislabelled, as *Carassius gibelio* has not been confirmed from Uvs Lake. The findings highlight long-standing taxonomic uncertainties in Mongolian *Carassius* noted by Kottelat (2006) [7] and suggest that western Mongolian populations may represent an undescribed lineage.

Molecular genetic tools-including mitochondrial DNA (mtDNA) markers, such as cytochrome b (Cytb) and the control region (D-loop), and nuclear microsatellite markers-have proven effective for resolving taxonomic ambiguities, revealing population structure, and assessing genetic diversity in freshwater fish [8]. The D-loop region evolves rapidly and is therefore useful for detecting recent divergence, whereas Cytb provides robust phylogenetic signal across deeper lineages. Microsatellite loci complement mtDNA analyses by enabling detection of fine-scale gene flow and population differentiation. Despite their ecological significance, the genetic diversity and population structure of *Carassius* species in Mongolia remain poorly studied and understood. Studies in neighboring regions have revealed distinct mitochondrial lineages and considerable intraspecific variation, often shaped by both sexual and asexual reproduction [3]. However, no comprehensive assessment combining both mitochondrial and nuclear markers has yet been conducted across the lakes and rivers of Mongolia.

This study aims to (1) clarify the taxonomic status of *Carassius* species in the Arctic Ocean (Selenge and Bulgan rivers) and Pacific Ocean (Buir lake, Kherlen and Onon rivers) basins of Mongolia, (2) investigate their population genetic structure using mtDNA (Cytb and D-loop) and microsatellite markers, and (3) explore their spatial distribution patterns across major river basins. By integrating mitochondrial and nuclear genetic data, we provide the first detailed assessment of *Carassius* genetic diversity in Mongolia, contributing valuable insights for conservation, management, and evolutionary studies of these freshwater fish.

MATERIALS AND METHODS

Study Area and Sampling

Samples for population genetic analysis were collected between 2022 and 2023 from Selenge and Bulgan rivers in the Arctic Ocean basin and Buir lake, Kherlen and Onon rivers along the Pacific Ocean basin, totaling 5 locations. Fish specimens were captured using nets sized ranging from

20×20 cm to 45×45 cm, which were set in stable tributaries of rivers and at depths of 1–3 meters in lakes.

In the mitochondrial analysis, DNA sequences from both highly variable and conserved regions were examined to assess genetic diversity. Of the 70 samples collected across seven locations, 45 met the required quality standards and were included in the final dataset.

For the microsatellite analysis, all 70 samples were successfully genotyped, as PCR amplification proceeded without any issue.

DNA Extraction

Each sample was carefully handled using disposable gloves and forceps, then placed into 1.5 ml plastic tubes containing 99.9% ethanol. The tubes were labeled with precise geographic coordinates and sample IDs, and stored at −20°C until DNA extraction.

Genomic DNA was extracted from tissue samples using the Zanaaspex DNA extraction kit, following the manufacturer’s protocol. The quality and integrity of extracted DNA were assessed by running samples on a 1% agarose gel via electrophoresis. DNA concentration was measured using a Mapada spectrophotometer.

Amplification of mtDNA D-loop Region

The mitochondrial DNA (mtDNA) regions Cytb and the D-loop were amplified using the polymerase chain reaction (PCR). To assess intraspecific genetic diversity, the D-loop region was targeted using the primers L15923 (5′-TTAAAGCATCGGTCTTGTAA-3′) and H16150 (5′-GCCCTGAAATAGGAACCAGA-3′), as described by[4] and[9]. For the Cytb region, the primers FishCytB F (5′-CCACCGTTGTTATTCAACTACAAGAAC-3′) and THR-Fish R (5′-ACCTCCGATCTTCGGATTACAAGACC-3′) were used, following [10].

PCR reactions were performed in a total volume of 25 µl, containing 1× PCR buffer, 200 µM dNTPs, 0.5 µM of each primer, 0.5 µl of template DNA, and 1.5 units of DreamTaq DNA polymerase. Thermal cycling was conducted on a BIOER Thermal Cycler under the following conditions: initial denaturation at 94°C for 4 minutes; followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds; and a final extension at 72°C for 5 minutes.

The amplified PCR products were verified by electrophoresis on a 1.5% agarose gel. Successfully amplified fragments were sent to Zanaaspex Co. for bidirectional sequencing using both forward and reverse primers.

Phylogenetic Tree Construction

Mitochondrial DNA (mtDNA) sequences of crucian carp (*Carassius spp.*), including 972 base pairs (bp) of the CytB gene and 316 bp of the D-loop region-totaling 1,288 bp-were processed using SeqMan 1.6 to assemble forward and reverse raw sequencing data. The consensus sequences were aligned and compared using MEGA [11]. The aligned concatenated sequences were further analyzed using the phylogenetic Chain Monte Carlo (MCMC) method [12] implemented in BEAUti & BEAST v1.6.1 to infer the phylogenetic relationships. The resulting phylogenetic tree was visualized, using FIGTREE v1.4.1.

Microsatellite Analysis Methodology

A comparative study was conducted on the molecular genetics and morpho-biological characteristics of *Carassius* populations distributed in the lakes and rivers of Mongolia.

Microsatellite loci were selected to assess genetic diversity in *Carassius* populations from the Onon river (n = 20), Buir lake (n = 19), Selenge river (n = 21), Bulgan river (n = 6), and Kherlen river (n = 4). The six loci were chosen based on Guo & Gui (2008), which validated these markers for *Carassius gibelio* strain identification. These loci consistently amplify across *Carassius* taxa, show high polymorphism, and are widely used in studies involving mixed-ploidy lineages. Fluorescently labeled primers (**Table 1**) were employed for PCR amplification of target loci.

Table 1. Information on Primers used in Microsatellite analysis.

№	Name (Fluorescent Dye)	Primer Sequence	Allele Size (bp)	Annealing Temperature (°C)
1	YJ0008 (HEX)	CCCTTTGGTTTTTGCGCGA GTGGAGGTGATTTTTTGGGG	228	53
2	YJ0009 (FAM)	GCGTCACAGTCCATCCAT AGCTCACAGCACAACCATC	182	53
3	YJ0020 (TAMRA)	CGAATCGATGTCAACCAATG TCCACGAGTCTCAGGCAGC	140-170	55-50
4	YJ0010 (FAM)	GATGGTTGTGCTGTGAGCT GAGTTCGTTTACATCTGGAC	150-202	53
5	YJ0002 (HEX)	CAAAAGCATGACAGAGTTAC TGAAGTTATTAGAAAGAGAG	82-106	55-50
6	YJ0033 (TAMRA)	CGGACACAAGAACGCCAAC GGACTGGGCTGAAACTGATG	172-190	55-50

PCR Conditions

PCR amplification was performed using the 2× Master Mix Kit from Choros-Onosh Company. Each 25 µl reaction mixture contained 2× Master Mix, 1 µM of each forward and reverse primer, and 50–200 ng of template DNA. PCR cycling conditions followed the manufacturer's protocol: initial denaturation at 94°C for 4 minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing for 30 seconds at the appropriate temperature, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 10 minutes.

For primers, a touchdown PCR protocol was applied. The annealing temperature was gradually decreased from 55°C to 50°C over the first 10 cycles (by 0.5°C per cycle), and maintained at 50°C for the remaining 20 cycles.

Fragment Analysis

The PCR products were sent to Zanaaspex Co. for fragment length analysis. Microsatellite allele sizes were determined using GeneMarker v3.0.1 software [14], calibrated with internal size standards based on fluorescence peak profiles.

Microsatellite genotype data were analyzed in R using the packages: polysat [15], poppr [16], adegenet [17], pegas [18] to calculate genetic diversity indices, including the number of observed alleles per locus. Ploidy inference and allele-copy ambiguity were resolved using the polysat, package which implements Bruvo's distance and accommodates uncertain allele dosage without forcing diploid assumptions. This approach is widely recommended for mixed-ploidy systems, as it avoids artificial inflation of genetic diversity metrics and allows unbiased estimation of heterozygosity, distance matrices, and AMOVA components. Genetic differentiation among populations was assessed using pairwise F_{st}, G_{st}, R_{st}, and Jost's D, as well as AMOVA and PERMANOVA tests based on Bruvo's distance [19], [20]. Population structure and spatial relationships were visualized through Principal Coordinate Analysis

(PCoA) and genetic network plots implemented in R.

RESULTS AND DISCUSSION

Mitochondrial DNA (mtDNA) Analysis Results

A phylogenetic tree was constructed using mitochondrial d-loop and cytochrome b (Cytb) gene sequences to evaluate relationships among populations of *Carassius*. For comparison, some reference sequences of crucian carp species - *Carassius carassius*, *Carassius gibelio*, and *Carassius auratus* (Dashdorj, 1964) - were retrieved from the NCBI database. Haplotype analysis was conducted based on Cytb gene sequences.

The ***Carassius*** populations from the Onon and Bulgan rivers (*Carassius auratus* – goldfish) are grouped into one clade (shown in light green), while the populations from Buir lake, Kherlen, Selenge, and Orkhon rivers (*Carassius gibelio* – Prussian carp) form a separate clade (shown in green).

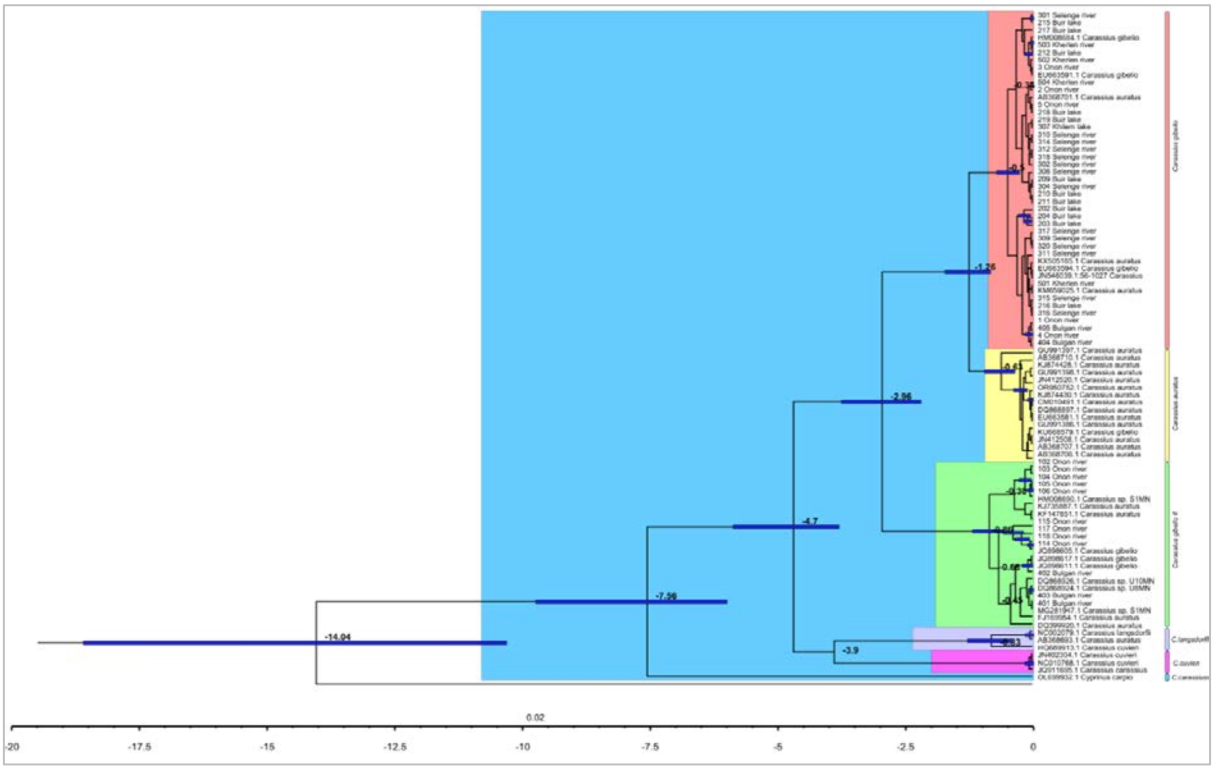

Figure 1. The phylogenetic tree.

The phylogenetic tree shows that no samples clustered with *Carassius carassius* (Crucian carp), suggesting that this species was not present in the sample collection (**Figure 1**). Instead, samples from the Selenge, Orkhon, Kherlen rivers, and Buir lake clustered with known *Carassius gibelio* sequences from the NCBI database (accession numbers HM008684.1, KX505166.1). Meanwhile, samples from the Onon and Bulgan rivers clustered with *Carassius auratus* sequences (accession numbers KF147851.1, DQ868924.1, KJ735887.1). This suggests that *Carassius* population in the Bulgan river may share a genetic lineage with those in the Onon river, likely indicating a relationship with goldfish (*Carassius auratus*).

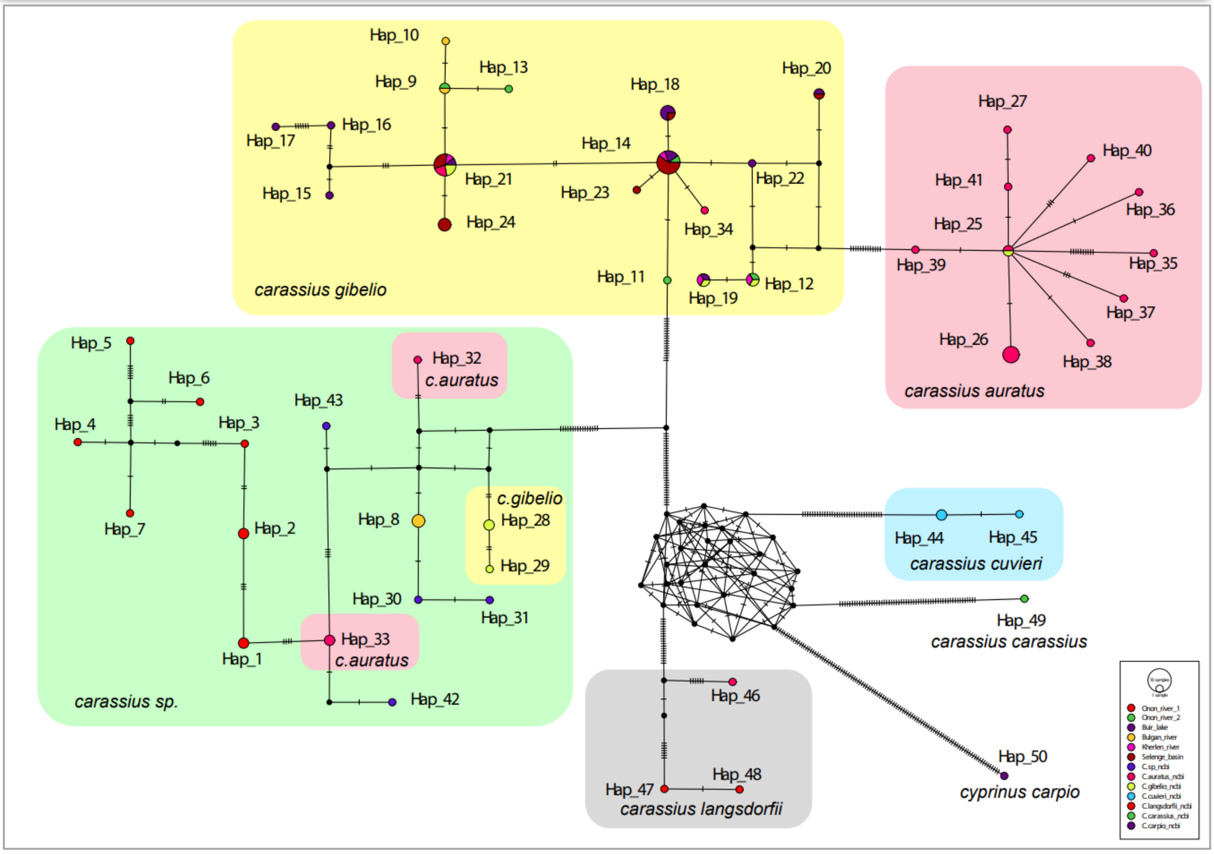

Figure 2. Haplotype network based on Cytb *gene sequences.*

Haplotype network of mitochondrial cytochrome b (Cytb) sequences of *Carassius* species and related taxa. Each circle represents a unique haplotype (Hap_1 to Hap_50), with circle size proportional to the number of individuals sharing that haplotype. Colors within each circle indicate sampling locality, as defined in the legend. Black lines represent mutational steps between haplotypes, and small black dots indicate hypothetical intermediate haplotypes. Colored boxes group haplotypes assigned to specific taxonomic units: *C. gibelio* (yellow), *C. auratus* (red), *Carassius* sp. (green), *C. cuvieri* (blue), *C. carassius* (purple), *C. langsdorfii* (gray), and *Cyprinus carpio* (white).

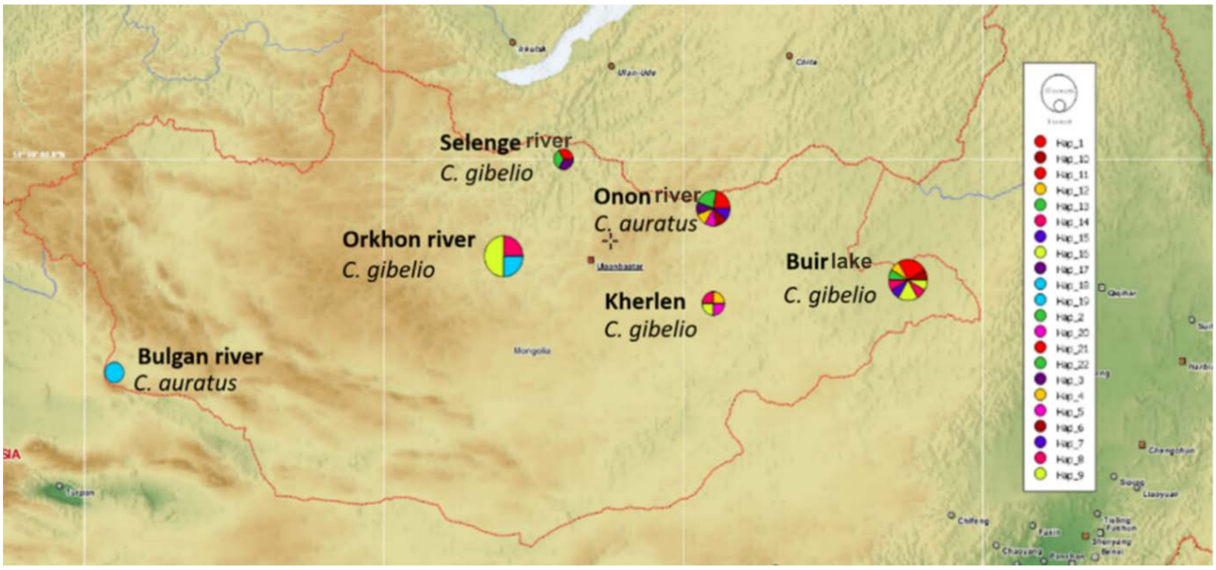

Figure 3. Haplotype distribution map based on Cytb *gene sequences.*

The haplotype distribution map provides a simple representation of the genetic diversity within populations. For *Carassius gibelio* (Prussian carp), Buir lake population exhibited the highest number of haplotypes, while the Orkhon and Selenge populations had relatively fewer haplotypes. For *Carassius auratus* (goldfish), the Onon river population showed relatively high genetic diversity with seven haplotypes detected, whereas the Bulgan river population had only one haplotype, indicating low genetic diversity.

This table presents genetic differentiation parameters calculated using 972 nucleotide positions of the *Cytb* gene for two populations, *C. auratus* and *C. gibelio*.

Table 2. Genetic differentiation based on Cytb and D-loop genes.

Parameter	***C. auratus***	***C. gibelio***	**Total**
Number of sequences	15	33	48
Sequence length (bp)	972	972	972
Number of polymorphic sites (S)	25	20	64
Average number of nucleotide differences (k)	9.543	3.735	21.844
Nucleotide diversity (Pi)	0.01	0.004	0.022
Number of haplotypes (h)	10	12	22
Haplotype diversity (Hd)	0.943	0.879	0.938
Average nucleotide difference between populations			43.77
Average nucleotide substitution rate (Dxy)			0.045
Net nucleotide difference (Da)			0.038

A total of 20 haplotypes were identified, divided into two haplogroups, with a haplotype diversity of 0.938, indicating relatively high genetic diversity. Additionally, the average nucleotide divergence between populations was 43.77, which reflects a relatively large genetic difference between *C. auratus* and *C. gibelio* species. This divergence suggests that these two species are genetically distinct and have undergone independent evolutionary development over a long period of time.

Microsatellite Results

Microsatellite loci was amplified successfully for all five *Carassius* populations. Depending on the genomic position of each marker and the karyotype of the individuals, one to three alleles per locus were detected. To minimize allele-calling errors, particularly stutter bands and shadow peaks common in polyploid microsatellite datasets, fragment analysis relied on fluorescence-based size calling with internal size standards. Additionally, locus YJ0008, which consistently produced a single peak corresponding to the mitochondrial control region, was used as a reference to detect and exclude ambiguous peak patterns. This safeguards against overestimating allelic dosage.

Table 3. Summary of allelic counts per locus and population.

	YJ0009	YJ0020	YJ0010	YJ0002	YJ0033
Onon river	2	10	14	6	4
Buir lake	2	19	15	1	7
Selenge river	2	13	16	6	5
Bulgan river	2	5	6	3	3
Kherlen river	2	8	7	4	3
Overall	2	22	21	11	7

Nuclear microsatellite genotyping revealed the coexistence of both diploid and triploid individuals across all five populations (Figure 4). Triploids were present at every site, with the highest frequency observed in Kherlen river population (≈56%) and the lowest in Bulgan river population (≈33%). Statistical analyses, including a binomial logistic regression (ΔDeviance = 2.13, df = 4, p = 0.71, AIC = 102.8) and Fisher's Exact Test (p = 0.745) indicated no significant differences among populations, suggesting that cytotype distribution is largely uniform across Mongolia.

In the logistic regression, the intercept represented the reference population, Buir lake population (log-odds = −0.606, p = 0.23), corresponding to an estimated triploidy probability of ~35%. Relative to this, triploidy probabilities were slightly higher in Kherlen river population (56%), Onon River population (50%), and Selenge river population (46%), and lower in Bulgan river population (33%), but none of these differences were statistically significant (all p > 0.23).

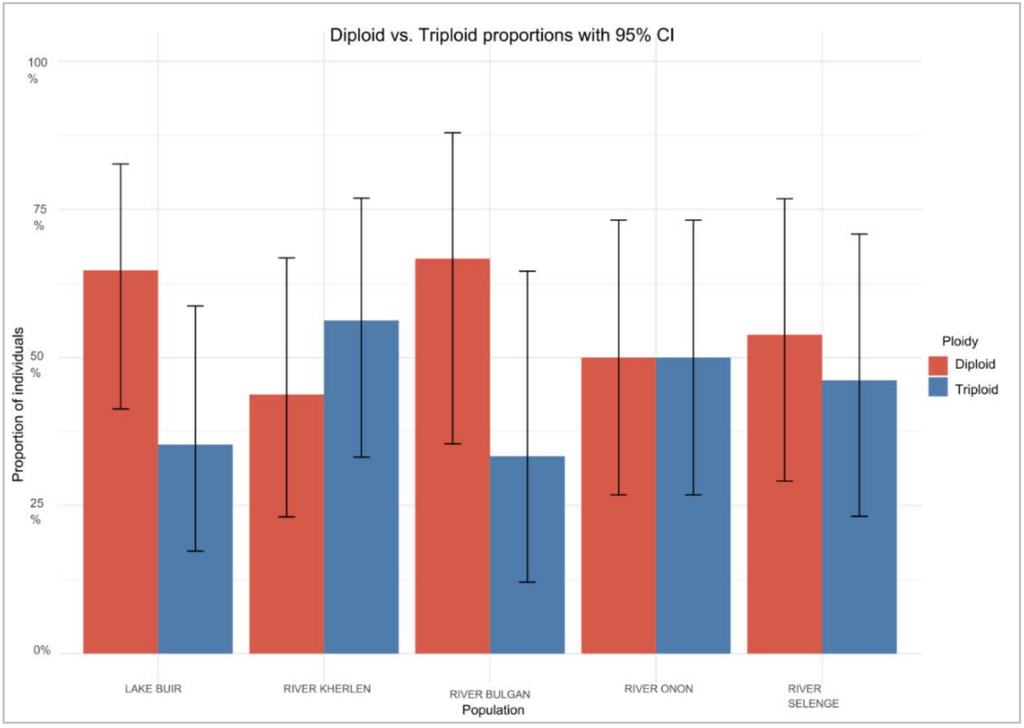

Figure 4. Proportion of diploid and triploid individuals (95% CI per population).

Genetic Diversity within Populations

Genetic diversity was estimated separately for diploid and triploid individuals using the *polysat* package in R. Observed heterozygosity (Ho) was uniformly high (Ho = 1.00) across both cytotypes, indicating that all genotyped loci were polymorphic. Expected heterozygosity (It ranged from **0.49 to 0.95** in diploids (mean ≈ 0.70) and from **0.50 to**

0.98 in triploids (mean ≈ 0.75), suggesting slightly greater allelic diversity among triploid individuals. Allelic richness (N_a) could not be directly estimated because allele dosage information was not fully resolved in triploids; however, multi-locus heterozygosity indicates comparable or higher diversity in the polyploid fraction of the populations.

Table 4. Heterozygosity values at six microsatellite loci in Carassius populations from rivers and lakes of Mongolia.

Cytotype	**Ho**	**He (range)**	**Mean He**
Diploid	1.000	0.49 – 0.95	~0.70
Triploid	1.000	0.50 – 0.98	~0.75

These findings indicate that triploid *Carassius* individuals maintain levels of genetic diversity comparable to or slightly higher than diploids, consistent with their hybrid or clonal–polyploid origin.

Genetic Diversity Among Populations

Global differentiation estimates confirmed these results: F_{ST} = 0.086, G_{ST} = 0.083, and Jost’s D = 0.343, each indicating moderate genetic structure. Bootstrap confidence intervals for pairwise GST (95% CI = 0.05–0.12) confirmed that inter-population differentiation was statistically significant for most pairs.

Parallel estimates based on G_{ST}, R_{ST}, and Jost’s D produced consistent rankings among populations, with Selenge and Kherlen rivers showing the highest inter-population distances (Table 5).

Table 5. Global and pairwise genetic differentiation metrics among populations.

Metric	**Global**	**Range (pairwise)**
F_{ST}	0.086	0.02–0.12
G_{ST}	0.083	0.01–0.09
Jost’s D	0.343	0.08–0.48
R_{ST}	0.09	0.02–0.15

F_{st} values ranged from 0.022 to 0.117, indicating low to moderate differentiation among populations (Table 6).

Table 6. Pairwise genetic differentiation (FST) among Carassius populations.

	Onon river	**Buir lake**	**Selenge river**	**Bulgan river**	**Kherlen river**
Onon river	0.000	0.033	0.081	0.056	0.043
Buir lake	0.033	0.000	0.044	0.039	0.022
Selenge river	0.081	0.044	0.000	0.051	0.051
Bulgan river	0.056	0.039	0.051	0.000	0.117
Kherlen river	0.043	0.022	0.051	0.117	0.000

The lowest pairwise FST values were observed between populations of Onon River and Buir lake (0.033) and Buir lake and Kherlen river (0.022), both below 0.05, suggesting substantial gene flow among these populations. The highest differentiation was detected between Bulgan river and Kherlen river populations (F_{ST} = 0.117), which likely reflects partial geographic isolation and reduced migration.

Table 7. Pairwise genetic differentiation (Jost's D) among Carassius populations.

	Onon river	**Buir lake**	**Selenge river**	**Bulgan river**	**Kherlen river**
Onon river	0.000	0.231	0.395	0.420	0.400
Buir lake	0.231	0.000	0.192	0.354	0.078
Selenge river	0.395	0.192	0.000	0.439	0.300
Bulgan river	0.420	0.354	0.439	0.000	0.479
Kherlen river	0.400	0.078	0.300	0.479	0.000

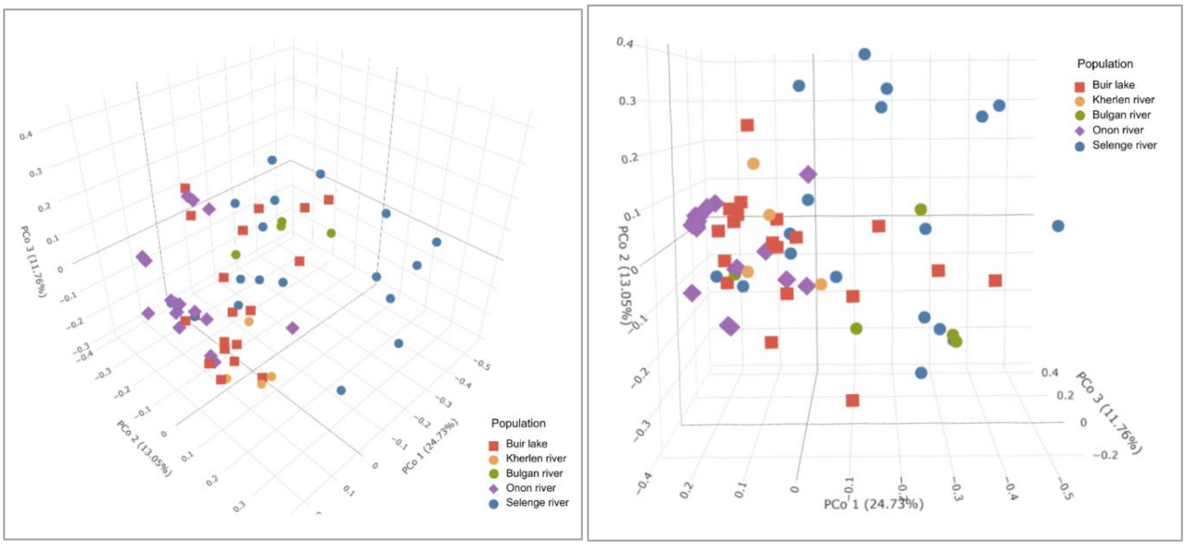

Figure 5. PCoA plot shows weak clustering of populations (based on Bruvo's distance). Red rectangle representing Buir lake population, yellow circle representing Kherlen river population, green circle representing Bulgan river population, Purple Diamond representing Onon river population, Blue Circle representing Selenge river population.

Population Structure and Interpretation

The PCoA (Figure 5) shows a weak clustering of the five sampling sites. The Bulgan ($n = 6$) and Kherlen ($n = 4$) populations appear isolated, but their small sample sizes limit confidence in these positions. Pairwise FST (0.022–0.117) and D values indicate moderate differentiation, consistent with recent divergence rather than deep phylogenetic separation.

Because Bulgan and Kherlen are represented by ≤ 6 individuals, the observed separation may reflect sampling variance; future work should increase sampling in these basins.

The low overall ΦST values obtained from AMOVA ($\Phi ST \approx 0.03$; $p > 0.05$; 10,000 permutations), performed with individuals nested within populations, indicate that the majority of genetic variation (~97%) occurs within populations rather than among populations. Variance components were 0.97 within populations and 0.03 among populations. Bruvo's distance was used to account for polyploid genotypes. PERMANOVA results corroborated this pattern ($R^2 = 0.031$, $F = 0.50$, $p = 0.75$), further supporting weak genetic differentiation across the Mongolian *Carassius* range.

Nuclear genetic structure and population clustering

Analysis of the microsatellite genotypes revealed distinct but partially overlapping genetic clusters among the *Carassius* populations. ADMIXTURE analysis, supported by the Evanno ΔK method [21], indicated that the optimal number of clusters was K=4 (**Figure 6**). Individuals largely grouped according to their sampling localities, but considerable admixture was observed among clusters. Each vertical bar represents one individual, and the proportion of colors corresponds to its membership coefficient in each cluster.

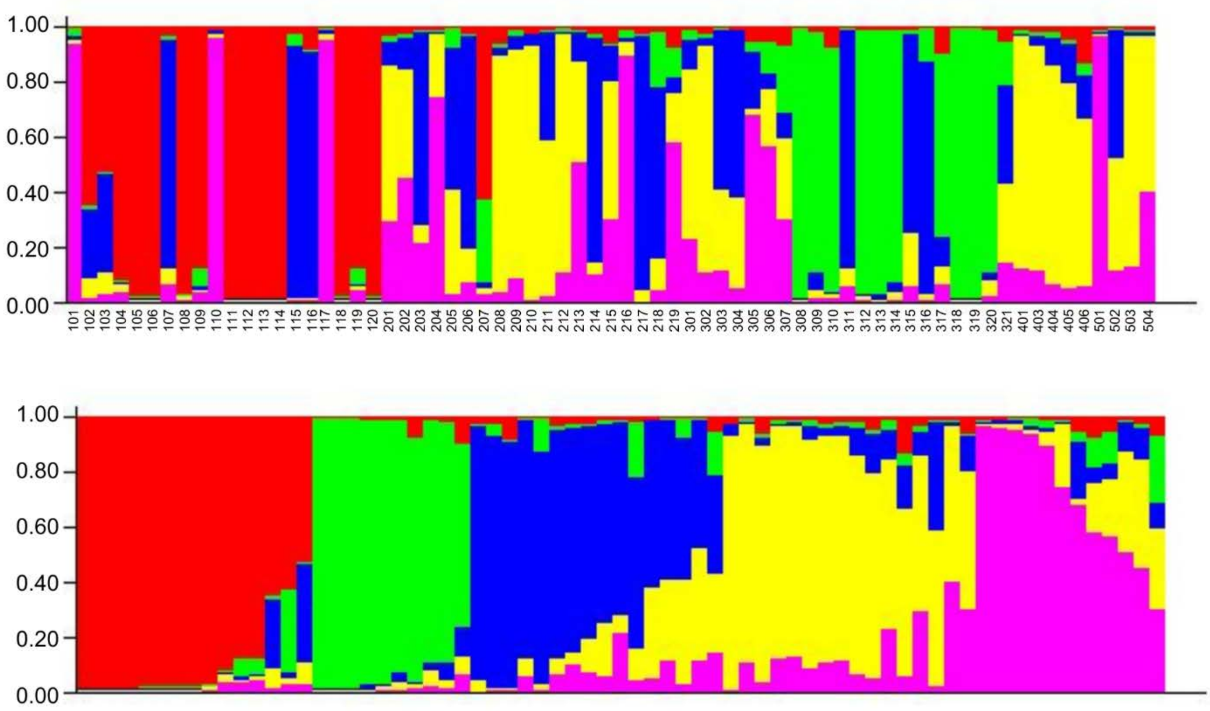

Figure 6. Population structure inferred from microsatellite data using the ADMIXTURE algorithm (K = 4) in Mongolian populations of Carassius.

The first cluster (red) dominated the Onon river population, while the second cluster (green) was prevalent in Bulgan river individuals. The third (blue) and fourth (yellow) clusters were most frequent in Buir lake, Kherlen river, and Selenge river populations, with some individuals showing mixed ancestry. This pattern indicates the presence of at least two major nuclear lineages and evidence of historical or ongoing gene flow among populations, consistent with hybridization or introgressive processes characteristic of *C. gibelio*.

Overall, the nuclear genetic data indicate moderate differentiation (global FST ≈ 0.086) with four genetic subgroups that partially correspond to geographic regions. The widespread admixture signals, together with the coexistence of diploid and triploid individuals, suggest complex reproductive dynamics involving both sexual and clonal lineages.

Molecular Genetic Characteristics

A total of 12 haplotypes were identified across both species. *C. gibelio* exhibited lower haplotype diversity (Hd = 0.533) compared to *C. carassius* (Hd = 0.733). Nucleotide diversity was also lower in *C. gibelio*. Pairwise F_ST values ranged from 0.418 to 0.672, indicating significant genetic differentiation. The phylogenetic tree revealed two well-supported clades corresponding to species, confirming molecular separation. Our net Cytb divergence (Da = 0.038; Dxy = 0.045) falls within published interspecific ranges for Cyprinidae (2–3%; [8]) and specifically between *C. auratus* and *C. gibelio* (3.5–5%[9]), supporting the interpretation that the two lineages present in Mongolia represent well-established maternal species.

The combined mitochondrial and nuclear data provide a coherent view of the genetic composition and structure of *Carassius* populations in Mongolia. Mitochondrial markers (Cytb and D-loop) clearly separated into two maternal lineages corresponding to *C. auratus* (Onon River and Bulgan river populations) and *C. gibelio (*Buir lake, Kherlen river, Selenge river and, Orkhon river populations). The relatively high haplotype diversity and substantial sequence divergence between these lineages indicate long-term historical isolation and independent evolutionary trajectories.

The absence of *C. carassius* haplotypes and the strong clustering of Onon River population and Bulgan river population sequences with *C. auratus* reference material confirm that only *C. auratus* and *C. gibelio* are represented in the current Mongolian populations. The reproductive biology of these taxa likely contributes to their observed genetic patterns: *C. gibelio* commonly exhibits gynogenetic reproduction, enabling clonal propagation and rapid range expansion [3]. Furthermore, the coexistence of diploid and triploid individuals across all sites is consistent with the polyploidy known from other Eurasian populations [4].

While mtDNA indicated strong maternal structuring, the nuclear microsatellite markers revealed only weak differentiation among population (PERMANOVA R^2 = 0.031). In the PCoA, individuals from Onon were widely scattered rather than forming a distinct cluster and Buir lake appeared more separated than Onon. This apparent discrepancy between marker types is expected due to their different inheritance modes. mtDNA reflects historical maternal lineages and clearly distinguishes *C.auratus* from *C.gibelio*, whereas microsatellites, which are biparentally inherited, are sensitive to recent admixture, triploidy, and gene flow. These processes homogenize nuclear variation and therefore mask the species-level divergence that is captured by mtDNA.

The nuclear microsatellite and ADMIXTURE analyses revealed moderate differentiation among populations (global FST ≈ 0.086) with four partially overlapping clusters (K = 4). This widespread admixture, especially among Buir lake, Kherlen river, and Selenge river populations, may reflect ongoing or historical hybridization, a phenomenon frequently observed between *C. auratus* and *C. gibelio* in other regions [1]. Such hybridization and introgression processes contribute to the blurring of species boundaries and enhance genetic diversity within populations.

Morphological and ecological adaptability also appears to play an important role in maintaining population connectivity. Both *C. auratus* and *C. gibelio* exhibit broad environmental tolerance and morphological plasticity, allowing them to thrive under varying environmental conditions [1]. *C. gibelio* in particular is recognized as an ecologically opportunistic species that can inhabit a wide range of freshwater ecosystems, from lowland lakes to river tributaries [22]. However, its clonal reproduction and invasiveness may pose a threat to native species through hybridization and competitive exclusion.

The presence of both diploid and triploid individuals in all sampling sites, with triploids comprising roughly one-third to one-half of each population, supports a mixed reproductive system combining sexual and clonal modes. Triploids maintained high heterozygosity, consistent with the gynogenetic reproductive mode of *C. gibelio*. The coexistence of multiple ploidy levels and partially admixed nuclear clusters suggests that hybridization and clonal propagation have shaped the genetic landscape more strongly than geographic isolation.

Although the number of microsatellite loci was limited, and allele dosage uncertainty may affect estimates in triploids, the congruence among mtDNA, ADMIXTURE, PCA, and clustering results strengthens the inference of two main maternal species with complex nuclear relationships. Future genome-wide studies will be required to disentangle the origins of triploids and quantify the extent of hybridization between *C. auratus* and *C. gibelio* lineages in Mongolia.

CONCLUSIONS

Mitochondrial and nuclear markers jointly demonstrate that Mongolian populations consist of two maternal species - *C. auratus* and *C. gibelio* - that are genetically distinct at the mitochondrial level but interconnected at the nuclear level. The moderate nuclear differentiation and mixed ancestry patterns indicate past or ongoing hybridization and gene flow, likely facilitated by the species' capacity for clonal and sexual reproduction.

The detection of both diploid and triploid individuals across all populations confirms that polyploidy is a stable and widespread feature within Mongolian *C. gibelio*.

Overall, the data point to a system shaped by historical divergence, secondary contact, and complex reproductive dynamics rather than strict isolation. Continued genomic monitoring and expanded sampling are essential to clarify the evolutionary relationships and assess the ecological consequences of clonal expansion in these populations.

Acknowledgements

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Ethical approval

There are no ethical issues associated with the publication of this article.

Author contribution

The authors confirm their contributions to the paper as follows:TD: Study conception and design, sample collection, mitochondrial DNA (mtDNA) analysis, writing, and review. AC: Study conception and design, investigation, review and editing, and supervision. GG: Investigation, sample collection. TA: Sample collection, writing, review, and editing. TN: Sample collection. BM: Statistical analysis, microsatellite analysis, writing, and editing. BC**:** Study conception and design, review, and supervision. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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