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RESEARCH PAPER

Genetic Diversity of Prussian Carp, *Carassius Gibelio* (Bloch 1782), Populations in Euphrates River Based on mtDNA and Microsatellites

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Abstract

Population genetic studies can be useful in understanding invasion scenarios of non-native species. Carassius gibelio is an invasive species that can be found in various river and lake systems around the world. In this study, species identification was made by mtDNA COI sequence analysis for C. gibelio populations along the Euphrates River system and the genetic diversity of the populations was revealed using mtDNA D-loop and microsatellite markers. A total of 19 alleles were detected as a result of four microsatellite markers. It has been determined that fish populations are not in Hardy-Weinberg equilibrium because they show inbreeding reproduction and gynogenetics. According to microsatellite data, there is no genetic structure that coincides with the geographical distribution of the populations. The Euphrates River ecosystem is exposed to the effects of invasive species as well as anthropogenic activities and the survival and diversity of natural fish populations in the rivers are endangered. If necessary precautions are not taken, natural and endemic species will not be able to compete and there will be a high probability that they will be replaced by the invasive species C. gibelio in the future.

Introduction

Freshwater biodiversity provides a wide range of services to humans and is vital to human well-being and livelihoods, the rapid increase in this loss of diversity creates risks and factors that threaten these natural benefits cause the ecological function in ecosystems to decrease (Reid *et al.*, 2019; Lynch *et al.*, 2023a). According to the International Union for Conservation of Nature (IUCN) 2022 Red List, one-third of freshwater species are at risk of extinction. However, these rates may be higher because this analysis does not take into account species for which data are missing (Lynch *et al.*, 2023a). Globally, more than a third of inland wetlands experienced declines from 1970 to 2015; this rate is three times more than the rate of deforestation (Darrah *et al.*, 2019; Convention on Wetlands, 2021). Persistent threats to freshwater biodiversity include habitat loss and degradation, pollution, river fragmentation, invasive species and changing climates (Strayer and Dudgeon, 2010; Reid *et al.*, 2019; Dudgeon, 2019; Koehnken *et al.*, 2020). The consequences of these changes are documented in an 84% decline in the Living Planet Index for freshwater vertebrate populations between 1970 and 2016, twice the rate of biodiversity loss across land and sea areas (WWF, 2020). Biodiversity loss and declining ecological function in freshwater ecosystems jeopardize the natural benefits that support human life (Cardinale, 2011; Cardinale *et al.*, 2012). Serious threats to freshwater species are likely to also

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put at risk the most vulnerable human populations that depend on freshwater biodiversity (Lynch *et al*. 2023a).

Similar threats are increasing day by day in the Euphrates River system, as in most freshwater ecosystems in the world. In recent years, due to the civil unrest in Syria, the Southeastern Anatolia Region has received significant immigration and freshwater fish have become an alternative protein source in response to terrestrial products that are insufficient to meet the protein needs of the increasing population (Parmaksız, 2020). Therefore, both production and fishing activities have increased in the region to meet the demands for freshwater fish. The Southeastern Anatolia Region ranks last in Turkey in terms of inland fisheries production (Ural and Canpolat, 2009) and the majority of freshwater fish consumed is obtained by fishing from the Euphrates River systems. As a result of fishing, there has been a pressure on species that have economic characteristics and the number of individuals in the populations of these species has decreased every year (Parmaksız, 2020). In addition to many threats such as overfishing, habitat degradation and the construction of dams, the introduction of invasive fish into the region in recent years has further increased the pressure on natural species (Parmaksız and Demir, 2022).

The Southeastern Anatolia Project (GAP), which is an integrated project covering development and services such as dams, hydroelectric power plants and irrigation facilities on the Euphrates River system. GAP has increased agricultural production capacity and made the region have a great aquaculture potential thanks to the Dam Lakes it created (Oymak, 2000; Oymak *et al*., 2011). In order to utilize this potential, restocking were carried out by releasing carp fry into the lake water. Thus, fishing activities were increased by adding fish fry from outside, as well as fish naturally living in the river systems. Thanks to these fisheries efforts, invasive fish species have settled in the region as well as the target species. Invasion of freshwater ecosystems by alien fish can lead to significant consequences for natural biodiversity, including local extinctions of endemic and native species (Gozlan *et al*., 2010; Mollot *et al*., 2017; Jackson *et al*., 2017).

One of the most invasive fish species is *Carassius* species which have become an important threat in inland waters, including endemic species in Turkey (Uğurlu and Polat, 2007). This threat significantly affects the habitats of natural species, causing the rapid decline of local species and the end of fishing activities in the basins (Leung *et al*., 2002). According to both the observations made for the Euphrates River system and the information received from experienced local fishermen who earn their living by fishing in the region; It has been determined that nearly half of the fish caught in the nets are *Carassius gibelio* and the number of individuals of this species is increasing rapidly day by day. It has been stated that if it continues like this, there is a high probability that only this fish will be encountered in the nets in the future (Parmaksız *et al*., 2022). This situation, that is, the rapid increase of the *Carassius gibelio* species, poses a great threat to the biodiversity of the Euphrates River system (Parmaksız, 2023). *Carassius gibelio* (Bloch, 1782) is a species belonging to the Cyprinidae family, originating from Far East Asia and due to its invasive nature, it is seen in many inland water systems in the world today (Rylková *et al*., 2013; Esmaeili *et al*., 2015; Ağdamar and Tarkan, 2019; Khosravi *et al*., 2020; Khosravi *et al*., 2022; Dürrani *et al*., 2023). The most basic biological threat in the invasive character of this fish species is its reproduction and due to its gynogenetic reproduction feature, it can quickly become dominant in the habitats (Ağdamar, 2017; Parmaksız *et al*., 2017). Depending on its biological and ecological characteristics, it negatively affects the population density of native fish species in the environments it enters (Tarkan *et al*., 2012). For this reason, identifying the species of invasive fish in the environment and developing a control program is important both scientifically and economically. These measures may include preventing multiple introductions and removing or controlling those in the environment, especially those with relatively low genetic diversity (Ferincz *et al*., 2016; Parmaksız *et al*., 2017; Ağdamar and Tarkan 2019; Parmaksız and Demir, 2022).

The aim of this study; To identify the species by mtDNA COI sequence analysis for C. gibelio populations located along the Euphrates River system and to reveal the genetic diversity of the populations using D-loop and microsatellite markers. Thus, the molecular identification of the target invasive species will be made and the level of genetic diversity will be determined. In line with the data obtained, answers will be sought to the question of where to start and how to direct the fight against this species.

Materials and Methods

Collection of Fish Samples and Total DNA Extraction

In our study, *C. gibelio* samples were taken from five localities in the Euphrates River system and all fish samples used were purchased from local fishermen. Geographical information about these localities is given in Figure 1 and localities according to the number of individuals are given in Table 1. These samples, which were obtained by fishermen in the region between 2022-2023, were kept in an ice container and transferred to the Zoology Laboratory of the Department of Biology, Faculty of Arts and Sciences, Harran University. After morphological identification of the species, approximately 1 g of muscle tissue was separated from each sample and placed in microcentrifuge tubes containing 95% ethanol and kept at -20°C until DNA extraction. Total DNA was isolated from muscle tissue using the GeneJET Genomic DNA Purification Kit (Thermo Scientific) according to the kit protocol.

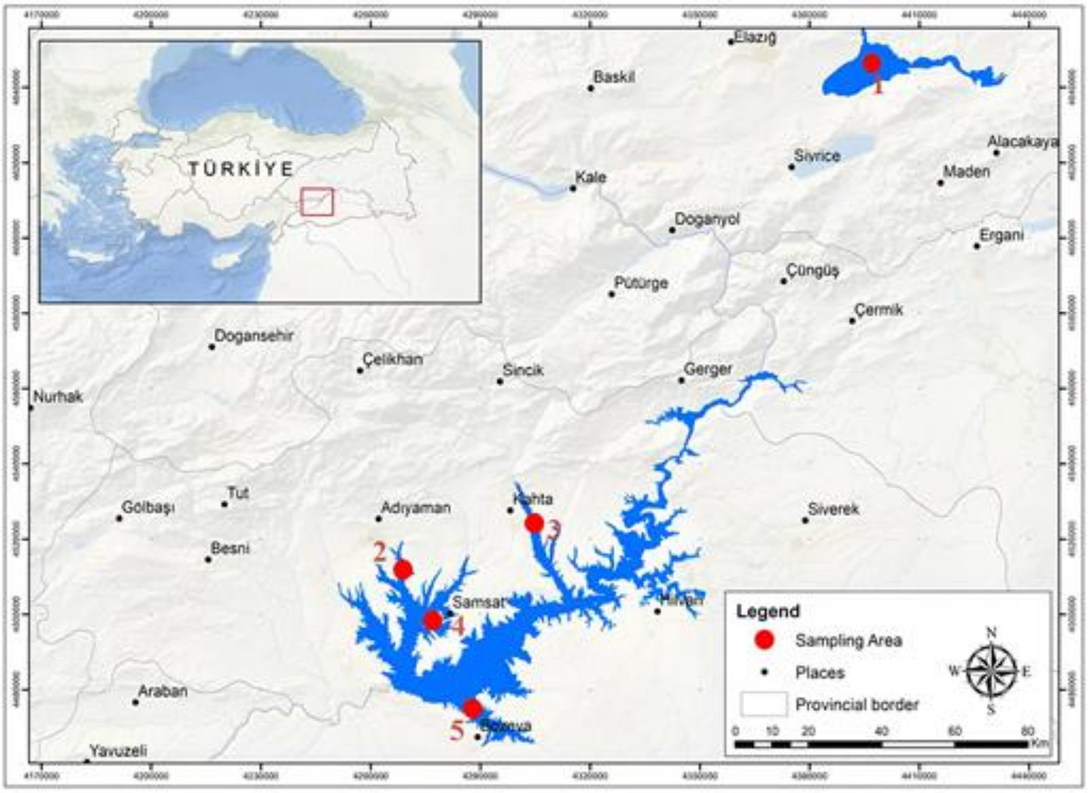

Figure 1. Sampling localities in the Euphrates River system (1-Elazığ, 2-Adıyaman, 3- Kahta, 4-Samsat, 5- Bozova)

Table 1. Number of individuals of *C. gibelio* according to sampling areas and studied markers

Localities	**Number of Individuals**		
	mtDNA COI	**mtDNA D-loop**	**Microsatellite**
Elazığ	30	30	20
Adıyaman	40	40	20
Kahta	25	25	20
Samsat	20	20	20
Bozova	20	20	20
Total	**135**	**135**	**100**

Amplification of Mitochondrial DNA COI, D-loop and Microsatellite

The studied fish were confirmed to be *C. gibelio* by using the mtDNA COI (Accesion number: OP242171) marker to make molecular diagnosis as well as morphological diagnosis of the species. mtDNA D-loop and microsatellite markers were used for genetic diversity analysis. D-loop is the most polymorphic region of mtDNA and therefore; It represents the maximum level of variation and is used as a marker to understand genetic diversity and population differentiation in various species (Lan *et al*., 2017). Therefore, it is often used as an effective tool to investigate the genetic diversity of populations of fish species belonging to the carp family (Wu *et al*., 2013). Since individuals of *C. gibelio* and *C. auratus* species belonging to the Carassius genus are morphologically similar, the mtDNA COI marker distinguishes them well genetically. Therefore, it was used in this study to provide precise information. In genetic diversity studies, mtDNA D-loop region and microsatellites which are nuclear DNA markers, were preferred. As for the selection of microsatellite markers, we selected the most polymorphic markers, taking into account our previous studies for the carp family. Information for each marker is shown in Table 2. PCR amplification of components were carried out in accordance with the methodology of the previous study with the same markers (Parmaksız and Batan, 2017; Parmaksız and Şeker, 2018; Parmaksız, 2020). Amplified mtDNA COI and D-loop fragments were sent to MEDSANTEK (Istanbul, TURKEY) for purification and sequencing.

This is due to the fact that the number of individuals according to locations in the study varies according to density while studying the mtDNA D-loop region. However, for microsatellite regions, all locations are fixed for 20 individuals. In addition, PCR was performed separately for four microsatellite regions. Capillary electrophoresis (ABI 3100 Genetic Analyzer) was used to determine the lengths of microsatellite regions as a result of PCR, and Applied Biosystems Peak Scanner™ Software v1.0 program was used for data analysis and correction from electropherograms.

Data Analysis

Raw data of mtDNA D-loop sequences belonging to a total of 135 individuals were evaluated using the FINCH TV 1.4.0 (Geospiza, Seattle, WA, USA) program and converted to FASTA format. The obtained sequences in FASTA format were aligned with the sequences of all individuals using the BioEdit software version v.7.2.5 program (Hall, 1999). mtDNA belongs to the D-loop region; the number of polymorphic regions, the number of haplotypes, haplotype-nucleotide diversity, Tajima D and Fu's statistics were calculated using the DnaSP 5.10.01 program (Rozas *et al.*, 2003). Median Joining was drawn to show the relationships between mtDNA D-loop haplotypes using the NETWORK 4.6.1.0 program Network (Bandelt, Forster, & Rohl, 1999).

To analyze the genotype data of microsatellite loci, allele numbers and observed and expected heterozygosity (Ho, He) values were calculated using the Arlequin version 3.5.1.3 (Excoffier and Lischer, 2010). F_{IS} (Weir and Cockerham, 1984) values were calculated between each population using FSTAT v.2.9.3.2 (Goudet, 1999). Fractional Correspondance Analyses (FCA) was applied in the Genetix 4.05.2 (Belkhir *et al.*, 2004) program to reveal the genetic differences between all individuals.

Results

Haplotype Distribution Based on mtDNA D-loop Analysis

The variable regions, frequencies and accession numbers of haplotypes for the D-loop of 26 variable regions and 4 haplotypes in total are shown in Table 3, and the analyzes of haplotypes according to localities are shown in Table 4. Haplotypes H1 and H2 are found in all localities. While H3 was found only in Samsat locality, H4 was detected only in Bozova. Thus, 73, 57, 4, 1 individuals from H1, H2, H3 and H4 haplotypes were calculated, respectively. The locality with the highest haplotype diversity (Hd) was Samsat (0.674) and the lowest was Elazığ (0.239). The locality with the highest nucleotide diversity (π) was Bozova (0.02659) and the locality with the lowest was Elazığ (0.01243). Positive values were calculated in Tajima's D values in all locations except Elazığ. Fu's Fs values were found to be positive in all studied populations, producing results similar to Tajima's D value.

In the Median-Joining Network haplotype, there is no central haplotype in the resulting network, indicating an evolutionary link (Figure 2).

Microsatellite Polymorphism and Genetic Diversity

Among the microsatellite markers, there are 5 alleles with a length of 176-194 bp for MFW1, 5 alleles with a length of 108-128 bp for MFW-9, 4 alleles with a length of 110-120 bp for Barbus-27 and 5 alleles with a length of 131-141 bp for Barbus-33 and totally 19 alleles have been identified. The average number of alleles for all loci was calculated as 4.75 (Table 5).

Genetic relatedness of individuals is depicted by a neighbor-joining tree using allele sharing distances between 100 individuals on four microsatellites (Figure 3). However, in many cases, even geographically distant individuals appear to exhibit high genetic similarity. Based on the loci and samples used in the study, there appears to be no consistent genetic differentiation among samples from five geographically distinct localities.

In Table 5, F_{IS} (inbreeding coefficient) all values; It was calculated as 0.859, 0.682, 0.720 and 0.625 for Elazığ, Adıyaman, Kahta, and Samsat, respectively ($P<0.001$) and were not in Hardy-Weinberg equilibrium, while for Bozova locality this value was calculated as 0.298 and was determined to be in Hardy-Weinberg equilibrium. When all microsatellite regions were considered for localities, it was determined that HW was not in balance in all except Bozova locality.

In Table 6, F_{ST} values between the populations were calculated between 0.010 and 0.3184, and the highest value was determined to be between Bozova and Elazığ. In addition, the genetic distances of the populations were calculated to be between 0.0808 and 0.3084, and the highest value was determined to be between Bozova and Elazığ, as in the F_{ST} value.

Discussion

Comparisons of Genetic Diversity

Considering the haplotypes for the mtDNA D-loop region in this study, it was determined that there were H3 and H4 haplotypes in Samsat and Bozova localities, unlike the others. Since the water is shallow and stagnant in Samsat and Bozova locations, there are many bays and many entrances, this has caused the C.gibelio species to be represented here with more individuals, strengthening the possibility of an increase in the number of haplotypes. Genetic differences as found in this study is important for better adaptation of populations to their habitats. While ten haplotypes for the *Capoeta trutta* species and four haplotypes for the *Carasobarbus luteus* species were detected in studies conducted for the same mtDNA D-loop gene region, which naturally lives in the Euphrates River and has a dominant feature, four haplotypes were observed in this study (Parmaksız, 2020; 2023). The number of haplotypes detected in the invasive species analyzed in this study was similar to that in the native species.

Table 2. Primer information for PCR amplification of mtDNA and microsatellite loci

Locus	Fluorescent label	Primer sequences	Repeat motif	Annealing temperature (°C)	Allele length (bp)	Denaturation 95°C (sec)	Annealing (sec)	Extension 72°C (sec)	Final Extension 72°C (sec)	Cycles	References
mtDNA COI	-	F:CAACCAACCACAAAGACATTGGCAC R:GACTTCTGGGTGGCCAAAGAATCA	-	62	600	30	45	45	10	35	Darabi *et al*., 2014
mtDNA D-loop	-	F:TTAAAGCATCGGTCTTGTAA R:GCCCTGAAATAGGAACCAGA	-	51	471	30	45	45	10	35	Iguchi *et al*., 1997 Inoue *et al*., 2000
MFW-1	FAM	F:GTCCAGACTGTCATCAGGAG R:GAGGTGTACACTGAGTCACGC	(CA)$_n$	55	175-212	35	45	60	10	35	Crooijmans *et al*., 1997
MFW-9	FAM	F: GATCTGCAAGCATATCTGTCG R:ATCTGAACCTGCAGCTCCTC	(CA)$_n$	59	92-144	35	45	60	10	35	Crooijmans *et al*., 1997
Barbus27	FAM	F: ATATCCAGCCACCCTTACCC R: TGCTTTAGCTGCCAGACAGA	(CA)$_n$	62	109-125	35	45	60	10	35	Gettova *et al*., 2013
Barbus33	HEX	F: TGAATGCATCATGGGCTAGA R: CAGAGCGAATCAAACATGGA	(CA)$_n$	56	101-154	35	45	60	10	35	Gettova *et al*.., 2013

Table 3. Variable regions and frequencies of the *C. gibelio* D-loop gene

Haplotype	Variable sites																										Frequency	Accesion number
	12	75	84	91	94	160	175	182	185	187	188	197	238	275	281	335	345	346	358	362	375	382	388	392	402	410		
H1	C	G	A	C	G	T	G	A	T	T	G	A	G	T	T	A	C	G	T	C	G	A	C	C	C	A	73	PP051501
H2	T	A	G	T	A	A	A	A	A	C	T	G	A	C	T	G	C	A	G	T	A	G	T	T	C	A	57	PP051502
H3	T	G	A	C	G	T	G	A	T	T	G	A	G	T	T	A	C	G	T	C	G	A	C	C	C	A	4	PP051503
H4	T	A	A	T	A	T	A	G	A	T	T	A	G	T	C	A	T	A	G	T	A	G	T	T	T	G	1	PP051504

Table 4. mtDNA D-loop haplotypes according to localities, Haplotype number (Nh), Haplotype (Hd) and nucleotide (π) diversity, Tajima's D and Fu's Fs values

Locality	n	Nh	Haplotype	Hd	π	Tajima's D	Fu's Fs
Elazığ	30	2	26H1, 4H2	0,239	0,01243	-0,18592	13,008*
Adıyaman	40	2	14H1, 26H2	0,467	0,02433	3,29234*	23,240*
Kâhta	25	2	18H1, 7H2	0,420	0,02205	2,13712*	17,768*
Samsat	20	3	8H1, 4 H2, 4H3	0,674	0,02634	3,03653*	14,229*
Bozova	20	3	7H1, 12H2, 1H4	0,542	0,02659	1,80795*	13,896
Total	**135**	4	4	0,532	0,02602	3,46960*	28,024

Significant difference at *P<0.05

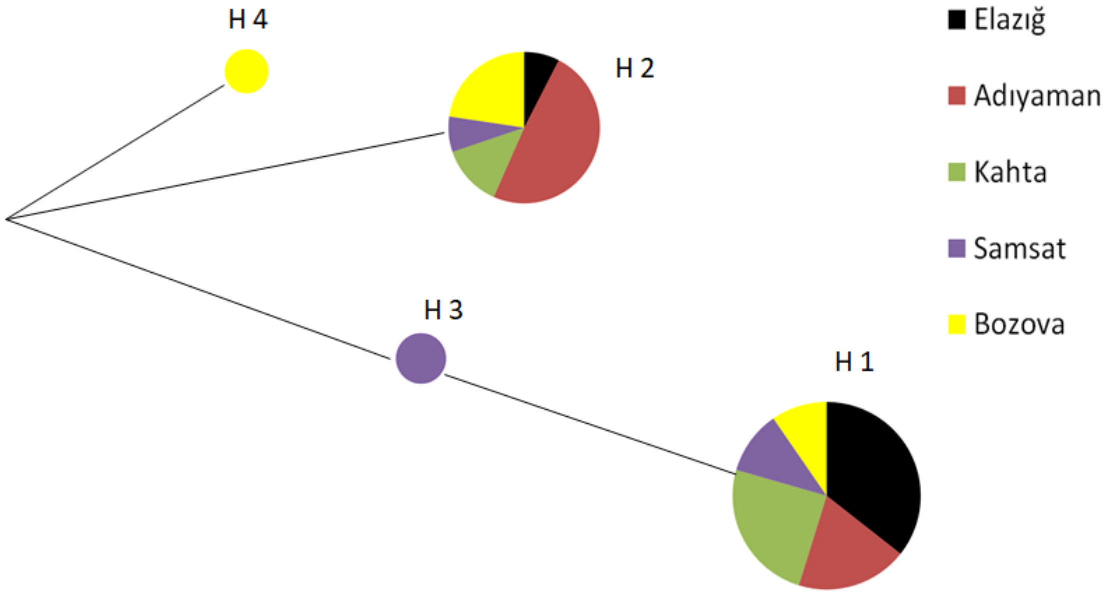

Figure 2. The median-joining network based on four haplotypes of *C. gibelio* found by sequencing D-loop gene of 135 individuals from five localities in Turkey. The colors represent the various populations, the size of a circle is proportional to the number of haplotypes represented.

In the study of Ağdamar and Tarkan (2019), they investigated the genetic diversity level of *C. gibelio* species by sampling in 18 different regions of Turkey, excluding the Euphrates River and determined 3 haplotypes for the COI region and 6 haplotypes for the D-loop gene region. It has been observed that there is no significant correlation between geographical distance and genetic distance of this invasive species, which lives in different regions of Turkey (Ağdamar and Tarkan, 2019). In this study, 1 haplotype for the mtDNA COI region and 4 haplotypes for the mtDNA D-loop region were detected. The level of genetic diversity of the populations of this invasive fish species based on the mtDNA D-loop region; it was determined to be higher in this study when compared to all populations in Turkey.

In addition, when the genetic diversity data of the D-loop region for other populations living in Turkey was examined, it was seen that haplotype diversity (Hd) was between 0 and 0.580, and nucleotide diversity (π) was between 0 and 0.0229, the number of haplotypes varied between 1 and 3 depending on the populations (Ağdamar, 2017). In this study, haplotype diversity was found to be between 0.239 and 0.674, and nucleotide diversity was found to be between 0.01243 and 0.02659, haplotype numbers were found to be between 2 and 3. It appears that *C. gibelio* populations living in the Euphrates River have higher genetic diversity for the mtDNA D-loop region compared to all Turkish populations.

In natural *C. gibelio* populations in China, a total of 14 haplotypes for the D-loop region were determined, with an average Hd value of 0.895 and a π value of 0.010 (Li and Gui, 2008). For this invasive species that lives in the Euphrates River, the average Hd value was determined as 0.532, the π value was 0.02602 and the number of haplotypes was four. Although the haplotype number and average haplotype diversity values in this study were lower than the natural population, the nucleotide diversity value was higher. High nucleotide diversity in a population can affect genetic adaptation in several ways. When the environment changes, a population with higher diversity is more likely to adapt to the new environment than a population with less diversity (Chung *et al.*, 2023). In the sampled localities, genetic diversity in natural species continues to decrease due to features such as global warming, habitat change and fishing. The fact that the *C. gibelio* species living in the Euphrates River and whose nucleotide diversity was found to be even higher than the natural population shows that the *C. gibelio* species will become more dominant in the region and cause the decrease of natural and indigenous species. In addition, high nucleotide diversity can be considered a sign that individuals of invasive species entering the river system studied come from different regions. This may be supported by the incidental introduction of *Cyprinus carpio* through government-based stocking practices across the country (Tarkan et al., 2015). The introduction of non-native domesticated species is one of the widely accepted methods for improving many natural waters with low fish productivity around the World (Tsipas et al., 2009).

A total of 19 alleles were detected for four microsatellite regions for the *C. gibelio* species living in the Euphrates River. As a result of the study conducted with the same microsatellite markers for the fish species *Cyprinion macrostomus*, which naturally lives in the Euphrates River; they detected a total of 40 alleles: 9 for MFW1, 17 for MFW9, 2 for Barbus27 and 12 for Barbus33 (Parmaksız and Batan, 2017). Compared to *C. macrostomus*, the genetic diversity of *C. gibelio* is lower. Low genetic diversity of non-native populations as a

Table 5. Genetic variability measures in Four microsatellite markers

LOCI	N_A	PIC	ELAZIĞ		ADIYAMAN		KAHTA		SAMSAT		BOZOVA		F_{IS}				
			H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	**ELAZIĞ**	**ADIYAMAN**	**KAHTA**	**SAMSAT**	**BOZOVA**
MFW-1	5	0.67	0.250	0.578	0.800	0.685	0.650	0.683	0.800	0.665	1.000	0.513	0.574	-0.174	0.050	-0.209	-1.000
MFW-9	5	0.52	0.000	0.5949	0.000	0.6821	0.000	0.4718	0.000	0.4308	0.000	0.5179	1.000***	1.000***	1.000***	1.000***	1.000***
BARBUS-2	4	0.40	0.0500	0.1885	0.0000	0.5590	0.0000	0.4923	0.0000	0.5077	0.0000	0.3846	0.740***	1.000***	1.000***	1.000***	1.000***
BARBUS-3	5	0.53	0.0000	0.7179	0.0500	0.6987	0.0000	0.6308	0.0000	0.4974	0.0000	0.0000	1.000***	0.930***	1.000***	1.000***	1.000***
MEAN	4.75	-	0.0750	0.5199	0.2125	0.6561	0.1625	0.5696	0.2000	0.5253	0.2500	0.3538	-	-	-	-	-
ALL													0.859***	0.682***	0.720***	0.625***	0.298
T			13		15		12		11		8						
A			3.25		3.75		3		2.75		2						

H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: inbreeding coefficient, n_a: allele number, T: total alleles, A: mean no. of alleles, ***$P<0.001$

result of genetic bottleneck is a general paradigm in the population genetics of biological invasions (Allendorf and Lundquist, 2003).

In Table 5, it was determined that the F_{IS} values calculated for four different microsatellite regions ranged between -0.209 and 1.000. It can be said that there is an insignificant excess of heterozygotes in the populations determined as negative. Since these fish populations generally show inbreeding growth and the F_{IS} value is significant, it is seen that they deviate from the Hardy-Weinberg equilibrium. In Figure 4, individuals from all populations are not completely separated from each other. Therefore, it cannot be said that there is a genetic diversity that coincides with their geographical distribution. Although the distance is long and there are some dams in the sampled locations, fish passage and gene exchange are possible. No study has yet been conducted on the existence of hybrid individuals of this invasive species with natural species.

In this study, it was determined that Fst values between Adıyaman-Kahta and Kahta-Samsat localities were statistically insignificant. The highest Fst value (0.3184) is between Elazığ and Bozova. All values of Elazığ locality among other localities were determined to be statistically significant. Since Elazığ and other localities are geographically distant and there are dams in between, the possibility of gene exchange is quite low. Therefore, these results are expected. Another possibility is that *C. gibelio* with a different genetic structure was released during fish farming for the Elazığ Dam Lake. Since there is no geographical barrier for other localities, gene exchange between localities is possible and it is expected that they will have similar genetic structure.

Haplotype Origin and Adaptation

The H1 haplotype belonging to the mtDNA D-loop region detected in this study has the same haplotype as the Georgian samples (Accession: KC243407) and shows 100% similarity. The H2 haplotype shows 100% similarity with samples from Russia (Accession: GQ985484) and samples from the Chinese region (Accession: JN790670). The H3 haplotype is a new haplotype and is not available in NCBI. The H4 haplotype shows 100% similarity with European and Chinese samples (Accession: FJ167413, DQ984994). The invasive *C. gibelio* species in the Euphrates River entered the system due to unnatural reasons, shows different haplotypes and is thought to have come from several different points. Divergent genetic structures among invasive populations is a common observation and is often attributed to multiple introductions from genetically distinct sources (Bock *et al.*, 2015). The high genetic diversity seen across Turkey for *C. gibelio* populations may be associated with multiple transitions, which may explain the high ecological tolerance that facilitates the invasion of the species into new environments (Ağdamar and Tarkan, 2019).

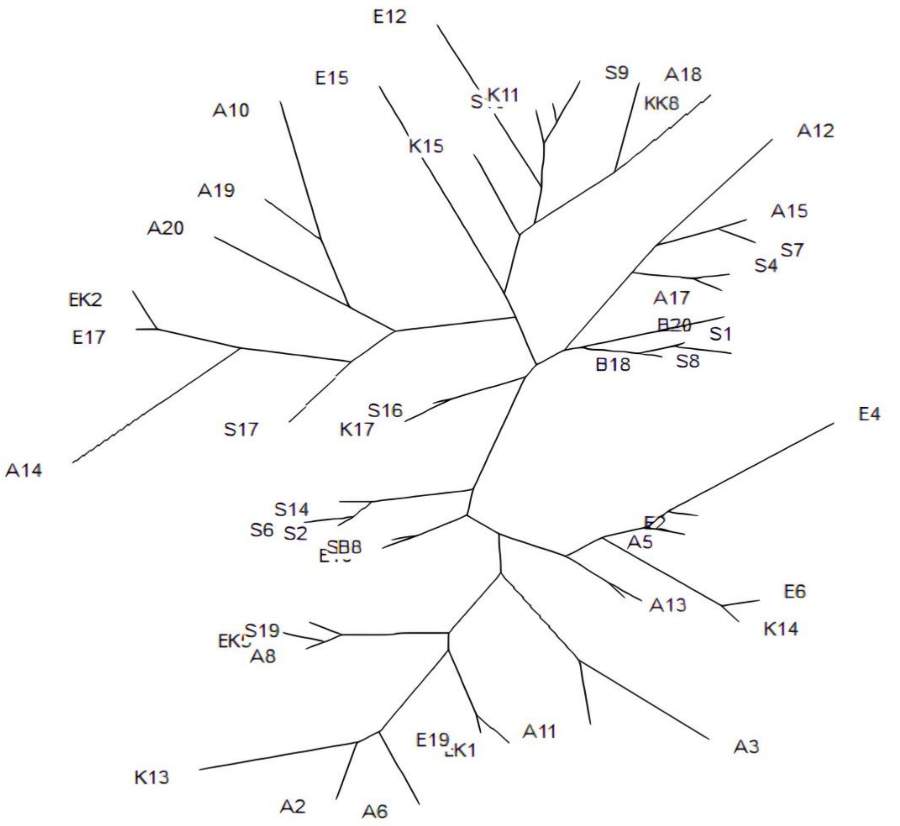

Figure 3. Neighbour joining tree where allele sharing distances among 100 samples on 4 microsatellites were used. A: Adıyaman, B: Bozova, E: Elazığ, K: Kahta, S: Samsat.

Table 6. Pairwise F_{ST} values (*belows diagonal*) and Nei's D_A genetic distances (*aboves diagonal*) based on 4 microsatellite loci samples from 5 different locality of Euphrates River

	ELAZIĞ	ADIYAMAN	KAHTA	SAMSAT	BOZOVA
ELAZIĞ	-	**0.088****	**0.118*****	**0.134*****	**0.3184*****
ADIYAMAN	*0.1612*	-	**0.0612**^{NS}	**0.108****	**0.2377*****
KAHTA	*0.0999*	*0.1009*	-	**0.010**^{NS}	**0.2490*****
SAMSAT	*0.1682*	*0.1783*	*0.0808*	-	**0.1386*****
BOZOVA	*0.3084*	*0.2956*	*0.2754*	*0.1213*	-

*** P<0.001; ** P<0.05

Conclusion

The Euphrates River ecosystem is exposed to the effects of anthropogenic activities such as environmental pollution, overfishing and the construction of dams are some of the challenges that threaten the survival and diversity of fish populations in the rivers (Parmaksız, 2020). Some natural, common, endemic and dominant species caught by fishermen are becoming very difficult to cope with these challenges and it is highly likely that they will be replaced by *C. gibelio*, an invasive species, in the future (Navodaru *et al*. 2002; Gaygusuz *et al*. 2007; Parmaksız and Demir, 2022; Parmaksız, 2020). If necessary precautions are not taken, especially for endemic species that have economic characteristics, the extinction of populations is inevitable. In parallel with the situation worldwide, Turkey's inland waters are also exposed to the introduction of species that cause habitat degradation and decline of endemic fish populations (Tarkan and Marr, 2015). It has been determined that the *C. gibelio* species, whose genetic diversity level is similar to the natural species living in the Euphrates River, has adapted to this river habitat and tends to become dominant by increasing the number of individuals day by day. If no precautions are taken, it is inevitable for this species to fully adapt to the river system and become dominant.

Ethical Statement

This study does not require any formal authorization. The authors declare that the present study was conducted in an ethical, professional and responsible manner.

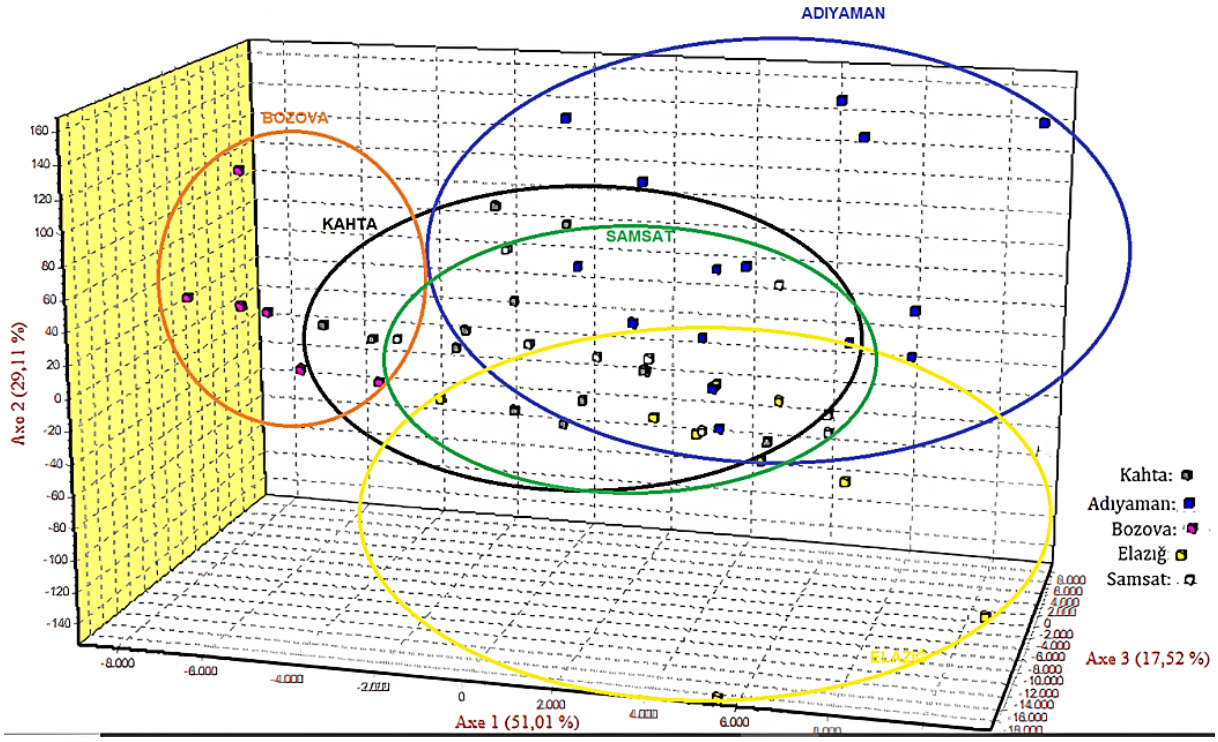

Figure 4. FCA results showing the relationship between individuals sampled from five localities.

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Author Contribution

All authors contributed to the study conception and design. First Author: Project administration, methodology, supervision and writing-original draft; Second Author: Investigation, data collection, visualization and writing review and editing; Third Author: Data curation, resources, writing-review and editing. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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