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Research Article

Analysis of mtDNA cyt b and mtDNA D-loop of *Carassius auratus* (Linnaeus, 1758) species living in Keban Dam Lake (Türkiye)

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Abstract: Keban Dam Lake is located within the boundaries Elazığ, Tunceli and Erzincan provinces of Türkiye and is an artificial lake where aquaculture production and fishing activities are carried out. In addition to native fish species, non-native and invasive fish that entered the dam lake later live in this lake. These invasive fish are a threat to native species and cause significant impacts to biodiversity. In this study, the genetic structure of *Carassius auratus*, one of these invasive fish, was investigated by sequence analysis of mtDNA cyt b and D-loop regions. DNA was extracted from muscle tissue using commercial kit. PCR stages performed using target primers and were subjected to sequence analysis. The haplotypes obtained for both gene regions were compared using the BLAST technique to reveal similarities in the gene bank. The information on genetic variation and population structure obtained for *C. auratus* in this study will be useful for planning effective strategies for the protection and rehabilitation of the species living in the dam lake.

Keywords: *Carassius auratus*, mtDNA, Invasive species, Alien species, Genetic diversity.

1. Introduction

Keban Dam Lake is the second largest artificial lake constructed within the borders of Türkiye following the Atatürk Dam Lake. Besides its primary purpose of electricity generation, the construction of the dam has also facilitated fishery production and agriculture within the reservoir, contributing to the country's economy (Güzel, 2022). A total of 28 fish species belonging to 7 families have been identified in Keban Dam Lake (Yıldırım et al., 2015). Among these species, *Carassius auratus*, *Carassius gibelio*, *Cyprinus carpio*, and *Oncorhynchus mykiss* are non-native fish species that have entered the reservoir later (Yıldırım et al., 2015).

The species *C. auratus* is a member of the Cyprinidae family and has a stocky body, typically measuring between 12 cm and 22 cm in length. Although its natural forms are silver or greyish, today it also has forms such as red and orange. The breeding season is between May and June.The native habitat of C. auratus is China, but it is widely distributed across the world (Xiong et al., 2015; Doğaç et al., 2016; Aktop, 2017; Pan et al., 2021). It is extensively distributed throughout much of China (Liu et al., 2017) and is one of the most important economic freshwater fish (Pan et al., 2021). Although the exact mechanism of its introduction into Keban Dam Lake is not fully understood, it is presumed that this fish was introduced into freshwater bodies by human activities during stocking, with records dating back to 2002 (Duman and Şen, 2002). This trend is steadily increasing as a result of human activities, causing adverse effects on numerous local ecosystems (Mack et al., 2000; Reid et al., 2005).

The presence of this invasive fish species has been reported in various regions of inland waters in Türkiye (Innal, 2011). The invasion of freshwater ecosystems by alien fish species can lead to significant consequences for natural biological diversity, including the local extinction of endemic and native species (Gozlan et al., 2010; Mollot et al., 2017; Jackson et al., 2017). This invasive species has become a significant threat to all native species, particularly economically important species, living in Keban Dam Lake. This threat significantly affects the habitats of native species, leading to a rapid decline in populations of local species and the cessation of fishing activities in the basins (Leung et al., 2002). Fish are considered the cheapest source of animal protein and minerals for poor families worldwide (Wu and Yang, 2012). Additionally, fish are an important source of essential fatty acids, vitamins, low levels of saturated fatty acids, and cholesterol (Vanlı et al., 2023). Therefore, ensuring the continuity of fishing activities in this reservoir is crucial for both nutrition and the employment of local people. Consequently, it is imperative to combat the invasive fish species, including *C. auratus*, in the environment. The characteristics of populations of this species should be investigated, and urgent measures should be taken to remove them from the environment to prevent their impact on native species.

This study aims to conduct sequence analyses of mtDNA cyt b and D-loop gene regions for the *C. auratus* population living in Keban Dam Lake, to perform species identification at the molecular level, and to determine the level of genetic diversity. Both the D-loop region and cyt b are utilized for genetic diversity and phylogenetic analysis in fish (Brahimi et al., 2016; Duong et al., 2019; Sun et al., 2019; Zhang et al., 2020). mtDNA cyt b gene region variations are used in genetic analysis studies of populations of fish species belonging to the Cypriniformes order (Fayazi et al., 2006), and it is a multipotential genetic marker that can be used especially for genetic variation analysis of fish (Saraswat et al., 2014). mtDNA D-loop site was used examining a greater number of populations and individuals. This site shows the greatest level of variation because it is the most polymorphic site of mtDNA (Li et al., 2015). Haplotype analysis of D-loop is used as a potent tool to research genetic diversity of cyprinid fish populations (Wu et al., 2013).

2. Materials and Methods

2.1. Collection of Fish Samples and Total DNA Extraction

Our study material consisted of 20 specimens of *C. auratus* species, which were collected by fishermen through fishing from the location shown in Figure 1 of Keban Dam Lake. A photograph of an organism belonging to the fish species examined is shown in Figure 2. The fork length of the individuals used in this study varies between 16.2 and 21.5 cm, and their weight varies between 52 and 126 g. The samples were randomly selected and preserved in ice until transferred to the Zoology Laboratory of the Faculty of Science and Arts, Harran University. Following morphological identification of the species, approximately 1 cm3 of muscle tissue was taken from each sample and placed in microcentrifuge tubes containing 95% ethanol, where they were kept at -20 °C until DNA extraction. Total DNA was extracted from muscle tissue using the GeneJET Genomic DNA Purification Kit (Thermo Scientific).

Figure 1. Location where fish samples were taken.

Figure 2. An organism belonging to the fish species studied.

2.2. PCR Stages of mtDNA cyt b and mtDNA D-loop Regions

Information regarding the primers used for amplifying the mtDNA cyt b and D-loop regions is provided in Table 1. The PCR steps represent the optimum values obtained after gradient and optimization. All PCR reactions were conducted in a total volume of 25 µl containing 0.5 mM of each primer, 0.2 mM of dNTPs, 1x PCR buffer, 2.5 mM MgCl2, 1 unit of Taq polymerase, and approximately 50 ng of DNA. Following the PCR stage, the products were electrophoresed on a 1.5% agarose gel at 100V for 30 minutes (Figure 3) and the resulting products were subsequently sent to a commercial company for DNA sequence analysis using the 3500 XL Genetic Analyzer instrument.

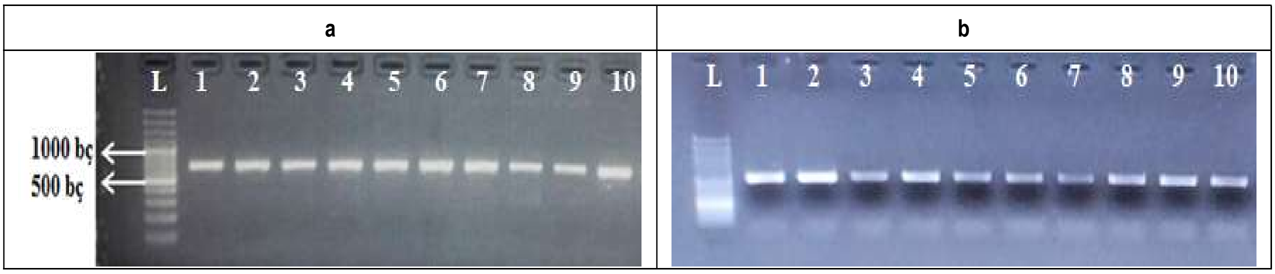

Figure 3. Agarose gel image of mtDNA; a) cyt b and b) D-loop PCR products (L: DNA Ladder).

2.3. Data Analysis

The raw data of mtDNA cyt b and D-loop sequences were evaluated using FinchTV 1.4.0 (Geospiza, Seattle, WA, USA), and all sequences of individuals were aligned using BioEdit software version 7.2.5. Sequences showing the highest similarity in the mtDNA cyt b and D-loop regions from the GenBank were included in the study. Neighbor joining tree phylogenetic analyses were conducted in MEGA X 10.2.4 (Kumar et al., 2018) program using the K2 parameter model, and a phylogenetic tree was constructed. Bootstrap test (1000 replicates) was used to test the reliability of the tree branches (nodes).

Table 1. PCR conditions and primer references.

Marker	Annealing	Denaturation 95 °C	Annealing (Tm)	Elongation 72 °C	Final Elongatiton 72 °C	Cycle	Reference
mtDNA cyt b	62°C	30 s	45 s	45 s	10 min	35	Briolay et.al., 1998
mtDNA D-loop	51°C	30 s	45 s	45 s	10 min	35	Iguchi et.al., 1997; Inoue et. al., 2000

3. Results

In this study, sequence analysis of mtDNA cyt b and D-loop loci was conducted for individuals of the *C. auratus* species, which have settled in Keban Dam Lake through unnatural means, exhibit invasive characteristics, and tend to be dominant in the lake. Since the number of individuals taken from only one location in this study is limited, it does not fully represent the Keban Dam Lake. However, it provides important data to gain an idea about the genetic characteristics of this invasive species. Sequences with average lengths of approximately 600 bp and 470 bp were obtained for the mtDNA cyt b and D-loop loci, respectively. All individuals collected from Keban Dam Lake exhibited the same haplotype for both gene regions, with no variation detected among individuals. Haplotypes obtained for both gene regions were compared with similarities in the GenBank database using the BLAST technique (Table 2).

Table 2. Comparison of the sequences obtained in this study with sequences in the NCBI database.

Marker	Accession Number	Country	Similarity Ratio (%)
mtDNA cyt b	OP056093	Türkiye	100
	MG281933	North America	99.82
	MG281847	North America	99.82
	KX688782	European	99.82
	KX688781	European	99.82
	MF443767	Chinese	99.82
	OR100497	Canadian	99.82
	GU991384	Czech	99,63
mtDNA D-loop	AB379916	Japan	100
	LC495699	Japan	100
	FJ167425	European	100
	KU146528	China	99.76
	AB379921	China	99.76
	AB379917	Japan	99.53

In the analysis of sequences from the cyt b region presented in Table 2, comparison with the sequences obtained in this study showed 100% similarity with the sequences of *C. auratus* individuals from the Atatürk Dam Lake on the Euphrates River, as reported in a study conducted by Parmaksız et al. (2022). It was determined that the sequences from this study represented a different haplotype from those recorded in the GenBank, and exhibited a similarity of up to 99.82% with individuals from other countries. Thus, the sequences obtained in this study were identified as a distinct and novel haplotype in the analysis of the cyt b region, recorded as OP056093 in the GenBank. Furthermore, the Neighbor Joining (NJ) tree constructed based on the cyt b sequences in Figure 4a illustrates that the haplotypes from this study and those from Bozova, Şanlıurfa, are distinct from other haplotypes. Regarding the D-loop region, as shown in Table 2, it was found to exhibit 100% similarity with sequences from Japan and European countries. In Figure 4b, According to their similarity, they are clustered in the neighbor joining tree.

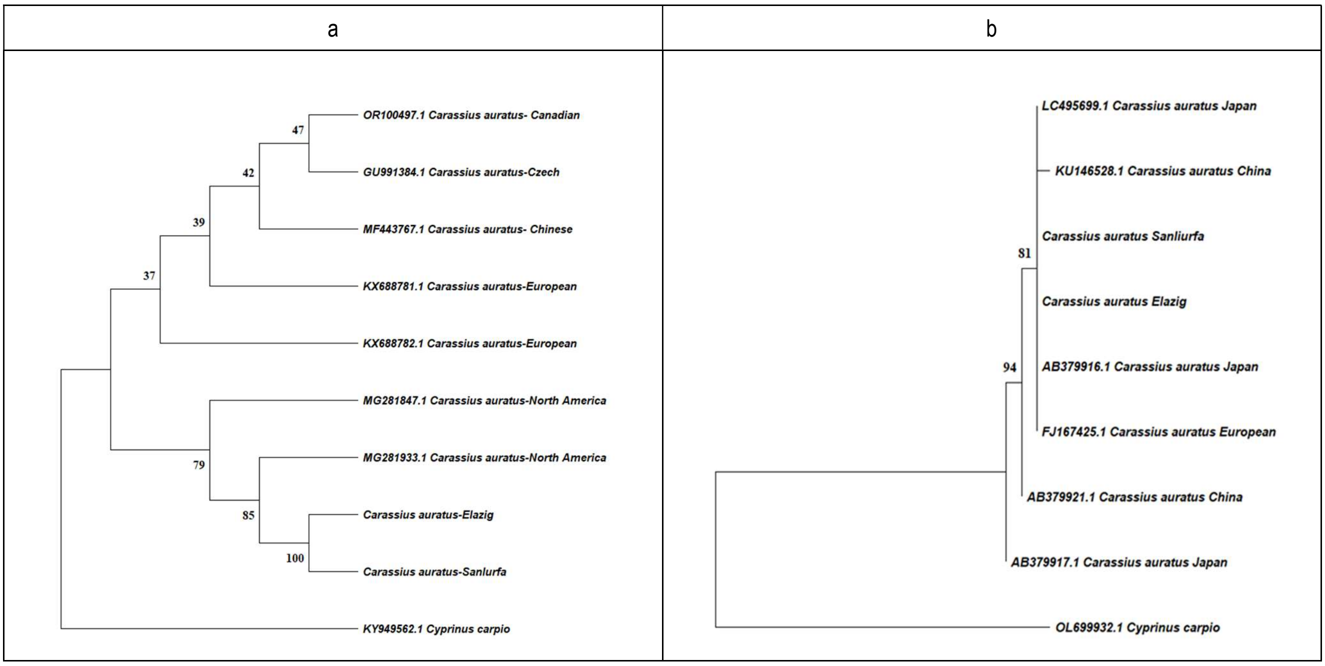

Figure 4. Neighbor-Joining (NJ) tree based on mtDNA; a) cyt b sequences, b) D-loop sequences.

4. Discussion

In light of these results, it has been determined that the genetic diversity of this species is low in the population of Keban Dam Lake, which is attributed to inbreeding and gynogenetic reproduction. Generally, a population entering a new environment tends to lose genetic diversity due to bottlenecks that can reduce fitness and evolutionary potential (Lee, 2002). In contrast to this study, high genetic diversity was detected in C. auratus for the mtDNA cyt b and D-loop regions in the vicinity of Hongze Lake in China, indicating the influence of human activities on the distribution and genetic structure of fish (Pan et al., 2021).

The presence of this species with invasive characteristics in Keban Dam Lake indicates the formation of the population as a result of the entry of a small number of individuals into the system due to anthropogenic effects. Biological invasion refers to the spread, reproduction, and persistence of a non-native species outside its natural range (Feng et al., 2023). Due to their high reproductive capabilities, these individuals rapidly increase in number and tend to become dominant in the new habitat, adapting quickly to the new environment. Furthermore, considering that the population of *C. auratus* in the studied reservoir likely originated from bottlenecked main populations, the low genetic diversity observed in this population is expected. Both observations and discussions with local fishermen have revealed an increase in the number of *C. auratus* individuals over time. Consequently, the proliferation of invasive species exacerbates the already pressured native species, further reducing their chances of survival due to problems such as habitat loss and overfishing. Additionally, in recent years, changes in climate and anthropogenic influences have caused fluctuations in the level of Keban Dam Lake, with climate change having a greater impact on the reservoir and leading to a significant decrease in water levels (Güzel, 2022). If this trend continues, the native, endemic, and economically important species living in the region will be increasingly affected over time, resulting in a reduction, or even extinction, of individuals belonging to economically important native species in the region. Consequently, the reservoir will transition into an environment dominated by non-economically important invasive species.

5. Conclusions

In a study by Tarkan et al. (2012), the population density of *Carassius gibelio* living in the Ömerli Reservoir in the Marmara Region was monitored over a six-year period. The study showed a significant decrease in the density of native species and an increase in the density of *C. gibelio* individuals. Similar results are expected for both *C. gibelio* and *C. auratus* in Keban Dam Lake. Therefore, a serious control program should be organized against invasive species. In particular, populations found in the reservoir should be identified, and efforts should be made to remove them from the environment through fishing before the breeding season begins.

Conflicts of Interests
Authors declare that there is no conflict of interests

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Statement contribution of the authors
This study's experimentation, analysis and writing, etc. all steps were made by the authors.

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