Research Article

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mtDNA COI and Cyt b Analysis of *Carassius auratus* (Linnaeus, 1758) Species Living in Atatürk Dam Lake (Turkey)

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ABSTRACT

Today, biological invasion is one of the leading threats to biodiversity and is an issue that is gaining more and more importance. In recent years, molecular techniques have been widely used in the identification and monitoring of invasive species in many parts of the world. This study aimed to detect the infested species, *Carassius auratus*, in Atatürk Dam Lake and to reveal the population status. For these purposes, 10 *Carassius auratus* specimens were randomly selected from Atatürk Dam Lake. After DNA isolation of the selected samples, mtDNA COI and cyt b gene regions were sequenced and analyzed. In this study, sequence analyzes of individuals belonging to the *C. auratus* species living in Atatürk Dam Lake were performed for the first time using mtDNA COI and cyt b markers. Analyzed sequence results were compared with databases and it was concluded that the results obtained for both mtDNA markers were compatible. No other variation was observed. When the results are evaluated as a whole, it is possible to say that this species has spread recently. In future studies, it is recommended to determine the invasive species populations that are intensely found in Atatürk Dam Lake and other lakes and to determine the necessary strategies to combat these invasive species.

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Introduction

Carassius auratus (Linnaeus, 1758) is a fish species whose homeland is China and is widely found in Japan, and it has been taken to almost all parts of the world [1,2]. *C. auratus* is a species belonging to the Cyprinidae family and varies greatly in size, body shape, fin configuration and color. They have formed a natural population in many countries around the world due to conscious vaccinations made to water resources and individuals escaping from breeding systems [2]. This species was first domesticated in China and has become one of the important and common farmed fish [3]. It was introduced in Europe and Japan in the 17th century and in the Americas in about 1850, then quickly spread all over the World [4].

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The *C. auratus* species can produce thousands of eggs and can cohabit and be reared in small areas. Periods of domestication have generated significant artificial and natural selection [5]. There are hundreds of varieties according to body shape, fins, eyes, and color of scales [3]. These different features make *C. auratus* a valid model for genetic, evolutionary, and biological research [6].

The presence of *C. auratus*, an invasive fish, has been reported in Turkish inland waters in different regions [7]. It is estimated that this species spread as a result of accidentally or deliberately releasing it into Turkish waters or escaping from fish production farms [8]. The Southeastern Anatolia Project has increased its agricultural production capacity and has become a great aquaculture potential thanks to the Atatürk Dam Lake [9,10]. Invasive fish species have settled in the lake water due to fisheries or other factors. The invasion of freshwater ecosystems by foreign fish can have important consequences for natural biodiversity, including local extinctions of endemic and native species [11, 12, 13].

In recent years, pond fish (Carassius sp.) have become an important threat in inland waters, including endemic species found in our country [14]. This threat significantly affects the habitats of natural species and causes the rapid decline of local species and the termination of fishing activities in the basins [15]. Natural fish species living in Atatürk Dam Lake; due to factors such as overfishing, the dominance of invasive species, and habitat loss, it is exposed to increasing pressures day by day, the number of individuals in economic species populations is decreasing, along with species losses [16]. Depending on their biological and ecological characteristics, invasive species negatively affect the population density of native fish species in the environments they enter [17]. Invasive fish species are a threat to both fisheries and biodiversity [18]. For this reason, it is important both scientifically and economically to determine the species of invasive fish in the environment and to put forward a control program. For this program, a certain genetic data must first be obtained. Sequences of COI and cyt b gene regions among mitochondrial genes are used for kinship relationships among fish.

The aim of this study; For *C. auratus*, by performing sequence analyses with mtDNA COI and cyt b markers, (i) species identification, (ii) upload the obtained sequences to the gene bank, and (iii) make predictions about where it came to Atatürk Dam Lake by comparing it with the data in the gene bank.

Material and Methods

In this study, 10 randomly selected *C. auratus* samples, which were sold at the stalls of local fishermen fishing in the Bozova region of Atatürk Dam Lake in December 2021, were used as material. The samples were brought to Zoology Laboratory of Harran University, Faculty of Science- Literature, Department of Biology by applying a cold chain. After the species were identified, muscle tissue was taken from the samples, placed in microcentrifuge tubes containing 90% ethanol, and stored at -20ºC until DNA was obtained.

DNA isolation and PCR

Total DNA isolation was performed from muscle tissue using the GeneJET Genomic DNA Purification Kit (Thermo Scientific) according to the protocol instructions. To check the presence of DNA after the protocol, DNA samples from all individuals were loaded into the wells of 0.8% agarose gel added to SYBR Green, carried out in electrophoresis, and visualized in a (UV) light device (Smart View Pro Imager System, Major Science).

In this study, PCR was performed in Thermal Cycler (BIO-RAD T100TM). The primer sequence used for amplification of the mtDNA COI gene region was reported by Darabi et al. (2014) (COI-625F: 5' TCA ACC AAC CAC AAA GAC ATT GGC AC-3'; COI-625R: 5' GAC TTC TGG GTG GCC AAA GAA TCA-3') [19]. PCR conditions and chemicals were applied according to the study of Parmaksız and Eskici (2018) and the product was obtained [20]. The primer sequence used for the mtDNA cyt b gene region was taken from Briolay et al., (1998) (L15267 F: 5ˈ GTT TGA TCC CGT TTC GTG TA-3ˈ; H15891 R: 5'AAT GAC TTG AAG AAC CAC CGT-3'), PCR conditions and chemicals were applied according to the study of Parmaksız and Şeker (2018) and the product was obtained [21, 22].

Data analysis

The obtained PCR products were sent to the commercial firm and sequence analysis was performed with the 3500 XL Genetic Analyzer device. Then, raw data of mtDNA COI and cyt b sequences were evaluated using the FinchTV 1.4 program. Furthermore, sequences of all individuals were aligned using BioEdit software version 7.2.5. The sequences of mtDNA COI and cyt b gene region of the target species in the gene bank were included in the study and similarity rates were determined.

Results and Discussion

In this study, sequence analyzes of the mtDNA COI and cyt b gene regions of individuals belonging to the *C. auratus* species, which live invasively in Atatürk Dam Lake and whose number of individuals have increased recently, were performed for the first time and compared with the data in the gene bank. An average of 600 bp (Figure 1) and 570 bp long sequences were obtained for the mtDNA COI and cyt b gene regions, respectively. Similarities were revealed by BLASTing these sequence results (Table 1).

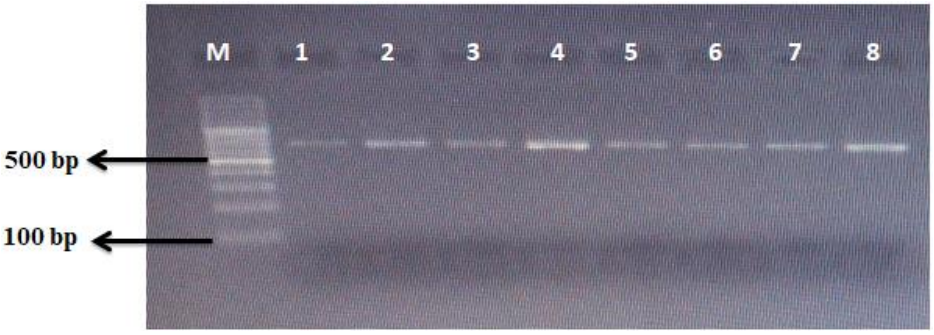

Fig 1. Image of mtDNA COI PCR Products (M: Marker; bp: Base Pair)

Table 1 Comparison of the sequences obtained in this study with the sequences in the NCBI database

Marker	Country	Access Number	Similarity Rate (%)	Source
COI	Bangladesh	MN171366.1	100	Unpublished
	Chinese	MF443771	100	Unpublished
	Chinese	KJ874430	100	Unpublished
	India	MN562051	100	Unpublished
	Japan	AB379921	100	Komiyama et al., 2009 [23]
	Australia	KF227945	100	Loh ve ark., 2014 [24]
	Korean	HQ536310	100	Unpublished
	United States	KF558297	100	Brandl et al., 2015 [25]
cyt b	America	MG281934	100	Halas et al., 2018 [26]
	Germany	KX688782	100	Knytl et al., 2013 [27]
	England	JN412524	100	Rylková et al., 2013 [28]
	Japan	AB379921	100	Komiyama et al., 2009 [23]
	Chinese	MT612437	100	Unpublished

In Table 1, the sequences of both mtDNA markers obtained from the *C. auratus* species caught from Atatürk Dam Lake are shown with 100% similarity to the sequences in the

Genbank. Accordingly, it is possible to say that this fish species is the same in terms of both markers in different continents and countries, does not show a different variation, and has recently spread. In other words, no different variation or mutation was detected between the fish in our study and the fish belonging to the same species in different countries shown in Table 1. It shows 100% similarity in terms of both markers. Thus, it raises the idea that all of these fish originated from the same ancestral fish. However, since the gene exchange is interrupted due to the difference in the environment, there is a high probability that different mutations and variations will occur in the future.

Biological invasions have caused significant disruption to natural ecosystems around the world through habitat change, competition and hybridization with native species. Especially species of the Carassius genus such as *C. auratus*, *C. carassius,* and *C. gibelio* have been transported to many inland water bodies throughout Turkey and are seen as a threat factor for native species [7]. The effects of these fish species, which form a continuous population, on local species and especially endemic species in the habitats should be investigated and awareness should be increased to prevent the human-induced spread of exotic species [8, 29]. In addition, in fisheries studies, only target species should be included in the study. Because it is seen that six aquarium fish species are spread in Turkish waters [8]. If species other than these aquarium fish enter the reservoir, it will be inevitable that natural species will be seriously affected. It will cause greater damage both economically and in terms of biodiversity.

In the study of Tarkan et al., (2012), the density of the *Carassius gibelio* species living in Ömerli Dam Lake in the Marmara Region, which was followed for six years, and the density of native and economic species were studied [30]. On the contrary, it was found to increase. It is estimated that similar results occurred for both *C. gibelio* and *C. auratus* in Atatürk Dam Lake. Therefore, a serious control program should be organized against invasive species. In particular, it should be ensured that the populations that are concentrated in the dam lake are detected and removed from the environment as much as possible before they enter the breeding period.

Conclusion

Since the reproductive capacity of invasive fish species is very high, the number of individuals is increasing rapidly. Therefore, in future studies, populations of *C. auratus* species should be determined along the Atatürk Dam Lake and Euphrates river, genetic

diversity levels of populations should be determined by using marker systems such as mtDNA D-loop and microsatellite, and the struggle should be started quickly to start with the population with the highest diversity recommended.

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