



Phylogenetic analysis of *Luciobarbus* Heckel, 1843 and *Barbus* Cuvier & Cloquet, 1816 species in the Euphrates River (Turkey) based on mtDNA COI gene sequences

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ABSTRACT

Natural fish species living in the Euphrates River System; It is subject to some pressures such as overfishing, competition with invasive species and habitat loss. As a result of these pressures, it leads to the decrease of endemic and native species. At the beginning of these species are the species belonging to the *Barbus* Cuvier & Cloquet, 1816 and *Luciobarbus* Heckel, 1843 genera, which have high economic importance. In this study, phylogenetic analysis of the species belonging to the genus *Barbus* Cuvier & Cloquet, 1816 and *Luciobarbus* Heckel, 1843, which live naturally in the Euphrates River, was carried out with mtDNA COI gene sequences. 17 fish samples belonging to five species from three localities belonging to the Euphrates river system (Turkey) were studied. Total DNA extraction was performed from muscle tissue using Commercial Kit. Then the mtDNA COI region was amplified by PCR and sequenced. Genetic distance values were calculated between 0.00201 and 0.15332, and it was determined that the closest species were *L. xanthopterus* and *L. esocinus*, and the most distant species were *B. lacerta* and *A. grypus*. In addition, phylogenetic analyzes of the target species were made and an phylogenetic tree was formed and the species were distinguished. In future studies, it is recommended to evaluate the data in this study, to determine the genetic characteristics of populations, and to carry out conservation studies at the population level.

Keywords: *Luciobarbus*, *Barbus*, mtDNA COI, Phylogenetic, Euphrates River

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Introduction

Populations in aquatic habitats are often threatened by the effects of human activities such as pollution, harvesting, fishing, alien species, tourism and urban expansion (Cognetti and Maltagliati, 2000). The destruction or change of habitats can lead to decreases in populations and species diversity and even the extinction of some species. The decline of individuals in natural populations may cause the disappearance of unique genotypes that cannot be found anywhere else, and when this genetic information is lost, it is almost impossible to recover (Parmaksız, 2020; Parmaksız, 2021). Genetic diversity is estimated to decrease faster than species diversity under increasing threats, but its spatial distribution remains poorly documented on a global scale (Manel et al., 2020). Genetic diversity directly reflects the ability of species or populations to adapt to environmental factors of alien environments (Frankham et al., 2002; Spielman et al., 2004).

Natural fish species in the Euphrates River System are exposed to pressures increasing day by day due to factors such as overfishing, dominance of invasive species and habitat loss. Invasion of freshwater ecosystems by alien fishes can have significant consequences for natural biodiversity, including local extinctions of endemic and native species (Gozlan et al. 2010; Jackson et al. 2017; Mollot et al. 2017). Recently, invasive species such as *Carassius gibelio* (Bloch, 1782) and *Carassius auratus* (Linnaeus, 1758) pose a great threat to native species in the Euphrates River (Turkey). Due to these dangers, the number of individuals, especially in the populations of economic species, is decreasing which consequently causes species loss. These species which have high economic value mainly belong to the *Barbus* Cuvier & Cloquet, 1816 and *Luciobarbus* Heckel, 1843 genera. Some of these species found in the Euphrates River (Turkey) and the dam lakes built on it are caught and sold by the fishermen of the region and sent to the neighboring cities. Ensuring the continuity of the populations of these species is very important both in terms of biodiversity and economy. Therefore, the identification of the species and their genetic structure of the populations is a matter that needs to be addressed with the utmost urgency.

Since the genus *Barbus* Cuvier & Cloquet, 1816 was separated from the genus *Luciobarbus* Heckel, 1843 very recently, there are usually problems in naming the species (Korkmaz, 2017). Morphological characters are widely used in studies such as identifying differences in fish taxonomy. In addition, studies in the recent years demonstrate that molecular data has been very successful in identifying species and that DNA barcoding is an essential marker for species identi-

fication (Rock et al., 2008). Advances in sequencing techniques have popularized the mtDNA (Liu and Zhou, 2016) which is widely studied as a significant data for predicting the genetic makeup of living things (Xu et al., 2011). Analysis of the mtDNA-*COI* region can be used as a reliable marker to identify fish species (Ward et al., 2005).

The aim of this study is to determine *Barbus* and *Luciobarbus* species based on mtDNA *COI* in Euphrates river basin in Turkey and revealing the status of the species in the dendrogram created based on this information. To observe genetic similarity between species, a dendrogram is usually prepared using a clustering algorithm. Being on the same branch in the phylogenetic tree reflects its genetic similarity.

Material and Methods

The fish samples used as material in this study were purchased from the fishermen of two locations on the Euphrates river system, and carried in an ice container when they were brought to the Zoology Laboratory of the Faculty of Science and Letters of Harran University. After the species were identified, muscle tissue was taken from the samples and placed in microcentrifuge tubes containing 90% ethanol and kept at -20°C until DNA was obtained.

Total DNA isolation was obtained from muscle tissue using the GeneJET Genomic DNA Purification Kit (Thermo Scientific). In order to check the presence of DNA after the protocol, DNA samples of all individuals were placed in the wells of 1% agarose gel added to SYBR Green, carried out in electrophoresis and visualized in a (UV) light-emitting device (Smart View Pro Imager System, Major Science). The primer used for amplification of the mtDNA *COI* gene region was adopted from Darabi et al. (2014) and PCR was applied.

PCR process was carried out with BIO-RAD T100TM Thermal Cycler device. For the PCR procedure, a total of 34 cycles were performed, including 3 minutes of initial denaturation at 95°C, 30 seconds of denaturation at 95°C, 30 seconds of bonding at 62°C, and 45 seconds of elongation at 72°C. The procedure was completed with keeping the samples at 72°C it for 10 minutes. The obtained PCR output was sent to a commercial firm for sequence analysis which was performed on the 3500 XL Genetic Analyzer (Thermo Fisher Scientific).

The raw data of the mtDNA *COI* sequences procured from the commercial company were evaluated using the ChromasPro v 2.0.1 (Technelysium Pty Ltd) program and converted into FASTA format. Sequences of all individuals in FASTA format were aligned using the BioEdit software version 7.2.5 program. Phylogenetic analyses between species

were carried out in the MEGA X program according to the Neighbor-joining tree model using the K2 parameter and a phylogenetic tree was created (Kumar et al., 2018). Bootstrap

test (1000 replicates) was applied to test the reliability of tree branches (nodes).

Table 1. Information on the fish species studied in the research

Fish No	Species Name	Location	Date
1	<i>Luciobarbus xanthopterus</i> Heckel, 1843	Adiyaman	September 2020
2	<i>Luciobarbus xanthopterus</i> Heckel, 1843	Adiyaman	September 2020
3	<i>Luciobarbus xanthopterus</i> Heckel, 1843	Adiyaman	September 2020
4	<i>Luciobarbus kersin</i> (Heckel, 1843)	Adiyaman	September 2021
5	<i>Luciobarbus kersin</i> (Heckel, 1843)	Adiyaman	September 2021
6	<i>Luciobarbus kersin</i> (Heckel, 1843)	Adiyaman	September 2021
7	<i>Arabibarbus grypus</i> (Heckel, 1843)	Adiyaman	September 2020
8	<i>Arabibarbus grypus</i> (Heckel, 1843)	Adiyaman	September 2020
9	<i>Arabibarbus grypus</i> (Heckel, 1843)	Adiyaman	September 2020
14	<i>Luciobarbus esocinus</i> Heckel, 1843	Adiyaman	September 2020
15	<i>Luciobarbus esocinus</i> Heckel, 1843	Adiyaman	September 2020
16	<i>Luciobarbus esocinus</i> Heckel, 1843	Adiyaman	September 2020
17	<i>Luciobarbus esocinus</i> Heckel, 1843	Şanlıurfa-Bozova	October 2020
18	<i>Luciobarbus esocinus</i> Heckel, 1843	Şanlıurfa-Bozova	October 2020
19	<i>Luciobarbus esocinus</i> Heckel, 1843	Şanlıurfa-Bozova	October 2020
20	<i>Barbus lacerta</i> Heckel, 1843	Adiyaman-Gölbasi	July 2020
21	<i>Barbus lacerta</i> Heckel, 1843	Adiyaman-Gölbasi	July 2020

Results and Discussion

In this study, the mtDNA *COI* gene region of individuals of *Luciobarbus*, *Barbus* and outgroup *Arabibarbus grypus* species in the Euphrates River, whose number of individuals have decreased considerably, were studied by conducting an average of 603 bp region sequence analysis, and phylogenetic analysis of the species were imaged by using the "Finch TV" program (Figure 1). A total of 106 polymorphic regions were identified for this region. The mean genetic distances between the species were calculated in the MEGA X program (Kumar et al., 2018) and are shown in Table 2.

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

Species Name	Accession No	Per. Ident %
<i>Luciobarbus xanthopterus</i>	KM590446	99.83
<i>Luciobarbus kersin</i>	MF599072	100
<i>Arabibarbus grypus</i>	KM590450	100
<i>Luciobarbus esocinus</i>	MF599073	100
<i>Barbus lacerta</i>	MF106166	100

In Table 2, similarity values are given by comparing the haplotypes of the mtDNA *COI* region of different species obtained in this study with the haplotypes in the NCBI GenBank

with Blast method. Information on species showing maximum similarity is presented. *Luciobarbus xanthopterus* species exhibits a different haplotype and the sequences of the other species studied are available in the GeneBank.

Genetic distance values of five species were calculated between 0.00201 and 0.15332 by analyzing according to the genetic distance estimation based on the Kimura parameter model. According to these calculations it was determined that the closest species were *L. xanthopterus* and *L. esocinus*, and the most distant species were *B. lacerta* and *A. grypus*.

In this study, the mtDNA *COI* region sequences and the neighbor joining tree were created with the MEGA X program as well (Kumar et al., 2018). The obtained NJ tree is given in Figure 2.

In Figure 2, it is seen that *A. grypus* is located on a separate branch, unlike other species. While *Barbus lacerta* species is located closer to *Luciobarbus* species, *Luciobarbus* species appear on separate branches at the species level. Although individuals belonging to the *Luciobarbus esocinus* species are close, they are divided among themselves because they were collected from two different localities and have different haplotypes

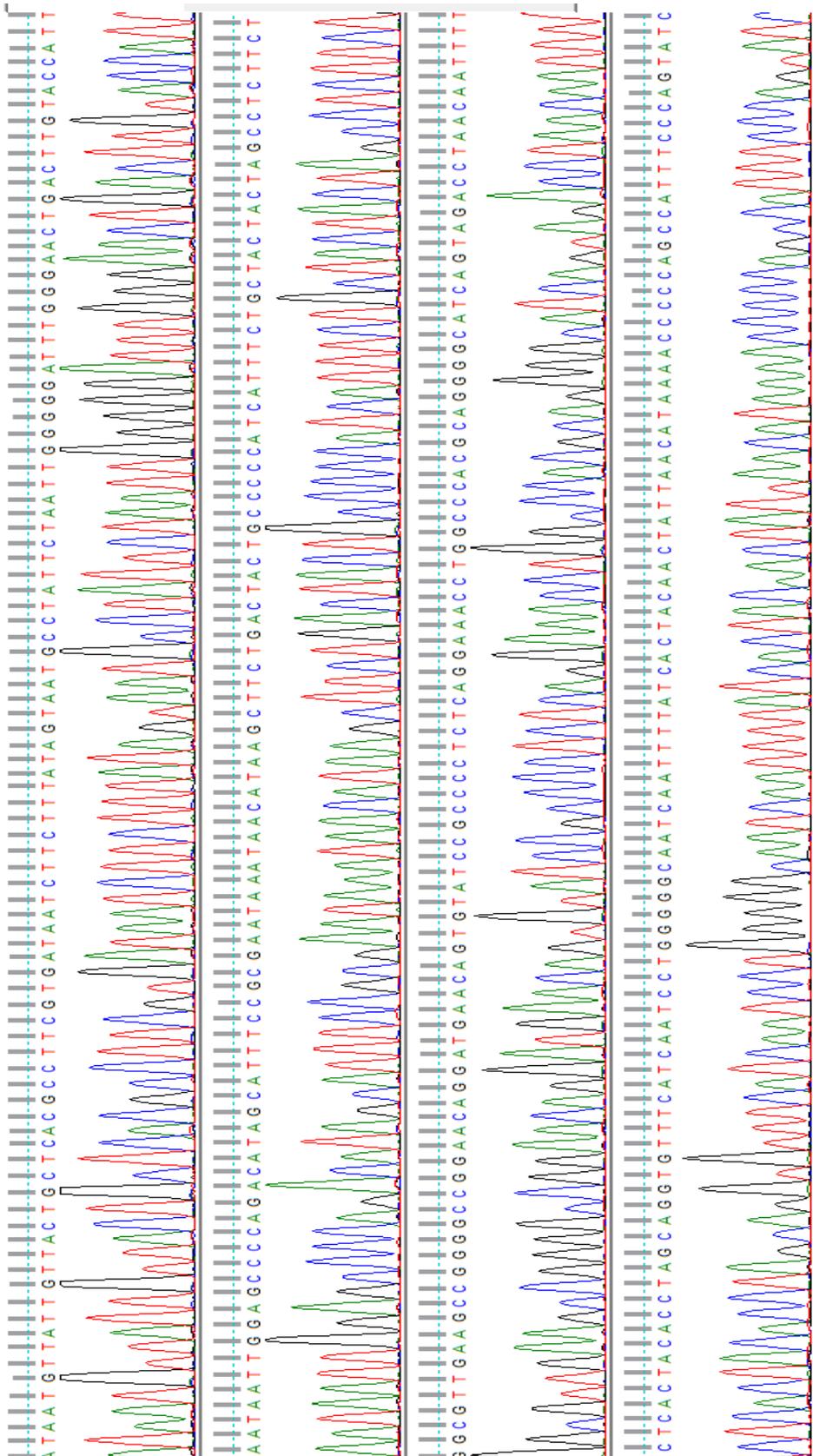


Figure 1. Chromatogram image of an exemplary sequence analysis of the mtDNA *COI* region.

Table 3. Means of genetic distance between studied species

Species	<i>L. xanthopterus</i>	<i>L. kersin</i>	<i>L. esocinus</i>	<i>B. lacerta</i>	<i>A. grypus</i>
<i>L. xanthopterus</i>	-				
<i>L. kersin</i>	0,02922	-			
<i>L. esocinus</i>	0,00201	0,02783	-		
<i>B. lacerta</i>	0,09258	0,10452	0,09108	-	
<i>A. grypus</i>	0,15107	0,13400	0,14947	0,15332	-

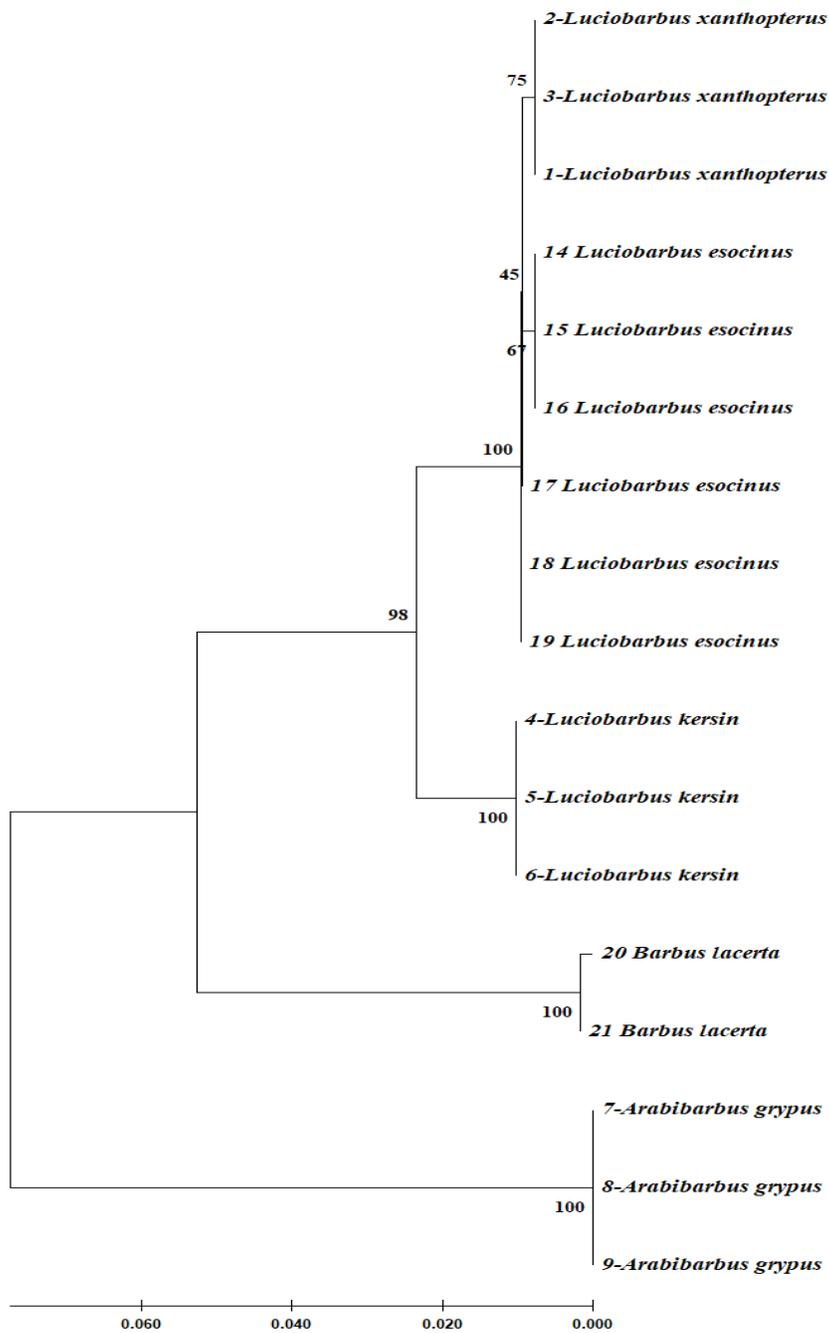


Figure 2. Neighbor Joining (NJ) tree of 5 species based on mtDNA *COI* sequences

Human activities have caused significant changes in the physical, chemical and biological composition of the Euphrates River systems. In addition, environmental factors such as industrial activities, intensive fishing and destruction of habitats will lead to the extinction of many species or the decrease of their populations (Kuru, 1986; Ünlü et al. 1997). Conservation of population size and genetic diversity is essential for the survival of the species. The decrease in the population results in deterioration of genetic diversity and poses a threat to survival of the population (Parmaksız, 2021). One of the most important things to be done in the study of populations is to differentiate the species genetically and morphologically. Once the species has been identified, the status of the populations should be determined and steps should be taken for future conservation strategies and habitat management of the target species. Especially in some localities unless the necessary precautions are taken, the level of genetic diversity will decrease, resulting in the degeneration of the feeding, reproduction, competition and adaptation abilities of the populations and the target organism will face the danger of extinction (Parmaksız, 2021).

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Conclusion

In this study, species whose numbers of individuals have decreased considerably due to environmental factors such as overfishing and habitat degradation in the Euphrates River systems were targeted for phylogenetic analysis and species differentiation. In future studies, it is recommended to determine the genetic characteristics of populations and to carry out conservation studies at the population level.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Ethics committee approval is not required for this study.

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