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Mitochondrial DNA sequence analysis of *Arabibarbus grypus* (Heckel, 1843) living in Great Zab (Erbil, Iraq)

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ABSTRACT

Arabibarbus grypus (Heckel, 1843), which naturally lives in the Euphrates and Tigris River systems and is endemic, is an economically important fish species consumed by humans. Since the population of this species, which is hunted by both fishermen and local people, is decreasing day by day, its genetic characteristics need to be determined. This study aims to determine the genetic characteristics of *A. grypus* samples living in Great Zab based on sequence analysis of mtDNA *cyt b* and mtDNA *d loop* gene regions. Total DNA isolation was performed from muscle tissue using the kit. Then, Polymerase Chain Reaction was applied through specific primers to mtDNA *cyt b* and mtDNA *d loop* gene regions, and the target regions were amplified. The products with the target length were sent to the commercial firm and sequence analysis was performed. Regarding these specimens living in Great Zab, two haplotypes were determined for the mtDNA *cyt b* gene region and five haplotypes for the mtDNA *d loop* gene region. These haplotypes were compared with the haplotypes in the gene bank and the results were evaluated. Some important data has been obtained regarding the conservation and management of this fish species. Since three new haplotypes were detected for the *d loop* region in this studied locality, it is important to include the samples in this locality in conservation studies.

Keywords: *Arabibarbus grypus*, mtDNA, Euphrates River, Tigris River

Introduction

Arabibarbus grypus (Heckel, 1843) is a member of the Cyprinidae family and is one of the most valuable fish in the world in terms of meat quality (Olgunoglu et al., 2011; Moradkhani et al., 2020). This fish, popularly known as Şabut (Shabout) fish, is an endemic species living in the Euphrates and Tigris river systems in Türkiye, Iraq, Iran, and Syria (Nikpey, 1996; Abdoli, 2000; Göçer, 2022). There are many studies on *A. grypus* living in this basin, some of which are as follows; growth, reproduction and heavy metal characteristics (Oymak et al., 2008; 2009); spermatological and hematological features (Dogu et al., 2014; Khodadadi et al., 2016), genetic features (Parmaksız et al., 2017; 2018). The delicious meat of the *A. grypus* species causes it to be preferred by the local people and increases its economic importance. Therefore, there is excessive hunting pressure on the populations of this species and it is known that its stocks are decreasing day by day (Parmaksız and Şeker, 2018). This species is sensitive in the "VU" (Vulnerable/Sensitive) category according to the 2020 IUCN criteria (Bayhan, 2021). Both the protection of these diminishing resources and the cultivation of this species have gained importance today. However, no measures have yet been taken to protect this species. To be evaluated, especially in terms of production, the individuals to be raised must adapt to the environmental conditions, be resistant to diseases, and the meat quality must be at an optimum level. Populations that will provide these characteristics must also have high genetic diversity. Therefore, first of all, populations in different locations should be evaluated genetically, and the similarities, differences and genetic diversity levels of the populations should be revealed. There are many genetic markers used in genetic studies, but in recent years, mtDNA markers have become popular with the developments in sequence analysis (Liu and Zhou, 2016). Therefore, mtDNA is used for population genetics in different species and can provide information about genetic structure.

In this study, it was aimed to establish genetic data by sequence analysis of mtDNA *cyt b* and mtDNA *d loop* gene regions in *A. grypus* individuals living in Great Zab, near Erbil province in Northern Iraq, and to compare them with the data in the gene bank to determine similarities and differences. Thus, an important data set will be created in terms of providing some of the preliminary information necessary for management and conservation studies.

Material and Methods

The material for this study was created by purchasing nine randomly selected fish from the fish caught by the fishermen living in the Great Zab close to the Erbil region. From these samples, 1 g of the muscle tissue at the base of the dorsal fins was taken and placed in microcentrifuge tubes containing 90% ethyl alcohol and stored in the refrigerator at +4°C until DNA isolation.

In this study, total DNA isolation was performed from muscle tissue using the GeneJET Genomic DNA Purification Kit (Thermo Scientific). Total DNA was obtained by applying the protocol in this kit. To control the presence of DNA, 2 µl of DNA samples from each individual were taken, and 2 µl of dye (2x loading dye) was added to 1% agarose gel added to SYBR Green, and placed in a tank containing 0.5x TBE (Tris/Boric acid/EDTA Buffer) solution. It was run at 120 V electrophoresis for 30 minutes and visualized in the device emitting ultraviolet (UV) light (Figure 1a).

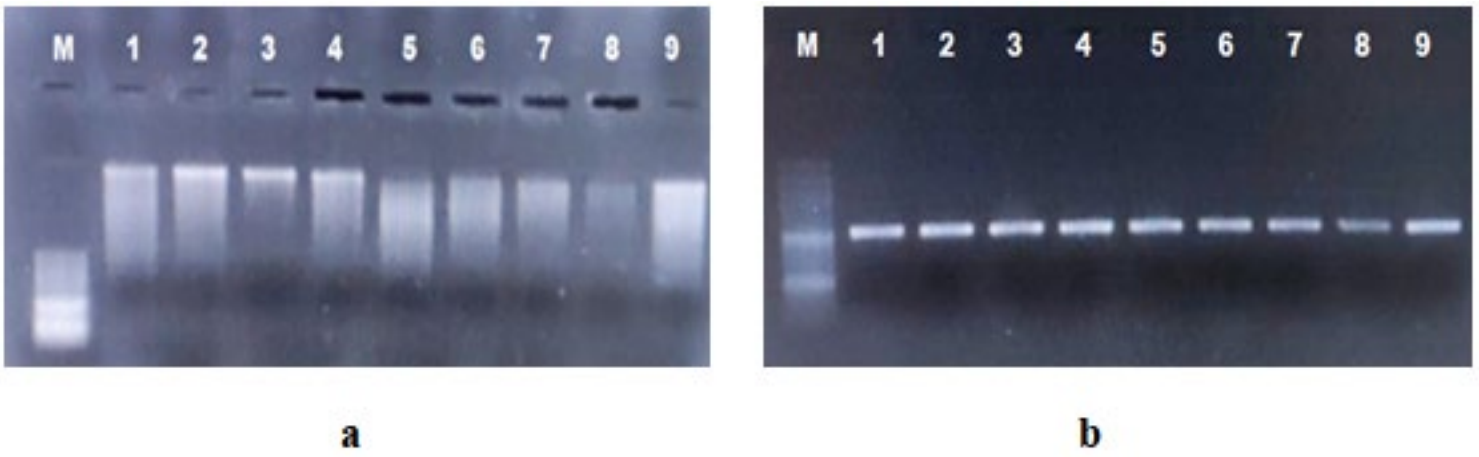

Figure 1. Individuals of the *A. grypus* species: a) Total DNA, b) Agarose gel image of mtDNA *cyt b* PCR products (M: Marker)

In this study, the Polymerase Chain Reaction (PCR) process was carried out in a thermal cycler (BIO-RAD T100™) device. The primer sequence used for the mtDNA *cyt b* gene region was taken from Briolay et al. (1998), and all PCR chemicals and conditions were made according to the criteria in the Parmaksız and Şeker (2018) study. The primer sequence used for amplification of the mtDNA *d loop* region is Iguchi et al. (1997), taken from Inoue et al. (2000) studies, and all PCR chemicals and conditions were adapted according to Oymak and Parmaksız (2018) study. PCR conditions; 3 minutes initial denaturation at 95 °C, and 35 cycles of 30 seconds at 95°C for denaturation, 30 seconds at 58°C for *cyt b* and 51°C for *d loop* annealing and 45 seconds at 72 °C for extension, and a final extension at 72 °C for 10 minutes. PCR mixture used to amplify *cyt b* and *d loop* regions are as follows; a total volume of 25 µL containing 1x PCR buffer, 1 unit Taq polymerase, 2.5mM MgCl2, 0.5 mM of each primer, 0.2 mM of each dNTP, and approximately 50 ng of template DNA. A 1.5% agarose gel was used to control the products formed after the PCR process. The agarose gel added to SYBR Green was placed in the tank containing 0.5x TAE solution and loaded into the wells with 5 µl of PCR products and 5 µl of dye, then run at 120 V electric current for 30 minutes and displayed on a UV light-emitting imaging device (Figure 1b). Target-length PCR products were sent to a commercial firm and sequence analysis was performed on the 3500 XL Genetic Analyzer (Thermo Fisher Scientific).

Data Analysis

Raw data of mtDNA *cyt b* and mtDNA *d loop* sequences were evaluated using the FinchTV 1.4 program, and sequences of all individuals were aligned using BioEdit software version 7.2.5. Those with the highest similarity to the sequences of the *cyt b* and *d loop* regions found in the NCBI Genbank were included in this study. Relationships between haplotypes and Neighbor-joining tree phylogenetic analyzes were performed in the MEGA X program according to the K2 parameter model and the phylogenetic tree was created (Kumar et al., 2018). A bootstrap test (1000 replicates) was used to test the reliability of tree branches (Nodes).

Results and Discussion

Sequences of the mtDNA *cyt b* region with an average length of 580 bp were obtained at the end of this study and the same species in the NCBI gene bank were evaluated together and are shown in Table 1.

As a result of the analysis of the sequences obtained from the *cyt b* gene region, it was seen that the samples taken from the Erbil locality consisted of individuals with H1 and H2 haplotypes. As a result of the analyzes of all individuals, a total of five variable regions and five haplotypes were determined for the cyt b gene region. The H1 haplotype has been seen in all countries and all localities, including Türkiye, Iraq, Iran, and Syria, and is the ancestral haplotype. The phylogenetic tree of haplotypes is shown in Figure 2.

In the phylogenetic tree created according to the analyzed *A. grypus cyt b* haplotypes, kinship relationships for a total of five haplotypes emerged. Accordingly, it is seen that the haplotypes H4 - H5 and H2 - H3 are located closer to each other.

The analysis of the sequences obtained from the *d loop* gene region, it was seen that the samples taken from the Erbil locality consisted of individuals with H1, H2, H3, H4 and H5 haplotypes. As a result of the analyzes of all studies, a total of twelve variable regions and seven haplotypes were determined for the *d loop* gene region (Table 2). The H4 haplotype was seen in all localities, including Türkiye and Iraq, and it can be said to be the ancestral haplotype. The phylogenetic tree of haplotypes is shown in Figure 3.

Table 1. Haplotypes and information about the mtDNA *cyt b* region of *A. grypus* species

Haplotype	Accesion Number	Observed Locations	Reference
H1	ON921337	Erbil, Siverek, Bozova, Çermik, Dicle; Iraq, Türkiye	Parmaksız and Şeker, 2018
	KF876028	Dayr az Zawr; Syria	Borkenhagen, 2014
	KF876025	Rūd-e Mand; Iran	Borkenhagen, 2014
	AF145945	Adıyaman, Diyarbakır; Türkiye	Durand et. al., 2002
	KF876027	Rūdkhāneh-ye Kheyrābād; Iran	Borkenhagen, 2014
H2	ON921338	Erbil, Dicle; Iraq, Türkiye	Parmaksız and Şeker, 2018
H3	ON921339	Bozova; Türkiye	Parmaksız and Şeker, 2018,
	KF876026	Rūdkhāneh-ye Kheyrābād; Iran	Borkenhagen, 2014
H4	ON921340	Çermik, Dicle; Türkiye	Parmaksız and Şeker, 2018
H5	ON921341	Dicle; Türkiye	Parmaksız and Şeker, 2018

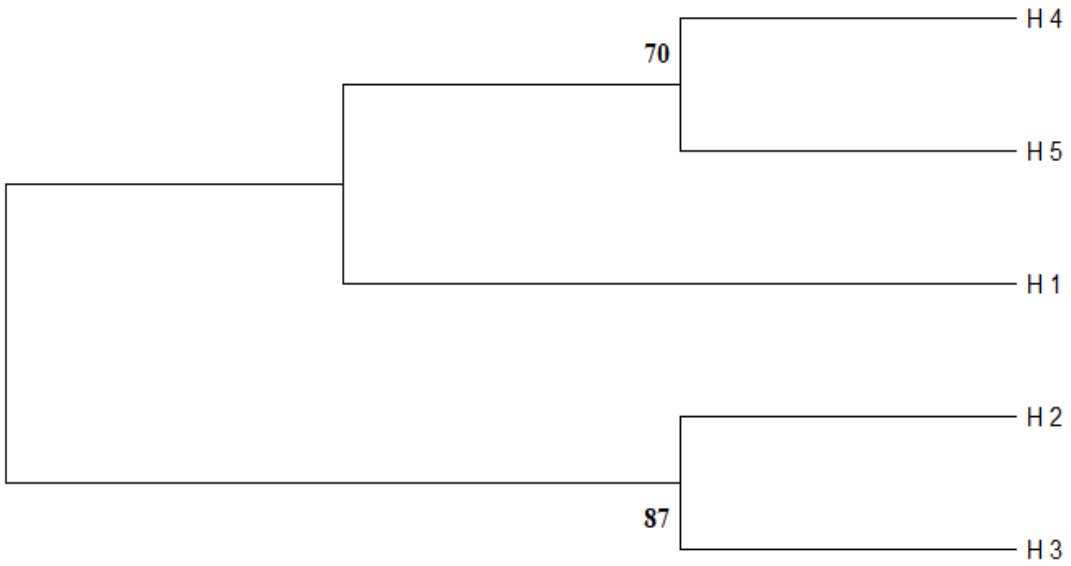

Figure 2. A neighbor-joining tree of *A. grypus* based on observed haplotypes of *cyt b* gene sequences

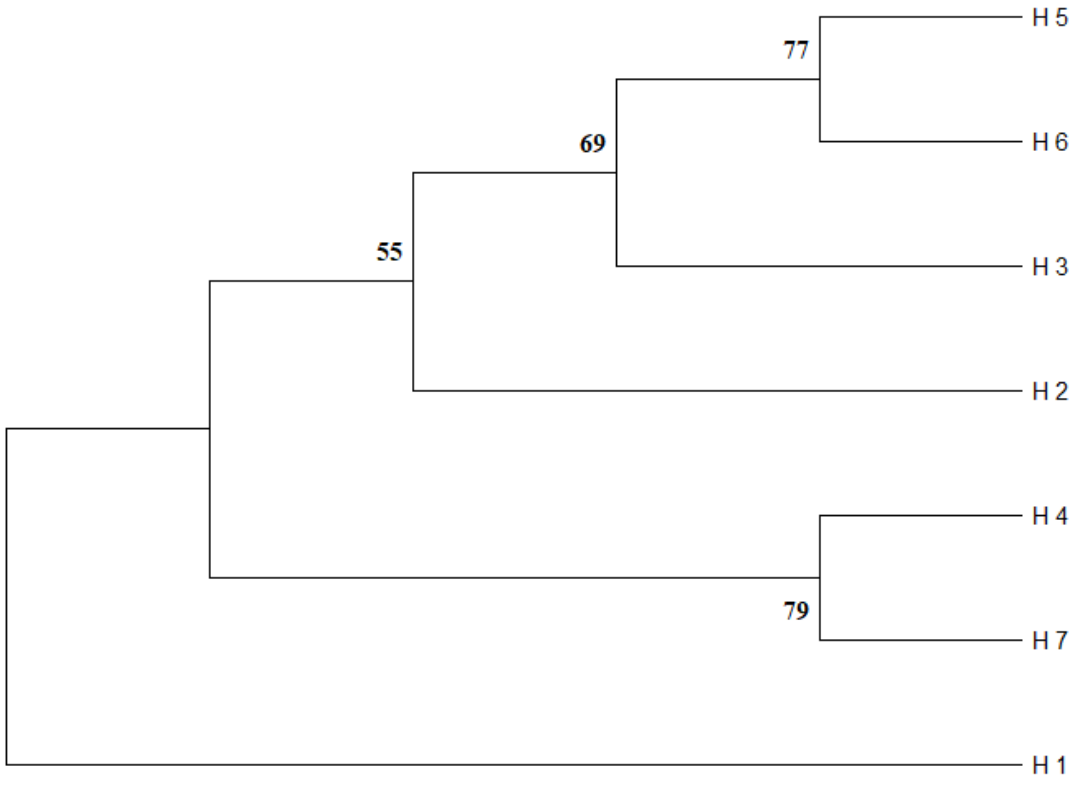

Figure 3. A neighbor-joining tree of *A. grypus* based on observed haplotypes of *d loop* gene sequences

Table 2. The haplotypes and information from the mtDNA *d loop* region of the *A. grypus* species

Haplotype	Accesion Number	Observed Locations	Reference
H1	OP234423	Erbil, Dicle; Iraq, Türkiye	This study, Oymak and Parmaksız, 2018
H2	OP234424	Erbil; Iraq	This study
H3	OP234425	Erbil; Iraq	This study
H4	OP234426	Erbil, Siverek, Bozova, Çermik, Dicle; Iraq, Türkiye	This study, Oymak and Parmaksız, 2018
H5	OP234427	Erbil; Iraq	This study
H6	OP234428	Bozova; Türkiye	Oymak and Parmaksız, 2018
H7	OP234429	Dicle; Türkiye	Oymak and Parmaksız, 2018

In the phylogenetic tree created according to the analyzed *A. grypus d loop* haplotypes, a total of seven haplotypes were related to each other. Accordingly, it is seen that haplotypes H5-H6 and H4 -H7 are located closer to each other.

The Great Zab locality, which was taken as an example in this study, is close to the city of Erbil and this fish species is caught by fishermen and local people. The local people consume the fish caught. Since the populations of these endemic fish species are decreasing day by day, breeding has gained great economic importance. To maintain the stocks of this fish species, which is, considered an alternative to carp or trout in inland fish farming (Gökçınar, 2010), its genetic diversity should be known very well to obtain high yields from these stocks. Because the degree of genetic diversity can be an indicator of the continuity of the population. For the future of this species, the unique genetic heritage found in different localities must be protected by taking necessary precautions. In addition, in the case of breeding this fish species, it is of great importance to take broodstocks from all localities and to create a population that will accommodate each haplotype. Although there is a no different genetic structure in terms of *cyt b* haplotype in the samples taken from the Erbil locality, it is noteworthy that the H2, H3 and H5 haplotypes for the *d loop* region are only in this region. Since phylogenetic analyzes were generally performed on this species, it was possible to find sequences related to *cyt b* in the gene bank. Since the *d loop* region is generally used in studies on population genetics and population genetics is limited for this species, it is expected that the *d loop* data in the gene bank will be low. For the *d loop* region detected only in Erbil, there is a strong possibility that some haplotypes will be found in similar localities if other localities are also studied.

Conclusion

The destruction or change of habitats as a result of anthropogenic effects, especially in localities close to city centers, may cause both populations and species diversity to decrease and even some species to disappear (Parmaksız et al., 2022). The decline of individuals in their natural populations can cause the disappearance of unique genotypes found nowhere else, and when this genetic information is lost, it is almost impossible to recover it (Parmaksız, 2020; 2021). Therefore, urgent measures should be taken for all factors that reduce the genetic diversity of susceptible species. Because genetic diversity directly reflects the ability of species or populations to adapt to environmental factors in alien environments (Frankham et al., 2002; Spielman et al., 2004). Therefore, it will be more beneficial for future studies to determine the genetic diversity of all populations of this fish, especially by using *d loop* and microsatellite markers, and to carry out detailed conservation and brodstock studies based on this data.

Compliance with Ethical Standards

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

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

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