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GENETIC DIVERSITY OF THE ENDEMIC SPECIES SHABBOUT (*Arabibarbus grypus* (HECKEL, 1843)) BASED ON PARTIAL CYTOCHROME B SEQUENCES OF MITOCHONDRIAL DNA

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ABSTRACT

Arabibarbus grypus (Heckel, 1843), a species endemic in river systems of Euphrates and Tigris, is an economically important freshwater fish. In this study, the genetic diversity of *Arabibarbus grypus* populations was determined basen on partial cytochrome b gene sequence of mtDNA. Totally 31 samples were collected from four localities and five polymorphic sites and five haplotypes were identified by carrying out mtDNA analysis. Mean haplotype (Hd) and nucleotide diversity (π) were calculated to be 0.348 and 0.00144 respectively. All values obtained following neutrality tests were found to be negative and statistically insignificant. Median joining network revealed that haplotype H1 was at the center of the network and was dominant. In the current survey, certain haplotypes (H2, H4, H5) identified for mtDNA cytochrome b gene are the new results to the literature and presented a novel data set for genetic diversity of this species.

Keywords: *Arabibarbus grypus*, Cytochrome b, Genetic diversity, Euphrates River, Tigris River

Introduction

Euphrates and Tigris Rivers are from important natural sources of fish diversity and fishing and also possess a considerable potential for meeting the need of food. Developed countries have started to conduct overall studies about species particularly with economic importance following the classification of fish in inland waters (Kaya, 2012). A majority of fish species inhabiting in basin of Euphrates and Tigris belongs to the family Cyprinidae. Because several species of this family are consumed for food, they possess economic importance (Parmaksız et al., 2016). The fish preferred most by local people thanks to its delicious meat is *Arabibarbus grypus* (Shabbout). The fish distributed in Iran, Turkey, Syria, and Iraq is an endemic species thriving in river systems of Euphrates and Tigris (Nikpei, 1996; Abdoli, 2000; Khodadadi et al., 2016). Endemic fish species of fish are important in terms of ecological aspects and assumed as gene banks of an ecosystem (Khodadadi et al., 2016).

Some studies conducted on this species include age, growth, and reproductive traits (Oymak et al., 2008); heavy metal concentration in tissues (Oymak et al., 2009); determination of spermatological and hematologic characteristics (Dogu et al., 2014); the relationships between sagittal otolith size and length of the fish (Dusukcan et al., 2015); investigation for concentration of mercury in edible muscle tissues (Asefi and Zamani-Ahmadm Mahmoodi, 2015); the effects of probiotics derived from *Lactobacillus* species on immunologic parameters of Shabbout (Mohammadian et al., 2016); sperm morphology, motility and composition of seminal plasma parameters (Khodadadi et al., 2016); determination of genetic diversity utilizing from gene sequences of mtDNA COI (Parmaksız et al., 2017).

Despite Oymak et al., (2009) stated in their study that Shabbout was abundant in the Euphrates, the number of individuals has decreased recently due to overhunting. It is crucial to know well about genetic diversity of this fish to ensure continuity of stocks and to obtain high yield from these stocks of the fish which is considered as an alternative to carp or trout for inland water fish farming (Gokcinar, 2010). There are several genetic markers based on DNA, however mtDNA studies have been made popular by developments of sequence analysis in recent years (Liu and Zhou, 2016). mtDNA, as an important and common molecular marker, has been used widely to estimate molecular variability and population genetics of numerous organisms (Xu et al., 2011). Different mtDNA gene sequences can be used to determine the variation in fish (Saraswat et al., 2014). Diversity in mtDNA cyt b gene is suitable for population genetic studies in cyprinid fishes (Fayazi et al., 2006).

The aim of this research is to determine genetic diversity of *A. grypus* populations in Euphrates and Tigris rivers via sequence analysis for mtDNA Cyt b fragment.

Materials and Methods

Collection of fish samples: A total of 31 individuals (15 from Euphrates and 16 from Tigris River) were collected via fishing method. 2 g of specimen was dissected from muscle tissue on the base of pectoral or dorsal fins of fish samples, held in refrigerator at 4°C inside micro centrifuge tubes with 1.5 mL volume, containing 95% ethanol until DNA isolation process.

DNA isolation: Total DNA was isolated from muscular tissue using GeneJET Genomic DNA Purification Kit (Thermo Scientific). Total DNA was obtained by practiced the protocol for the kit. To control the existence of DNA, 2 µl was taken from DNA samples of each individual, placed in to tank including 0.8% agarose gel, 0.5xTBE (Tris/Boric acid/EDTA Buffer) solution with the addition of 2 µl of stain (3x Loading dye) and SYBR Green, run in electrophoresis at 120 Volts for 30 minutes, then viewed in device giving off ultraviolet (UV) light (SmartView Pro Imager System, Major Science).

Amplification of target mtDNA site via polymerase chain reaction (PCR): Primers used for amplification of mtDNA Cytochrome b gene in the study (Briolay et al., 1998) were given below:

L15267: 5'-AATGACTTGAAGAACCACCGT-3'

H15891: 5'-GTTTGATCCCGTTTCGTGTA-3'

The PCR amplification process was carried out in a BIO-RAD T100™ Thermal Cycler under the following conditions: 3 minute at 95°C for initial denaturation and 30 seconds at 95°C for the second denaturation, 30 seconds at 58°C for annealing, 45 seconds at 72°C for extension, 35 cycles in total and a final extension at 72°C for 10 minutes. The amounts of DNA, concentrations of chemicals, and annealing temperatures of primers used in PCR amplification reactions were optimized by gradient PCR device. PCR mixture used in order to amplify this gene is as follows; a total volume of 25 µL containing 0.5 mM of each primer, 0.2 mM of each dNTP, 1x PCR buffer, 2.5mM MgCl₂, 1 unit Taq polymerase and approximately 60 ng of template DNA.

2% agarose gel was used to control final products of PCR process. Agarose gel which was included SYBR Green was run at 100 V electric current for 30 after placing in a tank with 0.5x TBE solution and loading 2 µl of PCR product and

2 μ L of stain in to wells, then monitored under UV device. (Figure 1).

Obtained PCR products were analyzed via 3500 XL Genetic Analyser (Thermo Fisher Scientific) by a commercial company.

Analysis of mtDNA cyt b sequences: Raw data of mtDNA sequences, which were delivered to us by commercial company, were evaluated and converted in to FASTA format by using Chromas Pro v 2.0.1 (Technelysium Pty Ltd). Resulting sequences in FASTA format were aligned utilizing BioEdit software version 7.2.5 program.

The number of polymorphic sites and haplotypes, diversity of haplotypes and nucleotides, Tajima D and Fu's statistics for the populations were identified by using DnaSP5.10.01 program (Rozas et al., 2003). The phylogenetic relationship between haplotypes was identified via Network version 5.0 program.

Results and Discussion

Genetic variation: Approximately 600 bp fragment of mtDNA Cytochrome b gene was sequenced from a total of 31 *A. grypus* samples in Euphrates and Tigris Rivers, 5 polymorphic sites and 5 haplotypes were identified. Nucleotide variations of this region were shown in Table 1.

Haplotype diversity (Hd) and nucleotide diversity (π) for each locality were given in Table 2.

In Table 2, H1 is the haplotype which has the highest frequency commonly seen in all localities. Haplotype H2 was observed only in Bozova locality, H3 in both Çermik and Dicle locality, haplotypes H4 and H5 in Dicle locality only. While Siverek locality had the lowest values in terms of both haplotype and nucleotide diversity, other localities had similar results. The locality with the highest nucleotide diversity is Bozova.

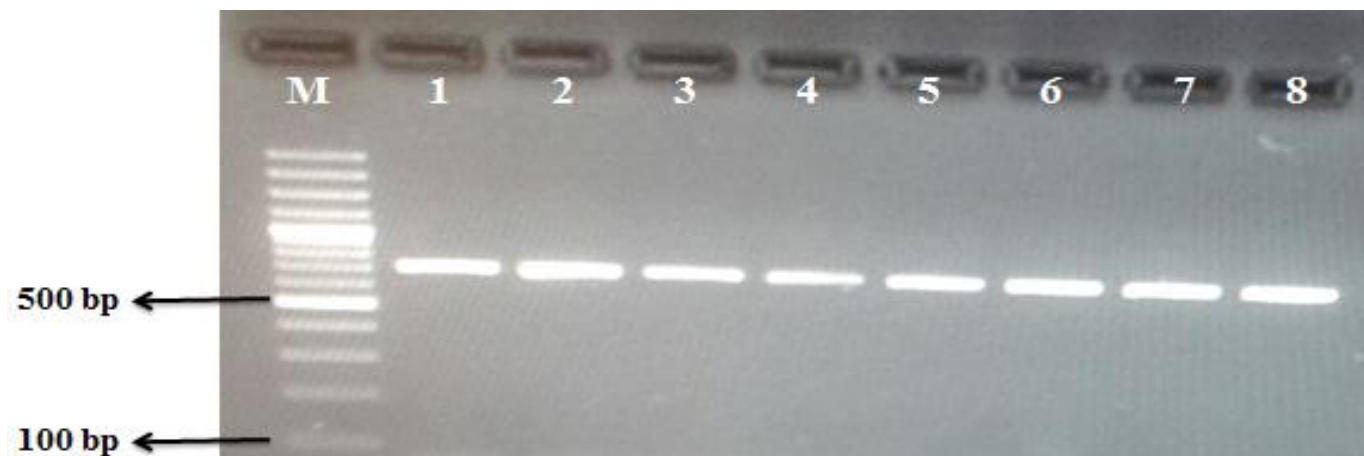


Figure 1. Image of PCR Products (M: Marker; bp: base pairs)

Table 1. Haplotypes and nucleotide variations of mtDNA Cytochrome b gene

Haplotypes	291	340	417	453	471
H1	G	T	G	C	G
H2	A	C	.	.	A
H3	A	C	.	.	.
H4	.	.	.	T	.
H5	.	.	A	.	.

Table 2. Genetic diversity of *A. grypus* localities based on mtDNA cytochrome b gene sequence and neutrality tests (N= number of individuals, Nh: number of haplotypes, Hd: haplotype diversity, π : nucleotide diversity)

River System	Locality	N	Nh	Haplotype frequency	Hd	π
Euphrates River	Siverek	5	1	H1 (1.0000)	0.000	0.00000
Euphrates River	Bozova	5	2	H1 (0.8000) H2 (0.2000)	0.400	0.00194
Euphrates River	Çermik	5	2	H1 (0.8000) H3 (0.2000)	0.400	0.00175
Tigris River	Dicle	16	4	H1 (0.7500) H3 (0.1250) H4 (0.0625) H5 (0.0625)	0.442	0.00135

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In Median-Joining Network created for 31 *A. grypus* samples analyzed 5 haplotypes were identified in total, resulting network includes existence of a central haplotype (H1) indicating an evolutionary connection. It is also likely to say that all other haplotypes are associated with haplotype H1 (Figure 2).

Neutrality tests: Neutrality tests (Tajima's D and Fu's Fs) were applied separately for each river. Tajima's D statistic was -0.94808 for Euphrates river and -1.26856 for Tigris river, -1.28294 in total, and found to be statistically insignificant ($p>0.05$). Fu' Fs values were determined as -0.006 for Euphrates river and -0.993 for Tigris river, -1.28294 in total, and found to be statistically insignificant ($p>0.05$).

In the present study, genetic diversity of populations was evaluated by conducting sequence analysis of approximately 600 bp of mtDNA cyt b. five polymorphic sites and five haplotypes were identified for this gene analyzed. Considering the fact that haplotype H1 was the most prevalent one with totally 25 individuals including 3 haplotypes (H1, H2, H3) in the Euphrates, 4 (H1, H3, H4, H5) in the Tigris, therefore it is possible to speculate that Haplotype H1 was ancestral because it was common in all populations. Even though haplotypes H1 and H3 were commonly seen in both river systems, haplotype H2 was observed only in the Euphrates, haplotypes H4 and H5 in only in individuals from the Tigris. Mean haplotype diversity (Hd) and nucleotide diversity (π) were calculated to be 0.442 and 0.00152 for individuals from Tigris River; 0.257 and 0.00138 for individuals from Euphrates River, respectively. Both values of Tigris River were higher. Therefore, it can be suggested to collect samples from Tigris River for the studies on aquaculture of this species. Mean haplotype diversity (Hd) and nucleotide diversity (π) were calculated to be 0.348 and 0.00144 for all of the individuals, respectively. Parmaksız et al., (2017) identified in their study on mtDNA COI gene of *A. grypus* that haplotype diversity and nucleotide diversity were 0.246 and 0.00045; respectively. The results in the present study were higher. Haplotype diversity was 0.642

and nucleotide diversity was 0.00138 in the study by Parmaksız and Eksi (2017) conducted for mtDNA COI gene in *Capoetta trutta* populations inhabiting in the same river systems, while nucleotide diversity was similar compared to the present survey, haplotype diversity was higher. Environmental heterogeneity and population size may support protection of high population diversity in populations (Nei, 1987; Avise, 1998). Haplotype diversity of *A. grypus* species was found to be lower because the number of individuals decreased thanks to overhunting.

This fish species is caught by fishermen and local people because all of the localities where samples of our research

were collected are near to residential areas. The fish caught are both consumed by locals and sold to neighboring provinces. Genetic diversity of *A. grypus* populations has been decreasing due to overhunting.

Median joining network analysis revealed that haplotype H1 was at the center of network and dominant, also all other haplotypes consisted of haplotype H1 indicating it was the ancestral one.

Some haplotypes identified in the present study possess new results for mtDNA cyt b gene, created an important data set for genetic diversity of this species. (Table 3).

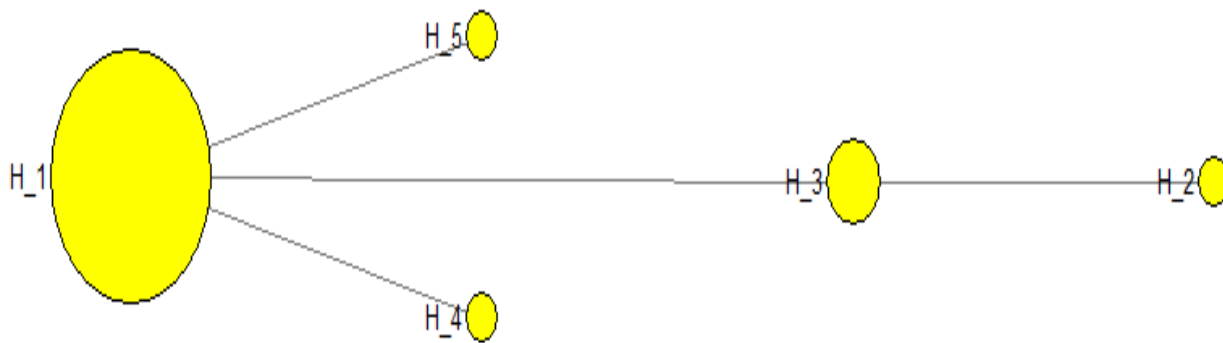


Figure 2. The model of *A. grypus* cyt b haplotypes

Table 3. Total cyt b haplotypes of *A. grypus* in the present study and GenBank

Haplotype	GenBank Data
H1	This study and KF876028, KF876027, AF145945
H2	This study
H3	This study and KF876026
H4	This study
H5	This study

Conclusions

This species is endemic and the most economically important species in the region. The population of this species have been influenced by pollution, destruction of habitat and especially over fishing exploitation. In this study, the sampling localities were only four localities. Further study based on microsatellite markers and mtDNA marker (D-loop) a comprehensive sampling collection is needed to extend for genetic diversity.

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