



Note

Genetic diversity of *Cyprinion macrostomus* (Heckel, 1843) populations in the Euphrates and Tigris rivers based on partial cytochrome b sequences of mitochondrial DNA

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ABSTRACT

Cyprinion macrostomus (Heckel, 1843), thriving in both Euphrates and Tigris river systems, is an economically important food fish species of the family Cyprinidae. It is important to know genetic structure of fish populations in terms of identification of genetic structure of stocks for facilitating sustainable fishing and conservation strategies. The present study, evaluated genetic structure of *C. macrostomus* populations in Euphrates and Tigris rivers using mtDNA cytochrome b sequence analysis. A total number of 44 *C. macrostomus* specimens were collected from Euphrates and Tigris rivers and total DNA was isolated from muscle tissue. Approximately 600 bp of mtDNA cyt b site was amplified by PCR and 8 polymorphic and 8 haplotypes were identified after sequence analysis. Mean haplotype and nucleotide diversity were calculated as 0.738 and 0.00253 respectively. Both populations had similar values in terms of haplotype as well as nucleotide diversity and neutrality tests were also found to be statistically insignificant.

Keywords: *Cyprinion macrostomus*, Euphrates River, Genetic diversity, mtDNA cyt b, Tigris River

The freshwater fish family Cyprinidae is the most important family in the Euphrates and Tigris river systems. *Cyprinion macrostomus* (Heckel, 1843) belongs to the family Cyprinidae with distribution in Turkey, Syria, Iraq and Iran (Kelle, 1978; Kuru, 1980; Taysi, 2014; Bilici *et al.*, 2015; Uckun *et al.*, 2015). *C. macrostomus* is an edible species and also valuable for sport fishing (Abdoli, 2000). This species also plays a therapeutic role in medical treatment (Undar *et al.*, 1990). Several workers carried out research on various aspects of the species *viz.*, morphological characteristics (Unlu, 1999), karyotype analysis (Gaffaroglu and Yuksel, 2004), age determination (Aydn *et al.*, 2009), haematology (Duman and Sahan 2014), length-weight relationships (Sedaghat and Hoseini, 2012; Bibak *et al.*, 2013), phylogeographical and phylogenetic relationships between *C. macrostomus*, *C. kais* and *Carasobarbus chantrei* in Euphrates and Tigris rivers (Durna *et al.*, 2012), selected morphometric characteristics (Kara and Gunes, 2015) and morphological differences (Bilici *et al.*, 2015).

For fishery management and conservation of fish species having economic importance, it is critical to understand genetic diversity and population structure (Ward, 2000; Ortega *et al.*, 2006). Mitochondrial DNA (mtDNA), which is an important and common molecular marker, has been widely used for estimating molecular variability and genetic structure of populations of

numerous organisms (Xu *et al.*, 2011). Recently, the mtDNA study has become more popular due to the advancement of molecular techniques for DNA sequencing and data analysis (Liu and Zhou, 2016). Among various mitochondrial genes, variation of cytochrome b (cyt b) gene has been used for population studies in cyprinids (Fayazi *et al.*, 2006).

The present study was undertaken to determine genetic diversity of *C. macrostomus* populations in the Euphrates and Tigris river systems, by sequence analysis of mtDNA cyt b gene.

The fish samples used in our study were collected from two different localities *viz.*, Adiyaman and Diyarbakir of Euphrates and Tigris river systems respectively, between February 2014 and January 2015. The samples were placed in ice container immediately after sampling and transported to the Zoology Laboratory of the Department of Biology, Faculty of Science and Arts, Harran University. Individuals were chosen randomly among the specimens which were decided to be the target species following identification. About 200 mg each of muscle tissue was dissected from a part closer to dorsal fin of the fish samples and placed in the microcentrifuge tubes containing 95% ethanol and kept at -20°C until DNA extraction. Total DNA was isolated using commercial kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific). Five microliters each of the isolated DNA

samples were run in 1% agarose gel at 100 V for 40 min, and results were examined.

Primers (Briolay *et al.*, 1998) used for amplification of mtDNA cyt b gene in the present study were :

L15267: 5'-AATGACTTGAAGAACCACCGT-3'

H15891: 5'-GTTTGATCCCGTTTCGTGTA-3'

Polymerase chain reactions (PCR) were performed at a total volume of 25 μ l comprising 0.5 mM of each primer, 0.2 mM of each dNTP, 1xPCR buffer, 2.5 mM MgCl₂, 1 unit Taq polymerase and approximately 90 ng of template DNA. PCR amplification was carried out in a BIO-RAD T100™ Thermal Cycler under the following conditions in a total number of 35 cycles: 3 min initial denaturation at 95°C, denaturation at 95°C for 30 s, annealing at 51°C for 30 s, extension at 72°C for 45 s, a final extension at 72°C for 10 min and terminated keeping the PCR products at 72°C for 5 s. PCR products (3 μ l each) were analysed on 2% agarose gel (1x TAE) together with a ladder and the most specific products were selected for sequencing. Amplified PCR products were purified using Favor/Prep gel/PCR purification mini kit (Favorgen, Vienna, Austria). Sequencing was done with an automated DNA sequencer (3500 XL Genetic Analyser, Thermo Fisher Scientific).

mtDNA cyt b sequences were evaluated and converted into FASTA format using Chromas Pro v 2.0.1 software (<http://www.technelysium.com.au/ChromasPro.html>). Resulting sequences of all individuals in FASTA format were aligned using BioEdit software version 7.2.5.

The number of polymorphic sites and haplotypes, diversity of haplotypes and nucleotides, Tajima D and Fu's statistics were estimated for the populations using DnaSP5.10.01 program. The phylogenetic relationships between haplotypes were identified using Network version 5.0 software.

An average number of 600 bp fragments of mtDNA cyt b gene were sequenced from 44 *C. macrostomus* specimens; totally 8 polymorphic sites and 8 haplotypes were identified. Nucleotide variations of this region are shown in Table 1. Haplotype diversity (Hd), the nucleotide diversity (π) and the neutrality tests for each of the populations are given in Table 2.

Diyarbakır population had greater values in terms of both haplotype diversity (Hd = 0.738) and nucleotide diversity (π = 0.00253). Tajima's D and Fu's Fs values, on the other hand, were negative for both the populations. Haplotypes H1, H2 and H4 were common for two populations, while H3 was found only in Adiyaman and H5, H6, H7 and H8 only in Diyarbakır. Haplotype H1 had a higher frequency which was observed in both populations.

Fig. 1 shows evolutionary network for 8 haplotypes identified on the Median-Joining Network (MJN) of haplotypes created for 44 samples analysed and resulting MJN shows presence of a central haplotype (H1) which indicates an evolutionary connection. Besides, it is possible to speculate that all of the other haplotypes were connected with haplotype H1.

Table 1. Haplotypes and nucleotide variations of mtDNA cyt b

Haplotypes	7	1	2	3	3	3	3	4
	8	3	5	0	3	3	6	8
		2	8	0	0	3	3	6
H1	C	A	A	G	A	A	G	A
H2	.	.	.	A
H3	.	.	G
H4	A	.
H5	.	G	.	.	.	G	.	.
H6	.	.	.	A	G	.	.	.
H7	G
H8	T	G

Table 2. *C. macrostomus* sampling site, size and their genetic diversity inferred from mtDNA cyt b gene sequence (n = Number of individuals, Nh: Number of haplotypes, Hd: Haplotype diversity, π : Nucleotide diversity)

River system	Locality	n	Nh	Haplotype frequency	Hd	π	Tajima's D	Fu's Fs
Euphrates River	Adiyaman	17	4	H1 (0.47) H2 (0.41) H3 (0.06) H4 (0.06)	0.640	0.00130	-0.43847	-0.828
Tigris River	Diyarbakır	27	7	H1 (0.49) H2 (0.07) H4 (0.07) H5 (0.07) H6 (0.04) H7 (0.07) H8 (0.19)	0.738	0.00253	-0.66327	-1.718

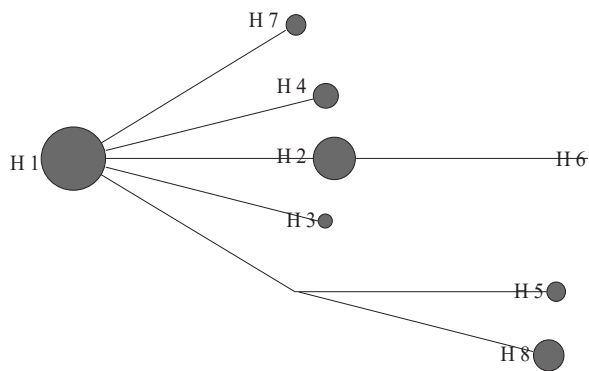


Fig. 1. Median-joining network showing relationships between 8 haplotypes of two *C. macrostomus* populations

Tajima's D and Fu's Fs tests were applied for each population. Tajima's D value was negative in both Adıyaman (-0.43847) and Diyarbakır (-0.66327) populations, and it was also negative in total (-0.91457) which was found to be statistically insignificant ($p > 0.05$). Fu's tests were negative in Adıyaman (-0.828) as well as Diyarbakır (-1.718) populations which was also negative in total (-2.357) and found to be statistically insignificant ($p > 0.05$).

Because Adıyaman Province is close to Euphrates and Diyarbakır Province to Tigris river, fishing activities are carried out by the people who live in these regions. *C. macrostomus* is one of the important fish species caught and consumed by local people as well as sold in the neighbouring provinces. Eight polymorphic sites and 8 haplotypes were identified from mtDNA cyt b gene consisting of 600 base pairs on an average. Four haplotypes (H1, H2, H3 and H4) were observed in Adıyaman population and 7 haplotypes (H1, H2, H4, H5, H6, H7 and H8) in Diyarbakır population. Haplotype H1 was seen in a total of 21 individuals and it appears that H1 is ancestral haplotype as it was found commonly in both populations and represented with the highest number of individuals. There are only two sequences of *C. macrostomus* species in GenBank (www.ncbi.nlm.nih.gov/genbank/) for the gene studied. As they were compared with haplotypes in the present study, they were identified to be Haplotype 2 (KP712263.1) and Haplotype 8 (AF180826.1). Thereby, haplotypes H1, H3, H4, H5, H6 and H7 were identified for the first time in this study. Mean haplotype diversity and nucleotide diversity were found to be 0.724 and 0.00221, respectively. Diyarbakır population had higher values than Adıyaman population in terms of both haplotype diversity and nucleotide diversity.

Mean haplotype and nucleotide diversity values based on mtDNA sequence analysis have been reported

for certain cyprinids living in Euphrates and Tigris river systems. The values determined by researchers in various fish species are: Hd: 0.642; π : 0.00138 in *C. trutta* populations (Parmaksız and Eksi, 2017), Hd: 0.246; π : 0.00045 in *Barbus grypus* populations (Parmaksız *et al.*, 2017) and Hd = 0.538 and π = 0.00345 for *Carasobarbus luteus* populations (Eskici, 2017). The mean haplotype diversity estimated in this study (0.724) was the maximum compared to those reported for other species. The value of nucleotide diversity recorded in *C. macrostomus* during the present study was higher in comparison with *B. grypus* and *C. trutta* species, while, it was found to be lower as compared to *C. luteus*. Population size and environmental heterogeneity contribute towards high Hd values for populations (Nei, 1987; Avise, 1998). Lower nucleotide diversity values are also indicative of low genetic diversity and population diversity is influenced by many factors, such as bottleneck effects, anthropogenic activity and habitation (Fennando *et al.*, 2000; Ma *et al.*, 2010).

Fixation index (F_{ST}) is used to determine the level of difference between two populations. In the present study, this value was calculated as 0.22724 ($p < 0.05$) and the difference between Adıyaman and Diyarbakır populations was found to be statistically significant.

In median joining network analysis, haplotype H1 was in the center of the network and the dominant one. All the other haplotypes also consisted of H1 (Fig. 1). Haplotype H1 appears to be ancestral since it is common in both the populations and dominant.

Tajima's D and Fu's Fs were applied separately and totally for both populations. All values were insignificant ($p > 0.05$) for overall population indicating that a possible population change occurred in the past.

In addition, it was determined that either of mean Tajima's D and Fu's Fs values were negative in the study by Parmaksız and Eksi (2017), on the contrary, both of them were positive in the study by Parmaksız and Eskici (2018). A positive Fu's Fs and Tajima's D test signifies lower levels for both low and high frequency polymorphisms, indicating a decrease in population size (Hu *et al.*, 2016). Considering the values in the neutrality tests in this survey, a decrease was not observed in the populations studied.

Haplotypes identified for mtDNA cyt b locus created a data set which is important for identifying the genetic diversity of *C. macrostomus* species that thrives in Euphrates and Tigris rivers. The data generated represent a significant tool for the characterisation of separate

populations for management and breeding programs. Further studies on a greater number of populations using diverse genetic markers will be more explanatory for assessing population genetics of this species.

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