Turk. J. Fish.& Aquat. Sci. 20(1), 79-86
http://doi.org/10.4194/1303-2712-v20_1_08

Turkish Journal of FISHERIES and AQUATIC SCIENCES

RESEARCH PAPER

Population Genetic Diversity of Yellow Barbell (*Carasobarbus luteus*) from Kueik, Euphrates and Tigris Rivers Based on Mitochondrial DNA D-loop Sequences

Arif Parmaksiz[1,*]

[1] Harran University, Faculty of Science-Literature, Department of Biology, Şanlıurfa, Turkey.

Article History
Received 08 May 2018
Accepted 14 January 2019
First Online 30 January 2019

Corresponding Author
Tel.: 04143183464
E-mail: aprmksz@gmail.com

Keywords
Carasobarbus luteus
Freshwater fish
Genetic diversity
Polymorphism
mtDNA, *D-loop*

Abstract

It is substantially important to know genetic structure of fish populations for long term fishery and with regards to conservation strategies. The present study was aimed to reveal the fundamental population genetic data for continuity and conservation of the Yellow barbell (Carasobarbus luteus) stocks, being considered as an economically important endemic fish species in Southeastern Anatolia Region. Therefore, a total number of 108 fish specimens were sampled from 9 localities of Kueik, Euphrates and Tigris river systems and subsequently, genetically analyzed based on mtDNA D-loop region sequencing. In total, 15 polymorphic nucloetide sites and 7 haplotypes were established during the analysis carried out. The mean haplotype (Hd) and nucleotide variation (π) were calculated as 0.373 and 0.00536. The highest values of haplotype and nucleotide variation was observed in Birecik (Hd=0,65152) and Adıyaman population (π =0,01094); respectively. In turns, none haplotype and nucleotide variation was observed in Kilis and Diyarbakır populations. Obtained FST values for examined populations ranged between -0.12389 and 0.15630. All FST values were found to be statistically insignificant for the populations within the same river and significant between some populations originated from different rivers. According to analysis of neutrality tests, Tajima's D values were statistically significant in 4 populations (Samsat, Bozova, Hilvan and Bismil). Obtained, under the present study information on genetic variation and population structure of C. luteus will be useful to plan an effective strategies for conservation and fishery supplementation of the species in the region.

Introduction

Freshwater fish have become an alternative source of protein in response to terrestrial products that are insufficient to meet protein needs of increasing human population of Southeastern Anatolia Region in the recent years. Fish are considered to be the cheapest source of animal protein and minerals for billions of poor families around the world (Wu & Yang, 2012). Both aquaculture and fishery activities have been enhanced in the region to meet the increasing demand for freshwater fish. Southeastern Anatolia Region ranks last in terms of amount of domestic aquaculture production in Turkey (Ural & Canpolat, 2009), where a great majority of consumed freshwater fish is obtained from river systems by fishery. Yellow barbell (*Carasobarbus luteus*) is amongst the most common and dominant fish within totally 46 fish species inhabiting Kueik, Euphrates and Tigris river systems, which are important natural resources for fish diversity and potential for fishery (Bilici, 2013). *C. luteus* is a common Cyprinidae species

endemic to Kueik, Euphrates and Tigris drainage, including natural and artificial lakes of Mesopotamia (Kuru, 1979; Ünlü, 1991; Gökçek & Akyurt, 2008; Coad, 2010). This fish species has particular economic importance since there is a high demand for it as food in Southeastern Anatolia Region (Borkenhagen, Esmaeili, Mohsenzadeh, Shahryari, & Gholamifard, 2011; Bilici, Ünlü, Çiçek, & Satici, 2016). Due to lack of reliable management plan in the region, natural populations of this species are exposed to overfishing by fishermen. Besides, environmental factors, such as: hydrotechnical development, rapidly increasing populations of invasive fish species, and destruction of habitats considerably influence on *C. luteus* populations and possess major risk for its future existence. Decreasing number of natural populations may lead to eradication of unique genotypes that are not found anywhere else. It is almost impossible to bring it back when a genetic data is lost. Hence, necessary precautions need to be taken to stop genetic loss and to conserve future of this species. Creditable data are required firstly for an effective conservation program. Analysis of population genetics is an influential tool in reasonable species' conservation strategy, enabling to obtain basal information for its management (Ryman, 1991; Ward, 2000). Molecular markers are effective methods to identify genetic diversity and population structure (Englbrecht, Freyhof, Nolte, Rassmann, Schliewen, & Tautz, 2000; Whitehead, Anderson, Kuivila, Roach, & May, 2003). Mitochondrial DNA markers are used in genetic studies for various species (Xia, Guo, Ye, Li, & Wu, 2016). Compared to nuclear DNA markers, mitochondrial DNA markers are preferred because of their specific characteristics, such as: maternal inheritance, slow evolutionary rate, and absence of recombinations (Barrette, Crease, Hebert, & Via, 1994).

Parmaksiz and Eskici (2018) utilized mtDNA *COI* sequences to evaluate genetic variation of *C. luteus*, the results provided some precious information to reveal genetic background of the species. Since genetic information obtained from a single mtDNA marker is limited, it shows that resolution power of genetic studies may be increased by the use of multiple genetic marker systems (Gruenthal, Acheson, & Burton, 2007). It is considered that *D-loop* is the most polymorphic site of mtDNA and, therefore; it represents the maximum level of the variation (Li *et al.*, 2015). Thus, haplotype analysis of D-loop is frequently used as an effective tool to investigate genetic diversity of cyprinid fish populations (Wu *et al.*, 2013).

The main goal of the present study is to identify genetic diversity of *C. luteus* populations, indigenously inhabit of Kueik, Euphrates, and Tigris rivers based on mtDNA *D-loop* sequence analysis. It was aimed that the results could contribute for conservation and sustainable management of the species *C. luteus* in the future

Material and Methods

Sample Collection

The fish samples used in the present study (n=108) were obtained from 9 localities of 3 diverse river systems, i.e. Kueik, Euphrates, and Tigris rivers during the period of January 2013 and December 2017. Geographical information of these localities was given in Table 1 and the map on Figure 1.

As soon as fish specimens were obtained, all samples were kept in ice container and transferred to Zoology Laboratory of Biology Department at the Faculty of Science & Arts of Harran University. Following the identification of species, approximately 1 g of muscle tissue was dissected from each sample and placed in the microcentrifuge tubes containing 95% ethanol and kept at -20 °C until DNA extraction.

DNA Extraction, D-loop mtDNA Region Amplification and Sequencing

In the present study, total genomic DNA was isolated from muscle tissue using GeneJET Genomic DNA Purification Kit (Thermo Scientific), according to the protocol provided by manufacturer.

Primer sequences used for amplification of mtDNA D-loop site were as follows; L15923: 5'-TTAAAGCATCGGTCTTGTAA-3' (Iguchi, Tanimura, & Nishida, 1997) and H16500: 5'-GCCCTGAAATAGGAACCAGA-3' (Inoue, Miya, Tsukamoto, & Nishida, 2000). All PCR reactions were performed in a total volume of 25 µl, containing: 2.5 µl (1x) PCR buffer, 2.0 µl (2.5 mM) $MgCl_2$, 0.5 µl (0.5 mM)

Tablo 1. Table 1. Details of sampling sites of C. luteus populations

Country	River	Locality	Geographic coordinates	Altitude	n
Turkey	Kueik	Kilis	37°17' 31.18"N, 37°34' 20.18"E	746	8
Turkey	Euphrates	Birecik	37°00' 18.58"N, 37°57' 48.41"E	338	12
Turkey	Euphrates	Adıyaman	37°40' 04.80"N, 38°20' 24.57"E	576	21
Turkey	Euphrates	Samsat	37°33' 48.07"N, 38°30' 44.08"E	534	10
Turkey	Euphrates	Bozova	37°25' 56.68"N, 38°31' 53.88"E	550	10
Turkey	Euphrates	Hilvan	37°41' 32.33"N, 38°52' 27.29"E	547	7
Turkey	Tigris	Diyarbakır	37°59' 55.15"N, 40°14' 44.92"E	590	7
Turkey	Tigris	Bismil	37°50' 51.18"N, 40°36' 09.18"E	545	24
Iraq	Tigris	Erbil	36°17' 05.45"N, 43°39' 16.12"E	249	9

of each primer, 0.5 µl (0.2 mM) dNTP, 0.1 µl of Taq DNA polymerase (5U/µl) and approximately 90 ng of template DNA. The PCR amplification was carried out in a BIO-RAD T100™ Thermal Cycler under the following conditions: 3 min initial denaturation at 95 °C, and 35 cycles of 30 s at 95°C for denaturation, 30 s at 51°C for annealing, and 45 s at 72 °C for extension, and a final extension at 72 °C for 10 min. Amplified DNA fragments were sent to MEDSANTEK (Istanbul, TURKEY) for purification and sequencing.

Data Analysis

The raw data of mtDNA *D-loop* sequences from 108 individuals were evaluated using Chromas Pro v 2.0.1 software and converted in to FASTA format. Resulting sequences of all individuals in FASTA format were aligned utilizing BioEdit software version 7.2.5. The number of polymorphic sites, the number of haplotypes, haplotype diversity (*Hd*) and nucleotide diversity (π) were established with DnaSP 5.10.01 (Rozas, Sanchez-DelBarrio, Messeguer, & Rozas, 2003) software. In order to indicate relationships between haplotypes, Median Joining Network (Bandelt, Forster, & Rohl, 1999) was drawn based on by using NETWORK 4.6.1.0 program. The neighbour-joining phylogenetic tree was constructed using the MEGA program (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) for envision genetic relationships among populations. The statistical robustness of test was assessed with 1000 bootstrap replicates. Genetic distances between populations were obtained by calculating pairwise F_{ST} values based on haplotype frequencies, with Arlequin 3.11 (Excoffier, Laval, & Schneider, 2005). Significance of results was tested with 1000 permutations.

Results

mt DNA D-loop Region Diveristy

An average of 471 base pairs (bp) length fragments of mtDNA D-loop region from 108 *C. luteus* specimens were sequenced; totally, 15 polymorphic sites and 7 haplotypes were detected. Nucleotide variations of this region are shown in Table 2. The average nucleotide composition for all the sequences was A: 36.5%, T: 31.2%, C: 19.1% and G: 13.2%. All the haplotype sequences were deposited in GenBank under accession numbers: MH727086-92.

Population Genetic Diversity and Haplotype Frequency

The frequencies of all identified haplotypes and the values of calculated genetic diveristy indicator are shown in Table 3. Haplotypes H1 (1.86%) and H2 (0.93%) were represented by small number of individuals only in Bismil population. The haplotype H3 (78.7%) was the most common within the studied fish group. H4 (6.48%) was observed in populations of Adıyaman, Bismil, Hilvan and Birecik; H5 (5.55%) in populations of Adıyaman, Samsat, and Birecik; H6 (5.55%) in populations of Adıyaman, Samsat, Bozova, and Birecik; H7 (0.93 %) only in population of Erbil. Accordingly, haplotypes H1, H2 and H7 seen only in populations of Tigris river, haplotypes H5 and H6 only in Euphrates populations. Populations of Kilis and Diyarbakır include haplotype H3 alone. All calculated values of haplotype (*Hd*) and nucleotide diversity (π) were in a range of 0.00000 – 0.65152 and 0.00000 – 0.01094; respectively. The highest values of haplotype and nucleotide variation was observed in Birecik (*Hd*=0,65152) and Adıyaman population (π =0,01094); respectively. In turns, none

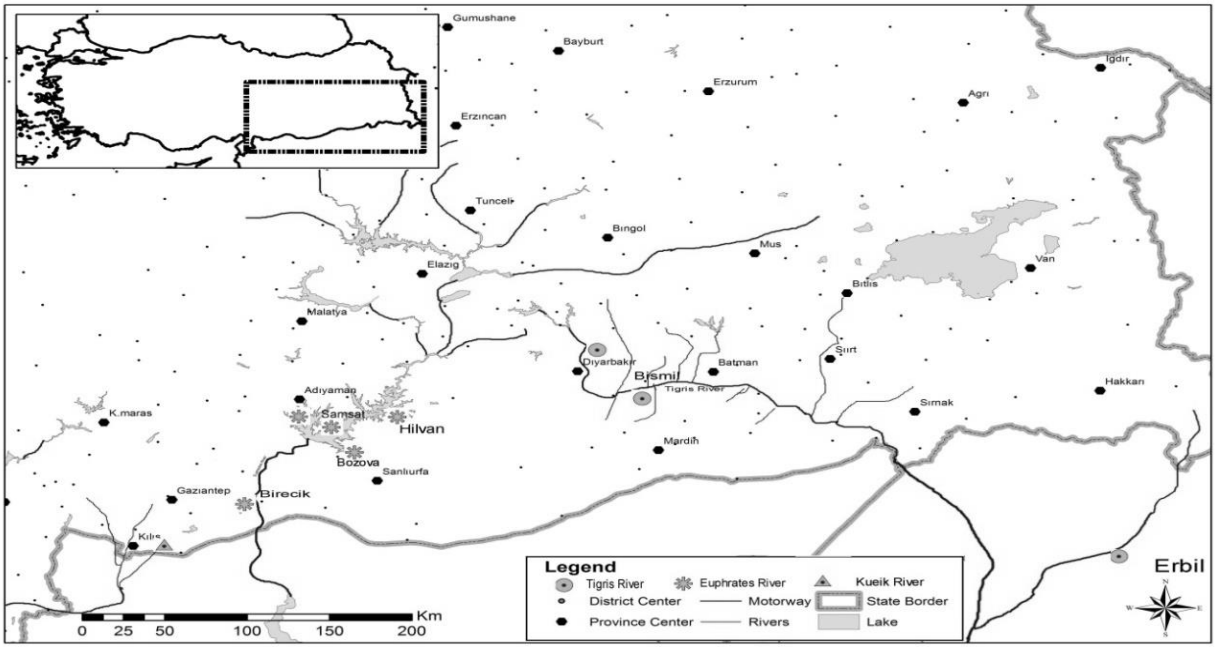

Figure 1. The locations of studied populations of *C. luteus* in Kueik, Euphrates and Tigris Rivers.

haplotype and nucleotide variation was observed in Kilis and Diyarbakır populations (Table 3).

Neutrality Tests

The results of Tajima's D and Fu's Fs neutrality tests are shown in Table 3. Tajima's D values were found to be statistically significant in four populations (Samsat, Bozova, Hilvan and Bismil) (P<0.05). Fu's Fs values, on the other hand, were statistically insignificant in all populations (P>0.05).

Population Genetic Structure

Pairwise F_{ST} results between populations based on haplotype frequencies are given in Table 4.

F_{ST} results were ranged between -0.12389 and 0.15630 for populations of *C. luteus*. F_{ST} values between Bismil and Birecik, Bismil and Adiyaman were 0.13731 (P<0.01) and 0.15630 (P<0.05), statistically significant, respectively; F_{ST} value between population of Kilis and Adıyaman, 0.13547 (P<0.05), was statistically significant.

Table 2. Variable sites of D-loop gene in *C. Luteus*

Haplotype	Variable sites															Accession
	133	149	151	160	168	169	171	251	284	292	298	333	341	351	374	number
H1	T	A	G	C	C	T	T	C	A	A	T	G	A	T	G	MH727086
H2	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	MH727087
H3	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	MH727088
H4	.	G	A	T	T	C	G	A	G	G	.	A	.	C	A	MH727089
H5	.	.	.	.	.	.	G	.	.	.	.	.	G	.	.	MH727090
H6	C	G	A	T	T	C	G	A	G	G	.	A	.	C	A	MH727091
H7	.	.	.		.	.	G	T	.	.	.	.	.	.	.	MH727092

Table 3. Information about nine localities of *C. luteus* samples (Hd: haplotype diversity, H: haplotype [individual's frequency], π: nucleotide diversity)

Locality	n	Haplotypes	Hd	π	Tajima's D	Fu's Fs
Kilis	8	H3(8)	0,00000	0,00000	0.00000	0.00000
Birecik	12	H3(7), H4(1), H5(2), H6(2)	0,65152	0,01084	0.78841	3.72918
Adıyaman	21	H3(12),H4(4), H5(3), H6(2)	0,63810	0,01094	1.52599	5.97313
Samsat	10	H3(8), H5(1), H6(1)	0,37778	0,00552	-1.97583*	2.67927
Bozova	10	H3(9), H6(1)	0,20000	0,00510	-1.96119*	4.58191
Hilvan	7	H3(6), H4(1)	0,28571	0,00667	-1.62257*	4.56086
Diyarbakır	7	H3(7)	0,00000	0,00000	0.00000	0.00000
Bismil	24	H1(2), H2(1), H3(20), H4(1)	0,30797	0,00261	-2.24520*	0.68856
Erbil	9	H3(8), H7(1)	0,22222	0,00047	-1.08823	-0.26348

Significant difference at *P<0.05

Table 4. Pairwise FST values and their significances between the populations on the basis of their mtDNA D-loop haplotype frequencies

Population	Birecik	Adiyaman	Hilvan	Samsat	Bozova	Bismil	Diyarbakir	Erbil	Kilis
Birecik	0.00000								
Adiyaman	-0.06569	0.00000							
Hilvan	-0.07746	-0.04283	0.00000						
Samsat	-0.02798	0.01344	-0.12002	0.00000					
Bozova	-0.02012	0.01885	-0.12389	-0.10619	0.00000				
Bismil	0.13731**	0.15630*	-0.01239	-0.02511	-0.03012	0.00000			
Diyarbakir	0.10056	0.12186	0.00000	-0.03960	-0.03960	-0.05802	0.00000		
Erbil	0.12386	0.14029	0.02242	-0.01724	-0.01770	-0.03176	-0.03067	0.00000	
Kilis	0.11814	0.13547*	0.02041	-0.02418	-0.02418	-0.04670	0.00000	-0.01408	0.00000

Significant difference at *P<0.05 **P<0.01

The analysis of molecular variance (AMOVA) of population structure revealed that percentage of variation between groups was 10.21%, among population within groups -2.53% and within population 92.31%. Fixation index was calculated to be 0.07687 (Table 5).

Totally 7 haplotypes were identified in Median-Joining Network which was established for 108 samples of *C. luteus* analyzed. Resulting Median-Joining Network showed the presence of a central haplotype (H3), indicating an evolutionary connection (Figure 2).

With respect to neighbour-joining tree (Figure 3), the observed genetic relationship of studied *C. luteus* populations, indicates to be a single lineage with no connection to geographical locations and exhibited no major genetic clustering of the genealogical relationship among haplotypes.

Discussion

Riverine ecosystems are very important harbour for aquatic organisms and the one habitat to live for numerous of them, including fish. Generally, fish species populations have diverse genetic structures or may display different geographical patterns because they were separated by geographical isolations; thus, they may have considerably distinguishable genetic structures even in neighboring rivers (Zhao, Erica, & Liu, 2018). The present study included sampling from 3 neighboring river systems (Kueik, Euphrates, and Tigris). Kueik river is 129 km long, emerging from southern parts of Gaziantep plateau, located in Southeastern Anatolia Region of Turkey and flowing through Halep city in the northern Syria (Kaya, Turan, & Bayçelebi, 2017). In turns, Tigris-Euphrates river system is a large watershed of southwestern Asia. It comprises Tigris and Euphrates rivers, which follow roughly parallel courses by way of the heart of the Middle East (Britannica, 2018). The aforementoned rivers have their sources in 50 miles of each other in eastern Turkey and tour southeast through northern Iraq and Syria to the head of the Persian Gulf (Britannica, 2018).

All of these river ecosystems are exposed to anthropogenic activities, such as: environmental pollution, overfishing, building dams and introduction of invasive-exotic species. Altogether circumstances are serious challenges threatening survival and diversity of fish populations in the region. Under such conditions,

Table 5. Analysis of molecular variance (AMOVA) among different *C. luteus* groups

Source of variation	d*f*	Sum of squares	Variance components	Percentage of variation	Fixation Indices	p-value
Among groups	2	10.073	0.14142 Va	10.21	FSC : -0.02813	0.74389+-0.01446
Among populations within groups	6	5.275	-0.03498 Vb	-2.53	FST : 0.07687	0.13685+-0.01141
Within populations	99	126.550	1.27828 Vc	92.31	FCT : 0.10213	0.06549+-0.00796
Total	107	141.898	1.38472			

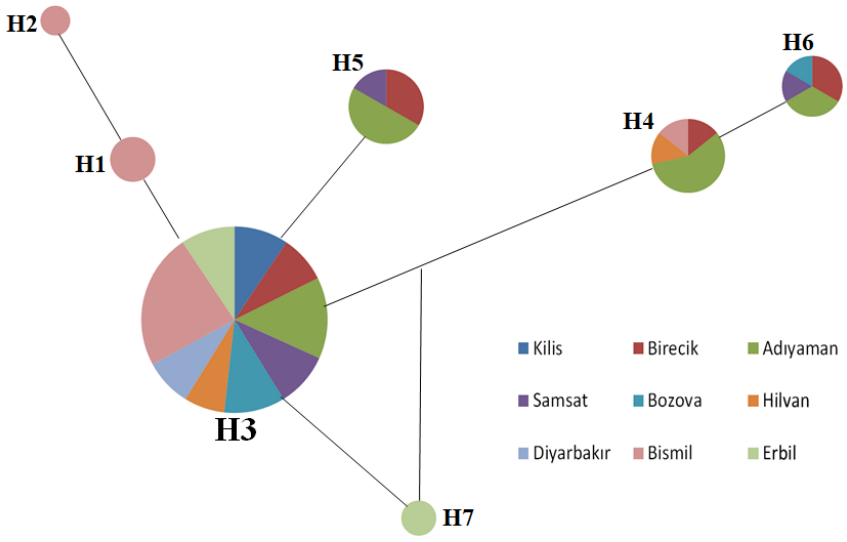

Figure 2. The median-joining network based on 7 haplotypes of *C. luteus* found by sequencing D-loop gene of 108 individuals from 9 populations in Turkey and Iraq. The colors represent the various populations, the size of a circle is proportional to the number of haplotypes represented.

the extinction of every endemic fish species populations is inevitable unless necessary precautions are taken. Invasive species in the future might take the place of this kind of natural, common, endemic and dominant species studied here. Therefore, it is needed to obtain the baseline data on genetic diversity and structure of studied fish species to conserve its future by application of apposite precautions, as genetic diversity is the basis and core of biodiversity, which guarantees species evolution (Gao *et al.*, 2017). High levels of genetic diversity ensure strong adaptation abilities and survival skills for populations (Barrett & Schluter, 2008).

Median joining network analysis revealed that haplotype H3 was in the center of network and all haplotypes were consisted of haplotype H3 (Figure 2). The fact that this haplotype is the most dominant and common (78.7% frequency) in all populations indicates it is the ancestral haplotype (Crandall, & Templeton, 1993). It is also possible to speculate that all other haplotypes are evolutionary related to H3 haplotype.

Accoding to obtained results Adıyaman and Birecik populations were the most similar. Both of them shared the same set of haplotypes (H3, H4, H5 and H6) and displayed similar levels of genetic diversity (Table 3). Because, these localities are fairly close, there is only a dam between them, for now transition of fish is possible and it is expected for them to have similar genetic structure since the dam has 20 years of background. In addition, nucleotide diversity of Tigris populations is substantially lower than those in Euphrates river. *C. luteus* species was not found in field studies in Hasankeyf locality placed in Tigris river. This is an indicator for decrease of the population in Tigris River.

Low nucleotide diversity was also established in mtDNA sequence analysis studies carried out in different countries for certain freshwater species with economic importance and having habitats disturbed by human activities, their results were in parallel to our study (Saraswat *et al.*, 2014; Khaefi, Esmaeili, Ansari, & Ebrahimi, 2018; Behera *et al.*, 2018; Zhao *et al.*, 2018). Observed low values of nucleotide diversity (π) may be an indicator of occurrence adverse effects affecting on populations fitness and survivability, such as: bottleneck effect, inbreeding, outbreeding and hybridization, which are mainly related with antrophogenetic activities (Ma, Cheng, Zhang, Zhuang, & Zhao, 2010; Fennando, Pfrender, Encalada, & Lande, 2000).

Genetic fixation index (F_{ST}) is the major criterion to estimate genetic differences between various geographical populations, at the same time it may also reflect the genetic correlation between populations (Gao *et al.*, 2017). Despite these values were low and insignificant for populations within the same rivers, they were statistically significant for certain populations in different rivers. The fact that F_{ST} results were significant between these localities indicates genetic differences despite they are neighboring rivers. F_{ST} value between rivers was also found to be insignificant in AMOVA analysis. According to the obtained results, there are some signs of genetic clustering between Tigris, Euphrates and Kueik rivers; therefore, all the three rivers should be considered as separate management units. Especially, selected regions/populations should be considered with caution during planning or carrying out any conservation management plans in the future, i.e. Bismil population representing Tigris river unit,

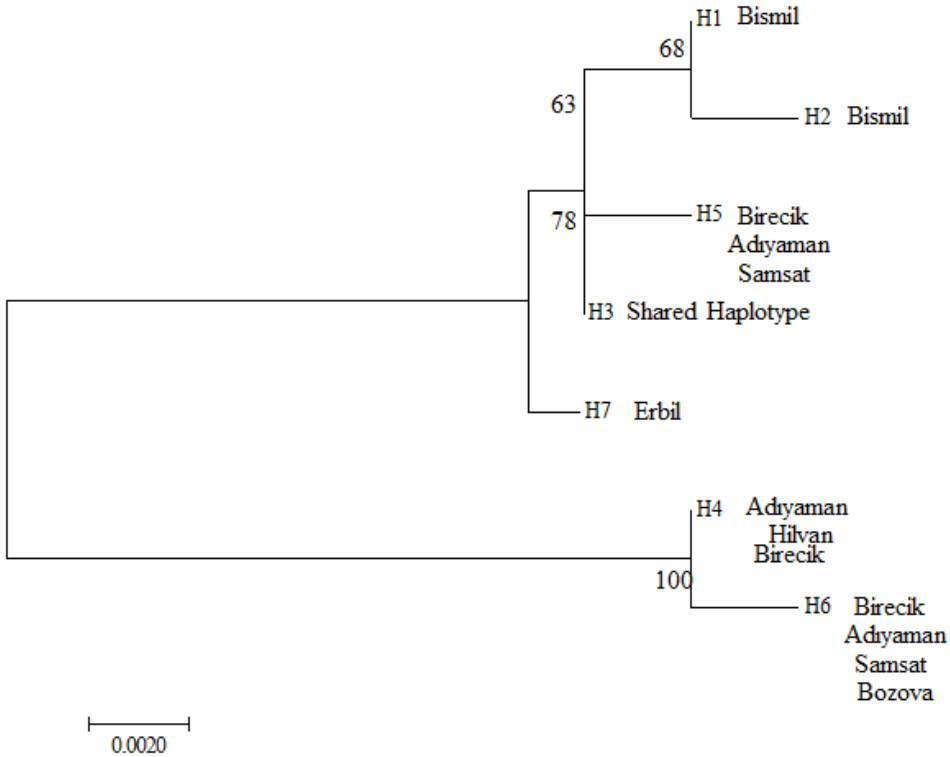

Figure 3. Neighbour-joining tree of *C. luteus* samples from 9 different localities based on observed haplotypes of D loop gene sequences

Adiyaman and Birecik populations representing Euphrates river unit as well as Kilis population representing Kueik river unit.

Despite Fu's Fs results were statistically insignificant after neutrality tests, Tajima's D values were both negative and statistically significant in 4 populations (Samsat, Bozova, Hilvan and Bismil). It is very likely that several haplotypes of these populations were eliminated and exposed to a bottleneck effect. Furthermore, this possibility was supported by low nucleotide diversity as well. Another study on mtDNA *COI* sequence analysis of *C. luteus* species in the same river system revealed a considerable divergence from neutrality (Parmaksız & Eskici, 2018). However, it was expected that genetic data resulted in greater details because the number of samples and localities were higher in this study.

The results obtained from the present study represent current genetic data on *C. luteus* and will contribute effective conservation and reasonable fishery. Bottleneck effect may lead to loss of genetic diversity and influence on population continuity. Presence of bottleneck in certain populations creates a risk for its future. Hence, it needs to be spotlight to conserve the data of genetic diversity. It is mandatory to prevent overfishing, particularly to prohibit fishing throughout reproductive season. It is aimed to extend and validate the study by using nuclear indicators in the future.

Acknowledgements

This study was funded by Harran University Research Fund (Project No: 17217). I would like to thank to Erhan Ünlü, Tarık Çiçek, Cüneyt Kaya, Necmettin Doğan, Yahia Radha Gül who did not withhold to help for collection of fish samples used in this study; Ahmet Oymak who identified the fish; Abdullah Akar who assisted in laboratory experiments and Mehmet Özcanlı who helping map drawing.

References

Bandelt, J., Forster, P. & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution, 16*(1), 37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barrett, R, & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, *23*, 38-44. https://doi.org/10.1016/j.tree.2007.09.008

Barrette, R.J., Crease, T.J., Hebert, P.D.N., & Via, S. (1994). Mitochondrial DNA diversity in the pea aphid *Acyrthosiphon pisum*. *Genome*, *37*(5), 858-865. https://doi.org/10.1139/g94-121

Behera, B.K, Baisvar, V.S., Kunal, S.P., Meena, D.K, Panda, D., Pakrashi, S., Paria, P., Das,

P., Bhakta, D., Debnath, D. (2018). Population structure and genetic diversity of Indian Major Carp, *Labeo rohita* (Hamilton, 1822) from three phylo-geographically isolated riverine ecosystems of India as revealed by mtDNA cytochrome b region sequences. *Mitochondrial DNA Part A, 29*(2), 199-205. https://doi.org/10.1080/24701394.2016.1267156

Bilici, S. (2013). *The investigations on the biology of Carasobarbus luteus, Capoeta trutta and Garra variabilis species Living in Tigris River*. (PhD Thesis), Dicle University, Diyarbakır, Turkey.

Bilici, S., Ünlü, E., Çiçek, T., & Satici, Ö. (2016). The reproductive biology of *Carasobarbus luteus* and *Capoeta trutta* in the Tigris River, Turkey. *Cybium, 40*(2), 147-153.

Borkenhagen, K., Esmaeili, H. R., Mohsenzadeh, S., Shahryari, F., & Gholamifard, G. **(**2011). The molecular systematics of the *Carasobarbus* species from Iran and adjacent areas, with comments on *Carasobarbus albus* (Heckel, 1843), *Environmental Biology of Fishes, 91*, 327-335. https://doi.org/10.1007/s10641-011-9787-1

Coad, B.W. (2010). *Freshwater Fishes of Iraq: A student guide.* Sofia, Moscow: Pensoft Publishers Press.

Crandall, K.A., & Templeton, A.R. (1993). Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*, *134*, 959-69.

Encyclopaedia Britannica (2018). *Tigris-Euphrates river system.* Retrieved from http://www.britannica.com/place/Tigris-Euphrates-river-system.htm

Englbrecht, C.C., Freyhof, J., Nolte, A., Rassmann, K., Schliewen, U., & Tautz, D. (2000). Phylogeography of thebullhead Cottus gobio (Pisces: Teleostei: Cottidae) suggests a pre-Pleistocene origin of the major central European populations. *Molecular Ecology*, *9*(6), 709-722. https://doi.org/10.1046/j.1365-294x.2000.00912.x

Excoffier, L., Laval, G., & Schneider, S. (2005). ARLEQUIN ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, *1*, 47-50. https://doi.org/10.1177/117693430500100003

Fennando, P., Pfrender, M. E., Encalada, S. E., & Lande, R. (2000). Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. *Heredity, 84*, 362-372. https://doi.org/10.1046/j.1365-2540.2000.00674.x

Gökçek, K. & Akyurt, I. (2008). Age and Growth Characteristics of Himri Barbel (*Barbus luteus* Heckel, 1843 in Orontes River, Turkey. *Turkish Journal Zoology, 32*, 461-467.

Gao, X., Mu, D., Lou, J., Hou1,C., Zhu, J., Wang, J., Wang, C., & Jin, S. (2017). Genetic Diversity and Structure Analysis of *Phascolosoma esculenta* in the Coastal Zone of South-eastern China Based on Mitochondrial Cyt b Gene. *Turkish Journal of Fisheries and Aquatic Sciences*, *18*, 519-528. https://doi.org/10.4194/1303-2712-v18_4_03

Gruenthal, K.M., Acheson, L.K., & Burton, R.S. (2007). Genetic structure of natural populations of California red abalone (*Haliotis rufescens*) using multiple genetic markers. *Marina Biology, 152*, 1237-1248.https://doi.org/10.1007/s00227-007-0771-4

Iguchi, K., Tanimura, Y., & Nishida, M. (1997). Sequence divergence in the mtDNA control region of amphidromous and landlocked forms of ayu. *Fisheries Science*, *63*, 901-905.

https://doi.org/10.2331/fishsci.63.901

Inoue, J.G., Miya, M., Tsukamoto, K., & Nishida, M. (2000). Complete mitochondrial DNA sequence of the Japanese sardine, *Sardinops melanostictus*. *Fisheries Science*, *66*,

924-932. https://doi.org/10.1046/j.1444-2906.2000.00148.x

Kaya, C., Turan, D., & Bayçelebi, E. (2017). Kueik Nehri'nin Güncel Balık Faunası, Kilis. *Proceedings of 3rd International congress on zoology and technology*. (pp. 51.) Afyon, Turkey, 117 pp.

Khaefi, R., Esmaeili, H.R., Ansari, M.H., & Ebrahimi, M. (2018). Genetic diversification and population structure of Barbus cyri De Filippi, 1865 (Teleostei: Cyprinidae) in northern Iran inferred from the mitochondrial D-loop gene sequence. *Environmental Biology of Fishes, 101*, 181-192. https://doi.org/10.1007/s10641-017-0690-2

Kuru, M. (1979). The fresh water fish of South-eastern Turkey-2 (Euphrates-Tigris sisteme). *Hacettepe Bulletin of Natural Sciences and Engineering, 7-8*, 105-114.

Li, W., Chen, X., Xu, Q., Zhu, J., Dai, X., & Xu, L. (2015). Genetic Population Structure of *Thunnus albacares* in the Central Pacific Ocean Based on mtDNA COI Gene Sequences. *Biochemical Genetics, 53*, 8-22. https://doi.org/10.1007/s10528-015-9666-0

Ma, C., Cheng, Q., Zhang, Q., Zhuang, P., & Zhao, Y. (2010). Genetic variation of *Coilia ectenes* (Clupeiformes: Engraulidae) revealed by the complete cytochrome b sequences of mitochondrial DNA. *Journal of Experimental Marine Biology and Ecology*, *385*, 14-19. https://doi.org/10.1016/j.jembe.2010.01.015

Parmaksız, A., & Eskici, H.K. (2018). Genetic Variation of Yellow Barbell (*Carasobarbus luteus* (Heckel, 1843)) From Four Populations Using Mitochondrial DNA COI Gene Sequences. *Applied Ecology and Environmental Research, 16*(2), 1673-1682. http://dx.doi.org/10.15666/aeer/1602_16731682

Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X., & Rozas, R. (2003). DnaSP DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, *19*, 2496-2497.https://doi.org/10.1093/bioinformatics/btg359

Ryman, N. (1991). Conservation genetics considerations in fishery management. *Journal of Fish Biology, 39*, 211-224.https://doi.org/10.1111/j.1095-8649.1991.tb05085.x

Saraswat, D., Lakra, W.S., Nautiyal, P., Goswami, M., Shyamakant, K., & Malakar, A. (2014). Genetic characterization of *Clupisoma garua* (Hamilton 1822) from six Indian populations using mtDNA cytochrome b gene. *Mitochondrial DNA, 25*(1), 70-77. https://doi.org/10.3109/19401736.2013.782014

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution,30*, 2725-2729.https://doi.org/10.1093/molbev/mst197

Ural, M., & Canpolat, İ. (2009). Doğu ve Güneydoğu Anadolu Bölgelerindeki Su Ürünleri Kooperatiflerinin Yapısı ve Av Araçları İle Balıkçı Teknelerinin Teknik Özellikleri. *Tübav Bilim Dergisi*, *2*(4), 372-384.

Xia, L., Guo, B., Ye, Y., Li, J., & Wu, C. (2016). Determination of genetic diversity of the cuttlefish *Sepiella japonica* inhabiting Chinese coastal waters using the mitochondrial D-loop region: The valuable inspiration to artificial releasing Project. *Biochemical Systematics and Ecology, 69*, 274-282. https://doi.org/10.1016/j.bse.2016.05.015

Ward, R.D. (2000). Genetics in fisheries management. *Hydrobiologia*, *420*, 191-201.

Whitehead, A., Anderson, S.L., Kuivila, K.M., Roach, J.L., & May, B. (2003). Genetic variation among interconnected populations of *Catostomus occidentalis*: implications for distinguishing impacts of contaminants from biogeographical structuring. *Molecular Ecology*, *12*, 2817-2833. https://doi.org/10.1046/j.1365-294X.2003.01933.x

Wu, X., Luo, J., Huang, S., Chen, Z., Xiao, H., Zhang, Y. (2013). Molecular Phylogeography and evolutionary history of *Poropuntius huangchuchieni* (Cyprinidae) in Southwest China. *PLoS One*, *8*(11), e79975. https://doi.org/10.1371/journal.pone.0079975

Wu, L., & Yang, J. (2012). Identifications of captive and wild tilapia species existing in Hawaii by mitochondrial DNA control region sequence. *PLoS One, 7*, e51731. https://doi.org/10.1371/journal.pone.0051731

Zhao, L., Erica, L.C., & Liu, Q. (2018). Population structure and genetic diversity of *Sinibrama macrops* from Ou River and Ling River based on mtDNA D-loop region analysis, China, *Mitochondrial DNA Part A, 29*(2), 303-311. https://doi.org/10.1080/24701394.2016.1278533