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Low Genetic Diversity in Turkish Populations of Wels Catfish *Silurus glanis* L., 1758 (Siluridae, Pisces) Revealed by Mitochondrial Control Region Sequences

Yusuf Bektaş[1,] *, Ismail Aksu[2], Gokhan Kalayci[2], Davut Turan[2]

[1]Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdogan University, 53100, Rize, Turkey.
[2]Department of Basic Sciences, Faculty of Fisheries, Recep Tayyip Erdogan University, 53100, Rize, Turkey.

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Corresponding Author
Tel.: +904642234093
E-mail: yusuf.bektas@erdogan.edu.tr

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Abstract

This study aimed to investigate the genetic diversity and population structure of Wels catfish *Silurus glanis* L. 1758 in Turkey using squences of the mitochondrial DNA control region The 887-*bp* fragment of *D-loop* was aligned for 112 *S. glanis* individuals from ten wild populations in Turkey, defined by 29 polymorphic sites comprising 16 haplotypes. The low haplotype diversity and nucleotide diversity within each population ranged from 0.000 to 0.378 and from 0.0000 to 0.0045, respectively. Analysis of molecular variance showed significant genetic differentiation among ten populations (F_{ST} =0.940; P<0.01). AMOVA revealed that the most of genetic variation was found between Thrace and Anatolia clades (74,07 %). The phylogenetic trees and haplotype network topologies were consistent with the results of AMOVA analysis. The non-significant negative *Tajima's D* (-0.875 P<0.05) and *Fu's Fs* (-0.381, P<0.02) values and mismatch distribution for *S. glanis* populations indicated no evidence for changes in population size. Furthermore, goodness-of-fit of the observed versus the theoretical mismatch distribution tested the sum of squared deviation (*SSD*; 0.00308, P>0.05), Harpending's raggedness index (*Hri*; 0,300, P>0.05) and Ramos-Onsins & Rozas (R2; 0,0771, P>0.05), supporting population neutrality.

Introduction

The family Siluridae contains 12 genera and about 100 species (CABI, 2020). The genus *Silurus* has 21 species (Fricke *et al.*, 2020), of which one is native to Euriasia: the wels catfish (*Silurus glanis*). The wels catfish, *Silurus glanis* (Linnaeus, 1758), is one of the largest and the economically important freshwater fish species belonging to the family Siluridae, which is widely distributed throughout Eurasia (Alp *et al.*, 2011; Bănărescu, 1989; Geldiay & Balık, 2007; Kottelat & Freyhof, 2007; Krieg *et al.*, 2000) including Turkey. Although the large size suggests high potential for dispersion, it suggests that natural distribution is slow and density dependent (Copp *et al.*, 2009). *Silurus glanis* is found in all inland waters of Turkey except for the Tigris and Euphrates River Basins (Çelikkale, 1994; Geldiay & Balık, 1988). Despite the increasing economic importance of *S. glanis* in aquaculture (Alp *et al.*, 2011; Arteni, 2009; Copp *et al.*, 2009; Doğan Bora & Gül, 2004; Linhart *et al.*, 2002; Muscalu *et al.*, 2010) and the recent stocking and fish farming activities in Turkey (Saygı & Güleç, 2019; Republic of Turkey Ministry of Agriculture and Forestry, Mediterranean Fisheries Research, Production and Training Institute, 2019), little is known about the genetic structure of cultivated or wild wels catfish stocks in Turkey. *S. glanis* is listed as Least Concern (LC) in the IUCN Red List of Threatened Species

(http://www.iucnredlist.org/). However, the ecology of its wild populations is not well known, probably due to the difficulty of sampling such a large species in large rivers or lentic ecosystems (Alp *et al.*, 2011; Carol *et al.*, 2007; Carol *et al.*, 2009; Copp *et al.*, 2009).

Mitochondrial DNA analysis has proved to be useful for studying population structure and genetic diversity in many animal species (Avise, 1994). The control region (CR), a non-protein-coding region, containing the replication origin of mitochondrial DNA, has been frequently used to reveal population structuring in fish; because: 1) it is not affected by selective pressure because it does not encode any protein, and it accumulates mutations faster than other genomic regions (Kumar *et al.*, 2012, Kumar *et al.*, 2014; Parmaksiz, 2020; Xiao *et al.*, 2009; Xue *et al.*, 2020). To date, the population structure and genetic diversity of some catfishes such as Amur catfish (Xue *et al.*, 2020), Asian catfish (Khedkar *et al.*, 2016), African catfish (Barasa *et al.*, 2016), the silver catfish (Nwafili & Gao, 2016), yellow catfish (Zhong *et al.*, 2013), neotropical catfish (Paixão *et al.*, 2018) and Mekong giant catfish (Phadphon *et al.*, 2019) were investigated by mitochondrial DNA control region. Populations covering of the whole range of *S. glanis* was investigated using PCR-RFLP analysis of four mitochondrial DNA regions, including the control region (Krieg *et al.*, 2000). Although there is a significant mitochondrial differentiation among the populations, a consistent geographic structuring model did not reveal and the nucleotide divergence among the *S. glanis* populations were low. Triantafyllidis *et al.* (2002) investigated the genetic structure of the natural distribution of *S. glanis* using SSR markers and found that levels of genetic diversity were much higher than in previous RFLP mitochondrial DNA analyzes. Despite the large genetic differentiation of the *S. glanis* populations, a consistent geographical structuring model has not emerged, unlike previous studies of European freshwater fish species. A pattern isolation by distance seems more likely, and the final distribution hypothesis has been proposed from only one glacial refugium around the Ponto-Caspian region. The genetic diversity and genetic structure of *S. glanis* populations in the northwestern Iran were evaluated using ISSR markers by Kamangar and Rostamzadeh (2015). The analysis of molecular variance results revealed significant differences between geographic regions in Iran. Palm *et al.* (2019) investigated the genetic structure of Swedish's European wels catfish populations using polymorphic microsatellite loci for a large sample group. Compared to data from a previous study of populations in other parts of Europe, they identified a relatively low-level effective population size and genetic diversity for Swedish populations. In line with the previous proposal of postglacial colonisation from a single refugium, it was reported that all Swedish populations were gathered on a common branch in a star-shaped dendrogram together with other European populations. On the other hand, It was indicated that even minor dispersal barriers may restrict gene flow for wels in running waters. The genetic assignment of specimens encountered in the brackish Baltic Sea and in lakes where the species did not occur naturally showed the presence of long-distance sea dispersal and illegal translocations. So far, the genetic structure of European (Krieg *et al.*, 2000; Triantafyllidis, Ozouf-Costaz *et al.*, 1999; Vittas *et al.*, 2011) and Iranian (Kamangar & Rostamzadeh, 2015) *S. glanis* populations were investigated using few mitochondrial or nuclear markers. However, currently, there is only one report about the genetic diversity, phylogenetic relationship and population genetic structure of wels catfish in Turkey.

Studying genetic diversity and population structure is essential for conservation of wels catfish, which depends on the knowledge of the amount of variation existing in natural populations. Therefore, this approach requires determining evolutionary units for wels catfish conservation and sustainable use. The purpose of this study is to uncover the population genetic structure of *S. glanis* using the control region of mitochondrial DNA (887 bp) from ten localities in Turkey.

Material and Methods

Sample Collections and DNA Extraction

Major river systems representing the natural distribution of Wels catfish in Turkey was chosen as sampling sites. A total of 112 catfish specimens were sampled by traps or trawls from commercial fishermen in ten populations of *S. glanis* throughout Turkey (Figure 1, Table 3). Fish sampling was performed by using multifilament trammel nets with between 20 and 140 mm streched mesh sizes. However, baited pelagic longline with different mouth opening sized hooks and fyke nets were used in fish samplings. Fishing operations was performed at depths varied from 2 to 30 m. Operational plans relating to fishing grounds for the trials were based on the prior experience of fishermen in the region. The number of samples taken for each location is given in Table 3. Fin-clips were immediately preserved in 95% ethanol and stored at 4°C until processed. Whole genomic DNA was extracted using Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI, U.S.A.) according to the manufacturer's protocol, followed by measuring the quality and quantity of total DNA using Nanodrop 2000C spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and subsequently its quality was checked by agarose gel electrophoresis. Extracted DNA was kept frozen at -20°C until gene amplification.

PCR Amplification and Sequencing of *Control Region*

The mitochondrial CR primers (SGDF: 5-GCGCCGGTCTTGTAATCCGG-3 and SGDR: 5-TAGTAAAGTCAGGACCAAGCC-3) were designed from

similar sequences determined by the BLAST search on the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) using the Primer3Plus interface (http://frodo.wi.mit.edu/). The mtDNA CR were amplified via polymerase chain reaction (PCR) in a 50 µl reaction volume with 10 µl of 5x *Taq* polymerase buffer (Promega), 4 µl of 10 mM each dNTP, 4 µl of 25 mM $MgCl_2$, 1 µl of 10 pmol of each primer, 0.2 µl *Taq* DNA polymerase (1U/µl; Promega Corp., Madison, WI), 26.8 µl distilled water and 3 µl template DNA (20 ng/µl) using T-100 Thermal Cyclers (Bio-Rad Laboratories Inc, USA). PCR cycling conditions consisted of an initial denaturation step at 94 ºC for 3 min, 35 cycles of 94 ºC for 45 sec, 53ºC for 45 sec and 72 ºC for 1 min, followed by a final extension of 7 min at 72°C and a 4 ºC hold. PCR products were visualized on EtBr-stained 1.2% agarose gel electrophoresis. PCR products were purified using ethanol precipitation and the purified PCR products were directly sequenced in both directions using PCR primers on an ABI 3730XL DNA Analyzer (Applied Biosystems) by Macrogen Inc. (Amsterdam, The Netherlands).

Data Analysis

Nucleotide sequences of the mitochondrial control region (CR) were trimmed and assembled using Seqman version 7 (Lasergene; DNASTAR). Multiple sequence alignments were created using Clustal W (Thompson *et al.*, 1994) as implemented in MEGA version X (Kumar *et al.*, 2018). The number of haplotype (h), haplotype diversity (Hd) and nucleotide diversity (π) were computed in DnaSP version 5.10.01 (Librado & Rozas, 2009) and Arlequin v 3.5 (Excoffier & Lischer, 2010).

To visualize the relationships among haplotypes, a haplotype network was constructed using the median joining (MJ) algoritm (Bandelt *et al.*, 1999) as implemented in the software PopART 5.0.1.1 (Leigh & Bryant, 2015). Phylogenetic inference analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The most suitable model of nucleotide substitution was calculated by the Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) approaches in the program jModeltest 2.1.1 (Darriba *et al.*, 2012). Maximum likelihood analysis was performed using the HKY+G model the programs GARLI v.2.0 (Zwickl, 2006) with 10 random sequence addition replicates, tree bisection reconnection (TBR) branch swapping with 500 replicates using PhyML 3.0 (Guindon & Gascuel, 2003). The Bayesian analysis was conducted with MrBayes v3.2 (Ronquist *et al.*, 2012) with the HKY+G model determined with jmodelTest 0.1.1 (Posada, 2008). The four-indepentend MCMC (Markov Chain Monte Carlo) analyses were run simultaneously for 2 million generations, with sampling every 1000 generations. The first 25% of generations were discarded as “burn in”. Lake Kastoria haplotypes (Genbank no: AM398435 and AM113592) from the Northern Greece, which represent to Europe, were used as comparison material in phylogenetic analysis. Trees were rooted using CR sequences of Mesopotamian catfish *S. triostegus* Heckel, 1843 (GenBank accession number MT183652-MT183656) were visualized using the MEGA X program.

Hierarchical analysis of molecular variance (AMOVA) with 10,000 permutations was performed to test the population genetic structure of *S. glanis* in Turkey. To quantify the genetic divergence, we computed pairwise F_{ST} (fixation index) values between populations with 10,000 permutations. AMOVA, and F_{ST} were all calculated using the software Arlequin v3.5.2.2 (Excoffier & Lischer, 2010).

Demographic History

We performed the neutrality tests *Fu's Fs* (Fu, 1997) and *Tajima's D* (Tajima, 1989), implemented in the program Arlequin version 3.5 (Excoffier & Lischer, 2010)

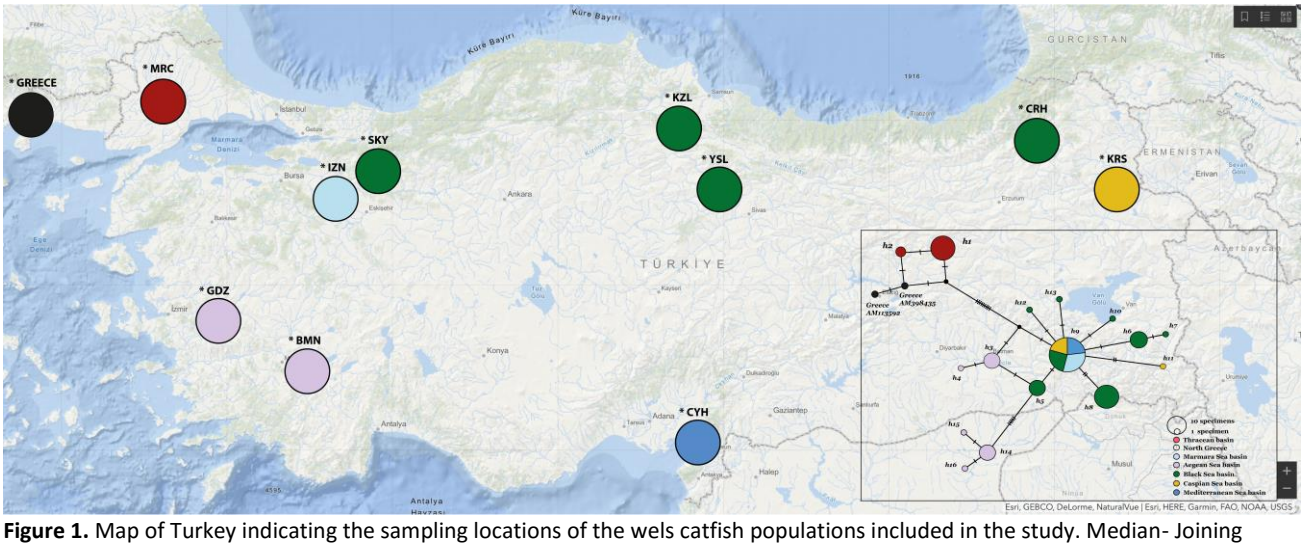

Figure 1. Map of Turkey indicating the sampling locations of the wels catfish populations included in the study. Median- Joining haplotype network of *S. glanis* CR haplotypes from Turkey. The size of circles are relative to the frequency of the haplotype. A branch represents a single nucleotide change.

to infer the demographic history of wels. Significance of these tests (*p* values) was calculated by developing 1000 simulations. The historical demographic events were also investigated with the mismatch distribution analysis using the program Arlequin v 3.5 (Excoffier & Lischer, 2010). The validity of the estimated demographic model was tested using the sum of squared deviations (*SSD*) of observed and expected mismatch (Rogers & Harpending, 1992) with a parametric bootstrapping approach using 10,000 replicates. Deviations from the estimated demographic model were evaluated using the tests of Harpending's raggedness index (*Hri),* (Harpending, 1994) and the *R2* statistic (Ramos-Onsins & Rozas, 2002) in program DnaSP (Librado & Rozas, 2009).

Results and Discussion

Genetic Diversity

A total of 112 specimens of *S. glanis* were sequenced for the mitochondrial CR (887 bp). The nucleotide content averaged 24.2% C, 31.3% T, 30.0% A, and 14.5% G. The *D-loop* was 61.4% A+T rich, which is consistent with the bias reported in other vertebrates (Alvarado Bremer *et al.*, 1997). In 112 specimens, the aligned sequences exhibited 29 variable sites (127 singleton variable sites, 17 parsimony informative sites, and 26 insertions/deletions). Overall, 16 different haplotypes were identified (GenBank accession numbers MT183636- MT183651, Table S1). Their distribution and relative frequencies by population are shown in Table S2. Eigth (50.0%) of these haplotypes occurred just once, one were shared by only two individuals, whereas the haplotypes 1, 3, 5, 6 and 14 were found in eigth or more individuals. Haplotypes 8 and 9, which were the most common haplotypes, were observed in two (CRH and YSL) and four populations (IZN, CYH, KRS and SKY), respectively. Generally, wels catfish samples displayed heterogeneity in haplotype frequencies (Table 2). However, the numerous populations in Anatolia (SKY, IZN, KRS, YSL, CRH and CYH) shared haplotypes 8 and 9 (Table S2), which implies that the presence of the newly isolated populations in Turkey. It is moreover known that the distribution and evolution of several species in Turkish freshwaters such as *Rhodeus* (Bektaş *et al.*, 2013), *Salmo* (Bardakci *et al.*, 2006) and *Capoeta* (Bektaş *et al.*, 2011; Parmaksiz & Eksi, 2017) were influenced by the last glacial periods in Eurasia.

Summary statistics including haplotype richness, haplotype diversity, and nucleotide diversity in each population are given in Table 2. Measures of haplotype and nucleotide diversity based on the CR sequences revealed that the haplotype (H) and the nucleotide diversities (π) within each population ranged from 0.000 to 0.378 and from 0.0000 to 0.0045, respectively (Table 2). Previous studies reported a low level of genetic diversity using different genetic markers such as RFLP (from 0.00 to 0.52 %, Triantafyllidis, Abatzopoulos, *et al.*, 1999) and mtDNA (from 0.00 to 0.91 %, Krieg *et al.*, 2000) in *S. glanis* populations from Europe and Middle East. On the other hand, the present study of mtDNA variation in Turkish *S. glanis* populations indicated low levels of diversity but high differentiation among these populations similar to that of *S. glanis* populations in Greece (Triantafyllidis, Abatzopoulos, *et al.*, 1999) and Europe (Krieg *et al.*, 2000). In contrast to the results of Krieg *et al.* (2000) and Triantafyllidis *et al.* (2002), there is a remarkable genetic distance of 1.3 ± 0.31% between the Thracean and Anatolian populations in Turkey. Although the Black Sea, which is considered as one of the most important refugies that freshwater fish colonize Anatolia and Europe (Bernatchez, 2001; Durand *et al.*, 1999; Kotlík *et al.*, 2004; Kotlík *et al.*, 2008; Triantafyllidis *et al.*, 2002), is thought to promote the diversification of freshwater fish lineages, apex predators with high tolerance to the environmental conditions such as catfish may exhibit a lower level of genetic diversity.

Genetic Relationships

The genetic associations among 16 CR haplotypes identified in eleven populations of *S. glanis*, most of which are separated by a mutational step, are illustrated by the Median joining network (MJ; Figure 1). Topology of the haplotype network obtained from the CR sequences showed a potent haplotypic structure with two distinct haplotype groups: Thracian (MRC) and Anatolian (the rest of all) separated by 8 mutational steps (Figure 1). Phylogenetic relationships are represented by ML and Bayesian trees of 16 haplotypes of *S. glanis* (Figure 2). The topology of ML and BI trees was congruent (Figure 1, 2). The analyses produced highly concordant trees, each revealing that *S. glanis* forms a monophyletic lineage with respect to the two clades (Thrace and Anatolia). In the phylogenetic trees (Figure 2), all haplotypes grouped into two main groups (Thrace: MRZ, and Anatolia: BMN, IZN, SKY, KZL, YSL; SYH, CRH, KRS and GDZ) with high values of posterior probabilities or bootstrap values (Figure 2). In the Anatolian clade, two subclades were seen consisting of Eastern and Western Turkish populations. The phylogenetic tree and haplotype network generally do not show a significant relationship between genetic distance and geography in Turkish populations, while there is a grouping tendency for wels catfish populations in the European (Thrace) and Asian (Anatolia) parts of Turkey. Based on the phylogenetic tree and haplotype network, the population structure of *S. glanis*, detected with two major clades, were observed in the some freshwater species in Turkey such as killifish (Triantafyllidis *et al.*, 2007), salmon (Bardakci *et al.*, 2006; Bernatchez, 2001), narrow-clawed crayfish (Akhan *et al.*, 2014), and fire-bellied toad (Alpagut-Keskin *et al.*, 2010). These two continental clades, "Europe" and "Asia" suggest a prevalence of intra-

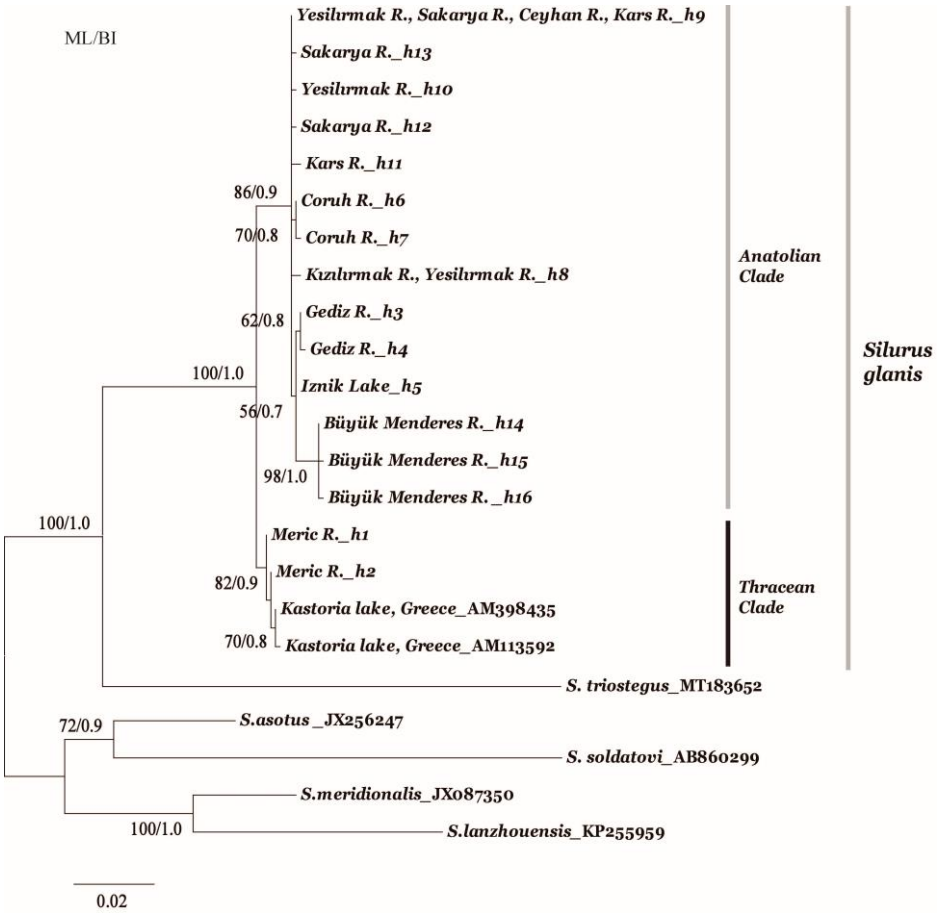

Figure 2. Phylogenetic relationships within *S. glanis* obtained from ML analysis of mtDNA CR sequences. Support values given as MP bootstrap/ML bootstrap/posterior probability: only support values above 50 are shown.

continental diversification of catfishes. Thus, the CR phylogeny identifies the well-known European-Asian relationships within catfishes (Sullivan *et al.*, 2006).

Demographic Neutrality and Population Genetic Structure

Demographic analysis was carried out in ten populations. Non-significant negative *Fu's Fs* (Fs = − 0.381; P<0.02) and Tajima's D test (D= − 0.875; P<0.05) values were detected in all populations except for IZN, KZL and CYH populations, indicating Tajima's D and Fu's Fs test were centered at zero (Table 2), which represents neutrality. The results of the mismatch distribution for Turkish Wels catfish populations (Figure 3) showed a tendency to multimodal and ragged shape, revealing demographic equilibrium (Ray *et al.*, 2003). The non-significant values for the neutrality tests indicate that the polymorphism occurring is coincident and is not under pressure. Both sum of squared deviation (*SSD* = 0.0030, p = 0.092, Table 2) and Harpending's raggedness index (*Hri* = 0.030, p = 0.595, Table 2) values were significant in all populations (p value > 0.05), confirming demographic stability of all population. In addition, the Ramos-Onsins and Rozas's R2 tests were not significant in populations (p value < 0.05), rejecting demographic expansion (R2 = 0.077, p = 0.013, Table 2). Overall, the multimodal mismatch distribution, non-significant negative neutrality index, genetic diversity values and the occurrence of only a few shared haplotypes among the populations showed evidence for demographic equilibrium in wels catfish located in present river systems in Turkey.

F_{ST} value, which is a measure of population differentiation, was calculated for all populations. The values of pairwise F_{ST} of eleven pairs of populations ranged from 0.008 (between KRS and CYH) to 1.0000 (among KZL, IZN and CYH), indicating high genetic differentiation among the populations except for a few population pairs (KZL and YSL, KRS and CYH, CYH and SKY, KRS and SKY) and were statistically non-significant (P≤0.05, Table 1). These results revealed statistically significant population differentiation (F_{ST} = 0.94052) throughout the range of *S. glanis* in Turkey (Table 3).

A hierarchical AMOVA was performed with the specimens divided into two "clades" Thrace and Anatolia populations of *S. glanis*. AMOVA analysis performed on the Turkish populations of *S. glanis* indicated that diversity among clades (F_{CT} = 0.740; P<0.001) explained 74.07% of the total molecular variance and 24.38% was explained by differences among populations within each groups (F_{SC} = 0.9405; P<0.001), whereas only 1.54% of the variance was expressed within the populations (F_{ST} = 0.984; P<0.001).

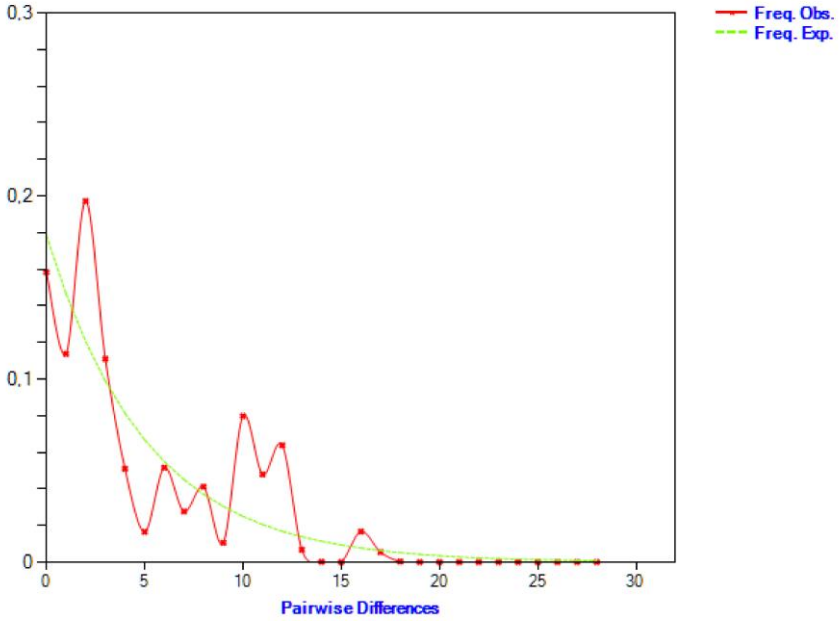

Figure 3. Mismatch distribution for CR haplotypes in *S. glanis* from 10 populations in Turkey under a model of population growth/decline model. The observed frequency is represented by a red line.

Table 1. Genetic distance between (upper diagonal) and within (diagonal) populations, and the fixation index considering genetic distances (F_{ST}) between populations (lower diagonal)

	MRC	GDZ	IZN	CRH	KZL	YSL	CYH	KRS	SKY	BMEN
MRC	0.016	1.168	1.277	1.289	1.396	1.402	1.159	1.181	1.183	1.900
GDZ	0.825	0.025	0.126	0.365	0.468	0.747	0.239	0.260	0.262	0.722
IZN	0.908	0.882	0.000	0.238	0.341	0.347	0.113	0.134	0.136	0.593
CRH	0.833	0.789	0.888	0.023	0.352	0.358	0.124	0.145	0.147	0.836
KZL	0.916	0.894	1.000	0.900	0.000	0.006	0.227	0.247	0.249	0.942
YSL	0.874	0.850	0.922	0.856	0.036	0.013	0.233	0.254	0.256	0.948
CYH	0.923	0.905	1.000	0.909	1.000	0.967	0.000	0.021	0.023	0.709
KRS	0.839	0.799	0.894	0.809	0.904	0.907	0.008	0.041	0.043	0.730
SKY	0.756	0.696	0.791	0.711	0.811	0.904	0.078	0.022	0.045	0.732
BMEN	0.756	0.696	0.791	0.711	0.811	0.974	0.827	0.724	0.622	0.045

Notes: CRH**:** Coruh River, Artvin, KRS: Kars Stream, Kars, YSL: Yesilirmak River, Tokat, KZL: Kizilirmak River, Kırıkkale, SKY: Sakarya River, Sakarya, CYH: Ceyhan River, Adana, IZN: Iznik Lake, Bursa, GDZ: Gediz River, Manisa, BMN: Adıgüzel Lake, Güney, Uşak, MRC: Meric River, Edirne**.** Significance level ^{ns} P≤0.05; * P>0.05.

The significant fixation index Fst in most populations and low haplotype frequencies revealed the fixation of particular haplotypes in the majority of populations. The genetic variations between populations are an indication that there is a low gene flow between populations or that these populations are isolated. Herein, low haplotype frequencies indicate that populations have sufficient time to accumulate unique mutations. Because similar results were also obtained from other fishes such as *Rhodeus* (Bektaş *et al.*, 2013), *Salmo* (Bardakci *et al.*, 2006) and *Capoeta* (Bektaş *et al.*, 2011; Parmaksiz & Eksi, 2017) in region. The analysis of molecular variance reveals clear genetic difference between the Thracean and Anatolian populations of *S. glanis* in Turkey (Table 3), in contrast to previous studies of wels catfish (Krieg *et al.*, 2000; Triantafyllidis *et al.*, 2002). The AMOVA analysis previously conducted for catfish (Krieg *et al.*, 2000) suggested that most of the genetic polymorphism (with 79%) was due to differences between natural populations. This is similar when hatchery populations were included or taking into consideration only the populations that were analyzed in a previous allozyme study (Triantafyllidis, Ozouf-Costaz *et al.*, 1999). In contrast to, the present study revealed that most of the molecular variance (74%) is among the predicted groups. This can be explained by the fact that wels catfish populations from two different continents (Europe and Asia), separated by a marine barrier, were included in the study, unlike previous studies. Even though the notable genetic differentiation of *S. glanis* populations, no meaningful model of geographical structuring was revealed (Figure 2a, 2b), contrary to previous studies on the Anatolian freshwater fish species. This phenomenon could possibly be explained by the fact that *S. glanis*, which is very tolerant to environmental changes, may have reached present its

Table 2. Statistical tests of neutrality and demographic parameter estimates with *P*-value (in parenthesis) for *Silurus glanis* populations. Population code (Pc), sample size (N), numbers of haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π), number of haplotypes (H), Tajima's D (D), Fu's (1997) *F*s (*F*s), sum of squares differences imn mistmach analysis (SSD), Harpending's (1994) raggedness index (H_{ri}), Ramos-Onsins and Rozas (R2) and GenBank Accession Numbers (GAN) are presented

		Diversity index			Neutrality test		Mistmatch distribution analysis			
Pc	N	h	H_d	π	D	*F*s	SSD (p)	H_{ri} (p)	R_2	GAN
MRC	14	h1-h2	0,143±0,119	0,00016±0,00013	-1,155 ^{NS}	-0,594 ^{NS}	0.00014 **	0,530 **	0,257^{NS}	MT183636, MT183637
GDZ	9	h3-h4	0,222±0,166	0,00025±0,00019	-1,088 ^{NS}	-0,263 ^{NS}	0.00046 **	0,358 **	0,314^{NS}	MT183638, MT183639
IZN	8	h5	0,000±0,000	0,00000±0,00000	0,000 ^{NS}	0.000 ^{NS}	0.00000 ^{NA}	0.000 ^{NA}	0,000^{NS}	MT183640
CRH	10	h6-h7	0,200±0,154	0,00023±0,00017	-1,111 ^{NS}	-0,339 ^{NS}	0.00041 **	0,400 **	0,300^{NS}	MT183641, MT183642
KZL	10	h8	0,000±0,000	0,00000±0,00000	0,000 ^{NS}	0.000 ^{NS}	0.00000 ^{NA}	0.000 **	0,000^{NS}	MT183643
YSL	18	h8-h10	0,111±0,096	0,00013±0,00011	-1,164 ^{NS}	-0,794 ^{NS}	0,00006 **	0,617 **	0,229^{NS}	MT183643-645
CYH	12	h9	0,000±0,000	0,00000±0,00000	0,000 ^{NS}	0.000 ^{NS}	0.00000 **	0.000 ^{NA}	0,000^{NS}	MT183644
KRS	11	h9, h11	0,182±0,144	0,00041±0,00032	-1,429 ^{NS}	0,506 ^{NS}	0,01810 **	0,735 **	0,287^{NS}	MT183644, MT183646
SKY	10	h9, h12-13	0,378±0,181	0,00045±0,00023	-1,400 **	-1,164 ^{NS}	0.00580 **	0,182 **	0,200^{NS}	MT183644, MT183647-648
BMN	10	h14-16	0,378±0,181	0,00045±0,00023	-1,400 **	-1,164 ^{NS}	0.00580 **	0,182 **	0,200^{NS}	MT183649-651
	112	16	0,842±0,018	0,00519±0,00052	-0,875 **	-0,381 ^{NS}	0.00308 **	0,030 **	0,077^{NS}	

^{NA}Not avaliable; ^{NS}Nonsignificant,*P<0.02, **P<0.05; =significant;

CRH**:** Coruh River, Artvin, KRS: Kars River, Kars, YSL: Yesilirmak River, Tokat, KZL: Kizilirmak River, Kırıkkale, SKY: Sakarya River, Sakarya, CYH: Ceyhan River, Adana, IZN: Iznik Lake, Bursa, GDZ: Gediz River, Manisa, BMN: Adıgüzel Lake, Uşak, MRC: Meric River, Edirne.

Table 3. Results of analysis of molecular variance (AMOVA) test on CR in different populations (Thrace and Anatolia) of *Silurus glanis* in Turkey

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation	Fixation Indices	
Among groups	2	124.122	4.27569 *V*a	74.07243	F_{CT}	0.74072***
Among populations within groups	7	122.369	1.40760 *V*b	24.38527	F_{SC}	0.98458***
Within populations	102	9.080	0.08902 *V*c	1.54220	F_{ST}	0.94052***
Total	111	255.571	4.85458			

F_{ST} fixation index within populations; F_{SC} fixation index among populations within groups; F_{CT} fixation index between groups. *** < 0.001

geographical range through the late Pleistocene Black Sea (25-11.7 kya, Würm period) characterized by relatively low salinity levels in coastal areas (Copp *et al.*, 2009; Froese & Pauly, 2020).

Conclusion

Wels catfish in Turkey exhibit similarly low genetic diversity to those in Europe. This low genetic diversity might reflect the presence of a single Pleistocene glacial refugia in Black Sea, followed by postglacial expansion and subsequent genetic isolation. However, it has been determined that Thracian populations gradually diverge from the genetically remaining Anatolian populations. *S. glanis*, which is estimated to have spread to the region from the Pontic-Caspian basin, may have started to make different choices in its gene pool during the adaptation period to the habitats in which they settled. In order to support commercial fish stocks, the Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TEGEM) has been carrying out the fisheries project of Turkish inland waters since 2013. In this project, 20 thousand juveniles obtained from the industrial production of Gölhisar origin wels catfish are left to the Gölhisart lake (Burdur), Iznik lake (Bursa), Porsuk dam (Kütahya), Kemer dam (Aydın) ve Hirfanlı dam (Ankara). Creating new stocks with hatchery breeding and release is an economically important strategy, but attention should be paid to maintaining the existence of genetic motifs in evolutionarily important units such as the Menderes, Iznik and Gediz populations.

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