Wiley
Journal of Applied Ichthyology
Volume 2024, Article ID 6851143, 11 pages
https://doi.org/10.1155/2024/6851143

WILEY

Research Article

Population Genetic Structure of *Petroleuciscus borysthenicus* (Kessler 1859) in Northwestern Türkiye Using Mitochondrial COX1 Gene

Sevan Ağdamar,[1] **Gülşah Saç**,[2] **Ümit Acar**,[1] **Özcan Gaygusuz**,[3] **Ersin Doğaç**,[4] **and Müfit Özuluğ**[2]

[1]*Canakkale Onsekiz Mart University, Bayramic Vocational School, Department of Forestry, Bayramic, Canakkale, Türkiye*
[2]*Istanbul University, Faculty of Science, Department of Biology, Vezneciler, Istanbul, Türkiye*
[3]*Istanbul University, Faculty of Aquatic Sciences, Department of Marine and Freshwater Resources Management, Laleli, Istanbul, Türkiye*
[4]*Mugla Sitki Kocman University, Faculty of Science, Department of Molecular Biology and Genetics, Kotekli, Mugla, Türkiye*

Correspondence should be addressed to Ümit Acar; umitacar@comu.edu.tr

Received 2 November 2023; Revised 11 June 2024; Accepted 22 June 2024

Academic Editor: Jorge Paramo

Petroleuciscus borysthenicus is a species with a wide distribution from the eastern, western, and northern Black Sea and Azov Sea basins, the Aegean Sea basin, and Northwestern Türkiye. However, there has been relatively little attention on the genetic variability of this species in their native distribution range, and nearly no study has been conducted in Türkiye despite its importance in biodiversity. The aim of this study was to elucidate the genetic variability and population structure of *P. borysthenicus* from Northwestern Türkiye using an integrated molecular method. A total of 72 specimens were collected from 13 localities in the Northwestern part of Türkiye. A total of six haplotypes were identified in all specimens. A relatively low level of genetic variability was found for *P. borysthenicus* throughout the study region based on the indices of genetic diversity including haplotype diversity and nucleotide diversity for the cytochrome oxidase subunit I ($h = 0.507$, $\pi = 0.0027$). The pairwise F_{ST} values between the samples ranged from −0.123 to 1.000. Furthermore, our results revealed no provable recent demographic expansion for *P. borysthenicus* from Northwestern Türkiye. However, more studies using additional geographic sampling and molecular analysis are needed to enhance our knowledge of the diversity and distribution of this species.

1. Introduction

There are nearly 300 native fish species in Anatolia, of which more than one-third are endemic, making it a biodiversity hotspot for freshwater fish species [1]. Several factors threaten this native fauna, including pollution, non-native species introductions, dam constructions, drainage, and overabstraction of water [2]. At this point, genetic diversity can help to estimate which species are most likely to adapt to future environmental changes [3]. Understanding how patterns of genetic variation change among fish can help identify species that may be particularly vulnerable to extinction and predict which species will likely adapt to future challenges [4].

Loss of genetic diversity is a major issue in conservation biology as it can inhibit a species from replying to natural selection and restrict its evolutionary influence [5]. Knowledge on genetic diversity helps authorities to make decisions on whether they should elaborate on the re-constitution of ideal habitat areas as stepping stones between present habitats, even on the conservation or expansion of current habitats to maintain genetic diversity and accordingly viability of the populations [6]. Translocation of the species or geographical isolation can affect the movement of

individuals and groups among native populations, altering the patterns of genetic differentiation due to gene flow. This can significantly impact the temporal and spatial dispersion of genetic diversity and the evolutionary advancement of native populations [7]. Isolation periods associated with environmental and geographical distances might create great demographic and genetic results for populations within species dispersion ranges [8]. Historical events of range extension and constriction may also change existing isolation impacts and influence genetic structure [9]. All these mechanisms may act alone or in synchrony, and their significance can change contingent on the spatial scale of the survey.

With the new techniques developed in the twenty-first century, the completion of morphology-based studies with molecular analyses allows significant changes in the characterization/classification of organisms. Species previously classified under the genus *Leuciscus* Cuvier, 1816, have been reclassified into the genus *Petroleuciscus* [10]. This genus, which extends eastward from the Black Sea and eastern Aegean drainages to Iran and Uzbekistan [11], now comprises five valid species [12, 13] including *Petroleuciscus aphipsi* (Aleksandrov, 1927), *Petroleuciscus borysthenicus* (Kessler, 1859), *Petroleuciscus ninae* [14], *Petroleuciscus smyrnaeus* (Boulenger, 1896), and *Petroleuciscus squaliusculus* (Kessler, 1872). Currently, three species of this genus are recognized in the inland waters of Türkiye. *Petroleuciscus borysthenicus* is found in the lotic and lentic systems of the Black Sea, Marmara, and northern Aegean Sea basins, and *P. smyrnaeus* and *P. ninae* are found in western Anatolia [14, 15]. Despite the known impact of habitat divergence and geographical isolation on speciation [16], studies on the genetic variation of *P. borysthenicus* remain limited [11, 14, 17, 18]. Therefore, this study aims to characterize the genetic variability and population structure among populations of *P. borysthenicus* in Northwestern Türkiye through the analysis of a partial mitochondrial cytochrome oxidase subunit I (COX1) gene region. Thus, constructing DNA sequence libraries and understanding genetic variability are essential to developing more effective management programs and conservation strategies for native fish species.

2. Materials and Methods

2.1. Study Area and Sampling. Fish samples were collected by electrofishing with SAMUS 725MP and 1000 portable electroshockers. The sampling sections, approximately 100 meters in length, were surveyed against the direction of water flow. Immediately after capture, fish specimens were anesthetized with clove oil and fixed caudal fin in 99% ethanol for molecular analyses. All the specimens were fixed with a 5% formalin solution for morphological analyses for further studies. The field surveys were conducted in a total of 13 sampling sites (shallow and small streams) encompassing the Marmara and Meriç-Ergene river basins (Figure 1, Table 1). In addition, one sampling site was on Gökçeada Island, an isolated ecosystem (Figure 1, Table 1). Each population's abbreviation is derived from its initials (e.g., "ES" for Efendi Stream), and these abbreviations are consistently used throughout the text. The map was created using the QGIS v. 3.4 software (available from https://qgis.org).

For molecular analyses, sampling sites were grouped according to the geographical position of the species by taking into account the geological history of the region [11, 19, 20]. Group 1 encompasses streams in the Marmara Basin that flow into the Black Sea without connection, Group 2 includes streams flowing into the Marmara Sea without interconnection, and Group 3 comprises Meriç-Ergene Basin streams that flow into the Aegean Sea (see Table 1).

2.2. DNA Extraction. For 72 collected specimens, total genomic DNA was isolated from muscle and caudal fin tissues for the specimens using a modified Lifton method [21]. DNA concentration and quality were checked using the NanoDrop ND-1000 spectrophotometer and visualized using 1% agarose gel electrophoresis. The final DNA concentration of all samples was set to 25 ng/μl. DNA samples were used either immediately in a polymerase chain reaction (PCR) or stored at −20°C.

2.3. PCR and Sequencing. The primer pairs were used for the amplification of the COX1 gene region: FishF1-5′TCA ACCAACCACAAAGACATTGGCAC3′, FishR1-5′TAGAC TTCTGGGTGGCCAAAGAATCA3′ [22]. DNA amplifications were performed in 25 μl volumes, each containing: 2 μl of 10X Taq Buffer with KCl (100 mM Tris-HCl, 500 mM KCl, pH 8.8), 2 μl of MgCl2 (25 mM), 1 μl of dNTPs (10 mM), 1 μl of each primer (10 pM/μl), 1 U of Taq polymerase (5 U/μl), and 2 μl of DNA (50 ng/μl). PCR amplifications were performed in an Eppendorf Mastercycler® with the following cycling conditions [23]: initial denaturation at 95°C for 5 min followed by 35 cycles consisting of denaturation at 95°C for 1 min, primer annealing at 56°C for 1 min, primer extension at 72°C for 1 min, and final extension step at 72°C for 10 min. To verify the size of the amplified region and assess potential contamination, all PCR products were visualized under UV light after electrophoresis on 1.5% agarose gels. Sanger sequencing was performed by BM Labosis (Ankara, Türkiye) using ABI 3730XL DNA Analyzer (Applied Biosystems, USA).

2.4. Data Analysis. Nucleotide sequences were trimmed and assembled using Geneious 8.1 [24]. Multiple sequence alignments were created using ClustalW algorithm in MEGA X [25] and deposited in GenBank database under accession numbers MZ350082-MZ350083, MZ350086-MZ350087, and MZ350799-MZ350800. The number of haplotypes (H), haplotype diversity (h), and nucleotide diversity (π) were determined using Arlequin 3.5 [26] and DnaSP 6 [27]. A haplotype network for COX1 was constructed to visualize interhaplotype relationships, employing the median-joining (MJ) method with PopART 1.7 [28]. This network incorporated previously identified haplotypes of *P. borysthenicus* from various global localities.

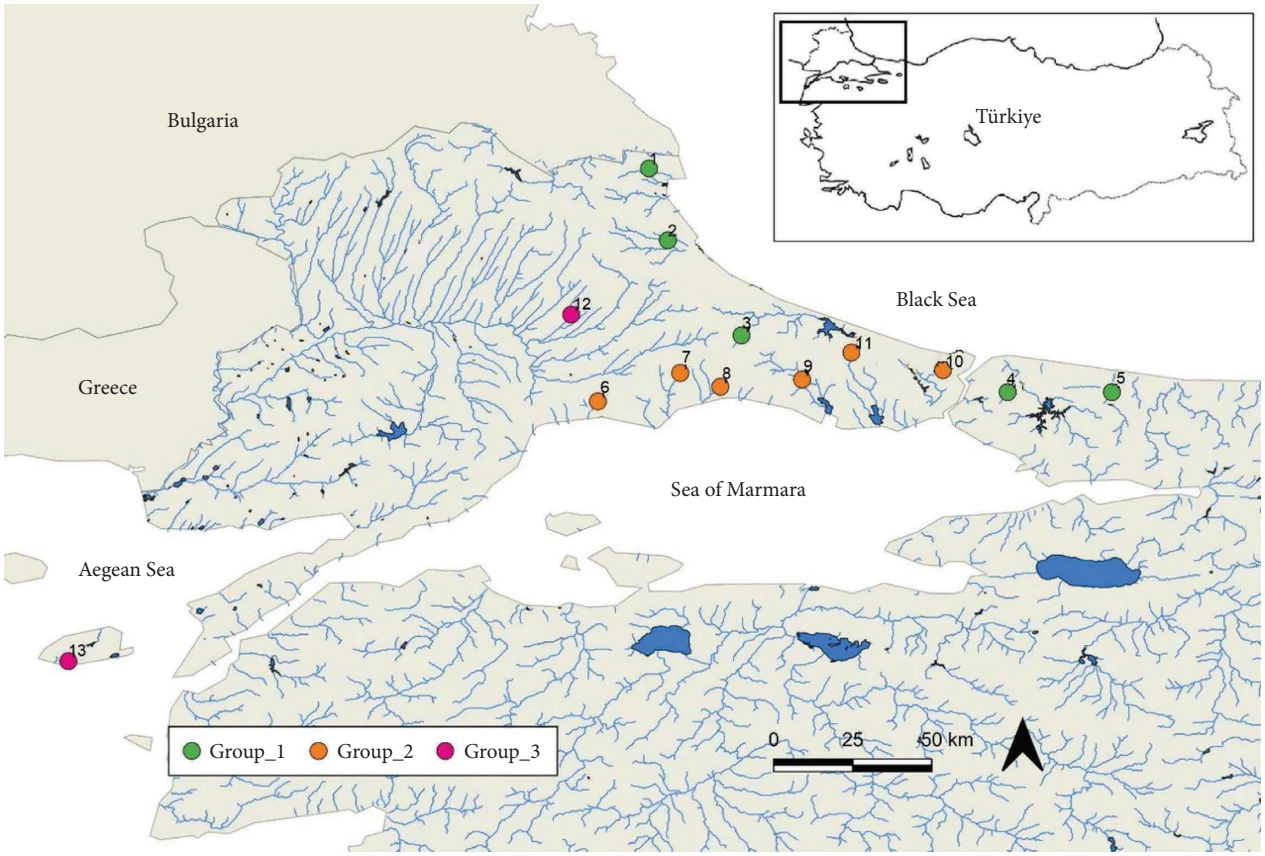

Figure 1: Sampling sites in the study area (Northwestern Türkiye).

Table 1: Data on the sampling sites of *Petroleuciscus borysthenicus* in Northwestern Türkiye.

Locality no.	Locality	Sample size	Coordinates	Province, basin	Sampling time
Group_1					
1	Efendi stream	5	41.927050, 27.908495	Kırklareli, MB-1	August 2017
2	Pabuç stream	5	41.663563, 27.977719	Kırklareli, MB-1	August 2017
3	Danamandıra stream	7	41.313644, 28.248347	İstanbul, MB-1	March 2013
4	Cumhuriyet stream	5	41.123792, 29.251743	İstanbul, MB-1	July 2017
5	Karaman stream	5	41.145747, 29.591917	İstanbul, MB-1	August 2016
Group_2					
6	Hacımurat stream	5	41.050463, 27.734301	Tekirdağ, MB-2	July 2017
7	Değirmenköy stream	5	41.175352, 28.023172	İstanbul, MB-2	April 2016
8	Kula stream	5	41.124713, 28.170686	İstanbul, MB-2	April 2016
9	Çatalca stream	5	41.151158, 28.471280	İstanbul, MB-2	April 2016
10	Bahçeköy stream	5	41.185990, 28.988924	İstanbul, MB-2	May 2017
11	Baklalı stream	5	41.250402, 28.652261	İstanbul, MB-2	April 2017
Group_3					
12	Teke stream	5	41.606913, 27.040950	Kırklareli, MEB	August 2017
13	Şahinkaya stream	10	40.115888, 25.774619	Çanakkale, AS	September 2019

AS: Aegean Sea, MB-1: Marmara Basin flows into the Black Sea, MB-2: Marmara Basin flows into the Marmara Sea, MEB: Meriç-Ergene Basin.

The analysis of molecular variance (AMOVA) with 5000 permutations was performed to test the population genetic structure of *P. borysthenicus* in Northwestern Türkiye. To quantify the genetic divergence, we computed pairwise F_{ST} (fixation index) values between populations with 5000 permutations. AMOVA and F_{ST} values were calculated in Arlequin 3.5 [26].

The neutrality tests *Fu's Fs* [29] and *Tajima's D* [30] were conducted in Arlequin 3.5 [26] to infer the demographic history of the species from the study area. The significance of these tests (p values) was calculated by developing 1000 simulations. Historical changes in effective population size were also investigated using the mismatch distribution (MMD) analysis in DnaSP 6 [27]. The validity of the

Table 2: Summary statistics of genetic variation parameters estimated with p value for *Petroleuciscus borysthenicus* populations.

Group	Population	Code	N	H	h	π	D	F_s	SSD	H_{ri}
Group 1	Efendi stream	ES	5	H1-H2	0.600 ± 0.031	0.0019 ± 0.00003	1.459^{NS}	1.688^{NS}	0.196^{*}	0.880^{NS}
	Pabuç stream	PS	5	H2	0	0	0^{NS}	NA	0^{NA}	0^{NA}
	Danamandıra stream	DS	7	H1	0	0	0^{NS}	NA	0^{NA}	0^{NA}
	Cumhuriyet stream	CS	5	H1	0	0	0^{NS}	NA	0^{NA}	0^{NA}
	Karaman stream	KS	5	H1	0	0	0^{NS}	NA	0^{NA}	0^{NA}
			27	2	0.399 ± 0.025	0.0013 ± 0.00002	0.292^{NS}	0.338^{NA}	0.039^{*}	0.176^{NS}
Group 2	Hacımurat stream	HS	5	H3	0	0	0^{NS}	NA	0^{NA}	0^{NA}
	Değirmenköy stream	DKS	5	H1	0	0	0^{NS}	NA	0^{NA}	0^{NA}
	Kula stream	KLS	5	H4	0	0	0^{NS}	NA	0^{NA}	0^{NA}
	Çatalca stream	CTS	5	H1-H4	0.400 ± 0.056	0.0025 ± 0.00008	-1.094^{NS}	2.202^{NS}	0.110^{NS}	0.680^{NS}
	Bahçeköy stream	BKS	5	H1	0	0	0^{NS}	NA	0^{NA}	0^{NA}
	Baklalı stream	BS	5	H1-H4-H5	0.800 ± 0.027	0.0044^{NA}	1.124^{NS}	1.220^{NS}	0.113^{NS}	0.280^{NS}
			30	4	0.637 ± 0.038	0.0031 ± 0.00005	0.005^{NS}	0.570^{NA}	0.037^{NS}	0.160^{NS}
Group 3	Teke stream	TS	5	H1	0	0	0^{NS}	NA	0^{NA}	0^{NA}
	Şahinkaya stream	SS	10	H1-H2-H6	0.378 ± 0.033	0.0010 ± 0.00002	-1.562^{*}	-0.459^{NS}	0.004^{NS}	0.222^{NS}
			15	3	0.590 ± 0.020	0.0018 ± 0.00001	-0.781^{NS}	-0.229^{NA}	0.002^{NS}	0.111^{NS}
Total			72	6	0.507 ± 0.052	0.0027 ± 0.0003	-0.006^{NS}	0.358^{NA}	0.033^{NS}	0.159^{NS}

N: number of sequences, H: number of haplotypes, h: haplotype diversity, π: nucleotide diversity, D: Tajima's neutrality test, F_s: Fu's neutrality test, *SSD*: sum of squares differences in mismatch analysis, H_{ri}: Harpending's raggedness index (^{NA}not avaliable; ^{NS}nonsignificant, $^{*}P < 0.05$; = significant).

estimated demographic model was tested using the sum of squared deviations (*SSD*) of observed and expected mismatch [31] with a parametric bootstrapping approach using 5000 replicates. Deviations from the estimated demographic model were evaluated using the tests of Harpending's raggedness index (H_{ri}) [32] in DnaSP 6 [27].

3. Results

A total of 72 *P. borysthenicus* specimens were sequenced for the mitochondrial COX1 barcode region (630 bp). In 72 specimens, six haplotypes were obtained, and the aligned sequences exhibited 7 variable sites (2 singleton and 5 parsimony informative sites).

Summary statistics of genetic variation parameters of all examined populations are given in Table 2. Haplotype diversity (h) ranged from 0 to 0.800 with the total entire value of 0.507 ± 0.052. Nucleotide diversity (π) among the populations varied from 0 to 0.004 with an overall value of 0.003 ± 0.0003. The total number of haplotypes (H), h, and π values for the three groups were $H = 2.00$, $h = 0.399 \pm 0.025$, and $\pi = 0.001 \pm 0.00002$; $H = 4.00$, $h = 0.637 \pm 0.038$, and $\pi = 0.003 \pm 0.00005$; $H = 3.00$, $h = 0.590 \pm 0.020$, and $\pi = 0.002 \pm 0.00001$, respectively.

The pairwise F_{ST} values of 13 populations ranged from −0.123 (between DKS and KLS; $p > 0.05$) to 1.000 (among BS, CS, DS, HS, KS, PS, TS, BKS, and CTS; $p < 0.05$; see Table 3). The results also indicated statistically significant population differentiation ($F_{ST} = 0.7446$).

The analysis of molecular variance (AMOVA), which was carried out with the collected specimens divided into three groups based on geographical positions that shaped their dispersal patterns. Partitioning of genetic diversity by AMOVA (Table 4) using grouped populations revealed that out of total genetic diversity, most of the COX1 diversity was distributed among populations (56.87%); remaining diversity was distributed within populations (21.75%) and among groups (21.138%), with fixation indices ($F_{ST} = 0.7446$, $F_{SC} = 0.1087$, and $F_{CT} = 0.7135$). AMOVA, which was performed with the specimens divided into three groups, revealed that a significant percentage of genetic variation (56.87%; $F_{SC} = 0.1087$; $p < 0.05$) in the COX1 barcode region of *P. borysthenicus* arose from genetic differences among populations within groups rather than variation within populations (21.75%; $F_{ST} = 0.7446$; $p < 0.05$) or among groups (21.38%; $F_{CT} = 0.7135$; $p < 0.05$).

Genetic relationships among the six observed haplotypes in 13 populations as well as the haplotypes from previous studies were determined using a median-joining haplotype network (Figure 2). Furthermore, the distribution and frequency of *P. borysthenicus* haplotypes in the studied populations were shown in the supplementary material. The results showed that two haplotypes (H5 and H6) detected in the studied Turkish samples were elucidated for the first time in this study, and the other four haplotypes (H1, H2, H3, and H4) were shared as well as the localities from the previous studies. Additionally, *P. borysthenicus* samples used in this study and the haplotypes and distributions of the samples registered in GenBank were presented in Table 5. Two haplotypes (H1 and H2) were the most common and widespread variants, seen in 79.17% of the specimens studied from Northwestern Türkiye.

The nonsignificant values for the neutrality tests (*Tajima's D* and *Fu's Fs*) revealed that the polymorphism resultant is COX1 coincidental and is not under pressure (Table 2). The results of the mismatch distribution for Turkish *P. borysthenicus* populations indicated demographic equilibrium (Figure 3). However, the sum of squared deviation (*SSD*) and Harpending's raggedness index (*Hri*) values rejected the demographic stability of all populations (Table 2). As a consequence, the multimodal mismatch distribution, nonsignificant neutrality tests, genetic variation

Table 3: Pairwise F_{ST} values among the studied *Petroleuciscus borysthenicus* populations based on COX1 data and the significance of population differentiation $^{*}p$ 0.05.

Group	Population	Code	ES	PS	DS	CS	KS	HS	DKS	KLS	CTS	BKS	BS	TS	SS
Group 1	Efendi stream	ES	—												
	Pabuç stream	PS	0.500	—											
	Danamandıra stream	DS	0.331	1.000*	—										
	Cumhuriyet stream	CS	0.250	1.000*	0.000	—									
	Karaman stream	KS	0.250	1.000*	0.000	0.000	—								
Group 2	Hacımurat stream	HS	0.250	1.000*	0.000	0.000	0.000	—							
	Değirmenköy stream	DKS	0.123	0.533*	0.304*	0.222	0.222	0.222	—						
	Kula stream	KLS	0.028	0.667*	0.073	0.000	0.000	0.000	−0.123	—					
	Çatalca stream	CTS	0.850*	1.000*	1.000*	1.000*	1.000*	1.000*	0.462	0.750*	—				
	Bahçeköy stream	BKS	0.800*	1.000*	1.000*	1.000*	1.000*	1.000*	0.417	0.692*	1.000*	—			
	Baklalı stream	BS	0.250	1.000*	0.000	0.000	0.000	0.000	0.222	0.000	1.000*	1.000*	—		
Group 3	Teke stream	TS	0.250	1.000*	0.000	0.000	0.000	0.000	0.222	0.000	1.000*	1.000*	0.000	—	
	Şahinkaya stream	SS	0.333	−0.084	0.815*	0.791*	0.791*	0.791*	0.523*	0.588*	0.901*	0.870*	0.791*	0.791*	—

Table 4: AMOVA for the three basin groups of *Petroleuciscus borysthenicus* from Northwestern Türkiye based on mitochondrial COX1.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation	Fixation indices
Among groups	2	10.776	0.1810 *Va*	21.38	F_{CT} 0.7135
Among populations	10	28.666	0.5389 *Vb*	56.87	F_{SC} 0.1087
Within populations	59	10.962	0.1967 *Vc*	21.75	F_{ST} 0.7446
Total	71	50.404	0.9165		

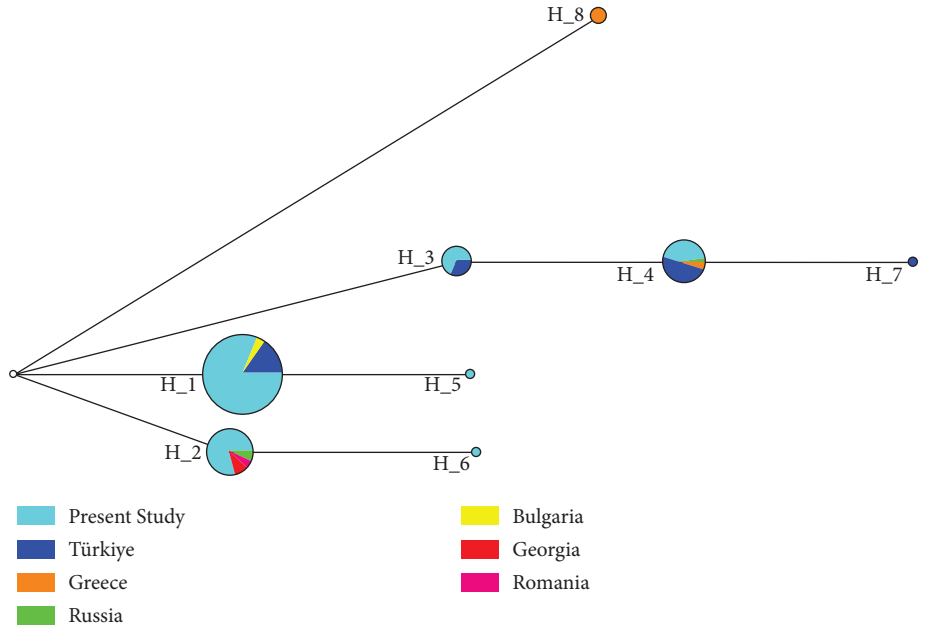

Figure 2: Haplotype network for COX1 sequences of *Petroleuciscus borysthenicus*. Haplotype names and their distributions by countries are presented in Table 5. Mutational steps are shown with hatch marks. Unique haplotypes determined in this study are shown underlined in Table 5.

Table 5: Novel and previously published GenBank accession numbers for the COX1 haplotypes of *Petroleuciscus borysthenicus* used in this study.

Haplotype	Country	Location	GenBank Acc. no.	References
H1	Türkiye	Efendi stream	MZ350082	Present study
		Danamandıra stream		Present study
		Cumhuriyet stream		Present study
		Karaman stream		Present study
		Değirmenköy stream		Present study
		Çatalca stream		Present study
		Bahçeköy stream		Present study
		Baklalı stream		Present study
		Teke stream		Present study
		Şahinkaya stream		Present study
		Karamandere stream	MF362192	[14]
		Bahçeköy stream	MF362193-MF362196	[14]
		Çamaşır stream	MF362197, MF362198	[14]
		Lake Sapanca	MG775382	[33]
	Bulgaria	Orljaska drainage	KJ554175, KJ554232	[17]
H2	Türkiye	Efendi stream	MZ350083	Present study
		Pabuç stream		Present study
		Şahinkaya stream		Present study
	Russia	Kherota River	MG775381	[33]
	Romania	Lake Somova	MG775380	[33]
	Georgia	Gubistskali River	KX516732, KX516733	[34]
H3	Türkiye	Hacımurat stream	MZ350086	Present study
		Ergene River	MF362190, MF362191	[14]

Table 5: Continued.

Haplotype	Country	Location	GenBank Acc. no.	References
H4	Türkiye	Kula stream		Present study
		Çatalca stream	MZ350087	Present study
		Baklalı stream		Present study
		Bakakak stream	HM560280	[11]
		Sakar stream	MF362183-MF362186	[14]
		Çınarcık stream	MF362188, MF362189	[14]
		Bakacak stream	MG775379	[33]
	Greece	Fotolivos stream	HM560281	[11]
H5	Türkiye	Baklalı stream	MZ350799	Present study
H6	Türkiye	Şahinkaya stream	MZ350800	Present study
H7	Türkiye	Çapraz stream	MF362187	[14]
H8	Greece	Strymon River	MG775383	[33]
		Agios Vjanis River	MG806855	[35]

Underlined haplotypes are detected for the first time.

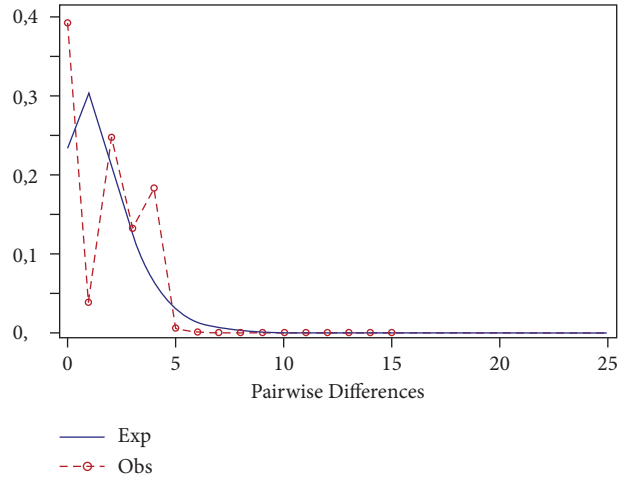

Figure 3: Mismatch distribution for COX1 haplotypes in *Petroleuciscus borysthenicus* under a model of population growth/decline. The observed and expected frequencies are represented by different colored lines.

values, and the occurrence of a few shared haplotypes among the populations indicated proof of a demographic equilibrium in *P. borysthenicus* located in the studied river basins in Türkiye. Estimates of *Tajima's D* ($D = -0.006$; $p > 0.05$) and Fu's Fs ($F_s = 0.358$; $p > 0.05$) were detected in some populations (i.e., BS, CTS, ES, and SR), but none of these deviations from neutrality were statistically significant (except the *D* value in SR; see Table 2). Results of the mismatch distribution analysis for the studied Turkish *P. borysthenicus* populations revealed a tendency to multimodal and ragged shape (Figure 3). Except for ES, estimates of both sums of squared deviation ($SSD = 0.033$) and Harpending's raggedness index ($Hri = 0.159$) were nonsignificant in the studied populations ($p > 0.05$; Table 2).

4. Discussion

In the present study, the genetic structures of *P. borysthenicus* populations from Northwestern Türkiye were investigated. There is only one recent study on the molecular phylogeny of *Petroleuciscus* species from Türkiye [18], where different *Petroleuciscus* species (*P. borysthenicus, P. smyrnaeus*, and *P. ninae*) were analysed for their mitochondrial cytochrome b (cyt b) gene variation, and a monophyletic group distinguishable from its sisters has been determined.

The observed values of mitochondrial COX1 variation including the haplotype and nucleotide diversity were relatively low ($h = 0.507$, $\pi = 0.0027$) for *P. borysthenicus* throughout the study area (Table 2). Consistent with our findings, low levels of COX1 variation have been reported in different freshwater fish species by several previous studies such as *Carasobarbus luteus* ($h = 0.534$, $\pi = 0.0037$; [36]), *Carassius gibelio* ($h = 0.500$, $\pi = 0.0127$; [37]), *Cyprinus carpio* ($h = 0.556$, $\pi = 0.0003$; [37]), and *Squalius aradensis* ($h = 0.435$, $\pi = 0.0008$; [38]). Habitat disruption and heavy fishing are widely recognized as principal factors reducing aquatic genetic variation [39]. The lower genetic variation values observed in some studied populations (e.g., CS, KS, DKS, and TS; see Table 2) might be associated with genetic bottleneck [40] or suggest that populations have been founded by a few individuals [41]. On the other hand, habitat

loss and destruction usually result in small, isolated populations fragile to environmental disruption and loss of genetic variation [42, 43]. The disturbance history (e.g., water pollution, habitat disruption) in the studied river basins could lead to bottlenecks compounding genetic drift due to fragmentation. Compared to other streams, CS and KS (in Group 1) exhibit low genetic variation and may be more susceptible to human-induced pressure and pollution owing to their proximity to human settlements. Likewise, the dam built on DKS (in Group 2), which has low genetic diversity, may have caused habitat destruction and decreased gene variation. Finally, the TS (in Group 3) flows into the Ergene River, where heavy pollution is observed, again under anthropogenic pressures [44]. Thus, habitat destruction occurs here as a result of pollution. This prediction can be confirmed by the fact that the SS (in Group 3) has higher genetic variation than other populations and, as an island locality, is less affected by human activities than other locations. In the meantime, dispersal patterns and significant transposition rates are required for conserving species' genetic variability [45].

The haplotype network did not indicate a significant phylogeographic structure in *P. borysthenicus* from Northwestern Türkiye. The glaciations of the Pleistocene played a significant role in the present European dispersal of the leuciscine, and more recent origins were identified for some leuciscine taxa after colonization from glacier refuges (e.g., Danube basin; see [46]). Although the other rivers of the Black Sea basin could have also played a role as a glacial refuge for freshwater fish fauna [11], it was argued that during its freshwater stage, the Black Sea did facilitate free dispersion of freshwater fishes now living in rivers entering the sea [19, 47–49]. On the other hand, molecular-based studies indicate that some fish species, such as *Rutilus frisii* [50] and *Vimba vimba* [51], were widely spread in the Black Sea basin during its freshwater stage and it was predicted that this was also the case for other species, such as *Abramis brama*, *Blicca bjoerkna*, and *Scardinius erythrophthalmus*, because these are currently widespread and inhabit in many rivers flowing into the Black Sea. It was very likely that these species were able to spread from one Black Sea tributary to the other, and their populations only became meshed from each other later on due to the increased salinity of the Black Sea water [52]. This dispersal pattern was also obvious in *Petroleuciscus* inhabiting the Black Sea basin. Therefore, the observed heterogeneity in the Turkish sequences of *P. borysthenicus* was anticipated. Although it is known that the study area, which comes to the lower limit of the natural range of the species, is very narrow and separated from each other by geographical barriers, this haplotype distribution is considered to be possible. Additionally, the fact that all haplotypes differ by only a single mutational step may indicate the limited nucleotide data for the species in GenBank. Consequently, no definitive conclusions can be drawn about the species' distribution pattern.

The pairwise F_{ST} values, which is an indicator of population differentiation, for 13 populations indicated high and significant genetic differentiations among the study populations except for some population pairs (e.g., between CS and DS, ES and KLS, HS and KS, PS and SR, DKS and KLS). The significant F_{ST} values and low genetic variability in most samples indicate the fixation of some haplotypes in the majority of samples (i.e., differences in haplotype frequencies). The presence of significant genetic variations among populations is an indication that there were low levels of gene flow between populations and/or that these populations were historically isolated [53]. High genetic structuring found in this study was not directly related to the geographical distance, but there is an obvious connection with the water basins where they entered. The same results were found for another freshwater fish, *Gambusia holbrooki* in Australia, where genetic diversity was low and there was proof of bottleneck events for some populations in a river basin posing obvious barriers to fish translocation [54]. Additionally, similar results were found in other freshwater fish species *Rhodeus amarus* [55] and *Paracapoeta trutta* [56] in Türkiye. Furthermore, AMOVA analysis confirmed obvious genetic differentiation (56.87%) among populations of *P. borysthenicus* in Northwestern Türkiye (Table 4). The AMOVA analysis also reported for *Pelasgus marathonicus* and *P. stymphalicus* [57] indicated that the genetic differentiation (52.11% and 58.34%, respectively) was due to differences among populations.

In conclusion, this study presents a genetic characterization survey for *P. borysthenicus* in Northwestern Türkiye. Genetic analysis of sampled populations suggested low genetic variability despite relatively high population structuring (due to the difference in frequency of haplotypes between localities). Due to the findings, COX1 variations of diversity in this species are thought to be the legacy of historical events. Further studies using additional sampling and molecular markers are needed to enrich our understanding of the diversity and distribution of this species. If the results of the present study are evaluated by public institutions, the management events can be better arranged on these native species. Building DNA barcode libraries and understanding species' genetic diversity could be used to develop more effective management programs for nonnative species and conservation strategies for native species. Additionally, future molecular studies on *P. borysthenicus* should consider a wider geographical scale with more populations, including founder populations and their distribution mechanisms over the countries.

Data Availability

All data generated or analysed during this study are included in this paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

SA substantially contributed in the concept and design of the study, contributed to field data collection, contributed to molecular and data analyses, and contributed to manuscript

preparation. GS, ÜA, and ÖG contributed to field data collection and manuscript preparation. ED contributed to molecular and data analyses and contributed to manuscript preparation. MÖ contributed to field data collection and contributed to data analyses and manuscript preparation. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The fish used in this study (except for the population of Gökçeada Island) were obtained from the TÜBİTAK project (Project Code: 3001, Project No: 116Z761), and the authors thank this project. This study was also conducted as out of scope with the permission of Çanakkale Onsekiz Mart University Committee of Animal Experiments Local Ethics (Decision No: 2021/02-16), and dead animal tissues were used (for the population of Gökçeada Island). The authors thank Sedat Ozan Güreşen for helping in sampling from Gökçeada Island. The authors also thank Dr. Çiğdem Gürsoy Gaygusuz and Dr. Nilgün Kaya for their contributions.

Supplementary Materials

Distribution and frequency of COI haplotypes in the groups of *Petroleuciscus borysthenicus* populations from Northwestern Türkiye. ES: Efendi Stream; PS: Pabuç Stream; DS: Danamandıra Stream; CS: Cumhuriyet Stream; KS: Karaman Stream; HS: Hacımurat Stream; Değirmenköy Stream: DKS; Kula Stream: KLS; Çatalca Stream: CTS; Bahçeköy Stream: BKS; Baklalı Stream: BS; Teke Stream: TS; Şahinkaya Stream: SS. (*Supplementary Materials*)

References

[1] R. Fricke, M. Bilecenoğlu, and H. M. Sarı, "Annotated checklist of fish and lamprey species (Gnathostomata and Petromyzontomorphi) of Türkiye, including a red list of threatened and declining species," *Stuttgarter Beiträge zur Naturkunde A Biologie*, vol. 706, pp. 1–172, 2007.

[2] A. S. Tarkan, S. M. Marr, and F. G. Ekmekçi, "Non native and traslocated freshwater fish species in Türkiye," *FishMed*, vol. 003, pp. 1–28, 2015.

[3] M. Minter, E. Nielsen, C. Blyth et al., "What is genetic diversity and why does it matter?" *Front Young Minds*, vol. 9, Article ID 656168, 2021.

[4] D. H. Reed and R. Frankham, "Correlation between fitness and genetic diversity," *Conservation Biology*, vol. 17, no. 1, pp. 230–237, 2003.

[5] F. Qamaruz-Zaman, M. F. Fay, J. S. Parker, and M. W. Chase, "The use of AFLP fingerprinting in conservation genetics: a case study of Orchis simia (Orchidaceae)," *Lindleyana*, vol. 13, pp. 125–133, 1998.

[6] M. Pfeifer and G. Jetschke, "Influence of geographical isolation on genetic diversity of Himantoglossum hircinum (Orchidaceae)," *Folia Geobotanica*, vol. 41, no. 1, pp. 3–20, 2006.

[7] A. J. Bohonak, "Dispersal, gene flow, and population structure," *The Quarterly Review of Biology*, vol. 74, no. 1, pp. 21–45, 1999.

[8] C. G. Eckert, K. E. Samis, and S. C. Lougheed, "Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond," *Molecular Ecology*, vol. 17, no. 5, pp. 1170–1188, 2008.

[9] S. Cushman, A. Storfer, and L. Waits, *Landscape Genetics: Concepts, Methods, Applications*, John Wiley & Sons, Hoboken, NJ, USA, 2015.

[10] N. G. Bogutskaya, "*Petroleuciscus*, a new genus for the *Leuciscus borysthenicus* species group (Teleostei: cyprinidae)," *Zoosystematica Rossica*, vol. 11, no. 1, pp. 235–237, 2002.

[11] S. Perea, M. Böhme, P. Zupancic et al., "Phylogenetic relationships and biogeographical patterns in Circum-Mediterranean Subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data," *BMC Evolutionary Biology*, vol. 10, no. 1, p. 265, 2010.

[12] M. Kottelat and J. Freyhof, *Handbook of European Freshwater Fishes*, Publications Kottelat, Cornol, 2007.

[13] R. Fricke, W. N. Eschmeyer, and R. Van der Laan, *ECoF. Eschmeyer's Catalog of Fishes: Genera, Species, References*, California Academy of Sciences, San Francisco, CA, USA, 2021.

[14] D. Turan, G. Kalaycı, C. Kaya, Y. Bektaş, and F. Küçük, "A new species of *Petroleuciscus* (Teleostei: Cyprinidae) from the Büyük Menderes river, southwestern Anatolia, Türkiye," *Journal of Fish Biology*, vol. 92, no. 4, pp. 875–887, 2018.

[15] N. G. Bogutskaya, "Contribution to the knowledge of leuciscine fishes of Asia minor. Part 1. Morphology and taxonomic relationships of *Leuciscus borysthenicus* (Kessler, 1859), *Leuciscus smyrnaeus* Boulenger, 1896 and *Ladigesocypris ghigii* (Gianferrari, 1927) (Cyprinidae, Pisces)," *Publicaciones Especiales Instituto Español de Oceanografía*, vol. 21, pp. 25–44, 1996.

[16] W. Eldridge, J. Myers, and K. Naish, "Long-term changes in the fine-scale population structure of coho salmon populations (*Oncorhynchus kisutch*) subject to extensive supportive breeding," *Heredity*, vol. 103, no. 4, pp. 299–309, 2009.

[17] M. F. Geiger, F. Herder, M. T. Monaghan et al., "Spatial heterogeneity in the mediterranean biodiversity hotspot affects barcoding accuracy of its freshwater fishes," *Molecular Ecology Resources*, vol. 14, no. 6, pp. 1210–1221, 2014.

[18] G. Kalaycı, "Molecular phylogeny and historical biogeography of *Petroleuciscus* (Teleostei: Leuciscidae) species in Türkiye," *Journal of Anatolian Environmental and Animal Sciences*, vol. 7, no. 1, pp. 88–95, 2022.

[19] P. M. Bănărescu, *Zoogeography of Fresh Waters, Distribution and Dispersal of Fresh Water Animals in North America and Eurasia*, AULA-Verlag, Wiesbaden, 1992.

[20] M. Kuru, S. Yerli, F. Mangıt, E. Ünlü, and A. Alp, "Fish biodiversity in inland waters of Türkiye," *International Journal of Fisheries and Aquaculture*, vol. 1, no. 3, pp. 93–120, 2015.

[21] W. Bender, P. Spierer, D. S. Hogness, and P. Chambon, "Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and bithorax complex in," *Journal of Molecular Biology*, vol. 168, no. 1, pp. 17–33, 1983.

[22] R. D. Ward, T. S. Zemlak, B. H. Innes, P. R. Last, and P. D. Hebert, "DNA barcoding Australia's fish species," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 360, no. 1462, pp. 1847–1857, 2005.

[23] E. Keskin, S. Ağdamar, and A. S. Tarkan, "DNA barcoding common non-native freshwater fish species in Türkiye: low genetic diversity but high population structuring," *Mitochondrial DNA*, vol. 24, no. 3, pp. 276–287, 2013.

[24] M. Kearse, R. Moir, A. Wilson et al., "Geneious Basic: an integrated and extendable desktop software platform for the

organization and analysis of sequence data," *Bioinformatics*, vol. 28, no. 12, pp. 1647–1649, 2012.

[25] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, and X. Mega, "Mega X: molecular evolutionary genetics analysis across computing platforms," *Molecular Biology and Evolution*, vol. 35, no. 6, pp. 1547–1549, 2018.

[26] L. G. L. Excoffier, G. Laval, and S. Schneider, "Arlequin (version 3.0): an integrated software package for population genetics data analysis," *Evolutionary bioinformatics online*, vol. 1, pp. 47–50, 2007.

[27] J. Rozas, A. Ferrer-Mata, J. C. Sánchez-DelBarrio et al., "DnaSP 6: DNA sequence polymorphism analysis of large data sets," *Molecular Biology and Evolution*, vol. 34, no. 12, pp. 3299–3302, 2017.

[28] J. W. Leigh and D. Bryant, "POPART: full-feature software for haplotype network construction," *Methods in Ecology and Evolution*, vol. 6, no. 9, pp. 1110–1116, 2015.

[29] Y. X. Fu, "Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection," *Genetics*, vol. 147, no. 2, pp. 915–925, 1997.

[30] F. Tajima, "Statistical method for testing the neutral mutation hypothesis by DNA polymorphism," *Genetics*, vol. 123, no. 3, pp. 585–595, 1989.

[31] A. R. Rogers and H. Harpending, "Population growth makes waves in the distribution of pairwise genetic differences," *Molecular Biology and Evolution*, vol. 9, no. 3, pp. 552–569, 1992.

[32] H. C. Harpending, "Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution," *Human Biology*, vol. 66, no. 4, pp. 591–600, 1994.

[33] J. Freyhof, C. Kaya, E. BayÇelebİ, M. Geiger, and D. Turan, "Generic assignment of *Leuciscus kurui* Bogutskaya from the upper Tigris drainage, and a replacement name for *Alburnus kurui* Mangit Yerli (Teleostei: Leuciscidae)," *Zootaxa*, vol. 4410, no. 1, pp. 113–135, 2018.

[34] R. Khaefi, H. R. Esmaeili, G. Sayyadzadeh, M. F. Geiger, and J. Freyhof, "*Squalius namak*, a new chub from lake namak basin in Iran (Teleostei: Cyprinidae)," *Zootaxa*, vol. 4169, no. 1, pp. 145–159, 2016.

[35] S. Schönhuth, J. Vukić, R. Šanda, L. Yang, and R. L. Mayden, "Phylogenetic relationships and classification of the holarctic family Leuciscidae (Cypriniformes: Cyprinoidei)," *Molecular Phylogenetics and Evolution*, vol. 127, pp. 781–799, 2018.

[36] A. Parmaksız and H. K. Eskici, "Genetic variation of yellow barbell (Carasobarbus luteus (Heckel, 1843)) from four populations using mitochondrial DNA COX1 gene sequences," *Applied Ecology and Environmental Research*, vol. 16, no. 2, pp. 1673–1682, 2018.

[37] G. Tsipas, G. Tsiamis, K. Vidalis, and K. Bourtzis, "Genetic differentiation among Greek Lake populations of *C. gibelio* and *C. carpio carpio*," *Genetica*, vol. 136, no. 3, pp. 491–500, 2009.

[38] N. Mesquita, B. Hanfling, G. R. Carvalho, and M. M. Coelho, "Phylogeography of the cyprinid Squalius aradensis and implications for conservation of the endemic freshwater fauna of southern Portugal," *Molecular Ecology*, vol. 14, no. 7, pp. 1939–1954, 2005.

[39] R. A. Beaumont and K. Hoare, *Biotechnology and Genetics in Fisheries and Aquaculture*, Blackwell Publishing, Oxford, UK, 2003.

[40] A. K. Sakai, F. W. Allendorf, J. S. Holt et al., "The population biology of invasive species," *Annual Review of Ecology and Systematics*, vol. 32, no. 1, pp. 305–332, 2001.

[41] B. Hänfling, "Understanding the establishment success of non-indigenous fishes: lessons from population genetics," *Journal of Fish Biology*, vol. 71, no. sd, pp. 115–135, 2007.

[42] J. Fischer and D. B. Lindenmayer, "Landscape modification and habitat fragmentation: a synthesis," *Global Ecology and Biogeography*, vol. 16, no. 3, pp. 265–280, 2007.

[43] R. A. Coleman, B. Gauffre, A. Pavlova et al., "Artificial barriers prevent genetic recovery of small isolated populations of a low-mobility freshwater fish," *Heredity*, vol. 120, no. 6, pp. 515–532, 2018.

[44] M. Özuluğ, Ö. Gaygusuz, Ç.G. Gaygusuz, N. Kaya, and G. Saç, "Fishes encountered in the Turkish thrace river systems (NW Türkiye)," *Inland Water Biol*, 2023.

[45] J. R. Freeland, *Molecular Ecology*, Wiley, South Sussex, UK, 2005.

[46] A. Levy, I. Doadrio, and V. C. Almada, "Historical biogeography of European leuciscins (Cyprinidae): evaluating the lago mare dispersal hypothesis," *Journal of Biogeography*, vol. 36, no. 1, pp. 55–65, 2009.

[47] J. D. Durand, H. Persat, and Y. Bouvet, "Phylogeography and post-glacial dispersion of the chub (*Leuciscus cephalus*) in Europe," *Molecular Ecology*, vol. 8, no. 6, pp. 989–997, 1999.

[48] J. Dominique Durand, Ü. Erhan, I. Doadrio, S. Pipoyan, and A. R. Templeton, "Origin, radiation, dispersion and allopatric hybridization in the chub *Leuciscus cephalus*," *Proceedings of the Royal Society of London. Series B: Biological Sciences*, vol. 267, no. 1453, pp. 1687–1697, 2000.

[49] C. S. Tsigenopoulos and P. Berrebi, "Molecular phylogeny of North Mediterranean freshwater barbs (genus Barbus: Cyprinidae) inferred from cytochrome b sequences: biogeographic and systematic implications," *Molecular Phylogenetics and Evolution*, vol. 14, no. 2, pp. 165–179, 2000.

[50] P. Kotlík, S. Marková, L. Choleva, N. G. Bogutskaya, F. G. Ekmekçi, and P. P. Ivanova, "Divergence with gene flow between Ponto-Caspian refugia in an anadromous scyprinid *Rutilus frisii* revealed by multiple gene phylogeography," *Molecular Ecology*, vol. 17, no. 4, pp. 1076–1088, 2008.

[51] B. Hänfling, C. Dümpelmann, N. G. Bogutskaya, R. Brandl, and M. Brändle, "Shallow phylogeographic structuring of *Vimba vimba* across Europe suggests two distinct refugia during the last glaciation," *Journal of Fish Biology*, vol. 75, no. 9, pp. 2269–2286, 2009.

[52] M. Özuluğ, M. F. Geiger, and J. Freyhof, "Alburnus goekhani, a new species of bleak from the Anatolian Black Sea basin (Teleostei: Leuciscidae)," *Zootaxa*, vol. 4425, no. 1, pp. 29–40, 2018.

[53] Y. Bektaş, I. Aksu, G. Kalayci, and D. Turan, "Low genetic diversity in Turkish populations of Wels catfish *Silurus glanis* l., 1758 (Siluridae, Pisces) revealed by mitochondrial control region sequences," *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 20, no. 10, pp. 767–776, 2020.

[54] R. M. Ayres, V. J. Pettigrove, and A. A. Hoffmann, "Low diversity and high levels of population genetic structuring in

introduced Eastern mosquitofish (*Gambusia holbrooki*) in the greater Melbourne area, Australia," *Biological Invasions*, vol. 12, no. 11, pp. 3727–3744, 2010.

[55] Y. Bektaş, A. O. Beldüz, and D. Turan, "The phylogenetic position of Turkish populations within the European bitterling, *Rhodeus amarus* (Osteichthyes: Cyprinidae)," *Zoology in the Middle East*, vol. 59, no. 1, pp. 39–50, 2013.

[56] A. Parmaksiz and E. Ekşi, "Genetic diversity of the cyprinid fish Capoeta trutta (Heckel, 1843) populations from Euphrates and Tigris rivers in Türkiye based on mtDNA COI sequences," *Indian Journal of Fisheries*, vol. 64, no. 1, pp. 18–22, 2017.

[57] N. Viñuela Rodríguez, R. Šanda, S. Zogaris, and J. Vukic, "Distribution and genetic diversity of two species of Pelasgus minnows (Leuciscidae) in southern Greece," *Knowledge and Management of Aquatic Ecosystems*, vol. 421, p. 27, 2020.