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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Population genetics of *Pomatomus saltatrix* (Linnaeus, 1766) in the seas of Türkiye based on microsatellite DNA

Türkiye denizlerindeki *Pomatomus saltatrix* (Linnaeus, 1766)'in mikrosatelit DNA tabanlı popülasyon genetiği

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Abstract: Bluefish, (*Pomatomus saltatrix* (Linnaeus, 1766)) a commercially important and highly migratory predatory fish species, is found along the coasts of Türkiye. Despite it's widespread presence along these coasts, there has been no detailed study on the genetic structure of bluefish populations in Türkiye. In order to protect the biological diversity of countries, the genetic diversity present in natural resources must be identified. In this study, we examined the population structure of bluefish in the coastal regions of Türkiye; We aimed to identify bluefish samples collected from 14 regions along Türkiye coast by analyzing microsatellit DNA. For the microsatellite analysis, eight loci (ELF 17, ELF 37, ELF 49, ELF 19, ELF 39, ELF 46, ELF 44, ELF 50) were analyzed. A total of 433 samples from 14 populations were studied. In total 207 alleles and 61 specific alleles were identified across all populations and all loci. The highest observed (Ho) heterozygous values are ELF 50 (Ho: 0,991) while the lowest value is ELF 19 (Ho: 0.716). The highest expected (He) heterozygous values are ELF 39 (He: 0.952) while the lowest value is ELF 50 (He: 0.518). According to the Hardy-Weinberg analysis results, it was determined that there was a significant deviation in all populations. When bluefish populations are clustered according to their phylogenetic lineages by applying principal coordinate analysis (PCoA), the first three axes show 94% of the total genetic variation. The highest variation values and eigenvalues were found on the 3rd axis. When the analysis results are examined, it is clearly seen that the Mersin bluefish population is clustered differently from other populations. According to the Mantel test, a low correlation (R^2 = 0.3061, P = 0.01) was detected between genetic and geographical distance. Admixed individuals and low genetic differentiation were observed in all populations.

Keywords: Bluefish, loci, microsatellite, allele, Fst

Öz: Lüfer (*Pomatomus saltatrix* (Linnaeus, 1766)), ticari açıdan oldukça önemli ve göç eden bir yırtıcı balık türü olup, Türkiye kıyılarında bulunmaktadır. Buna rağmen, Türkiye'deki Lüfer popülasyonlarının genetik yapısı hakkında detaylı bir çalışma yapılmamıştır. Ülkelerin biyolojik çeşitliliğini korumak için doğal kaynaklarda bulunan genetik çeşitliliğin belirlenmesi gerekmektedir. Bu çalışmada, Türkiye'nin kıyı bölgelerindeki Lüfer popülasyon yapısını inceledik; Türkiye kıyılarındaki 14 bölgeden toplanan Lüfer örneklerini mikrosatelit DNA analizi yaparak tanımlamayı amaçladık. Mikrosatelit analizi için sekiz lokus (ELF 17, ELF 37, ELF 49, ELF 19, ELF 39, ELF 46, ELF 44, ELF 50) analiz edildi. Toplam 14 popülasyondan 433 örnek incelendi. Toplamda tüm popülasyonlarda ve tüm lokuslarda 207 alel ve 61 spesifik alel tanımlanmıştır. Gözlemlenen en yüksek (Ho) heterozigot değerler ELF 50 (Ho: 0,991) iken en düşük değer ELF 19'dur (Ho: 0,716). Beklenen en yüksek (He) heterozigot değerler ELF 39 (He: 0,952) iken en düşük değer ELF 50'dir (He: 0,518). Hardy-Weinberg analiz sonuçlarına göre tüm popülasyonlarda önemli bir sapma olduğu belirlenmiştir. Lüfer popülasyonları filogenetik soylarına göre temel koordinat analizi (PCoA) uygulanarak kümelendirildiğinde ilk üç eksen toplam genetik varyasyonun %94'ünü göstermektedir. En yüksek varyasyon değerleri ve öz değerler 3. eksende bulunmuştur. Analiz sonuçları incelendiğinde Mersin lüfer popülasyonunun diğer popülasyonlardan farklı olarak kümelendiği açıkça görülmektedir. Mantel testine göre genetik ve coğrafi mesafe arasında düşük bir korelasyon (R^2 = 0,3061, P = 0,01) tespit edilmiştir. Sonuç olarak Tüm popülasyonlarda karışık bireylerin olduğu ve düşük genetik farklılaşma gözlenmiştir.

Anahtar kelimeler: Lüfer, lokus, mikrosatelit, allel, Fst

INTRODUCTION

Bluefish (*Pomatomus saltatrix* (L., 1766)) are predatory fish that spread in temperate and warm waters around the world, generally on the continental margin, and migrate to warm waters between seas (Briggs, 1960; Wilk, 1977; Juanes et al., 1996). Adult bluefish are fast-swimming fish that migrate in response to seasonal changes (Wilk, 1977). Although bluefish prefer sandy substrates, they are also found in clayey and muddy ground (Bal, 2015).

Bluefish are fish that migrate between seas to warm waters. Bluefish are capable of long-distance movement, and in geographically isolated populations bluefish are known to undertake extensive seasonal migrations (Van der Elst, 1976).

Since Türkiye is located in the temperate climate zone of the world, bluefish can be found in all four of our regional seas (Bal et al., 2018). In Türkiye waters, it is known that bluefish migrates from the Sea of Marmara and The Aegean Sea after September (Türgan, 1959; Akşiray, 1987).

When we look at genetically bluefish population structure, there are very few studies on bluefish in literature. There is no detailed genetic study on Türkiye seas. According to the results

of Pardinas et al. (2010) study; They reported that the populations in the Eastern Atlantic Ocean (including the Eastern Mediterranean) and the populations in the Western Atlantic Ocean did not share any haplotypes. They also revealed complete genetic isolation between the two sides of the North Atlantic Ocean. In addition to; Miralles et al. (2014b) reported that genetically, there are two barriers, one in the middle of the Atlantic Ocean and the other in the Mediterranean, but regional permeability and migration occur in both. Miralles et al. (2016) also revealed a mixture of Eastern and Western Mediterranean strains in the farm located in Guardamar in the Western Mediterranean. They also reported that although most of the individuals caught around the facility genetically belonged to the local population, 7.14% to 11.9% of the individuals belonged to the genetic population living in Turkish waters.

Despite their complicated migration patterns, there are no compherensive molecular studies on bluefish in Türkiye, although some limited research has been conducted globally.

In the study aims to determine the genetic structure of the bluefish, an economically valuable species found in Türkiye seas, through microsatellite analysis. Thus, the study was conducted contribute to the studies aimed at revealing the biological diversity of Türkiye.

MATERIALS AND METHODS

Sampling

Samples were collected from 14 stations along the Türkiye coast: Hopa, Trabzon, Giresun, Samsun, Sinop, Ereğli, İğneada, Rumeli Feneri (İstanbul), Çanakkale, Erdek (Marmara sea), Bodrum, İzmir, Mersin and Adana (Figure 1). A 2-3 cm^2 of caudal fin tissue was sampled from a sufficient number of fish at each station and stored in 96% ethanol at room temperature.

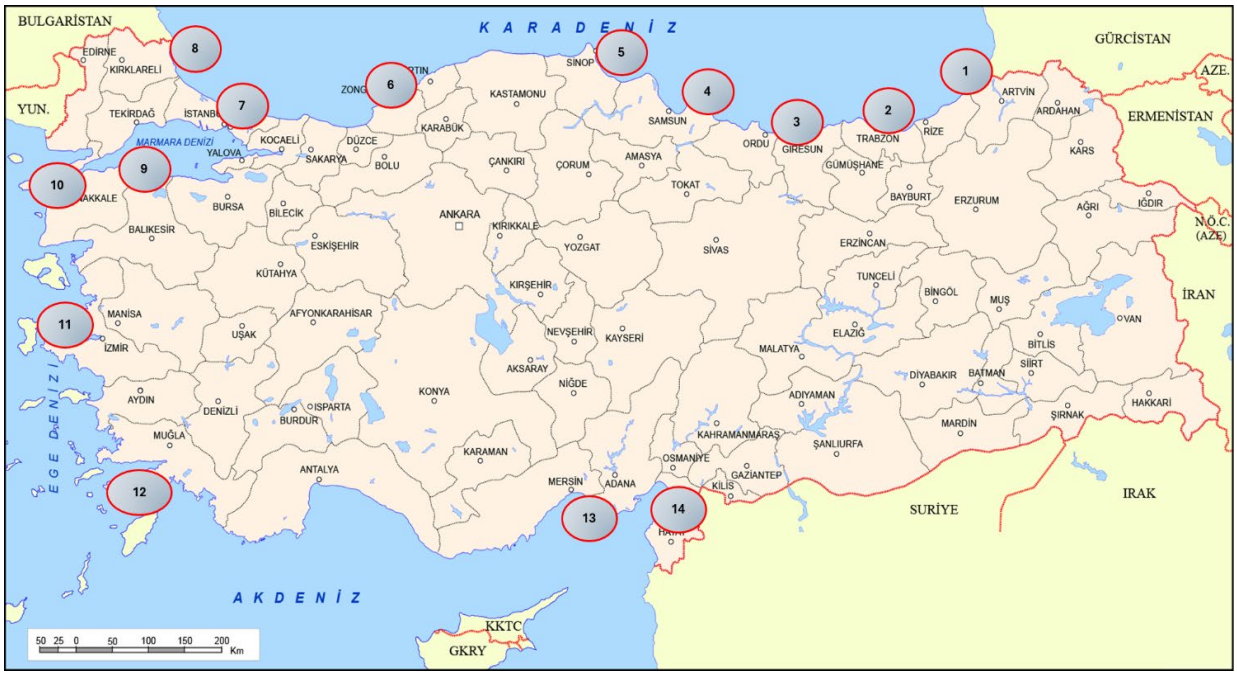

Figure 1. Map of sampling locations for *P. saltatrix* in coastal of Türkiye (1-Hopa, 2-Trabzon, 3-Giresun, 4-Samsun, 5-Sinop, 6-Ereğli, 7-Rumeli Feneri, 8-İğneada, 9-Erdek, 10-Çanakkale, 11-İzmir, 12-Bodrum, 13-Mersin, 14-Adana)

DNA extraction, amplification and microsatellite analysis

Genomic DNA was extracted using the kit (QIAamp DNA HT, Qiagen®, Germany) following the protocol suggested by the manufacturer. The samples were visualized through electrophoresis on 1,5 % agarose gel, dyed with SafeViewTM (NBS Biologicals, UK) and visualized under ultraviolet light.

Multiplex PCR was performed with 2x Master Mix (Type-it Microsatellite PCR Kit; 2000, Qiagen®, Germany), 10µl Forward Primer (10µM), 1µl Reverse Primer (10µM), 1µl, DNA (~50 ng/ml), 1µl Nuclease-free water and up to 20µl) with using primers in literature (Table 1) and the products were checked on agarose gel. Then, optimized the annealing temperatures that were created in three groups designated as 55-56-58°C.

In the next step, microsatellite locus-specific primers labelled with different fluorescently labelled dyes to determine allele sizes for multiplex PCR were synthesized (Table 1).

The resulting products of the PCR reaction and the displayed samples were diluted 1/50 in preparation for fragment analysis. After 0.5 µl of the diluted product was taken and 9.5 µl of formamide and 0.05 µl standard (LIZ-600 Genescan Size Standard, Applied Biosystems™, Lithuanian) were added.

It was placed on ice after being denatured at 95°C for 5

minutes. Afterwards, fluorescently labelled PCR products were electrophoresed along on the ABI 3500 Sequencer (Applied Biosystems) and allele lengths obtained for the microsatellite locus were determined on each samples.

Table 1. Microsatellite loci and primers (Dos Santos et al., 2008) used in the multiplex PCR study

Group	Lokus	Primer sequence (5'–3')	GenBank Accession Number	Fluorescent dye	Repeat Motif	Allele References Range (bp)
Group 1 Annealing 55 °C	ELF 17	F:TTCCACTTCCTCCTACTTTC R:GCAGGCTAATAATCGTTGAC	EU289407	FAM-Blue	$(TATC)_{21}$	136-216
	ELF 19	F:GCGACGGCTCTGTCTATGTG R:GAGGCTGAGACGGGTTCTTG	EU289408	PET-Red	$(TATC)_{22}$	234-394
	ELF 37	F:TGCTCGGCTACAATAACG R:GACCTGTCAGTGGAGATTC	EU289409	VIC-Green	$(TATC)_{28}$	216-324
	ELF 49	F:TACACCATGAGTGAACAAAG R:ATGAGAAGAAGGAAGCTAAG	EU289413	NED-Yellow	$(TATC)_{14}$	158-234
Group 2 Annealing 58 °C	ELF 39	F:TAGTGGTTCTGGGCAACAGG R:TATCCGGGCTGTACTGTTGG	EU289410	FAM- Blue	$(TATC)_{30}$	157-285
	ELF 44	F:ACTTGGGGTTGGGCAATATG R:ATTTCACAGCACAGGAAGAC	EU289411	VIC- Green	$(TATC)_{34}$	216-320
	ELF 46	F:TCAGATTACCCTCCCTGTTC R:TGTAGATGTGCTGGTGATCC	EU289412	NED- Yellow	$(TATC)_{25}$	268-376
Group 3 Annealing 56 °C	ELF 50	F: CTGCACAGGAACACGTCAGT R: ATCTGCCCCAAAACAGACAC	EU289414	FAM- Blue	(TATC)09	130-218

Microsatellite data analysis

The raw data obtained from fragment analysis were processed using the Convert program (Glaubitz, 2004) and analyzed with the Genemarker (Soft Genetics LLC) to determine allele sizes and frequencies.

Null-allele, allele overlap (stuttering) and allele loss (large allele dropout) were determined using Microchecker v2.2.3 (Van Oosterhout et al., 2004), and null allele frequencies were determined using the maximum likelihood method ML-NULLFREQ (Kalinowski and Taper, 2006) program.

The compliance of genotypic ratios with the Hardy-Weinberg (HW) equilibrium was determined by the "exact test" method of the GENEPOP v.4.2 (Rousset, 2008) program. To control the false discovery rate (FDR), new probability threshold values (Threshold P) were calculated and adjusted with the Bonferroni method (Benjamini and Hochberg, 1995). Probability values (P value) were determined based on 10,000 demomorizations, 500 batches, and 5,000 repetitions for each batch.

Total number of alleles (NA), expected heterozygosity (He), observed heterozygosity (Ho) and Polymorphic information content (PIC) were calculated with the Cervus 3.0.7 (Kalinowski et al., 2007) program.

Pedigree coefficient (FIS) and Allelic Richness (AR) values were calculated with the FSTAT v.2.9.3 (Goudet, 1995) program. The presence of unique alleles (private alleles) in the populations and the allele frequency were determined and calculated with the GenAlEX 6.5 program. Due to the possible presence of null allele, FST and null allele frequency were recalculated with 25 000 replicates in the Freena (Chapuis and Estoup, 2007) program.

To identify genetic differences between populations, interpopulation fixation indices (FST) based on allele frequency variation of loci (Weir and Cockerham, 1984) GENEPOP 4.2. It was calculated in the program (Raymond and Rousset, 1995).

The presence of genetically different populations in the data set was investigated using the Bayesian multi-locus clustering method in the STRUCTURE v.2.3.4 (Falush et al., 2003) program. Geographical and genetic distance relationships were examined with the Mantel test in the GenAlEx 6.5 (Mantel, 1967). To determine the most appropriate number of clusters, default clusters between 2-7 were tested in three independent repetitions. MCMC searches were created with a total of 108 steps, 107 of which were burning. The probable K value was determined with the delta K (ΔK) statistics using the online software Structure Harvester.

And principal coordinate analysis (PCoA) was performed with the same program to obtain more information about the relationship between populations (Liu and Muse, 2005)

The Analysis Molecular Variance (AMOVA) was performed on Arlequin (Excoffier et al., 1992) software to detect differences between populations.

RESULTS

Polymorphism of microsatellite loci

Eight microsatellite loci (ELF 17, ELF 37, ELF 49, ELF 19, ELF 39, ELF 46, ELF 44, ELF 50) were analyzed, Of the 433 samples from 14 populations seven loci were polymorphic, while one locus (ELF 44) was monomorphic. The polymorphic loci exhibited moderate to high polymorphism, and with PIC values ranging from 0.400 to 0.949 (average 0.730, Table 2). A total of 207 alleles were identified across all loci. The most

common allele was stated in Table 3. The highest observed heterozygosity (Ho: 0.991) occurred at ELF 50 while the lowest (Ho: 0.716) was at ELF 19. Expected heterozygosity ranges from 0.518 (ELF 50) to 0.952 (Table 2).

According to the results of the Hardy-Weinberg analysis, it was determined that there was a significant deviation in bluefish populations (Table 3' blue colors).

At the population level, the average NA (number of alleles) number varies from 9.5 for the Mersin population to 14.25 for Çanakkale (average 12.4). The highest average number of alleles was observed in Çanakkale and İzmir populations (14.3-14.1). The average He (expected heterozygosity) and Ho (observed heterozygosity) for each population range were calculated between 0.730 and 0.716, respectively (Table 4).

In all populations, the highest observed heterozygosity (0.781) was determined in the R. Feneri population, while the lowest observed heterozygosity (0.647) was determined in the Sinop population (Table 4).

Genetic differentiation and structure among populations

61 specific alleles were identified in all populations. The populations and numbers where special alleles were seen were as follows. 9 alleles in Adana, 8 alleles each in Çanakkale and İzmir, 7 alleles in R. Feneri, 5 alleles in Mersin and Sinop, 4 alleles in Bodrum, 3 alleles each in İğneada and Erdek, 2 alleles in Ereğli, Samsun, Trabzon and Hopa and 1 allele in Giresun (Table 5). When allele frequencies were compared between populations, 3 loci (ELF 49, ELF 19 and ELF 50) were found to deviate significantly from HWE in all populations ($p < 0.01$) in Table 5.

Table 2. Parametric properties of microsatellite loci (NA: number of alleles; HObs: observed heterozygosity; HExp: expected heterozygosity; PIC: Polymorphic Information Content; HW; deviate significantly from Hardy-Weinberg Equilibrium, F(null) null allele frequency, Fis: Coefficient of nobility, Fit: Fixation index of Individual relative to gametes of the Total Population, Fst: fixation indices, NS: Non Significant Value, ND: Not Detected)

No	Locus	Na	HObs	HExp	PIC	HW	F (Null)	Fis	Fit	Fst
1	ELF 17	22	0.755	0.831	0.814	NS	0.0448	0.066	0.098	0.034
2	ELF 37	47	0.804	0.946	0.942	ND	0.0801	0.127	0.148	0.024
3	ELF 49	27	0.767	0.912	0.904	***	0.0867	0.132	0.152	0.023
4	ELF 19	29	0.716	0.923	0.916	***	0.1262	0.194	0.220	0.033
5	ELF 39	52	0.857	0.952	0.949	NS	0.0514	0.068	0.098	0.032
6	ELF 46	24	0.845	0.924	0.918	NS	0.0442	0.054	0.085	0.032
7	ELF 44	1	0.000	0.000	0.000	ND	ND	0.000	1.000	1.000
8	ELF 50	5	0.991	0.518	0.400	***	-0.3195	-0.888	-0.852	0.019

*** P<0.001 Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium

Table 3. Stations and loci with significant deviations according to the HW analysis results

	ELF 17	ELF 19	ELF 37	ELF 39	ELF 44	ELF 46	ELF 49	ELF 50
Adana		■	■	■		■		■
Mersin	■	■		■				■
Bodrum	■	■	■	■		■		■
Çanakkale			■					■
İzmir		■	■				■	■
Erdek			■	■				■
Rumeli Feneri			■					■
İğneada	■	■	■			■	■	■
Ereğli	■		■	■		■		■
Sinop	■	■	■	■		■	■	■
Samsun		■						■
Giresun			■				■	■
Trabzon			■	■		■		■
Hopa		■	■				■	■

Table 4. Heterozygosity and polymorphism in population (N: Analyzed number of the Samples, Na:Average alllels number, Ne: Number of effective alleles, F: Fixation Index, I: Information Index, UHe: Unbiased Expected Heterozygosity, HO: observed heterozygosity, HE: expected heterozygosity)

Population	N	Na	Ne	I	Ho	He	uHe	F
Çanakkale	32	14.3	8.957	2.047	0.758	0.744	0.756	-0.070
Erdek	32	13.4	8.717	2.011	0.773	0.740	0.752	-0.093
R. Feneri	32	12.5	8.367	1.955	0.781	0.733	0.745	-0.125
Giresun	32	12.0	8.513	1.957	0.758	0.736	0.748	-0.094
Samsun	32	13.1	8.135	1.928	0.711	0.719	0.730	-0.059
Trabzon	32	12.4	7.474	1.918	0.766	0.729	0.740	-0.113
Hopa	32	13.0	8.117	1.957	0.711	0.733	0.744	-0.036
İğneada	32	11.0	7.188	1.818	0.680	0.713	0.725	-0.011
İzmir	32	14.1	9.079	2.040	0.719	0.741	0.752	-0.038
Adana	32	13.1	8.952	2.029	0.688	0.745	0.757	0.007
Sinop	34	12.9	7.607	1.903	0.647	0.718	0.729	0.031
Ereğli	32	10.8	6.698	1.776	0.668	0.703	0.714	-0.003
Bodrum	32	11.3	7.982	1.920	0.672	0.732	0.743	0.014
Mersin	15	9.5	6.759	1.830	0.700	0.738	0.764	0.053

Table 5. Specific allele frequencies seen in populations (Note: The same allele is specific because it is at different loci and was observed only at that station)

Population	Locus	Allele	Frequency	Population	Locus	Allele	Frequency
Çanakkale	ELF 17	132	0.016	Mersin	ELF 39	459	0.033
Çanakkale	ELF 17	183	0.016	Mersin	ELF 50	158	0.233
Çanakkale	ELF 37	210	0.016	İzmir	ELF 37	242	0.031
Çanakkale	ELF 49	379	0.016	İzmir	ELF 37	248	0.016
Çanakkale	ELF 39	466	0.016	İzmir	ELF 37	283	0.016
Çanakkale	ELF 46	240	0.047	İzmir	ELF 37	287	0.016
Çanakkale	ELF 46	257	0.016	İzmir	ELF 37	324	0.016
Çanakkale	ELF 46	269	0.031	İzmir	ELF 39	401	0.016
Erdek	ELF 37	292	0.016	İzmir	ELF 39	414	0.016
Erdek	ELF 49	214	0.016	İzmir	ELF 39	418	0.016
Erdek	ELF 39	411	0.016	Adana	ELF 17	114	0.031
R. Feneri	ELF 37	221	0.016	Adana	ELF 17	139	0.016
R. Feneri	ELF 37	281	0.016	Adana	ELF 37	229	0.047
R. Feneri	ELF 49	168	0.016	Adana	ELF 37	279	0.016
R. Feneri	ELF 49	198	0.016	Adana	ELF 49	204	0.031
R. Feneri	ELF 49	210	0.016	Adana	ELF 49	286	0.016
R. Feneri	ELF 39	446	0.031	Adana	ELF 39	387	0.031
R. Feneri	ELF 39	456	0.031	Adana	ELF 39	393	0.031
Giresun	ELF 37	215	0.016	Adana	ELF 46	303	0.031
Samsun	ELF 37	202	0.016	Sinop	ELF 37	264	0.015
Samsun	ELF 37	291	0.016	Sinop	ELF 37	366	0.015
Trabzon	ELF 39	455	0.016	Sinop	ELF 49	148	0.015
Trabzon	ELF 46	304	0.016	Sinop	ELF 49	297	0.015
Hopa	ELF 49	259	0.016	Sinop	ELF 49	365	0.015
Hopa	ELF 49	318	0.016	Ereğli	ELF 37	234	0.016
İğneada	ELF 37	256	0.016	Ereğli	ELF 37	318	0.016
İğneada	ELF 46	258	0.063	Bodrum	ELF 37	299	0.016
İğneada	ELF 46	278	0.063	Bodrum	ELF 49	244	0.016
Mersin	ELF 17	147	0.067	Bodrum	ELF 39	403	0.047
Mersin	ELF 17	162	0.033	Bodrum	ELF 46	261	0.031
Mersin	ELF 37	185	0.033				

AMOVA analysis determined that genetic diversity was 1% between populations and 95% within populations. (Table 6, $p < 0.01$).

Bluefish populations were clustered according to their phylogenetic lineages by applying principal coordinate analysis (PCoA) with Genalex 6 software. The first three axes show 94% of the total genetic variation. The highest variation values and Eigen values were found on the 3rd axis. The analysis results clearly show that the Mersin population is clustered differently from other populations (Figure 2).

Null allele frequencies were estimated, and the effect of null alleles on the fixation index FST was calculated with and

without excluding null alleles. Patterns of genetic differentiation between populations at all locations are shown using pairwise Fst analyses. Pairwise FST comparisons showed low levels of genetic differentiation (Fst < 0.05) in Table 7.

Table 6. Analysis of molecular variance between populations

Source of variation	df	SS	MS	Est. Var.	%
Between Populations	13	69.071	5.313	0.036	1
Between Individuals	419	1286.958	3.071	0.102	4
Within Individuals	433	1241.500	2.867	2.867	95
Total	865	2597.529		3.006	100

The results of the pairwise population matrix of genetic similarity between populations in this study are presented in Table 8. The pairwise population matrix value between Trabzon and Mersin is 0.208, which is the highest of the pairwise genetic similarity indices between populations.

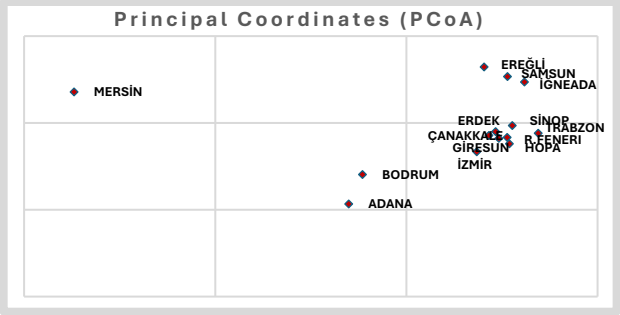

Figure 2. PCoA graph created using FST for bluefish populations

Table 7. FST (Weir and Cockerham, 1984) estimates using correction and without ENA correction for pairwise comparisons of allele distributions

FST	Çanakkale	Erdek	R. Feneri	Giresun	Samsun	Trabzon	Hopa	İğneada	İzmir	Adana	Sinop	Ereğli	Bodrum	Mersin
Çanakkale	0.0000													
Erdek	-0.001414	0.0000												
R. Feneri	-0.001755	-0.000658	0.0000											
Giresun	0.001223	0.005226	0.004029	0.0000										
Samsun	0.009449	0.012652	0.012223	0.002972	0.0000									
Trabzon	0.005123	0.009768	0.006411	-0.00217	0.004514	0.0000								
Hopa	0.006620	0.009483	0.008797	0.000440	0.011084	0.001306	0.0000							
İğneada	0.007795	0.007690	0.008048	0.010640	0.009168	0.011146	0.012595	0.0000						
İzmir	0.006098	0.010181	0.010064	0.002823	0.009323	0.010264	0.007655	0.013887	0.0000					
Adana	0.013216	0.014386	0.014646	0.012057	0.032007	0.017460	0.013414	0.027085	0.008713	0.0000				
Sinop	0.004559	0.004318	0.001901	0.004145	0.013752	0.003923	0.003326	0.007444	0.009646	0.020337	0.0000			
Ereğli	0.012014	0.008945	0.012176	0.010770	0.012389	0.012959	0.012495	0.001500	0.016499	0.032589	0.001704	0.0000		
Bodrum	0.013955	0.016440	0.015401	0.013428	0.025682	0.019623	0.014360	0.028764	0.007778	0.008690	0.014042	0.022788	0.0000	
Mersin	0.032540	0.034522	0.036222	0.033867	0.035409	0.040540	0.037184	0.038030	0.033751	0.025459	0.036032	0.032222	0.024779	0.00
FST+ENA	Çanakkale	Erdek	R. Feneri	Giresun	Samsun	Trabzon	Hopa	İğneada	İzmir	Adana	Sinop	Ereğli	Bodrum	Mersin
Çanakkale	0.0000													
Erdek	-0.000763	0.0000												
R. Feneri	-0.001842	-0.000420	0.0000											
Giresun	0.001941	0.005292	0.004086	0.0000										
Samsun	0.010623	0.012924	0.012471	0.002584	0.0000									
Trabzon	0.005927	0.010405	0.007225	-0.00097	0.005912	0.0000								
Hopa	0.006927	0.009754	0.009272	0.001297	0.011338	0.002531	0.0000							
İğneada	0.007683	0.007449	0.008017	0.009134	0.009432	0.010822	0.011950	0.0000						
İzmir	0.006235	0.009545	0.009186	0.002938	0.009143	0.010323	0.007740	0.012709	0.0000					
Adana	0.013820	0.014192	0.015301	0.013028	0.032532	0.019085	0.015384	0.025337	0.009647	0.0000				
Sinop	0.004709	0.004026	0.001609	0.003725	0.012646	0.005107	0.003125	0.008754	0.007752	0.020739	0.0000			
Ereğli	0.011008	0.006689	0.010201	0.009979	0.012256	0.012500	0.011500	0.002122	0.014976	0.030881	0.003514	0.0000		
Bodrum	0.012791	0.015716	0.014958	0.013348	0.025576	0.019527	0.013527	0.025484	0.006502	0.011025	0.012861	0.020120	0.0000	
Mersin	0.033667	0.032988	0.034723	0.034321	0.037267	0.042072	0.036800	0.036669	0.033012	0.027898	0.037110	0.033573	0.026624	0.0000

Table 8. Pairwise population matrix of the proximities of populations to each other

	Çanakkale	Erdek	R. Feneri	Giresun	Samsun	Trabzon	Hopa	İğneada	İzmir	Adana	Sinop	Ereğli	Bodrum	Mersin
Çanakkale	0.000													
Erdek	0.042	0.000												
R. Feneri	0.039	0.042	0.000											
Giresun	0.049	0.061	0.055	0.000										
Samsun	0.072	0.080	0.077	0.052	0.000									
Trabzon	0.059	0.073	0.061	0.037	0.055	0.000								
Hopa	0.067	0.075	0.071	0.047	0.076	0.048	0.000							
İğneada	0.066	0.065	0.065	0.073	0.068	0.074	0.080	0.000						
İzmir	0.067	0.079	0.076	0.055	0.072	0.076	0.071	0.085	0.000					
Adana	0.092	0.094	0.093	0.086	0.147	0.101	0.091	0.129	0.078	0.000				
Sinop	0.057	0.056	0.048	0.055	0.081	0.054	0.054	0.063	0.073	0.108	0.000			
Ereğli	0.075	0.066	0.074	0.071	0.075	0.076	0.077	0.046	0.090	0.142	0.046	0.000		
Bodrum	0.091	0.098	0.092	0.088	0.122	0.105	0.091	0.131	0.072	0.077	0.086	0.108	0.000	
Mersin	0.189	0.193	0.194	0.189	0.186	0.208	0.202	0.194	0.194	0.169	0.191	0.167	0.160	0.000

According to the Mantel test, a low correlation (R^2 = 0.3666, P = 0.01) was detected between genetic and geographical distance (Figure 3). The K values for the mixed model analysis were chosen between 2-5 and the calculations were repeated 20 times. The graph depicting the assignment of individuals to ancestral populations, based on the barcode method, is shown in Figure 4. For K = 2 and K = 3, individuals from all 14 regions could not be assigned to a different cluster (Figure 4).

The accuracy of the analysis for each K in structure is indicated by the selection of K based on LnP(D) (Taki et al., 2021). The value of LnP(D) is almost maximized when K=3 (Figure 5).

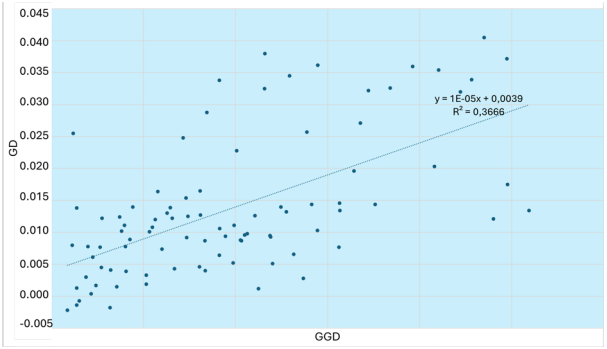

Figure 3. Mantel test between genetic and geographic distance of bluefish populations

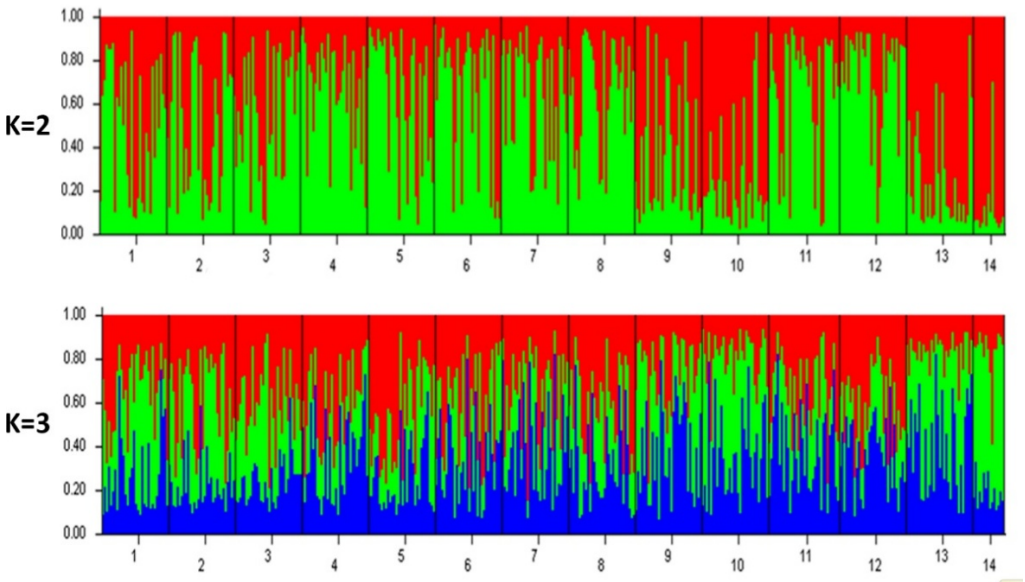

Figure 4. Population structure of bluefish for K = 2 and K = 3 (A thin vertical line represents each individual, and each color represents the probability of belonging to one of the genetic clusters. Black lines separate individuals from different sampling areas) (1-Çanakkale, 2-Erdek, 3-R. Feneri, 4-Giresun, 5-Samsun, 6-Trabzon, 7-Hopa, 8-İğneada, 9-İzmir, 10-Adana, 11-Sinop, 12-Ereğli, 13-Bodrum, 14-Mersin)

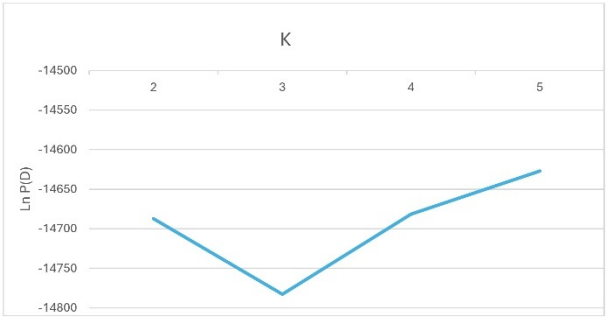

Figure 5. In STRUCTURE, LnP (D) was almost maximized at K=3

DISCUSSION

Genetic studies on bluefish are very limited worldwide, with only a few studies focusing samples taken from İstanbul and Çanakkale in Türkiye.

Miralles et al. (2014a) analyzed eight loci from a total of 120 *Pomatomus saltatrix* samples collected from 2004 and 2009, they calculated microsatellite allele sizes, null alleles, conformity to Hardy-Weinberg equilibrium, microsatellite variation (such as the number of alleles per locus, allelic richness, and observed and expected heterozygosity). According to Bayesian analysis (K=3), bluefish belong to three different genetic units with moderate admixture between them as indicated by genetic distances (K = 3, STRUCTURE test), mitochondrial and nuclear FST results and AMOVA analysis, which revealed some permeability between these genetic units). Their findings confirmed that bluefish populations belongs to the distinct genetic unit. However our results are inconsistent with this conclusion as we observed mixed individuals and low genetic differentiation across all clusters.

Miralles et al. (2014b) reported values close to our AMOVA test results, with (among population variation at 2,96 and within population variation at 96,3) Table 6. Furthermore their study suggests that the Strait of Gibraltar does not act as a barrier to gene flow for *Pomatomus saltatrix* as Spanish samples from both sides of the strait (Cadiz and Barcelona) showed no significant genetic differences.

Miralles et al. (2016) compared the genetic diversity of adult bluefish caught around an aquaculture farm in the Spanish waters of the Western Mediterranean with reference individuals from offshore stocks in the Eastern and Western Mediterranean. According to the study results, bluefish collected from around the fish farm exhibited very high genetic diversity in terms of both microsatellite and mitochondrial DNA, showing that the high genetic diversity of the bluefish caught in the farm is due to the mixing of populations (the Eastern and Western Mediterranean)although most of the individuals caught around the facility belonged genetically to the local population, 7.14% to 11.9% of the individuals belonged to the genetic population living in Türkiye waters. It was revealed that there was some degree of hybridization between the Eastern and Western Mediterranean bluefish stocks in the farm located in Guardamar in the Western Mediterranean. It confirms that individuals from a single population are mixed in all the stations we found.

Dos Santos et al. (2008) also investigated the genetic structure of bluefish populations that are widely distributed from Mozambique to Namiba on the coast of South Africa. They reported high polymorphic differences loci in eight polymorphic regions in their applied studies.

It is seen that eight loci are sufficient to distinguish polymorphic differences between bluefish populations. It is seen that the same loci and the study we examined revealed monomorphism in only one locus and polymorphic differences in the other seven.

When the principal coordinate analysis results are – examined, it is clearly seen that the Mersin population is clustered differently from other populations. However, according to the Mantel test, a low correlation (R^2 = 0.3666, P= 0.01) was detected between genetic and geographical distance. According to the structure analysis results, individuals in 14 regions could not be assigned to a different cluster for K = 2 and K = 3. It was observed that there were mixed individuals in all clusters and there was low genetic differentiation as a structure analysis result.

Reid et al. (2016) examined bluefish samples collected from the Africa coast to the Indian Ocean, analyzing variation in 15 polymorphic microsatellite loci. Contrary to our results, their results showed that; both sequence and microsatellite data showed population partitioning between southern Africa and other locations (South Africa, East Atlantic and another location), which could be explained by the Benguela upwelling as a barrier to gene flow. The absence of subgroups among bluefish populations in Türkiye seas indicates no barrier to gene flow between locations reported in the literature (Miralles et al., 2014a; Reid et al., 2016).

CONCLUSION

Our results showed that, contrary to the literature that;

- Loci showed highly significant deviations from the Hardy-Weinberg equilibrium.

- Pairwise FST comparisons revealed low levels of genetic differentiation (Fst < 0,05).

- For K= 3 structure analysis, individuals in 14 regions could not be assigned to dictinct cluster. (Figure 4).

- Mersin population was different from others according to principal coordinate analysis (Figure 2) but According to the Mantel test, a low correlation (R^2 = 0,3666, P = 0,01) was detected between genetic and geographical distance (Figure 3).

- AMOVA analysis showed that variation within populations was greater than variation between populations (Table 6).

In conclusion, our study reveals a high degree of genetic mixing among bluefish populations, contrary to previous findings that suggested distinct genetic units. The presence of

mixed individuals across all clusters and low genetic differentiation indicates that gene flow is extensive and barriers to genetic exchange are minimal. The results emphasize the need for further studies incorporating broader geographic sampling and advanced genomic techniques to fully understand the population structure and genetic connectivity of bluefish. These findings have significant implications for conservation and fisheries management, underscoring the importance of considering genetic diversity and connectivity when developing sustainable management strategies.

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AUTHORSHIP CONTRIBUTIONS

İlyas Kutlu: Conceptualization, investigation, methodology, writing -original draft; Zehra Duygu Düzgüneş: Analysing statistic, writing -review and editing; Şirin Firidin: Writing -review and editing; Melike Alemdağ: Writing -review and editing; Ayşe Cebeci: Visualization, writing -review and editing; İbrahim Turan: Supervision, writing- review and editing.

CONFLICT OF INTEREST

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

ETHICS APPROVAL

All experiments were carried out considering the ethical rules of the authorities, with the approval coded as 325.04.02-12 by the Ethical Committee of Animal Experiments of Central Fisheries Research Institute.

DATA AVAILABILITY

For questions regarding datasets, the corresponding author should be contacted.

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