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RESEARCH PAPER

Genetic Diversity of Bluefish Population in Türkiye Coastal

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Abstract

Bluefish (*Pomatomus saltatrix*) is a commercially important, highly migratory predatory fish species distributed along the coasts of Türkiye. Conservation and sustainability of bluefish populations requires knowledge of the genetic structure of the population, but There are no genetic studies on bluefish in Türkiye. In this study, the population structure of Bluefish in the coastal regions of Türkiye was investigated by sequence analysis of mitochondrial DNA gene regions (Cytochrome b and Displacement-loop) and nuclear gene region (Rhodopsin). We tried to identify bluefish samples collected from 14 regions in the coastal region of Türkiye by analyzing two mtDNA gene sequences (Cyt b and D-loop) and nuclear gene region (Rhodopsin). While a total of 35 haplotypes were found in the Cytb gene region, a total of 132 haplotypes were found in the D-loop (control region) gene region. A single haplotype was detected in all stations for Rhodopsin. Also, the maximum likelihood trees based on Cyt b and D-loop sequences suggested relationship of Bluefish populations from Türkiye and the world. Results showed a low level of genetic differentiation between Bluefish populations in the study area, but Aegean and Mediterranean populations are more diverse than other populations due to migratory trait of this species.

Introduction

While today's technological developments have brought significant dynamism to the fishing industry, they have also become one of the main sources of human destruction in the marine ecosystem. Conservation of fish species is vital not only for aquatic ecosystems, but also for humans. In order to achieve sustainable fisheries management, various biological characteristics of the selected fish species must be determined.

Bluefish is the only member of the Pomatomidae family, which generally spreads on the continental margin and is found in temperate and warm waters around the world (Briggs, 1960). In addition, Bluefish (*Pomatomus saltatrix*) is an important species that is found in all seas of Turkey, has high economic value and needs to be protected (Akşıray, 1987; Demirsoy, 1999). Most of the taxonomic studies carried out to date on bluefish populations in Turkey are based on differences and similarities in morphometric and meristic characteristics (Turan et al., 2006; Habib, 2015). Other studies are on reproductive biology, hunting and the characteristics of hunting tools (Ceyhan et al., 2007; Öztekin et al., 2018; Özdemir & Erdem, 2018).

When we look at the literature, there are very limited genetic studies on Bluefish, which spread around the world, and a few of them include genetic studies conducted with samples taken from our country, Istanbul and Çanakkale (Pardinas et al., 2010; Miralles et al., 2014 a/b).

As a result of rapid developments in DNA sequencing technology in recent years, DNA-sequence analysis has significantly expanded the possibilities of phylogenetic studies and in many cases has replaced or supplemented more traditional morphometric methods

(Hillis et al., 1990). DNA barcoding based on sequencing is now widely used in species identification and mitochondrial DNA gene regions or nuclear DNA gene regions are used as markers (Hajibabaei et al., 2006).

As one gene of mtDNA, Cyt b have been widely used for phylogenetic analysis at the species and family levels (Kumar et al., 2017;Lalitha and Chandavar, 2018). The non-coding control region of mtDNA (D-loop) has hypervariable sites and a faster mutation rate to analyze the population structure and intraspecific phylogenetics(Shamblin et al., 2015;Chan et al., 2016). Hybridization could cause a problem for barcoding since mitochondrial marker gene derives from the maternal parent of any barcoded specimen and therefore, any hybrid fish will be wrongly diagnosed as its maternal species. It is suggested that DNA barcoding should be accompanied by the analysis of known species-specific nuclear DNA alleles to clarify its status (Ward et al., 2009). As a nuclear gene, rhodopsin has been recommended for teleost fishes, and applied in different phylogenetic analyses (Sevilla et al., 2007; Chan et al., 2016).

The aim of this study is to assess the genetic diversity and population structure of bluefish (P. saltatrix) in Turkish waters by analyzing mitochondrial (Cyt b, D-loop) and nuclear (rhodopsin) gene sequences. The data obtained will facilitate the determination of the level of genetic differentiation between populations, the identification of potential breeding areas and migration routes, which are crucial elements for the development of effective conservation strategies for this economically valuable species.

Material and Methods

Sampling

It was sampled to fish in 14 stations: Hopa, Trabzon, Giresun, Samsun, Sinop, Ereğli, İğneada, R. Feneri (Istanbul), Çanakkale, Erdek (Marmara sea), Bodrum, İzmir, Mersin and Adana (Figure 1). Caudal fin clips (2-3 cm²) were from 32 of fish at each station and stored in 99% ethanol at room temperature.

DNA Extraction, Amplification and Sequence Analysis

The total genomic DNA was extracted from each sample using the QIAamp DNA HT (Qiagen®) kit following the protocol suggested by the manufacturer. The isolated DNA were visualized through electrophoresis in 1.5% agarose gel stained with a fluorescent dye SafeViewTM (NBS Biologicals, UK) and visualized under ultraviolet light. Two mitochondrial DNA markers, Cytochrome b (Cyt b) and D-loop gene region and nuclear gene Rhodopsın were screened as potential markers for species identification and genetic diversity evaluation in this study.

The mitochondrial Cyt b and D-loop gene regions (for Cyt b: ~580 bp, D-loop: ~720 bp and Rhodopsın: ~520 bp) and nuclear gene Rhodopsın were amplified via PCR in using the primers in seen Table 1.

The reactions were performed with a final volume of 10 µl which comprised: 5 µl of 2× Master Mix (Hibrigen®), 0.5 µl F (forward) and R (reverse) primers

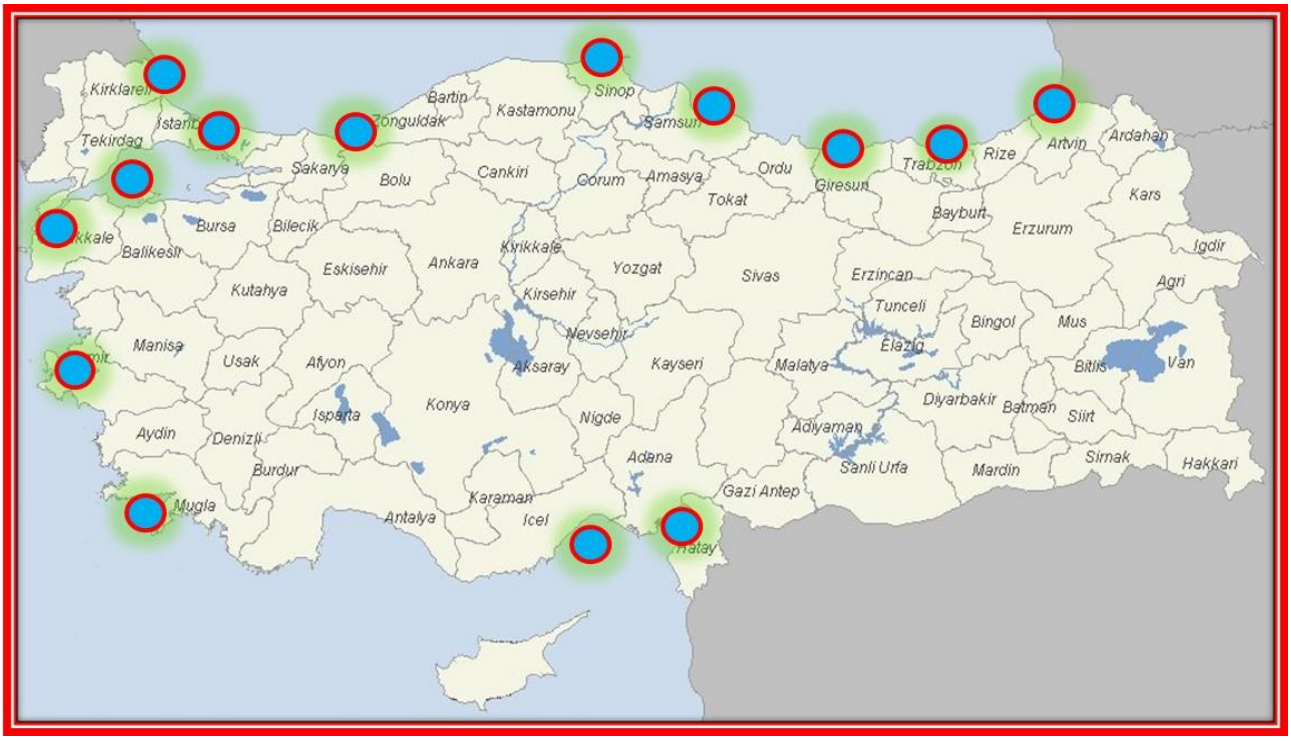

Figure 1. Map of sampling locations for *P. Saltatrix* in coastral of Türkiye.

(10mM), 1 µl DNA (50 ng/µl) and 3 µl of DNAase free water. The amplification of DNA by PCR was optimized separately for each primer pairs (Table 1). The amplification formed an initial step of 3 min of denaturation at 94°C, the second step is 1 min of denaturation at 94°C, 45 s of hybridization between 55-65°C, and 1 min at 72°C by 30-35 cycles followed by a final polymerization at 72°C for 10 minutes (ABI Veriti). Amplifications were verified by agarose gel electrophoreses as mentioned above. PCR products were stored at 4°C until sequencing (Firidin et all., 2020).

After PCR product was precipitated using EDTA/Ethanol precipitation (Fujikura, 2015) prior to sequencing PCR. The samples were sequenced in forward and reverse directions using the kit Bigdye Terminator v3.1 Cycle Sequencing kit and forward or reverse primer. The reactions were performed with a final volume of 10 µl which comprised: 5xSequencing Buffer (ABI), 0.4 µl forward or reverse primers (10mM), 0,5 µl BigDye Terminator (ABI), 1 µl amplified DNA and 6,1 µl of ultrapure water) were used for one experimental run. The PCR program included an initial 1-min denaturation step at 96°C and 40 cycles of denaturation at 96°C for 10 s, annealing at 50 to 55°C for 5 s and primer extension at 60°C for 150 s. Aftter sequencing PCR DNA was precipitated using EDTA/Ethanol precipitation (Fujikura, 2015) again and the samples sequencing using the automatic genetic analyzer ABI 3500 (ABI).

Data Analysis

Raw sequences data of Cyt b, D-loop and Rhodopsin gene regions were arranged with BioEdit program (Hall,1999) and sequences of each genes (Cyt b, D-loop and Rhodopsın) were aligned using ClustalW (Thompson et al., 1994) from the BioEdit (Hall, 1999). Low-quality sequences were removed from alignment. Each sequence was compared to existing sequences in the database at NCBI GenBank using BLAST mode (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The genetic diversity indexes of each locations for Bluefish population in Türkiye coastral area: Haplotype numbers (h), haplotype diversity (Hd), nucleotide diversity (Pi), number of variable regions (S) were calculated by using program DNAsp v5 (Librado and Rozas, 2009).

The program Arlequin v3.0 (Excoffier et al., 2005) carried out an analysis of molecular variance each locations for Bluefish populations (AMOVA) and to calculate pairwise FST values (Wright, 1969). Haplotype network maps were generated with PopART (Leigh and Bryant, 2015).

Data obtained from both DNA gene regions were used to create trees examining phylogenetic relationships and to reveal the genetic structure. Mega X program (Kumar et al., 2018) program was used to determine the most suitable base exchange model.

Phylogenetic tree dendrograms were drawn in the Mega X program with the select-link technique with 1000 repetitions using the Maximum Likelihood method. Using the obtained DNA sequences as well as the records in the NCBI GenBank database, the most appropriate nucleotide change model was decided in accordance with the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) for the Cyt b, D-loop and Rhodopsın gene regions data set. While there are plenty of Cyt b, D-loop and Rhodopsın gene regions sequences of Bluefish available in the GenBank database for comparison, genetic data for other members of the Pomatomidae family are not available in NCBI (since the only member of the family is Bluefish). Thus, *Thunnus thynnus* was used as the outgroup terminal in forming the phylogenetic tree.

All protocols of this study were approved by Agricultural Research and Policy General Directorate (TAGEM). The samples of legal-sized Blue fish were collected from the commercial catches on board. Thus, this study does not require animal ethic.

Results

The 582 bp portion of the Cyt b gene region of 382 samples from all stations was analyzed and a total of 35 haplotypes were detected. The most shared haplotypes in terms of number of samples are hp-3 (in 249 samples from 12 stations), hp-2 (in 29 samples from 9 stations), hp-32 (in 24 samples from 1 station). As the least common 21 haplotypes were found in one sample each.

The 719 bp portion of the D-loop gene region of 237 samples from all stations was analyzed and a total of 132 haplotypes were detected. The most shared haplotypes in terms of number of samples are hp-26 (in 25 samples from 6 stations), hp-6 (from 6 stations in 7 samples), hp-8 (from 6 stations in 8 samples). As the least common 108 haplotypes were found in one sample each. Haplotype values according to stations are given in Table 2.

Nucleotide substitution models were selected based on AIC and BIC criteria for the Cyt b and D-loop for the set of newly obtained sequences in this study, which were combined with reference sequences from the NCBI GenBank database. The HKY+G model was determined to be most suitable for the Cyt b gene (Figure 2), while the T92+G+I model was selected for the D-loop gene (Figure 3). A maximum likelihood phylogenetic tree was constructed for the D-loop and Cyt b genes using the Bootstrap method with 1000 replicates.

The 513 bp portion of the Rhodopsin gene region of 427 samples from all stations was analyzed and a single haplotype was detected in all stations. By adding the records in the NCBI GenBank database together with the DNA sequences we obtained, K2P was selected as the most appropriate nucleotide change model according to the AIC and BIC criteria in the data set obtained for the Rhodopsin gene region (Figure 4). Also

Table 1. Primers Used in the Study.

Gene Region	Primer Name	Primer Sequence	Annealing Tm	References
D-Loop	M. Fish D-Loop-F	AGCACCGGTCTTGTAAACCG	63°C	Cheng et al., 2012
	M. Fish D-Loop-R	GGGCTCATCTTAACATCTTCA	63°C	Cheng et al., 2012
Cyt b	Cyt b-F	CGATTCTTCGCATTCCACTTCCT	54°C	Sevilla et al., 2007
	Cyt b-R	GGTCTTTGTAGGAGAAGTATGGGTGGAA	54°C	Sevilla et al., 2007
Rhodpsin	Rod-F2B	GTCTGCAAGCCCATCAGCAACTTCCG	62°C	Sevilla et al., 2007
	Rod-5R	GGTGGTGATCATGCAGTGGCGGAA	62°C	Sevilla et al., 2007

Table 2. mt DNA Gene Regions (Cyt b and D-loop) Haplotype Values.

Cyt b							Sampling Location	D LOOP						
n	**h**	**Hd**	**Pi**	**k**	**S**	**Eta**		**n**	**h**	**Hd**	**Pi**	**k**	**S**	**Eta**
31	10	0.770	0.00396	2.30108	12	12	Adana	24	21	0.989	0.01528	10.98551	51	53
22	4	0.333	0.00088	0.51082	3	3	Ereğli	18	13	0.961	0.00824	5.92157	24	24
21	10	0.886	0.00393	2.28571	9	9	Bodrum	15	10	0.933	0.00877	6.30476	18	18
24	4	0.370	0.00199	1.15580	6	6	Çanakkale	12	9	0.939	0.01041	7.48485	20	20
31	2	0.065	0.00033	0.19355	3	3	Giresun	10	6	0.844	0.00677	4.86667	15	15
32	3	0.284	0.00111	0.64718	4	4	Hopa	11	10	0.982	0.00723	5.2000	19	19
30	5	0.308	0.00080	0.46207	6	6	İğneada	20	11	0.895	0.00514	3.69281	16	16
31	4	0.598	0.00271	1.57419	5	5	İzmir	20	14	0.937	0.01037	7.45263	28	28
26	3	0.280	0.00159	0.92615	5	5	Erdek	24	14	0.935	0.00667	4.79710	19	19
22	5	0.468	0.00208	1.20779	6	6	Mersin	19	15	0.977	0.00922	6.62573	29	30
20	2	0.189	0.00033	0.18947	1	1	R. Feneri	15	8	0.790	0.00604	4.34286	17	17
31	4	0.243	0.00085	0.49462	4	4	Samsun	15	14	0.989	0.00908	6.52747	26	26
34	7	0.326	0.00111	0.64528	10	11	Sinop	17	12	0.949	0.00843	6.05882	28	28
27	4	0.214	0.00076	0.44444	6	6	Trabzon	17	11	0.934	0.00708	5.08824	20	20
382	**35**	**0.563**	**0.00213**	**1.23902**	**27**	**29**	**Total**	**237**	**132**	**0.982**	**0.01052**	**7.56649**	**80**	**85**

(**n:** Number of samples, **h:** Number of haplotypes, **Hd:** Haplotype diversity, **Pi:** Nucleotide diversity, **k:** Nucleotide difference values, **S:** Number of variablesites, **Eta:** Total number of mutations)

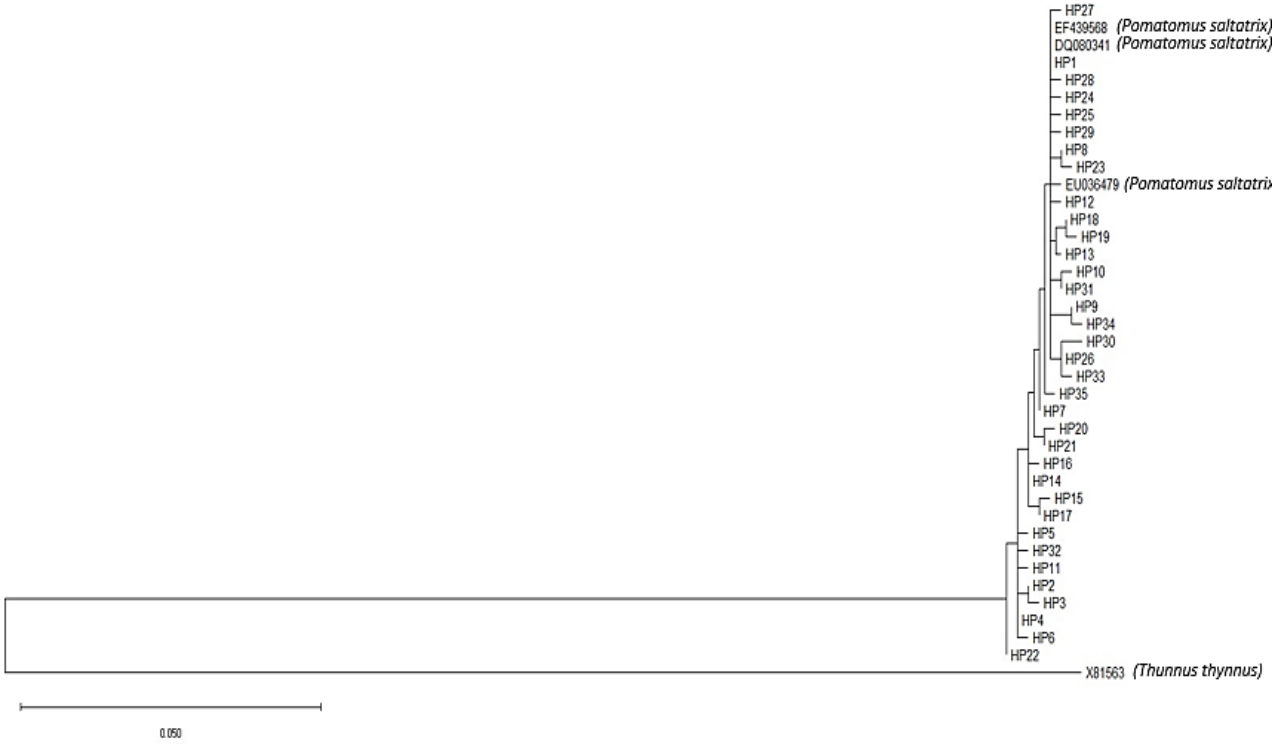

Figure 2. Maximum Likelihood Phylogenetic Tree Obtained for the Cyt b Gene Region.

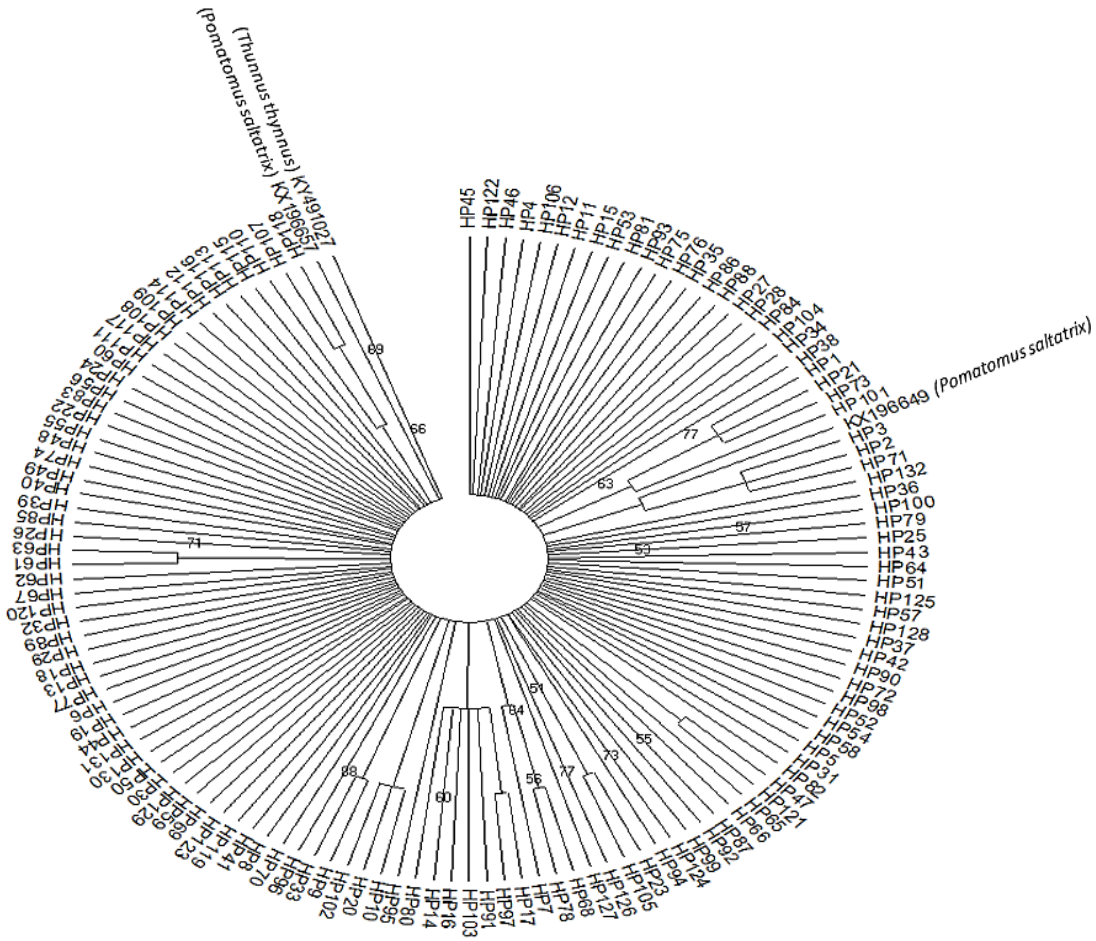

Figure 3. Maximum Likelihood Phylogenetic Tree Obtained for the D-loop Gene Region.

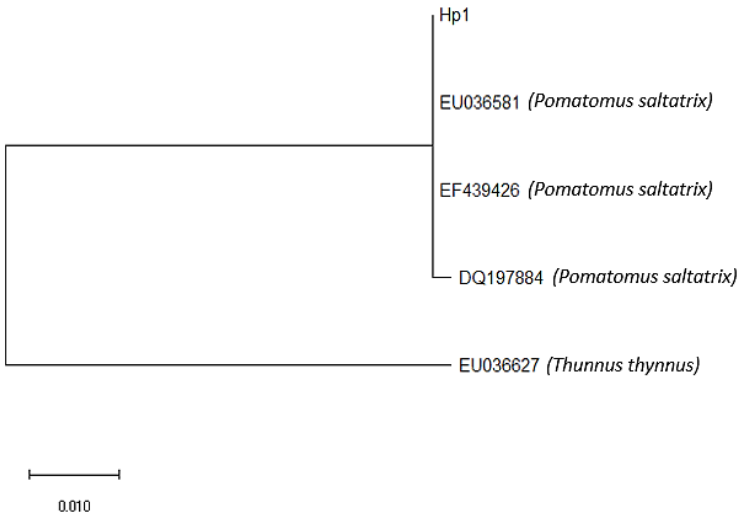

Figure 4. Phylogenetic Tree Obtained for the Rhodopsin Gene Region.

Haplotype network were constructed for haplotypes of D-loop and Cyt b gene regions (Figure5a-b).

The graph of statistical values obtained from FST values, which express the degree of genetic distance between populations belonging to the Cyt b and D-loop gene regions of mtDNA, is given in Figure 6 a-b. In the AMOVA analysis performed to determine the distribution of genetic diversity in Cyt b and D-loop gene regions data. Results of both Cyt b and D-loop gene region AMOVA analysis reveal that among population variation is less than within population variation. Differences in variation analysis results for populations for both Cytb and D-loop gene regions are seen in Table 3.

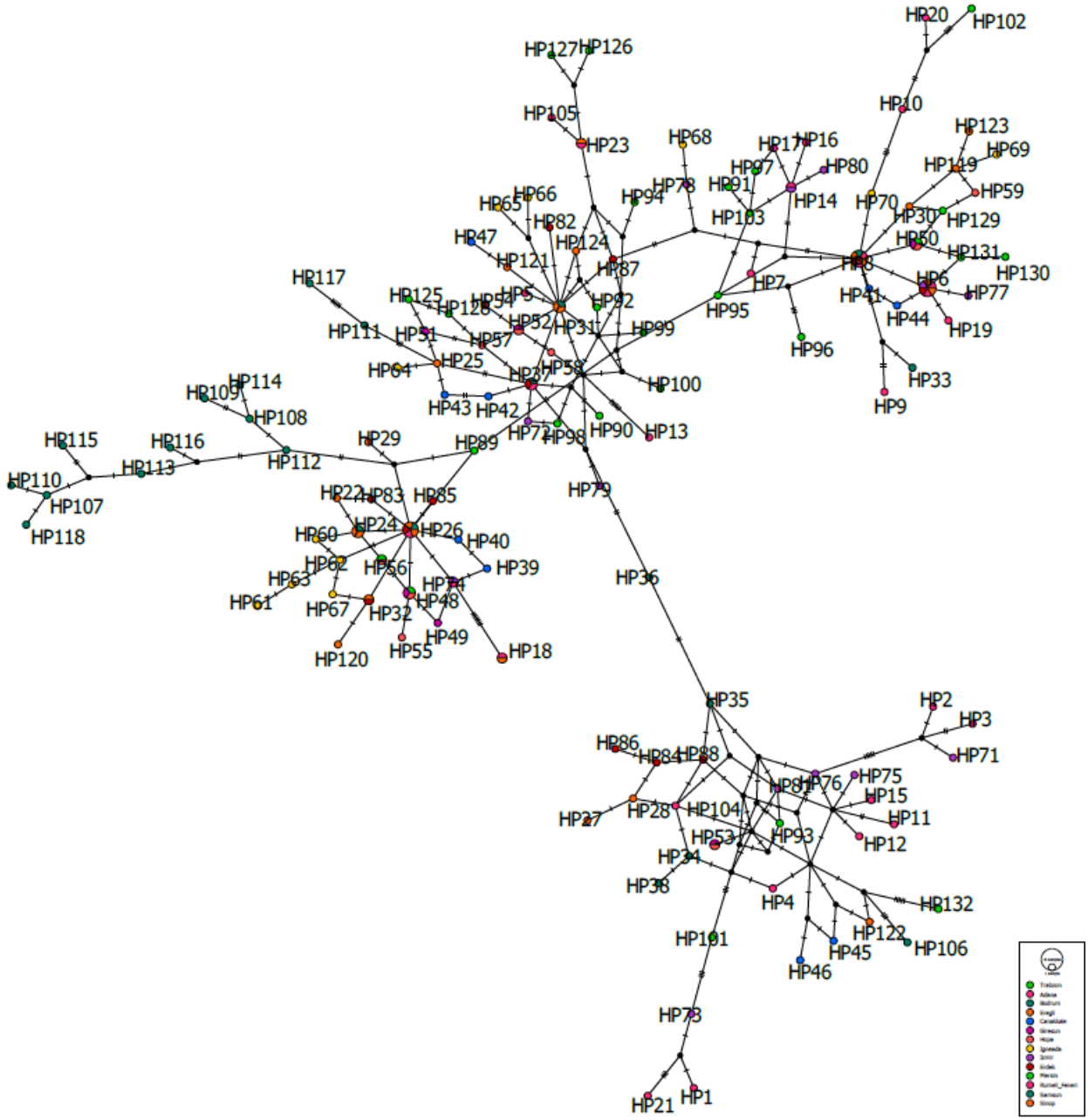

Figure5a. Haplotype network for haplotypes of D-loop gene region

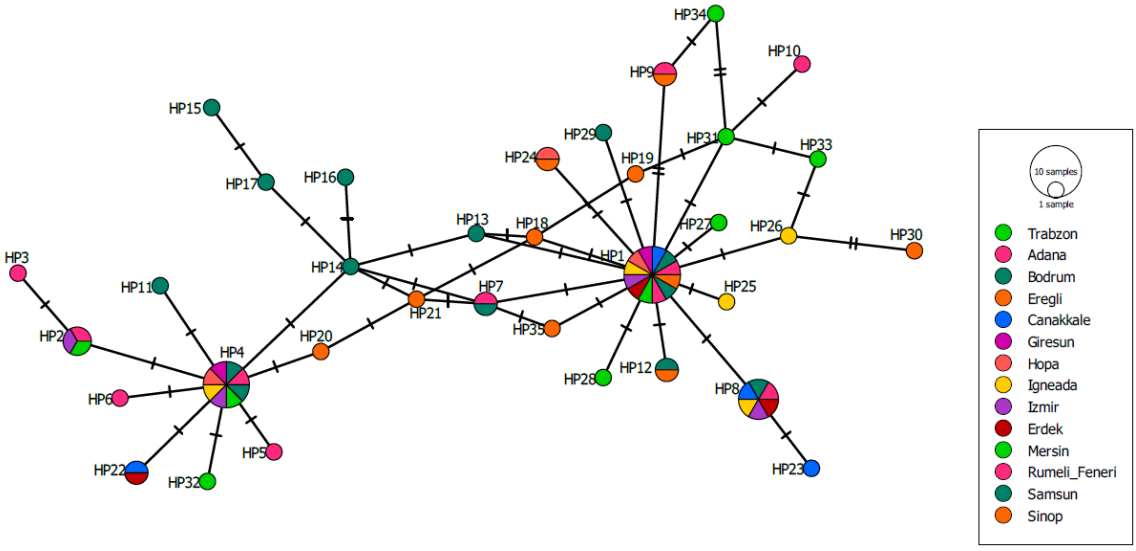

Figure5b. Haplotype network for haplotypes of Cyt b gene region

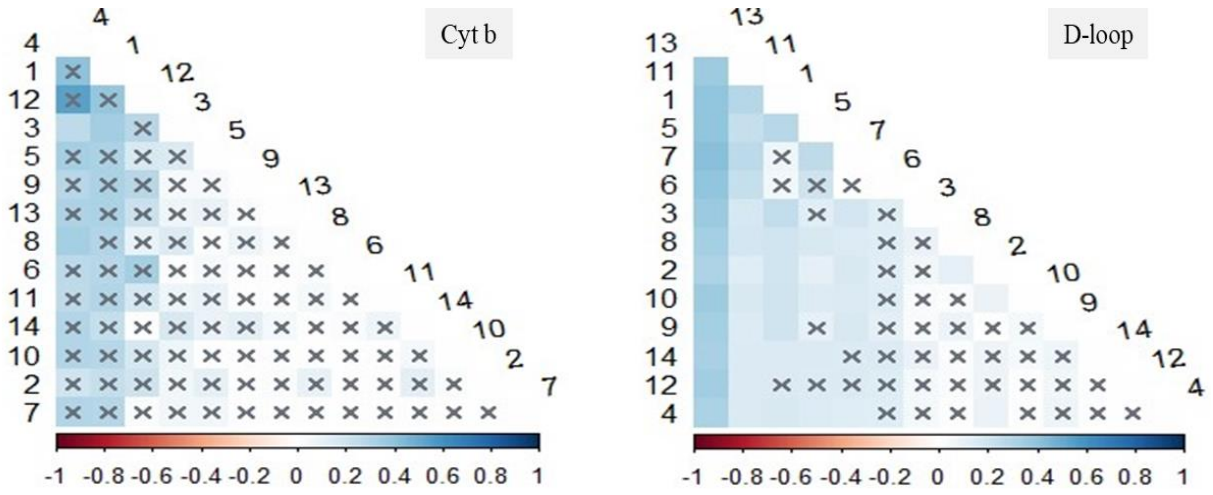

Figure 6. mtDNA gene regions FST statistical values graph (**x**; Non-significant difference) (**1**-Trabzon, **2-** Adana, **3-** Bodrum, **4-** Ereğli, **5-** Çanakkale, **6-** Giresun, **7-** Hopa, **8-** İğneada, **9-** İzmir, **10-** Erdek, **11-** Mersin, **12-** R. Feneri, **13-** Samsun, **14-**Sinop)

Table 3. Global AMOVA results of mtDNA (Cyt b and D-loop) data sets

Cyt b			**Source of Variation**	**D-loop**		
Sum of Squares	Variance Components	% Variation		Sum of Squares	Variance Components	% Variation
37.93	0.178	**12.29**	Among Pop.	263.86	0.701	**16.73**
150.06	1.271	**87.71**	Within Pop.	1130.2	3.488	**83.27**
188.000	1.450	**100.00**	Total	1394.1	4.189	**100.00**

Discussion

In this study, we investigated the genetic structure and relationships among Bluefish populations sampled from stations along the studied coast. The genetic structure and relationships among populations were analyzed by using two mitochondrial markers and one nuclear marker. Results indicated that two mitochondrial markers were effective, but the selected nuclear marker was not, because it did not show differences.

De Queiroz-Brito et al. (2022) obtained approximately 570 bp sequence from the COI gene region from samples collected at the Venezuelan station and analyzed with 154 COI sequences from international databases (Western North Atlantic; Gulf of Mexico, Caribbean Sea; Western South Atlantic, Mediterranean; Marmara Sea: Eastern South Atlantic; Indian Ocean, compared to the South Pacific). Comparing our results of the Cyt b and D-loop gene with the AMOVA analysis, it was found that variation among populations was lower than variation within populations, as shown in Table 3. This finding aligns with the results of De Queiroz-Brito et al. (2022).

Mirales et al., (2014b); according to the AMOVA analysis results of their study by sequence analysis of the COI and Cyt b gene regions of a total of 120 *Pomatomus saltatrix* samples collected from eight different locations between 2004 and 2009 the variation within populations is less than the variation between populations (29.3 % within the population and 70.6 % between populations) and is similar to our results as seen in Table 3. In the same study, 120 samples from all regions, 29 haplotypes for the Cyt b gene region were revealed, characterized by high haplotype diversity and low nucleotide diversity. Our results for both gene regions showed similar patterns (Table 1-2). Additionally, the study reported that the Strait of Gibraltar was not a barrier to gene flow for Pomatomus saltatrix since the Spanish samples on the two sides of the strait (Cadiz and Barcelona) did not show significant differences.

In another study Mirales et al., (2014a) reported that pairwise F_{ST} comparisons revealed clusters of three different genetic branches for both mitochondrial and nuclear markers. For mtDNA in this study, The position of the branch in the similarity tree containing the Spanish samples (Cadız and Barcelona), clustering with the Eastern Mediterranean branch (Türkiye samples İstanbul and Çanakkale) and the American branch for microsatellites, reported that Bluefish occur in trans-Atlantic migration such as Shark and Tuna, as well as trans-Mediterranean migration. In conclusion, They reported that this explains the lack of genetic separation and thus the continued existence of a single species throughout the Atlantic Ocean and in the family Pomatomidae.

Sabates et al. (2012) reported that due to the increasing surface water temperature in the Northwest Mediterranean in the 1980s and 1997s, the Bluefish distribution area in the Western Mediterranean expanded towards the north and the species reproduced in new distribution areas.

Miralles et al. (2016) conducted a sequence analysis of seven microsatellite loci and mtDNA COI on 159 adult Bluefish samples caught between 2004 and 2005 around an aquaculture farm (coming to aquaculture cages to hunt farmed fish) in the Spanish waters of the Western Mediterranean. According to the results of the study in which reference individuals of Mediterranean stocks and reference individuals of Western Mediterranean stocks were compared with reference data; Bluefish collected around the fish farm display very high genetic diversity in terms of both microsatellite and mitochondrial DNA, shows that the high genetic diversity of farm-caught Bluefish is due to the mixture of populations (coming from different genetic units previously described in the Mediterranean Basin; Pardiñas et al. 2010; Miralles et al. 2014b). Although most of the individuals caught around the facility genetically belong to the local population, 7.14% to 11.9% of the individuals belong to the genetic population living in Türkiye waters. The farm in Guardamar in the Western Mediterranean revealed a degree of hybridization between Eastern and Western Mediterranean Bluefish stocks.

Pardinas et al. (2010) performed a sequence analysis of the Cyt b gene region from Bluefish samples collected between 2006 and 2008. They reported that populations in the Eastern Atlantic Ocean (including the Eastern Mediterranean) and those in the Western Atlantic Ocean did not share any haplotypes, and that there was complete genetic isolation between the two sides of the North Atlantic Ocean. In addition to no significant differences were found between the eastern Atlantic Ocean (Cadiz and Canary Islands) and Mediterranean samples, indicating that *P. saltatrix*, like other species, can overcome biogeographic barriers. Although the haplotypes of the populations we detected were similar to the haplotypes found in the Mediterranean basin and the African coast, it was observed that they were not shared with any haplotypes detected in the American continent.

It appears that there is no effective marker gene for Rhodopsin in determining inter-population genetic diversity.

Finally, Habib Bal, in PhD thesis (2015), examined 27 metric and 6 meristic characters in a total of 131 Bluefish from the Eastern Black Sea, Western Black Sea, Marmara and Aegean Seas. When the data were analyzed, it was concluded that the morphological similarity rates of the samples taken from different regional seas were high, there were no subspecies and it was a migratory species among our seas.

In conclusion; according to genetic studies published about Bluefish in the world and the results we obtained from genetic studies of Bluefish samples collected from the coasts of Türkiye; Although there are nucleotide differences, genetic differences between populations have been found to be very low. It has been observed that the Aegean and Mediterranean populations are more diverse than other populations, that there are mixed individuals in all populations. Therefore, it can be said that the Bluefish is represented by a single species as a genetically do not have any subspecies in Türkiye seas.

Ethical Statement

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.

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Author Contribution

İlyas KUTLU: Conceptualization, Investigation, Methodology, Writing -original draft; Ayşe CEBECİ: Visualization, Writing -review and editing; Melike ALEMDAĞ: Writing -review and editing; Şirin FİRİDİN: Writing - review and editing; Zehra Duygu DÜZGÜNEŞ: Writing -review and editing.

Conflict of Interest

The authors 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.

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