Turk. J. Fish.& Aquat. Sci. 25(3), TRJFAS26010
https://doi.org/10.4194/TRJFAS26010

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Population Genetic Diversity of Bluefish (*Pomatomus saltatrix* L, 1766) in Coastal of Türkiye

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How to Cite

Kutlu, I., Firidin, S., Duzgunes, Z. D., Alemdag, M., Cebeci, A., Turan, I. (2025). Population Genetic Diversity of Bluefish (*Pomatomus saltatrix* L, 1766) in Coastal of Türkiye. *Turkish Journal of Fisheries and Aquatic Sciences*, *25(3), TRJFAS26010.* https://doi.org/10.4194/TRJFAS26010

Article History
Received 26 April 2024
Accepted 14 November 2024
First Online 29 November 2024

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Keywords
COI
16S rRNA
Bluefish
Phylogenetic
Pomatomus saltatrix

Abstract

Bluefish, is a commercially important fish species distributed the Türkiye coastal area. The high migration rates of Bluefish may prevent indirect speciation across its broad geographic distribution. The conservation and sustainability of the Bluefish populations require knowledge of the population's genetic structure. There are no genetic studies on Bluefish in Türkiye and there are only a few studies in the world. In this study we investigated the population structure of this species in Türkiye coastal areas. *COI* and *16S rRNA* gene sequences were performed in Bluefish samples collected from 14 regions. All stations are displayed low number of haplotypes for *COI* and *16S rRNA*. In the *COI* gene region, total of 20 haplotypes was found whereas in the *16S rRNA* gene region, total of 8 haplotypes was found. The results of both *COI* and *16S rRNA* AMOVA analysis revealed that within population variation is less than among population variation It has been observed that Aegean and Mediterranean populations are more diverse than other populations. It is seen that the genetic differentiation among Bluefish populations in coastal Türkiye is low level and there are mixed individuals in all populations

Introduction

In a country, plant and animal species are considered the biological wealth of both that country and the world (Demirayak, 2002). Türkiye is a very rich country in terms of biodiversity (Aksoy and Atasagun, 2023). Türkiye's coastal and marine areas are of global importance (Özyanık et al., 2010).

The natural environment is under threat from human activities, leading to habitat loss, loss of biodiversity and spread of invasive species (Kahrić et al., 2022). In order for human activities to continue, genetic diversity among and within populations of all species is necessary for people and nature to survive and thrive in a changing world (Hoban et al., 2023).

In order to protect biological diversity in a geographical region, it is necessary to first determine the genetic diversity present in natural resources. There is a need to conserve and maintain the genetic diversity of these valuable resources for sustainable food security (Salgotra and Chauhan, 2023). The investigation of the population structures of species is a very important step towards the protection of their genetic heritage (Keskin, 2013). In particular, genetic research on fish populations is important for establishing fisheries policy and for the protection of shared stocks and threatened species.

There are many species with different economic values in Türkiye seas. The Bluefish, which is the only species of its family, is distributed in many parts of the world, usually on the edges of continents, in temperate and warm waters. The predatory fish species that is known to make long-distance migrations between seas in response to seasonal changes. Since Türkiye is located in the temperate climate zone in the world, Bluefish also

spreads in the Türkiye's seas (Bilecenoglu et al., 2002). Bluefish is a species that attracts great attention in terms of fishing due to its high commercial value and is hunted in all Türkiye's seas. According to the data of the last 20 years, the average Bluefish production in Türkiye is 8718 tons/year (Arslan and Yıldız, 2021).

Basic molecular markers commonly used to determine genetic diversity include minisatellites, RFLP, RAPD, AFLP, SNP, mtDNA and microsatellite analyzes (Liu, 1998). DNA barcoding has become the most popular approach to species identification in recent years (Raupach et al., 2022). Among different taxonomic methods, DNA barcoding is a common technique used to identify fish species using a short genetic marker (Haldar and Nath, 2020).

There is no study reporting comprehensive genetic diversity on Bluefish (*Pomatomus saltatrix*) populations in Türkiye. This study was conducted in order to contribute to the studies aimed at revealing the biological diversity of Türkiye.

The genetic diversity and genetic structure of the Bluefish (*Pomatomus saltatrix* L, 1766) population in the coastal region of Türkiye were evaluated using two mtDNA (*COI* and *16s rRNA*) markers.

Material and Method

Sampling

A total of 14 stations on the Turkish coastal were selected as the study area: Hopa, Trabzon, Giresun, Samsun, Samsun, Sinop, Ereğli, İğneada, R. Feneri (İstanbul), Çanakkale, Erdek (Marmara Sea), Bodrum, İzmir, Mersin and Adana (Figure 1). Legal size Bluefish samples were collected from commercial fishing vessels. It was carried out in accordance with the work permit numbered 325.04.02-12 of the Ethics Committee of Animal Experiments of the Central Fisheries Research Institute. Approximately 2-3 cm^2 of tail fin tissue was sampled from each Bluefish and stored at room temperature in 96% ethanol.

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 recommended by the manufacturer. The DNA samples were controlled by electrophoresis in 1,5% agarose gel colored with SafeViewTM (NBS Biologicals, UK). The concentrations of DNA samples were measured with NanoDrop™8000 Spectrophotometer. In the study, DNA samples with a ratio of 260/280=1,8 and a concentration between 50-100ng/µl were used for amplification.

Two mitochondrial DNA markers were screened as potential markers for species identification and population genetic diversity assessment in this study (*cytochrome c oxidase* subunit I; *COI* and *16S rRNA*). The mitochondrial *COI* and *16S rRNA* genes regions (for *COI*: ~700 bp, 16S: ~520 bp) were amplified via PCR using the forwad (F) and reverse (R) primers (Table 1).

The reaction was carried out in a final volume of 10 µl (Table 2). The amplification of DNA by PCR was optimized separately for primer pair. The amplification formed an initial step of 3 minutes of denaturation at 94°C, the second step is 1 minute of denaturation at 94°C, 45 second of hybridization between 50-64°C, and 1 minute at 72°C by 30-35 cycles followed by a final polymerization at 72°C for 10 minutes (ABI Veriti).

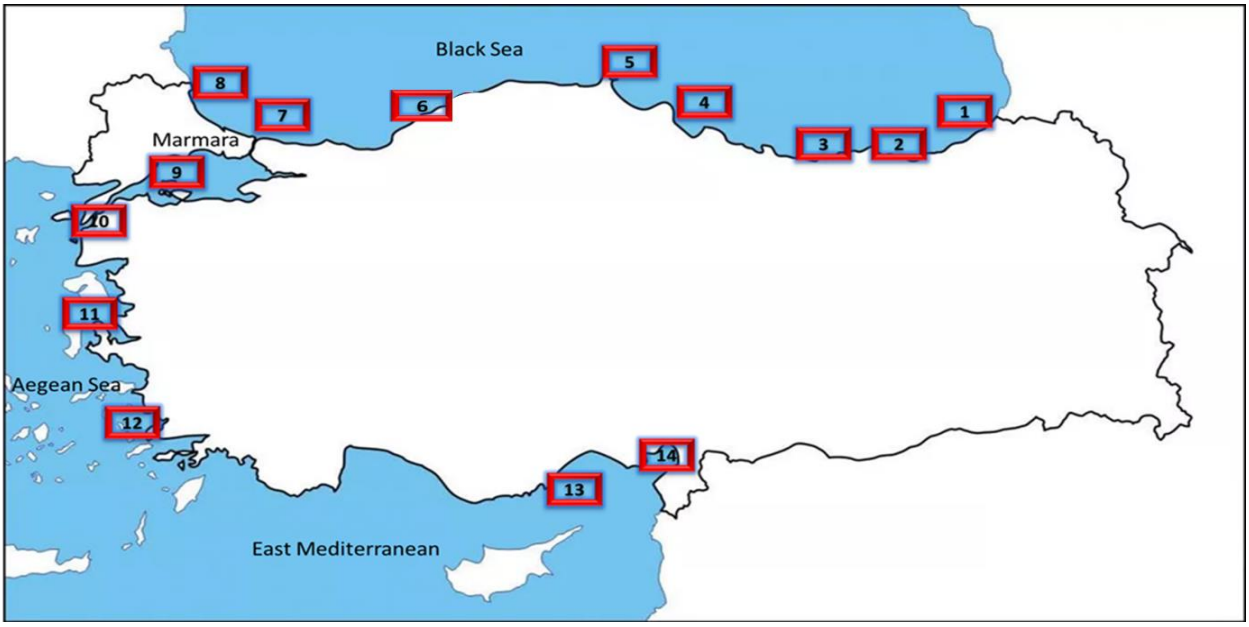

Figure 1. Map of sampling locations for *P. Saltatrix* in coastal of Türkiye (1-Hopa, 2- Trabzon, 3- Giresun, 4- Samsun, 5- Sinop, 6- Batı Karadeniz (Ereğli), 7- Rumeli Feneri, 8- İğneada, 9- Marmara Sea (Erdek), 10- Çanakkale, 11- İzmir, 12- Bodrum, 13- Mersin, 14- Adana).

Amplifications were verified by agarose gel electrophoreses as mentioned above. PCR products were stored at 4°C until sequencing (Firidin et al., 2020). After PCR product was precipitated using EDTA/Ethanol precipitation (Fujikura, 2015) prior to sequencing PCR.

The reactions of sequencing PCR were performed with a final volume of 10 µl. The sequencing protocol using Bigdye Terminator v3.1 Cycle Sequencing kit was performed as a Table 3.

After the sequencing PCR, they were prespited again and drying. After drying, the samples were dissolved in 10 µl of HI-DI formamide and sequenced using an automated genetic analyzer ABI 3500 (ABI).

Data Analysis

The raw sequences data of *COI* and *16S rRNA* were arranged using BioEdit Sequence Alignment Editor (Hall, 1999) and sequences of each mtDNA genes (*COI* and *16S rRNA*) were aligned using ClustalW (Thompson et al., 1994) from the BioEdit Sequence Alignment Editor (Hall, 1999). Low-quality sequences were removed from the alignment.

Each sequence was identified and 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 Türkiye coastal area; haplotype numbers (h), haplotype diversity (Hd), nucleotide diversity (Pi), number of variable regions (S) were calculated by using program DNAsp version 5 (Librado and Rozas, 2009).

The program Arlequin version 3.0 (Excoffier et al., 2005) carried out an Analysis Molecular Variance (AMOVA) in or Bluefish populations and to calculate pair-wise FST values (Wright, 1969). Haplotype network maps were generated with PopART (Leigh and Bryant, 2015).

Data from both mtDNA gene regions were used to construct phylogenetic trees that examined phylogenetic relationships and revealed genetic structure. Mega X program (Kumar et al., 2018) was used to determine the most appropriate base exchange model program. 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 *COI* and *16S rRNA* gene region data set. While there are plenty of *COI* and *16S rRNA* 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, ***Scomber scombrus*** (KX782959: Germany-Bremen) was used as the outgroup terminal in forming the phylogenetic tree.

Results

In this study, a dataset of *COI* and *16S rRNA* sequences of Bluefish was obtained. A sequence of approximately 704 bp of the *COI* gene region was analyzed from all stations. Sequence data of 360 samples taken from all stations were included in the calculation and a total of 18 haplotypes were detected.

Table 1. Primers Used in the Study.

COI	(Ward et al., 2005)	F	5`-TCAACCAACCACAAAGACATTGGCAC-3`
		R	5`-TAGACTTCTGGGTGGCCAAAGAATCA-3`
16S rRNA	(Palumbi, 1996)	F	5`-CGCCTGTTTATCAAAAACAT-3`
		R	5`- CCGGTCTGAACTCAGATCACGT-3`

Table 2. PCR reaction conditions.

2× Master Mix (Hibrigen®)	:	5 µl
10mM Forward primers	:	0.5 µl
10mM Reverse primers	:	0.5 µl
DNA (50 ng/µl)	:	1 µl
Ultrapure water	:	3 µl

Table 3. Sequence Protocol and PCR reaction conditions.

Sequence PCR Mix Components Per Sample		**Thermal Cycle Steps**		
5x Sequencing Buffer	2 µl	96°C	1 minute	
10mM Forward or Reverse primers	0,4 µl	96°C	10 second	35-40
Bigdye Terminator v3.1	0,5 µl	55°C	5 second	cycles
Ultrapure water	6,1 µl	60°C	4 minute	
Template DNA	1 µl	4°C	∞	

The generated *COI* and *16S rRNA* sequences were deposited at NCBI GenBank (Access numbers: ***COI*; PP758548-PP758565**, ***16S rRNA***; **PP734702-PP734709**).

It was observed that 320 of the total 360 data sets shared the most common haplotype of *COI*-Hap-1 in all stations. Eleven haplotypes were seen in only one sample. Haplotype diversity (Hd) for *COI* varied between 0,071-0,476 and for the *16S rRNA* gene region 0,065-0,157. Nucleotide (Pi) diversity for *COI* varied between 0,0001-0,00078 and for the *16S rRNA* gene region values between 0,00012-0,00031.

The highest haplotype diversity value (Hd=0,476) for the *COI* gene region was observed in the Mersin population. The highest haplotype diversity value (Hd=0,476) for the *16S rRNA* gene region was found in the Adana population. On the other hand, the highest value of nucleotide diversity (Pi) was calculated for the Adana population for both *COI* and *16S rRNA* gene regions (seen in Table 2).

Comparison of *COI* and *16S rRNA* sequences obtained from sampling sites with the GenBank database was performed. Query Cover values were max: 100 and min: 98,31 for *COI*. It varied between 100 and 97,85. Another gene regıon *16S rRNA* values are Query Cover values were max: 100 and min: 89 for *16S rRNA*. It varied between 99,81 and 98,82 per identification.

A sequence of approximately 521 bp of the *16S rRNA* gene region was analyzed from all stations. The sequence data of 303 samples taken from all stations were included in the calculation and a total of 8 haplotypes were detected. It was observed that 292 of the total 303 data sets shared the most common haplotype of *16S rRNA*-Hap-2 and six haplotypes were seen in only one sample. The sampling area genetic diversity parameters for mtDNA marker (*COI* and *16S rRNA*) are given in Table 4. We obtained the phylogenetic tree of the *COI* gene region by adding the sequence records from the GenBank database (Figure 2). We also obtained the phylogenetic tree of the *16S rRNA* gene region by adding the sequence records from the GenBank database (Figure 3).

AMOVA analysis was performed to determine the distribution of genetic diversity in *COI* and *16S rRNA* data. The results of both *COI* and *16S rRNA* AMOVA analysis reveal that within population variation is less than among population variation. The differences in the results of variation analysis for populations for both *COI* and *16S rRNA* are seen in Table 5.

The genetic distances calculated using Mega X between Bluefish populations (*COI* and *16S rRNA* gene regions) are given in Tables 6 and 7. The pairwise FST values ranged from 0,000 to 0,00652 for *COI*. The maximum value was shown for Ereğli population (Table 6). The pairwise FST values ranged from 0-0,00258 for *16S rRNA* (Table 7). The maximum value are for Ereğli and Trabzon populations can be seen Table 7. However, none of these values were statistically significant.

According to the *COI* gene region haplotype analyses, 18 haplotypes were revealed from 360 sample sequences belonging to 14 stations. 8 haplotypes were detected for *16S rRNA* from 303 samples. According to the haplotype network analysis, the most common haplotype of *COI* (Hap-1) is shared by samples taken from most sampling points, and there are at most 2 nucleotide differences between the haplotypes determined for the *COI* gene region (Figure 4a). The most common haplotype of *16S rRNA* (Hap-1) is shared by samples taken from all sampling sites and there are at most 3 nucleotide differences between the haplotypes determined for the *16S rRNA* gene region (Figure 4b).

Table 4. Genetic diversity parameters are COI and 16S rRNA genes regions of Bluefish populations

COI							*16S rRNA*				
n	**h**	**Hd**	**Pi**	**S**	**Haplotype Distribution**	**Collection Site**	**Haplotype Distribution**	**n**	**h**	**Hd**	**Pi**	**S**
21	4	0,414	0,00078	4	1-2-3-4	Adana	1-2-3	25	3	0,157	0,00031	2
23	2	0,166	0,00024	1	1-7	Ereğli	2-4	14	2	0,143	0,00027	1
23	4	0,249	0,00037	3	1-3-5-6	Bodrum	2	17	1	-	-	-
20	2	0,189	0,00027	1	1-7	Çanakkale	2	16	1	-	-	-
27	3	0,145	0,00021	2	1-7-8	Giresun	2-5	31	2	0,065	0,00012	1
30	4	0,193	0,00028	3	1-7-9-10	Hopa	2	21	1	-	-	-
29	3	0,135	0,00020	2	1-7-9	İğneada	2	20	1	-	-	-
30	4	0,251	0,00037	3	1-3-11-12	İzmir	2-6	29	2	0,069	0,00013	1
23	2	0,087	0,00012	1	1-13	Erdek	2-5	17	2	0,118	0,00023	1
22	6	0,476	0,00076	5	1-2-3-6-9-14	Mersin	2	14	1	-	-	-
27	5	0,279	0,00042	3	1-10-15-16-17	R. Feneri	2-5-7	30	3	0,131	0,00026	2
28	2	0,071	0,00010	1	1-7	Samsun	2	22	1	-	-	-
31	3	0,127	0,00018	2	1-7-18	Sinop	2-5	21	2	0,095	0,00018	1
26	2	0,212	0,00030	1	1-7	Trabzon	2-5-8	26	3	0,151	0,00030	2
360	**18**	**0,209**	**0.00032**	**16**		**Total Stations**		**303**	**8**	**0,071**	**0,00014**	**7**

(**n:** Count of samples, **h:** Count of haplotypes, **Hd:** Haplotype diversity, **Pi:** Nucleotide diversity, **S:** Number of Variable Regions)

Discussion

This study present a phylogenetic analysis of the Bluefish populations in the coastal of Türkiye based on mtDNA sequence analysis. According to mtDNA analysis results, Black Sea, Marmara, Aegean and Mediterranean populations showed mixed genetic diversity and exhibited low numbers of haplotypes for *COI* and *16S rRNA*.

According to our results of the calculated Amova variation analysis, it was found that the among population variation was less than the within population variation (Table 2). These are similar to results of the study published by Queiroz-Brito with coauthors in 2022; they performed a sequence analysis of the *COI* gene region of Bluefish samples collected from the coast of Venezuela. They compared this to 154 reference sequences from the NCBI gene bank. According to the results of the AMOVA analysis, it was determined that the source of variation was 77,34% within groups, 4,03% between populations and 18,63% within populations. Another study by Miralles et al. (2014a), according to the AMOVA analysis they performed from the *COI* and *Cyt-b* gene region sequence results of 123 Bluefish samples collected from eight stations, the source of variation was calculated as 70,61% between groups and 29,30% within the population.

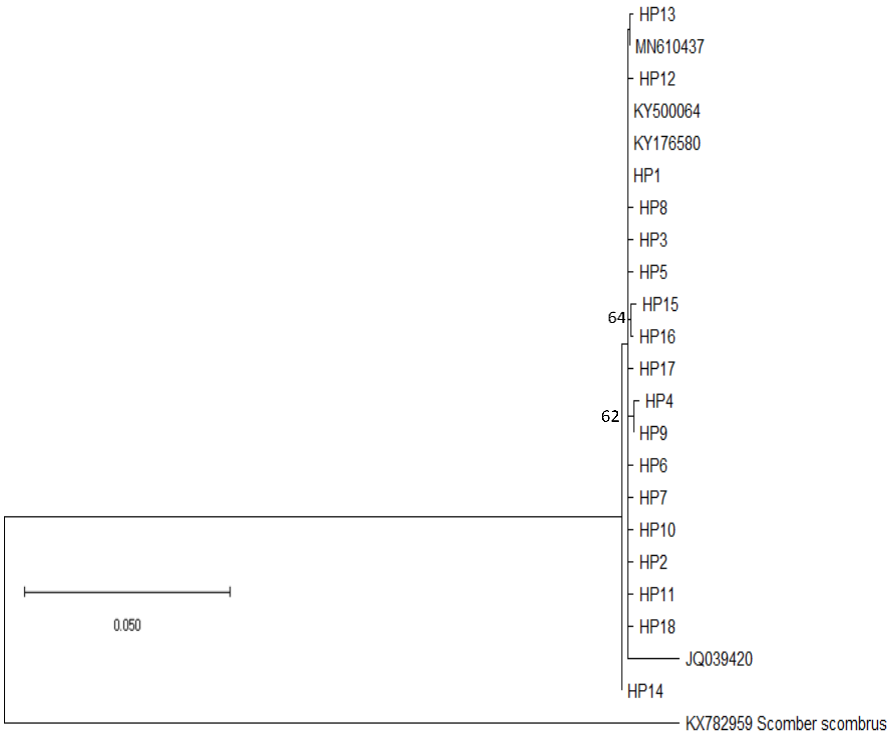

Figure 2. *COI* Maximum Likelihood Phylogenetic Tree. NOTES:

1- Maximum Similarity select and connect values are higher than 50% are shown.

2- **MN610437: *P. Saltatrix*-Blacksea, KY176580: *P. Saltatrix*-İstanbul, KY500064: *P. Saltatrix*-Tunisia, JQ039420: *P. Saltatrix*-Mediterranean Sea-Spaın, KX782959: *Scomber scombrus*-Germany. Bremen**)

Figure 3. *16S rRNA* Maximum Likelihood Phylogenetic Tree. NOTES:

1- Maximum Similarity select and connect values are higher than 50% are shown.

2- **DQ532941: *P. Saltatrix*-USA: New York, EU410419:*P. Saltatrix*-İstanbul, KJ128898: *Scomber scombrus*-Sweden**

Table 5. Global AMOVA results of mtDNA data (*COI* and *16S rRNA*) sets.

COI			**Source of Variation**	***16S rRNA***		
Sum of Squares	Variance Components	% Variation		Sum of Squares	Variance Components	% Variation
1.5240	0.00167	**1.49**	Among Pop.	6.520	0.03989	**9.95**
38.832	0.11095	**98.51**	Within Pop.	13.000	0.36111	**90.05**
40.356	0.11262	100	Total	19.520	0.40101	100

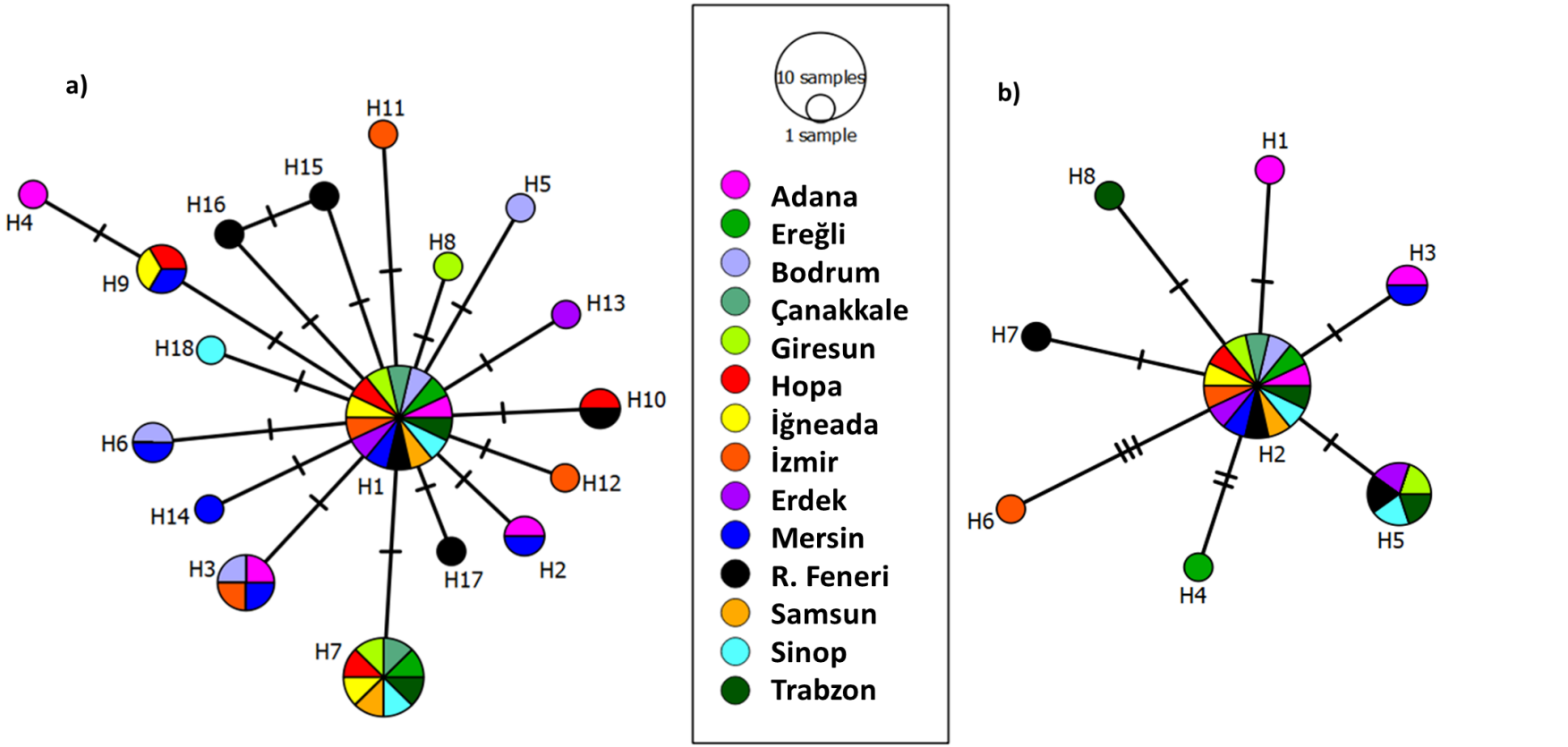

Figure 4. Haplotype networks generated based on the (a) *COI* and (b) *16S rRNA* sequences. The numbers and every circle symbolize unique haplotypes; circle size is proportional to their frequency, and colors show populations; short lines in branches symbolize the diffrent nucleotid number.

Bluefish populations are expected to mix due to the Bluefish's high migratory capacity. According to our findings, the fact that 13 stations in the *COI* gene region and 14 stations in the *16S rRNA* gene region share the same haplotype confirms this expectation. It may be a coincidence that the most common haplotype detected for the *COI* gene region is not found at Ereğli station. There are articles that support this idea: Pardinas with coauthors reported in 2010 same results; when they compared the sequence analyzes of the *Cyt-b* gene region of samples collected from the Western North Atlantic, Eastern North Atlantic and Mediterranean seas. No significant differences were found between the Eastern Atlantic Ocean (Cadiz and Canary Islands) and the Mediterranean samples, although the all populations from different continents shared no haplotypes.

Another paper by Mirales et al., published in 2016; they compared the genetic diversity of adult Bluefish caught at an aquaculture farm in the Spanish sea. They reported that the Bluefish population caught around the farm had a higher genetic diversity (mtDNA *COI* gene was analyzed) than all reference samples collected in the Mediterranean basin. These results also show that two genetic units (Bluefish populations in the Western and Eastern Mediterranean) are mixed. This can be explained by the mating of both populations with each other (Mirales et al., 2016).

In accordance with Sabates et al. (study published in 2012); it was found that the Bluefish distribution area in the Western Mediterranean expanded northward due to the increasing surface water temperature in the Northwestern Mediterranean in the 1980s and 1997s (Rixen et al., 2005). So that it has been reported that the species breeds by mixing in new distribution areas.

Although Mirales et al. (2014b) defined 3 different haplogroups (Northwest Atlantic, Western Mediterranean and Eastern Mediterranean groups) according to the phylogenetic tree obtained from the *COI* gene region in their study, our study found that there was no different genetic unit in the Mediterranean basin.

If we examine the population genetic studies conducted on marine fish in different regions of the world in addition to the studies that supporting the admixture genetic unit we found, there are also studies with genetic sub-haplogroups.

According to the study conducted by Nomura et al. (2014), the genetic population structure of Pacific Bluefin Tuna and Yellowfin Tuna in the North Pacific Ocean was compared by analyzing the mtDNA gene region. Nomura with coauthors reported that there was no genetic differentiation between the geographical populations of the Pacific Bluefin Tuna (a migratory species such as Bluefish) or the Yellowfin Tuna on opposite coasts of the ocean for their species.

Table 6. Pairwise genetic distance between geografic regions based on *COI* sequences

ADANA													
MERSIN	-0,01381												
BODRUM	0,02647	-0,01867											
CANAKKALE	0,04904	0,02308	0,02158										
EREGLI	0,05223	0,02433	0,01818	-0,4797									
GIRESUN	0,04884	0,01830	0,00185	-0,01142	-0,01579								
TRABZON	0,06522	0,03923	0,03828	-0,04500	-0,03813	0,00405							
HOPA	0,03850	0,00980	0,00139	-0,00768	-0,01102	-0,01484	0,00758						
IGNEADA	0,04282	0,01097	0,00293	-0,00778	-0,01291	-0,01811	0,00816	-0,2811					
IZMIR	0,03158	-0,01016	-0,01358	0,02890	0,02542	0,00991	0,04552	0,00985	0,01095				
ERDEK	0,04777	0,01536	-0,00000	0,04002	0,03030	-0,00168	0,05481	-0,00388	-0,00203	0,00741			
R. FENERI	0,04072	0,01663	0,00471	0,02347	0,02066	0,00641	0,04010	-0,00357	0,00759	0,01348	0,00374		
SAMSUN	0,05931	0,02358	0,00477	-0,00854	-0,01682	-0,02436	0,00845	-0,01873	-0,02437	0,01201	0,00078	0,00865	
SINOP	0,05616	0,02326	0,00406	-0,00422	-0,01010	-0,01728	0,01213	-0,01305	-0,01689	0,01025	-0,00217	0,00881	-0,02421

Table 7. Pairwise genetic distance between geografic regions based on *16S rRNA* sequences

ADANA													
MERSIN	-0,01381												
BODRUM	0,02647	-0,01867											
CANAKKALE	0,04904	0,02308	0,02158										
EREGLI	0,05223	0,02433	0,01818	-0,4797									
GIRESUN	0,04884	0,01830	0,00185	-0,01142	-0,01579								
TRABZON	0,06522	0,03923	0,03828	-0,04500	-0,03813	0,00405							
HOPA	0,03850	0,00980	0,00139	-0,00768	-0,01102	-0,01484	0,00758						
IGNEADA	0,04282	0,01097	0,00293	-0,00778	-0,01291	-0,01811	0,00816	-0,2811					
IZMIR	0,03158	-0,01016	-0,01358	0,02890	0,02542	0,00991	0,04552	0,00985	0,01095				
ERDEK	0,04777	0,01536	-0,00000	0,04002	0,03030	-0,00168	0,05481	-0,00388	-0,00203	0,00741			
R. FENERI	0,04072	0,01663	0,00471	0,02347	0,02066	0,00641	0,04010	-0,00357	0,00759	0,01348	0,00374		
SAMSUN	0,05931	0,02358	0,00477	-0,00854	-0,01682	-0,02436	0,00845	-0,01873	-0,02437	0,01201	0,00078	0,00865	
SINOP	0,05616	0,02326	0,00406	-0,00422	-0,01010	-0,01728	0,01213	-0,01305	-0,01689	0,01025	-0,00217	0,00881	-0,02421

MtDNA *D-loop* gene sequencing was used to investigate the genetic structure of 11 bonito populations in the Black Sea, Marmara, Aegean, Mediterranean and Adriatic Sea by Turan et al. (2015). Although low genetic diversity was observed within the population, they reported that the Black Sea and Marmara Sea populations of Türkiye constitute one genetic unit, the Aegean and Mediterranean coast populations constitute a genetically different second unit, and the Adriatic Sea population also differ from genetically these two units.

Finally, in Habib BAL's in Phd (2015), 27 metric and 6 meristic characters were studied in a total of 131 Bluefish found in 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, that there was no subspecies and that there was a migratory species between our seas.

As a result; according to the published genetic studies about Bluefish, it has been found that although there are nucleotide differences, the genetic differences between populations are very low. It has been observed that Aegean and Mediterranean populations are more diverse than other populations, and there are mixed individuals in all populations.

If so, it can be said that the Bluefish is represented by a single species, since there are no genetical subspecies in the 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.

Funding Information

This study was funded by the Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies Agricultural (Funding No: TAGEM/ HAYSÜD /Ü/20/A6/P3/1814).

Author Contribution

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

Acknowledgements

The authors would like to thank Assoc. Prof. Dr. Rafet Çağrı Öztürk for his valuable contributions in writing the article.

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