



Genetics Structure Analysis of Turbot (*Scophthalmus maximus*, Linnaeus, 1758) in the Black and Mediterranean Seas for Application of Innovative Management Strategies

Cemal Turan^{1*}, Petya Pavlova Ivanova², Violin Stoyanov Raykov², Mevlut Gurlek³, Deniz Erguden³, Deniz Yaglioglu⁴, Serpil Karan³, Servet Ahmet Dogdu³, Ali Uyan³, Bayram Ozturk⁵, Venelin Nikolov², Ivan Dobrovolov^{2†}, Antonina Khanaychenko⁶ and Vitaly Giragosov⁶

¹ Marine Science Department, Iskenderun Technical University, Hatay, Turkey, ² Marine Biology and Ecology Department, Institute of Oceanology, Bulgarian Academy of Sciences, Varna, Bulgaria, ³ Fisheries Sciences Department, Iskenderun Technical University, Hatay, Turkey, ⁴ Department of Biology, Duzce University, Düzce, Turkey, ⁵ Faculty of Fisheries, Istanbul University, Istanbul, Turkey, ⁶ Department of Aquaculture and Marine Pharmacology, Institute of Biology of the Southern Seas, Sevastopol, Russia

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*Correspondence:

Cemal Turan
turancemal@yahoo.com

[†] Deceased

Specialty section:

This article was submitted to
Marine Fisheries, Aquaculture and
Living Resources,
a section of the journal
Frontiers in Marine Science

Received: 29 May 2019

Accepted: 13 November 2019

Published: 28 November 2019

Citation:

Turan C, Ivanova PP, Raykov VS, Gurlek M, Erguden D, Yaglioglu D, Karan S, Dogdu SA, Uyan A, Ozturk B, Nikolov V, Dobrovolov I, Khanaychenko A and Giragosov V (2019) Genetics Structure Analysis of Turbot (*Scophthalmus maximus*, Linnaeus, 1758) in the Black and Mediterranean Seas for Application of Innovative Management Strategies. *Front. Mar. Sci.* 6:740. doi: 10.3389/fmars.2019.00740

Keywords: mtDNA, microsatellite, turbot, *Scophthalmus maximus*, population genetics

INTRODUCTION

The turbot, *Scophthalmus maximus*, is a commercially important flatfish and has remarkable attention with respect to fisheries and aquaculture (Iyengar et al., 1999) from Northeast Atlantic to the Arctic Circle and commonly found in the Baltic and some part of north Mediterranean including the Marmara and the Black Sea, but the Aegean Sea (Turan et al., 2007). *S. maximus* has restricted spatial migrations and live at depth of 20 up to 100 m, predate on the small fish, crustaceans and other benthic marine animals (Karapetkova, 1980; Ivanov and Beverton, 1985). The turbot is considered as vulnerable (VU) under the current IUCN Red List criteria (IUCN, 2019). The natural populations of turbot are subject to strong anthropogenic pressure.

The species in the Black Sea is under catch quota following the multiannual management plan for turbot fisheries in the Black Sea. A total allowable catch (TAC) is set at 644 tons for 2018–2019, and each authorized vessel shall not exceed a maximum number of 180 fishing days per year. In addition, the status of the turbot stock must be regularly assessed and the level of current fishing mortality established. Hence, the implementation of a TAC, assuming a 100% curb of IUU fishing, would allow the stock to quickly recover to large biomass values with a relatively low probability of SSB falling below B_{LIM} (11.6% in 2030). These results provide grounds for the continuation of fishing activities at levels that are acceptable for both the population and the fishery, provided the adequate management measures are adopted (FAO, 2018). The total turbot catches in the Black Sea accounted for 661, 1,444 tons IUU included (GFCM, 2018), the prevailing landings belong to Russia and Turkey for 2016. Despite its economic importance and wide range of distribution, limited literature is available on the population structure of *S. maximus* using molecular markers (Atanassov et al., 2011; Nikolov et al., 2015).

Over-exploitation of this resource is a factor for the complete disappearance of local fish populations worldwide (Dulvy et al., 2003). Therefore, globally increased level of exploitation of fisheries resources requires urgent measures to establish the impact on the genetic diversity and population genetic structure of commercial populations in order to improve the strategy for long-term management, which would ensure the conservation of fisheries resources (Karahana et al., 2014).

The genetic tools deliver significant advances for fisheries management in the short term that the determination of fishery stock structure is important for defining stock boundaries to underpin sustainable fishery management that is one of the priority areas relevant for fisheries management under the E.U. Common Fisheries (Ovenden et al., 2015). The suggestions mentioned above are based on the immediate needs emerging from the CFP regulation (EC, 2013) and genetic approaches that appear to be feasible, practicable and cost-efficient (Casey et al., 2016).

Microsatellite markers have been determined in a large variety of fish species and are commonly used in population genetic studies (Iyengar et al., 2000). Recently, microsatellite markers have progressively been used to get knowledge on population structure and interaction between populations of a given species (Chen et al., 2017). Due to its maternal inheritance mode and relative lack of recombination, mitochondrial DNA symbolizes a useful marker system for use in population genetic studies (Hurst et al., 1999).

Numerous studies of the genetic structure of turbot have been conducted on North-eastern Atlantic and western Mediterranean countries (Pardo et al., 2005), however, there has been limited study conducted on genetic structure of that species in the Black Sea. Therefore, there is a need for elucidation of the population genetic structure of turbot to find appropriate management strategies for Black Sea countries.

Therefore, the aim of this study is to elucidate population genetic structure of turbot (*S. maximus*) from the Black and Marmara Seas and to implement it into management strategies as a key element for rational exploitation and conservation of fish populations.

MATERIALS AND METHODS

Sample Collection

S. maximus samples (fin clips) from 50 individuals in total (10 from each site) were collected at fishing ports (commercial landings) from Turkish marine waters [Trabzon (TRB), Duzce (DUZ), and Marmara Sea (MAR)], from Bulgaria-Varna coastal waters (VAR) and from Russia Sevastopol coastal waters (SVT) between 2013 and 2015 (Figure 1). The samples were taken to the laboratory and frozen at -30°C until DNA extraction.

DNA Extraction

The total DNA was isolated by using a phenol-chloroform method (Sambrook et al., 1989). After DNA extractions, DNA product was visualized in 0.7% agarose gels and quantitation of the DNA was completed using a spectrophotometer.

Microsatellites

Gel Analyzer 2010a (1-D Gel Analysis) software was applied to score alleles visualized by electrophoresis. Five microsatellite primers originally developed for *S. maximus* (Table 1) were applied after the optimization and determination of polymorphisms. A Polymerase Chain Reaction (PCR) was carried out using reaction volume of 15 μl including 1 U Taq polymerase (Thermo scientific), 2 μM of each primer, 200 mM

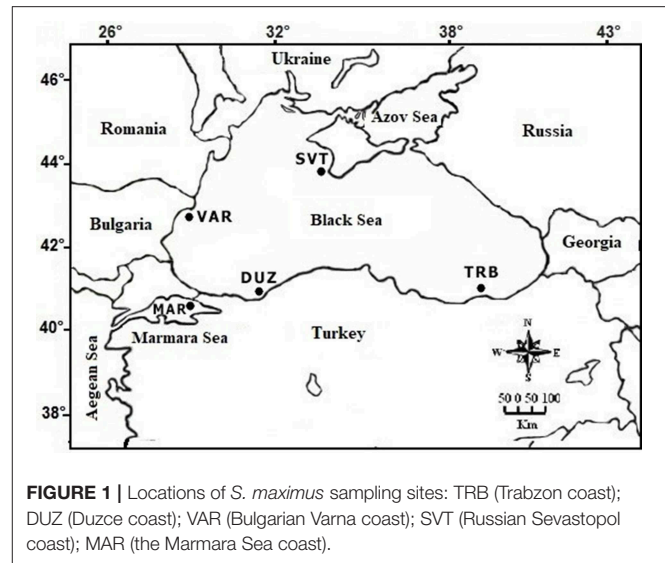


FIGURE 1 | Locations of *S. maximus* sampling sites: TRB (Trabzon coast); DUZ (Duzce coast); VAR (Bulgarian Varna coast); SVT (Russian Sevastopol coast); MAR (the Marmara Sea coast).

dNTPs, 25 mM MgCl_2 , 10 mM Tris-HCl pH 8.8, 50 mM KCl, and 1 μl template DNA ($\approx 10\text{--}25$ ng). It was performed with two stages: pre-denaturation at 95°C for 1 min followed by five denaturation cycles at 94°C for 20 s, annealing at 55 s, extension at 72°C for 2 s that was followed by the second stage of 25 denaturation cycles in 20 s at 94°C and final extension in 20 s at 72°C .

Statistical Analyses

Deviations of Hardy-Weinberg genotypic distributions (HWE), observed (HO), and expected (HE) heterozygosities were analyzed with Arlequin v3.5.1.3 (Excoffier and Lischer, 2010) to test for excess heterozygosity and deficiency of microsatellite data. A stepwise mutational model based on allelic identity (F_{ST}) was used to elucidate genetic differentiation between populations while the estimation of allelic size (R_{ST}) is less reliable than F_{ST} when <20 microsatellite loci are used (Gaggiotti et al., 1999). The Sequential Bonferroni technique was applied for detected significance levels with multiple tests (Rice, 1989). The Garza-Williamson Index (GWI), which measures the ratio of the observed number of alleles relative to the total number of possible allelic states at the locus over the allelic range, was used to detect bottlenecks (Garza and Williamson, 2001). PCAGEN, SPSS, and SYSTAT software were used for Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) of microsatellite data. A neighbor-joining dendrogram (Saitou and Nei, 1987) was constructed to reveal genetic relationships among the populations (Cavalli-Sforza and Edwards, 1967) with PHYLIP 3.57 (Felsenstein, 1989). Pairwise genetic distance matrices (F_{ST}) and geographic distance (km) were tested whether gene flow was geographically restricted and followed the isolation-by-distance model using the Mantel test (Mantel, 1967).

mtDNA Sequence Analysis

The complete mtDNA COIII gene region was amplified via PCR reactions, which was carried out according to the protocol given

TABLE 1 | Set of primers and amplification conditions for the 5th microsatellite loci and universal COIII primers of mtDNA.

Name	Primer	Core sequence	Annealing t°C	References
COIII-F	5'-AGC CCA TGA CCT TTA ACA GG-3'		49°C	Valles-Jiménez, 2005
COIII-R	5'-GAC TAC ATC AAC AAA ATG TCA GTA TCA-3'			
Smax-02F	GGAGGATGTATTGAAAGTGT	(TG) ₁₆	56°C	Bouza et al., 2002
Smax-02R	AGAGCAGGTCATTATACAGC			
Sma1-125INRA F	CACACCTGACAAAGCTCAAC			Estoup et al., 1998
Sma1-125INRA R	GCTGAACATTTTCATGTTGATAG	(TAGA) ₁₁ -(TG) ₄	58°C	
Sma3-12INRA F	CACAATGAATCAGGATG	(TG) ₂₁	58°C	Estoup et al., 1998
Sma3-12INRA R	GCCACCACTGCGTAACAC			
B12-I GT14 F	GTGATGGAAGATTGTACCAG	(GT) ₁₄	56°C	lyengar et al., 2000
B12-I GT14 R	CACAATAAAGGATAGACCAG			
3/9CA15 F	AGAGTGAAGAACGTACCTGC	(CA) ₁₅ 60°C		lyengar et al., 2000
3/9CA15R	CAATGGAGAGGCAGTATCGG			

by Turan et al. (2019). The mitochondrial cytochrome c oxidase subunit III (COIII) gene was amplified using the universal primers (Table 1). The mtDNA sequence analysis was performed according to Sanger et al. (1977) on ABI 3130 XL genetic analyzer.

Statistical Analyses

After sequence alignment, the best model for sequence divergences were calculated using Mega v5, and the molecular phylogenetic tree was also constructed using MEGA v5 (Tamura et al., 2011). Neighbor joining (NJ) tree (Saitou and Nei, 1987) was applied to visualize relationship among the populations. The statistical robustness in the nodes of the resulting tree was determined by 1,000 bootstrap replicates. Tajima's D test (Tajima, 1989) was carried out to tests the conformity of DNA sequence evolution to neutrality. The sequences have been deposited in the GenBank with accession numbers, MN062299—MN062348.

RESULTS

Microsatellite

In the microsatellite analysis, a total of 108 alleles were detected from five microsatellite loci and the allele numbers for each microsatellite locus within the *S. maximus* populations ranged from 3 to 14. The expected and observed heterozygosity in *S. maximus* populations varied between 0.32 and 0.88 and 0.07 and 1.00, respectively. The genotypic structuring at overall loci revealed statistically significant deviation from HWE ($P < 0.001$). The highest expected heterozygosity was 0.88 at 3/9CA15 locus while the lowest expected heterozygosity was 0.32 at Sma02 locus. Alleles numbers within samples at each microsatellite locus ranged from 3 at locus B12-I GT14 and 3/9CA15 in TRB, DUZ, and MAR sample to 14 at locus Sma02 and 3/9CA15 in VAR and DUZ samples (Table 2).

Average alleles number overall loci ranged from 6.0 at the TRB and MAR populations to 9.60 at the SVT population with an average allele number of 7.32. The Garza-Williamson index was found as the lowest at 3/9CA15 locus in the SVT population, and the highest at B12-I GT14 and 3/9CA15 loci in the TRB and VAR populations, respectively (Table 2). The average GWI across

loci was highest at the TRB population and lowest at the SVT population, indicating a possible bottleneck effect.

Genetic diversity values based on microsatellites within populations was the lowest in the TRB population (3.00735). However, the highest genetic diversity was observed in the SVT population (4.17306). Genetic diversity for mtDNA was zero in TRB and DUZ populations (0.0).

The mean pairwise differentiation (F_{ST}) within population was found to be the lowest in the Trabzon population and the highest in the Sevastopol population (Table 3). In pairwise comparison of populations, the F_{ST} values ranged from 0.08792 to 0.36059 with an average value 0.249246 (Table 3). The Varna and Sevastopol samples showed the lowest genetic distance (0.08792), whereas the Trabzon and Marmara samples showed the highest, 0.36059. The pairwise F_{ST} values showed that all samples were significantly distinct from each other ($P < 0.001$). The Mantel test showed non-significant ($P > 0.05$, $r = 0.43$) isolation-by-distance for the geographically separated turbot populations.

Principal component analysis of microsatellite data revealed five principal components (PCs) of which 31 and 24% of genetic variation were presented in the first and second PCs. Plotting the first two PCs (Figure 2) revealed that B12-I GT14 showed highest contribution on population differentiation on the first PC, and Sma02 and Sma03 highly contributed on population differentiation on the second PC. The use of five PCs in DFA revealed overlapping distribution of 95% confidence ellipses of populations (Figure 3). The Russia and Marmara populations were overlapped on all the populations, but the Trabzon and Duzce populations clearly separated from each other.

On the other hand, the Neighbor-Joining tree demonstrated that population structuring is associated with geographic separations. The Bulgaria and Russia populations were clustered close to each other (Figure 4) while the Marmara Sea population showed the most distinguishing population.

mtDNA Sequencing

After alignment, the COIII region consisted of 565 bp fragments which were contained 2 bp parsimony informative sites. The

TABLE 2 | Descriptive statistics for five microsatellite loci between the turbot populations.

Samples		Locus					Average across loci
		SMA1	SMA02	SMA3	B12-I GT14	3/9CA15	
TRB	n	10	10	10	10	10	
	a	6	4	7	3	10	6.0
	Ho	0.12***	0.66**	0.58***	1.00***	0.09	0.49
	He	0.59***	0.32***	0.82***	0.39***	0.86***	0.60
	G.W. index	0.12	0.66	0.58	1.00	0.09	0.49
	Allelic size range	49	5	11	2	110	35.40
DUZ	n	10	10	10	10	10	
	a	8	5	4	3	14	6.80
	Ho	0.15***	0.41***	0.80***	0.50***	0.10***	0.39
	He	0.80***	0.54***	0.60***	0.33***	0.88***	0.63
	G.W. index	0.15	0.41	0.80	0.50	0.10	0.39
	Allelic size range	50	11	4	5	130	40.0
VAR	n	10	10	10	10	10	
	a	9	14	9	5	4	8.20
	Ho	0.18***	0.12***	0.60***	0.12***	1.00***	0.40
	He	0.76***	0.78***	0.75***	0.60***	0.74***	0.73
	G.W. index	0.18	0.12	0.60	0.12	1.00	0.40
	Allelic size range	49	114	14	39	3	43.80
SVT	n	10	10	10	10	10	
	a	11	11	11	9	6	9.60
	Ho	0.22***	0.11***	0.64***	0.09***	0.07***	0.23
	He	0.87***	0.84***	0.85***	0.80***	0.78***	0.83
	G.W. index	0.22	0.11	0.64	0.09	0.07	0.23
	Allelic size range	49	114	14	39	3	43.80
MAR	n	10	10	10	10	10	
	a	8	7	5	7	3	6.0
	Ho	0.16***	0.08***	0.41***	0.43***	0.42***	0.30
	He	0.84***	0.77***	0.76***	0.61***	0.46***	0.69
	G.W. index	0.16	0.08	0.41	0.43	0.42	0.30
	Allelic size range	47	86	11	15	6	33.0

***Significant probability values ($P < 0.001$) after Bonferroni correction. n, number of samples; a, number of alleles; Ho, observed heterozygosity and He, expected heterozygosity. TRB, Trabzon; DUZ, Duzce; MAR, Marmara Sea; VAR, Bulgaria-Varna coastal waters; SVT, Russia Sevastopol coastal waters.

TABLE 3 | Values of microsatellite pairwise F_{ST} (below diagonal) and average number of pairwise differences within population (diagonal elements).

Samples	TRB	DUZ	MAR	VAR	SVT
TRB	3.00735				
DUZ	0.33107***	3.18367			
MAR	0.36059***	0.30969***	3.47592		
VAR	0.28864***	0.25297***	0.24455***	3.65551	
SVT	0.22431***	0.19686***	0.19586***	0.08792***	4.17306

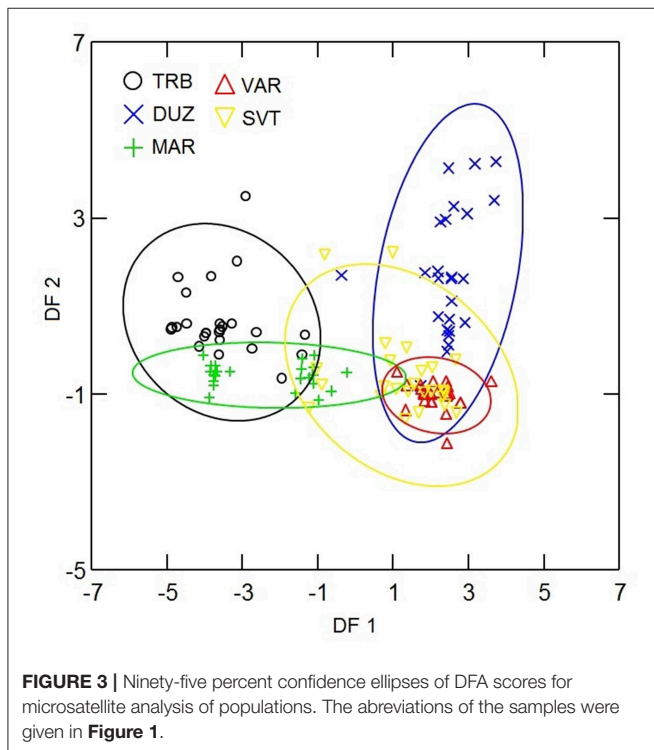
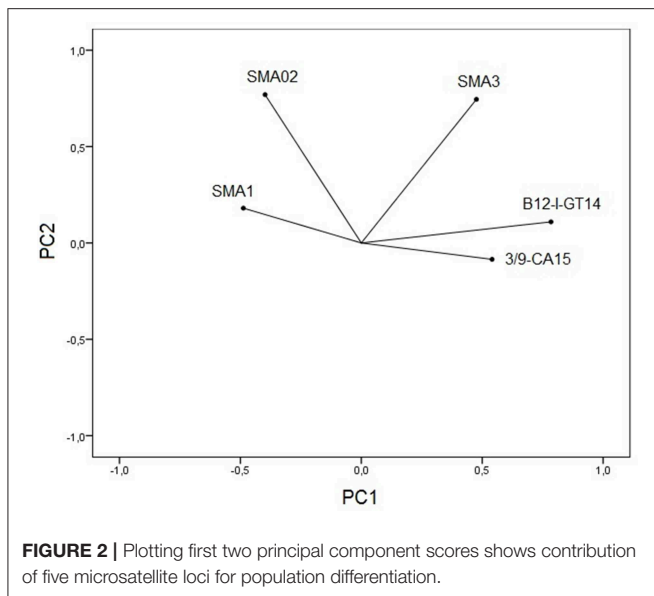
*** $P < 0.0001$. TRB, Trabzon; DUZ, Duzce; MAR, Marmara Sea; VAR, Bulgaria-Varna coastal waters; SVT, Russia Sevastopol coastal waters.

mean composition of nucleotides for thymine (T), cytosine (C), adenine (A), and guanine (G) were as 28.7, 28.6, 23.0, and 20.5%, respectively. Jukes and Cantor model (Jukes and Cantor, 1969) was chosen as the best method for intra and interspecific variations on our dataset after running ModelTest (Posada and

Crandall, 1998). DNA sequencing of the COIII exhibited four different haplotypes. The result of mean haplotype diversity between populations was 0.380 (Table 4). The average intra-population genetic diversity was found to be 0.000204. The detected genetic divergence between populations are given in Table 5. Average genetic divergence was found to be 0.00081. Clustered to the NJ tree, Trabzon and Duzce populations clustered together as one group with sister clustering the Sevastopol and Varna, respectively. The Marmara population was different from all other populations (Figure 5). Tajima's D for the populations of *S. maximus* was found to be 0.045756 (Table 6) which indicate that the expected heterozygosity is lower than that observed.

DISCUSSION

In this study, genetic analysis of *S. maximus* populations in the Black and Marmara Seas was investigated by using



microsatellite and mtDNA sequencing analysis. On the bases of the two molecular markers, *S. maximus* can be divided into genetically separated populations. For microsatellite analysis, all the geographically separated populations revealed genetic differences from each other. For mtDNA sequencing analysis, only the Marmara Sea (MS) population constitute a genetic unit, while the others comprise genetically different second unit. There is a contradiction between the markers, but the genetic

differentiation of the Marmara Sea population was supported by the microsatellite and mtDNA sequencing analyses.

Microsatellite and mtDNA markers indicated that there are restricted gene flows between populations which indicate ongoing processes of genetic differentiation. The Mantel test indicated that the genetic heterogeneity was not related to geographic proximity of the samples for both molecular markers.

Marine species are generally genetically more adaptable than anadromous and freshwater species (DeWoody and Avise, 2000), and therefore, have less genetic differentiation between populations (Ward, 2002). This is thought to mirror their higher effective population size and less restricted migration (DeWoody and Avise, 2000). In the present microsatellite analysis, a total of 108 alleles ranging from 3 to 14 for each locus were detected within the *S. maximus* populations which were similar to the previous studies (Pardo et al., 2005; Florin and Höglund, 2007; Navajas-Pérez et al., 2012). Karan (2015) examined the number of alleles per locus for *S. maeoticus* populations and found to be the lowest in the 3/9CA15 locus as four and the highest in *Sma1* locus as 11. Rodríguez-Ramilo et al. (2007) observed that allele number of *Sma1* locus was eight in *S. maximus* populations. Moreover, pairwise F_{ST} analysis demonstrated that the lowest genetic distance between Sevastopol (SVT) and Varna (VAR) populations for microsatellite (0.08792), whereas mtDNA pairwise genetic differences analysis indicated that the highest genetic distance (0.001522) between the Bulgarian and Marmara populations. Karan (2015) analyzed *S. maeoticus* populations with microsatellite marker and stated that the lowest genetic distance was between the Duzce and Marmara Sea populations (0.19549), whereas the highest was between the Trabzon and Marmara Sea populations (0.21755). Nielsen et al. (2004) reported that the largest pairwise F_{ST} value of *S. maximus* populations between any of the pooled samples was 0.032 (between North Sea and Northern Baltic Sea) even though there was no significant differentiation between the Northern Baltic and the Southern Baltic samples. Florin and Höglund (2007) reported the highest pairwise F_{ST} of *P. maxima* populations in the Baltic Sea as 0.0156 between the Åland and Gotland populations.

The detected expected and observed heterozygosity in each microsatellite locus of *S. maximus* populations are also reported by similar studies, having more sample size (from 22 to 48) than that in our study (Liu and Cordes, 2004; Pardo et al., 2005; Florin and Höglund, 2007; Rodríguez-Ramilo et al., 2007; Navajas-Pérez et al., 2012). Karan (2015) reported that expected and observed heterozygosity of *S. maeoticus* populations varied from 0.64 to 0.88 and from 0.08 to 0.72, respectively.

In the mtDNA sequencing analysis of COIII region, the detected haplotype diversity of *S. maximus* in our study showed four different haplotypes and was found low. Suzuki et al. (2004) found 28 haplotypes for the phylogeographic analysis of *Psetta maxima*. Atanassov et al. (2011) detected 36 haplotypes with average haplotypes diversity of 0.47 among *Psetta maxima* populations from Bulgarian and Romanian in the Black Sea. Karan (2015) identified 10 haplotypes with average haplotype diversity of 0.6345 between *S. maeoticus* populations from the Trabzon, Duzce and Marmara.

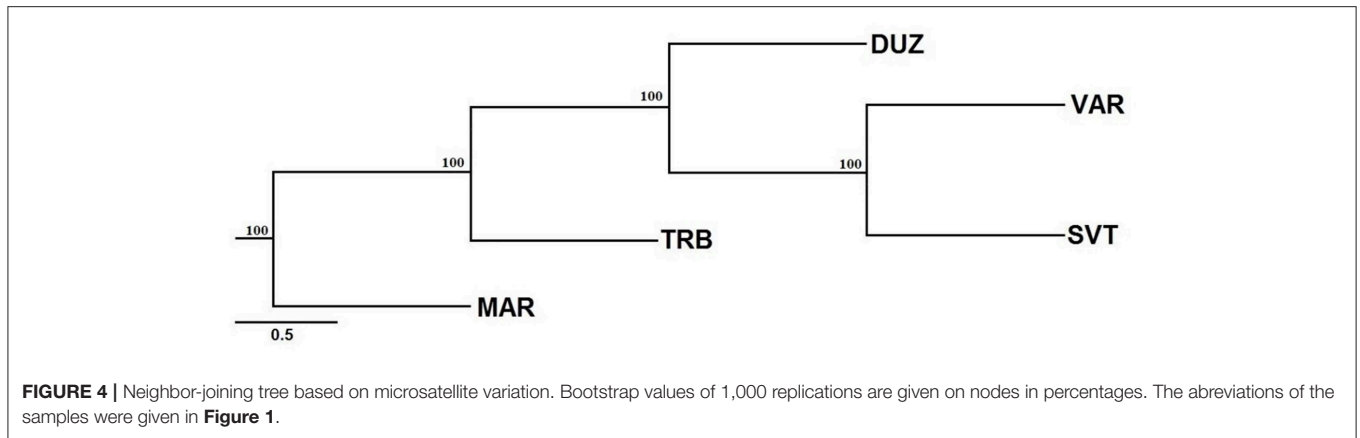


TABLE 4 | Distribution and frequency of COIII haplotypes of *S. maximus* populations.

Haplotype	TRB	DUZ	MAR	VAR	SVT	Total
Hap 1	10	–	4	7	8	29
Hap 2	–	10	6	–	–	16
Hap 3	–	–	–	2	–	2
Hap 4	–	–	–	1	2	3
Total	10	10	10	10	10	50

TRB, Trabzon; DUZ, Duzce; MAR, Marmara Sea; VAR, Bulgaria-Varna coastal waters; SVT, Russia Sevastopol coastal waters.

TABLE 5 | Pairwise genetic distance based on sequence analysis of the COIII region between populations of *S. maximus* (below diagonal), and genetic diversity within samples (transversal diagonal as given in bold).

Samples	TRB	DUZ	MAR	VAR	SVT
TRB	0				
DUZ	0	0			
MAR	0.001061***	0.001061***	0.000943		
VAR	0.000885	0.000885	0.001522*	0.001456	
SVT	0.000354	0.000354	0.001416**	0.001027	0.000629

Statistically significance levels; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. TRB, Trabzon; DUZ, Duzce; MAR, Marmara Sea; VAR, Bulgaria-Varna coastal waters; SVT, Russia Sevastopol coastal waters.

The lowest genetic distance (0.0) was detected between the Duzce and Trabzon populations, and the highest value (0.001522) was found between Bulgarian and Marmara populations based on mtDNA analyses. In the pairwise comparison of the genetic differences of the samples, only the Marmara sample differed significantly from all other samples with different levels of significance (**Table 5**) which is also supported by NJ tree. However, the pairwise F_{ST} and DFA of the microsatellite data indicated genetic separation of Trabzon and Duzce populations (**Figure 3**), and *B12-I GT14* locus plays an important role in this differentiation (**Figure 2**). Moreover, small sized 95% confidence ellipses of the Bulgarian population also indicate

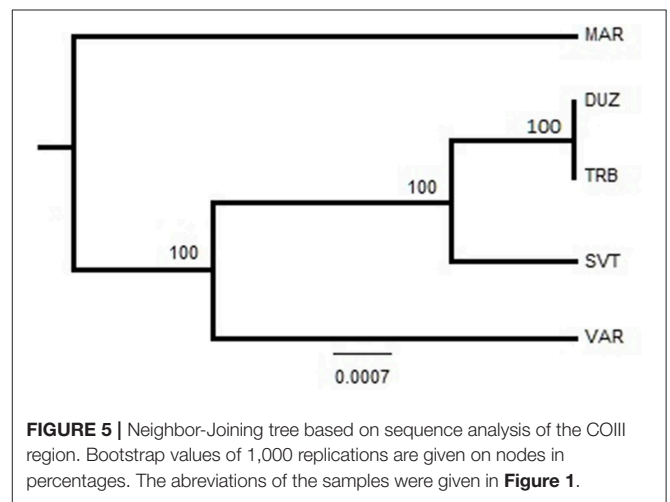


TABLE 6 | Neutrality tests and the estimated parameters of mismatch distribution for populations of *S. maximus* from Turkish waters.

m	S	p_s	Θ	π	D
50	2	0.003534	0.000789	0.000809	0.045756

m, number of sequences; *S*, number of segregating sites; p_s , polymorphic site rate; Θ , population mutation rate; π , average pairwise distance; *D*, Tajima's *D*.

its' very low genetic diversity (**Figure 3**) that be important for management purposes. Tajima's *D* (Tajima, 1989) for the *S. maximus* populations was found to 0.045756, indicating that the observed heterozygosity is higher than that expected (**Table 6**). The detected rare alleles at low frequencies may indicate that the populations were undergone a balancing selection and sudden population contractions. Karan (2015) found Tajima's *D* for *S. maeoticus* populations as -2.114293 which was related to a recent bottleneck effect while the rare alleles were found at high occurrences. The positive and low *D* value in the present study may indicate low levels of both low and high frequency polymorphisms, indicating a decrease in population size and/or balancing selection for *S. maximus*.

The overfishing drives the decay of genetic diversity across a wide range of marine fishes (Pinsky and Palumbi, 2014). Reduced population size enhances genetic drift, which in turn causes a higher loss of genetic variability per generation. Increased genetic drift augments the loss of variability and the ability of adaptation (Hauser et al., 2002; Spielman et al., 2004, according to Madduppa et al., 2018). Reductions of genetic diversity in some of the world's most abundant species may lead to a long-term impact of fishing on their evolutionary potential, particularly if abundance remains low and diversity continues to decay (Pinsky and Palumbi, 2014). Therefore, the low genetic diversity observed for both markers may be explained with excessive fishing pressure on Trabzon population and high geographic separation of this population from the other populations which may cause limited interspecific gene flow. Moreover, this case has pointed out possibility of which these populations could have gone through a bottleneck in the recent past.

In the present study, microsatellite and mtDNA results were not congruent to indicate same pattern of differences between the populations which can be explained that mtDNA and microsatellite markers may show different temporal genetic patterns due to differences in their rate and pattern in mutation (Suzuki et al., 2004), and based on the allele frequency distributions, the differences of microsatellite and mtDNA results may be caused by the using of single marker of mtDNA vs. five markers of microsatellite (Larssoni et al., 2009). The results of the present study support restricted gene flow between populations. On the other hand, based on both microsatellite and mtDNA analysis, two major genetic breaks were observed for *S. maximus*; the TRB, DUZ, the VAR, and SVT populations constitute one genetic unit and the MAR population is the second genetically different unit. Different rates of gene flow, mutation or natural selection of mtDNA and microsatellites also could give rise to greater differentiation of microsatellites than mtDNA. Different parts of mtDNA evolve at different rates, thus different parts of mitochondrial DNA could be considered for future studies to find higher-level population differentiation (Avice, 1992).

The current status of the turbot populations in the Black Sea characterize the stocks as unsustainably exploited and at risk of collapse and “overexploited” and “in overexploitation” (GFCM, 2018). The conservation and sustainability of the turbot populations requires knowledge for the population genetic structure and constant monitoring of its biodiversity. Turbot stock identification and stock boundaries are still not well defined and for the time being the turbot population in the Black Sea is assessed as a single stock (GFCM, 2018). According to the GFCM (2018), an adequate scientific monitoring of the status of turbot and associated species in GSA 29 should be provided. Because, data collection is limited in scope and the national systems in the Black Sea region

lack of harmonization, existing gaps (GFCM, 2018) including those covering turbot population-genetic monitoring should be appraised.

The results from the present study show consistency of the detected differentiation that may reflect its temporal and spatial integrity and thus would also require its consideration as separate populations for the fishery management purposes. Based on that, we propose to include the genetic population data analyses as a new indicator of biological monitoring.

Genetic approaches to detect stock structure will continue to be an indispensable part of fisheries management. Some developments in this field will increase the analytical power by boosting sample sizes and numbers of DNA markers. In the future, there is likely to be a shift in emphasis toward greater integration of genetics into multidisciplinary assessments of stock structure (Ovenden et al., 2015). The approaches described in the present paper can be of value to the effective integration of genetic information into the fisheries management decision-making process.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in GenBank with accession numbers MN062299 - MN062348.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study, because, the turbot (*Scophthalmus maximus*) is considered as Vulnerable (VU) under the current IUCN Red List criteria. The species in the Black Sea is under catch quota following the multiannual management plan for turbot fisheries in the Black Sea, approved in 2017 by the General Fisheries Commission for the Mediterranean (GFCM), (COM/2018/710 final). The study was based on landings sampling of species. The tissues needed for genetical analyses were obtained non lethally (e.g., fin clipping) and with minimal handling. No other authorization or ethics board approval was required to conduct the study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This research was supported by the Scientific & Technological Research Council of Turkey (TUBITAK-112O920), Bulgarian Academy of Sciences (BAS).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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