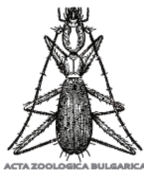

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Evaluation of Molecular and Phenotypic Markers for Phylogeographic Analysis of the Black-Sea Turbot *Scophthalmus maeoticus* (Pallas, 1814) (Actinopterygii: Scophthalmidae)

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Abstract: Molecular genetics and phenotypic markers were evaluated for detecting phylogeographic difference in the populations of the Black-Sea turbot *Scophthalmus maeoticus* occurring in the Black Sea and the Marmara Sea. Microsatellites of nuclear DNA and the COIII region of mitochondrial DNA (mtDNA) were used for the genetic analyses. Morphometrics, meristics, otolith shape and otolith chemistry were applied for the phenotypic analyses. The mean number of alleles was 14.4, using five polymorphic microsatellite loci; the lowest genetic distance (0.19549) was observed between the Marmara (MS) and the West Black Sea (BS2**)** populations; the highest genetic distance (0.21755) was observed between the Marmara Sea population and East Black Sea (BS1) population. MtDNA analysis revealed an overall genetic diversity of 0.00148. The smallest (0.001416) genetic distance was observed between BS2 and BS1 and the highest (0.001593) was the genetic distance between BS1 and MS populations. Discriminant function analysis of morphometric and meristic characters discriminated only BS1 population from the other populations, which overlapped with one another. The otolith shape analysis also supported the morphometric and meristic analyses that the BS1 population was differentiated from the other two populations.

Key words: Black Sea turbot, *Scophthalmus maeoticus*, population genetics, molecular markers, phenotypic markers.

Introduction

Molecular genetics approaches have been progressively used in the fisheries management and ecology over the last six decades (Waples et al. 2008, Penaluna et al. 2016). This resulted into significant insights in the population genetics of exploited marine species, with the latest molecular approaches changing our perception of marine environment and resources (Hauser & Carvalho 2008, Pereira et al. 2013). The genetic structure of natural populations is potentially influenced by a mix of evolutionary forces such as genetic drift, migration, mutations and selection. Molecular genetics techniques are applied to describe stock structure of marine species. Microsatellite markers are increasingly involved in identifying genetic differences between stocks (Liu & Cordes 2004, Takeshima et al. 2017). The mitochondrial DNA (mtDNA) is a preferred marker for population studies because of its compact size, fast evolutionary rate and exclusive maternal mode of inheritance (Brown et al. 1979, Harrison 1989). Studies on morphological variations among fish populations continue to have a significant role in stock identification and stable differences in shape between populations may demonstrate different growth, mortality or reproductive rates between populations (Swain & Foote 1999, Cadrin et al.

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2013). However, marine fish species usually demonstrate phenotypic plasticity that is the degree to which the expression of a genotype varies in response to environmental change (Thompson 1991). Therefore, morphological variation between fish populations may not have purely a genetic basis and is influenced by a mixture of environmental factors that include but are not limited to temperature, salinity, radiation, dissolved oxygen, water depth and current flow (Lindsey 1988, Turan 2000). Morphometrics and meristics are frequently used to describe populations of exploited marine fish species (Murta 2000, O'Reilly & Horn 2004, Turan 2004). One of the most rapidly developing markers for otolith techniques is the otolith chemistry analysis, commonly used for fish population discrimination (Turan 2006, Veinott et al. 2012). The chemistry of the otolith is influenced by various environmental factors but also shows consistent and temporally stable differences between populations and suggests genetic control. Otolith chemistry and shape are commonly used as markers in fisheries biology to measure discreteness and relationships among fish populations and to identify different stocks (e.g. Turan 2000, 2006, Bergenius et al. 2005).

The Black-Sea turbot *Scophthalmus maeoticus* (Pallas, 1814) is an economically important flatfish of the family Scophthalmidae, which is a subject of considerable interest with respect to fisheries and aquaculture (Karan et al. 2016). It is one of the three species of the genus *Scophthalmus* Rafinesque, 1810 occurring in European waters. The geographical range of this species is restricted to the Black-Sea basin. It is frequently considered as a subspecies or as a synonym of *Scophthalmus maximus* (L., 1758), which is widespread in the Mediterranean Sea and the Eastern North Atlantic (Froese & Pauly 2019). To date, no microsatellite and mtDNA markers have been applied for the Black-Sea turbot. However, there population genetic studies have been carried out on *S. maximus*. Florin & Höglund (2007) detected significant genetic differentiation among *S. maximus* in the Baltic Sea by using eight polymorphic microsatellite markers. Nielsen et al. (2004) studied populations of *S. maximus* in the Northeast Atlantic with eight highly polymorphic microsatellite loci and found significant genetic differentiation among samples. Iyengar et al. (2000) developed highly polymorphic microsatellite markers for turbot *S. maximus* with potential to use them for several other flatfish species and reported characterisation of an entire suite of novel polymorphic microsatellite markers. Suzuki et al. (2004) explored the phylogeographic relationship of populations of *S. maximus* within the Mediterranean basin using mtDNA haplotype variation and revealed two distinct genetic lineages, western Mediterranean and "eastern secluded Mediterranean", with the latter lineage exhibiting many haplotypes from the Sea of Azov being endemic and more than half of them derived from a single ancestral haplotype shared among all the eastern Mediterranean areas.

The aim of the present study is to explore the genetic structure of populations of *S. maeoticus* from the Marmara Sea and the Black Sea coasts of Turkey by applying both molecular genetic (microsatellite and mtDNA sequencing) and morphological (morphometric, meristics, otolith chemistry and otolith shape) markers.

Materials and Methods

Sampling

We collected 25 samples from three populations originating from the Eastern Black Sea (BS1), Western Black Sea (BS2) and the Sea of Marmara (MS) (Table 1). Each sample was placed into a plastic bag and kept frozen at -20°C until transportation. Diagnostic description of species and systematic discrimination followed Akşiray (1987), Fischer et al. (1987) and Turan et al. (2007). In the laboratory, tissue samples for genetic analysis were placed in 98% ethanol and stored at +4°C. Sagittal otoliths were carefully removed, cleaned and stored in dry envelopes. Total DNA was extracted from the muscle using the standard phenol – chloroform – isoamyl alcohol procedure (Sambrook et al. 1989). After DNA extractions, DNA product was controlled in 0.7% agarose gels.

Microsatellite analysis

Microsatellite primers were originally developed for *S. maximus* in previous studies (Iyengar et al. 2000, Estoup et al. 1998, Bouza et al. 2002) and subsequently optimised for *S. maeoticus* (Karan et al. 2016). Primers B12-I GT14 (Iyengar et al. 2000), 3/9CA15 (Iyengar et al., 2000), SMA1-125INR (Estoup et al. 1998), SMA-02 (Bouza et al. 2002) and SMA3-12INR (Estoup et al. 1998) were used for routine analyses. Each microsatellite locus was optimised at different reaction conditions. Annealing temperatures for each locus were 56-54°C, 58-60°C, 58°C, 54-56°C and 54-58°C, respectively. Polymerase chain reaction (PCR) was carried out using a reaction volume of 15 µl containing 1 U Taq polymerase (Thermo Scientific), 2 µM of each primer, 200 mM dNTPs, 25 mM $MgCl_2$, 10 mM Tris-HCI pH 8.8, 50 mM KCI and

Table 1. Sampling details of Scophthalmus maeoticus used in this study

Sampling area	Abbreviation	Sample size	Sex (F/M)	Sampling area coordinates	Sampling date	Gear	Mean total lenght
East Black Sea (BS1)	BS1	25	15/10	41^0 22’36”N 39^0 44’23”E	20.12.2014	Gill net	36.7 (4.7)
West Black Sea (BS2)	BS2	25	13/12	41^0 25’ 29”N 30^0 59’06” E	22.10.2013	Trawl	25.7 (10.0)
Marmara Sea (MS)	MS	25	15/10	40^0 43’23”N 28^0 36’48”E	19.09.2014	Trawl	38.5 (4.5)

1 µl template DNA (≈10-25 ng). PCR temperature profile consisted of two stages. In the first stage, pre denaturation at 95°C for 1 min was followed by 5 cycles of denaturation at 94°C for 20 s, annealing at primer-specific temperature for 55 s, extension at 72°C for 2 s. The second stage included 25 cycles of denaturation at 94°C for 20 s and final extension at 72°C for 20 s.

The forward primers were fluorescently labelled with FAM. The quality of the PCR amplifications was checked by subjecting the PCR products to electrophoresis in 1.5 % agarose gels. Allele size was determined in an ABI-PRISM 3100 sequencer (Applied Biosystems, Foster City CA, U.S.A.). Raw data from the sequencer were processed with the PEAK SCANNER1.0 software for peak identification and fragment sizing (Applied Biosystems).

MtDNA Sequencing

In order to determine the genetic structure of *S. maeoticus* populations, mtDNA COIII gene was used. After DNA extraction, mtDNA COIII gene was amplified using PCR (Saiki et al. 1988). Polymerase chain reactions were at a reaction volume of 25 µl containing 5 units of *Taq* polymerase (Thermo Scientific), 2 mM of each primer, 10 mM dNTPs (Thermo Scientific), 25 mM $MgCl_2$ (Thermo Scientific), 10 mM Tris-HCl pH 8.8, 50 mM KCI and 1 µl template DNA (~10-25 ng). The amplification was performed with a profile of 1 cycle of denaturation at 94ºC for 5 min, followed by 40 cycles of strand denaturation at 94ºC for 1 min, annealing at 50ºC for 1 min and primer extensions 72ºC for 1 min 30 sec and 1 cycle of final elongation at 72ºC for 5 min. After PCR amplification, a 3 µl sample of each PCR product was controlled in 1.5% agarose gels. At last, all samples were sequenced in the forward direction with an automated sequencing machine. PCR amplification of the mitochondrial COIII gene was carried out using the universal primers: COIII-F:
5’-AGC CCA TGA CCT TTA ACA GG -3’(Tm 47˚C) and COIII-R: 5’- GAC TAC ATC AAC AAA ATG TCA GTA TCA -3’ (Tm 49˚C).

The partial COIII nucleotide sequences were aligned using Clustal W (Thompson et al. 1994) and the last alignment was done manually with BioEdit (Hall 1999). In the analyses of genetic data, MEGA*v*5 (Tamura et al. 2011), DNA SP (Librado & Rozas 2009) and Arlequin 3 (Excoffier et al. 2005) software packages were used.

Morphological and chemical analyses

The morphometric distances were determined by using images and applying an image processing tool especially developed for morphometric measurements and analysis of fish populations (Fig. 1a). Meristic characters commonly used to describe scophthalmid species such as dorsal fin, caudal fin, anal fin, ventral fin, pectoral fin, back pectoral fin, gill rakers number and vertebrae number were examined.

Most of the variability in morphological characters is due to size differences (Junquera & Perez-Gandaras 1993, Turan 1999).Shape analysis should be free from the effect of size to avoid misinterpretation of the results (Strauss 1985). Therefore, the allometric-size correction method (Elliott et al. 1995) was used to remove the effect of size variation. Principal component analyses (PCA) were used for detecting morphological characters exhibiting differences among populations. Discriminant function analyses (DFA) were used for distinguishing morphological differences between populations (TURAN 1999). Univariate analysis of variance (ANOVA) was used to compare the variation between size-adjusted characters. SPSSv23 and SYSTATv13 were used for all statistical analysis.

The left sagittal otolith was cleaned before examination and otoliths images were acquired with a digital camera and then analysed (Fig. 1b). For otolith chemistry, one otolith (sagitta) from each fish was carefully washed of surface contaminants and cleaned with analytical grade 1% nitric acid (HNO_3). Otoliths were first soaked in ultra-pure water to hydrate biological residue adhering to the surface of the sample, then were soaked in 3% hydrogen peroxide for 5 min to dissolve remaining biological residue

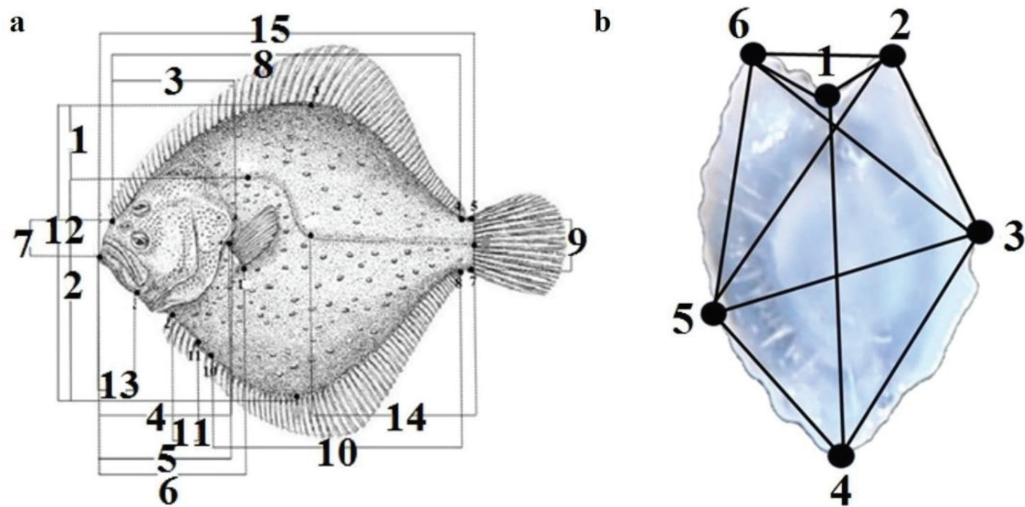

Fig. 1. a. Morphometric measurements of *S. maeoticus*. b. Locations of the six landmarks (●) for constructing the truss network on the otoliths of *S. maeoticus*.

and immersed for 5 min in 1% nitric acid to remove surface contamination. Otoliths were then rinsed in ultra-pure water for 5 min to remove the acid. Finally, otoliths were dried and stored in an incubator at 120°C. Before instrumental analysis, each otolith was weighed and dissolved in 10 mL of 37 % nitric acid. Elemental concentrations of the samples were determined using solution-based inductively coupled plasma-atomic emission spectrometry (ICP-AES). Evaluated elements (Ba, Mg, Mn, Sr, Na, K and Ca, Fe) in the otolith were measured.

Results

Microsatellite analysis

Observed allele number for all loci varied between 10 and 18. The highest allele number was 18 at SMA1 locus and the lowest allele number was 10 at 3/9CA15 locus (Table 2). The highest expected heterozygosity was 0.81 at SMA1 locus, while the lowest expected heterozygosity was 0.68 at SMA3 locus. When considered all loci, the expected heterozygosity was higher than the observed heterozygosity values.

Mean observed heterozygosity values were the lowest in the BS2 (0.25) and highest in BS1 and MS (0.40) populations (Table 2). Allele number of each microsatellite locus was found to be the highest at SMA1 locus in BS1 population whereas the lowest was at 3/9CA15 locus in BS1 population. The Garza-Williamson index was the lowest in MS population at the SMA1 locus and the highest in MS population at the B12-I GT14 locus. Samples from BS1 population at the 3/9CA15 locus were in Hardy-Weinberg equilibrium ($P>0.05$) while the others were not with varying statistical significance, which may be associated with the low sample size. Moreover, observed heterozygosity in all populations was lower than expected (Table 2).

The pairwise comparisons of genetic differences between populations indicated that all the populations were significantly different from each other ($P<0.001$) and the lowest distance was between the MS and BS2 populations (0.19549), while the highest was between the MS and BS1 populations (0.21855) (Table 4).

The lowest and highest genetic diversity within a population were seen in the BS1 (0.716082) and MS (0.748735) populations, respectively (Table 3). According the neighbour-joining tree, BS1 population was separated from the BS2 and MS populations, which were on the same node (Fig. 2).

MtDNA sequencing

In the mtDNA sequencing analysis, the partial COIII gene sequences consisted of 566 bp fragments in which there were 556 bp conserved sites, 10 bp as variable sides, 1 bp parsimony informative side and 9 bp singleton side. The average nucleotide composition of adenine (A), thymine (T), cytosine (C) and guanine (G) were 23.2%, 28.8%, 28.4% and 19.6%, respectively. The best model in accordance with our data was the Jukes-Cantor model (Jukes & Cantor 1969), which was used in the genetic diversity and divergence analyses.

The average value of genetic diversity within the populations was 0.001481. The smallest and highest genetic diversities were observed in the BS2 (0.001337) and MS populations (0.001692). Average haplotype diversity between populations was

Table 2. Descriptive statistics for the five microsatellite loci between populations of S. maeoticus. Abbreviations: n, sample size; a, number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; G.W. index, Garza-Williamson index; *, P < 0.05; **, P < 0.01; ***, P < 0.0001.

Samples		Locus					
		SMA1	SMA02	SMA3	B12-I GT14	3/9CA15	Average Across Loci
BS1	N	25	25	25	25	25	
	A	11	6	5	7	4	6.60
	Ho	0.52***	0.58***	0.12***	0.16***	0.72	0.40
	He	0.88***	0.70***	0.66***	0.72***	0.72	0.72
	G.W. index	0.24	0.75	0.55	0.35	0.57	0.49
	Allelic size range	44	7	8	19	6	44
BS2	N	25	25	25	25	25	
	A	7	8	5	9	7	7.20
	Ho	0.32***	0.56***	0.12***	0.68***	0.32***	0.40
	He	0.78***	0.75***	0.69***	0.78***	0.74***	0.75
	G.W. index	0.14	0.33	0.62	1.00	0.70	0.56
	Allelic size range	49	23	7	8	9	
MS	N	25	25	25	25	25	
	A	8	7	8	8	5	7.20
	Ho	0.52***	0.16***	0.24***	0.24***	0.08***	0.25
	He	0.78***	0.70***	0.70***	0.81***	0.64***	0.73
	G.W. index	0.19	0.44	0.36	0.89	0.63	0.50
	Allelic size range	41	15	21	8	7	
	Observed Allele Number	18	15	16	13	10	14.4
	Observed Heterozy-gosity	0.45	0.43	0.48	0.36	0.37	0.42
	Expected Heterozy-gosity	0.81	0.72	0.68	0.77	0.7	0.74

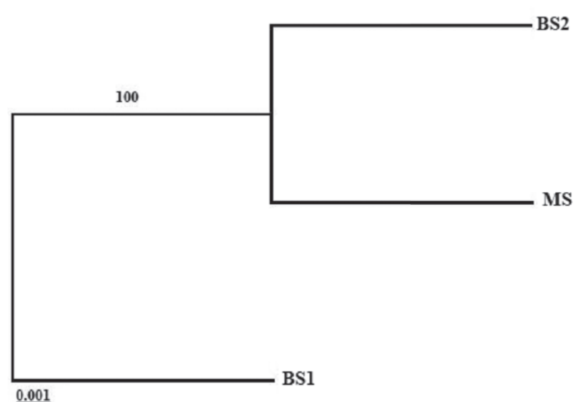

Fig. 2. Neighbour-joining trees based on the microsatellites loci. Numbers of nodes indicate the bootstrap values.

found to be 0.6345 and 10 different haplotypes were observed (Table 4). Haplotype 1 had equal distribution in all populations. Additionally, Haplotype 7 was shared between the BS2 and MS populations.

The average divergence between comparisons of all populations was 0.001485. For inter-population comparison, the highest genetic distance value (0.001593) was observed between MS population and BS1 population, and the lowest value (0.001416) was detected between BS1 and BS2 populations. Statistically significant (P<0.001) genetic differences were found between the MS and BS1 populations (Table 3). Tajima's D, which tests the conformity of DNA sequence evolution to neutrality, was found to be -2.114293. The neighbour-joining algorithm showed that BS1 population was the most distinct population compared to BS2 and MS populations which were on the same node.

Morphology

Principal component analysis (PCA) was used for eliminating variations due to differences in the age and length (allometry) of samples and determining of possible morphometric and meristic characters, which can make the difference between populations of *S. maeoticus*. In PCA, 28 principal components were produced: the first principal component (PC1)

Table 3. Microsatellite pairwise F_{ST} (below diagonal) based on Reynold's distance (1983), mtDNA sequencing pairwise F_{ST} (above diagonal) based on the Jukes-Cantor model (1969). Average number of pairwise differences within population for microsatellite (bold diagonal elements) and average number of pairwise differences within population for mtDNA (italic diagonal elements). ***, P < 0.0001.

Populations	BS1	BS2	MS
BS1	0.716082/ 0.001416	0.001416	0.001593***
BS2	0.21015***	0.748735/ 0.001337	0.001452
MS	0.21755***	0.19549***	0.725388/ 0.001692

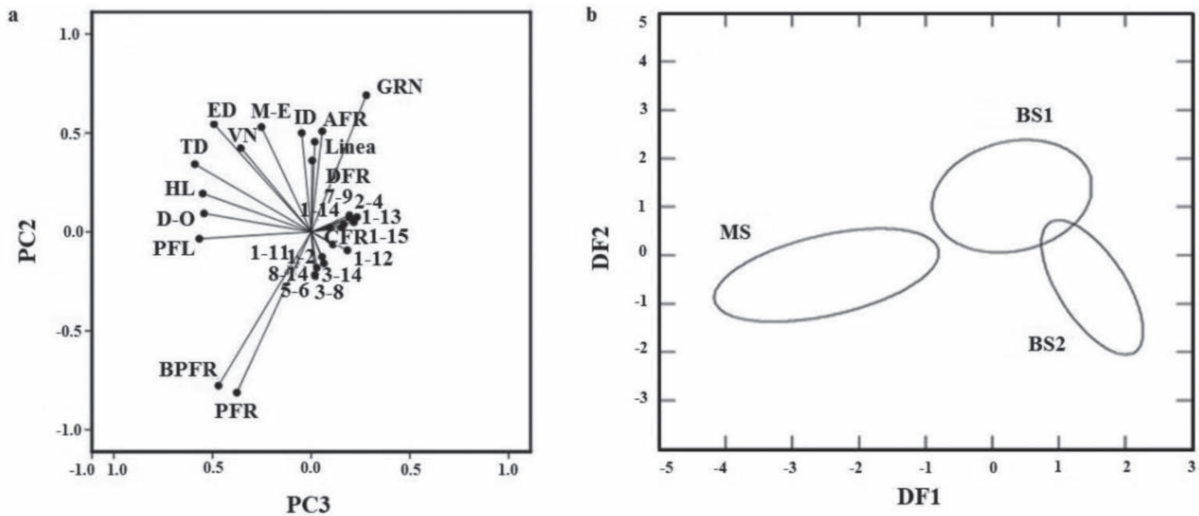

Fig. 3. a. Distribution of characters according to relevant contributions to the main components. b. Morphometric and meristic differentiations of the stocks of S. maeoticus in the discriminant space.

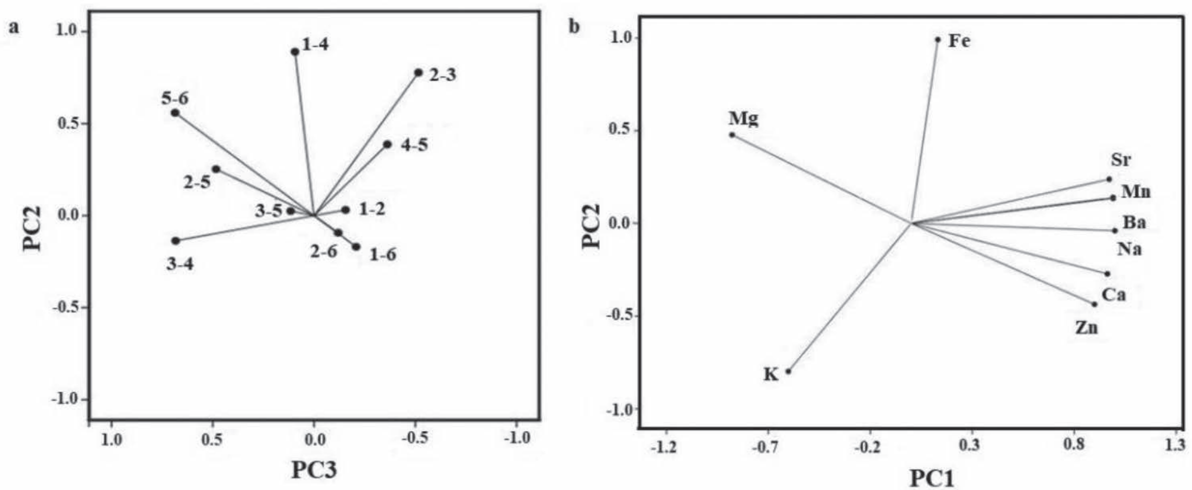

Fig. 4. a. Output of principal component analysis: the distribution of species according to the specifications of the main components that played a role in distinguishing from each other. b. Output of principal component analysis: the distribution of species according to the specifications of the main components that played a role in distinguishing from each other.

accounted for 48% of the total variance and PC2 accounted for 8% of the total variance. Plotting PC1 and PC2 showed a clear separation of characters which take role to separate the populations (Fig. 3a).

As a result of discriminant function analysis (DFA), four functions were produced and the first two discriminant functions (DFs) explained 100% of between-group variability. Plotting DF1 (73%) and DF2 (27%) showed a clear between-population differentiations of the MS population (Fig. 3b). A correct classification of individuals into their original population showed the highest classification of the BS1 with 84%, which indicated that BS1 population was the most distinct popula-

Table 4. Distribution and frequency of COIII haplotypes of *S. maeoticus* populations.

Haplotypes	BS1	BS2	MS
Hap 1	6	6	6
Hap 2	1	-	-
Hap 3	1	-	-
Hap 4	1	-	-
Hap 5	1	-	-
Hap 6	-	1	-
Hap 7	-	2	2
Hap 8	-	1	-
Hap 9	-	-	1
Hap 10	-	-	1

tion from the other populations. Examination of vectorial distribution of the morphometric characters showed that the distance between mouth and eye and eye diameter measurements, pectoral fin ray number, back pectoral fin ray number and gill raker number were detected to be the most effective characters for discriminating between the populations (Fig. 3a).

In PCA, 9 principal components (PCs) were produced and 43% of the total variance was presented in the first (20%) and second (20%) PCs. Based on the vectorial distribution of the characters in the PCA, the most significant otolith measurements were 1-4 and 2-3 characters (Fig. 4a).

In discriminant function analysis, two DFs were produced and the first and the second DFs accounted for 65% and 35% of the between-group variability. Discriminant graphic could not be plotted due to the low number of DFs. The correct classification of individuals into their original population varied between 60% and 80% according DFA and 69% of the individuals could be classified in their correct a priori grouping. The proportion of correctly classified BS1 samples into their original group was the highest (80%).

Univariate Analysis of Variance showed significant differences between means of all the samples for all otolith elements ($P<0.001$). In PCA, 6 principal components were produced and 75% and 23% of the total variance were explained by the first and second PCs. In the first PC, Na, Ba, Mn, Sr, Ca, Zn and Mg elements were observed to be effective for distinguishing species whereas in the second PC, Fe and K elements were effective for discriminating populations (Fig. 4b).

In DFA, two functions were produced and the first and second discriminant functions accounted for 82% and 18% of the total variation. The correct classification of individuals into their original populations was 100% for all populations that indicated significant differentiation among the BS1, BS2 and MS populations.

Discussion

The population structure of *S. maeoticus* from the Marmara Sea and the Black Seas was studied for the first time on the basis of genetic data, such as microsatellite and mtDNA sequencing, and morphology, such as morphometric, meristic, otolith chemistry and shape analyses. All analyses showed similar pattern of differentiation with varying degree, which reflected the sensitivity of the markers for distinguishing different populations. Several genetic studies have previously shown variable degree of genetic differentiation for *S. maximus*; however, there are no any genetic studies for *S. maeoticus*. Do Prado et al. (2018) analysed the genetic structure of *S. maximus* populations from the Baltic Sea, Atlantic Ocean, Mediterranean Sea and Black Sea and found genetic differentiation of 0.090. Turan et al. (2019) examined molecular systematic structure of three turbot species (*S. maeoticus*, *S. maximus* and *S. rhombus*) with mtDNa sequence analysis of COI-II region and confirmed genetically *S. maeoticus*, *S. maximus* and *S. rhombus* as distinct species.

All genetic and morphological markers were in accordance and showed that the BS1 population revealed morphological and genetic discreteness from the BS2 and MS populations. BS2 sample was significantly different from the other two populations with microsatellites and otolith chemistry as a marker, which should be enough to discriminate it as a different stock. MS sample was also significantly different based on microsatellite and morphometric-meristic data and otolith chemistry markers, which also deserve to be treated as a different management unit.

The average observed and expected heterozygosity was found to be 0.42 and 0.74, respectively, with the number of alleles varying between 10 and 18 in the present study. We observed relatively low heterozygosity, which might be related to the overfishing of this species and other anthropogenic factors such as pollution (Liu et al. 2014). Coughlan et al. (1998) analysed the wild and cultured populations of *S. maximus* by using microsatellite technique and found that mean heterozygosity was 0.66 for wild and cultured populations in Ireland and 0.71 for wild Norwegian populations. Pardo et al. (2005) found expected heterozygosity values of *S. maximus* populations between 0.13 and 0.91 with allele number per locus between 2 and 15. Lui et al.

(2006) studied populations of *S. maximus* using five polymorphic microsatellite loci and found observed heterozygosity between 0.76 and 0.90 and expected heterozygosity between 0.63 and 0.83. Florin & Höglund (2007) found mean heterozygosity of *S. maximus* between 0.64 and 0.72. Navajas-Pérez et al. (2012) found expected heterozygosity between 0.021 and 0.951 for *S. maximus*. The studies on *S. maximus* showed that the heterozygosity is generally low, which could be related to the short-distance migratory behaviour of turbot species that may cause a reduced emigration/immigration rate between populations. However, factors such as overfishing and other anthropogenic activities may also reduce heterogeneity and cause irreversible genetic changes.

In our study, the average haplotype diversity between populations was 0.6345, with overall ten different haplotypes. Suzuki et al. (2004) reported 28 haplotypes for the phylogeographic analysis of *S. maximus* in the Mediterranean basin. Atanassov et al. (2011) identified 36 haplotypes with average haplotype diversity of 0.47 between populations of *S. maeoticus* (mentioned as *S. maximus*) from Bulgarian and Romanian waters of the Black Sea. The haplotype diversity and number of haplotypes in their study seem to be lower compared to the study of Suzuki et al. (2004). The low diversity observed for *S. maeoticus* in the present study might be due to the high overfishing pressure in Turkish marine waters.

In general, marine species demonstrate low genetic differentiation due to deficiency of major geographical barriers to dispersal and gene flow (Avise et al. 1987). The mean genetic divergence among populations of the Black Sea turbot *S. maeoticus* were 0.207 and 0.0014 for microsatellite and mtDNA, respectively. In the present study, the differentiation as evidenced by the microsatellite analysis was higher than that revealed by the mtDNA analysis. Patton et al. (1997) examined genetic variation of microsatellite and mtDNA markers in broad whitefish (*Coregonus nasus*) and found that it was lower in mtDNA compared to that in the microsatellite loci. McLean & Taylor (2001) studied the population structure of the eulachon (*Thaleichthys pacificus*) and recorded higher genetic differentiation value for microsatellites than that observed with mtDNA. A lower level of differentiation of mtDNA than of microsatellite loci is contrary to the expectation based on the more rapid differentiation of mtDNA than nuclear genes because the smaller effective gene number of haploid, maternally inherited mtDNA (Desalle et al. 1987, McLean & Taylor 2001). On the other hand, relatively few males would mate with many females and thus the effective gene numbers may be greater for mtDNA than for nuclear genes, which might lead to more rapid genetic drift of nuclear loci. Moreover, different rates of gene flow, mutation or natural selection of mtDNA and microsatellites may also generate greater differentiation of microsatellites than of mtDNA (Karl & Avise 1992, Slatkin 1995).

In the present study, relatively higher genetic differentiation was found among populations of *S. maeoticus* in comparison to the former studies on *S. maximus* populations. Florin & Höglund (2007) studied population structure of turbot (*S. maximus*) in the Baltic Sea and found relatively low microsatellite genetic differentiation (0.004). Bouza et al. (2002) studied genetic diversity in natural and domestic population of *S. maximus* by using microsatellite technique and found very low genetic divergence (0.002). Nielsen et al. (2004) found genetic divergence between populations of *S. maximus* of 0.032 based on microsatellite analysis. The detected high genetic differentiation between the samples in the present study may be due to the detected low genetic and haplotype diversity within the samples, which might be caused by overfishing, high geographic separation and environmental differences between regions.

In otolith chemistry, all elements were observed to be effective for distinguishing geographically separated populations. The otoliths grow by stratification and may incorporate dissolved chemical compounds from the environment. Accordingly, the otoliths can also incorporate water pollutants (Campana & Neilson 1985, Campana 1999). Furthermore, the industrialised shores of the West Black Sea may also contribute to the detected differences so that iron-steel factories in the West Black Sea region and the discharge of waste of other industries into the sea affects the mineral composition of seawater in this region (Atac 1997). Turan et al. (2014) reported that Sr, Mg, Mn and Na were effective for differentiation of *S. maximus* among the BS1, BS2 and MS populations. The observed differentiation ability of otolith chemistry for *S. maeoticus* and *S. maximus* showed that otolith chemistry could be an effective tool for identification of populations of the demersal fish species, since it has been supported also by the genetic analysis.

Morphometric and meristic analyses could only separate the MS population from the other populations which indicated lower discriminant ability of morphometric and meristic characters for turbot species. The closed structure of the Marmara Sea and its environmental differences from the Black Sea could generate the detected morphometric and meristic differences of the MS population.

Otolith shape analysis was the weakest morphological marker for detecting differences between populations of turbot, which could be related to the demersal behaviour of turbot species. Moreover, the sampled populations were on the same parallel, which suggested temperature similarity between the regions. The temperature and parallel effect on the otholith shape differentiation was reported by Turan (2000).

Conclusion

Identification of fishery management units is very crucial for the effective management of marine resources and vital to indicate anthropogenic impacts on the abundance of species (Palsbøll et al. 2007). Significant genetic and morphological differentiations were found between geographically separated populations of *S. maeoticus* in Turkish marine waters. *Scophthalmus maeoticus* populations in the Marmara Sea, Western Black Sea and Eastern Black Sea should be recognised as different management units compatible with their demographic independence. In order to protect these economically important discontinuous stocks, the fishing pressure should be kept down and additional regulations should be conducted for sustainability of this species in the Black Sea and the Marmara Seas.

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