www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 18: 301-311 (2018) DOI: 10.4194/1303-2712-v18_2_09

RESEARCH PAPER

Genetic Differentiation of Three *Spicara* (Pisces: Centracanthidae) Species, *S. maena, S. flexuosa* and *S. smaris*: and Intraspecific Substructure of *S. flexuosa* in Turkish Coastal Waters

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Received 11 April 2017
Accepted 28 June 2017

Abstract

Interspecific and intraspecific differentiation of the picarel in Turkish coastal waters were examined using partial 16S rRNA (1021 bp) and whole cytochrome b gene (1141 bp) sequences of 244 samples from 7 picarel populations across their geographical distribution. Genetic distances and phylogenetic tree topologies revealed that three *Spicara* species were distinctly separated from each other and *S. flexuosa* and *S. maena* are genetically more closely related than *S. smaris*. Low-level genetic diversity and a star-like haplotype network can be attributed to the founder effect associated with demographic bottleneck in late Pleistocene Mediterranean basin. AMOVA of the genetic substructure for *S. flexuosa* does not support any grouping in the Turkish waters. The divergence time between the Mediterranean and Black Sea populations (0.015%, cyt b) of *S. flexuosa* estimated based on the mtDNA clock calibration of 2% sequence per million years suggests that the Mediterranean samples with reduced genetic diversity has colonized into the Black Sea through straits during the early Holocene (about 7500 kya). The current distribution and demographic pattern of *Spicara* species in the Eastern Mediterranean basin are shaped by climatic events during late Pleistocene and mid-Holocene (about 25-5 kya) and the biological and ecological characteristics specific to *Spicara* species.

Keywords: Centracanthidae, *Spicara*, species discrimination, population structure, mtDNA.

Introduction

Spicara is a centracanthid genus inhabiting shallow, rocky, and muddy bottoms throughout the Mediterranean and the Black Sea, in eastern coastal water of the Atlantic including Portugal and Morocco, and around the Canary Island (Froese & Pauly, 2017). Moreover, *Spicara* genus is represented by three species [*Spicara maena* (Linnaeus, 1758); *Spicara flexuosa* (Rafinesque, 1810); *Spicara smaris* (Linnaeus, 1758)] in the Turkish coastal waters of the Mediterranean and Black Sea (Tortonese, 1986; Golani, Ozturk, & Basusta, 2006; Turan, 2011; Froese & Pauly, 2017). *S. maena* and *S. flexuosa* are morphologically quite similar and their distribution areas mostly overlap, causing identity problems in this region. Morphological similarity can be attributed to the color change resulting from sexual maturation and the coloration diversity associated with sexual dimorphism (Pollard & Pichot, 1971). However, metric and meristic characters of *Spicara* species have proved to be quite essential for identification purposes (Tortonese, 1986; Fischer, Bauchet, & Schneider, 1987; Rizkalla, 1996) despite the problems mentioned above. In fact, Minos, Imsiridou, & Katselis (2013) showed that the identification of both species (*S. maena* and *S. flexuosa*) could easily be done depending on the external morphological characteristics.

The *Spicara* genus has been genetically examined previously as part of the ongoing efforts aimed at genealogy of the Sparid family based on partial 16S rRNA (Hanel & Sturmbauer, 2000; Orrell & Carpenter, 2004) and cytochrome b gene (Orrell, Carpenter, Musick, & Graves, 2002; Orrell & Carpenter, 2004; Chiba, Iwatsuki, Yoshino, & Hanzawa, 2009) sequences of mitochondrial DNA. Later, only a few genetic studies targeting the genus *Spicara* were directly conducted. At first, using mitochondrial 16S rRNA sequences, Imsiridou, Minos, Gakopoulou, Katsares, and Karidas (2011) have precisely separated *S. maena* from *S. flexuosa*, which had long been debated whether they were congeneric (Tortonese, 1975; Eschmeyer, 2013; Froese & Pauly, 2017) or not (Vasiliev, 1980; Papakonstantinou, 1988; Golani et al., 2006; Vasileva, 2007). Second, by using mitochondrial DNA partial 16S rRNA sequences Turan (2011) has

tried to explain the monopyly of Centracanthidae family and the phylogenetic relationships of three *Spicara* species (*S. smaris*, *S. maena*, and *S. flexuosa*), distributing in Turkish coastal waters. He suggested that *S. flexuosa* differs from both *S. maena* and *S. smaris* that do not show identified genetic differences and may thus belong to the same species. Finally, Georgiadis, Sandaltzopoulos, Stergiou, and Apostolidis (2014) genetically discriminated three species of the genus *Spicara* from each other by using melt-curve MHS-PCR method developed.

Despite all these efforts, genetic data of *Spicara* species stored in genetic databases are incompatible with each other probably due to the morphologically incorrect identification of three *Spicara* species. These inconsistencies produced by the previous phylogenetic studies based on mitogenome data suggest that the genetic discrimination and identification of *Spicara* species are still problematic. Therefore, in the present study, the interspecific and intraspecific mitochondrial DNA variation of three *Spicara* species inhabited in Turkish coastal waters were examined using mitochondrial 16S rDNA (Infante, Catanese, & Manchado, 2004; Orrell & Carpenter, 2004; Pardo et al., 2005) and cytochrome b gene (Orrell & Carpenter, 2004) sequences that were previously proven their usefulness to illuminate the phylogenetic complexities.

Material and Methods

Sample Collection

Two hundred and fourty-three of picarel samples were collected from seven localities along the coastal regions of Turkey from 2009 to 2015 (Figure 1; Table 1) by helping local fisherman. Due to the common phenotypic plasticity, the morphological

Figure 1. Map showing sampling area and stations. (●) *S. flexuosa*, (■) *S. smaris*, (▲) *S. maena.*

Table 1. Frequency and distribution of 16S rRNA and cytochrome b haplotypes of *Spicara* species sampled from Turkish coastal waters

Haplotypes	16S rRNA															Haplotypes	cytochrome b													
	S. maena					*S. flexuosa*							*S. smaris*				*S. maena*					*S. flexuosa*							*S. smaris*		
	IST	CAN	IZM	ANT	ADN	RZE	SNP	IST	CAN	IZM	ANT	ADN	IZM	ANT	ADN		IST	CAN	IZM	ANT	ADN	RZE	SNP	IST	CAN	IZM	ANT	ADN	IZM	ANT	ADN
Sms1	14	16	14	15	12											Smc1	14	16	14	11	12										
Sms2	1															Smc2	1														
Sms3			1													Smc3			1												
Sms4			1													Smc4			1												
																Smc5				4											
Sss1													22	16	17	Ssc1													22	15	16
Sss2														3		Ssc2														3	
																Ssc3														1	
																Ssc4															1
Sfs1						20	12	16	15	15	14	14				Sfc1						3		3							
Sfs2								2								Sfc2						17	12	15	15	13	10	14			
Sfs3										2						Sfc3										1					
Sfs4											1					Sfc4										1					
Sfs5											1					Sfc5										1					
																Sfc6										1					
																Sfc7											6				
																Sfc8															
	15	16	16	15	12	20	12	18	15	17	16	14	22	19	17		15	16	16	15	12	20	12	18	15	17	16	14	22	19	17

identifications of picarel were performed by examining some morphological features (Vidalis, Markakis, & Tsimenides, 1997; Karidas, Argiridis, & Minos, 2009; Minos et al., 2013) under expeditionary conditions.

DNA Extraction, PCR Amplification and DNA Sequencing

Fin clips of 244 specimens were sampled and stored in 96% ethanol at −20°C until DNA extraction. DNA was extracted from fin tissues of each picarel using Qiagen DNeasy Blood & Tissue Kit (Qiagen, USA) and purified. The obtained DNA were stored in -20°C for PCR applications. The quality and quantity of isolated DNA were verified using NanoDrop 2000c Spectrophotometer (Thermo Scientific, USA). The PCR amplifications of full cytochrome b gene and partial 16S rDNA regions were performed using following pimer pairs: SpcrCytbF: 5'-CAA GCC TTC GAA ARA CAC ACC C-3'; and SpcrCytbR: 5'-ACY TTR TTT TCT AWA GTT GCT GC-3'), Spcr16SF: 5'- GTA TGG GCG ACA GAA AAG G-3' and Spcr16SR: 5'-ACT GAC CTG GAT TAC TCC GG-3'). PCR reactions were performed in 50 µl reaction volume containing 5µl 10x PCR buffer, 1,5 mM $MgCl_2$, 0.5 mM dNTPs mix, 0,5 mM of each primer, 50 ng template DNA, and 1 U *Taq* DNA polymerase (Fermentas, Thermo Fisher Scientific Inc). The PCR amplifications were performed under following conditions using a gradient thermal cycler Biorad T100^{TM} (Bio-Rad, Hercules, CA, USA): initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing (at 59 °C for cyt b and 50 °C for 16S rDNA) for 45 second, primer extension at 72 °C for 1 min through 35 cycles and a final extension at 72 °C for 7 min. The PCR products were run on 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL), in TAE buffer, and were visualized under UV Quantum-Capt ST4 system (Vilber Lourmat, France). The concentrations of purified PCR products were estimated by NanoDrop 2000C UV-Vis spectrophotometer (Thermo Fisher Scientific, USA). Double stranded PCR product were purified using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA), and then were bidirectionally sequenced on an ABI Prism 3730x1 automatic DNA sequencer (Applied Biosystems, USA) using related PCR primers. The obtained sequences of haplotypes were deposited in GenBank under accession numbers (MF149882-MF149892 of 16S rRNA haplotypes; MF149896-MF149912 of cytochrome b haplotypes).

Data Analysis

The obtained sequences were edited and aligned using software Bioedit (Hall, 1999), and genetic distances were calculated according to Kimura (1980) method. The molecular diversity indices such as number of haplotypes (H), polymorphic sites, nucleotide diversity (π) and haplotype diversity (*Hd*) were computed for each species using the program DnaSP version 5.10.1 (Rozas, 2009). Intraspecific genealogy based on network inference of haplotypes were reconstructed by means of a median-joining (MJ) algoritm implemented in Network 4.6.1.3 software (Bandelt, Forster, & Röhl, 1999, www.fluxus-engineering.com). Detecting of the hierarchical population structure within *S. flexuosa* dispersed throughout Turkish waters was performed using hierarchical analysis of molecular variance AMOVA (Excoffier, Smouse & Quattro, 1992) implemented in Arlequin version 3.5 (Excoffier & Lischer, 2010) with 10,000 permutations. The phylogenetic analyses of *Spicara* haplotypes were performed through neighbour joining (NJ), maximum-parsimony (MP) and maximum- likelihood (ML) analyses. MP analyses were conducted in software PAUP v. 4.0b10 (Swofford, 2003) using a heuristic search, with the tree-bisection and reconnection (TBR) branch swapping and 100 random addition replicates. On the other hand, ML analyses were performed by software PHYML version 3.0 (Guindon & Gascuel, 2003), with 100 bootstrap repetitions using the TIM2+G (for both markers) substitution models, as selected according to AICc in jModelTest 0.1.1 (Posada, 2008). The robustness of nodes was assessed with 100, 500 and 1000 replicates in ML, MP and NJ, respectively. In order to construct the phylogeny of genus *Spicara*, *Centrachanthus cirrus* (GenBank accession number: MF149893-MF149895 of 16S rRNA haplotypes; MF149913-MF149916 of cytochrome b haplotypes) and *Pagrus major* (GenBank accession number: AP002949) were used as outgroups because they are closely related to *Spicara* species.

Results

Genetic Diversity

The full mitochondrial DNA cytochrome b (1141 bp) and partial 16S rRNA (1021 bp) sequences were analyzed for 244 individuals of three *Spicara* species from seven geographic populations natively distributed in Turkish territorial waters (Figure 1; Table 1). The nucleotide frequences for mitochondrial DNA cytochrome b were identified as 33.31% A, 22.16% T, 23.46% C, and 21.07% G. The overall transistion/transversion bias was R=2.612. A total of 17 mtDNA cyt b haplotypes (1141 base pairs) were defined; five from *S. maena* (N=74), eight from *S. flexuosa* (N=112) and four from *S. smaris* (N=58) (Table 1). In total, 149 variable sites were identified, 140 of which were polymorphic. On the other hand, the nucleotide frequences identified for partial 16S rRNA were found as 24.32% A, 30.78% T, 29.28% C, and 15.63% G. The overall transistion/transversion bias was R=5.293. A total of 11 mtDNA 16S rRNA

haplotypes (1021 bp) were defined, four from *S. maena* (N=74), five from *S. flexuosa* (N=112) and two from *S. smaris* (N=58) (Table 1). The aligned partial sequences of 16S rRNA included 63 variable sites, including 54 parsimony informative.

Population Structure

The obtained molecular findings pointed out that both mitochondrial cytochrome b and 16S rRNA sequences of the individuals of *S. maena*, *S. flexuosa* and *S. smaris* of *Spicara* genus in Turkish coastal waters showed low level of haplotype and nucleotide diversity [(Sm: *Hd*= 0.1791 and π=0.00016; *Hd*=0.07997 and π=0.00013), (Sf: *Hd*=0.2768 and π=0.00026; *Hd*=0.1044 and π=0.00029) and (Ss: *Hd*=0.1654 and π=0.00020; *Hd*=0.09982 and π=0.00020)], respectively (Table 2).

The mutational distance-based relationships between the mitochondrial cytochrome b and 16S rRNA haplotypes were utilized in order to elucidate the genetic structure of *Spicara* populations through a MJ (median joining) network analysis with few unique haplotypes linked by one or two mutations (Figure 2). The haplotype network revealed a star-like structure containing several unique haplotypes, linked to dominant haplotypes (Smc1:67, Sfc1:105, and Ssc1:53 for cyt b; Sms1:71, Sfs1:106, Sss1:55 for 16S rRNA) with one or two mutational steps in each *Spicara* species that clearly separated from each other by at least 28 mutational steps (Figure 2).

AMOVA revealed that most of the total molecular variance 84.69% can be attributed to regional differences among individuals within the population, while 13.93% was apportioned among the population within the group and 1.38% among groups for cytochrome b (Table 3). Genetic variations within *S. flexuosa* populations (fixation index within populations F_{SC} = 0.14121, $p < 0.001$) is higher than that among populations within groups (fixation index among populations within groups F_{ST} = 0.15306, $p < 0.001$) and among groups (fixation index between groups F_{CT} = 0.01380, $p < 0.001$) (Table 3). For 16S rRNA, AMOVA revealed that most of the total molecular variance 98.65% can be attributed to regional differences among individuals within the population, while 0.58% was apportioned among the population within the group and 0.77% among groups (Table 3). Genetic variations within *S. flexuosa* populations (fixation index within populations F_{SC} = 0.00583, $p < 0.001$) is higher than that among populations within groups (fixation index among populations within groups F_{ST} = 0.01351, $p < 0.001$) and among groups (fixation index between groups F_{CT} = 0.00733, $p < 0.001$) (Table 3). AMOVA and fixation indices did not suggest any hierarchical regional structuring among populations within group or among groups.

Species Divergence and Phylogenetic Relationships

The phylogenetic trees obtained from different methods such as NJ, MP, and ML highlighted the existence of similar tree topologies with high reliability (71-100) (Figure 3, Figure 4) for both mtDNA markers. *Spicara* species in the coastal water surrounding Turkey were subdivided into two main clusters. The first cluster was composed of two main haplogroups, corresponding to *S. flexuosa* and *S. maena*, indicating that they are more closely related

Table 2. Main values of mtDNA variability in Turkish *Spicara* populations. Number of samples (N) and haplotypes numbers (H), haplotype diversity (*Hd*), nucleotide diversity (π) values

		16S rRNA			cytochrome b	
Species, Locality, Sea	N	H	*Hd*	π	H	*Hd*	π
S. maena							
Istanbul, Sea of Marmara	15	2	0.13333	0.00026	2	0.1333	0.00012
Canakkale, Sea of Marmara	16	1	0.00000	0.00000	1	0.0000	0.00000
Izmir, Aegean Sea	16	3	0.24167	0.00035	3	0.2416	0.00020
Antalya, Mediterranean Sea	15	1	0.00000	0.00000	2	0.4190	0.00037
Adana, Mediterranean Sea	12	1	0.00000	0.00000	1	0.0000	0.00000
	74	4	0.07997	0.00013	5	0.1791	0.00016
S. smaris							
Izmir, Aegean Sea	22	1	0.00000	0.00000	1	0.0000	0.00000
Antalya, Mediterranean Sea	19	2	0.28070	0.00089	3	0.3684	0.00040
Adana, Mediterranean Sea	17	1	0.00000	0.00000	2	0.1176	0.00021
	58	2	0.09982	0.00020	4	0.1654	0.00020
S. flexuosa							
Rize, Black Sea	20	1	0.00000	0.00000	2	0.2684	0.00024
Sinop, Black Sea	12	1	0.00000	0.00000	1	0.0000	0.00000
Istanbul, Sea of Marmara	18	2	0.20915	0.00020	2	0.2941	0.00026
Canakkale, Sea of Marmara	15	1	0.00000	0.00000	1	0.0000	0.00000
Izmir, Aegean Sea	17	2	0.22059	0.00022	5	0.4246	0.00041
Antalya, Mediterranean Sea	16	3	0.24167	0.00025	2	0.5000	0.00044
Adana, Mediterranean Sea	14	1	0.00000	0.00000	2	0.1428	0.00013
	112	5	0.10440	0.00029	8	0.2768	0.00026

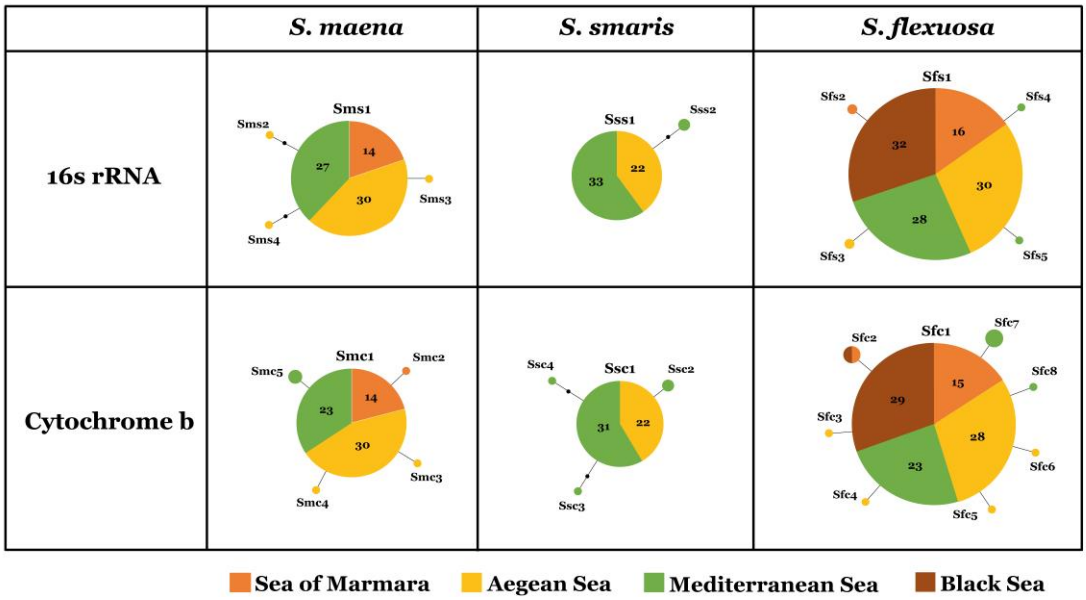

Figure 2. Median-joining network for the mtDNA 16S rRNA and cytochrome b haplotypes of *Spicara* spp. from Turkish coastal waters. Mutation differences between haplotypes are indicated in the branches. Circle size is proportionate to the number of individuals carrying the same haplotype.

Table 3. Analysis of molecular variance (AMOVA) for cyt b and 16S rRNA sequences of *S. flexuosa* distributed throughout Turkish waters

Genes	Source of variation	d.f.	Variance components	Variance components	Percentage Variation
cytochrome b	Among groups	3	1.873	0.00244 Va	1.38
	Among populations within groups	3	1.576	0.02459 Vb	13.93
	Within populations	105	15.700	0.14952 Vc	84.69
	Total	111	19.149	0.17654	
	F_{SC}:0.14121, F_{ST}: 0.15306, F_{CT}: 0.01380				
16S rRNA	Among groups	2	0.174	0.00165 Va	3.77
	Among populations within groups	4	0.135	-0.00059 Vb	-1.35
	Within populations	105	4.494	0.04280 Vc	97.58
	Total	111	4.802	0.04386	
	F_{SC}: -0.01404, F_{ST}: 0.02415, F_{CT}: 0.03766				

than *S. smaris*. Second cluster included only *S. smaris* haplotypes (Figure 3, 4).

The pairwise sequence divergence among three *Spicara* species ranged from 7.99 to 9.41% for cytochrome *b* gene and 2.90 to 4.44% in 16S rRNA (Table 4). *S. flexuosa* and *S. maena* appear more closely related compared to *S. smaris*. Assuming that a 2% cytochrome b divergence rate per million years (Grant & Bowen, 1998; Bargelloni, Alarcon, Alvarez, Penzo, Magoulas, Reis, & Patarnello, 2003), the differences measured among the samples of *S. flexuosa* from both Mediterranean and Black Sea (0.015%, Table 4) illustrated the precise isolation since the early Holocene (7500 years ago).

Discussion

Geographical Distribution of *Spicara* Species

The boundaries of current distribution areas of picarel species through field studies conducted during the present study were estimated. In addition, as in most marine species (Leppakoski, 1991), we speculated that the salinity level of the seawaters determines the limits of the geographical distribution of *Spicara* species. The Black Sea basin is partially isolated and thus differs from the Mediterranean in terms of ecological barriers such as the salinity factor. The salinity level of the Black Sea surface waters (16.5-18.5 psu, Oguz, Özsoy, Latif, Sur, & Ünlüata, 1990; Altiok, Sur, & Yuce, 2012) is quite lower than Mediterranean Sea (36.5-39 psu) (Topper & Meijer, 2015). For this reason, it is speculated that from south to north, the geographical distribution of picarel is limited with Dardanelles strait for *S. smaris* and Bosphorus strait for *S. maena*. Because *S. flexuosa* are able to tolerate higher salinity levels (%17-39) (Lleonart, 2008), compared to both *S. maena* and *S. smaris*, we have only observed the existence of *S. flexuosa* in present-day Black Sea, which is

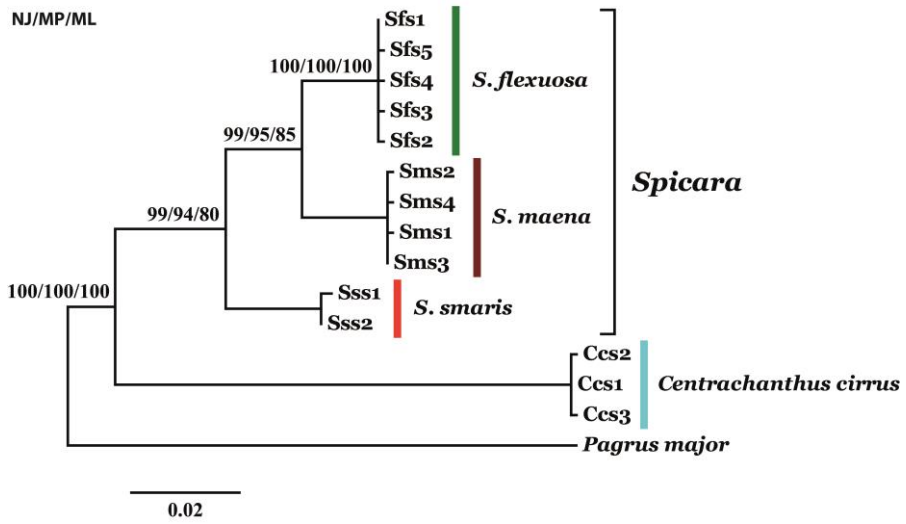

Figure 3. Maximum likelihood tree of the partial 16S rRNA gene sequences of three picarel species. The neighbor joining, maximum parsimony and maximum likelihood analysis resulted in similar and congruent trees. Bootstrap (only 50 % or higher) values are shown above nodes on trees.

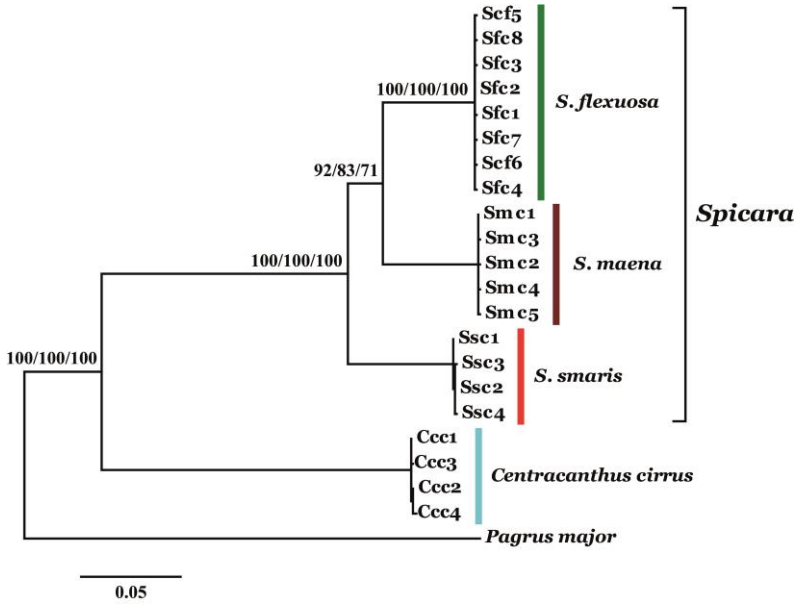

Figure 4. Maximum likelihood tree of cytochrome b gene sequences of three picarel species. The neighbor joining, maximum parsimony and maximum likelihood analysis resulted in similar and congruent trees. Bootstrap (only 50 % or higher) values are shown above nodes on trees.

characterized as brackish.

Species Divergence and Phylogenetic Relationships

The genetic distances (7.99-9.41%, cyt b and 2.90-4.44%, 16S rRNA) among the *Spicara* species in Turkish coastal waters revealed a precise genetical divergence when compared to the findings [%6.21'lik (16S+Cytb) (Orrell & Carpenter, 2004) and 10.5-22% (cyt b) (Junhong, 2007)] previously reported for some other Sparid taxa. In addition, upon the analysis of partial 16S rRNA sequences, a much higher interspecific variation (2.90-4.44%) level was determined when compared to that of reported (0-0.5%) by Turan (2011), who previously investigated the systematic status of the Mediterranean *Spicara* species with the same marker. On the other hand, although Turan (2011) previously reported no genetic distance among *S. maena* and *S. smaris*, the existence of a 4.44% genetic distance based on the mitochondrial DNA 16S rRNA gene sequences was determined with this study. Similarly, the phylogenetic tree topologies obtained from the mitochondrial cyt b and 16S rRNA gene sequence analyses (Figure 3, 4) also supported a closer genetic relationship between *S. flexuosa* and *S. maena* than to

Table 4. Genetic distance (percentage) calculated with the kimura 2 parameter (K2P) model based on mitochondrial sequences of cyt b and 16S rRNA for *Spicara* spp.

	Cytochrome b					16S rRNA		
Spp	*Sm*	*Sf*	*Ss*			*Sm*	*Sf*	*Ss*
Sm	0.16				*Sm*	0.13		
Sf	7.99	0.21			*Sf*	2.90	0.10	
Ss	9.30	9.41	0.20		*Ss*	4.44	4.35	0.20
		BS	MS	MEDS		BS	MS	MEDS
Sf	BS	0.011			BS	0.000		
	MS	0.010	0.006		MS	0.006	0.004	
	MEDS	0,015	0,013	0.013	MEDS	0,008	0,007	0.002

Note: The lower diagonal shows the interspecies and interpopulation divergences for *Spicara* spp. and *S. flexuosa*, respectively. The intraspecific and intrapopulation distances shaded.

S. smaris, confirming the results reported by previous genetic (Georgiadis et al., 2014) and morphological studies (Minos et al., 2013) on three different *Spicara* species. It is thus hypothesized that although being supplied from almost the same locations in Turkey's coastal waters, the current discrepancies related to the reports on the interspecific relationships in *Spicara* genus is most likely due to the inaccurate morphological descriptions from previous studies.

Population Structure of *S. flexuosa*

The potential distribution of planktonic larvae and eggs, and high gene flow mostly caused by the lack of geographical barriers among populations (McCusker & Bentzen, 2010) contributes to low genetic differentiation in marine fish (Wang, Meng, Liu, Zhang, & Lin, 2011). Contrary to these phenomena, there are three events favorable to the genetic differentiation for *Spicara* populations.

First, the picarel have a relatively low distribution potential in the early and adult stages of life cycles. Second is the presence of geographical barriers among the Turkish picarel populations (Mediterranean-Marmara-Black Sea) such as the Dardanelles and the Istanbul Straits. The distribution potential of picarel is low due to the presence of demersal eggs and the semi-pelagic forms of adults (Francour, Pollard, Bizsel, Kara, Yokes, & Goren, 2011). Thirdly, picarel individuals are protogynous hermaphrodite (Zei, 1941, 1949; Tortonese, 1975). For this reason, this reproduction pattern may be casual reason of the low level diversity of mtDNA (Richardson & Gold, 1997) in *S. flexuosa*, which are distributed throughout the coastal waters of Turkey.

Therefore, contrary to what is observed in some other marine fish such as anchovy (Grant, 2005) and sprat (Debes, Zachos, & Hanel, 2008), it is expected that there will be a remarkable genetic differentiation among the populations because picarel tend to live as dependent on a particular region in early and adult stages of life. The expectations related to the low genetic similarity is more understandable for the Aegean and Mediterranean populations in the absence of significant geographical barriers. On the other hand, high proportion of genetic differentiation is expected for the Mediterranean, Aegean, Marmara and Black Sea populations, which are currently dealing with the both geographical (Bosphorus and Dardanelles straits) and some ecological obstacles (temperature and salinity differences). Despite these biotic and abiotic barriers that cause population differentiation through low gene flow, it is difficult to explain the low genetic differences [0.010-0.015% for cyt b; 0.006-0.008% for 16S rRNA (Table 4)] among groups (especially between the Mediterranean and the Black Sea, which are separated by a geographical barriers) of *Spicara* species by the ongoing gene flow. Moreover, as similar to the previously reported findings for anchovy (Grant, 2005) and horse mackerel (Bektas & Belduz, 2008) in the Mediterranean Sea and Black Sea, the low haplotype and nucleotide diversity (Table 2) and star-like network topologies (Figure 2) identified for Spicara species may be the main indicator of a founder effect associated with demographic bottleneck in Mediterranean basin during the late Pleistocene. AMOVA results showed that there was no significant difference in hierarchical levels among groups (Mediterranean Sea, Sea of Marmara and the Black Sea) (Table 3), separated by geographical obstacles such as Dardanelles and Bosphorus straits. In particular, the identified this genetic uniformity among the *S. flexuosa* populations distributing throughout the Turkish coastal waters point out to the phylogeographic history of the *Spicara* genus in the region unlike the ongoing gene flow.

Phylogeographic History of *S. flexuosa* in Turkish Coastal Waters

The geographical and climatic factors occurred during the Pleistocene glaciers (1.8 mya-11.5 kya) are the main factors responsible for the present genetic structure of existing marine life (Hewitt, 2000). Phylogenetic studies on aquatic organisms provide information on biotic responses to palaeohydrological dynamics associated with climatic oscillations (Faria,

Weiss, & Alexandrino, 2012). The Mediterranean ichthyofauna is characterized by the formation of habitats that are unsuitable for many marine organisms, developed during the Pleistocene glacial events (1,8 mya-present), such as sea-level fluctuations and the Messinian salinity crisis (Wilson, 2006). In this sense, changes in the Mediterranean Sea level related to the Pleistocene glaciation resulting from global climate changes have been more effective on the hydrological budgets of semi-enclosed or marginal sea basins such as the Black Sea (Ryan, Major, Lericolais, & Goldstein, 2003; Lericolais, Bulois, Gillet, & Guichard, 2009).

To support this hypothesis, the dropping of the global sea level during the last glacial period of Pleistocene (25-11.7 kya, Würm period), has led to the breakthrough of saline flow from the Mediterranean into the Black Sea through the straits (Popescu, Lericolais, Panin, Normand, Dinu, & Le Drezen, 2004; Lambeck & Purcell 2005, 2007; Lericolais, Bulois, & Gillet, 2006; Panin & Popescu, 2007). Afterwards, the ancient Black Sea (new euxinian) has become a low saline lake (salinity <5 psu) isolated from the Mediterranean Sea due to the contribution of large rivers for thousands of years (Nevesskaya, 1963, 1965; Grigor'ev, Isagulova, & Fedorov, 1984; Mudie, Rochon, Aksu, & Gillespie, 2002; Lericolais, Popescu, Guichard, Popescu, & Manolukakis, 2007). These new environmental conditions, which destroyed the halophile Mediterranean fauna in the Black Sea, were probably not suitable for *S. flexuosa* that are eligible to tolerate to high salinity (17-39%) (Lleonart, 2008), similar to other marine life forms such as horse mackerel (*Trachurus* spp., Bektas & Belduz, 2008) and anchovy (*E. engrasicolus*, Grant, 2005) except for several estuarine forms such as Pipefish (Wilson & Veragut, 2010) and sprat (Debes et al., 2008).

After the Würm glacial period (between 7-5.3 kya), the significant rise in Mediterranean Sea level caused by the interchange of marine waters between Atlantic and Mediterranean Sea led to the establishment of marine connection between the Black Sea and the Mediterranean (Ryan et al., 2003; Ryan & Pitman, 2000; Lericolais et al., 2007). The Neouxinian lake (present-day Black Sea) has thus been colonized by the Mediterranean immigrants due to the gradual increase in salinity (Ryan et al., 1997; Ballard, Coleman, & Rosenberg, 2000; Reid & Orlova, 2002; Cristescu, Hebert, & Onciu, 2003; Boissin et al., 2016) following this reconnection of the Black Sea to Mediterranean Sea. The separation time calculated based on the genetic distances between the Mediterranean and Black Sea populations of *S. flexuosa*, coincided with the latest gradual re-colonization process of the Black Sea by marine organisms that took place since about 7 kya (Ryan et al., 1997). For the last 10,000 years, marine organisms have invaded the Black Sea, and it is clear that this immigration still continues. Moreover, a slight increase in the salinity of the Black Sea has been observed in recent years, resulting in the invasion of several marine species, a process known as "mediterranization" (Gomoiu, 1981; Cvetkov & Marinov, 1986) of the Black Sea fauna.

Acknowledgments

Thanks to Caglar Oruc from IKCU for the map design. This work was funded in part by the scientific research funds of Recep Tayyip Erdogan University (RTEÜ BAP, Project No: 2014.103.01.04).

References

Altiok, H., Sur, H.İ., & Yuce, H. (2012). Variation of the cold intermediate water in the Black Sea exit of the Strait of Istanbul (Bosphorus) and its transfer through the strait. *Oceanologia*, 54, 233-254. http://dx.doi.org/10.5697/oc.54-2.233.

Ballard, R.D., Coleman, D.F., & Rosenberg, G.D. (2000). Further evidence of abrupt Holocene drowning of the Black Sea shelf. *Mar. Geol*., 170, 253-261. http://dx.doi.org/10.1016/S0025-3227(00)00108-0.

Bandelt, H.J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.*, 16, 37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Bargelloni, L., Alarcon, J.A., Alvarez, M.C., Penzo, E., Magoulas, A., Reis, C., & Patarnello, T. (2003). Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic-Mediterranean divide. *J. Evol. Biol*., 16, 1149-1158. https://doi.org/10.1046/j.1420-9101.2003.00620.x

Bektas, Y., & Belduz, A.O. (2008). Molecular phylogeny of Turkish *Trachurus* species (Perciformes: Carangidae) inferred from mitochondrial DNA analyses. *J. Fish Biol*., 73(5), 1228-1248. https://doi.org/10.1111/j.1095-8649.2008.01996.x

Boissin, E., Micu, D., Janczyszyn-Le, G.M., Neglia, V., Bat L., Todorova, V., ... Planes, S. (2016). "Contemporary genetic structure and postglacial demographic history of the black scorpionfish, *Scorpaena porcus*, in the Mediterranean and the Black Seas.". *Mol. Ecol*., 25, 2195-209. https://doi.org/10.1111/mec.13616.

Chiba, S.N., Iwatsuki, Y., Yoshino, T., & Hanzawa, N. (2009). Comprehensive phylogeny of the family Sparidae (Perciformes: Teleostei) inferred from mitochondrial gene analyses. *Genes and Genetic Syst*., 84, 153-170. http://doi.org/10.1266/ggs.84.153

Cristescu, M.E.A., Hebert, P.D.N., & Onciu, T.M. (2003). Phylogeography of Ponto-Caspian crustaceans: a benthic-planktonic comparison. *Mol. Ecol*., 12, 985–996. https://doi.org/10.1111/mec.13616.10.1046/j.1365-294X.2003.01801.x

Cvetkov, L.P., & Marinov, T.M. (1986). Faunistic enrichment of the Black Sea and changes in its benthic ecosystems. *Hidrobiyologiya*, 27, 3-21.

Debes, P. V., Zachos, F. E., & Hanel, R. (2008). Mitochondrial phylogeography of the European sprat (*Sprattus sprattus* L., Clupeidae) reveals isolated climatically vulnerable populations in the Mediterranean Sea and range expansion in the

Northeast Atlantic. *Mol. Ecol*., 17, 3873-3888. https://doi.org/10.1111/j.1365-294X.2008.03872.x

Eschmeyer, W.N. (2013). Catalog of Fishes. California Academy of Sciences (http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version 25 March 2013.

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 13, 479-491. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Res*., 10 (3), 564-567. https://doi/10.1111/j.1755-0998.2010.02847.x

Faria, R., Weiss, S., & Alexandrino, P. (2012). Comparative phylogeography and demographic history of European shads (*Alosa alosa* and *A. fallax*) inferred from mitochondrial DNA. *BMC Evol. Biol*., 12, 194. https://doi.org/10.1186/1471-2148-12-194.

Fischer, W., Bauchet M.L., & Schneider, M. (1987). Fishes FAO identification des especes pour les besoins de la peche. Mediterrance et Mer Noire. Zone de peche 37 vol II. Vertebres FAO&C.E.E. project. FAO. 761-1530.

Francour, P., Pollard, D., Bizsel, C., Kara, M.H., Yokes, B., & Goren, M. (2011). *Spicara smaris*. The IUCN Red List of Threatened Species 2011: e.T170283A6736201. Downloaded on 07 October 2016.

Froese, R., & Pauly, D. (Eds). (2017). FishBase. World Wide Web electronic publication. www.fishbase.org, version (accessed February 2017).

Georgiadis, A., Sandaltzopoulos, R., Stergiou, K.I., & Apostolidis, A.P. (2014). Melt-curve-multiplex-haplotype-specific-PCR, a valuable tool for biological studies: Application in congeneric species discrimination assay. *Biochem. Syst. Ecol*., 56, 271-277. http://dx.doi.org/10.1016/j.bse.2014.07.013

Golani, D., Ozturk, B., & Basusta, N. (2006). Centracanthidae. In Fishes of the Eastern Mediterranean (Golani, D., Ozturk, B. & Basusta, N., eds), pp. 168–169. Istanbul: Turkish Marine Research Foundation.

Gomoui, M.T. (1981). Distribution of *Mya arenaria* L. populations in the Western part of the Black Sea. Cercet. Mar. IRCM. 14, 145-158.

Grant, W.A.S., & Bowen, B.W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. J. Hered. 89, 415–426. https://doi.org/10.1093/jhered/89.5.415

Grant, W.S. (2005). A second look at mitochondrial DNA variability in European anchovy (*Engraulis encrasicolus*): assessing model of population structure and the Black Sea isolation hypothesis. *Genetica*, 125, 293 –309. https://doi.org/10.1007/s10709-005-0717-z

Grigor'ev, A.V., Isagulova, E.Z., & Fedorov, P.V. (1984). Chetvertichnaia sistema. (Quaternary system). In: Shnyukov, E.F. (Ed.), Geologiia shel'fa USSR: Stratigrafiia. Shel'f i poberezh'ia Chernogo moria (Geology of the Ukrainian Shelf: Stratigraphy. Shelf and Coast of the Black Sea). Naukova Dumka, Kiev, pp. 153-166 (in Russian).

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol*., 52, 696–704. https://doi.org/10.1080/10635150390235520

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hanel, R., & Sturmbauer, C. (2000). Multiple recurrent evolution of trophic types in northeastern Atlantic and Mediterranean seabreams (Sparidae, Percoidei). *J. Mol. Evol*., 50, 276-283. https://doi.org/10.1007/s002399910032.

Hewitt, G.M. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405, 907-913. https://doi.org/10.1038/35016000.

Imsiridou, A., Minos, G., Gakopoulou, A., Katsares, V., & Karidas, T. (2011). Discrimination of two picarel species *Spicara flexuosa* and *Spicara maena* (Pisces: Centracanthidae) based on mitochondrial DNA sequences. *J. Fish Biol*., 78 (1), 373-377. https://doi.org/10.1111/j.1095-8649.2010.02858.x.

Infante, C., Catanese, G., & Manchado, M. (2004). Phylogenetic relationships among ten sole species (Soleidae, Pleuronectiformes) from the Gulf of Cadiz (Spain) based on mitochondrial DNA sequences. *Mar. Biotechnol*., 6, 612–624. https://doi.org/10.1007/s10126-004-3081-6

Junhong, X. (2007). Identification standard of Sparid species based on cytochrome b sequences. South *China Fish. Sci.*, 3(1), 37-43.

Karidas, T., Argiridis, N., & Minos, G. (2009). Sex identification on hermaphrodite picarel *Spicara flexuosa* (Rafinesque, 1810) based on external characteristics and length frequency analysis. p. 128-129. In: Proceedings of the 31st Scientific Conference of Hellenic Association for Biological Sciences, Patra, 14-16 May 2009. (In Greek and English).

Kimura, M. (1980). "A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences". *J. Mol. Evol*., 16(2), 111–120. https://doi.org/10.1007/BF01731581

Lambeck, K., & Purcell, A. (2005). Sea-level change in the Mediterranean Sea since the LGM: model predictions for tectonically stable areas. Quaternary Science Reviews 24, 1969-1988. http://dx.doi.org/10.1016/j.quascirev.2004.06.025

Leppäkoski, E. (1991). Introduced species - resource or threat in brackish-water seas? Examples from the Baltic and the Black Sea. Mar. Poll. Bull. 23, 219–223, http://dx.doi.org/ 10.1016/0025-326X(91)90678-L

Lericolais, G., Bulois, C. & Gillet, H. (2006). Forced Regression deposits shaped before the Black Sea Flood. Pseudo-3d very high resolution seismic data interpretation, European Geosciences Union, General Assembly 2006, Vienna (AT), N° CL038-1FR4O-003, Paper N° EGU06-A-01686.

Lericolais, G., Popescu, I., Guichard, F., Popescu, S.M., & Manolukakis, L. (2007). Water level fluctuation in the Black Sea since the Last Glacial Maximum, in The Black Sea Flood Question: Changes in

Coastline, Climate, and Settlement, edited by V. Yanko-Hombach et al., pp. 437–452, Springer, New York. http://dx.doi.org/10.1007/978-1-4020-5302-3

Lericolais, G., Bulois, C., Gillet, H., & Guichard, F. (2009). High frequency sea level fluctuations recorded in the Black Sea since the LGM. *Global Planet. Change*, 66(1-2), 65-75. https://doi.org/10.1016/j.gloplacha.2008.03.010.

Lleonart, J. (2008). Review of the state of Mediterranean and Black Sea fishery resources. In: Basurco B. (ed.). The Mediterranean fisheries sector. A reference publication for the VII meeting of Ministers of agriculture and fisheries of CIHEAM member countries (Zaragoza, Spain, 4 February 2008). Zaragoza: CIHEAM / FAO / GFCM, p. 57-69. (Options Méditerranéennes: Série B. Etudes et Recherches; n. 62). 7. Meeting of Ministers of Agriculture and Fisheries of CIHEAM Member Countries, 2008/02/04, Zaragoza (Spain). http://om.ciheam.org/om/pdf/b62/00800737.pdf.

McCusker, M.R., & Bentzen, P. (2010). Positive relationships between genetic diversity and abundance in fishes. *Mol. Ecol.*, 19, 4852–4862. https://doi.org/10.1111/j.1365-294X.2010.04822.x.

Minos, G., Imsiridou, A., & Katselis, G. (2013). Use of morphological differences for the identification of two picarel species *Spicara flexuosa* and *Spicara maena* (Pisces: Centracanthidae). *Mediterr. Mar. Sci*., 14, 26–31 http://dx.doi.org/10.12681/mms.423.

Mudie, P. J., Rochon, A., Aksu, A. E., & Gillespie, H. (2002). Dinoflagellate cysts, freshwater algae and fungal spores as salinity indicators in Late Quaternary cores from Marmara and Black seas. *Mar. Geol.*, 190, 203–231. http://dx.doi.org/10.1016/S0025-3227(02)00348-1

Nelson, J.S. (1994). Fishes of the World. Third Edition. John Wiley & Sons, Inc., New York. 600.

Nevesskaya, L.A. (1963). Guide to the identification of bivalves from Quaternary marine sediments of the Black Sea). Trudy Paleontologicheskogo Instituta Akademii Nauk SSSR [Transactions of the Paleontological Institute, Academy of Sciences USSR] Moscow, 96, 211 pp (in Russian).

Nevesskaya, L.A. (1965). Pozdnechetvertichniye Dvustvorchatie Molluski Chernogo Morya. *ikh Sistematika i Ecologia*. (Late Quaternary Bivalvia of the Black Sea, their Systematics and Ecology). Nauka, Moscow (in Russian).

Oguz, T., Özsoy, E., Latif, M.A., Sur H.I., & Ünlüata, Ü. (1990). Modeling of hydraulically controlled exchange flow in the Bosphorus Strait. *J. Phy. Oce*., 20, 945-965. http://dx.doi.org/10.1175/1520-0485(1990)020<0945:MOHCEF>2.0.CO;2.

Orrell, T.M., Carpenter, K.E., Musick, J.A., & Graves, J.E. (2002). Phylogenetic and biogeographic analysis of the Sparidae (Perciformes: Percoidei) from cytochrome b sequences. *Copeia*. 618-631. https://doi.org/10.1643/0045-8511(2002)002[0618:PABAOT]2.0.CO;2.

Orrell, T.M., & Carpenter, K.E. (2004). A phylogeny of the fish family Sparidae (porgies) inferred from mitochondrial sequence data. *Mol. Phylogenet. Evol.*, 32, 425-434. http://dx.doi.org/10.1016/j.ympev.2004.01.012.

Panin, N., & Popescu, I. (2007). The northwestern Black Bea: climatic and sea levelchanges in the Upper Quaternary. In: Yanko-Hombach, V., Gilbert, A.S., Panin, N., Dolukhanov, P.M. (Eds.), The Black Sea Flood Question: Changes in Coastline, Climate, and Human Settlement. Springer, NewYork, pp. 387-404.

Papakonstantinou, C. (1988). Checklist of marine fishes of Greece. *National Centre for Marine Research, Hellenic Zoological Society*, Athens, 257.

Pardo, B.G., Machordom, A., Foresti, F., Porto-Foresti, F., Azevedo, M.F.C., Banon, R., ... Martinez, P. (2005). Phylogenetic analysis of flatfish (order Pleuronectiformes) based on mitochondrial 16S rDNA sequences. *Sci. Mar*., 69, 531–543.

Pollard, D.A., & Pichot, P. (1971). The systematic status of the Mediterranean fishes of the genus *Spicara* and in particular *S. chrselis* (Val.) as indicated by electrophoretic studies of their eye-lens proteins. *J. Biol.*, 3, 59-72.

Popescu, I., Lericolais, G., Panin, N., Normand, A., Dinu, C., & Le Drezen, E. (2004), The Danube Submarine Canyon (Black Sea): Morphology and sedimentary processes. *Marine Geol*., 206, 249–265. https://doi.org/10.1016/j.margeo.2004.03.003

Posada, D. (2008). jModelTest: phylogenetic model averaging. *Mol. Biol. Evol*., 25, 1253–1256. https://doi.org/10.1093/molbev/msn083

Reid, D.F., & Orlova, M.I. (2002). Geological and evolutionary underpinnings for the success of Ponto-Caspian species invasions in the Baltic Sea and North American Great Lakes. *Can. J. Fish. Aquat. Sci*., 59, 1144–1158. https://doi.org/10.1139/F02-099.

Richardson, L.R., & Gold, J.R. (1997). Mitochondrial DNA diversity in and population structure of red grouper, *Epinephelus morio*, from the Gulf of Mexico. *Fish. Bull. U.S*., 95, 174–179.

Rizkalla, S.I. (1996). A comparative study on the morphometric characters of fishes belonging to family: Centracanthidae in the Egyptian Mediterranean waters. *J. King Abdulaziz Univ. Mar. Sci*., 7 (1), 255-261.

Rozas, J. (2009). DNA Sequence Polymorphism Analysis using DnaSP. 337-350. https://doi.org/10.1007/978-1-59745-251-9_17.

Ryan, W.B.F., Pitman III, W.C., Major, C.O., Shimkus, K., Moskalenko, V., Jones, G., ... Yuce, H. (1997). An abrupt drowning of the Black Sea shelf. *Marine Geol*., 138, 119-126. https://doi.org/10.1016/S0025-3227(97)00007-8

Ryan, W.B., & Pitman, W.C. (2000). Noah's Flood: The new scientific discoveries about the event that changed history, Simon & Schuster, ISBN 0-684-85920-3.

Ryan, W.B.F., Major, C.O., Lericolais, G., & Goldstein, S.L. (2003). Catastrophic flooding of the Black Sea. *Annu. Rev. Earth Pl. Sc*., 31, 525–554. https://doi.org/10.1146/annurev.earth.31.100901.141249

Swofford, D.L. (2003). PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods) Version 4. Sinauer Associates; Sunderland, MA. https://doi.org/10.1111/j.0014-3820.2002.tb00191.x

Topper, R.P.M., & Meijer, P.Th. (2015). Changes in Mediterranean circulation and water characteristics due to restriction of the Atlantic connection: a high-resolution ocean model. *Clim. Past*., 11, 233-251. https://doi.org/10.5194/cp-11-233-2015.

Tortonese, E. (1975). Fauna d'Italia, Vol. 11. Bologna:

Edizioni Calderini.

Tortonese, E. (1986). Carangidae in Fishes of the North-Eastern Atlantic and the Mediterranean. In: P.J.P. Whitehead, ML., Bauchot, JC., Hureau, Nielsen J. and Tortonese E. (eds.) Unesco, Paris. 908-911.

Turan, C. (2011). The systematic status of the Mediterranean Spicara species (Centracanthidae) inferred from mitochondrial 16S rDNA sequence and morphological data. *J. Black Sea Mediterranean Environment*, 17 (1), 14-31.

Vasileva, E.D. (2007). Fish of the Black Sea. Key to Marine, Brackish-water, Euryhaline, and Anadromous Species With Color Illustrations, Collected by S. V. Bogorodsky. Moscow: VNIRO Publishing (in Russian, with English abstract).

Vasiliev, V.P. (1980). The selective mortality of different chromosome morphs in *Spicara flexuosa* Raf. *Genetica*, 52/53 (1), 327-331.

Vidalis, K., Markakis, G., & Tsimenides, N. (1997). Discrimination between populations of picarel (*Spicara smaris* L., 1758) in the Aegean Sea, using multivariate analysis of phenetic characters. Fish. *Res.*, 30(3), 191-197. https://doi.org/10.1016/S0165-7836(96)00571-1

Wang, L., Meng, Z., Liu, X., Zhang, Y., & Lin, H. (2011). Genetic diversity and differentiation of the orange-spotted grouper (*Epinephelus coioides*) between and within cultured stocks and wild populations inferred from microsatellite DNA analysis. *Int. J. Mol. Sci*., 12(7), 4378-94. https://doi.org/10.3390/ijms12074378.

Wilson, A.B. (2006). Genetic signature of recent glaciation on populations of a near-shore marine fish species (*Syngnathus leptorhynchus*). *Mol. Ecol*., 15, 1857–71. https://doi.org/10.1111/j.1365-294X.2006.02911.x

Wilson, A.B., & Veragut, I.E. (2010). The impact of Pleistocene glaciation across the range of a widespread European coastal species. *Mol. Ecol*., 19, 4535–4553. https://doi.org/10.1111/j.1365-294X.2010.04811.x.

Zei, M. (1941). Studies on the morphology and taxonomy of the Adriatic species of Maenidae. *Acta Adriat*., 2, 1–189.

Zei, M. (1949). Typical sex reversal in Teleosts. *Proc. Zool. Soc*., 119, 1920–1971.