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Genetic and morphological impact of the cultured gilthead sea bream (*Sparus aurata* Linnaeus, 1758) populations on wild stocks

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ABSTRACT

Sparus aurata is a very important fish, in fisheries and aquaculture. In this study, the genetic impact of cultured gilthead sea bream *S. aurata* was examined on wild stocks in the Iskenderun Bay (Northeastern Mediterranean) by genetic and morphological analyzes. Morphometric traits of gilthead sea bream were investigated using a traditional set of measurements and network system. In total, 91 morphometric characters were measured for the Biomorph v3 of measurements and 14 for the truss network system. Results of discriminant function analysis (DF), three functions were produced, and the first two discriminant functions (DFs) explained 90.1% of between-group variability and plotting DF1 (55.2%) and DF2 (34.8%). Mitochondrial DNA sequencing of 16S rRNA region was found to be 830 base pair and 21 bp variable and 3 bp parsimony informative sites between populations. The nucleotide composition was found to be 21.1%, 24.1%, 32.9% and 21.1% for T, C, A and G, respectively. The average value of genetic diversity and genetic distance within populations was found to be 0.00316 and -0.00088, respectively. Haplotype diversity of the cultured population was found to be 0.6710 while the wild population was found to be 0.8333. In the present study, the morphological and molecular analysis identified cultured fish escapees in the wild data set sampled in the aquaculture sites. Despite a mixed gene pool in the wild population, as a result of a long-term culture period of fish originating from Mediterranean broodstocks, the molecular genetics tools identified potential cultured escapees.

INTRODUCTION

Gilthead seabream, *Sparus aurata* (Linnaeus, 1758) is common along the coasts of the eastern Atlantic Ocean, the British Isles, Canary Islands and throughout the Mediterranean Sea **(Bartley, 2006; Rossi *et al.* 2006; Froese and Pauly, 2021)**.

The family Sparidae exhibits a variety of mechanisms for sex determination. There are both gonochoristic species and a hermaphrodite. The latter are sequential hermaphrodites and include species that are protandrous hermaphrodites. *S. aurata* most individuals revert to female sex by 2 years of age, through development and maturation of the ovarian portion of the gonad and regression of the testicular region (**Franch *et al.* 2006**). Gilthead seabream production in the world reached 201.502 tons by aquaculture

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(FishStatJ, 2015). In our country, total *S. aurata* production reached 99.730 tons as of 2019 (**TUİK, 2021**). 29 fish farms breed gilthead sea bream in our country that is mainly distributed in the Aegean Sea and the Mediterranean Sea.

The distribution of wild and cultured *S. aurata* populations is needed to establish appropriate guidelines for establishing and maintaining culture populations. The effective size of founder populations is due to the constraints of agriculture, resulting in few individuals being used as breeding material. This practice can lead to erosion of the genetic diversity of the stocks, thus affecting industrial performance (**Alarcon *et al.* 2004**). Because of its special properties, the analysis of mitochondrial DNA (mtDNA) is a very useful marker for molecular genetic studies **(Meyer *et al.* 1990; Billington and Hebert, 1991)**. Therefore, mtDNA variation for the identification of potential genetic differentiation of fish species has been widely investigated **(Papasotiropoulos *et al.* 2002; Turan, 2015; Uyan and Turan, 2017)**. mtDNA can be used as a dominant technique in few sampling studies in which genetic variation, genetic differences and genetic influences on wild stocks of genetic stocks such as alloenzyme and microsatellite molecular dominant techniques **(Iguchi *et al.* 1999; Sekino *et al.* 2002; Romana-Eguia *et al.* 2004)**. The utility of the mitochondrial DNA (mtDNA), especially the 16S ribosomal RNA (16s rRNA) and control region (D-Loop) as a marker has been widely recognized **(Meyer, 1994; Avise, 2012)**.

In this study, we determined the genetic impact of cultured gilthead sea bream *S. aurata* on wild stocks in the Iskenderun Bay (northeastern Mediterranean) by mtDNA sequencing analysis using 16S rRNA region and morphological network system.

MATERIALS AND METHODS

1. Sampling Area

Gilthead seabream, *S. aurata* wild samples were collected from Iskenderun (36°37'23.0" N -36°05'00.9" E) and Muğla Bay (37°15'39.1" N - 27°21'41.7"E) using commercial fishing trawler. Thirty samples were collected from each region. Cultured population samples thirty samples for each population were collected from the Mazman Culture offshore cage in the Iskenderun Bay (36°30'34.5" N - 35°49'12.1"E) and Kılıç seafood market in the Muğla-Milas. Samples were placed in plastic bags and kept frozen (-20℃) until transport to the laboratory.

2. Morphological Analysis

Morphometric analyses of gilthead sea bream were studied using a traditional set of measurements and a grid system **(Uyan and Turan, 2017)**. A total of 19 morphometric characters (Table 1) were measured using the Biomorph v3 of measurements and 14 using the truss network system (Figure 1). This combined method was used to ensure maximum extraction of morphometric differences between wild and cultured individuals.

Table 1. Truss network point and traditional set of measurements of *S. aurata* populations

Code	Vector	Truss Point	Character
1_2	Nape Length	**1**	Tip of premaxillary
1_3	Pre-Dorsal Length	**2**	Nape
1_4	Post-Dorsal Length	**3**	Anterior insertion of dorsal fin
1_6	Standard Length	**4**	Posterior insertion of dorsal fin
1_8	Post-Anal Length	**5**	Dorsal point at least depth of caudal peduncle
1_9	Pre-Anal Length	**6**	The posterior extremity of the lateral line
1_10	Pre-Pelvic Length	**7**	Ventral point at least depth of caudal peduncle
1_11	Pre-Pectoral Length	**8**	Posterior insertion of anal fin
1_13	Pre-Orbital Length	**9**	Anterior insertion of anal fin
3_4	Dorsal fin base length	**10**	Anterior insertion of pelvic fin
3_10	Maximum body height	**11**	Operculum
5_7	Caudal peduncle width	**12**	Dorsal insertion of pectoral fin
8_9	Anal fin base length	**13**	The beginning of eye
12_14	Postocular distance	**14**	The end of the eye
13_14	Eye diameter		
TL	Total Length		
HL	Head Length		
D	Height of dorsal fin		
h	Minimum body height		

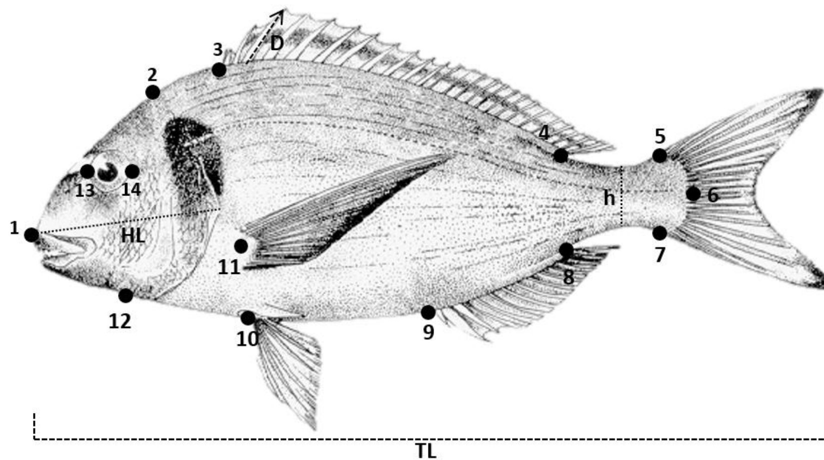

Figure 1. Locations of the 14 markers used to construct the truss network on S. aurata picture as black dots and morphometric distance measures.

The photos from the samples have been obtained with a high-quality digital camera. The truss distances at the photos have been analyzed the usage of BioMorph Pro 3.1.15 **(Kutlu and Turan, 2018)** the image processing tool specially developed for morphometric measurements and analysis of populations.

The variability in the morphological characters is due to size (**Turan, 1999**). For that reason, analysis of shape should be free from the effect of size to avoid misreading of results **(Strauss and Bookstein, 1982)**. Principal component analysis (PCA) was used to remove the effect of shape mass. This technique removes the first component as an isometric size factor so that subsequent components can be interpreted as a summary of shape variation dependent on size and variation among the individuals studied. As a result of PCA, analyses were used in discriminant function analysis (DFA) using SPSS (v20.0). DFA combines a selection of body measures in a linear fashion to produce a mathematical function that can be used to classify species. Species were assigned to groups using the DFA and the percentage of fish correctly assigned was an additional measure of variation between stocks.

3. Genetic Analysis of 16s rRNA

mtDNA was extracted from the fish muscle according to **Sambrook *et al.* (1989)**. PCR amplification using to 16s rRNA gene region was conducted. Using the following universal primers:

16S rRNA-A: 5'-CG (CT) AAG GGA A (ACT) G CTG AAA-3'

16S rRNA-B: 5'-CCG GTC TGA ACT CAG ATC ACG TAG-3'

PCR was at using a volume of 50 ml containing 2 mM of each primer, 10 mM dNTPs, 25 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCI and 2 µl template DNA (10-25 ng) with 5 units of Taq polymerase. The amplification was implemented with as; 1 cycle of denaturation at 92ºC for 5 min, 35 cycles of strand denaturation at 95ºC for 1 min, annealing at52.5ºC for 1 min and primer extensions 70ºC for 1 min 30 s, and 1cycle of final elongation at 70º C for 5 min.

4. Sequence Alignment and Genetic Analysis

The partial 16S rRNA nucleotide sequences were made straight using BioEdit **(Hall, 1999)**. The mtDNA sequence data were analyzed to assess the level of pairwise nucleotide variation and to determine the nucleotide composition for each population using MEGA X (**Kumar *et al.*, 2018).** Model Test **(Posada and Crandall, 1998)** pointed out that the best model for nucleotide substitution and genetic diversity is the analyses of the Jukes-Cantor model (**Jukes and Cantor, 1969**). Haplotype diversity of populations was analyzed using DnaSP 6 (**Rozas *et al.*, 2017**).

RESULTS

1. Morphological Results

In the principal components analysis (PCA) containing the percentage of the total variance of all variables, the allometric size factor was presented and excluded from the analyses, and the subsequent components were used in the DFA. Pooled correlations within groups between discriminant variables and discriminant functions (DFs) showed that morphometric traits, i.e., predorsal length, standard length, preanal length, and prepectoral length, were more efficient in discriminating populations.

In the discriminant function analysis (DFA), three functions were created, and the first two discriminant functions (DF) explained 90.1% of the variability between groups, drawing DF1 (55.2%) and DF2 (34.8%) (Table 2). In figure 2 showed a clear differentiation between wild and cultivated populations of Iskenderun. However, it was observed that the wild and cultivated populations of Mugla were intertwined.

Table 2. Discriminant function analysis scores

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	16.539[a]	55.2	55.2	0.971
2	10.433[a]	34.8	90.1	0.955
3	2.971[a]	9.9	100.0	0.865

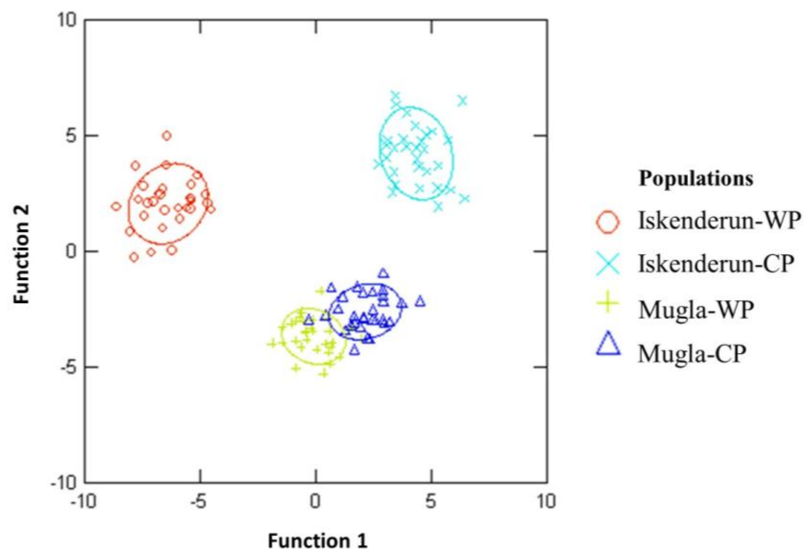

Figure 2. Distribution of characters according to relevant contributions to the main components. Morphometric and meristic differentiation of a population of *S. aurata* in the discriminant space.

2. Genetic Analyses of 16s rRNA

DNA extract was successfully obtained only from Iskenderun wild and culture populations. DNA could not be obtained from Muğla samples, it was not included in genetic analysis. After alignment, the partial of 16S rRNA gene sequences consisted of 830 bp. The average nucleotide composition as 21.9 %thymine (T), 24.1 %cytosine (C), 32.9 % adenine (A), and 21.1 % guanine (G) were obtained. Sequence analysis of 16S rRNA revealed 12 different haplotypes (Table 3). Average haplotype diversity between populations was found to be 0.7204. Haplotype diversity of the cultured population was found to be 0.6710 while the wild population was found to be 0.8333. Haploit_1 was found common haplotype of which 13 were in the cultured population and 4 were in the wild population.

Table 3. Distribution and frequency of 16S rRNA haplotypes of *S. aurata* populations.

	Populations	
Haplotypes	**Aquaculture**	**Wild**
Hap_1	13	4
Hap_2	1	-
Hap_3	5	-
Hap_4	1	-
Hap_5	1	-
Hap_6	1	-
Hap_7	1	-
Hap_8	-	1
Hap_9	-	1
Hap_10	-	1
Hap_11	-	1
Hap_12	-	1
Total	22	9

Genetic Distance, Genetic diversity and Haplotype diversity within populations are given in Table 4. Genetic diversity and genetic distance within populations were observed -0.00088 and 0.00316, respectively.

Table 4. Genetic Distance, Genetic diversity and Haplotype diversity within populations of *S. aurata*.

	Population	
	Aquaculture	**Wild**
Genetic Distances	0.00109	0.00526
Genetic Diversity	0.00108	0.00525
Haplotype Diversity	0.6710	0.8333

In the UPGM tree analysis with out-group (LK022698.1) was observed only two samples from the native population different from those of the culture population. The UPGMA tree consisting of two main branches shows the genetic differences of the samples collected under 10 different clusters (Figure 3).

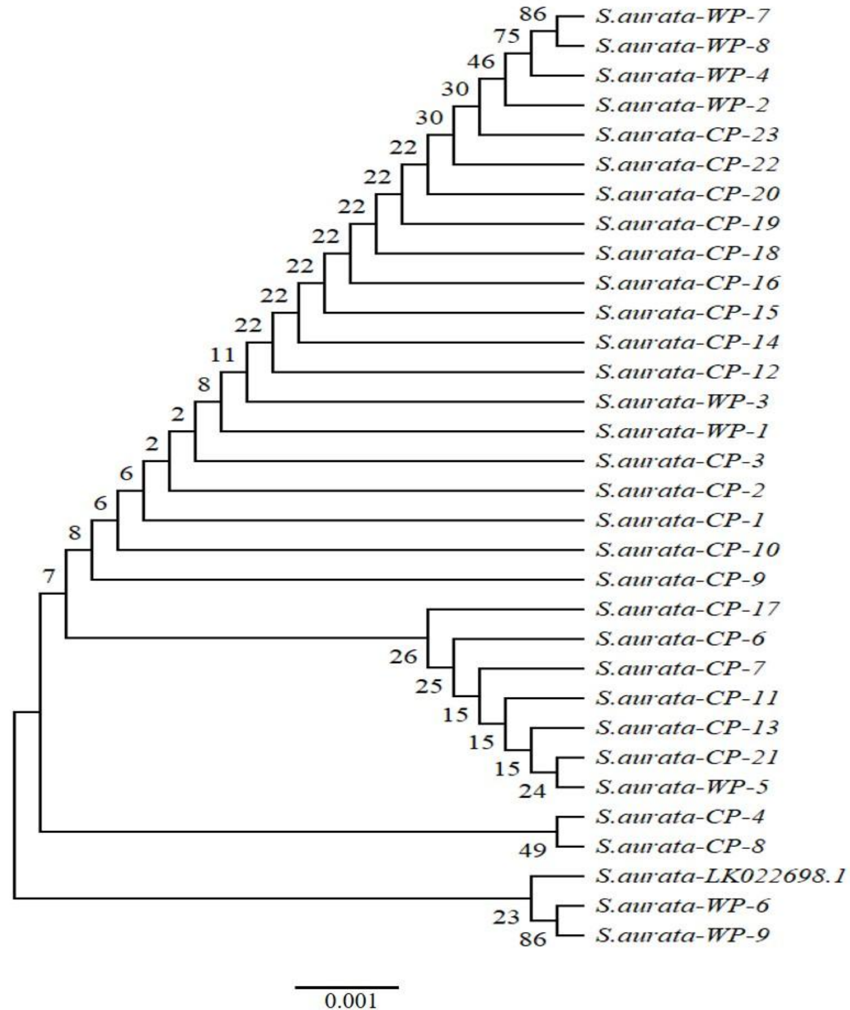

Figure 3. UPGM tree of *S. aurata* samples. Bootstrap values of 1000 replications are shown on nodes in percentages (WP- Wild Population, CP- Cultured Population).

DISCUSSION

In this study, we examined morphological differences of *S. aurata* cultured population on wild stocks in the Iskenderun Bay (northeastern Mediterranean) and Muğla (Aegean Sea) by using Biomorph v3.

Morphological differences between wild and cultured species were significant (t-test, $p < 0.05$). Cultured *S. aurata* were characterized by a body with a head and short fins in comparison to wild specimens, which had an extended body with less body dept **(Šegvić-Bubić *et al.* 2014)**. Biomorph measurements revealed significant differences in pectoral fin sizes, dorsal fin height, body size, anal distance, and body and eye diameters. The truss system identified measurements of pectoral fin length, total length, anal fin length, prepectoral length and caudal peduncle width as those of primary importance in distinguishing wild from cultured gilthead sea bream **(Šegvić-Bubić *et al.* 2014)**.

Morphological characters are influenced by habitat, collection period, broodstock or selective breeding programs applied in a fish farm **(Karaiskou *et al.* 2009, Rogdakis *et al.* 2011)**. Comparative morphology studies between wild and cultured *S. aurata* in the Mediterranean show that the majority of producers aim for a body shape with a higher body height **(Hurtado-Rodriguez *et al.* 2010; Rogdakis *et al.* 2011; Arechavala-Lopez *et al.* 2012)**. The indirect result of selection may be the reduction of anal and trunk length in cultured fish, which consequently leads to a longer caudal fin.

Fin dimensions in the cultured fish are related to their rearing conditions and influence swimming performance (**Basaran *et al.* 2007; Hanson *et al.* 2007**). The volume and hydrodynamics of floating marine cages, stocking density, absence of predators, and daily food availability result in lower use of the fin repertoire.

In this study, head characteristics of wild and cultured gilthead sea bream differed by head profile rather than head length; cultured fish were characterized by a cramped head shape compared to wild gilthead sea bream. Different breeding strains have shown different head shapes throughout the Mediterranean, i.e. with a rounded snout with slight prognathism **(Grigorakis *et al.* 2002)**, a longer head **(Rogdakis *et al.* 2011)** and a smaller snout **(Arechavala-Lopez *et al.* 2012)** compared to wild fish. These forms may be related to different parental phenotypes.

Haplotype diversity studies on wild and culture populations, **Iguchi *et al.* (1999)** analyzed of *Plecoglossus altivelis* haplotype diversity was observed wild populations maximum 1.0 culture populations maximum 0.867. **Sekino *et al.* (2002)** analyzed of *Paralichthys olivaceus* the maximum haplotype diversity of cultural populations 0798, wild populations 0.998. **Coscia *et al.* (2012)** was observed *S. aurata* haplotype diversity as 0.7196 using the mtDNA control region. We have observed the cultured population of

haplotype diversity 0.6710 and wild population of 0.8333. As in the haplotype diversity of cultural stocks, the low number of populations in our population may be due to a limited number of rootstocks in culture populations. A low level of haplotype variation may be indicative of the fact that cultural populations enter the genetic bottleneck. In other marine fish studies with the 16S rRNA gene, the low haplotype diversity may be characteristic of the species and the characteristic of the 16S rRNA gene as well as environmental and human factors (**Palumbi, 1994; Uyan and Turan, 2017**). In future studies, it is necessary to work with different gene regions.

Analysis by Jukes-Cantor model, mean genetic distance values culture and wild populations were found to be 0.00109 and 0.00526, respectively. Genetic diversity of culture population and wild population were observed 0.00108 and 0.00525, respectively. **Wang *et al.* (2011)** studied *Epinephelus coioides* from the South China Sea using the microsatellite technique. They have observed the genetic diversity of cultured and wild population 0.689 and 0.748, respectively. **Loukovitis *et al.* (2014)** reported genetic diversity of the *Dicentrarchus labrax* culture population (0.640) and wild population (0.738) in the Greek and France Seas. **Kumar *et al.* (2016)** also reported the genetic diversity of the culture and wild population of *Lates calcarifer* in the Indian Sea. It was reported of cultured population 0.0010, wild population 0.0052. **Loukovitis *et al.* (2012)** reported the genetic diversity of the culture and wild population of *S. aurata* in Greece. It was reported genetic diversity ranged from 0.5554 to 0.8128 in the cultured stocks.

Mean genetic diversity for the entire populations was found at 0.00229 and mean interpopulation genetic diversity was found at -0.00088. Estimates of genetic divergence between populations were detected 0.00316. **Dogankaya and Bekcan (2012)** studied *S. aurata* culture and wild populations in the Turkish Mediterranean coast using mtDNA 12s rRNA, COII and CytB gene regions and reported that 0.00594 in the 12s rRNA, 0.00358 in the COII and 0.00845 in the CytB region. All results and **Dogankaya and Bekcan (2012)** study showing that the lowest genetic diversity between populations, it is thought that the continuity of populations in the future is a significant signal of danger.

In the study, created of the UPGM tree showing that wild population were intertwined in Iskenderun Bay with cultural populations (Figure 3). Only two samples from the wild population different from the culture population. These two samples may not interfere with the culture population and may be indicative of no gene flow from them.

In the light of all genetic results obtained mtDNA sequence analysis of 16S rRNA with the Gulf of Iskenderun in the cultured population and wild population of sea bream, it can be said that low genetic diversity. The reason for this is thought that the rate of individuals who escaped from the cultivation cages and interfered with the wild populations is high. It is thought that the most important reason for inbreeding for the

genetic diversity in cultural stocks is five times lower than the wild stocks. It is thought that the status of the aquaculture fish stock is very critical because fish production facilities do not perform rootstock resource management, continue to traditional breeding stock management, take rootstock resources from the same region and the annual renewal rate of breeding stocks is low.

CONCLUSION

Morphological and molecular analyses in the present study identified cultured fish escapees in the wild dataset sampled at aquaculture facilities. Despite a mixed gene pool in the wild population as a result of a long-term breeding period of fish derived from Mediterranean broodstocks, the molecular genetic tools identified potential cultured escapees. Morphological data showed that it is not a good indicator for the effect of cultured population on the wild stock. Morphological data shows the potential to discriminate against recently escaped individuals within wild populations, as the method is based on traits influenced by the environment. But, its accuracy can be proven when supported by genetic analysis.

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