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Research Article

Examination of Genetic and Morphologic Structure of Sea-Bass (*Dicentrarchus labrax* L., 1758) Populations in Turkish Coastal Waters

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Abstract: In this study genetic and morphologic structure of the sea-bass, *Dicentrarchus labrax*, in Turkish coastal waters were studied. Total 120 individuals were sampled in equal numbers from the Black, Marmara, Aegean and North-eastern Mediterranean Seas. In genetic analyses, four-enzyme systems (*G3PDH*, ME*, MDH*, PGI**) were assayed, representing 9 loci (*G3PDH-1*, G3PDH-2*, MDH-1*, MDH-2*, MDH-3*, ME*, PGI-1*, PGI-2*, PGI-3**), 2 of which were polymorphic (*G3PDH-2*, PGI-3**). Fisher's exact test revealed that there were no genetic differences between populations using 9 loci. Nei's genetic distance was 0.0001 between the Black Sea and Mediterranean samples. Genetic identity was also found to be 0.9999 between the Black and Mediterranean Sea samples.

In canonical discriminant function analysis, a high degree of morphologic differentiation was detected between populations. Proportions of correctly classified Aegean Sea (100%) and Black Sea (97%) samples to their original group were highest. Plotting discriminant function 1 and discriminant function 2 separated all the populations from each other, showing a high degree of morphometric differentiation among populations.

Key Words: *Dicentrarchus labrax*, stock structure analyses, genetic, morphologic, Turkish coastal waters

Türkiye Denizlerinde Bulunan Deniz Levreği (*Dicentrarchus labrax* L., 1758) Populasyonlarının Genetik ve Morfolojik Yapılarının İncelenmesi

Özet: Bu çalışmada, Türkiye denizlerinde bulunan deniz levreği (*Dicentrarchus labrax*) populasyonlarının genetik ve morfolojik yapısının incelenmesi amaçlanmıştır. Karadeniz, Marmara, Ege ve Kuzeydoğu Akdeniz'den eşit sayıda olmak üzere 120 birey toplanmıştır. Genetik analizde dört enzim sisteminde (*G3PDH*, MDH*, ME*, PGI**) toplam dokuz losi (*G3PDH-1*, G3PDH-2*, MDH-1*, MDH-2*, MDH-3*, ME*, PGI-1*, PGI-2*, PGI-3**) incelenmiş ve bunlardan sadece 2 losi (*G3PDH-2*, PGI-3**) polimorfik olarak bulunmuştur. Dokuz losi kullanarak gerçekleştirilen Fisher'in testi sonucunda populasyonlar arasında genetik bir farklılaşma gözlenmemişti. Karadeniz ve Akdeniz örnekleri arasında Nei'nin genetik mesafe katsayısı 0.0001 olarak bulunmuştur. Genetik benzerlik katsayısı ise yine Karadeniz ve Akdeniz örnekleri arasında 0.9999 olarak bulunmuştur.

Kümelerarası korelasyon analizinde populasyonlar arasında yüksek derecede morfolojik farklılıklar tespit edilmiştir. Kümelerarası korelasyon analizi sonucunda kendi grubuna doğru olarak sınıflandırmada en yüksek, Ege Denizi (% 100) ve Karadeniz (% 97) populasyonları bulunmuştur. Birinci ve ikinci varyasyon değişkenleri grafiklendirildiğinde populasyonlar arasındaki varyasyonun % 99'u ifade edilmiş ve populasyonlar arasındaki farklılığın yüksek derecede olduğu gözlenmiştir.

Anahtar Sözcükler: *Dicentrarchus labrax*, stok yapı analizi, genetik, morfolojik, türkiye kıyısal suları

Introduction

The sea-bass, *Dicentrarchus labrax* L., 1758, has a widespread distribution and ranges from Turkish coasts of the Black Sea, Sea of Marmara, Aegean Sea, Mediterranean and Atlantic coasts from Spain, Portugal, Morocco coast and North Sea, Baltic Sea and North America (1,2). *D. labrax* is an important commercial fish species along the Mediterranean and Atlantic coastline in both for fishing and aquaculture. In order to manage a fishery effectively, it is important to know the identity of stock structure of the species, as each stock must be managed separately to optimise their yield (3). Disregard of stock structure and ineffective fishery management can lead to dramatic changes in the biological attributes

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and the productivity of a species (4). Allozyme electrophoresis is still one of the commonly applied genetic techniques for stock identification within a species (5-9). Morphologic characters as morphometrics and meristics have also been widely used as to delineate stocks (10-14). Although morphometric and meristic characters may be influenced by environmental factors, they can be valuable in indicating stock discreetness (15).

There has been no remarkable study on genetic and morphological structure of *D. labrax* in Turkish coastal waters. Therefore this preliminary study aims to investigate genetic and morphologic structure of *D. labrax* populations throughout the distributional ranges in Turkish coastal waters.

Materials and Methods

In the present study, 30 samples from each population were collected from the South-eastern Black Sea (Trabzon), Sea of Marmara (İstanbul), central Aegean Sea (İzmir) and North-eastern Mediterranean (İskenderun) (Figure 1). Sampling locations, sampling gear, sample size and standard lengths of samples are depicted in Table 1. Samples were placed individually into plastic bags, and kept frozen at –20 ºC until transportation. In the laboratory, muscle tissues of samples were dissected quickly on the lateral line and stored at –80 ºC until examined for allozymes. Morphometric measurement and meristic counts were examined after taking allozyme samples.

Allozyme

Horizontal Starch gel (12%) electrophoresis (16,17) was performed to screen genetic variation. The nomenclature used to designate loci and alleles was that proposed by Shaklee et al. (18). After screening program, four enzyme systems comprising nine putative loci produced well-resolved staining patterns were routinely examined. The enzymes used were glycerol-3-phosphate dehydrogenase (EC 1.1.1.8; *G3PDH**), malate dehydrogenase (EC 1.1.1.37; *MDH**), malic enzyme (EC 1.1.1.40; *ME**), and phosphoglucose isomerase (EC 5.3.1.9; *PGI**). Muscle samples were used for all enzyme systems, which were run using Tris citrate buffer (pH 8). Alleles were scored according to their mobility relative to the most commonly observed allele, which was designated as *100. Allelic frequencies, deviation from the Hardy-Weinberg equilibrium, mean number of alleles per locus, proportion of polymorphic loci at 95% level, observed (Ho) and expected (He) heterozygosity, Nei's genetic distance (D) and genetic identity (I) (19) were calculated using the BIOSYS-1 (Release 1.7, 20) and Popgen32 (21) program packages.

Morphology

The truss network system described for fish body morphometrics (22) was used to construct a network on sea-bass body. Thirteen landmarks determining 29 distances were chosen and measured on the body (Figure 2). Fish were thawed, placed on their right side on acetate sheets, and body posture and fins were teased into a natural position. Each landmark was obtained by piercing the acetate sheet with a dissecting needle,

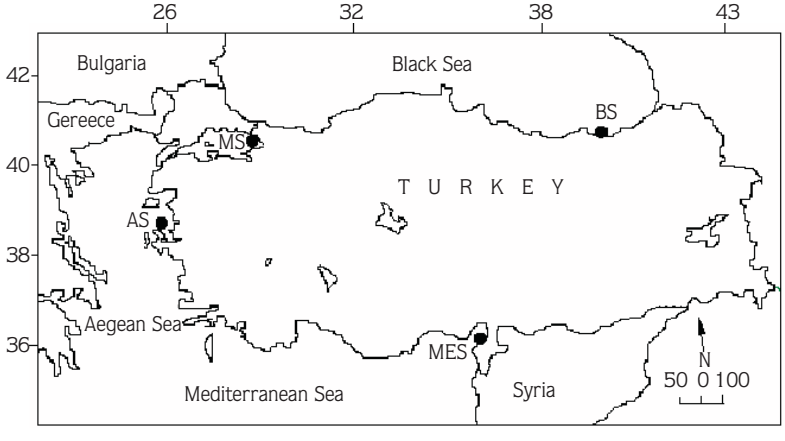

Figure 1. The map of sampling of sea-bass (*Dicentrarchus labrax)*. • sampling locations.

Table 1. Location and biological features of sea-bass populations. Standard deviations of mean standard length (STL) of samples are given in brackets.

Sample	Locations	Collection Date	Gear	Sample Size	Mean STL (cm) (± SD)
Black Sea (BS)	41° 10' N 39° 36' E	04.04.2002	Gill net	30	25.6 (±1.37)
Marmara Sea (MS)	41° 01' N 29° 09' E	20.04.2002	Gill net	30	28.7 (±1.42)
Aegean Sea (AS)	26° 85' N 38° 35' E	30.04.2002	Gill net	30	23.0 (±1.37)
Mediterranean (MES)	36° 35' N 36° 11' E	18.03.2002	Gill net	30	22.0 (±1.61)

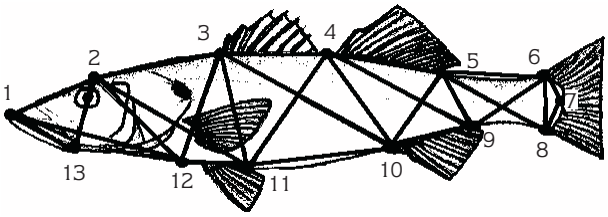

Figure 2. Locations of the 13 landmarks for constructing the truss network on fish. Landmarks (•) refer to (1) anterior tip of snout at upper jaw, (2) most posterior aspect of neurocranium (beginning of scaled nape), (3) origin of dorsal fin, (4) posterior insertion of first dorsal fin and origin of second dorsal fin, (5) insertion of second dorsal fin, (6) anterior attachment of dorsal membrane from caudal fin, (7) posterior end of vertebrae column, (8) anterior attachment of ventral membrane from caudal fin, (9) insertion of anal fin, (10) origin of anal fin, (11) insertion of pelvic fin, (12) origin of pectoral fin, (13) posteriomost point of maxillary.

defining thirteen landmarks (Figure 2). Additional data, such as eye diameter, head width were also recorded. Only undamaged fish were included in the analyses.

Five meristic characters commonly used to describe sea-bass were examined: first and second dorsal fin rays (DFR I and DFR II), ventral fin rays (VFR), anal fin rays (AFR) and pectoral fin rays (PFR). All meristic counts were carried out under a binocular microscope.

Multivariate analyses

Multivariate techniques were used to exhibit pattern of differentiation among samples that simultaneously consider the variation in several characters and thereby assess the similarities between samples. Principal component analysis was used to remove size effect from the shape measures (23). This method extracts a first component as isometric size factor, allowing the subsequent components to be interpreted as summarising shape variation independent of size and random variation among the sampled individuals. The subsequent principal components were used in canonical discriminant function analysis (DFA) using SPSS*v*9.0 and graphs were generated using SYSTAT*v*5.0. Population centroids with 95% confidence ellipses derived from the DFA were used to visualise relationships among the individuals of groups. Individuals were assigned to the samples using the canonical functions. The percentage of correctly assigned fish was an additional measure of differentiation among samples. Univariate analysis of variance (ANOVA) was used to compare the variation among samples for truss measurements and meristic counts.

Results

Allozyme

Allele frequency distributions of each population are shown in Table 2. Only 2 loci were found to be polymorphic, *G3PDH-1** and *PGI-3**, using 95% criterion, and the other loci were monomorphic. Genotypic frequencies were in Hardy-Weinberg equilibrium in all populations.

Estimates of Nei's genetic distance (D) and genetic identity (I) were calculated using the allele frequencies. The Nei's distance was 0.0001 between the Black Sea and Mediterranean populations. Genetic identity was also found to be 0.9999 between the Black Sea and Mediterranean populations. The genetic distances between the other populations were 0. Fisher's exact test revealed no genetic differences between the populations.

Morphology

In principal component analysis (PCA), 37 principal components (PC) which contains percentage of total variance of all variables were produced and 60% of the variation was presented in the first PC, which presents

Table 2. Allele frequencies at polymorphic loci in sea-bass populations. Sample sizes were identical for monomorphic loci.

Loci	Allele	BS	MS	AS	MES
*n		30	30	30	30
MDH-1*	A	1.000	1.000	1.000	1.000
	B				
MDH-2*	A	1.000	1.000	1.000	1.000
	B				
MDH-3*	A	1.000	1.000	1.000	1.000
	B				
G3PDH-1*	A	1.000	1.000	1.000	0.9833
	B				0.0167
G3PDH-2*	A	1.000	1.000	1.000	1.000
	B				
PGI-1*	A	1.000	1.000	1.000	1.000
	B				
PGI-2*	A	1.000	1.000	1.000	1.000
	B				
PGI-3*	A	0.9833	1.000	1.000	1.000
	B	0.0167			
ME*	A	1.000	1.000	1.000	1.000
	B				

*n: Sample size

allometric size factor and was excluded from the analyses. The subsequent components were used in canonical variation analysis and explain 40% of the total variance of all variables.

In DFA, the first DF accounted for 67% and the second (DF2) accounted for 32% of the between-group variability among populations. Plotting DF1 and DF2 explained 99% of the between-group variation and separated all the populations from each other, showing high degree of morphologic differentiation among populations (Figure 3). In DFA, the overall random assignment of individuals into their original population was high (87.5%) (Table 3). The proportions of correctly classified Aegean Sea (100%) and Black Sea (97%) samples to their original group were highest. Moreover, proportions of correctly classified individuals into their

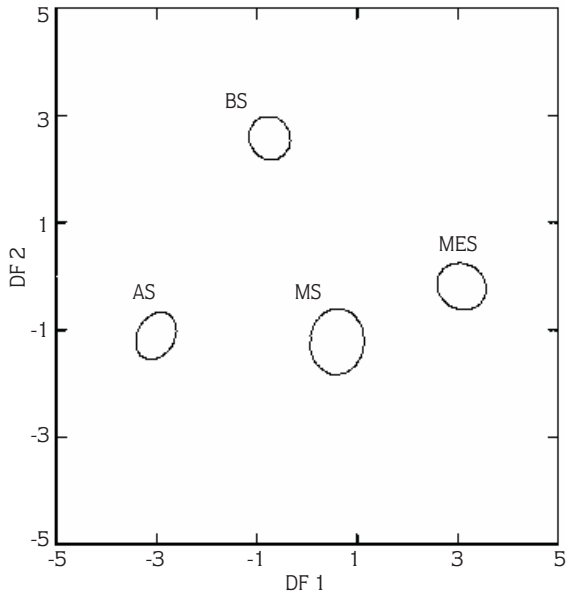

Figure 3. Canonical variation scores of morphologic characters. The distance of each morphometric character from the centre of the components indicates the relative contribution of the character to the geographic separation.

original groups for the Mediterranean and Marmara populations were moderate (83% and 70%, respectively).

Univariate statistics (ANOVA) showed that 27 of 38 truss measurements were significantly different among samples with varying degrees (Table 4).

Discussion

In present study highly morphologic differentiation among *D. labrax* populations was detected. However, this morphologic differentiation was not supported by genetic analysis. It is likely that the apparent genetic homogeneity in the present study arises from high number of monomorphic loci analysed (24,25). Only two loci were found to be polymorphic. If a high number of polymorphic loci had been used in the present study enhanced genetic variation might have been found among the populations. In the present study, 17 enzyme systems were screened, and after screening, four enzymes comprising nine putative loci produced well-resolved staining patterns. Therefore, in future studies, analysing a higher number of polymorphic enzymes would increase genetic heterogeneity among the sea-bass populations, which may support the detected phenotypic

Table 3. Correct classification of individuals (82.5%) into their original populations.

	Sample	BS	MS	AS	MES	Total
Original count	BS	29	0	1	0	30
	MS	2	21	1	6	30
	AS	0	0	30	0	30
	MES	0	5	0	25	30
%	BS	97	0	3	0	100
	MS	7	70	3	20	100
	AS	0	0	100	0	100
	MES	0	17	0	83	100

Table 4. Univariate statistics (ANOVA) testing sex differences between the sea bass samples from all truss and body measurements. Significance levels; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Characters	F	Significance
1_2	1.044	0.309
1_13	8.984	0.003**
2_13	4.400	0.038*
1_12	10.321	0.002**
2_12	15.117	0.000***
13_12	6.531	0.012*
2_3	18.313	0.000***
12_11	2.003	0.160
2_11	11.779	0.001**
3_12	7.039	0.009**
3_11	4.866	0.029*
3_4	6.231	0.014*
4_10	3.099	0.081
11_10	10.260	0.002**
3_10	6.268	0.014*
4_11	8.888	0.003**
4_5	5.068	0.026*
5_9	4.541	0.035*
10_9	3.720	0.056
5_10	5.707	0.018*
4_9	0.481	0.489
5_6	8.664	0.004**
8_9	2.474	0.118
5_8	6.452	0.012*
6_9	5.133	0.025*
6_8	4.218	0.042*
6_7	13.812	0.000***
7_8	14.678	0.000***
1_7	9.118	0.003**
PFL	13.769	0.000***
EY	2.129	0.147
HD	7.501	0.007**
DFR I	4.636	0.033*
DFR II	0.671	0.414
VFR	1.263	0.263
AFR	3.108	0.081
PFR	3.862	0.052

differentiation. Similar studies from different seas have been conducted with allozyme electrophoresis for population structure of sea-bass. A few studies have found genetic differences between populations. Benharrat et al. (26) compared Atlantic and Mediterranean basses using starch-gel electrophoresis and observed low level of genetic differentiation between populations. Child (27) found significant differences in allele frequency only at the PGM locus between sea-bass populations in the Irish Sea. Martinez et al. (25) showed significant genetic differences between populations from Tinamenor (Cantabria) and Puerto Real (Cadiz).

A fish stock can be defined as local population adapted to a particular environment, having some degree of genetic differences from other stocks as a consequence of this adaptation (28,29). Although genetic differences between stocks are a condition of this definition, stock identification has often relied on phenotypic characters rather than direct measures of genetic differentiation. Morphometric and meristic variation was assumed to be purely genetic in early studies (30). At the present it is accepted to have both environmental and genetic components (31). Therefore morphological differences between groups of fish do not necessarily indicate genetic differences. The time may be not enough to generate genetic component of detected morphological differentiation. For that reason, the morphologic differentiation should be taken in consideration for the perpetuation of the stocks and species.

Consequently this preliminary study revealed that sea-bass populations in four different seas were morphologically different which was not genetically supported with genetic analysis. On the other hand, it

should be emphasised that application of more powerful genetic techniques such as mitochondrial and microsatellite DNA (32,33) or use of higher number of polymorphic loci would be very beneficial to support the detected phenotypic differentiation.

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