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Original Article

The morphometric, meristic and genetic characteristics of European sprat (*Sprattus sprattus*) in the Black Sea

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Abstract
The European sprat (*Sprattus sprattus*) represents the unit stock shared among the Black Sea countries. It has a key role in the Black Sea ecosystem and is determined by its importance from both a commercial and an ecological point of view. Up to now, there are few studies on the meristic, morphometric and genetic characteristics of this species. In this study, the species were specified and studied for meristic, morphometric and genetic characteristics. Mitochondrial gene regions which 16S rRNA and cytochrome c oxidase subunit I (COI), were studied and compared with some of the family Clupeidae species in the National Center for Biotechnology Information (NCBI) and the phylogenetic relationship was examined. A total of 14 haplotypes were determined for the mitochondrial COI gene region and 3 haplotypes were determined for the 16S rRNA gene region. This study will contribute to the sustainable management of this species as well as be a basis for future studies.

Keywords: Black Sea; COI; genetics; meristic; morphometric; sprat

1 | INTRODUCTION

The European sprat (*Sprattus sprattus*, Linneaus, 1758) is the second abundant fish species after the anchovy in the Black Sea fisheries (TSI 2022). Ecologically, it provides a flow of energy from planktonic organisms to predators in the food chain (Bat *et al.* 2008). This species is used as raw material in fish meal-oil factories for industrial purposes therefore it is not used for human consumption in the Turkish fish market on the contrary to other countries (Flintegård 1987). European sprat is pelagic-neritic and oceanodromous (Riede 2004) species distributed in the Northeast Atlantic (North Sea, British Isles, Baltic Sea and Morocco), northern Mediterranean (Gulf of Lion and the Adriatic Sea) and Black Sea (Froese and Pauly 2021). It lives in marine to brackish waters as an inshore schooling at 10 – 50 m depth ranges (Muus and Dahlstrøm 1989). It migrates between winter months for feeding and summer months for spawning (Flintegård 1987). European sprat feeds on planktonic crustaceans (Flintegård 1987). The most of the studies were realised on the sprat fishery (Avşar 1994; Zengin *et al.* 2011; Totoiu *et al.* 2016; Balık 2018; Özdemir *et al.* 2018) although there are no studies on the morphometric, meristic and genetic characterisation except of limited information about the family (Akşıray 1955; Whitehead 1985).

Therefore, there is a need for comprehensive information about this species in order to create a well-designed fisheries / stock management. In this study, detailed morphometric, meristic and genetic characteristics were examined for both male and female individuals of European sprat *S. sprattus* from the south-eastern Black Sea.

2 | METHODOLOGY

One hundred and twenty European sprat (60 female and 60 male) individuals were sampled from the Black Sea for morphometric and meristic measurements. The individuals were sampled using mid-water trawl. The specimens were weighed and measured following the measuring scheme shown in Figure 1. All length measurements were made with a digital calliper, to the nearest 1 mm precision. The morphometric results were considered statistically significant at $p < 0.05$. The data were analysed in Microsoft Excel 2019.

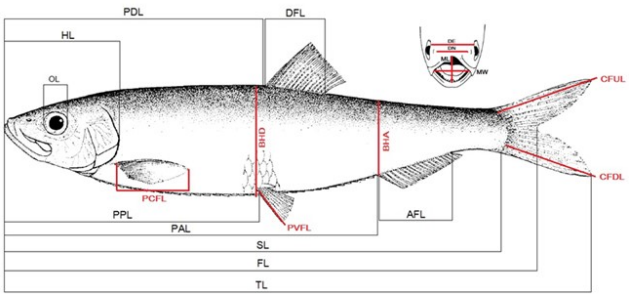

FIGURE 1 Morphometric measurements of *Sprattus sprattus* (modified from Whitehead 1985 by YY Kasapoglu). TL, total length; FL, fork length; SL, standard length; PAL, pre-anal length; PDL, pre-dorsal length; PPL, pre-pelvic length; BHD, dorsal fin based body height; BHA, anal fin based body height; DFL, dorsal fin length; PCFL, pectoral fin length; PVFL, pelvic fin length; AFL, anal fin length; CFUL, caudal fin upper lob length; CFDL, caudal fin down lob length; HL, head length; NL, nostril length; DN, distance between nostrils; OL, orbital length; DE, distance between eyes; ML, mouth length; MW, mouth width.

For genetic studies, the caudal fin samples of species were collected from Trabzon (30 units) and they were preserved in 98% ethanol. Total DNA isolation was performed using the QIAamp 96 DNA QIAcube HT commercial kit (QIAGEN, USA). The estimation of concentration and purity of DNA samples was made using Nanodrop (NanoDrop 8000, USA) by reading its optical density at 260 and 280 nm wavelengths. The mitochondrial DNA markers which are cytochrome c-oxidase subunit I (COI) and 16S rRNA were used as potential markers for species identification and genetic diversity assessment. The Mitochondrial DNA gene regions were amplified using primer pairs U.COI (5′TTCTCAACTAACCAYAAAGAYATYGG3′ and 5′TAGACTTCTGGGTGGCCRAARAAYCA3′) and barcoding 16Sar (5′CGCCTGTTTATCAAAAAACAT3′ and 5′CCGGTCTGAACTCAGATCACGT3′). PCR was prepared as a total volume of 25 µl of reaction solution contained 10 µl of 2X Master mix (Qiagen), 1 µM of each primer (F and R), 90 – 150 ng of DNA and ddH_2O. The cycling conditions were as follows: 94°C for 5 minutes; then 35 cycles at 95°C for 1 minute, at 58°C for 90 seconds and 72°C for 1 minute; the final step 10 minutes at 72°C. PCR was performed at the same conditions for the COI gene and 16S rRNA gene reaction solution.

For the sequencing of all gene regions, the samples were purified with the BigDye v.3.1 Terminator Cycle Sequencing Kit on the ABI 3500 Genetic Analyser (ThermoFisher, USA). The raw sequences of the COI and 16S rRNA regions were arranged and aligned using the ClustalW algorithm (Thompson *et al.* 1994) in BioEdit v.7.2.5 (Hall 1999). Low quality sequences were discarded. Species identification was performed by comparing sequence similarity with the reference dataset (Wong and Hanner 2008). Separation regions, haplotype number, nucleotide diversity (π) and haplotype diversity (Hd) and Tajima-D statistics were used for populations by DnaSP v.5.0 (Librado and Rozas 2009). FST pairwise values and genetic heterogeneity were generated using ARLEQUIN version 5 (Schneider *et al.* 2000). The haplotype distribution was created using the PopART programme (Leigh and Bryant 2015).

3 | RESULTS

The morphometric and meristic characters were estimated for female and male samples shown in Table 1 and 2. The European sprat has 15 – 16 fin rays in the dorsal fin, 16 – 19 fin rays in the pectoral fins, 6 – 8 fin rays in the pelvic fins and 18 – 20 fin rays in the anal fin. The scale numbers in the lateral line varied between 48 and 51; vertebral bones were counted as 46 – 48 and gill rakers were counted as 30 – 41. The European sprat's last two anal fin rays are not enlarged and it is distinguished from other cluponella species by these features. The ratio of all morphometric measurements of females were greater or bigger than males except for the distance between eyes and mouth length (*t*-test; $p < 0.05$).

TABLE 1 Meristic counts of European sprat *Sprattus sprattus* ($n = 120$).

Meristic characters	Number
Vertebral counts	46 – 48
Dorsal fin rays	15 – 16
Anal fin rays	18 – 20
Pectoral fin rays	16 – 19
Pelvic fin rays	6 – 8
Gill rakers	30 – 41
Scale along lateral line	48 – 51

In total, 14 haplotypes were determined for mitochondrial cytochrome c oxidase (COI) gene region and 3 haplotypes for 16S rRNA gene region. They were deposited into NCBI database (COI; OL614340-OL614353. 16S rRNA; OL614146-OL614148). The haplotype diversity and nucleotide diversity indices were calculated as 0.947 and 0.00852 for the mtDNA COI gene region and 0.511 and 0.00357 for the 16S rRNA gene region. A total of 24 variable sites (11 singleton and 13 parsimony informative sites)

for COI and 5 variable sites (2 singleton and 3 parsimony informative sites) for 16S rRNA were detected.

As a result of haplotype network analysis, 146 base differences for COI and 53 bases for 16S rRNA were found between the reference sequences from NCBI and *S. sprattus sequences*. The phylogenetic tree formed by the COI and 16S gene sequences of the species belonging to the Clupeidae family in GenBank was divided into two clades in the southern and northern hemisphere. In this study, sprat samples created with reference sequences were found to be similar to North Sea, Baltic Sea and Black Sea specimens (Figures 2 and 3).

TABLE 2 Morphometric measurements (mm) and statistics of European sprat *Sprattus sprattus* (TL: total length; FL: fork length; SL: standard length; PAL: pre-anal length; PDL: pre-dorsal length; PPL: pre-pelvic length; BHD: dorsal fin based body height; BHA: anal fin based body height; DFL: dorsal fin length; PCFL: pectoral fin length; PVFL: pelvic fin length; AFL: anal fin length; CFUL: caudal fin upper lob length; CFDL: caudal fin down lob length; HL: head length; NL: nostril length; DN: distance between nostrils; OL: orbital length; DE: distance between eyes; ML: mouth length; MW: mouth width).

Measures	Female			Male			*p-value*
	$\bar{x}$ ± SE	Range	TL (%)	$\bar{x}$ ± SE	Range	TL (%)	
TL	100.47±1.819	75.81 – 116.44		86.69±2.639	67.95 – 101		0.008
FL	94.60±1.666	71.56 – 110.51	94.16	82.084±2.396	66.17 – 96	94.69	0.009
SL	85.378±1.541	63.91 – 100.05	84.98	73.590±2.240	58.98 – 85	84.89	0.007
PAL	62.732±1.039	50.83 – 75.55	55.07	53.165±1.743	41.46– 63.24	53.93	0.001
PDL	46.977±0.789	36.75 – 53.15	73.56	39.651±1.176	31.48 – 46.03	72.21	0.001
PPL	47.572±0.933	36.20 – 55.36	55.71	41.167±1.303	31.58 – 48.23	55.95	<0.001
BHD	16.914±0.429	10.74 – 20.07	19.76	13.828±0.636	10.31 – 18.39	18.69	<0.001
BHA	12.784±0.368	8.23 – 15.13	14.92	10.726±0.419	8.33 – 13.43	14.54	0.001
DFL	12.001±0.255	9.38 – 14.48	14.11	10.322±0.351	7.88 – 12.45	14.04	<0.001
PCFL	13.372±0.278	10.86 – 15.82	15.69	11.979±0.419	8.56 – 14.33	16.26	0.007
PVFL	7.781±0.204	5.79 – 10.01	9.13	7.322±0.289	5.40 – 9.32	9.93	0.190
AFL	12.666±0.441	7.47 – 15.53	14.76	11.190±0.535	6.90 – 14.73	15.13	0.041
CFUL	15.409±0.343	11.74 – 19.55	18.04	13.308±0.449	10.84 – 15.94	18.08	0.001
CFDL	15.440±0.339	11.64 – 19.55	18.07	13.303±0.434	10.82 – 15.75	18.08	<0.001
HL	21.014±0.417	15.57 – 24.06	21.01	18.948±0.467	15.80 – 21.47	18.95	0.003
			HL (%)			**HL (%)**	
NL	10.213±0.316	6.81 – 13.06	48.58	8.415±0.452	5.70 – 12.49	44.56	0.002
DN	3.391±0.165	2.32 – 4.84	16.07	2.966±0.162	1.79 – 3.95	15.68	0.089
OL	5.716±0.112	4.64 – 6.75	27.29	5.153±0.195	3.57 – 6.32	27.21	0.011
DE	4.575±0.223	3.15 – 7.89	21.71	4.879±0.342	3.12 – 6.69	25.69	0.441
ML	8.324±0.336	5.46 – 11.68	39.53	8.894±0.373	6.52 – 11.57	46.97	0.276
MW	5.00±0.252	3.61 – 7.50	23.74	4.737±0.234	3.86 – 7.04	25.06	0.475

4 | DISCUSSION

The morphometric and meristic characters in fish are important for the reveal of differences between fish populations / stocks. These characters are affected by environmental factors as well as genotype (İnnal and Katselis 2019). This study demonstrates morphometric, meristic and genetic characteristics of European sprat.

The number of dorsal soft rays and anal soft rays were found as 15 – 16 and 18 – 20 respectively and these values were within the values recorded by Whitehead (1985). The number of pelvic fin rays was found 6 – 8 and it was also similar to Whitehead (1985). Most of the morphometric characters in female were longer than male individuals except for mouth length and distance between eyes. There is no previous study that enabled us to compare our findings. Avşar (1994) reported that, based on the morphometric and meristic discrimination analysis, there is only one sprat stock unit for the Turkish Black Sea Coast.

There is a lack of genetic studies on this species from the Black Sea and the Mediterranean basin. Therefore, we investigated European sprat's genetic characters within the concept of national registration of the fish species by the Ministry of Agriculture and Forestry of Turkish government. Canales-Aguirre *et al.* (2021) studied the origin and monophylesis of the genus *Sprattus* with a phylogenetic approach based on DNA sequences from five mitochondrial genome regions (mtDNA) for species in the Clupedae family. It has been observed that *Sprattus* species is a polyphyletic group, closely related to *S. sprattus* and the genus *Clupea* in the Northern hemisphere, and the rest of the Southern wing *Sprattus* members are

closely related to *Strangomer bentincki* and *Ramnogaster melanostoma*. In our study, we compared them with some Clupediae species found in Turkish waters, using a phylogenetic approach based on DNA sequences from two mtDNA. When the COI data were examined, it was seen that the Black Sea *Sprattus* haplotype is similar to the *Sprattus* haplotypes in the North Sea and Baltic Sea (northern hemisphere clade).

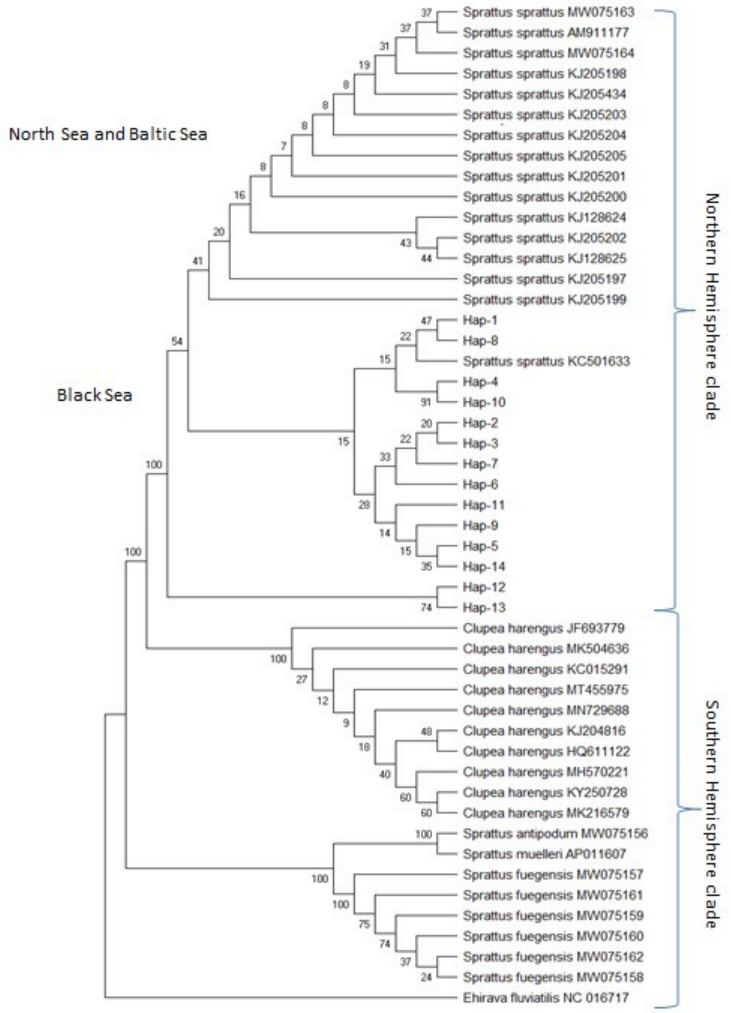

FIGURE 2 Evolutionary history was inferred using the UPGMA method based on COI haplotypes of *Sprattus sprattus* and available reference sequences of *Sprattus fuegensis*, *Sprattus muelleri*, *Sprattus antipodum*, *Clupea harengus* and *Ehireva fluviatilis* from the GenBank database.

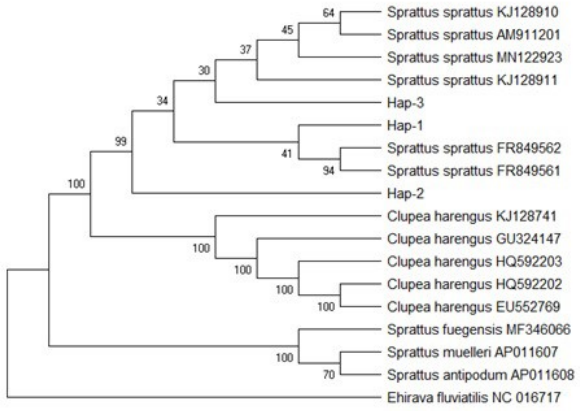

FIGURE 3 Evolutionary history was inferred using the UPGMA method based on 16S rRNA haplotypes of *Sprattus sprattus* and available reference sequences of *Sprattus fuegensis*, *Sprattus muelleri*, *Sprattus antipodum*, *Clupea harengus* and *Ehireva fluviatilis* from the GenBank database.

5 | CONCLUSIONS

There is no descriptive study on the European sprat from the Turkish Black Sea coasts until now. Therefore, this study is the first comprehensive one in terms of morphometric, meristic and genetic characters of the species. The results of this study will contribute to the determination of European sprat traits. In addition to this, it may be used as a base for future studies of this economically and ecologically important species.

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CONFLICT OF INTEREST
The author declares no conflict of interest.

AUTHORS' CONTRIBUTION
All author equally contributed to the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on a reasonable request from the corresponding author.

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