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Research Article

Morphological and genetic characteristics of garfish *Belone belone* (L., 1760) (Belonidae, Teleostei) population from the southern Bulgarian Black Sea coast

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Abstract

This study was conducted to investigate genetic and some morphometric and meristic characteristics of garfish *Belone belone* from Nesebar in the Bulgarian Black Sea coast. Twelve morphometric characters were measured, and six meristic characters were counted for each individual. Based on both sexes' morphological and meristic analyses, no statistically significant sexual differences were observed. Additionally, DNA barcoding was done. The fragment of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA was sequenced to supplement the species identification and population diversity study. Two haplotypes were found out of 39 sequences, indicating a low level of haplotype diversity (0.146±0.072). Nucleotide diversity was also found to be low (0.00023±0.00011). The Nesebar population of *B. belone* requires conservation efforts, due to the highly decreased mtDNA genetic diversity.

Key words: *Belone belone*, Bulgarian Black Sea coast, genetic, meristic analysis, morphometric

Introduction

The Belonidae family, represented by a total of 10 genera and 44 nominal species in marine and freshwater ecosystems, constitutes one of the important groups of the ichthyofauna of the Eastern Atlantic, the Mediterranean and the Black Sea (Fricke et al. 2022; Öztürk 2023). Three subspecies have been recognized (Collette and Parin 1986): *B. belone belone* (Linnaeus, 1760) – restricted to the north-eastern Atlantic, *B. belone gracilis* Lowe, 1839 - distributed from the south of France in the Mediterranean Sea to the Canary Islands in the Atlantic, and *B. belone euxini* Günther, 1866 - which is found in the Black Sea and the Sea of Azov. The garfish *Belone belone euxini* (Gunther, 1866), distributed in the Black Sea and Azov Sea (Zaitsev and Mamaev 1997), is one of the most important pelagic fish species in the Black Sea artisanal fishery. A few studies concerning some aspects of biometric, biological, and electrophoretic analysis (Dobrovolov et al. 1980; Prodanov

1982; Dorman 1988), were carried out for populations that inhabited the Bulgarian Black Sea. In spite of its wide distribution in the Black Sea, the knowledge of biology and ecology of this species is still scarce and for genetics absent.

Morphometrics and meristics characters are the two types of morphologic characters that have been most frequently used to describe populations of exploited marine fish species (Turan 2004; Uyan and Turan 2017). In addition, morphometric parameters of a fish species have a major role to ensure whether there is any disparity between the same species of different geographic regions (Naeem and Salam 2005).

Genetic diversity is the basis of species adaptability and evolution, and there is a positive linear relationship between intraspecific genetic diversity and the adaptability of the species to the environment (Cruz et al. 2013; Turan et al. 2016). Nucleotide diversity and haplotype diversity are important indicators in terms of revealing mtDNA genetic variation in populations (Liu and Zhang 2009; Jiang et al. 2019). Among the most common mitochondrial genes used in detecting genetic diversity and population structure, the mtDNA cytochrome c oxidase subunit I (COI) gene represents useful genetic marker to assess cryptic diversity and population genetic diversity and structure in many fish species (Ivanova et al. 2021; Wei et al. 2023). So far mtDNA COI is mainly used for species identification and phylogeny of *Belone belone* from North Atlantic and Mediterranean (Turkish waters) (Knebelsberger et al. 2014; Turan et al. 2023), and there have been no reports about genetic diversity and population structure of garfish populations based on COI gene. In addition, except for one study of allozymes (Dobrovolov et al. 1980), no other molecular markers have been used in the genetic diversity analysis of different Black Sea populations of *B. belone*. The studies of population genetic structure and genetic diversity could provide guidance for the establishment of fishing quotas to prevent overharvesting (Zhao et al. 2019).

The aim of this paper is to represent for the first time the morphological and genetic diversity of one *Belone belone* population along the Bulgarian Black Sea coast, which as a commercially important fish species could aid in its conservation.

Methods

Morphological study

B. belone specimens were collected in December 2022 from the Bulgarian Black Sea coast (Nesebar) (Fig. 1). A number of 40 garfish individuals were used for morphological study and 39 of them were additionally used for molecular analyses. Biometric measurements were performed using BioMorph (Kutlu and Turan 2018) on the fresh fish. Twelve morphometric and six meristic body characters were examined. The analyzed morphometric and meristic characters are presented in Fig. 2 and in Tables 1, 2, respectively. The total and standard lengths were measured to the nearest 0.1 cm (Fig. 3). The rest of the morphometric characters were measured to the nearest 0.01 mm. Measurements of the head were expressed as percentages of the head length whereas other body measurements were expressed as percentages of the standard length. Statistical analyses were performed with the SPSS 5.5 software package and level of significance of α=0.05 was accepted. The determination of the sex was made by direct examination of the gonads after opening the abdominal cavity.

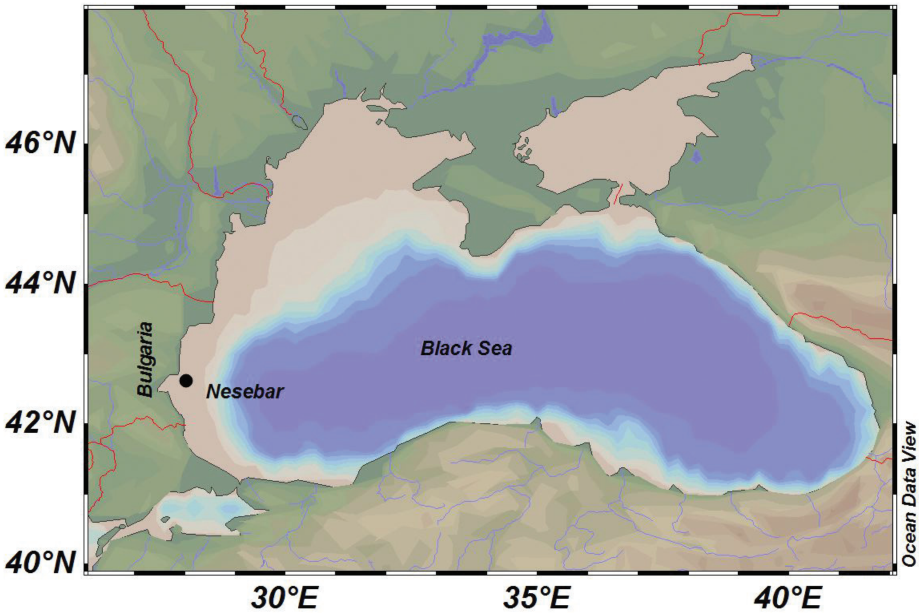

Figure 1. Location of the sampling station.

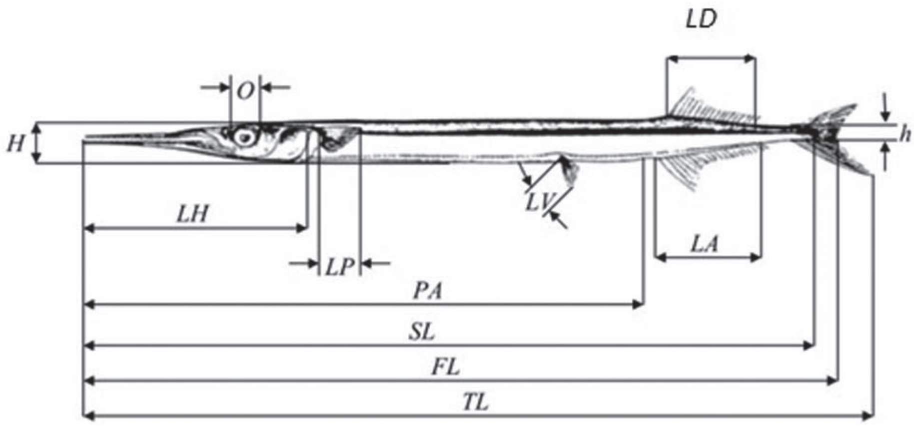

Figure 2. Morphometric measurements of garfish. Figure is from Collette (2016).

DNA extraction and PCR amplification

Tissue sample from the pectoral fin was cut and preserved in 96% ethanol at 4 °C. The genomic DNA was isolated using the DNeasy Blood & Tissue Kit (QIAGEN), and the target DNA was amplified with universal set of mitochondrial primers – cytochrome c oxidase subunit I (COI) FishF2: 5'TCGACTAATCATAAAGATATCGGCAC3' and FishR2: 5'ACTTCAGGGTGACCGAAGAATCAGAA3' (Ward et al. 2005). The polymerase chain reaction (PCR) was carried out in a reaction volume of 50 µl containing 1 µl of each primer, 25 µl of the mastermix (MyTaqTM HS Mix) and 2 µl of the target DNA. The PCR conditions included the following parameters: 95 °C for 1 min, and 95 °C for 30 sec, 54 °C for 30 sec, 72 °C for 1 min (35 cycles), 72 °C for 10 min. A quality control of the PCR product was performed by electrophoresis on 1% agarose gel. The DNA sequencing was performed by Macrogen Europe B.V. The obtained haplotype sequences were submitted to GenBank under the accession number OR554252 and OR554253.

Table 1. Relative relationships of measured body proportions of *Belone belone* in the Bulgarian Black Sea coast. n-number of fish studied, $\bar{X}$ -mean value, SD-standard deviation; SE-standard error of mean value; CV-coefficient of variation.

Relation	Sex	n	Range	$\bar{X}$ ±SD	$\bar{X}$ ±SE	CV (%)
FL/TL	♀	26	32.7±39.9	35.3±1.79	35.3±0.35	5.08
	♂	14	30.0±37.8	34.7±2.15	34.7±0.57	6.21
	Total	40	30.0±39.9	35.1±1.91	35.1±0.30	5.46
SL/TL	♀	26	31.1± 39.4	34.1±1.80	34.1±0.35	5.29
	♂	14	25.8± 36.6	33.2±2.88	33.2±1.80	8.69
	Total	40	25.8±39.4	33.8±2.25	33.8±0.35	6.65
PA/TL	♀	26	24.1±30.3	48.9±1.32	48.9±0.26	5.01
	♂	14	23.2±28.0	26.2±1.24	26.2±0.33	4.72
	Total	40	23.2±30.3	26.3±1.28	26.3±0.20	4.86
HL/TL	♀	26	10.1±11.5	19.5±0.39	19.5±0.07	3.73
	♂	14	9.5±10.9	10.3±0.33	10.3±0.09	3.25
	Total	40	9.5±11.5	10.4±0.37	10.4±0.06	3.60
O/LH	♀	26	0.8±1.0	0.9±0.06	0.9±0.01	7.03
	♂	14	0.7±0.9	0.9±0.06	0.9±0.02	7.01
	Total	40	0.7±1.0	0.9±0.06	0.9±0.01	7.19
H/TL	♀	26	1.5±1.9	1.7±0.12	1.7±0.02	7.02
	♂	14	1.5±1.9	1.7±0.12	1.7±0.03	7.18
	Total	40	1.5±1.9	1.7±0.12	1.7±0.02	6.99
h/H	♀	26	0.7±0.8	0.7±0.04	0.7±0.01	5.59
	♂	14	0.7±0.8	0.7±0.03	0.7±0.01	3.78
	Total	40	0.7±0.8	0.7±0.04	0.7±0.01	5.53
LD/TL	♀	26	4.3±5.3	4.8±0.03	4.8±0.06	6.93
	♂	14	4.3±5.1	4.2±0.03	4.7±0.08	6.44
	Total	40	4.3±5.3	4.7±0.32	4.7±0.05	6.94
LP/TL	♀	26	1.8±2.4	2.2±0.13	2.2±0.02	5.89
	♂	14	2.0±2.4	2.2±0.11	2.2±0.03	4.96
	Total	40	1.8±2.4	2.2±0.12	2.2±0.02	5.55
LA/TL	♀	26	5.2±6.3	6.1±0.36	6.1±0.07	5.84
	♂	14	5.1±6.2	6.4±0.37	6.4±0.10	5.78
	Total	40	5.1±6.3	6.2±0.36	6.2±0.06	5.82
LV/TL	♀	26	1.4±1.9	1.7±0.10	1.6±0.02	6.19
	♂	14	1.4±1.9	1.7±0.12	1.7±0.03	6.91
	Total	40	1.4±1.9	1.7±0.11	1.7±0.02	6.37

Figure 3. The captured specimen of garfish *Belone belone*.

Table 2. Meristic characteristic of garfish from Bulgarian Black Sea. n-number of fish studied, $\bar{X}$ -mean value, SD-standard deviation; SE-standard error of mean value; CV-coefficient of variation.

Meristic characteristic	Sex	n	Range	$\bar{X}$ ± SD (%)	$\bar{X}$ ±SE (%)	CV (%)
No rays in dorsal fin (*D*)	♀	26	16–19	17.8±0.65	17.8±0.13	3.67
	♂	14	16–19	17.6±0.75	17.6±0.20	4.30
	Total	40	16–19	17.7±0.68	17.7±0.11	3.88
No rays in pectoral fin (*P*)	♀	26	12–19	13.0±0.20	13.0±0.04	1.51
	♂	14	13–19	13.0±0.01	13.0±0.01	0.01
	Total	40	12–19	13.0±0.16	13.0±0.03	1.22
No ray in anal fin (*A*)	♀	26	19–23	21.9±1.02	21.9±0.20	4.64
	♂	14	19–23	21.6±1.01	21.6±0.27	4.66
	Total	40	19–23	21.8±1.01	21.8±0.16	4.63
Upper teeth	♀	26	13–15	14.0±0.28	14.0±0.05	2.02
	♂	14	14–14	14.0±0.01	14.0±0.00	0.01
	Total	40	13–15	14.0±0.23	14.0±0.03	1.62
Gill	♀	26	31–34	33.1±0.88	33.2±0.17	2.65
	♂	14	31–34	32.9±0.83	32.9±0.22	2.52
	Total	40	31–34	33.1±0.86	33.1±0.14	2.60
Vert.	♀	26	80–81	80.0±0.20	80.0±0.04	0.24
	♂	14	80–81	80.2±0.36	80.2±0.10	0.45
	Total	40	80–81	80.1±0.27	80.1±0.04	0.33

Results

The total length of female varied between 30.0 cm±37.8 cm (TL_{min}-TL_{max}) and 32.7 cm± 39.9 cm for males. No statistically significant difference was noted between sexes (KOLMOGOROV-SMIRNOV Test, P<0.05) for all morphometric characters: total length P (T<=t) =0.54, fork length P (T<=t) =0.50, standard length P (T<=t) =0.29, pre-anal distance P (T<=t) =0.74, head length P (T<=t) =0.29, eye diameter P (T<=t) =0.18, maximum body height T<=t) =0.81, minimum body height P (T<=t) =0.24, length of the dorsal fin basis base P (T<=t) =0.63, length of anal fin basis P (T<=t) =0.75, length of pectoral fin P (T<=t) =0.44, length of pelvic fin P (T<=t) =0.82. The fork length, standard length, pre-anal distance, length of anal fin, length of dorsal fin, maximum body height, length of pelvic fin, minimum body height was found as 87.5%, 84.4%, 65.8%, 14.5%, 11.9%, 4.3%, 4.1% and 1.8% of the total length of the fish respectively (Fig. 4A). Head length, length of pectoral fin, and eye diameter were found as 26.1%, 5.9% and 2.2% (Fig. 4B). Of all investigated specimens 65% were females and 35% were males.

The coefficient of variation CV was relatively low (CV<9%) for all morphometric measured body proportions of *Belone belone*. The lowest value was recorded for the relationship of length head and total length (LH/TL) in male (CV= 3.25%), while the highest value was registered for relationship (SL/TL) of standard and total length in male (CV= 8.69%) (Table 1).

The dorsal fin is long, unbranched with 16–19 spiny rays. The pectoral and anal fin comprised of 13–19 and 19–23 fin rays respectively. The coefficients of variation CV were relatively low for all meristic characteristics. The lowest value was recorded for the pectoral fin rays in total (CV= 0.01%), while the highest value was registered for anal fin rays in male (CV= 4.66%). No statistical difference was observed between sexes (Table 2).

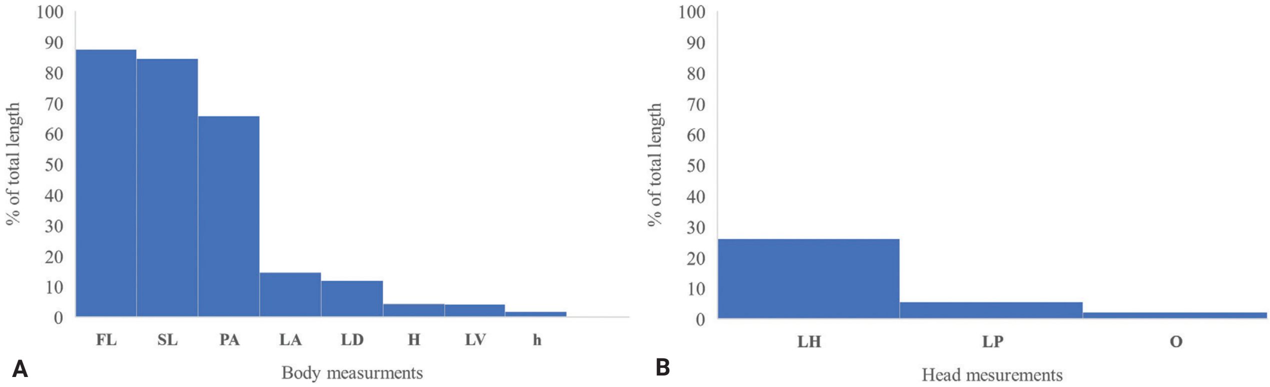

Figure 4. Morphometric measures of garfish in relation to the **A** total length and **B** head length of the fish in percentage.

Some length-length relationships (FL/TL, PA/TL, SL/FL, SL/TL, LH/TL) were fitted well by linear regression and show high determination (R^2) with the exception of the relationships:

(H/TL, LV/TL, LD/TL, h/H, LP/TL, LH/ O), which displayed very small values of R^2 (Fig. 5A–K).

The obtained COI gene fragment (633 bp) showed high percent identity (100% for haplotype 1 and 99.84% for haplotype 2) with *B. belone* sequences deposited in GenBank (KJ204729 North Sea, KJ128428 Sweden), supporting the species identification. From the 39 garfish samples analyzed, only two haplotypes were found, indicating low level of haplotype (0.146 ±0.072) diversity. The first haplotype (H1) was dominant, presented in 36 of the samples, whereas the second one (H2) was found only in 3 of them. Low nucleotide diversity (0.00023 ±0.00011) was also found.

Discussion

The range of TL observed in the present study (31–39.90 cm) was generally congruent with results from Bosphorus area (31–59 cm, Yüce 1975; 29–58 cm, Samsun et al. 2006) and previous investigation from the Black Sea (32–57 cm, Samsun et al. 1995; Samsun 1996; 34–52 cm, Erkoyuncu et al. 1994), with the exception of the upper limit of total length, which is significantly higher than that given in this research (Table 3). For garfish in the Black Sea, Prodanov (1982) and Kaya and Saglam (2017) reported the total length 25.60–57.90 cm and 49.5 cm, respectively. Other authors have received slightly higher estimates of TL, between 50.5 and 60.3 cm (Samsun 1995; Bilgin et al. 2004; Samsun et al. 2006; Polat et al. 2009).

The observation of infrequent growth in total length could be a result of hereditary factors (Borges 2001) or may be associated with the variations in morphologic characteristics (Bauer 1961). Fish populations exposed to high fishing pressure react to this pressure by reproducing at smaller ages and sizes (Helfman et al. 2009).

The feminine rate of garfish population is dominant with 65% and was higher than that reported for southern Ireland (58.3%: Dorman 1989), for Swedish waters (38.6%: Dorman 1991) and also for the Black Sea (53.4%: Samsun et al. 1995; 51.5%: Samsun 1996). Hemida (1987) explained this dominance by high natural mortality in males.

It was noted that R^2 values from the trends (Fig. 5) were above 0.70. R^2 values of the total length against morphometric parameters increase in the order

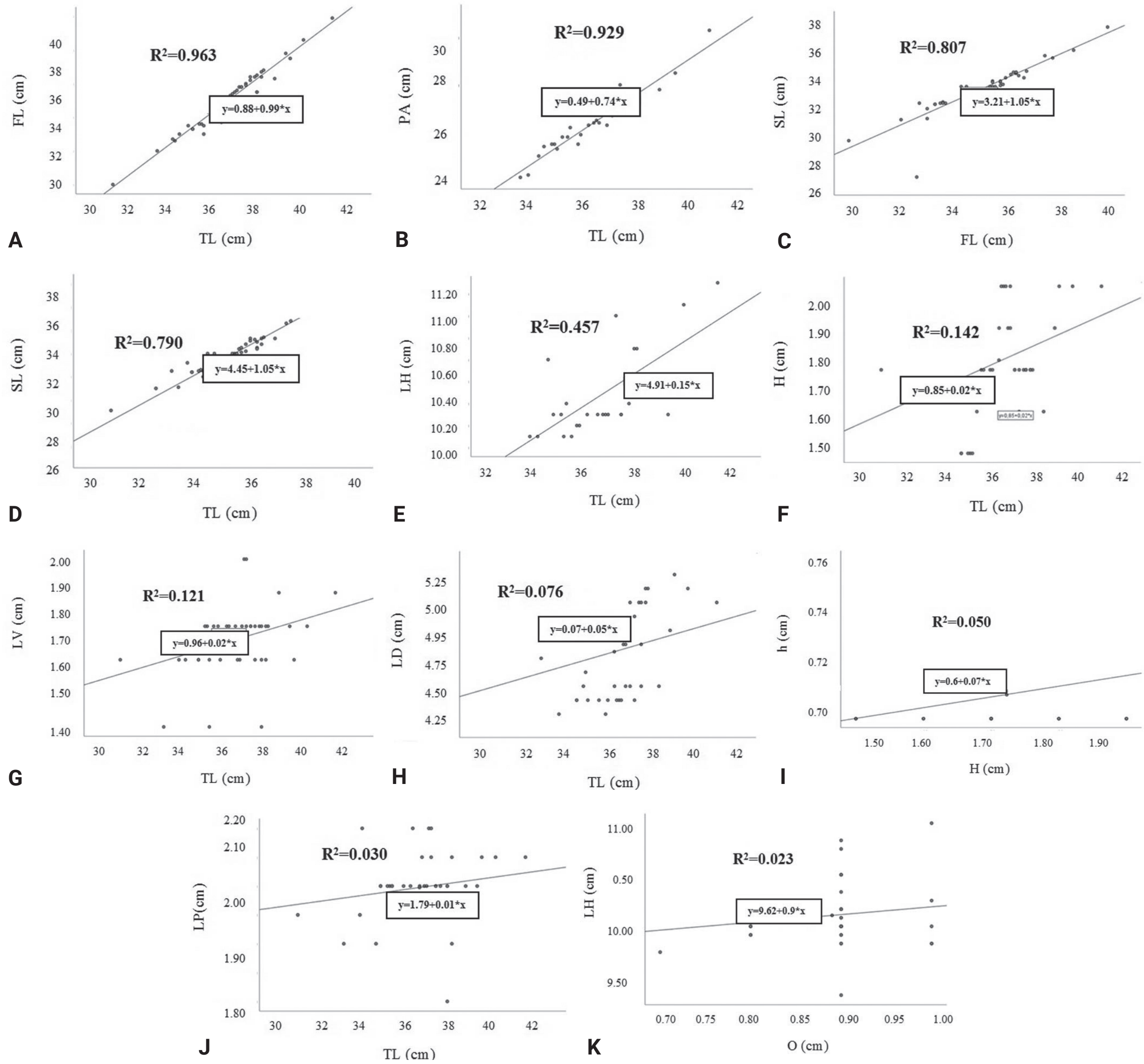

Figure 5. Length-length regression and coefficient of determination R^2.

FL > SL > PA. This entails that below 80% of changes in the total length were predicted by the selected morphometric parameters selected for this study.

Data from the literatures on morphometric relations are comparable since they refer to total body length. However, a study carried out by Fehri-Bedoui and Gharbi (2004) showed lower value for morphometric relationships of fork and total and standard and total length (FL/TL= 70% SL/TL=78%). The present findings show that both relationships (FL/TL= 87.5%, SL/TL=84.4%) are close to data reported by Zorica and Čikeš Keč (2011) (FL/TL=96.2% SL/TL=93.8%) and Sinovčić et al. (2004) (SL/TL=82%). The differences between this study with previous and other studies in different regions may have been caused by the dissimilarities of habitats, sampling time, differences in population and fishing gears used in sampling (Kalayci and Yeşilçiçek 2012).

Zorica and Čikeš Keč (2011) reported that the head constitutes 22.6% of the total body length in *Belone* from the Adriatic Sea. We determined that the head makes up 26.05% of the standard body length in sample of Bulgarian

Table 3. Comparison of the maximum length recorded in different areas.

Area	Authors	Length (cm)	Length type	Fishing gear
Black Sea, Turkey	Bilgin et al. 2014	65.1	TL	Surrounding net
Black Sea, Turkey	Polat et al. 2009	60.3	TL	Surrounding net
Black Sea, Turkey	Bilgin et al. 2004	58.0	TL	Purse seine, Surrounding net
Black Sea, Turkey	Samsun et al. 2006	58.0	TL	Purse seine, Surrounding net
Black Sea, Turkey	Samsun 1995	52.2	TL	Surrounding net
Black Sea, Turkey	Kalayci and Yeşilcicek 2012	50.5	TL	Purse seine, Gillnet
Black Sea, Turkey	Kaya and Saglam 2017	49.5	TL	Trammel net
Bosporus, Turkey	Yuce 1975	57.5	FL	Unknown
Aegean Sea, Greece	Koutrakis and Tsikliras 2003	27.0	TL	Beach seine
Mediterranean Sea, Tunisia	Ben Smida et al. 2014	40.1	TL	Unknown
Black Sea, Bulgaria	Prodanov 1982	57.9	TL	Unknown
Black Sea, Bulgaria	Present Study	39.9	TL	Trammel net

Table 4. Comparison of the maximum length recorded in different areas.

Area	Authors	No rays in Dorsal fin (*D*)	No rays in Pectoral fin (*P*)	No rays in Anal fin (*A*)	*Vert.*
Black Sea	Bănărescu (1964)	14–16		II/18–20	
Black Sea	Svetovidov (1964)	II/14–16	9–11	II/18–20	
Black Sea	Prodanov (1982)	II/14–18	II/11–12	II/17–21	74–81
Mediterranean, Atlantic, Black Sea	Colette and Parin (1986)	16–20	11–14	19–23	75–84
Adriatic Sea	Jardas (1996)	16–19	11–14	19–23	80–81
Present study		II/16–19	I/13–19	II/19–23	80–81

Black Sea coast. According to the Tortonese (1970) and Jardas (1996) data about maximum body height H/TL, relationships have been calculated slightly lower as follows H/TL=0.06%. According to the Tortonese (1970) and Jardas (1996) data about LH/TL relationships have been calculated as LH/TL=28.6. In this study values of the relative relationship between length head and total length (LH/TL=26.05%) were in agreement with the observations given by Zorica and Čikeš Keč, (2011) LH/TL=22.6%) and Tortonese (1970) and Jardas (1996). Several researchers reported the number of dorsal fin rays in Black Sea between 14–18 and 16–20 for the Mediterranean and Adriatic Sea (Bănărescu 1964; Svetovidov 1964; Prodanov 1982; Collette and Parin 1986; Jardas 1996). The meristic characters reported for species in the Adriatic Sea show the same result with respect to dorsal and anal fin rays (Table 4). The result of dorsal and pectoral fin rays showed low variability compared with results given by previous studies from other research areas. Similarly, to the Bănărescu (1964) and Svetovidov (1964), we found the presence of spines in anal and dorsal fins (Table 4). According to Svetovidov (1964), the presence or absence of the upper teeth on the hard palate, found in the present research, could be a reason for subspecies differentiation. Lindsey and Harrington (1972) found that the number of spines and pectoral fin rays in some fishes depended on the temperature of the water in which the fertilized egg develops, with increasing temperature and the number of indicated meristic characters improving. With regard to the latter, the number of vertebrae and rays in the pectoral fins is determined for 4 and 8 days, respectively. These data show that a number of abiotic environmental factors, especially during the development of fertilized eggs, play a major role in the formation of plastic and meristic characters (Prodanov 1982).

Genetic diversity is an important part of biodiversity, and its level reflects the environmental adaptability, evolutionary potential, and viability of species. Haplotype and nucleotide diversity are two important indicators for evaluating population genetic diversity. Low haplotype (0.146) and nucleotide (0.00023) diversity of COI found in Nesebar population could be a result of population decline, probably caused by overfishing. The results indicated that some measures should be taken to protect the resources of *Belone belone* along the Bulgarian Black Sea coast. The information on genetic diversity and population structure of *B. belone* are critical for phylogenetic relationships, resource conservation and fisheries management. Suggesting future studies, the genetic diversity analysis of other genetic markers should be supplemented.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Data curation: YR. Formal analysis: PI, MY, ND. Investigation: ND, MY, PI, VR. Methodology: VR, PI, MY, ND. Software: MY. Visualization: MY. Writing - original draft: ND, PI, VR, MY. Writing - review and editing: MY, VR, PI, CT, ND.

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Data availability

All of the data that support the findings of this study are available in the main text.

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