



## Existence of *Belone svetovidovi* Collette & Parin, 1970 in the Marmara Sea and Black Sea Coasts of Türkiye

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### Abstract

The first morphologic and genetic documentation of the short-beaked garfish *Belone svetovidovi* Collette & Parin, 1970 from the coast of the Marmara Sea (Yalova) and Black Sea (Akçakoca) is reported in the present study. The morphological characters and genetic (mtDNA COI) analyses confirmed the existence of this species both in the Marmara and Black Seas. *B. svetovidovi* is genetically distinct from the another species of this genus *Belone belone*. All morphologic measurements, counts, and colour descriptions of *B. svetovidovi* agree with its previous descriptions.

### Keywords:

*Belonidae*, short-beaked garfish, first record, existence, Marmara Sea, Black Sea

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### Introduction

The short-beaked garfish *Belone svetovidovi* Collette & Parin, 1970 belongs to the *Belone* genus and represents the *Belonidae* family. This family is comprised of three species (*Belone belone*,

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*Belone svetovidovi* and *Tylosurus acus*) on the coast of Türkiye (Collette & Parin 1986; Karataş et al., 2021).

The short-beaked garfish *B. svetovidovi* is a benthic fish species and prefers tropical and temperate waters, and found between depths of 1 to 20 m (Froese and Pauly, 2023) in the Eastern Atlantic and Mediterranean, where its range extends from southern Ireland, Spain, Portugal, Israel and Turkey (Akşiray, 1987; Golani, 2006; Froese and Pauly, 2022). In Turkish marine waters, *B. svetovidovi* was first time recorded in the Aegean Sea (Meriç and Altun, 1999), and then this species was reported from the Turkish coast of the Eastern Mediterranean (Iskenderun Bay) by Dalyan & Eryılmaz (2005), and taken place in the Turkish checklists (Fricke et al., 2007; Karataş et al., 2021).

In the present study, the existence of the short-beaked garfish *B. svetovidovi* from the Marmara and Black Sea coasts of Türkiye is given for the first time with both morphologic and genetic evidence. Therefore, the finding of *B. svetovidovi* herein presented constitutes the first well-documented record with taxonomic and molecular identification of the species from the Turkish coasts of the Marmara Sea and Black Sea. It also contributes significantly to the inclusion of this species in the Turkish marine fish checklist with evidence of the presence of this species from the Marmara Sea and Black Sea.

## Materials and Methods

### *Sampling and Morphological Analysis*

The five short-beaked garfish *Belone svetovidovi* specimens were collected as gill net at the Yalova coast on November 11, 2022, in the Marmara Sea (40.682854 N, 29.268145 E), depth 10 m), and five specimens were captured at the coast of Akcakoca in the Black Sea (41.122554 N, 31.122507 E) on September 2022 (Figure 1). All captured specimens were immediately frozen and transported to the laboratory for detailed morphological and genetic analysis. Each body length ( $\pm 0.1$  mm) and total body weight (W) ( $\pm 0.01$  g) were measured. Sex and gonad maturity stages were assessed macroscopically. All specimens of *B. svetovidovi* (Figure 2) were carefully examined and identified using field guides and ichthyological fauna (Collette and Parin, 1986; Golani et al., 2006).

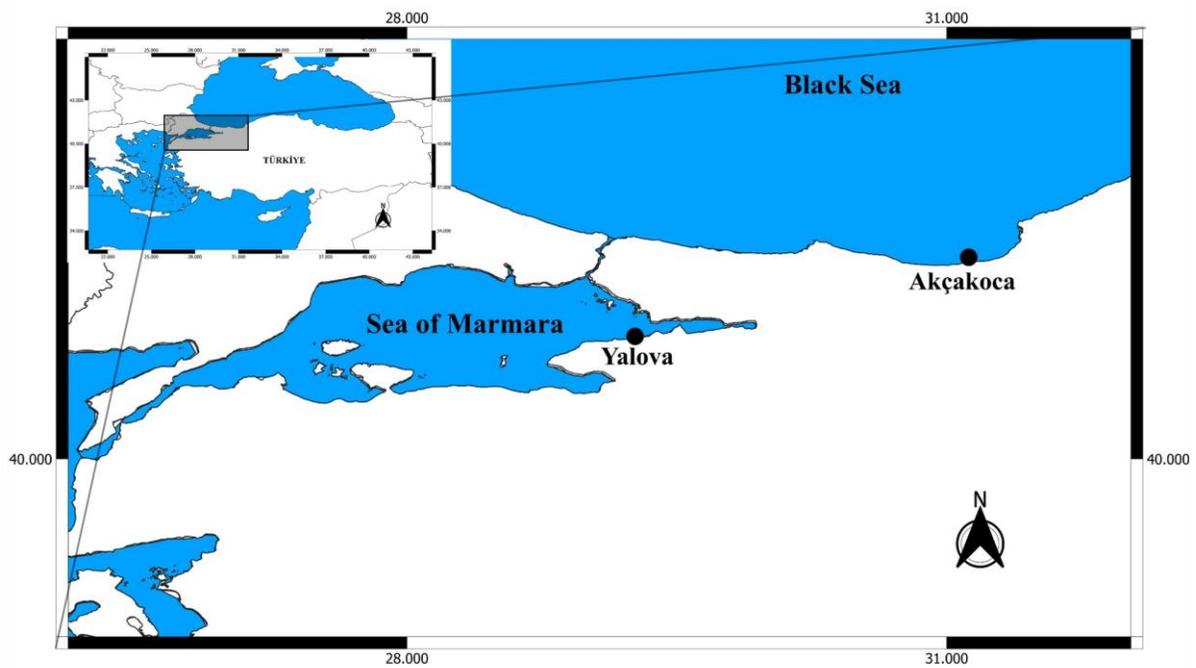


Figure 1. Sampling locations of *Belone svetovidovi*.



Figure 2. *Belone stevetovidovi* (a: General view, b: Beak and head structure, c: The back, anus and caudal fin).

### **Molecular Analysis**

Total genomic DNA from five specimens of each sea was extracted from the muscle and fin samples using the DNeasy Blood and Tissue Kit (Qiagen, USA). The manufacturer's protocols were used during all steps. The mtDNA COI gene region was amplified through PCR with universal primers (Ward et al., 2005). Fish\_F: 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-'3' -Fish\_R: 5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-'3'.

The PCRs were conducted in a 50 µl total volume with 0.4 µM of each primer, 0.2 mM of dNTP and 1.25U of Taq DNA polymerase in a PCR buffer that included 20 mM of Tris-HCl (pH 8.0), 1.5 mM of MgCl<sub>2</sub>, 15 mM of KCl and 1-2 µl template DNA. The denaturation step was at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s for 30 cycles and followed by a final extension for 7 min at 72 °C. The PCR products were visualized using electrophoresis on 1.5 % agarose gel. DNA sequencing was attempted to determine the order of the nucleotides of the mtDNA COI gene region. The chain termination method by Sanger et al. (1977) was applied with Bigdye Cycle Sequencing Kit V3.1 and ABI 3130 XL genetic analyzer. The initial alignments of partial COI sequences were performed with the Clustal W program (Thompson et al., 1994), and the final alignment was completed manually with BioEdit (Hall, 1999). The best-fit substitution model (HKY + G) was provided by the MEGAX software (Kumar et al., 2018). After sequence alignment, MEGA X was used to determine the genetic diversity and sequence divergences and to construct the phylogenetic tree (Kumar et al., 2018). Sequences of *Belone belone* and *Sphyraena sphyraena* were obtained from Genbank and Bold System.

### **Results**

*Belone svetovidovi* has a body with a more compressed and narrower inter-orbital width. The main diagnostic characters and morphometric measurements of the captured specimens of *B. svetovidovi* are given in centimetres: the five specimens were 33.8-42.8 cm in total length and 32.5-41.3 cm in standard length, and 47.76-97.64 g in the total weight for Marmara Sea (Yalova) and 20.4-46.2 cm in total length and 18.6-43.7 cm in standard length, and 8.51-111.50 g in the total weight for Black Sea (Düzce-Akcakoca).

The meristic characters: Dorsal fin rays 15-18, anal fin rays 19-23, pectoral fin rays 10-13 and gill rakers number 38-49 for Marmara and Black Seas specimens. All the morphometric measurements and meristic characters of *Belone svetovidovi* from the Marmara and Black Sea regions are given in Table 1.

Table 1. Morphometric measurements (cm) and meristic characters of *Belone svetovidovi* were collected in the Marmara and Black Sea regions.

<i>Characters</i>	<b>Measurements (cm)</b>	
	<b>Marmara Sea (Yalova)</b>	<b>Black Sea (Düzce-Akçakoca)</b>
<i>Morphometric</i>	<b>Range (Mean±SD) (n=5)</b>	<b>Range (Mean±SD) (n=5)</b>
Total length (TL)	33.8-42.8 (37.08±3.46)	20.4-46.2 (30±10.06)
Fork length (FL)	33.2-42 (36.06±3.46)	19.6-44.6 (29.06±9.69)
Standard length (SL)	32.5-41.3 (35.94±3.36)	18.6-43.7 (27.84±9.78)
Head length (HL)	14.07-17.81 (15.43±1.44)	8.49-19.23 (12.48±4.19)
Eye diameter (ED)	1.29-1.64 (1.42±0.13)	0.78-1.77 (1.14±0.38)
Body depth (BD)	2.10-2.66 (2.30±0.21)	1.27-2.88 (1.87±0.62)
Length of dorsal fin	5.65-7.15 (6.19±0.57)	3.41-7.72 (5.01±1.68)
Length of anal fin	6.69-8.48 (7.34±0.68)	4.04-9.15 (5.94±1.99)
Length of pectoral fin	2.54-3.21 (2.78±0.25)	1.53-3.47 (2.25±0.75)
Length of ventral fin	1.94-2.45 (2.12±0.19)	1.17-2.65 (1.72±0.33)
<i>Meristic</i>		
Number of rays in dorsal fin (D)	15-18	15-17
Number of rays in pectoral fin (P)	10-12	10-13
Number of rays in ventral fin (V)	I+5	I+5
Number of rays in anal fin (A)	20-22	19-23
Gill rakers number (First-gill arch)	39-49	38-48
Vertebrae numbers	72-76	72-78
Teeth count (teeth within a section of the middle of the upper jaw equaling the eye diameter)	13-20	14-21

After sequencing of the mtDNA COI gene region, there were 13 variables, 13 parsim-info and 557 conservative nucleotides, of which 93 were parsimony informative over 570 bp sequences. All sequences were submitted from Genbank and accession numbers were received as OR234691.1-OR234700.1.

The Neighbour-Joining (NJ) and Maximum Parsimony (MP) phylogenetic tree analyses were given in Figure 3 and Figure 4. NJ and ML trees indicated similar tree topologies. *B. belone* and *B.*

*svetovidovi* were separated from the two branches and the outgroup *Sphyræna sphyraena* was branched as a separate branch. According to NJ and MP trees, *B. Belone* and *B. svetovidovi* were clustered in different sets.

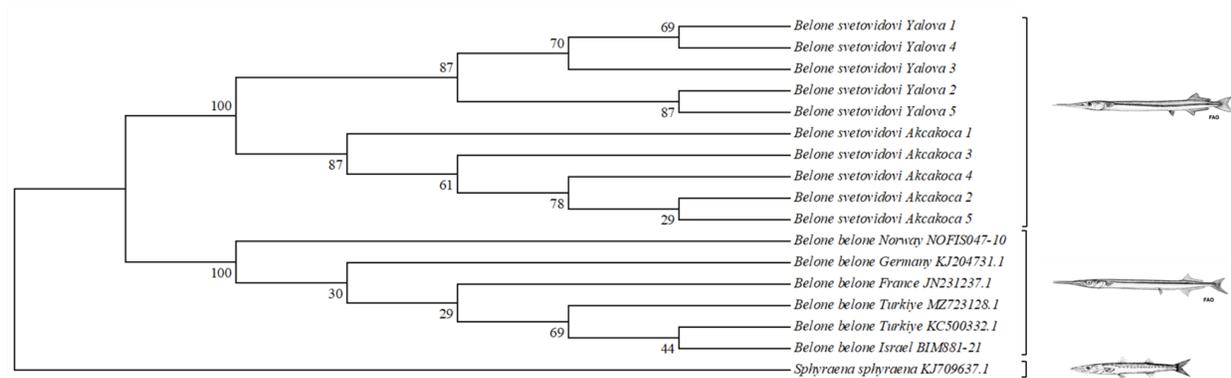


Figure 3. Neighbour-Joining tree (NJ) of garfish species with GenBank and Bold system sample references. The numbers above branches indicate bootstrap values among 1000 replicates. Fish sketches were given from FAO.

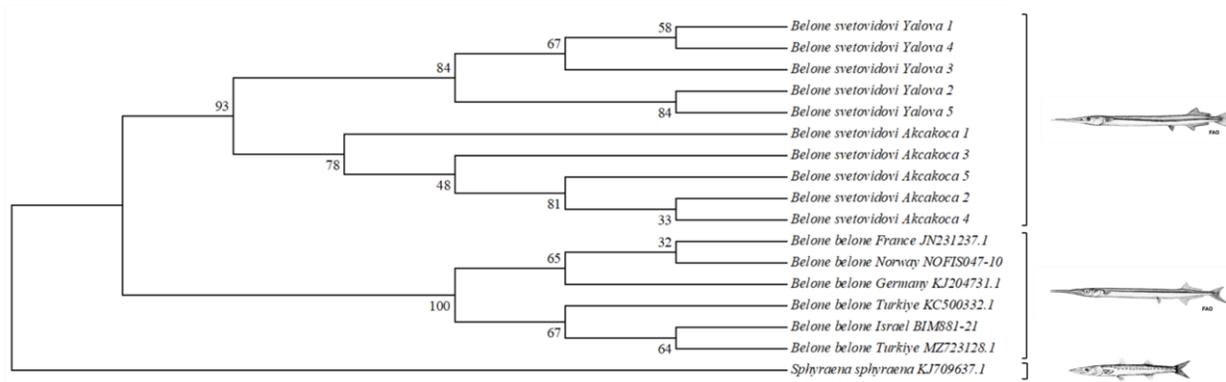


Figure 4. Maximum Parsimony tree (MP) of garfish species with GenBank and Bold system sample references. The numbers above branches indicate bootstrap values among 1000 replicates. Fish sketches were given from FAO.

### Discussion

The phenotypic appearance of *B. sveovidovi* is quite similar to that of *Belone belone* and, in our belief, has thus far been confused with *B. belone*. The body is green or blue on the back and silvery white on the lower sides and belly of *B. svetovidovi*. At first glance, it differs from *B. belone* in the size and density of its beak teeth. This species can be distinguished from *B. belone* by the following characteristics: Its smaller, more delicate and features 13-21 teeth within a section of the middle of

the upper jaw equaling the diameter of its eye and teeth present on vomer and total gill rakers on first-gill arch 38-52 (Akşiray, 1987; Collette and Parin 1990; Dalyan and Eryılmaz, 2005).

In this study, general shape, morphometric measurements, meristic counts and colouration recorded in the present specimens are in total agreement with Collette and Parin (1990). All metric measurements and meristic data of garfish specimens are given in Table 1.

*Belone svetovidovi* was considered a synonym for *Belone belone* for many years. These two species are morphologically similar, which causes misidentification. We have revealed the genetic difference between these species by mtDNA COI sequencing analysis. The species of *B. belone* and *B. svetovidovi* concerned were separately clustered in our studies and the same has also been reported in previous studies on systematics and phylogeny of needlefishes using different mtDNA gene regions as 16s rRNA, Cyt B (Lovejoy, 2000; Lovejoy et al., 2004). The present and previous studies show that the COI gene has been used effectively in the genetic description of problematic species, and the mtDNA COI region provides a useful tool to identify species and to detect possibly cryptic species or new species (Turan et al., 2017; 2020; Ghouri et al., 2020; Doğdu and Turan, 2021).

In the present study, the first record of *B. svetovidovi* in the Marmara Sea and Black Sea and the first genetic and morphological confirmation of the garfish species in Türkiye are presented. New morphologic and genetic findings confirm the validity of including the short-beaked garfish *B. svetovidovi* in the Turkish ichthyofauna of the Marmara and Black Seas. The sample numbers obtained show that this species is widespread and settled in the waters of two sea regions of Turkey.

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### **Conflict of Interest**

The authors declare that they have no competing interests.

### **Author Contributions**

C.T. performed all the experiments and drafted the main manuscript text. D.Y., S.A.D. and D.E. collected samples. C.T. and S.A.D. performed genetic analysis. C.T., D.Y., S.A.D., D.E., P.P.I. and V.S.R. performed morphological analysis. All authors reviewed and approved the final version of the manuscript.

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