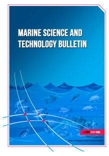
Mar. Sci. Tech. Bull. (2023) 12(2): 156-161
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1254345



Marine Science and Technology Bulletin

PRENSIP
https://prensip.gen.tr/

RESEARCH ARTICLE

Molecular and phenotypic characteristics of short-beaked garfish *Belone svetovidovi* Collette and Parin, 1970 in a new location, the Sea of Marmara

Dilruba Seyhan Öztürk[1]*

[1] *İzmir Katip Celebi University, Faculty of Fisheries, Department of Basic Sciences, 35620, Izmir, Türkiye*

ARTICLE INFO

Article History:
Received: 21.02.2023
Received in revised form: 26.04.2023
Accepted: 28.04.2023
Available online: 20.06.2023

Keywords:
Belonidae
Mediterranean Sea
Short-beaked garfish
Sea of Marmara

ABSTRACT

There are two species of garfish belonging to the genus *Belone* (garfish *B. belone* and short-beaked garfish *B. svetovidovi*) in the coastal regions of Türkiye. It is known that *Belone belone* is distributed all around the Turkish coasts, and *Belone svetovidovi* is distributed only in the Mediterranean and Aegean coasts of Türkiye. These species' morphological similarities and mainly overlapped morphological characters led to confusions for years. The present study represents the first detailed morphological and molecular analysis including twenty-two body portions and DNA barcode analysis of *B. svetovidovi* and reports the newly established populations in the Sea of Marmara with molecular and morphological evidences.

Please cite this paper as follows:

Seyhan Öztürk, D. (2023). Molecular and phenotypic characteristics of short-beaked garfish *Belone svetovidovi* Collette and Parin, 1970 in a new location, the Sea of Marmara. *Marine Science and Technology Bulletin*, *12*(2), 156-161. https://doi.org/10.33714/masteb.1254345

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).

In the last checklist of the marine fish species of Türkiye, the existence of three species is mentioned; *Belone belone* (Linnaeus, 1761), *Belone svetovidovi* Collette & Parin, 1970 and *Tylosurus acus* (Lacepède, 1803) (Bilecenoğlu et al., 2014). According to this checklist, while *Belone svetovidovi* and *Tylosurus acus* species are distributed only in the Mediterranean and Aegean coasts of Türkiye, it is known that *Belone belone* is distributed in all around Turkish coasts (Bilecenoğlu et al., 2014).

The short-beaked garfish *Belone svetovidovi* was first described by Collette & Parin (1970) from Galicia, Spain then was reported by Collette & Parin (1986) in the north-eastern Atlantic (Southern Ireland, Spain and Portugal), in the eastern Mediterranean (Israel) by Golani (1996), and in the Aegean Sea by Meriç & Altun (1999). After its Aegean Sea record, only one

---

* Corresponding author
E-mail address: seyhandilruba@gmail.com (D. Seyhan Öztürk)

specimen of *B. svetovidovi* was recorded from the north-eastern Levantine Sea by Dalyan & Eryılmaz (2006). According to Collette & Parin (1970), *B. svetovidovi* differs from *B. belone* by having more closely spaced and smaller teeth on the jaws, by lacking vomerine teeth (mostly present in adult *B. belone*), and by having more numerous gill-rakers (Dorman, 1987; Meriç & Altun, 1999).

Although there are a couple of previous studies using limited metric and meristic characters (Dorman, 1987; Meriç & Altun, 1999), there is no integrative study that includes molecular and morphological analysis that can shed light on molecular and phenotypic characteristics of this commercial species.

The aim of this paper is to represent the first detailed morphological and molecular analysis and report the newly established populations of *Belone svetovidovi* in the Sea of Marmara with both molecular and morphological evidences.

Material and Methods

Samplings

B. svetovidovi specimens were collected directly from the local fishing boats in the Sea of Marmara, which used trammel nets (Figure 1). Ten specimens were sampled from the Northern coast of the Sea of Marmara (the Bosporus/Istanbul) and nine specimens were sampled from Southern coast of Sea of Marmara (Gemlik District). Pectoral fin clips were fixed in 96% ethanol for genetic analyses and then the specimens were fixed in 4% formalin solution for morphological analysis. The collected specimens were deposited in the Fish Collection Centre of İzmir Katip Çelebi University (IKC PIS 1271-1289).

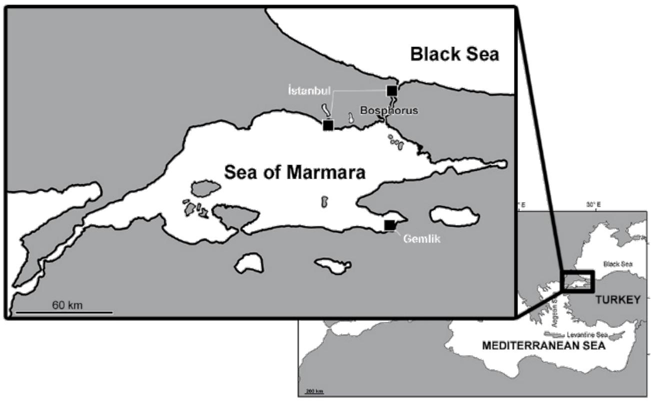


Figure 1. Sampling locations of short-beaked garfish *Belone svetovidovi* in the Sea of Marmara

Morphological Analysis

Morphological methods follow Zorica & Čikeš Keč (2011) with some additional characters (SL% of body depth at anal fin pelvic fin and dorsal fin origins; head depth, interorbital distance, counts of gill-rakers and teeth, the teeth count within a section of the middle of the upper jaw equalling the eye diameter) were added (Figure 2). Totally 22 different body proportions were used (7 of which were related to head length) in morphological analysis (Table 1).

Table 1. Morphometric and meristic characters of *Belone svetovidovi* (n=19)

Characters	**Range (mean±S.D.)**
SL (cm)	26-35.7 (30.03±3.2)
SL%	
HL/SL	20.4-35.3 (28.5±4)
Pre-dorsal/SL	76.4-82.9 (79.9±1.6)
Pre-pelvic/SL	61.1-69.4 (65.7±2.3)
Pre-anal/SL	74.5-80.4 (77.8±1.4)
Pre-pectoral/SL	21.5-36.3 (29.7±4.3)
Pelvic to anal/SL	10.5-14.3 (12.1±1.1)
Pectoral length/SL	5.2-7 (6.0±0.5)
Pelvic length/SL	10.5-14.3 (12.1±1.1)
Caudal fin length/SL	9.3-12.6 (10.5±0.9)
Caudal peduncle depth/SL	1.8-2.7 (2.0±0.2)
Depth at dorsal fin orj/SL	4.6-6.4 (5.5±0.4)
Depth at pelvic fin orj/SL	4.8-6.6 (5.7±0.5)
Depth at anal fin orj/SL	4.6-6.5 (5.6±0.5)
Dorsal fin base/SL	11.5-14.8 (13.1±0.9)
Anal fin base/SL	13.6-16.9 (15.3±0.8)
Pectoral fin base/SL	5.2-7 (6.0±0.5)
Pelvic fin base/SL	3.4-5.3 (4.4±0.5)
HL (cm)	5.6-10.6 (8.6±1.6)
HL%	
Head depth/HL	13.2-23.8 (16.8±3.4)
Pre-orbital/HL	48.2-74 (63.8±7.4)
Post-orbital/HL	18.3-37.5 (24.9±5)
Eye diameter/HL	8.4-15.5 (11.0±2.4)
Interorbital/HL	6.5-12.7 (8.9±1.9)
Meristic Counts	
Dorsal fin rays	15-19 (16.7±1.1)
Anal fin rays	18-23 (21.1±1.3)
Pectoral fin rays	11-15 (12.3±1)
Pelvic fin rays	6-7 (6.1±0.3)
Gill-rakers	40-49 (43.5±3.2)
Teeth count within a section of the middle of the upper jaw equalling the eye diameter	14-20 (16±1.94)

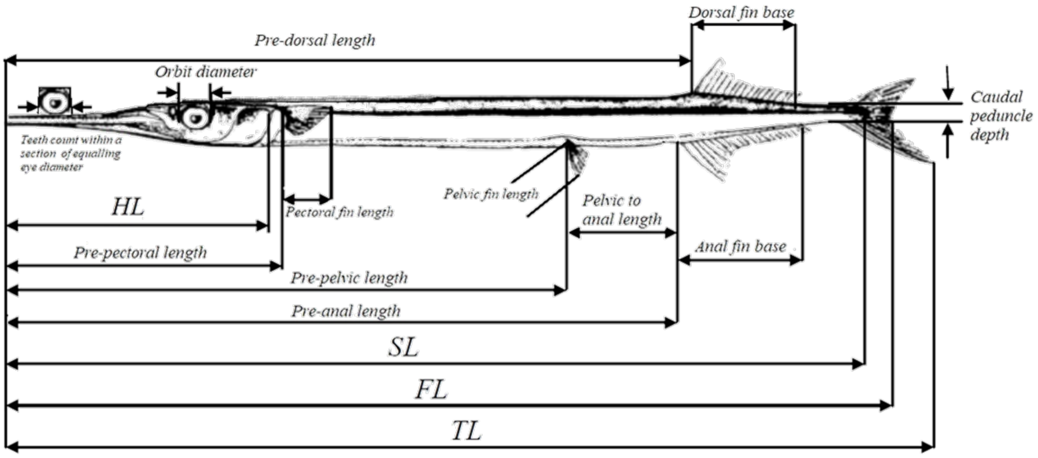


Figure 2. Drawing of body proportions of short-beaked garfish modified from Zorica & Čikeš Keč (2011)

Molecular Analysis

Total genomic DNA was isolated from the fin clips according to the manufacturer's instructions with a DNA isolation kit (AMBRD Laboratories, Türkiye). FishF2/FishR2 primers described by Ward et al. (2005) were used to amplify Cytochrome c Oxidase I, which is also known as the barcode region using the 5x FIREPol Master Mix (Solis Biodyne; www.sbd.ee). The thermocycler profile consisted of a step 1 min at 94°C, five cycles of 94°C for 30 s, 50°C for 40 s, and 72°C for 1 min, followed by 35 cycles of 94°C for 30 s, 54°C for 40 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were checked with 2% agarose gel. Sequencing was carried out by Macrogen Inc., Seoul, South Korea. Nucleotide sequences were aligned using ClustalW (Thompson et al., 1994) implemented in MEGA version X (Kumar et al., 2018) and edited with BioEdit (Hall, 1999). Aligned sequences of Sea of Marmara samples were submitted to GenBank with accession numbers: OQ329406- OQ329424. Maximum Likelihood (ML) and Neighbour Joining analysis were performed with the MEGA version X using the T92 (Tamura-3) + Gamma model due to the lowest Bayesian Information Criterion (BIC) values. Uncorrected *p* and T92 distances were used to compare the barcode data. A bootstrap test with 1000 replicates was performed to verify the robustness of the tree.

Results

Morphological Analysis

Nineteen specimens of *B. svetovidovi* have been obtained of which 12 were male and seven were female. All measured specimens varied in total length from 28.4 to 39 cm (mean±SD: 32.9±3.2 cm). The total length of males varied between 30.6-39 cm (mean±SD: 34.5±3 cm), whereas for females varied between 28.4-33.1 cm (mean±SD: 30.3±1.6 cm). Meristic counts and the results of the morphometric proportion of the body measurements expressed in percentages are given in Table 1. All examined specimens lacked vomerine teeth and featured 14-20 teeth within a section of the middle of the upper jaw equalling the eye diameter.

Genetic Analysis

A total of 625 bp of COI fragments of 31 short-beaked garfish were analysed COI clades of the specimens were well supported in the ML and NJ phylogenetic analysis (Figure 3, Figure 4). Since there are no COI barcode data of *B. svetovidovi* available in both GenBank and BOLD system, the Aegean Sea sequences of this species (which were also generated in this study) were used to compare the data. The sequence of the COI fragment, presented here for the first time for *B. svetovidovi.* Both uncorrected *p* and T92 distances have shown low intra-specific distance (0.4% SE=0.001) between analysed sequences. The interspecific distance between *B. svetovidovi* and *B. belone* species is 3% SE=0.006 (for both uncorrected *p* and T92 distances).

Discussion

There are two species of garfish belonging to the genus *Belone* (garfish *B. belone* and short-beaked garfish *B. svetovidovi*) on the coastal regions of Türkiye (Bilecenoğlu et al., 2014). While the garfish *B. belone* is distributed over a wide area of the Atlantic Ocean from Iceland and Norway in the north, the western coast of Africa in the south and also in the Mediterranean Sea, the short-beaked garfish *B. svetovidovi* is distributed North-East Atlantic (Southern Ireland, Spain,

Figure 3. Maximum Likelihood tree of analysed *Belone* species. The tree is drawn to scale, with branch lengths (above the branches) measured in the number of substitutions per site Numbers next to branches indicate bootstrap values for 1000 replicates

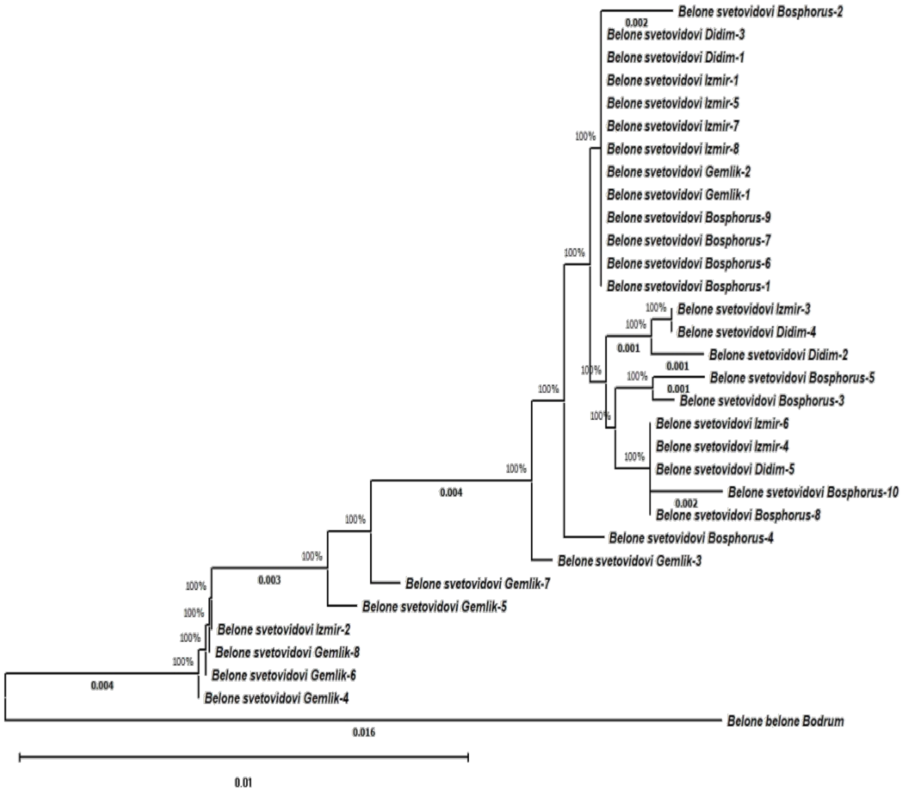


Figure 4. Neighbour joining tree of analysed *Belone* species. The tree is drawn to scale, with branch lengths (above the branches) measured in the number of substitutions per site Numbers next to branches indicate bootstrap values for 1000 replicates

Portugal) and in the Mediterranean Sea (Collette & Parin, 1986; Golani, 1996; Meriç & Altun 1999; Dolgov & Zabavnikov, 2021). These species' very similar appearance and mainly overlapped morphological characters led confusions for years (Dorman, 1987; Meriç & Altun, 1999; Dolgov & Zabavnikov, 2021). *B. svetovidovi* differs from *B. belone* by having more closely spaced and smaller teeth on the jaws, by lacking vomerine teeth (mostly present in adult *B. belone*); and by having more numerous gill-rakers (Dorman, 1987; Meriç & Altun, 1999). Additionally, Meriç & Altun (1999) also remarked that the teeth count within a section of the middle of the upper jaw equalling the diameter of its eye can distinguish these two species. Due to this, they reported that while *B. svetovidovi* has 11-21 teeth in this section, *B. belone* has 5-10 teeth within a similar-sized area. In this study, *B. svetovidovi* featured 14-20 teeth within a section of the middle of the upper jaw equalling the eye diameter. The number of the gill-rakers of *B. svetovidovi* was reported as a mean of 45.20 and 48.7, respectively, by Dorman (1987) and Meriç & Altun (1999) while it is determined as 43.5±1.8 in this study. These counts are noticeably higher than those reported for *B. belone* [mean 32.22 (Meriç & Altun, 1999; mean 35.6 Dorman, 1987)].

In addition to morphological analysis, DNA barcoding technique was used to confirm the identification of *B. svetovidovi*. DNA barcoding technique has a proven efficacy for species identification throughout fish taxa (Ward et al., 2005, 2008; Hubert et al., 2008; Steinke et al., 2009). According to the DNA barcoding concept; if the inter-specific distance is greater than intra-specific distance and there is no overlap between these thresholds, this would indicate a taxonomic identity among samples (Hebert et al., 2004; Wiemers & Fiedler, 2007). Genetic distances between *B. svetovidovi* and *B. belone* were on average 7.5-fold higher than within species. The results of the barcoding analysis represented that *B. svetovidovi* is clearly distinct from analysed *B. belone*. This study showed that barcoding analysis in proximate and mixed species is a powerful aid in making taxonomic distinctions.

Conclusion

Present study represents the first detailed morphological and molecular analysis of *Belone svetovidovi*. The sequence of the COI fragment, presented here for the first time and findings of morphological analysis highlighted that the number of the gill-rakers and the teeth count within a section of the middle of the upper jaw equalling the diameter can distinguish these two species. This study also reports,*Belone svetovidovi* has extended its distribution of from the Aegean Sea to the Sea of Marmara in Turkish coasts.

Acknowledgements

This study has been supported by TÜBİTAK (Grant No:121 Y 553). I also would like to thank Ugur Asan for introducing me to local fishermen.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statements

The author confirms that the data supporting the findings of this study are available within the article.

References

Bilecenoğlu, M., Kaya, M., Cihangir, B., & Çiçek, E. (2014). An updated checklist of the marine fishes of Turkey. *Turkish Journal of Zoology, 38*(6), 901–929. https://doi.org/10.3906/zoo-1405-60

Collette, B. B., & Parin, N. V. (1970). Needlefishes (Belonidae) of the eastern Atlantic Ocean. *Atlantide Report, 11*, 7-60.

Collette, B. B., & Parin, N. V. (1986). Belonidae (pp. 604-609). In Whitehead, P. J. P., Bauchot, M. L., Hureau, J. C., Nielsen, J., & Tortonese, E. (Eds.), *Fishes of the North-eastern Atlantic and the Mediterranean* Vol 2. UNESCO.

Dalyan, C., & Eryılmaz, L. (2006). Two new fish records from Turkish coast of the eastern Mediterranean: the garfish, *Belone svetovidovi* Collette and Parin, 1970; the spiny gurnard, *Lepidotrigla dieuzeidei* Audoin in Blanc and Hureau, 1973. *Journal of Black Sea/Mediterranean Environment, 11*(3), 277-280.

Dolgov, A. V., & Zabavnikov, V. B. (2021). New data on the occurrence of garfish *Belone belone* (Belonidae) in the Norwegian, Barents and the White Seas. *Journal of Ichthyology, 61*(5), 787–790. https://doi.org/10.1134/S0032945221050039

Dorman, J. A. (1987). *Belone svetovidovi* Collette and Parin: A species of garfish new to northern Europe. *Journal of the Marine Biological Association of the United Kingdom*, *67*(3), 679-685. https://doi.org/10.1017/S0025315400027387

Fricke, R., Eschmeyer, W. N., & Van der Laan, R. (Eds.). (2022). Eschmeyer's Catalog of Fishes: Genera, Species, References. Retrieved on March 21, 2022, from http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version.

Golani, D. (1996). The marine ichthyofauna of the eastern Levant—History, inventory, and characterization. *Israel Journal of Ecology and Evolution*, *42*(1), 15-55. https://doi.org/10.1080/00212210.1996.10688830

Hall, T. A. (1999). BioEdit: Q user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Hebert, P. D., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLOS Biology*, *2*(10), e312. https://doi.org/10.1371/journal.pbio.0020312

Hubert, N., Hanner, R., Holm, E., Mandrak, N. E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., & Bernatchez, L. (2008). Identifying Canadian freshwater fishes through DNA barcodes. *PLOS ONE, 3*(6), e2490. https://doi.org/10.1371/journal.pone.0002490

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

Meriç, N., & Altun, Ö. (1999). The garfish, *Belone svetovidovi* Collette and Parin, 1970, new to the Aegean Sea. *Israel Journal of Zoology*, *45*(3), 423-426. https://doi.org/10.1080/00212210.1999.10689009

Steinke, D., Zemlak, T. S., Boutillier, J., & Hebert, P. D. N. (2009). DNA barcoding of Pacific Canada's fishes. *Marine Biology, 156,* 2641–2647. https://doi.org/10.1007/s00227-009-1284-0

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research, 22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Ward, R. D, Costa, F. O., Holmes, B. H., & Steinke, D. (2008). DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence for most taxa, but *Zeus faber* and *Lepidopus caudatus* each probably constitute two species. *Aquatic Biology, 3*, 71–78. https://doi.org/10.3354/ab00068

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Wiemers, M., & Fiedler, K. (2007): Does DNA barcoding gap exist? A case study in blue butterflies (Lepidoptera: Lycaenidae). *Frontiers in Zoology, 4,* 8. https://doi.org/10.1186/1742-9994-4-8

Zorica, B., & Čikeš Keč, V. (2011). Phenotypic characteristics of garfish *Belone belone* (Linnaeus, 1761) in the Adriatic Sea. *Acta Adriatica, 52*(2), 269-278.