Parasite fauna of garfish *Belone belone* collected from Sinop coasts of the Black Sea, Turkey

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Abstract

Parasite fauna of garfish *Belone belone* was determined in 16 fish specimens collected from Turkish coasts of the Black Sea in January and February 2013. A total of 6 parasite species was identified:*Ceratomyxa beloneae*, *Sigmomyxa sphaerica*, *Axine belones*, *Hysterothylacium aduncum*, *Diphyllobothrium* sp. procercoids and *Trichodina* sp. Their morphometric measurements, infection prevalences, intensities and their sites of infection were presented. *C. beloneae* and *S. sphaerica* are new parasite records for Turkey and *A. belones, H. aduncum, Trichodina* sp.and *Diphyllobothrium* sp. are reported in *B. belone* in Turkish coasts of the Black Sea for the first time.

Introduction

The garfish *Belone belone* (L. 1761) is an epipelagic teleost fish distributed to the NE Atlantic, Mediterranean and the Black Seas and has economical significance in the countries surrounding both seas. There have been several studies on its parasite fauna including myxozoans, monogeneans, nematodes, cestodes and protozoans. Myxosporea represent a major group of fish parasites and their impact on wild and cultured fish is significant (Kent et al., 2001). Only two myxosporean species have so far been reported from the gall bladder of garfish: *Myxidium sphaericum* Thélohan, 1895 and *Ceratomyxa beloneae* Lubat et al., 1989 in the Black and Adriatic Seas (Pogoreltseva, 1964; Lubat et al., 1989; Yurakhno, 2004; Mladineo et al., 2009). However, Karlsbakk and Køie (2012) redescribed *M. sphaericum* from *B. belone* and showed that this species did not belong in genus *Myxidium* and was not closely related to *Myxidium* spp. from gadids, a host which previously reported. Based on the morphological and phylogenetic distinctness from genus *Myxidium sensu stricto*, they proposed a novel genus *Sigmomyxa* for *M. sphaericum*. Karlsbakk and Køie (2012) also reported that *Sigmomyxa sphaericum* in garfish used the polychaete *Nereis pelagica* L. as alternate host in its life cycle. Some monogeneans are known to be strictly host specific and *Axine belones* Abildgaard, 1794 is a microcotylid specific to the gills of *B. belone* (Strelkov, 1953; Euzet and Lopez-Roman, 1973; Popjuk, 2009; Gaevskaya et al., 2010). *Hysterothylacium aduncum* (Rudolphi, 1802) is one of the anisakid nematodes that lives as sexually mature adults in the digestive tracts of marine teleosts. Its larvae are known to occur in marine invertebrates and in fish (Køie, 1993). This ani-

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sakid has a cosmopolitan and circumpolar distribution. It is found mainly in marine teleosts in temperate and cold waters (Berland, 1991) and has been reported in *B. belone* (Grabda, 1981; Popjuk, 2009; Gaevskaya et al., 2010). *Trichodina inversa* Dogiel, 1948 is the only trichodinid species reported from *Belone belone* in the Black Sea (Gaevskaya et al., 1975). So far, there is no report on the presence of any species of *Diphyllobothrium* from *B. belone*. In the present study, we provide data on parasitic fauna of *Belone belone* in Turkish coasts of the Black Sea for the first time and provided more data on parasite morphology based on light microscopy.

Materials and methods

A total of 16 garfish *Belone belone*, 30 – 59 cm in length, were caught by hook and line by a commercial fisherman in Sinop coasts of the Black Sea in January (n: 9) and February (n: 7) in 2013 and examined for parasites. Skin, gills, gall bladder, stomach, intestine, kidney, gonads and liver were examined. All the organs were dissected and placed separately in petri dishes to determine infected organ and numbers of parasites. Whole content of the stomach and intestine were emptied and tissues were scraped by a scalpel. Fresh parasites were examined by Olympus BX51with phase-contrast attachment and photographed by DP-25 digital camera using data-processing software. Species identification were in accordance with Strelkov (1953), Yamaguti (1963), Euzet and Lopez-Roman (1973) for *Axine beloneae*; Lom and Dykova (1992) for *Trichodina* sp.; Lubat et al., (1989), Mladineo et al., (2009), Karlsbakk and Køie (2012) for myxospores; Moravec (1994) for *Hysterothylacium aduncum*; personal communication with Prof. Vadim V. Kornyushin from I.I. Schmalhausen Institute of Zoology in Kiev, Ukraine for *Diphyllobothrium*. Infection prevalence (%) and mean intensity follow the recommendations of Bush et al. (1997).

Results

Ceratomyxa beloneae (Lubat et al., 1989)

Site of infection: Gall bladder

Infection prevalence: 18.8%

Intensity: Tens and hundreds plasmodia in smear

Vegetative stages: Numerous young plasmodia with and without spores were observed (Fig. 1A). Vegetative stages are disporic plasmodia (Figure 1A and 1B). The measurement data on the size of plasmodia is provided in Table 1.

Spore description: Typical mature spores were

Table 1. Measurements (in micrometres) of *Ceratomyxa beloneae* from *Belone belone* in the Black Sea compared with those published previously from *B. belone.*

	Lubat et al. (1989)	**Mladineo et al. (2009)**	**Our data (n: 10) (Sevastopol)**	**Our data (n:20) (Sinop)**
Plasmodia size	17	-	-	21.4 ± 3.5 (16.7-30.0) x 19.3 ± 3.1(14.0 – 24.7)
Spore length	6.75 – 7.5	11.88	5.4 – 7.9	7.4 ± 0.6 (6.7 – 8.3)
Spore thickness	14.0 – 18.0	28.4	13.0 – 23.0	16.2 ± 0.7 (14.7 – 17.3)
Polar capsule length	2.75	4.77	3.5	4.0 ± 0.2 (3.7 – 4.3)
Polara capsule width	2.5	4.61	2.0 – 3.0	2.7 ± 0.3 (2.3 – 3.7)

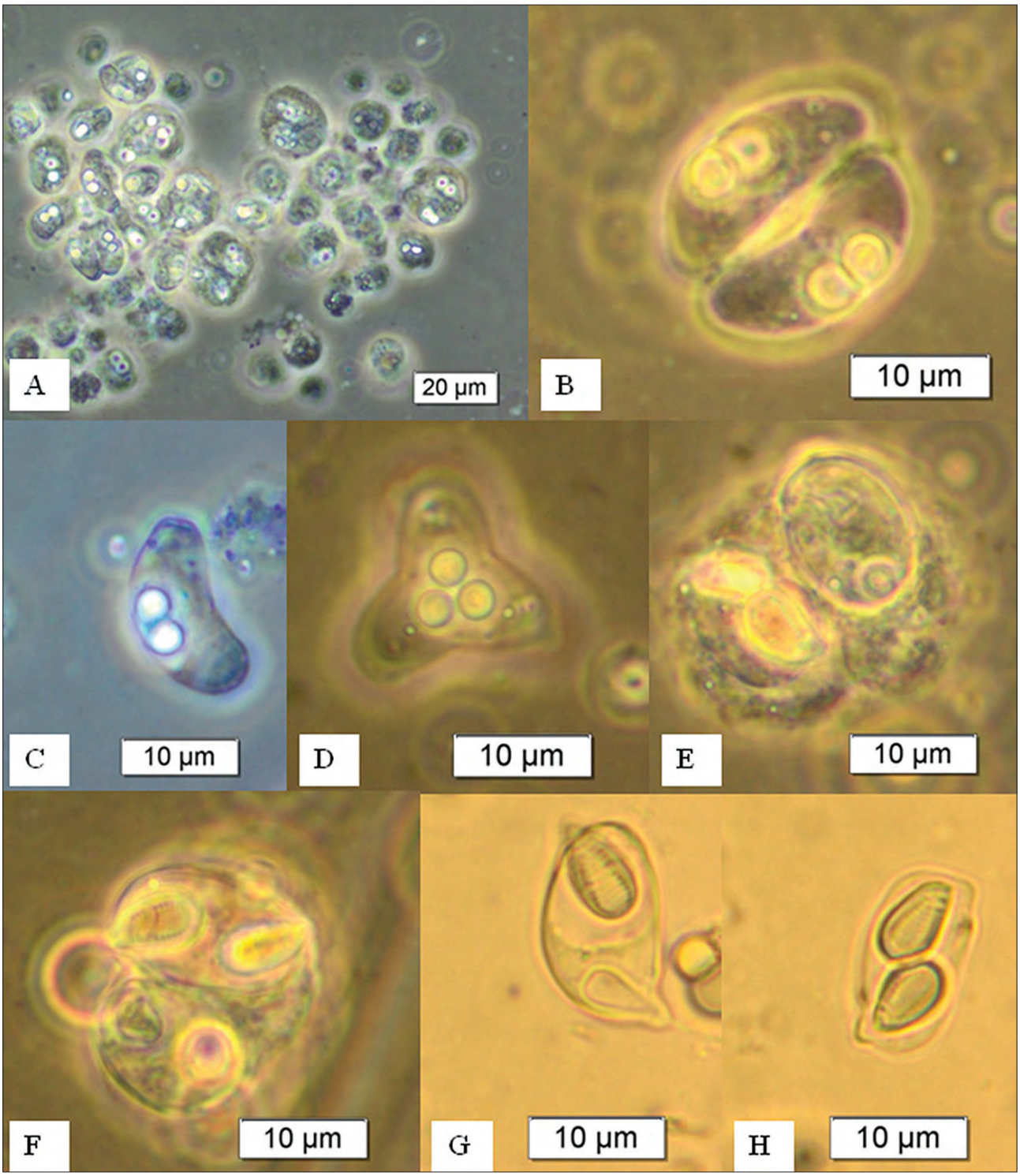

Figure 1. A. Vegetative stages of *Ceratomyxa beloneae*, **B.** Disporic plasmodium, **C.** Spore, **D.** Aberrant spore of *Ceratomyxa beloneae,* **E. F.** Disporic plasmodia of *Sigmomyxa sphaerica,* **G.** Spore, **H.** Nine polar flament coils inside capsules

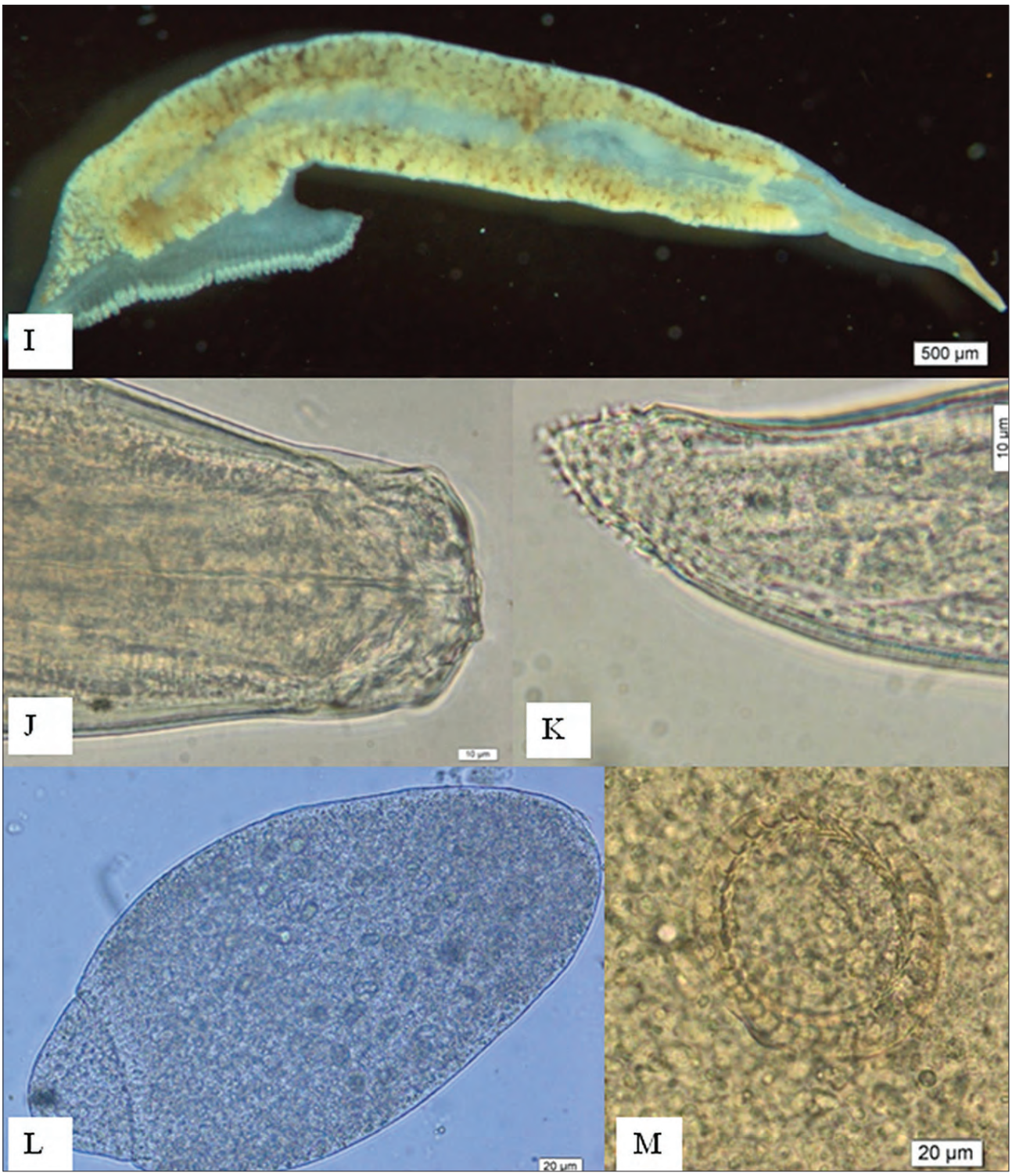

Figure 1 continued. I. *Axine belones*, **J.K.** Head and tail of *Hysterothylacium aduncum*, **L.** *Diphyllobothrium* sp. procercoid, **M.** *Trichodina* sp.

crescent-shaped in the frontal view, with a convex anterior end and flat or curved posterior (Figure 1C). Shell valves were smooth with rounded ends. Polar capsules were subspherical to oval. The polar filament formed 5 turns arranged along the longitudinal axis of the capsule. Sometimes abnormal shapes with three polar capsules and three shell valves were observed among the spores (Figure 1D).The measurement data on the size of spore and polar capsules are provided in Table 1.

Sigmomyxa sphaerica (Thelohan, 1895)
Site of infection: Gall bladder
Infection prevalence: 6.3%
Intensity: Several plasmodia in smear
Vegetative stages: Vegetative stages are disporic plasmodia (Figure 1E and 1F). The measurement data on the size of plasmodia is provided in Table 2.
Spore description: Typical mature spores were crescent-shaped in the frontal view, with a convex anterior end and flat or curved posterior (Figure 1G). Shell valves were smooth with rounded ends. Polar capsules were subspherical to oval. The polar filament formed 9 turns arranged along the longitudinal axis of the capsule (Figure 1H).The measurement data on the size of spore and polar capsules are provided in Table 2.

Axine belones (Abildgaard, 1794)
Site of infection: Gills
Infection prevalence: 6.3%
Intensity: 1
Parasite: The body of *Axine belones* is flat (Figure 1L) and resembles a narrow triangle the short side of which bears the attaching disc with a number of attaching clamps. The length of the body was 8.5 mm, the width 2.5 mm. The asymmetrical attaching disc occupies the posterior end of the body and attaching clamps are distributed in one row along the edge of the disc. The number of clamps was 52. The length of clamps was 0.065 mm and the width was 0.040 mm.

Hysterothylacium aduncum (Rudolpi, 1802)
Site of infection: Intestine
Infection prevalence: 56.3%
Intensity: 1-27
Parasite: The fourth-stage larvae had 3 distinct lips with short interlabia and a spinous tail, a feature sometimes referred to as a cactus tail (Fig. 1J,K). The larvae averaged 14.30 (10.05-17.44) mm in length and 0.20 (0.13-0.26) mm in width at the nerve ring. The mean length of the esophagus was 1.23 (0.77-1.45) mm, the intestinal caecum 0.18 (0.10-0.25) mm, and the ventricular appendix 0.40 (0.37-0.49) mm. The nerve ring averaged 0.37 (0.32-0.43) mm from the anterior extremity.

Diphyllobothrium sp.
Site of infection: Intestine
Infection prevalence: 12.5%
Intensity: 5-23
Parasite: Parasite specimens were found at the stage of procercoid (Figure 1L). Scolex and proglottid formation was not observed, however, one segment at the tip of the parasite probably reflecting the formation of scolex was evident (Figure 1L). It has a whole body in size from 0.30 mm to 0.37 mm in length and in width from 0.16 mm to 0.17 mm in length.

Trichodina sp.
Site of infection: Gills
Infection prevalence: 6.3%
Intensity: 3

Table 2. Measurements (in micrometres) of *Sigmomyxa sphaerica* from *Belone belone* in the Black Sea compared with those published previously from *B. belone.*

	Thélohan (1895)	Pogoreltseva (1964)	Lubat et al. (1989)	Mladineo et al. (2009)	Karlsbakk and Køie (2012)	Our data (n:10) (Sinop)
Plasmodia size	20.0 – 22.0	22.0	20.0 – 22.0	44.0	20.0 – 37.0	31.7 ± 1.1 (30.0 – 33.0) x 25.1 ± 1.0 (24.0 – 27.0)
Spore length	15.0 – 20.0	14.0 – 18.0	14.0 – 20.0	12.95	16.7 – 19.4	24.6 ± 0.9 (23.0 – 26.0)
Spore width	7.0 – 8.0	5.0 – 7.0	7.0 – 10.0	13.16	10.2 – 12.8	11.7 ± 0.5 (11.0 – 12.5)
Spore thickness	-	-	-	8.17	7.9 – 9.1	-
Polar capsule length	-	-	5.0	5.89	6.3 – 9.3	10.5 ± 1.0 (9.0 – 12.0)
Polara capsule width	-	-	3.0	2.83	3.5 – 4.9	4.8 ± 0.7 (4.0 – 6.0)
Polar filament length	60.0	-	-	-	-	115.0-125.0

Parasite: Parasite had characteristic disc-shape with denticulate ring typical to genus *Trichodina*. Unstained specimens were measured about 0.09 mm in diameter (Figure 1M).

Discussion

Studies on the parasite fauna of garfish *Belone belone* are limited (Grabda, 1981; Popjuk, 2009; Gaevskaya et al., 2010) and have mainly focused on one group of parasites, such as myxosporeans (Lubat et al., 1989; Yurakhno, 2004; Mladineo et al., 2009; Karlsbakk and Køie, 2012) or monogeneans (Strelkov, 1953; Euzet and Lopez-Roman, 1973). Our study, however, presents comprehensive data on the parasite fauna of *B. belone* including new parasite records.

Two coelozoic myxosporeans, *Ceratomyxa beloneae* Lubat et al., 1989 and *Sigmomyxa sphaerica* (Thélohan, 1895), are the only myxosporean parasites reported to be specific to gall bladder of *B. belone* in the Mediterranean, Adriatic, Baltic and Black Seas (Lubat et al., 1989; Yurakhno, 2004; Mladineo et al., 2009; Karlsbakk and Køie, 2012). *Sigmomyxa sphaerica* (Syn. *Myxidium sphaericum*) was described first time from *B. belone* in French waters of the Mediterranean (Banyuls) and Atlantic Ocean (Thélohan, 1895). *C. beloneae* was found as a new species in the Montenegro waters of Adriatic Sea (Bay Boka Kotorska) (Lubat et al., 1989). First registration of *S. sphaerica* in the Black Sea was made by Pogoreltseva (1964) in Ukrainian waters. Yurakhno (2004) reported both *C. beloneae* (January, 2001; prevalence 20 %) and *S. sphaerica* (December, 2000; prevalence 25 %) simultaneously in the same host near Sevastopol. It was the first finding of *C. beloneae* in the Black Sea. *S. sphaerica* was also reported by Yurakhno (2009) in 7 % of *B. belone* near nature reserve Karadag (eastern coast of Crimea) in June. This study is also the first report of these species in Turkish coastal zone (Sinop). By descent *C. beloneae* and *S. sphaerica* are Mediterranen aliens in the Black Sea. Form and measurements of *C. beloneae* spores and plasmodia in our data correspond to primary source (Lubat et al., 1989) and the smaller measurements of Mladineo et al., (2009) (see Table 1). The shape of *S. sphaerica* correspond to literature data, measurements are similar

or slightly longer than measurements in other studies (see Table 2).

Studies on monogenean parasites of *B. belone* are limited. Many monogeneans are known to be host specific and *Axine belones* has been reported from *B. belone* in the Mediterranean and Black Seas (Strelkov, 1953; Euzet and Lopez-Roman, 1973; Popjuk, 2009; Gaevskaya et al., 2010). Morphometric data measured here are in accordance with those in Euzet and Lopez-Roman (1973) but larger than those in Strelkov (1953). This is the first report of *A. belones* in *B. belone* in Turkish coasts of the Black Sea. Gaevskaya et al., (2010) reported infection prevalence values differing from 36% to 56% and mean intensities of 0.5 – 3.0 in the years between 2007 and 2010 in the Black Sea. Popjuk (2009) also reported infection prevalence of 16% and intensities between 1-30 specimens per fish. The infection intensities were lower in our study.

The anisakid nematode *Hysterothylacium aduncum* is widely distributed species in the Black Sea and known from a number of fish species. It was reported in *B. belone* in Baltic Sea and the Black Sea (Grabda, 1981; Popjuk, 2009; Gaevskaya et al., 2010; Gaevskaya et al., 2012). It has also been reported in different fish species, except *B. belone,* in Turkish waters of the Aegean Sea by Akmirza (1997), in the Sea of Marmara by Oğuz (1995) and in the Black Sea by Doğanay (1994), Avşar (1997), and Özer et al., (2000, 2007). We have found *H. aduncum* for the first time in *B. belone* in Turkish coasts of the Black Sea. The infection intensities in the present study are similar to Gaevskaya et al., (2012) and lower than reported by Popjuk (2009) in *B. belone*. It lives as sexually mature adults in the digestive tracts of marine teleosts and its larvae are known to occur in marine invertebrates and in fish, including *B. belone*. *Hysterothylacium aduncum* is one of the nematodes that may cause anisakidosis (Shih and Jeng, 2002) and is responsible for human infections caused by the consumption of raw, undercooked, not adequately salted, pickled, or smoked seafood (Ruitenberg et al., 1979; Ward et al., 1997).

In the present study, several cestode procercoids in the stage of recently shed by copepod host were found in 2 specimens of garfish (prevalence 12.5%). The condition of our material precluded identification at specific level. However, based on morphological resemblances and geographical evaluation, our samples corresponds likely to *Diphyllobothrium* sp. Fish are intermediate hosts of *Diphyllobothrium* and plerocercoids may survive in their body from several months up to a few years (Dick et al., 2001). Scholz et al., (2009) reported several *Diphyllobothrium* species in their comprehensive review as a human infecting marine parasite as raw or undercooked fish consumption.

In Turkey, studies on the trichodinid ciliophorans are few and increasing in number recently. As a result, several species of trichodinid ciliophorans representing the genera *Trichodina* Ehrenberg, 1838, *Paratrichodina* Lom, 1963, and *Trichodinella* Sramec-Husek, 1953 have been reported from different freshwater, brackish and marine fishes in Turkish waters (Özer and Erdem, 1998; 1999; Özer, 2000; 2003a,b; 2007; Özer and Öztürk, 2004; Öztürk and Özer, 2008). However, there is only one record on a trichodinid, namely *Trichodina inversa,* on garfish *B. belone* in the Black Sea so far (Gaevskaya et al., 1975). Based on morphological resemblances, our trichodinid was obviously a

member of genus *Trichodina*. However, the measurement data obtained from unstained specimens in the present study reveals that our species is different from *T. inversa*, our species being four times larger. Unfortunately, it was not possible to identify this parasite to species level as a result of low numbers encountered.

In conclusion, this study yielded new information on the parasite fauna of garfish *B. belone* and a total of 6 parasite species were identified, two only in genus level. *Ceratomyxa beloneae* and *S. sphaerica* are new parasite species for Turkish fauna and *A. belones, H. aduncum, Trichodina* sp. and *Diphyllobothrium* sp. are reported in *B. belone* in Turkish coasts of the Black Sea for the first time.

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