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RESEARCH PAPER

Metazoan Parasite Fauna of the Red Mullet, *Mullus barbatus ponticus* Essipov, 1927 in the Sinop Coasts of the Black Sea

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Abstract

In the present study, a total of 330 specimens the red mullet, *Mullus barbatus ponticus* caught in the Black Sea coasts near Sinop, Turkey were investigated throughout a 1-year period for their metazoan parasite fauna. Thirteen metazoan parasite species, *Loma* sp., *Proctotrema bacilliovatum, Galactosomum lacteum* (metacercariae), *Progrillotia dasyatidis, Nybelinia* sp., *Scolex pleuronectis* (pleurocercoid), *Hysterothylacium fabri, H. aduncum, Ascarophis valentina, Capillaria* sp. *Contracaecum* sp., unidentified larval nematode (L2) and *Acanthocephaloides irregularis* were identified. The overall infection prevalence and mean intensity values were 97.88% and 34.12±5.23 parasites per infected fish, respectively. *Proctotrema bacilliovatum* was the core species with infection prevalence of 70.61% and mean intensity value of 29.09±7.01 parasites per infected fish. The infection indice values for each parasite species were also determined and discussed according to season, length classes and sex of fish. This paper is the first report on metazoan parasite fauna of the red mullet in Turkey.

Keywords: Mullus barbatus ponticus, metazoan parasites, Black Sea.

Introduction

The family Mullidae is a commercially important fish group which can be seen throughout the world's seas. They are demersal fishes in coastal water, including estauries, over sandy or muddy bottoms of temperate and tropical region. The genus Mullus is represented by two species (M. barbatus L., 1758 and M. surmuletus L., 1758) and one subspecies (M. barbatus ponticus Essipov, 1927). Mullus barbatus and M. surmuletus are distributed in the Mediterranean Sea, the eastern North Atlantic Ocean and the Black Sea. Mullus barbatus ponticus Essipov, 1927 is taxonomically classified as a subspecies of Mullus barbatus in the Black Sea (Hureau, 1986; Sahin & Akbulut, 1997; Turan, 2006). Since the red mullet is both economically valuable and an export product of Turkish fisheries, it is among the important demersal fish of the Black Sea. In spite of their ecological and economical importance in the region, the research on the parasitic fauna and parasite ecology of the fish are still not enough. There are many parasitological studies on Mullus barbatus from the Mediterranean and Atlantic Seas (Essafi, Raibaut Boudaoud-Krissat, 1983; Abo-Esa, 2007, & Paradiznik & Radujkovic, 2007; Ramdane & Trilles, 2010; Carreras-Aubets, Repulles-Albelda, Kostadinova & Carrassón, 2011; Carreras-Aubets, Montero, Kostadinova & Carrassón, 2012; Debenedetti, Madrid & Fuentes, 2013; Radujkovic & Sundic, 2014). On the other hand, studies on the parasite fauna of *Mullus barbatus* or *M. barbatus ponticus* in Black Sea are limited (Ovcharenko & Yurahno, 2006; Gaevskaya, 2012).

The aim of the present study is to reveal the parasite fauna of the red mullet, their infection prevalences and intensities in relation to season and length classes as well as sex of fish. This study is the first to provide data on metazoan parasites and their infection indices in red mullet collected from the Turkish Black Sea coast.

Materials and Methods

Fish specimens were collected from commercial fishing vessels off Sinop coast in the Black Sea in Turkey ($42^{\circ}00'05''$ N, $35^{\circ}08'59''$ E). Samplings were carried out monthly throughout a one-year period. A total of 330 fish specimens were investigated for parasites. At necropsy, total length and sex were recorded for each individual. The length of fish varied between 8.0 and 21.0 cm during whole sampling

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period. Seasonally, the average length of the fish was recorded as Lsp: 14.2 cm in spring, Lsu; 13.5 cm in summer, Lau: 13.9 cm in autumn, Lwi: 14.5 in winter. In addition fish were grouped according to the following size classes after total lengths had been measured: <13 cm (up to 13.0), 13-15 cm (13.0-14.9), and >15 cm (15.0-21.0). The three size classes were chosen according to the approximate age of the species suggested by Aydın and Karadurmuş (2013). External (gills, skin, fins, eyes) and internal organs (heart, liver, kidney, gall bladder, muscle) of fishes were examined for parasites and the number of parasites was counted individually and the site of infection was recorded.

Fish were then examined for parasites using standard methods. Digenean parasites were fixed in Bouin's solution, nematodes were fixed in hot (70-80 C) 70% ethanol, cleared in Amann lactophenol, acanthocephalans and cestode were fixed in alcohol 70% and studied by direct examination between slide and cover slip. The parasites were identified based on morphological criteria. Parasite preparations were conducted according to methods indicated by Bray, Gibson and Jones (2008) for digeneans, Petter, Lebre and Radujkovic (1984); Ferrer et al. (2005) for nematodes, Beveridge, Neifar and Euzet (2004) for cestodes, Amin, Oğuz, Heckmann, Tepe and Kvach (2011) for acanthocephala. Morphological diagnostic features of parasite specimens were identified using light microscope (Olympus BX53) fitted with digital camera attachment (DP50).

The prevalence and mean abundance values of *Hysterothylacium aduncum* and *H. fabri* were given as *Hysterothylacium* spp. for pooled data rather than by each *Hysterothylacium* species.

The prevalence, mean intensity, abundance and intensity range (minimum and maximum parasites number) were determined according to Bush, Lafferty, Lotz and Shostak (1997). The standard error (SE) for mean intensity and abundance given. While the differences in the mean intensities values of each parasite species for length classes of fish and the seasons was tested by the Kruskal-Wallis test (nonparametric ANOVA), the difference between parasite loads in male and female fish was tested by the Mann-Whitney U test. Chi square was used on the prevalence of parasites. The analyses were carried out using the computer programme GraphPad Instat 3.0 and "Minitab 16 statistical software. The significance level was P<0.05.

Results

A total of 13 metazoan parasite species were found (Table 1). These are; Loma sp. (Figure 1), Proctotrema bacilliovatum, Galactosomum lacteum (metacercariae) (Figure 2), Progrillotia dasyatidis, Nybelinia sp., Scolex pleuronectis (pleurocercoid) (Figure 3), Hysterothylacium fabri, H. aduncum, A. valentina, Capillaria sp., Contracaecum sp. (Figure 4), an unidentified larval nematode (L2) and Acanthocephaloides irregularis (Figure 5). Table 1 illustrates identified parasite list with indications of prevalence, mean intensity, site of infection (microhabitat) and parasite range. No parasite species was detected in the muscle of examined fish. The infection prevalence (%) and mean intensity values were 97.88% and 34.12±5.23 parasites per infected fish, respectively. Parasite component communities in M. barbatus ponticus comprised 13 parasite species; of these, 6 were rare by having prevalence values less than 10% in the entire dataset (Table 1).

Seasonal prevalence and mean intensity of all parasite species are reported in Table 2. The overall mean intensity values of parasite species on M. barbatus ponticus varied significantly among the seasons (P<0.05) and the highest mean intensity occurred in summer. Proctotrema bacilliovatum, P. dasyatidis, Hysterothylacium spp. (H. fabri, H. aduncum), A. valentina, Capillaria sp., Contracaecum sp., and an unidentified larval nematode (L2) were recorded in fish at all seasons. The mean intensity values of Ρ. bacilliovatum, Ρ. dasyatidis, Hysterothylacium spp., Capillaria sp., Contracaecum sp., and an unidentified larval nematode (L2) varied significantly depending on the seasons (P<0.05, Table 2).

The infection parameters of parasite species in

Table 1. Infection site (microhabitat), prevalence (%),mean intensity (MI \pm SE:) and mean abundance (MA \pm SE) values of parasite species found in red mullet, *Mullus barbatus ponticus*

	Microhabitat	Pr (%)	MI ±SE	$MA \pm SE$	Min-Max
Loma sp.	rectum	0.91	96.67 ± 76.56	0.88 ± 0.76	7-249
Galactosomum lacteum	gills	0.61	3.00 ± 0.00	0.018 ± 0.015	1-5
Proctotrema bacilliovatum	intestine and rectum	70.61	29.09 ± 7.01	20.54 ± 5.00	1-1187
Progrillotia dasyatidis	gall bladder, stomach	38.79	3.30 ± 0.49	1.28 ± 0.21	1-46
Nybelinia sp.	gall bladder, stomach	1.21	1.00 ± 0.00	0.006 ± 0.004	1-1
Scolex pleuronectis	intestine	2.12	1.57 ± 0.30	0.033 ± 0.014	1-3
Hysterothylacium spp.	intestine, body cavity, gall bladder, stomach	56.36	4.56 ± 0.45	2.57 ± 0.28	1-46
Ascarophis valentina	esophagus, stomach	3.94	3.31 ± 1.21	0.13 ± 0.06	1-16
Capillaria sp.	stomach	39.39	3.39 ± 1.21	1.34 ± 0.15	1-26
Contraceacum sp.	intestine, body cavity, gall bladder, stomach	40.0	4.77 ± 0.51	1.91 ± 0.24	1-45
unidentified larval nematode (L2)	Pyloric ceace	43.33	10.78 ± 1.97	4.67 ± 0.90	1-170
Acanthocephaloides irregularis	intestine	1.21	2.75 ± 1.75	0.033 ± 0.025	1-8
OVERALL		97.88	34.12 ±5.23	33.40 ± 5.62	1-1187

Pr.: prevalence, SE: standard error



Figure 1. Photomicrographs of *Loma* sp. A. spores of *Loma* sp, fixed in Bouin's fixative; B. xenomas of *Loma* sp.; C. part of the intestine wall containing cysts of *Loma* sp. (young and mature xenomas), (original).



Figure 2. Photomicrographs of two digenean species. A. *Proctotrema bacilliovatum*, Odhner, 1911. B. *Galactosomum lacteum* (Jagerskiold, 1896) excysted metacercaria, (*Os*: oral sucker; p; pharynx, Ov, ovary, Vs: ventral sucker, t, testis, Cs; cirrus sac) (fresh material, original).

three length classes of fish are given in Table 3. *Loma* sp., *G. lacteum, Nybelinia* sp. and *A. irregularis* were found only in some length classes of fish, *Loma* sp. in the middle and large and *Nybelinia* sp. only in the largest. Although large fish had higher mean intensity value than did small fish, the difference among the fish length classes was statistically insignificant (P>0.05, Table 4).

Both overall infection prevalence and mean intensity values were higher in females than in males (Table 4). However, the difference between the mean values was not statistically significant (P>0.05).

The only microsporidian parasite detected was *Loma* sp. in the rectum and intestine of fish with a prevalence of 0.91%. The digeneans, *Proctotrema*

bacilliovatum and *Galactosomum lacteum* occurred in the digestive tract as adults and metacercaria, respectively. *Proctotrema bacilliovatum* was the most abundant and had 70.61% infection prevalence and mean intensity values of 29.09±7.01 parasites per infected fish. On the contrary, *Galactosomum lacteum* was the least (Table 1). *Progrillotia dasyatidis, Nybelina* sp. and *Scolex pleuronectis* cestodes were found in the gall bladder, stomach and intestine.

Six nematode species, *Hysterothylacium* aduncum, H. fabri, Ascarophis valentina, Capillaria sp., Contracaecum sp. and unidentified larval nematode (L2), were identified. While Ascarophis valentina and Capillaria sp. were adults, Contracaecum sp. was third stage larvae (L3) and H.



Figure 3. Photomicrographs of the three cestode species: A. *Progrillotia dasyatidis* Beveridge Neifar & Euzet, 2004; B. tentacles of *P. dasyatidis*, C. *Nybelinia* sp., D. tentacle of *Nybelinia* sp. E. *Scolex pleuronectis* (plerocercoid), F. bothrial margin of *S. pleuronectis* (original).



Figure 4. Photomicrographs of the four nematode species. A. anterior end of *Hysterothylacium fabri* (Rudolphi, 1819), B. anterior end of *H. aduncum* Rudolphi, 1802, C. *Contracaecum* sp. (encysted), D. tail (female) of *Ascarophis valentina* Ferrer *et al.*, 2005, E. tail of (male) of *A. valentina*, F. gravid female of *A. valentina*, G. tail (female) of *Capillaria* sp., H. tail (male) of *Capillaria* sp., I. developed egg of *Capillaria* sp. (original).

aduncum and H. fabri were at their third and fourth larval stage. Adult *Acanthocephaloides irregularis* specimens were isolated from the intestine at a low infection rate.

Discussion

The present study is the first report on the parasite fauna of the red mullet captured from the Black Sea coast of Turkey and provides some new data about its parasite fauna and their intensities in relation with season and some host factors.

So far, members of the genera Aponurus, Holorchis, Derogenes, Lecithaster, Lasiotocus, Proctotrema and Anisocladium have been reported in Mullus barbatus in the Mediterranean Sea (Bray, 1985; Bartoli & Bray, 1996; Bartoli, Gibson & Bray, 2005; Carreras-Aubets et al., 2011; Carreras-Aubets et al., 2012; Debenedetti et al., 2013; Radujkovic & Sundic, 2014). Expect Proctotrema, the absence of



Figure 5. Photomicrographs of *Acanthocephaloides irregularis* Amin, Oğuz, Heckmann, Tepe & Kvach, 2011. A. female specimen, B. male specimen, C. anterior regio, D. Proboskis, E. posterior regio (original).

these digenean parasites in this study could be resulted from the lack of the final and intermediate hosts in our sampling area. Proctotrema bacilliovatum which was previously recorded in Mullus surmuletus in the Sea of Marmara and the Aegean Sea, had not been previously recorded in M. barbatus ponticus from the Black Sea coasts of Turkey. The mean intensity of P. bacilliovatum was higher in summer than in winter in the present study. Water temperature is one of the most important factors determining release of digenean cercariae from the first host snail. Moreover, transmission to the fish host is highly temperature-dependent. It's could be due to effect of seasonal temperature. To date, Galactosomum lacteum metacercaria has been reported in marine teleosts from the Black Sea and the Mediterranean Sea (Lushchina, 1985; Culurgioni, D'Amico Figus, 2007). It is noticeable that this digenean species identified here is reported for the first time in this fish species from Turkey.

Progrillotia dasyatidis has been recorded in gobiid fish in the Sea of Marmara (Oğuz & Bray, 2008), and in various teleosts including M. barbatus in the Black Sea coast of Turkey (Tepe, Oğuz & Heckmann, 2014). Marine cestodes such as Progrillotia dasyatidis use planktonic copepods as first and, sometimes, second intermediate hosts. Zander, Strohbach and Groenewold (1993) reported that, in benthic fish, larval cestodes are presumably acquired during the period of the preadult stages, as a result of the ingestion of planktonic crustaceans. The results of this study show that infection with P. dasyatidis increases with fish length, so that parasites are still being acquired after the fish have apparently ceased to feed on copepods.

Nematoda was the most represented taxa with two adult (Ascarophis valentina, Capillaria sp.) and four larval (Hysterothylacium fabri, H. aduncum, Contracaecum sp. and unidentified larval nematode) parasites in M. barbatus ponticus in the present investigation. Among nematodes, H. fabri and H. aduncum showed consistently high prevalence as they were found commonly in Mullus surmuletus and M. barbatus in the Mediterranean Sea (Radujkovic & Raibaut, 1989; Le Pommelet, Bartoli & Silan, 1997; Carreras-Aubets et al., 2012). Moreover, several fish species have also been reported to act as intermediate and/or paratenic host for H. fabri and H. aduncum in the Mediterranean and Black Sea (Carreras-Aubets et al., 2012; Tepe & Oğuz, 2013). In the present study, seasonal changes in the prevalence of *Hysterothylacium* spp., Capillaria sp. and Contracaecum sp. and an unidentified larval nematode (L2) were determined. Statistical analysis has releaved the presence of significant changes among seasons for these parasite species (Table 3). Generally the highest values of the prevalence were recorded in spring and summer. Similarly, Gordon (1977) observed a decline in prevalence during cold months and the highest prevalence in July/August in whiting. Moreover, Ismen and Bingel (1999) found that the prevalence and intensity of H. aduncum were significantly higher in the warm season (July/August) than in the colder period (January/February). There is good agreement between the results obtained in the present study and those found by Gordon (1977) and Ismen and Bingel (1999).

Capillariid nematodes use usually oligochaetes and rarely fish as intermediate hosts. Especially, oligochaetes have been reported by Moravec, Prokopic and Shlikas (1987) as act as transport hosts and is important for the transmission of capillarids. *Mullus barbatus* is a benthic predator and mainly feeds on small crustaceans and polychaetes (Chérif *et al.*, 2011). It has been reported that the composition of the ingested prey varied with the size of predator fish and cephalopods occurred exclusively in the diet of larger specimens than smaller ones, and diets varied seasonally; decapods were commonly consumed in summer, whereas amphipods were commonly consumed during winter and spring (Labropoulou, Machias, Tsimenides & Eleftheriou, 1997). The

	Prevalence (%)				Mean Intensity \pm SE			
	Spring (n: 78)	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Parasites species		(n: 82)	(n: 80)	(n: 90)	(Lsp: 14.2 cm)	(Lsu: 13.5 cm)	(Lau: 13.9 cm)	(Lwi: 14.5 cm)
Loma sp.	1.28	0.0	0.0	2.22	7.00 ± 0.00	0.0	0.0	141.50 ± 107.50
P. bacilliovatum	51.28	79.27	62.50	86.67	11.90 ± 2.73^{a}	77.37 ± 23.98^{b}	10.60 ± 3.23^{a}	9.51 ± 1.37^{a}
G. lacteum	0.0	0.0	2.53	0.0	0.0	0.0	$.00 \pm 2.00$	0.0
P. dasyatidis	37.18	36.59	28.75	51.11	4.03 ± 1.55	3.67 ± 1.11	3.48 ± 1.07	2.50 ± 0.36
Nybelinia sp.	0.0	4.88	0.0	0.0	0.0	1.00 ± 0.00	0.0	0.0
S. pleuronectis	5.13	2.44	0.0	0.0	1.25 ± 0.25	2.50 ± 0.50	0.0	0.0
Hysterothylacium spp.	53.85	41.46	70.00	60.00	2.74 ± 0.35^{a}	3.79 ± 0.85^{a}	7.68 ± 1.25^{b}	3.22 ± 0.41^{a}
A. valentina	1.28	6.09	3.75	4.44	1.00 ± 0.00	4.60 ± 2.87	3.67 ± 2.19	2.00 ± 1.00
<i>Capillaria</i> sp.	46.15	58.54	37.50	17.78	2.50 ± 0.33^{ac}	4.90 ± 0.71^{bc}	$2.87\pm0.43^{\rm ac}$	1.88 ± 0.30^{a}
Contraceacum sp.	66.67	28.05	3.75	53.33	3.17 ± 0.55^{a}	$6.61 \pm 2.01^{\rm ac}$	$2.56\pm0.97^{\rm a}$	$6.02 \pm 0.74^{\rm bc}$
larval nematod (L2)	33.33	53.66	52.50	34.44	4.58 ± 2.24	7.93 ± 1.83	14.67 ± 4.95	14.74 ± 5.12
A. irregularis	0.0	4.88	0.0	0.0	0.0	2.75 ± 1.75	0.0	0.0
TOTAL	96.15	97.56	67.50	100	14.60 ± 2.32^{a}	75.54 ± 19.57^{b}	$19.30 \pm 3.09^{\rm ac}$	$23.32 \pm 3.99^{\circ}$

Table 2. Seasonal infection prevalence (%) and mean intensity (MI \pm SE) values of parasites found in M. barbatus ponticus (n: number of examined fish, SE: standard error, Means followed by the same superscript letter are not significantly different (P<0.05)

Lsp, Lsu; Lau, Lwi: seasonally, the average length of the fish

Table 3. Infection prevalence	(%) and mean intensit	v (MI±SE) levels of	parasites founded in <i>M. barbatus</i>	<i>ponticus</i> according to fish lenght classes

		Prevalence (%)			Mean Intensity±SE	
Parasites species	<13 cm	13-15 cm	>15 cm	<13 cm	13-15 cm	>15 cm
	(n: 60)	(n: 193)	(n: 77)	(n: 60)	(n: 193)	(n: 77)
Loma sp.	-	0.52	2.60	-	34.0 ± 0.00	128.0 ± 121.0
P. bacilliovatum	60	72.54	74.02	41.39 ± 20.52	31.97 ± 10.29	14.23 ± 3.61
G. lacteum	-	1.04	-	-	3.00 ± 2.00	-
P. dasyatidis	48.33	25.23	40.26	2.93 ± 0.82	3.25 ± 0.55	3.74 ± 1.45
Nybelinia sp.	-	-	5.19	-	-	1.00 ± 0.00
S. pleuronectis	3.33	1.04	3.89	1.00 ± 0.00	1.50 ± 0.50	1.33 ± 0.33
<i>Hysterothylacium</i> spp.	48.33	58.55	57.14	3.86 ± 0.72	4.49 ± 0.58	5.20 ± 1.12
A. valentina	3.33	3.63	5.19	1.00 ± 0.00	1.86 ± 0.55	7.00 ± 3.34
<i>Capillaria</i> sp.	51.67	38.86	31.17	3.13 ± 0.65	3.16 ± 0.31	4.46 ± 1.12
Contraceacum sp.	36.67	36.27	51.95	4.18 ± 1.05	5.06 ± 0.77	4.58 ± 0.82
unidentified larval nematode (L2)	48.33	46.11	32.41	8.38 ± 2.97	12.84 ± 2.92	6.20 ± 2.50
A. irregularis	3.33	1.04	-	1.00 ± 0.00	4.50 ± 3.50	-
TOTAL	98.33	97.41	98.07	36.05 ± 12.74	37.25 ± 7.81	24.87 ± 4.88

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Table 4. Infection prevalence (%) and mean intensity (MI \pm SE) values of parasites found in female and males of *M*. *barbatus ponticus*

	Prevale	ence (%)	Mean Intensity \pm SE		
Parasites species	Female	Male	Female	Male	
	(n:266)	(n:64)	(Lf: 14.27)	(Lm: 13.11)	
<i>Loma</i> sp.	0.75	1.56	128.0 ± 121.0	34.0 ± 0.00	
P. bacilliovatum	72.18	64.06	30.11 ± 7.62	24.29 ± 17.85	
G. lacteum	0.38	1.56	1.00 ± 0.00	5.00 ± 0.00	
P. dasyatidis	40.6	31.25	3.57 ± 1.26	1.80 ± 0.35	
<i>Nybelinia</i> sp.	1.13	1.56	1.00 ± 0.00	1.00 ± 0.00	
S. pleuronectis	1.88	3.12	1.80 ± 0.74	1.00 ± 0.00	
H. aduncum H. fabri	56.77	54.67	4.58 ± 0.53	4.46 ± 0.76	
A. valentina	3.38	3.12	3.56 ± 1.62	2.75 ± 1.75	
Capillaria sp.	40.6	34.38	3.59 ± 0.37	2.41 ± 0.34	
Contraceacum sp.	42.11	31.25	4.71 ± 0.54	5.05 ± 1.42	
unidentified larval nematode (L2)	42.11	48.44	7.34 ± 1.43	23.19 ± 7.13	
A. irregularis	1.13	1.56	3.33 ± 2.33	1.00 ± 0.00	
TOTAL	98.1	96.88	34.12 ± 5.78	34.11 ± 12.33	

Lf: the average length of the female fish, Lm: the average length of the male fish

prevalence value of *Capillaria* sp. was higher in summer than that of in winter (Table 2). These values were also negatively correlated with increasing fish size (Table 3). Moreover, larger fish (Lwi: 14.5 cm) caught during winter had lower parasite intensity than smaller fish (Lsu: 13.5) caught during summer. Seasonal average lenght of fish in summer has higher and this clearly explanied its lower presence in other seasons. Thus our results indicated that the prevalence of infection had a relation with host size.

Ascarophis valentina was first identified in Mullus surmuletus from the Mediterranean Sea (Ferrer et al., 2005). So far, some species of Ascarophis genus have been described in mullid species; Ascarophis mullusi in M. surmuletus from the Aegean Sea and in M. barbatus from the Adriatic Sea. Indeed, A. valentina has not been recorded outside the Mediterranean Sea (Le Pommelet et al., 1997; Ferrer et al., 2005; Klimpel, Kleinertz & Palm, 2008) and this study is the first report of A. valentina in M. barbatus ponticus from the Black Sea.

In conclusion, the metazoan parasite fauna of *M. barbatus ponticus* in Turkish Black Sea coasts firstly reported with this study. We report *Proctotrema bacilliovatum* and *Ascarophis valentina* to be new records for Turkish parasite fauna. *Ascarophis valentina* is also a new record in Black Sea. Thus, we can say that the geographical distribution of this parasite has extended.

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