

## Metazoan parasites of common sole (*Solea solea*) and scaldfish (*Arnoglossus laterna*) (Pleuronectiformes) from Sinop coast of Black Sea

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### Summary

The metazoan parasites were investigated of two flatfish the common sole, *Solea solea* (n:140) and the scaldfish, *Arnoglossus laterna* (n: 22) in the Sinop coast of Black Sea from June 2015 to June 2017. A total of 15 metazoan parasite species belonging to Digenea (6), Cestoda (3), Acanthocephala (1), Nematoda (4) and Isopoda (1) taxonomic groups were identified. *Solea solea* was found to be infected by *Condyllocotyla pilodora*, *Proctoeces maculatus*, Opecoelidae gen. sp., *Metadena* sp., *Stephanostomum* sp., *Progrillotia* sp., *Capillaria gracilis*, *Cucullanus campanae*, *Solearhynchus rhytidotes* and *Nerocila orbignyi*. *Arnoglossus laterna* was found to be infected by *Lecithochirium musculus* and *Grillotia erinaceus*. *Scolex pleuronectis*, *Hysterothylacium aduncum* and *Dichelyne minutus* were determined in both flatfish. Infection prevalence and mean intensity values were recorded for each parasite species. Infection values for each parasite species in relation to season and fish size were also determined and compared as comparatively. This study is the first one assessing the metazoan parasites both of *S. solea* and *A. laterna* collected from the Turkish coast of Black Sea. While *Condyllocotyla pilodora* and *Capillaria gracilis* are new parasite records for *S. solea*, *Grillotia erinaceus* is new parasite record for *A. laterna*. Moreover, this paper is the first report on occurrence of Opecoelidae gen. sp., *Metadena* and *Stephanostomum* genera in *S. solea*.

**Keywords:** Common sole; Scaldfish; Metazoan parasite; Sinop; Black sea

### Introduction

Pleuronectiformes, also called flatfish, are characterized by oval-shaped, flattened bodies, unique among fishes in being asymmetrical with both eyes on one side in adults. Flatfish fishery is among the most profitable demersal types of activity, since these species are economically and ecologically important part of benthic ecosystems in the world (Diaz de Astarloa, 2002; Gibson, 2005). The common sole, *Solea solea* (Linnaeus, 1758) and the scaldfish, *Arnoglossus laterna* (Walbaum, 1792) are flatfish inhabiting sandy or muddy marine bottoms of the sea floor. They feed on small bottom-dwelling invertebrates, including polychate, bivalves

and crustaceans. They are to be found in the Black Sea and in the Mediterranean Sea along the west coast of Europe to Norway (Nielsen, 1973). *A. laterna* is caught in commercial trawlers, but it is usually discarded because of this fish species generally not being preferred for human consumption. Although it is not a commercially important species, it is important ecologically as it serves within the food chain for other predator fish. However, *S. solea* is a species with high commercial interest, moreover it has been shown as suitable species for aquaculture (Fanelli, 2008). Parasites are a natural part of all ecosystems and they represent important component of the biodiversity of shallow coastal areas (Combes, 2001). Besides they play an important role for their host,

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as they give information about both physiology and ecology (etc. growth, condition, survival, fecundity, behaviour and population structure) of their hosts (Combes, 2001; MacKenzie and Abaunza, 2005). Parasitism is an important factor that could substantially affect on host biology, ecology and phylogeny (Marques *et al.*, 2011). In reared fish species, it is extremely important to know which parasites are found in the target fish species. The aim of present study was to reveal the metazoan parasites of two flatfish, *S. solea* and *A. laterna* in Sinop region of the Black Sea. The diversity of parasites in relation to seasonal and size classes of the two flatfish were investigated and described for the first time in the Turkish coast of the Black Sea, in order to contribute to the knowledge on parasitofauna of demersal fish health.

### Materials and Methods

Fish samples were collected from the southern Black Sea off Sinop (N 42° 01' 55" E 35° 16' 36") (Fig. 1). A total of 162 flatfish; common sole *S. solea* (n:140), 22 and sculdfish *A. laterna* (n:22) were collected between June 2015 – June 2017 with gill nets and beach seine. Fish were transferred to the laboratory and some part of them examined freshly and some part of them frozen to be examined later. At necropsy, total length and total weight of fish were measured. Fish were grouped in three size classes after total lengths has been measured: class 1: <14.0 cm, class 2: 14.0-15.9 cm, class 3: >15.9 cm. The flatfish were dissected to check

for metazoan parasites using conventional methods; assessing smears of skin, fins, gills, eyes (lens and vitreous humour), kidney, liver, stomach and intestine of fish under the dissecting microscope and then counting parasite specimens. Parasite species were identified by Gibson, 2002, Bray, 2005a;b, Miller and Cribb, 2008, Pearson, 2008 for digeneans, Lebre and Petter, 1984, Køie, 1993, Køie, 2001a, Arai and Smith, 2016 for nematodes, Campbell and Beveridge, 1994, Beveridge *et al.*, 2004, Beveridge and Campbell, 2007 for cestodes, Belofastova, 2006 for Acanthocephala and Al-Zubaidy and Mhaisen, 2013 for Isopoda. All parasite species were examined using a stereo Olympus microscope (BX53) equipped with a digital camera (DP50). The terms prevalence (percentage of fish infected), mean intensity (mean number of parasites per infected fish), and mean abundance (mean number of parasites per examined fish) were calculated following the definitions of Bush *et al.* (1997). The significance of the host-parasite relationship was determined according to the abundance values; core species were those represented by >2 parasite species, secondary species by 0.6-2, satellite species by 0.2 – 0.6 and rare species 0.2 (Zander, 2003). The mean infracommunity was characterized as the mean number of parasite species per host individual (Zander, 2004). The differences in the mean intensities values of each parasite species for length classes of fish and seasons were tested by the Kruskal-Wallis test (Nonparametric ANOVA). All statistical analysis were done using Graph Pad InStat Software. Statistical significance level was evaluated at 0.05.

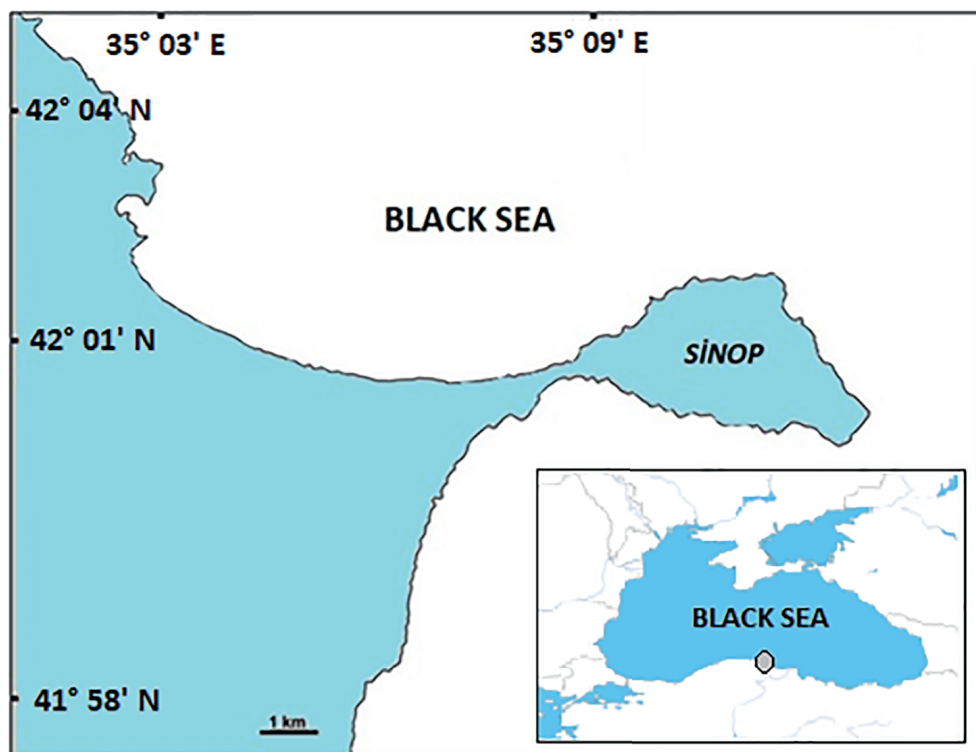


Fig. 1. The southern Black Sea off Sinop.

Table 1. Parasite list with indications of infection site (microhabitat), prevalence (%), mean intensity (MI) and abundance (A) in both flatfish host

Parasites	Solea solea (n: 140)				Arnoglossus laterna (n: 22)			
	Infection Site	P (%)	MI	A	Infection Site	P (%)	MI	A
<b>Digenea</b>								
<i>Lecithochirium musculus</i>					intestine, stomach	81.82	26.39	21.59
<i>Protoeces maculatus</i>	intestine	2.14	1.00	0.02				
<i>Condylotyla pilodora</i> met.	brain	2.14	2.00	0.04				
Opcoelidae gen. sp. met.	gill	18.57	3.00	0.56				
<i>Stephanostomum</i> sp. met.	gill	4.29	1.33	0.06				
<i>Metadena</i> sp. met	gill, eye, kidney	23.57	10.43	2.47				
<b>Cestoda</b>								
<i>Scolex pleuronectis</i> pl.	intestine	5.71	18.62	1.06	intestine	81.82	85.72	70.14
<i>Grillotia erinaceus</i>					intestine	77.27	15.65	12.09
<i>Progrillotia</i> sp.	intestine, gall bladder	6.43	5.44	0.35				
<b>Nematoda</b>								
<i>Hysterothylacium aduncum</i>	intestine	3.57	2.40	0.09	intestine	45.45	5.10	2.32
<i>Capillaria gracilis</i>	intestine,	37.86	3.57	1.35				
<i>Cucullanus campanae</i>	intestine	43.57	5.36	2.34				
<i>Dichelyne minutus</i>	intestine	72.14	26.09	19.05	intestine	9.10	5.00	0.45
<b>Acanthocephala</b>								
<i>Solearthynchus rhytidotes</i>	intestine	28.57	3.67	0.81				
<b>Isopoda</b>								
<i>Nerocila orbignyi</i>	Fins, skin	3.57	2.00	0.31	-	-	-	-
<b>Overall</b>		81.43	26.70 <sup>a</sup>	21.74 <sup>a</sup>		100	106.59 <sup>b</sup>	106.59 <sup>b</sup>

## Ethical Approval and/or Informed Consent

The research related to animals use has been conformed with all the particular national regulations and institutional policies for the care and use of animals. The study protocol no. 19 of 13/07/2015 was approved by Republic of Turkey, Sinop University Experimental Animals Local Ethics Committee.

## Results

In this study a total of 15 metazoan parasites were identified: five species of Digenea; *Lecithochirium musculus* (Hemiuridae), *Proctoeces maculatus* (Fellodistomidae), *Condyllocotyla pilodora* met. (Heterophyidae), Opacoelidae gen. sp. met. (Opacoelidae), *Stephanostomum* sp. met. (Acanthocolpidae), *Metadena* sp. met. (Cryptogonimidae), three of cestodes; *Scolex pleuronectis* pl. (Tetraphyllidae incertae sedis), *Grillotia erinaceus* (Lacistorhynchidae), *Progrillotia* sp. (*Progrillotiidae*) four of Nematoda; *Hysterothylacium aduncum* (Raphidascarididae), *Capillaria gracilis* (Capillariidae), *Cucullanus campanae* and *Dichelyne minutus* (Cucullanidae), one of Acanthocephala; *Solearhynchus rhytidotes* (Echinorhynchidae) and one of Isopoda; *Nerocila orbigny* (Cy-mothoidae). Table 1 summarises parasite list with indications of infection site (microhabitat), prevalence (%), mean intensity (MI) and abundance (A) in both flatfish host. While the common sole was infected with 13 parasite species, the scaldfish was infected with only 5 parasite species. The cestode *Scolex pleuronectis*, the nematodes *Hysterothylacium aduncum* and *Dichelyne minutus* were determined in both host. *Lecithochirium musculus* and *Grillotia erinaceus* were found only in the scaldfish; *Proctoeces maculatus*, *Condyllocotyla pilodora*, Opacoelidae gen. sp. met., *Metadena* sp., *Stephanostomum* sp., *Progrillotia* sp., *Capillaria gracilis*, *Cucullanus campanae*, *Solearhynchus rhytidotes* and *Nerocila orbigny* were found only in the common sole (Table 1). Seven species, *L. musculus*, *Metadena* sp., *S. pleuronectis*, *G. erinaceus*, *H. aduncum*, *C. campanae* and *D. minutus* are designated as core species in the metazoan parasite faunas of two flatfish. In the common sole, the dominant species was *D. minutus*, while in the scaldfish, it was *S. pleuronectis* (Table 1).

Seasonal prevalence and mean intensities of metazoan parasite species in flatfish are showed in Table 2. The species composition and the parasite species richness of the two flatfish were found to be different from each other and varied among the seasons. The overall mean intensity values of parasite species on the common sole varied significantly among the seasons ( $P < 0.05$ ) and the highest mean intensity occurred in spring. *D. minutus* and *S. rhytidotes* were detected in all seasons and maximum infection prevalence was 90.2 % in spring for *D. minutus* and 45.5 % in autumn for *S. rhytidotes*. Metazoan parasite diversity differed between seasons. When evaluating the diversity of the parasite species depending on the seasons in the common sole, maximum value was found in spring encompassing thirteen parasite species. For the scald-

fish, the parasite species richness showed no difference among seasons, except for summer. However, overall infection parameters were similar in all seasons except for summer in the scaldfish (Table 2).

The infection parameters of parasite species in selected three size classes of flatfish are given in Table 3. Opacoelidae gen. sp. met. and *Stephanostomum* sp. were absent in the smallest ( $< 14.0$  cm) and *P. maculatus* and *C. pilodora* were found only in the largest class ( $> 15.9$  cm) of common sole. The largest fish length class had higher overall mean intensities than the other length classes in the common sole, showing statistically significant difference ( $P < 0.05$ ). In the scaldfish, species richness, the overall mean intensity and prevalence values were found to be independent from fish length and showed no significant differences (Table 3). A similar pattern was also seen in the mean infracommunity values between the size classes. The minimum number of parasite species was found in the smallest size class ( $< 14.9$  cm) in the common sole (Table 3).

## Discussion

This study is the first on metazoan parasites of scaldfish from Turkish coast of Black Sea and *G. erinaceus* and *D. minutus* are first geographical report for scaldfish in the Black Sea. There are few studies on metazoan parasite fauna of common sole in Turkey, it is also very limited especially in Turkish coast of Black Sea. To date, only ten metazoan parasite species have been reported in the common sole in Turkish waters according to data from different authors. These are; one species of Isopoda, *Nerocila orbigny* (Kayış and Ceylan, 2011) and one species of Myxozoa, *Sinuolinea rebae* (Özer et al., 2015) in Black Sea, three species of Arthropoda, *Caligus solea* in North Eastern Mediterranean (Demirkale et al., 2014), *Caligus brevicaudatus* and *Caligus apodus* in Eastern Mediterranean (Özak et al., 2013), three species of Cestoda, *Bothriocephalus scorpii*, *Grillotia* sp. and *Scolex pleuronectis* in the Marmara Sea (Keser et al., 2007), one species of Digenea, Hemiuridae metacercaria in the Marmara Sea (Keser et al., 2007), one species of Nematoda, *Hysterothylacium aduncum* in the Marmara Sea (Keser et al., 2007) and in Eastern Mediterranean Sea (Keskin et al., 2015). Other studies of the metazoan parasites of common sole showed that *Hysterothylacium aduncum*, *S. pleuronectis*, *Solearhynchus rhytidotes* (syn: *S. kostylewi*) and *Nerocila orbigny* were previously reported. We found 13 parasite species; 6 of these had already been mentioned in published studies, but so far, 7 species, *Condyllocotyla pilodora*, *Proctoeces maculatus*, Opacoelidae gen. sp. met., *Metadena* sp., *Stephanostomum* sp., *Capillaria gracilis* and *Progrillotia* sp. haven't been reported in the common sole, and this is their first report. But, *C. pilodora*, *P. maculatus* and *Stephanostomum* sp. have been detected in low intensity and is being registered as rare species during this investigation. Therefore, we consider that their presence in the common sole is accidental and that they are parasites of other fish species in the study area. In this study, Digenea was the most represented taxa with a total

Table 2. Seasonal prevalence and mean intensities of metazoan parasite species in flatfish.

Parasites	Solea solea (n: 140)						Arnoglossus laterna (n: 22)					
	Spring (82)		Summer (38)		Autumn (9)		Spring (3)		Summer (2)		Autumn (11)	
	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI
<i>Lecithochirium musculus</i>	3.7	1.0	-	-	-	-	100	92.7	100	23.5	84.6	13.5
<i>Proctoeces maculatus</i>												1.0
<i>Condylomyxa pilodora</i>	3.7	2.0	-	-	-	-						
<i>Opcoelidae</i> gen. sp. met.	31.7	3.0	-	-	-	-						
<i>Stephanostomum</i> sp. met.	6.1	1.4	2.6	1.0	-	-						
<i>Metadene</i> sp. met	35.4	11.4	7.9	4.7	-	-						
<i>Scolex pleuronectis</i> pl.	8.5	14.3	-	-	-	-	66.6	138.5	-	-	92.3	84.9
<i>Grillotia erinaceus</i>							33.3	35.0	-	-	92.3	14.6
<i>Progrillotia</i> sp.	8.5	5.9	-	-	-	-						
<i>Hysterothylacium aduncum</i>	6.1	2.4	-	-	-	-	33.3	9.0	-	-	53.9	5.4
<i>Capillaria gracilis</i>	37.8	3.19	44.7	3.7	45.5	5.4						33.3
<i>Cucullanus campanae</i>	43.9	5.5	65.8	5.2	-	-						
<i>Dichelyne minutus</i>	90.2	26.6 <sup>a</sup>	81.6	7.4 <sup>b</sup>	72.7	19.3 <sup>a</sup>	-	-	-	-	7.7	2.0
<i>Solearhynchus rhytidotes</i>	26.8	3.2	5.26	10.0	45.5	4.0						33.3
<i>Nerocila orbignyi</i>	2.4	1.5	23.7	4.1	18.2	2.0						
<b>Overall</b>	98.8	35.9 <sup>a</sup>	86.8	14.9 <sup>bc</sup>	100	18.2 <sup>ac</sup>	100	199.7	100	23.5	100	106.3
<b>Species richness</b>	13		7		4		4		1		5	
<b>Mean infracommunity</b>	3.06		2.67		1.91		2.3		1.00		3.31	
<b>Mean fish length</b>	15.9 <sup>a</sup>		14.0 <sup>bc</sup>		15.2 <sup>ac</sup>		15.9		15.5		14.1	

Means followed by the same superscript letter are not significantly different.



6 species, 2 adults (*L. musculus* and *P. maculatus*) and 4 metacercariae stage (*C. pilodora*, Opecoelidae gen. sp. met., *Stephanostomum* sp. and *Metadena* sp.). Among the Digeneans, only the *L. musculus* was detected in the sculdfish, the other 5 digenea parasites were detected in the common sole. Except *L. musculus*, other digeneans were observed in spring and summer seasons. Release of digenean cercariae from the snail host and successful transmission to the fish host is highly temperature-dependent. In this study the prevalence of digenean species in spring and summer may be explain with water temperature.

Fish size had an effect in both species diversity and mean intensity of digenean species. In general, mean intensity values were positively correlated with fish size in the common sole (Table 3). *Proctoeces maculatus*, *C. pilodora*, *Stephanostomum* sp. and Opecoelidae gen. sp. met. were absent in the smallest fish size class (<14.0 cm). The absence of these parasites can be explained by dietary differences related to fish length groups. Studies of dietary composition show that the food of the common sole consists of bottom-dwelling invertebrates, such as Polichaeta, Crustacea, Mollusca. The variety of habitats, for example site type, e. g. shallow coastal areas, continental shelf, estuarine systems and the range of fish lengths account for dietary dissimilarities (Muus and Dahlström 1991; Banaru and Harmelin-Vivien, 2009; Cabral 2000).

Four nematode species, *Hysterothylacium aduncum*, *Capillaria gracilis*, *Dichelyne minutus* and *Cucullanus campanae* were determined in two flatfish during the present investigation. The common sole was found to be infected with above mentioned four nematode species, the sculdfish was infected with *H. aduncum* and *D. minutus*. Among recorded nematodes, *D. minutus* and *C. campanae* as core species, *C. gracilis* as secondary species and *H. aduncum* as rare species were determined in the common sole. Whereas in the sculdfish, the *H. aduncum* was detected as core species, and *D. minutus* as satellite species (Table 1). *Cucullanus campanae* is specific parasite of the common sole (Koie, 2000). Similarly, *D. minutus* is a typical and common parasite of demersal fishes such as flounders and gobies (Moravec, 1994; Koie, 2001a). *Dichelyne minutus* uses usually the polychaete *Hediste diversicolor* as an obligate intermediate host, and members of gobiid and flatfish are the definitive hosts of this nematode (Koie, 2001a). *H. diversicolor* has been indicated as the first intermediate host of this nematode in also Black Sea and mature individuals of *D. minutus* were reported in various species of fish (Pronkina *et al.*, 2017). In the present study, seasonal changes in the mean intensity and prevalence of *D. minutus* were determined. The highest prevalence value was recorded in spring (Table 2). Similarly in spring, Pronkina *et al.* (2017) reported high prevalence from the definitive hosts in the Black Sea. The results obtained in the present study are in good agreement with Pronkina *et al.* (2017).

*Capillaria gracilis* which is known mainly as a parasite of gadoid fish, uses usually oligochaetes and rarely fish as the intermediate hosts. It has been reported that gobies act as obligatory intermediate hosts (Koie 2001b). The prevalence value of *C. gracilis* was

higher in summer than in other seasons and it was not detected in winter (Table 2). The mean intensity values were also positively correlated with increasing fish size (Table 3). Seasonal average length of fish in winter is lower and this clearly explains its absence in winter. Thus our results indicated that the infection prevalence had a relation with host size.

*Scolex pleuronectis* (pleuroceroid), *Grillotia erinaceus* and *Progrillotia* sp. were the cestod parasites determined in the present study. *S. pleuronectis* and *G. erinaceus* were the core and dominant species for the sculdfish as seen in overall infection values (Table 1). However *S. pleuronectis* has been previously reported in certain fish species in the same sampling region (Güneydağ *et al.* 2017). Similarly, *G. erinaceus* has been reported in *Merlangius merlangus* in the same sampling area (Özer *et al.*, 2014). Two cestodes species are reported in the sculdfish first time with this study. Moreover, the prevalence and mean intensity values obtained were significantly higher for both cestode species compared with previous reported in the region. Therefore, we suggest that the sculdfish is the principal second intermediate host for both cestode.

*Solearhynchus rhytidotes* is a rare parasite of the common sole. It is redescribed and reported in the common sole captured from the Sea of Marmara by Kvach and Oğuz, 2010.

*Nerocila orbignyi* was detected from fin and skin in the common sole. The presence of *N. orbignyi* has been previously reported in the common sole from Black Sea by Kayış and Ceylan, 2011. In this study, detailed data related with infection values both *S. rhytidotes* and *N. orbignyi* were presented for the first time in Turkey. Although the sculdfish had lower number of parasite species then the common sole, the highest prevalence and intensity values (100 % and 106.59) were found in sculdfish (Table 1). The results of this study showed that many parasite species prefer the common sole rather than the sculdfish. It could be due to effect of dietary preferences. The sculdfish, *Arnoglossus laterna* is a highly motile and selective predatory, it feeds on small fishes and invertebrates (Fanelli *et al.*, 2009). Conversely, the common sole, *Solea solea* is sedentary predatory, it feeds upon worms, mollusks, and small crustaceans that most of them are infected with parasites (Cabral 2000).

*Solea solea* has different prey groups compared to *A. laterna* and this study shows that this different diet compositions may affect parasite species diversity. Although this is a factor influencing parasite load and parasite diversity, diet diversity alone cannot fully explain it. Additionally our results indicated that the number of parasite species had a relation with host size in particular *S. solea*. It can be due to the composition and diversity of the ingested prey varied with the size of predator fish and increased of the diet of larger specimens than smaller ones.

In conclusion, the data presented in this study contribute to the knowledge on metazoan parasites in two flatfish and add some valuable information on its occurrence on seasonal level and the relation with hosts size.

Table 3. The infection parameters of parasite species in selected three size classes of flatfish.

Parasites	Solea solea (n: 140)						Arnoglossus laterna (n: 22)					
	< 14.0 cm (n:30)		14.0 – 15.9 cm (n:62)		15.9 cm (n:48)		< 14.0 cm (n:11)		14.0 – 15.9 cm (n:5)		15.9 cm (n:6)	
	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI
<i>Lecithochirium musculus</i>	-	-	-	-	-	6.3	63.6	8.57 <sup>a</sup>	100.0	41.4 <sup>b</sup>	100.0	34.7 <sup>b</sup>
<i>Proctoeces maculatus</i>	-	-	-	-	-	6.3						
<i>Condyllocotyla pilodora</i> met.	-	-	-	-	-	6.3						
Opoeceidae gen. sp. met.	-	-	8.1	1.8	43.8	3.3						
<i>Stephanostomum</i> sp. met.	-	-	3.2	2.0	10.4	1.2						
<i>Metadena</i> sp. met	3.3	1.0	11.3	3.9	52.1	12.7						
<i>Scolex pleuronectis</i> pl.	6.7	46.0	1.6	2.0	10.4	11.0	90.9	77.1	60.0	60.3	100.0	98.5
<i>Grillotia erinaceus</i>							90.9	16.3	40.0	16.5	100.0	11.7
<i>Progrillotia</i> sp.	10.0	14.0	1.6	10.0	10.4	1.2						
<i>Hysterothylacium aduncum</i>	3.3	3.0	4.8	2.7	2.1	1.0	45.5	1.8	-	-	100.0	7.0
<i>Capillaria gracilis</i>	30.0	1.9	40.3	3.1	39.6	5.0						
<i>Cucullanus campanae</i>	40.0	5.3	51.6	3.9	35.4	8.2						
<i>Dichelyne minutus</i>	83.3	8.15	88.7	21.4	83.3	28.2	9.1	8.0	-	-	16.7	2.0
<i>Solearhynchus rhytidotes</i>	16.7	6.8	27.4	1.9	20.8	4.7						
<i>Nerocila orbignyi</i>	6.7	4.0	14.5	3.3	4.2	3.0						
<b>Overall</b>	90.0	17.2 <sup>a</sup>	98.4	24.4 <sup>b</sup>	95.8	40.8 <sup>c</sup>	100.0	91.9	100.0	84.2	100.0	152.2
<b>Species richness</b>	9		11		13		5		3		5	
<b>Mean infracommunity</b>	2.19		2.60		3.37		3.00		2.00		3.67	

Means followed by the same superscript letter are not significantly different.

## Conflict of Interest

Authors state no conflict of interest

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