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**Parasite infection in *Serranus cabrilla* (Perciformes, Serranidae):
histopathological aspects and new host record for nematode genus *Philometra*
from Aegean Sea, Turkey**

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ABSTRACT. This study was conducted to determine infection with nematode parasite *Philometra* sp. in gonads of *Serranus cabrilla* recorded in Izmir Gulf between October 2016 and July 2017. The overall prevalence was 14.46% and the mean intensity of infection 1.2 parasites per fish. The occurrence of philometrid infection on comber hosts was assessed according to several risk factors (fish length class, sex and season). Our findings suggest that the highest rates of parasite infection occur in larger sized fish, hermaphrodite individuals and during April month.

The present report also revealed that marked hyperemia was the major findings of infected gonads of *S. cabrilla*, where parasitic nematodes caused a marked inflammatory reaction at the histopathological examination.

As far as we know, this paper represents the first mention of genus *Philometra* in *S. cabrilla* from Turkey and the first presence of philometrid parasites in Aegean coast of Turkey. Furthermore, the present work is the first record of the effects of *Philometra* sp. parasitism on a serranid species in Izmir Gulf.

Keywords: gonad, histopathology, infection, *Philometra*, *Serranus cabrilla*

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INTRODUCTION

The gonad-infecting parasites of *Philometra* (Nematoda: Phylometridae) occur in marine waters, causing harmful effects to their hosts (Perdikaris et al., 2003). These nematodes have a wide distribution in Atlantic, Indian and Pacific Oceans, damaging wild or cultured commercially important fish (Moravec et al., 2008). According to Moravec and de Buron (2013), the members of the Phylometridae family are the most important group of dracunculoid nematodes parasitizing in the teleost fish.

The presence of four species of *Philometra* parasite genus has been already documented for helminthofauna of seven marine perciformes from Turkey: *P. filiformis* (Stossich, 1896) in Common pandora *Pagellus erythrinus* (Linnaeus, 1758); *P. globiceps* (Rudolphi, 1819) in Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner, 1868) and Stargazer *Uranoscopus scaber* (Linnaeus, 1758); *P. lateolabracis* (Yamaguti, 1935) in White grouper *Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817), Dusky grouper *Epinephelus marginatus* (Lowe, 1834) and Mottled grouper *Mycteroperca rubra* (Bloch, 1793); *P. saltatrix* (Ramachandran, 1973) in Bluefish *Pomatomus saltatrix* (Linnaeus, 1766); additionally, unnamed helminthes *Philometra* sp. were found in Goldblotch grouper *Epinephelus costae* (Steindachner, 1878) from Mediterranean Sea (Moravec and Genc, 2004; Öktener, 2014). Except the *P. globiceps* founded in the Black Sea, the other three knowing species of *Philometra* in Turkey were encountered in the Mediterranean Sea (Öktener, 2014).

The comber, *Serranus cabrilla* (Linnaeus, 1758) is a perciform species with minor commercial value, occurring in the Eastern Atlantic and the Mediterranean Sea (Ilhan et al., 2010). Along the Turkish coast of Aegean Sea, an important region for fishing industry, *S. cabrilla* is one of the main species captured by fishing trawlers and the most abundant comber encountered in the Turkish Sea, unlike its congeners (Brown comber *S. hepatus* Linnaeus, 1758; painted comber *S. scriba* Linnaeus, 1758) (Torcu-Koc et al., 2004). This member of Serranidae family was previously registered in Izmir Bay, Central Aegean Sea (Özaydin et al., 2007; Ilhan et al., 2010).

The comber was analysed before in many aspects, concerning: growth and reproduction (Ilhan et al., 2010); feeding habits (Çakir and Koç, 2002); or spatio-temporal patterns of abundance and biomass (Özbek et al., 2016). However, there is still a lack

of knowledge regarding the parasites of *S. cabrilla*. As Genc et al. (2005) postulated, since Serranids are predators, the prevalence of infestation in those fish deserves a great deal of attention.

To date, only few parasite species have been reported worldwide on the comber host: the trematode *Lecithochirium musculus* (Paradižnik and Radujković, 2007); the isopods *Ceratothoa steindachneri* (Öktener et al., 2007), *Gnathia* sp. (Alaş et al., 2009) and *Nerocila orbigny* (Özcan et al., 2015); the anisakid nematodes such as *Anisakis* and *Hysterothylacium* (Kassem and Bowashi, 2015); the copepod *Anchistrotos laqueus* n. sp. (Leigh-Sharpe, 1935) and monogenoidean species *Megalocotyle hexacantha* and *Protolamellodiscus serranelli* (Strona et al., 2010). Among the 14 nominal recognized species of nematodes *Philometra* parasiting the gonads of marine fishes, only *P. serranellicabrilla* Janiszewska, 1949 was described in comber, from the Adriatic Sea and from Mediterranean Sea, in southern Corsica (Moravec et al., 2006).

Until now, in Aegean coast of Turkey no *Philometra* species was founded. Also, there is not yet any evidence of philometrid nematode parasitizing *S. cabrilla* in Turkey. Therefore, this study aimed to investigate for the first time the occurrence of nematode *Philometra* sp. in comber from Aegean coast of Turkey and the aspects of pathogenity within the host.

MATERIALS AND METHODS

Overall, 83 comber fishes were collected on seasonally basis, during October 2016 and July 2017 along the coasts of Izmir City. Two stations (1) Foça (38°36'58.N; 26°42'59.E) and (2) Sığacık (38°09'19.N; 26°42'29.E) were selected and samplings were carried out by using fishnets of mesh size of 20-24 mm. Each fish individual was examined to evaluate the parasite community.

The total body length of *Serranus cabrilla* was measured and three length-groups were established: 10-14.9, 15-19.9 and 20-25 (cm). Sex of each host was determined at necropsy by macroscopic investigation. During the dissection, internal organs (gastrointestinal tract, liver, kidney, heart, swim bladder, gallbladder and gonads), and body surfaces were examined separately under a dissecting microscope. Fixation, staining and preparation process of the determined parasites was done according to Pritchard and Kruse (1982). The parasites were identified up to

genus level using selected identification keys (Chaubaud, 1975; Moravec, 2004, 2006).

Prevalence (Pr %), expressed as the percentage of hosts infected with a particular parasite species or taxonomic group and mean intensity (Int), defined as the total number of individuals of a parasite species per individual infected host were calculated following Bush et al. (1997). Infection rates and statistical analyses were conducted by using Quantitative Parasitology 3.0 web application (Rozsa et al., 2000), Excel programme and SPSS 15. The χ^2 -test was performed to test for significant differences between the infection rates over the three length classes. A Kruskal–Wallis test was applied to find significant differences in the mean intensity of the parasite species for host fish size, sex and seasons. Differences were considered to be significant when $p \leq 0.05$.

During the necropsy of comber, gonad samples were collected and fixed in 10% neutral formalin solution for histopathological assessment. After routinely preparation by an automatic tissue processing equipment (Leica ASP300S, Leica Microsystem, Nussloch, Germany), samples were embedded in paraffin and 5 μ m sections were taken by a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch, Germany). Then sections were stained with hematoxylin and eosin (HE) and examined under the 40X objective of an Olympus CX41 light microscope. Morphometric evaluation and microphotography was performed using the Database Manual cellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

RESULTS

Among the total number of 83 specimens of *Serranus cabrilla* examined for parasites, only 12 fish were infected with nematode *Philometra* sp. (Table 1). The prevalence of infection was 14.46% (sexes combined), while the mean intensity of infection during the study was 1.2 parasites per fish. The highest mean intensity (1.3 parasites/fish) was recorded in hermaphrodite host individuals. Overall, the nematode infection was higher in female fishes whilst no worm was found in comber male individuals.

There were significant differences ($P \leq 0.05$) in the infection rates of *S. cabrilla* between length groups, sexes and seasons. The risk factors associated with the highest prevalence of philometrid infection in comber hosts were assessed as follows: hermaphrodite sex of examined individuals, length-group 20-25 cm and spring season (April 2017), respectively. The lowest infection rate was found in autumn (October 2016), and in small comber hosts (15-19.9 cm length-group), respectively. No infection was registered in the warmest month (July 2017). Also, no *Philometra* nematode was detected in the smallest length-group of hosts (10-14.9 cm).

In addition to *Philometra* parasites, in the gonads of some fish individuals were found larvae of anisakid nematodes.

Table 1. Seasonal prevalence and intensity of *Philometra* sp. infestation in *Serranus cabrilla* from Izmir Gulf (2016-2017) (N=total number of hosts examined; N'= number of infected fishes; NP=number of collected parasites; Pr=prevalence; Int=mean intensity of infection)

Parameters	N	N'	Pr (%)	NP	Int
Length class (cm)					
10.0-14.9	16	0	0	0	0
15.0-19.9	44	3	6.82	3	1
20.0-25	23	9	39.13	11	1.2
Sex					
Female	52	8	15.38	9	1.1
Male	6	0	0	0	0
Hermaphrodite	25	4	16	5	1.3
Season					
October	27	2	7.41	2	1
February	12	1	8.33	1	1
April	36	9	25	11	1.2
July	8	0	0	0	0
Total	83	12	14.46	14	1.2

With respect to histopathological findings, it was generally noticed that parasites were attached to the fibrous capsule around the gonads of *S. cabrilla*. Some nematodes were localized inside of the gonads. Hyperemia was the prominent finding. When *Philometra* sp. parasites were localized in the gonadal tissue, they caused a marked inflammatory reaction while slight inflammatory reaction was observed around the parasites disposed the gonads (Fig. 1-3).

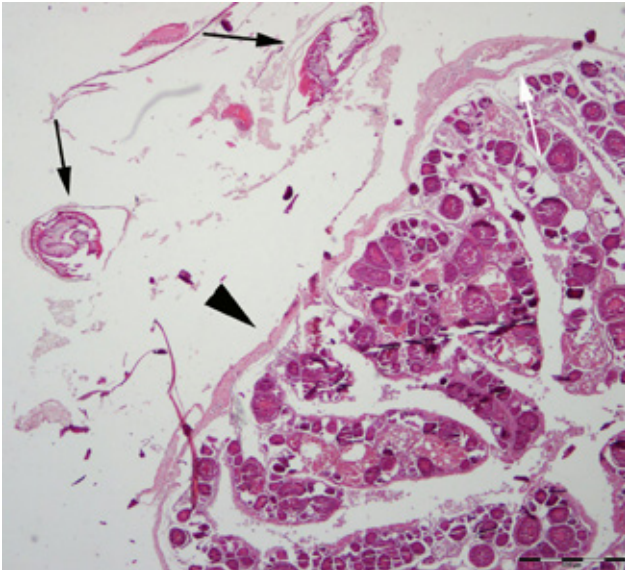


Figure 1. Histopathological appearance of the *Philometra* sp. parasites (black arrows) located gonad of *S. cabrilla* (arrow head). Marked hyperemia (white arrow) at the capsular vessels, HE, Bar=200µm.

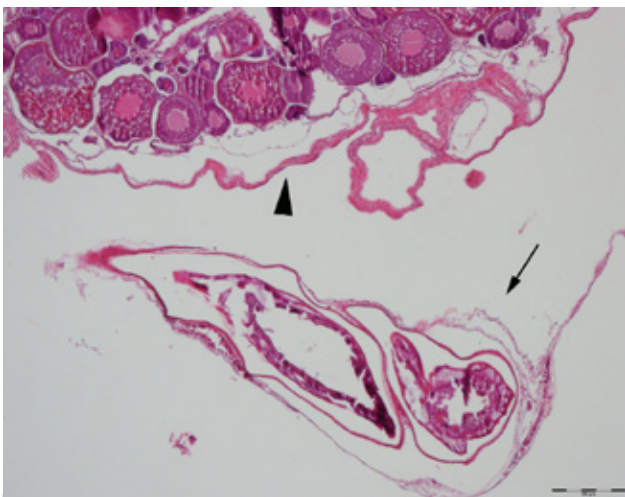


Figure 2. A parasite section (arrow) localized outside of the gonad capsule (arrow head), HE, Bar=200µm.

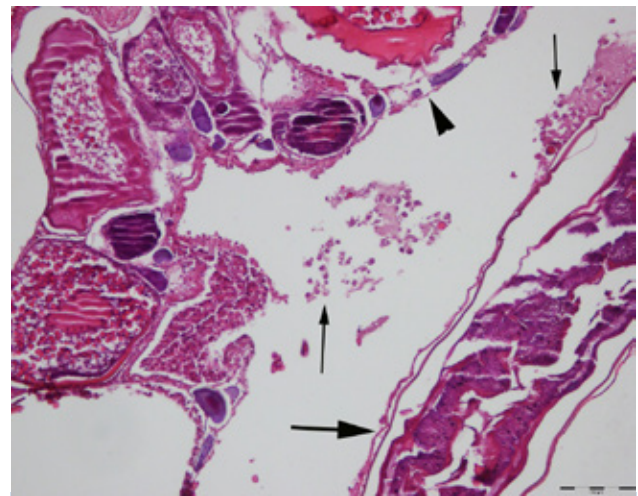


Figure 3. Inflammatory cell infiltrations (thin arrows), between *Philometra* sp. parasite (thick arrow) and gonad of *S. cabrilla* (arrow head), HE, Bar=200µm.

DISCUSSION

The fact that both values of prevalence and mean intensity in nematode infections increase with the body length of the host has been noticed before by various authors who suggested that as fish growth, chances of infection are higher either due to the exposure time or the increase of the internal organs of the fish that a parasite can use to attach itself (Moravec and Scholz, 1995; Al-Zubaidy, 2009; Ahmad et al., 2018). Our data are also consistent with some studies that revealed the relation between the sexually mature fish and occurrence of nematodes, younger fish being less infected or even not infected at all than older fish (Bergmann and Motta, 2004; Szostakowska et al., 2005; Perez et al., 2009). It has been postulated that *S. cabrilla* individuals reach sexual maturity at 3 years old, having an estimated standard length of 15.2 cm at first maturity, and a maximum life span ranged between 4 and 9 years (Torcu-Koc et al., 2004). Taking into account these growth features, we could conclude that the highest prevalence (39.13%) of philometrid infection was reached in sexually mature comber specimens from Izmir Gulf. The reduced percentages of prevalence of *Philometra* infection registered in October and February could be correlated with the fact that these months are out of the reproductive season for the host, since it was assumed that the spawning season on *S. cabrilla* from Mediterranean lasts from April to August, with a mass period in April (García-Días et al., 1997). In agreement with this statement, in the present study, the highest prevalence of parasitic nematodes (25%) was recorded in April. On the one hand, the absence of nematodes in

fish collected during July, in full spawning season can be explained by the type of fish reproduction in the spawning season, when host repetitively expels in the water column its own eggs, along with those belonging to the parasite (Cháves and Oliva, 2011). On the other hand, prevalence of infection categorized as none in July 2018 may be underestimated, as a result of a reduced number of fish collected in this month from the study area and further studies based on larger samples size are needed to clarify this aspect. A possible explanation for the limitations of using a small sample size in the parasitological analyses is given by Marques and Cabral (2007). It was already shown that prevalence and intensity of philometrid infections varies with many factors, including fish species, fishing area, food intake or season (Ali and Afsar, 2018). A relation between the prevalence of infection with *Philometra* adult female worms and the spawning season of its fish host has been suggested by various parasitologists (Genc et al., 2005; Perez et al., 2009; Selvakumar et al., 2015).

Moreover, the lack of parasitic infection in males of *S. cabrilla* could be attributed to the absence of a female-specific physiological trigger responsible for molting larval *Philometra* into adults, as Perez et al. (2009) suggested. The highest prevalence (16%) attributed to hermaphrodite comber individuals may be explained by the reproductive nature of *S. cabrilla*. As García-Días et al. (1997) pointed out, this fish is a synchronous hermaphrodite species, meaning that the ovarian and testicular tissue mature simultaneously.

Factors that influence the prevalence of *Philometra* sp. nematodes have not been documented before in comber host from Aegean Sea. Our data suggest that host body size, sex and season are important risk factors for parasite infection on *S. cabrilla* from Izmir Gulf.

Similar host response was registered by Ali and Afsar (2018), who found that a range of signs and symptoms as inflammation, necrosis and destruction of gonadal tissues are caused by the penetration of nematodes into gonadal wall. Genc et al. (2005) also noticed hyperemia and edema in the ovary of groupers serranid fish from Iskenderun Bay, Northeast Mediterranean Sea, infected with *Philometra lateolabracis*. Study of parasitic nematodes infecting the fish gonad is important, due to the fact that, sometimes, the host is an economically important species. Potential pathogenic effects of parasites belonging to the family Philometridae could result in significant

damage to the gonads, including severe changes of reproductive fitness of the host (Cháves and Oliva, 2011; Bakenhaster et al., 2014; Selvakumar et al., 2015). In massive infections, when *Philometra* nematodes feed on ovarian fluid, the parasitic castration and loss of reproductive function in female hosts may occur (Ali and Afsar, 2018).

However, Bakenhaster et al. (2014) have not detected negative effects of *P. floridensis* on the overall health of the red drum *Sciaenops ocellatus*, mentioning that the question regarding how philometrid nematodes affects reproduction in their fish hosts is still a subject to be elucidated.

Concerning the presence of anisakid parasites in the gonads of *Serranus cabrilla*, it must be mentioned that this fish species has been recognize as host for these nematodes by various authors. Thus, Figus et al. (2005) registered adult forms of anisakid genus *Hysterothylacium* from comber fish from southwestern Mediterranean Sea. Kasem and Bowashi (2015) recorded larvae of anisakid parasites belonging to *Anisakis*, *Contracaecum*, *Hysterothylacium* and *Pseudoterranova* taxa in *S. cabrilla* and other marine fishes sampled on Lybian coast.

CONCLUSIONS

The present paper provides first evidence for the nematode parasites of genus *Philometra* in comber fish from Turkey. Overall, the occurrence of parasite infection was prevalent in the gonads of hermaphrodite and female sexually mature individuals of *Serranus cabrilla*. Our histopathological results show that marked hyperemia occurs when infection caused a marked inflammatory reaction. Since pathological changes in gonadal tissues induced by parasites, both philometrids and anisakids could lead to severe consequences of reproduction of their host, representing also a major concern for fisheries and home cooking, further studies are needed to assess the presence of nematodes and associated zoonoses in fish populations of Izmir Gulf and Aegean coast of Turkey.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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