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# Seasonality and host-parasite interrelationship of *Hysterothylacium aduncum* (Nematoda) in whiting *Merlangius merlangus* off the southern and northern coasts of the Black Sea

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Article info	Summary
Received March 11, 2016	In the present study, we investigated the comparative infection levels, ecology and host-parasite interrelationship of a nematode <i>Hysterothylacium aduncum</i> (Rudolphi, 1802) in whiting, <i>Merlangius merlangus</i> (L., 1758) in southern (Sinop) and northern (Balaklava Bay) coasts of the Black Sea for the first time. Fish were collected throughout a period from May 2011 to March 2014 from local fishermen. A total of 690 fish specimens near Sinop and 423 fish near Balaklava Bay were examined for parasites. <i>Hysterothylacium aduncum</i> was the only nematode species identified in the digestive tract of the Black Sea whiting <i>Merlangius merlangus</i> . Prevalence of infection, mean intensity and mean abundance values were determined according to season, length classes and sex of fish at both localities. These infection indices were always higher in Sinop samples than those of Balaklava Bay samples.
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## Introduction

*Hysterothylacium aduncum* (Rudolphi, 1802) is a generalist nematode registered from many marine fish species (Andersen 1993) and it occurs in around 30 fish species, including the whiting, *Merlangius merlangus* (L., 1758), in the Black Sea (Kornyychuk & Zavjalov, 2005; Öztürk, 2005; Özer, 2007; Gaevskaya *et al.*, 2010; Özer *et al.*, 2012; Özer & Olguner, 2013; Tepe & Oğuz, 2013). It lives as sexually mature adults in the digestive tracts of marine teleost fishes (Navone *et al.*, 1998) and its larvae are known to occur in marine invertebrates and in fish (Koie, 1993). The 3rd-stage larvae have been found encapsulated in the mesentery and viscera of a wide range of fishes that act as transport hosts (Berland, 1961; Koie, 1993). The first intermediate hosts for *H. aduncum* are crustaceans, such as copepods, amphipods, decapods, shrimps, euphasiids, and isopods (Koie, 1993; Marcogliese, 1996). Gadoids are generally believed to be the main final hosts (Berland, 1961). Predatory fish, like gadoids, largely acquire and accumulate different stages of *H. aduncum* by ingesting crustaceans, chaetognaths and small fish species that are infected and serve as carriers (Klimpel *et al.*, 2003; Klimpel & Rückert, 2005). Whiting is a commercially important fish species and widely distributed in the eastern North Atlantic Ocean, the northern Mediterranean, western Baltic and the Black Sea (ICES, 2006). It is particularly distributed along Romanian, Bulgarian, Turkish, Ukrainian, Russian and Caucasus coasts in the Black Sea (Burdak, 1960; Svetovidov, 1964; Gönener & Bilgin, 2010; Özer *et al.*, 2014).

The objective of the present study is to investigate the infection levels of *H. aduncum* in *M. merlangus* from the southern and northern Black Sea coasts and to provide first detailed comparative ecological analysis of *H. aduncum* infection of whiting at both localities.

#### **Materials and Methods**

#### Study area and explanatory variables

Whiting samples were collected throughout a period from May 2011 to March 2014 from commercial fishing vessels in the southern Black Sea off Sinop (N 42° 05' 68" E 35° 10' 55") and northern Black Sea in Balaklava Bay (N 44° 49' 54" E 33° 59' 48"). In total, 690 fish from Sinop and 423 from Balaklava Bay were measured to the nearest cm of total length and then examined for parasites. Fish were transferred to the laboratories at Sinop University, Faculty of Fisheries and Aquatic Sciences and the A.O. Kovalevsky Institute of Marine Biological Research of RAS in Sevastopol and then all fish were examined for parasites within 24 h of sampling. Examinations were conducted on liver, stomach, caeca, intestine and its contents, swim bladder and gonads in accordance with Navone et al. (1998). All specimens of nematodes at different developmental stages (L3, L4 and adults) were collected and counted by screening whole smears of digestive tract under a dissecting microscope and identified at x40 magnification after washing the obtained larvae and adults repeatedly in 0.9 % saline solution, fixing in 70 % ethanol and clearing with lactophenol. Taxonomic features indicated by Berland (1961), Petter and Maillard (1988), Koie (1993) and Navone et al. (1998) in discriminating parasites to developmental stages and species level were followed and identification was conducted using Olympus microscope (BX53). Water temperature (°C) values were measured monthly using a YSI Professional Plus water quality instrument during one year period of 2012 - 2013.

#### Statistical analyses

All developmental stages were lumped and the prevalence, mean intensity and mean abundance values were determined according to Bush et al. (1997). Quantitative Parasitology 3.0 software (Reiczigel & Ryzsa, 2005) was used to calculate Sterne's exact 95% confidence limits for prevalence, bootstrap 95 % confidence limits (number of bootstrap replications=2.000) for mean abundances, mean intensity, median intensity, variance to mean ratio and exponent of the negative binomial (k). Difference in prevalence values between two fish populations from two sampling localities and between two fish sexes were determined by the exact unconditional test that maintains the prescribed type I error rate, which has a higher statistical power in a small sample size (N<100). Differences in prevalence values between sampling seasons, age and length categories were determined using the Fisher's exact test while the differences in mean abundance and intensity were performed by the bootstrap two-sample t test.

#### Results

Hysterothylacium aduncum (Rudolphi, 1802) was the only nematode identified as 3<sup>rd</sup> stage larvae encysted in mesentery and viscera and as 4<sup>th</sup> stage larvae and adults in the digestive tract of whiting *Merlangius merlangus* collected from Sinop coasts and Balaklava Bay in the Black Sea. Infection parameters of the 690 fish from Sinop and 423 fish from Balaklava Bay were determined (Table 1). Prevalence of infection, mean intensity and mean abundance values in Sinop samples were higher than those in Balaklava Bay samples.

Seasonal prevalence, mean intensity and mean abundance values of H. aduncum infecting M. merlangus at both localities were determined (Table 2). Seasonal value of prevalence was between 72.7 % and 88.3 %, mean intensity 5.3 and 14.4, and mean abundance 4.3 and 11.8 in the fish samples from Sinop coasts. When the samples from Balaklava Bay were evaluated in terms of these infection indices, seasonal value of prevalence was found to be between 11.3 % and 46.1%, mean intensity 1.5 and 3.7, and mean abundance 0.2 and 1.7. Seasonal differences in prevalence and median intensity (variable) of whiting with H. aduncum in Sinop and Balaklava Bay samples were compared (Table 3): there was statistically significant differences in prevalence (p=0.001) and median intensity (p=0.012) between seasons in Sinop samples and similar significant differences were also obtained in prevalence (p=0.000) and median intensity (p=0.011) between seasons in Balaklava Bay samples. Seasonal differences in prevalence, mean/median intensity and mean abundance of H. aduncum between Sinop and Balaklava Bay samples were compared (Table 4). Statistically significant differences between sampling locations were determined in prevalence (p<0.05) values for all seasons; mean intensity (p<0.05) values in spring and winter; median intensity (p<0.05) values in all seasons except summer and mean abundance (p<0.05) values in all seasons except autumn.

Prevalence, mean intensity and mean abundance values of H. aduncum infecting three length classes of M. merlangus at both localities were determined (Table 2). Prevalence was between 70.9 % and 88.7 %, mean intensity 10.3 and 20.6, and mean abundance 8.3 and 18.1 in the Turkish coast. On the other hand, for fish samples from Balaklava Bay, prevalence was between 24.8 % and 43.6 %, mean intensity 1.8 and 5.0, and mean abundance 0.6 and 2.2. The differences in prevalence and median intensity (variable) of H. aduncum between Sinop and Balaklava Bay samples in terms of fish length classes are compared (Table 3); there was statistically significant differences in prevalence (p=0.000) and median intensity (p=0.000) between length classes in Sinop samples and similar significant differences were also obtained in prevalence (p=0.007) and median intensity (p=0.000) between length classes in Balaklava Bay samples. The differences in prevalence, mean/median intensity and mean abundance of H. aduncum between Sinop and Balaklava Bay samples in terms of fish length classes are compared (Table 4). Statistically significant differences between sampling localities are determined in prevalence (p<0.05) values in all fish length classes; however, these significant differences in mean intensity (p<0.05), median intensity (p<0.05) and mean abundance (p<0.05) values were limited to larger fish length classes 15 – 18 cm and >18 cm at both localities.

Infection prevalence (%)	Infection Intensity	Abundance	Sampling Area	Author(s)	Remarks
			Georgia – Batumi	Chulkova (1939)	as Contracaecum clavatum
68.7	1 – 12 (mean = 4.6)	3.2	Ukraine – Sevastopol	Osmanov (1940)	as Contracaecum clavatum
36	7 – 9		Ukraine – Novorossiysk, Karadag	Pogoreltceva (1952)	as Contracaecum aduncum
53.3	1 – 3		Ukraine – Odessa Gulf	Chernichenko (1955)	as Contracaecum clavatum
			Bulgaria	Margaritov (1960)	as Contracaecum clavatum
41	1 - 11		Black Sea	Nikolaeva and Shramova (1975)	as Contracaecum aduncum
16.8 - 88.2			Bulgaria	Dimitrov, 1989	as Contracaecum aduncum
			Ukraine – Alushta, Karadag, Sevastopol	Mange (1993)	
			Turkey – Samsun	Doğanay (1994)	
21.8 – 54.8	0.5 – 1.45 (mean intensity)		Turkey - Turkish Black Sea coasts	Ismen and Bingel (1999)	
60.3	$7.5 \pm 0.1$		Turkey - Sinop	Özer et al. (2000)	
62.5 (adult); 25.0 (larvae)		1 – 6 (adults); 2.0 (larvae)	Ukraine – Karadag	Miroshnichenko (2004)	as Thynnascaris adunca
60.3	7.48 ± 0.9	$6.62 \pm 0.9$	Turkey - Sinop	Özer et al. (2009)	
91.2 (Sinop)	41.79 ± 10.03 (Sinop)		Turkey – Sinop; Ukraine – Sevastopol	Özer et al. (2012)	
40.5 (Sevastopol)	1.99 ± 0.52 (Sevastopol)		Turkey – Sinop; Ukraine - Sevastopol	Özer et al. (2012)	
4-91 (larvae); 2-74 (adult)			Ukraine-Sevastopol, Sudak, Kalamitskiy Bay	Zavyalov (2013)	
2 – 100	1 – 24		Ukraine – Streletskaya Bay-Sevastopol	Skuratovskaya et al. (2013)	
88.5	$7.5 \pm 0.1$		Turkey – Sinop	Özer and Olguner (2013)	
37.5	1 – 8 (mean=3.8)		Turkey - Eastern Black Sea coasts	Pekmezci et al (2013)	
56.0	9.4 (mean intensity)	10.0	Turkey – Artvin, Rize, Trabzon	Tepe and Oğuz (2013)	
80.3 (77.1 – 83.1)	11.5 (9.6 – 17.2)	9.3 (7.7 – 13.9)	Black Sea – Sinop	This study	
32.9 (28.5 – 37.6)	3.1 (2.5 – 4.0)	1.0 (0.8 – 1.4)	Black Sea- Sevastopol	This study	

Season/fish length/sex	z	Fish Length±SE	Prevalence (%) Cl	Mean I CI	Mean A Cl	m/v	k*
Sinop Samples							
Seasons							
Summer (June – August) (20.4 – 26.1 °C)	26	$15.5 \pm 0.2$	80.8 (61.7 – 92.1)	5.3 (3.2 – 9.1)	4.3 (2.5 – 7.5)	9.5	0.711
Autumn (September – November) (15.2 – 21.4 °C)	222	$15.5 \pm 0.2$	88.3 (83.4 – 91.9)	14.4 (9.0 – 33.1)	11.8 (8.0 – 29.5)	227.7	0.548*
Winter (December – February) (8.3 – 13.1 °C)	222	$15.2 \pm 0.1$	79.7 (73.9 – 84.6)	11.9 (9.3 – 17.1)	9.5 (7.5 – 13.4)	48.3	0.481
Spring (March – May) (9.5 – 17.2 °C) Fish Lendth Classes	220	$15.0 \pm 0.1$	72.7 (66.4 – 78.2)	9.7 (7.8 – 12.4)	7.0 (5.6 – 9.0)	24.4	0.454
	000		700/65 6 75 7)	11 7 0 00 11 7 11	02/5/ 105/	1000	*Ucc U
1.1.3 cm 15	326	1 1	(1.0.7 (00.00) / 0.0 / 0	10.3 (8.9 – 29.1) 10.3 (8.9 – 12.8)	0.0 (0.4 – 10.0) 0 1 (7 0 – 11 3)	74.1	0000.0
>18 cm	41	I	87 8 (74 6 – 95 1)	20 6 / 14 0 - 31 5)	18 1 (12 0 - 20 1)	37.0	0.682
Fish Sav	-				1	2	100.0
	000	11 5 - 0 0	7/7/686 700/	106/E0 376)	70/1/ 210/	301 2	*775 0
	277		(4.1 (00.0 - 13.3)		() (+.+ - 2  ) 0 0 0 1 - 2 0 0	041.0	
Female	461	$15.6 \pm 0.1$	83.1 (79.3 – 86.3)	11.9 (10.4 – 14.4)	9.9 (8.5 – 12.0)	34.4	0.568*
Overall	690	$15.2 \pm 0.1$	80.3 (77.1 – 83.1)	11.5 (9.6 – 17.2)	9.3 (7.7 – 13.9)	115.7	0.487*
Balaklava Bay Samples							
Seasons							
Summer (June – August) (21.7 – 25.2 °C)	40	$15.1 \pm 0.2$	17.5 (8.4 – 32.3)	1.7 (1.1 – 2.1)	0.3 (0.1 – 0.6)	1.7	0.254
Autumn (September – November) (13.9 – 21.6 °C)	97	$18.6 \pm 0.3$	11.3 (6.1 – 19.5)	1.5(1.1 - 1.6)	0.1 -	1.5	0.216
Winter (December – February) (8.2 – 11.0 °C)	80	$17.0 \pm 0.2$	32.5 (23.0 – 43.7)	2.2(1.5 - 3.8)	0.7(0.4 - 1.3)	4.5	0.328
Spring (March – May) (8.4 – 16.4 °C)	206	$15.7 \pm 0.2$	46.1 (39.3 – 53.2)	3.7 (2.9 – 4.8)	1.7 (1.3 – 2.4)	8.0	0.331
	ļ				0	0	
<15 cm	117	I	43.6 (34.6 – 53.0)	5.0 (3.7 – 7.2)		9.9	0.249
15 – 18 cm	181	I	31.5 (25.1 – 38.7)	1.8 (1.5 – 2.3)	0.6 (0.4 – 0.8)	2.2	0.490
>18 cm	125	I	24.8 (17.9 – 33.2)	2.3(1.9 - 3.7)	- I	4.1	0.211
Fish Sex							
Male	69	$14.8 \pm 0.2$	33.3 (23.1 – 45.6)	3.4 (2.2 – 5.6)	1.2 (0.7 – 2.0)	6.9	0.214
Female	354	$16.9 \pm 0.2$	32.8 (27.9 – 37.8)	3.1(2.5 - 4.1)	1.0 (0.8 – 1.4)	7.7	0.232
Overall	423	16 5 + 0 2	32 9 (28 5 - 37 6)	31/25-10	10/08 11/	7 6	0 228

Fish samples	Variable	Prevalence (%)	Mean Intensity	Median Intensity	Mean Abundance
Sinop	Seasons	0.001*	-	0.012*	-
	Length classes	0.000*	-	0.000*	-
	Sex	0.009*	0.712	0.010*	0.574
Balaklava Bay	Seasons	0.000*	-	0.011*	-
	Length classes	0.007*	-	0.000*	-
	Sex	1.000	0.668	1.000	0.700

Table 3. Comparative statistical differences in prevalence, mean intensity, median intensity and mean abundance of *H. aduncum* in Sinop and Balaklava Bay samples relative to season, fish size and sex

\* level of significance with p<0.05

Prevalence, mean intensity and mean abundance values of H. aduncum infecting M. merlangus different sexes at both localities were determined (Table 2). Of the above mentioned infection indices, only prevalence and median intensity values between female and male whiting in Sinop samples had statistically significant differences (p<0.05) (Table 3); on the other hand, there was no statistically significant (p>0.05) difference in none of the infection indices between female and male whiting in Balaklava Bay samples (Table 3). Differences in prevalence, mean/median intensity and mean abundance of H. aduncum between Sinop and Balaklava Bay samples in terms of the sex of fish were compared (Table 4). The differences were statistically significant between sampling locations in prevalence (p<0.05) and median intensity (p<0.05) values in females as well as in males. However, mean intensity and mean abundance did not change in female and male fish groups from both of the localities (Table 4).

#### Discussion

The present study has revealed the seasonality and host-parasite interrelationships of *H. aduncum* found in the digestive tract of whiting *M. merlangus* and compared its occurrences between two geographically distant southern and northern localities of the Black Sea. In 3-year period of our research study, *H. aduncum* was the only nematode species found in the Black Sea whiting *M. merlangus*, at both larval and adult stages. Black Sea whiting is one of numerous hosts for *H. aduncum* and it is a representative of cods feeding on small fish and planktonic crustaceans such as copepods, amphipods, decapods, shrimps, euphasiids, and isopods which are the intermediate hosts of this parasite species. It was reported by Loboda and Khvorov (2004) that 12.5 % of *Sagitta setosa* in the Black Sea acted as an additional host in the life cycle of *H. aduncum* and nematode larvae are able to grow

Table 4. Comparative statistical differences in prevalence, mean intensity, median intensity and mean abundance of H. aduncum
between Sinop and Balaklava Bay samples relative to season, fish size and sex

Variable	Prevalence	Mean	Median	Mean
	(%)	Intensity	Intensity	Abundance
Seasons				
Spring	0.000*	0.000*	0.000*	0.000*
Summer	0.000*	0.067	0.084	0.051*
Autumn	0.000*	0.313	0.001*	0.281
Winter	0.000*	0.002*	0.000*	0.000*
Length classes				
<15 cm	0.000*	0.253	0.086	0.206
15 – 18 cm	0.000*	0.000*	0.000*	0.000*
>18 cm	0.000*	0.011*	0.013*	0.000*
Sex				
Female	0.000*	0.000*	0.000*	0.000*
Male	0.000*	0.374	0.042*	0.377

\* level of significance given as p<0.05

in sagittas. Gaevskaya et al. (2012a) also reported that copepods Pleurobrachia rhodopis and Pseudocalanus elongates and chaetognath S. setosa are the first and second intermediate hosts, respectively, for H. aduncum in the Black Sea and all year round presence of above mentioned species have been reported and reviewed extensively in Sinop coasts of the Black Sea where this study was conducted (Bat et al., 2007a,b, 2011). Therefore, fish may have become infected with H. aduncum larvae by consuming those previously infected intermediate hosts (Klimpel et al., 2003; Klimpel & Rückert, 2005). Overall infection values determined in the present study were about 3 times higher in Sinop samples than that of Balaklava Bay samples, and the differences between localities were statistically significant. Previous studies (see Table 1 for details) on H. aduncum infections in M. merlangus in Sinop coasts of the Black Sea reported high infection prevalence levels from 37.5 % to 100.0% and mean intensities from 3.8 to 12.8 per infected fish (Özer et al., 2000; Özer et al., 2009; Özer & Olguner, 2013; Tepe & Oğuz, 2013; Pekmezci et al., 2013). Skuratovskaya et al. (2013) reported infection prevalence levels of H. aduncum ranging between 46 – 65 % and 1 – 12 per infected *M. merlangus* collected from Crimean coast of the Black Sea. It is known that the Black Sea whiting is divided into different populations due to their reproductive isolation which is determined by spatially isolated spawning areas (Burdak, 1960), and regional differences were expected in the component structure of the Black Sea whiting parasites. Thus, we can assume that 3 times higher infection levels in Sinop samples than that of Balaklava Bay samples are resulted from this situation. Moreover, more than 85 % of total amount of whiting harvest in the Black Sea is caught in Turkey, mainly near Sinop, while other Black Sea countries use only small part of whiting stocks. Thus, it can be assumed that whiting stocks near Sinop coasts might be affecting the infection pattern with H. aduncum by having more intermediate hosts available along with relatively higher seasonal temperature values facilitating food consumption of fish in Sinop coastal areas. Wide range of infection levels reported for H. aduncum in many different fish hosts investigated in different geographical parts of the Black Sea show that this parasite species has not only the ability of wide dispersion to different host species but also different geographic populations in the Black Sea. However, given the high level of infection throughout three-year period, it is clear that *M. merlangus* serve as at least one of main hosts for H. aduncum. Parasites have been useful in separating fish populations in other geographical regions (MacKenzie, 2002; Timi et al., 2005) and future studies should focus on genetic differences of parasites for further evidence in discriminating fish stocks in the southern and northern zones of the Black Sea.

*Hysterothylacium aduncum* occurred all year-round in both Sinop and Balaklava Bay samples, autumn in Sinop samples and spring in Balaklava Bay samples were the most appropriate season for both its prevalence of infection and intensities. Despite determined statistically significant differences among seasons at both localities, obtained results indicate that *H. aduncum* has the ability of

all year-round infection capability which is not so affected from environmental factors, especially from temperature that is accepted to be one of the triggering factors for food consumption of fish host over infected intermediate hosts. In the literature, the highest prevalence and mean intensity (54.8 % and 1.40, respectively) for this nematode in Black Sea whiting was in the warm months of July/August when collected along the continental shelf of Turkish coast of the Black Sea (İşmen & Bingel, 1999). On the other hand, in a study conducted by Andersen (1993), it was reported that H. aduncum infected cod, Gadus morhua, through all-year period with some peaks in colder seasons. Valero et al. (2000) reported minimum and maximum infection parameter in winter and spring, respectively, in blue whiting Micromesistius potassou collected from the western Mediterranean Sea, off southern Spain. Adroher et al. (1996) also reported a year-round infection for H. aduncum in horse mackerel Trachurus trachurus in the Mediterranean Sea with several peaks at moderate water temperature values. Kalav et al. (2009) also stated high prevalence and mean intensity levels of H. aduncum invasion of Mediterranean fishes Sparus aurata and Diplodus vulgaris during the period of moderate water temperature (June and March, respectively). The results of this study also correspond with the fact that H. aduncum is a moderate water temperature parasite better surviving at 16 °C (Adroher et al., 1996). As Smith (1983) stated that anisakid nematodes disperse their eggs via final hosts throughout the year, and they may develop and hatch at any time and seasonal variations in infection levels of anisakid nematodes were reasoned due to changes in the population of infected zooplankton serving as intermediate hosts (Smith & Wootten, 1978). One of the most important factors influencing the composition of the parasite fauna is age and size of a host (Özer & Öztürk, 2005). The results of this study indicated that larger hosts had more infection and parasite load and similarly in a study by Adroher et al. (1996) increasing infection levels of H. aduncum were reported in close association with increasing size of horse mackerel. Rello et al. (2008) reported increases, though not statistically significant, at prevalence and intensities of infection of H. aduncum by length of Sardina pilchardus from the southern and eastern coasts of Spain. Anderson and Gordon (1982) reported that the higher parasitism observed in larger fish over smaller ones might be a result of change in diet of the fish and higher activity to search for food. Morsy et al. (2011) also found similar results indicating that larger fish were able to compete better for food than the smaller fish groups which meant that when there is more contact with food there is a higher tendency of getting infected with parasites, and this could be due to an accumulation of parasites in the host throughout its life (Bussmann & Ehrich, 1979).

Parasites may infect both sexes differently, because male and female fish often have different feeding habits (Rohde, 1993). In our study, host sex did not have a significant influence on the infection parameters of *H. aduncum* in *M. merlangus*, suggesting that habitat use and diet are similar for both sexes of this species at both sampling zones. In detail, the infection parameters calculated for female fish was higher in Sinop samples and the situation was the reverse in Balaklava Bay samples in which male fish had higher infection values, both without any significance, except prevalence of infection in Sinop samples.

In conclusion, H. aduncum infected the digestive tract, mesentery and viscera of whiting. It occurred all year-round in both Sinop and Balaklava Bay, though the most appropriate seasons at both sampling localities were different. Larger length classes of Black Sea whiting had more infection values at both localities; however, this situation was reverse in the effect of host sex over its occurrence. In the present study, higher infections obtained from Sinop samples than that of Balaklava Bay samples may reflect that whiting populations at both sampling localities are different as a result of their reproductive isolation determined by spatially isolated spawning areas, and as a result, regional differences can be expected in infection structure of the Black Sea whiting parasites. Future studies should focus on genetic differences of parasites for further evidence in discriminating fish stocks in the southern and northern zones of the Black Sea. Whiting is a fish species frequently consumed by of Black Sea region and thus far, there is no evidence that H. aduncum larvae are pathogenic in humans and Huang (1988) reported that this nematode cannot survive at human body temperature.

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