New record of larval *Hysterothylacium* sp. (Nematoda: Raphidascarididae) in pick handle barracuda (*Sphyraena jello*) from the Persian Gulf, Iran

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Abstract

Pick handle barracuda (*Sphyraena jello*) is one of the most important commercial species in the Persian Gulf. In order to investigate prevalence and intensity of nematoda, 150 *S. jello* from three sites (Khuzestan, Bushehr and Hormozgan provinces) of the Iranian coast of the Persian Gulf were monthly investigated in the period 2012-2013. The nematodes belonging to the genus *Hysterothylacium* (family Raphidascarididae) were isolated from the abdominal cavity and digestive tract with a prevalence of 12% and mean intensity of 4.8. Intensity of infestation was increased with size of the host ($P<0.05$). This study is the first report of presence of the third and fourth- stage of an unreported type of *Hysterothylacium* larvae in *S. jello* from the Persian Gulf.

Keywords: *Sphyraena jello*, *Hysterothylacium* sp., nematodes, Persian Gulf.

Introduction

The Persian Gulf has a vast potential of marine fish commercial production. Three coastal provinces of Iran have important fisheries on their side of the Persian Gulf: Khozestan in the northwest, Hormozgan in the northeast and Boushehr in the center of the Persian Gulf. The pickhandle barracuda (*Sphyraena jello*) is a member of the Sphyraenidae family that includes 21 species. It grows to 150 cm in length and maximum weight of about 11.5 kg and feeds mainly on fishes. The mentioned species is widely distributed in marine and brackish water being one of the most commercially important coastal fish in the Persian Gulf (Randall, Allen & Steene 1997; Hoese, Bray, Paxton & Allen 2006).

The anisakid nematodes can be transmitted to man, and fish can act as intermediate, paratenic or definitive hosts (Anderson 2000). In humans, the ingestion of their third-stage larvae through consumption of lightly cooked or raw marine fish and invertebrates infested with anisakid larvae can cause anisakiasis (Nagasawa 2005). *Hysterothylacium* larvae under natural conditions can reach sexual maturity in the digestive tract of bony fish (Køie 1993) or in marine mammals (Deardorff & Overstreet 1982). Although, they may be less of a hazard for humans compared to other genera, some species have been considered as being of zoonotic interest (Deardorff & Overstreet 1981b). They have been recorded from several freshwater and marine fish (Deardorff & Overstreet 1981a,b) or both (Brizzola & Tanzola 1995), in several parts of the world, Likewise H. punctati was isolated from the freshwater fish spotted snake head, Channa punctata (Lakshmi 1995b), and H. japonicum was reported from the marine fish slender ribbonfish, Trachipterus ishikawae in Japan (Moravec & Nagasawa 1998). *Hysterothylacium* sp. was recovered from a cage-cultured coho salmon, Oncorhynchus kisutch in Chile (Carvajal

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& González 1990). In Brazil, large-tooth flounder, Paralichthys isosceles was recognized as new host for *Hysterothylacium* larvae (Felizardo, Knoff, Pinto & Gomes 2009). Several studies on *Hysterothylacium* sp. have been undertaken from the Persian Gulf (Kardousha 1992; Petter & Sey, 1997; Al-Behbehani 2003; Bagherpour, Afsharnasab, Mobedi, Jalali & Mesbah 2011). The present study investigated presence of the third and fourth- stage of an unrecorded type of *Hysterothylacium* larvae in *S. jello* from the Persian Gulf demonstrating that this fish can act as intermediate or definitive host for this parasite.

No human anisakiasis is yet recorded from Iran but with an increasing trend to use undercooked or raw fish among Iranians, the prevalence of anisakiasis cases is expected to merge. As anisakiasis is considered as an emerging zoonosis, anisakid larvae require more attention of investigation of their morphology, biology and life cycle.

Materials and Methods

A total number of 150 *S. jello* were sampled from three sampling sites (Khuzestan, Bushehr and Hormozgan provinces) along the Iranian coast of the Persian Gulf from August 2012 to December 2013. Fish were collected from fish harbors randomly. The specimens were 20-80 cm in length and 200-1200 g of weight. They were examined for anisakid larvae infection, transported to the Veterinary Organization laboratories and eviscerated. Abdominal cavity was washed under running water into a 50 mesh sieve to remove adhering larvae. Skin, abdominal cavity, stomach, sub- serous tissues, stomach and intestine contents, liver, spleen and gonads were examined macroscopically using a stereomicroscope to isolate anisakid larvae. Larvae (Fig. 1) were counted, fixed in 70% ethanol, cleared in lactophenol for 48 h for identification. Schematic drawing was prepared by camera lucida with drawing tube (ZIESS-West Germany). Identification was carried out using available keys (Gibbons 2010). Prevalence and intensity were calculated according to Bush, Lafferty, Lotz & Shostak (1997) and data analysis of the parasites and hosts were carried out using SPSS, version 16. t-test was used for comparison of the means and to determine the relation between size of fish and parasitic intensity, at significance level of 0.05. Voucher specimens have been deposited in the Collection of the National Museum of Parasitology, University of Tehran, Tehran, Iran, ID: 763, *Hysterothylacium* sp.

Results

Out of 150 investigated fish, in 18 specimens *Hysterothylacium* sp. Ward & Magath, 1917 were identified with a prevalence of 12% and mean intensity of 4.8. Intensity of infestation was increased with the size of the host ($P<0.05$). In the present study, identified larvae represented L3 and L4 stages of *Hysterothylacium* sp. and were removed from the abdominal cavity and digestive tract of *S. jello* and none of the parasites were found in the liver, spleen and gonad (The measurements are given in Table 1).

Discussion

Description based on 39third-stage larvae

They had a dorsal and two ventro-lateral lips and boring tooth was absent (Fig. 2). Excretory pore was at the level of nerve ring. Ventriculus had nearly oval shape, intestinal caecum was extended anteriorly and ventricular appendix was projected posteriorly. Four rectal glands were present. Conical tail was tipped with the arranged spines in a circle (Fig. 3).

Description based on 19 fourth-stage larvae

All of the mentioned characteristics were observed in four-stage larvae, except for the presence of the more developed lips and cactus tail with multi-spinous structure in the posterior end (Fig. 4), its features in having cavernous alaes and extended chords at the cross section approved the results (Fig. 5).

The ascaridoidea naturally parasitize fish, cephalopods, marine mammals and piscivorous birds. Humans can become accidental hosts by ingesting raw marine fish and invertebrates infested with third stage larvae (Doupe′, Lymbery, Wong & Hobbs 2003). The larvae invade the gastrointestinal mucosa and cause abdominal pain, vomiting, nausea, and

Table1 Measurements (mm) of *Hysterothylacium* sp.

Structures (mm)	Minimum size	Maximum size	Mean±SD
Body length	20.5	28.8	24.5±2.43
Body width	0.9	1.3	1.1±0.13
Esophagus L.	1.385	2.88	2.1±0.44
Esophagus W.	0.19	0.395	0.285±0.05
Intestinal Caecum L.	0.45	1.05	0.72±0.19
Intestinal Caecum W.	0.15	0.32	0.23±0.05
Ventriculus L.	0.21	0.45	0.32±0.07
Ventriculus W.	0.18	0.375	0.27±0.05
Ventricular appendix L.	2.9	5.85	4.3±0.97
Ventricular appendix W.	0.165	0.375	0.275±0.06
Spineous end	0.015	0.027	0.02±0.004

L.- Length, SD.- Standard Deviation, W.- width.

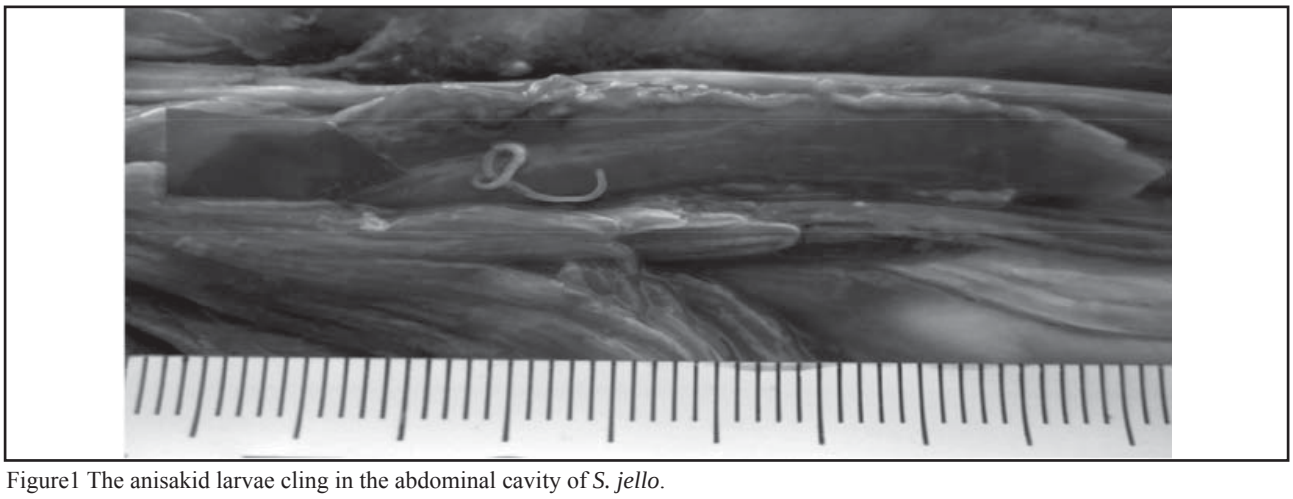

Figure1 The anisakid larvae cling in the abdominal cavity of *S. jello*.

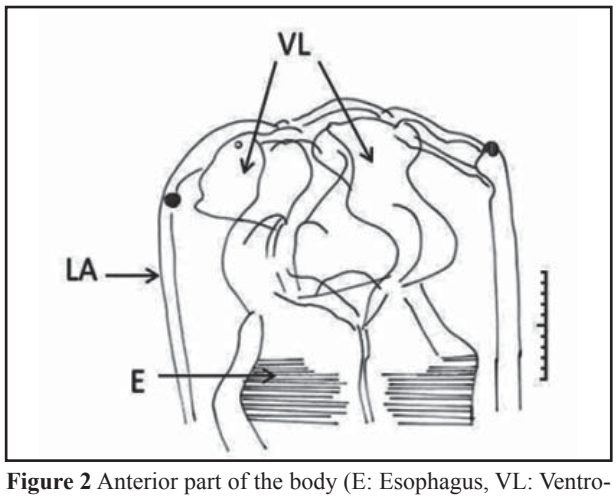

Figure 2 Anterior part of the body (E: Esophagus, VL: Ventro-lateral Lips, LA: Lateral alae). Scale bar=100 µm.

different gastrointestinal lesions (Kim, Choi, Lee & Choi 2006). The nematodes that cause anisakiasis are larvae of Anisakis sp. in most cases followed by larvae of Pseudoterranova sp. Other anisakid larvae, such as Contracaecum sp. and *Hysterothylacium* sp. are hardly ever found in humans (Schaum & Müller 1967; Ishikura 2003), however, Yagi, Nagasawa, Ishikura, Nagagawa, Sato, Kikuchi & Ishikura (1996) reported a case of human infection with H. aduncum in Japan and experimental reports of their hemorrhagic lesions were recorded in the rhesus monkeys stomach (Overstreet & Meyer 1981). Although, some species do not have any record of causing disease in humans, their presence in the viscera and flesh may impact upon visual aesthetics and the market value, and parasite removal only adds to product cost while further reducing its attraction to consumers (Doupe′ et al. 2003).

The third- stage of *Hysterothylacium* larvae have been infected some fish species of the Persian Gulf (Kardousha 1992; Petter & Sey 1997; Al-Behbehani 2003; Bagherpour et al. 2011) whilst it is the

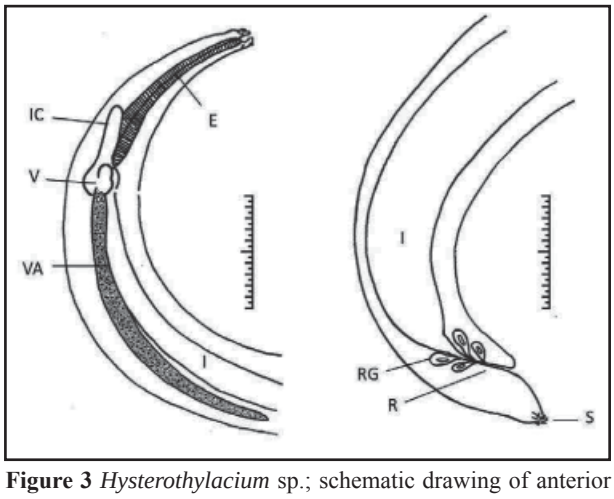

Figure 3 *Hysterothylacium* sp.; schematic drawing of anterior and posterior parts of the body (E: Esophagus, I: Intestine, IC: Intestinal Caecum, R: Rectum, RG: Rectal Glands, S: Spines, V: Ventriculus, VA: Ventricular Appendix). Scale bars=1 mm.
Figure 4 The caudal multi-spinous of cactus-tail of four-stage larvae. Scale bar=100 µm.

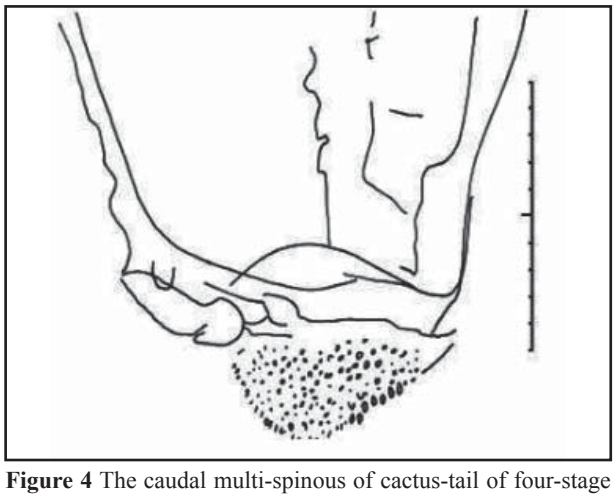

Figure 4 The caudal multi-spinous of cactus-tail of four-stage larvae. Scale bar=100 µm.

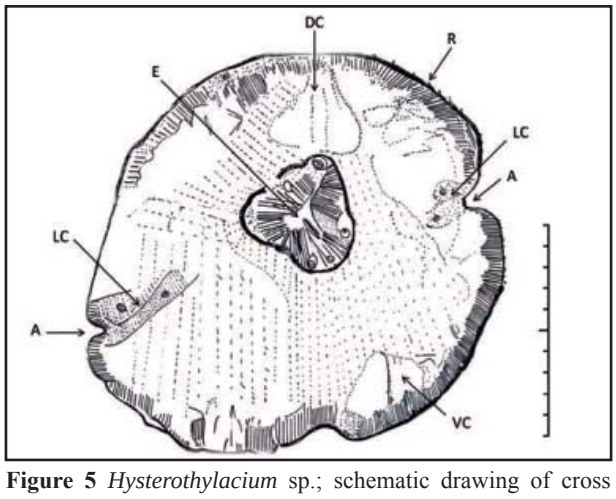

Figure 5 *Hysterothylacium* sp.; schematic drawing of cross section (A: Alae, E: Esophagus, DC: Dorsal cord, LC: Lateral cord, R: Ridges, VC: Ventral cord). Scale bar=1 mm.

first record of the presence of its fourth- stage from the Persian Gulf fishes which appears that L4 stage larvae show a narrower host- specificity, however, more host species need to be investigated to support this proposal. Accordingly, presence of L3 and L4 stages of *Hysterothylacium* sp. in *S. jello* demonstrates that this fish can act as intermediate or definitive host for this parasite.

Third-stage larvae studied , differ from *Hysterothylacium* sp. type MB collected in *S. jello* by Kardousha (1992) in which a small boring tooth is present and tail ends with a terminal process.

Petter & Sey (1997) classified *Hysterothylacium* larvae in six different types (KA-KF) and reported types KA and KB in *S. jello*. There are morphological similarities between the present specimens and type KD in having the arranged spines in the posterior extremity and ratio of esophagus length to ventricular appendix length (e/a) or intestinal caecum length (e/c). Comparing these features revealed that ventricular appendix was much longer than esophagus in the present specimens but their lengths were almost the same in type KA. In spite of the present types, ventricular appendix is shorter than twice the esophagus length and tail is tipped with a single terminal spine in type KB. So it can be concluded that the present type is different from types KA and KB, previously described in *S. jello*.

According to other research conducted in Kuwaiti fishes by Al-Behbehani (2003) only one female of silver pomfret, Pampus argenteus was found infected (6.7%) and the higher infection rate was recorded in malabar blood snapper, Lutjanus malabaricus in summer (20.0% male and 22.2% female) and their third-stage larvae were similar to type KD of Petter & Sey (1997). Bagherpour et al. (2011) reported H. aduncum in black sole fish, Brachirus orientalis from the Persian Gulf with the highest prevalence in spring (56%), while in this study it occurred in summer thus further studies are required to assess its seasonal incidence. In the present study, larvae were only isolated from the abdominal cavity, although previously in some species were reported from the intestine and stomach of some fish (Lakshmi 1995 a,b). The intensity of the infestation was correlat-

ed with the size of the host in our study ($P<0.05$), Aloo, Anam & Mwangi (2004) reported that large hosts can provide more appropriate habitats for parasites than small ones, on the other hand, as the fish grows, the amount of its food consumption increases, including the amount of larval stages consuming along.

The parasitation site of third stage larvae can confirm that studied fish acts as their paratenic hosts. In spite of the low infection rate of *Hysterothylacium* larvae, its occurrence might cause a serious public health problem. Therefore, consumption of infected fish if it is not properly cooked and ingestion of the immature worm may lead to anisakiasis. No human anisakiasis is yet reported from Iran, this issue could be attributed to cuisine habit of fish in studied areas as well as other parts of the country. The existence of *Hysterothylacium* larvae in *S. jello* in this region is of great concern for the health of these fish as hosts and human as consumers. Therefore, it increases the necessity to provide more information about ichthyoparasitoses and prophylactic approaches with the improvement of sanitary educational programs at various levels.

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ثبت جدید لارو انگل هیستروتیلاسیوم از نماتودهای رافیدآسکاریدیده در ماهی کوتر ساده (*Sphyraena jello*) از خلیج فارس

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چکیده

ماهی کوتر ساده یکی از مهمترین گونه‌های تجاری خلیج فارس می‌باشد. به منظور شناسایی آلودگی به نماتودها ، تعداد ۱۵۰ عدد کوتر از سه سایت خوزستان، بوشهر و هرمزگان واقع در آب‌های خلیج فارس مورد بررسی قرار گرفتند. در این بررسی نماتودهای متعلق به جنس هیستروتیلاسیوم از محوطه شکمی و لوله گوارشی ماهی با شیوع ۱۲ درصد و شدت آلودگی ۴/۸ جداسازی گردیدند. همچنین شدت آلودگی با افزایش اندازه میزبان افزایش را نشان می داد. این مطالعه اولین گزارش حضور لارو نماتود هیستروتیلاسیوم در ماهی کوتر ساده از خلیج فارس می‌باشد.

واژه‌های کلیدی: هیستروتیلاسیوم، کوتر ساده، نماتود، خلیج فارس.

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