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Nematodiasis in some marine fishes from Red Sea and its public health significance

Heba, I. Abdel-Mawla* and Nesreen, S. I. Yousef **

Fish Diseases Department, Animal Health Research Institute, Ismailia branch*, Dokki**, Giza, Egypt.

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Abstract

A total number of 200 marine fish 50 *Pomadasys stridens*, 40 *Saurida undosquamis*, 40 *Trachurus indicus*, 25 *Sscombermorus commerson* and 45 *Nemipetrus japonicus* which collected from Red Sea, Suez Canal area. The infected fishes revealed no clinical abnormalities except *Pomadasys stridens* showed slight abdominal swelling and appearance of big sized nematode worm out from the vent. The postmortem findings showed paleness, stretched and the empty intestine occupied by nematodes in infected *Pomadasys stridens*. Paleness or congestion and presence of encapsulated or free larvae of Anisakid nematodes in abdominal cavity, on the visceral organs especially in liver, gonads and in the musculature of infested *Saurida undosquamis*. The total prevalence of nematodes in the examined marine fishes was 28%. The highest infection rate of nematodes was recorded in *Saurida undosquamis* 42.5% (*Anisakis larvae* 32.5% and *Hysterothylacium* 25%) followed by *Nemipetrus japonicas* (28.9%), *Trachurus indicus* (27.5%) and *Scombermorus commerson* (24%) while the lowest infection was in *Pomadasys stridens* (18%). The isolated nematodes were identified as *Anisakis simplex, Hysterothylacium analarum, Hysterothylacium sp., Hysterothylacium reliquens, Contracaecum rudolphii* and *Camallanus cotti.*

The morphometric characters of isolated nematodes were discussed. Some larvae sample from *Saurida undosquamis* was confirmed by using PCR targeting ITS region *Anisakis sp.* and showed specific band at 1000 bp. Further molecular approach based on restriction profile PCR- RFLP obtained after digestion of ITS region with restriction enzymes *HinfI* showing *Anisakis simplex* pattern. The public health importance of the isolated parasites were discussed according to published records

Key words: *Marine fish, Red Sea, nematodes, Anisakid larvae, public health importance, Molecular identification.*

Introduction

Nematodes comprise one of the largest and most diverse groups of helminthes mostly in wild fresh water, brackish water, and marine fishes; these points to harmful effects induced by them, heavily infected fish show emaciation, imbalanced swimming, and reduction of their vitality (**Morsy *et al.* 2013** and **Morsy *et al.* 2015**).

Most adult nematodes are found in the intestine of fish but its larval stages are present in the flesh and viscera causing disease and economic problems. The occurrence of anisakid nematodes in the fish muscle is also unappealing to consumers because they can reduce the value of the product. Humans can become accidental hosts by consuming raw or undercooked fish or seafood that contains the third-stage larvae. Humans might subsequently suffer by two distinct clinical entities, namely gastrointestinal anisakiasis and allergic anisakiasis **(Shih *et al.*, 2010).** Several species of fish-borne nematodes are recognized as causative agents for human diseases**,** Anisakids are nematodes from super family *Ascaridoidea* (families: *Anisakidae* and *Raphidascarididae).* Especially those belong to genera (*Anisakis, Pseudoterranova, Con-*

tracecum and Hystrothylacium) are of biological, economic importance in the aquatic environment and well known as human pathogens. **Szostakowska *et al.* (2005), Cross and Belizario (2007) and El-Asely *et al.* (2015).**

A. simplex is a fish borne parasitic infection, broad worldwide with no conspicuous variety in morphology. Human are accidental host in the life cycle, and the parasites almost never developed further within the human but may penetrate the alimentary tract causing a range of pathological effects. Moreover, owing to the thermostability of *Anisakis simplex* allergens, the ingestion of safely cooked fish containing dead parasites can also be potentially dangerous and can cause severe allergic reactions such as contact dermatitis, facial oedema and asthma. Meanwhile, studies are now in progress to identify the fish species that is responsible for human infection. This information is crucial for initiating prevention measures against human anisakiasis Therefore, molecular methods were applied for distinguishing species level of anisakid nematodes in fish which is caused by an accidental infection with larvae belonging to the family *Anisakidae* when consuming raw, undercooked, or improperly processed marine fish and cephalopods, resulting in sudden gastrointestinal disor- ders usually leading to severe pain, nausea, vomiting, diarrhea, or allergic reactions. **El- Daly *et al.* (2004); Audicana and Kennedy (2008); Umehara *et al.* (2008); Al-Zubaidy (2010); Mohamed and Abd El-Ghany (2011); Sohn *et al.* (2014); Kassem and Bowashi (2015); El -Asely *et al.* (2015); Mehrdana *et al. (*2014) and Buchmann and Mehrdana (2016).**

D'Amelio *et al.* (2000); Fang *et al.* (2010); Setyobudi *et al.* (2011) and Pekmezci *et al.* (2014) recorded that anisakiasis, an important fish borne zoonotic disease caused primarily by nematodes of the genera *Anisakis* and *Pseudoterranova*, has been recognized as an important parasitic disease in areas of the world where people eat raw fish. To date, diagnosis based on polymorphism in deoxyribonucleic acid (DNA) sequences has been considered very useful for the definitive identification of clinically obtained worms; and several methods such as PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism), PCR-SSCP (single-strand conformation polymorphism), PCR random amplified polymorphic DNA (RAPD), and multiplex PCR have been developed for identification of anisakid species.

The aim of the current study is to determine the existence of nematodes and Anisakid larvae that affect some marine fishes from Red Sea. Investigation of both clinical and postmortem lesions are also considered with their prevalence and public health importance. PCR and PCR-RFLP have been used for accurate identification of larval stages of nematodes.

Materials and Methods

Fish:

The present investigation was done using 200 marine fishes samples (50 *Pomadasys stridens*, 40 *Saurida undosquamis*, 40 *Trachurus indicus*, 25 *Scombermorus commerson* and 45 *Nemipetrus japonicus*) of different weights and randomly collected from Red Sea at Suez Governorate. The collected fish were transported to the lab in plastic bags partially filled with its natural water within a short time according to **Langdon and Jones (2002).** With aid of all parameters of living transfer related to oxygen, temperature, fish count and PH.

Clinical picture:

The collected fish samples were examined carefully, externally and internally for detection of any abnormalities. Musculatures, stomach, intestine and internal organs were submitted for thorough examination for nematodes according to **Conroy and Hermann (1981).**

Parasitological examination:

The collected nematodes were washed in distilled water and kept in 70% ethanol glycerin for both morphological identification and DNA extraction or fixed in hot alcohol-glycerin 5%

until evaporation of ethyl alcohol then cleared in lactophenol and mounted in glycerin- gelatin according to **Meyer and Olsen (1992).**

DNA extraction and PCR-RFLP analysis
DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, worms were washed with 1% SDS, then 180 µl of ATL buffer was added to 25 mg of the sample and 20 µl QIAGEN protease. For homogenization of samples, tubes were placed into the adaptor sets, which were fixed into the clamps of the Qiagen tissue Lyser. Disruption was performed in 2 minutes high-speed (30 Hz) shaking step. Then samples were incubated at 56˚C till lysis. After lysis, 200 µl of the lysate was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 72^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from **Metabion (Germany)** are listed in Table (1).

PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gelpilot 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Anisakis ITS	GTA GGT GAA CCT GCG GAA GGA TCA TT TTA GTT TCT TTT CCT CCG CT	1000	94˚C 5 min.	94˚C 30 sec.	52˚C 40sec.	72˚ 1min.	72˚C 10min.	**Cavallero *et al.*, (2012)**

Preparation of restriction Master Mix according to Thermo FastDigest® *hinf*I Cat. No. FD0804

Component	Volume/reaction
10X FastDigest Green buffer	2 µl
*hinf*I	1 µl
PCR product	10 µl
Water, nuclease -free	17 µl

The reaction was done at 37^{o}C for 5 min in a thermoshaker (Biometra). The digested products was analyzed by electrophoresis in 1.5% agarose gel containing ethidium bromide and visualized under UV light.

Results and discussion

Clinical picture:

The clinical signs of most examined fish revealed no pathognomonic abnormalities (no external visible signs) except in infected *Pomadasys stridens* showed slight abdominal swelling and appearance of big sized nematode worm out from vent. **(Plate 1; A)** The postmortem findings showed paleness, stretched and the empty intestine occupied by nematodes in infected *Pomadasys stridens*. **(Plate 1; B, C).** Paleness or congestion in internal organs of infected fish **(Plate 4A &E)** and presence of Anisakid nematodes in abdominal cavity, on the visceral organs especially in liver, gonads and in the abdominal muscles of infected *Saurida undosquamis*. Some cases found anisakid larvae encapsulated or free attached in the mesentery or in the body cavity forming coiled appearance in a thin walled cyst **(Plate 2A, B & C)** These postmortem findings are similar to that recorded by **Shager and El-Ashram (2007), El-Ashram and Shager (2008), Al-Zubaidy (2010), Abdel-Mawla and Abo-Esa (2011), Mohamed (2013), Soewarlan *et al.* (2014)** and **El-Asely *et al.* (2015).**

Parasitological finding: Identification of the parasites was carried out according to their morphometric features (**Moravec , 1994**):

Class : *Chromadorea*
Order: *Rhabditida*
Superfamily: *Ascaridiodea*
Family: *Anisakidae* (**Railliet and Henry, 1912)**
Subfamily : *Anisakinae*
Genus: *Anisakis*
Species : *Anisakis simplex* (Rudolphi 1809)

Isolated larvae from the abdominal cavity and intestine of *Saurida undosquamis***.** Body thickest posteriorly, tapering gradually towards the anterior; width at ventriculus 0.33 +0.03 mm (0.25-0.40 mm), boring tooth prominent 0.010 ± 0.002 mm high (0.007-0.015 mm), 3 lips. Inconspicuous, excretory pore ventral between rudimentary sub-ventral lips; nerve ring 0.29 + 0.02 mm (0.274-0.320 mm) from anterior end; muscular oesophagus (pharynx) 1.99 ± 0.21 mm long (1.57-2.34 mm), followed by a glandular ventriculus 0.69 + 0.09 mm long (0.47-0.85 mm), no caeca or diverticula, 3 anal glands, anus 0.12 ± 0.01 mm (0.09-0.15 mm) from the tip of the tail, tail short, rounded, mucron distinct 0.023 ± 0.004 mm long (0.015 -0.030 mm), cuticle with transverse striations 0.007-0.008 mm apart, irregularly wrinkled near the tail. **(Plate 2D, E &F)**. This description was coincided with that recorded by **El-Ekiaby (2011); Pekmezci *et al.* (2014) and Abdel-Mawla *et al.* (2017).**

Class: *Chromadorea*
Order : *Rhabditida*
Superfamily: *Ascaridoidea*
Family: *Raphidascaridinae*
Genus: *Hyserothylacium*
Species: *Hyserothylacium analarum* (Rye and Baker, 1984)

The worm isolated from the intestine of *Pomadasys stridens* which characterized by the body cuticle with narrow transverse striations and wide, irregularly spaced transverse folds. Lateral alae absent. Narrow caudal alae present. Cephalic lips equal in length and width. Dorsal lip with two double papillae, subventral lips each with one double papilla, one single papilla and one amphid. Intestinal caecum and ventricular appendix present. Excretory pore at level of nerve ring. Tail tip of both sexes covered with numerous blunt spines. Males: 18.1 (13.5–19.1) long. Intestinal caecum 1.19 (0.54 –2.30) and ventricular appendix 0.300 (0.225–0.325) long. Excretory pore 0.390 (0.325–0.475) and nerve ring 0.390 (0.325–0.440) from anterior end . Tail conical 0.240 (0.160–0.190) long. Posterior half of tail with four pairs of papillae: two pairs lateral and two pairs subventral . Anterior half of tail with three pairs of subventral papillae. Anterior lip of anus with one unpaired papilla. Pre-cloacal region with 18 (13 to 21) pairs of subventral caudal papillae extending anteriorly. Pre-cloacal subventral region with numerous cau-

dal muscles. Spicules equal, 0.580 (0.450–0.625) long, alate, rounded distally, with blunt capitulum . Gubernaculum absent. **(Plate 3A& B)** This agrees with that described by **El-Ekiaby (2011)**; **Mohamed and Abd El-Ghany (2011); Al-Zubaidy *et al.* (2012) and Morsy *et al.* (2013).**

***Hyserothylacium spp*. (Ward and Magath, 1917)**

Isolated from the stomach and intestine of *Saurida undosquamis.* Rather large nematodes. Lips well developed approximately equal in size, bearing transparent cuticular flanges on lateral margins, flanges with or without indentations, internal plp sually pedunculated , dentigerous ridges absent . interlabia present. Lateral alae distinct or indistinct. Oesophagus muscular, provided with nearly spherical ventriculus, posteriorly directed ventricular appendix present. anterior intestinal caecum present. excretory pore at or near level of nerve ring. Spicules similar, alate, equal or slightly unequal in length. genital papillae numerous. Vulva anterior to mid-body. Uterus didelphic, opisthodelphic. Oviparous. Tail conical, tip with or without ornamentation. **(Plate 3C, D &E).** This description was similar to that recorded by **Al-Zubaidy *et al.* (2012) and El-Asely *et al.* (2015)**

Species: *Hysterothylacium reliquens* (Deardorff and Overstreet, 1981)

Isolated from the intestine of *Nemipetrus japonicas.* Body reaching greatest width near midbody. Cuticle with inconspicuous annulations and minute lateral alae. Alae become more apparent at levels behind rectum. Lips approximately equal in size, all longer than wide, flanges constricted near middle of lip, pulp pedunculated. Interlabia with height equal to or slightly greater than width at base. Interlabial grooves absent . Preventriculus 11–13% of body length. Ventriculus narrower than widest level of preventriculus. Usually longer than broad, ventricular appendix departing without angulation from posterior portion of ventriculus. Nerve ring located at anterior 16–28% of preventriculus. Excretory pore at or immediately posterior to level of nerve ring. Tail with spined conical mucron. Males: body 25–40 long by 0.5–0.9 wide. Preventriculus 3.3–4.5, ventriculus 0.141–300, ventricular appendix 1.2–1.7, and intestinal caecum 0.315–598 long. Spicules 1.7–2.4 long, relative length varies: spicule ratio 1:0.6– 1.3. Caudal papillae 27 to 33 pairs: 23 to 29 pre–cloacal pairs, four to five post–cloacal pairs with 3rd pair from tail end doubled, para-anal papillae lacking. Distinct medio-ventral organ, papillated. Tail reflexed ventrad, 0.123– 0.185 long, including multispinous process. Females: body 21–44 long by 0.5–1.1 wide. Preventriculus 2.9–5.0, ventriculus 0.142–0.286, ventricular appendix 1.0–2.9, and intestinal caecum 0.394–0.788 long. Vulva without protruding lips, opens 6.5–17.0 from anterior end. Ovaries rarely extend beyond anterior level of vulva. Tail 0.370–0.574 long, including multispinous process.

(Plate 3F, G & H). This agrees with that described by **Shager and El-Ashram (2007), Mohamed (2013)** and **Abo-Esa and Abdel-Mawla (2012)**

Class: *Chromadorea*
Order: *Rhabditida*
Superfamily: *Ascaridoidea*
Family: *Anisakidae*
Subfamily: *Contracaecinae*
Genus: *Contracaecum* (**Railliet and Henry, 1912)**
Species: *Contracaecum rudolphii*. (Hartwich, 1964)

Isolated from the stomach and intestine of *Trachurus indicus.* The body was light-coloured, with a very fine, dense transverse striation of the cuticle. The cephalic end was provided with a distinct dorsal larval tooth 3 long; the tail was 39–45 long, with a rounded tip. The oesophagus was light-coloured, not well visible, 87–96 long, encircled by the nerve ring located 51–57 from the anterior extremity. The small, thin-walled ventriculus was 9 long and 9 –12 wide, the ventricular appendix was poorly developed, short. The intestine was filled with numerous small brownish granules, no intesti-

nal caecum was present. A small oval genital primordium located postequatorially on the ventral side was visible in some larvae. Intestinal caecum considerably broader than ventricular appendix, the length ratio of intestinal caecum and ventricular appendix 3:1. Distance of nerve ring 0.14- 0.15 mm in advanced larvae. The tail is conical and it has not a mucron (**Plate 4A, B, C & D**). This in agreement with that described by **Moravec (2009), Abo-Esa and Abdel-Mawla (2012), El-Asely *et al.* (2015)** and **Al-Moussawi (2017)** in its general morphological characteristics.

Class: *Chromadorea*
Order: *Rhabditida*
Superfamily: *Camallanoidea*
Family: *Camallanidae*
Genus: *Camallanus*
Species: *Camallanus cotti* (Fujita, 1927)

Isolated from the intestine of *Scombermorus commerson*. Medium-sized nematodes with finely transversely striated cuticle and large orange-brown buccal capsule typical of genus. Mouth aperture slitshaped, surrounded by four submedian cephalic papillae and two lateral amphids. Valves of capsule roughly pentagonal in lateral view, internally bearing smooth longitudinal ridges, some incomplete. Anterior outer surface of each valve with two longitudinally elongate sclerotized plates. Narrow, sclerotized ring present at bottom of capsule. Tridents large, only moderately surpassing posterior border of buccal capsule. Excretory pore somewhat posterior to level of nerve ring. Deirids small, slightly asymmetrical, located at about two thirds of muscular oesophagus. Female : Body length of7.00–9.44 (6.62) mm, maximum width 367–490 (286). Buccal capsule including basal ring 147–174 (153) long, maximum width 129–150 (141); basal ring 18 (18) long, 81–90 (81)Wide, length of tridents 135–156 (141). Each valve of capsule with 21–22 (21) ridges, 5–7 (5) incomplete. Muscular oesophagus 517–653 (530) long, 105–117 (102) wide, glandular oesophagus 571–666 (571) long, 99–111 (108) wide, length ratio of both parts of oesophagus 1:1.09–1.29 (1:1.08). Buccal capsule and oesophagus representing 15–19 (19) % of total bodylength. Nerve ring, excretory pore and deirids 249–286 (252), 297 –375 (291) and 411–557 (402), respectively, from anterior extremity. Tail very elongate, 1.27–1.39 (1.12) mm long, representing 12–18 (17) % of body length, with rounded tip without any processes. Posteriorlip of anus with characteristic dotted sculpture . Vulva slightly postequatorial , 3.60–5.15 (3.17) mm from anterior end of body, with elevated lips. Vagina muscular, directed posteriorly from vulva. Uterus extending posteriorly into tail, filled with numerous larvae. **(Plate 4F, G & H)**. This description was similar to that recorded by **Moravec** and **Justine(2006).**

Prevalence of the detected nematodes parasites:

In the present study, the total prevalence of infection in some marine fish (*Pomadasys stridens, Saurida undosquamis, Trachurus indicus, Scombermorus commerson and Nemipetrus japonicas*) was (28%). Which was nearly similar to the results obtained by **El-Ashram and Shager (2008), Kassem and Bowashi (2015)** in which the percentages were 34.4, 38.4% respectively. Meanwhile, it was lower than that obtained by **Badawy (2001); Shager and El-Ashram (2007); Shamsi *et al.* (2011); Mohamad and Abd El-Ghany (2011); El-Ekiaby (2011)** and **Morsy *et al.* (2015)** who recorded that the total prevalence of nematodes infested marine fish were 43.75% from some marine fish, 45.9% wild marine fishes from the Red Sea, 75% from fish of Southern Australian waters, 65.81% from fish markets at Sharkia Province, 62.97% from frozen fish at Sharkia, and 75% *Saurida undosquamis* respectively.

Table (2) showed the prevalence of nematodes in *Pomadasys stridens* was (18%) which was nearly similar to the results obtained by **Badawy (2001)** 19.8% in some Red Sea fish**, El-Ekiaby (2009)** 13.79% in *Scomberomorus commerson* and **Abo-Esa and Abdel-Mawla**

(2012) 21.9% in *Lutjanus spp*. This difference may be attributed to the locality from which fish samples obtained and the species of examined fish.

The total prevalence of nematodes in *Saurida undosquamis* was 42.5% which nearly the same results was recorded by **Shager and El-Ashram (2007)** 41.7% *Carangoides bajad* and **El-Ekiaby (2009)** 41.9% *Saurida undosquamis* but differ from **Shager and El-Ashram (2007)** 50% *Scomberomorus maculatus* and **El-Ekiaby (2011)** 53.9% *Trachurus trachurus* Meanwhile, the prevalence of Anisakid larvae in *Saurida undosquamis* was 32.5%, this result coincided to that obtained by **Morsy *et al.* (2015)** *Saurida undosquamis* 35%, **Shager and El-Ashram (2007)** *Mulloides flavolineatus* 33.9%**, Al-Zubaidy (2010)** in *Carangoides bajad* 34.9% and higher than that obtained by **Abdel-Mawla *et al.* (2017)** 16.7% from *Mugil cephalus,* **Koinari *et al.* (2013)** *7.6%* from marine fish of Papua New Guinea, **Shamsi *et al.* (2011)** in *Platycephalus bassensis* (sand flathead) 20% and **Al-Zubaidy (2010)** *Lethrinus lentjan* 22.4%, *L. nebulosus* 22.7% and *Variota louti* 18.3%. While it was lower than the finding recorded by **Dadar *et al*. (2016)** from greater lizardfish (*Saurida tumbil)* 90.3%, **Shamsi *et al.* (2011)** *Aldrichetta forsteri* (yellow-eye mullet) 90%, *Neoplatycephalus richardsoni* (tiger flathead) 100%, *Sardinops sagax* 90%. This variation may not only be attributed to the change in the climatic conditions, but also to the differences in host species, size and sampling periods.

In addition, the prevalence in *Trachurus indicus, Scombermorus commerson* and *Nemipetrus japonicas* were 27.5%, 24% and 28.9% respectively. These results were nearly similar to **El-Ekiaby (2009)** who recorded the prevalence in *Argyrosomus regius* 26.47%, **Abo-Esa and Abdel-Mawla (2012)** *Lethrinus lentijan* 26.7 %.

Molecular analysis by PCR- RFLP

In the present study, the morphological identification of anisakid larvae isolated from *Saurida undosquamis* was identical to be *Anisakis simplex* larvae. So the PCR-RFLP method used for accurate identification and confirmed that the isolated larvae were *Anisakis simplex.*

The larvae sample was identified by PCR-RFLP analysis (Approximately 1000bp fragment was produced after amplification of the rDNA region). Digestion of the PCR product using H*infI* produced strong bands (620, 250 and 100 bp), the pattern of these fragments corresponds to species of *Anisakis simplex* (**Fig. 5**). This finding was nearly similar with those previously reported by **D'Amelio *et al.* (2000); Setyobudi *et al.* (2011); Cavallero *et al.* (2012); Sohn *et al.* (2014) and Pekmezci *et al.* (2014).**

It is also worth indicating that ansakids may not be host specific at the larval stages, which means that a wide range of fish species can act as their intermediate or paratenic hosts. It has been shown that larval anisakids can pass through several fish species via predation and can be accumulated in larger fish. Hence, fish of different species can play an important role in the distribution of anisakids in the environment. Different species of fish are not only the source of infection to humans, but also infect a broad range of marine mammals and piscivorous birds. **Shamsi *et al.* (2011) and El-Asely *et al.* (2015).**

Research focused subsequently on how worms could be rendered inactive and non-infective in addition to control measures by **FDA (2001); Choi *et al.*, (2009); Buchmann and Mehrdana (2016) and Topuz and Gökoğlu (2017)** who recommended the evisceration of fish as soon as possible, avoid eating the whole small fish or abdominal region, avoid insufficient cooking and temperature must be more than 60 °C for 1 min, well frozen fish at -35°C or below for 15h or -23°C for at least 7days kills the larvae of nematodes. Freezing, cooking, salting, marinating, irradiation, high hydrostatic pressure and chemicals have been reviewed.

Table (2). The prevalence and intensity of nematodes among the examined marine fish

Fish species	Common names	No. of exam. fish	No. of inf. Fish	%	Parasitic isolate	Site of infect. & intensity
Pomadasys stridens **(shokhrom)**	**Striped piggy**	50	9	18	- *H. analarum*	- intestine (1- 5/F)
Saurida un-dosquamis **(Makarona)**	**Brushtooth lizardfish**	40	17	42.5	- *A. simplex* 32.5% - *Hysterothylacium* 25%	- Abdominal cavity& intestine (2- 10/F) - stomach & intestine (1- 6/F)
Trachurus indicus **(Bagha)**	**Arabian scad**	40	11	27.5	- *C. rudolphii*	- stomach & intestine (4- 9/F)
Scombermorus commerson **(Derak)**	**narrow-barred spanish mackerel**	25	6	24	- *C. cotti*	- intestine (2- 3/F)
Nemipetrus japoni-cas **(sarahea)**	**Trieadin braems**	45	13	28.9	- *H. reliquens*	- intestine (1- 3/F)
Total		200	56	28		

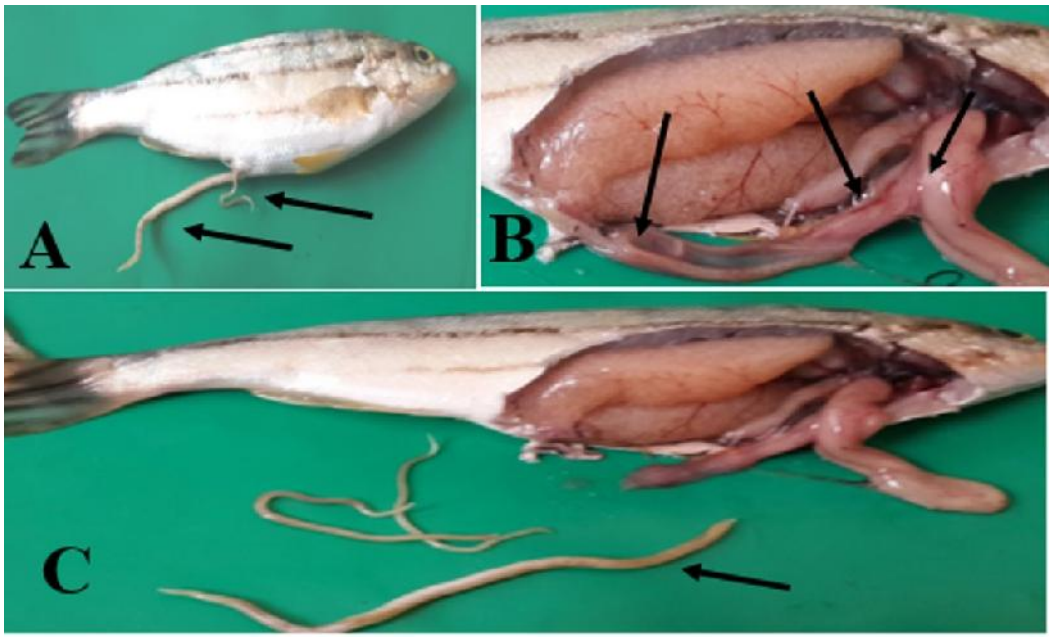

Plate (1): The infected *Pomadasys stridens* with *Hysterothylacium analarum*, showed:
A: presence of big sized nematodes worm out from the vent.
B: the intestine was empty, paled and occupied by nematodes worms.
C: Numbers of *Hysterothylacium analarum* and its size in relation to infected fish.

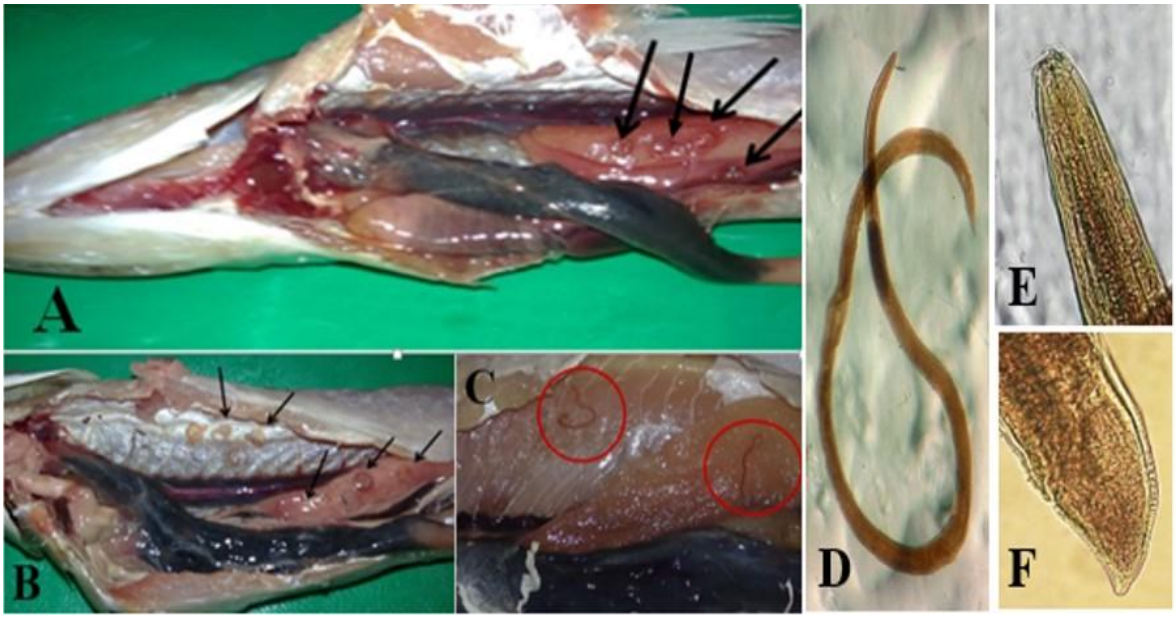

Plate (2): The infected *Saurida undosquamis* with *Anisakis simplex*, showed:
A: presence of Anisakid nematodes encapsulated in abdominal cavity and internal organ as liver and gonads.
B: the internal musculature and organs infected with Anisakid coiled nematodes larvae and encapsulated.
C: free *Anisakis simplex* larvae within the musculature and gonads of *Saurida undosquamis.*
D: *Anisakis simplex* third-stage larva (X10)
E: anterior end (X40)
F: posterior end (X40)

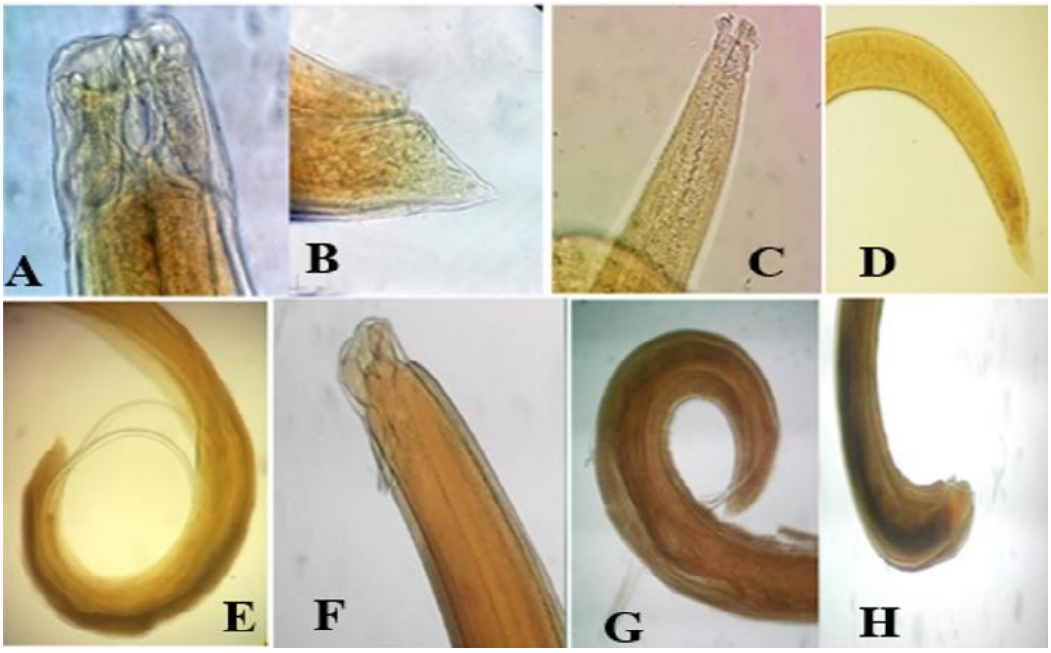

Plate (3):
A: *Hysterothylacium analarum* male (Anterior end) (X40)
B: Posterior end of male(X40)
C: Anterior end of *Hysterothylacium spp*. (X20)
D: Female Posterior end (X20)
E: male Posterior end**.** (X20)
F: Anterior end of *Hysterothylacium reliquens*. (X20)
G: male Posterior end**.** (X20)
H: female Posterior end**.** (X20)

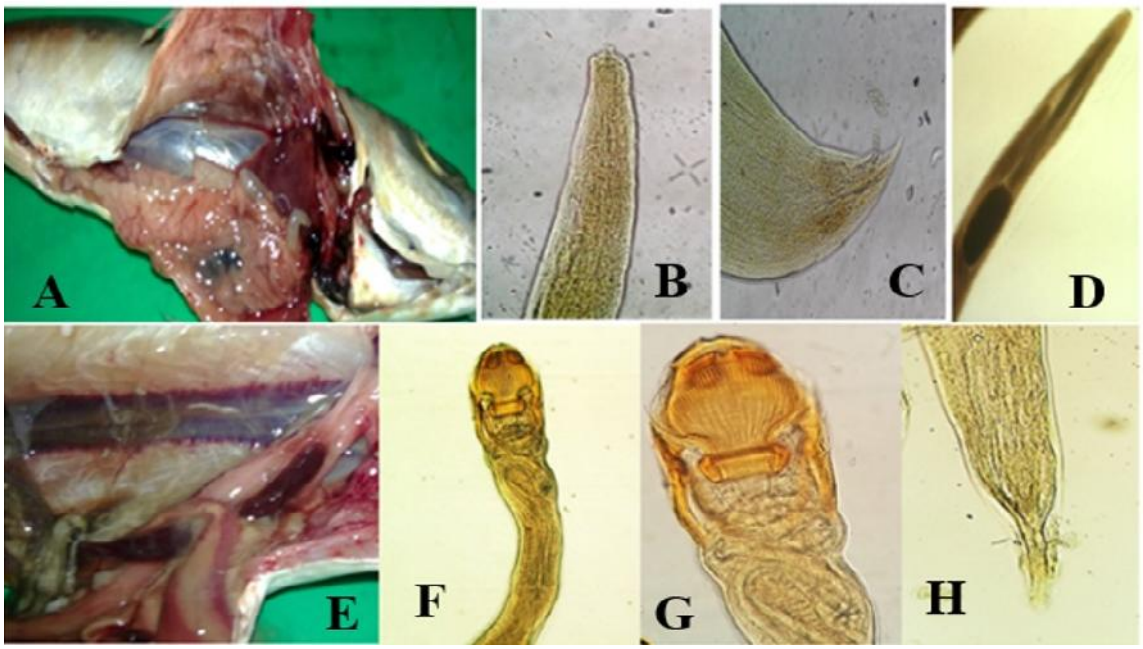

Plate (4): A: The infected *Trachurus indicus* showed congested liver and paleness of the intestine
B: Anterior end of *Contracaecum rudolphi*. (X40)
C: Posterior end (X40)
D: Anterior part showed the intestinal-ventricular junction level. (X10)
E: The intestine of *Scombermorus commerson* was pale and congestion in some internal organs.
F: *Camallanus cotti* femal (Anterior end) (X20)
G: high magnification of head region. (X40)
H: Posterior end of female. (X40)

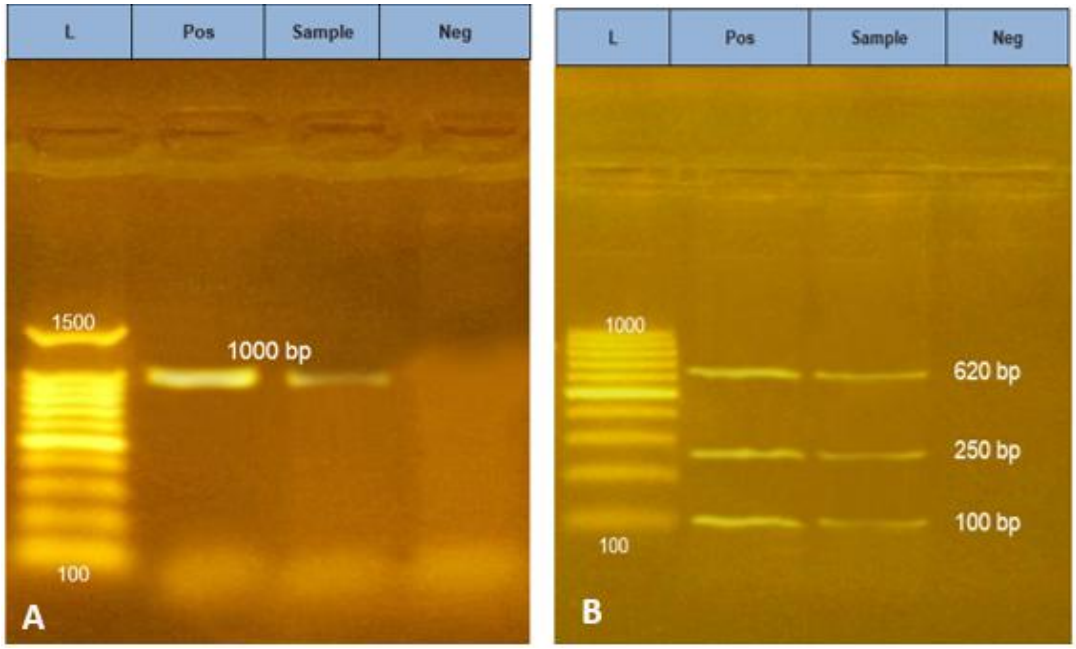

Fig (5):

A: Molecular identification of examined sample using PCR ITS region showed positive identification of *Anisakis* species at (1000 bp).
L: 100 bp ladder

B: RFLP profiles obtained by digestion of ITS region with the restriction enzymes HinfI showing *Anisakis simplex* pattern.
L: 100 bp ladder

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