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ROLE OF SEA BREAM FISH *P. PAGRUS* (FAMILY: SPARIDAE) IN HARBORING SOME LARVAL ASCARIDOIDS (FAMILY: ANISAKIDAE) IN CAIRO GOVERNORATE, EGYPT

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Abstract

The study dealt with anisakid nematode larvae (L_3) in a commercially important marine fish *Pagrus pagrus* purchased from different fish markets at 3 localities: El-Matareya, El-Zaitoun and, Saray El-Kobba, Cairo. Anisakid nematodes are zoonotic fish-borne parasites that may transmit to human by consuming raw fish containing the third stage larvae encapsulated in the fish muscles caused a serious disease Anisakidosis. The symptoms of that disease are nausea, diarrhea, vomiting and severe abdominal pain. Four species of L_3 larvae were detected; *Hysterothylacium* sp., *Raphidascaris* sp., *Contracaecum* sp. and *Terranova* sp. Identification of the larvae depended basically upon the structure of the cephalic region, oesophogus shape, ventricular appendage, tail end and presence or absence of mucron. Two-hundred and thirty-three larvae were isolated from 80/140 (56.4%) examined fish. The hosts were collected from November 2019 to April 2020. Larvae were measured in relation to host's weight, sex and infection prevalence. The results statistically showed that larvae prevalence and intensity were varied significantly in relation to host's sex and weight.

Key words: Third-stage larvae, Anisakidae, *Pagrus pagrus*, Fish-borne zoonoses

Introduction

Anisakiasis (Anisakidosis) is a fish-borne zoonotic parasite with larval stages of the ascaridoid nematodes belonging to family Anisakidae and probably Raphidascarididae. The Anisakids have two different hosts in life cycles: 1- Vertebrates including aquatic mammalians, birds, reptiles, and fish as definitive hosts. 2- Aquatic invertebrates and fish as paratenic or intermediate hosts (Murrell and Fried, 2007). The nematodes are transmitted to fish by microcrustaceans or any aquatic invertebrates and within the fish larvae changed to the third-stage larvae (Oshima, 1972). It may be transmitted several times from fish to fish before reaching the final host invading various tissues and organs as digestive canal, gonads, somatic musculature, liver, blood vessels, fins and eyes (Moravec, 1994; Dezfuli *et al,* 2007). The common sea bream *P. pagrus* (Linnaeus, 1758) was one of the most important and marketable food-fish. It lives in shallow water of Atlantic Ocean and the Mediterranean Sea. Severe inflammatory reactions, tissue deformation, cellular infiltration, nodules in the serosa of intestine and hemorrhage resulted from anisakids larvae infection (Marci *et al,* 2010; Levsen and Berland, 2012). Few histopathological studies on *P. pagrus* infected with *Anisakis* larvae are available: Eiras and Rego (1987) found a reduction in size of liver and spleen of infected fish. The fish-borne parasitic zoonoses were limited to populations who live in the Far East Developing Countries for increasing international markets improved transportation systems and demographic changes such as population movements (Chai *et al,* 2005). Yet, zoonoses were responsible for large numbers of human infections worldwide. The knowledge of public health significance of zoonoses, their relations to cultural traditions, poverty, environmental degradation, and no control ways increased (WHO, 2004). Human is an accidental host in the life cycle acquire the anisakid infection by consuming row fish or seafood infected with larval stages in the flesh, viscera or body cavity (Audicana and Kennedy, 2008). Within human body, the nematodes never develop to adult, but penetrate the alimentary tract and associated organs causing severe pathological effects involved gastrointestinal disorders, vomiting, na-

usea and allergic re-actions (Murrell and Fried, 2007). During the past three decades about 20.000 cases of zoonotic anisakidosis were worldly reported (Hochberg and Hamer, 2010). The increased human infection was attributed to high distribution of anisakid in almost all oceans and sea, increase in populations conservation measures, human migratory movements and globalization and the use of faster cooking tools a microwave (Audicana *et al,* 2003).

Ascaridoid nematode larvae from fish and cephalopods by Levsen *et al.* (2005), they detected *Anisakis simplex* larvae in the North Atlantic marine fishes. Al-Zubaidy (2010) from Red Sea fishes in Yemen Coast. Soares *et al.* (2014) detected 24 larvae of anisakid species: *Contracaecum, Hysterothylacium, Raphidascaris* from 7 fish out of 36 of *P. pagrus* in Rio de Janeiro, Brazil. Chen *et al.* (2018) detected third stage larvae of anisakid from *Conger myriaster* in China. Dadar *et al.* (2016) reported anisakis type III from some fish species in Persian Gulf. In Egypt, there were few reports about fish anisakid larva. Abdou (2005) identified *Terranova* sp. from Red Sea fishes. Abo-Mazyd *et al.* (2005) reported zoonotic anisakid in Fayoum Governorate. Rizk *et al.* (2015) reported it some marine fish in Damietta. Morsy *et al.* (2015) recorded three juvenile nematodes: *Anisakis* sp. type Π, *Hysterothylacium patagnense* and *Echinocephalus overstreeti* from *Saurida undosquamis* in the Red Sea. Younis *et al.* (2017) found *Contracaecum* larva from teleostean species in Lake Nasser.

Anisakis sp., *Raphidascaris* and *Terranova* type 1 were identified as the major causative agents of human anisakiasis, genus *Anisakis* included nine species; 2 species of *Anisakis simplex* complex: *A. simplex sensustricto* (s.s) and *A. pegreffii* were associated with human infections (Audicana and Kennedy, 2008). Genus *Hysterothylacium* Ward and Magath, 1917 comprised more than 50 species of nematodes of teleost fish whereas larval stages live in various tissues of fish species and in >100 invertebrates of different phyla (Deardorff and Overstreet, 1980). Eiras and Rego (1987) in Brazil reported them as adult worm in liver of *P. pagrus*.

The present study aimed to determine occurrence, prevalence, and mean intensity of anisakid nematode larvae in a frequently consumed important zoonotic fish *P. pagrus* collected from different areas in Cairo.

Materials and Methods

Collection of specimens: Fish specimens (n=140) of the common seabream or red porgy *Pagrus pagrus* (Family: Sparidae) were collected from different fish markets at three localities; El-Matareya, El-Zaitoun and, Saray El-Qobba at Cairo, Egypt during the period from November 2019 to April 2020. Fishes were identified (Burgees *et al,* 2000; Schultz 2003). Weight and length of each one ranged from 35.5-75gm by 14.6-18.8cm respectively. Fishes were differentiated to sex from outward confirmed to be 50 males & 90 females. They were dissected to the internal organs such as digestive canal, liver, kidney, heart and gonads. Then, they washed very well in physiological solution 0.7% NaCl. Alimentary canal was removed, sectioned into its main parts (stomach, small and large intestine). Internal organs and body cavity were examined for nematode larvae. Isolated larvae were counted, washed several times in saline solution, fixed in 7% warm formalin for 24hrs, then cleared and mounted by lactophenol for morphological study. Specimen measurement was an important feature. Systematic characters were identified based on documented Keys (Cannon, 1977; Al-Zubaidy, 2010; Shamsi *et al,* 2018). Photomicrographs were done with a digital camera microscope and drawn using a camera Lucida. All measurements were in millimeters unless otherwise stated. Mean measurements were included with range in parentheses.

Statistical analysis: SPSS version-19.0 soft ware was used to data analysis. Nonparametric Spearman test was used to find the correlation between host's weight and infection intensity. Independent samples T-test

and Chi-Square test correlated between intensity and prevalence of males and females.

Results

Seventy-nine (56.4%) out of 140 fish *P. pagrus* were found infected with 233 third-stage larvae. Larvae were isolated from body cavity as attached to the mesenteries of digestive canal. Total parasite abundance was 1.66. The larvae were identified as; *Hysterothylacium* sp. (n=112), *Contracaecum* sp. (n=36), *Raphidascaris* sp. (n=68), and five larvae were *Terranova* sp. (Dia. 1).

Total mean intensity of nematodes (number of larvae per host) = 1.66±1.94. The parasite abundance = 2.95. SDE (standard error) = 0.16, Variance =3.7, Range =11. Mean intensity was higher 2.3±1.87 in males than 1.3±1.9 in females of the infected fish (Tab. 2). Prevalence of infection in males was higher (70%) than in females (48.9). Chi-Square test was used to analyze the correlation between the infection and the sex of the host. Data showed a high significant difference between them P<0.01 (Tab. 3).

Table 1: Number of infections in male and female fish

Count	Infection		
	Infected	Non-infected	Total
Male	35	15	50
Female	44	46	90
Total	79	61	140

Table 2: Prevalence and mean intensity of infection in both sexes

Sex	No.	P%	Mean	Std. Deviation	Std. Error	Variance	Median	Range
Male	50	70	2.30	1.876	0.265	3.5	2	6
Female	90	48.9	1.31	1.905	0.201	3.63	0.0	11

Table 3: Chi-Square Test

Pearson Chi-Square	Value	df	Asymp. Sig-(2-sided)
	5.826	1	0.016

Table 4: Independent samples test relation between intensity of infection and host's sex

Intensity	Variances equality		t-test for Equality of Means				
	F	Sig.	t	df	Sig. (t-tailed)	Mean Difference	Std. Error Difference
Equal variances assumed	1.149	.286	2.958	138	0.004	.988	0.334
Equal variances not assumed			2.971	102.699	0.004	.988	0.332

The relationship between intensity of infection and host's sex was statistically analyzed by independent samples t-test. The results showed that males have higher significant intensity than females P<0.004 (Tab. 4). Fishes weight ranged from 35.5 to 75gm. Prevalence of infection was higher in small sizes than larger ones in both males and females. Statistical analysis (Non-Parametric Spearman Test) ensured a significant correlation at the 0.01 level (2-tailed) between infection rate and the host size. Independent samples t-test was used to analyze the correlation and showed that a negative relationship between the weight of the host and infection as the infection increases in small weights of hosts than in the larger weights (Tab.5).

Table 5: Independent samples t-test showed relation between host weight and infection

Weight	Variances equality		t-test for Equality of Means				
	F	Sig.	t	df	Sig.(tailed)	Mean Difference	Std. Error Difference
Equal variances assumed	1.406	.238	-12.907	138	.000	-16.57159-	1.283
Equal variances not assumed			-12.722	121.363	.000	-16.57159-	1.302

All nematodes were larvae third stage development (L3).

Hsyterothylacium sp. (Ward and Magath, 1917) Family: Raphidascarididae: Small to medium in size, whitish color, total length 5.023±0.136 (4.7-5.2) by 0.14±0.01 wide (0.13-0.15), cuticle lightly striated longitudinally and transversely striated posteriorly. Three prominent lips, lip length 0.019±0.005 (0.014-0.025), Nerve ring 0.23±0.028 (0.21-0.25) from anterior end, excretory pore at ventral side immediately behind nerve ring level. Muscular oesophogus 0.75±0.12 long (0.64-0.88) by 0.05±0.01 wide (0.04-0.06), ventricular appendix 0.503±0.07 long (0.42-0.56) by 0.057 ±0.009 wide (0.05-0.07).

Ventriculus present between muscular oesophogus, appendage measured 0.05±0.01 long (0.04-0.06). Intestinal caecum longer and wider than ventricular appendage, gonads as filament-like structure & vulva not visible, Anus located at 0.07±-0.01 (0.06-0.08) from posterior extremity. Tail conical shaped with 2 developed caudal papillae (Figs. A, B, & 1-4).

Contracaecum sp. Railliet and Henry, 1912; Family: Anisakidae: White in color and small sized body, 4.48±0.46 long (4.05-5.1) by 0.18±0.02 wide (0.17-0.22). Cuticle transversely striated par at posterior body part. Mouth triangular surrounded by three inconspicuous lips 1 dorsal and 2ventrolateral, each lip measures 0.013± 0.0026 long (0.01-0.02), Cephalic capsule 0.04±0.01 long (0.03-0.05). Nerve ring measured 0.22± 0.04 (0.17-0.25) from anterior extremity and oesophogus 1.33±0.2 long (1.1-1.5). Ventricular appendix measured 0.57±0.07 long (0.52-0.65), Ventriculus 0.06± 0.01 long (0.05-0.07). Anus measured 0.1±0.015 (0.09-0.12) from posterior end. Tail 0.15± 0.01 long (0.14-0.16) with blunt end lacked a mucron and two caudal papillae at anus level (Figs. C-D & 9-10).

Raphidascaris sp. Railliet and Henry, 1915: Family: Raphidascarididae: Medium sized larva, body length 5.7±0.45 (5.18-6.23) by 0.14±0.017 wide (0.13-0.17), Muscular oesophogus 0.55-±0.05 long (0.5-0.6) by 0.066±0.02 wide (0.05-0.09). Ventriculus 0.06±0.01 long (0.05-0.07), Ventricular appendix 0.4± 0.027 (0.37-0.42), Nerve ring 0.17±0.04 from anterior extremity (0.12-0.2). Anus measured 0.09±0.01 from posterior end (0.08-0.1), Tail ends with sharp mucron 0.007±0.002 (0.001) (Figs. E, F & 5-8).

Terranova sp. Linton, 1901, Family: Anisakidae: Medium sized larva, body cylinder and thin, measured 5.17± 0.8 long (4.5-6.3) by 0.16±0.01 wide (0.15-0.17). Cephalic capsule 0.05±0.015 long (0.04-0.07), Lips inconspicuous with a prominent tooth on cephalic region extreme anterior end & with four small papillae: two dorsolateral & two ventrolateral. Cuticle greatly transversely striated particularly on tail region. Muscular oesophogus measured 0.6±-0.2 long (range 0.4-0.8) by 0.06±0.01 wide (0.05-0.07), but glandular one 1.13±0.25 long (range 0.9-1.4) by 0.04±0.007 (0.04-0.05) wide, Ventriculus measured 0.04±0.01 (range 0.03-0.05), intestine long wider than oesophogus, and opens in anus by a short rectum. Gonads not developed, excretory pore measured 0.056± 0.015 (0.04-0.07) from anterior extremity, Tail annulated, measured 0.06±0.153 (0.05-0.08) long (Figs. G-H &11-12).

Discussion

The anisakids constitute the ascaridoids with an aquatic definite host (mammal, bird, reptile, & fish) which transmission depended on water and involves aquatic invertebrate and fish as intermediate host (Anderson, 2000). At least 20 different genera of anisakids have been described. Hartwich (1974) defined one family only within (Anisakidae), with 3 subfamilies: Anisakidae; Geoziinae and Raphidascaridinae. Fagerholm (1991) divided Anisakids into 2 families: Anisakidae that included 2 subfamilies: Anisakinae (Contracaecinae) and Raphidascarididae. They were extensive distributed parasites in Europe (The United Kingdom, France, The Netherlands, Germany, Italy, & Spain), Asia (Iran, Japan, & Korea), Africa (Egypt) and Americas (Canada, Alaska, & Hawaii) (Daddar *et al,* 2016).

The present study revealed 4 species of anisakid nematode third-stage larvae (*Hysterothylacium* sp., *Raphidascaris* sp. *Contracaecum* sp. and *Terranova* sp.) attached to the mesenteries of internal organs and encapsulated on the wall of the stomach and intestine of the Sea-Bream fish *P. pagrus*. There was a scarce information concerning distribution of these anisakids in fishes especially those have a commercial important and mostly consumed by human particularly in Egypt. In the present study, total infection rate was (56.4%), higher than (19.4%) reported by Soares *et al.* (2014) from *P. pa-*

grus in State of Rio de Janeiro, Brazil. They detected 24 larval of *Anisakid* sp., *Contracaecum* sp., *Hysterothylacium* sp. and *Raphidascaris* sp. and higher than (34%) by Shamsi *et al.* (2018) in Australia, than (21.05%) by Rezk *et al.* (2015) in Damietta Governorate, Egypt. Also, it was higher than (33.7%) by Debenedetti *et al.* (2019) in At-l-antic and Mediterranean Regions; they reported that larvae were higher in viscera than musculature. But, Morsy *et al.* (2015) recorded 3 juvenile nematodes; *Anisakis* sp. type Π, *H. patagonense* and *Echinocephalus overstreeti* from *Saurida undosquamis* in the Red Sea (75%) that was higher than that in the present study. The high prevalence levels of infection by L_3 larvae was reported (Chen *et al,* 2018), they identified *Anisakis pegreffii*, *A. typica, A. simplex* and five species of *Hysterothylacium* and *Raphidascaris* sp. from *Conger myriaster* in China (100%). The present result was distinctly less than (100%) reported in Korea (Setyobudi *et al,* 2011) from Salmon fish. These variations between the present result and the other previous might be due to different factors as geographical region, size and age of the host, seasonal variations, feeding habits, type of water supply, and abundance of final host (Dione *et al,* 2014).

The present study showed that male was significantly more infected by anisakid larvae than female. These agreed with Aliyua and Solomon (2012), and may be due to the fact that males were known to be more sensitive to parasitic infection than females due testosterone hormone effect which may exert a decreased immune competency (Bichi and Yelwa, 2010). A negative significant was found between host's weight and prevalence of infection. High value of intensity was recorded in the small sized fishes than in the larger ones. This disagreed with Kassem *et al.* (2015). It could be explained by that the small fishes were more activity moving within aquatic environment consequently have long exposure time to be infected by feeding. The zoonotic fish-borne nematodes have an economic significance and growing global health concern (Shamsi *et al,* 2018), caused by accidental infection with *Anisakis* L3 larvae. Oshima (1972) stated that the ascaridoid nematode larvae were capable of invasion of man. Several countries where raw fish was commonly consumed suffered from infection as Korea, Japan Taiwan, Europe, South Africa and North America (Buchmann and Mehrdana, 2016). Thus, zoonoses problem was reliable global behavior and feasible control and prevention programs were a must (Chai *et al,* 2005). Anisakids infection affect fish by increasing the susceptibility to predation, decreasing host density, reducing marketability of fish mainly when larvae were located in muscles caused economical loss for fish industry (Angot and Brasseur, 1995). Genus *Hysterothylacium* Ward and Magath, 1917 (Anisakidae, Rhaphidascaridinae) parasitized the digestive system of marine teleosts in temperate and cold waters (Deardorff and Overstreet, 1980). The genus is characterized by oesophogus divided into anterior muscular part and posterior glandular part separated by valvular structure and by conical tail and caudal papillae. The present *Hysterothylacium* sp. size agreed with Navone *et al.* (1998) for *H. aduncum* from the mesenteries of fishes *Engraulis anchoita* and *Merluccius hubbsi* in the South West Atlantic, and with Moravec *et al.* (1985) from fishes in Japan.

In the present study, infection by L3 larva *Hysterothylacium* was high (48%) than the other discovered larvae, and may be attributed to food habits of the host during ilife span (Navone *et al,* 1998). The occurrence of *Contracaecum* sp. Railliet and Henry, 1912 in the present fishes was (15.4%), lower than (35.6.5; 82%; 100%) from *Oreochromis niloticus, Hydrocynus forskahlii* and *Lates niloticus* respectively in Lake Nasser (Younis *et al,* 2017), than (23%) by Moravec *et al.* (2016) who presented *Contracaecum* sp. for the first time from freshwater fish *Sandelia capensis* in South Africa. they stated to be with low pathogenicity. But, it

was higher than (0.14%) in *Tilapia galilaea*. *Contracaecum* 3rd stage larva of the present study was morphologically similar to *Contracaecum* larval type Ⅱ of Cannon (1977) from Queensland fishes and, in length, width and oesophogus. The third stage larva of *Raphidascaris* was 29.18% of all anisakid larvae in the present study. It was lower than (63%) by Valtonen *et al.* (1994) from roach (*Rutilus rutilus*) in Finland. This genus is distinct by pointed tail (mucron), well-developed lips that bear double papillae without interlabia, and triangular mouth opening. It was described from marine and freshwater fish (Malta *et al,* 2020). Genus *Terranova* sp. was represented by the lowest infection rate (2.14%) that was less than (19%) by Abdou (2005) in Hurghada. It was distinct by a boring tooth and well developed mucron with strongly transversally striated posterior extremity, with high specificity to the final host and fish body cavity represented the most favorable medium for infection (Andrada *et al,* 2008).

In the present study, larva *Terranova* sp. was recorded from body cavity and mesenteries of *P. pagrus* as new host record. Shamsi and Suthar (2016) reported that all *Terranova* larvae cause zoonotic Pseudoterranvosis, they were similar in shape and cannot be distinguished by morphology. The variety of the larval infection in *P. pagrus* fish in the present study may be either due to the feeding habits of this host or abundance of definitive host in the collected area.

Anisakids were identified essentially due to some important taxonomical characters as cephalic region, the presence of boring tooth or lips, the presence or absence of mucron (caudal spine or cactus tail), position of excretory pore, length of ventriculus (Anderson, 2000; Shin and Jengo, 2002).

Some recommendations were in considered to avoid infection such as cooking fish by heating to 60℃ for some minutes and freezing to -20℃ for 24hr enough to kill larvae (Wharton and Aalders, 2002). But, domestic freezers didn't provide a low homogenous temperature and thus, not able to inactivate larvae (Debenedetti *et al,* 2019). Albendazole ® & Thiabendazole® treated anisakid in man and herbs (Pacios *et al,* 2005) and Mirazid® (Abo Mazyad *et al,* 2004). Prevention, proper identification, and effective therapy dramatically improve the health and productivity of affected fish (Yanong, 2002).

Conclusion

The results revealed that the marine fish *P. pagrus* representing a proper environment for the harboring the anisakid third stage larvae.

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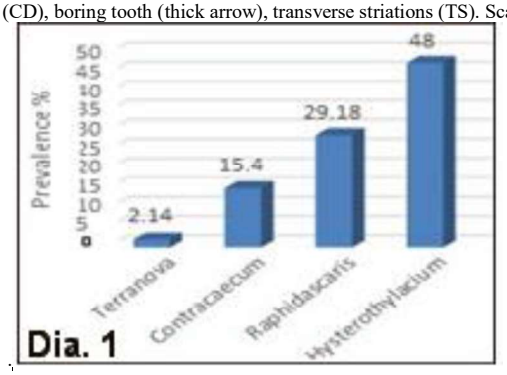
Explanation of figures

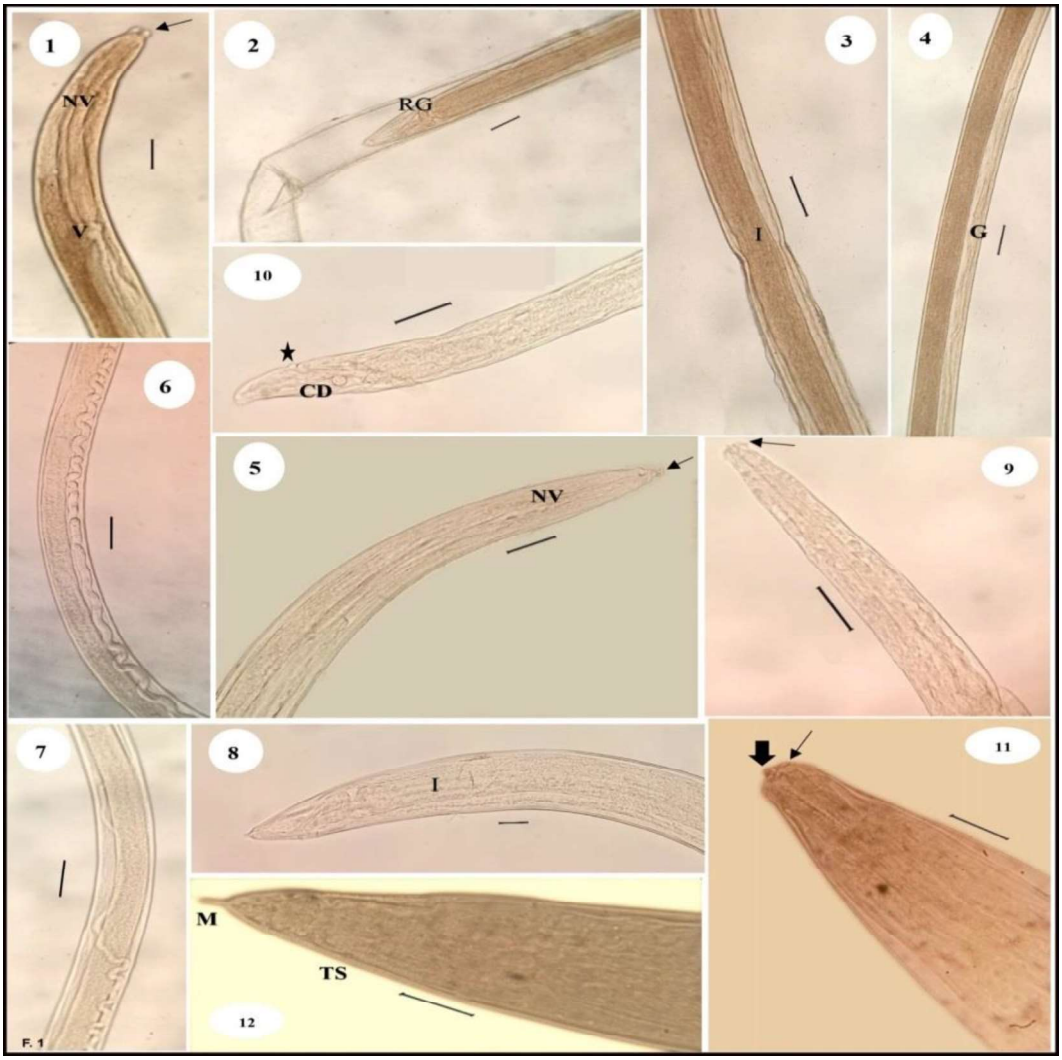
Dia. 1: Prevalence of nematode larvae in the examined fishes

Fig. 1:A-H: Anterior and posterior extremities of Anisakid nematode larval morphotypes isolated from *P. pagrus* in Cairo, Egypt. A&B, *Hysterothylacium* sp., C&D, *Contracaecum* sp., E&F, *Rhaphidascaris* sp. and G&H, *Terranova* sp.

Fig. 2:1-12: Larval stages (L3) of nematodes. 1-4: *Hysterothylacium* sp. 5-8: *Rhaphidascaris* sp. 9-10: *Contracaecum* sp. and 11-12: *Terranova* sp. showed venterolateral lip (arrow), nerve ring (NV) ventriculus (V), rectal glands (RG), intestine (I), gonad (G). mucron (M), rectum (R), anus (star), caudal papillae (CD), boring tooth (thick arrow), transverse striations (TS). Scale bars= 0.01 mm.



Dia. 1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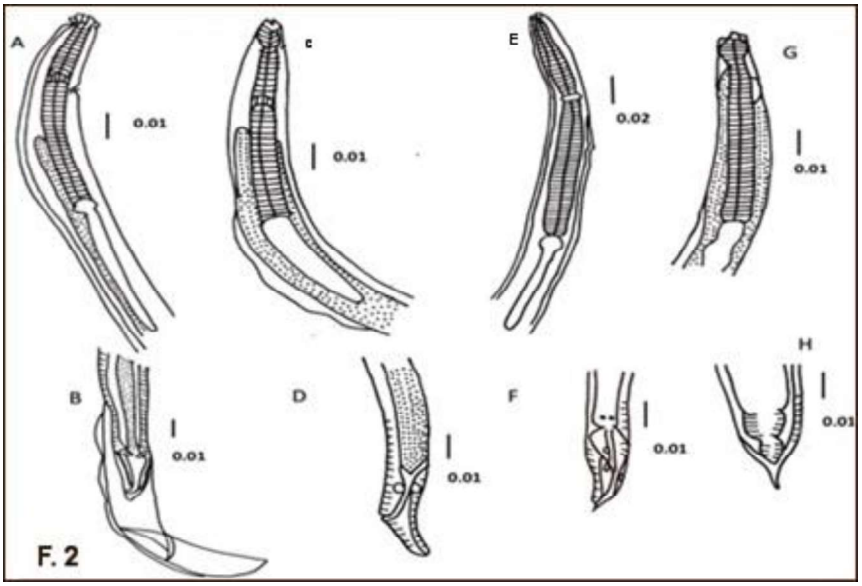
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