Received: 26 August 2023, Accepted: 04 October 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 8, 1678-1683

Occurrence and Public Health Importance of Ascaridoid Nematodes in Red Porgy, *Pagrus pagrus* (Perciformes: Sparidae)

Amany M. Abd El-Ghany[1*], Abdallah F.A. Mahmoud[2], Adel I.M. El-Atabany[2], Salwa M.A. Attia[3]

[1]Parasitology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519 Sharkia, Egypt.

[2]Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519 Sharkia, Egypt.

[3]Private Veterinarian, Sharkia province, Egypt.

***Correspondence**
Corresponding author: Amany M. Abd El-Ghany
E-mail address:amanyabdelghany13@gmail.com

Abstract

Anisakid nematodes have been found in a variety of marine fishes worldwide and they are known to cause anisakiasis and allergic symptoms in human. One-hundred and five fish specimens of different sizes of red porgy (*Pagrus pagrus*) were collected randomly from Sharkia fish markets originating from the Gulf of Suez, Red Sea, Egypt and examined for the presence of ascaridoid nematodes. The overall prevalence of ascaridoids was 36.19% (38/105). Four morphotypes belonging to two genera of anisakid nematodes (*Anisakis* type I and *Terranova* type II larvae) as well as one genus of raphidascarid; *Hysterothylacium* (larvae and adult) were reported during this study. *Hysterothylacium* larvae were the most predominant species (23.81%) and abundance value =1.68. Interestingly, *Pagrus pagrus* was found as new host and locality records for larvae of *Terranova* type II. The results revealed a significant statistical correlation between fish length and number of the recovered ascaridoid nematodes (rs= 0.244, P < 0.05). There was a positive correlation between the host length and presence of *Anisakis* type I L_3 (rs=0.276, P < 0.001). Significant differences were observed between the occurrence of *Terranova* type II L_3 and *Hysterothylacium* L_3 occurrence (rs= 0.388, P < 0.001) and adult (rs= 0.442, P < 0.001). In addition, a highly significant relationship was observed between the presence of *Hysterothylacium* L_3 and adult (rs= 0.428, P < 0.001). Furthermore, the obtained results were lower than the recommended Egyptian standards (<100 parasites/fish), demonstrating that human health risk through consumption of fish flesh is not possible.

KEYWORDS
Pagrus pagrus, *Terranova*, *Hysterothylacium*, *Anisakis*, Risk.

INTRODUCTION

Fish consumption has concurrently increased with its nutritional and therapeutic significance. Fish is a high protein diet that usually contains all the essential amino acids, high levels of vitamins, minerals and omega-3 fatty acids required for human life. Nevertheless, consumption of fish products has been related to approximately 56 million cases of parasitic infections (WHO, 2012). Parasitic nematodes comprise one of the largest and most diverse groups of parasitic helminths in fresh, brackish, and marine water fishes.

Nematodes of the superfamily Ascaridoidea (Nematoda: Chromadorea) include the following families: Anisakidae, Raphidascarididae, Ascarididae, Heterocheilidae, Quimperiidae and Toxocaridae. Family Anisakidae includes several genera as *Anisakis, Pseudoterranova* and *Contracaecum*. While family Raphidascarididae encompasses nine genera including *Hysterothylacium*. Although *Hysterothylacium* is now classified under the Raphidascarididae family (Fagerholm, 1991; Nadler and Hudspeth, 2000; Li *et al*., 2018), it is still identified as an anisakid nematode rather than raphidascarids in some publications (Luque *et al*., 2007; Lopes *et al*., 2011; Abdel-Ghaffar *et al*., 2015).

Among these nematodes, the anisakid larvae, the causative agents of anisakiasis or anisakidosis that pose a major problem for commercial fish industry, and possible risks to human health, (Hochberg and Hamer, 2010), as well as a potential food-borne allergen (Audicana and Kennedy, 2008; Figueiredo *et al*., 2013; Polimeno *et al*., 2021; Audicana, 2022).

Fish-borne anisakids and raphidascarids are widely distributed throughout the world's oceans and seas in a wide range of marine fish, cephalopods and aquatic invertebrates that act as intermediate, paratenic, or definitive hosts in their life cycle (Kuhn *et al*., 2011). Parasitological surveys of anisakid nematodes, particularly larval stages, in consumed fish and seafood are crucial to ensure food safety. The presence of these parasites in the flesh, body cavity or visceral organs of fish should be regarded as a major threat to public health due to the possibility of post-mortem migration of these larvae to fish flesh (Cipriani *et al*., 2015). According to Egyptian standards No. 889 of 1991, the number of parasites or worms which can be seen with the naked eye in each fish shall not exceed 100, and the percentage of fish containing these parasites shall not exceed 20% of the examined specimens (ES, 1991).

The distribution of marine ascaridoid nematodes is influenced by a wide range of abiotic and biotic factors. The effect of biotic factors such as body size, weight, sex and age of fish on the prevalence of different anisakid genera has been reported in various fish species (Adroher *et al*., 1996; Cruz *et al*., 2005; Valero *et al*., 2006; Barcala *et al*., 2018; Debenedetti *et al*., 2019). Also, the effect of abiotic factors such as specific oceanographic or

ISSN: 2090-6277/2090-6269/

ecological factors at the fishing area has also been demonstrated (Molina-Fernández *et al*., 2015; Gazzonis *et al*., 2017; Barcala *et al*., 2018; Debenedetti *et al*., 2019).

Pagrus pagrus (*P. pagrus*) Linnaeus, 1758 (Perciformes: Sparidae), popularly known as red porgy or common sea bream, is a demersal fish species in which larval stage of several genera of anisakid nematodes (*Anisakis*, *Pseudoterranova*, *Contracaecum*) and larval stages of raphidascarids *Hysterothylacium* as well as adult stage of *H. aduncum* have been recorded (Paraguassú *et al*., 2002; Saad and Luque, 2009; Morsy *et al*., 2013; Soares *et al*., 2014; Soares and Luque, 2015; Soares *et al*., 2018). The third-stage larvae of *Anisakis* spp. have been reported in *P. pagrus* from the state of Rio de Janeiro, Brazil (Mattos *et al*., 2014; Soares and Luque, 2015).

The purpose of this research was to investigate the prevalence, abundance, intensity and distribution of ascaridoid nematodes infecting the common seabream or red porgy fish sold in Sharkia fish markets, as well as to determine the possible health risks associated with fish consumption. Additionally, the effect of biotic factor (fish length) on the prevalence and number of ascaridoid nematode infection was studied.

MATERIALS AND METHODS

Sample collection and processing

A total of 105 samples of *P. pagrus* originally fished from the Suez Gulf, Red Sea, Egypt was collected randomly from Sharkia fish markets between October 2018 and the end of May 2019. The fish samples were measured in centimeters (cm) for their total length, and then dissected. The body cavity and visceral organs were examined and carefully inspected with a stereo microscope for the presence of ascaridoids at the Parasitology Department laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt. The musculature was investigated using artificial tissue digestion technique (Garcia, 2001). The detected ascaridoids were collected, counted and the site of infection was noted in each specimen. Most of the nematodes obtained were examined directly under a light microscope but some ascaridoids were fixed in 70% ethanol for morphological analysis under light microscope. Some larvae were individually cleared in lactophenol and mounted for morphological identification (Kruse and Pritchard, 1982). The taxonomic identification followed Cannon (1977), Deardorff and Overstreet (1981), Smith (1983), Olson *et al*. (1983), Ishii *et al*. (1989), Shih (2004), Morsy *et al*. (2013) and Shamsi *et al*. (2018). Parasitological indices (prevalence, abundance, and mean intensity) were calculated following Bush *et al*. (1997). The protocol was carried out according to guidelines of the Institutional Care and use Committee, and ethical approval was obtained from Zagazig University, Egypt (ZU-IACUC/2/F/4/2023).

Statistical analysis

The statistical analyses were carried out using SPSS version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24. Armonk, NY). Spearman's rho correlation was applied to test type and strength of relationship between the fish length with the prevalence and number of parasites observed. Chi-square test was run for evaluating association between categorical variables. Logistic regression and odds ratio were used to predict the presence of the parasite and the length of the fish. Differences were considered significant when $p < 0.05$.

RESULTS

Ascaridoid nematodes were found in 36.19% (38/105) of the dissected red porgy. A total of 306 ascaridoids collected from the examined fish were divided morphologically into four types: *Anisakis* type I larvae (3.81%) (Plate I Figs. 1-3), *Terranova* type II larvae (22.86%) (Plate I Figs. 4-7), *Hysterothylacium* spp. larvae (23.81%) (Plate II Figs. 1-2) and *Hysterothylacium* spp. adult (9.52%) (Plate II Figs. 3-4 female and Figs. 5-7 male). The mean intensity for the respective parasites was 1.25, 3.5, 7.04 and 4.1 (Table 1). The distribution and number of recovered ascaridoid nematodes in different organs of *P. pagrus* were shown in Fig. (1). The percentage of single infection with one ascaridoid nematode

Table 1 Parasitizing parameters of ascaridoid nematodes in red porgy (*P. pagrus*).

Infection parameters	*Anisakis*^{l} type I	*Terranova*^{l} type II	*Hysterothylacium*^{l}	*Hysterothylacium*^{a}
No. of infected fish	4	24	25	10
Prevalence	3.81%	22.86%	23.81%	9.52%
No. of parasites recovered	5	84	176	41
Mean intensity (Min.-Max.)	1.25 (1-2)	3.5 (1-8)	7.04 (1-32)	4.1 (1-7)
Abundance	0.05	0.8	1.68	0.39

^{l} larval stage, ^{a} adult stage.

Table 2 Single and mixed infection of ascaridoid nematode in *P. pagrus* in relation to host size.

Host size class (cm)	No. examined	No. infected	Prevalence (%)	Single infection No. of infected fish (%)			Mixed infection with two parasite species No. of infected fish (%)				Mixed infection with three parasite species No. of infected fish (%)	
				A.	*T.*	*H.*^{l}	*A.* + *T.*	*T.* + *H.*^{l}	*T.* + *H.*^{a}	*H.*^{l} + *H.*^{a}	*A.* + *T.* + *H.*^{l}	*T.* + *H.*^{l} + *H.*^{a}
13-14.9	16	4	25	-	-	1	-	2	1	-	-	-
15-17.9	47	14	29.79	-	3	-	-	4	1	1	-	5
18-20.9	26	10	38.46	-	2	6	-	-	-	1	-	1
21-23.9	16	10	62.5	2	3	3	1	-	-	-	1	-
Total	105	38	36.19	2	8	10	1	6	2	2	1	6
				20 (19.05%)			11 (10.48%)				7 (6.66%)	

A: *Anisakis* type I larvae; T: *Terranova* type II larvae; H: *Hysterothylacium*; ^{l} larval stage; ^{a} adult stage.

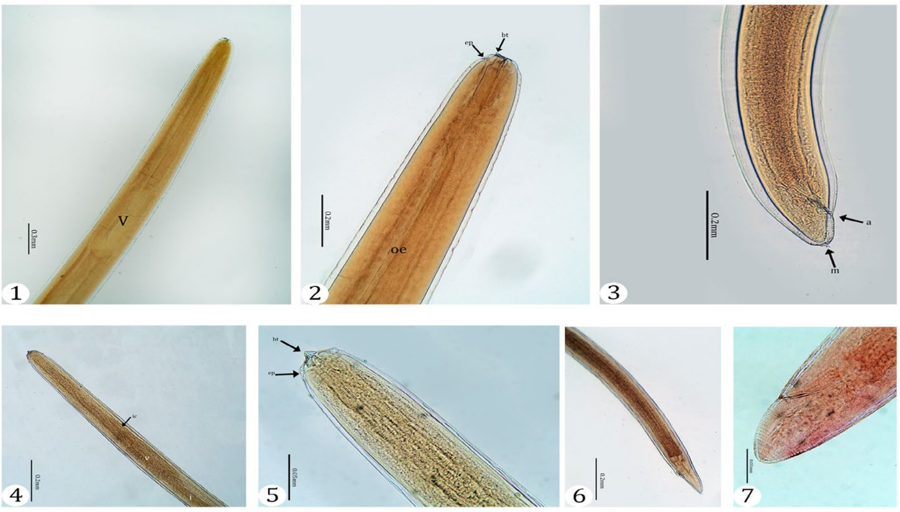

Plate I. Figs. 1-7. Photomicrographs of *Anisakis* type I larva showing 1-2 anterior end possessing boring tooth (bt), esophagus (oe), excretory pore (ep), ventriculus (V). 3 posterior end showing mucron (m) and anus (a). 4-5 photomicrographs of *Terranova* type II larva showing high magnification of anterior end which have boring tooth (bt), excretory pore (ep), intestinal caecum (ic), ventriculus (V) and intestine (i). 6-7 posterior end of *Terranova* type II larva with strongly annulated conical tail.

was 19.05 while, mixed infection with two or three ascaridoids species was reported in 17.14% of the examined samples (Table 2).

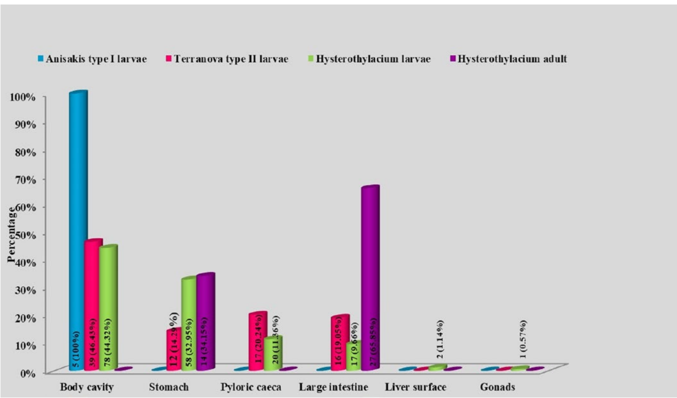

Fig. 1. Distribution of different types of ascaridoid nematodes in organs of red porgy (*P. pagrus*).

The host length was divided into four categories (13-14.9, 15-17.9, 18-20.9, and 21-23.9 cm). The prevalence of overall ascaridoid infection was observed in relation to host size. There is a gradual increase in infection rate with increasing length of fish (Fig. 2 and Table 2). The fish length was significantly correlated with number of the recovered ascaridoid nematodes and this relationship is positive and weak (rs= 0.244, P < 0.05). Furthermore, there was a positive correlation between the host length and presence of the third stage larvae of *Anisakis* type I by using Spearman's rank correlation coefficient (rs=0.276, P < 0.001) and by using logistic regression model analyses [Odds ratio (OR) = 1.73 with confidence interval (1.10-2.73), P < 0.05]. In contrast, the fish length was not correlated with the presence of the third stage larvae of *Terranova* type II (rs =0.039, P > 0.05), *Hysterothylacium* L_3 (rs =0.099, P > 0.05), and *Hysterothylacium* adult (rs =- 0.056, P > 0.05) (Table 3). There was no significant correlation existed between the site of infection and type of ascaridoid nematodes (rs= 0.123, P > 0.05). Additionally, the site of infection was statistically non-significant compared to the number of parasites (rs= 0.145, P > 0. 05).

No significant differences were observed between the occurrence of *Anisakis* type I and *Terranova* type II larvae (χ^2= 1.737, rs= 0.129, P > 0.05) and the occurrence of *Hysterothylacium* L_3 (χ^2 = 0.003, rs= 0.006, P > 0.05). On the contrary, significant differences were detected between the occurrence of *Terranova* type II larvae and *Hysterothylacium* L_3 occurrence (rs= 0.388, P < 0.001) and adult (rs= 0.442, P < 0.001). In addition, a highly significant

Table 3. Spearman's rank correlation coefficient (rs) and logistic regression model used to evaluate possible relationships among the total length of *P. pagrus*, and prevalence of ascaridoid nematodes.

Parameters	r_s	*P*-value	OR	CI	*P*-value
Length and presence of *Anisakis* type I larvae	0.28	< 0.001**	1.73	1.10-2.73	< 0.05*
Length and presence of *Terranova* type II larvae	0.04	> 0.05^{NS}	1.03	0.87-1.21	> 0.05^{NS}
Length and presence of *Hysterothylacium* larvae	0.10	> 0.05^{NS}	1.06	0.89-1.25	> 0.05^{NS}
Length and presence of adult *Hysterothylacium*	-0.06	> 0.05^{NS}	0.86	0.65-1.14	> 0.05^{NS}

OR (odds ratio) with 95% confidence interval (CI), NS non-significant, *Significant difference, **highly significant difference.

Table 4 Co-occurrences of ascaridoid nematodes pairs in *P. pagrus* using Spearman correlation coefficient values (rs) and chi-square values (χ^2).

Anisakid nematodes pairs	r_s	*P*	χ^2	P-value of Chi-square test	P-value of Phi and Cramer's V
Terranova[l] type II and *Anisakis*[l] type I	0.13	> 0.05^{NS}	1.74	> 0.05^{NS}	(0.13) > 0.05^{NS}
Terranova[l] type II and *Hysterothylacium*[l]	0.39	< 0.001**	15.81	< 0.01**	(0.39) < 0.01**
Terranova[l] type II and *Hysterothylacium*[a]	0.44	< 0.001**	20.47	< 0.01**	(0.44) < 0.01**
Anisakis[l] type I and *Hysterothylacium*[l]	0.01	> 0.05^{NS}	0.00	> 0.05^{NS}	(0.01) > 0.05^{NS}
Hysterothylacium[l] and *Hysterothylacium*[a]	0.43	< 0.001**	19.24	< 0.01**	(0.43) < 0.01**

[l] larval stage, [a] adult stage, NS non-significant, **highly significant difference.

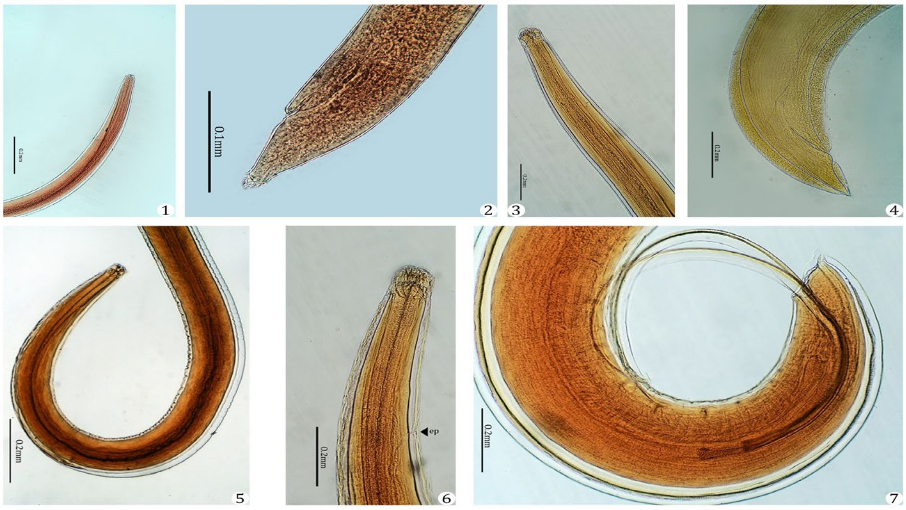

Plate II. Fig. 1-7. Photomicrographs of *Hysterothylacium* spp. showing 1&2 anterior and posterior ends of *Hysterothylacium* spp. larva which ends with cactus tail and anal opening. 3 anterior end of *Hysterothylacium* spp. female being equipped by three lips with interlabium in between and provided with papillae. 4 posterior end of *Hysterothylacium* spp. female with short cactus tail. 5-6 *Hysterothylacium* spp. male anterior end showing excretory pore (ep). 7 posterior end of *Hysterothylacium* spp. male with two spicules and short cactus tail.

relationship was observed between the presence of *Hysterothylacium* L_3 and adult (rs= 0.428, P < 0.001) as shown in Table 4.

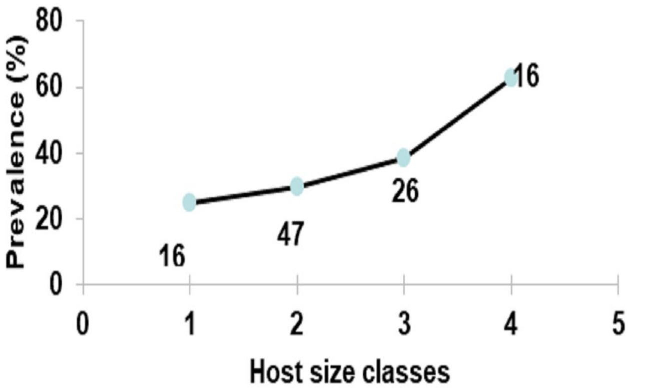

Fig. 2. The prevalence of ascaridoid nematodes in *P. pagrus* in relation to host size. Sample size is indicated for each data point. The host size classes are 1: 13-14.9 cm, 2: 15-17.9 cm, 3: 18-20.9 cm and 4: 21-23.9 cm.

DISCUSSION

A wide variety of nematodes was found in fishes from the Gulf of Suez (Nada and Abd El-Ghany, 2011; Morsy *et al*., 2013; Abd El-Ghany *et al*., 2023). The current study showed that a wide variety of ascaridoid nematodes was found in *P. pagrus*. Only *Hysterothylacium* spp. was previously observed in this host from the same area for the first time (Morsy *et al*., 2013). This diversity could be related to the feeding habits of this host, which feeds on a wide range of benthic and other demersal fish as well as invertebrates (Haimovici *et al*., 1994), and may therefore become subjected to various possible intermediate and paratenic hosts of ascaridoids nematodes. Several studies on infection of red porgy with anisakid and raphidascarid nematodes have been recorded worldwide, including Egypt (Nada and AbdEl-Ghany, 2011; Morsy *et al*., 2013) and Brazil (Paraguassú *et al*., 2002; Saad and Luque, 2009; Soares *et al*., 2014; Soares and Luque, 2015; Soares *et al*., 2018). The present study revealed that 38 fish specimens (36.19 %) were positive for one or more ascaridoid nematodes, which were detected as single or mixed infections. Some studies showed a higher prevalence in this fish; 88% (São Clemente *et al*., 1994), 59% (Paraguassú *et al*., 2002), 59.09% (Nada and Abd El-Ghany, 2011), 53.7% (Soares *et al*., 2014) and 58% (Soares and Luque, 2015). These results probably varied because of geographical factors, the availability of the parasites' potential intermediate and final hosts, environmental conditions and variations in the number and size of fish samples studied.

A total of 306 ascaridoid nematodes were isolated from 38 *P. pagrus*. Based on morphological characters, it was observed that these ascaridoids represented four different morphotypes belonging to three different genera: *Anisakis*, *Terranova* and *Hysterothylacium*. Only five individuals of these recovered anisakids were morphologically classified as *Anisakis* type I larvae and 84 individuals were identified as *Terranova* type II larvae. In addition, two different morphotypes of *Hysterothylacium* spp. (Raphidascarididae) were identified, comprising 176 individuals of *Hysterothylacium* larval type as well as 41 individuals of the adult stage. The most prevalent ascaridoid nematode was *Hysterothylacium* spp. larvae with a prevalence of 23.81% and a mean intensity of 7.04 (intensity 1-32). The predominance of *Hysterothylacium* spp. larvae in the same fish host was agreed with Soares *et al*., 2014; Soares and Luque, 2015; Soares *et al*., 2018 who reported a high prevalence of 90, 92 and 89.19%, respectively from the state of Rio de Janeiro, Brazil. Likewise, Morsy *et al*. (2013) recorded a higher infection (44.2%) in the intestine of *P. pagrus* at coasts of the Gulf of Suez and Hurgada City at Red Sea, Egypt. On the other hand, lower prevalence (1.74%) was found in different species of Sparid fishes (Genc, 2002). This anisakid genus was also reported from Sparid fish, *Pagellus acarne* (Petter and Cabaret, 1995). Paraguassú *et al*. (2002) isolated *Contracaecum* spp. with a percentage of 93.3. Nevertheless, Soares *et al*. (2014) reported that the authors incorrectly identified the larvae of *Hysterothylacium* as *Contracaecum* larvae based on the location of excretory pore. Additionally, *Hysterothylacium* spp. adult was detected in stomach and large intestine of ten fishes (9.52%), while *H. aduncum* was recovered from intestine only of the same fish species (Morsy *et al*., 2013).

Concerning the public health significance, all the detected anisakid nematodes (larvae or adults) identified during this survey were detected in the body cavity or visceral organs that are not consumed by humans, and no larvae were observed in the musculature. This finding was in accordance with Cruz *et al*., 2005, and may be attributed to the feeding habits of the host. The present data revealed a marked prevalence of ascaridoid nematodes in body cavity (39.87%) of infected fish. Smith, 1984 claimed that the distribution of anisakid larvae is primarily controlled by the conditions encountered within host tissues and may be linked to the availability of nutrients. Although no larvae have been found in the musculature of the examined fish, their significance as a possible source of human infection cannot be ignored (Smith and Wootten, 1975), as the flesh can be readily contaminated when the fish samples are eviscerated and handled. According to Cipriani *et al*. (2015) *Anisakis* larvae have the potential to migrate from the body cavity and/or visceral tissues to the flesh after the fish dies. The distribution pattern of these ascaridoid larvae (*Anisakis* type I and *Terranova* type II and *Hysterothylacium* spp.) in the body cavity or visceral organs indicating that this fish species could act as intermediate or paratenic host for these nematodes (Smith, 1983). In addition, *Hysterothylacium* spp. adult was found in the stomach and intestine, suggesting that *P. pagrus* may serve as their definitive host (Køie, 1993; Shih and Jeng, 2002).

In the present study, single infection was higher than mixed infection in the fish under investigation. Mixed parasitic infections revealed 6 different associations of the identified ascaridoid nematodes (Table 3). These associations varied from double to triple parasitic mixed infections. This result was disagreed with Kassem and Bowashi, 2015 who observed that mixed infection was higher than single infection in all fishes.

With regard to *Anisakis* type I larvae infection, the rate of infection of *Anisakis* type I larvae in common sea bream or red porgy was 3.81% with intensity of (1-2) and all larvae were found in body cavity. Meanwhile, Paraguassú *et al*. (2002); Mattos *et al*. (2014); Soares *et al*. (2014); Soares and Luque (2015) and Soares *et al*. (2018) had a higher prevalence of 7.7, 22.22, 40, 23 and 16.22%, respectively. Moreover, a total of 84 *Pseudoterranova* larvae were found to be parasitizing *P. pagrus*. All of them were identified as *Terranova* type II larvae (prevalence: 22.86%; abundance: 0.80; intensity: 1-8). Lower prevalence (6.6%) was detected by Paraguassú *et al*. (2002). On the other hand, Soares *et al*. (2014); Soares and Luque (2015) and Soares *et al*. (2018) did not detect this parasite. Interestingly, *Terranova* larval type II has not been previously reported in Red Sea fish; thus, this study is a new geographical record; nevertheless, previous publications have described specimens simply as *Terranova* sp. or *Pseudoterranova* sp. larvae with no further clarification as to type (Nada and AbdEl-Ghany, 2011; Al-Zubaidy *et al*., 2012). This species has been reported by Shamsi *et al*. (2015, 2018) in several fish species in New Caledonia.

The fish length was an influential factor for parasitization by ascaridoid nematodes in the fish species examined, indicating that larger specimens may pose a higher risk of infection. A significant positive correlation between the host length and the number of ascaridoid nematodes was observed in our study (rs= 0.244, $P < 0.05$) which can be due to the cumulative effect of frequent parasite infections, acquired over a longer lifetime for larger (older) fish and constant dietary reinfection. Likewise, Karpiej *et al*. (2013) found that the number of parasites increased with the fish length (rs= 0.300; $P < 0.005$). A similar positive correlation was observed also in *P. pagrus* from Rio de Janeiro, Brazil by Paraguassú *et al*. (2002) and Soares *et al*. (2014). In addition, Pulleiro-Potel *et al*. (2015), Barcala *et al*. (2018) and Gaglio *et al*. (2018) reported an affirmative association between standard length and prevalence in the examined fish samples ($p < 0.05$).

The presence of a significant association between *Anisakis* type I L_3 prevalence and length (rs= 0.276, $p < 0.001$) was in accordance with Young's (1972) hypothesis; where, the distributions of *Anisakis simplex* L_3 in fish are expected to be primarily influenced by fish size. Similarly, a recent study considered fish size as a significant predictor of *Anisakis* spp. occurrence in most fish species except for mackerel (Levsen *et al*., 2018). The size of the host is directly related to the host age and therefore, the high level of parasitization in older specimens may be attributed to the cumulative parasitization over time through the diet and the higher probability of infection, leading to higher rates of abundance and prevalence (Strømnes and Andersen, 2000). Logistic regression model analysis, carried out to examine the effect of host length upon infection with different ascaridoid nematodes of red porgy, showed that the risk of infection by *Anisakis* type I larvae increases 1.73 times for every additional cm in the length of the red porgy, as previously reported by Molina-Fernández *et al*. (2015) in sardines (Sardina pilchardus) from Iberian waters, southwestern Europe. On the other hand, the fish length had no significant effect on occurrence of *Pseudoterranova* L_3 (rs =0.039, $P > 0.05$), *Hysterothylacium* L_3 (rs =0.099, $P > 0.05$), and *Hysterothylacium* adult (rs =- 0.056, $P > 0.05$) in *P. pagrus*, which is perhaps unexpected. Although our data on body length of *P. pagrus* did not show a significant association to larvae of *Hysterothylacium* spp. infection [Odds ratio (OR) = 1.06 with confidence interval (0.89-1.25), $P > 0.05$], the body length of the same host from Rio de Janeiro, Brazil demonstrated to be a risk factor associated to the *Hysterothylacium* infection (Soares *et al*., 2014).

CONCLUSION

The results of this study suggest that red porgy, *P. pagrus* caught in Suez Canal are susceptible to parasitization by many ascaridoid nematodes and can act as intermediate or paratenic host as well as definitive host for these nematodes. Positive correlations between numbers of anisakid, *Anisakis* type I larvae prevalence and fish length were observed in our study. These results provide important basic knowledge about the occurrence and infection parameters of ascaridoid nematodes in this marine fish. In conclusion, a higher prevalence of the parasite in the viscera and body cavity was found rather than in the flesh, indicating that consumption of fish flesh does not pose a risk to human health as well as the quick evisceration after catching is suggested as an effective preventive measure.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdel-Ghaffar, F., Abdel-Gaber, R., Bashtar, A.R., Morsy, K., Mehlhorn, H., Al Quraishy, S., Saleh, R., 2015. *Hysterothylacium aduncum* (Nematoda, Anisakidae) with a new host record from the common sole *Solea solea* (Soleidae) and its role as a biological indicator of pollution. Parasitol. Res. 114, 513-22. doi: 10.1007/s00436-014-4213-1.

Abd El-Ghany, A.M., Nada, M.S.M., Nadler, S.A., 2023. Morphological and molecular characterization of larval *Echinocephalus* sp. (Spirurida: Gnathostomatidae), a parasite of the greater lizard fish (*Saurida undosquamis*) and red porgy or common seabream (*Pagrus pagrus*). Parasitol. Res. 122, 2405–2411. Doi: 10.1007/s00436-023-07942-z.

Adroher, F.J., Valero, A., Ruiz-Valero, J., Iglesias, L., 1996. Larval anisakids (Nematoda: Ascaridoidea) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada (Spain). Parasitol. Res. 82, 253-256.

Al-Zubaidy, A.B., Mhaisen, F.T., Abker, M.A.M., 2012. Occurrence of five nematode species from some Red Sea fishes, Yemen. Mesopotamian Journal of Marine Science 27, 140-156.

Audicana, M.T., 2022. *Anisakis*, Something Is Moving inside the Fish. Pathogens 11, 326. https://doi.org/10.3390/pathogens11030326.

Audicana, M.T., Kennedy, M.W., 2008. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. Clin. Microbiol. Rev. 21, 360-379. doi: 10.1128/cmr.00012-07.

Barcala, E., Ramilo, A., Ortega, N., Picó, G., Abollo, E., Pascual, S., Muñoz, P., 2018. Occurrence of *Anisakis* and *Hysterothylacium* larvae in commercial fish from Balearic sea (Western Mediterranean Sea). Parasitol. Res. 117, 4003-4012. doi: 10.1007/s00436-018-6110-5.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. J. Parasitol. 83, 575-583.

Cannon, L.R.G., 1977. Some larval ascaridoids from south-eastern Queensland marine fishes. Int. J. Parasitol. 7, 233-243.

Cipriani, P., Acerra, V., Bellisario, B., Sbaraglia, G.L., Cheleschi, R., Nascetti, G., Mattiucci, S., 2015. Larval migration of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in European anchovy, Engraulis encrasicolus: implications to seafood safety. Food Control 59, 148-157. doi: 10.1016/j.foodcont.2015.04.043.

Cruz, C., Vaz, A., Saraiva, A., 2005. Larval anisakids from horse mackerel in Portugal. Helminthologia 42, 3-7.

Debenedetti, Á.L., Madrid, E., Trelis, M., Codes, F.J., Gil-Gómez, F., Sáez-Durán, S., Fuentes, M.V., 2019. Prevalence and risk of anisakid larvae in fresh fish frequently consumed in Spain: an overview. Fishes 4, 13. doi:10.3390/fishes4010013.

Deardorff, T.L., Overstreet, R.M., 1981. Review of *Hysterothylacium*, and *Iheringascaris* (both previously: Thynnascaris) (Nematoda: Anisakidae) from the northern Gulf of Mexico. Proc. Biolog. Soc. Wash. 93, 1035-1079.

ES (Egyptian Standards), 1991. The Egyptian Standards for frozen fish, No. 889/1991 (standard of parasitic content for frozen fish).

Fagerholm, H.P., 1991. Systematic implications of male caudal morphology in ascaridoid nematode parasites. Syst. Parasitol. 19, 215-228.

Figueiredo, I., Vericimo, M.A., Cardoso, L.R., São Clemente, S.C., do Nascimento, E.R., Teixeira, G.A., 2013. Cross-sectional study of serum reactivity to *Anisakis simplex* in healthy adults in Niterói, Brazil. Acta Parasitol. 58, 399-404. https://doi.org/f5cz.

Gaglio, G., Battaglia, P., Costa, A., Cavallaro, M., Cammilleri, G., Graci, S., Marino, F., 2018. *Anisakis* spp. larvae in three mesopelagic and bathypelagic fish species of the central Mediterranean Sea. Parasitol. Int. 67, 23-28. doi: 10.1016/j.parint.2017.09.010.

Garcia, L.S., 2001. Diagnostic Medical Parasitology 4th (ED) ASM Press, Washington, DC., U.S.A.

Gazzonis, A.L., Cavallero, S., Zanzani, S.A., Olivieri, E., Malandra, R., Ranghieri, V., D'Amelio, S., Manfredi, M.S., 2017. *Anisakis* sp. and *Hysterothylacium* sp. larvae in anchovies (*Engraulis encrasicolus*) and chub mackerel (*Scomber colias*) in the Mediterranean Sea: Molecular identification and risk factors. Food Control 80, 366-373. https://doi.org/10.1016/j.foodcont.2017.05.004.

Genc, E.R., 2002. The endoparasites and histopathologies found in some commercial teleosts in the Bay of Iskenderun. PhD Thesis, Department of Fisheries, Institute of Natural and Applied Sciences, Adana, Turkey. Dissertation, University of Cukurova.

Haimovicil, M., Martins, A.S., Figueiredo, J.L., Vieira, P.C., 1994. Demersal bony fish of the outer shelf and upper slope of the southern Brazil Subtropical Convergence Ecosystem. Mar. Ecol. Prog. Ser. 108, 59-77.

Hochberg, N.S., Hamer, D.H., 2010. Anisakidosis: perils of the deep. Clin. Infect. Dis. 51, 806-812. doi: 10.1086/656238.

Ishii, Y., Fujino, T., Weerasooriya, M.V., 1989. Morphology of Anisakine larvae. In: Ishikura H., Namiki M. (eds) Gastric anisakiasis in Japan. Springer, Tokyo. Doi: https://doi.org/10.1007/978-4-431-68290-5_4.

Karpiej, K., Dzido, J., Rokicki, J., Kijewska, A., 2013. Anisakid nematodes of greenland halibut *Reinhardtius hippoglossoides* from the Barents Sea. J. Parasitol. 99, 650-654. doi: 10.1645/GE-2987.1.

Kassem, H.H., Bowashi, S.M., 2015. Prevalence of anisakid nematode larvae infecting some marine fishes from the Libyan coast. J. Egypt. Soc. Parasitol. 240, 1-8.

Køie, M., 1993. Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Can. J. Zool. 71, 1289-1296.

Kruse, G.O.W., Pritchard, M.H., 1982. The collection and preservation of animal parasites. Univ. Nebraska, Lincoln and London, pp. 141.

Kuhn, T., García-Màrquez, J., Klimpel, S., 2011. Adaptive radiation within marine anisakid nematodes: A zoogeographical modeling of cosmopolitan, zoonotic parasites. PLoS ONE 6, e28642. https://doi.org/10.1371/journal.pone.0028642

Levsen, A., Svanevika, C.S., Cipriani, P., Mattiucci, S., Gayd, M., Hastiee, L.C., Bušelićg, I., Mladineo, I., Karlh, H., Ostermeyerh, U., Buchmann, K., Højgaardk, D.P., González, A.F., Pascual, S., Pierce, G.J., 2018. Survey of zoonotic nematodes of commercial key fish species from major European fishing grounds-introducing the FP7 PARASITE exposure assessment study. Fish Res. 202, 4-21. https://doi.org/10.1016/j.fishres.2017.09.009.

Li, L., Lü, L., Nadler, S.A., Gibson, D.I., Zhang, L.P., Chen, H.X., Zhao, W.T., Guo, Y.N., 2018. Molecular phylogeny and dating reveal a terrestrial origin in the early carboniferous for ascaridoid nematodes. Syst. Biol. 67, 888-900. doi: 10.1093/sysbio/syy018.

Lopes, L.P.C., Pimpão, D.M., Takemoto, R.M., Malta, J.C.O., Angela, M.B., Varella, A.M.B., 2011. *Hysterothylacium* larvae (Nematoda, Anisakidae) in the freshwater mussel *Diplodon suavidicus* (Lea, 1856) (Mollusca, Unioniformes, Hyriidae) in Aripuanã River, Amazon, Brazil. J. Invertebr Pathol., 106, 357-359. https://doi.org/10.1016/j.jip.2010.12.002.

Luque, J.L., Bannock, L.M., Lagrue, C., Poulin, R., 2007. Larval *Hysterothylacium* sp. (Nematoda, Anisakidae) and trematode metacercariae from the amphipod *Paracorophium excavatum* (Corphiidae) in New Zealand. Acta Parasitol. 52, 146-150. 10.2478/s11686-007-0022-3.

Mattos, D.P.B.G., Lopes, L.M.S., Verícimo, M.A., Alvares, T.S., São Clemente, S.C., 2014. Anisakidae infection in five commercially important fish species from the State of Rio de Janeiro, Brazil. Rev. Bras. Med. Vet. 36, 375-379. http://rbmv.org/index.php/BJVM/article/view/553.

Molina-Fernández, D., Malagón, D., Gómez-Mateos, M., Benítez, R., Martín-Sánchez, J., Adroher, F.J., 2015. Fishing area and fish size as risk factors of *Anisakis* infection in sardines (*Sardina pilchardus*) from Iberian waters, southwestern Europe. Int. J. Food Microbiol. 203, 27-34. doi: 10.1016/j.ijfoodmicro.2015.02.024.

Morsy, K., Bashtar, A., Abdel-Ghaffar, F., Mostafa, N., 2013. New host and locality records of two nematode parasites Dujardinnascaris mujibii (Heterocheilidae) and *Hysterothylacium aduncum* (Anisakidae) from the common seabream *Pagrus pagrus*: a light and scanning electron microscopic study. Parasitol. Res. 112, 807-815. doi: 10.1007/s00436-012-3270-6.

Nada, M.S.M., Abd El-Ghany, A.M., 2011. Anisakid nematodes in marine fishes. Journal of American Science 7, 1000-1005.

Nadler, S.A., Hudspeth, D.S.S., 2000. Phylogeny of the Ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: hypotheses of structural and sequence evolution. J. Parasitol. 86, 380-393.

Olson, A.C., Lewis, M.D., Hauser, M.L., 1983. Proper identification of Anisakine worms. Am. J. Med. Technol. 49, 111-114.

Paraguassú, A., Luque, J., Alves, D.R., 2002. Community ecology of the metazoan parasites of red porgy, *Pagrus pagrus* (L., 1758) (Osteichthyes, Sparidae), from the coastal zone, state of Rio de Janeiro, Brazil. Maringá 24, 461-467.

Petter, A.J., Cabaret, J., 1995. Ascaridoid nematodes of teleostean fishes from the eastern North Atlantic and seas of the North of Europe. Parasite, 2, 217-230.

Polimeno, L., Lisanti, M.T., Rossini, M., Giacovazzo, E., Polimeno, L., Debellis, L., Ballini, A., Topi, S., Santacroce, L., 2021. *Anisakis* Allergy: Is Aquacultured Fish a Safe and Alternative Food to Wild-Capture Fisheries for *Anisakis simplex*-Sensitized Patients?. Biology 10, 106. https://doi.org/10.3390/biology10020106

Pulleiro-Potel, L., Barcala, E., Mayo-Hernández, E., Muñoz, P., 2015. Survey of anisakids in commercial teleosts from the western Mediterranean Sea: infection rates and possible effects of environmental and ecological factors. Food Control 55, 12-17. https://doi.org/10.1016/j.foodcont.2015.02.020.

Saad, C.D., Luque, J.L., 2009. Larval Anisakidae in musculature of *Pagrus pagrus* from the State of Rio de Janeiro, Brazil. Rev. Bras. Parasitol. Vet. 18, 71-73..

São Clemente, S.C., Uchoa, C.M.A., Freire, N.M.S., 1994. Larvas de anisakideos em *Pagrus pagrus* (L.) e seu controle através de baixas temperaturas. Revista Brasileira de Ciência Veterinária 1, 21-24.

Shamsi, S., Poupa, A., Justine, J.L., 2015. Characterisation of ascaridoid larvae from marine fish off New Caledonia, with description of new *Hysterothylacium* larval types XIII and XIV. Parasitol. Int. 64, 397-404. https://doi.org/10.1016/j.parint.2015.05.014.

Shamsi, S., Chen, Y., poupa, A., Ghadam, M., Justine, J., 2018. Occurrence of anisakid parasites in marine fishes and whales off New Caledonia. Parasitol. Res. 117, 3195-3204. doi: 10.1007/s00436-018-6018-0.

Shih, H., 2004. Parasitic helminth fauna of the cutlass fish, Trichiurus lepturus L., and the differentiation of four anisakid nematode third-stage larvae by nuclear ribosomal DNA sequences. Parasitol. Res. 93, 188-195. doi: 10.1007/s00436-004-1095-7.

Shih, H.H., Jeng, M.S., 2002. *Hysterothylacium aduncum* (Nematoda: Anisakidae) infecting a herbivorous fish, Siganus fuscescens, off the Taiwanese coast of the northwest Pacific. Zool. Stud., 41, 185-189.

Smith, J.W., 1983. *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) (Nematoda: Ascaridoidea): morphology and morphometry of larvae from euphausiids and fish, and a review of the life history and ecology. J. Helminthol. 57, 205-224.

Smith, J.W., 1984. The abundance of *Anisakis simplex* L_3 in the body cavity and flesh of marine teleosts. Int. J. Parasit. 14, 491-495.

Smith, J.W., Wootten, R., 1975. Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda:ascaridida) into the flesh of herring, Clupea harengus L. Int. J. Parasitol. 5, 133-136.

Soares, I.A., Luque, J.L., 2015. Seasonal variability of the composition and structure of parasite communities of red porgy, *Pagrus pagrus* (Perciformes: Sparidae) off Brazil. Helminthologia 52, 236-243. doi: 10.1515/helmin-2015-0038

Soares, I.A., Vieira, F.M., Luque, J.L., 2014. Parasite community of *Pagrus pagrus* (Sparidae) from Rio de Janeiro, Brazil: evidence of temporal stability. Braz. J. Vet. Parasitol. Jaboticabal. 23, 216-223. doi: http://dx.doi.org/10.1590/S1984-29612014047.

Soares, I.A., Lanfranchi, A.L., Luque, J.L., Haimovici, M., Timi, J.T., 2018. Are different parasite guilds of *Pagrus pagrus* equally suitable sources of information on host zoogeography?. Parasitol. Res. 117, 1865-1875. https://doi.org/gdkjjx. 57.

Strømnes, E., Andersen, K., 2000. "Spring rise" of whaleworm (*Anisakis simplex*; Nematoda, Ascaridoidea) third stage larvae in some fish species from Norwegian waters. Parasitol. Res. 88, 619-624. doi:10.1007/pl00008541.

Valero, A., Paniagua, M.I., Hierro, I., Díaz, V., Valderrama, M.J., Benítez, R., Adroher, F.J., 2006. Anisakid parasites of two forkbeards (Phycis blennoides and Phycis phycis) from the Mediterranean coasts of Andalucía (Southern Spain). Parasitol. Int. 55, 1-5.

WHO, 2012. Soil-transmitted Helminths. World Health Organization. Accessed on 31-03-2019 from. http://www.who.int/intestinal_ worms/en/.

Young, P.C., 1972. The relationship between the presence of larval anisakine nematodes in cod and marine mammals in British home waters. J. Appl. Ecol. 9, 459-485.