Original article

Investigation of *Philometra* spp. Nematodes Infecting the Gonads of *Pagrus pagrus* Fish in Sirte, Libya

Ahmed Almashai

Department of Zoology, Faculty of Science, Sirte University, Sirte, Libya Corresponding email. <u>Ahmedalifeeda@gmail.com</u>

Abstract

The Libyan city of Sirte is distinguished by an important geographical location overlooking the coast of the Mediterranean Sea. This coast is full of many fish species, which negatively affect the quality of the sea and are considered an important indicator of pollution in the region. Among the important fish species in this region and in great demand among the population is the Pagrus pagrus fish. Parasite worms infect many species of fish and cause them many damages that negatively affect the growth of these fish and consequently their weight and the amount of meat in them, as well as affecting their reproduction, especially when these parasitic worms infect the gonads of these fish. This study was aimed to verify and describe the presence of *Philometra sp* in *Pagrus pagrus* fish in the marine waters of the city of Sirte and isolate these worms. This study was conducted from May 2022 to October 2023. About 110 samples of Pagrus pagrus fish were collected from the seashore of the city of Sirte, Libya. These samples were transferred to the Zoology Laboratory, Science Faculty, Sirte University. Various measurements were taken for each sample. After that, each sample was dissected using special dissection tools. After the dissection was completed and the sample was opened, the internal viscera as well as the gonads of each sample were taken out, and the gonads were placed in a Petri dish containing a saline solution with a concentration of 5%. The gonads were dissected and examined under appropriate lighting, and nematode parasite worms (Philometra sp) were extracted. The results indicated that 27 fish out of a total of 110 examined were infected, with female fish being more infected than males, with an infection rate of 22 out of a total of 40, while 5 infected males out of a total of 70 fish were examined.

Key words: Nematoda, Pagrus pagrus, Philometra sp, Waters of Sirte.

Introduction

The negative effects of pollution on environmental parameters, which can directly affect the fish, and the emergence and overextension of fish parasites can cause a supplementary synergic direct negative effect transposition in mas fish deaths due to the deterioration of host immunity and to the wounds induced in fish where bacterial, viral, and fungal pathogens can be effective [1]. Fish have substantial social and economic importance as they act as a vital source of food for people. They are considered a single source of high-quality protein, providing 16% of the animal protein consumed by the world's population. Fishes are also zoonotic important, since several diseases are transmitted to humans by fish helminth parasites, including Anisakiasis. In addition, several species of fish parasites have been identified as harmful [2]. Fish parasites are important biological indicators indicating the separation and location of fish for example, in the case of fish migration the seasons of the year from one place to another, every place visited by fish acquires a type of parasite that indicates the environment and thus parasites have become evidence of annual migrations and knowledge of habits, behavior, reproduction and life cycles for fish [3]. Although they are the oldest and most diverse members of the subphylum, the fishes have relatively few nematode parasites in comparison with other vertebrate classes, it is hypothesized that this paucity of parasite species has occurred because nematode parasites first evolved in terrestrial hosts and only a few lines of these parasites were able to transfer to fish after the appearance of heteroxeny (use of intermediate hosts) and paratenesis (use of transport hosts [4].

Parasitic nematodes (Nematoda) represent an important group of fish parasites; many species are highly pathogenic, often infecting fish hosts. The significance of recognizing these parasites increases with the development of aquaculture in many countries and with transcontinental transfers of fish. A prerequisite for developing effective control measures in fish culture is the exact identification of these parasites, as well as a knowledge of their frequently complicated host-parasite environment relationship [5].

Nematodes, or roundworms, infect many different species of aquaculture and wild fish, small numbers of nematodes often occur in healthy fish, but high numbers cause illness or even death, in aquaculture systems, brood stock infected with a small number of nematodes may not even show signs of illness, but they often have reduced reproductive capacity, on the other hand, juvenile fish infected by small numbers of nematodes are more likely to show signs of illness and also have reduced growth rates [6].

Members of the *Philometridae* represent the most important group of dracunculoid nematodes parasitizing fishes. In his monograph treating the Dracunculoidea Moravec (2006) reported a total of 11 genera and 105 species of philometrids parasitizing freshwater, brackish-water, and marine fishes, However, during the last six years (2007-2012), an additional 42 new species of *philometridae* have been described, representing a 40% increase of the number of nominal species [7]. This study aims to identify the extent of infection of *Pagrus pagrus* fish gonads with the parasite *Philometra spp* and to isolate and describe this parasite.

Methods

A total of 110 samples of coral fish were collected from the period of May 2022 to October 2023 from the shore of the city of Sirte, Libya, which is located on the coast of the Mediterranean Sea and is 400 Km west of the capital, Tripoli, to the west. The samples were transferred to the Laboratory of the Department of Zoology, Faculty of Science, University of Sirte, and various measurements were taken of the samples. After that, the sample was externally examined, and then the dissection process began in the known scientific method and using special dissection tools.

Dissection begins with the excretory opening of the fish using dissecting scissors, then moving the scissors forward, then moving them up against the gills, after which the fish is opened. After that, the internal cavity of the fish was examined, and the sex of the fish was identified through the gonads (testis and ovaries). The gonads were extracted and placed in a Petri dish containing a saline solution with a concentration of 5%. The fish gonads were examined with the naked eye, then dissected, and the nematodes of the genus *Philometra sp* were extracted using a fine brush. These extracted worms are placed on a slide, then a drop of lactophenol is added to them, and they are examined well under a microscope to describe their external and internal parts, and different pictures of all their areas were taken at different magnification powers. The fish gonads were examined with the naked eye, then dissected, and the nematodes of the genus *Philometra sp* were extracted using a fine brush. These extracted under a microscope to describe their external and internal parts, and different pictures of all their areas were taken at different magnification powers. The fish gonads were examined with the naked eye, then dissected, and the nematodes of the genus *Philometra sp* were extracted using a fine brush. These extracted worms are placed on a slide, then a drop of lactophenol is added to the naked eye, then dissected, and the nematodes of the genus *Philometra sp* were extracted using a fine brush. These extracted worms are placed on a slide, then a drop of lactophenol is added to them, and they are examined well under a microscope at different magnification.

Various examinations of these worms were conducted under a microscope to describe their external and internal parts, and different pictures of all their areas were taken at different magnification powers.

Results

In this study,110 samples of *Pagrus pagrus* fish were collected. After dissection and careful examination of them, especially the gonads, it was found that 27 of the 110 fish were infected (Figure 1). The number of infected males was 5 out of 70 fish examined (Figures 2 and 3), while the number of infected females was 22 out of 40 fish examined (Figures 2 and 4).

Philometra sp, Long, thread-shaped worms, brown-colored, and the cuticle is smooth, transverse striations are evident all over the body. The mouth is small and surrounded by indistinct cephalic papillae, and the mouth opens into along esophagus divided into two parts, an anterior muscular, bulbous part, followed by a glandular part, which opens into a long brown intestine that ends with the anus at the end of the body. The nerve ring is located at the anterior end of the body (Figures 5 and 6). Males are shorter than females, and females have a long uterus located in the posterior region of the body, which is where the eggs and larvae of the worm are located (Figure 7). The movement of these nematodes was observed under the microscope after they were extracted alive by moving the anterior and posterior parts in both directions.



Figure 1. The overall infection rate.



Figure 2. A comparison between the rate of infection of males (Philometra sp) and females.



Figure 3. The number of infected males (Pagrus pagrus) from healthy ones.



Figure 4. The number of infected females (Pagrus pagrus) from healthy ones.



Figure 5. Anterior region of philometra sp. a: Mouth, b: Cephalic papillae, c: esophagus.



Figure 6. Anterior region of philometra sp. a: Nerve ring, b: Muscular esophagus, c: Glandular esophagus, d: Intestine.



Figure 7. Posterior region of female Philometra sp. a: Anus, b: Uterus.

Discussion

In this study, it was discovered that (27) samples of *Pagrus pagrus* fish were infected with the *Philometra sp* parasite out of (110) samples collected from the beach of the city of Sirte, Libya. The number of infected males of these fish was (5), while the number of infected females was (22) infected fish.

Nematoda belonging to the family *Philometridae* are frequently found in various organs, tissues, and body cavities of a wide variety of fishes [8]. Worm carcasses were consistently found in both male and female gonads throughout the year, with an overall average prevalence of 86.4%. In some cases, these carcasses filled the entire gonad, including ovarian cavity and sperm ducts, extending to the area near the cloaca, potentially impeding ovulation and sperm release [9]. *Philometra cephalus* is highly host-specific and infects both the testis and the ovary of *Valamugil cunnesius* fish. The prevalence of infection was 41.7% which was much higher in females (48.3%) than in males (28.2%) [10]. A new nematode species, *Philometra terapontis. sp. (Philometridae*), is described from male and female specimens found in the ovary of the jarbua terapon *Terapon jarbua* from the Bay of Bengal off the eastern coast of India [11]. The parasite *Philometra lateolabracis* was extracted from *Pagrus auratus* fish [12].

The *Philometra lateolabracis* was extracted from the gonads of *Glaucosoma hebvaicum* fish, and mature female fish were more infected than mature males [13]. Examination of 203 adult blue fish (*Pomatomus saltatrix*) from Long Island, New York, in 2002 and 2003 and 66 from the Outer Banks, North Carolina, in 2003 revealed the presence of dracunculoid nematodes (*Philometra saltatrix*) in the ovaries of female fish [14]. A new species of *Philometra* has been discovered and named *P. philippinensis sp. nov*, and was extracted from the gonads of big eye *Barracuda sphyraena* fish caught in the West Philippine Sea [15].

A recent examination of *Philometra* nematodes from mangrove red snapper *Lutjanus argentimaculatus* from the Karachi coast, Kemari was done in the years 2021-2022, the present study reported three new species of genus *Philometra*, *P.kolachii*, *P.lutjani* and *P. kemarii*, total of 140 fish samples were examined, and 76 were infected [16]. Examinations of some marine fish in Japan revealed that, in addition to being infected with some parasites, eight species of *Philometra* worms were found in the ovaries of the fish, and the infection was only in female fish [17].

Philometroides seriolae is a large nematode parasite in the skeletal musculature of Japanese amberjack *Seriola quinqueradiata. Philometra* nematodes generally use copepods as the intermediate host. However, the life cycle of *P. seriolae* remains unknown, including its intermediate host [18]. The *Philometra* was extracted from the ovaries of *Pennahia anea, Panna micrdon,* and *Johnius argentatus*. The infection rate was 3% [19]. The male and sub-gravid female of *Philometra serranellicabrillae* collected from the gonads of *Serranus cabrilla* off Tunisia are described for the first time based on light and scanning electron microscope studies [20].

Based on light and electron microscopical studies of males and mature females, two new gonad infecting species of *Philometra* Costa, 1845 (Nematoda: *Philmetridae*) are described from the ovary of groupers, *Epinephelus spp.* (Perciformes; *Serranidae*), in the Mediterranean Sea off Tunisia [21]. Based on light and electron microscopical studies, the following four species of *Philometra Costa*, 1845 are described from marine fishes from off Basrah, southern Iraq [22]. Based on light and scanning electron microscopical studies, three new gonad-infecting species of *Philometra Costa*, 1845 are described from marine fishes from off Basrah, southern Iraq [22]. Based on light and scanning electron microscopical studies, three new gonad-infecting species of *Philometra Costa*, 1845 are described from marine fishes of the genus *Lutjanus* Bloch in the northern Gulf of Mexico: *P. longispicula* sp. n. from the ovary of the northern red snapper *L. campechanus* and silk snapper *L.vivanus*; *P. latispicula sp. n.* from the ovary and rarely testes of the grey snapper *L. griseus*; and *P. synagridis sp. n.* (only males available) from the ovary of the lane snapper *Lutjanus synagris* [23]. The four species (only females available) of the *Philometridae* (Nematoda: *Dracunculoidea*) were recorded from freshwater fishes of Lake Turkana, northwestern Kenya in 2007-2008 [24].

The nematode *Philometra jordanoi* was described by Lopez-Neyra (1951) from the females found in the ovary of the dusky grouper *Epinephelus gigas* [25]. Three gonad-infecting species of *Philometra Costa*, 1845 were, for the first time, recorded from perciform fishes from estuarine and marine waters in South Carolina and Georgia, USA: *Philometra charlestonensis sp. nov.* from the scamp *Myccteroperca phenax* (Jordan et Swain) (Serranidae), *P. saltatrix* Ramachandran, 1973 from the bluefish *Pomatomus saltatrix* (Linnaeus), and *Philometra sp.* from the Atlantic croaker Micropogonias undulates (Linnaeus) (*Sciaenidae*) [26]. Examinations of marine fishes off New Caledonia, South Pacific, carried out in 2003-2006, yielded some nematodes of the genus *Philometra Costa*, 1845, including the following three new species: *P. cyanopodi sp.nov*.(males and subgravid females) and *P. lethrini sp.nov*.(males and subgravid females) from the gonads of *Epinephelus cyanopodus* (Richardson) (*Serranidae*) and *Lethrinus genivittatus* Valenciennes(*Lethrinidae*), respectively, and *P.lagocephali sp. nov*. (gravid female) from the abdominal cavity of *Lagocephalus sceleratus*(Gmelin) (*Tetraodontidae*) [27].

Morphological data and molecular analyses are used to describe the taxonomy of philometrid nematodes of the genus *philometra Costa*, found in the gonads of marine fishes in Japan. A new *Philometra* species, *P. sawara sp. n.*, is described based on male and female specimens collected from the gonads of *Scomberomorus niphonius* (Cuvier) (Japanese Spanish mackerel). Two additional species, *Philometra nemipteri* Luo, 2001, and *Philometra sciaenae* Yamaguti, 1941, are confirmed as valid species and are

redescribed based on specimens collected from the gonads of *Nemipterus virgatus* (Houttuyn) (golden threadfin bream) and *Pennahia argentata* (Houttuyn) (silver croaker), respectively [28].

Two new nematode species, *Philometra strongylurae sp. n.* and *Philometra tylosuri sp. n.*, are described from female specimens collected from needlefishes (*Belonidae*, Beloniformes) off Fao coast, Basrah, Persian Gulf, in Iraq. *Philometra strongylurae* (gravid females with larvae) was recorded from the subcutaneous tissue, musculature, and the subcutaneous tissue of *Tylosurus crocodilus* [29]. Two nematode species of the genus *Philometra Costa*, 1845 (only females), *P. ocularis sp. nov.* and *P. managatuwo* Yamaguti, 1941, were recorded from the ocular cavity and the gonad, respectively, of the srranid fish (grouper) *Epinephelus septemfasciatus* (Thunberg) from the coast of the Tsushima Island, Nagasaki Prefecture, Japan [30].

Conflict of interest. Nil

References

- 1. Ahmed O, Doru B. Ecological interdependence of Pollution, Fish Parasites, and Fish in Freshwater Ecosystems of Turkey. J Water. 2023;15(7):1385. doi:10.3390/w15071385.
- 2. Bilqees FM, Khatoon N, Hadi R. A new species of the genus *Stromaturus* from a fish, *Otolithus argenteus* of Karachi coast, India. J Exp Zool. 2005;8(2):435-439.
- 3. Marcelo, Cormona. Nematodes of Elasmobranch fishes from the southern coastal of Brazil. J Oswaldo Cruz. 2001;96(1):81-87.
- 4. Roy C. Why do fish have so few roundworm (nematode) parasites? Environ Biol Fish. 1996;46(1-5).
- 5. Moravec F. Parasitic Nematodes of Freshwater Fishes of Europe. Kluwer Academic Publishers; 1994:470.
- 6. Roy PE. Nematode (Roundworm) Infections in Fish. Univ Florida IFAS Ext. 2002.
- 7. Moravec F, Buron I. A synthesis of our current knowledge of philometrid nematodes, a group of increasingly important fish parasites. Folia Parasitol. 2013;60(2):81-101. doi:10.14411/fp.2013.010.
- 8. Karl M, Tomoyoshi Y, Kazuo O. Taxonomical study into two new species of *Philometra* (Nematoda: *Philometridae*) previously identified as *Philometra lateolabracis* (Yamaguti, 1935). Folia Parasitol. 2008;55:29-41.
- 9. Yuki T, Keitaro K, Youhei W, Sho S. Infections of *Philometra madai* (Nematoda: Philometridae) in the Gonads of a High-growth Broodstock Population of Red Seabream *Pagrus major. Fish Pathol.* 2024;59(2):71-79. doi:10.3147/jsfp.59.71.
- S R, M B, GR D, Tresa R. Philometra cephalus (Nematoda) infection in the gonads of the long-arm mullet Valamugil cunnesius: host-parasite relation. Indian J Fish. 2010;57(2):33-37. <u>http://www.cmfri.org.in/html/cmfriljF01.html</u>.
- 11. Moravec F, Ayyaru G, Mayalagu R, Ayyapan S, Sinduja K. A new gonad-infecting species of Philometra (Nematoda: *Philometridae*) from the marine fish *Terapon jarbua* off the eastern coast of India. *Syst Parasitol.* 2011;80:23-33. doi:10.1007/s11230-011-9310-2.
- 12. Angela D, Sharples C, Evans W. Metazoan parasites of the snapper, *Pagrus auratus* in New Zealand. 1. Prevalence and abundance. NZ J Mar Freshwater Res. 2010;29(2):195-201. doi:10.1080/00288330.1995.9516.
- 13. SA H, Hobbs RP, Potter IC. Infection of gonads of *Glaucosoma hebvaicum* by the nematode *Philometra lateolabracis*: occurrence and host response. *J Fish Biol.* 2002;60(3):663-673. doi:10.1111/j.1095-8649.2002.tb01692.x.
- 14. Lora M, Alistair D, David O. Prevalence, intensity, and effect of a nematode (*Philometra saltatrix*) in the ovaries of bluefish (Pomatomus saltatrix). *Fish Bull*. 2006;104(1):118-125.
- 15. Karl Q, Tomoyoshi Y. Gonad-infecting philometrid *Philometra philippinensis sp. nov.* (Nematoda, *Philometridae*) from the big eye Barracuda *Sphyraena forsteri* Cuvier (Sphyraenidae) off Mariveles, Bataan Province, Philippine archipelago. *Acta Parasitol.* 2013;58(4):504-514. doi:10.2478/s11686-013-0165-3.
- 16. Ghafar R, Hassan H, Kamil N, et al. Occurrence of new *Philometra sp.* in mangrove red snapper *Lutjanus argentimaculatus* from the Karachi coast, Pakistan. *Braz J Biol.* 2023;84. doi:10.1590/1519-6984-269504.
- 17. Moravec F, Kazuya N, Masato N, Atsushi T. New records of philometrids (Nematoda: *Philometridae*) from marine fishes off Japan, including description of *Philometra kidakoi sp. n.* and *Congerinema japonicum* gen. et sp. n. *Folia Parasitol.* 2019;66:021. doi:10.14411/fp.2019.021.
- 18. Kazuo O, Masaaki A, Satoko H, Mutsuki U, Sho S. *Philometroides seriolae* Recovered from the Swim bladder of Japanese Amberjack Seriola quinqueradiata. Fish Parasitol. 2023;58(2):71-73. doi:10.333147/jsfp.58.71.
- 19. Subrina S, Nasima KK, Anirbar S, et al. Parasite diversity and histopathological changes due to infection on three croakers (Pennahia Anea, Panna Microdon and Johnius Argentatus) of Saint Martins Island, Bay of Bengal. *Biores Commun.* 2023;9(2):1297-1304. doi:10.3329/brc.v9i2.67081.
- 20. Nessrine G, David G, Lamia G. First description of the male and sub gravid female of *Philometra* serranellicabrillae Janiszewska, 1949 (Nematoda: *Philometridae*), a gonad-infecting parasite of the comber Serranus cabrilla (Linnaeus) (Serranidae) off Tunisia. Syst Parasitol. 2019;96:673-679. doi:10.1007/s11230-019-09879-3.
- 21. Moravec F, Amira C, Lassad N, Delphine G, Jean J. Descriptions of *Philometra aenei n.sp.* and *P. tunisiensis n.* sp. (Nematoda: *Philometridae*) from *Epinephelus spp*. Off Tunisia confirm a high degree of specificity of gonad-infecting species of Philometra Costa, 1845 in groupers (Serranidae). *Syst Parasitol.* 2016;93:115-128. doi:10.1007/s11230-015-9610-z.
- 22. Moravec F, Atheer HA. Additional observations on *Philometra spp.* (Nematoda: *Philometridae*) in marine fishes off Iraq, with the description of two new species. *Syst Parasitol.* 2014;87:259-271. doi:10.1007/s11230-014-9475-6.

- 23. Moravec F, Micah B, Emma J, Fajer A. Three new gonad-infecting species of *Philometra* (Nematoda: *Philometridae*) parasitic in *Lutjanus spp.* (*Lutjanidae*) in the northern Gulf of Mexico off Florida, USA. *Folia Parasitol.* 2014;61(4):355-369. doi:10.14411/fp.2014.041.
- 24. Moravec F, Harrison C, Jirka M. *Philometridae* (Nematoda: *Philometridae*) from Lake Turkana, Kenya, including two species of *Philometra* and erection of *Afrophilometra gen. n. Folia Parasitol.* 2009;56(1):41-54.
- 25. Moravec F. Systematic status of *Philometra jordanoi* (Lopez-Neyra, 1951) and some other congeneric species previously identified as *Philometra lateolabracis* (Yamaguti, 1935) (Nematoda: *Philometridae*). *Folia Parasitol.* 2008;55(2):159-160.
- 26. Moravec F, Isaure B, Tiffany B, David G. Some gonad-infecting species of *Philometra* (Nematoda, *Philometridae*) from offshore fishes of South Carolina and Georgia, USA, including *Philometra charlestonensis sp. nov.* from the scamp *Mycteroperca phenax. Acta Parasitol.* 2008;53(4):382-391. doi:10.2478/s11686-008-0049-0.
- 27. Moravec F, Jean J. Some philometrid nematodes (*Philometridae*), including four new species of *Philometra*, from marine fishes off New Caledonia. *Acta Parasitol*. 2008;53(4):369-381. doi:10.2478/s11686-008-0050-7.
- 28. Karl MA, Quiazon TY, Kazuo O. *Philometra sawara sp. n.* and a redescription of *Philometra sciaenae* (Yamaguti, 1941) and *Philometra nemipteri* (Luo, 2001) (Nematoda: *Philometridae*): a morphological and molecular approach. *Folia Parasitol.* 2008;55:277-290.
- 29. Moravec F, Atheer HA. Two new species of *Philometra* (Nematoda: *Philometridae*) from needlefishes (Belonidae) in Iraq, with a Key to *Philometra spp.* parasitic in the hosts subcutaneous tissue, fins and musculature. *Folia Parasitol.* 2005;52:267-273.
- 30. Moravec F, Ogawa K, Suzuki M, Miyazaki K, Donai H. On two species of *Philometra* (Nematoda, *Philometridae*) from the serranid fish *Epinephelus septemfasciatus* in Japan. *Acta Parasitol.* 2002;47(1).