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# Molecular characterization and morphology of *Philonema onchorhynchi* (Nematoda: Philometridae), a parasite infecting the Nile perch (*Lates niloticus*) in Egypt with some of its histopathological effects.

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**ABSTRACT**

The present study aimed to detect the helminth parasites from the Nile Perch *Lates niloticus* Linnaeus, 1758 collected from Manzala lake in Damietta province, Egypt. Twenty-six (34.7%) of 75 fish samples were found infected with one species of nematode *Philonema onchorhynchi* Kuitunen-Ekbaum, 1933 (Family: Philometridae). The mean intensity was (0.51±0.79) and relative density (0.50) with no significant difference ($P$>0.05) in the infection between male and female hosts. Partial fragments of the first internal transcribed spacer (ITS-I) region and small subunit ribosomal RNA (SSU rRNA) 28S gene of the nematode were amplified, sequenced, and available in GenBank under accession number MZ766122. Molecular data confirmed the identification of the species as *P. onchorhynchi* belonging to the family Philometridae. The obtained nematode was described using light and scanning electron microscopy which revealed it is characterized by inconspicuous small and large papillae distributed on the cephalic region surrounding the mouth opening with no true lips and four pseudolapia. The histopathology of the intestine of the infected hosts was also investigated.

## INTRODUCTION

Manzala lake is a shallow lagoon with depth ranges from 0.7-1.5 m. It is the most productive lake for fisheries and considered as the most productive fishery resources **(Rashad and Abdel-Azeem, 2010; Bahnasawy *et al*., 2011)**. It is highly dynamic aquatic system due to chemical, physical, and biological changes during the last century. It has been gradually transformed from a largely marine to brackish water system that contains different fish species of marine and freshwater because of different aspects of human impacts. Fishes are zoonotically important, since several diseases are transmitted to human via the consumption of undercooked or row fish infected by parasite larvae where they play an essential role as either intermediate or definitive hosts for many parasites **(Shaukat, 2008).** Helminth infections in fish have a great impact on its production in relation to fish health such as declining of the host`s immune system due to the pathogeneic effects of secondary infections, depriving fish of essential nutrients,

leading to morbidity, mortality, and subsequent economic loss **(Nguyen *et al.*, 2021; El-Seify *et al.*, 2015)**

The Nile Perch *L. niloticus* Linnuaeus 1758 (Family: Centropomidae, Subfamily: Latidae) is a widespread species with high value throughout world's rivers and in brackish water for commercial aquaculture **(Namulawa *et al.*, 2015).** It is of genuine economic and food security importance in Esat Africa **(Chrètien *et al.*, 2016)** but its population is threatened due to the excessive harvest and parasitic diseases **(Katurole and Wadanya, 2012)**. Although that, few helminthological studies were done on that species **(Emere, 2000; Nkechi Esther *et al.*, 2010; Elseify *et al.*, 2015).** The nematode *Philonema* Kuitunen-Ekbaum, 1933 (Family: Philometridae) was recorded from different localities: **Rumyantsev (1965)** stated that *P. sibirica* harbour different whitefish in the USSR, whereas, according to **Meyer's study (1960),** *P. agubernaculum* occurs primarily in salmonids and seldom in whitefish. **Sobecka and Piasecki (1993)** recorded nematode *Philonema* from *Salvelinus alpinus* in Norway and **Korenchenko (1994)** isolated larvae of *P. sibrica* from *Cyclops gracilis* in lake Gekovo, Russian. *P. onchorhynchi* redescribed by **Moravec and Nagasawa (1999)** from three species of anadromous Pacific salmon; *Oncorhynchus kisutch*, *O. keta*, and *O. nerka* from Japan and by **Berg (1995)** from Sockeye salmon *Oncorhynchus nerka*. Also, *Philonema margolis* was described by **Moravec *et al*. (2017)** from *Epinephelus morio* in Mexico.

The aim of the present study is to provide morphological and molecular characteristics of the nematode parasite *Philonema onchorhynchi* Kuitunen-Ekbaum, 1933 found in *Lates niloticus* Linnaeus, 1758 as a new host record from Manzala lake in Egypt. In addition to investigate the histopathological changes in the intestine of the natural infected hosts. Also, more understanding about the characteristics of this parasite that infect one of the most commercial fish species *L. niloticus* in Manzala lake will aid in the prediction of its distribution in other areas rich in this fish.

## MATERIALS AND METHODS

**Study area:** Manzala lake is the largest coastal lake in Egypt with an area of $192x10^3$ acre, its water depth ranges from 50-120 cm. **(Thomas and El-Kariony, 1995).** It is located in the North of the Nile Delta between 31˚ 35□N latitude and 31˚ 45□N and 33 ˚ 15□E longitudes. It is surrounded by Mediterranean Sea in the North, at East Suez Canal, in the Northwest Damietta province and Dakahlia in the Southwest **(Rashad and Abdel-Azeem, 2010).** The samples were gathered by local fishermen from Manzala lake in Damietta province, Egypt in February of year 2021.

**Parasitological study:** Seventy-Five (30 males & 45 females) of *Lates niloticus* Linnaeus, 1758 (Centropomidae) were collected, transferred to laboratory, their sexes were identified externally, dissected, and their body cavities were inspected for intestinal

helminths. For the detected nematode parasite, all measurements were in millimeters unless otherwise stated with means in parentheses. The parasites were identified according to **(Yamaguti, 1961, Anderson *et al*., 2009; Madhavi and Bray 2018).** Parasite specimens were deposited in the helminths collection in the Zoology Department, Faculty of Education, Ain Shams University, Cairo, Egypt. For Scanning Electron Microscopy, the nematodes were fixed in 2 % glutaraldehyde in 0.1 M. sodium cacodylate buffer (PH 7.2) then dehydrated in ethyl alcohol, dried at critical point, mounted then coated with gold, photographed by Joel Scanning Electron Microscope (Jeol. JSM-5400), Atomic Energy Agency, Nasr City, Cairo, Egypt.

**Molecular study:** In the present study the molecular identification of *Philonema onchorhynchi* was confirmed by Genetic Engineering Research Institute, Agriculture Research Center (ARC), Giza, Egypt. Purification and DNA extraction were made according to DNeasy Blood & Tissue kits (QLAGEN-Germany). ITS (non-coding region) was amplified and sequenced using ITS1 and ITS4 primers (Table, 1). The PCR amplification was performed in a total volume of 50 ul, containing 1X reaction buffer, 1.5 mM $MgCl_2$, 1U *Taq* DNA polymerase (promega), 2.5mM dNTPs, 30 poml of each primer and 30 ng genomic DNA. PCR amplification was performed in a Perkin-Elmer / GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. The amplification products were analyzed by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. A 100bp DNA ladder was used as a molecular size standard (Fig.1).

**ITS Sequencing analysis:** ITS-I was sequenced using ITS1 and ITS4 primers **(Table 1)**. BLAST sequence analysis led to the identification of *P. onchorhynchi* (GenBank under accession number MZ766122).

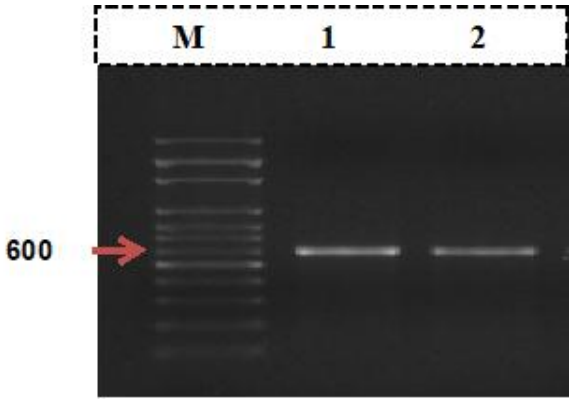



**Fig. 1.** Agarose gel electrophoresis of amplified ITS-I of ribosomal RNA in *P. onchorhynchi* Kuitunen-Ekbaum, 1933.

**Table 1.** Primer code and its nucleotide sequence

| Primer Code | Sequence | Product Size |
|---|---|---|
| *(ITS-1)* F | 5′- TCCGTAGGTGAACCTGCGG -3′ | 600bp |
| *(ITS-4) R* | 5′- TCCTCCGCTTATTGATATGC-3′ | |

**Histopathological study:**

Intestine of infected and noninfected host *L. niloticus* was dissected out from each tested specimen and washed very well in 0.7% saline solution then samples were preserved in the fixative 10% formalin for 24 hours. The samples were prepared and mounted according to **Bancroft and Gamble (2002)**. The slides were examined and photographed using an Olympus C X 31 microscope and an Olympus digital camera E-330-ADU1X Japan to determine the histopathological effects of *P. onchorhynchi* on the intestinal tissues.

**Statistical analysis:** SPSS 19.0 for windows (Independent sample T-test) was applied. *P* value <0.05 was considered.

## RESULTS

**Table 2.** Prevalence of infection and mean intensity of *P. onchorhynchi* in both male and female hosts

| **Character** | ***L. niloticus*** | |
|---|---|---|
| | Male | Female |
| **Total N.** | 30 | 45 |
| **Infection N.** | 10 | 16 |
| **Prev. (%)** | 33.3 | 35.5 |
| **M±SD** | 0.57±0.93 | 0.47±0.69 |
| **R.** | (2-4) | (1-3) |

**M±SD Mean intensity ± Standard Deviation - R. (Range)**

**1. Host-Parasite Data:** 26 spcimens of Nile Perch out of 75 (34.7%), were found infected with *Philonema onchorhynchi* Kuitunen-Ekbaum, 1933. Mean intensity was 0.51±0.79, Abundance 0.5, Range (1-3). Infection rate in females was higher (35.5%) than in males (33.3%) with no significance difference (*P* >0.05) **(Table 2)**.

## 2. Description:

As appeared by light and Scanning Electron Microscopy: Body is white, cylindrical, medium to large sized, females are larger than males. The body has broad rounded cephalic with cuticular striations anteriorly (Figs. 2 &3 a). Mouth is triangular, thick walled free from lip structures. The cephalic end is provided with cuticular folds and inconspicuous small and large papillae distributed on cephalic region surrounding mouth opening and four pseudolapia (Fig. 3, c&d). Two amphids are present each one has a pair of rounded papillae and some pores (Fig. 3, c). An excretory pore on the ventral side was located near the cephalic region (Fig. 3, a). Esophagus is cylindrical and divided into anterior short muscular part and posterior long glandular part. **Male**: (n=3) Body 2.5–2.8 (2.65) long and 0.8–0.85 (0.825) cm. wide. Muscular esophagus 0.35-0.43 (0.39) long represents 14.7% of total body length (TBL) and 0.082-0.1 (0.091) wide. Glandular esophagus 0.97-1.35 (1.16) long represents 43.7% of (TBL) and 0.13-0.14 (0.135) wide. Nerve ring 0.13-0.14 (0.135) from anterior extremity. The cloaca from posterior extremity 0.1-0.114 (0.107). Two equal sclerotized spicules present, they are not protruded to outside in some specimens, spicule 0.22-0.24 (0.23) long and 0.02-0.03 (0.025) wide (Fig. 2, b). The posterior end has a pair of horn-like protrusion near the cloacal lip as well as cuticular ornamentation on its caudal extremity (Fig. 3, I). Gubernaculum 0.165-0.178 (0.171) long and caudal end 0.056 from cloaca to the posterior extremity. **Female**: (n=7) Body 3.6-4 (3.8) long and 0.8-1 (0.9) cm. wide. Muscular esophagus 0.56-0.62 (0.59) long represents 15.5% of (TBL) and 0.081-0.11 (0.095) wide. Glandular esophagus 0.72-0.86 (0.79) long represents 20.8% of (TBL) and 0.13-0.15 (0.14) wide. Nerve ring 0.124-0.128 (0.126) from anterior extremity. Two opposite short ovaries, short vagina and two long uteri were present. The vulva is atrophied, and tail is ending by sharp pointed tip (Fig. 3, h). The anal opening is surrounded by two rectal glands and one pair of adanal papillae (Fig. 2, c). The distance from anus to posterior extremity 0.18-0.19 (0.185). **(Table 3)** showing the measurements of the present specimen in comparison with the closest species.

**3. Molecular analysis of *Philonema*:** The BLAST analysis of nematode specimens (*Philonema*) revealed high homology (99.03%) with sequences of *Philonema* sp. deposited by **Frisse *et al.* (1999)** in GenBank under accession number U81574.1 and high similarity (98.38%) with *P. onchorhynchi* that presented by **Wijova *et al*. (2006)** with accession number DQ442670.1 deposited in GenBank Database **(Table 4)**. The partial sequence of 28S r-RNA gene of the present adult nematode was deposited in the GenBank database https://www.ncbi.nlm.nih.gov under accession number MZ766122 (Fig. 4).

**Table 3.** A comparison between the present description of *P. oncorhynchi* Kuitunen-Ekbaum, 1933 and the more related species.

| Character | *P. sibirica* (Bauer, 1946) Rumyantsev (1965) | *P. oncorhynchi* Kuitunen-Ekbaum, 1933 (Moravec and Nagasawa, 1999) | *P. oncorhynchi* Kuitunen-Ekbaum, 1933 (Present Work) |
|---|---|---|---|
| **Body L.** ♂<br>♀ | 11.2<br>30 | 13.91-30.16<br>102.8-185 | 2.5-2.8<br>3.5-4.0 |
| **Body W.** ♂<br>♀ | 1.6<br>3.6 | 2.06-4.12<br>0.9-1.48 | 0.8-0.85<br>0.8-1 |
| **Entire esophagus L.** ♂<br>♀ | 1.04<br>1.17 | 1.66-2.9<br>1.83-2.31 | 1.32-1.78<br>1.28-1.48 |
| **L. of Muscular esophagus** ♂<br>♀ | 0.49<br>0.49 | 0.44-0.76<br>0.43-1.09 | 0.35-0.43<br>0.56-0.62 |
| **L. of Glandular esophagus** ♂<br>♀ | ---<br>--- | 1.22-2.16<br>1.27-2.02 | 0.97-1.35<br>0.71-0.86 |
| **Nerve ring from anterior extremity**♂<br>♀ | ---<br>--- | 0.26-0.39<br>0.25-0.45 | 0.13-0.14<br>0.12-0.13 |
| **Anus from posterior end** | --- | --- | 0.18-0.184 |
| **Spicule L.**<br>**W.** | 0.25<br>0.006 | ---<br>--- | 0.23<br>0.025 |
| **Gubernaculum** | Absent | Absent | Present |
| **Hosts** | White fish | *Oncorhynchus nerka* & *O. kisutch* | *Lates niloticus* |
| **Infection site** | Body cavity | Abdominal cavity | Body cavity mesenteries, and intestine |
| **Distribution** | **USSR** | **Japan & North Pacific Ocean** | **Manzala lake, Egypt** |

**Abbreviations: L.=length; W.=width, ♀=female, ♂= male**

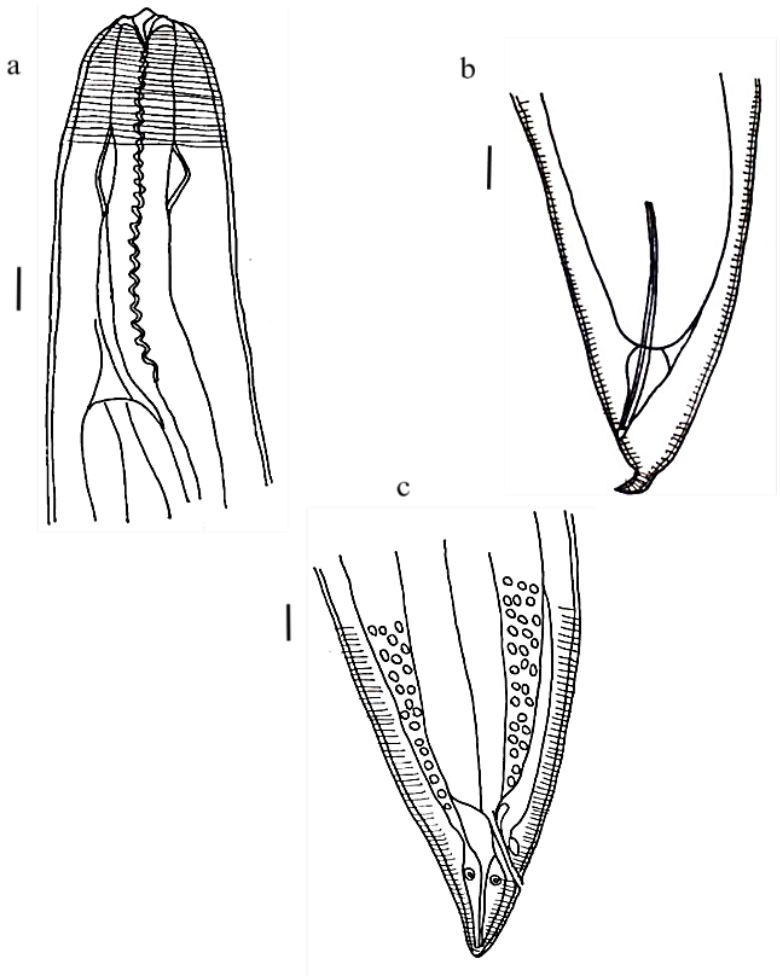



**Fig. 2.** Light Microscope drawing of *P. onchorhynchi* Kuitunen-Ekbaum, 1933. **a:** Anterior part of adult female. **b:** Posterior part of adult male showing spicules sheathed inwards. **c:** Posterior end of female worm showing rectal glands and a pair of adanal papillae. Scale bars= 0.1 mm.

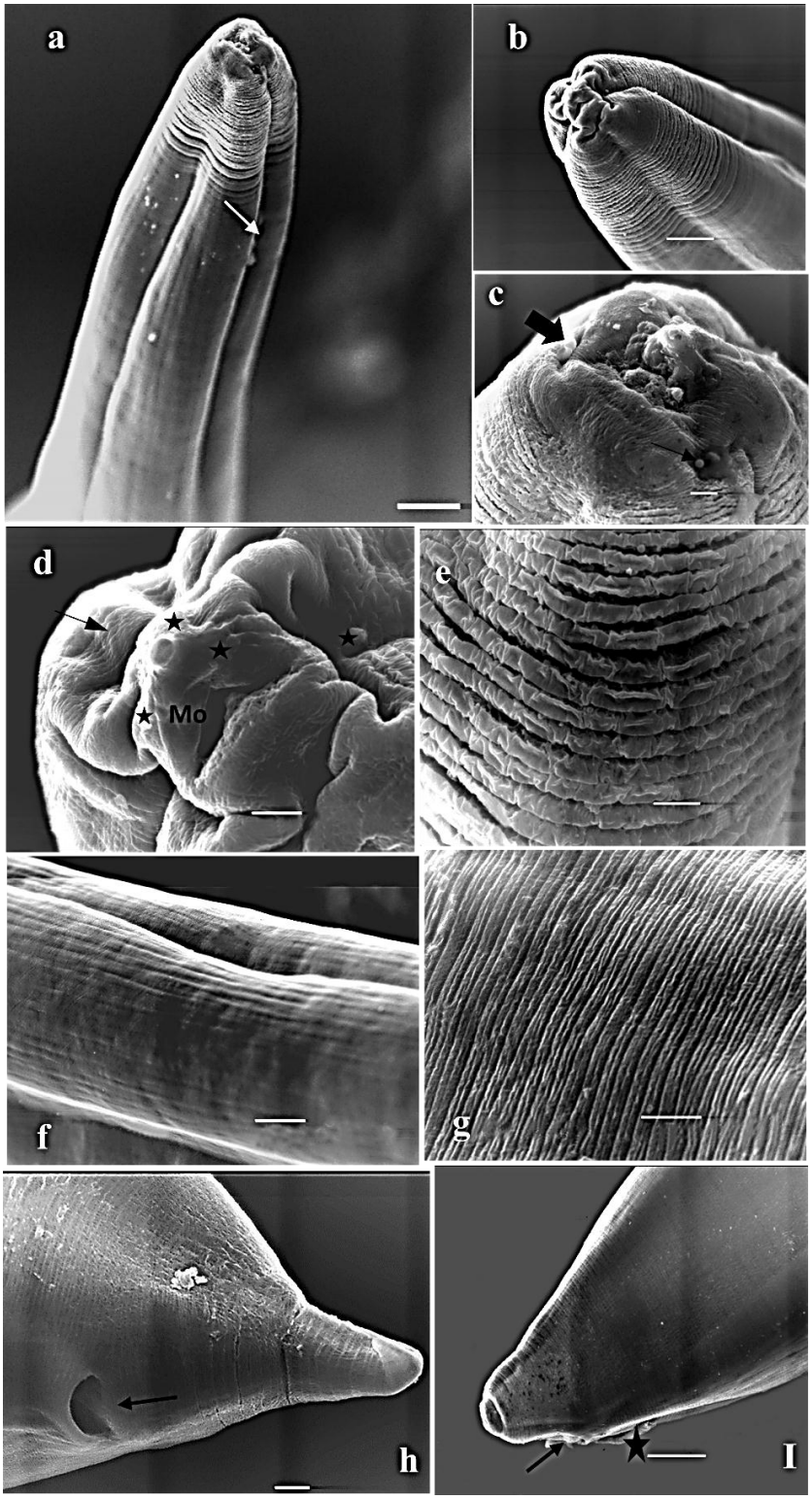



**Fig. 3.** Scanning Electron Micrographs of *Philonema oncorhynchi* Kuitunen-Ekbaum, 1933. (a) Anterior end of the adult female worm showing the excretory pore (arrow). (b) Cephalic region of female worm. (c) Cephalic end of female showing amphid (thick arrow), papilla (narrow arrow). (d) Cephalic end showing, triangular mouth opening (M), pseudolapia (arrow), papillae (stars). (e) Ventral surface of anterior end of male showing distinct transverse striations separated by narrow grooves. (f) Middle part of female showing a large depression. (g) Middle part of female worm showing regular transverse cuticular striations. (h) Posterior extremity of female showing the anal pore (arrow) and tail is ending by sharp pointed tip. (I) Posterior extremity of male showing horn-like protrusion on the cloacal lip. Scale bars: a, f & I=100 µm. b=50 µm. c, d, e, g, h=10 µm.

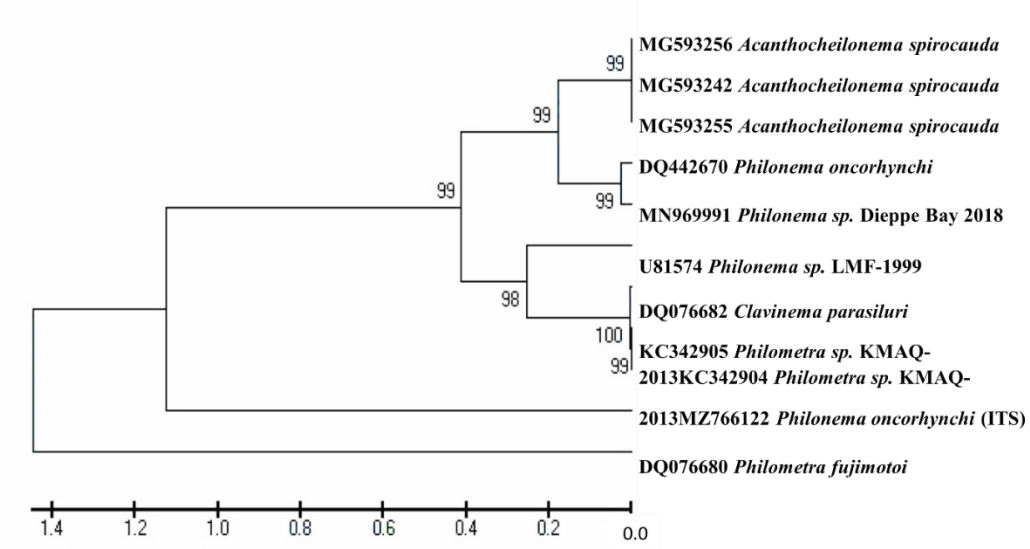



**Fig.4.** Phylogenetic tree for the relationships of *P. onchorhynchi* to other related species that analyzed with the aid of the NCBI taxonomy database.

**Table 4.** Sequences producing significant alignments from GenBank database of *Philonema spp.* and related nematode species: National Center for Biotechnology Information (nih.gov):

| Scientific Name | % Identity | ACC. Len | Accession |
|---|---|---|---|
| ***Philonema* sp. LMF-1999 18S r RNA gene** | 99.03% | 1749 | U81574.1 |
| ***Philonema onchorhynchi* rRNA gene** | 98.38% | 1670 | DQ442670.1 |
| ***Philonema* sp. Dieppebay 2018 rRNA gene** | 94.06% | 1733 | MN969991.1 |
| ***Philometra* sp. KMAQ-2013 *isolate 3* 18S rRNA gene** | 91.48% | 1145 | KC342905.1 |
| ***Philometra* sp. KMAQ-2013 isolate 2 18S rRNA gene** | 91.48% | 1147 | KC342904.1 |
| ***Clavinema parasiluri* 18S rRNA gene** | 91.49% | 927 | DQ076682.2 |
| ***Philometra fujimotoi* 18S rRNA gene** | 91.71% | 924 | DQ076680.2 |
| ***Acanthocheilonema spirocauda* isolate P-Pr-13-104 rRNA gene** | 91.44% | 823 | MG593256.1 |
| ***Acanthocheilonema spirocauda* isolate DO-5476 r RNA gene** | 91.44% | 853 | MG593255.1 |
| ***Acanthocheilonema spirocauda* isolate P-Pr-11-007 rRNA gene** | 91.44% | 820 | MG593242.1 |

## 4. Histological study:

The normal histological structure of the intestine in Nile Perch fish is composed of mucosal layer of simple columnar epithelium lining the intestine with many of lymphocytes and goblet cells found in the intestinal lamina. The submucosal layer is thin with connective tissue extending to the whole length of villi. Tunica muscularis consists of outer longitudinal layer and inner circular layer of smooth muscle. The histopathological effects in the intestine of infected host that were recorded in the present study included destruction of villi, necrosis, inflammatory reaction appeared in the infiltration of macrophages and neutrophils. Also, in some specimens an increasing of goblet cells and degenerated nuclei were observed. Moreover, in other specimens a severe damage including atrophy in muscular layers and submucosa with hemorrhage was noticed **(Fig. 5).**

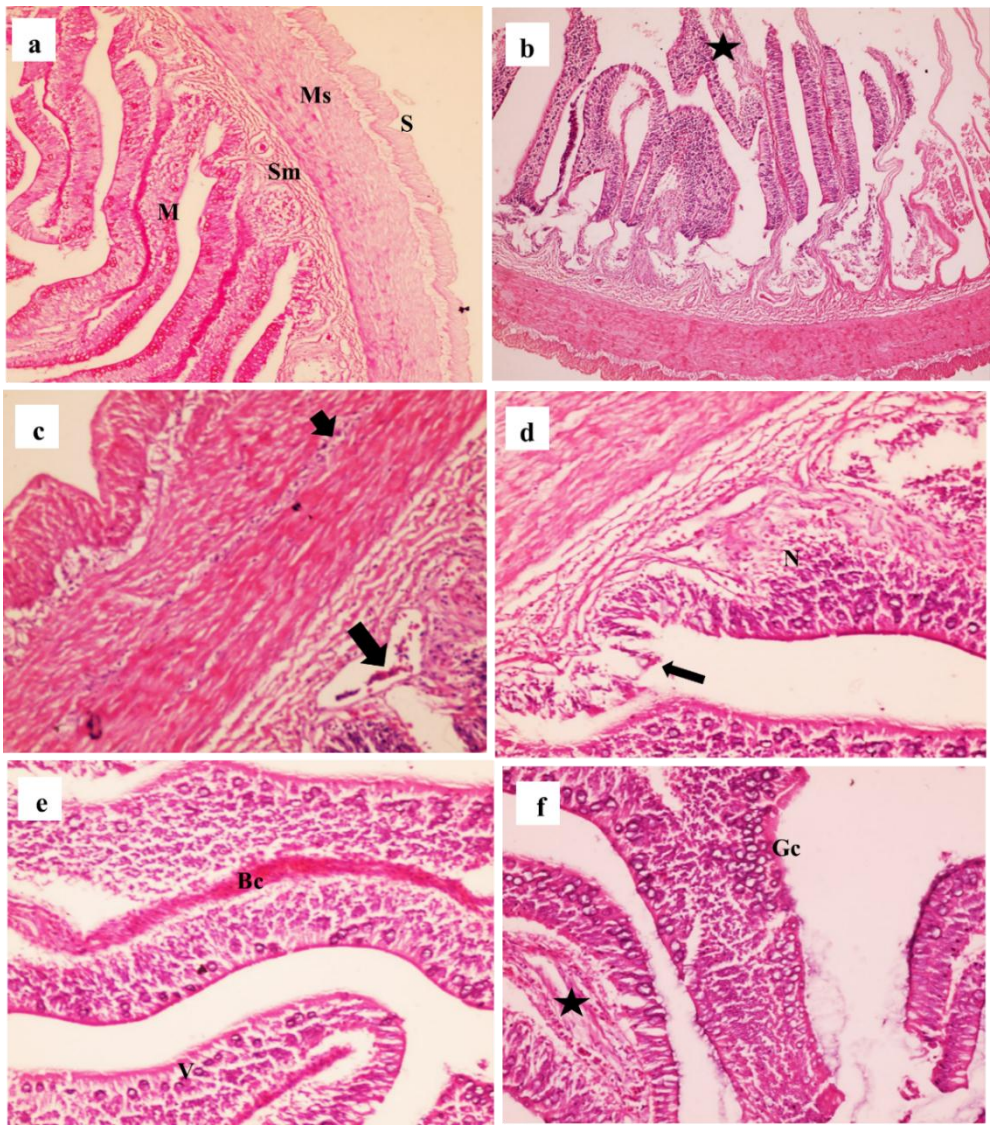
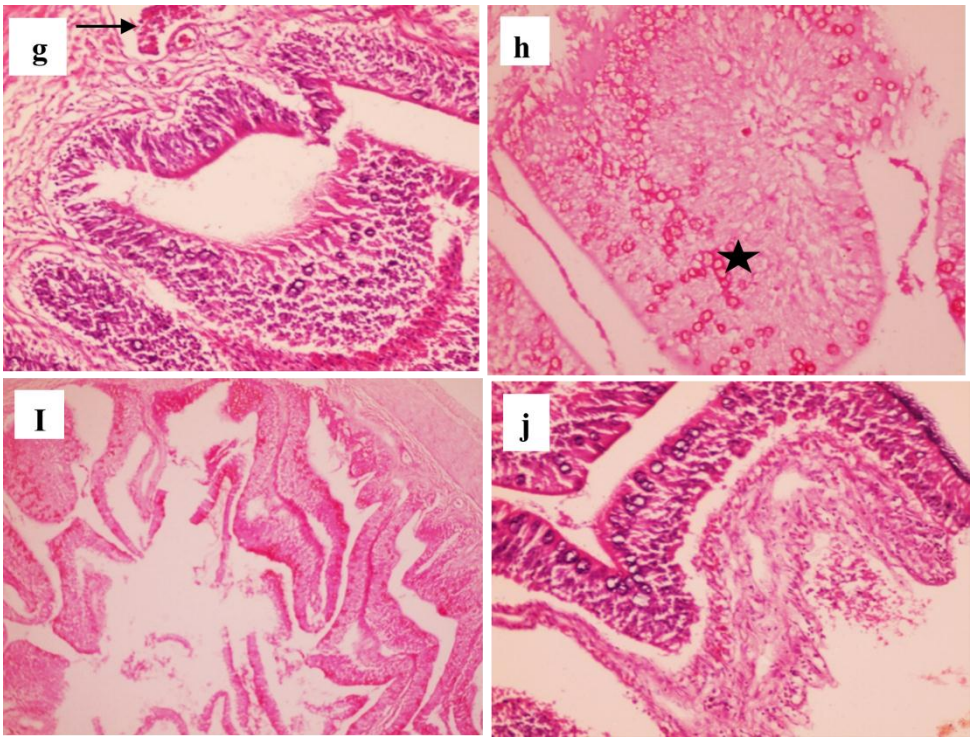


**Fig. 5. (a-j):** Photomicrographs of T.S in intestine of noninfected **(a)** and infected **(b-j)** *Lates niloticus* fish with nematode *P. onchorhynchi* Kuitunen-Ekbaum, 1933. **a:** serosa (S), muscularis (Ms), submucosa (Sm), mucosa (M). **b:** Destruction of villi (star) & necrosis. **c:** Infiltration of macrophages and neutrophils in the longitudinal muscle layer and sub-mucosa (arrows). **d:** Degenerated mucosal folds (arrow) & necrosis. **e:** Blood congestion (Bs), degeneration of mucosal membrane of villi (V). **f:** Increasing in goblet cells (Gc), hemorrhage (star). **g:** Blood congestion (arrow), destruction of submucosa and necrosis. **h:** Broken of outer membrane of mucosal folds, aggregation of goblet cells (star). **I:** Fused and disturbance of microvilli**. j:** Severe damage in intestinal mucosa.




## DISCUSSION

The nematode *Philonema* (Nematoda, Philometridae) was discovered originally by Kuitunen-Ekbaum, 1933 from the body cavity of sockeye salmon *Oncorhynchus nerka* for the type species *P. oncorhynchi*. It is mainly characterized by simple mouth without true lips, very short ovaries with atrophied vulva in female, two equal spicules in adult male worm, and four pseudolapia. These features indicated that it is belonging to genus *Philonema*. The present description agrees with *P. oncorhynchi* that described by **Moravec and Nagasawa (1999)** in the measurements of female body width, and in the number of papillae at cephalic end. However, it differs in the longer body, shorter entire esophagus length and in the presence of anal opening which missed in the latter species. Moreover, the rectal glands are highly reduced in *P. oncorhynchi* by **Moravec and Nagasawa (1999),** while they are well developed herein. However, it differs from *P.*

*sibirica* Rumyantsev, 1965 in larger body length, while it is showed a similarity in the length of spicules. The presence of papillae of various sizes on the cephalic region is a distinct character for the present species. Morever, **Platzer and Adams (1967)** mentioned that the oral and tail papillae are obscure in adults *Philonema* and this is in corresponds to the present specimens in the disappearance of caudal papillae. Also, the present nematode is similar to *P. onchorhynchi* described by **Moravec and Nagasawa (1999)** in the presence of pair of horn-like protrusions. On the other hand, they revealed a median pre-anal papilla on the cloacal lip which is not appeared in the current specimens. In the present study, it was observed that the vulva is atrophied which agrees with the findings of **Moravec and Nagasawa (1999).** Two amphids were found on cephalic region of the worm as they are chemosensory, and some are photo respective with an associated gland **(Naem *et al.*, 2013)**. In the present study most of recovered nematodes were found in body cavity of the new host, Nile perch *Lates niloticus* from new locality in Egypt, Manzala lake. The prevalence of its infection was (34.7%) which is lower than (99.6%) that presented by **Berg (1995)** for *P. onchorhynchi* in the host *Onchorynchus nerka* (sockeye salmon). Meanwhile, it was higher than (10%) by **Rumyantsey (1965)** for *P. sibirica* in the host Coregonus albula (European cisco) and by Korenchenko (1994) (0.12%), (2.85%), (1.6%) for *P. sibirica* from three hosts: *Acanthcyclops* sp., *Cyclops gracilis* and *Heterocope borealis.* Also, the obtained data showed slightly higher prevalence (35.5%) in females than (33.3%) in males with no significance difference ($P>0.05$). These results in accordance with that presented by Moravec *et al*., (2017) for *P. margolis*. **Platzer and Adams (1967)** stated that infection by *Philonema* worms is correlated with host's sex hormones. The differences in the infection between males and females may be regarded to genetic predisposition, the diet, physiological status of female that may increase its susceptibility to infection **(Abiyu *et al.*, 2020).** The phylogenetic study revealed that the present species showed a high phylogenetic relationship with *Philonema* sp. deposited by **Frisse *et al*. (1999)** under accession number U81574.1 with (0.97%) nucleotide difference and *P. onchorhynchi* that analyzed by **Wijova *et al.* (2006)** with accession number DQ442670.1 and nucleotide difference (1.62%) available in GenBank. Recently, molecular techniques have been used by scientists as new approach for confirming identification at species level and to utilize these parasites further in large scale of ecological survey and environmental assessments **(Avo *et al.*, 2017).** Our data contribute to the morphological characters for more understanding of the evolution of species of the family Philometridae. Few studies were carried out on the molecular analysis of members of that family so no more data are available in the GenBank related to the sequences of genes of the present nematode. In the present work several histopathological effects were observed in the intestine of the infected host. The main changes were destruction and degeneration of mucosal villi with increasing in goblet cells and necrosis. The main function of these villi is improving absorption process **(Farrag *et al.*, 2021). Alabssawy *et al.* (2019)** estimated that the

normal histological structure of intestine in fishes is correlated with their diet and feeding behavior. Also, inflammatory reaction was appeared in the infiltration of macrophages and neutrophils in muscular layer and submucosa. This agrees with results of **Dezfuli *et al*. (2017)** on the intestine of Mullet fish infected by helminths. They observed several mucous, mast, rodlet cells and macrophages as a cellular immune response of the host as these cells have a great role in mucosal immunity of intestine especially in the submucosa. **Namulawa *et al*. (2015)** studied the detailed structure of intestinal tract of *L. niloticus* by TEM, they observed that the epithelium is composed of columnar enterocytes, rodlet cells, goblet cells and migratory lymphocytes. Numerous goblet cells are noticed in the present work due to nematode infection. This condition is like that recorded by **Hassan *et al*. (2019)** in the intestine of freshwater fish *Channa micropeltes* infected with cestode *Senga rostallarae*. The increasing in goblet cells indicating a defense mechanism against infection. The major change that caused by *Philonema* was a destruction of mucosal epithelia and villi which lead to a disruption of the normal organization of intestine. This alters the physiological status of fish like nutrition and digestion processes **(Kaur *et al*., 2012).** Because of the large size of the present nematode, it may cause blocking in intestinal lumen consequently disrupt the absorption efficiency that affect the growth and the development of the host.

## CONCLUSION

The present study showed the effect of helminth parasites on fish of considerable importance because of their wide occurrence and being as intermediate hosts in the food chain. The present findings provide new criteria related to morphological and molecular studies of *Philonema onchorhynchi* infecting *Lates niloticus* in Egypt so, more studies are required for avoiding and controlling of these parasitic diseases.

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