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Morphological and Ultrastructural Redescription of *Philometra cyanopodi* from *Epinephelus chlorostigma* and *Variola louti* in the Red Sea, Egypt

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Abstract: This study provides a detailed morphological and ultrastructural redescription of the nematode parasite Philometra cyanopodi Moravec and Justine, 2008, collected from the gonads of two serranid fish species, Epinephelus chlorostigma and Variola louti, in the Red Sea, Egypt. A total of ten fish (five of each species) were examined, revealing a 40% infection rate in both hosts. The parasites were characterized using light microscopy and scanning electron microscopy (SEM), revealing distinct morphological features, such as a small, filiform body, fine transverse striations, and specific cephalic and caudal structures. Comparative analysis with previously described specimens highlighted both similarities and notable differences in morphological measurements. This redescription confirms the presence of P. cyanopodi in new host species and geographical locations, expanding our understanding of its taxonomy, host-parasite relationships, and geographic distribution. The study underscores the importance of detailed taxonomic analyses for accurate species identification and provides insights into the host specificity and ecological interactions of this nematode parasite.
Keywords: Philometra cyanopodi, Epinephelus chlorostigma, Variola louti, Red Sea, Scanning Electron Microscopy (SEM).

1. Introduction

The family Philometridae, described by Baylis and Daubney in 1926, comprises filamentous, viviparous nematodes that inhabit various locations within fish, including the body cavity, fin rays, subcutaneous tissue, and gonads [1,3]. Taxonomic differentiation within this family relies on characteristics such as the structure of the esophagus, the number, arrangement, and type of cephalic papillae in females, cuticular ornamentations, and tail morphology [4]. Philometridae includes three subfamilies found in marine fishes: Neophilometroidinae Moravec, Salgado-Maldonado, and Aguilar-Aguila, 2002; Phlyctainophorinae Roman, 1965; and Philometrinae Baylis and Daubney, 1926 [5]. The genus Philometra, established by Costa in 1845, is characterized by several distinctive features. Female Philometra are larger than males, with filiform bodies and rounded anterior and posterior extremities. The mouth lacks lips, and tail papillae are present. Ovaries are located at each end of the body [6]. Males possess equal and slender spicules, a gubernaculum, and a uterus that occupies most of the body [7]. Previous research by Moravec and Justine in 2008 documented the presence of Philometra cyanopodi parasitizing *Epinephelus chlorostigma* (brown spotted grouper) and *Variola louti* (yellow-edged lyretail) in the Red Sea waters of Egypt. This marked the first record of this species in the region, expanding our understanding of its geographic distribution.

2. Materials and Methods

2.1. Sample Collection

A total of five *E. chlorostigma* (3 males and 2 females) and five *V. louti* (2 males and 3 females) were collected from Hurghada in the Red Sea, Egypt, between April 2019 and May 2021. The fishes were captured and immediately transported to the Parasitology Laboratory, Zoology Department, Faculty of Science, Sohag University, Egypt. Fish identification was conducted based on established criteria [8, 10] with further confirmation obtained from information available on the Fish Base website [11].

2.2. Macroscopic Examination

The gastrointestinal tract was carefully untangled, and the entire digestive system and other viscera were longitudinally opened. Both macroscopic and microscopic examinations of various organs were performed to detect any nematode parasites. The collected parasites were cleaned by washing several times with an isotonic saline solution of 0.9% [12]. Encountered nematodes were fixed in warm (60°C) 70% ethanol, then preserved in bottles containing a mixture of 70% ethanol and 5% glycerol until ready for examination [13].

2.3. Light Microscopic Examination

For the microscopic study, nematode parasites were mounted on a glass slide with a few drops of lactophenol solution and covered with a thin glass coverslip using one drop of glycerin jelly [14]. The mounted nematode parasites were photographed and drawn using a camera lucida. Measurements were expressed in millimeters (mm), with measurements of nongravid specimens in parentheses. Samples were identified using keys for vertebrate nematode parasites **[6, 15-17].**

2.4. Scanning Electron Microscope (SEM) Examination

For SEM examination, specimens were fixed for six hours at 4°C in 3% buffered glutaraldehyde, washed several times in 0.1 M sodium cacodylate buffer, dehydrated in ascending ethanol concentrations, and transferred to pure acetone. Samples were then processed in a Bomer-900 critical point drier with Freon 13. The samples were sputter-coated with gold in a Technics Hummer V and studied using a JEOL JSM-5400LV SEM operated at 15 kV in the electron microscopy unit of Assiut University, Egypt [18].

3. Results and Discussion:

3.1. Morphological Description:

The present study focused on characterizing and diagnosing *P. cyanopodi* Moravec and Justine, 2008, collected from the gonads of the Brown-spotted grouper (*E. chlorostigma*) and the Yellow-edged lyretail (*V. louti*) in the Red Sea, Egypt. *P. cyanopodi* was found in 2 out of 5 specimens of *E. chlorostigma* (3 males, 2 females) and 2 out of 5 specimens of V. louti (2 males, 3 females), representing a 40% infection rate in both hosts. The mean intensity of infection was 4 and 6 worms per infected fish, respectively. The fish sizes ranged from 29 to 44 cm (0.165 to 0.1013 gm) for *E. chlorostigma* and 36 to 48 cm (0.429 to 1.206 gm) for *V. louti*. The nematode *P. cyanopodi* was characterized by its small, filiform, whitish body with bluntly rounded ends, widest slightly posterior to the end of the esophagus. The cuticle was thin with fine transversal striation. The cephalic end was rounded, with a small, triangular oral aperture without lips, and a narrow, long esophagus with slight inflation at the anterior end. The esophageal gland had a large nucleus in the middle of the esophagus, and the excretory pore was in the posterior half of the esophagus. The intestine was dark brown, simple, straight, and ended blindly, attached by a long ligament ventrally near the caudal end. The tail was simple and rounded in both sexes. Male: The male body measured 1.900-3.510 mm in length by 0.040-0.111 mm in maximum width. The cephalic and caudal ends were 0.029-0.070 mm and 0.022-0.058 mm wide, respectively. The esophagus was 0.470-0.654 mm long, representing 15-19% of the body length, and 0.022-0.040 mm in maximum width. The esophageal nucleus was 0.233-0.332 mm from the anterior extremity. The nerve ring and excretory pore were 0.130-0.190 mm and 0.148-0.220 mm from the anterior extremity, respectively. The posterior end was blunt with a broad, U-shaped mound. Spicules were brownish, needle-like, and 0.100-0.210 mm long, representing 5.2-7.3% of the body length. The gubernaculum was 0.095-0.165 mm long, with a dorsally bent anterior portion, representing 36-39% of its total length, and had distinct transverse lamella-like structures on the dorsal side. The ratio of gubernaculum to spicules length was 1:1.05-1.30.Female: The female body was longer, measuring 15.670 mm in length by 0.304 mm in maximum width. The cephalic and caudal ends were 0.210 mm and 0.147 mm wide, respectively. The esophagus, including anterior bulbous inflation, was 1.350 mm long, representing 9% of the body length, and 0.133 mm wide. The esophageal gland nucleus was 0.923 mm from the anterior extremity. The nerve ring was 0.250 mm from the anterior extremity. The ventriculus measured 0.030 mm in length by 0.070 mm in width. The intestine was attached by a 0.075 mm long ligament near the caudal end. The vulva and anus were absent. Two short, thick ovaries were located near the anterior and posterior extremities, with the uterus occupying most body space and filled with numerous eggs. Intrauterine eggs were spherical, thick-walled, and 41-50 µm in diameter. The caudal end had two small, lateral papilla-like projections.

3.2. Ultrastructure

The cephalic end was rounded in both sexes, with a small, triangular oral aperture surrounded by 14-minute cephalic papillae in two circles: an outer circle of four submedian pairs and an inner circle of six single papillae (four submedian and two lateral). A pair of small lateral amphids was located posterior to the lateral cephalic papillae. The cuticle had numerous inflated bosses, irregularly distributed on the body, and was densely transversely striated. The male's posterior extremity was blunt with a broad U-shaped mound lateral and dorsal to the cloacal opening, with one pair of small, very flat precloacal papillae and two postcloacal papillae on the mound. Phasmids were present in the middle of each mound arm. The female's posterior extremity was rounded with two small sublateral papillae-like caudal projections. Uterine eggs were oval and surrounded by a thin-walled shell.

3.3. Taxonomic Identification

Specimens were identified as belonging to the family Philometridae Baylis and Daubney, 1926, characterized by an elongated body, a simple mouth without lip-like structures, the absence of a vulva, vagina, and anus in adults, and relatively short ovaries at opposite ends of the body [6]. The males were smaller than females with equal spicules and a present gubernaculum. The uterus was viviparous, containing two species. The family Philometridae includes three subfamilies from marine fishes: Neophilometroidinae Moravec, Salgado-Maldonado, and Aguilar-Aguila, 2002; Phlyctainophorinae Roman, 1965; and Philometrinae Baylis and Daubney, 1926 [5, 19]. The current specimens belonged to the subfamily Philometrinae, which includes 11 genera, identified by the following criteria: larger females, filiform body, rounded anterior and posterior extremities, a mouth without lips, and the presence of head, tail papillae, and ventriculus [6]. Males had equal, slender spicules, a present gubernaculum, and a uterus occupying most of the body [7].

3.4. Comparative Analysis

Several species of Philometra have been reported from the gonads of serranid fishes, including *P. managatuwo* from *E. septemfasciatus* off Japan [20], *P. serranellicabrillae* [21], *P.* jordanoi from Serranus cabrilla and *E. marginatus* [22], and others from various hosts and regions [23, 34]. Although the present specimens shared some morphological features with other gonad-infecting Philometra species, distinct differences were noted. These differences included variations in the caudal papillae, the shape of the caudal mound, and specific measurements Table 1.

The redescription of *P. cyanopodi* from new fish hosts holds several significant implications for the fields of parasitology and fish biology. Here are some key points highlighting the importance of this study:

1. Taxonomic Confirmation: This study provides a detailed

Table 1: Comparison between *P. cyanopodi* (Moravec and Jus-tine, 2008) of the present specimens and previously described forms (all measurements are in mm unless mentioned in µm).

Characteristic	Moravec and Justine, 2008 [31]	Present Study
Fish host(s)	*E. cyanopodus* (Family: Serranidae)	*E. chlorostigma* and *V. louti* (Family: Serranidae)
Locality	off Nouméa, New Caledonia	Hurghada, Egypt, Red Sea
Measured parasite number	19 (9♂, 10♀)	20 (18♂, 2♀)
Site of infection	Gonads (ovaries)	Gonads (ovaries)
Male measurements:		
Body length	2.72-3.59 mm	1.900-3.510 mm
Maximum body width	54-82 µm	0.040-0.111 mm
Esophagus length	654-765 µm	0.470-0.654 mm
Esophagus length/body length (%)	19-24%	15-19%
Maximum esophagus width	30-36 µm	0.022-0.040 mm
Esophageal nucleus from anterior extremity	408-558 µm	0.233-0.332 mm
Nerve ring from anterior extremity	195-243 µm	0.130-0.190 mm
Excretory pore from anterior extremity	233-286 µm	0.148-0.220 mm
Length of spicules	183-228 µm	0.100-0.210 mm
Spicules length/body length (%)	6-8%	5.2-7.3%
Gubernaculum length	129-162 µm	0.095-0.165 mm
Anterior part of the gubernaculum length	39-63 µm	35-65 µm
Anterior part of gubernaculum length/Entire gubernaculum length (%)	30-39%	36-39%
Gubernaculum and spicules length ratio	1:1.4	1:1.05-1.30
Female measurements:		
Body length	12.85-16.43 mm	15.670 mm
Maximum body width	0.274-0.367 mm	0.304 mm
Esophagus length	1.07-1.31 mm	1.350 mm
Esophagus length/body length (%)	7-8%	9%
Maximum esophagus width	0.107-0.126 mm	0.133 mm
Esophageal nucleus from anterior extremity	0.86-1.03 mm	0.923 mm
Nerve ring from anterior extremity	0.2-0.24 mm	0.250 mm
Ventriculus length	0.03 mm	0.030 mm
Ventriculus width	0.07 mm	0.070 mm
Egg diameter	40-45 µm	41-50 µm
Uterus	Fills the entire body	Fills the entire body
Intestine	Brown	Dark brown

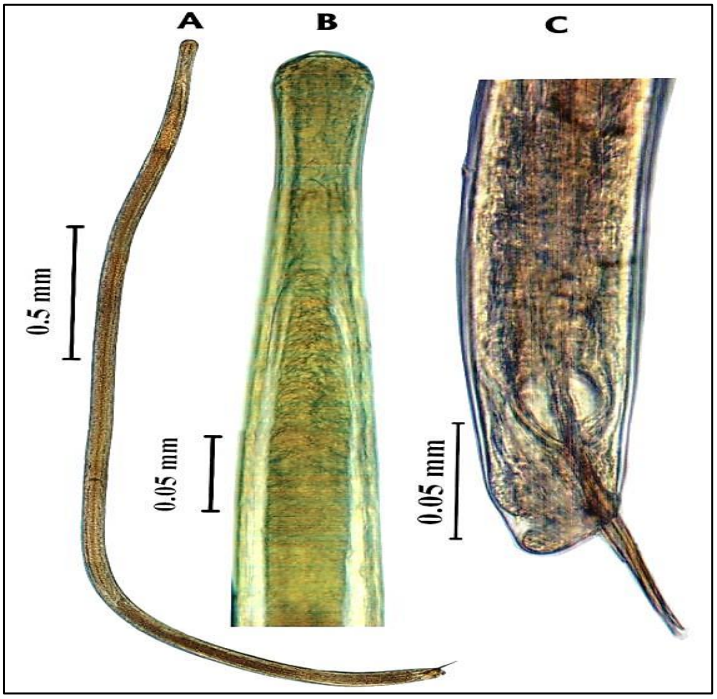

Fig.1: Photomicrographs of an adult male of P. cyanopodi infecting V. louti. A. Whole nematode. B. Lateral view of the anterior extremity of the nematode. C. Lateral view of the posterior extremity of the nematode.

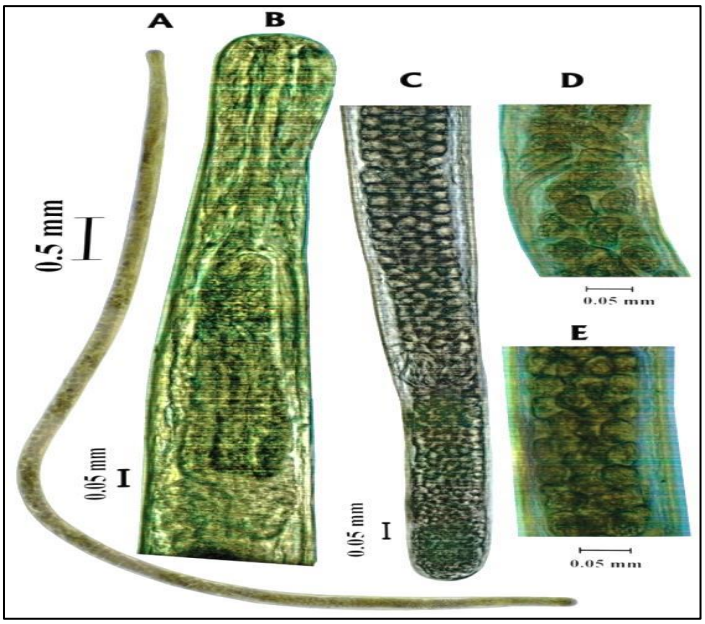

Fig. 2: Photomicrographs of subgravid female of *P. cyanopodi* infecting *V. louti*. A. Whole nematode. B. Lateral view of the anterior extremity of the nematode. C. Lateral view of the posterior extremity of the nematode. D., E. Lateral view of the uterus filled with eggs.

and updated taxonomic description of *P. cyanopodi*. By redescribing this nematode parasite, the research enhances the accurate identification and classification of the species, improving our understanding of its morphological characteristics and life cycle. This detailed description is crucial for *distinguishing P. cyanopodi* from other closely related species.

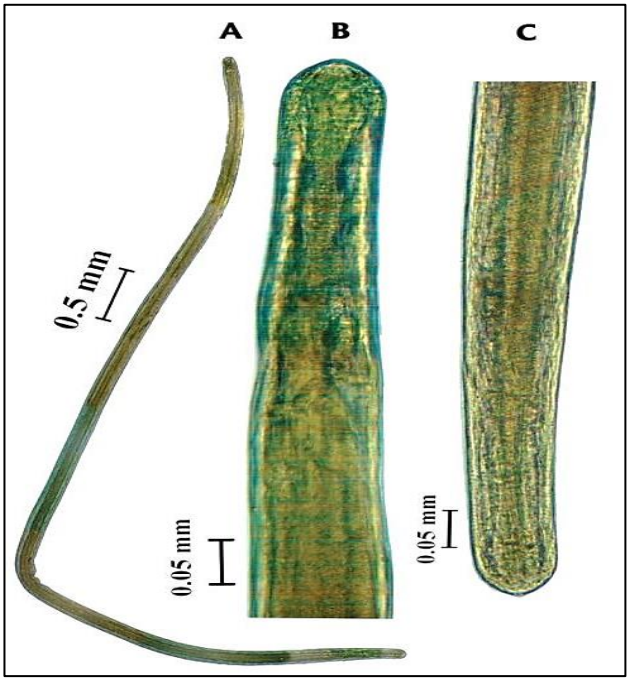

Fig. 3: Photomicrographs of nongravid female of *P. cyanopodi* infecting *V. louti*. A. Whole nematode. B. Lateral view of the anterior extremity of the nematode. C. Lateral view of the posterior extremity of the nematode.

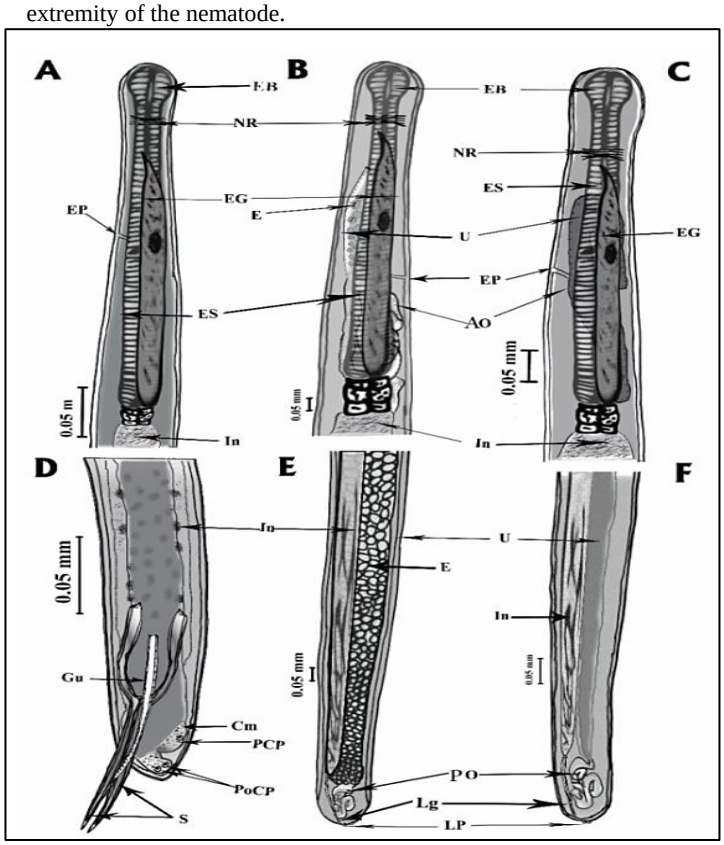

Fig. 4: Camera lucida drawing of *P. cyanopodi* infecting *V. louti*. A. Lateral view of the male anterior extremity. B. Lateral view of the subgravid female anterior extremity. C. Lateral view of the non-gravid female anterior extremity. D. Lateral view of the male posterior extremity. E. Lateral view of the non-gravid female subgravid female posterior extremity. F. Lateral view of the non-gravid female posterior extremity. (EB) Esophageal Bullous, (NR) Nerve Ring, (ES) Esophagus, (EG) Esophageal Gland, (EP) Excretory Pore, (E) Egg, (U) Uterus, (AO) Anterior Ovary, (In) Intestine, (Gu) Gubernaculum, (Cm) Caudal mound, (PCP) Pre-Cloacal Papilla, (PoCP) PostCloacal Papilla, (PO) Posterior Ovary, (Lg) Ligament, (S) Spicule, (LP) Lateral Papilla.

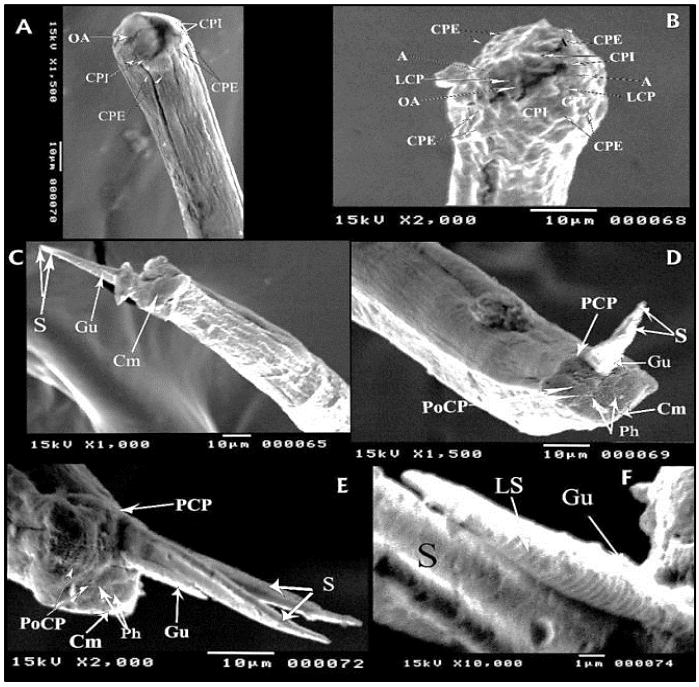

Fig. 5: SEM photomicrographs of the adult male of *P. cyanopodi* infecting *V. louti*. A. Lateral view of anterior extremity of the male. B. Apical view of high magnification of the cephalic end. C. Lateral view of the posterior extremity of the male. D. Ventral view of posterior extremity of the male. E. High magnification of ventral view of the caudal mound & spicules of the male. F. High magnification ventrolateral view of the lamellate-like structure and part of the spicule of the male.

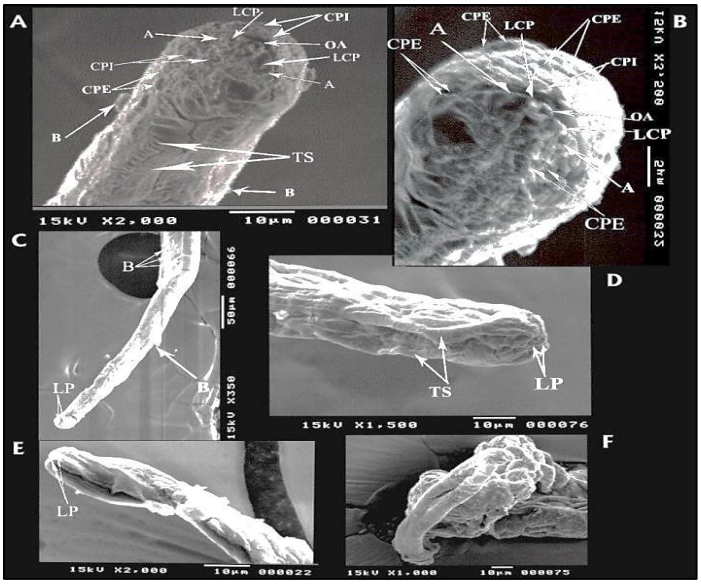

Fig. 6: SEM photomicrographs of the subgravid & nongravid females of *P. cyanopodi* infecting *V. louti*. A. Lateral view of the subgravid female anterior extremity. B. Apical view of high magnification of the subgravid female cephalic end. C. Lateral view of the subgravid female posterior extremity. D. High magnification of ventral view of posterior extremity of the subgravid female. E. Lateral view of the non-gravid female posterior extremity. F. Section of the uterus of the

subgravid female. (CPI) Cephalic Papilla Internal, (CPE) Cephalic Papilla External, (OA) Oral Aperture, (A) Amphid, (LCP) Lateral Cephalic Papilla, (PCP) Pre-Cloacal Papilla, (S) Spicule, (Gu) Gubernaculum, (Cm) Caudal mound, (PoCP) PostCloacal Papilla, (Ph) Phasmids. (LS) Left spicule, (B) Bosses, (TS) Transverse Striations, (LP) Lateral Papilla.

2. Host-Parasite Relationship: Identifying *P. cyanopodi* in new fish hosts, *E. chlorostigma* and *V. louti*, broadens our knowledge of host-parasite interactions. It confirms the ability of *P. cyanopodi* to parasitize a wider range of fish species, providing valuable insights into its host specificity and ecological interactions. Understanding these relationships is essential for comprehending the parasite's adaptability and the potential impacts on different fish populations.

3. Geographic Distribution: Reporting the presence of *P. cyanopodi* in the Red Sea, specifically in Hurghada, Egypt, expands our understanding of the parasite's geographic distribution. This information is crucial for mapping the distribution patterns of parasitic organisms and studying the factors that influence their prevalence and abundance in various regions. Knowledge of geographic distribution helps in assessing the risk of parasitic infections in different fish populations.

4. Ultrastructural Features: This study provides enhanced details on the morphological description and fine structure of *P. cyanopodi* using Scanning Electron Microscopy (SEM). These ultrastructural features contribute to a more comprehensive understanding of the parasite's anatomy, aiding in accurate identification and comparison with other species. The detailed morphological data also facilitate future studies on the functional biology of the parasite.

5. Host-Parasite Dynamics: Discovering *P. cyanopodi* in *E. chlorostigma* and *V. louti* contributes to our understanding of host-parasite dynamics within the Red Sea ecosystem. It sheds light on the susceptibility of specific fish species to parasitic infections and provides insights into the potential impacts of the parasite on the health and ecology of the host populations. Understanding these dynamics is vital for managing fish health and conserving biodiversity in marine environments. Future studies focusing on the ecological implications, pathogenicity, and host-specificity of *P. cyanopodi* would be valuable in assessing its role and significance within the Red Sea marine environment. Continued efforts in taxonomic investigations and parasite-host relationships will enhance our understanding of the complex interactions between parasitic nematodes and their fish hosts, ultimately contributing to the field of marine parasitology and the overall health of marine ecosystems. The observed morphological variations in *P. cyanopodi* found in the new host species, *E. chlorostigma* and *V. louti*, from the Red Sea region, can provide valuable insights into nematode adaptation and evolution. Here are some ways these findings can inform our understanding:

6. Host-Driven Adaptation: The morphological variations observed in *P. cyanopodi* compared to the original description may reflect the parasite's ability to adapt to the specific environmental conditions and host characteristics of the new fish species. Nematodes can exhibit phenotypic plasticity, allowing them to modify their morphology to better suit the resources and microhabitats available within different host species [35, 36].

7. Diversification and Speciation: The morphological differences could suggest the potential for divergence and speciation within the *P. cyanopodi* species complex. Parasites may undergo adaptive radiation and evolve distinct morphological forms to exploit new host resources, eventually leading to the emergence of new species or subspecies [37, 38].

8. Geographical Isolation and Genetic Differentiation: The discovery of *P. cyanopodi* in a new geographical region, the Red Sea, may have resulted in genetic and morphological differentiation due to isolation from the originally described populations. Geographical barriers can promote the accumulation of genetic and phenotypic variations over time, leading to the observed morphological variations [39, 40].

9. Niche Expansion and Ecological Adaptations: The ability of *P. cyanopodi* to infect new host species suggests its capacity for niche expansion and ecological adaptations. The morphological changes may reflect the parasite's efforts to overcome host defenses, optimize resource utilization, and enhance transmission success in the new host environments [41, 42].

10. Evolutionary Trajectories and Coevolution: The observed variations in *P. cyanopodi* can contribute to our understanding of the evolutionary trajectories and coevolutionary dynamics between parasitic nematodes and their fish hosts. These interactions can drive the selection of specific morphological traits that confer advantages in exploiting new host resources or evading host immune responses [43, 44].

4. Conclusion

The morphological and ultrastructural characterization of *P. cyanopodi* from the gonads of E. *chlorostigma* and *V. louti* in the Red Sea, Egypt, has been provided. This study marks the first record of *P. cyanopodi* in these hosts and geographical regions, expanding the known distribution and host range of this nematode species. The observed morphological variations compared to the original description underscore the importance of detailed taxonomic studies for accurate species identification. In conclusion, the redescription of *P. cyanopodi* from new hosts in the Red Sea offers significant contributions to taxonomy, host-parasite relationships, geographic distribution, ultrastructural characterization, and the dynamics of parasitic infections. These findings are essential for enhancing our knowledge of parasitic nematodes and their interactions with marine fish hosts, contributing to the broader field of marine parasitology and ecosystem health.

Credit authorship and contribution statement:

Author Contributions: M.E. and Z.A.; methodology, software, R.K.; validation, R.K. and T.H.; formal analysis, M.E., and Z. A.; investigation, Z.A.; resources, M. E. and Z.A.; data curation, M.E. and Z.A.; writing-original draft preparation, T.H.; writing review and editing, R.K. and T. H.; visualization, R. K., T.H., and M. E.; supervision, M. E. and Z.A.; project administration, M.E. and Z.A.; funding acquisition, Sohag University. All authors have read and agreed to the published version of the manuscript."

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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