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Light Microscopic Description of *Neocamallanus singhi* and *Buckleynema buckleyi* third Larval Stages Infecting Labridae and Atherinidae Hosts of The Red Sea in Egypt

Sahar El-Ganainy¹, Zeinab Adam², Asmaa Adel^{3*}, Kareem Morsy² and Manal Ahmed¹

1-Departments of Zoology, Faculty of Science, , Minia University, Minya, Egypt

2-Departments of Zoology, Faculty of Science, Cairo University, Cairo, University

3- Zoology Department, Faculty of Science, South Valley University, Qena, Egypt

E-mail: asas_zoology@yahoo.com

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ABSTRACT

During a survey on helminth parasites infecting marine fishes captured from water locations at Abu-Qalawa and Saad reef regions along Hurgada coasts of the Red Sea in Egypt during the period from June to December 2017, two different species of nematode parasites were isolated as a new host and locality records. These were *Neocamallanus singhi* (L3, f: Camallanidae) and *Buckleynema buckleyi* (L3, Quimperidae) isolated from *Cheilinus undulatus* (Family: labridae, no. 34) and *Atherina boyeri* (Family: Atherinidae, no. 18) respectively. The parasites were described morphologically by light microscopy. They were compared with similar species isolated previously from different hosts worldwide. The study concluded that marine fishes species of the Red Sea should receive much attention for parasitic species infecting fishes in order to collect a lot of data concerning parasitic worms which could be transferred to humans causing dangerous diseases.

INTRODUCTION

Rapidly growing populations and food shortage constitute the most important problems all over the world especially in Africa, so that scientists still trying to find solutions for these problems (Morsy *et al.*, 2013). Fishes play an important role in human nutrition and fish farming, apparently offers a solution to the problem of the increasing human population (Maghrabi *et al.*, 2010), it is one of our most valuable sources of protein food. Animal protein is the most valuable element of nutrition due to its content of nearly all of the essential amino acids necessary for man and animals (Morsy *et al.*, 2013). Fisheries in the Red Sea are of considerable socio-economic importance to the Red Sea countries in terms of national food security and income generation for rural communities (Hossam, 2012). The study of fish parasites has attracted the attention of many parasitologists, since they not only cause injuries or even death to the fish (Davis *et al.*, 1961), but also do some human health problems (Williams and Jones, 1976). Nematode parasites are common parasites in freshwater and marine fish. It is worth mentioning that, fish nematodes represent an important public health problem, because fishes may be a source of serious nematode diseases to man as anisakid worms. These constitute one of the earliest known groups of helminthes in fishes and they infest marine, brackish and fresh-water fishes. Although nematodes have been collected from many tissues and organs within fishes, adult nematodes are moderately site-specific within fishes.

Adult cucullanid, gnathostomatid and anisakid nematodes are typically found in the digestive tract (Olsen, 1974; Moravec and Nagasawa, 2000). Members of the genus *Buckleyinema* are parasitic in the intestine of siluroid fishes. The genus *Buckleyinema* was erected by Ali and Singh (1954). Sood (1988) listed a few species of the genus *Buckleyinema* namely *Buckleyinema buckleyi*, Ali and Singh (1954) from the intestine of *Mystuscavasius* from Hyderabad, Andhra Pradesh; *B. singhi* from the stomach of *Mystus seenghala* from Gorakhpur; U. P., was added by; *B. channaif* from the intestine of *Channa striatus* from Bihar; *B. notopterif* from the intestine of *Notopterus notopterus* from Bihar. Other species *B. baylisif* from *Heteropneustes fossilis*; *B. eutropiichthysi* Gupta and Naqvi (1986) from *Eutropiichthys vacha*; *B. kharei* Misra and Tewari (1986) from *Bagarius bagarius* are also known. Taxonomy of members of Camallanidae Railliet and Henry, 1915 based mainly on the presence of tridents, was created by Railliet and Henry (1915). Ali (1957) established the genus *Neocamallanus* mainly on the basis of the absence of tridents or rods. Yeh (1960), however, did not accept the validity of the latter genus, instead preferred to split *Camallanus* into four genera, *Camallanus*, *Piscillania*, *Serpinema*, and *Zeylanema*, of which only the first and last were accepted by Kalyankar (1971), and Kalyankar and Palladwar (1977); and the first and third by Chabaud (1975), and Petter (1979). This paper documented for the first time the prevalence of two nematode parasites belonging to families Camallanidae and Quimperiidae isolated from marine fishes of families Labridae and Atherinidae in Egypt. Also, morphological identification of these species was carried out by means of light microscopy.

MATERIALS AND METHODS

A total of fifty-two specimens of the marine fishes *Cheilinus undulatus* (Family: labridae, no. 34) and *Atherina boyeri* (Family: Atherinidae, no. 18) were collected

by fishermen at Abu-Qalawa and Saad reef regions at Hurghada City of the Red Sea in Egypt during the period from June to December 2017. Fishes have transported a life or in an insulated icebox filled with ice immediately to the National Institute of Oceanography and Fisheries (N.I.O.F) at Hurghada city or some times to the Parasitology Laboratory, Zoology Department, Faculty of Science, Cairo University, Cairo. Fishes were identified and classified according to (Froese and Pauly, 2003). In the laboratory, the stomach and intestine of the collected fishes were dissected, opened longitudinally in 0.7 % NaCl saline solution, and examined for the presence of any Nematode parasites under a Leica S6D stereozoom microscope (Leica, Wetzlar, Germany). Hot 70% ethyl alcohol was the best fixative for nematodes. Following 12 hours, the fixative was replaced by Lactophenol which is poured to cover the preserved nematodes and left for at least 24 hours allow clearing. This chemical is a useful clearing and treating reagent where it is an amount of high refractive index for temporary preparations and is used for detailed nematode examination without staining. measurements of worms were expressed as a range in millimeters (mm) with a mean \pm SD in parentheses.

RESULTS

Neocamallanus singhi (L3) Ali, 1957

Description:

The body of the recorded larvae measured a total length of 0.437–0.514 (0.480 \pm 0.002) mm in length and 0.192–0.281 (0.213 \pm 0.002) mm in width. The cuticle was thin with indistinct transverse striations. The anterior end was relatively wide, hemispherical in outline without dorsal tooth. The anterior end of the oesophagus was covered with a thin hyaline, sclerotized "cap" which enlarged in older larvae to form abell- shaped formation giving rise to buccal capsule. The oesophagus was 0.072–0.189 (0.131 \pm 0.002) mm long, not distinctly divided into muscular and glandular parts.

The posterior part of oesophagus was slightly broadened. The oesophagus intestinal junction was guarded by distinct valves. The nerve ring and excretory pore lied at a distance of 0.041–0.047 (0.043 ± 0.002) mm and 0.032–0.037 (0.033 ± 0.002) mm, respectively, from the anterior end. The intestine was wide and straight without orange coloration. The rectum was a thin-walled, narrow tube with distinct rectal glands. The tail was conical and measured a length of 0.043–0.069 (0.052 ± 0.002) mm with a distinct anus which was 0.031–0.035 (0.032 ± 0.002) mm from the posterior part. The genital primordium was not detected.

Taxonomic summary:

Family: Camallanidae Railliet and Henry, 1915

Host: (humphead wrasse) *Cheilinus undulatus* (Family: labridae)

Infection site: intestine.

Type locality: Coasts of Hurghada city along the Red Sea, Egypt.

Prevalence: 17 out of 34 fish specimens with a percentage of (50%) were found to be naturally infected. The infection was generally increased during summer (7/11 (63.63%)) and decreased during winter (10/23 (43.47%)).

***Buckleynema buckleyi* (L3)** Ali and Singh, 1954

Description:

Body was 4.91–7.11 (5.14 ± 1.01) mm long and 0.163–0.198 (0.174 ± 0.002) mm wide; the head was bluntly rounded, with cuticular cephalic inflation; the mouth was bounded by triangular cuticular rim; cephalic papillae and "amphids" was not prominent; submedians prominent appearing on the head like knobs between which amphids situated laterally; oesophagus was 0.284–0.354 (0.315 ± 0.002) mm long, club-shaped, and with a pair of uninucleate glands with granular cytoplasm in its posterior half; nerve ring was 0.128–0.181 (0.143 ± 0.002) mm, and excretory pore was 0.48–0.62 (0.58 ± 0.02) mm from the anterior end; the tail was 0.17–0.28 (0.23 ± 0.02) mm long, sharply pointed with slightly developed caudal alae; gubernaculum "V" shaped; caudal papillae on membranous flap projected from the anterior border of anal aperture were observed.

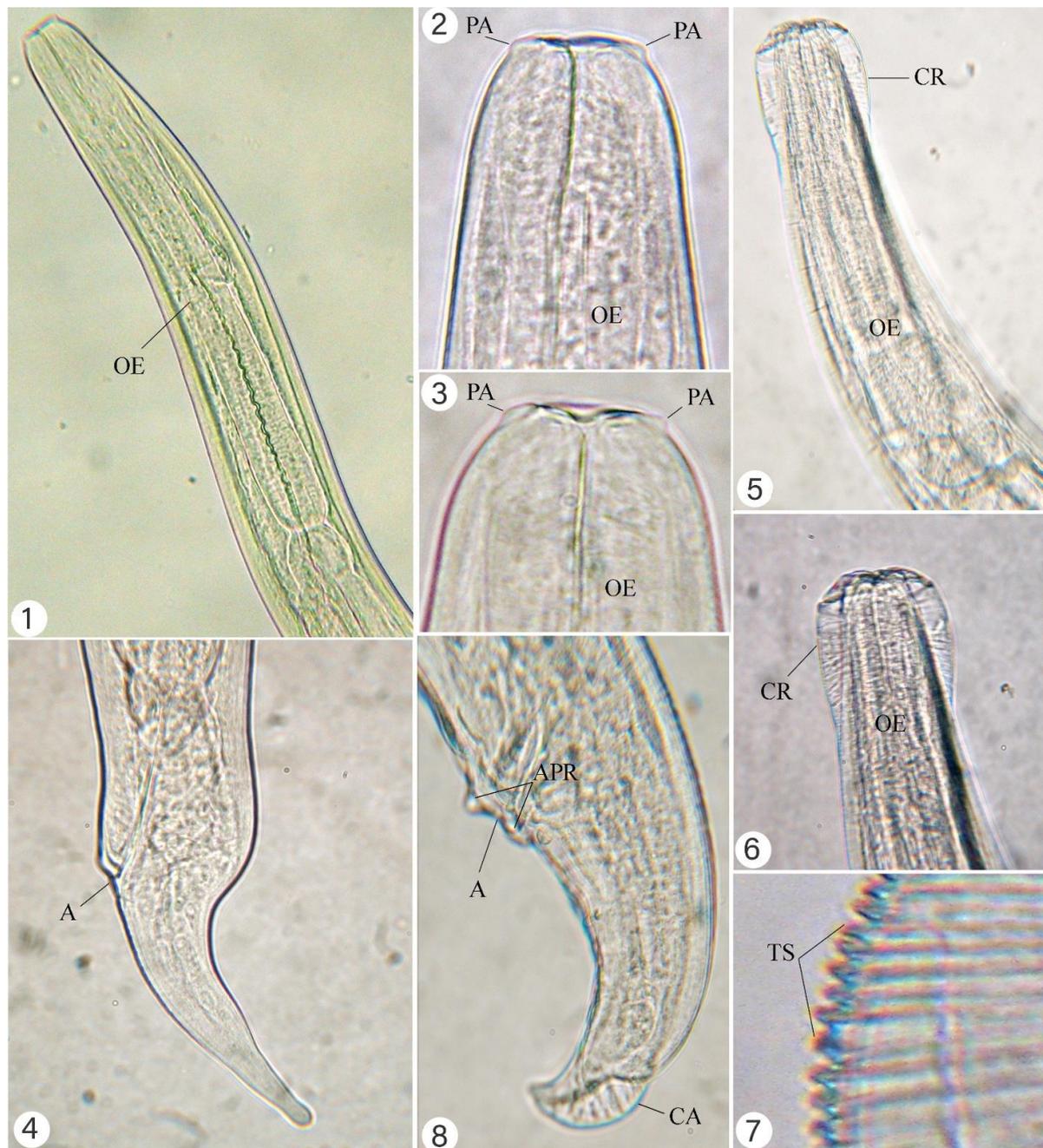
Taxonomic summary:

Family: Quimperiidae

Host: (big-scale sand smelt) *Atherina boyeri* (Family: Atherinidae)

Type locality: Coasts of Hurghada city along the Red Sea, Egypt.

Prevalence: 7 out of 18 fish specimens with a percentage of (38.88 %) were found to be naturally infected. The infection was generally increased during winter (6/13 (46.15%)) and decreased during summer (1/5 (20%)).



Figs. 1-4: Photomicrographs of the *Neocamallanus singhi* third larval stage infecting the humphead wrasse fish *Cheilinus undulatus* showing high magnification of: **1:** most of the worm body from the anterior part with a prominent oesophagus (OE). **2, 3:** the anterior part with a prominent papilla (PA) and oesophagus (OE). **4:** posterior part of the worm with the anus (A).

Figs. 5-8: Photomicrographs of *Buckleynema buckleyi* larvae infecting the big-scale sand smelt *Atherina boyeri* showing high magnification of: **5, 6:** the anterior part of the worm supported by the cuticular rim (CR) and an oesophagus (OE). **7:** transverse striations of cuticle (TS). **8** posterior end of the worm terminated at a caudal ala (CA) and anal pore (A) was surrounded by anal process (APR).: (Figs.1, 2, 3X240;4, 5, 6X60; 7X300; 8X160.).

DISCUSSION

Neocamallanus singhi isolated from the intestine of (humphead wrasse) *Cheilinus undulatus* recovered from the Red Sea water at Hurghada City in Egypt. The described parasite is consistent with *Neocamallanus singhi* larvae studied by De *et al.* (1984) from *Channa striata* in Burdwan, West Bengal, India. Both are characterized by a thin cuticle with indistinct transverse striations, the anterior end was relatively wide, hemispherical in outline without dorsal tooth; a short, weakly sclerotized buccal tube, the posterior part of oesophagus was slightly broadened, the tail was conical and the genital primordium was not detected. Comparing between the two species, it was observed that both species more or less similar the difference may be related to the pressure occurred during preparation. The present parasite is considered as new in host and locality records in Egypt. *Buckleyinema buckleyi* isolated from the intestine of the big-scale sand smelt *Atherina boyeri*. It has all the characteristic features of genus *Buckleyinema* in the following criteria, the head was bluntly rounded, with cuticular cephalic inflation; the mouth was bounded by triangular cuticular rim; cephalic papillae and "amphids" was not prominent; submedians prominent appearing on the head like knobs between which amphids situated laterally. Since there is no available data on the species of this genus, the only species that resemble the present nematode are *B. buckleyi* and *B. channai*. The present specimens resemble *B. channai* in the presence of 3 pairs of teeth in buccal cavity, club-shaped oesophagus, postequatorial vulva, and pointed tail but differ in the length of oesophagus, location of nerve ring, excretory pore, and body measurements. It is morphologically & morphometrically similar to the *B. buckleyi* in the presence of oesophagus which was club-shaped with a pair of uninucleate glands with granular cytoplasm in its posterior half; the tail was long, sharply pointed with slightly developed

caudal alae; gubernaculum "V" shaped; caudal papillae on membranous flap projected from the anterior border of anal aperture were observed. Because of the non-availability of male-specific identification is not possible. Hence the present specimens are regarded as *Buckleyinema* sp. is new host records for this genus from Egypt.

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ARABIC SUMMARY

وصف بالمجهر الضوئي لليرقة الثالثة من طفيلي الديدان الخيطية نيوكامالانس سينجاي وبوكلينيما بوكلي التي تصيب الاسماك البحرية من البحر الأحمر في مصر

سحر الجنائني¹، زينب آدم²، أسماء عادل³، كريم مرسي²، منال أحمد¹
¹ قسم علم الحيوان كلية العلوم جامعة المنيا،
² قسم علم الحيوان كلية العلوم جامعة القاهرة،
³ قسم علم الحيوان كلية العلوم جامعة جنوب الوادي

في الدراسة الحالية تم عمل مسح طفيلي للديدان الخيطية التي تصيب تصيب الاسماك البحرية التي تم تجميعها من المياه في منطقتين على طول سواحل الغردقة بالبحر الأحمر في مصر خلال الفترة من يونيو إلى ديسمبر 2017. تم عزل نوعين مختلفين من طفيليات النيماطودا كأنواع جديدة في إصابتها لهذة الأنواع من الاسماك ولقد تم في هذة الدراسة تجميع عدد 34 سمكة من كينيليس انديولاتس حيث وجدت الإصابة ببرقات نيوكامالانس سينجاي في 17 (50%) من هذه الاسماك. وجمع عدد 18 من سمك الأزرينا بوري حيث وجدت الإصابة ببرقات بوكلينيما بوكلي في 7 (38.88%). تم وصف الطفيليات شكليا بالمجهر الضوئي. تمت مقارنتها مع الأنواع المماثلة التي تم عزلها سابقاً من عوائل مختلفة. وخلصت الدراسة إلى أن أنواع الاسماك البحرية في البحر الأحمر يجب أن تحظى باهتمام كبير للأنواع الطفيلية التي تصيب الاسماك وذلك لجمع الكثير من البيانات المتعلقة بالديدان الطفيلية التي يمكن أن تنتقل إلى الإنسان مسببة الأمراض الخطيرة.