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Light and Scanning Electron Microscopy on *Cucullanus aliyaii* Akhtar and Mujib (2012) (Nematoda: Cuculanidae) From the Rabbitfish *Siganus canaliculatus* of The Red Sea, Egypt

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ABSTRACT

In the present study, new host and locality records of *Cucullanus aliyaii* Akhtar and Mujib (2012), a cuculanid nematode isolated from the intestine of the rabbitfish, *Siganus canaliculatus* collected from the Red Sea, coasts of Hurghada city, Egypt during the period from January to September 2017. Fifteen out of 55 (27.3%) of the examined fish were infected. Worms were recorded by naked eyes on the flesh, stomach, intestines, as well as body cavities of the infected fish. Light and scanning electron microscopy revealed that the isolated worms possessed the important criteria characteristic for members of family Cuculanidae: dimensions of oesophagus, spicules, mucron, size of pseudobuccal capsule, eggs, the position of deirids, excretory pore and vulva, numbers and arrangement of caudal papillae, host groups and zoogeographical regions. The recorded parasite was whitish and small-sized, female worms were 9.40 ± 2 (7.4-10.3) x 2.5 ± 0.2 (2.00-2.60) mm while male worms were 6.30 ± 0.02 (6.00-8.30) mm x 1.60 ± 0.02 (1.59-1.73) mm. Oral aperture was dorsoventrally elongate, slit-like, surrounded by distinct collarette, beared one row of small denticles on its inner surface; four submedian cephalic papillae and a pair of prominent lateral amphids present. Oesophagus was of two distinct portions: anterior pseudobuccal capsule measured 0.50 ± 0.02 (0.47-0.053) mm in length, narrowing immediately below nerve ring; and posterior with a club-shaped muscular structure measured 0.45 ± 0.02 (0.41-0.49) mm in length

INTRODUCTION

Nematodes represent the most frequent and the most important parasites of fishes in the freshwater, brackish-water and marine environments throughout the world (Moravec, 2007). Although many species were recorded worldwide, the progress in the research of fish nematodes has been achieved during recent years. Adult nematodes are found in the intestine of fish, while larval stages are sometimes found on the flesh and viscera causing severe diseases and economical losses (Moravec *et al.*, 2005, Timi & Lanfranchi, 2006 and Moravec *et al.*, 2008). The larval stages are infective to humans and have the greatest impact on consumer acceptance of fish as a source of protein (Moravec, 1994 and Dick & Choudhury, 1995).

The genus *Cucullanus* Müller (1777) includes over 100 nominal parasitic species in marine and freshwater fishes, and in aquatic turtles (Dick & Choudhury, 1995, Costa *et al.*, 2004, Moravec *et al.* 2005, 2007, Timi & Lanfranchi 2006 and Kinoshita *et al.* 2014). Taxonomically, the morphological characters such as dimensions of the body, oesophagus, spicules, size of pseudobuccal capsule, eggs, the position of deirids, excretory pore, vulva, numbers, and arrangement of caudal papillae, host groups and zoogeographical regions are important criteria for differentiating and identifying *Cucullanus* species (Moravec *et al.*, 1993, 1997, 2005, Caspeta-Mandujano *et al.*, 2000 and Li *et al.*, 2015). During a recent survey on parasitic helminths infecting marine fishes, nematode parasites were isolated from the rabbitfish *Siganus canaliculatus* (Perciformes: Siganidae) from the Red Sea, coasts of Hurghada city, Egypt during the period from January to September 2018. They were identified morphologically and morphometrically by light and scanning electron microscopy.

MATERIALS AND METHODS

A total of fifty-five specimens the rabbitfish *Siganus canaliculatus* (Perciformes: Siganidae) were randomly collected from the Red Sea, coasts of Hurghada city, Egypt during the period from January to September 2017. Fish were transported alive to the laboratory using small containers containing seawater with aeration. Fish were dissected and examined thoroughly for nematode endoparasites using a stereomicroscope. Worms were isolated and washed in a physiological saline 0.6 % followed by relaxation and fixation in 70% hot ethyl alcohol. They were cleared and then mounted in lactophenol. Photomicrographs were taken using Zeiss Axiovert 135 microscope supplied with a Canon Digital Camera. Measurements were presented as mean±S.D. (range). For scanning electron microscopy, specimens were fixed in 4% buffered glutaraldehyde, washed in

cacodylate buffer, and dehydrated in alcohol. After passing through an ascending series of Genosolv-D, specimens were processed in a critical point drier “Bomer-900” with freon 13, sputter-coated with gold-palladium in a Technics Hummer V, and examined with an Etec Autoscan at 20 kV Jeol scanning electron microscope.

RESULTS

Cucullanus aliyaii Akhtar and Mujib (2012)
Nematoda: Cuculanidae

15 out of 55 specimens (27.3%) of the examined fish were found to be naturally infected with nematode parasites. The infection was recorded in the intestine of the host fish. The adult worms were relatively small, slender, whitish with a broad anterior end. Oral opening dorsoventrally elongated, surrounded by narrow membranous flange or collarete and supported by a ring of numerous teeth (Figs.1,2,8,9). Four submedian cephalic papillae and a pair of prominent lateral amphids were observed (Figs.1, 8). Esophagus consisted of two distinct portions: anterior end with sclerotized lining expanded anteriorly to form a pseudobuccal capsule, narrowed immediately below the nerve ring; while the posterior end was muscular with a claviform aspect and opened into the intestine through valves. The pseudobuccal capsule or esophastome was wider than the posterior part of esophagus. The entire esophagus of female worms measured 0.95 ± 0.02 (0.83-1.00) mm in length, length of the pseudobuccal capsule was 0.50 ± 0.02 (0.47-0.053) mm with a maximum width was 0.11 ± 0.02 (0.07-0.13) mm, while in males, the entire esophagus was 0.65 ± 0.02 (0.54-0.89) in length, terminated at a posterior club-shaped muscular part measured 0.45 ± 0.02 (0.41-0.49) mm in length and 0.12 ± 0.02 (0.10-0.15) mm in width. Body covered by cuticle which was transversely striated (Figs.3,11).

Male:

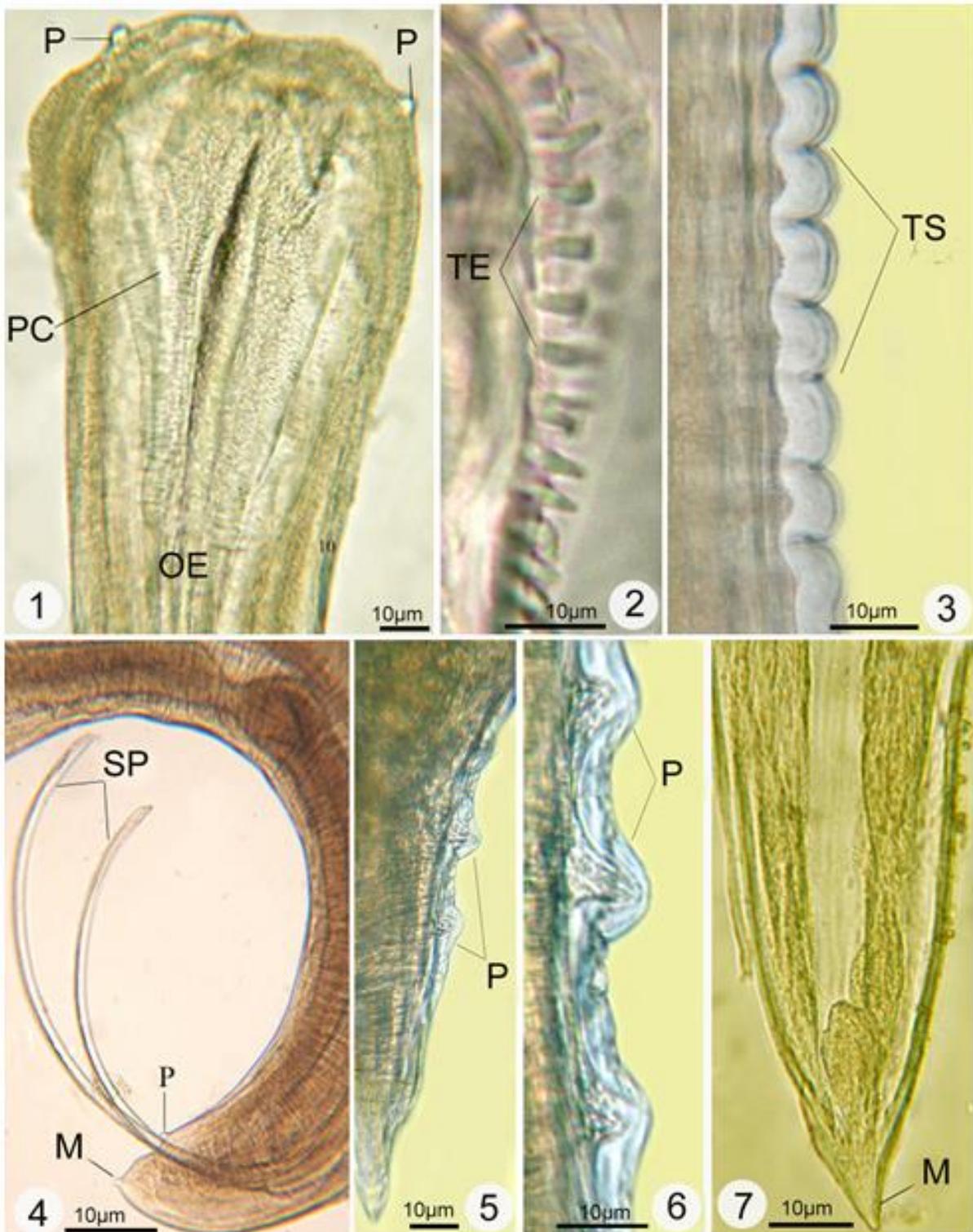
Body smaller than those of female worms, it was about 6.30 ± 0.02 (6.00-8.30) mm long and 1.60 ± 0.02 (1.59-1.73) mm wide with

two sub-equal spicules measured 1.20 ± 0.02 (1.18-1.2) mm long (Fig. 4). Terminal papillae were observed (Fig. 5, 6, 12).

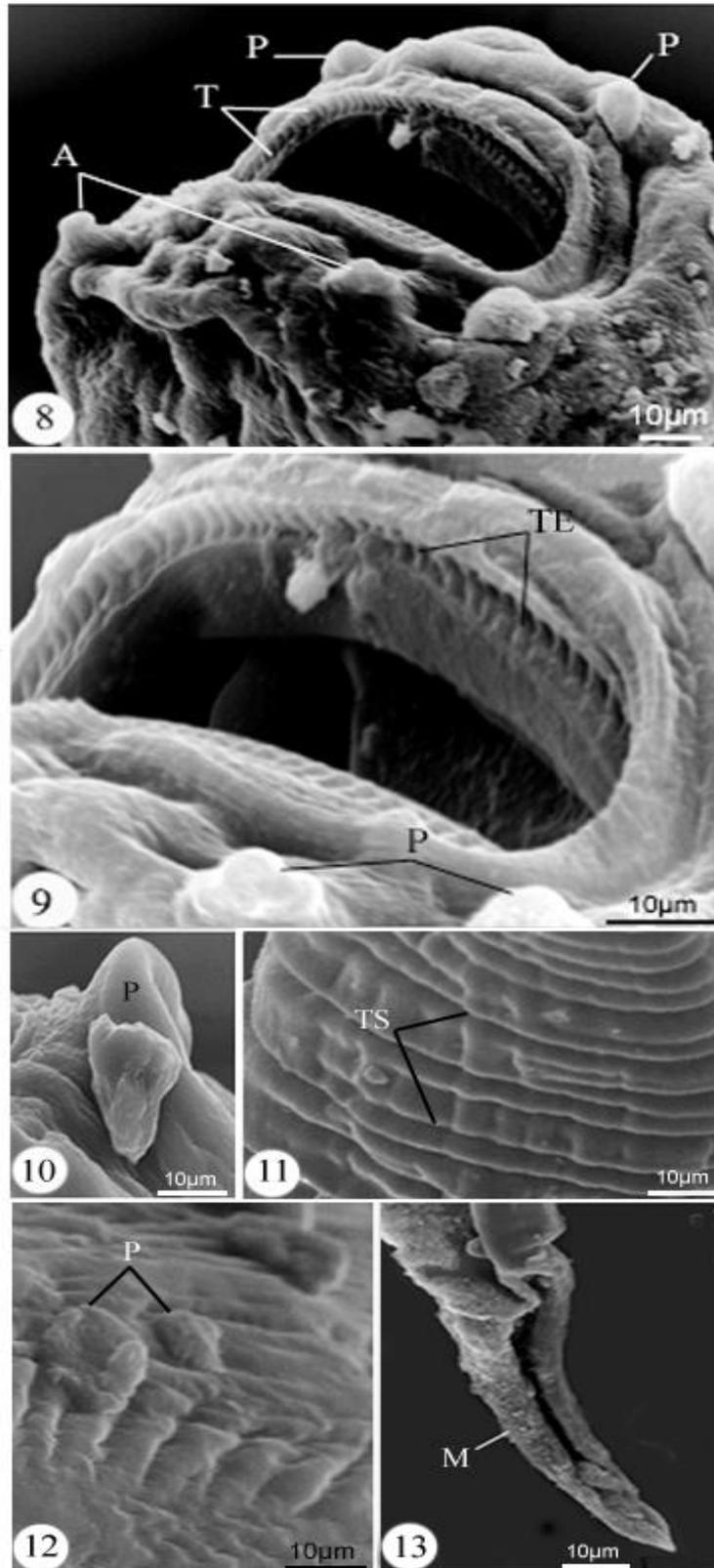
Female:

Body long with pointed posterior end and a broad anterior end measured 9.40 ± 2 (7.4-10.3) mm in length and 2.5 ± 0.2 (2.00-2.60) mm in width, with broadly conical tail or

mucron (Figs. 7, 13). The vulva was post equatorial, with vulvar lips elevated at a distance of 3.80 ± 0.02 (3.82-4.10) mm from the anterior extremity. Vagina long, with muscular tube and directed upward. Uterus thin-walled contained numerous relatively large, thin-shelled eggs.



Figs. 1-7: Photomicrographs of the nematode *Cucullanus aliyai* (F: Cucullanidae) infecting the rabbitfish *Siganus canaliculatus*. **1:** The broad anterior part of the worm as a club shaped pseudobuccal capsule (PC), equipped with cephalic papillae (P), a muscular oesophagus abbreviated as (OE). **2-7:** High magnifications of: **2:** pseudobuccal capsule teeth (TE). **3:** Transverse striations of cuticle (TS); **4:** Posterior end of male with two long spicules (SP) and a short mucron (M); **5, 6:** Caudal papillae (P) of male; **7:** Posterior end of an adult female terminated at a mucron (M).



Figs.8-12: Scanning electron micrographs of the parasite showing high magnifications of: **8, 9:** The anterior part of the worms showing the row of teeth (TE) lining the pseudobuccal capsule. Also, four cephalic papillae (P) and amphids (A) were observed; **10:** Papilla (P); **11:** Transverse striations (TS) of cuticle; **12:** papillae (P) at posterior end of male; **13:** Posterior end of female terminated at a mucron (M).

DISCUSSION

Cucullanidae Cobbold (1864) includes intestinal nematodes characterized by a highly developed buccal cavity formed by the esophagus as described by Berland (1970), and males with or without a pre-cloacal sucker harboring 10–15 pairs of caudal papillae (Maggenti, 1971). About 100 species of *Cucullanus* have been described from fishes of different orders of which at least 70 species were collected from marine or brackish water fishes (González-Solís *et al.*, 2007 and Moravec *et al.*, 2008). Yamaguti (1961) listed 60 species within the genus *Cucullanus* including the genotype. In addition, Gupta and Masoodi (1982) described *C. sootai* and listed another 30 species, including *C. rituali* (Karve, 1952); *C. jaiswali* (Ali, 1956); *C. indica* (Agrawal, 1965); *C. pseudotropi* (Agrawal, 1967); *C. arabianse* (Petter, 1974); *C. tharaponi* (Rasheed, 1968); *C. pangasius* (Soota and Chaturvedi, 1971); *C. jalnaensis*; *C. alii*; *C. malvanae*; *C. tachysuri* (Kalyankar, 1971); *C. bilqeesi*, (Bilqees *et al.*, 1971, Petter, 1974); *C. carioca* (Vicente and Fernandes, 1973); *C. rougetae* (Vicente and Santos, 1974); *C. bagrae* (Petter, 1974); *C. karachii* (Zaidi and Khan, 1975); *C. olivaceus* (Akram, 1976); *C. guerrori* (Arya and Jhonson, 1975); *C. ariusi* (Srivastava and Gupta, 1976); *C. sciaenai* (Gupta and Gupta, 1979); *C. rivulatus* (Soota and Dey Sarkar, 1980); *C. trichiurisi*; *C. simhai* (Gupta and Naqvi, 1983); *C. thapari*; *C. mastacembeli* (Gupta and Srivastava, 1984). Later on more species have been described both from freshwater and marine fishes of various localities including *C. rhamphichthidis* (Moravec, 1979); *C. campanae* (Labre and Petter, 1984); *C. fugianensis* (Wang, 1984); *C. brevispiculus* (Moravec *et al.*, 1993); *C. riograndensis*; *C. fabrigasi* (Fortes *et al.*, 1993a, b); *C. mexicanus* (Capseta-Mandujanoi, 2000). The fish fauna of the Red Sea is highly enriched and nearly 500 species of bony, as well as cartilaginous fish, were recorded from different coasts. The study of the Fish parasites in the Red Sea

waters has been evoked recently for the last three decades. Many investigations have been triggered from the coasts of the Arabian Gulf as (Bayoumy *et al.*, 2008, Khalil *et al.*, 2014 *et al.*, 2016 and Abdel-Baki *et al.*, 2017). According to Moravec *et al.* (1993), the genus *Cucullanus* includes several species that bear many similarities. Due to minute differences in the morphology and because of inadequate descriptions, a detailed comparison among species is very complicated. Therefore, some authors prefer to deal with these parasites according to their host groups (Petter, 1974, Moravec *et al.*, 2005, 2008, González-Solís *et al.*, 2007 and López-Caballero *et al.*, 2009) or their zoogeographical region (Moravec *et al.*, 1997, Sardella *et al.*, 1997, Caspeta-Mandujano *et al.*, 2000, Daniel *et al.*, 2002 and Lafranchi *et al.*, 2004). The data obtained in the present study by light and scanning electron microscopy revealed that the pseudobuccal capsule (oesophastome) of the recorded parasite is wider than the posterior part of the oesophagus, with a continuous row of teeth. Also, the surface nature and morphology of parasitic worms seem to be important in the intricate relationship between these organisms and their hosts, the cuticle has rugae or folds that described as transverse ridges and an external raised incomplete annulations; branched and interrupted on the cuticle surface. The female tail has a pair of sensory papillae situated in a ventrolateral position, which represent the phasmids and they are considered to be comparable to the amphids seen on the head and may have both a glandular and sensory function in agreement with (Melarn, 1976). By comparison with similar previously recorded species such as *C. longicollis* Bayoumy *et al.* (2008) infecting *Mullus surmuletus*, it was observed that it has markedly longer spicules; 2 pairs of triangular teeth and a row of sensitive papillae along the body, which are not observed in the parasite recorded (Petter 1974, Ivashkin & Khromova 1976 and González-Solís *et al.*,

2007). The chitinous triangular teeth are probably used during penetration into and migration through the intestinal wall of the fish host; while the row of sensitive papillae served for orientation during sexual intercourse. Also, in comparison with *C. aliyai* (Akhtar and Mujib (2012) recorded from *Otolithus ruber*, the parasite recorded in the present study had a body dimension larger than those of *C. aliyai* but they are much similar with it in the shape of the mouth opening, arrangement of teeth and cephalic papillae, these features guide the classification of the present species belonging to genus *Cucullanus*.

Conclusion

The parasite recorded in the present study possessed all of the characteristics features of family Cucullanidae, and by comparison with the most similar species to the present parasite, it was observed that it is similar in morphology to *C. aliyai* and *C. longicollis* in general and it is closely similar to *C. aliyai*. So, it is concluded that the present parasite should be classified as *C. aliyai* with new host and locality records in Egypt.

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