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A new species of *cucullanus* (Nematoda: Cucullanidae) from marine edible fish *Otolithus ruber* (sciaenidae) based on light and Scanning Electron Micrscopy.

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ABSTRACT

Based on light and scanning electron microscopical observations, a new species of *Cucullanus* Müller, 1777 (Nematoda: Cucullanidae) is described from the intestine of marine edible fishes *Otolithus ruber* (Schneider, 1801), were collected (February 2006 to July 2007) from fresh landing of Karachi coast, Pakistan. Detailed light and scanning electron microscopy revealed some important taxonomical features like, Broader anteriorly, posterior end pointed, provided with small bifurcated spike at the tip of the tail. Lateral alae absent. Oral opening dorsoventrally elongate, surrounded by narrow membranous flange or collarette supported by row of numerous teeth. Three submedian cephalic papillae and a pair of prominent lateral amphids are present. Pseudobuccal capsule or esophastome wider than posterior part of esophagus. Deirids simple, just anterior to esophagus and intestinal junction. Tail broadly conical, bifurcated at the tip.

Key words: Parasitic nematode; new species; *Otolithus ruber;* fish; Intestine, Karachi coast; Pakistan.

INTRODUCTION

During a survey of nematodes from fishes of Karachi coast, Pakistan, a new species of the nematode of the family Cuculanidae Cobbold 1864 from the intestine of *Otolithus ruber* (Sciaenidae) was recovered, during the year 2006 to 2007. This species is described here in detail and is regarded a new species.

MATERIALS AND METHODS

Nematodes collected from the intestine of *Otolithus ruber* (2 male and 4 female specimens) were processed for light and electron microscopy.

Examination of specimens for nematode parasites

A total of 20 fish specimens were subjected to parasitological examination. The body cavity of fish was opened, and the gut and liver were removed by cuts in the region of the anus and the division between esophagus and anterior stomach. The section of the gut in a large Petri dish was opened with a longitudinal cut, and the whole inner surface lightly scraped to remove the parasites with mucus. The nematode recovered were washed in physiological saline and preserved in 70% ethanol. For light microscopy the nematodes were cleared in glycerin. Diagrams were prepared with a camera Lucida E 200 Nikon Drawing Tube. Measurements are given length by width in millimeters. Specimens for scanning electron microscopy were fixed in cold 4% glutaraldehyde in buffer (pH=7.2) and kept in it for 24 hours, then dehydrated through a graded series of alcohols, infiltrated with amyl acetate, after critical point drying mounted on stubs, coated with gold and photographs were taken with the help of SEM. Joel Japan JSM 6380A at an accelerating voltage of 15KV at Karachi University, central laboratory. The SEM measurements are in micrometers.

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RESULTS

A total of 20 fish specimens were subjected to parasitological examination. From three infected fish specimens, a total of six nematodes including (2 male and 4 female) were collected. These nematodes were recovered from the intestine. The new nematode species was identified and compared with the available literature (Yamaguti, 1961; Sood, 1989;Bilqees *et. al*. 2005; Akhtar & Bilqees, 2006 and Bilqees, 2007).

Taxonomic summary:

	Cucullanus aliyaii n. sp. (Fig.1-2)
Order:	Spiruridea Diesing 1861
Family:	Cuculanidae Cobbold 1864
Sub family:	Cucullaninae Yorke & Maplestone 1926
Genus:	Cucullanus Muller, 1777
Type host:	*Otolithus ruber*(Schneider, 1801)
Site of infection:	Intestine
Type locality:	Fish harbor, Karachi coast Pakistan
Prevalence:	15 % (20 fish examined / 3 fish infected)
Intensity:	2 (with 2 male and 4 females).
Holotype(male):	JUW. N. 40
Allotype(female):	JUW. N. 41

Male: (2 specimens including holotype): Length of body 2.02-2.29, greatest width 0.35-0.40, entire esophagus 0.36-0.38 in length. Its minimum width is in the middle 0.10, Length of esophastome is 0.18-0.20, maximum width at esophastome 0.13-0.15, posterior club- shaped part of esophagus in 0.34-0.37 in length and 0.09-0.11 in width .Nerve ring at a distance of 0.22-0.29 from the anterior extremity. Testis situated in posterior half of body or slightly anteriorly. Precloacal sucker is absent but few weak muscle fibers can be send. Spicules sub equal and 0.59-0.61 and 0.70-0.77 in length. Gubernaculums is absent. 12 pairs of caudal papillae are present including, 7 pairs preanal, 4 pairs are post anal and

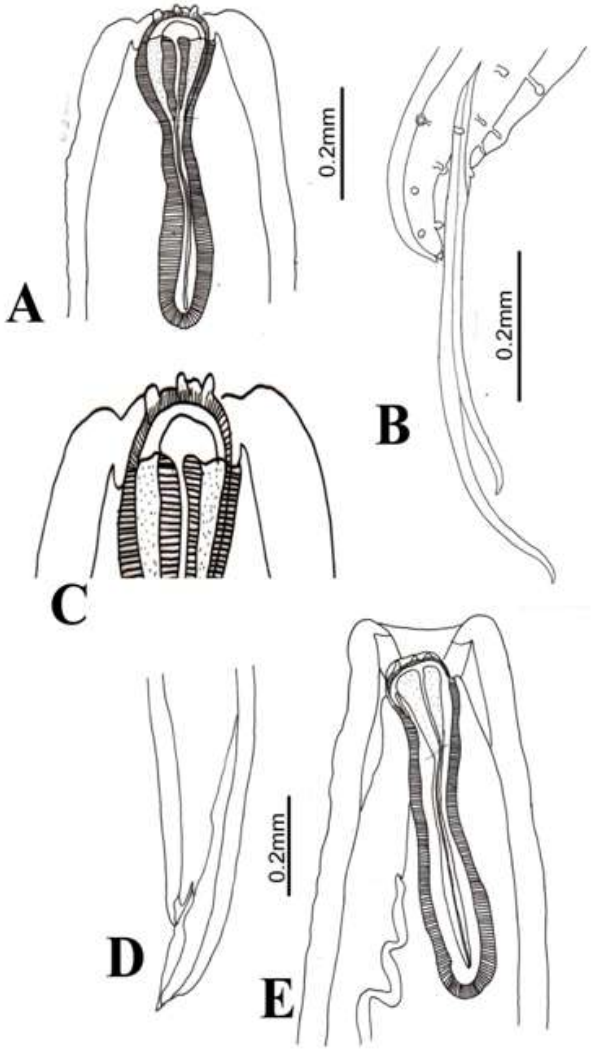

Fig.1. (A-E) *Cucullanus aliyaii* **n.sp.**
A. Anteior end of male (holotye)
B. Psterior end of male
C. Head of male
D. Anterior end of female
E. Posterior end of female

Female: (4 specimens including allotype): Length of body is 3.24, maximum width is 0.30. Entire esophagus is 0.50 in length, Greatest width 0.15 at esophastome , minimum width almost at the middle. Posterior club-shaped portion 0.15 in widths. Distance of nerve ring from the anterior extremity 0.30. Vulva 0.17-0.18 post equatorial, prominent, vulvar lips elevated at a distance of 1.80 from the anterior extremity. each containing elongated gland cells, extruding out of body wall. Vagina long, muscular tube and is directed upward. Uterus thin- walled,

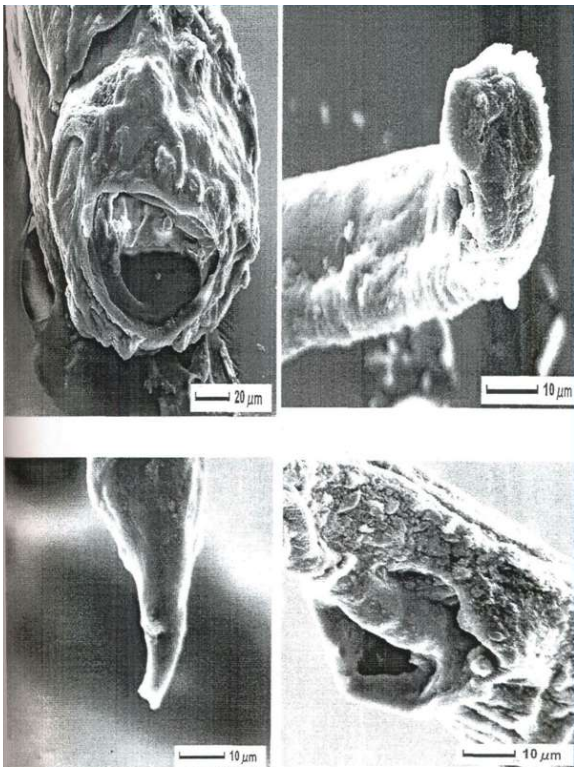

Figure 2. *Cucullanus aliyaii* **n.sp. allotype**
A. Cephalic end(Enface View)
B. Anterior region showing head and deirids
C. Tail region (showing bifurcation)
D. Anal Region

containing numerous relatively large, thin- shelled eggs 0.07x0.04. Tail is 0.011 in length.

REMARKS

The genus *Cucullanus* Muller, 1777(Cucullanidae) contains a large number of species parasitizing various fresh water and marine water fishes around the world. Due to minute differences in the morphology, a detail comparison among species is very complicated. Therefore the present new species *C. aliyaii* is compared with the species recovered from South Asia, especially Pakistan and some related species from other parts of the world. The species reported from marine fishes of Pakistan are all from fishes of Karachi coast. These include *C.armatus* (Yamaguti, 1954) reported by Rasheed (1968) from *Tachysurus serratus*; *C. diminutus* (Rasheed, 1968) from *Stromateus niger.* Rasheed (1968) also reported *C.hians* (Dujardin,1945) from *Lates cacarifier, Sciaena* sp., *Belone strongylurus, Acanthopagrus berda* and *Sparus* sp. At the same time Rasheed described *C. identatus* from *Polynemus tetradactylus* and *Pristopoma hasta*. He also redescribed *C fastigatus* (Chandler, 1935) from *Pristopona hasta*. *C. theraponi* (Rasheed, 1968) was described from *Hilsa* sp., and *Therapon* sp., *C. bilqeesi* (Petter, 1974) was originally described by Bilqees *et al*., (1971) as *C.elongatus* from *Erethistes elongate* and Petter renamed it as the species name was pre-occupied. *C. quadrii* (Bilqees, & Fatima,1980) from *Arius serratus*; *C. olivaceus* (Akram, 1975-1976) was described from *Tachysurus serratus* and *Pomadosys olivaceus*. Akram (1975-1976) also described *C. sparus* from *Sparus spinifer* and *Arius dussumieri* and *C. tachysuri* from *Tachysurus serratus* and *T. dussumieri*. *Indocucullanus karachii* (Zaidi & Khan, 1975) from the fish *Engraulis indica* was transferred to the genus *Cucullanus* by Soota (1983) with a combination as *C. karachii.*

Petter (1974) regarded *C. tachysurusi* as *C. arabiense* (Ali & Kalyankar, 1967) from Maharshtra, Mangalore and Karnataka. Petter (1974) restricted its occurrence in Siluriform hosts and gave only three pair of preanal caudal papillae for the species.

Yamaguti, (1961) has listed 60 species in the genus *Cucullanus* including the genotype. In addition to this Gupta and Masoodi (1982) described *C. sootai* and listed another 30 species including *C. ritali* (Karve, 1952); *C. jaiswali* (Ali, 1956); *C.indica* (Agrawal, 1965); *C. pseudotropi* (Agrawal,1967); *C. arabianse* (Ali and Kalyankar,1967; Petter, 1974); *C. theraponi* (Rasheed,1968); *C.pangasius* (Soota and chaturvedi., 1971); *C. jalnaensis*; *C. alii* Kalyankar, (1971); *C. malvanae* Kalyankar, (1971); *C. tachysuri* (Kalyankar, 1971); *C. bilqeesi*, (Bilqees *et al*., 1971; Petter, 1974); *C. carioca* (Vicente and Fernandes, 1973)*;C. rougetae* (Vicente and Dos, 1974); *C. bagrae* (Petter,1974); *C. karachii* (Zaidi & Khan, 1975); *C.olivaceus* (Akram, 1975-1976); *C. guerrori* (Arya and Jhonson, 1975); *C. ariusi* (Srivastava and Gupta, 1976); *C. sciaenai* (Gupta and Gupta, 1979); *C. rivulatus* (Soota and Sarkar,

1980); *C trichiurisi* (Gupta and Naqvi, 1983); C. *simhai* (Gupta and Naqvi, 1983); *C. thapari* (Gupta and Srivastava, 1984) and *C. mastacembeli* (Gupta and Srivastava, 1984).

Later on more species have been described both from fresh water and marine fishes of various localities including *C.* (Truttaedacnitis) *truttae* (Moravec, 1979); *C. campanae* (Labre and Petter, 1984); *C. fugianensis* (Wang, 1984); *C. brevispiculus* (Moravec *et al*., 1993); *C. riograndensis* (Fortes *et al*., 1993a); *C. fabrigasi* (Fortes *et al*., 1993b); *C. mexicanus* (Capseta-Mandujanoi,2000); *C. marplatensis* (Ingrid *et al*., 2002); *C.oceaniensis* (Moravec *et al*.,2005); *C.pedroi (*Timi and Lanfranchi, 2006). David *et al*., (2007) describe *C. pargi* from the grey snapper *Lutjanus griseus* of the southern coast of Quintana Roo, Mexico. Moravec *et al*., (2008) also describe *C. maldivensis* from marine fishes of Maldive Islands.

The present species *C.aliyaii* is different from *C. Alti* (Kalyankar, 1971) Peter, 1974; *C. arabianse* (Ali & Kalyankar, 1967; Petter, 1974); *C. ariusi* (Srivastava & Gupta, 1976) Soota, (1983); *C. armatus* (Yamaguti, 1954); *C. bulbosa*(Lane,1916) Barreto, 1918; *C. chrysophrydis* (Gendre, 1927); *C. guerreroi* (Arya & Johnson, 1975) Soota, 1983; *C. jalnaensis* (Kalyankar, 1971) Petter, 1974; *C. karachii* (Zaidi & Khan, 1975) Soota, 1983; *C. malvanae* (Kalyankar, 1971) Petter, 1974; *C. pangasius* (Soota & Chaturvedi, 1971); *C. ritai* Karve, 1952; *C. rivulatus* Soota & Sarker, 1980; *C. sciaenai* (Gupta & Gupta, 1979) Soota, 1983; *C. theraponi* (Rasheed, 1968); *C. indica* (Agrawal, 1965); *C. tachysuri* (Kalyankar, 1971) Petter, 1974; *C. mystusi* (Gupta & Naqvi, 1985) Gupta & Masoodi, 1986; *C. vinodae* (Gupta & Naqvi, 1985) Gupta & Masoodi, 1986; *C. schizothoraxi* Gupta & Masoodi, 1986; *C. sootai(*Gupta & Masoodi, 1986); *C. fujianesis* (Wang, 1984); *C. baylisi* (Lakshmi, 2000); as all these species have a gubernaculum. While gubernaculums is absent in the present new species. Species described by Moravec *et al*., (1993a-b, 1997) also have gubernaculums. Similarly gubernaculums is present in *C. riograndensis* (Fortes *et al*., 1993a) and *C. fabrigasi* (Fortes *et al*., 1993b).

Present species *C. aliyaii* is also different from *C. marplatensis* (Ingrid *et al*.,2002); *C.bonaerensis* (Lafranchi *et al*., 2004) ; *C. oceaniensi* (Moravec *et al*., 2005); *C. pedroi* (Timi and Lanfranchi,2006) ; *C. pargi* (David *et al*., 2007) and from *C. maldivensis* (Moravec *et al*., 2008) in variable combination of characters, specially in size of esophagus, spicule length and number and position of caudal papillae in male. But the present species *C. aliyaii* resemble in the presence of deirids with *C. oceaniensis* (Moravec et al.,2005) .*C. bilqeese* (Bilqees *et al*., 1971) Petter, 1974 and *C. olivaceus* are described only by females. These are also different from the present species in the size of body, esophagus and eggs. The presence of deirids are not reported previously from Pakistan. As mentioned the morphological characters and presence of deirids, the present nematodes of the genus Cucullanus appear an undescribed species for which the name C. aliyaii n. sp., is proposed.

Soota (1983) regarded *C. quadrii* as synonym of *C. armatus*. This species was originally described by Yamaguti (1954) from *Tachysurus* sp., (*Arius* sp.) in Borneo and later on by Rasheed (1968) from *T. serratus*. Even if *C. quadrii* is considered synonym to *C. armatus*, the present species is different having different host, lacking a gubernaculums and precloacal sucker, longer subequal spicule (0.59-0.61 and 0.70-0.77) in length), vulvular lips are prominent elevated and protruded. The caudal papillae in the present species are 12 in number and in *T. armatus* caudal papillae are(9-10) pairs. Characters such as the presence of deirids, absence of gubernaculums, precloacal sucker, number of caudal papillae, spicules length, and morphology of vulva, egg sizes and tail length are considered valuable differentiating diagnostic features. Therefore, present specimens are regarded a new species and its name *C. aliyaii* n.sp. is proposed. Present species resemble in some morphological features with *C .pakistanensis* Bilqees *et al*., 2005, including sub equal spicules, length of tail and bifurcation at its tip, absence of gubernaculums and precloacal sucker.

But body size, length of esophagus, presence of deirids and morphology of vulva is different from *C. pakistanensis*.

The species in which gubernaculums is absent such as *C. bengalensis* (Gupta and Masoodi, 1985), But the caudal papillae are same (12 in numbers) as in the present species.

Gubernaculum is present in *C. armatus* (Yamaguti, 1954); *C. bulbosa* (Barreto, 1918); *C. indica* (Agrawal, 1965); *C. tachysuri* (Kalyankar, 1971) Petter, (1974); *C. mystusi*, (Gupta and Masoodi, 1986); *C. riograndensis*, (Fortes *et al*; 1993a) *C. fabrigasi* (Fortes *et al*; 1993b); *C. baylist* (Lakshmi, 2000). Lakshmi (2000) described *C. baylisi* from the related host *Arius thalassinus* in India. Present new species is also separated from *C baylisi* which has a gubernaculums and much smaller spicules (0.237- 0.198mm in length), smaller eggs (0.069 x 0.048) and smaller tail (0.348 in female and 0.16-0.18in male). in addition a large number of sessile caudal papillae are also present in *C. baylisi.* Lakshmi probably did not consider *C. quadrii* a synonym of *C. armatus* or was unaware about this.

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