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Contracaecum otolithi in the intestine of *H. Fossilis*

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Abstract
Although nearly every organ of fish can be infected by parasitic nematodes, the intestinal tract of catfish was used in this investigation. Due to several physical differences in the body, the genus *Contracaecum* is categorized on the basis of microscopic morphological characteristics. These characteristics include pharyngeal, buccal, and cephalic structures, cuticular features and the excretory pore's interlabial placement. During the survey of helminth parasites of freshwater fishes in the Muzaffarnagar district of India, a known species of Nematode parasite, belonging to genus *Contracaecum* was isolated from the gut of *Heteropneustes fossilis* (a member of the Heteropneustidae family). The morphological description of *Contracaecum otolithi*, is provided. Light microscopic illustrations and Motic microscope photomicrographs corroborate the species' morphological description.

Keywords: Nematode, *Contracaecum*, Fish, *Heteropneustes* etc.

Introduction
Anisakidosis is a zoonotic parasitosis induced by members of the family Anisakidae. The anisakid genera includes *Anisakis*, *Pseudoterranova*, *Hysterothylacium* and *Contracaecum*. With a complex life cycle, marine animals are the ultimate hosts for these nematodes. Humans are unintentional hosts for these nematode parasites, which exploit various fish and crab species as intermediate hosts. Consuming sea foods, especially fish, contaminated with the parasite's infectious stage (third stage larvae [L3]), results in human anisakiasis, an infection brought on by members of the genus *Anisakis* (Shamsi & Butcher 2011) [19].
Contracaecum is the genus of parasitic nematodes from the family Anisakidae (Railliet & Henry 1912), Subfamily Filocapsulariinae (Railliet & Henry 1915) [16]. These nematodes are parasites of warm-blooded, fish-eating animals, i.e. mammals and birds, as sexually mature adults. It is the only genus in family Anisakidae which can infect terrestrial, marine and freshwater animals.
The adults live as parasite in stomach of piscivorous birds and mammals. As third-stage larvae, they adhere to the fish species' stomach, feeding on the prey of their ultimate host. The larvae of *Contracaecum* undergo two moults to become adult males and females, and then release their eggs into the water through the host's excrement. When the intermediate host fish is consumed, parasite reaches the warm stomach of predator of fish.
The larvae in the fish host pierce the intestinal wall to access the organs and body cavity. In response, the fish immune system creates a connective tissue capsule that encases the larvae and holds them there for the duration of the fish's life. The capsule encasing the larvae is broken down upon consumption by another fish or the removed intestines of a cleansed fish, allowing the larvae to proceed to the next phase of their life cycle.
It is known that many nematode species parasitize fish, but only a small number of these species can infect man (Adams *et al*., 1997). In freshwater fish, among the nematode families that cause most zoonoses, the Anisakidae family (Audicana *et al*., 2002) [3] and Dioctophymatidae family (Spalding and Forrester, 1993 [18]; Narr *et al*.; 1996) [13] stand out. Abdallah *et al*., 2012 [2] also recorded zoonoses by the nematode parasites of the Anisakidae family from different fresh water fish in Brazil.
The current study investigated *Contracaecum* sp. in the fish *Heteropneustes fossilis*, the only freshwater and estuarine member of the Heteropneustidae family of catfish.

Material and methods
For collection of parasites, fishes were brought to the laboratory from different natural water resources and nearby fish markets available in Muzaffarnagar area in India. Unlike water-breathing fish, air-breathing fish can be easily stored and transported in living condition. The collected fishes were brought to the laboratory in the plastic containers with proper handling method to reduce stress and mortality and were maintained in suitable tanks and glass aquaria. The classic works of Day (1958) [7] were utilised to identify fish.
The fish viscera were removed. Under a dissecting microscope, the organs were teased with fine forceps or dissecting needles in saline water, and the parasite collection was done with considerable caution. The parasites were fixed using the Eiras *et al*. (2000) [8] approach.
For morphological study and identification, diagrams were drawn with the help of camera lucida and photographic print taken by the Motic microscope. Different measuring scales were used for measurement.

Observation and description
Only 01 fish host out of 441 fish host belonging to the species *Heteropneustes fossilis* was found infected with this parasite and only 01 female parasite was collected from the intestinal region. This parasite is rarely found in freshwater fishes.

Description

The identified parasite belongs to the nematode group and the genus is *Contracaecum*. The parasite of genus *Contracaecum* is non-segmented and yellowish - white in colour. The cuticle is thick and finely striated, and entire body is irregularly enlarged in some areas. Annulations emerge on the anterior body. As the genus' name suggests, these nematodes have two oppositely-directed caecae as part of their digestive system (Shamsi, 2020) [20]. They also have an excretory pore located at their anterior end. These should be considered the most important morphological traits for distinguishing *Contracaecum* species from other anisakid worms since they are persistent throughout the developmental cycle. Other important features with taxonomic significance in adult *Contracaecum* species include the presence of an interlabia and labia, absence of labial denticulation, rounded eggs with smooth shells, presence of two spicules, conical tails in both male and females (which are shorter in males) and presence of post- and pre-cloacal papillae in males. Species within the genus can be differentiated based on variations in these features (Mozgovoi, 1953 [12]; Hartwich, 1964) [9].

Female: (Plates 1-2) [measurements based on 01 specimen]. This is a slender yellow worm; Body measures 1.96 mm x 0.1 mm. Head is 0.03 mm wide. Lips are 0.02- 0.022 x 0.01 mm. Interlabia is 0.015 mm long. Oesophagus is 0.16 x 0.02 mm. Ventriculus is 0.02 x 0.01 mm, it has solid ventricular appendix that measure 0.17 x 0.04 mm. Intestinal caecum is 0.13 x 0.01 mm. Tail is conical, 0.045 mm long and tip is covered with numerous minute spines.

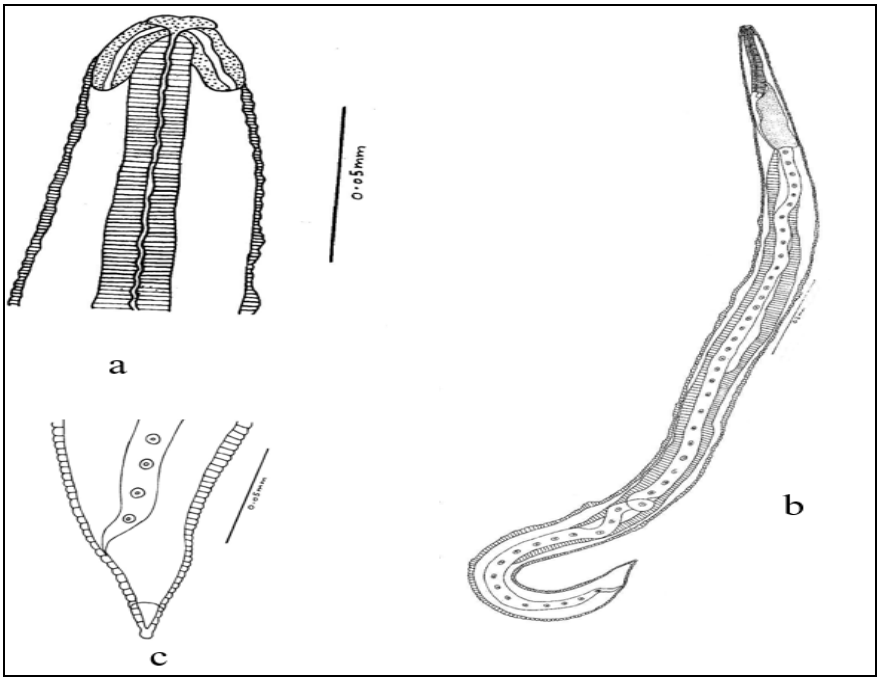

Plate 1: Contracaecum otolithi (female):- a. Anterior region (1000X) b. whole mount and c. Posterior region (1000X)

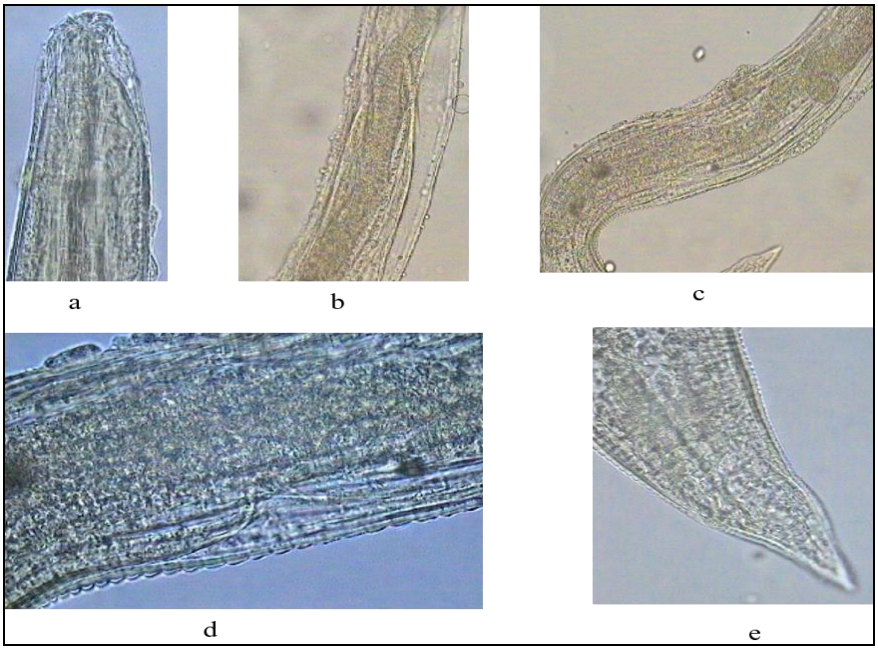

Plate 2: Contracaecum otolithi (female):- a. Anterior region (1000X) b-c Middle region (400X) d. Middle region (1000X) e. Posterior region (1000X)

Discussion

Contracaecum sp. is distributed throughout the alimentary tract of the host. These nematodes are mainly found in the body cavity but some are extracted from the stomach, Intestine, liver and pancreas of fish. The mean count of the parasite is highest from the body cavity (70.45±24.45) in the larger weight fishes. (Priyanka Rani *et al*., 2019)

Contracaecum otolithi was first described by Bilqees and Rashid (1982) [6] from Karachi in the host *Otolithus argenteus C.* Nematodes of the family Anisakidae, and particular species of the genera *Anisakis*, *Pseudoterranova*, and *Contracaecum*, are of medical and socioeconomic concern globally as they are the causative agents of a fish-borne zoonosis called anisakidosis (Bao *et al*., 2019) [4]. Besides the presence of anisakids in fish, the occurrence of other ascaridoid nematodes belonging to the family Raphidascarididae, i.e. *Hysterothylacium aduncum* (Rudolphi, 1802) [17] is also very common (Klimpel and Rückert, 2005) [10]. *Hysterothylacium* species use fish as the final host, whilst *Anisakis* spp., *Pseudoterranova* spp., and *Contracaecum* spp. use cetaceans, seals, and seals/fish-eating birds, respectively in their life cycle (Berland, 1991) [5]. The genus *Hysterothylacium* (Ward and Magath, 1917) [21] is frequently mistaken with the genus *Contracaecum* (Railliet and Henry, 1912). Genus *Contracaecum* possesses an excretory pore next to the ventral interlabium, in genus *Hysterothylacium* this pore is located on the nerve ring region (Lopes *et al*., 2011) [11]. The present specimen is collected from the host *Heteropneustes fossilis* at Muzaffarnagar region. The specimen at disposal of the writer exhibits more variations in the measurements of some body parts, due to small size. In previous specimen, the body is 63 – 70 mm long and 8.71 mm wide while the present specimen is 1.96 mm long and 0.1 mm wide. In previous specimen, head is 0.36 – 0.39 mm wide while in the present specimen 0.03 mm wide. In previous specimen, oesophagus is 5.60 – 5.72 mm long and 0.36 – 0.39 mm wide while in the present specimen 0.16 mm long and 0.02 mm wide. In previous specimen, ventriculus is 0.26 mm while in the present specimen 0.02 mm long and 0.01 mm wide. In previous specimen, ventricular appendix is 1.7 – 1.9 mm long and 0.11 – 0.12 mm wide while in the present specimen 0.17 mm long and 0.04 mm wide. In previous specimen, intestinal caecum is 0.80 – 0.90 mm long and 0.32 – 0.34 mm wide while in the present specimen 0.13 mm long and 0.01 mm wide. In previous specimen, tail is 0.05 -0.052 mm long while in the present specimen 0.045 mm long.

It is therefore, briefly re-described here as such. The re-description is based on the fresh material collected by the author. Difference in various measurement of body of the present worm from worms described earlier is given in

Table 1: Showing measurements of various body parts of *Contracaecum otolithi*

Body parts	***Contracaecum otolithi***	***Contracaecum otolithi***
	Bilqees and Rashid, 1982 (measurements in mm)	Present study (measurements in mm)
Host	*Otolithus argenteus C.*	*Heteropneustes fossilis*
Locality	Karachi	Muzaffarnagar (India)
Body	63-70 x 8.71	1.96 x 0.1
Head	0.36 - 0.39	0.03
Lips	-	0.02-0.022 x 0.01
Interlabia	-	0.015
Oesophagus	5.60-5.72 x 0.36-0.39	0.16 x 0.02
ventriculus	0.26	0.02 x 0.01
Ventriculur appendix	1.7-1.9 x 0.11-0.12	0.17 x 0.04
Intestinal caecum	0.80-0.90 x 0.32-0.34	0.13 x 0.01
Vulva	-	-
Eggs	0.050-0.052 x 0.039-0.049	-
Tail	0.050-0.052	0.045

(Bilqees and Rashid, 1982) [6] (Female)

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Authorship contributions-

Ruchi Sharma: Conceptualization of ideas; formulation of research questions; hypothesis development; taking pictures and parasite identification; data/evidence collection; writing of the initial draft.

Nisha Siwal: Writing of the initial draft and editing and finalizing final draft

Anju Panwar: Writing of the initial draft; research supervision and editing and finalizing final draft

Yougesh Kumar: Conceptualization of ideas; formulation of research questions; hypothesis development; research supervision.

Conflict of interest

The corresponding author declares that there is no conflict of interest on behalf of all authors.

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