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Research Article

Light microscopic characterization of camallanid nematode parasitizing *Tor putitora* (Hamilton, 1822) in Mahakali River, Nepal

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Abstract
Helminth parasites infect a wide variety of fish, with nematodes being widely distributed pathogens, especially in wild fish. The present study aimed to document the morphological characteristics of camallanid nematode infecting the golden mahseer (*Tor putitora)* fish in the Mahakali River along the Nepal-India border of Kanchanpur district, Nepal. Fish samples (n=157) were collected from Khalla and Bhujela areas in the river and nearby markets from March 2023 to February 2024. As revealed by light microscopy, the morphology of the present nematode species closely resembled the Asian freshwater fish nematode *Camallanus cotti* Fujita, 1927. Notable features included translucent red color in the fresh specimen, a pale yellow buccal capsule with longitudinal ridges and lateral valves, two amphids, eight cephalic papillae, two sclerotic plates, a basal ring separating the oesophagus from the buccal cavity, prongs of tridents extending beyond the basal ring, inconspicuous nerve ring in the muscular oesophagus, deirids at the beginning of glandular oesophagus, oesophagus with short anterior muscular and long posterior glandular portion, pre equatorial vulva and uterus confined in the posterior part of the body containing first stage larvae in gravid female, long tail and finely striated cuticle. The study, thus, confirmed that the camallanid nematode parasitizing freshwater fish golden mahseer is *C. cotti*.

Keywords: *Camallanus cotti,* Fish nematode, Golden mahseer, Helminth parasite

INTRODUCTION

Tor putitora (Hamilton, 1822*),* commonly known as golden mahseer, is an endangered (Jha *et al*., 2018) heritage fish in Mahakali River, a boarding river between Far Western Province of Nepal and Uttarakhand of India. It is a dominant fish in Mahakali River (Joshi and Joshi, 2021). This fish has a high sport and food value (Nautiyal, 2014). This fish is susceptible to parasitic worms. However, little is known regarding the *Bothriocephalus* tapeworm (Joshi and Joshi, 2024) and *Camallanus* nematode (Joshi and Joshi, 2023) parasitizing the golden mahseer.

Camallanus Railliet and Henry,1915*,* a common endoparasite nematode, has 150 parasitic species in the gastrointestinal tract of vertebrates. More than 100 species are reported in fish and 40 species in herpetofauna, especially turtles and tortoises (Jeyathilakan *et al*., 2015). *C. cotti* parasitizes tropical freshwater feral and cultivated fish species (Rigby *et al*., 1997). It has been reported in more than 58 fish species belonging to 51 genera, 20 families and 8 orders (Moravec and Justine, 2006). It yet lacks detailed evidence to support the assertion that camallanid nematodes infect golden mahseer fish. Thus, this research aimed to document the morphological characteristics of camallanid nematode parasitizing freshwater fish golden mahseer in Mahakali River, Nepal.

MATERIALS AND METHODS

Fish sampling location

Specimens of golden mahseer (n = 157) were collected using the gill nets from Khalla (29º63''17.28'N

80º89''17.16'E) and Bhujela (28º58''45.44'N 80º69''48.96'E) areas in Mahakali River, Nepal in between March 2023 to February 2024 under the permission of local government–Bheemdatt Municipality (Ref No. 3404/2023/024), Kanchanpur district, Far Western Province, Nepal.

Processing, identification and measurements of parasites

The fish samples were handled in fresh condition in the Laboratory of Central Department of General Science, Far Western University, Nepal. After examining the external body surface, fish samples were dissected through the abdomen using a fine-tipped scissor. The digestive organs were removed from the body and put in a Petri plate containing 0.9% Sodium Chloride solution. The surfaces of the internal organs, body cavity and mesenteries were examined with the help of a hand lens. The alimentary tract was separated into stomach and intestine, split lengthwise, cleaned several times and observed thoroughly first under simple dissecting microscope and then binocular microscope (model number: Motic® BA210 LED) for the presence of helminth parasites (Joshi and Joshi, 2023). The retrieved parasites were preserved, dehydrated, stained, cleared and mounted for identification following Thatcher (2006).

The nematodes were identified under a light microscope (model number: Motic® BA210 LED) based on morphological characteristics of buccal capsule and associated structures, number of cephalic papillae and sclerotic plates around buccal capsules, nature of tridents, size of oesophagus, position of vulva, nature of tail and striations in cuticle (Yeh, 1960; Rigby *et al*., 1997; Menezes *et al*., 2006; Moravec and Justine, 2006; Thatcher, 2006; Rigby and Rigby, 2014; Svitin *et al*., 2019). Parasites were measured by ocular micrometer under a Binocular microscope (model number: Motic® BA210 LED) and photographed by 12 MP 1/2.3'' Sony CMOS Sensor Microscope Camera (Model number: COSUSB12000) under 5X, 10X, 45X magnification. Scale bars were drawn with the help of ImageJ software. The nematodes were collected in March, June, October, November, and December 2023. Voucher specimens have been deposited in the museum of the Far Western University (FWUFP Nos 185, 207, 302, 402, 472, 513).

Data analysis

The prevalence and mean intensity of the worm were calculated after Bush *et al*. (1997).

RESULTS AND DISCUSSION

Six golden mahseer fish (Family: Cyprinidae, Order: Cypriniformes) were found infected by *C. cotti* Fujita 1927 (Family: Camallanidae, Order: Spirurida). A total of six female specimens and one first-stage larva were recovered from the intestine of infected fish in Bhujela area of Mahakali River, Nepal. The prevalence of adult nematodes was 3.82%, and the mean intensity was 1.

Description

Adult female

The parasite was characterized by translucent red color in life, pale yellow buccal capsule with 20 smooth longitudinal ridges, of them only 14 reached up to basal ring (Fig. 1b); buccal capsule without denticles; two amphids; eight cephalic papillae (four large and four small); two sclerotic plates; two lateral valves with smooth inner surface; basal ring; distinct oesophageal cup just behind the basal ring separating the muscular oesophagus from buccal cavity; a pair of tridents with three unequal prongs extending beyond basal ring, the middle one was larger than laterals; two small and simple deirids asymmetrically located in anterior part of glandular pharynx; oesophagus with short anterior muscular and long posterior glandular portion; vulva preequatorial with raised front lip (Fig. 1d); vagina was oriented backwardly from vulva; uterus and anus were confined in the posterior half of body; anus was provided with preanal and postanal papillae, subventral papillae; long blunt tail without caudal projections (Fig. 1e, f) and finely transversely striated cuticle. The nerve ring was inconspicuous and excretory pore was not observed.

First stage larva: Buccal capsule was without longitudinal ridges, sclerotic plates and cephalic papillae; feebly developed lateral valves; oesophagus was not differentiated into muscular and glandular parts; total length 480 µm; length of buccal capsule 92 µm, breadth of buccal capsule with lateral valve 99 µm.

Measurements (based on four female specimens)

The nematode parasites recovered from golden mahseer were medium-sized. Their morphometric characters are presented in Table 1.

The present nematode species closely resembled by morphology and measurements in total length, buccal structures, tridents, size of oesophageal parts, position of deirids, vulva, vagina and uterus, nature of tail, and finely transversely striated cuticle with the *C. cotti* Fujita, 1927 (Table 1) as described in other host fish species by Rigby *et al*. (1997), Menezes *et al*. (2006), Moravec and Justine (2006) and Raja *et al*. (2018). The number of papillae and sclerotic plates was similar to the *C. cotti* as explained by Moravec and Justine (2006).

In the present nematode, the width of the body and muscular oesophagus were slightly smaller. Glandular oesophagus and tail were shorter than *C. cotti*. The nerve ring was somewhat nearer, but deirids were far

Table 1. Morphometric data for the nematode parasite observed in this study and comparison with the published description of nematode *Camallanus cotti* Fujita, 1927

Morphological characters	Measurements (µm) of present nematode recovered from golden mahseer	Measurements (µm) of sub-gravid and gravid female specimens of *C. cotti* Fujita, 1927 (Moravec and Justine, 2006)
Total length	6211–-7050	6620–9440
Maximum breadth	221	279–490
Length of buccal capsule with basal ring	133–155	120–174
Breadth of buccal capsule with lateral valve	112–129	129–150
Length of cephalic papilla	27–30	–
Breadth of cephalic papilla	8–9	–
Length of sclerotic plate	27–28	–
Breadth of sclerotic plate	30–32	–
Length of lateral valves of buccal cavity	136–158	–
Breadth of lateral valves	14–19	–
Length of basal ring	12–18	12–18
Breadth of basal ring	86–95	64–90
Length of prongs of tridents	101–115	97–156
Length of muscular pharynx	509–522	428–563
Breadth of muscular pharynx	74–97	102–117
length of glandular pharynx	496–541	571–736
Breadth of glandular pharynx	90–120	99–111
Nerve ring (from anterior end)	171	230–375
Excretory pore (from anterior end)	Not observed	249–286; Not reported in some nematode parasites
Deirids (from anterior end)	711–824	402–557; Not reported in some nematode specimens
Vulva (from anterior end)	2979–3765	3170–5150
Anus (from anterior end)	5319–6158	–
Tail length	795–892	1120–1390

- Indicating not mentioned when used alone

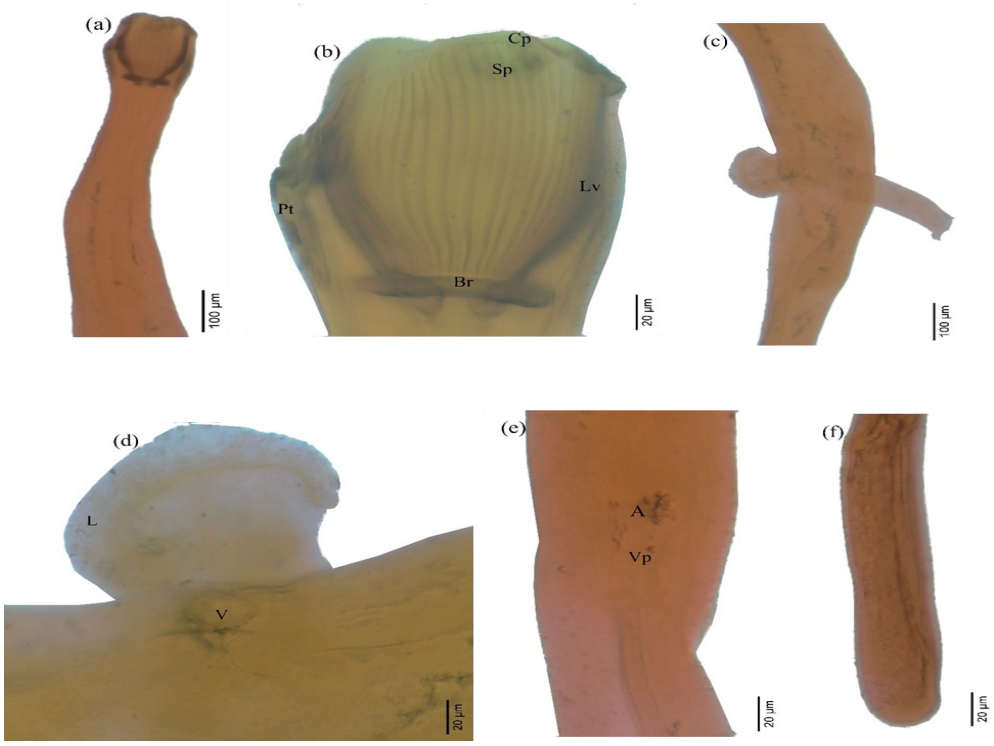

Fig. 1. *Camallanus cotti (a) anterior end (ventro-lateral view), (b) enlarged view of anterior end, (c) middle part showing vulva and larva (lateral view), (d) enlarged view of middle part with vulva and larva, (e) anus (lateral view), (f) tail of female (ventral view). Note: Cp = cephalic papilla; Sp = sclerotic plate; Lv = lateral valve; Pt = prongs of trident; Br = basal ring; V = vagina; L = larva; A = anus; Vp = ventral papilla.*

located from the anterior end (Table 1). These differences might be due to the maturity stages of the observed nematodes. Morphometric variations and the inconspicuousness of excretory pore and deirids in some *C. cotti* nematode parasites with other host fish species are also reported by Moravec and Justine (2006).

On the basis of the abovementioned differences and resemblances, it was concluded that the present nematode species is *C. cotti*. In this study, only female nematodes were observed, probably due to either the low infection rate of the parasite or not capturing the fish specimens infected with male parasites during random sampling.

Conclusion

This study identifies and characterizes the morphology of Asian fish nematode *C. cotti* parasitizing golden mahseer (*Tor putitora*) in Mahakali River, Nepal. The important diagnostic characteristics were medium sized body with transversely finely striated cuticle, buccal capsule with smooth longitudinal ridges, oesophagus nearly 15% of total body length and divided into short anterior muscular and longer posterior glandular portions, preequatorial vulva and long tail without mucrones.

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Conflict of interest

The authors declare that they have no conflict of interest.

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