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PK Prasad

Ecological Parasitology and
Tropical Biodiversity
Laboratory, Department of
Zoology, Kannur University,
Mananthavady Campus,
Wayanad, Kerala, India

Sherin Chacko

Ecological Parasitology and
Tropical Biodiversity
Laboratory, Department of
Zoology, Kannur University,
Mananthavady Campus,
Wayanad, Kerala, India

Clinostomum cupani n. sp. metacercaria infecting the freshwater fish, *Pseudosphromenus cupanus* from the Western Ghats, Wayanad region, India

PK Prasad and Sherin Chacko

Abstract

Clinostomum cupani n. sp. (Digenea, Clinostomidae) from the operculum and body cavity of the freshwater fish *Pseudosphromenus cupanus* collected from water bodies in the Wayanad region of the Western Ghats is described and illustrated. *C. cupani* n. sp. is new to the genus and is separated from its congeners on the basis of differences in morphology and morphometry. Detailed comparison of the present species with *C. complanatum* Rudolphi, 1814 and *C. marginatum* Rudolphi, 1819 is made in this study. *C. cupani* n. sp. differs from all other species of *Clinostomum* Leidy, 1856 in the shape of its uterus and the presence of pigmented eyespots. It is named after the host *P. cupanus*. The prevalence of infection of *C. cupani* n. sp. is 50%, the intensity of infection is 12.33 and the mean abundance is 6.16.

Keywords: *Clinostomum cupani* n. sp., digenea, metacercaria, second intermediate host, fish

1. Introduction

The genus *Clinostomum* Leidy, 1856 (Digenea: Clinostomidae) has heteroxenous life cycle. Adults are found in the buccal cavity and esophagus of birds, reptiles and mammals [1]. The freshwater snails are their first intermediate hosts and freshwater fishes or amphibians as their second intermediate hosts [2, 3]. The metacercariae of *Clinostomum* are usually known as yellow grubs. This fish-borne zoonotic trematode causes acute pharyngitis or laryngitis in those who eat raw fish meat with metacercarial infection [4]. Human infections have been reported by many workers [4-9].

Because of the high degree of morphological inconsistency within the same species, *Clinostomum* had been subjected to several taxonomic reviews [1, 2, 10-13], of which Ukoli [1] was one of the pioneer reviewers, who synonymized 20 *Clinostomum* species with *C. complanatum* and documented 13 valid species. Several species were then added to or detracted from the genus *Clinostomum* with the support of molecular and conventional taxonomic studies. Now this cosmopolitan genus encompasses 29 valid species [14].

The present paper describes the morphological features and taxonomic status of a new species of digenetic trematode metacercaria, *C. cupani* n. sp. infecting operculum and body cavity of freshwater fish *P. cupanus* from the Wayanad region of the Western Ghats.

2. Materials and Methods

2.1 Study area

The study was carried out in the Wayanad region of the Western Ghats, one of the hottest hot-spots of biodiversity in the world due to its incredible biological diversity and a high degree of endemism. The geographical location of Wayanad region is 11° 27' to 15° 58' north latitude and 75° 47' to 70° 27' east longitude.

2.2 Methods

Host fish specimens collected from different water bodies using sweep net were kept alive, brought to the laboratory, maintained in clean glass jars or aquarium and occasionally fed with cooked rice or fish food. Fish were sacrificed and body surface, fins, abdominal cavity, gill filaments, eyes and muscles were carefully examined for the presence of metacercariae. Internal organs were dissected out, placed in separate Petri dishes containing

Correspondence

PK Prasad

Ecological Parasitology and
Tropical Biodiversity
Laboratory, Department of
Zoology, Kannur University,
Mananthavady Campus,
Wayanad, Kerala, India

0.75% saline, macerated and observed under the binocular LABOMED Luxeo 4Z stereo zoom microscope. The live encysted meta cercariae were transferred to 0.75% saline, excysted by rupturing the cyst wall with a fine needle. Studies on meta cercariae were carried out on live and fixed specimens, without supravital staining or staining with neutral red or methylene blue, under Nikon ECLPSE Ni-U research microscope. Permanent whole mounts were prepared after fixing them in 5% formalin under slight cover glass pressure, and then staining with acetocarmine, following the procedure outlined by Cantwell [15]. Measurements (in μm) were taken with the support of Nikon NIS Elements Imaging software. Sketches were prepared with the Nikon Y- IDT drawing tube attached to the Nikon ECLPSE Ni-U research microscope and Photographs were taken with Nikon Y-TV55 camera attached to a Nikon ECLPSE Ni-U research microscope with the support of Nikon NIS Elements Imaging Software. Prevalence, intensity and mean abundance of infection were measured following Bush *et al.* [16].

3. Results

3.1 *Clinostomum cupani* n. sp. (Fig. 1)

Body linguiform, 2188.00-3090.00 (2716.36) long and 648.96-951.24 (815.73) wide. Slightly ovoid anterior part bears a small sub-terminal oral sucker, 138.14-207.55 \times 149.34-222.57 (171.33 \times 195.87), surrounded by oral collar 235.08-319.95 \times 372.94-562.15 (270.14 \times 443.66). Cuticle aspinose. Ventral sucker large, well developed, round at the posterior region, 296.61-551.09 \times 306.48-524.04 (397.91 \times 384.13). Distance between suckers 299.19-398.97 (342.04). Short pre-pharynx, 52.88-84.12 \times 11.76-26.77 (69.74 \times 18.32). Pharynx rudimentary, 58.32-85.48 \times 40.78-74.68 (69.63 \times 59.26).

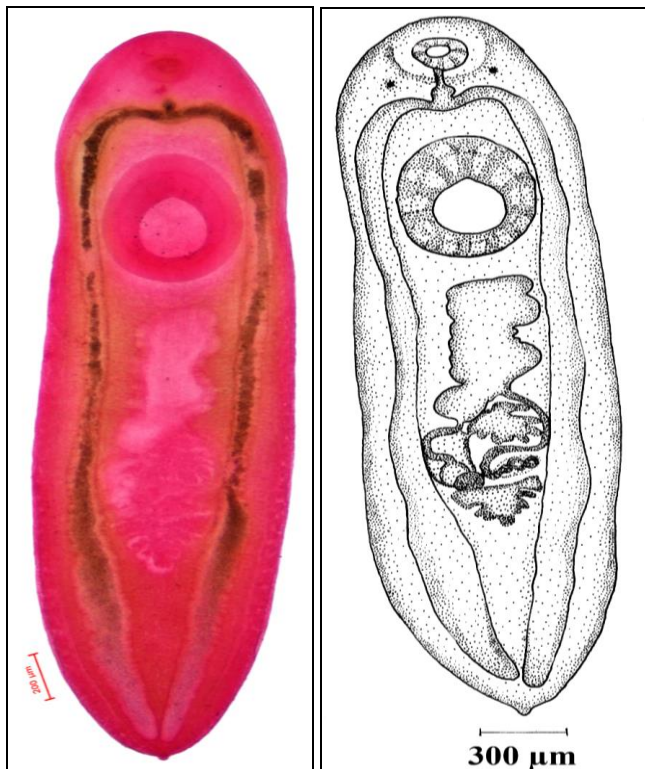


Fig 1: *Clinostomum cupani* n. sp. Metacercaria (Photograph and line drawing).

Eyespots at the level of the anterior part of pharynx, anterior to caecal shoulders. Esophageal bulb absent. Intestinal caeca

1979.74-2957.25 \times 125.52-274.13 (2506 \times 181.65), broad at the postacetabular region, lateral pouches are not prominent, extending up to the posterior end of the body. Testes deeply lobed; anterior testis irregular, close to the right margin of left caecum. 143.36-242.49 \times 142.93-301.07 (181.70 \times 215.44). Posterior testis slightly triangular, inter-caecal, 74.24-228.43 \times 106.80-366.26 (151.34 \times 232.02). Distance between testes 107.11-178.96 (147.23). Cirrus pouch large, slightly ovoid, 113.48-254.90 \times 77.05-166.86 (177.88 \times 107.75), in the inter-testicular space close to the left margin of the right caecum. Ovary small, slightly oval, 57.81-100.16 \times 55.19-91 (78.65 \times 69.01). Ootype at the inter-testicular space between ovary and the left intestinal caecum. Uterine sac with lateral evaginations, 209.08- 606.43 \times 106.60-324.69 (429.96 \times 218.69) partially covers the intercaecal space in that region. Excretory bladder V-shaped.

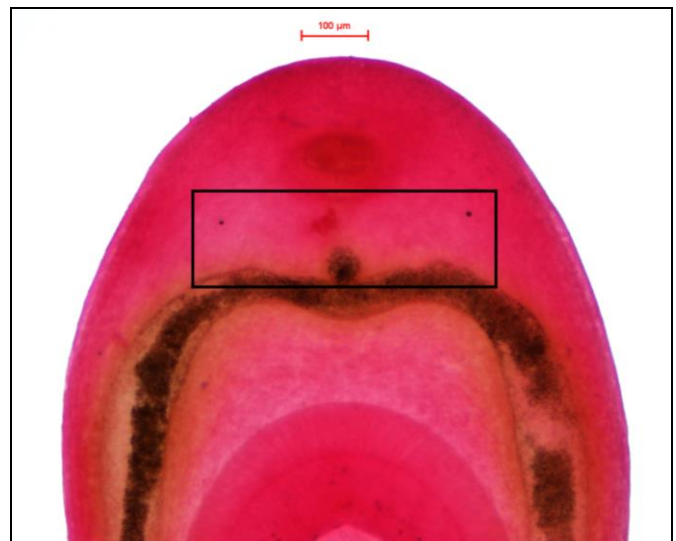


Fig 2: Eyespots of *Clinostomum cupani* n. sp. metacercaria marked with a rectangular box.

3.2 Taxonomic summary

Type specimen: Holotype (No. Z-TM 42) deposited in the helminth parasite collections, Ecological Parasitology and Tropical Biodiversity Laboratory, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India.

Type host: *P. cupanus* ((No. Z-FTM 147) deposited in the Ichthyology collections, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India.

Type locality: Moolithod, Wayanad District, Kerala, India.

Site of infection: Operculum and body cavity.

Period of collection: June 2018 to January 2019

Prevalence: Three of six fishes (50%) screened were infected. Intensity: Thirty seven *C. cupani* n. sp. recovered from three infected fish (12.33).

Mean abundance: Thirty seven *C. cupani* n. sp. recovered from a total of six fish examined and, therefore, the mean abundance is 6.16.

Of the six *P. Cupanus* examined, three were found infected with a total of 37 parasites. The prevalence of infection was 50%, while the intensity of infection stood at 12.33.

4. Discussion

The present study showing a detailed morphological description of the metacercaria of *C. cupani* n. sp.,

emphasizes the presence of pigmented eyespots (Fig. 2) and large uterus with lateral pouches. According to Ukoli ^[1] the significant characters which are not likely to vary within the species and which are, therefore, most reliable for the purposes of classification of *Clinostomum* species are the presence of lateral evaginations in the uterine sac and the

anterior extent of the vitellaria. The characters such as the position of gonads, genital pore & cirrus pouch and the shape of testes, which vary between meta cercariae and adults and all other characters that are likely to change due to external influences.

Table 1: Comparison of morphometric characters (in μm) of metacercariae of the *C. marginatum* Rudolphi, 1819, *C. complanatum* Rudolphi, 1814 ^[11] and *C. cupani* n. sp.

Characters	<i>Clinostomum marginatum</i> Rudolphi, 1819 (n = 10) min-max (mean)	<i>Clinostomum complanatum</i> Rudolphi, 1814 (n = 11) min-max (mean)	<i>Clinostomum cupani</i> n. sp. (n = 7) min-max (mean)
Body L \times B	Oval, elongate, wider in gonadal region 4215.00–6346.00 \times 979.00–1532.00 (5402.00 \times 1329.00)	Stout, oval, elongate, wider in gonadal region 4495.00–7874.00 \times 1635.00–2434.00 (5741.00 \times 1934.00)	Oval, elongate 2188.00–3090.00 \times 648.96–951.24 (2716.36 \times 815.73)
Body L/B	3.05–4.40 (4.03)	2.20–4.37 (2.99)	3.10–3.52 (3.29)
Oral collar B	502.00–874.00 (721.00)	686.00–1030.00 (820.00)	372.94–562.15 (443.66)
Oral sucker L \times B	238.00–599.00 \times 194.00–586.00 (312.00 \times 290.00)	259.00–337.00 \times 284.00–507.00 (294.00 \times 401.00)	138.14–207.55 \times 149.34–222.57 (171.33 \times 195.87)
Oral sucker B/body B	0.82–1.05 (0.92)	1.06–1.67 (1.36)	0.22–0.27 (0.23)
Ventral sucker L \times B	Larger than oral 510.00–757.00 \times 571.00–804.00 (669.00 \times 708.00)	Larger than oral 637.00–910.00 \times 766.00–952.00 (795.00 \times 839.00)	Larger than oral 296.61–551.09 \times 306.48–524.04 (397.91 \times 384.13)
Ventral sucker B/oral sucker B	0.99–1.12 (1.06)	1.78–2.69 (2.14)	1.70–2.46 (1.95)
Ventral sucker B/body B	0.47–0.62 (0.53)	0.39–0.49 (0.44)	0.42–0.55 (0.465)
Distance between suckers	959.00–1491.00 (1243.00)	860.00–1115.00 (1020.00)	299.19–398.97 (342.04)
Anterior testis L \times B	231.00–413.00 \times 264.00–498.00 (307.00 \times 389.00)	316.00–957.00 \times 273.00–559.00 (484.00 \times 412.00)	143.36–242.49 \times 142.93–301.07 (181.70 \times 215.44)
Anterior testis B/L	0.68–1.72 (1.29)	0.46–1.22 (0.90)	0.76–1.34 (1.18)
Posterior testis L \times B	233.00–457.00 \times 295.00–492.00 (327.00 \times 405.00)	245.00–441.00 \times 408.00–602.00 (328.00 \times 493.00)	74.24–228.43 \times 106.80–366.26 (151.34 \times 232.02)
Posterior testis B/L	0.89–1.52 (1.25)	1.09–1.88 (1.54)	1.19–2.00 (1.52)
Distance between testes	196.00–393.00 (320.00)	214.00–527.00 (353.00)	107.11–178.96 (147.23)
Ovary L \times B	85.00–142.00 \times 62.00–97.00 (115.00 \times 80.00)	135.00–164.00 \times 97.00–178.00 (149.00 \times 129.00)	57.81–100.16 \times 55.19–91.00 (78.65 \times 69.01)
Ovary B/L	0.43–0.97 (0.71)	0.59–1.09 (0.87)	0.65–1.05 (0.88)
Cirrus sac L \times B	Well developed, crescent shaped, compressed against left anterior margin of anterior testis 172.00–343.00 \times 119.00–153.00 (256.00 \times 137.00)	Well developed, extending from inter testicular space to posterior right margin of anterior testis 209.00–405.00 \times 124.00–197.00 (296.00 \times 157.00)	Large and slightly ovoid, in the inter-testicular space close to the left margin of right caeca 113.48–254.90 \times 77.05–166.86 (177.88 \times 107.75)
Cirrus sac L/body L	0.03–0.06 (0.05)	0.03–0.07 (0.05)	0.05–0.08 (0.06)
Eyespots	Not observed	Not observed	Located at the level of the anterior part of pharynx, anterior to the caecal shoulders
Uterine sac L \times B	Tubular proximal uterus. No lateral digitations	Tubular proximal uterus. No lateral digitations	Large and wider uterus. Uterine sac with lateral evaginations 209.08–606.43 \times 106.60–324.69 (429.96 \times 218.70)
Pharynx	Not observed	Not observed	Rudimentary 58.32–85.48 \times 40.78–74.68 (69.63 \times 59.27)
Pre-pharynx	Not observed	Not observed	Slightly long 52.88–84.12 \times 11.76–26.77 (69.74 \times 18.32)
Oesophagus	Very short. oesophageal bulb present	Oesophageal bulb well developed	Oesophagus and oesophageal bulb absent
Intestinal caeca	Lateral to ventral sucker, characterized by visible intestinal pouches especially in postacetabular part	Lateral to ventral sucker and genital complex, with prominent lateral pouches	Lateral pouches in intestinal caeca are not prominent Caeca more bulbous at postacetabular region

The present species has some general morphological similarities with the *C. marginatum* (Table 1). But it exhibits some distinct morphological features such as the presence of pigmented eyespots, large sacculated uterus, pre-pharynx,

rudimentary pharynx; absence of esophagus, positioning of the cirrus pouch and absence of cuticular spines. The described species also exhibits changes in morphometry such as distance between suckers, the ratio between oral sucker

width and body width and distance between testes.

The present species displays some similarity with *C. complanatum* (Table 1). Detailed study revealed that the species differs from *C. complanatum* [11] on many morphological features and morphometry like presence of pigmented eyespots, pre-pharynx, rudimentary pharynx, difference in the distance between suckers, ratio between oral sucker width and body width, size of anterior testis, distance between testes and shape & size of uterine sac. Neither *C. complanatum* nor *C. marginatum* has lateral digitation of the uterine sac [17] and presence of pigmented eyespots distinguishes the described species from these two species.

The species described under the genus *Clinostomum* from India documented by Pandey and Agrawal [18] in their work 'The metacercarial fauna of India' included *C. piscidium* Southwell and Prasad, 1918, *C. prashadi* Bhalerao, 1942, *C. gideoni* Bhalerao, 1942, *C. dasi* Bhalerao, 1942, *C. indicum* Bhalerao, 1943, *Clinostomum sp.* Srivastava, 1950, *C. schizothoraxi* Kaw, 1950, *C. microstomum* Singh, 1955, *C. giganticum* Agarwal, 1955, *C. macrosomum* Jaiswal, 1957, *C. mastacembeli* Jaiswal, 1957, *C. progonum* Jaiswal, 1957, *C. orientale* Mukherjee, 1967, *C. lucknowensis* Pandey, 1968, *C. trichogasteri* Pandey, 1969 and *Clinostomum sp.* Rekharani and Madhavi, 1985. The presence of pigmented eyespots and lateral evaginations in the uterine sac are not reported in any of these species.

Concerning the other species described, at the metacercarial stage, the morphological features observed in the present species differ consistently from *C. cutaneum*, which has a unique Y-shaped uterine sac [10], *C. phalacrocoracis* metacercaria that bears fan-shaped testes and straight uterus running from ventral sucker to anterior testis [19]. Although the presence of lateral digitations in the uterine sac is a common feature in *C. detruncatum* and the present species [17] the former lacks eyespots. The present species varies from *C. tilapiae* in which body surface is armed with numerous cuticular spines and straight uterus running from ventral sucker to anterior testis [20]. The species described is different from *C. caffarae* on the nature of testes, from *C. arquus* on the shape of testes & nature of uterine sac and from *C. Cichlidorum* on the absence of pharyngeal bulb [14].

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