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OBSERVATION AND DESCRIPTION OF *CLINOSTOMUM PISCIDIUM* SOUTHWELL AND PRASHAD, 1918 RECOVERED FROM THE BODY CAVITY OF *CHANNA PUNCTATUS* IN INDIA

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ABSTRACT

Present communication deals with morphological description of parasitic trematodes, *Clinostomumpiscidium* Southwell and Prashad, 1918, collected from the body cavity of *Channapunctatus* (Siluriformes, Channidae) Muzaffarnagar. During a survey of helminth parasites of freshwater fishes in Muzaffarnagar region a known species of Digenea belonging to genus *Clinostomum* was isolated. Morphological description of the species is supported by light microscopic drawings and photomicrographs.

Keywords: Digenea; *Clinostomum*; Metacercariae; Fish; *Channapunctatus*.

INTRODUCTION

Parasites are often described as an ideal biological model for ecological study (De Meeus, et. al. 1998). Flukes, digeneans (formerly digenetic trematodes) form another class of flatworms. Flukes reproduce as adults and again as larvae, hence the name “di-genetic” or two births. They cause serious and fatal diseases in many animals including humans (Schell, 1970).

Leidy (1856) established the genus *Clinostomum*. After that number of species have been described within this genus from various parts of the world. As we know, Southwell and Prashad (1918) described the metacercaria of *Clinostomum piscidium* for the first time in India from *Nandus nandus* (Hamilton, 1822) and *Colisafasciata* (= *Trichogaster fasciatus*) (Bloch and Schneider, 1801). Subsequently, this larval trematode was

reported at several occasions by Bhalerao (1942) from *N. nandus* at Poona and Jabalpur (Singh, 1959). Pandey and Baugh (1969) found this parasite in *C. fasciata* and *N. nandus* at Lucknow. Specific determination of larval stages using morphological traits is often difficult and uncertain. Information on geographical variation of *Clinostomum* species is limited and some reported variation clearly requires further analysis.

MATERIALS AND METHODS

Collection and identification of host: -

Fishes were collected from local fish markets and water bodies of the Muzaffarnagar region. They were transported to the laboratory and maintained in aquaria. Identification of the fishes was carried out with the help of Classical works of Day (1958).

Dissection: - The fishes were taken out from water and then anaesthetized with clove oil in another container. The dissection and examination for parasites were performed under dissecting microscope using standard methods. The fishes were dissected by opening the body cavity along the ventral midline of the body from anus to head to expose the alimentary canal. The entire digestive tract and the stomach were removed and placed separately in petridishes containing physiological saline. The internal organs were cut open lengthwise and the parasites were allowed to come out of the lumen.

Collection and fixation of parasites: - A thorough examination of alimentary canal was made for the presence of parasites. These parasites were isolated carefully and picked up with the help of fine glass dropper and transferred to cavity blocks. - The collected parasites were fixed by the method suggested by Eiras et al. (2000) and Marcogliese (2004). Digeneans were transferred to a small glass Petri dish. Excess saline or water was removed and hot 10% formalin or AFA was added. Thicker worms were flattened under a cover slip and flooded with warm fixative.

Dehydration: - The fixative was thoroughly removed from specimens prior to staining. The formalin was washed in distilled water and AFA was washed in 70% alcohol.

Staining: - Digeneans were stained in

Semichon's Acetocarmine.

Destaining:- (a). The excess stain was washed off in two changes of 70 % ethanol (5-10 minutes each for change).

(b) 70 % acid alcohol was used for destaining. Specimens were observed using a dissecting microscope and destain until parenchyma and muscles were nearly free from stain (light pink) while internal organs were well stained (red).

(c) Destaining was stopped by replacing acid alcohol with two changes of basic alcohol (5-10 minutes each).

(d) Dehydration was done in 95 % ethanol for 15 minutes and two changes of 100 % ethanol for 10-15 minutes each.

Clearing: - The parasites were cleared in xylene for at least 10 minutes. Specimens were ready for mounting.

Transferring: - After clearing, specimen were transferred to a slide using a clean camel hair brush. Specimens were oriented in the centre.

Mounting: - For the study of morphology, digeneans were mounted in Canada balsam. The cover slip was placed gently to prevent the entry of air bubbles and sufficient mounting medium was added to fill space under the cover slip and then sealed with the help of nail paint all around it.

Observations:- Slides were observed under Olympus OIC 41231 light microscope using 5x, 10x, 12x and 15x eyepieces and 10x, 40x, and 100x objectives and in motic microscope using W10x /18 eye piece and 4x, 10x, 40x, and 100x objectives as per size and requirement of the specimen.

Morphological and morphometrical observation:- The diagrams were drawn with the help of camera lucida, (prism type) and motic microscope to support the morphological observations. Various body parts of parasites under observation were measured using a measuring scale in the eyepiece of the light microscope and through the photographic print of measuring scale in motic microscope.

Photography: - Pictures of the mounted parasites were obtained with the help of Motic DMB1-223ASC-B High Resolution digital

compound microscope (Motic images plus 2.0) using W10x/18 oculars and 4x, 10x, 40x, and 100x objectives as per size and requirement of the specimen. The original photographs were reduced and trimmed as was necessary to include them in this work.

OBSERVATION AND DISCUSSION

Family: Clinostomatidae (Luhe, 1901)

Sub-family: Clinostominae (Pratt, 1902)

Genus: *Clinostomum* (Leidy, 1856)

Species: *Clinostomum piscidium* (Southwell and Prasad, 1918)

Generic Diagnosis: Clinostomatidae :

Clinostominae : Body spined, stout, linguiform, convex dorsally and concavo ventrally. Oral sucker surrounded by collar like fold when retracted. Oesophagus swollen bulbously at posterior end without forming the typical pharynx. Caeca with sinuous wall, opening into excretory vesicles by a narrow passage. Acetabulum usually in the anterior third of the body. Testes at about middle of hind body or near posterior extremity. Cirrus pouch anterior to ovary, or white half of anterior testis, containing winding seminal vesicles and ejaculatory duct. Prostatic complex apparently absent. Genital atrium opening on the right of medium line between two testes. Uterus may or may not reach to acetabulum. Vitellaria may extend into fore body, confluent behind posterior testis. Excretory plexus extending in peripheral parenchyma; excretory vesicle, small, V-shaped with dorsoterminal pore. Parasitic in buccal cavity and oesophagus of birds. Larva encysts in fish, frogs, salamanders, and also in land snail (Yamaguti, 1958).

Description: -Body small, convex dorsally width fairly uniform although wider in the region of the ventral sucker. Metacercarial body appears yellowish with brownish excretory system and intestinal caeca. Body is thick and tongue-shaped, with round anterior and posterior ends. Body cuticle is beset with minute backwardly directed spines. Oral sucker is oval in outline; the ventral sucker is well developed, large, strongly muscular structure much larger than the oral sucker. Longitudinal excretory ducts extend anteriorly to the region of the ventral sucker. Digestive system

has bulbous part at the end of a short oesophagus. Intestinal caeca arises from behind the bulbous structure and runs posteriorly along the sides, up to the hind end of the body where they end blindly. Intestinal caeca are club shaped, being narrow anteriorly but broad posteriorly in the hind end of the body. Testes are triangular in shape with smooth, crenated margins. Anterior and posterior testes are roughly equal or subequal in size. Anterior testis in posterior region of middle third of body, roughly triangular, offset to left, with apex pointing towards midline. Posterior testis is more deeply lobed and larger than anterior testis, in anterior end of posterior third of body, uniformly triangular, median, with apex pointing posteriorly. Cirrus sac is oval to elliptical in shape, immediately anterior to the ovary. Ovary is globular, subglobular or even and located slightly on the right in the inter-testicular field, immediately behind the cirrus sac. Excretory bladder is V shaped, small and located at the posterior end of the body. It opens outside by a terminal excretory pore.

DISCUSSION: (FIGS-1, PICS-1-6)

Leidy (1856) created the genus *Clinostomum* for Rudolphi's worm *Distomacomplanatum* (1809). Southwell and Prasad (1918) gave an update list of species of *Clinostomum* described from Indian region viz, *Clinostomum piscidium* in *Trichogaster fasciatus* and *Nandus nandus* from lake of Kashmir, syn. of *Clinostomum microstomum* Singh (1955), syn. of *Clinostomum marginatum* Agarwal (1960), syn. of *C. complanatum*. Bhalerao (1942) reported the morphology of *C. piscidium*. Singh (1959) reported the morphology and life history of *C. piscidium*. Pandey and Baugh (1969) described and illustrated *C. piscidium* after a thorough restudy of specimens. In the Rybinsk reservoir, U.S.S.R. Shigin (1957) found adult Clinostomes only in the spring, and not in other season.

The specimen at disposal of the writer exhibits some variations in the measurements of some body parts. 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

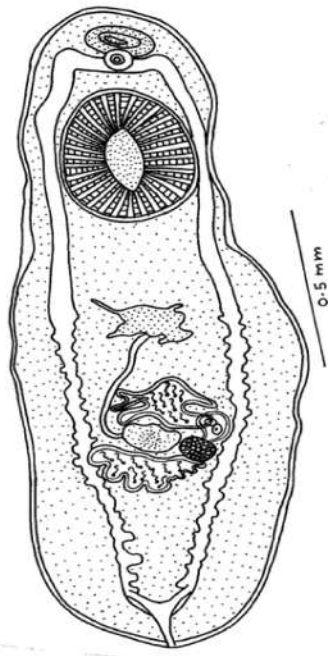
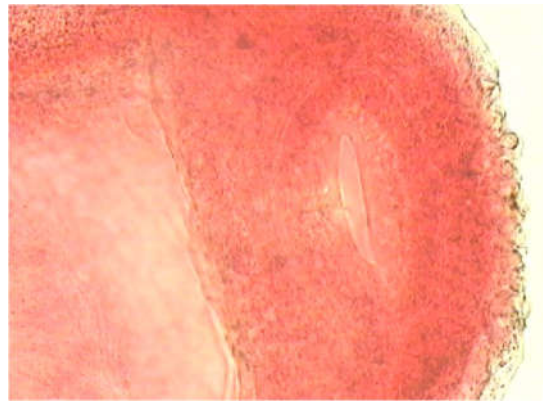
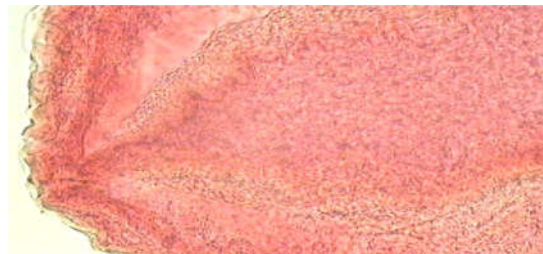


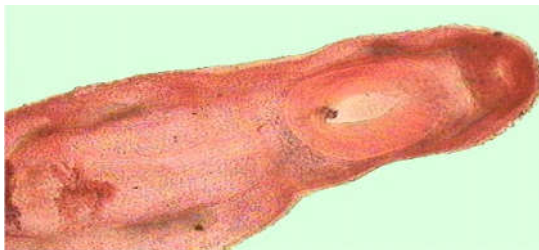
Figure 1. *Clinostomum piscidium* Southwell and Prashad, 1918.



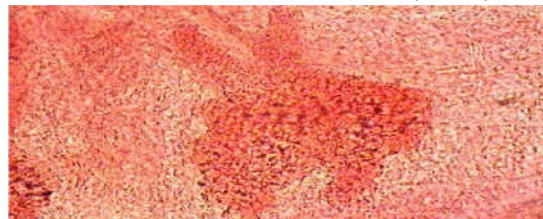
PIC -3. ORAL SUCKER (400 X)



PIC -4. EXCRETORY PORE (400 X)



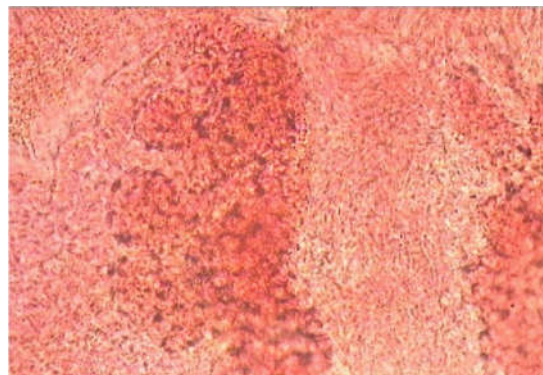
PIC- 1. ORAL AND VENTRAL SUCKER (100x)



PIC -5. ANTERIOR TESTIS (400 X)



PIC -2. ANTERIOR AND POSTERIOR TESTIS, CIRRUS SAC AND OVARY (100X)



PIC -6. POSTERIOR TESTIS (400 X)

Table1: Showing measurements of various body parts of *Clinostomum. piscidium* (Southwell and Prashad, 1918)

Body parts	<i>C. piscidium</i> Southwell and Prashad, (1918)	<i>C. piscidium</i> Bhalerao (1942)	<i>C. piscidium</i> Pandey and Baugh (1969)	<i>C. piscidium</i> Singh, Chaudhary and Halajian (2010)	<i>C. piscidium</i> Present study
Body size	2.8-5.2 x 1.4-1.8	3.8-1.96	2.26-4.57x0.64-1.6	2.27-3.36 x 1.17-1.26	2.05 x 0.78
Oral sucker	-	2.8-0.18	0.12-0.22x 0.31-0.60	0.14-0.18 x 0.23- 0.30	0.09 x 0.19
Ventral sucker	-	0.6-0.48	0.31-0.60 x 0.49-0.69	0.60-0.68 x 0.70-0.80	0.43 x 0.36
Oesophagus	-	-	0.30-0.70	0.20-0.24	0.07 x 0.08
Anterior testis	-	-	0.10-0.22 x 0.15-0.31	0.15-0.22 x 0.25-0.35	0.17 x 0.28
Posterior testis	-	-	0.10-0.22 x 0.18-0.43	0.12-0.20 x 0.30-0.40	0.13 x 0.32
Cirrus sac	-	0.35	0.15-0.22 x 0.07-0.15	0.14-0.20 x 0.07-0.15	0.15 x 0.09
Ovary	-	-	0.04-0.06 x 0.04-0.09	0.08-0.12 x 0.10-0.14	0.07 x 0.1

measurement of body of the worm from worms described earlier given in **Table 1**.

Number of specimens recovered- 02

Host- *Channa punctatus*

Site of infection- Body cavity

Locality- Muzaffarnagar region

Number of fish examined- 473

Number of fish infected- 01

Prevalence and intensity- 01 fish infected/ 473 fish examined: 02 digenean.

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