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Morphological Evaluation of a Helminth Parasite *Isoparorchis hypselobagri* Recovered From Freshwater Bottom Dwelling Fishes of Kolong River, Assam, India

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Abstract The present investigation deals with the study of morphological features of a helminth parasite Isoparorchis hypselobagri recovered from three bottom dwelling fishes viz . *Mastacembelus armatus, Notopters notopterus* and *Wallago attu* of Kolong river, Assm. This study included to record the light, microscopic morphology and histological analysis of morphological features of the helminth parasite. Histological crossectional study conducted revealed surface and internal morphological features of *I. hypselobagri* for the first time hitherto unknown. Present study reveals that shape of the body and morphology of internal organs of the parasite is different from other known species of the genus *isoparorchis.*

Keywords *Isoparorchis*, Helminth, *Mastacembelus armatus, Notopterus notopterus, Wallago attu.*

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Introduction

Southwell in 1913 described the prevalence of *Isoparorchis hypselobagri*, a digenetic trematode parasite from the freshwater catfish, *Wallago attu*. The parasites were recovered from the fishes caught at a tank at Bankipur, Bengal. All the adult *W. attu* fishes examined contained cent percent infection with *I. hypselobagri*. He also opined that, *Isoparorchis hypselobagri* is a widely spread trematode parasite, adults are found in the swim bladder of freshwater fishes (*Wallago attu, Hypselobagrus* sp., *Tandanus tandanus, Pseudobagrus aurantiacus, Barbus tor, Parasilurus asotus*) in Australia, Indonesia, India, Japan, China and Siberia.

Immature stages encyst in different body parts of various fishes. Bhalerao in 1936 recorded the encysted form of *Isoparorchis hypselobagri* in various fishes from different parts of India. The encysted forms were found in liver, body cavity and subcutaneous tissue of *Anabasis nana* at Poona, in mesentery and liver of Notopterus notopterus, in muscles of *Gobius giuris*, *Ophiocephalus marulius, O.gachua, O. punctatus* and *Mastacembalus armatus* at Hyderabad and in the muscles and coelomic cavity of *Ophiocephalus striatus* at Nagpur.

Materials and Methods

For the morphological study of the helminth, three species of bottom dwelling fishes viz. *M. amatus, N.notopterus* and *W. attu* were collected from seven different sampling centres across the Kolong river of Assam during the period of May 2014 to April 2015. Helminth parasites encountered in the present investigation were collected to study their light microscopic morphology leading to establish their taxonomic identity and the tegumentary ultrastructure. For light microscopic morphological studies parasites were collected during post-mortem examination in the laboratory using a camel hair brush. They were put in clean, flat bottom screw capped glass tubes (*Borosil*, O.D.25 × Length 57 mm) in normal saline solution. After collection they were gently washed several times with double distilled water to remove any tissues or debris attached on the surface of the body. Parasites were put in glass petri dishes (*Borosil*, O.D. 100 × Height 17 mm) containing distilled water. A few drops of 70% alcohol were mixed into it. With the help of a fine camel hair brush, the parasites were gently stirred till the body muscles were gradually relaxed. When they died, the parasites were divided into two groups and processed differently. After collection and cleaning, a few representative parasites were individually put in between two glass slides and compressed till their body became flattened revealing some of their internal structures. The slides were then tightened with rubber bands. The slides containing the parasites were put into 10% formalin for 48 h. When the parasites were fixed, both the glass slides were removed and the flattened parasites were put into 10% formalin and preserved. Some flattened parasites were subjected to staining with alcoholic borax carmine stain following the method described by Roberts [1]. Accordingly, the fixed parasites were first washed overnight in flowing water to remove the traces of fixatives. After washing, the parasites were dehydrated in ascending grades of ethyl alcohol, i. e., 30%, 50%, and 70%, 2 changes in each for 15 minutes. Dried parasites were stained for overnight in alcoholic Borax carmine stain. They were destained in 1% acid alcohol. The destaining was observed taking the overstrained parasites under a stereo microscope. When the internal structures of the parasites could be differentiated, the destaining step was stopped. Thereafter, the stained parasites were again dehydrated in 70%, 80%, 90%, 95% and 100% ethyl alcohol, 2 changes in each for 15 minutes. The dehydrates parasites were momentarily put in clove oil and then cleared in xylene. Finally they were mounted on microslides (HIMEDIA, 25.4 mm × 76.2 mm (1″ × 3″), thickness 1.0-1.2 mm, Comer : 90°) using DPX and covered with round cover glass (Blue Star, 18 mm diameter) or rectangular cover glass (Blue Star, size 22X 30 mm) as and when necessity demanded. The stained and mounted permanent slides were observed under a trinocular research microscope (Nikon, Model : Eclipse E 200) with an image analyzer (Image pro Express Ver. 6.0) to record the taxonomic identity using key morphological features as described by Yamaguti [2] and Shimazu et al. [3].

Results and Discussion

In the present study trematode parasites could be recovered from different parts, organ/location of the body of Mastacembelus armatus, Notopterus notopterus and Wallageo attu from the study site. These parasites were subjected to morphological studies by light microscopy after staining them with alcoholic borax carmine stain. Longitudinal sections of the parasites were prepared, stained with routine hematoxylin and eosin stain to study the internal organs of the parasites.

Length and breadth of 6 uncompressed parasites, which were freshly recovered from the swim bladder of Wallago attu were recorded. The average length × breadth was found to be (9.9 ± 0.44 mm × 5.05 ± 0.48 mm with their range being (8.2-11.0) mm ×

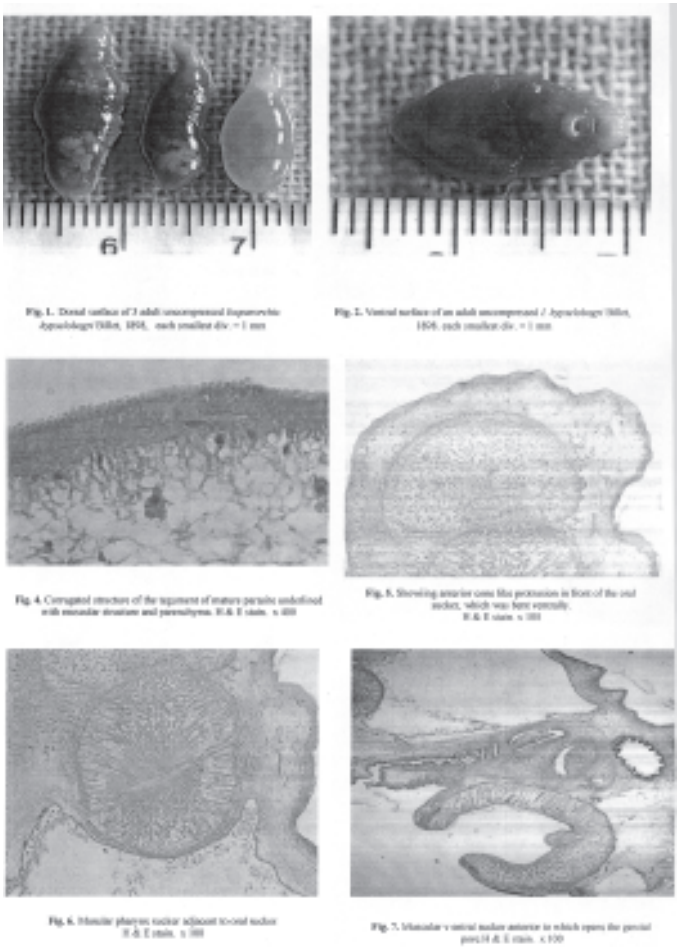

Fig. 1. Dorsal surface of 3 adult uncompressed *Isoparorchis hypselobagri* billet, 1898, each smallest div.= 1mm. **Fig. 2**. Ventral surface of an adult uncompressed *I. hypselobagri* billet. 1898. each smallest div. = 1 mm. Fig. 4. Corrugated structure of the tegument of mature parasite underlined with muscular structure and parenchyma. H and E stain × 400. **Fig. 5.** Showing anterior cone like protrusion in front of the oral sucker, which was bent ventrally. H and E stain × 100. **Fig. 6.** Musular pharynx sucker adjacent to oral sucker. H and E stain × 100. **Fig. 7**. Muscular v entral sucker anterior to which opens the genital pore. H and E stain × 100.

(4.0-6.5) mm. The parasites were robust, fleshy, dorsoventrallay flattened, foliate, oval or oblong with a translucent tegument, through which the internal structures of the parasites could be visualized (Fig. 1 and Fig.2). Freshly collected parasites were reddish brown in color. In case of compressed preparation of the parasite the average length × breadth was found to be (17.48 ± 0.37) mm × (8.93 ±0.34) mm with their range being (16.5-18.7) mm × (8.0-9.9) mm.

The adult specimen had a fore body, which was conical in shape. The hind body was broad and slightly rectangular or elliptical. The oral sucker was ventral and sub-terminal with a small cone like structure at the anterior end. There was a distinct shoulder at the level of which was placed the ventral sucker, which was muscular and bigger than the oral sucker. Pharynx was well developed and muscular but small. Oesophagus was short and muscular leading to a well developed Drusenmagen on both sides. Intestinal caeca was bifurcated, simple, undulating into about 8 times, reaching the posterior part of the body. Tes-

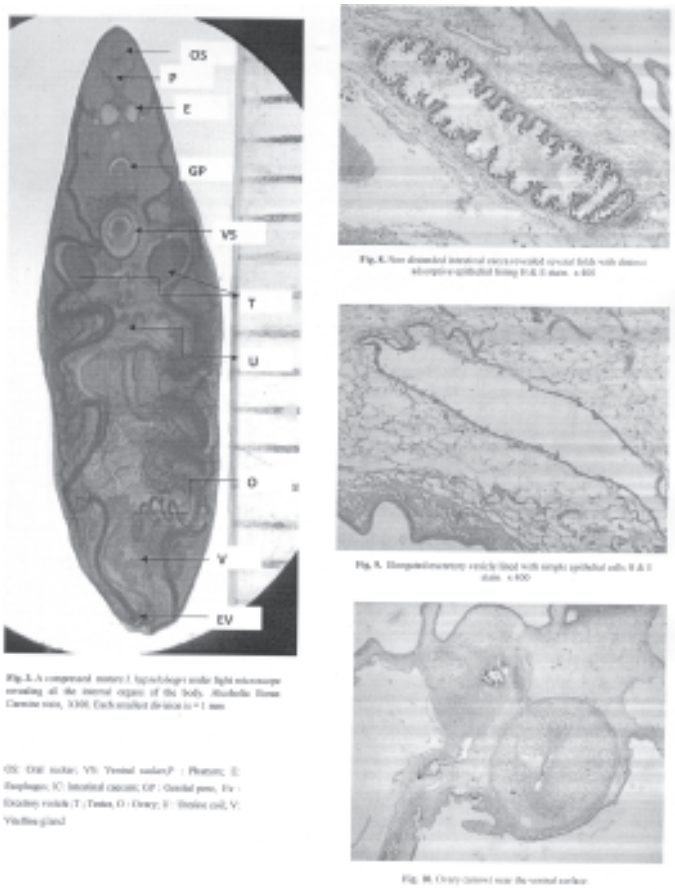

Fig. 3. A Compressed mature *I. hypselobagr*i under light microscope revealing all the internal organs of the body. Alcoholic borax carmine stain × 100. Each smallest division is = 1 mm. **Fig. 8**. Non distended intestinal caeca revealed several folds with distinct adsorptive epithelial lining H and E stain × 400. **Fig. 9.** Elongated excretory vesicle lined with simple epithelial cells H and E stain × 400. **Fig. 10.** Ovary (arrow) near the ventral surface H and E stain × 400. OS : Oral sucker; VS : Ventral sucker P : Pharynx ; E : Esophagus ; IC : Intestinal caecum; GP : Genital pore ; Ev : Excetroy vesicle ; T : Testes ; O : Ovary; U : Uterine coil ; V : Vitelline gland.

tes were entire, horizontally placed, situated just behind the ventral sucker. Vitelline glands were situated at the posterior 3rd of the body, anterior to which lies the ovary, which was a tortuous pipe like structure. Uterine coil occupied the middle field of the body ; the common genital pore opened anterior to ventral sucker and just opposite to intestinal bifurcation. The excretory duct aroses from near the oral sucker in both the sides, which met at the equatorial plane and then continued in a single zig-zag tube to the posterior 3rd of the body, where it emptied into a long and tubular excretory vesicle (Fig. 3).

Studies on the longitudinal section of the mature parasites revealed corrugated structure of the tegument underlined with muscular structures and parenchyma (Fig. 4). There was an anterior cone like protrusion in front of the oral sucker, which was bent ventrally (Fig. 5). Oral sucker was muscular adjacent to which situated pharynx (Fig. 6). Ventral sucker was muscular (Fig. 7) anterior to which opened the genital pore. The tegumentary structure of the ventral sucker was simple. Longitudinal sections of the non-distended intestinal caeca revealed several folds withdistinct absorptive epithelium lining (Fig. 8) . Excretory vesicle was elongated and lined with simple columnar epithelium (Fig. 9). Ovary was located towards the ventral surface (Fig. 10). Uterus contained numerous ova inside it (Fig. 11).

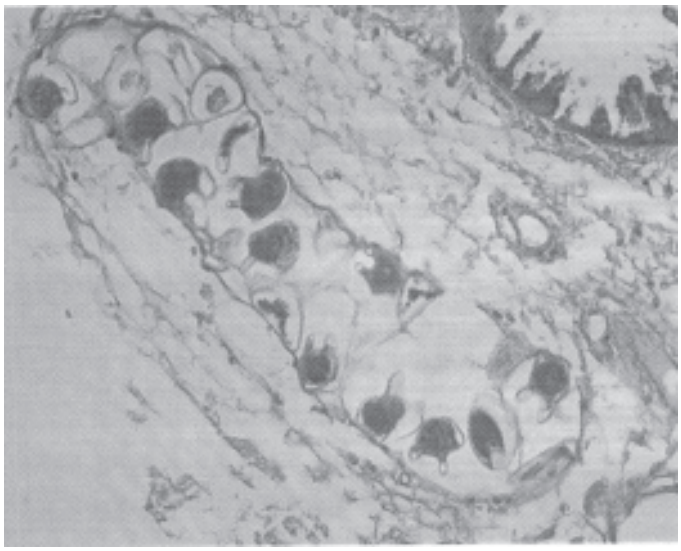

Fig. 11. Uterus showing numerous ova inside H and E stain × 400.

In the present study morphometrical and morphological studies of the helminth parasite recovered from *M. armatus, N. notopterus* and W. attu was conducted . This consisted of gross morphological studies on uncompressed parasites, preparation of compressed specimen using borax carmine stain to study the internal morphology, and preparation of longitudinal sections to study the location of the internal organs.

The findings of morphometrical and morphological studies of the present studies when compared with the earlier records of Southwell [4], Yamaguti [2] and Shimazu et al. [3] revealed that all the helminth specimens recovered from the three differnt host species was morphologically similar to the species *Isoparorchis hypsalobagri,* Billet [5] with little variations in morphometry. Earlier Southwell [4] recovered these trematodes from the gas bladder of silurid fish *W. attu* and nomenclatured as *I. trisimilitubis*. Later on Ejsmont [6] proved the earlier collection of Southwell [4] and commented as synonym of *I hypsalobagris.* Bhalerao [7] recorded the parasite from the gas bladder of *W. attu* and Pandey [8] studied the morphology and development of the parasite. Bashirullah [9] described the morphological features of the parasite recovered from *W. attu* and *Channa puntatus*. Dhole et al. [10] has recently recorded a new species of the genus *Isoparorchis* from *M. armatus* in Maharashtra and nomenclatured as *I. maharahtrensis* (n.sp.). Perusal of literature indicate paucity of information on the occurrence of *I. hypsalobagri* from this part of the country; hence, this finding has been considered as first record of recovery of *I. hypsalobagri* from *W. attu, N. notopterus* and *M. armatus* from Assam.

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