International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.04, March 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

SEM study of *Diplozoon kashmirensis* (Monogenea, Polyopisthocotylea) from Crucian Carp, *Carassius carassius*

Shabina Shamim, Fayaz Ahmad

Department of Zoology, University of Kashmir, Srinagar – 190 006, Kashmir, J&K, India

ABSTRACT

Using Scanning Electron Microscopy the external morphology of the helminth parasite Diplozoon kashmirensis (Monogenea, Diplozoidae) from the fish Carassius carassius is described herein for the first time. The present study is a part of the parasitological work carried out on the fishes of Jammu and Kashmir. These fish helminthes are ectoparasites, blood feeding found on gills of fishes. They have extraordinary body architecture due to their unique sexual behavior in which two larval worms fuse together permanently resulting in the transformation of one X shaped duplex individual. Oral sucker of the prohaptor has a partition giving it a paired appearance. The opisthohaptor present on hind body contains four pairs of clamps on each haptor of the pair, a pair of hooks and a concave terminal end. Body is composed of tegmental folds to help the worms in fixing to the gills. This type of strategy adapted for parasitic life in which two individuals permanently fuse into a single hermaphrodite individual without any need to search for mating partner and presence of highly sophisticated attachment structures, shows highest type of specialization of diplozoid monogeneans. In this study we used SEM to examine the surface topography of Diplozoon kashmiriensis, thereby broadening our existing knowledge of surface morphology of fish helminthes.

Key Words* – *Carassius carassius, Diplozoon kashmirensis, Monogenea, Opisthohaptor, SEM.

I INTRODUCTION

Monogenea is one of the largest classes within the phylum Platyhelminthes and they usually possess anterior and posterior attachment apparatus that are used for settlement, feeding, locomotion and transfer from host to host [1, 2, 3]. Species of *Diplozoon* Nordmann, 1832 *(*Monogenea, Diplozoidae*)*are blood-feeding ecto-parasites mainly parasitizing the gills of cyprinid fishes, where they can cause mechanical damage to the gill filaments, initiating the development of secondary infections (bacteria, mycotic) and anemia [4,5]. Numerous studies have already targeted their life cycle and pairing process [6-9]. They have a direct & unique life cycle during which eggs are laid in the gills of host fish, hatch into free swimming oncomiracidia then a diporpa larvae stage and adult. Diporpa will remain in that stage unless two larvae come together. Then the two larvae will go metamorphosis and become fused together [10]. A diporpa juvenile can live for several months, but it cannot develop further until encountering another diporpa; unless this happens, the diporpa usually will die. When one diporpa finds another, each attaches its sucker to the dorsal papilla of the other. This begins one of the most intimate associations of two individuals of the animal kingdom. The complete fusion of two worms stimulates maturation, producing a typical X shaped *Diplozoon* body resulting in cross fertilization [11]. They are oviparous and have extraordinary body morphology. It is considered a 2 headed parasite due to fusion of two bodies. The present study is the first demonstration of the surface topography of this parasite via SEM.

Diplozoon species spend their whole life attached to the gills of fishes so they have developed a number of adaptations for successful parasitic mode of life. An important part of monogenean body is the posterior portion of the body which is modified to form ophisthohaptor, a disc like structure and a powerful adhesive organ comprising a pair of central hooks and four pairs of clamps. The clamps are thought to provide major role in the attachment while the relatively small hooks most likely function only during the initial stage of attachment [12]. All the internal organs are present in the anterior half of the body and the reproductive organs are located in the posterior half of body. *Diplozoon* is regarded as being completely monogamous [13]. This study is a part of the parasitological work carried out on the fishes of Jammu and Kashmir. The great advantage using the SEM for topography measurements is the high flexibility to zoom from low magnification and locating interesting areas to high magnification of down to nanometer large surface features within seconds. It provides a strong tool for investigating different aspects of parasite's morphology and adaptive mechanism.

II MATERIAL & METHOD

Parasites were collected from the gill filaments of Crucian Carp, *Carassius carassius* from Dal Lake Srinagar. For Scanning Electron Microscopy the worms collected were washed thoroughly to get the body surface cleared from mucus and debris. After washing parasites were fixed in 2.5% glutaldehyde in 0.1 M Sodium cocodylate buffer for 24 hours at 4°C. .After fixation the specimens were washed in sodium cocodylate buffer followed by post fixation in 1% Osmium tetraoxide ($OsO_{4)}$ for 1hour. The material was subsequently dehydrated through graded ethanol series ranging from 70-100%, dried in HMDS (Hexamethyl disilazine) by putting the material in 50&100% HMDS (1/2 an hour each), mounted on Aluminum stubs with double sided adhesive tape, coated with gold and examined in a JSM- IT300 Scanning Electron Microscope. Pictures were taken at desired magnification & measurements of body and other structures were noted.

III RESULTS

They are small worms, elongated, dorsoventrally flattened, bilaterally symmetrical measuring 1.708 to 3.22 mm in size including haptor. Adults permanently united in copulating pair & always occur in the shape of 'X' as two flukes join in posterior half of the body (Fig 1). Fore body longer than hind body, measures 1.102-2.3 x 0.499 - 0.82 & hind body measures 0.606- 0.92 x 0.209-0.5 mm. Comparison of two bodies of the pair reveals slight difference. SEM observation revealed rough body surface with absence of spines and papillae. Both the fore body and hind body is distinctly ridged. Tegument of both the bodies shows transverse ridges and folds which are discontinuous at lateral margins but continuous at the region of fusion. Anterior part of hind body is also

IJARSE
ISSN: 2319-8354

covered with folds and ridges. Both prohaptor and opisthohaptor are prominent. The region where the organisms are fused 'fusion bridge' is wide and shows the opening of uterus on the ventral surface (Fig 2). Ventral side of the fore body bears a large muscular mouth terminal in position on each of the two fore bodies (Fig 3). Around mouth the surface of tegument bears numerous sensory structures, pits & brush border used to grab on the gills of the fish (Fig 4). Fore body bears a pair of prominent rounded well developed buccal suckers in the buccal cavity as shown (Fig 4). Pharynx is just behind the region. Hind body is highly modified for attachment. Opisthohaptor bears four pairs of clamps on ventral side of hind body organized in two parallel rows, rectangular in shape, facing outwards (Fig 5). Opisthohaptor slightly widened in area of clamps and concave dorsally. Each haptor is symmetrical with two rows of the four clamps, with a thin layer of tegument, and a pair of central hooks which were not observed through SEM (Fig 6). Each of the clamps has a flattened bowl like structure composed of sclerites and each clamp consists of two jaws, hinged to each other. Size of clamps varies from individual to individual. Starting from anterior most pair as first pair, last pair of the clamps is the smallest (Fig 7). Dorsal side of opisthohaptor at terminal end is concave with three lobed structures, one central and two laterals (Fig 6). The part of the hind body above and including haptor is not covered with any tegumentary folds. Organs of reproduction located in anterior part of hind body, at the region of fusion. Eggs were not observed. The measurements of clamps are given below:

1st pair : 0.10x0.028 mm
2nd pair : 0.11x0.037mm
3rd pair : 0.141x0.052mm
4th pair : 0.109x0.027mm


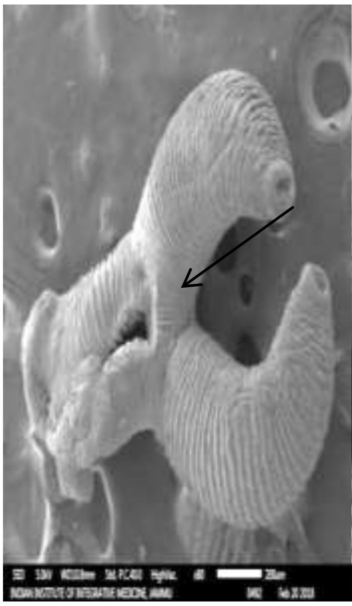
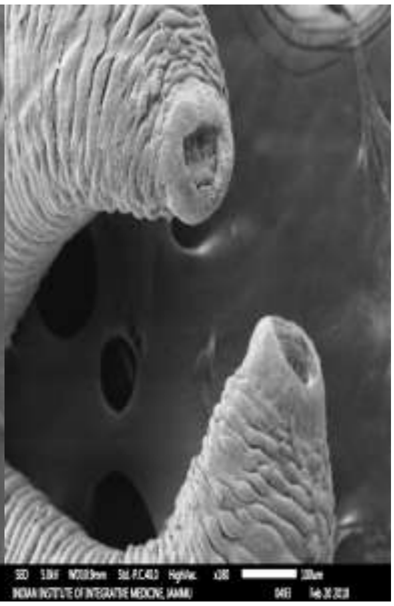

figure 1 **figure 2** **figure 3**

figure 4

figure 5

figure 6

Fig 1: Scanning electron micrographs of two fused individuals of *Diplozoon kashmirensis*. **Fig 2:** Full organism in union showing transverse tegumentary annular ridges, Uterus opening (arrowed) and fusion bridge between the two organisms. **Fig 3**: Anterior ends of fore body of the individual with open mouths on each of the two bodies. **Fig 4**: Detail of buccal cavity showing buccal suckers with a partition in between them and various digitations on the surface of mouth. **Fig 5**: detail of the flattened disc of hind body showing 4 pairs of clamps organized in two rows used for attachment to the host. **Fig 6:** Hind body of the individual with both ventral and dorsal sides showing clearly the concave terminal end & three lobed structures of dorsal side, one central and two lateral.

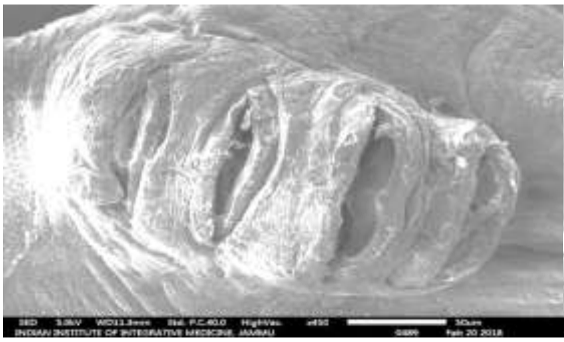

figure 7

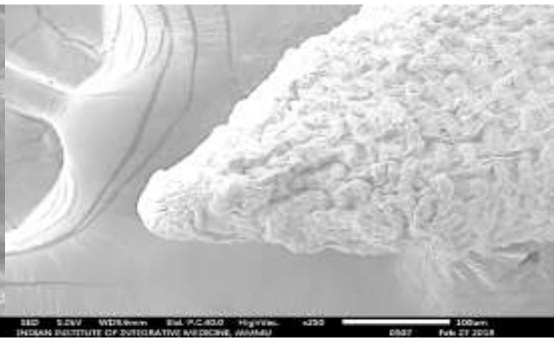

figure 8

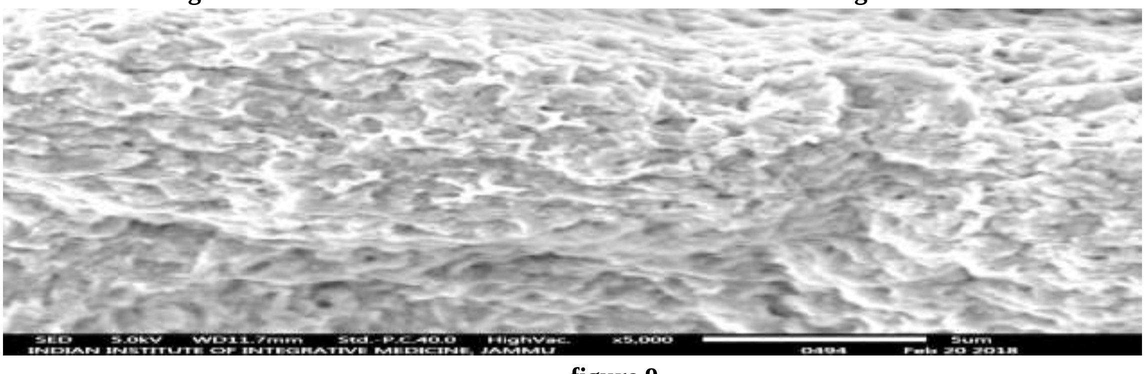

figure 9

Fig 7: Magnified image of opisthohaptor, a rectangular foot like structure, showing clamps, each with open jaws. **Fig 8::** Dorsal anterior side of fore body showing rough tegumentary surface. **Fig 9:** Magnified image of body surface with 5µm scale- bar.

IV DISCUSSION AND CONCLUSION

The specimen under discussion belongs to the genus *Diplozoon* Nordmann, 1832. . Of the species which occur in Kashmir, this species described here is most similar to *Diplozoon kashmirensis* (Kaw, 1950). It was first of all reported by Kaw (1950) from gills of Schizothorax sp from Dal Lake. Subsequently, Fotedar & Parveen, (1987) Ahmed & Chisti (1997) and Zargar et al (2013) have also reported it from different fish hosts. . In this study SEM has shown much more details in organization and morphology of individual structures. It shows resemblance with *Diplozoon kashmirensis* in size of clamps, general body length, and presence of plicae on hind body and absence of papillae like protuberances. After detailed morphological and comparative studies revealed the worm closely resembles *Diplozoon kashmirensis* with certain differences which are insufficient. This paper describes the morphological features that are present on the surface of ectoparasite using SEM, especially the attachment structures. In conclusion this study brings in detail the major structures necessary for attachment to the host for ectoparasitic life style The ridges and extensions present on the body are important for attachment to host. Buccal cavity with muscular buccal suckers, covered with various digitations clearly shows that the parasite is well equipped for blood sucking & attachment on the gills of its host**.** Besides, most important is the haptor equipped with clamps indicates their significant role in attachment and movement on the host. Comparisons of surface structures of various body parts revealed slight differences. During the examination a highly sophisticated adaptation system, to secure their attachment onto their hosts, is confirmed which is characteristic to an ectoparasitic lifestyle for diplozoid monogeneans.

V ACKNOWLEDGEMENTS

This work was done in Indian Institute of Integrative Medicine Jammu. The author is very much thankful to Director IIM. Dr Ram A Vishwakarma and Dr Parduman Raj Sharma (Principal Technical Officer, Cancer Pharmacology) for their helpful and kind approach in providing facilities required for Scanning Electron Microscopy.

REFERENCES

1. Bychowsky, B. E. (1957). *Monogenetic Trematodes, Their Systematics and Phylogeny*. Moscow/ Leningrad: Academy of Sciences of the U.S.S.R. [in Russian]. (English translation by W. J. Hargis and P. C. Oustinoff, 1961). Washington DC: American Institute of Biological Sciences. **Google Scholar**
2. Yamaguti, S. (1963). *Systema Helminthum. IV. Monogenea and Aspidocotylea*. New York, NY: John Wiley and Son **Google Scholar**
3. Kearn, G. C. (1998). *Parasitism and the Platyhelminths*. New York, NY: Chapman and Hall. **Google Scholar**

4. Kawatsu H.Studies on the anemia of fish-IX. Hypochromic microcytic anemia of Crucian carp causing infestation with a trematode, *Diplozoon nipponicum. Bull Japan Soc Sci Fish. 1978; 44:1315-9.*
5. Buchmann K, Bresciani J. Monogenea (Platyhelminthes). *Fish Dis Disorders. 2006; 1:297-344.*
6. Hirose H, Akamatsu h, Hibiya T, on the development of clamps and pairing of diporpain *Diplozoon nipponicum* (Monogenea). *Bull Japan Soc Sci Fish.1887; 53(6); 953-957.*
7. Kamegal S, Ichigara a, Kato K, Nonobe h, Machida M. *Diplozoon nipponicum* goto, 1891 Part 1: Morphological observations on the worms obtained from Cyprinus carpio, *Month Rep Meguro Parasitol Mus. 1966; 83-84: 1-9. Japanese.*
8. Schabussova I, Koubkova B, Gelnar M. Schabuss M, Horak P. Surface carbohydrates of *Eudiplozoon nipponicum pre- and post-fusion. J Helminthol 2004; 78:68-68. Pmid: 14972039.*
9. Hodova I, MatejusovaI, Geinar M, The Surface topography of *Eudiplozoon nipponicum* (Monogenea) developmental stages parasitizing carp (Cyprinus carpio L.), *Cent Eur J Biol, 2010; 5(5):702-709*.
10. Kagel M., Taraschewski H. 1993. Host-parasite interface of *Diplozoon paradoxum* [Monogenea] in naturally infecting bream *Abramis brama* [L.] *J. Fish Dis. 16:501-506.*
11. Richard A. Heckmann, Nguyen Van Ha,Atif m.ElNaggar 2012 electron Optics Study (SEM, EDXA) of *Diplozoon paradoxum* (Nordman, 1832) (Diplozoidae, Trematoda) from the common carp, *Cyprinus carpio* L. (Cyprinadae, Osteoichthyes) in Vietnam with comments on potential host fish. *Sci parasitol 13[3]:109-117.*
12. Owen, I. L. (1963a) The attachment of the monogenean *Diplozoon paradoxum* to the gills of *Rutilus rutilus* L. II. *Structure and mechanism of the adhesive apparatus. Parasitology 53, 463-468.*
13. Wey Lim Wong and Stanislav N. Gorb, 2013 Attachment ability of a clamp-bearig fish parasite *Diplozoon paradoxum* (Monogenea) on gills of the common bream *Abramis brama, The Journal of Experimental Biology*.