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Ultrastructural studies of *Puntius shalynius* an endemic and threatened hill stream fish

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Abstract

Aim : Shalynai fish, *Puntius shalynius* is an inhabitant of freshwater, endemic and threatened hill stream fish of North-East India. The present work aimed to study the ultrastructural and functional characteristics of *P. shalynius* intestine, as addition to studies on nutritional requirement. This fish is considered an important species for ornamental exploitation.

Methodology : A total of five samples were collected from Umiam river, Meghalaya and transported to the laboratory for Transmission Electron Microscopy. Samples from intestine were cut into small pieces, fixed in Karnovsky's fixative, washed in 0.1M sodium cacodylate buffer and examined under an electron microscope.

Results : The electron microscopy of the anterior intestine from five individuals indicated that the enterocytes were elongated resting on basement membrane and covered apically with uniform microvilli. The apical cytoplasm, just below the microvilli, had no cellular organelles and formed the terminal web. Numerous mitochondria were seen along with other cell organelles such as ribosomes and nucleus. Cellular ultrastructural features were associated with digestive functions.

Interpretation : The ultrastructural characteristics of *P. shalynius* intestine showed that a functional specialization exist in this fish also like other teleostean species, a common approach for active digestion in the lack of definite enzymes. The existence of microvilli seems to be important for absorptive function of the cells and enterocytes seems to function primarily in lipid absorption.

Ultrastructure of the intestine region in *Puntius shalynius*

Introduction

Puntius shalynius (Yazdani and Talukdar, 1975), a minor carp belonging to the order Cypriniformes and family Cyprinidae is a hill stream, endemic and economically important species for its ornamental value. This species has restricted distributional range found in Meghalayan rivers namely, Umiam, Umkhen, Umtrew, Umryleng Myntdu, Myntang, Umshing and Kynshi. These rivers belong to four different districts of Meghalaya viz. Ribhoi, Jaintia Hills, East Khasi Hills and West Khasi Hills. It is a small hill stream fish where the maximum growth of the adult is about 6.3 - 7.0 cm in length. The body of the species is characterized by short, compressed dorsal profile little convex than the ventral profile. Minute white tubercles present on the head of males are distinguishing features from that of females (Manorama and Ramanujam, 2016). The habitat of the fish is situated in between 1000 to 1400 msl which might be the possible reason for restricted distributional range of the species (Manorama *et al.*, 2014). *P. shalynius*, previously an abundant fish found wild in Meghalaya, has now been categorized as "vulnerable" (IUCN, 2015). Earlier it was reported that *P. shalynius* feeds on animal matter, detritus and algae (Manorama and Ramanujam, 2011), but there is no information on the ultrastructure of this fish which is crucial for diagnosing intestinal diseases and formulating suitable feeds of this fish. Fish digestive tract shows remarkable differences in morphology and functions in various species and plays an important role in the digestion and absorption of dietary nutrients (Murray *et al.*, 1996). The overall gastrointestinal morphology is related to different feeding habits including the nature of the food and frequency of food intake, as well as taxonomy, body size and shape (Boglione *et al.*, 1992; Domeneghini *et al.*, 1999). The alimentary canal of teleostean fishes has been widely studied (MacDonald, 1987; Kuperman and Kuzmina, 1994; Domeneghini *et al.*, 2002; Khojasteh *et al.*, 2009; Camacho *et al.*, 2011) and described morphologically to determine the function of many specialized anatomical structures (Cataldi *et al.*, 1987). In many fish species, the anatomy of intestine has been studied which are important for aquaculture, recreation and aquarium (Caceci and Hrubec, 1990).

In the present study, the anatomical structures of *P. shalynius*, collected from Umiam river, Meghalaya, were studied for ascertaining digestive functioning. To the best of our knowledge, limited work has been reported on ultrastructure of digestive tract of *P. shalynius* having ornamental and food value. The objective of the present study was to describe the structure of the intestine of *P. shalynius*, an important hill stream and threatened species in Meghalaya, India.

Materials and Methods

Sample collection : *P. shalynius*, measuring 6.0 to 7.0 cm in length comprising of both sexes, were collected from Umiam river, Meghalaya, India. The study area is situated at an altitude of

1020 msl and falls at an intersection of 25°40'N latitude and 91°54'E longitude. The samples were collected with the help of local fishermen and transported to the fish laboratory in live condition. After being taken to laboratory, fish were sacrificed, dissected and the entire digestive tract was exposed. The samples of intestinal region were taken out for further study.

Due to poor picture quality of histological sections, we excluded histology from the present study.

Transmission electron microscopy : Five individuals were used for Transmission Electron Microscopy (TEM). Samples from anterior and posterior intestine were cut into small pieces (2 mm) with the help of simple microscope and sharp scissor. Extreme care was taken in order to cut tiny pieces of the intestine and fixed at 4°C in modified Karnovsky's fixative for 4 hrs. The fixed samples were washed in 0.1M sodium cacodylate buffer (Watson, 1958). Post fixation was carried out in 1% osmium tetroxide in the same buffer for 1 hr at 4°C. The samples were dehydrated in ascending grades of acetone and cleared in propylene oxide. It was then embedded in Araldite medium. Ultra-thin sections of 70 nm thickness were collected on copper grids and stained with 50% alcoholic saturated uranyl acetate for ten minutes at room temperature followed by lead citrate solutions. Here, lead citrate was used for staining ultrathin sections because it is stable for long time and does not contaminate the sections with unnecessary precipitate (Reynolds, 1963). The samples were analyzed under an electron microscope with JEM 2100.

Results and Discussion

Study on the digestive tract and different feeding habits of fish residing in various water depth provide useful ecological and biological information. In the present study, the intestinal sample of *P. shalynius* was examined following the standard protocol and anatomy resembles that of other teleostean fishes. Similar studies were reported by Arellano *et al.* (2002) on the anterior and posterior intestine of *Solea Senegalensis*; Dai *et al.* (2007) made a detail ultrastructural study of the digestive tract of *Monopterus albus*; Khojasteh *et al.* (2009) worked on transmission electron microscopy collected from different fragments of intestine in *Oncorhynchus mykiss* and Namulawa *et al.* (2015) described transmission electron microscopy of the intestine of *Lates niloticus*.

TEM of the intestine revealed that the columnar epithelial cells or enterocytes were elongated resting on the basement membrane (Fig. 1A). Findings on enterocyte of the intestine in *P. shalynius* are congruent with that of previous report in *Salvelinus alpinus* (Olsen *et al.*, 1999). The apical cell membrane showed numerous regularly arranged cytoplasmic projections, microvilli, which formed the brush border (Fig. 1C). These microvilli were covered by glycocalyx and the upper parts of some of them were dense and formed spherical bleb called capitulum (Fig. 1B). The

existence of microvilli is related to the absorptive function of the cells (Grau *et al.*, 1992). The tip of microvilli in *P. shalynius* possess capitula which may increase the apical surface and intensify penetration. (Athikesavan *et al.*, 2006). Similar observations were reported in the intestine of *Hypophthalmichthys molitrix* and *Labeo niloticus* (Naguib *et al.*, 2011). The apical cytoplasm just below the microvilli had no cellular organelles and formed the terminal web. Invaginations of the apical plasma membrane were frequently seen among the microvilli forming the endocytotic channels. The enterocytes consisted of a few numbers of endocytotic channels which helps in uptake of protein macromolecules. This result is in confirmation with that of Khadse and Gadhihar (2017). The enterocytes were joined at the apical surface by typical junctional complexes

including the desmosomes (Fig. 1B). Kiliaan *et al.* (1996) suggested that the junctional complexes of the enterocytes of *Carassius auratus* may prevent the paracellular leakage of ions and macromolecules. It is well established that enterocytes are involved in lipid absorption (Houssain and Dutta, 1998). The mitochondria were numerous and polymorphic in shape (rounded, oval and elongated) distributed in the cytoplasm of epithelial cells. The inner mitochondrial membrane formed numerous lamellar cristae. The free ribosomes were abundant and scattered throughout the cell cytoplasm (Fig. 1D). The nuclei of enterocytes were large and oval in shape. The nuclear envelope was double membrane consisting of outer and inner nuclear membranes. Euchromatin was represented by pale granular areas of nucleoplasm, whereas heterochromatin was

Fig. 1 : TEM micrograph of cell types present in the intestine of *P. shalynius*; (A.) Inner layer of intestine with lumen (L), nucleus (N) and microvilli (MV).; (B.) Micrograph showing apical junctional complex desmosomes (D) with long microvilli (MV), capitulum (C), endocytotic channels (EC) and numerous scattered mitochondria (M) ; (C) Micrograph showing long structure of microvilli (MV) and (D) Micrograph showing nuclear envelope (NE), nucleus (N), heterochromatin (H), euchromatin (E), mitochondria (M) and free ribosomes (FR)

represented by small dark granular patches (Fig. 1D).

Stomach is absent in *P. shalynius*, which is similar to other cyprinid fish such as loaches (Venkat Appa Rao and Shukla, 2013) and gobies (Wolczuk *et al.*, 2015). In fish, the absence of a stomach does not limit trophic preference leading to identical alimentary morphology susceptible of digesting many diets (Day *et al.*, 2011). Unal *et al.* (2001) stated that true stomach of *Chalcalburnus tarichi* was absent and instead there was an expansion at intestine called the post-oesophageal swelling. Mir and Channa (2010) reported the presence of intestinal bulb in *Schizothorax curvifrons* which lacks stomach. The presence of intestinal bulb in *P. shalynius* is similar to the findings of other teleostean fish such as *Anarhichas lupus* (Helberg and Bjerkas, 2000), *Misgurnus mizolepis* (Park and Kim, 2001) and *Mystus cavasius*, *Oreochromis niloticus* and *Gudusia chapra* (Ghosh and Chakrabarti, 2015). It is worth mentioning that the intestine of teleostean fish exhibits the site of food digestion and absorption.

In conclusion, the present study on anatomical structures of *P. shalynius* provide information on digestion and absorption of nutrients in relation to feeding habits. More studies should be carried out for better understanding of the processes of digestion in *P. shalynius*, highly endemic and threatened fish species of North-East India. The results presented in this study may be considered as a baseline for subsequent studies on the intestinal bulb of different teleosts.

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