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Histological and ultrastructural study of intestine of Asiatic knife fish, *Notopterus notopterus*

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Abstract
The purpose of the present study was to study the histological and ultrastructural description of the intestine of the teleost fish *Notopterus notopterus*. Knowledge about morphology, histology and ultrastructural studies is essential for deeper understanding of the physiology and pathology of fish intestine. The intestinal morphology of the fish can be influenced by feeding habits. *Notopterus notopterus*, a carnivorous fish, shows typical intestinal morphology. Interestingly the intestine of the fish is very short. Histologically, the intestinal wall of the investigated species comprised of mucosa, sub-mucosa, muscularis and serosa. Stratum compactum and stratum granulosum layers found in the sub mucosa. Numerous villi lined with simple columnar epithelium along with absorptive cells, intraepithelial lymphocytes and goblet cells were observed which were numerous in the proximal region of intestine as compared to distal region of intestine of *N. notopterus*. Eosinophilic granular cells (paneth cell) which show a protective role in fish immunity were observed in the mucosa, sub-mucosa and lamina propria of intestine. Ultrastructural studies of intestine showed the presence of enterocytes with regular microvilli, junctional complex, lysosomes, endoplasmic reticulum, microfilaments and polymorphic mitochondria.

Keywords: Histology, ultrastructure, intestine, teleost, *Notopterus notopterus*

1. Introduction
The fish digestive tract shows a marked diversity of both morphology and function [5, 9]. In general intestinal morphology of fish can be influenced by feeding habits, frequency of food intake as well as by body size and shape [4, 7]. Depending on diet, the fish intestine can vary morphologically from short straight to coiled and complexly arranged [25]. It is suggested that there is a general relationship between the length of the intestine and feeding habits [16]. The intestine in the carnivorous fish is shorter than that of omnivores and herbivores. It is suggested that the histological studies on fish have a tool to provide extensive information on fish gastrointestinal tract [28]. The intestine of the fish plays an important role in digestion absorption of dietary nutrients and also involved in immunological functions [9, 27]. As the contribution to the development of the fish farming and a rational use of natural resources, the interest to approach the histological study of these structures resides in the application of the knowledge more about the pathology of fishes [23]. By the light and ultrastructural studies recent reports are made on the anatomical, morphological description of gut of fishes [3].
Notopterus notopterus (Asiatic Knifefish) is a carnivorous and predatory fish. Intestine of the carnivorous fishes is short or more or less straight, because meaty foods can be digested more readily than vegetable ones. This fish accepts most kind of live frozen food. Some specimen accepts pellets and dry food. It is distributed through out the India, Pakistan, Bangladesh, Nepal, Thailand, Malaysia and Indonesia [29]. Body is highly compressed, dorsal and ventral profile almost equally convex.
Hence in the present investigation, general histological and ultrastructural studies were carried out. The histological studies of the gut region across species of fish are becoming more valuable as the interest in fish culture is expanding and will be helpful with respect to feeding and nutrition.

2. Material and Methods
2.1. Sample collection
Six live adult *Notopterus notopterus* of either sex with body length ranging from 19-22 cm, weighing 80-100 gm were collected from fresh water pond Chatri Talao, Amravati,

Maharashtra, India (20^0 56' N, 77^0 47' E). Fishes were anaesthesized with 2-phenoxy ethanol (1:2000) for 10-15 mins in the anaesthetized tank. After being anaesthesized, the fish was sacrified and processed for histological and transmission electron microscopic studies.

2.2. Light microscopy
For histological study, small pieces of proximal and distal parts of intestine of *Notopterus notopterus* were removed and fixed in Bouin's fluid for 24 hours. The tissues were washed under tap water for 18-24 hours. After fixation tissues were passed through graded alcohol 30%, 50%, 70%, 90% and 100% (absolute). After dehydration tissues were cleared with xylene and processed for paraffin embedding. Sections were cut at 6-8µm thick in a rotary microtome and proceed for Haematoxylin and Eosin staining (HE) [16]. Microphotographs were taken under Olympus microscope.

2.3. Transmission electron microscopy
For the transmission electron microscopy, small pieces of proximal and distal parts of intestine were excised and fixed in 3% glutaraldehyde for 6-8 hours and then transferred in 1M sodium cacodylate buffer (pH 7.4), post fixed with 1% Osmium tetroxide (OsO_4) in the same buffer for hours at 4^0C, dehydrated in alcohol and acetone. The tissue were transferred in araldite with 2,2-Dimethoxy Propane (DMP) then embedded in as resin beam capsule. This capsule cut with the ultramicrotome (Leica ultracut) into semithin sections (1 µm thick).The semithin sections were stained with 1% toluidine blue and observed under light microscope. Pale gold section (80-90 nm thickness) were mounted on copper grids. The ultrathin sections were stained with 5% uranyl acetate and lead citrate [26] and observed under transmission electron microscope (JOEL 1010).Images were digitally acquired by using a CCD camera (Camera Erlanghen ES 500 voltage, Gatan model no.782) attached to the transmission electron microscope (JASLOK, Mumbai).

3. Results
3.1. Histology of intestine
The histological structure of proximal and distal intestine of *N. notopterus* showed that the basic organization of intestinal wall was similar to that in other teleost and vertebrates forming by four layers, mucosa, sub-mucosa, muscularis and serosa (fig.1 and fig.6).

The mucosal surface forms numerous highly branched, elongated, compact, deep finger like folds called villi in proximal portion (fig.1) and few short villi in distal portion of intestine lined by simple columnar epithelium (fig.6).

This epithelium consist of absorptive cells and goblet cells. The goblet cells appear like swollen, ovoid and flask shaped scattered among the absorptive cells (fig.2,5,6 and 7).The number of goblet cells became lesser towards the distal portion of the intestine. The eosinophilic granular cells (EGCs) were detected in the lamina propria, sub-mucosa and at the base of the epithelium (fig.3 & 4).The simple columnar epithelium rests on a thin basement membrane and possesses a intraepithelial lymphocytes (IELs) (fig.5).The lamina propria consists of small blood vessels, smooth muscle fibres, EGCs and lymphocytes (fig 8 & 9). Beneath the lamina propria stratum granulosum and stratum compactum are observed which contain EGCs (fig.11).The muscularis layers comprises of outer longitudinal muscle layer (LML) and inner circular muscle layer (CML) and were thicker in the proximal intestine as compared to the distal intestine. Nerve plexus (Auerbach's plexus) were observed between the outer LML and inner CML (fig.10). The serosa constituted to be the extremely thin layer of simple squamous epithelium (fig.6).

3.2. Ultrastructural study of intestine
The ultrastructural studies of the proximal and distal intestine revealed presence of tall columnar enterocytes and goblet cells. The apical region of the columnar cells bear numerous microvilli covered by glycocalyx (fig.12, 17 and 18).The enterocytes are joined to form junctional complex (fig.12 and 17).

Numerous polymorphic mitochondria, lysosomes, rough endoplasmic reticulum, pepsinogen granules, microfilament, chylomicron and some lipid droplets were evident in the apical cytoplasm of the enterocyte (fig.12, 13, 14, 17 and 18). The polymorphic heterochromatin and euchromatin nuclei with one or more nucleoli were situated at the center or base of the cell covered by nuclear envelope (fig.14, 15 and 18). In proximal portion of intestine, pyramidal shaped EGC or paneth cell was observed which showed electron dense granules in the apical cytoplasm and irregular shape nucleus at the base of the cell. The Golgi apparatus were detected between the nucleus and apical cytoplasmic granules (fig.16). Mature oval and flask shaped goblet cells filled mucinogen granules at the swollen apical cytoplasmic part and irregular shaped nucleus and few mitochondria were located at the end of elongated part of the goblet cell (fig.19).

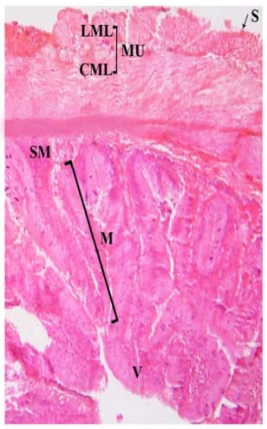
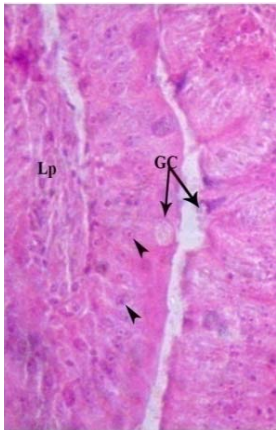


Fig 1



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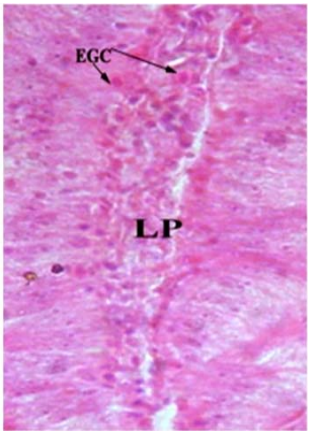

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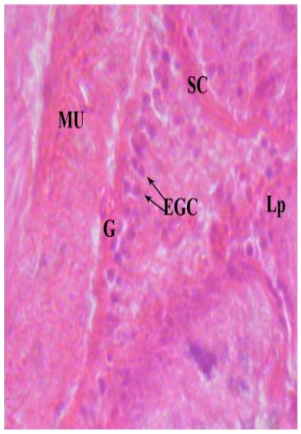

Fig 4

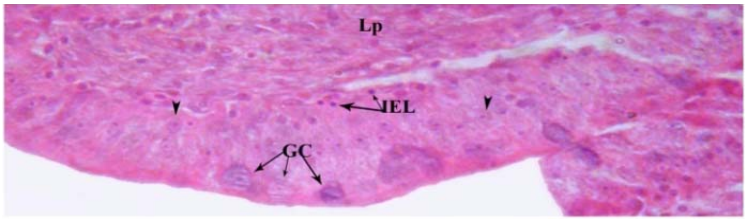

Fig 5

Fig 1-5: Proximal intestinal wall of *N. notopterus* (H-E staining). **Fig 1:** Proximal intestine with villi (V), mucosa (M), sub mucosa (SM), muscularis (MU), longitudinal muscle layer (LML), circular muscle layer (CML), serosa (S). (HEX100). **Fig 2:** Mucosa of proximal intestine with goblet cell (GC), absorptive cell (arrow head) and lamina propria (Lp). (HE X400).**Fig 3:** Enlarged part of villus with eosinophilic granular cell (EGC) (arrow) and lamina propria (Lp). (HEX 400). **Fig 4:** Muscularis (MU) and stratum granulosum (G) lies beneath the lamina propria (Lp) and stratum compactum (SC) and contains many eosinophilic granular cells (EGC).(HEX400). **Fig 5:** Half part of the villus with goblet cell (GC), intraepithelial lymphocytes (IEL), absorptive cells (arrow head) and lamina propria (Lp).(HEX 400).

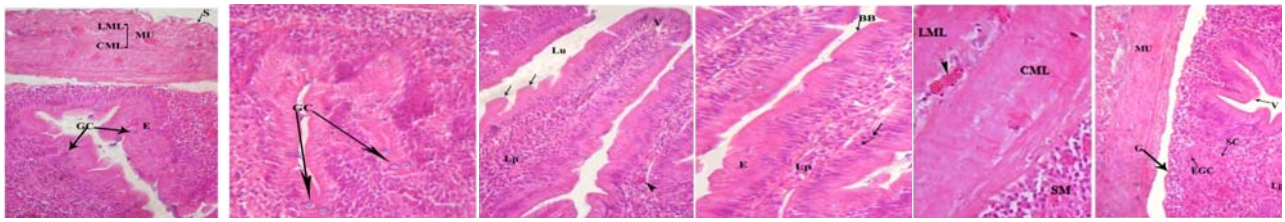

Fig 6 **Fig 7** **Fig 8** **Fig 9** **Fig 10** **Fig 11**

Fig 6-11: Distal intestinal wall of *N. notopterus* (H-E staining).**Fig 6:** Distal intestine with serosa (S), muscularis (MU), longitudinal muscle layer (LML), circular muscle layer (CML), sub mucosa (SM), Epithelium (E), goblet cell (GC). (HEX400). **Fig 7:** Enlarged portion of villi with goblet cells (GC). (HEX 400). **Fig 8:** Single villi (V) with primary fold (arrow), lamina propria (Lp), lumen (Lu) and blood vessels (black arrowhead). (HEX400). **Fig 9:** Enlarged portion of villus showing epithelium (E), lamina propria (Lp), smooth muscle fibres (white arrow head), brush border (BB) and lymphocytes (black arrow).(HEX 400). **Fig 10:** Nerve plexus (arrow head) lies between the longitudinal muscle layer (LML) and circular muscle layer (CML). (HXE400). **Fig 11:** Stratum granulosum (G) lies beneath the lamina propria (Lp) and stratum compactum (SC), villi (V) and muscularis (MU).(HEX400).

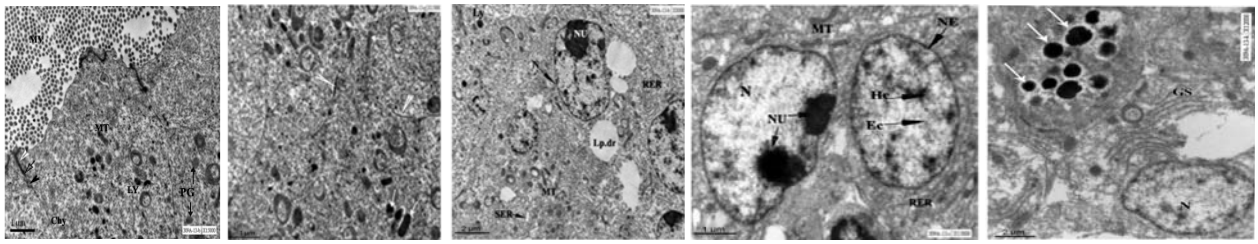

Fig 12 **Fig 13** **Fig 14** **Fig 15** **Fig 16**

Fig 12-16: Ultrastructure of proximal intestine of *N. notopterus*. **Fig 12:** Mucosa of proximal intestine with microvilli (MV), junctional complex i.e tight junction (white arrow head), adherens junction (black arrow), desmosomes (black arrow head) between the enterocytes, mitochondria (MT), lysosomes (Ly), pepsinogen granules (PG) and chylomicron (chy).(X 15000, 1µm). **Fig 13:** Enterocyte cytoplasm with polymorphic mitochondria (white arrow) and microfilament (white arrow head) (X 15000, 1 µm). **Fig 14:** Middle epithelium with nucleus(N), nucleolus(Nu), mitochondria (MT), lysosomes (Ly), smooth endoplasmic reticulum (SEM), rough endoplasmic reticulum (RER) and lipid droplets (Lp.dr).(X8000, 2µm). **Fig 15:** Enterocyte with nucleus (N) with two nucleoli (Nu), heterochromatin euchromatin, nuclear envelope (NE), mitochondria (MT) and rough endoplasmic reticulum (RER). (X5000,1 µm). **Fig 16:** Eosinophilic granular cell or paneth cell with numerous electron dense grannule at apical part and basally located nucleus (N), golgi stacks lies between them. (X 10000, 1µm).

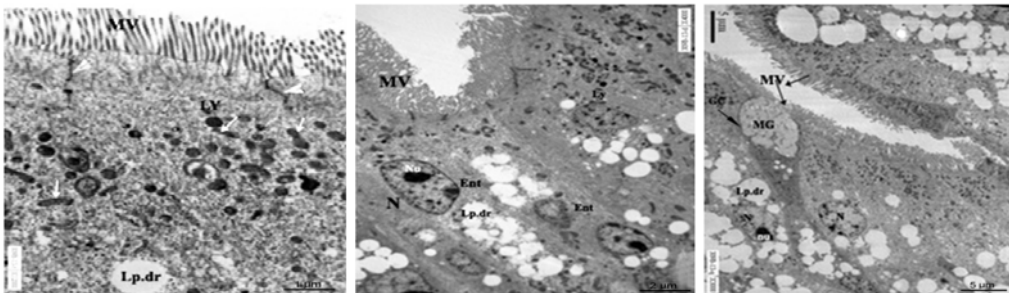

Fig 17 **Fig 18** **Fig 19**

Fig 17-19: Ultrastructure of distal intestine of *N. notopterus*. **Fig 17:** Mucosa of the distal intestine with apical junctional complex (white arrow head) separating the enterocytes, microvilli (MV), lysosome (Ly), mitochondria (white arrow) and lipid droplets (Lp.dr), (X 12000, 1µm). **Fig 18:** Distal intestinal mucosa with the enterocytes (Ent), microvilli (MV) apically and basally located nuclei (N) with one or two nucleoli (Nu), lysosomes(Ly) and lipid droplet (Lp.dr) (X 4000, 2µm). **Fig 19:** Mucosa of the distal intestine with microvilli (MV), Lipid droplets (Lp.dr), goblet cell (GC) having apical swollen part with mucus granules, basal elongated part and cytoplasm contains nucleus(N) with nucleolus (Nu).(X 3000,5µm).

4. Discussion

4.1. Histology

The histological studies of the gastrointestinal tract across the species of fish are more beneficial as the interest in the fish culture spreads and more information is needed with regards to feeding and nutrition. In the present study the wall of the intestine of *N. notopterus* histologically comprised of four different layer, mucosa, submucosa, muscularis and serosa were similar with other teleost fishes like *Clarius batrachus* [24], *Salmo salar* [18], *Hypophthalmichthys nobilis* [12].

The present study revealed presence of short intestine with elongated mucosal folds which became shorter towards distal intestine lined by columnar epithelial cells and goblet cells. Corresponding studies carried out by authors revealed correlation of relative length of intestine with feeding habit. Similar observations were recorded in Benthic feeding fish species [14], *Oncorhynchus mykiss* [5], *Clarius batrachus* [24], *Hypophthalmichthys nobilis* [12], *Clarius batrachus* [10].

In the present study the number of the goblet cells were observed to decrease towards the distal intestine. This results are in agreement with those found in *Mugil cephalus* [13]. Whereas in other species of fishes like *Clarius batrachus* [10], *Monopterus albus* [11], *Schilbe mystus* [2], the goblet cells were seen to increase towards distal intestine. The stratum compactum and stratum granulosum layers were observed in the submucosa of intestine of *N. notopterus* in the present study. Similar findings have been reported in some other teleosts fishes [6] but only stratum compactum layer was found in *Hypophthalmichthys nobilis* [12].

4.2. Ultrastructural study

In the present investigation ultrastructural studies revealed presence of enterocytes with apical microvilli which increase the surface area and intensify the penetration in proximal and distal intestine of *N. notopterus*. Similar results were reported in *Schilbe mystus* and *Labeo niloticus* [2]. The enterocytes showed presence of polymorphic mitochondria, junctional complex, lysosomes, microfilaments and numerous large lipid droplets. Similar findings were reported in several other fishes *Schilbe mystus* and *Labeo niloticus* [2], *Salvelinus alpines* [22], *Clarius batrachus* [10], *Oncorhynchus mykiss* [5], *Anabus testudineus* [20].

At the base of the intestinal enterocyte of *N. notopterus*, the euchromatin and poorly developed heterochromatin and nucleus with one or two apically, centrally and basally located nucleoli were observed. This structure was similar to those found in species such as centrally located nucleoli in *Labeo niloticus* [2], either centrally or basally located in *Mugil cephalus* and *Sparus auratus* [13]. The mucosa of the intestine contained goblet cells filled with mucus granules with different sizes and densities which were similar to those found in *Labeo niloticus* [21] and *Schilbe mystus* [2].

Another peculiar features of the intestine of *N. notopterus* was the presence of pyramidal shaped paneth cells or eosinophilic granular cell which are considered to be similar to mammalian mast cell in *Tamandua tetradactyla* [15].

5. Conclusion

Histological studies carried out will help in understanding the cellular structures of intestine of this carnivorous fish which are similar to other fishes. However few differences were observed like decrease in number of goblet cells towards the distal part of the intestine. Presence of numerous lymphocytes in the mucosal epithelium of intestine which confirmed the role of intestine in protecting fish from pathogenic organism. Ultrastructural investigations were similar to as that of other fishes. But with a peculiar feature as compared to other fishes i.e Paneth cell or Eosinophilic granular cells (EGCs) were observed at the base of the epithelium of intestine which help in protecting the fish from pathogenic organism. These studies could be extended to study for the immunology of this fish.

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