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THE DIGESTIVE TRACT OF THE FALSE UPSIDE DOWN CATFISH (*Synodontis nigrita*) FROM RIVER BENUE NIGERIA: A MICRO-MORPHOLOGICAL INVESTIGATION

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Abstract: The digestive tract histology of the false upside down catfish (*Synodontis nigrita*) was investigated to fill knowledge gap and provide data for further studies. The jaws were lined by stratified squamous epithelium containing eosinophilic club cells, mucous cells and lymphocytes. This epithelial covering may be related to protective function from pathogens and predators. The oesophagus presented abundant mucous cells in stratified squamous epithelium. These mucous cells help lubricate the tract during passage for food materials as the teleost lack salivary glands. The stomach was lined by simple columnar cells containing intra-epithelial lmphocytes which contribute to local immune response. The intestine was sacculated and mucosa was lined by simple columnar epithelium containing abundant goblet cells and lymphocytes. An intestine-rectal valve separated the rectum from the intestine. In conclusion, the digestive tract of the false upside down catfish (*Synodontis nigrita*) is adaptive to its feeding and lifestyle in the aquatic wild environment.

Keywords: taste bud, pharyngeal pad, gastric glands, sacculations

INTRODUCTION

The biology of teleosts from Nigerian waters has continued to elicit good research interest especially the digestive system (Agbugui, 2013; Ikpegbu et al., 2018). This may be attributed to the need to understand its biology to help serve the growing aquaculture industry. The increasing aquaculture practice is a direct response to depleting fish in water bodies due to over fishing, climate change, increasing population and water pollution (Garcia and Rosenberg, 2010; Krkošek, 2017).

The *Synodontis nigrita* is a teleost of growing importance as possible species for polyculture are widely advocated for to improve fish pond ecosystem. As a possible species for aquaculture, it becomes important to understand its digestive tract, as this system is central for food digestion and conversion (Delashoub et al., 2010). Also, as the fish may gradually become commercially aquaculture, it will soon be impacted with factors associated with domestication such as diseases, stocking density optimization and need to improve feed conversion efficiency (Krkošek, 2017). Hence, it becomes imperative to provide baseline data to establish normal digestive tract histology of *Synodontis nigrita* as there is dearth of information from available literature. This will also help further investigative studies and help pathologists in its disease diagnosis.

MATERIALS AND METHODS

Seven adult *Synodontis nigrita* fish sourced from River Benue, in north central Nigeria were used for the study. They weighed an average of 133.6g and measured a standard body length of 12.2cm. The fish were humanely immobilized by chloroform euthanasia. The oro-pharyngeal cavity was cut open through the membrane between the upper and lower jaws, and the specimens dissected out. The samples under study – lips, tongue, pharyngeal pads and cavity walls were excised, but the lips and pharyngeal pads were decalcified according to Good and Stewart, 1932; before subjecting to routine histological procedure of dehydration in graded concentrations of ethanol, clearing in xylene and embedding in paraffin wax.

Sections 5μm thick were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination (Bancroft and Stevens, 1990). Photomicrographs were taken with – Motican 2001 camera (Motican UK) attached to Olympus microscope.

RESULTS

Upper jaw: The upper jaw was lined by stratified squamous epithelium containing eosinophillic club cells, mucous cells and lymphocytes (Fig.1). While the mucous cells were superficially located, the eosinophillic club cells and lymphocytes were basally located in the epithelium. The club cells were spherical to pear-shaped with centrally located nucleus. Some of the club cells were bi-nucleated. The club cell cytoplasm was filled with eosinophilic material. The lamina propria/submucosa layer contained dense regular connective tissue beneath the epithelium while loose fibres were placed in deeper aspects. The layer also contained blood vessels, adipocytes and melanophores (Fig.1). The melanophores were arranged in a mostly single layer in close contact with the epithelial basement membrane. sa contained loose connective tissue fibres and adipocytes. Beneath the submucosa was a rectangular layer of bone containing large collagen fibres and few osteocytes? Closely associated with this bone tissue was a circular layer of hyaline cartilage and adipose tissue.

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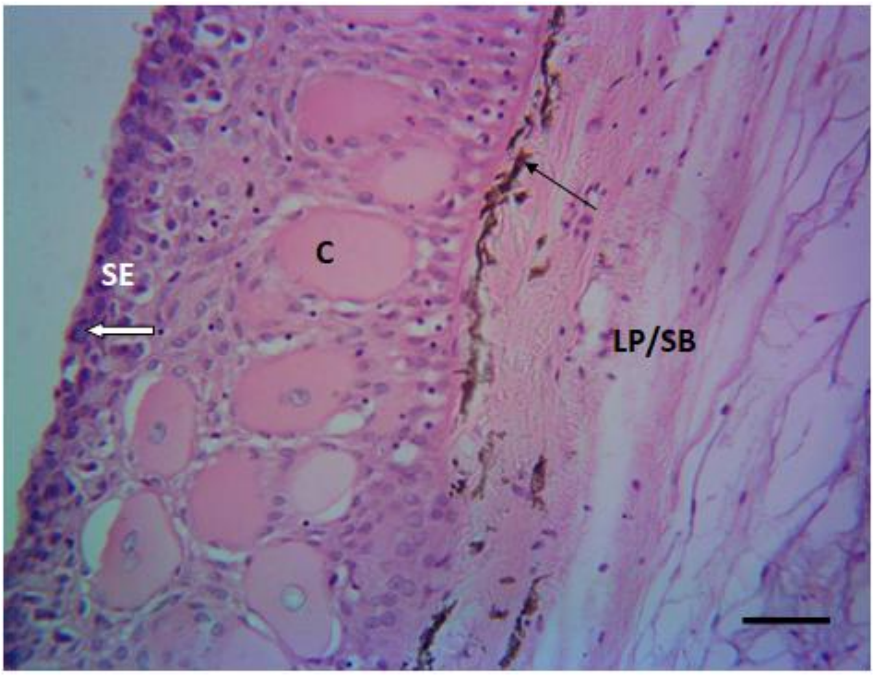

Fig. 1. Section of the Upper jaw lined by stratified squamous epithelium containing eosinophilic club cells C, lymphocytes, and mucous cells (white arrow). Note melanophores (black arrow) in the laminar propria/submucosa (LP/SB). Note the wide submucosa. H & E. (Scale bar = 40µm).

Lower jaw: The lower jaw was lined by stratified squamous epithelium containing mucous cells, eosinophilic club cells and lymphocytes (Fig.2). The lamina propria/submucosa contained dense regular and loose connective tissue and blood vessels. Bars of elastic cartilage were scantly observed in the submucosa (Fig.3). These elastic cartilages provided points of origin and insertion for the lower jaw skeletal muscle fibres. The skeletal muscle bundles had circular, longitudinal and oblique orientation at various locations.

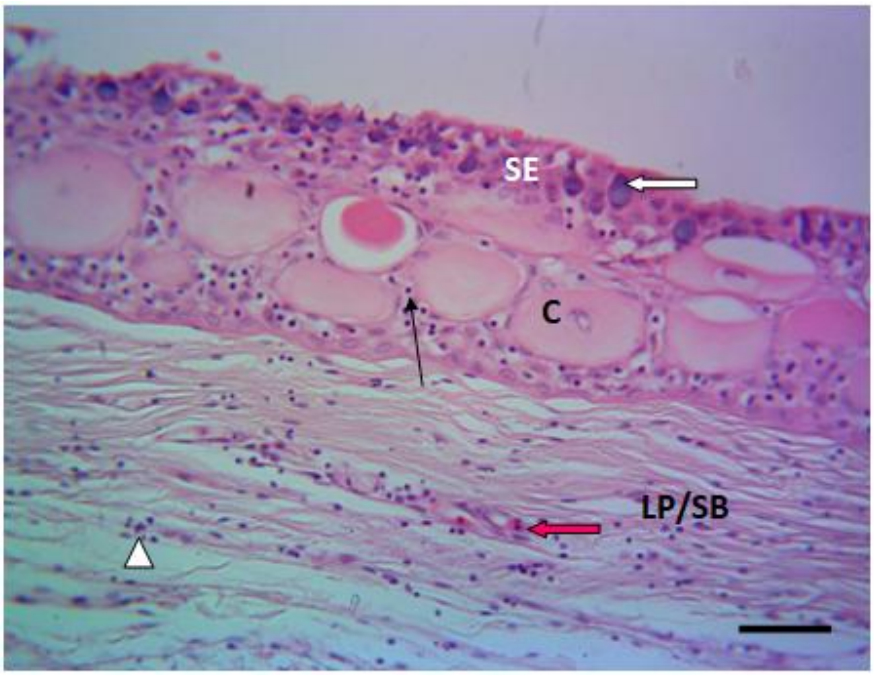

Fig. 2. Section of the lower jaw lined by stratified squamous epithelium containing eosinophilic club cells (C), lymphocytes, and mucous cells (white arrow). Note large infiltrating lymphocytes (white arrow head) and blood vessel (red arrow) in the laminar propria/submucosa (LP/SB). H & E, (Scale bar = 40µm).

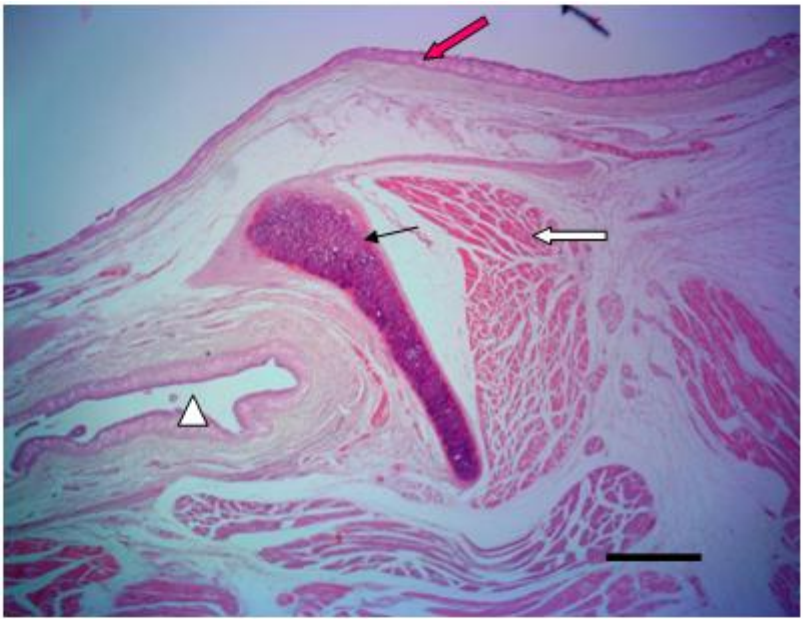

Fig. 3. Section of the lower jaw showing the epithelium (red arrow), prominent elastic cartilage (black arrow) and skeletal muscle fibres (white arrow). Note the cavity lined by epithelial in-folding (white arrow-head). H & E, (Scale bar = 40µm).

Tongue: The tongue was lined by stratified squamous epithelium containing mucous cells, eosinophilic club cells, lymphocytes and taste buds (Fig.4). Whereas the mucous cells, club cells and taste buds were superficially located, the lymphocytes were mostly basally located. The lamina propria contained thin layer of dense regular collagen fibres with lymphocytic infiltration. The submucosa contained loose connective tissue, blood vessels and adipose tissue. Beneath the submucosa were located layers of hyaline cartilage, bone tissue and highly vascularised skeletal muscle. The bone tissue provided points of origin and insertion for the highly vascularised skeletal muscle.

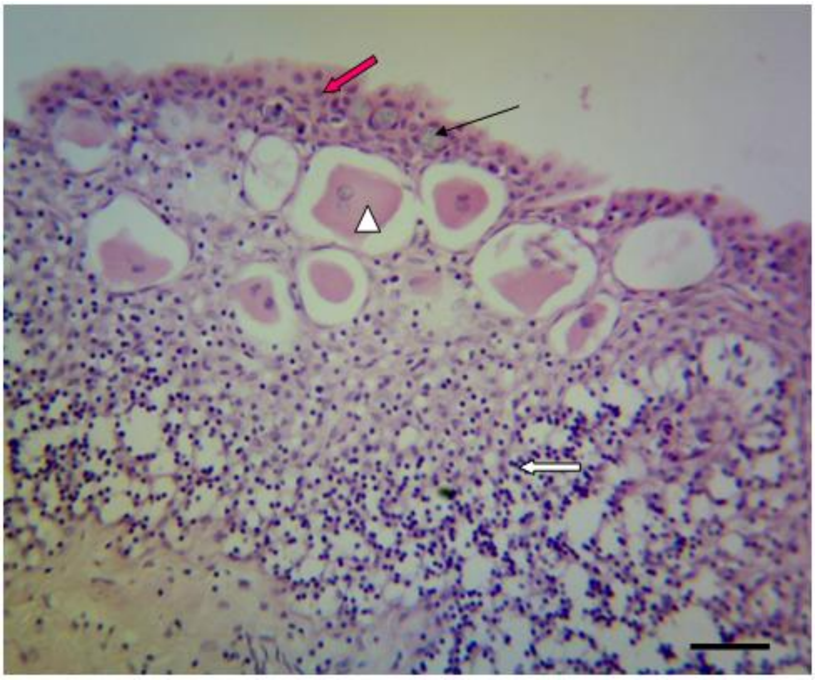

Fig. 4. Section of the tongue lined by stratified squamous epithelium (red arrow) containing eosinophilic club cells (white arrow head), and mucous cells (black arrow). Note large infiltrating lymphocytes (white arrow). H & E, (Scale bar = 40µm).

Pharyngeal pad: The pharyngeal pad was lined by stratified squamous epithelium containing mucous cells, club cells and taste buds (Figs. 5 & 6). Some of the taste buds reached the epithelial surface, while others were embedded within the epithelium. Aggregates of mucous cells were also observed. The highly vascularised lamina propria contained teeth with some erupted to the epithelial surface. The pharyngeal pad teeth were of caniform and molariform types. Beneath the tunica mucosa were bone tissue, hyaline cartilage and skeletal muscles. The bone tissue provided alveoli for the teeth roots.

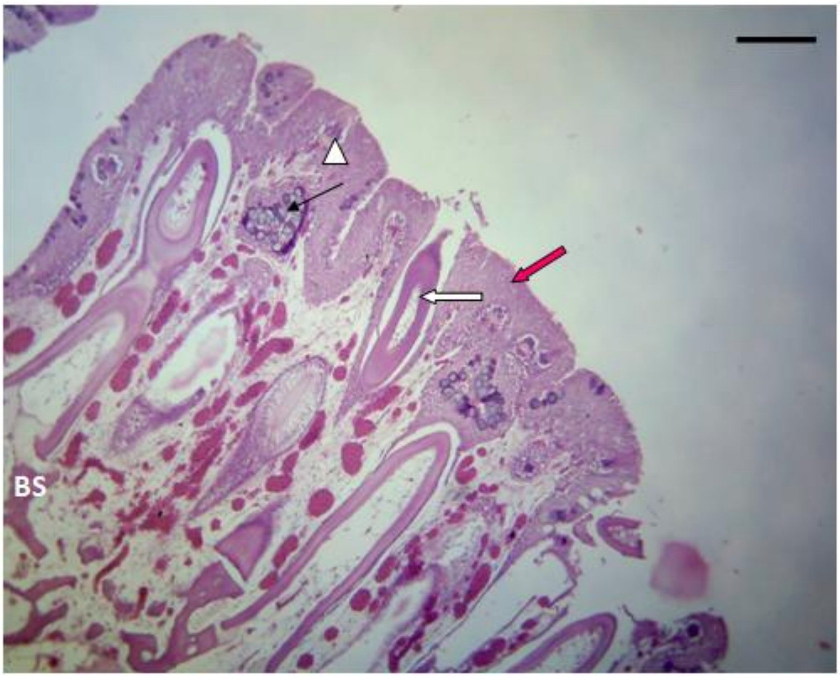

Fig. 5. Section of the Pharyngeal pad lined by stratified squamous epithelium (red arrow) containing taste buds (white arrow head) and mucous cells (black arrow). Note the cuneiform teeth erupting at the epithelial coat (white arrow) and supporting bone spicles (BS). H & E, (Scale bar = 40µm).

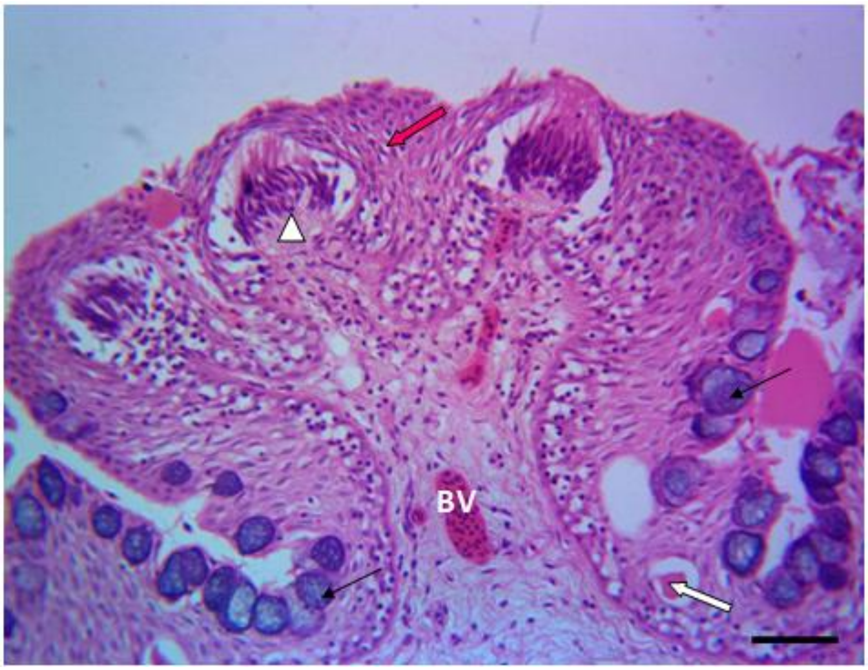

Fig. 6. Section of the Pharyngeal pad epithelium with higher magnification showing stratified squamous cells epithelium (red arrow) containing taste buds (white arrow head), eosinophilic club cells (white arrow), and mucous cells (black arrow). Note the blood vessels (BV). H & E, (Scale bar = 40µm).

Oesophagus: The oesophageal mucosa was modified into longitudinal folds of outer epithelium and lamina propria core (Figs. 7 & 8). Some longitudinal folds were linear-shaped, some mound to rectangular-shaped, while others were branched (Fig.7). The longitudinal folds were lined by stratified squamous epithelium containing club cells and mucous cells and lymphocytes. The lamina propria core contained connective tissue fibres, blood vessels and lymphocyte. These structures were also seen in the submucosa. The skeletal muscle of the tunica muscularis was of outer circular and inner longitudinal in orientation. The tunica serosa contained loose connective tissue, several nerve plexues and blood vessels.

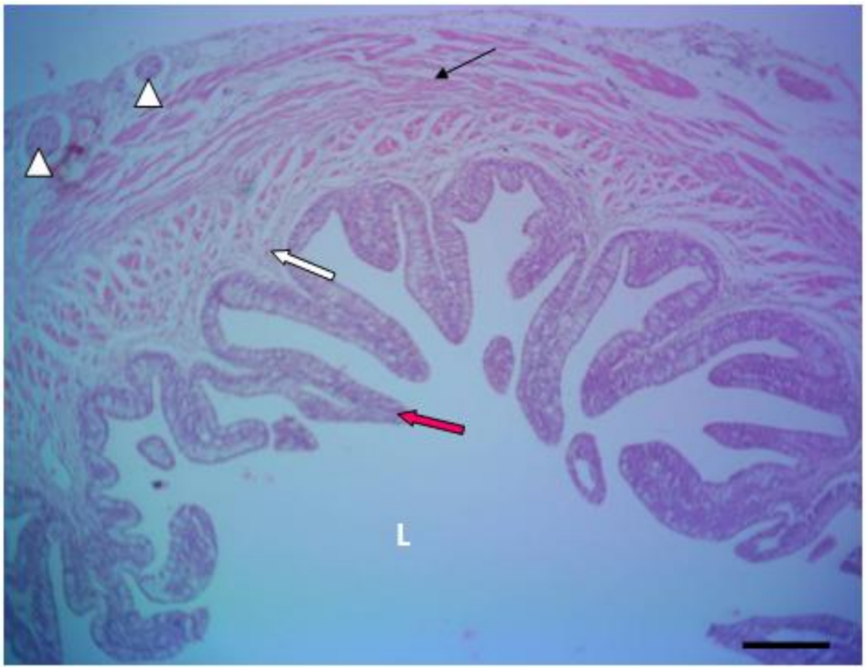

Fig. 7. Transverse section of the oesophagus showing mucosal folds lined by stratified mucous epithelium (red arrow) pointing into the lumen (L). Note the submucosa (white arrow), tunica muscularis containing skeletal muscle fibres (black arrow) and sub-serosal nerve plexus (white arrow head). H & E, (Scale bar = 4µm).

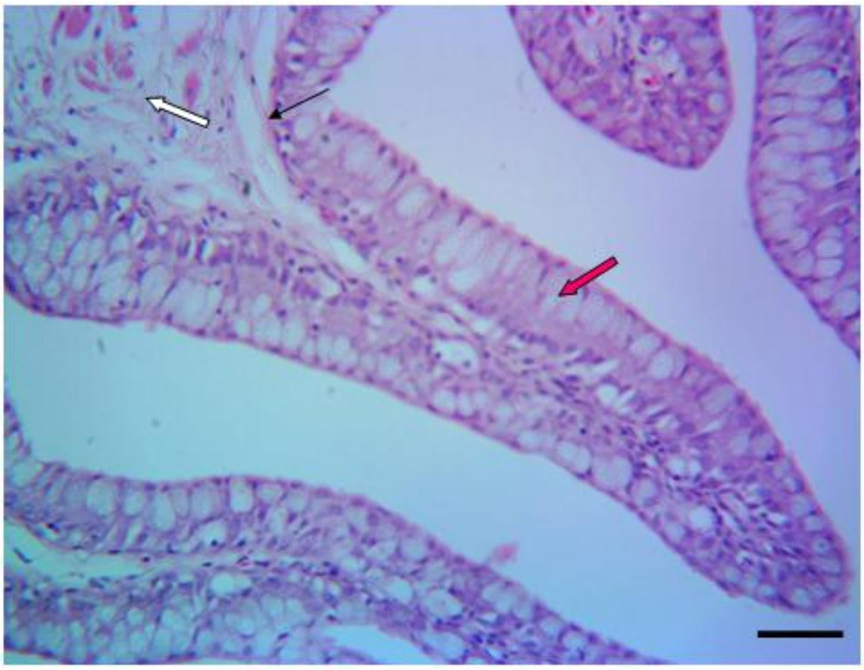

Fig. 8. Transverse section of the oesophagus showing mucosal folds lined by stratified mucous epithelium (red arrow) with lymphocytic infiltration. Note the muscularis mucosae (black arrow) and submucosa (white arrow). H & E, (Scale bar = 40µm).

Stomach: The stomach cardia was lined by simple columnar epithelium containing lymphocytic infiltration (Fig.9). The lamina propria containing gastric glands was compartmentalized into rectangular shape by collagen fibres. The muscularis mucosae were smooth muscle fibres. The submucosa contained loose collagen fibres, blood vessels and lymphocytes. The smooth muscle fibres of the tunica muscularis were arranged into inner circular and outer longitudinal fibres. Tunica serosa was present. The histology of the fundic region was very similar to that of the cardia except that the lamina propria gastric gland compartments of the fundic region were smaller in size and alveolar shaped (Fig. 10). These alveolar shaped glands were surrounded by well developed smooth muscle cells of the muscularis mucosae. In the pyloric region, the histology was similar except the absence of gastric glands in its lamina propria (Fig.11).

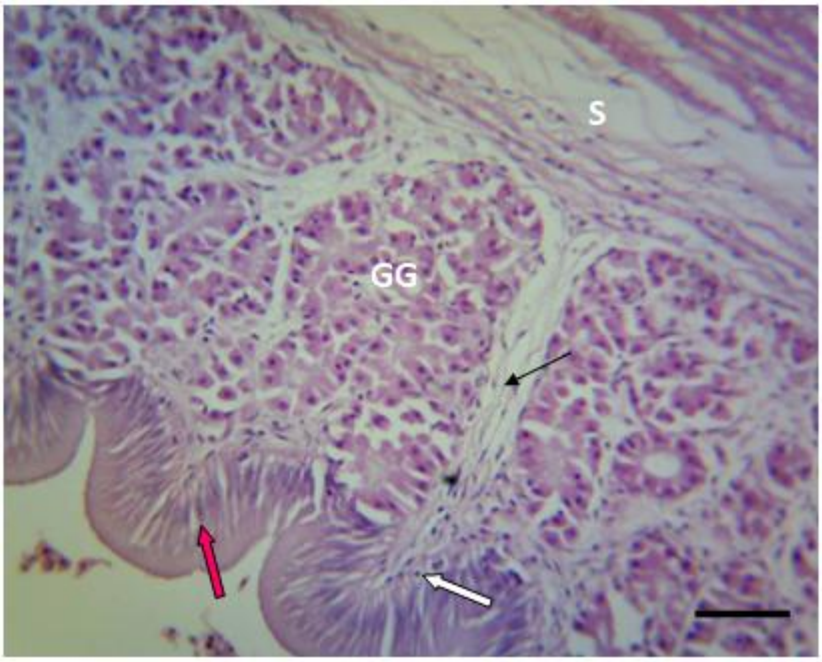

Fig. 9. Transverse section of the cardiac stomach lined by simple columnar epithelium (red arrow) containing lymphocytes (white arrow). Note the gastric glands (GG), in the laminar propria; muscularis mucosae (black arrow) and submucosa (S). H & E, (Scale bar = 40µm).

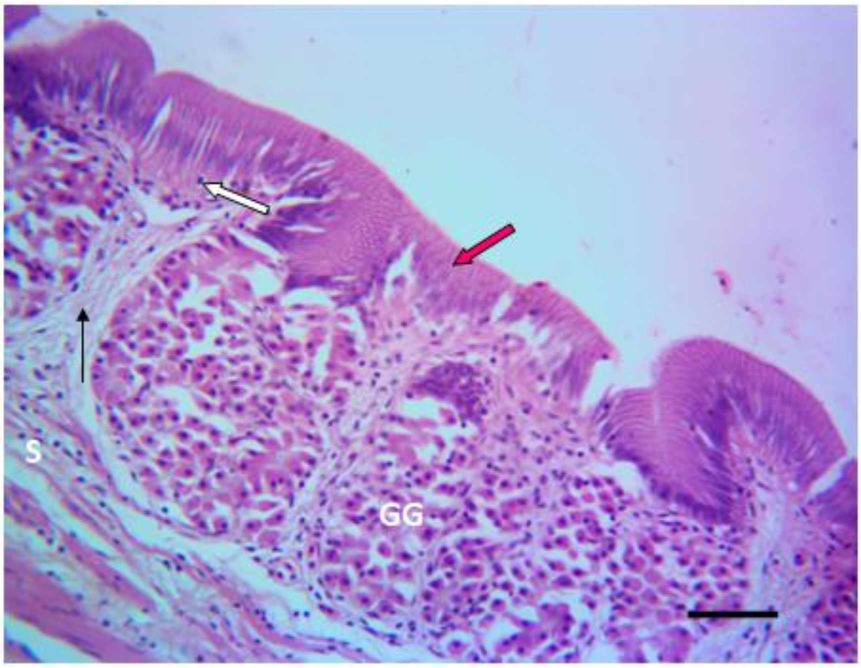

Fig. 10. Section of the fundic stomach lined by simple columnar epithelium (red arrow) containing lymphocytes (white arrow). Note the gastric glands (GG), in the laminar propria; muscularis mucosae (black arrow) and submucosa (S). H & E, (Scale bar = 40µm).

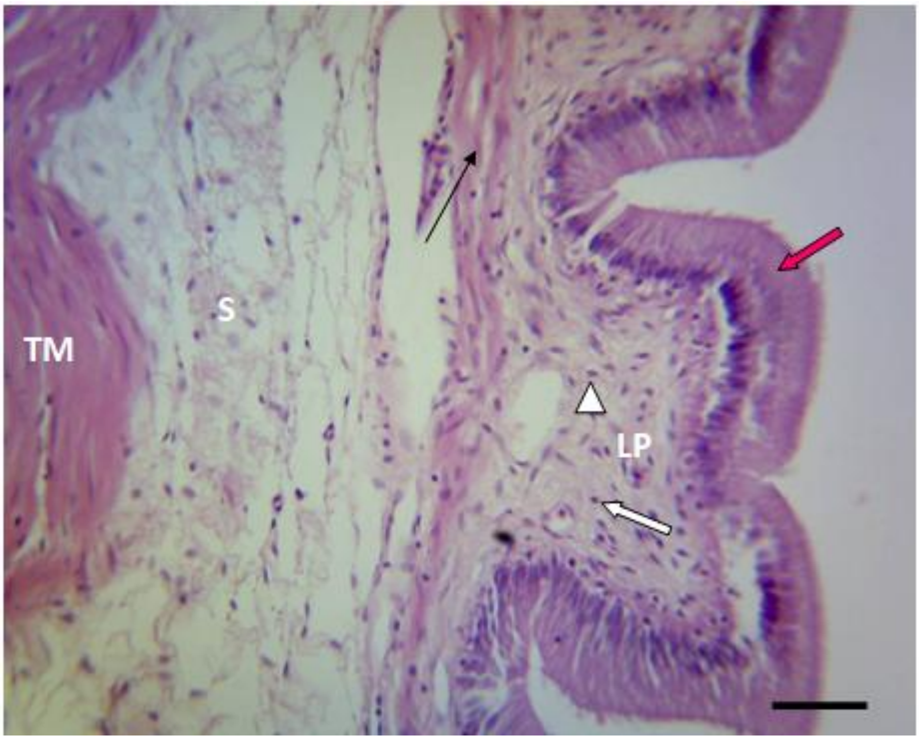

Fig. 11. Section of the pyloric stomach lined by simple columnar epithelium (red arrow). Note the abundant lymphocytes (white arrow) and fibroblasts (white arrow head) in the laminar propria (LP). Observe the presence of muscularis mucosae (black arrow), submucosa (S), and tunica muscularis (TM). H & E, (Scale bar = 40µm).

Intestine: The proximal intestinal tunica mucosa was modified into longitudinal folds. These folds on low magnification were seen as branched and anastomosed and almost covered the intestinal lumen (Fig.12). The longitudinal folds were lined by simple columnar epithelium containing goblet cells and lymphocytes (Fig.13). The lamina propria core of the longitudinal fold contained collagen fibres, blood vessels and lymphocytes. The muscularis mucoae was of smooth muscle cells. The submucosa contained loose connective tissue, blood vessel and abundant lymphocytes. The smooth muscle cells of the tunica muscularis were arranged in an inner longitudinal and outer circularly oriented fibre. Myenteric nerve pleus was observed. Tunica serosa was present.

The histology of the middle intestine was very similar to that observed in the proximal intestine except that the longitudinal folds were simple and the number of goblet cells increased. The smooth muscle cells of the tunica muscularis were arranged in inner circular and outer longitudinal circularly oriented fibres (Fig.14).

In the distal intestine, the histology was very similar to that observed in the middle except that the outer longitudinal smooth muscle cells of the tunica muscularis were bigger than the inner circular muscle (Fig.15). Also the inner circular muscles bulged at regular intervals into the submucosa. These bulgings were onion-bulb shaped.

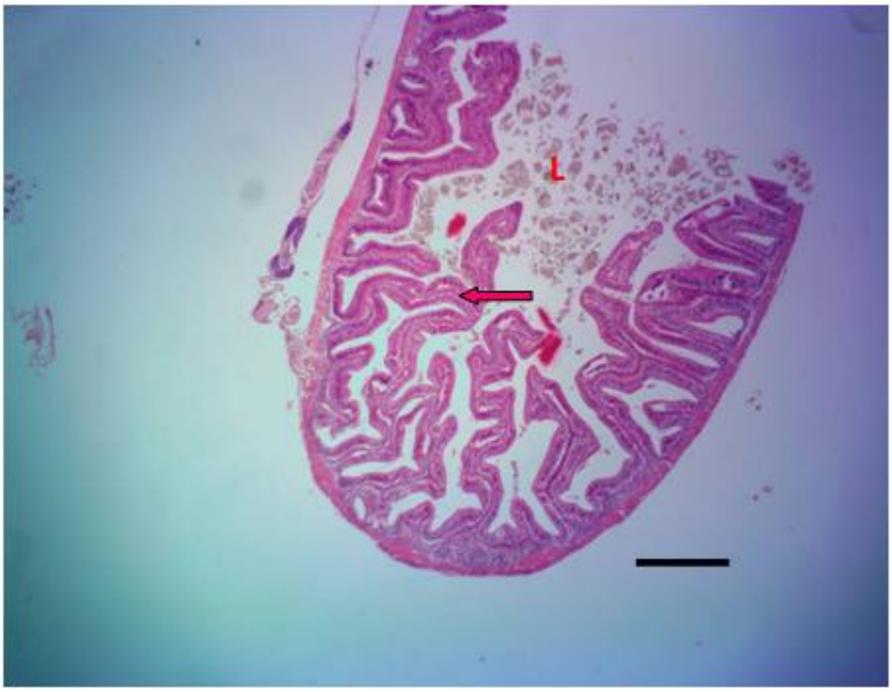

Fig. 12. Transverse section of the proximal intestine showing mucosal folds (red arrow), branched, anastomosed and almost covered the intestinal lumen (L). H & E, (Scale bar = 4µm).

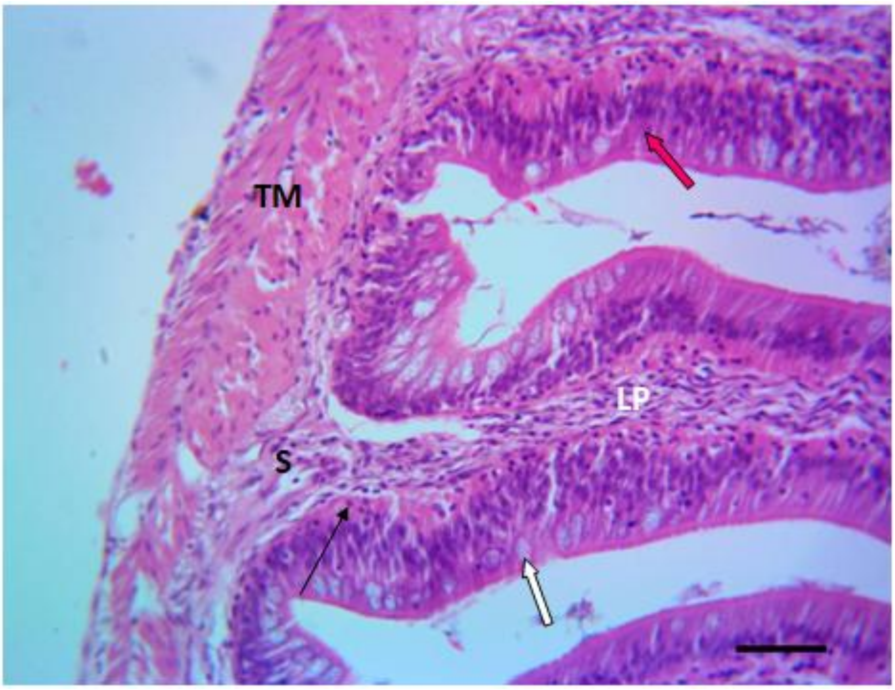

Fig. 13. Section of the proximal intestine lined by simple columnar epithelium (red arrow) containing mucous cells (white arrow) and lymphocytes (black arrow). Note the laminar propria (LP), submucosa (S) and tunica muscularis (TM). H & E, (Scale bar = 40µm).

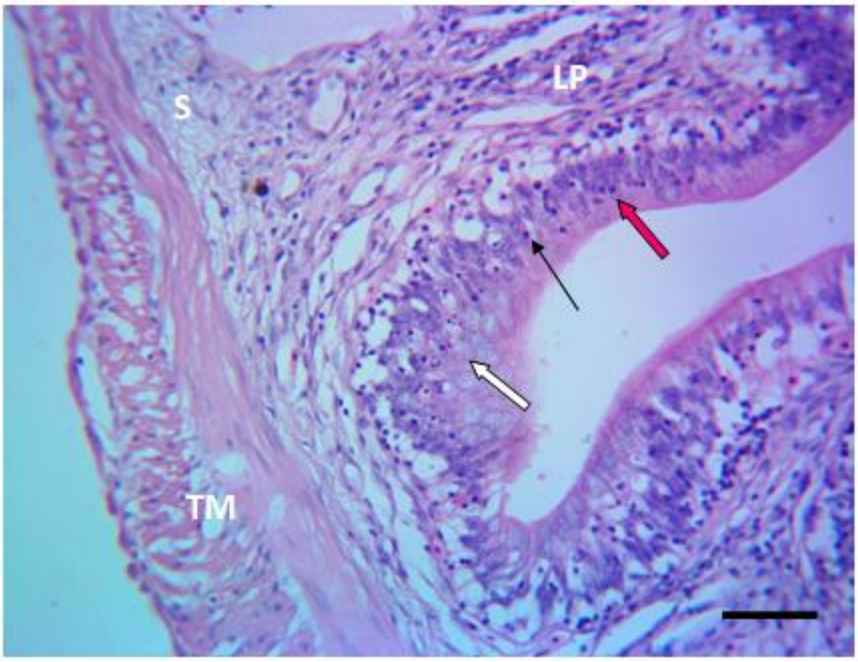

Fig. 14. Section of the middle intestine lined by simple columnar epithelium (red arrow) containing mucous cells (white arrow) and lymphocytes (black arrow). Note the laminar propria (LP), submucosa (S) and tunica muscularis (TM). H & E, (Scale bar = 40µm).

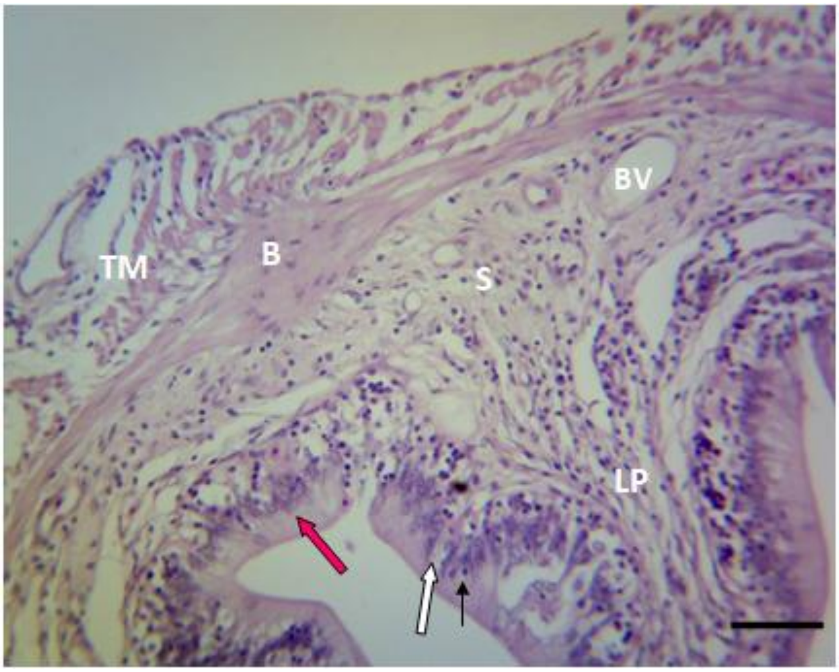

Fig. 15. Section of the distal intestine lined by simple columnar epithelium (red arrow) containing mucous cells (white arrow) and lymphocytes (black arrow). Note the laminar propria (LP), submucosa (S) and tunica muscularis (TM) showing onion shaped bulging of inner circular muscles (B) . H & E, (Scale bar = 40µm).

Rectum: The rectum was demarcated from the intestine by the intestine-rectal valve. The rectum grossly was characterized by several sacculations. Histologically, the rectum was lined by simple columnar epithelium containing goblet cells and lymphocytes (Fig. 16). The lamina propria contained blood vessels, collagen fibres and lymphocytes. The muscularis mucosae were of smooth muscle fibres. The submucosa contained loose connective fibres, fibroblasts, blood vessels and pockets of skeletal muscles. The smooth muscle cells of the tunica muscularis was arranged in inner circular and outer longitudinal fibres. Myenteric nerve plexus was seen.

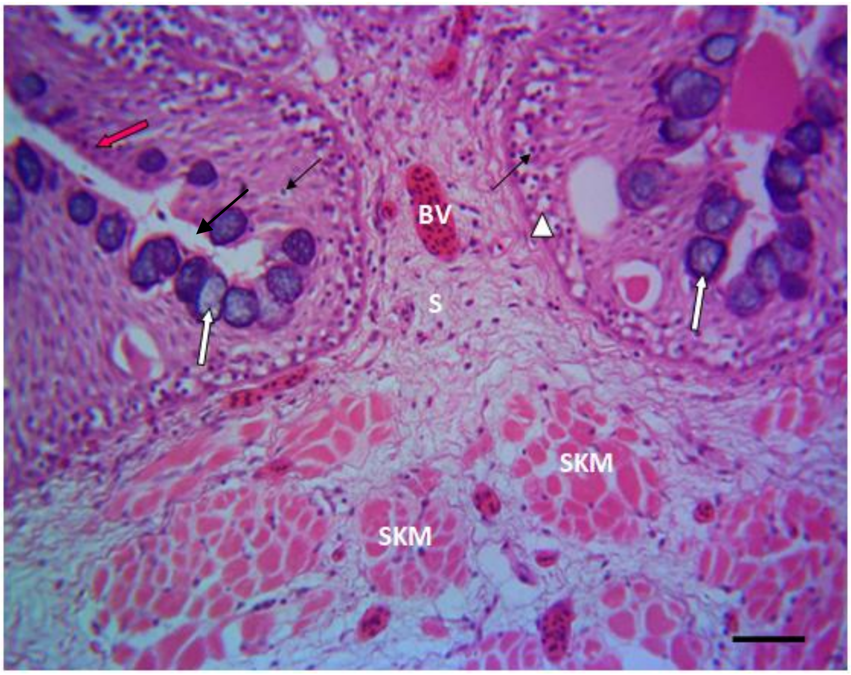

Fig. 16. Section of the rectum lined by stratified squamous epithelium (red arrow) containing mucous cells (white arrow) and lymphocytes (black arrow). Note the laminar propria (white arrow head), submucosa (S) containing pockets of skeletal muscle fibre (SKM) and blood vessels (BV). H & E, (Scale bar = 40µm).

DISCUSSION

The anatomical features of fish digestive tracts have been reported to adapt to some factors like feeding habit, general body conformation and taxonomical groupings (Scocco et al., 1997; Domeneghini et al 2006; Purushothaman et al., 2016). In the Synodontis nigritis under study, the oro-pharyngeal cavity showed adaptation for food selection and possible transient increase in volume during food acquisition as was demonstrated with well developed taste buds in the tongue and pharyngeal pads; and presence of elastic cartilage in the lower jaw respectively (Hobbler and Merchant, 1983; Linser et al., 1998). The cavity's epithelial lining protects the fish from mechanical abrasion by means of several layers of squamous cells and sandwiched mucous cells (Agrawal and Mittal, 1991; Ikpegbu et al., 2014a). Also the abundant lymphocyte confers local immunological defense against invading pathogens (Park et al., 2003; Banan-Khojasteh et al., 2009). The large presence of eosinophilic club cells shows the need to quickly respond attacks by predators in the large river with lots of animals at different levels of the food chain, as these cells have been variously described as alarm cells (Diaz et al., 2006; Cao and Wang, 2009). The anterior extremity of the jaws (lips) was lined with stratified squamous epithelium for protection during foraging in the river as the absence of taste buds is very suggestive that all potential food materials are ingested into the cavity where selection is carried out. The presence of lip taste buds have been reported in the Leporinus friderici and L. taeniofasciatus, and this was associated with food selection prior to ingestion (Albrecht et al., 2001). The highly vascularized tongue skeletal muscles are very suggestive of an active organ involved in food selection and caudally pushing food towards the pharyngeal pad and oesophagus. The pharyngeal pad apart from being the most adapted structure for food trituration, it may be the principal organ actively selecting food for acceptance or rejection. This speculation is drawn from the prominent teeth co-localized with taste buds on the pads (Linser et al., 1998). The caniform teeth present on the pad may suggest that the fish is involved in micro-predation but more work should be done to ascertain the veracity of this claim.

The oesophageal mucosa lined by protective stratified squamous cells containing mucous cells was modified into longitudinal folds helps to increase surface area for mucous cells secretion that lubricates the tube during deglutition since teleosts lack salivary glands (Tibbetts, 1997; Scocco et al., 1997; Murra et al., 1994). Oesophageal mucosa that was modified into villi and lined by tall columnar epithelium has been reported in the Persian tooth-carp Aphanium persicus (Monsefi et al., 2010).

The stomach that was lined by simple columnar cells, which may have absorptive functions, has been reported in the African catfish (Ikpegbu et al., 2014b). The presence of gastric glands in the cardia and fundic regions only may be related to the need to reduce effect of acidic content as the food moves from the pylorus that lacked these glands into the intestine. This will enable the pancreatic juice and intestinal enzymes to function optimally in an alkaline environment. The absence of gastric glands in the pylorus as seen in this study has also been documented in the Yellow Catfish Petteobagrus fulvidraco (Cao and Wang, 2009). But the presence of gastric glands in all the three stomach regions has been reported in the Oreochromis niloticus (Caceci et al., 1997). The alveolar shaped glands in the fundic region were seen ensheathed by smooth muscle cells of the muscularis mucosae. These muscle cells may be having contractile effect that will help express the gland secretions, hence may increase digestion time in the stomach. This morphological adaptation may relate to need to forage as much material as possible in short time since predation will be enormous in this large body of water but more work should be done to verify this claim. Whilst smooth muscle cells were seen in the stomach tunica muscularis suggestive of involuntary control; skeletal muscles have been reported in the Nile tilapia Orechromis niloticus and was speculated to assist the fish in digesting it principal plant based feed materials (Caceci et al., 1997).

The proximal intestine lined by absorptive simple columnar epithelium presented a mucosa modified into branched anastomosing longitudinal folds. This branching as reported in the African catfish was associated with increasing surface area for nutrient absorption (Ikpegbu et al., 2013). The increasing goblet cell number caudally towards the distal intestine and rectum may indicate increasing need for more lubrication as fecal material is formed (Monsefi, 2010). Goblet cell secretions have also been related to protection from pathogenic agents (Pedini at al., 2006). This observation of more goblet cells in distal intestine has been reported in the Bighead carp Hypopthalmichtys nobilis (Delashoub et al., 2010). The onion-bulb shaped inner circular smooth muscles seen in the intestinal tunica muscularis may be helping with feed trituration in the intestine. It may also be potentiating rthymic contractions along the intestinal tract. This speculative function may help explain the presence of the intestino-rectal valve which may help slow down food passage time into the rectum. The valves in digestive tracts have also been attributed to be involved in food retention time in the intestine thereby increasing absorptive time and preventing reflux of materials anteriorly into the distal intestine (Jaroszewska et al., 2008).

AUTHOR CONTRIBUTIONS

Conceptualization, Ikpegbu E. and Nlebedum U.C.; Methodology, Ikpegbu E., Nlebedum U.C and Ibe C. S.; Nlebedum U.C and Ikpegbu E. Data validation; Data processing Nlebedum U.C.; Writing—original draft preparation, Ikpegbu E. and Ibe C.S.; Writing—review and editing, Ikpegbu E., Nlebedum U.C and Ibe C.S.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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