Histological Structure and Histochemistry of the Digestive Tract of the Striped Tiger Nandid Fish, *Pristolepis fasciata*

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Abstract: The striped tiger nandid fish, *Pristolepis fasciata*, is widely distributed in Thailand. However, its diet composition has been intensively investigated. The histological profile remains an exciting and challenging issue. Therefore, the objectives of this study are to describe the histological structure and histochemistry of the digestive tract of the striped tiger nandid fish. Fifteen adult fish were collected from Songkhla province, Thailand. The digestive tract was fixed in Bouin's solution, followed by basic paraffin techniques. The 5 μm sections were stained with hematoxylin and eosin (H&E), periodic acid Schiff's (PAS), alcian blue (AB) pH 2.5 and pH 1.0, and Masson's trichrome (MT). The results revealed that the digestive tract wall comprised four layers: mucosa, submucosa, muscularis, and serosa. The mucous and goblet cells in the esophagus exhibited positive staining with PAS, AB pH 2.5, and pH 1.0. The stomach was divided into three parts: cardiac, fundic, and pyloric stomach. The epithelium of the cardiac stomach showed positive staining with PAS and weak staining with AB pH 1.0. The fundic cells showed strong positive staining with PAS but weak staining with AB pH 2.5 and 1.0. In contrast, the cells lining the fundic and cardiac glands showed positive staining with PAS. The pyloric epithelium revealed positive staining with PAS but did not contain a gland. In the anterior, middle, and posterior intestines, glands were absent. Goblet cells exhibited intense labeling with PAS and AB pH 2.5 and 1.0 in each portion. The intestinal coefficient was 0.62±0.01, indicating an omnivorous fish.

Keywords: Gastrointestinal tract; gastric gland; histology; histochemical study; intestinal coefficient

1. Introduction

The digestive tract is one of the largest systems in fish, which is related to their feeding habitat, environment [1, 2], type of food, and behavior [3]. Additionally, the digestive tract plays an important role in growth and nutrition [1, 2]. Al-Abdulhadi [4] mentioned that the digestive tract exhibits a diversity of morphology and function. The digestive tract of many teleosts has been studied through gross anatomy, histology, and histochemical analysis, such as the South American catfish *Rhamdia quelen* [5], short mackerel *Rastrelliger brachysoma* [6], spotted snakehead fish *Channa punctata*, striped snakehead fish *C. striata* [7], pebbly fish *Alestes baremoze* [8], large yellow croaker *Larimichthys crocea* [9], lizardfish *Synodus variegatus* [10] and banded tilapia *Tilapia sparrmanii* [2]. However, the gastrointestinal tract in these fish exhibits a marked difference in

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characteristics, reflecting their evolution, diet, and environment [2, 11]. Herbivorous fish primarily consume plants and have a small stomach and a long intestine. Omnivorous fish, feeding on animals and plants, exhibits a large stomach and a long intestine [2, 12]. On the other hand, carnivorous fish, specializing in animal consumption, possess a distensible stomach and the shortest intestine [13].

In categorizing feeding habits, numerous studies utilize the intestinal coefficient (IC) to identify fish feeding types [14]. Omnivorous fish typically have an IC ranging from 0.6 to 8.0, while carnivorous fish exhibit an IC in the range of 0.2 to 2.5. Additionally, herbivorous fish generally have an IC between 0.8 and 15.0. Notably, some herbivorous fish species may have a shorter intestine and a lower IC [9]. Despite the fish having various diets, the digestive wall's characteristics consist of four tunics when considering histology. These are arranged from the innermost to the outermost tunic, i.e., (I) the mucosa, including the layer of epithelial tissue, lamina propria, and muscularis mucosae, (II) the submucosa being the layer of connective tissue, blood vessels, and nerve plexus, (III) the muscular tunic that subdivided into two layers (inner circular and outer longitudinal layers), and (IV) the layer of connective tissue that known as the serosa [6].

The striped tiger nandid fish, *Pristolepis fasciata* is a member of the family Nandidae. This species is distributed widely from Burma to Indonesia, inhabiting areas with aquatic or emergent plants in marshes, lakes, swamps, and rivers, typically slow-running or still water [15]. In the previous study, Sangpradub and Chutima [16] investigated the diet composition of striped tiger nandid fish in Kaeng Lawa, Thailand, identifying as the significant groups of prey (in the phylum Arthropoda and Mollusca) and other foods (plants, algae, and Euglenozoa). Furthermore, several studies on the diet of striped tiger nandid fish yield consistent results. The striped tiger nandid fish is reported to feed on aquatic insects, crustaceans, filamentous algae, submerged plants [15], fish fry, and aquatic insects [17]. Despite numerous studies on the diet of striped tiger nandid fish, there remains a scarcity of information on the histology and histochemical analysis of the digestive tract.

The information about the structure and characteristics of the digestive tract in fish is important because it can serve as a guideline for understanding the pathology, culture, conservation, and management and gaining insights into the structure and physiology of the fish's digestive system. In the present work, the histological structure and histochemistry of the digestive tract of the striped tiger nandid fish were deeply investigated.

2. Materials and Methods

Fifteen adult striped tiger nandid fish were collected by trawling from Pak Ro subdistrict, Singhanakhon district, Songkhla province, Thailand (7°15'05.3"N 100°26'24.0" E) from February to November 2023. The sampling was collected every two months. Fifteen specimens (mean snout-vent length approximately 15.91±0.82 cm (Table 1)) were anesthetized with fish anesthesia (tricaine methanesulfonate, MS 222, and solution by using a dosage of 100 mg/L). The digestive tract was removed for photography after a longitudinal incision in the abdominal wall. The intestinal length, with a mean total length of 9.79±0.59 cm (Table 1), was measured to calculate the IC [14].

$$IC = \frac{\text{Intestinal length}}{\text{Snout} - \text{vent length}}$$

The digestive tract was cut into small pieces, which were fixed in Bouin's solution for 48 hours and placed in 70% alcohol. Subsequently, they underwent basic paraffin techniques, including dehydration using a graded series of ethyl alcohol (70%, 80%, 95%, and absolute alcohol), clearing with xylene, infiltration, and embedding with Paraplast Plus® (Sigma-Aldrich, USA), and sectioning was performed with a rotary microtome. The 5 μm serial sections were stained with hematoxylin and eosin (H&E) for general characteristics, periodic acid Schiff's – hematoxylin (PAS) for neutral glycoproteins/mucopolysaccharides, alcian blue (AB) pH 2.5 – nuclear fast red (NF) for carboxylated acid glycoproteins/mucopolysaccharides, AB pH 1.0 – NF for sulphated acid glycoproteins/mucopolysaccharides and Masson's trichrome (MT) for the connective tissue and muscle [18]. The sections were observed under a light microscope. This research was

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approved by the Animal Ethics Screening Committee, Thaksin University (Permit number: TSU 2022-008. IACUC No. 0008).

3. Results and Discussion

3.1 Gross morphology

The gross morphology of the digestive tract of striped tiger nandid fish is shown in Figure 1, and the length of the digestive tract is presented in Table 1. The esophagus was found to be a short, straight, muscular tube connecting to the white J-shaped stomach. At the end of the stomach, it curved and connected to the intestine. Meanwhile, the intestine exhibited a marked decrease in size from the anterior to posterior portions, according to other fish species [2, 19, 20].
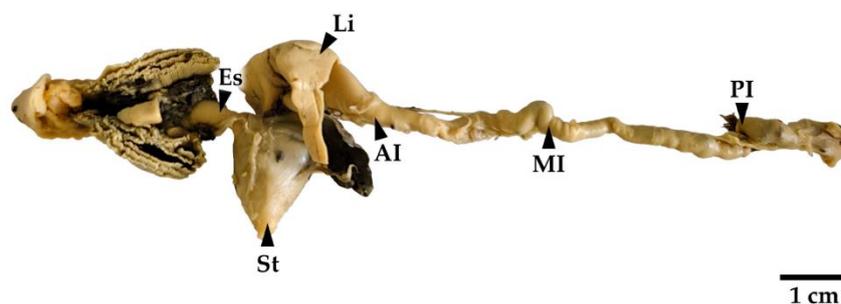
Figure 1. Photograph showing the components of the digestive tract and liver (Li), consisting of the esophagus (Es), stomach (St), and intestinal part. The intestine was divided into three parts: the anterior intestine (AI), the mid-intestine (MI), and the posterior intestine (PI).

Table 1. Mean and standard deviation of snout-vent length, gastrointestinal tract length, intestinal length, and intestinal coefficient (IC) (N=15).

	Snout-vent length (cm)	Gastrointestinal tract length (cm)	Intestinal length (cm)	Intestinal coefficient (IC)
Mean ± standard deviation	15.91 ± 0.82	13.43 ± 0.67	9.79 ± 0.59	0.62 ± 0.01

3.2 Histological structures and histochemistry

The histological structure of the digestive tract in striped tiger nandid fish revealed general characteristics similar to those found in other fish and vertebrates, with the wall comprising four layers: the mucosa (epithelium, lamina propria, and muscularis mucosae), submucosa, muscularis, and serosa, whereas some portions lacked muscularis mucosae. However, the characteristics of the four tunics displayed no discernible difference in each fish corresponding to those observed in spotted pimelodid *Pimelodus maculatus* [21] large yellow croaker *L. crocea* [9] and banded tilapia *T. sparrmanii* [2]. However, some species lacked muscularis mucosae in all parts, such as pike characin *Oligosarcus hepsetus* [22]. In addition, the muscular layers of all portions in striped tiger nandid fish were involved in the motility of food, mixing food with digestive enzymes [22], and transporting food.
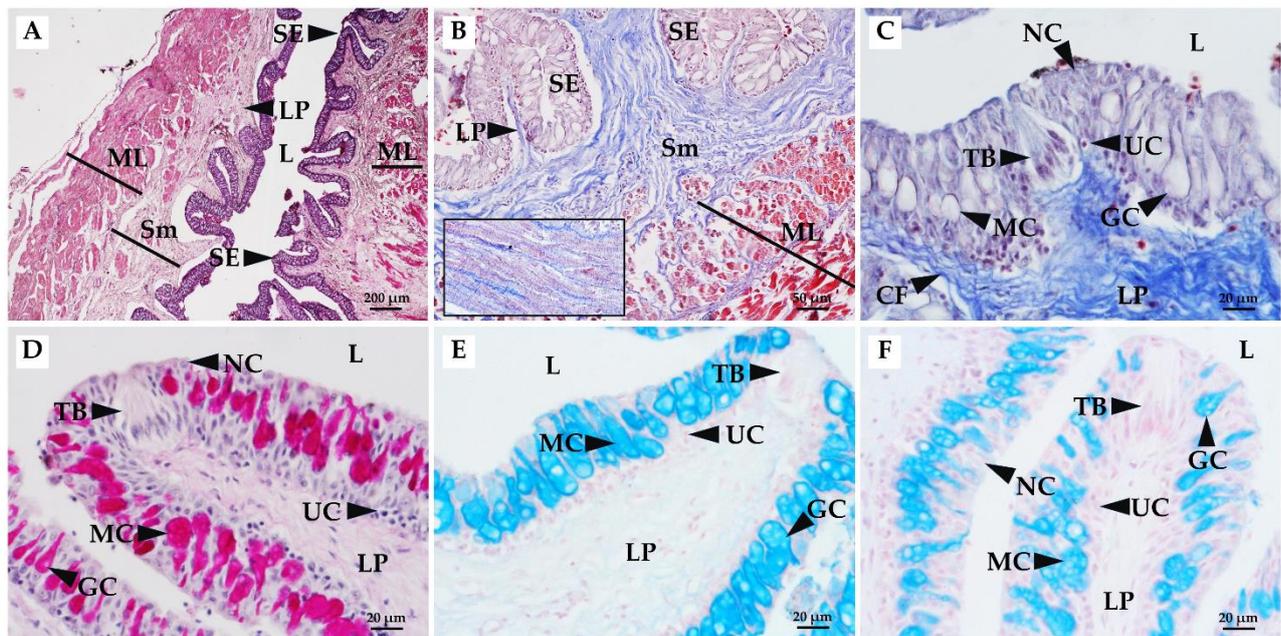
3.2.1 Esophagus

The mucosa exhibited numerous longitudinal folds (Figure 2A) with a mean fold height of 313.84±36.46 μm and a fold width of 236.36±51.56 μm (Table 2). The longitudinal fold of the esophagus in striped tiger nandid fish helped to increase the luminal diameter and distension during the food transported to the stomach, similar to other fish species [2, 21]. Furthermore, Okuthe and Bongile [2] stated that the number of folds was related to the digestive capacity, with more folds leading to the increased efficiency. The epithelium of the esophagus was lined by non-keratinized stratified columnar epithelium. These consisted of

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four cell types: non-mucous, mucous, undifferentiated, and goblet cells (Figures 2C-2F). The stratified epithelium was similar to the esophagus of many fish such as sea bream *Mylio cuvieri* [4], pike characin *O. hepsetus* [22], killifish *Anablepsoides urophthalmus* [23], and banded tilapia *T. sparrmanii* [2]. However, the epithelium of the esophagus of striped tiger nandid fish was columnar, which differed from the large yellow croaker *L. crocea* [9]. In contrast, the esophagus of a large yellow croaker, *L. crocea*, was divided into two regions: the frontward part with stratified squamous epithelium and the hindmost region with stratified columnar epithelium [9]. Additionally, the esophagus of striped tiger nandid fish differed from pike characin, *O. hepsetus*, which had stratified squamous epithelium [22], and from Asian seabass, *Lates calcarifer* [24], which had simple columnar epithelium. The present study and previous reports indicated the variability in esophageal epithelium types. The stratified epithelium, as observed in the esophagus of striped tiger nandid fish, was suggested to have a role in the renewal and protection of the surface from abrasion or abrasion resistance to protect the epithelium from injury [4]. Furthermore, the stratified epithelium was believed to protect the esophagus from pathogens [4, 25].

The non-mucous cells of the esophagus were low-columnar to columnar with round nuclei. The cytoplasm showed positive staining with eosin but negative staining with PAS (Figure 2D), AB pH 2.5 (Figure 2E), and AB pH 1.0 (Figure 2F). The mucous cells interspersed between non-mucous cells had round nuclei and a columnar shape. The cytoplasm showed strongly positive staining with PAS (Figure 2D), AB pH 2.5 (Figure 2E), and AB pH 1.0 (Figure 2F). These results demonstrated the secretion of neutral, carboxylated, and sulphated acid mucins, respectively. These results were consistent with the previous reports suggesting that neutral mucous with low viscosity functioned in lubricating the luminal epithelium [22] and helped to protect the esophageal epithelium from chemical and mechanical injury [24, 26]. Reifel and Anthony [27] stated that numerous mucous cells in the esophagus were mostly found in carnivorous and omnivorous fish but decreased in herbivorous fish, functioning similarly to salivary glands in mammals [28]. Meanwhile, acid mucous with high viscosity was believed to have the function of trapping particles to enter the esophagus [29].

Near the basement membrane, undifferentiated cells were found, characterized by clear cytoplasm and round nuclei (Figure 2C). These cells showed negative staining with PAS (Figure 2D), AB pH 2.5 (Figure 2E), and AB pH 1.0 (Figure 2F). Between the mucous cells, the goblet cells showed strongly positive staining for PAS (Figure 2D), AB pH 2.5 (Figure 2E), and AB pH 1.0 (Figure 2F). Goblet cells showed positivity to AB pH 2.5, indicating the synthesis of carboxylated acid mucins. The benefit of acid mucins was the increase in the viscosity of mucus for the lubrication of food during swallowing and protecting the epithelium from bacterial invasion, as noted in other studies [2, 30]. Meanwhile, neutral mucins secreted from goblet cells might be involved in transporting food from the esophagus to the stomach, protecting the wall from mechanical damage [2, 9]. Furthermore, the striped tiger nandid fish's anterior esophagus had taste buds between the epithelial cells. The taste buds were round-shaped and comprised of columnar taste cells with basal nuclei and supporting cells (Figures 2C-2F). The discovery of taste buds indicated that striped tiger nandid fish could perceive taste and reject unswallowed food.

The thick muscular layer consisted of striated muscle (Figures 2A-2B). These results were similar to damsel fish *Stegastes fuscus* [31], Pantanal eartheater *Satanoperca pappaterra* [32], pike characin *O. hepsetus* [22], Asian seabass *L. calcarifer* [24], large yellow croaker *L. crocea* [9], and killifish *A. urophthalmus* [23]. The striated muscle might be useful for controlling the swallowing of food. Furthermore, Kalhoro *et al.* [9] stated that the striated muscle might help in luminal expansion or assist the fish in ejecting unswallowed food [33].

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Figure 2. A-F: Photograph of the esophagus of *P. fasciata*. A: Photograph showing distinct layers, including stratified epithelium (SE), lamina propria (LP), submucosa (Sm), and the muscularis layer (ML). B: An enlarged picture of the muscularis and submucosa layers, depicting inner circular and outer longitudinal muscles and loose connective tissue in the submucosa. The box highlights the striated muscle of the esophagus. C: An enlarged view of the mucosa, consisting of epithelium and lamina propria. The epithelium comprises non-mucus cells (NC), mucus cells (MC), undifferentiated cells (UC), and goblet cells (GC). Taste buds (TB) were visible between the epithelial cells. Beneath the epithelium, positive staining with aniline blue of Masson's trichrome reveals collagen fibers (CF). D: Photograph illustrating mucus cells and goblet cells containing neutral mucins. E: Photograph illustrating mucus cells and goblet cells containing carboxylated acid mucins. F: Photograph illustrating mucus cells and goblet cells containing sulphated acid mucins. Lumen (L). A: H&E, B-C: MT, D: PAS-H, E: AB pH 2.5-NF, F: AB pH 1.0-NF.

3.2.2 Stomach

The stomach of striped tiger nandid fish had basic morphology similar to many teleosts [22]. In the present study, the stomach appeared to be a J-shape, similar to omnivorous fish [34], such as characin *Astyanax bimaculatus* [35]. However, it differed from the U-shaped stomach of armored catfish *Hypostomus pusarum* [36], gangetic mystus *Mystus cavasius* [19], whitemouth croaker *Micropogonias furnieri* [37], and large yellow croaker *L. crocea* [9], which was the characteristic of carnivorous fish. Other reports mentioned that the morphological differences in the stomach of teleosts could indicate the food, feeding habits, body shape, and environment [19]. The mucosal fold (Figure 3A) of the stomach of striped tiger nandid fish increased the surface area for digestion and food storage [2, 38]. Okuthe and Bongile [2] also suggested that the mucosal folds might delay food during transport through the stomach and facilitate the mixing with digestive enzymes.

The stomach was divided by histological characteristics into three parts: cardiac, fundic, and pyloric stomach. The epithelium of the three portions was simple columnar epithelium (Figures 3, 4, 5). These results were similar to other fish species [4, 8, 19, 22, 24]. Beneath the laminar propria, the muscularis mucosae, the smooth muscle band was found (Figures 3B, 4B-4D, 5A-5B). The muscularis mucosae aided in the elimination and generated the force for the compression of gastric glands. The submucosa was loose connective tissue of collagen fibers and blood vessels (Figures 3B, 4B, 5B). Surrounding the submucosa, two muscular layers were observed: inner circular and outer longitudinal layers. The muscular layer of the stomach of striped tiger nandid fish was similar to other fish species [19, 21, 22, 32, 35].

The stomach could be divided into three portions, with two portions having glands (cardiac and fundic stomach), also called the glandular stomach (Figures 3, 4), and another portion not having glands (pyloric stomach) or the non-glandular stomach (Figure 5). The first portion was the cardiac stomach, which connected to the esophagus. The mean mucosal fold height was 1,744.94±226.06 μm, and the mucosal fold width was 1,301.16±284.60 μm (Table 2). Beneath the epithelium, the lamina propria contained gastric glands that were simple tubular glands. The cardiac glands were surrounded by thin connective tissue. The cells lining the glands were simple cuboidal epithelium with a central nucleus (Figure 3). The gland cells secreted the content to the lumen through the gastric crypts (pits). The epithelium of the cardiac stomach showed positive staining with PAS (Figure 3D) and weak staining to AB pH 1.0 (Figure 3F). The cardiac glands showed positive staining with PAS (Figure 3D) but were negative to AB pH 2.5 (Figure 3E) and AB pH 1.0 (Figure 3F). The thickness of the cardiac glands was 408.24±72.89 μm and the cardiac stomach had a mean muscle tissue layer thickness of 918.25±49.81 μm (Table 2).

The fundic stomach had fundic glands in the lamina propria, which were simple tubular glands lined by simple cuboidal to low columnar epithelium (Figures 4A-4E). The fundic glands open to the lumen by secreting granules through gastric pits. The epithelial cells showed strong positive staining with PAS (Figure 4D) but showed weak staining to AB pH 2.5 (Figure 4E) and AB pH 1.0 (Figure 4F). The cells lining the fundic glands showed positive staining with PAS (Figure 4D) but were negative to AB pH 2.5 (Figure 4E) and AB pH 1.0 (Figure 4F). In contrast, the gastric pits showed weakly positive staining with PAS (Figure 4D) and AB pH 1.0 (Figure 4F). The thickness of the fundic glands was 159.36±12.12 μm, the mean mucosal thickness was 240.88±10.96 μm, and the mean muscle tissue layer thickness of the fundic stomach was 86.30±5.15 μm (Table 2).
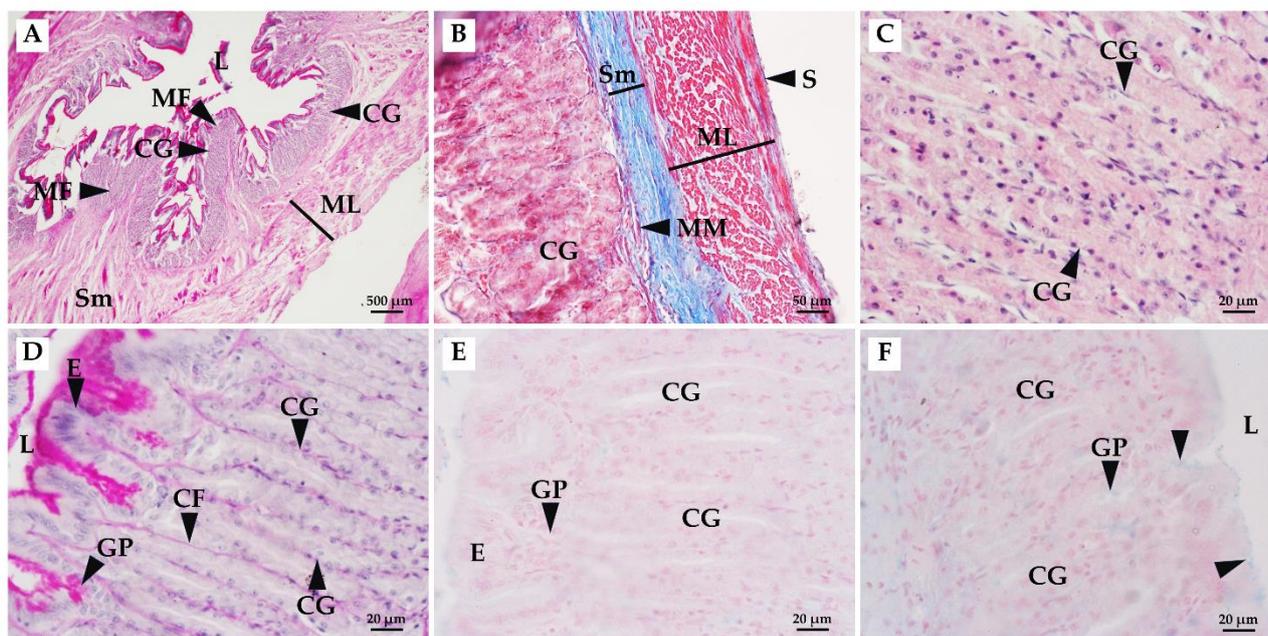
The pyloric stomach had a simple columnar epithelium; the submucosa had rich blood vessels and loose connective tissue. The muscular layer comprises inner circular and outer longitudinal smooth muscle (Figures 5A-5D). The results of histochemistry revealed positive staining with PAS (Figure 5D) but showed weak staining to AB pH 2.5 (Figure 5E) and AB pH 1.0 (Figure 5F). However, any gland was invisible, increasing the width of the lumen. This led to the assumption that the pyloric region served as the site for food storage [24]. This configuration increased the lumen's diameter and helped extend digestion time [24]. The pyloric stomach had a mean mucosal thickness of 126.60±19.19 μm and a mean muscle tissue layer thickness of 170.08±13.95 μm (Table 2).

The cardiac and fundic glands were simple tubular glands, similar to tilapia fish *T. spilurus* [4], but different from European eel *Anguilla anguilla* [39], stripped weakfish *Cynoscion guatucupa* [30], and pike characin *O. hepsetus* [22]. Rotta [40] suggested that branched tubular glands were found in carnivorous fish. In this study, we found cardiac and fundic glands in the cardia and fundus, respectively, indicating that these portions played a role in gastric digestion, corresponding to other studies [2, 19]. Vieira-Lopes *et al.* [22] stated that the gastric glands consisted of oxynticopeptic cells, which played a role in hydrochloric acid (HCl) and pepsinogen secretion. These cells were similar to principal and parietal cells in mammals, respectively. Both cardiac and fundic glands found in this study were common features of carnivorous and omnivorous fish, correlating with protein digestion [26]. Similarly, based on the previous research, striped tiger nandid fish were omnivorous, sometimes consuming invertebrate animals. Therefore, the presence of gastric glands might be related to their feeding habits.

The simple columnar epithelium of the cardiac stomach showed weak staining to AB pH 1.0 (Figure 3F). In contrast, the fundic and pyloric stomach showed weak staining to AB pH 2.5 (Figures 4E, 5E, respectively) and AB pH 1.0 (Figures 4F, 5F, respectively). These findings demonstrated that the epithelium primarily secreted neutral mucins, protecting the epithelium from stomach enzymes and HCl [19, 24] and promoting food transport to the intestine [22]. Meanwhile, acid mucins might be a barrier against microorganisms [41]. The cardiac and fundic glands of striped tiger nandid fish showed a positive reaction only with PAS, similar to tilapia fish *T. spilurus* [4], Gangetic mystus *M. cavasius*, nile tilapia *Oreochromis niloticus* [19]. Meanwhile, the gastric pits in the cardiac and fundic stomachs reacted to AB pH 1.0, demonstrating that the gastric pit cells secreted sulphated mucopolysaccharides, as observed in banded tilapia *T. sparrmanii* [2].

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Table 2. Mean and standard deviation of musosal fold height (MFH), musosal fold width (MFW) of the esophagus and stomach, cardiac gland thickness (CGT), muscular layer thickness (MLT), mucosal thickness (MuT) and fundic gland thickness (FGT) of the stomach (µm) (N=15).

	Esophagus		Stomach								
			Cardiac				Fundic			Pyloric	
	MFH	MFW	MFH	MFW	CGT	MLT	MuT	FGT	MLT	MuT	MLT
Mean ± standard deviation	313.84 ± 36.46	236.36 ± 51.56	1,744.94 ± 226.06	1,301.16 ± 284.60	408.24 ± 72.89	918.25 ± 49.81	240.88 ± 10.96	159.36 ± 12.12	86.30 ± 5.15	126.60 ± 19.19	170.08 ± 13.95

Figure 3. A-F: Photograph of the cardiac stomach of *P. fasciata*. A: Photograph showing distinct layers and mucosal folds (MF) consisting of cardiac glands (CG) in the lamina propria and muscularis layer (ML). B. A picture highlighting the muscularis mucosae (MM), submucosa (Sm), muscularis layer and serosa (S). C: An enlarged picture of cardiac glands, revealing a simple cuboidal-low columnar epithelium. The gland cells exhibit eosin-positive granules. D: An enlarged view of PAS-positive cuboidal-columnar cells and a PAS-positive gastric pit (GP). The cardiac gland displays weak PAS-positive cells. E: Photograph illustrating the negative staining with AB pH 2.5 of cardiac gland cells and gastric pit. F: Photograph illustrating AB pH 1.0-positive epithelial cells (arrowhead) and gastric pit. Collagen fiber (CF), epithelium (E), lumen (L). A, D: PAS-H , B: MT, C: H&E, E: AB pH 2.5-NF, F: AB pH 1.0-NF.

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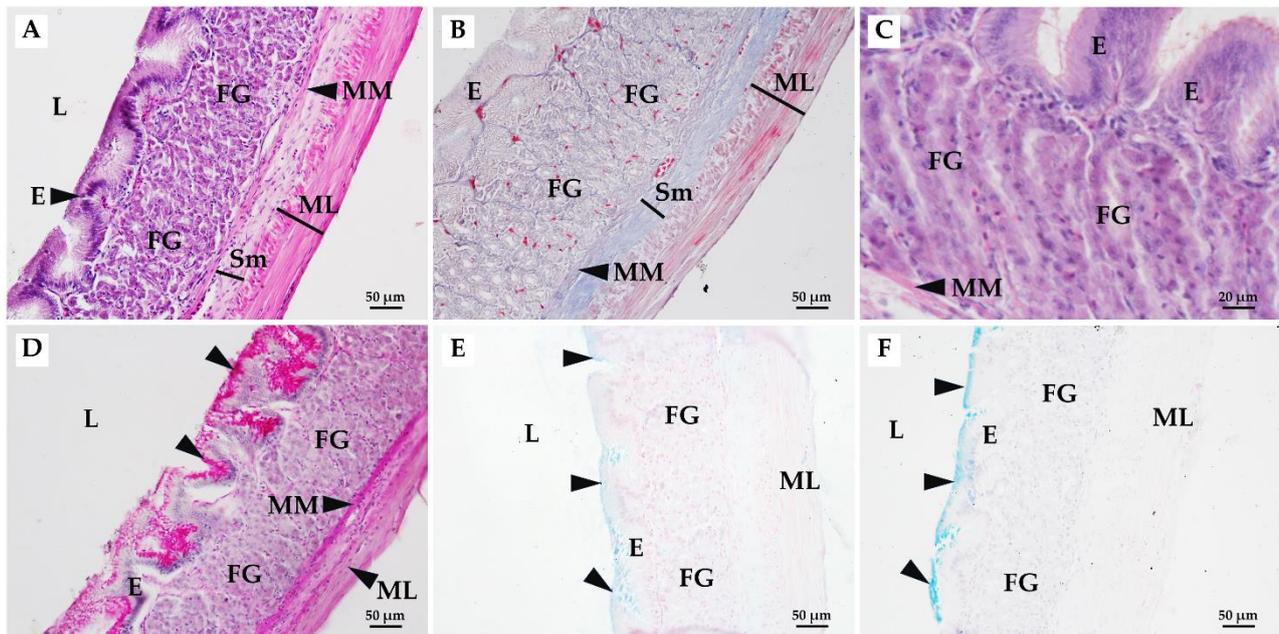
Figure 4. A-F: Photographs of the fundic stomach of *P. fasciata*. A-B: Photographs showing distinct layers of mucosa, consisting of epithelium (E), and fundic glands (FG), as well as the muscularis mucosae (MM). These pictures represent the two distinct muscularis layer (ML) layers, consisting of inner circular and outer longitudinal smooth muscle bundles. C: An enlarged picture of the mucosa showing simple columnar epithelium (E). The fundic gland cells exhibit eosin-positive granules and thin muscularis mucosae. D: Photograph showing PAS-positive columnar epithelial cells (arrowheads) and a PAS-positive gastric pit. The fundic glands show weak PAS-positive cells. E-F: Photographs illustrating the representation of AB pH 2.5 and AB pH 1.0 positive fundic epithelial cells, respectively (arrowheads). Lumen (L), Sm (submucosa). A, C: H&E, B: MT, D: PAS-H, E: AB pH 2.5-NF, F: AB pH 1.0-NF.

3.2.3 Intestine

The IC of striped tiger nandid fish was 0.62±0.01 (Table 1), indicating an omnivorous fish. The intestine could be divided into three portions (Figure 6): anterior, middle, and posterior intestines. The intestine wall had four layers, similar to other digestive tract regions. The epithelium was lined by a simple columnar epithelium consisting of absorptive cells (enterocytes) and goblet cells (Figure 6). The enterocyte had a brush border at the apical domain. The nucleus of the goblet cell was found at the basal portion of the cell, while the mucus was found in the cytoplasm (Figure 6). In some sections, exocytosis released content from the goblet cell's apical domain. This was similar to other teleosts [2, 9, 22, 23, 24, 32, 35]. The enterocytes showed positive staining with PAS (Figures 6B, 6F, 6J), similar to Asian seabass *L. calcarifer* [24]. The submucosal layer comprises loose connective tissue with collagen fibers and blood vessels. All three parts of the intestine had no glands (Figures 6A, 6E, 6I), the similarity observed in Asian seabass *L. calcarifer* [24], large yellow croaker *L. crocea* [9], and killifish *A. urophthalmus* [23]. The muscularis layer consisted of the inner circular layer and the outer longitudinal layer. The outermost layer was the mesothelium and loose connective tissue (Figures 6A, 6E, 6I).

The mucosal fold of the intestine, also known as the villi, consists of the epithelium and the lamina propria. Differences between each portion were defined by mucosal fold height, width, and muscular thickness. The anterior intestine had a mean fold height of 1,046.15±75.08 µm, a mean fold width of 157.28±16.34 µm, and a mean muscular layer thickness of 96.22±18.11 µm. The mid-intestine had a mean mucosal fold height of 814.22±95.15 µm, a mean fold width of 95.42±15.42 µm, and a mean muscular layer thickness of 17.82±3.33 µm. The posterior intestine had a mean fold height of 753.11±64.44 µm, a mean fold

width of 74.16±6.13 μm, and a mean muscular layer thickness of 99.93±5.56 μm (Table 3). The results showed that the anterior intestine's mucosal fold/villi were the tallest than the middle and posterior intestine. This finding was similar to pike characin *O. hepsetus* [22], pebbly fish *A. baremoze* [8], and killifish *A. urophthalmus* [23], leading to the suggestion that the anterior and middle intestine of striped tiger nandid fish were the main regions for digestion and absorption [23, 35]. The mucosal folds/villi reduced food speed as it flowed through the intestine for absorption. These folds could promote an increase in absorptive efficiency due to the presence of numerous enterocytes or absorptive cells [2].
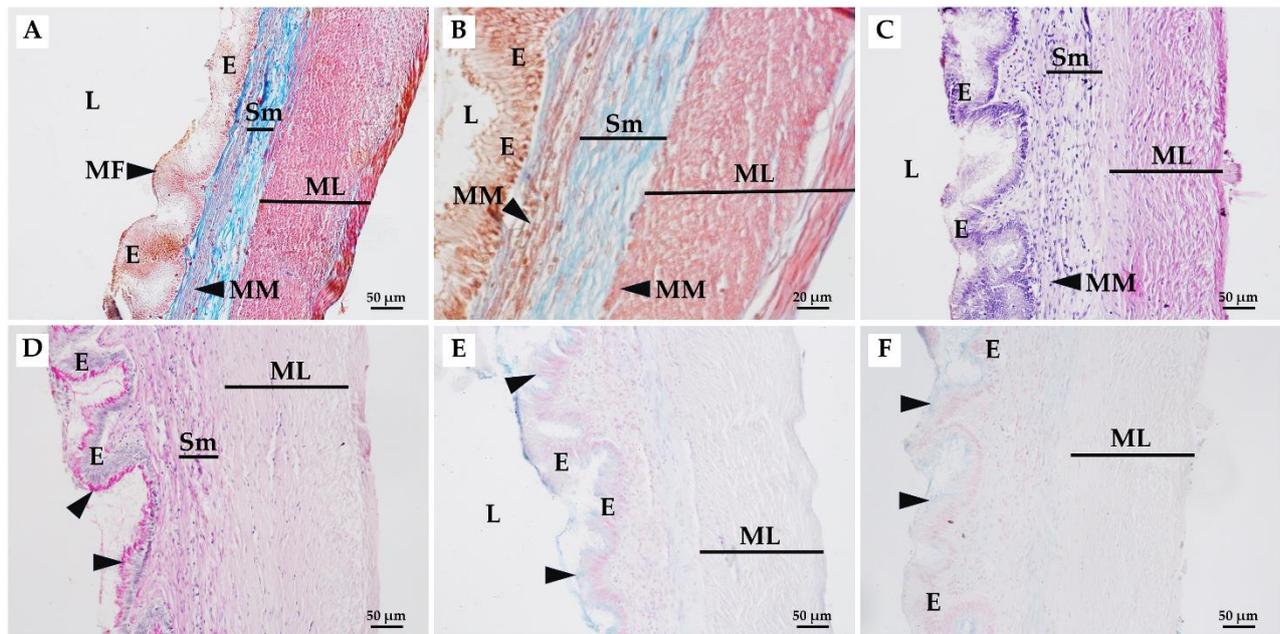
Figure 5. A-F: Photograph of the pyloric stomach of *P. fasciata*. A-C: Photograph showing distinct mucosa layers consisting of simple columnar epithelium (E), lamina propria, and muscularis mucosae (MM). Pictures A and B represent the distinct muscularis layer layers (ML). D: Photograph showing PAS-positive columnar epithelial cells (arrowheads). E-F: Photograph illustrating the representation of AB pH 2.5 and AB pH 1.0 positive epithelial cells, respectively (arrowheads). Lumen (L), mucosal fold (MF), submucosa (Sm). A-B: MT, C: H&E, D: PAS-H, E: AB pH 2.5-NF, F: AB pH 1.0-NF.

Additionally, the goblet cells were numerous in the posterior intestine of striped tiger nandid fish, similar to flower fish *Pseudophoxinus antalyae* [1], characin *A. bimaculatus* [35], Asian seabass *L. calcarifer* [24], pebbly fish *A. baremoze* [8] and large yellow croaker *L. crocea* [9]. Machado *et al.* [26] suggested mucous from goblet cells helped defend the intestine lining and support waste or feces inclusion. The goblet cells in each portion revealed intense labeling with both PAS (Figures 6B, 6F, 6J) and AB pH 2.5 (Figures 6C, 6G, 6K) but weak reaction to AB pH 1.0 (Figures 6D, 6H, 6L). This finding was also found in other species, such as flower fish *P. antalyae* [1], common dentex *Dentex dentex* [42], characin *A. bimaculatus* [35], and Asian seabass *L. calcarifer* [24]. These results indicated they secreted mainly neutral and carboxylated acid glycoproteins/mucopolysaccharides. The neutral mucous from goblet cells might function in protecting the epithelium from gastric juices from the stomach, providing co-factors useful to nutrient enzymatic breakdown [43], emulsification of nutrients [44], absorption [45], and protection of the intestinal epithelium from HCl [40]. In addition, Carrassón *et al.* [42] stated that neutral mucous had a lubrication function for transporting nutrients. Meanwhile, carboxylated acid mucins shielded the luminal surface from glucosidase deprivation [42]. This work also found sulphated acid mucins secreted from goblet cells. These mucins might help prevent microorganism invasion or infection, similar to large yellow croaker *L. crocea* [9], and trapping particles.

However, the co-secretion of neutral and acidic mucins probably represented the sequential steps of mucous synthesis [24].
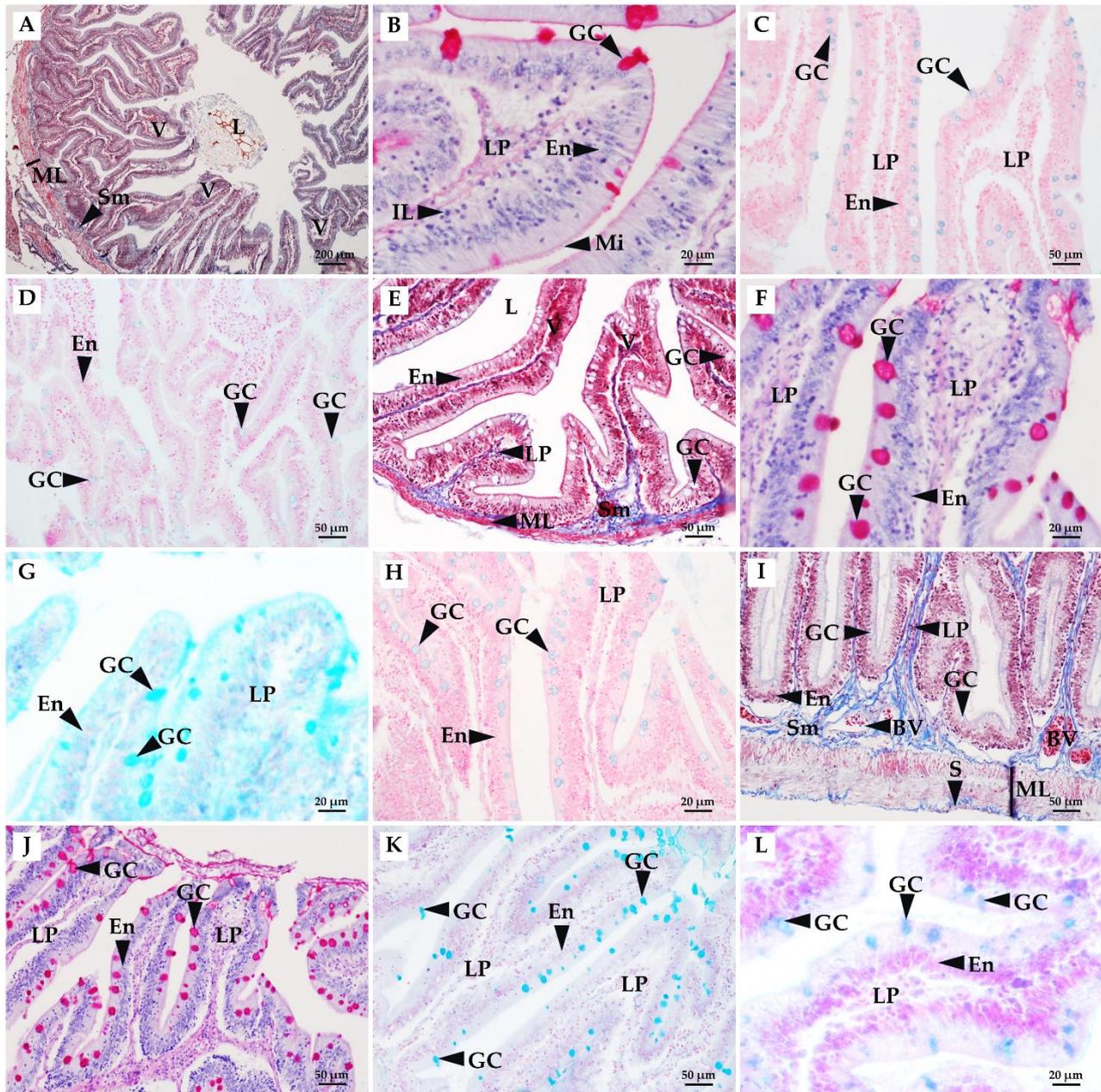
Figure 6. A-D: Photograph of the anterior intestine of *P. fasciata*. E-H: Photograph of the mid-intestine. I-L: Photograph of the posterior intestine. Pictures A, E, and I show distinct layers of mucosa, consisting of simple columnar epithelium with enterocytes (En) and goblet cells (GC), lamina propria (LP), and also the submucosa (Sm) with blood vessels (BV). Underneath the submucosa, circular and longitudinal smooth muscles were observed. Pictures B, C, and D show villi (V) of the anterior intestine, with PAS-positive, AB pH 2.5-positive, and AB pH 1.0-positive goblet cells, respectively. Pictures F, G, and H depict the presence of PAS-positive, AB pH 2.5-positive, and AB pH 1.0-positive goblet cells, respectively. Pictures J, K, L: Photograph illustrating the representation of PAS-positive, AB pH 2.5-positive, and AB pH 1.0-positive goblet cells, respectively. Intraepithelial leukocytes (IL), lumen (L), microvilli (Mi), muscularis layer (ML), and serosa (S). A, E, I: MT, B, F, J: PAS-H, C, G, K: AB pH 2.5-NF, D, H, L: AB pH 1.0-NF.

Table 3. Mean and standard deviation of musosal fold height (MFH), musosal fold width (MFW) and muscular layer thickness (MLT) of the intestines (μm) (N=15).

	Anterior intestine			Mid-intestine			Posterior intestine		
	MFH	**MFW**	**MLT**	**MFH**	**MFW**	**MLT**	**MFH**	**MFW**	**MLT**
Mean ± standard deviation	1,046.15 ± 75.08	157.28 ± 16.34	96.22 ± 18.11	814.22 ± 95.15	95.42 ± 15.42	17.82 ± 3.33	753.11 ± 64.44	74.16 ± 6.13	99.93 ± 5.56

4. Conclusions

This research represented the first description and report on the histology and histochemistry of the gastrointestinal tract of the striped tiger nandid fish. The histological characteristics of their gastrointestinal tract were consistent with those of fish consuming both plants and animals (omnivore). The short esophagus was the muscular tube connecting to the stomach, the sac containing fundic and cardiac glands. In the pylorus of the stomach, no glandular structures were found. Similarly, glandular structures were not found in any of the three parts of the intestine; however, the enterocyte and goblet cells were present. In the future, this research may be used to develop food and farm management strategies, as the morphology of the gastrointestinal tract is closely related to feeding habits. Furthermore, it can aid in distinguishing abnormal tissue from normal tissue.

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References

[1]   Cinar, K.; Nurgül, S. Histological and histochemical characterization of the mucosa of the digestive tract in flower fish (*Pseudophoxinus antalyae*). *Anat. Histol. Embryol*. **2006**, *35*, 147–151.

[2]   Okuthe, G.E.; Bongile, B. Morphology, histology and histochemistry of the digestive tract of the banded tilapia, *Tilapia sparrmanii* (Perciformes: Cichlidae). *Zoologia* **2020**, *37*, 1-14.

[3]   Wainwright, P.C.; Richard, B.A. Predicting patterns of prey use from morphology of fishes. *Environ. Biol. Fishes*. **1995**, *44*, 97-113.

[4]   Al-Abdulhadi, H.A. Some comparative histological studies on alimentary tract of tilapia fish (*Tilapia spilurus*) and sea bream (*Mylio cuvieri*). *Egypt. J. Aquat. Res*. **2005**, *31*, 387-39.

[5]   Hernández, D.R.; Pérez Gianeselli, M.; Domitrovic, H.A. Morphology, histology and histochemistry of the digestive system of south American catfish (*Rhamdia quelen*). *Int. J. Morphol.* **2009**, *27*, 105-111.

[6]   Senarat, S.; Jes, K.; Wannee, J.; Niwat, K. Structural classifications in the digestive tract of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851) from upper gulf of Thailand. *Songklanakarin J. Sci. Technol*. **2015**, *37*, 561-567.

[7]   Borman, M.; Ismot, A.; Mohammad, K.; Md Rafiqun, N. Histo-morphology of the alimentary canal in two freshwater snakehead fish *Channa punctata* and *Channa striata*. *J. Fish.* **2015**, *3*, 297-300.

[8] Kasozi, N.; Gerald, I.D.; Julius, M.; Charles, D.K.; Majid, K.; Akisoferi, O.W.; Godfrey, K.; Victoria, T.N. Histomorphological description of the digestive system of pebbly fish, *Alestes baremoze* (Joannis, 1835). *Sci. World J.* **2017**, https://doi.org/10.1155/2017/8591249.

[9] Kalhoro, H.; Shengli, T.; Lei, W.; Ying, H.; Josie, A.V.; Qingjun, S. Morphological study of the gastrointestinal tract of *Larimichthys crocea* (Acanthopterygii: Perciformes). *Zoologia* **2018**, *35*, 1-9.

[10] Alabssawy, A.N.; Hassan, M.M.K.; Ahmed, A.G. Anatomical and histological adaptations of digestive tract in relation to food and feeding habits of lizardfish, *Synodus variegatus* (Lacepède, 1803). *Egypt. J. Aquat. Res.* **2019**, *45*, 159–165.

[11] Santos, M.L.D.; Fábio, P.A.; Tiago, C.P.; José, E.S. Morphological, histological and histochemical analysis of the digestive tract of *Trachelyopterus striatulus* (Siluriformes: Auchenipteridae). *Zoologia* **2015**, *32*, 296–305.

[12] Smith, L.S. *Introduction to Fish Physiology*, Argent Laboratories Press: Washington, DC., The United States of America, **1991**.

[13] Qu, M.; Shaoxiong, D.; Xiaojing, X.; Minghui, S.; Yingzhe, Y.; Yongquan, S. Ontogenetic development of the digestive system and growth in coral trout (*Plectropomus leopardus*). *Aquaculture* **2012**, *334*, 132–141.

[14] Angelescu, V.; Franscisco, S.G. Adaptaciones del aparato digestivo al régimen alimenticio en algunos peces del Río Uruguay y Río de La Plata. *Rev. Inst. Nac. Invest. Nat.* **1949**, *1*, 161-281.

[15] Rainboth, W.J. *Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes*, FAO: Rome, Italy, **1996.**

[16] Sangpradub, N.; Chutima, H. Diet composition of *Pristolepis fasciata* (Bleeker, 1851) (Family Nandidae) and *Puntius brevis* (Bleeker, 1849) (Family Cyprinidae) in Kaeng Lawa, Thailand. *Chiang Mai J. Sci.* **2017**, *44*, 839-846.

[17] Uk-katawewat, S. *Illustration of Thai Fish and Aquatic animals*, Kdurusapa Ladprao Printing Press: Bangkok, Thailand, **2002**.

[18] Bancroft, J.D.; Marilyn, G. *Theory and practice of histological techniques*, Churchill Livingstone: London, U.K., **2002**.

[19] Ghosh, S.K.; Padmanabha, C. Histological and histochemical characterization on stomach of *Mystus cavasius* (Hamilton), *Oreochromis niloticus* (Linnaeus) and *Gudusia chapra* (Hamilton): comparative study. *The Journal of Basic and Applied Zoology* **2015**, *70*, 16–24.

[20] Smith, B.J.; Smith, S.A.; Bundit, T.; Terry, A.L. Gross morphology and topography of the adult intestinal tract of the tilapian fish, *Oreochromis niloticus* L. *Cells Tissues Organs* **2000**, *166*, 294–303.

[21] Santos, M.D.; Lima, C.L.P.; Sandra, R.; Carvalho, B.S.M. Metazoan parasite fauna of *Pimelodus maculatus* La Cépède, 1803 (Siluriformes, Pimelodidae) from the Guandu river, Rio de Janeiro Rio State, Brazil. *Acta Sci. Biol. Sci.* **2007**, *29*, 101-107.

[22] Vieira-Lopes, D.A.; Nadja, L.P.; Armando, S.; Adriana, V.; Francisco, G.A.; Iracema, D.G.; Aparecida, A.N. Immunohistochemical study of the digestive tract of *Oligosarcus hepsetus*. *World J. Gastroenterol*. **2013**, *19*, 1919-1929.

[23] Nascimento, W.S.; Glaucia, M.M.S.; Lenilda, T.T.; Naisandra, B.S.; Sathyabama, C. Histology of the digestive tract of *Anablepsoides urophthalmus* from Brazilian oriental Amazonia. *Journal of Aquaculture and Marine Biology* **2018**, *7*, 39-42.

[24] Purushothaman, K.; Doreen, L.; Jolly, M.S.; Syed, M.S.; Declan, P.L.; Shubha, V.; László, O. Morpho-histological characterisation of the alimentary canal of an important food fish, Asian seabass (*Lates calcarifer*). *PeerJ*. **2016**, DOI 10.7717/peerj.2377.

[25] Dias, A.C.M.I.; Castelo, B.C.W.; Lopes, V.G. Estudo da dieta natural de peixes no reservatório de Ribeirão das Lajes, Rio de Janeiro, Brasil. *Acta Sci. Biol. Sci.* **2005**, *7*, 355–364.

[26] Machado, M.R.F.; Helena, O.S.; Valderes, L.S.; Alexandre, A.; Roberto, G.; André, D.B. Morphological and anatomical characterization of the digestive tract of *Centropomus parallelus* and *C. undecimalis*. *Acta Sci. Biol. Sci.* **2013**, *35*, 467–474.

[27] Reifel, C.W.; Anthony, A.T. Structure and carbohydrate histochemistry of the stomach in eight species of teleosts. *J. Morphol* **1978**, *158*, 155–67.

[28] Pedini, V.; Cecilia, D.; Parillo, F.; Paola, S. A lectin histochemical study of the oesophagus of Shi Drum. *J. Fish. Biol.* **2004**, *64*, 625–631.

[29] Sibbing, F.A.; Uribe, R. Regional specialisation in the oro-pharyngeal wall and food processing in carp (*Cyprinus carpio* L.). *Neth. J. Zool.* **1985**, *35*, 377–422.

[30] Díaz, A.O.; Alocia, M.C.; Adriana, L.G. Glycoconjugates in the mucosa of the digestive tract of *Cynoscion guatucupa*: a histochemical study. *Acta Histochem.* **2008**, *110*, 76–85.

[31] Canan, B.; Wallace, S.N.; Naisandra, B.S.; Sathyabama, C. Morphohistology of the digestive tract of the damsel fish *Stegastes fuscus* (Osteichthyes: Pomacentridae). *The Scientific World Journal* **2012**, doi:10.1100/2012/787316.

[32] Rodrigues da Silva, M.; Maria, R.M.N.; Norma, S.H. Histology of the digestive tract of *Satanoperca pappaterra* (Osteichthyes, Cichlidae). *Acta Sci. Biol.* **2012**, *34*, 319-326.

[33] Cao, X.J.; Wang, W.M. Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. *Anat Histol Embryol*. **2009**, *38*, 254-261.

[34] Eckert, R.; Randall, D.; Augustine, G. *Animal Physiology; Mechanisms and Adaptations,* WH Freeman and Company: New York, The United States, **1988**.

[35] Cardoso, N.N.; Enely, M.S.F.; Iracema, D.G.; Aparecida, A.N.; Armando, S.; Francisco, G.A. Histochemical and immunohistochemical study on endocrine cells (5HT, GAS, and SST) of the gastrointestinal tract of a teleost, the characin *Astyanax bimaculatus*. *Acta Histochem*. **2015**, *117*, 595-604.

[36] Pessoa, E.K.R.; Naisandra, B.S.; Naithirithi, T.C.; Arrilton, A.S.; Sathyabama, C. Morfologia comparativa do trato digestorio dos peixes *Hoplias malabaricus* e *Hypostomus pusarum* do acude Marechal Dutra, Rio Grande do Norte, Brasil. *Biota Amazôn.* **2013**, *3*, 48–57.

[37] Andrade, I.M.; Juliana, P.G.; Matheus, M.R.; Renata, B.M. Morphology of the digestive tract of the whitemouth croaker *Micropogonias furnieri* (Desmarest, 1823) (Perciformes: Sciaenidae). *Acta Zool.* **2016**, doi: 10.1111/azo.12156.

[38] Legler, J.M. Morphology and physiology of the Chelonia. *Australian Biological Recourses Study, Caberra* **1993**, 108–119.

[39] Domeneghini, C.; Silvana, A.; Giuseppe, R.; Giampaolo, B.; Veggetti A. Histochemical analysis of glycoconjugate secretion in the alimentary canal of *Anguilla anguilla* L. *Acta Histochem*. 2**005**, *106*, 477-87.

[40] Rotta, M.A. *Aspectos gerais da fisiologia e estrutura do sistema digestivo dos peixes relacionados à piscicultura*, Corumbá Embrapa: Brazil, **2003**.

[41] Ringø, E.; Reidar, M.; Terry, M.M.; Rolf, E.O. Bacterial translocation and pathogenesis in the digestive tract of larvae and fry. *Aquaculture* **2007**, *268*, 251–264.

[42] Carrassón, M.; Grau, A.; Dopazo, L.R.; Selena, C. A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). *Histol. Histopathol.* **2006**, *21*, 579-593.

[43] Anderson, T.A. Histological and cytological structure of the gastrointestinal tract of the luderick, *Girella tricuspidata* (Pisces, Kyphosidae), in relation to diet. *J. Morphol.* **1986**, *190*, 109–119.

[44] Clarke, A.J.; Witcomb, D.M. A study of the histology and morphology of the digestive tract of the common eel (*Anguilla anguilla*). *J. Fish. Biol.* **1980**, *16*, 159-170.

[45] Gartner, L.P.; Hiatt, J.L.; De Souza, L.F.; Sales, Md.G.F. *Atlas colorido de histologia*, Guanabara Koogan LTDA: Rio de Janeiro, Brazil, **2002**.