

FIRST REPORT ON THE ANATOMY AND HISTOLOGY OF THE ALIMENTARY CANAL OF AN IMPORTANT ESTUARINE FOOD FISH, *OXYURICHTHYS TENTACULARIS* (VALENCIENNES, 1837)

Fiona Paulose¹ and *Sherly Williams E²

¹Department of Zoology, Fatima Mata National College (Autonomous), Research centre, University of Kerala, Kollam, Kerala, India

²Department of Environmental Sciences, University of Kerala, Kariavattom Campus, Thiruvananthapuram, Kerala, India

*Author for Correspondence: sherlyrobin@rediffmail.com

ABSTRACT

The differences in food and feeding habits in fishes are often correlated to the adaptations in anatomical and histological structures of the digestive tract. The results indicated *Oxyurichthys tentacularis* possess small and villiform recurved canines along with well-developed pharyngeal teeth which helps to crush the food and prevents the regurgitation of food. It was found that anatomically, the alimentary canal of *O. tentacularis* is a straight tube without a distinct stomach and long intestine. Based on histological analysis, the following segments of the alimentary canal were distinguished: pharynx, esophagus, residual stomach, intestine and rectum. Between the small intestine and other segments there are two well-developed sphincters: pyloric and ileorectal. The esophagus is characterized by a large number of goblet cells. The stomach showed greatest variation by the total absence of mucus cells and presence of gastric glands. The relative length of gut ranges from 0.68 to 0.86 cm. The intestine is long, coiled tube with long mucosal folds. Based on the findings of the anatomical and histological investigations, it may be concluded that there is a correlation between the adaptations in the digestive tract structure of the Arrow Fin Gobi, *O. tentacularis*, and their omnivorous feeding habits.

Keywords— Anatomy, Histology, Alimentary Tract, Gobiidae, Mucus-Secreting Goblet Cells

INTRODUCTION

Because of the complexity of features related with eating, the digestive system of teleosts has piqued the interest of many researchers. As a result, the gross morphology and histology of the gastrointestinal system has long been a subject of research, either commonly understood (Al-Hussaini, 1946) or connected to dietary habits (Girgis, 1952). A study of the anatomy of the eating and digestive organs is required for an accurate comprehension of a fish's feeding habits. Anatomical and histological studies provide information for the characterisation of the digestive tract, thus enabling the understanding of the physiology, feeding habits and management of fish species (Germano *et al.*, 2014).

The alimentary tract in fishes displays structural and functional heterogeneity, according to Santos *et al.* (2015), which is connected to food demands, feeding behaviours, phylogeny, body weight and shape, and sometimes even sexes (Al-Hussaini, 1949; Day *et al.*, 2014). At both the macroscopic and microscopic levels, the length of the gut, the degree of its anatomical complexity, and the histological features of its numerous divisions have all revealed considerable changes (Kalhor *et al.*, 2018). The Gobiidae family is one of the largest acanthomorph fish families, with about 1,950 species found in marine, brackish, and freshwater settings across the temperate and tropical zones (Thacker and Roje, 2011). Oxudercinae subfamily species have also adapted well to amphibian life (Polgar *et al.*, 2010; Thacker and Roje, 2011). Carnivores, herbivores and omnivores are all represented (Geevarghese, 1983; Wu *et al.*, 2010). A

diversity of food preferences as well as the environmental conditions in which they live affected the evolution of a number of adaptational features in the architecture of the Gobiidae digestive system (Geevarghese, 1983; Kobegenova and Dzhumaliev, 1991). The absence of a well-developed stomach distinguishes this species of fishes (Geevarghese 1983; Kobegenova and Dzhumaliev, 1991). Several experts confused this fish family for stomachless due to the morphological uniqueness of the fish alimentary canal (Barton, 2007; Jaroszewska *et al.*, 2008). There is currently a lack of convincing evidence that not all Gobiidae are stomachless in terms of histological and physiological features of the oesophagogastric segment, despite the fact that not all research agrees (Geevarghese, 1983; Hur *et al.*, 2005; Wu *et al.*, 2010). The majority of these results were derived based on anatomical data (Geevarghese, 1983; Pogoreutz and Ahnelt, 2014; Wilson and Castro, 2010).

Despite the extensive literature on the alimentary tract in teleost fish, the alimentary tract in gobioid fish has received comparatively less attention (Wilson and Castro, 2010). However, only a few species, especially from the Gobiinae and Gobionellinae subfamilies, have had their alimentary tracts studied histologically (Kobegenova and Dzhumaliev, 1991; Hur *et al.*, 2005; Jaroszewska *et al.*, 2008; Wołczuk *et al.*, 2015). In *Glossogobius giuris* (Ham), Mohsin (1962) discovered a link between the histomorphology of the digestive organs and eating patterns. Hur *et al.* (2005) investigated the morphology of the giurine goby, *Rhinogobius giurinus*, digestive tract and goblet cells. Remya *et al.* (2017) studied the reproductive biology of *Oxyurichthys tentacularis*, Hur *et al.* (2016) studied the alimentary canal of monkey goby. The alimentary tract of the Oxudercinae subfamily is poorly understood. The lack of understanding of the architecture and function of the alimentary tract in Oxudercinae contributed to the various perspectives on the existence or absence of the stomach in these fishes. The current findings can only be compared to closely related *Gobius* species because the histology of *O. tentacularis* alimentary tract has never been investigated before. Wołczuk *et al.* (2015) investigated the anatomical, histological, and histochemical characteristics of the alimentary canal of the monkey goby, *Neogobius fluviatilis*, as well as histological, histochemical, and ultrastructural analyses in the freshwater tubenose goby, *Proterorhinus semilunaris*.

Since the frequency of food items is typically linked to their availability in the environment, interpreting eating habits only on the basis of gastrointestinal contents analysis may result in incorrect conclusions. As a result, learning the anatomy of a fish's feeding and digesting organs is essential for understanding its diet and eating habits. As a result, the present study details the anatomical and histological alterations in the digestive system of the Arrow Fin Gobi, *Oxyurichthys tentacularis*, as well as the morphology of various cells lining its digestive system. This description adds to our understanding of the structure of the Gobiid fish's alimentary canal, which is currently restricted.

MATERIALS AND METHODS

A total of 30 specimens (15 males and 15 females) of *Oxyurichthys tentacularis*, (Valenciennes, 1837), ranging between 10 and 17 cm in total length and 6–15 gm in weight were collected from Ashtamudi Estuary, Kerala, South India, during the period from October to December 2018. Fresh samples of the digestive tract and liver were fixed in 10% formalin for 24 hours for histological examinations. Following fixation, the tissues were adequately dehydrated using an ascending series of ethanol, then cleared in xylene and embedded in paraffin wax with a melting temperature of 56-58°C for one hour in a thermostat vacuum paraffin embedding bath. The tissue was sectioned at 4-5 µm thickness using a Yorco rotary microtome and then stained using hematoxylin and eosin. The sections were examined by a light microscope (Primostar Zen Cam, Ver. 2.3.71) and the required photomicrographs were taken. Total length of each individual was measured to the nearest 0.01(cm) from tip of snout to the end of caudal fin and the gut length (the distance from oesophagus to anus) was also measured to the nearest 0.01(cm) using measuring scale. Relative Length of Gut (RLG) was calculated by dividing the gut length by total

length of fishes. The relationship between Gut Length (GL) and Total Length (TL) was estimated by the formula given by Al Hussaini (1949) as $RLG=GL/TL$

Table 1. Relative length of gut (Average \pm SD) of the Arrow Fin Gobi, *Oxyurichthys tentacularis* collected from Ashtamudi Lake, Kerala, during the period from October to December 2018

Total length (cm)	No of fish examined	Gut length		Relative length of gut
		Range	Average	
10	3	7.5-9	8.4 \pm 0.65	0.68
11	3	8.5-10	9.33 \pm 0.76	0.86
12	3	9.1-11	9.86 \pm 1.00	0.78
13	6	8.5-11.5	10.28 \pm 1.26	0.81
14	5	7.4-13	10.34 \pm 2.54	0.73
15	4	11.5-13	12.25 \pm 0.86	0.81
16	3	13.5-15	13.66 \pm 0.28	0.87
17	3	12.5-15	14 \pm 1.32	0.86
Total	30	7.5-15	11.01	0.80 \pm 0.06

RESULTS

Anatomy of Alimentary Tract

The alimentary canal of *O. tentacularis* consists of mouth, pharynx, oesophagus, stomach, intestine, and rectum (Figure 1 & 2). The division of the alimentary tract from mouth to anus was demarcated by the presence of a pyloric sphincter and an ileorectal valve.

Mouth: The mouth of *O. tentacularis* is wide, protractile, slightly subterminal with large lips and without barbels is the characteristic feature of bottom feeding and bottom dwelling fishes. The upper jaw is slightly longer than the lower, so that mouth opening is distinctly downwards (Figure 3 & 4). The lower jaw is large, and the mouth is slanted, producing a 45° angle with the body axis (Figure 5). Both the jaws are provided with well-developed sharp, small, villiform conical teeth which prevent the escape of the prey from the mouth. Tongue tip rounded. Strong canines make up the upper jaw's teeth, which are roughly double the size of the lower jaw's outer row teeth. The teeth are not used for grinding. The moist skin of the head is turned over both the jaws, forming well developed movable lips which are highly important for food catching (Figure 6). The compressed, flat buccal chamber is reached by the tiny, crescentic mouth.

Buccal Cavity: The oral cavity was well developed, dorsoventrally compressed and spacious. Typically, 5 pairs of gill arches are present. The gill rakers are long, pointed, wide, teeth like arranged in interlocking fashion and attached to the branchial arches through their base. The gill rakers form efficient sieve-like structure when two gill arches came closer and act as rasping organ. The pharyngeal dental apparatus consists of a ventral triangular plate that is placed in the caudal region of the oropharynx cavity (Figure 7). A white muscular cushion pad was placed on the roof of the buccal cavity, which was covered by a translucent mucus membrane. This mucus is released to assist lubrication of the food and make swallowing easier. A non-protruding, non muscular tongue emerges from the floor of the buccal cavity as a moderately sized fold devoid of teeth (Figure 5). The buccal cavity leads into the pharynx from the behind.

Pharynx: It was compressed dorsoventrally, wide laterally with flat floor and arched roof. The lateral wall of the pharynx was perforated by five pairs of gill slits separated by four pairs of gills. Pharynx is provided with pharyngeal pads on the roof towards the entrance of the oesophagus. On the floor, the teeth

were located on the two V – shaped elevated close-set structure borne by the fifth gill arch. The inferior pharyngeal teeth were situated just opposite to the dorsal pair of pharyngeal pads and formed an effective grinding surface to crush the food material finely. The function of pharyngeal teeth was to prevent the regurgitation of food or prey when once it has crossed into the gullet. Its posterior part opens into the oesophagus.

Oesophagus: Oesophagus of *O. tentacularis* is short, thick-walled elastic anterior region of alimentary canal which helps to engulf food. The oesophagus is a short tube between the pharynx and the stomach (Figure 1). It is very short in length, narrow and moderately thick-walled tube. The oesophagus was completely covered by the liver. Posteriorly the oesophagus leads into stomach.

Stomach: In *O. tentacularis*, a sac like swollen stomach is present. It constitutes a short section situated between the oesophagus and the intestine (Figure 1 & 2). It acts as a food storage organ where breakdown of food material takes place. In *O. tentacularis* pyloric valve was observed as posterior part of the intestine that reserves the food material. The pyloric valve in *O. tentacularis* is a projection of the submucosa and of the inner muscular layer, separating the stomach from the intestine. The presence of such a valve contributes to control the passage of the digested food to the intestine.

Intestine: The intestine forms the longest part of the alimentary canal with a straight tube (Figure 1 & 2) of uniform diameter throughout its length leading to the rectum as in most omnivorous fishes. It originates from right anterolateral position of stomach. The lumen of the intestine was more or less uniform. Though no macroscopic differences can be observed in the long-coiled intestine, histologically it was divided into anterior, middle and posterior. The proximal intestine was thicker than the middle as well as the distal region. Functional separation of two intestinal region was possible due to the presence of a prominent ileorectal sphincter.

Rectum: In *O. tentacularis* rectum was the posterior part of the intestine. Rectum was not distinguished from intestine (Figure 2).

Relative length of gut (RLG): Results in Table 1 showed that the relative length of gut of *O. tentacularis* ranges from 0.68 to 0.86 cm with an average of 0.80 ± 0.06 cm. It less than one (<1) suggesting that it is omnivorous.

Histology of digestive tract

Pharynx: The pharyngeal area histologically has the conventional vertebrate layers: mucosa, submucosa, muscularis externa and serosa. The mucous cells and stratified squamous epithelial cells constitute the mucosal folds. Taste buds, while present, are sparse in number; the mucosa is supported by a separate lamina propria. The sub mucosa is well developed. The serosa is thin (Figure 8 & 9).

Oesophagus: Histologically, the oesophagus consists of the following layers: mucosa, sub mucosa, muscularis and serosa (Figure 11). The mucosa formed longitudinal branching folds and its epithelial lamina was formed by the stratified squamous epithelium. The basal layer of epithelium consisted of the prismatic cells with a round centrally placed nucleus. Within the epithelium numerous, large goblet cells were observed (Figure 12). They were sac-like structures that spanned the total thickness of the epithelium. Collagenous fibres made up the majority of the submucosa. This layer also contained bundles of striated longitudinal muscles. The muscularis was formed by an outer layer of circular organized muscles and the inner layer of longitudinally arranged bundles of muscle fibres. There is a definite change in the epithelium of the mucosa at the gastro-oesophageal junction. The boundary between these two areas was conspicuous, with short non-glandular mucosal area at oesophageal-gastric connection and a transition from squamous epithelium with goblet cells in the oesophagus to a simpler columnar epithelium lacking goblet cells in the gastric region (Figure 10). The oesophagus was equipped with well-developed longitudinal folds to permit distensibility with a wide lumen. The fine longitudinal folds of the oesophagus became coarse folds in the stomach. In the transition zone between the oesophagus and stomach, in the muscularis, smooth muscles can be observed. From the outside, the oesophagus wall is covered by a thin layer of serosa.

Stomach: The wall of the stomach, similar to the oesophagus was composed of the mucosa, sub mucosa, muscularis and serosa (Figure 13). The mucosa of the stomach was formed by the longitudinal folds. The surface layer of the mucosa was made up of the simple columnar epithelium. The lamina propria of mucosa sub divided into a glandular layer and connective tissue layer. Under the glandular layer, there was a thin layer of connective tissue containing parallel collagen fibres and numerous blood vessels (Figure 14). The submucosa is made up of fibres of connective tissue innervated with blood vessels. The muscularis of the stomach was composed of the inner circular and the outer longitudinal smooth muscles. The total thickness of the muscularis was clearly thinner in comparison with the oesophagus. A little way further, the arrangement of the two layers changes, the inner layer has a circular arrangement and the outer layer has a longitudinal arrangement. At the end of the stomach, the circular muscle layer thickens significantly and forms the pyloric sphincter (Figure 15). The outside of the stomach is covered with a thin layer of serosa which was very thin and made up of mesothelium and loose connective tissue. Total absence of gastric glands was noticed in the transitional zone between the oesophagus and stomach and in the area of the pyloric sphincter whereas they are located at the base of large folds in a cluster of four to six glands.

Pyloric Sphincter: In this region the cells of the inner circular layer of the muscularis change their direction, running towards the lumen of the organ towards the rectum (Figure 16). At the intestinal wall, the muscular cells become organized again. The valve is a projection towards the lumen of the organ, where an abrupt change on the epithelial cells takes place.

Intestine: The intestine proper of *O. tentacularis* is a long straight tube of uniform diameter throughout its length leading to the rectum as in most omnivorous fishes.

Anterior Intestine/Foregut: Long and slender mucosal folds characterised the mucosa of the anterior intestine, more or less parallel to each other with round tips and directed towards the lumen. The epithelium is made up of columnar epithelial cells. Mucous cells are numerous and appeared circular or oval in shape. The height of the mucosal folds varies greatly, but their distal points are either tapering or conical. Columnar epithelial cells have nuclei that are positioned basally. A well-developed lamina propria is present. A large number of vesicles, lymphocytes and granular cells are also seen in the mucosa. A vast number of capillaries, lymphocytes, vesicles, and granular cells constitute the sub mucosa, which is made up of loose connective tissue. The muscularis is made up of two layers of smooth muscles: an inner circular layer and an outer longitudinal layer. In comparison to the circular layer, the longitudinal layer is considerably thinner. Although the serosa is thin, it is well developed (Figure 17 & 18).

Middle Intestine/Midgut: No boundaries were found between the anterior and mid intestine of *O. tentacularis*. However, some histological characteristics can be easily distinguished. At the middle region of the intestine, mucosal folds are short and the intestinal lumen is very large. The folds are not compactly arranged and there is space between the folds. The mucous cells are large. The lamina propria is highly vascularised and is not very compact. The sub mucosa consists of loose connective tissue. The muscularis consists of circular and longitudinal muscles of almost equal thickness. The luminal tunica mucosa was modified into mucosal folds. These mucosal folds appear finger-like outgrowths into the lumen on a transverse section with a covering epithelium and a lamina propria core (Figure 19). A brush border was present at the apical region of the simple columnar epithelium containing goblet cells (Figure 20). The lamina propria core contained collagen fibres and blood vessels. The serosa is well formed and was made up of simple squamous cells.

Posterior Intestine/Hindgut: The lumen in this area is modest in size, and the mucous glands (flat or round) are somewhat smaller in size and are positioned near the distal end of the mucosal folds. The folds are made up of tall columnar epithelial cells and mucous glands. The sub mucosa is formed of loose connective tissue, and it is also vascularised. The muscularis is two layered with an outer longitudinal layer and inner circular layer, which are of almost equal thickness. The luminal mucosal fold of this

segment was broader and shorter than that observed in the middle intestine (Figure 21). It contained simple columnar epithelium with brush border and intraepithelial lymphocytes. The goblet cells were few in number. Lamina propria is highly vascularized with its core containing collagen fibres, blood vessels and leucocytes. The submucosa contained collagen fibres, smooth muscle, leucocytes, and blood vessels (Figure 22). The serosa is thin but well formed.

Ileo-rectal valve/Intestino-rectal valve: The ileo-rectal valve (Figure 23 & Figure 24) is a projection of the internal circular muscular layer, displayed towards the rectum, forming a thick fold that separates the distal intestine from rectum. The epithelial lining of the valve has the same characteristics.

Rectum: The rectal region can be easily distinguished by the shallowness and the greater thickness of the mucosal folds. The lamina propria of the rectum was wider than in other portions of the intestine, and the epithelium was well developed (Figure 25). The columnar cells and the goblet cells resemble those of the intestine. The wall of the rectum was made up of mucosa, submucosa, muscularis and serosa. The mucosa was covered with columnar epithelium, which was similar to the oesophagus and proximal intestine. In comparison to the proximal intestine, mucus cells occur in greater density. Below the epithelium, there was lamina propria which consists of loose connective tissue. The muscularis was well developed and significantly thicker than in the small intestine. But, unlike in the small intestine, the rectum's inner layer of circular muscles is thicker than outer longitudinal one. From the posterior intestinal portion to the rectum oval shaped goblet cells were observed (Figure 26 & Figure 27). The muscularis externa consists of two layers of smooth muscle cells. The thickness decreased from anterior to posterior intestine. The inner circular layer was more developed than the outer longitudinal layer. Unlike that of the intestine the villi are greater in number and are long and slender. Mucous cells were abundant. The lumen of the rectum on transverse section was compartmentalized due to the outgrowth of the whorl-like shaped mucosal folds (Figure 25). The epithelium of simple columnar cells also contained goblet cells and leucocytes. The lamina propria core of the mucosal fold was significantly larger because of the presence of dense regular collagen fibres. Blood vessels and leucocytes were also present in the lamina propria. The submucosa contained smooth muscle cells, loose collagen fibres, leucocytes and blood vessels. Serosa forms a very thick layer and contained loose connective tissue fibres and blood vessels. In teleosts that possess well defined rectum as seen in this study, there is a change in the diameter of the intestine and the presence of valve like structure at the beginning of the rectum.



Figure 1. showing digestive tract in-vivo. ES- Oesophagus, ST- Stomach, GB- Gall Bladder, SP: Spleen, FG: Foregut, MG: Midgut, HG: Hindgut.

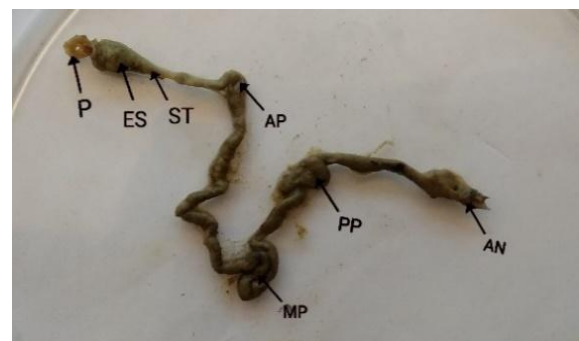


Figure 2. showing in-vitro. P- Pharynx, ES- Oesophagus, ST- Stomach, AP- Anterior intestine portion, MP- Mid intestine portion, PP- Posterior intestine portion, SP- stomach portion, AN- Anus



Figure 3. showing Upper Jaw bearing sharp teeth

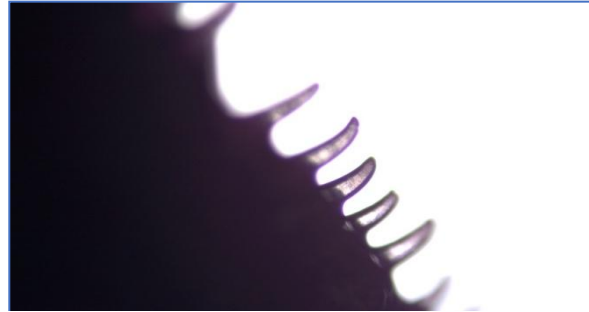


Figure 4. showing lower Jaw bearing sharp teeth



Figure 5. Showing wide mouth of *O. tentacularis* with non-protrusible, non-muscular tongue.



Figure 6. Showing well developed movable lips.



Figure 7. Floor of the oral cavity (1) and oropharynx (2) showing: branchial arches (I to V), pharyngeal teeth apparatus (arrow) (bar = 1 cm), tongue (asterisk).

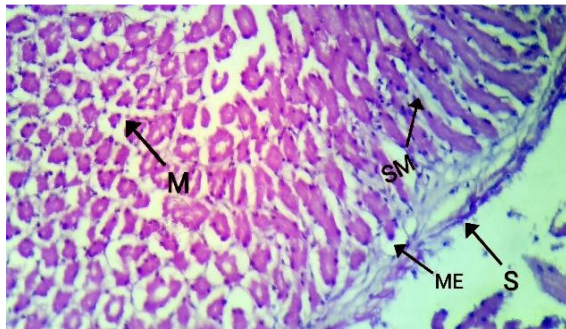


Figure 8. C. S of Pharynx showing Serosa (S), Mucosa (M), Submucosa (SM) and Muscularis Externa (ME). (HE×10x)

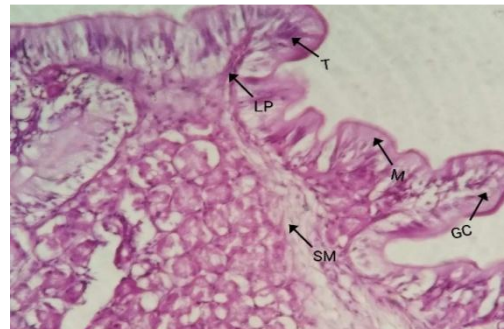


Figure 9. T. S of Pharyngeal mucosa showing Goblet Cells (GC), Lamina Propria (LP), Mucosa (M), Submucosa (SM), Taste bud (T). (HE×40X)

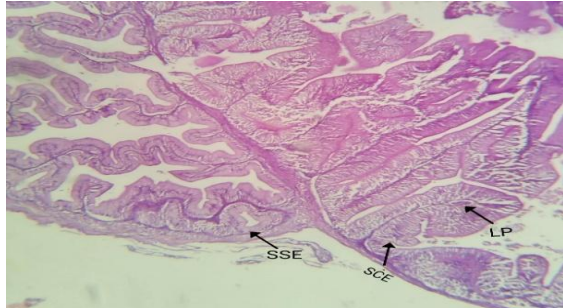


Figure 10. Longitudinal section showing transition of epithelium from the oesophagus to stomach: Stratified Squamous Epithelium (SSE) to Simple Columnar Epithelium (SCE), Lamina Propria (LP). (HE×10X)

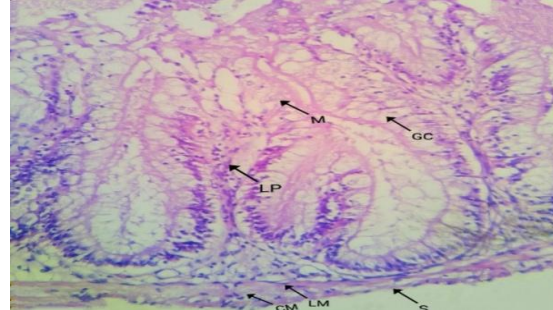


Figure 11. C. S of the oesophagus showing Mucosa (M), Lamina Propria (LP), Circular Muscle (CM), Longitudinal muscle (LM), Serosa (S). (HE×40X)

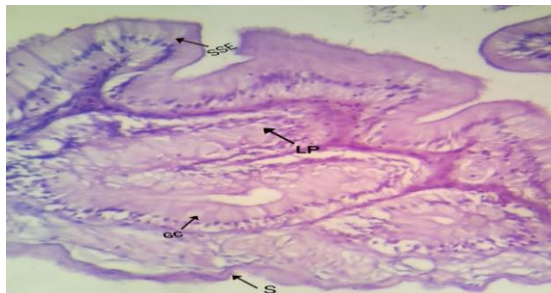


Figure 12. L. S of esophagus showing Serosa (S), Lamina Propria (LP), Goblet Cells (GC), Stratified Squamous Epithelium (SSE). (HE×40X)

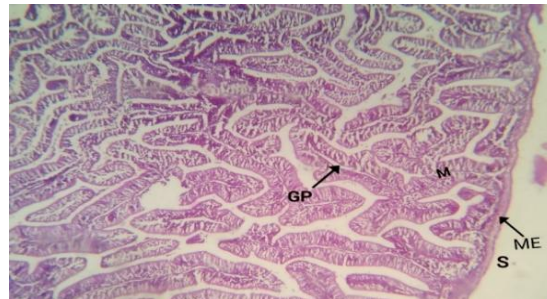


Figure 13. L.S of stomach region of *O. tentacularis* showing Serosa (S), Muscularis Externa (ME), Mucosa (M), Gastric Pit (GP). (HE×10X)

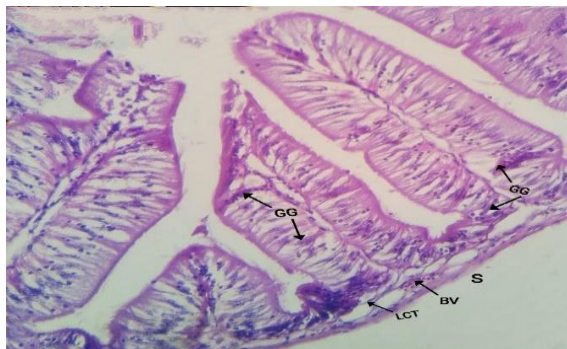


Figure 14. C. S of stomach region of *O. tentacularis* showing Loose Connective Tissue (LCT), Gastric Glands (GG), Blood Vessel (BV), Serosa (S). (HE×40x)

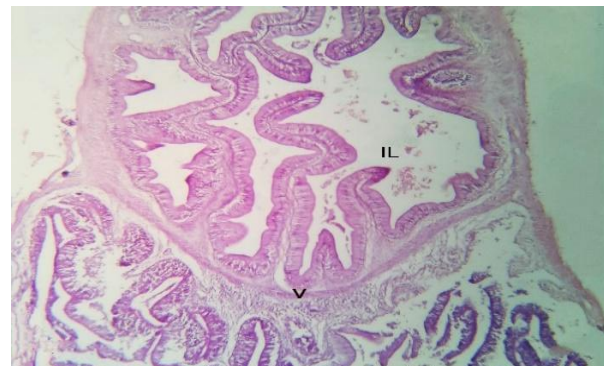


Figure 15. L. S of pyloric sphincter showing Intestinal Lumen (IL), Valve (V) and Stomach (ST). (HE×10X)

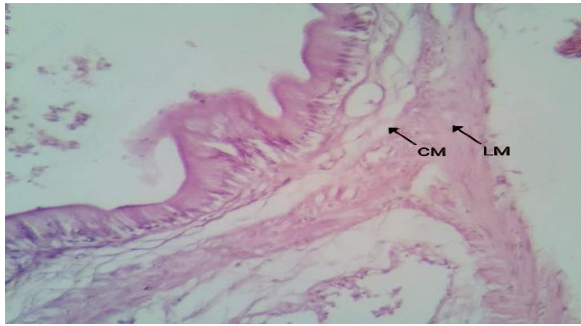


Figure 16. showing arrangement of muscles in the sphincter region, Circular Muscle (CM), Longitudinal Muscles (LM). (HE×40x)

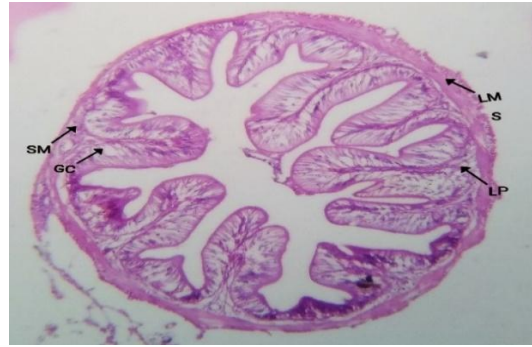


Figure 17. C. S of the foregut showing the Serosa (S), Submucosa (SM), Longitudinal Muscle (LM), Goblet Cell (GC), Lamina Propria (LP). (HE×10X)

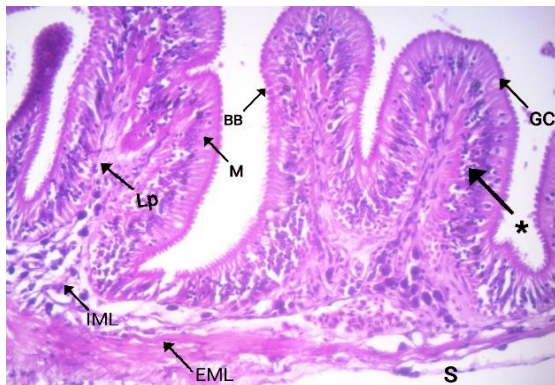


Figure 18. C. S of the foregut showing the Mucosa (M), Lamina Propria (LP), Internal Muscular Layer (IML), External Muscular Layer (EML), Goblet Cell (GC), Lymphocyte (asterisk), Brush Border (BB). (HE×40X)

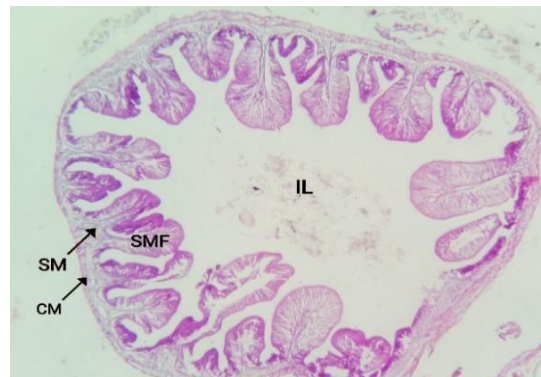


Figure 19. C. S of the midgut showing Intestinal Lumen (IL), Simple Mucosal Folds (SMF), Submucosa (SM), Circular Muscle (CM). (HE×10X)



Figure 20. C. S of the midgut mucosa showing Brush Border (BB) and Goblet Cell (GC). (HE×40x)



Figure 21. C. S of the hindgut showing Mucosa (M), Submucosa (SM), Simple Columnar Epithelium (SCE), Lumen(L), Lamina Propria (LP), Serosa (S). (HE×10X)

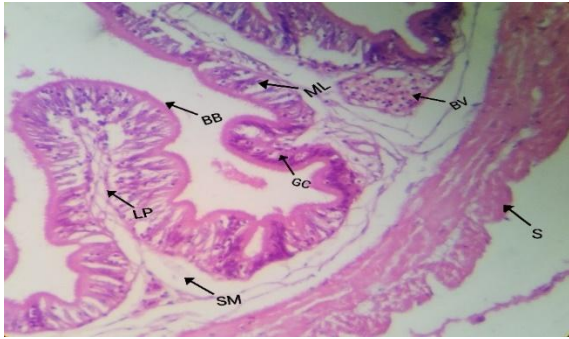


Figure 22. C. S of the hindgut showing Brush Border (BB), Submucosa (SM), Lamina Propria (LP), Serosa (S), Blood Vessel (BV) and Migratory Leukocytes (ML). (HE×40X)



Figure 23. T. S of the ileorectal valve showing Rectal Lumen (RL), Intestinal Lumen (IL), Valve (V). (HE×10X)

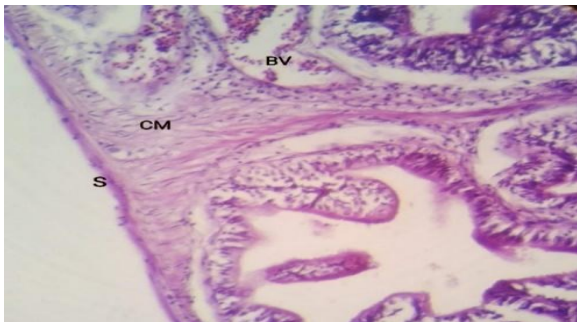


Figure 24. T. S showing Circular Muscle (CM), Serosa(S) and Blood vessel (BV). (HE×40X)

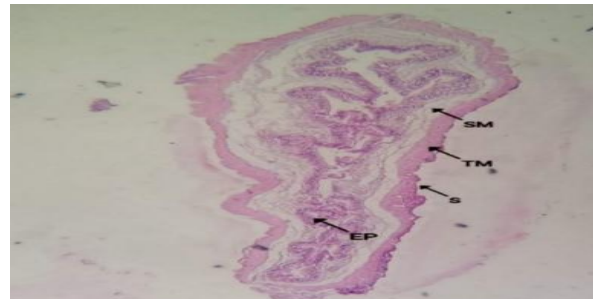


Figure 25. C.S of rectum showing Epithelium(EP), Serosa(S), Submucosa(SM), Tunica Mucosa (TM). (HE×4X)

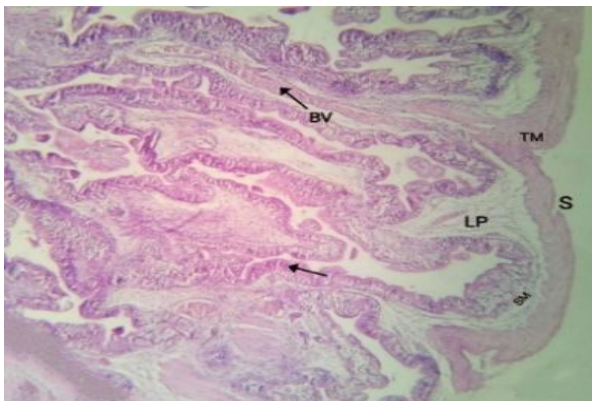


Figure 26. T. S of rectum showing Serosa (S), Submucosa (SM), Lamina Propria (LP), Blood Vessel (BV), vacuolated Columnar Epithelium (arrow). (HE×10X)

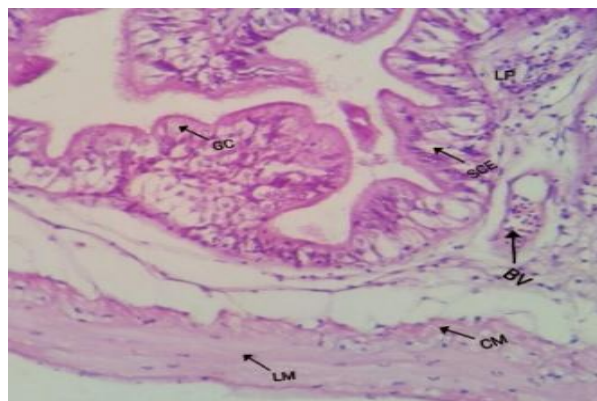


Figure 27. C. S of rectum showing Longitudinal Muscle (LM), Circular Muscle (CM), Goblet Cell (GC), Blood Vessel (BV), Lamina Propria (LP) and vacuolated Simple Columnar Epithelium (SCE). (HE×40X)

DISCUSSION

As interest in fish culture increases and more knowledge on feeding and nutrition is needed, histological studies of the alimentary canal across species of fish are becoming increasingly important. The structure of a fish's alimentary canal is altered to suit the nature of its food and eating patterns, and this modifies the alimentary canal's nature as well (Yadav and Singh, 2013). In terms of shape, size, structure, abundance, and limitation of microscopic elements such as teeth, mucous cells, taste buds, digestive glands, and muscular coat in the fish oesophagus, stomach and intestine, the adaptations of fish digestive organs to their regular diet are generally satisfactory. All of these features are amenable to a variety of minor and major modifications, which support their eating and feeding habits (Khalaf-Allah, 2013; Shalaby, 2017).

Geevarghese (1983) distinguished among the Gobiidae three trophic types, including: herbivores, whose relative length of the alimentary canal is above 1.25; omnivores with a tendency to herbivory, whose relative length of the alimentary canal is 1.0-1.25; and omnivores with a tendency to carnivory, whose relative length of the alimentary canal is below 1.0. In the present study, the relative length of the gut of *Oxyurichthys tentacularis* ranges from 0.68 to 0.86 cm, with an average of 0.80 ± 0.06 cm. Hence, from the value, it was clear that *O. tentacularis* is an omnivore with a tendency to carnivory. According to Khojasteh (2012), other factors that influence intestinal length besides nutrition include fish size (mass and length), body shape, recent feeding history, ontogeny and phylogeny.

Histological analysis revealed that the alimentary tract in *O. tentacularis* consisted of the buccal cavity, pharynx, oesophagus, stomach, intestine, and rectum. The sections were clearly separated by sphincters: the pyloric sphincter, which connects the stomach to the intestine, and the ileorectal valve, also known as the prerectal (Oliveira-Ribeiro and Fanta, 2000) or intestinorectal (Ezeasor and Stokoe, 1980) valve, which connects the intestine to the rectum. Both structures are also found in other species from the subfamily Gobiinae, i.e., *Mesogobius batrachocephalus*, *Neogobius ophiocephalus* (Kobegenova and Dzhumaliev, 1991) and *Neogobius gymnotrachelus* (Jaroszewska *et al.*, 2008). Well-developed sphincters control food transportation between segments of the alimentary canal, prevent food regurgitation, and divide the alimentary tract into functionally different compartments.

In the present study, the oesophagus of the omnivorous fish, *O. tentacularis*, was a short, narrow and distensible muscular tube. It was found that the wall of the oesophagus composed of innermost mucosa, submucosa, muscularis and outermost serosa. Similar observations were made by Canan *et al.* (2012) in the damselfish *Stegastes fuscus*. The mucosa constitutes the bulk of the oesophageal wall and it was characterised by the presence of numerous mucosal folds, muscularis mucosae, goblet cells, stratified squamous epithelium and tunica propria. Similar observations were made by Murray *et al.* (1996); Jaroszewska *et al.* (2008) in *Pseudopleuronectes amaricanus*, *Limanda ferruginea*, and *Babka gymnotrachelus* and Silva *et al.* (2012) in *Satanoperca pappaterra*. The submucosal layer consists of a thick layer of loose connective tissue, blood, and lymph-like vessels. Similar results were obtained by Senarat *et al.* (2015) and Silva *et al.* (2012). The inner layer of the oesophageal wall consists of longitudinal bundles of muscle fibres separated by connective tissue. A similar structure to the muscularis was observed in other gobies (Kobegenova and Dzhumaliev, 1991; Jaroszewska *et al.*, 2008; Wołczuk *et al.*, 2015). It reinforces the oesophagus, which is subjected to forceful constricting and stretching while food is propelled (Oliveira-Ribeiro and Fanta, 2000; Wołczuk *et al.*, 2015).

The intestinal anatomy of invertebrates is largely conservative, although the stomach shape varies greatly. Depending on the type of food consumed, several changes occur in the digestive tract, especially in the stomach. The nature of the food and the size of the prey are known to influence the size and capacity of a fish's stomach (Gosh and Chakrabarti, 2015b). Several freshwater and marine fish families, including the Cyprinidae, Labridae, and Gobiidae, have lost their stomachs (Al-Hussaini, 1949; Barton and Bond, 2006). In *O. tentacularis*, the true stomach was found as the widening of the lumen of the anterior portion of the alimentary canal, separated from the intestine by the pyloric sphincter. It was characterised by the

presence of gastric glands that produce hydrochloric acid and pepsinogen. The muscular and distensible stomach in *O. tentacularis* was highly developed for the omnivorous mode of life. Fish that ingest mud or other minute particles on a regular basis only require a small stomach, if any at all. In *O. tentacularis*, the oesophagus to stomach passage is marked by a form of surface epithelium that transitions from stratified squamous to simple columnar cells. The same was also described in other goby fishes (Kobegenova and Dzhumaliev, 1991; Jaroszevska *et al.*, 2008; Wołczuk *et al.*, 2015). An important feature of the surface epithelium of the stomach of *O. tentacularis* was the lack of goblet cells. Similar results were reported in other goby species (Kobegenova and Dzhumaliev, 1991; Hur *et al.*, 2005; Jaroszevska *et al.*, 2008; Wołczuk *et al.*, 2015). The presence of mucus in the stomach is thought to be linked to the mucosa's defence against the harsh effects of hydrochloric acid and pepsin, according to many researchers (Al-Hussaini, 1949; Barrington 1957; Reite 2005). It can be supposed that hydrochloric acid was not produced in the stomach of *O. tentacularis*. Gastric glands in fish have the shape of tubules and alveoles (Wilson and Castro, 2010) and their growth and distribution are influenced by food intake (Kapoor *et al.*, 1976; Diaz *et al.*, 2008; Gosh and Chakrabarti, 2015a). The tubular gastric glands in *O. tentacularis* were sparsely distributed in the gastric mucosa and absent in the pyloric sphincter. Similar observations were made by many researchers (Kobegenova and Dzhumaliev, 1991; Hur *et al.*, 2005; Jaroszevska *et al.*, 2008; Wołczuk *et al.*, 2015). The inner circular and outer longitudinal layers of the muscularis in the stomach change their position relative to the arrangement present in the oesophagus, i.e., the circular layer of the muscles was situated internally while the longitudinal one was situated externally. A similar structure of the muscularis has been described in other Gobiidae fish (Kobegenova and Dzhumaliev, 1991; Jaroszevska *et al.*, 2008; Wołczuk *et al.*, 2015). The inner layer of the muscles in the rear region of the stomach was significantly thicker, producing a sphincter. The presence of such a thick layer of muscularis aids in the management of digested food flow to the gut. These findings were consistent with those of Abdel-Wahab *et al.* (2017).

The intestine is divided into three portions: the anterior, middle, and posterior intestine. The present results were in accordance with those of Hassan (2013) and El-Deeb *et al.* (2016). During the present investigation, the observation of histological sections of the intestine of *O. tentacularis* revealed that it was formed of four layers: the innermost mucosa, sub mucosa, muscularis, and outermost serosa. The mucosa of the intestine is characterised by muscularis mucosae, goblet cells, epithelium and tunica propria. The intestinal wall was lined by columnar epithelium with numerous mucus-secreting goblet cells. The large and many goblet cells imply that they may provide viscosity for both food and faeces (Mabrouk, 2015; El-Deeb *et al.*, 2016; Chirde and Gadhikar, 2014). The mucosal folds in the anterior intestine of *O. tentacularis* were very long and numerous, with round tips. Such structures are fewer in number towards the middle intestine and much thicker in the posterior intestine. This pattern of organisation of simple columnar epithelium throughout the intestine of *O. tentacularis* revealed as an intensely absorbing surface, which is important for the process of absorption of nutrients. The histology revealed distinct characteristics between regions of the intestine that are related to different functions in the digestive physiology of this species. The brush border seen at the apical margin of the intestine's simple columnar epithelium serves to increase the amount of surface area accessible for digestion and absorption (Jaroszevska *et al.*, 2008; Banan Khojasteh *et al.*, 2009). Long-folded and well-developed intestinal microvilli with a large diameter and thick muscles are found in the anterior section of the intestine, indicating that this is the primary site for food digestion and absorption in this species. The anterior intestine is assumed to be the principal location of food digestion and absorption in many other fish species that lack stomachs (Cao *et al.*, 2011). However, in *O. tentacularis*, the anterior and middle intestinal regions promote digestion and absorption. In *Neogobius gymnotrachelus* and *Hypophthalmichthys nobilis*, intestinal mucosal folds with decreasing height and increasing breadth caudally, as well as a denser collagen fibre core from proximal to distal gut, have been identified (Jaroszevska *et al.*, 2008; Delashoub *et al.*, 2010). Apart from a decrease in mucosal fold height, a

broadening of the mucosal fold breadth, and an increase in goblet cell population from the proximal to distal gut, no regional differences in *Oncorhynchus mykiss* and *Glyptosternum maculatum* have been reported (Banan-Khojasteh *et al.*, 2009; Xiong *et al.*, 2011). In *O. tentacularis* an increased concentration of mucus-secreting cells appearing from the anterior to the posterior region followed by the rectum was seen. It implies that mucus production assists in intestinal lining defence as well as waste exclusion (Machado *et al.*, 2013). Furthermore, the presence of intraepithelial lymphocytes (IEL) in the same species as epithelial cells and mucus-producing cells suggests that IEL play a role in the teleost gut immune response. They control the number of lymphocytes in the intestinal mucosa, indicating that a mucosal immune system exists (Miura *et al.*, 2012). The anatomy of the gastrointestinal tract of *O. tentacularis* was compatible with omnivorous eating habits based on our findings. Food retention duration in the gut has also been connected to digestive tract valves, which improve absorption time and prevent anterior reflux of materials into the distal intestine.

The rectum of *O. tentacularis* was histologically identical to the midgut of teleosts. There is a change in the diameter of the intestine in teleosts with a well-defined rectum, as demonstrated in this study, as well as the existence of a valve-like structure at the commencement of the rectum. Only certain teleost fishes have an ileorectal valve. A muscular sphincter empties the rectum into the vent. When the intestino-rectal valve is present, it prevents the reflux of posterior intestinal contents (Jaroszewska *et al.*, 2008). In *O. tentacularis* it is present between the middle and posterior intestine and has a small diameter and flattened folds. Under the electron microscope, such a well-defined rectum comprised many annular mucosal folds that looked like a stack of caudally-directed funnels, according to Ezeasor and Stokoe (1980). Significant folds in the posterior gut (rectum) are unusual in teleosts (Grau *et al.*, 1992). The mucosal and submucosal layers of the valve and the ileum are related in this study. The circular layer of the muscularis is present in the valve, though. These findings are in line with Gorman's findings (1982).

CONCLUSION

The anatomy and histology of the digestive tract of *O. tentacularis* have identified as an omnivore. The structure of the oesophagus, stomach, and intestine was found to be adapted to food and feeding strategies. The understanding of the feeding habits is essential to emphasise the related functional mechanisms of the fish digestive system. Interpretations of feeding habits based only on analysis of gastrointestinal contents might lead to incorrect conclusions. This research contributes to a better understanding of the digestive system of the examined species and offers histological data that can be used as a reference for understanding biological alterations that may impact the fish digestive tract. They will also be beneficial for comparative analysis within the group, providing information for future evolutionary research. There should be more research on the processes of nutrient digestion, which is important for human feeding and professional fishing. It is important to highlight that there have been no studies done so far on the digestive tract morphology of this species. Hence, the results of the present study seem to be applicable to the management of this species.

ACKNOWLEDGEMENTS

The authors are grateful to Kerala University, Thiruvananthapuram for the financial assistance (JRF) provided. The authors are also thankful to the management of Fatima Mata National College for providing the necessary facilities.

REFERENCES

Abdel-Wahab S, El-Deeb RM, Khalaf-Allah HM, Abu-Zaid MM and Shalaby WT (2017). Morphological and histological adaptations in the oesophagus and stomach of some coral reef fishes in Hurghada, Red Sea, Egypt in relation to feeding strategy. *International Journal of Environmental Science and Technology*, **8** 1–14.

Al-Hussaini AH (1946). The anatomy and histology of the alimentary tract of the bottom-feeder, *Mulloides auriflamma* (Forsk.). *Journal of Morphology*, **78**(1) 21–153. <https://doi.org/10.1002/jmor.1050780108>

Al-Hussaini AH (1949). On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: Anatomy and histology. *Journal of Cell Science*, s3-**90**(10) 109-139. <https://doi.org/10.1242/jcs.s3-90.10.109>

Banan-Khojasteh SM, Sheikhzadel F, Mohammadnejad D, and Azani A (2009). Histological, histochemical and ultrastructural study of the intestine of rainbow trout (*Oncorhynchus mykiss*). *World Applied Sciences Journal*, **6** 1525-1531.

Barrington E (1957). The alimentary canal and digestion in fish. In Brown, M. E. (Ed.): *The Physiology of Fishes*, 109-161. Academic Press, New York.

Barton, M. (2007). *Bond's Biology of Fishes*, third ed. Thomson, Belmont, CA.

Barton M and Bond CE (2006). *Bond's biology of fishes*. 3rd edition. California, USA: Thomson Brooks Cole, Thomson.

Canan B, Nascimento WS, Silva NB and Chellappa S (2012). Morphohistology of the digestive tract of the damselfish *Stegastes fuscus* (Osteichthyes: Pomacentridae). *The Scientific World Journal*, **2012** 1-9. doi: 10.1100/2012/787316

Cao XJ, Wang WM and Song F (2011). Anatomical and histological characteristics of the intestine of the Top mouth culter (*Culter alburnus*). *Anatomia, Histologia, Embryologia*, **40**(4) 292-298. <https://doi.org/10.1111/j.1439-0264.2011.01069.x>

Chirde SG and Gadhikar YA (2014). Histology, histochemical and ultrastructural studies on Intestine of Indian catfish, *Clarias batrachus* (Linn. 1758). *Asian Journal of Biology and Biotechnology*, **3**(3) 1-9.

Day RD, Tibbetts IR and Secor SM (2014). Physiological responses to short-term fasting among herbivorous, omnivorous, and carnivorous fishes. *Journal of Comparative Physiology B*, **184**(4) 497-512. <https://doi.org/10.1007/s00360-014-0813-4>

Delashoub M, Pousty I and Khojasteh SM (2010). Histology of bighead carp (*Hypophthalmichthys nobilis*) Intestine. *Global Veterinaria*, **5**(6) 302-306.

Diaz AO, Garca AM, Figueroa DE and Goldemberg AL (2008). The mucosa of the digestive tract in *Micropogonias furnieri*: A light and electron microscope approach. *Anatomia, Histologia, Embryologia*, **37**(4) 251-256. <https://doi.org/10.1111/j.1439-0264.2007.00837.x>

El-Deeb RMA, Abdel-Wahab SM, Khalaf-Allah HM, Abu-Zaid MM and Shalaby WTS (2016). Comparative study on the histological structures of the intestine in some coral reef fishes in Hurghada, Red Sea, Egypt. *International Journal of Environmental Science and Technology*, **7** 103–112.

Ezeasor DN and Stokoe WM (1980). Scanning electron microscopic study of the gut mucosa of the rainbow trout *Salmo gairdneri* Richardson. *Journal of Fish Biology*, **17**(5) 529-539. <https://doi.org/10.1111/j.1095-8649.1980.tb02784.x>

Geevarghese C (1983). Morphology of the alimentary tract in relation to diet among gobioid fishes. *Journal of Natural History*, **17**(5) 731-741. <https://doi.org/10.1080/00222938300770571>

Germano RM, Stabile SR, Mari RB, Pereira JNB, Faglioni JRS and Miranda-Neto MH (2014). Morphological characteristics of the *Pterodoras granulosus* (Valenciennes, 1821) digestive tube (Osteichthyes, Doradidae). *Acta Zoologica*, **95**(2) 166-175. <https://doi.org/10.1111/azo.12016>

Ghosh SK and Chakrabarti P (2015a). Histological and histochemical characterization on stomach of *Mystus cavasius* (Hamilton), *Oreochromis niloticus* (Linnaeus) and *Gudusia chapra* (Hamilton): Comparative study. *The Journal of Basic & Applied Zoology*, **70** 16-24. <https://doi.org/10.1016/j.jobaz.2015.04.002>

Ghosh SK and Chakrabarti P (2015b). Histological, surface ultrastructural, and histochemical study of the stomach of red piranha, *Pygocentrus nattereri* (Kner). *Archives of Polish Fisheries*, **23**(4) 205-215. <https://doi.org/10.1515/aopf-2015-0023>

- Girgis S (1952).** On the anatomy and histology of the alimentary tract of an herbivorous bottom-feeding cyprinoid fish, *Labeo horie* (Cuvier). *Journal of Morphology*, **90**(2) 317-362. <https://doi.org/10.1002/jmor.1050900207>
- Grau A, Crespo S, Sarasquete MC and Canales ML (1992).** The digestive tract of the amberjack *Seriola dumerili*, Risso: A light and scanning electron microscope study. *Journal of Fish Biology*, **41**(2) 287-303. <https://doi.org/10.1111/j.1095-8649.1992.tb02658.x>
- Groman DB (1982).** Histology of the striped bass. Monog.3, ISSN0362-1715, Bet., Mary., USA.
- Hassan MN, Rahman M, Hossain MM, Newsad AAK and Hossain MB (2013).** Post-Harvest Loss and Shelf Life of Traditionally Smoked Shrimp Products Produced in Bangladesh. *World Journal of Fish and Marine Science*, **5**(1) 14-19. DOI: 10.5829/idosi.wjfms.2013.05.01.6595
- Hur S, Song Y, Lee C, Lim B and Lee Y (2005).** Morphology of digestive tract and its goblet cells of Giurine goby *Rhinogobius giurinus*. *Fisheries and aquatic sciences*, **8**(2) 83-89. <https://doi.org/10.5657/fas.2005.8.2.083>
- Hur S, Kim S, Kim D, Lee B, Park S, Hwang H, Jun J, Myeong J, Lee C and Lee Y (2016).** Digestive physiological characteristics of the gobiidae. *Development & Reproduction*, **20**(3) 207-217. <https://doi.org/10.12717/dr.2016.20.3.207>
- Jaroszewska M, Dabrowski K, Wilczyńska B and Kakareko T (2008).** Structure of the gut of the racer goby *Neogobius gymnotrachelus* (Kessler, 1857). *Journal of Fish Biology*, **72**(7) 1773-1786. <https://doi.org/10.1111/j.1095-8649.2008.01870.x>
- Kalhor H, Tong S, Wang L, Hua Y, Volatiana JA and Shao Q (2018).** Morphological study of the gastrointestinal tract of *Larimichthys crocea* (Acanthopterygii: Perciformes). *Zoologia*, **35** 1-9. <https://doi.org/10.3897/zoologia.35.e25171>
- Kapoor BG, Smit H and Verighina, A (1976).** The alimentary canal and digestion in teleosts. *Advances in Marine Biology*, **13** 109-239. [https://doi.org/10.1016/S0065-2881\(08\)60281-3](https://doi.org/10.1016/S0065-2881(08)60281-3)
- Khalaf Allah HM (2013).** Morphological adaptations of digestive tract according to food and feeding habits of the Broomtail wrasse, *Cheilinus lunulatus*. *Egyptian Journal of Aquatic Biology and Fisheries*, **17**(1) 123-141. <https://dx.doi.org/10.21608/ejabf.2013.2162>
- Khojasteh SMB (2012).** The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. *International Journal of Aquatic Science*, **3**(2) 71-88.
- Kobegenova SS and Dzhumaliev MK (1991).** Morphofunctional features of the digestive tract in some Gobioidei, Vopr. Ikhtiol. **31** 965–973 (in Russian).
- Mabrouk RTM (2015).** Effect of environmental factors on larval growth and development of Gilthead seabream (*Sparus aurata*). M. Sc. Thesis, Zool. Dep., Fac. Sci., Al-Azhar University, Cairo, Egypt, p. 236.
- Machado MR, Souza HD, Souza VL, Azevedo AD, Goitein R and Nobre AD (2013).** Morphological and anatomical characterization of the digestive tract of *Centropomus parallelus* and *Centropomus undecimalis*. *Acta Scientiarum. Biological Sciences*, **35**(4) 467-474. <https://doi.org/10.4025/actascibiolsci.v35i4.14352>
- Miura S, Hokari R and Tsuzuki Y (2012).** Mucosal immunity in gut and lymphoid cell trafficking. *Annals of Vascular Diseases*, **5**(3) 275-281. doi: 10.3400/avd.ra.12.00059
- Mohsin SM (1962).** The morphology and histology of the alimentary canal of *Glossogobius giurus* (Ham.). *Canadian Journal of Zoology*, **39**(5) 605-613. <https://doi.org/10.1139/z61-065>
- Murray HM, Wright GM and Goff GP (1996).** A comparative histological and histochemical study of the post-gastric alimentary canal from three species of pleuronectid, the Atlantic halibut, the yellowtail flounder and the winter flounder. *Journal of Fish Biology*, **48**(2) 187-206. doi:10.1111/j.1095-8649.1996.tb01112.x

- Oliveira Ribeiro CA and Fanta E (2000).** Microscopic morphology and histochemistry of the digestive system of a tropical freshwater fish *Trichomycterus brasiliensis* (Lütken) (Siluroidei, Trichomycteridae). *Brazilian Journal of Zoology*, **17**(4) 953-971. <https://doi.org/10.1590/S0101-81752000000400007>
- Pogoreutz C and Ahnelt H (2014).** Gut morphology and relative gut length do not reliably reflect trophic level in gobiids: A comparison of four species from a tropical Indo-Pacific seagrass bed. *Journal of Applied Ichthyology*, **30**(2) 408-410. doi: 10.1111/jai.12295
- Polgar G, Sacchetti A and Galli P (2010).** Differentiation and adaptive radiation of amphibious gobies (Gobiidae: Oxudercinae) in semi-terrestrial habitats. *Journal of Fish Biology*, **77**(7) 1645-1664. <https://doi.org/10.1111/j.1095-8649.2010.02807.x>
- Reite OB (2005).** The rodlet cells of teleostean fish: their potential role in host defence in relation to the role of mast cells/eosinophilic granule cells. *Fish and Shellfish Immunology*, **19**(3) 253–267. doi: 10.1016/j.fsi.2005.01.002.
- Remya Mohan S, Harikrishnan M and Sherly Williams E (2017).** Reproductive biology of a gobiid fish *Oxyurichthys tentacularis* (Valenciennes, 1837) inhabiting Ashtamudi Lake, S. India. *Journal of Applied Ichthyology*, **34** (5) 1099-1107. <https://doi.org/10.1111/jai.13739>
- Santos ML, Arantes FP, Pessali TC and Santos JE (2015).** Morphological, histological and histochemical analysis of the digestive tract of *Trachelyopterus striatulus* (Siluriformes: Auchenipteridae). *Zoologia (Curitiba)*, **32**(4) 296-305. <https://doi.org/10.1590/S1984-46702015000400005>
- Senarat S, Kettratad J, Jiraungkoorskul W and Kangwanrangsan N (2015).** Structural classifications in the digestive tract of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1865) from Upper Gulf of Thailand. *Songklanakarin Journal of Science and Technology*, **37**(5) 561-567.
- Shalaby WTS (2017).** Comparative study on the structural adaptation due to feeding strategy in some coral reef fishes. M.Sc. Thesis, Zool. Dep. Fac. Sci., Al–Azhar Univ., Egypt, pp. 241.
- Silva MR, Natali MR and Hahn NS (2012).** Histology of the digestive tract of *Satanoperca pappaterra* (Osteichthyes, Cichlidae). *Acta Scientiarum. Biological Sciences*, **34**(3) 319-326. <https://doi.org/10.4025/actasciobiolsci.v34i3.8956>
- Thacker C and Roje D (2011).** Phylogeny of Gobiidae and identification of gobiid lineages. *Systematics and Biodiversity*, **9**(4) 1-19. <http://dx.doi.org/10.1080/14772000.2011.629011>
- Valenciennes A (1837).** In: *Histoire Naturelle des Poissons*, Cuvier, G. L. and Valenciennes, A. (Ed.). Levrault, Paris, pp.1-507.
- Wilson JM and Castro LFC (2010).** Morphological diversity of the gastrointestinal tract in fishes. In: Grosell M, Farrell AP, Brauner CJ (eds) *The multifunctional gut of fish, fish physiology*, vol 30. Academic Press, Burlington, pp 1–55.
- Wolczuk K, Nowakowska J, Płachocki D and Kakareko T (2015).** Histological, histochemical and ultrastructural analysis reveals functional division of the oesophagogastric segment in freshwater tubenose goby *Proterorhinus semilunaris* Heckel, 1837. *Zoomorphology*, **134** 159–168. DOI: 10.1007/s00435-014-0250-7
- Wu R, Hong W & Zhang Q (2010).** Digestive enzyme activities in mudskipper *Boleophthalmus pectinirostris* and Chinese black sleeper *Bostrichthys sinensis*. *Chinese Journal of Oceanology and Limnology*, **28** 756–761. <http://dx.doi.org/10.1007/s00343-010-9111-5>
- Xiong D, Zhang L, Yu H, Xie C, Kong Y, Zeng Y, Huo B and Liu Z (2011).** A study of morphology and histology of the alimentary tract of *Glyptosternum maculatum* (Sisoridae, Siluriformes). *Acta Zoologica*, **92**(2) 161-169. <https://doi.org/10.1111/j.1463-6395.2010.00458.x>
- Yadav SK and Singh BB (2013).** Morphological and histochemical study of digestive system in relation to feeding habits of *Chanda ranga*. *The Asian Journal of Animal Science*, **8**(2) 125-133.