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Original Article

Structural classifications in the digestive tract of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851) from Upper Gulf of Thailand

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Abstract

This study was the first attempt on the classification of the gross anatomy with histological structures of the digestive tract in the *Rastrelliger brachysoma.* Based on gross anatomical analysis, the digestive tract (65.68±7.06 cm, n = 25) is distinctly composed of four parts; oesophagus, stomach, pyloric caeca and intestine, respectively. Base on the histological analysis of the digestive tract, it is composed of four layers: mucosa, submucosa, muscularis and serosa. The epithelial lining of anterior oesophagus was shown to be a simple squamous epithelium whereas posterior oesophagus, stomach and intestine were simple columnar epithelium. The surface of this epithelium positively stained with both Periodic Acid Schiff (PAS) and alcian blue (AB). The posterior oesophagus exclusively showed high longitudinal fold with numerous gastric glands in the mucosal layer, which is similar to the stomach structure (cariac and pylorus). Numerous pyloric caeca about 220-225 pieces, were detected between the stomach and intestine regions. Finally, the intestinal coefficient (IC) was 3.69±0.47 cm. The anterior intestine presented either various longitudinal folds or various goblet cells. The short longitudinal folds of posterior intestine were in the higher number of goblet cell than the previous part.

Keywords: histology, histochemistry, digestive tract, *Rastrelliger brachysoma*, Thailand

1. Introduction

The digestive tract has been exclusively examined either gross anatomy or histological aspectin many teleost such as, *Clarias batrachus, Serrasalmus nattereri* (Raji and Norouzi 2010), *Puntius stoliczkanus* (Senarat *et al*., 2013a), *Hemibagrus filamentus* (Senarat *et al*., 2013b), *Seriola dumerili* (Grau *et al*., 1992), *Gnathonemus petersii*, *Scyliorhinus canicula* (Genten *et al*., 2008), and *Gadus morhua* (Morrison, 1987). Based on previous studies, the arrangement of this organ varies among teleost fish, but its histologically constituted into four layers. (i) The mucosa consists of epithelial lining, lamina propria and muscularis mucosae. (ii) The submucosa contains a less cellular connective tissue, blood vessel and nerve plexus. (iii) The muscularis is divided into two sub layers including inner circular and outer longitudinal layers. Externally, (iv) the serosa is mainly composed of loose connective tissue (Genten *et al*., 2008).

Rastrelliger brachysoma (Bleeker, 1859) belongs to the family Scombridae. It is an epipelagic fish and commonly found in both tropical and subtropical oceans. This fish is found along the coastal areas of the Central Indo-West Pacific Ocean from the eastern Andaman Sea to Thailand, Indonesia, Papua New Guinea, the Philippines, the Solomon Islands, and Fiji (FAO, 2010). Currently, *R. brachysoma* has been under consideration as a potential marine aquaculture

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fish in Thailand because of the decreasing number of the wild population of *R. brachysoma*, mainly due to the over fishing and the deterioration of the environment. The major source of this fish has been only form the capture fishery, which is not enough to meet for consumer demand (Dhebtaranon and Chotiyaputta, 1972; Personal communication). Thus, the Samut Sakhon Fishery, Samut Sakhon Province, Thailand has set up a breeding project for this fish. The goals of this project are to increase the fish population as well as to optimize fisheries management especially in the natural habitat of this species. However, to optimize these manipulative procedures, a good understanding of basic knowledge of the functional structures, especially digestive systems, is needed early during the development of diet and weaning success for this fish. Therefore, the histological study of the structure of the digestive tract in adult *R. brachysoma* is vital in the propagation of this species. To address the knowledge gap, we investigated the gross morphology, histology and histochemistry of the digestive tract in *R. brachysoma*.

2. Materials and Methods

2.1 Fish collection and gross anatomical study

Twenty-five normal and healthy adult *R. brachysoma* with a standard length about16-19 cm (mean 17.16 cm) and weighing about 70-90 g (mean 78.9 g) were collected. The fish were sampled during the reproductive season (December, 2013 to February, 2014) by bamboo strake trap from Samut Songkram Province, in the Upper Gulf of Thailand (13°16' 18.4" N, 100°02'13.4" E). All fish were euthanized by rapidly cooling shock (Wilson *et al*., 2009). The lengths of digestive tracts (starting oesophagus to intestine) were measured as to both digestive tract (DT) and intestine length (L_i). Especially, the intestine tissues were also calculated to analyze the intestinal coefficient (IC = L_i/L_s; L_i = intestine length and L_s = standard length). Then, all specimens of the digestive tract were completely dissected and rapidly fixed in Davidson's fixative for about 36-48 hrs at room temperature. The digestive tracts were transferred and changed into 70% ETOH for gross morphological study under stereoscopic dissecting binocular microscope.

2.2 Histological and histochemical analyses

All parts of the digestive tracts (starting at oesophagus to intestine) were processed by standard histological technique (Humason, 1979). The paraffin blocks were cut at 5-6 µm thickness and stained with Hematoxylin and Eosin (H&E). Other sections were specially stained by Masson's trichrome (MT), Periodic Acid Schiff (PAS), aniline blue (AB), reticulin (Rt) and alcian blue (Ac) pH 2.5 (Bancroft and Gamble, 2002; Humason, 1979). The histological structures of digestive tract were investigated in detail under light microscope.

3. Results

3.1 Gross anatomy

The gross anatomy of the digestive tubes (DT) of *R. brachysoma* was observed from the tubular oesophagus to the intestine. The length of DT was estimated as 65.68± 7.06 cm (Mean±SD, n = 25). In dorsal view, the longitudinal tube of the oesophagus was observed, before joining with the stomach as a J-shape. The stomach was also anatomically separated into two parts: cardiac (approximate ¾ of total length) and pyloric (approximate 1/4 of total length). Various pyloric caecum, about 220-225 pieces was found at the junction of stomach andintestine (Figure 1A). Moreover, the intestine of this species was very long and anatomically divided into two parts: anterior and posteriorparts (Figure 1A).The intestinal coefficient (IC) was estimated as 3.69± 0.47 cm (Mean±SD, n = 25). In ventral view, pyloric caecum and intestine were also observed (Figure 1B).

3.2 Histological and histochemical observations

In our investigation, the digestive tract structures were classified in adult *R. brachysoma* by using histological and histochemical analyses. Cross and longitudinal sections of the digestive tract from the oesophagus to intestine consisted of four layers: mucosa, submucosa, muscularis and serosa which are described as follows (Figure 2 to 4; Table 1);

3.2.1 Oesophagus

Oesophagus could also be classified into two portions: anterior and posterior portions (Figure 4A). In the anterior

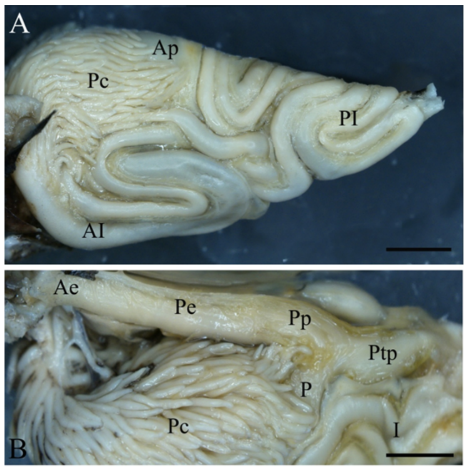

Figure 1. Picture showing of the gross anatomy of *Rastrelliger brachysoma* digestive tract: A, dorsal and B, ventral views; A-B = 1 cm*; Ae= anterior oesophagus, AI = anterior intestine, Ap = anterior portion, I = intestine, P = pyloric part, Pc = pyloric caecea, Pe = posterior oesophagus, PI = posterior intestine, Ptp = posterior portion.*

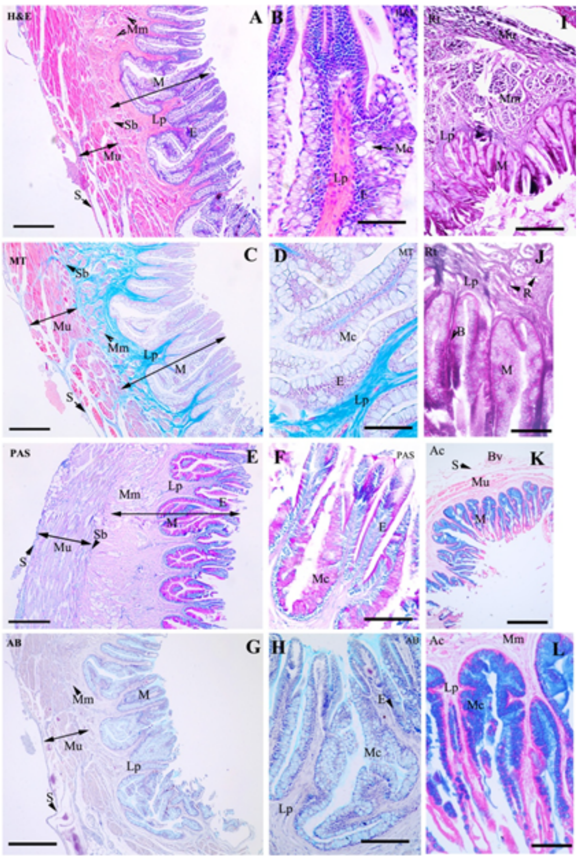

Figure 2. Light photomicrograph of anterior oesophagus; A-C-E-G-I-K = 200 µm; B-D-F-H-J-L = 30 µm; *B = basement membrane, Bv = blood vessel, E = epithelium, Lp = lamina propria, M = mucosa, Mc = mucous cells, Mm = musculoris mucosae, Mu = muscularis, R = reticular fibers, S = serosa, Sb = submucosa. (H&E = Hematoxylin and Eosin, MT = Masson's trichrome, PAS = Periodic Acid Schiff, AB = aniline blue, Rt = reticulin, Ac = alcian blue).*

part, the mucosal layer formed numerous longitudinal folds which were covered by stratified squamous epithelium with non-ciliated and numerous goblet cells (Figure 2A and 2B). The upper part of epithelialand goblet cells were positively stained with PAS, Ac and AB as shown in Figure 2C to 2F, 2G to 2H, and 2K to L, respectively. Their epithelium was lined on basement membrane with positive to reticulin staining. Moreover, this basement membrane also separated between epithelium and lamina propria (Figure 2I and 2J). The lamina propria was composed of loose connective tissue with collagen fibers (greenish with MT, Figure 2C and 2D) and small blood vessels. Muscularis mucosae were dominantly observed throughout the anterior oesophagus. The sub-mucosal layer slightly consisted of loose connective tissue and numerous blood vessels. The muscular layer consisted of thick smooth muscular layers and circular layers. Externally, the serosa was observed as simple squamous epithelium (Figure 2A). In the posterior portion, the epithelial cells changed to simple columnar epithelium with microvilli. Their epithelial cells exhibited oval nuclei in shape and were located near the basement membrane (Figure 3A and 3B). The surface of epithelial cells was also intensively stained with PAS and AB similar to the anterior oesophagus (Figure 3C and 3D). Surprisingly, the lamina propria in this species was well developed due to the fact that it contained a great amount of gastric gland. The epithelium of each tubular gland was lined by a high cubic volume of epithelium that contained the oval nuclei and eosinophilic cytoplasm (H&E) (Figure 2B). Noticeably, the muscularis layer of this part also gradually decreased from the anterior to posterior portion.

3.2.2 Stomach

The stomach of this fish was distinctly classified into two parts: cardiac and pyloric, based on localization, histological structures and different cell types (Figure 3E to 3P;

Table 1. Major variation from oesophagus to intestine.

Organs	Mucosal epithelum	Goblet glands	Gastric gland	Muscularis mucosae	Submucosa	Layer of muscularis
Anterior oesophagus	Simple squamous	Absent	Absent	Extremely present	Present	One
Posterior oesophagus	Simple columnar	Absent	Extremely Present	Rarely present	Present	One
Cardiac stomach	Simple columnar	Absent	Rarely present	Absent	present	Two
Pyloric stomach	Simple columnar	Absent	Extremely present	Absent	present	Extremely present:Two
Pyloric caecum	Simple columnar	Present	Absent	Absent	Rarely present	Two
Anterior intestine	Simple columnar	Extremely Present	Absent	Absent	Rarely present	Two
Posterior intestine	Simple columnar	Present	Absent	Absent	present	Two

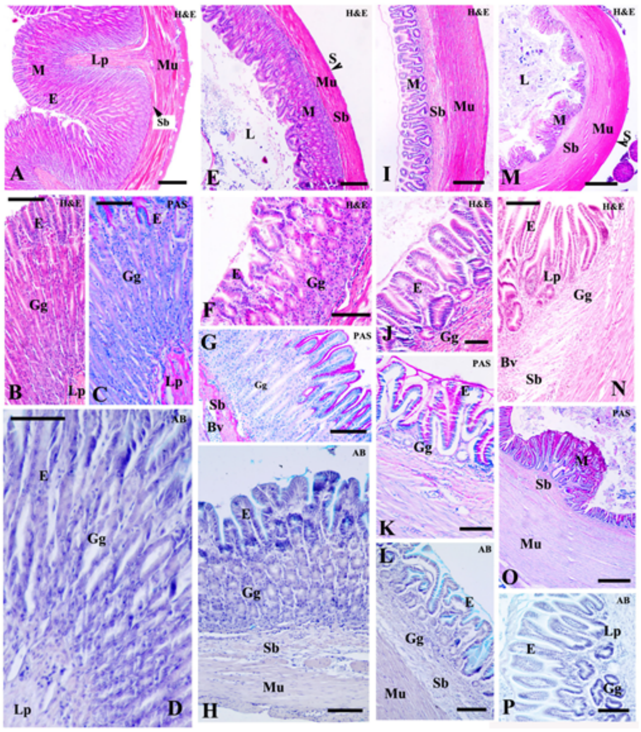

Figure 3. Light photomicrograph of posterior oesophagus to pyloric part; (A-D)the posterior oesophagus with showing gastric glands; (E-H) the anterior portion of cardiac stomach; (I-L) the posterior portion of cardiac stomach with showing gastric glands; (M-P) the pyloric part of stomach with showing numerous of gastric glands; A= 200 µm, E-I-M = 300 µm; B-C-D-F-G-H = 30 µm; *Bv = blood vessel, E = epithelium, Gg = gastric gland, L = lumen, Lp = lamina propria, M = mucosa, Mc = mucous cells, Mm = musculoris mucosae, Mu = muscularis, S = serosa, Sb = submucosa. (H&E = Hematoxylin and Eosin, MT = Masson's trichrome (MT), PAS = Periodic Acid Schiff, AB = aniline blue, Rt = reticulin, Ac = alcian blue).*

4B and 4C). In the cardiac part, it could also be classified into two subportions: anterior and posterior subportions. The anterior portion displayed shallow gastric pits. The mucosal layer was covered by a single layer of simple columnar epithelium with microvilli. It was similar to the posterior part of the oesophagus (Figure 3E and 3F). PAS and AB positive reactions were clearly shown (Figure 3G to 3H). It indicated that these cells produced glycoprotein and mucopolysaccharides. The lamina propria was well developed and containing various cluster of gastric glands. It is worth note that the gastric glands were observed continuously from the oesophagus. The submucosal layer consisted of a thick layer of loose connective tissue (stained as pink with PAS), blood and lymph-like vessels. In this part, the layer of striated smooth muscular tissue in both the thick circular internal layer and the thin longitudinal external layer were well developed. The serosa was similar to the one that was described in the esophagus. The posterior part revealed that gastric glands were gradually replaced by muscularis (Figure 3I to 3L). Finally, in terms of the characterization, the pyloric part was similarly formed with the cardiac region, except that the largest of the muscularis was obviously seen than the previous part (Figure 3M to 3P).

3.2.3 Pyloric caeca

Numerous pyloric caeca were present between stomach and intestine.The longitudinal fold of each tube, about 100-120 µm was lined with a single layer of simple columnar epitheliums. This epithelium contained oval nucleus and was surrounded by basophilic cytoplasm (H&E stain) (Figure 5A and 5B). Based on histochemistry, the surface of these cells positively stained with PAS and AB (Figure 5C and 5D). It confirmed that they acted to produce both glycoprotein and mucopolysaccharine. In addition, goblet cells were also detected, both negatively stained to H&E and intensively stained to PAS and AB. Three layers of thin submucosa, thick muscularis (as greenish, Figure 5E) and serosa were also exhibited.

3.2.4 Intestine

The two segments of intestine were composed of the anterior and the posterior intestine, which showed differences in localization and histological structures (Figure 5F to 5P). In the anterior intestine, the epithelial lining structure of the mucosal folds was similar in pyloric caeca. The layer of the lamina propria and the submucosa were thin and complexly divided. In other observations, two thin layers of inner circular and outer longitudinal layers of smooth muscle were noticeable. In the posterior intestine, both histological and histochemical observations of their mucosal layer were similar to the anterior intestine structure, except the longitudinal folds of mucosal layer were less numerous than in the anterior part and the globelt cells were more numerous than in the anterior intestine.

4. Discussion

Several investigations of digestive tract in teleostean have been reviewed (Morrison, 1987; Grau *et al.*, 1992; Genten *et al.*, 2008, Raji and Norouzi, 2010; Senarat *et al.*,

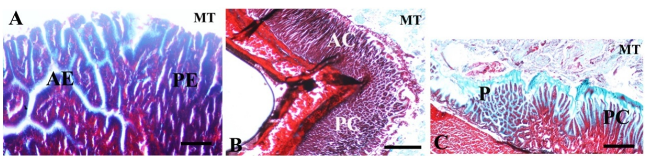

Figure 4. Light photomicrograph of the junction between anterior oesophagus to pyloric stomach; A = 20 µm; B-C = 200 µm; *AC = anterior cadiac, AE = anterior oesophagus, P = pylorus, PC = posterior cardiac, PE = posterior oesophagus. (MT = Masson's trichrome).*

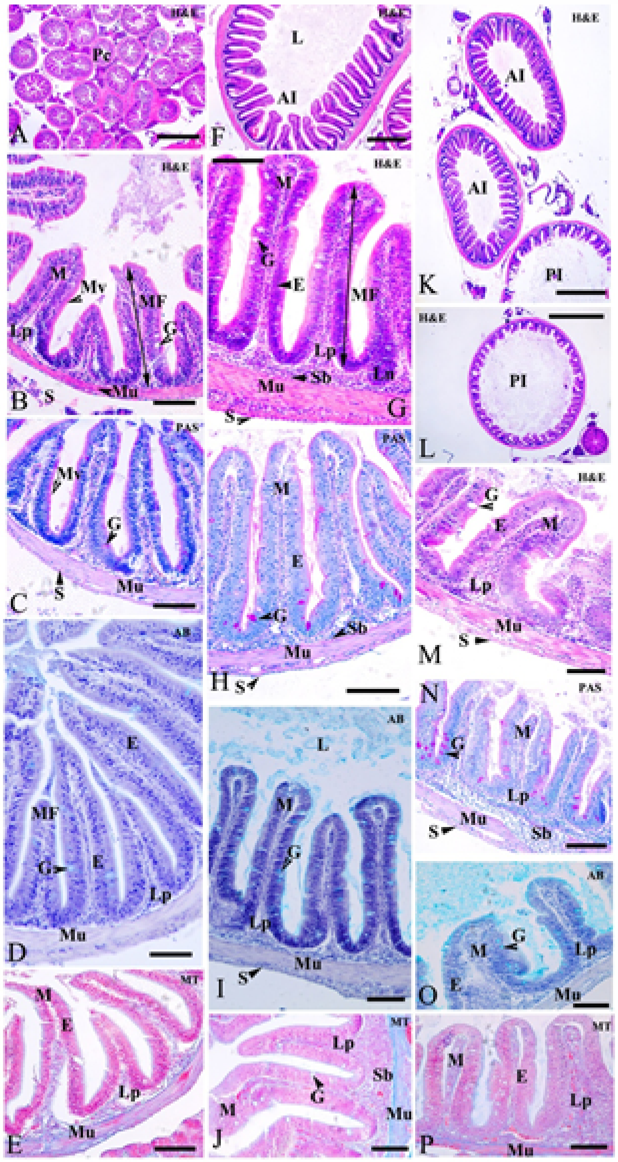

Figure 5. Light photomicrograph of pyloric caecum (Pc) to posterior intestine (PI); (A-E)The pyloric caecum with showing various globelt cells; (F-K)The anterior intestine; (AI); (K-P)the posterior intestine with showing also gastric glands; A-F-K-L = 200 µm; B-C-D-E-G-H-I-J-M-N-O-P = 20 µm; *E = epithelium, G = goblet cells, L = lumen, Ln = lymphatic nodule, Lp= lamina propria, M = mucosa, Mc = mucous cells, MF = mucosal fold, Mm = musculoris mucosae, Mu = muscularis, Mv= microvilli, S = serosa, Sb= submucosa. (H&E = Hematoxylin and Eosin, MT = Masson's trichrome, PAS = Periodic Acid Schiff (PAS), AB = aniline blue, Rt = reticulin (Rt), Ac = alcian blue).*

2013a). Histologically, the epithelial lining structure of *R. brachysoma* anterior oesophagus was similar to the results in both marine fish (*Astatotilapia burtoni* (Genten *et al.*, 2008)) and freshwater fish (*Hemibagrus filamentus* (Senarat *et al.*, 2013b)). However, some investigators found different structures that showed stratified cuboidal epithelium including in chondricthyes, *Raja clavata* (Holmgren and Nilsson, 1999) and *Squalus acanthias* (Leake, 1975). The surface of these epithelial cells in mucosal layer was positively stained with PAS, Ac and AB stains. These confirm that these cells play apart in producing or releasing acid mucopolysaccharides, neutral and acidic glycoprotein as mucus-secreting function. It is believed that these secretions play a part in the transferring food to the stomach (Harder, 1975; Cataldi *et al.*, 1987; Grau *et al.*, 1992). Additionally, the function of the oesophageal epithelium in fish includes protected against damage and bacterial infection (Grau *et al.*, 1992; Albrecht *et al.*, 2001). In some fish, histological esophagus also contained the taste buds in the mucosal epithelium (Grau *et al.*, 1992) including *Gadus morhua* (Martin and Blaber, 1984; Morrison, 1987). However, the taste buds were not seen in this fish, like in *Sp. auratus* (Elbal and Agulleiro, 1986) and *Solea senegalensis* (Arellano *et al.*, 2001). As mention above, the anterior oesophagus *R. brachysoma* was similar to other teleost. However, the histological features of the posterior oesophagus were easily observed because the mucosal epithelium changed from simple squamous epithelium into simple columnar epithelium which contained various gastric glands. We discussed the possibility that this structure may help to increase the breakdown of ingested food before entering the stomach. Because it contained acidophilic granules and oxynticopeptic cells, so it played a role in producing both pepsin and hydrochloric acid (oxyntopeptidic cell) as digestive enzymes (Kapoor *et al.*, 1975; Genten *et al.*, 2008).

In teleost fish, it is well known that the function of stomach concernsboth the storage and digestion of food (Genten *et al.*, 2008). The anatomy of the stomach in both marine and freshwater fishes varies in both shape and structure, depending on food habitats because some structures are absent in some families (Cyprinidae, Labridae, Gobiidae, Scaridae, Cyprinodontidae, and some Poeciliidae) (Genten *et al.*, 2008). In contrast, in other species it was presented in different shapes. A U-shape stomach was present in *Anguilla anguilla* (Clarke and Whitcomb, 1980), *Sp. aurata* (Cataldi *et al.*, 1987) and *Seriola dumerili* (Grau *et al.*, 1992) which indicated carnivorous feeding type. However, the stomach of *R. brachysoma* was relatively large and presented in a J-shape. This also occurs in some other fishes (Raji and Norouzi, 2010). This shape may be considered as herbivorous feeding type. Based on histology, the mucosal structures of *R. brachysoma* contained the gastric glands in both cardiac and pylorus. It was similar to those of *Sp. aurata* (Cataldi et al., 1987), *Se. dumerili* (Grau *et al.*, 1992), *Schilbe mystus*, *Petrocephalus microphthalmus*, *Perca fluviatilis* (Genten *et al.*, 2008) and *H. filamentus* (Senarat *et al.*, 2013b). The functional structures of these gastric glands are related with producing the digestive enzyme which helps in digesting food (Genten *et al.*, 2008). Although, the structure of this fish is similar to that of other fishes, some details deserve attention. The layer of muscular is in this species gradually increased in size, while the gastric gland also gradually decreased in size. We believe that the function of this structure may help in digesting some food elements before they proceed to the complete digestion in other parts of the digestive system.

At the junction of stomach and intestine of this fish, there were various pyloric caecum which are similar to those present in other fish, including *Clarias batrachus* and *Serrasalmus nattereri* (Raji and Norouzi, 2010). It is believed that this structure is related to the natural habitat. From histological feature, several goblet cells in mucosal layer in this species were detected, like in other teleost fishes (Raji and Norouzi, 2010). It has been believed that this cell is associated with increasing the effectiveness of the absorption surface area (Evans, 1998). The intestine length is used to explain the trophic level in nutritional ecology or feeding habits. Therefore, the values of IC are classified into three groups within fishes, including low IC values (0.5-0.6 in *Sp. aurata* (Cataldi *et al.*, 1987) and 0.8 in *Glyptosternum maculatum* (Xiong *et al.*, 2011)) as carnivores or piscivores; intermediate values (1.25 in *Leporinus friderici*; 1.14 in *L. taeniofasciatus* (Albrecht *et al.*, 2001) and above 2.0 in *Ctenopharyngodon idellus* (Nie and Hong, 1963)) as omnivores and high values (above 3.0 in *Puntius stoliczkanus* (Senarat*et al.*, 2013a)) as herbivores. The IC value of *R. brachysoma* indicated a typical herbivorous. The length of IC can be explained by the fact that digestion is slower and requires either longer period or wider exposure (Albrecht *et al.*, 2001; Silva *et al.*, 2012). The number of goblet cells in the posterior part was greater than the anterior part which was similar to that of other fishes (Canan *et al.*, 2012; Kapoor *et al.*, 1975; Senarat *et al.*, 2013a) including *Pseudophoxinus antalyae* (Cinar and Senol, 2006). It is well known that these cells play an importance role in lubrication, absorption and transportation of macromolecule (Arellano *et al.*, 2002). Therefore, the increase of goblet cells may be involved in mucosal protection and lubrication for fecal expulsion (Murray *et al.*, 1994). In the mucosal intestine, the epithelium of *R. brachysoma* showed a stratified columnar structure with goblet cells that was similar to pyloric caecum epithelium. The feature of the intestine of this fish was similarly reported in freahwater fish, *P. stoliczkanus* (Senarat *et al.*, 2013a) and marine species, *Sq. acanthias* (Leake, 1975).

In conclusion, we have produced the first report of the details of the gross anatomy, histology and histochemistry of the digestive tract in *R. brachysoma*. The structural classification of the digestive tract in this fish was similar to that of most fishes which are composed of mucosa, submucosa, muscularis, and serosa. The results of this study will provide the basic structure of the digestive tract, which will be needed for further studies, including the ultrastructural level and immunohistochemistry of this species.

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