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Histological Study of the Intestine of Stoliczkae's Barb *Puntius stoliczkanus* (Day, 1871) (Cypriniformes: Cyprinidae)

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ABSTRACT

The histological and histochemical characteristics of the digestive tract of Stoliczkae's barb *Puntius stoliczkanus* (Day, 1871) were examined. This fish had an intermediate gut length with the nonexistence of the stomach. The histology revealed that the intestine consisted of four different layers: mucosa, submucosa, muscularis and serosa. The mucosa comprised the various villi and simple, columnar epithelia which were lying on the lamina propria. Muscularis mucosae and mucosal tubular glands were not present. Eosinophilic granular cells were observed along the intestine, mainly beneath the lamina propria. Histochemical analysis showed the goblet cells and eosinophilic granular cells react positively with periodic acid-Schiff. This research reports on the histological study of the alimentary tract of *P. stoliczkanus* for the first time.

Keywords: histochemistry, histology, intestine, periodic acid-Schiff (PAS), *Puntius stoliczkanus*

INTRODUCTION

Stoliczkae's barb *Puntius stoliczkanus* (Day, 1871), belonging to the family Cyprinidae (order Cypriniformes), are caught as juveniles and adults for sale in the ornamental fish trade in Thailand because of their unique characteristics. Thus, it is considered an interesting species for commercial aquaculture in Thailand. Nonetheless, little is known about the biology, ecology and morphology of this fish. This basic information could be valuable for the development of aquaculture in the country.

Knowledge of the digestive tract of normal histological structures is essential for a better understanding of the physiology and pathology of fish. The intestine of fish is a complex multifunctional organ which is crucial for the fluid and electrolyte balance (sodium and chloride) and for regulating the endocrine level and metabolism and immunity, among other things (Buddington *et al.*, 1997). The morphological characters of the different digestive tracts in fish are related to the physical characteristics of food, feeding habits and taxonomy. The digestive tract of teleost has been widely studied and morphological descriptions have been provided of the function of many specialized anatomical structures in relation to different feeding adaptations (Cinar and Senol, 2006).

However, to date, no such information is available for *P. stoliczkanus*. Therefore, the intestine of *P. stoliczkanus* was selected and described using the principles of histological and histochemical analyses.

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MATERIALS AND METHODS

Twenty pooled samples of male and female adult *Puntius stoliczkanus* were used in this study, which were gathered from 2011 to 2012 in two periods, one each in the rainy (November, 2011) and dry (April, 2012) seasons, with 10 individuals per sampling from the Tapee river, Chawang district, Nakhon Sri Thammarat province, Thailand (8°28.10N, 99°29.45E). Each sample was euthanized by the rapid cooling method (Wilson *et al*., 2009). The body of each fish was dissected and the digestive tract was removed. The intestine was measured and then fixed in Davidson's fixative for 48 hr. The specimens were processed using standard histological techniques. Sections 5 μm thick were cut by rotary microtome and stained with Harris haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Afterward, a light microscope was used to observe the histological structure of the intestine of *P. stoliczkanus*.

The intestinal coefficient (IC) was calculated, which is the ratio of the intestinal length (L_{IT}) to the body length (L_S), given by IC = L_{IT} / L_S. The relation between L_S and L_{IT} was tested by correlation analysis. All length measurements were reported as a mean ± SD.

RESULTS

Histological study

Microscopically, *Puntius stoliczkanus* had a mean standard body length of 5.35 ± 0.46 cm and an intermediate intestine length of 10.25 ± 0.99 cm with the absence of the stomach. Histologically, this intestinal organ was divided into proximal and distal regions by the presence of distinct characteristics based on the histological structure and cell types. The histological technique showed that the intestinal wall of *P. stoliczkanus* was composed of four different tunical layers: mucosa, submucosa, muscularis and serosa (Figures 1A, 1B and 1D) after staining with H&E). Each of these layers is described below.

Tunica mucosa: the tunica mucosa was composed of the epithelium layer, lamina propria and muscularis mucosae. The epithelium layer was lined by simple columnar epithelia that exhibited the tall columnar cells or enterocytes of acidophilic cytoplasm. The mucosal surface had several villi that became shorter and wider from the anterior to the posterior intestine. Goblet cells presented a supranuclear region as basophiclic cells. The number of goblet cells was higher in the posterior part of the intestine than in the anterior part. The lamina propria was situated below the epithelium layer and contained blood and lymph vessels and nerve. The mucularis mucosae and mucosal tubular gland were not observed.

Tunica submucosa: this layer generally consisted of a thin, loose, connective tissue layer and a small blood layer.

Tunica muscularis: this was a layer of smooth muscle which was thicker than the posterior part. There were two layers of smooth muscle; a thick, inner, longitudinal layer and a thinner, outer, circular layer, lined by many nerve plexuses between them. The inner, longitudinal muscle layer in the anterior part was thicker than the posterior part.

Tunica serosa: this was the most external part of the intestine. It consisted of dense, irregular, connective tissues that contained collagen fibers.

Histochemical study

Two types of cells of the intestine of *Puntius stoliczkanus* showed a positive reaction to the PAS technique: (i) goblet cells, which were acidic cells in the intestinal epithelia and (ii) eosinophilic granular cells observed between the lamina propria and the submucosa (Figure 1C).

DISCUSSION AND CONCLUSION

Although there are differences in the histology of the intestinal tract among the different fish species, the wall of the intestine of

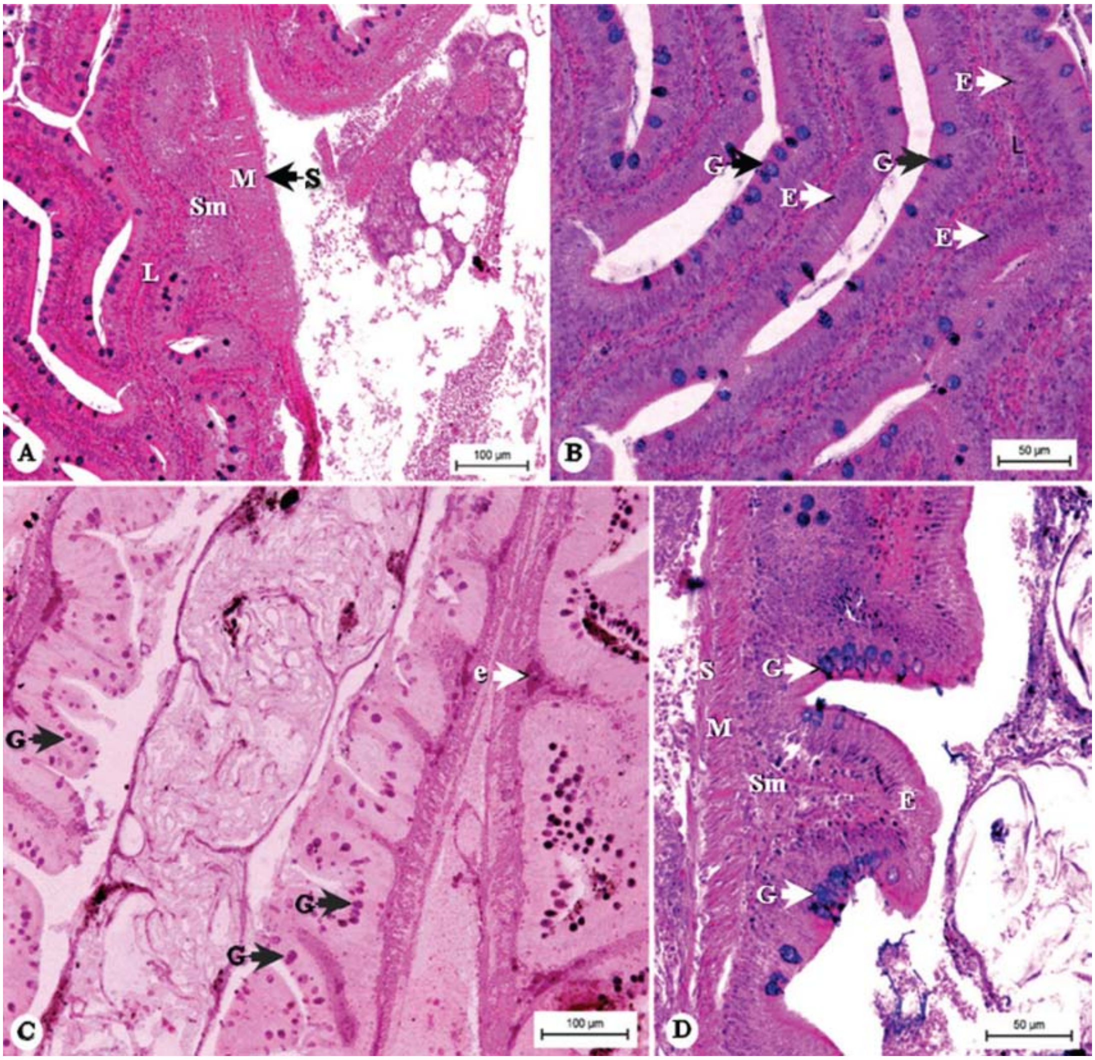

Figure 1 Intestine of *Puntius stoliczkanus*: (A) and (B) Anterior; (C) Periodic acid-Schiff positive; (D) Posterior. (Abbreviations: E = Epithelium/enterocyte, e = Eosinophilic granular cell, G = Goblet cell, L = Lamina propria, M = Muscularis, S = Serosa, Sm = Submucosa. Staining with Harris haematoxylin and eosin.

Puntius stoliczkanus is classified into four layers, which is fairly common in vertebrates such as mammals, birds, amphibians and reptiles (Andrew and Hickman, 1974; Kuperman and Kuzmina, 1994). Although the intestines of fish have the same elementary organs as other vertebrates, the intestine is modified for more limited and specialized nutrients (Moffatt, 2001). The stomach was not present in this fish, which is similar to other cyprinid fish (carp) and other fish such as loaches (Cobitidae), killifish (Cypridontidae), clingfish (Gobiesocidae) and gobies (Gobioidae) according to Harder (1975).

The length of the intestine is also mainly associated with feeding habits— detritivores, herbivores, carnivores and omnivores—and previous research has reported that the length of the intestine of herbivorous fish species was generally longer than in carnivorous species (Canan *et al*., 2012). The digestive tract in the current study was long, which might have been caused by the low energy diet, which required slower intestinal transit for digestion, absorption and utilization. On the other hand, the reduced size of the intestine was probably justified by the fact that the organ served as a brief passage for obtaining the nourishment from the food as it passed (Silva *et al*., 2012).

In general, the intestinal organ, through which the diet passed and where alkaline digestion and nutrient absorption took place, was tubular. The intestinal coefficient is used extensively to categorize species into different trophic levels (Rodrigues *et al*., 2010; Canan *et al*., 2012). According to the results of the current study, the segment of the digestive tract of *P. stoliczkanus* was moderately long (L_{IT}; 10.25 ± 0.99 cm), which was longer than its body length (L_S; 5.35 ± 0.46 cm); consequently the average value of IC was 1.93 ± 0.21. Moreover, there was a positive correlation ($P < 0.05$) between L_{IT} and L_S from this study. The intestinal length of *P. stoliczkanus* was about twice as long as the body length; therefore it is possible that this fish could be classified as a typical herbivorous fish, with its intestinal structure possibly being adapted to plant feeding. This result is also similar to that reported in other researches; for example, the intestine of the cichlid fish (*Satanoperca pappaterra*) was relatively long (27.78 ± 2.48 cm) according to Silva *et al*. (2012). It was suggested that its tract could be adapted to detritivore-invertivore feeding (Hahn and Cunha, 2005). In addition, a functional enlargement of the long digestive tract of herbivorous fish suggested that some of the food was slow to be ingested and required both a longer period of time and wider exposure (Silva *et al*., 2012).

From a histological aspect, the overall structure of the intestine in *P. stoliczkanus* was similar to other fish (Cinar and Senol, 2006). The goblet cells could be determined during the differentiation of the intestinal mucosa of fish but in some species like European catfish, there were rare goblet cells on the intestinal villi (Petrinec *et al*., 2005). The current study found that localization and distribution of goblet cells were greater in the posterior part of intestine than in the anterior part, which was similar to other fish (Canan *et al*., 2012). The increased number of goblet cells may imply the need for increased mucosa protection and lubrication for fecal expulsion (Murray *et al*., 1994). According to the current study, the intestine of *P. stoliczkanus* contained several goblet cells which gave a positive reaction to the PAS technique (neutral mucopolysaccharides). This result depended on the degree of maturation of the goblet cells. The goblet cells were able to synthesize a mixture of neutral and acidic glycoconjugates especially sulphate; the variation of these secretions was possibly caused by changes in environmental conditions that may in turn sustain functional alterations of the digestive apparatus (Domeneghini *et al*., 1998).

In this study, eosinophilic granule cells were observed. The role of these cells was believed analogous with the mast cell. Eosinophilic granule cells contained antimicrobial peptides and their degranulation could increase the vascular permeability and promote neutrophil adhesion; furthermore, Powell *et al*. (1993) speculated that they were intimately involved in innate immunity and inflammation.

The study of the structural level of the tissue and the histochemistry of the digestive tract of *P. stoliczkanus* presented here is considered to be the first such report. The fundamental data may elicit better understanding of this species' intestinal mechanisms of digestion and also foster further studies in other fish species and other aspects such as behavior and life history and bionomics.

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