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Histochemical and immunohistochemical study on endocrine cells (5HT, GAS, and SST) of the gastrointestinal tract of a teleost, the characin *Astyanax bimaculatus*

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ABSTRACT

Endocrine cells secrete hormones through the mucosa of the gastrointestinal tract (GIT) and act on the overall regulation of digestive processes such as nutrient absorption, gut motility and intestinal blood flow. This study aimed to determine regional distribution and frequency of endocrine cells secretory of serotonin (5-HT), somatostatin (SST) and gastrin (GAS) in the GIT of a small-bodied widespread characin *Astyanax bimaculatus* using histological, histochemical and immunohistochemical techniques. Fragments of the stomach and gut fixed for 8 h in Bouin liquid were subjected to histological processing and immunohistochemical routine. For the histological analyses, the technique of staining with hematoxylin and eosin (HE) was used, whereas for the histochemical analyses Gomori's trichrome, periodic acid + Schiff (PAS) and Alcian blue pH 2.5 (AB) were used to further immunohistochemical processing. The stomach has a mucosa lined with a simple columnar epithelium with mucus-secreting cells; the glandular region (proximal and distal portions) has folds and pits, whereas the non-glandular region has pits only. The intestinal epithelium is simple with plain cylindrical grooved and goblet cells. The anterior region has thin folds with few goblet cells, and the posterior region with thick folds and many goblet cells. The regional distribution and frequency of endocrine cells varied across regions of the GIT with the stomach showing the highest amount of immunoreactive (IR) cells. Only the 5-HT was found in the stomach (epithelia and glands) and gut regions, with comparatively higher frequency in the stomach. SST-IR cells were found in the stomach (epithelia and gastric glands) with higher frequency in the glandular region, whereas GAS-IR were found in the gastric glands only. The stomach was the only organ to have all the three types of endocrine cells, indicating that this organ is the main site of digestion of food in this species.

Introduction

Anatomy of the gastrointestinal tract (GIT) of fishes is highly variable, changing according to feeding habits, phylogenetic position and lifestyles (Santos et al., 2011; Castro et al., 2003). Overall, the histological architecture of the GIT includes a layer of mucus-secreting cells, observed by histochemical techniques in various studies of teleosts (Vieira-Lopes et al., 2013). Secretory cells play an important role in lubricating the organ and protecting against proteolytic degeneration and pathogenic microorganisms (Reid et al., 1988). Moreover, several functions of the different GIT segments are controlled by endocrine cells that form a complex system disseminated among the epithelial components, with the ability to secrete physiologically active polypeptide hormones and amines (Carvalheira et al., 1968). According to Deveney and Way (1983), gastrointestinal hormones secreted by endocrine cells have important functions in the overall regulation of the digestive process, such as nutrient absorption, gut motility and intestinal blood flow. The presence of endocrine cells in the fish GIT can vary in frequency and distribution depending on the fish species (Pan et al., 2000a; Çinar and Diler, 2002; Bosi et al., 2004; Ku et al., 2004; Tarakçi, 2005; Min et al., 2009).

The small-bodied characin *Astyanax bimaculatus* is a fish species with omnivorous feeding habit, widely distributed in rivers and reservoirs of Southeastern Brazil, and plays an important role in trophic web because it is preyed by a number of large-sized species,

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thus transferring matter and energy through the trophic web. This species also has potential to be used as bait sport fishing, human consumption and ornamental purposes (Sato et al., 2006). However, there is no available information on its digestive physiology, such as the distribution of endocrine cell thought the gastrointestinal tract. These immunoreactive cells play an important role on the regulation and digestion of several aminoacids and glucose during the food processing (Pan et al., 2000a; Vieira-Lopes et al., 2013). Therefore, in the present study, we describe histological and histochemical aspects of the gastrointestinal tract of *A. bimaculatus* and the regional distribution of endocrine cells in the tract by immunohistochemistry using three types of immunoreactive cells: the serotonin (5-HT), somatostatine (SST) and gastrin (GAS).

Materials and methods

Study area

Fish were collected from a tropical reservoir in Southeastern Brazil (22°30′ S, 44°45′ W), in winter (July–August) 2011. The reservoir (Funil Reservoir) has a 40 m^2 area, and is located at ca. 440 m above the sea level. Hydraulic residence time varies between 10 and 50 days, according to seasonal variation in precipitation. Rainfall averages 500 mm in winter and 1500–2500 mm in summer (Branco et al., 2002; Soares et al., 2008). Annual temperature averages 21 °C, with means of 24 °C in summer and 17 °C in winter.

Fish collection and histological procedure

Thirty-six adult (total length 9.8–12.9 cm, total weight 10–30 g) specimens were used in this study without sexual distinction. Immediately after collection, all fishes were anaesthetized in benzocaine hydrochloride (50 mg/l), and then rapidly killed by hypothermia, measured (in mm) and weighted (g). Then, the fish were dissected, and fragments of two stomach regions (glandular and non-glandular) and two intestinal regions (anterior and posterior) were obtained from each specimen (Fig. 1), fixed for 8 h in Bouin's fluid and then placed in 70% alcohol. These materials were processed for routine histological techniques, being dehydrated, diaphanized in xylol and embedded in paraffin. Consecutive transverse sections 5 μm of thickness were cut and mounted on glass slides and stained in hematoxylin and eosin (HE). Two slides obtained from each specimen were prepared for each one of the seven protocols (HE, Gomori's trichrome, periodic acid Schiff – PAS, Alcian blue-AB, 5-HT, SST and GAS), for each sectioned regions, resulting in 2016 samples (7 protocols × 2 slides × 36 individuals × 2 organs × 2 regions).

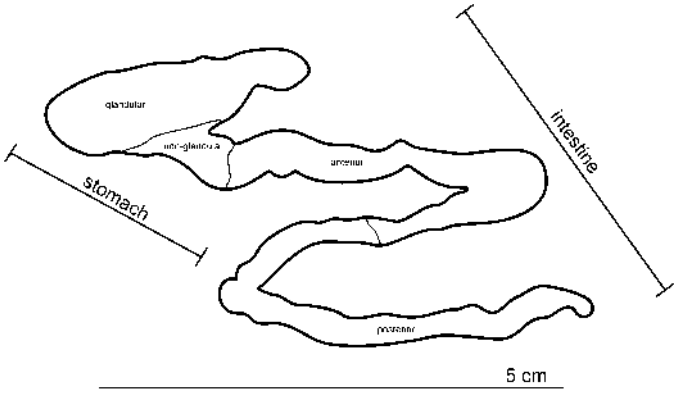

Fig. 1. Anatomic diagram of the digestive tract of *Astyanax bimaculatus*.

Table 1
List of primary antisera used in the present study.

Antiserum	Code	Dilution	Source
Anti-gastrin	G 0785	1:1000 μl	Sigma–Aldrich, Inc.
Anti-serotonin	S 5545	1:8000 μl	Sigma–Aldrich, Inc.
Anti-somatostatin	A 0566	1:300 μl	Dakocytomation
Kit ABC	PK-6200	–	Vector
DAB	K-047	20 μl:1 ml	Diagnostic Biosystems
Antibody diluent	ADS-125	–	Spring Bioscience
Poly-L-lysine	10:90 ml	Sigma–Aldrich, Inc.	Sigma–Aldrich, Inc.

Histochemical and immunohistochemical analysis

Histochemical procedure comprised Gomori's trichrome, periodic acid Schiff (PAS) and Alcian blue (AB) pH 2.5 staining to reveal the neutral and acid glycoconjugates (GCs), respectively. For the immunohistochemical (IHC) procedure, 5 μm thick sections were cut by microtome (model Spencer-820) and mounted on glass slides precoated with 0.1% poly-L-lysine, after being dewaxed and dehydrated by the routine protocol. Samples were incubated in citrate buffer (pH 6.0–0.01 M) and placed in a microwave oven for 15 min to recover the antigen. Then, they were incubated with a solution of 3% H_2O_2 in methanol for 15 min to block any endogenous peroxidase. Subsequently, the sections were incubated at room temperature in a humid chamber (incubation tray for IHC, EP-51-05022, Erviegas®) with a 1:100 μl dilution of bovine serum albumin in phosphate buffered saline (PBS) solution for 30 min.

The sections were first incubated overnight at 4 °C with the primary antisera against the individual gastrointestinal hormones (Table 1). Then, the sections were incubated with biotinylated "Universal" secondary antibody diluted to 1:200 μl for 30 min at room temperature, and then with avidin–biotin–peroxidase complex, diluted at 1:200 μl for 30 min at room temperature. Subsequently, the peroxidase label was revealed by reaction with Stable DAB/Plus, prepared according to the kit's instructions. All dilutions and thorough washes between stages were performed using PBS (pH 7.4). The sections were counterstained with Harris hematoxylin, rinsed with deionized water, dehydrated through a series of ethanol and methylcyclohexane solutions and mounted using Entellan (Chem Supplier).

Because of the difficulty of obtaining antibodies to *A. bimaculatus* against the proteins studied, and because of these proteins are conserved in the phylogenetic scale (Buddington and Krogdahl, 2004; Nelson and Sheridan, 2006; Daza et al., 2012; Pereira et al., 2015), we used polyclonal antisera produced in mammals against 5-HT, SST and GAS. Several earlier studies used antibodies produced in mammals and obtained consistent results in fishes, proving the affinity of the antibodies (e.g., Ku et al., 2004; Hernández et al., 2012; Vieira-Lopes et al., 2013). According to Langer et al. (1979), the cross-reactivity strongly suggests that peptides in teleosts fishes share similar or even identical epitopes with their equivalents in higher vertebrates.

To investigate the specificity of the reactions, negative and positive controls were used. Sections of bat intestine were used as positive controls, because they yielded positive marking in previous studies (e.g., Santos et al., 2008a,b; Machado-Santos et al., 2009). The negative control was prepared by replacement of the primary antibody with non-immune serum and PBS (pH 7.4).

Observation and photomicrography

Photomicrographs of all samples from each of the thirty-six specimens were obtained with a digital camera Sony Cybershot DSCW 230 attached to a microscope Olympus BX41. The evaluation of the frequency and distribution of immunoreactive endocrine

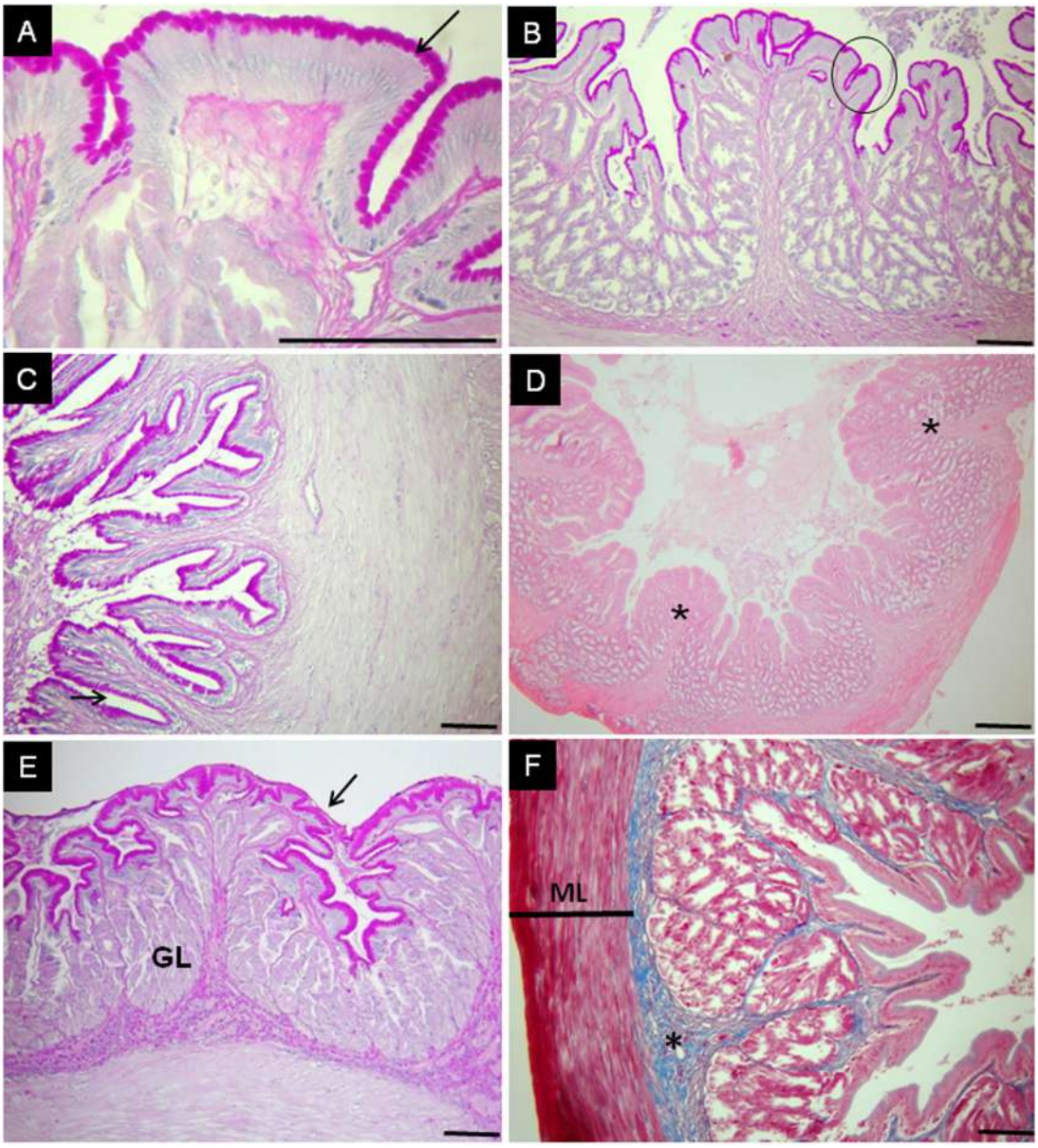

Fig. 2. Photomicrographs of transversal sections of the stomach. (A) Epithelial lining of the stomach, being single cylindrical secreting mucus (arrow). (B) Foveolar glandular region with shallow (circle). (C) Non-glandular foveolar region with great depth (arrow). (A–C) Periodic acid–Schiff stain. (D) Overview of the glandular region with emphasis on the presence of short and thick folds (*). Hematoxylin and eosin (HE) stain. (E) Layer mucosa showing neutral glycoconjugates (GCs) (arrow) in the mucus-secreting cells of the epithelium and the presence of gastric glands (GL) in the lamina propria. Periodic acid Schiff (PAS) stain. (F) Submucosa layer showing the presence of collagen tissue (*) and muscular layer (ML). Gomori's trichrome stain. Scale bar: 100 μm.

cells for each antiserum (expressed as the average number of immunoreactive cells) was assessed by examining 5 random fields of non-overlapping areas from the 2 regions of the stomach and intestine in 2 slides prepared from each one of the 36 specimens. In total, we observed 1440 samples (5 fields × 36 fishes × 2 organs 2 regions × 2 slides).

The photomicrographs were analyzed and the relative frequency of endocrine cells immunoreactive (IR) measured using a computerized image analyzer (Image-J software). The frequency of immunoreactive cells is expressed as mean + SD (standard deviation) per unit area (mm^2) of mucosa.

The intensity of the marking histochemical was visually analyzed and further described according to the observed intensity of color reaction, i.e., (−), no staining; (+), low; (++), medium; and (+++), strong staining.

Statistical analysis

The mean number of endocrine cells in the gastrointestinal tract immunoreactive to 5-HT-, SST- and GAS-antisera were compared in each organ and region of the TGI of *A. bimaculatus* using the Kruskal–Wallis non-parametric test. The significance level was set at 0.001.

Results

Histological and histochemical study

The following layers were observed in the gastrointestinal tract (GIT) of *A. bimaculatus*: mucosa, submucosa, muscular and serosa. The muscularis mucosae is absent in this species.

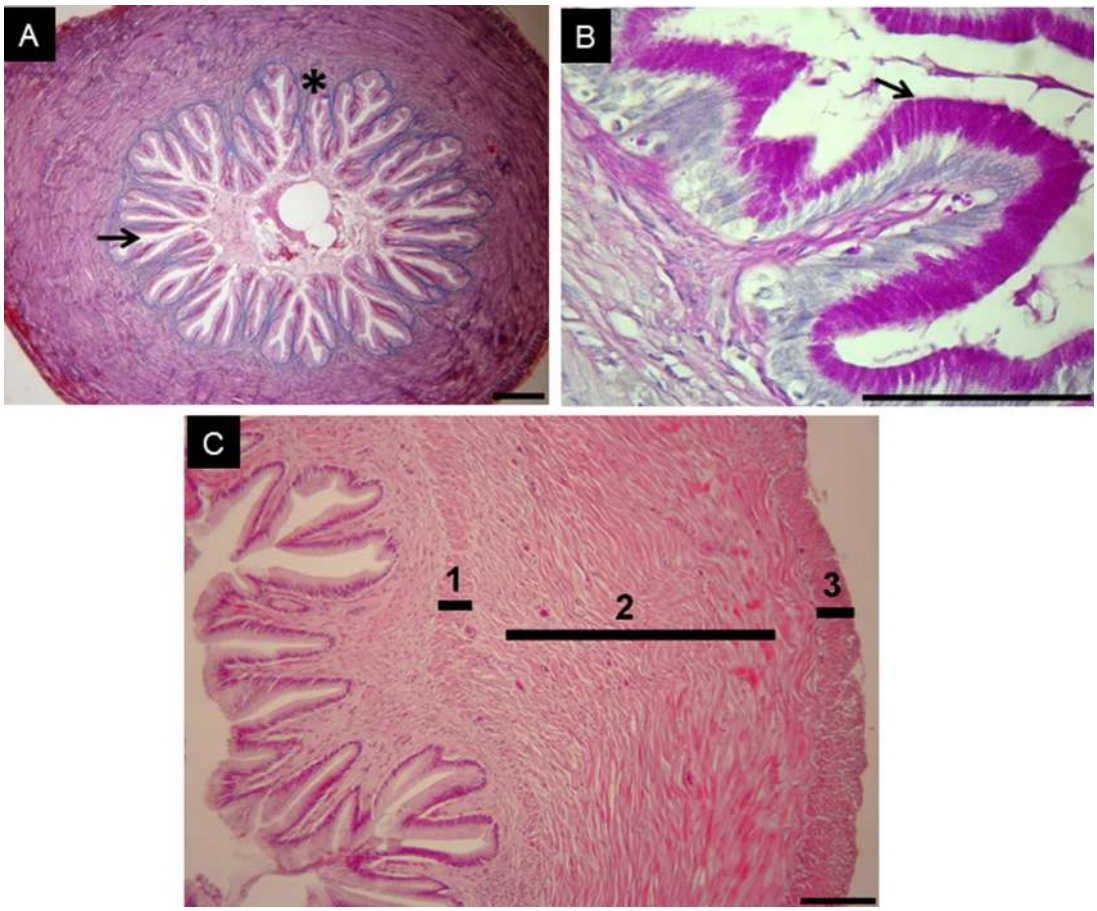

Fig. 3. Photomicrographs of the non-glandular region stomach. (A) Overview of the non-glandular region showing deep pits (arrow) and absence of gland in the lamina propria (*). Mallory's trichrome stain. (B) Epithelium and the presence of neutral glycoconjugates. (GCs) (arrow) in the mucus-secreting cells of the epithelium. Periodic acid Schiff (PAS) stain. (C) Muscular layer emphasizing the division of the three sub-layers with fibers in oblique direction (1), circular (2) and longitudinal (3). Hematoxylin and eosin (HE) stain. Scale bar: 100 μm.

Stomach

According to the structural characteristics observed in this study, the stomach of *A. bimaculatus* was divided in two regions: a glandular region with folds and shallow pits and a non-glandular region with deep pits only.

The mucosa is lined with a simple columnar epithelium layer composed of mucus-secreting cells with basal nuclei (Fig. 2A). The stomach epithelium forms crypts along the gastric mucosa (Fig. 2B and C). The mucosa layer projects toward the organ's lumen, forming various thick and rounded gastric folds longitudinally arranged. In the non-glandular region, the submucosa and muscular layers accompany the mucosa, making the lumen very small.

The glandular region is characterized by having a well-developed tubular gastric glands, composed of oxynticopeptic cells, occupying the entire lamina propria (Fig. 2D). The mucus-secreting cells were reactive to PAS but not to AB, revealing the presence of only neutral GCs (Fig. 2E). The muscular mucosa is absent in this species. The submucosa layer is composed by loose connective tissue, with abundant connective fibers and blood vessels. The muscular layer is composed of smooth muscle fibers arranged in two directions: internal circular and external longitudinal (Fig. 2F). This region contains myenteric plexuses arranged in sparse groups, composing the enteric nervous system and located between the muscular sub-layers. A serous layer surrounds these structures.

In the non-glandular region, differently from the glandular region, the mucosa and the submucosa do not form longitudinal folds, and present deep pits lacking gastric glands in the lamina propria (Fig. 3A). In this region, there is no muscular layer of the mucosa. A submucosa layer is comprised of loose connective tissue and blood vessels. The mucus-secreting cells of simple columnar epithelium as well as in the anterior region reacted positively to PAS only (Fig. 3B). The muscular layer is thicker when compared with the glandular region (Fig. 3C). This well-developed muscular layer is comprised by three sub layers of smooth muscular fibers: in oblique direction, circular and longitudinal (Fig. 3C). A serous membrane forms the outermost layer of these structures.

Intestine

The histological analysis of the intestine revealed that the pattern of folds varies, characterizing two distinct regions: anterior and posterior. The folds of the mucosa layer in the anterior segment are more numerous, thinner and elongated compared with the posterior segment (Fig. 4A) and have less goblet cells (Fig. 4B). In the posterior segment, the folds are thicker and fewer (Fig. 4C).

The intestinal mucosa is lined by a simple columnar epithelium with a striated border and goblet cells (Fig. 4D). The use of histochemical techniques PAS and AB enables to observe in the two segments the mucus-secreting cells were positive to PAS and AB staining with pink (PAS) and blue (AB) indicating the presence of neutral and acid GCs, respectively in the anterior segment (Fig. 5A and B) and in the posterior segment (Fig. 5C and D) of the intestine. However, in the stomach (Table 2), only the mucus-secreting cells were reactive to PAS.

The boundaries between the lamina propria and submucosa are not evident and can be seen only the presence of loose connective tissue and blood vessels in these regions. In the segments of the

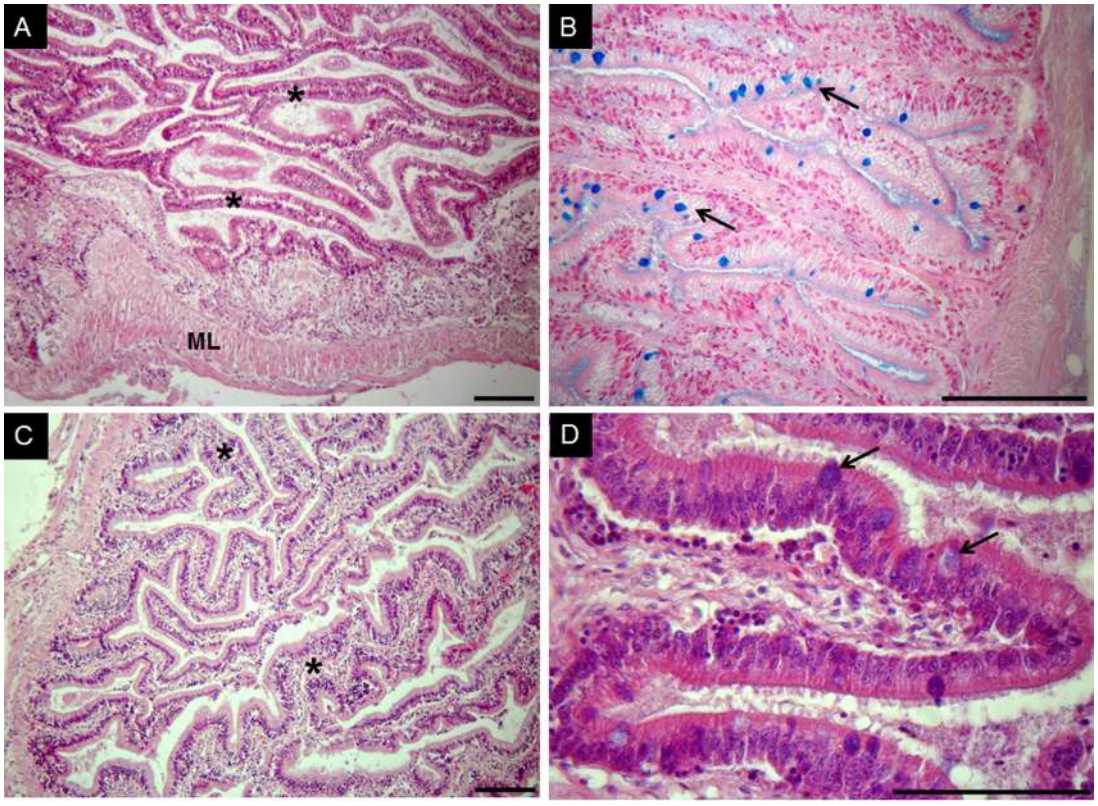

Fig. 4. Photomicrographs of the anterior and posterior intestine region. (A) Overview of the anterior intestine with emphasis for slender folds (*). Hematoxylin and eosin stain (HE). (B) Anterior intestine with presence of few goblet cells (arrow). Alcian blue stain. (C) Overview of the posterior intestine region characterized by thick folds (*). Hematoxylin and eosin stain (HE). (D) Mucosa layer showing simple columnar epithelium with goblet cells and striated flatness. Periodic acid Schiff (PAS) stain. Scale bar: 100 μm.

intestine, the muscular layer follows the same pattern of organization seen in the glandular stomach with an inner circular layer and outer longitudinal consisting of smooth muscle fibers, forming a thinner layer in the posterior segment of the intestine (Fig. 4C) when compared to the anterior segment (Fig. 4A). In the posterior segment of the intestine, we observed presence of parasites in approximately 60% of the cases (Fig. 5E). The serous layer is located in the outer position.

Immunohistochemical study

The 5-HT-, SST- and GAS-immunoreactive (IR) cells were identified in the GIT. The regional distribution and frequency of the different types of endocrine cells varied according to their location in the GIT (Table 3).

Sero tonin immunoreactivity

5-HT-IR cells were observed in the glandular (Fig. 6A and B) and non-glandular region of the stomach (Fig. 6C and D) and, in comparatively lower frequency in the anterior and posterior regions of the intestine (Fig. 6E). Regarding the morphology of immunoreactive cells, two types of cells were found in the whole GIT: closed-type cells and open-type cells (Fig. 6A and B).

Table 2
Intensity of histochemical marking of glycoconjugates (GCs) in gastrointestinal tract of the *A. bimaculatus*.

Technique	Stomach		Intestine	
	Glandular	Non-glandular	Anterior	Posterior
AB pH 2.5	–	–	++	+++
PAS	+++	+++	++	+++

AB, Alcian blue; PAS, periodic acid Schiff.
Intensity: (–), no staining observed; (+), low; (++), medium; (+++), strong.

Somatostatin immunoreactivity

SST-cells were observed in the glandular and non-glandular region of the stomach only. Closed-type and open-type immunoreactive cells were found in both stomach regions (Fig. 7A–D).

Gastrin immunoreactivity

GAS-cells were observed only in the glandular region of the stomach. Only closed-type immunoreactive cells were found in this region (Fig. 8A and B).

Discussion

The histological analysis showed that the stratification of the wall of the GIT of *A. bimaculatus* has similar organization to other teleosts. In this study, we observed four layers (mucosa, submucosa, muscular and serous), similar to that described by Chaves and Vazzoler (1984) for *Semaprochilodus insignis*, another Characiformes species. These four layers also have been reported for two Siluriformes species, namely *Pimelodus maculatus* by Santos et al. (2007) and *Rhamdia quelen* by Hernández et al. (2009). These authors reported that muscle tissue between the lamina propria and submucosa aids in the elimination of the substances produced by the glands.

The stomach mucosae layer is lined by a simple cylindrical mucus-secreting epithelium with basal nuclei. This type of stomach lining epithelium in *A. bimaculatus* has been similar to observed in the majority of other teleosts (Díaz et al., 2003, 2008; Domeneghini

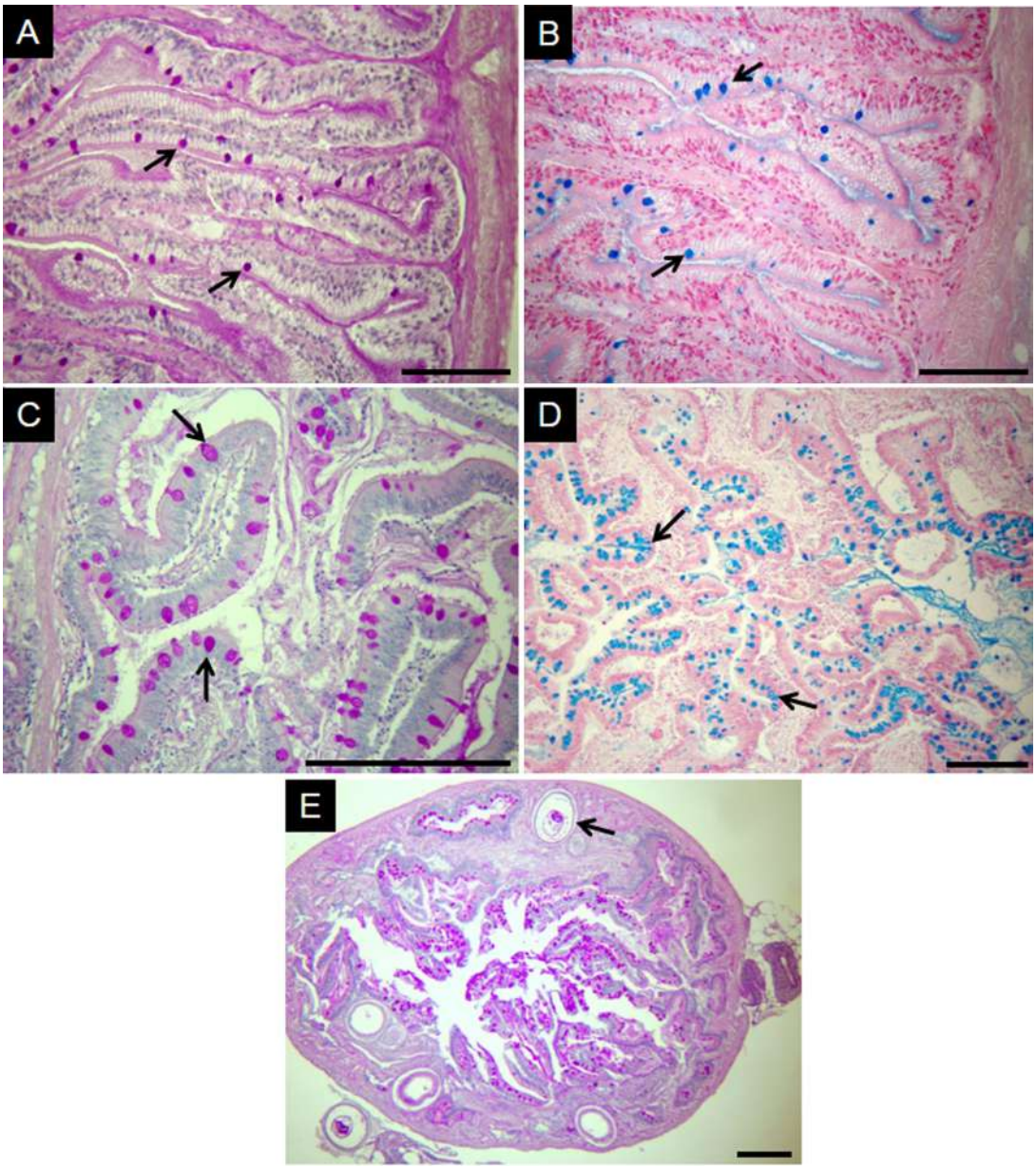

Fig. 5. Photomicrographs of the anterior and posterior regions of the intestines. (A) Overview of the anterior intestine with presence of the neutral glycoconjugates (GCs) (arrow) in the mucus-secreting cells of the epithelium. Periodic acid Schiff (PAS) stain. (B) Anterior intestine with presence of the acid glycoconjugates (GCs) in the mucus-secreting cells of the epithelium (arrow). Alcian blue stain. (C) Posterior intestine presence of many neutral glycoconjugates (GCs) (arrows) in the mucus-secreting cells of the epithelium. Periodic acid Schiff (PAS) stain. (D) Posterior intestine presence of many acid glycoconjugates (GCs) (arrows) in the mucus-secreting cells of the epithelium. Alcian blue stain. (E) Overview of the posterior intestine region highlighting the presence of the parasite (arrow). Periodic acid Schiff (PAS) stain. Scale bar: 100 μm.

et al., 2005; Carrassón et al., 2006; Santos et al., 2007), except for *Epinephelus marginatus* (Borges et al., 2010), that has the anterior stomachal epithelium squamous and cubic type, becoming simple columnar in the posterior region. In this layer one can see pits progressively deeper toward the non-glandular region. Similar pattern was found by Castro et al. (2003) for the Characiformes *Prochilodus marggravii*, *Salminus brasiliensis* and *Leporinus reinhardti*.

According to Stoskopf (1993), gastric glands in fish are not comprised of principal and parietal cells, as in mammals' stomach that display oxynticopeptic cells secreting hydrochloric acid and

Table 3
Frequency (means ± SD) and distribution of the endocrine cells of the gastrointestinal tract of *A. bimaculatus*. *N*, number of samples (5 fields × 2 slides × 36 fishes = 360). H, Kruskal–Wallis statistics.

Organ	Region	5-HT	SST	GAS	H	*P*
Stomach	Glandular	2.66 ± 2.38 (*N* = 360)	2.20 ± 1.32 (*N* = 360)	3.13 ± 3.22 (*N* = 360)	4.15	0.125
	Non-glandular	1.86 ± 1.92 (*N* = 360)	0.53 ± 0.74 (*N* = 360)	0 (*N* = 360)	13.43	0.001*
Intestine	Anterior	0.33 ± 0.60 *N* = 360)	0 (*N* = 360)	0 (*N* = 360)	2.08	0.352
	Posterior	0 (*N* = 360)	0 (*N* = 360)	0 (*N* = 360)	–	–

* Significant at $P < 0.01$.

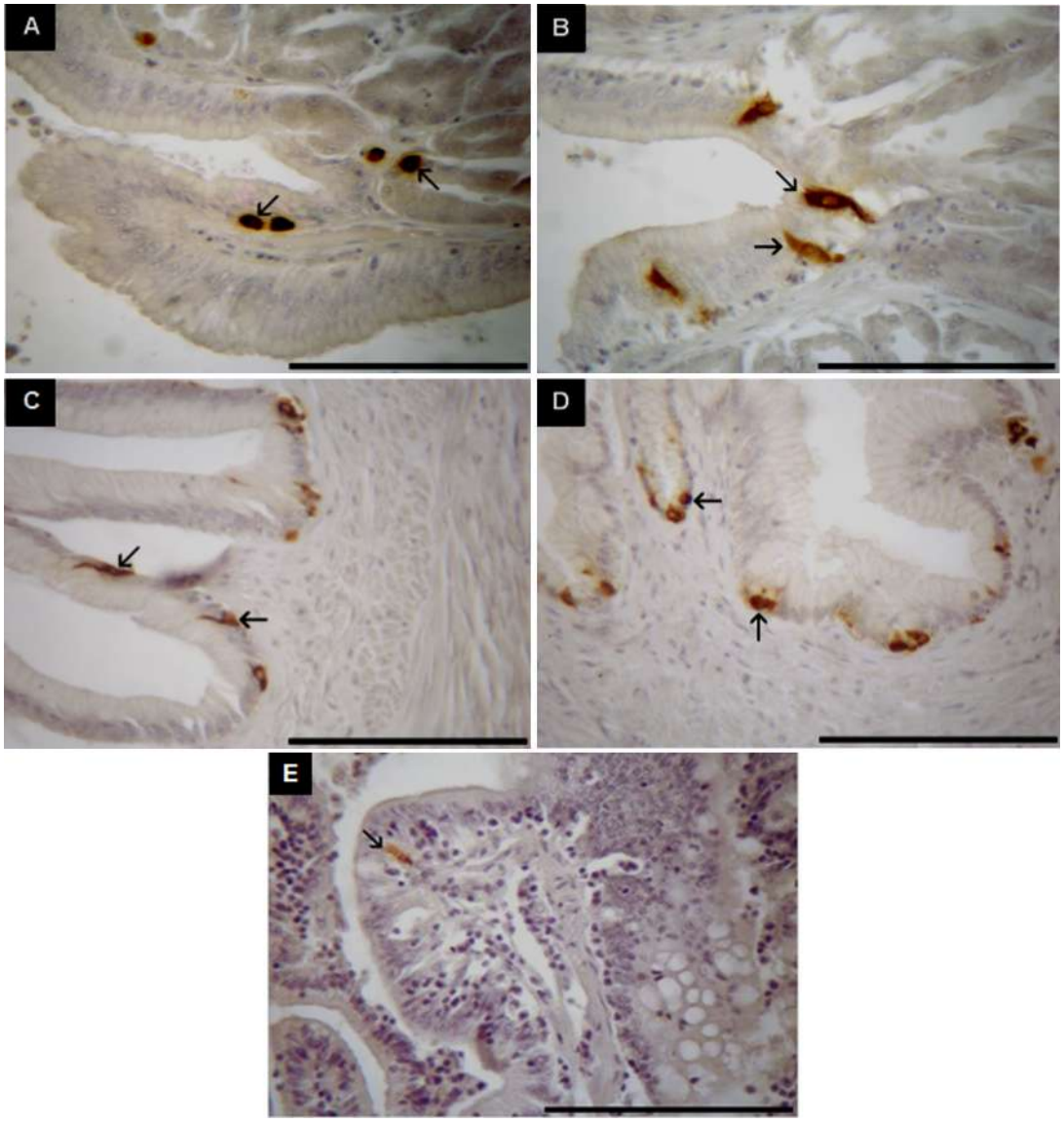

Fig. 6. Photomicrographs of serotonin-immunoreactive cells in the gastrointestinal tract. (A and B) Glandular region of the stomach. (A) Highlighting the existence of closed cell types (arrow) in both the epithelium and in the glands. (B) Featured, open-type cells (arrow) interspersed in the epithelium. (C and D) Non-glandular region of the stomach. (C) Existence of open-type cells (arrow). (D) Featured closed-type cells (arrow). (E) Region of intestine anterior intestine with serotonin immunoreactive cell (arrow). Scale bar: 100 μm.

digestive enzymes, and endocrine cells that produce hormones. Menin and Mimura (1992) comparing three omnivorous teleost fishes stated that the presence of thick folds in the stomach region has the function of storing large volumes of food ingested by these fish. It is likely that in *A. bimaculatus*, another omnivorous species, the thick folds in the stomach had similar function.

The mucus-secreting epithelial cells are very reactive to PAS, but not to AB and this findings match others reported elsewhere (e.g., Castro et al., 2003; Hernández et al., 2009; Vieira-Lopes et al., 2013). According to Grau et al. (1992), secretion of neutral mucosubstances in the stomach epithelium may be related to the absorption of easily digestible molecules, which can be related to the species feeding habit. On the other hand, these mucus substances may facilitate circulation of large food particles as well serve as protection of the mucosa against mechanic injuries (Petrinec et al., 2005).

In this study, no mucosa muscular was found for *A. bimaculatus*. This coincides with the findings of Moraes et al. (1997) and Castro et al. (2003) for other Characiformes species. The submucosa layer of loose connective tissue is rich in connective fibers and blood vessels but lacks glands. On the other hand, the muscular layer is comprised by two layers of smooth muscular fiber, with a circular inner layer and a longitudinal outer layer, similarly to reported for *P. maculatus* (Santos et al., 2007) and *Oligosarcus hepsetus* (Vieira-Lopes et al., 2013).

The submucosa layer is thinner than the muscular layer, and this coincides with the findings of Castro et al. (2003) for three Characiformes species with different feeding habits. This pattern seems no to be associated to feeding habits according to Borges et al. (2010) that reported thinner submucosa layer for the carnivores *E. marginatus*.

The muscular layer in the non-glandular region of the stomach of *A. bimaculatus* is well developed. According to Moraes et al. (1997), well-developed circular muscular layer indicates its role in the maceration of the food bolus. Yet, Castro et al. (2003) reported that this pattern is associated to possible control of carrying and separation of the digested material toward the intestine.

Histologically, the intestine of *A. bimaculatus* is similar to description of other freshwater species such as the *Anguilla anguilla*

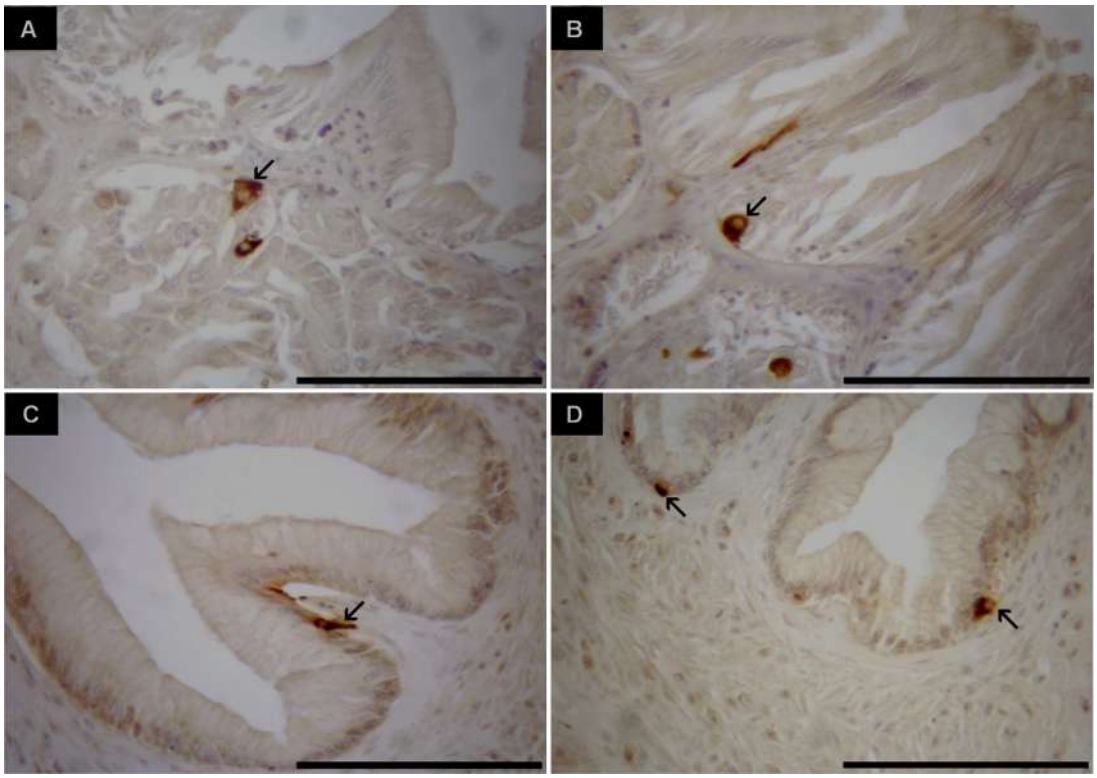

Fig. 7. Photomicrographs of somatostatin-immunoreactive cells in the stomach. (A and B) Glandular region. (A) Highlighting the existence of closed-type cells (arrow) in the glands. (B) Featured, closed-type cells (arrow) in both the epithelium (C and D) non-glandular region. (C) Existence of open-type cells (arrow). (D) Featured closed-type cells (arrow). Scale bar: 100 μm.

(Clarke and Witcomb, 2006) and *Salminus affinis* (Atencio et al., 2008). Regional differences in the distribution of endocrine cells in intestine (anterior/posterior) is associated to absorption of different nutrients, with the anterior intestine being the main local of nutrients absorption with numerous elongated folds. According to Vernier (1990), most lipids and proteins are absorbed in anterior intestine of teleosts, whereas macromolecular proteins are absorbed in the posterior intestine. Moreover, the morphological arrangement of the digestive tract of *A. bimaculatus* is similar to other species of fish with omnivorous habits that have the stomach in "j-shape" and the intestine in "n-shape" (Kapoor et al., 1975; Eckert et al., 1988). The intermediary size of the intestine is also another indication of the onivory in this species.

According to the observed folds in the intestine of *A. bimaculatus* there is a slight change in the distribution pattern in each segment, being more complex in the anterior portion of this organ. According to Stoskopf (1993), the posterior region of the intestine is difficult to identify, however posterior mucosa protrusion is more simple than the anterior mucosa.

Simillaly to other teleosts (Hernández et al., 2009; Vieira-Lopes et al., 2013), *A. bimaculatus* also have a great number of goblet cells in the posterior intestine. Increasing in goblet cells in the posterior region of the intestine may be related to ions and fluids assimilation that occur in this part of the GIT as reported by Petrinec et al. (2005). Other studies (e.g., Khanna and Mehrotra, 1971) also suggested that the higher number of these cells could facilitate elimination of the food bolus.

The use of histochemical techniques of PAS and AB, enable to observe two intestinal segments (anterior and posterior) for *A. bimaculatus*. The mucus-secreting cells, responsible by the production of mucus for lubrication of mucosa surface have granules secretion positive to PAS and AB, suggesting large amount of neutral

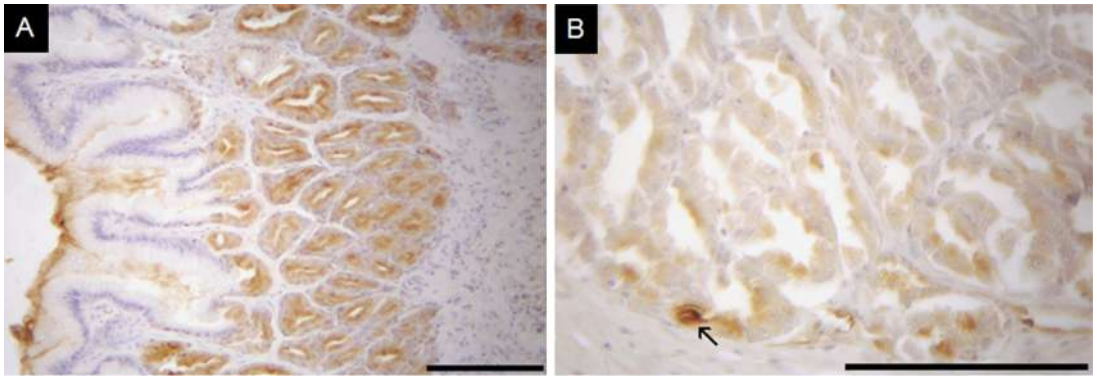

Fig. 8. Photomicrographs of gastrin-immunoreactive cells in the stomach. (A and B) Glandular region. (A) Presence of gastrin-immunoreactive cells only in cells of mucous glands. (B) Highlighting the existence of closed cell types (arrow) in the cells of the mucous glands.

and acids GCs, respectively. However, in the stomach, the muco-secreting cells were positive to PAS only.

In the posterior region of the intestine, we observed the presence of a parasite in approximately 60% of the examined species. This contributed to disorganization of the histological pattern in this region. According to Abdallah et al. (2004), low prevalence of parasites can occur in GIT of characins in impacted systems, and there is a relationship between parasite diversity and environmental quality. According to Castro (1992), GIT of several fish species are favorable environment for establishing and growth of pathogenic organisms (e.g. helminthes endoparasites), thus inducing inflammation and alteration of local tissues function.

Two patterns of endocrine cells were visualized by immunohistochemical techniques, the open and closed-type cells. The "open-type" cells have apices reaching the mucosa surface, responding to chemical stimulus and pH alteration of the lumen. In the "closed-type" cells, the apices did not reach the mucosa surface, and respond to hormonal stimulus from the blood flow and or mucosa stretching from digestion (Rodrigues et al., 2005).

The stomach was the most important site for lodgment of the three studied hormonal activities. According to Pan et al. (2000a), the 5-HT-IR immunoreactive cells have strong effect in regulation and digestive function. Frequency and distribution of 5-HT-IR were higher in stomach of *A. bimaculatus* compared with the catfish *Ictalurus punctatus* (Min et al., 2009) and the Cyprinidae *Garra rufa* (Kuru et al., 2010) where the highest frequency 5-HT-IR cells occur in the anterior region of the intestine. Coinciding with the findings of present study, a higher number of 5-HT-IR cells in the stomach was reported for the Characiforms *Colossoma brachypomum* and for the Perciforms *Tilapia nilotica* by Pan et al. (2000b).

The frequency of 5-HT-IR cells in the intestine for *A. bimaculatus* found in this study was comparatively lower than the reported by Pan et al. (2000b) for the snakehead *Channa argus*, a Perciformes fish and for the Amur catfish *Silurus asotus*, a Siluriformes fish. Likewise, Ku et al. (2004) also found high frequency of 5-HT-IR cells for the Freshwater minnow *Zacco platypus*, a Cypriniformes fish. The absence of 5-HT-IR cells in the posterior region of the intestine may be associated with the presence of parasites that may alter the wall structure. According to Fairweather (1997), parasites can seriously alter the gut wall structure and interrupt the communication between the nervous system and the endocrine system.

Somatostatin is expected to exert inhibitory effects on different body parts, such as inhibition of gastric acid secretion, gastric and duodenal motility, pancreatic exocrine secretion, biliary vesicle contraction and nutrient absorption (Rodrigues et al., 2005). Its secretion may be stimulated by factors such as the presence of acid in the stomach and nutrients in the gut (Nelson and Sheridan, 2005). In this study, the presence of somatostatin was restricted to the both stomach regions. Vieira-Lopes et al. (2013) found that this hormone can result in the inhibition of various substances such as GAS, CCK, GLUC, INS, as well as control and absorption of amino acids and glucose as it operates as mechanisms for efficient food processing.

SST-IR cells were not found in intestine of *A. bimaculatus* and the absence of this hormone was also reported by Vieira-Lopes et al. (2013) for *O. hepsetus*, other Characiformes species. On the other hand, other fish species are reactive to these cells both in the stomach and in the intestine, such as the largemouth black bass *Micropterus salmoides* (Pan et al., 2000b), the minnow *Pseudophoxinus antalyae* (Çinar et al., 2006), the channel catfish *I. punctatus* (Min et al., 2009) and the carp *G. rufa* (Kuru et al., 2010).

GAS-IR cells were restricted to the glandular stomach region in *A. bimaculatus*. Its occurrence in tetrapods is limited to the central mucosa of the stomach, whereas the expression in fish GAS-IR cells constantly vary from species to species (Vigliano et al., 2011). The occurrence of only close-type cells in *A. bimaculatus*, coincided with other studies that also found only this type of cells in *C. argus* and in the yellow catfish *Pelteobagrus fulvidraco* (Pan et al., 2000b). Moreover, no GAS-IR cells were found in the stomach of the catfish *R. quelen* that was present only in the intestine (Hernández et al., 2012), indicating that the distribution of this hormone can vary depending on the species examined.

In brief, the three types of endocrine cells (5-HT, SST and GAS) in GIT of *A. bimaculatus* occurred in the stomach regions, which suggest that this organ is the main site of food digestion for this species. This study is a step for understanding the digestive physiology of this species and provide a basis for comparisons with other Neotropical fishes.

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