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Histology of the digestive tract of *Satanoperca pappaterra* (Osteichthyes, Cichlidae)

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ABSTRACT. This study aimed to locate, describe and characterize the esophagus, stomach and intestine of *Satanoperca pappaterra* using histological techniques. The species presents detritivore-invertivore feeding habit and is widely distributed in Neotropical continental waters. The esophagus is short, the stomach is small with saccular form and the intestine is long. The histological sections were stained using hematoxylin/eosin and Periodic Acid Schiff. Throughout the digestive tract, the gastric wall is composed by four different tunicae: mucosa, submucosa, muscularis externa and serosa, besides distinct intrinsic innervation represented by the submucosal and myenteric ganglionated plexuses. Depending on the organ, several characteristics were peculiar such as the external muscle layer of the esophagus that permeates the submucosa; a single sphincter between the stomach and intestine; stomach without differentiated regions; intestine histologically and physiologically divided into two regions (proximal and distal), considering the villi height and spacing besides the density of the goblet cells.

Keywords: freshwater fish, Cichlidae, histological methods, digestive tract.

Histologia do trato digestivo de *Satanoperca pappaterra* (Osteichthyes, Cichlidae)

RESUMO. Este estudo teve por objetivo localizar, delimitar e caracterizar histologicamente o esôfago, estômago e intestino de *Satanoperca pappaterra*. A espécie possui hábito alimentar detritívoro-invertívoro e é amplamente distribuída em águas continentais neotropicais. Macroscopicamente, o esôfago é curto, o estômago é pequeno e com formato sacular e o intestino é longo. Os cortes histológicos foram corados com hematoxilina/eosina e ácido periódico de Schiff. Ao longo de todo o tubo digestório, nota-se quatro túnicas distintas compondo a parede: mucosa, submucosa, muscular externa e serosa e distinta inervação intrínseca representada pelos plexos ganglionados submucoso e miontérico. Dependendo do órgão ficaram evidentes características peculiares, tais como, a muscular externa do esôfago que permeia a submucosa; um único esfíncter entre o estômago e o intestino; estômago sem regiões diferenciadas; intestino dividido histológica e fisiologicamente em duas porções (proximal e distal), considerando a altura e espaçamento das vilosidades e a densidade das células caliciformes.

Palavras chaves: peixe de água doce, Cichlidae, método histológico, tubo digestório.

Introduction

The Cichlidae presents the highest species richness, with about 1,300 described species, one of the greatest among vertebrates (KULLANDER, 2003). In South America, this family is represented by approximately 50 genera and an estimative of 450 species (6 - 10% of freshwater fish worldwide) (KULLANDER, 1988). This is an evolutionarily interesting group because presents peculiar morphological traits that reflect specializations in the diet (RÜBER; ADAMS, 2001; LÓPEZ-FERNÁNDEZ et al., 2005; SAMPAIO; GOULART, 2011).

Despite the high diversity and the great ecological importance of this group, little is known about Neotropical cichlids. The most studied representatives of feeding or trophic morphological aspects of this

family in Neotropical water are the *Cichla* (COCHRAN-BIEDERMAN; WINEMILLER, 2010; HELLIG et al., 2010; SANTOS et al., 2011) and *Oreochromis* species (CACECI et al., 1997; MORRISON; WRIGHT, 1999; NJIRU et al., 2004), due to the importance for aquiculture and species of the subfamily Geophaginae (LÓPEZ-FERNÁNDEZ et al., 2005).

Satanoperca pappaterra (Heckel, 1840), also identified as *Geophagus pappaterra* until the review by Kullander (1986) apud Kullander and Ferreira (1988), is a cichlid widely distributed in continental water from Brazil, Bolívia and Paraguai (KULLANDER, 2003), however its autecology is scarcely known. Hahn and Cunha (2005) described the digestive tract and the diet of this species and

ascribed the detritivore-invertivore feeding habit to the constant presence of detritus and benthic organisms in the stomach content. The authors observed that the stomach is similar to a small sac and is positioned as a lateral “diverticulum” at the beginning of the long intestine. This pattern does not resemble the morphology described to most teleosts, although the shape above mentioned is reported in the literature for other cichlids, as *Oreochromis niloticus* (MORRISON; WRIGHT, 1999), and *Retroculis lapidifer* (MOREIRA; ZUANON, 2002).

Besides the function of digestion and absorption, the stomach and intestine may play other functions, e.g., assist breathing as in *Plecostomus* sp., *Ancistrus* sp., *Hoplosternum* sp. and *Loricariichthys* sp. (GIAMAS et al., 2000; SILVA et al., 1997; ZAVALA-CAMIN, 1996) and in the osmoregulation as in *Anguilla* sp. and *Trichomycterus* sp. (HERNANDEZ-BLAZQUEZ, 1995; RIBEIRO; FANTA, 2000), sufficient indication of the plasticity of these organs to change their structure and physiology. In these cases, when the organ shape creates uncertainties about the real function, histological studies are an excellent tool. According to Brett and Groves (1979), the routine of histological sections assists on the quantification, through quantitative morphometrical techniques, and also allow the researcher, through a more detailed analysis, to obtain data for physiological and/or biochemical tests, which favor the correlation between structure and function.

Therefore, regarding the shape, position and size of the stomach from *S. pappaterra* and the disproportion with the intestine length, the present study aimed to locate, describe and characterize the regions of the digestive tract from this species, using histological techniques. Additionally, we intend to contribute to broaden the knowledge about the species and consequently about Neotropical cichlids.

Material and methods

Individuals of *S. pappaterra* were gathered in September 2007, using gill nets, at two environments from the upper Paraná river floodplain, Porto Rico County, Paraná State, Brazil (Garças Lake, 22°43'27.18"S; 53°13'4.56"W), located in the right bank of the Paraná river and (Leopoldo Backwater, 22°45'24"S; 53°16'79.8"W), situated in Mutum Island, both directly connected to the river. Voucher specimens were deposited in the Ichthyological Collection from the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia - State University of Maringá, Paraná State, Brazil), under the number 1841.

After the sampling, five adult specimens were selected, with mean length of 16.17 and standard deviation of ± 1.46 cm. Afterwards, we removed the digestive tract of the individuals, modeled freehand and delimited the regions between the esophagus and intestine to be processed histologically (Figure 1).

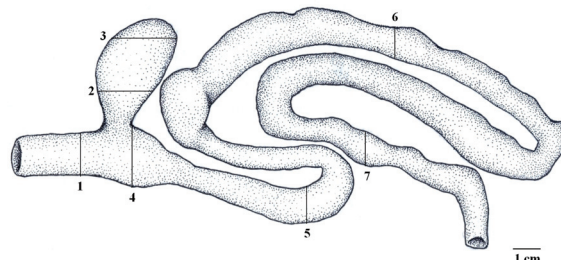


Figure 1. Digestive tract of *S. pappaterra*: esophagus (1), stomach (2, 3), transition region (4) and intestine (5, 6 and 7). Drawing: Gabriel Deprá

For the histological analysis, the samples were fixed using Bouin's solution for 48 hours, dehydrated at increasing alcohol concentrations, cleared in xylene, paraffin-embedded and sectioned transversely and sagittally with a thickness of 7 μ m. After that, the sections were stained with hematoxylin-eosin (HE) to characterize the general morphology of the organs, and then we performed the histochemical reaction, which evidences the mucus-producing cells, through the Periodic Acid Schiff (PAS) (BEÇAK; PAULETE, 1976).

The analyses were carried out with optical microscopy and the photo documentation with optical microscope BX 41, coupled to an image capture system.

Results

The complete digestive tract of *S. pappaterra* is long, with mean length of 27.78 and standard deviation of ± 2.48 cm, with approximately twice the mean standard length of the animal.

The histological analyses evidenced, along the entire digestive tract, the presence of four distinct tunicae composing the wall: mucosa, submucosa, muscularis externa and serosa, besides distinct intrinsic innervation represented by the submucosal and myenteric ganglionated plexuses, in accordance to the organ considered.

Macroscopically, we observed anatomical structures similar to a sphincter delimiting the stomach from the intestine (transition region), which was corroborated through microscopic analysis. At this region (base of the stomach) there is confluence of striated muscle fibers and collagen

fibers, which suggest a control mechanism. This is a true pyloric sphincter since is composed by fibromuscular folds of the wall, facing the intestinal lumen, with components of smooth and striated muscle. (Figure 2).

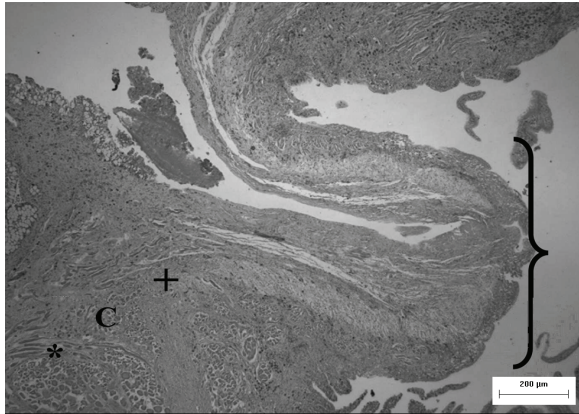


Figure 2. Transition region of the digestive tract from *S. pappaterra*, evidencing the pyloric sphincter fold (curly bracket) with confluence (C) of the striated muscle fibers (*) and collagen fibers (+) HE.

The esophagus is short, about 0.5 cm long, wide, straight and is connected to the swim bladder through the pneumatic duct. Microscopically, this organ is composed by several folds, lined with mucus-secreting epithelium and lamina propria that presents esophageal glands (Figure 3A). We did not observe the muscularis mucosae. The submucosa is compounded by moderately dense connective tissue, presenting cells with acidophilic granules. This layer is permeated by the muscularis externa, which is composed by two well-developed layers of striated muscle (Figure 3B), lined with a poorly-defined serosa.

The stomach is sac-like and very small, and is positioned laterally to the intestine, similar to an

appendix. The cell composition is uniform throughout the wall extension, and for this reason without division into distinct regions. The mucosa exhibits tall and uniform folds, with evident borders, is completely glandular and presents simple cylindrical mucus-secreting epithelium, revealed by the PAS positive reaction (Figure 4A). At the lamina propria, we observe tubular gastric glands formed by a single cell type (oxintopeptic) with central nucleus. These glands are permeated by collagen and smooth muscle fibers that are organized to form the muscularis mucosae. The submucosa presents several collagen fibers, developed blood vessels, beyond the presence of numerous cells with acidophilic granules that have infiltrated into the epithelium (Figure 4B). The muscularis externa tunica is compounded by smooth muscle fibers arranged in two layers, the inner is thick, formed by fibers in the transverse direction (inner circular) and the outer is reduced, composed by fibers in the longitudinal direction (outer longitudinal).

A very thin serous layer forms the outer lining of the entire stomach. This is constituted by a thin layer of connective tissue lined with simple squamous epithelium (mesothelium) (Figure 5).

Macroscopically, the intestine is tubular, long, with mean standard length of 26.78 ± 2.48 cm, presenting four folds that form loops. The inner lining of the intestine presents simple cylindrical epithelium compounded by enterocytes and typical goblet cells, which lines the intestinal villi in all the extent, and we did not verify crypts. The enterocytes show columnar form with a cylindrical nucleus arranged in the basal region forming borders facing the lumen. The goblet cells (mucus-producing), evidenced by PAS histochemical method, are arranged along the entire length of the intestinal tract; besides that their frequency are sharply higher at the final portion of the tract.

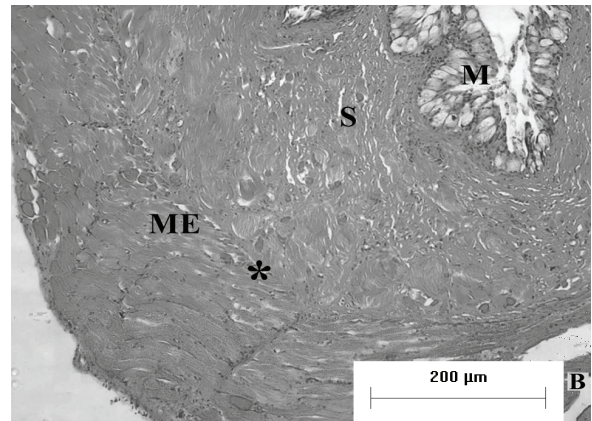
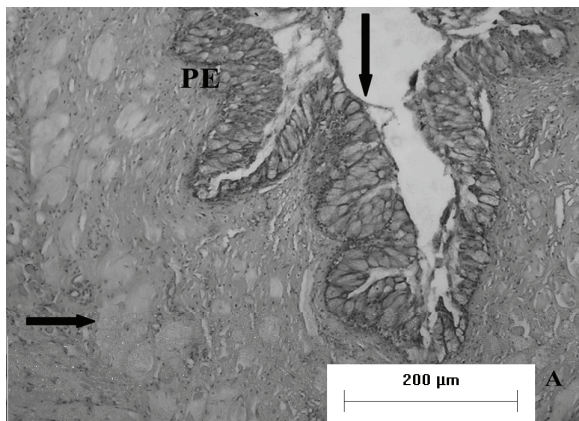


Figure 3. Esophagus of *S. pappaterra*, evidencing A: esophageal fold (PE), mucus-secreting epithelium (vertical arrow); and esophageal glands (horizontal arrow) PAS; B: mucosa (M), submucosa (S) and striated muscle fibers (*) compounding the tunica muscularis externa (ME) HE.

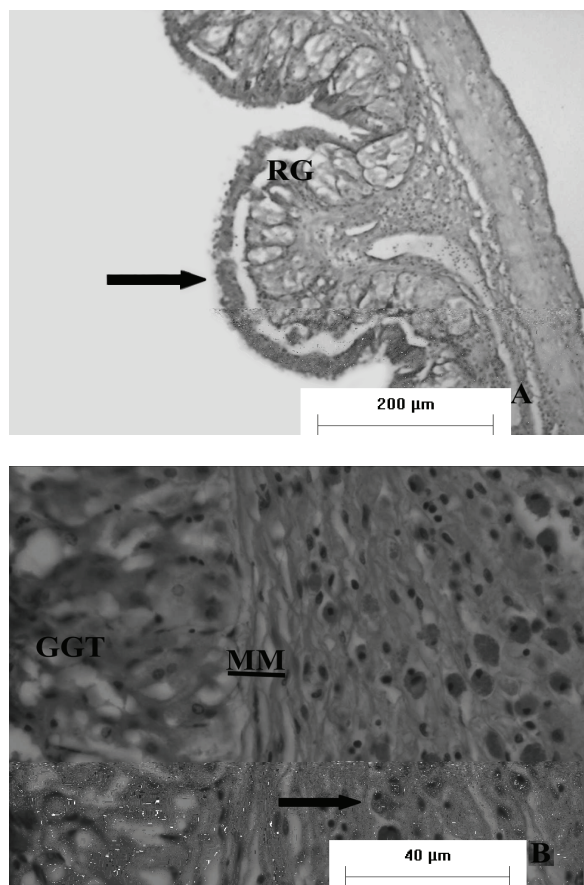


Figure 4. Stomach of *S. pappaterra* - A: simple cylindrical mucus-secreting epithelium – (arrow) and glandular region (RG) - PAS; B: evidencing the lamina propria with tubular gastric glands (GGT); muscularis mucosae (MM) and submucosa with acidophilic cells – arrow (HE).

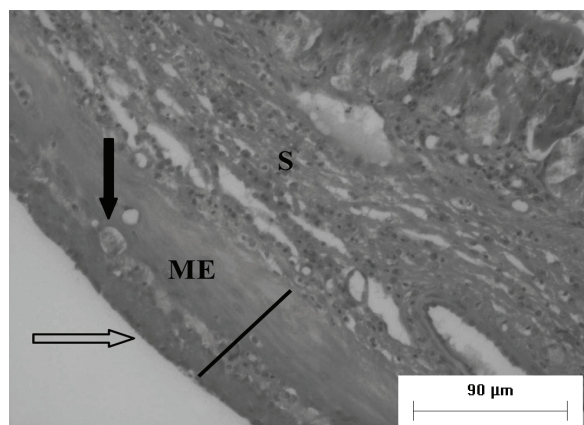


Figure 5. Stomach of *S. pappaterra*, evidencing the submucosa (S); the smooth muscle layers of the muscularis externa tunica (ME - bar); myenteric ganglionated plexus (full arrow) and serous tunica (empty arrow) (HE).

Histologically this organ was divided into proximal and distal regions by the presence of distinct characteristics. At the proximal portion, the villi are tall, numerous, next to each other and with

foliaceous aspect (Figure 6A), whereas at the distal region, the villi have reduced height, less numerous and with digitiform aspect (Figure 6B). The lamina propria is composed by loose connective tissue and small caliber blood vessels. Smooth muscle fibers compound a thin layer, the muscularis mucosae, which are projected toward the lamina propria and also permeate the submucosa tunica. The latter is relatively developed, compounded predominantly by collagen fibers, blood and lymphatic vessels.

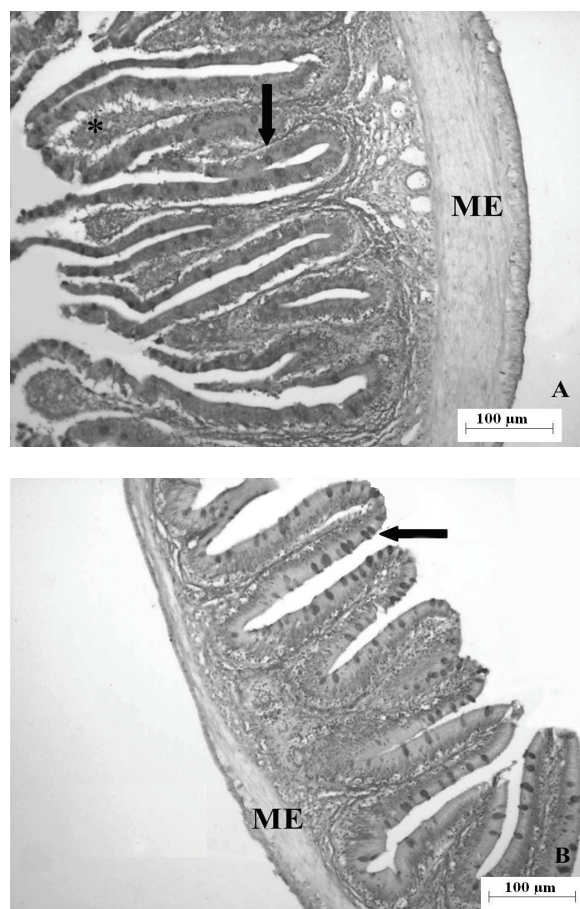


Figure 6. Intestine of *S. pappaterra*, evidencing: A – proximal intestine: villi tall and close to each other (*), goblet cells (arrow) and thick muscularis externa (ME). B – distal intestine: low and sparse villi (*), goblet cells (arrow) and reduced muscularis externa (ME) PAS.

The muscularis externa tunica is formed by two layers of smooth muscle fibers, oriented internally in the transverse direction and externally in longitudinal direction, and at the proximal portion, the muscle layer is thicker than the distal. The fourth and the outer tunica of the intestine wall, the serous tunica, is extremely reduced, with mesothelial lining (Figure 7).

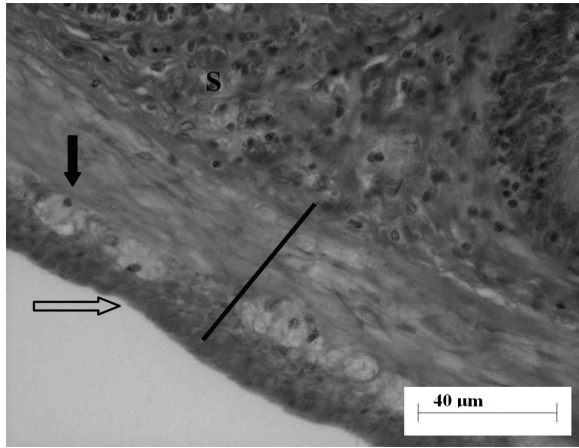


Figure 7. Proximal intestine of *S. pappaterra*, evidencing the submucosa tunica (S), the muscularis externa (bar), the myenteric plexuses (full arrow) and serous tunica (empty arrow) (HE).

Discussion

The morphological and anatomical structure from the digestive tract of *S. pappaterra*, suggests an adaptation to the detritivore-invertivore feeding habit (HAHN; CUNHA 2005; CASSEMIRO et al., 2008). The reduced size of the stomach is probably justified by the fact that this organ serves to brief passage of food, where the chemical digestion occurs. Otherwise, the intestine is long due to the low-energy food that requires slower intestinal transit for digestion, absorption and utilization.

The esophagus morphology is similar to the description by Gargiulo et al. (1996) and Morrison and Wright (1999), for tilapias, which observed stratified epithelium with mucus-producing cells and striated muscle from the beginning to the end of the organ wall. The folds of the esophagus described for other teleosts (TAKASHIMA; HIBIYA, 1995; RANDAL; KUZ'MINA, 2000; SANTOS et al., 2007) were also observed in *S. pappaterra*.

The position that the sphincter occupies in the digestive tract and the histological characteristics (mainly the striated muscle at the muscularis externa tunic) suggest that the sphincter controls the entry of recently swallowed food until the stomach. Probably this prevents that the food passes directly into the intestine without chemical digestion, and then releases the digested content from the stomach to the intestine. Morrison and Wright (1999) also mention a type of valve between the pyloric region of the stomach and the intestine of tilapia, which despite histologically similar to that of *S. pappaterra*, is anatomically in different region.

The histological analyses confirm that this organ is restricted only to the saccular structure, described by Hahn and Cunha (2005). The most remarkable

difference in the stomach from this species refers to the histological uniformity of the wall, not consistent with previous studies with other cichlids (MORRISON; WRIGHT, 1999; OSMAN; CACECI, 1990) and several other teleosts (ALBRECHT et al., 2001; BICCA et al., 2006; SILVA et al., 2005), which observed three histologically distinct regions: cardiac, intermediary or middle and pyloric. We may deduce that the absence of these differences as well as the presence of well developed folds of the gastric mucosa suggest adaptation to increase the internal surface and perform exclusively function of chemical digestion. For Osman and Caceci (1990), the mucosal folds may allow delay in the food passage through the stomach into smaller portions, permitting an efficient mixing of the food bolus with digestive fluids.

The presence of mucus-secreting mucosa should have the task of facilitating the food passage through the organ, adjust the pH, as well as protecting from the acid content, as suggested by Osman and Caceci (1990) and Morrison and Wright (1999), for *Tilapia nilotica* and *Oreochromis niloticus*, respectively; and moreover, under normal conditions the secreted mucus functions as a barrier against microorganisms in larvae and juvenile fish (RINGØ et al., 2007). Tubular gastric glands with oxintopeptic cells, which were observed throughout the entire lamina propria, produce simultaneously pepsinogen and hydrochloric acid, corroborating the descriptions by Osman and Caceci (1990), Rotta (2003), Chatchavalvanich et al. (2006) and Santos et al. (2007) in studies with different fish species.

The presence of several cells with acidophilic granules suggests that are mast cells, which mediate immune responses. According to Osman and Caceci (1990) the presence of immune cells in the mucosa and submucosa of tilapia reflects a competent defense system of fish against invading pathogens. Agius and Roberts (2003), verified an increase in the number of other immune cells, such as macrophages, in fish inhabiting polluted water. For Bernstein et al. (1998), the presence of these cells in the digestive tract, comparable to that found in mammals, is related to the first line of defense of fish, as described by, Peretti and Andrian (2008) for different fish groups, including a cichlid species.

The intestinal length of *S. pappaterra* is directly related to the species feeding habit, corresponding to studies carried out by several authors (FUGI et al., 2001; RODRIGUES; MENIN, 2008) that related the relative length of intestine with the feeding habit, which is shorter in carnivores, and longer in herbivore and detritivore. In accordance with

Hidalgo and Alliot (1987), the intestine length influences extremely the quantitative aspects of digestion and absorption of food. The well-developed structure of intestinal mucosa, characterized in the present study, due to the presence of villi long and close to each other in the anterior region of the intestine, was also described by Manjakasy et al. (2009) as directly involved in absorption processes. Indeed, the intestinal length together with the villi presence increase the number of enterocytes, cells that have the function of final digestion, absorption of nutrients, proteins and lipids (proximal portion) as well as water, electrolytes and protein macromolecules (distal portion) (TAKASHIMA; HIBIYA, 1995). For Seixas Filho et al. (2001), the arrangement of mucosal folds and the speed of food transport within the intestine, slow the food advance in aboral direction, which allow longer digestive period and consequently better utilization of the nutrients. The gradual increase in the number of goblet cells from proximal to distal intestine, found in *S. pappaterra* is supported by the descriptions from Rotta (2003) for different teleost species, including cichlids, from Çinar and Şenol (2006) for *Pseudophoxinus antalyae*, from Peretti and Andrian (2008) for *Asiyanax altiparanae* and from Manjakasy et al. (2009) for two piscivore species from the order Beloniformes. In accordance to Rotta (2003), the mucus produced by these cells, plays a key role in protecting the intestinal mucosa against the hydrochloric acid from the stomach, besides lubricating the food bolus. Nevertheless, the increase in quantity of these cells towards the distal region of the intestine, does not match the description of Takashima and Hibiya (1995) for *Oncorhynchus mykiss*, which recorded higher density of goblet cells in the proximal portion.

According to Takashima and Hibiya (1995), the histological structure of the wall from the alimentary tract of fish is generally similar to the other vertebrates; the difference is in the extraordinary morphological diversity observed in the different fish groups, which according to Santos et al. (2007) reflects the different positions in the food chain that these animals occupy. This fact is partially due to the constant changes physical, chemical and biological that the organisms are subjected in the environment.

For Kullander (2003), behavioral and morphological characteristics are important factors explaining the high diversity of cichlids, including among them, anatomical changes in different feeding mechanisms. Therefore, the fish, in general, are adapting somehow to environmental stimulus more convenient that can ensure better utilization of ingested food and, consequently, higher

performance. Thus it is justified the great variety of feeding habits and the high morphophysiological diversity of the digestive tract in fish.

The histological characterization of the digestive tract of *S. pappaterra*, presented here, serves as theoretical and practical bases for further studies about trophic behavior of cichlids.

Conclusion

The histological characterization of the digestive tract of *S. pappaterra*, presented here, serves as theoretical and practical bases for further studies about trophic behavior of cichlids.

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