Received: 9 August 2021 | Accepted: 8 November 2021

DOI: 10.1111/ahe.12763

ANATOMIA HISTOLOGIA EMBRYOLOGIA JOURNAL OF VETERINARY MEDICINE WILEY

ORIGINAL ARTICLE

Comparative analysis of the small and large intestines of Acará *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (Pisces: Cichlidae)

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Funding information
Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Grant/Award Number: APQ03232-15; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 306946/2016-5 and 407719/2016-4; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: 001

Abstract

Geophagus brasiliensis, popularly known as acará, is a common fish in lentic freshwater environments in South America. This species has a detritivorous-iliophagous or omnivorous feeding habit, with high food plasticity; however, there are no studies describing its intestinal tract histologically. Therefore, the present study analysed through histological and histochemical techniques the intestines of the acará. Adult specimens were collected with gillnets, anaesthetized and euthanized. Then, the fish were submitted to biometry and dissection to remove fragments of intestines. The samples were fixed in Bouin liquid for 12 hours and subjected to histological and histochemical techniques. Histologically, all samples of intestines were organized into four layers: mucosa, submucosa, muscular and serosa. The small intestine (foregut and midgut) was characterized by the presence of intestinal villi covered by simple prismatic epithelium with a striated border and goblet cells supported by the connective lamina propria. In the large intestine (hindgut), there was an absence of villi and an abundance of goblet cells. Positive reaction to Periodic Acid–Schiff (PAS) and Alcian Blue (AB) pH 2.5 reactions were detected in goblet cells, indicating the presence of mucosubstances. No lipids were detected in the intestine cells due to the negative reaction to the Sudan Black B. The results of the present study provide subsidies for a better understanding of the intestinal morphology of teleosts and provide valuable information for phylogenetic studies.

KEYWORDS
enterocytes, goblet cells, lymphocytes, mucosubstances, teleosts

1 | INTRODUCTION

The digestive system of fish presents marked morphological and functional variations, which reflect the wide diversity of this group and its different feeding habits (Díaz, García, Devincenti, & Goldemberg, 2003). The general morphology of the digestive tract is mainly related to the nature of the feeding habits, the environment in which the fish inhabit, and the size and shape of the body (Andrade, Guimarães, Rotundo & Mari, 2017). In teleosts, it has some basic structural similarities, such as its anatomical division, consisting of the buccopharyngeal cavity, oesophagus, stomach, intestines, anus and accessory organs such as the liver, gallbladder and pancreas (Santos, Arantes, Santiago & Santos, 2015). However, depending on the feeding habit, the gastrointestinal tract can vary between species. For example, the size of the stomach and intestines, the presence of pyloric cecum in the

intestines and the content of secretion produced by some cells (Baldisserotto et al., 2014).

The small intestine, also subdivided in foregut and mid gut, is a tubular organ in which food transits and is responsible for alkaline digestion, absorption of nutrients, water and electrolytes, and help the immune system (Nachi et al., 1998). This section of the gastrointestinal tract contains specialized structures such as villi, microvilli and pyloric cecum that increase the absorption area (Santos et al., 2015). The presence of longitudinal folds, and the inner circular and outer longitudinal muscle layers, together promote a peristaltic activity that facilitates the transit of food (Genten et al., 2009).

The large intestine, also called hindgut, located in the caudal region does not present villi and is related to the absorption of water and electrolytes, mucus secretion and faeces elimination. This section presents specializations such as intestinal absorptive cells and numerous goblet cells (Amorim et al., 2009).

Geophagus brasiliensis (Quoy & Gaimard, 1824), popularly known as acará, cará or papa-terra, belongs to the order Cichliformes. It is widely distributed in lentic environments of coastal watersheds in Uruguay and eastern and southeastern Brazil (Buckup et al., 2007; Graça & Pavanelli, 2007; Meschiatti, 1995; Reis et al., 2003). Acará is a territorial species, found in backwaters, close to the bottom, presenting daytime activity, visual guidance and parental care, including storing eggs in a nest or substrate (Barlow, 2000). This species has a great potential for ornamental aquaculture due to its ease of reproduction in captivity, attractive colour and behaviour (Assis et al., 2014; Bizerril & da Silveira Primo, 2001).

The acará has a high degree of food plasticity and has been described at different trophic levels, which reflects its ability to adapt and survive in different environments (Abelha & Goulart, 2004; Bastos et al., 2011; Nunes et al., 2014). Some authors describe its feeding habits as omnivorous, with a high incidence of insects and microcrustaceans (Sabino & Castro, 1990; Stefani, 2006). Others define this species as detritivorous-iliophagous (Meschiatti, 1995). According to Lazzaro (1991), specimens with less than 4.0 cm in size feed mainly on insect larvae, while specimens longer than 4.0 cm have a more diversified diet, with the main foods being insects and gastropods. Other foods are arthropod fragments, plant fragments, sediments, algae and other items (Azevedo et al., 2006).

Due to its ecological and economical importance, and the lack of studies on the gastrointestinal description of *Geophagus brasiliensis*, this study aims to describe the intestines of this species using histological, histochemical and histometric techniques, generating important data for phylogenetic studies and the conservation of the species.

2 | MATERIALS AND METHODS

2.1 | Sampling

The sampling occurred between October and December 2019 in a small lake from the Sao Francisco River basin. Forty ($n = 40$) adult fish were collected (20 males and 20 females). The fish were anaesthetized with Eugenol (30 mg/L) and euthanized by the cross section of the cervical medulla following the ethical principles established by the National Council for the Control of Animal Experiments (CONCEA, 2013). The specimens were transported to the Ichthyology Laboratory of the Postgraduate Program in Vertebrate Biology at PUC Minas, where biometrics and dissection of specimens were performed. The research was approved by the Ethics Committee on the Use of Animals (CEUA PUC Minas protocol No. 26/2019).

2.2 | Light microscopy, histochemical and histometric analysis

For histological analysis, small (foregut and midgut) and large (hindgut) intestine fragments were transversally sectioned and fixed in Bouin's fluid for 8 hrs. Subsequently, the fragments were embedded in paraffin, cut 5 μm thick in the microtome and the histological slides were stained with haematoxylin–eosin (HE) and Gomori's trichrome (TG).

To determine the histochemical content of cells and structures of the intestines, the following techniques were used: periodic acid–Schiff (PAS) for detection of glycogen, neutral glycoproteins and sialomucins; salivary amylase (30 min at 37°C) for glycogen digestion, followed by PAS (Amylase +PAS); Alcian blue pH 2.5 (AB pH 2.5) for carboxylate acid and sulphate glycoconjugates including sialomucins; Alcian blue pH 0.5 (AB pH 0.5) for sulphated glycoconjugates; and Sudan black B (SB) for lipids in general. Histological and histochemical slides were analysed and photographed in an Olympus—BX50 microscope coupled with an Olympus SC-30 camera.

For the histometric analysis, sections on histological slides were analysed and photographed with a magnification of 4X and 20X in five randomly selected fields. Then, villi, epithelium and goblet cells were measured. Measurements were performed using the Olympus CELL software, with an Olympus SC-30 camera coupled to an Olympus—BX50 microscope.

3 | RESULTS

The total length of the specimens in the present study ranged between 15 and 23 cm, with an average of 19.91 ± 2.52 cm. Intestine length ranged between 12.7 and 28 cm. The mean intestine length ratio to body size was 1.00±0.13. Macroscopically, it was not possible to distinguish different sections of the intestine of *G. brasiliensis*.

Histologically, we have identified two distinct regions throughout the intestine. The most cranial region (foregut and midgut) presented the same cellular characteristics, called the small intestine, and the caudal region (hindgut) presented some differences in relation to the others, called in the present study the large intestine.

The small intestine of *G. brasiliensis* was organized in four layers: mucosa, submucosa, muscular and serosa. The mucosa covers the intestinal villi, consisting of simple prismatic epithelium with a striated

or brush border and goblet cells supported by the basal membrane and connective lamina propria (Figure 1a). The epithelium is in contact with the lumen of the digestive tract, and the prismatic cells, called enterocytes, are absorptive. The submucosa layer, located just below the mucosa, was made up of highly vascularized loose connective tissue (Figure 1b). The muscle layer was formed by two muscle bundles along the entire length of the intestine: the inner circular and outer longitudinal layers, both consisting of smooth muscle fibres (Figure 1c). The serosa layer was formed by squamous epithelial cells, mesothelium and a thin layer of loose connective tissue. In the villi, abundant lymphocytes were also observed (Figure 1d).

The large intestine of *G. brasiliensis* was also histologically organized into four layers: mucosa, submucosa, muscular and serosa without the presence of intestinal villi (Figure 2). In the mucosa, intestinal absorptive cells and a large number of goblet cells were observed.

In both intestines, it was possible to detect neutral glycoproteins in the basal membrane due to the positive reaction to the PAS technique and in goblet cells sialic acid-rich glycoproteins, the sialomucins, due to the positive reactions to the PAS and AB pH 2.5 (Figures 1e,f, and 2e,f). No intestine structure showed a positive reaction to the SB and AB pH 0.5 (Table 1).

The histometry of the small intestine structures showed the following values (mean ± standard deviation): villous length =320.18±116.0 µm, height of the lining epithelium=44.18±12.13 µm and diameter of goblet cells = 10.88 ± 1.95 µm. Large intestine histometry showed the following values (mean ± standard deviation): height of the lining epithelium = 52.58 ± 12.30 and diameter of goblet cells = 9.17 ± 1.93.

4 | DISCUSSION

The small and large intestines of *G. brasiliensis* were made up of four distinct layers along their entire length, a common pattern to the intestine of most Actinopterygii (Albrecht et al., 2001; Mello et al., 2019; Wilson & Castro, 2010; Xiong et al., 2011). Studies of these layers indicate that the main differences between them occur in the epithelium and are related to eating habits, type of food ingested, absorption and production of mucous substances (Albrecht et al., 2001; Germano et al., 2014).

Even with no histological and/or histochemical differences, the intestine of teleosts is often subdivided into three segments, based on the metabolic function of each one: foregut, with cells specialized

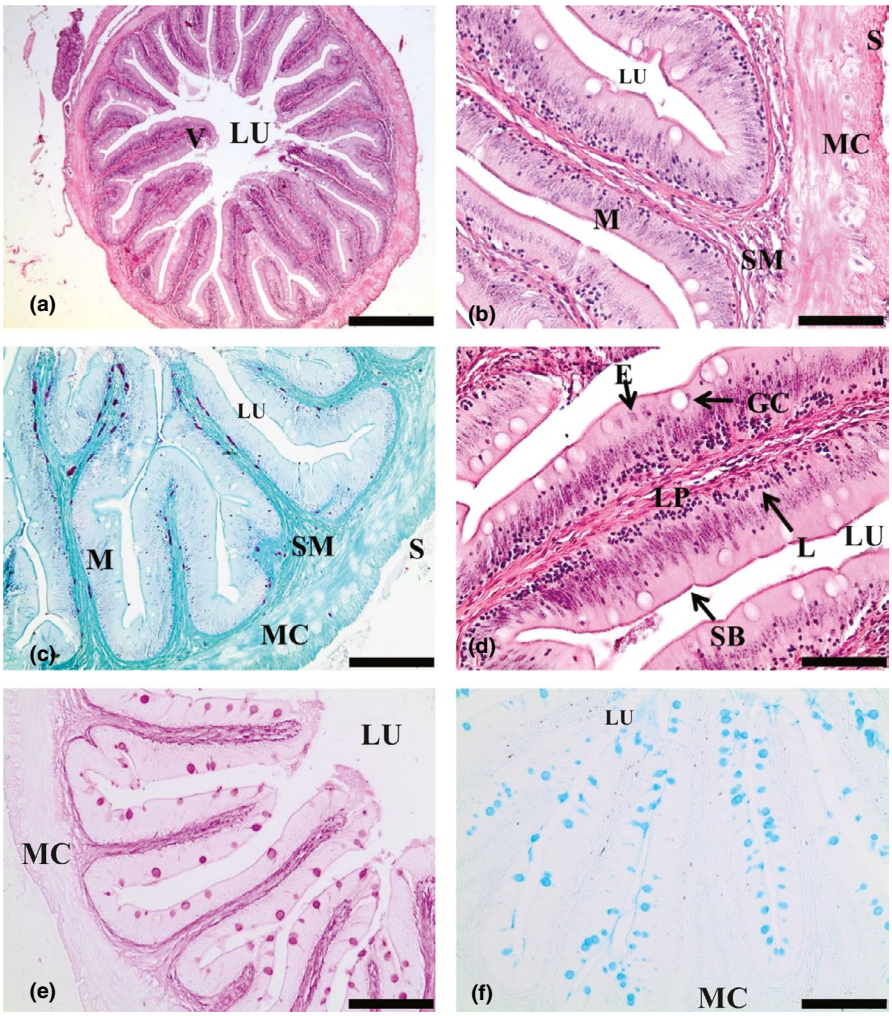

FIGURE 1 Cross sections of the small intestine of *G. brasiliensis*: (a) Panoramic view of the small intestine showing intestinal villi (V) stained in HE; scale bar = 300 µm; (b) mucosa (M), submucosa (SM), muscle (MC) and serosa (S) layers, stained in HE; scale bar = 60 µm; (c) mucosa (M), submucosa (SB), muscle (MC) and serosa (S) layers, stained in TG; scale bar = 150 µm; (d) detail of villi showing connective lamina propria (LP), goblet cells (GC), lymphocytes (L), enterocytes (E) and striated border (SB), stained in HE; scale bar = 60 µm; (e) Positive PAS reaction in goblet cells; scale bar=150 µm; (f) Positive AB pH 2.5 reaction in goblet cells; scale bar = 150 µm.

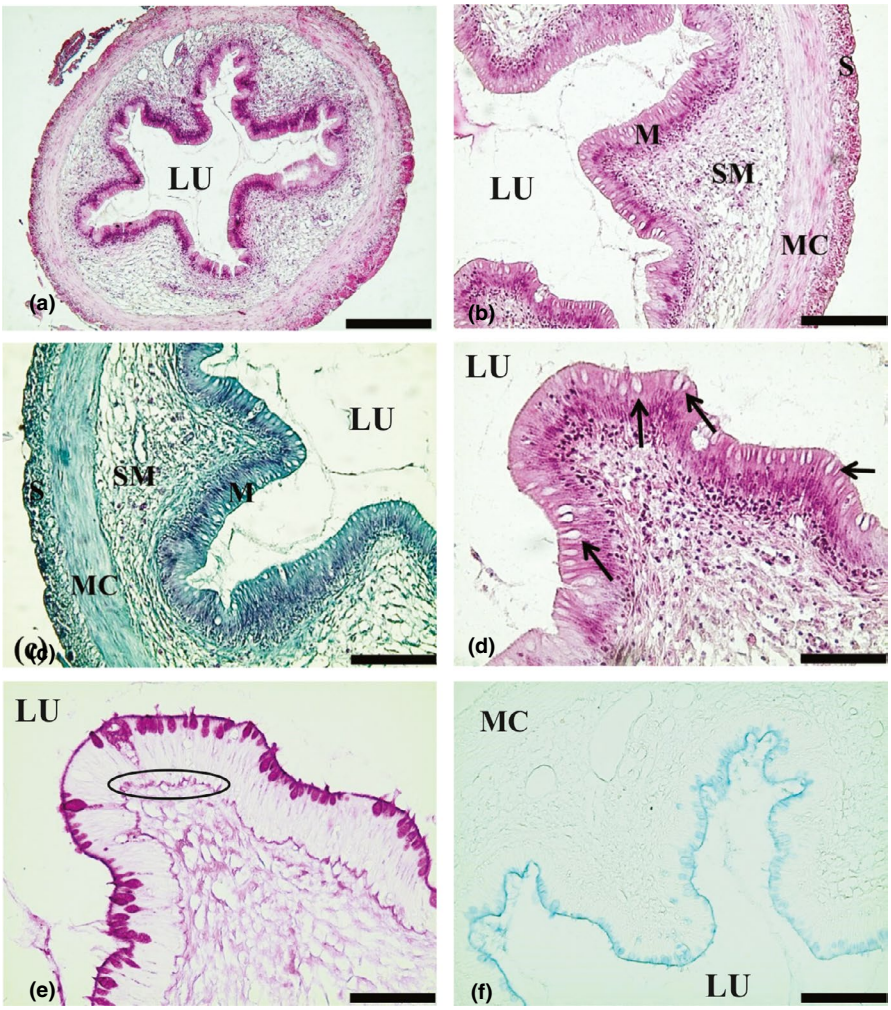

FIGURE 2 Cross sections of the large intestine of *G. brasiliensis*: (a) Panoramic view of the large intestine showing absence of villi stained in HE; scale bar = 250 μm; (b) mucosa (M), submucosa (SM), muscle (MC) and serosa (S) layers, stained in HE; scale bar = 140 μm; (c) mucosa (M), submucosa (SM), muscle (MC) and serosa (S) layers, stained in TG; scale bar = 140 μm; (d) detail of the mucosa full of goblet cells (arrows) stained in HE; scale bar = 70 μm; (e) positive PAS reaction in goblet cells; scale bar = 70 μm; (f) positive AB pH 2.5 reaction in goblet cells; scale bar = 70 μm.

TABLE 1 Histochemical reactions in goblet cells, enterocytes and basal membrane in the small and large intestine of *G. brasiliensis*

	Goblet cells	Enterocytes	Basal membrane
PAS	+	–	+
AB pH 2,5	+	–	–
AB pH 0,5	–	–	–
SB	–	–	–

Abbreviations:: PAS, Periodic acid–Schiff; AB pH 2.5, Alcian Blue at pH 2.5; AB pH 0.5, Alcian Blue at pH 0.5; SB, Sudan Black B; +, positive; -, negative.

in lipid absorption; midgut, responsible for the absorption of proteins and hindgut, whose function is the absorption of water and electrolytes (Clements & Raubenheimer, 2006; Noaillac-Depeyre & Gas, 1979; Stroband & Van Der Veen, 1981). However, from a histological and histochemical point of view, the organization of intestinal segments may be different in taxonomically close species. For example, the analysis of the intestine of *Amatitlania nigrofasciata* showed a homogeneous morphology in all segments (Hopperdietzel et al., 2014). In the present study, the intestines were histologically divided into two distinct segments: the foregut and midgut regions were grouped in the small intestine, and the hindgut was called the large intestine, similar to the procedures by Amorim et al. (2009).

Lymphocytes observed in the intestinal villi and large intestine of *G. brasiliensis* were related to a regulatory function, suppressing the immune system response to intestinal antigens and simultaneously inducing an immune response in the lamina propria (Pabst, 1987). The large number of lymphocytes found in the intestinal mucosa of fish suggests the existence of a local immune system (Rombout et al., 1989). Furthermore, these cells have great potential to detect environmental impacts based on their morphology (Younis et al., 2013).

The goblet cells observed in the present study are generally found in the digestive tract of all vertebrates (Kapoor et al., 1976; Kardong, 2011), releasing sialic acid-rich glycoproteins, the sialomucins, as observed in *G. brasiliensis*. These mucosubstances have epithelial lubrication functions, facilitating the passage of food and macromolecules, in addition to mechanically protecting against bacterial contamination (Mello et al., 2019; Petrinec et al., 2005; Santos et al., 2015; Yashpal & Mittal, 2014). The abundance of goblet cells, and the amount and composition of mucus, may vary depending on environmental conditions and type of food ingested

(Burkhardt-Holm, Schumacher, Welsch & Storch, 1989). The presence of neutral mucosubstances combined with alkaline phosphatase is involved in the digestion and emulsification of food to chyme (Clarke & Witcomb, 1980).

The histometric analysis performed in the present study indicated an increase in the number of goblet cells in the large intestine compared to the small intestine, as observed in studies with other Neotropical species, such as *Lophiosilurus alexandri* (Mello et al., 2019), *Prochilodus scrofa* (Nachi et al., 1998) and *Serrasalmus nattereri* (Raji & Norouzi, 2010). The increase in the concentration of goblet cells in the final region of the intestine is related to the need for greater protection of the mucosa and an increase in its viscosity for faecal liberation (Al-Hussaini, 1949).

Histological, histometric and histochemical data obtained in this study provided important subsidies for understanding the digestive physiology of *G. brasiliensis*. In fact, morphological studies of the digestive tract in fish are considered effective tools for a better understanding of the species' relationship with its habitat (Al-Hussani, 1949; Angelescu & Gneri, 1949; Xiong et al., 2011), in addition to elucidating the mechanisms of ingestion, digestion and absorption of food (Germano et al., 2014).

5 | CONCLUSION

The use of histological, histochemical and histometric techniques proved to be effective in the analysis of the morphological organization of the intestines in fish since the use of macroscopic anatomical differentiation alone does not allow to precisely define the regions of the intestines and their respective characteristics and physiological properties. The two regions detected in the present study, the small and large intestine, were differentiated by the presence or absence of intestinal villi, the height of the lining epithelium, and the diameter and amount of goblet cells.

ACKNOWLEDGEMENTS

The authors want to thank Rogério Matos for mounting the histological slides and PUC Minas for the logistical support. This work had the financial support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) under grant (Finance code-001); Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq under grant (306946/2016-5 and 407719/2016-4); and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) under grant (APQ03232-15).

CONFLICTS OF INTERESTS

The authors declared that there is no potential conflict of interest regarding the research, authorship and publication of this article.

DATA AVAILABILITY STATEMENT

Data supporting the results of this study are available upon request to the author for correspondence.

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How to cite this article: Chaves, A. P. G. D. M., Oliveira, M. G., Paschoalini, A. L., Bazzoli, N., & Santos, J. E. D. (2022). Comparative analysis of the small and large intestines of Acará *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (Pisces: Cichlidae). *Anatomia, Histologia, Embryologia*, 51, 79–84. https://doi.org/10.1111/ahe.12763