Micron 64 (2014) 10–19

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Morphology and histochemistry of the digestive tract in carnivorous freshwater *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae)

Claudemir Kuhn Faccioli[a,b], Renata Alari Chedid[a,c], Antônio Carlos do Amaral[a], Irene Bastos Franceschini Vicentini[a], Carlos Alberto Vicentini[a,*]

[a] Department of Biological Sciences, Faculty of Sciences, São Paulo State University – UNESP, Bauru, SP, Brazil
[b] Institute of Biosciences, Letter and Exact Sciences, São Paulo State University – UNESP, São José do Rio Preto, SP, Brazil
[c] Aquaculture Center of UNESP – CAUNESP, Jaboticabal, SP, Brazil

ARTICLE INFO

Article history:
Received 5 February 2014
Received in revised form 18 March 2014
Accepted 22 March 2014
Available online 1 April 2014

Keywords:
Digestive tract
Ultrastructure
Mucin
Catfish
Hemisorubim platyrhynchos

ABSTRACT

The aim of this study was to characterize the morphology and histochemistry of the digestive tract of *Hemisorubim platyrhynchos*, a freshwater carnivorous catfish found in Neotropical region, using gross anatomy, light microscopy and transmission electron microscopy. This species presented a short and tubular esophagus with thick longitudinal folds. The esophageal mucosa was lined by stratified squamous epithelium containing epithelial cells, club cells and also numerous goblet cells, which secreted acidic and neutral mucins to protect and lubricate the epithelium. The stomach was a J-shaped saccular organ consisting of the cardiac, fundic and pyloric regions. The cardiac and fundic regions contained tubular gastric glands, whereas these glands were absent in the pyloric region. The gastric epithelial cells presented apical secretions that predominantly consisted of neutral mucins. The gastric musculature was, therefore, likely designed for retaining prey and the mechanical preparation of food. The intestine consisted of four regions: anterior, middle, posterior and rectal. The anterior intestine possessed thick folds to increase the surface area for absorption, the middle intestine was coiled and the posterior intestine presented thin folds and a thick musculature. The intestinal epithelium consisted mainly of enterocytes and goblet cells. Enterocytes were columnar cells with a PAS-positive brush border that contained lysosomes in the posterior intestine. Goblet cells were more numerous in the posterior intestine and secreted acidic and neutral mucins important for lubricating and protecting the epithelium. The rectum was lined by columnar epithelium with goblet cells and epithelial cells containing apical acidic and neutral mucins.

1. Introduction

The digestive tract of fishes exhibits morphological and functional variations. According to Wilson and Castro (2010), morphological data of the digestive tract are important for understanding fish nutrition. In addition, morphological studies of the gut are necessary to understand pathological or physiological alterations (Carrassón et al., 2006).

Studies on Neotropical fishes have demonstrated anatomical differences between regions of the digestive tract. In general, these fishes have a short esophagus and a stomach that can be saccular or cecal (Menin and Mimura, 1992; Moraes et al., 1997; Peretti and Andrian, 2008; Rodrigues and Menin, 2008; Hernández et al., 2009). Variations have also been reported in the length of the intestine and the pattern of the intestinal loops (Moraes et al., 1997; Seixas Filho et al., 2000, 2001; Peretti and Andrian, 2008; Rodrigues and Menin, 2008; Hernández et al., 2009).

Although significant differences can be observed macroscopically, the basic histological structure of the digestive tract is similar among species. The esophagus normally consists of a stratified epithelium that is composed mainly of epithelial cells and secretory cells (Menin and Mimura, 1993; Abaurrea-Equisoaín and Ostos-Garrido, 1996; Arellano et al., 2001; Hernández et al., 2009; Cao and Wang, 2009; Fishelson et al., 2011; Xiong et al., 2011; Germano et al., 2013). The stomach presents a simple epithelium consisting of mucus-secreting columnar cells, whereas the intestine is composed of absorptive cells and goblet cells (Menin and Mimura, 1993; Albrecht et al., 2001; Santos et al., 2007; Hernández et al., 2009;

* Corresponding author at: Department of Biological Sciences, Faculty of Sciences, UNESP, Av. Luiz Edmundo Carrijo Coube, 14-01, CEP 17.033-360 Bauru, SP, Brazil. Tel.: +55 14 31036078; fax: +55 14 31036092.
E-mail address: carlosav@fc.unesp.br (C.A. Vicentini).

http://dx.doi.org/10.1016/j.micron.2014.03.011
0968-4328/

Xiong et al., 2011; Løkka et al., 2013). In general, mucus-secreting cells are observed in the epithelial lining of the digestive tract of fish and present wide distribution and histochemical differences (Domeneghini et al., 2005). The large amount of mucus produced by these cells is critical for maintaining the mucosa of the digestive tract. Although studies have shown that mucins are produced in fish (Tibbetts, 1997; Domeneghini et al., 1998; Park and Kim, 2001; Pedini et al., 2001; Marchetti et al., 2006; Cao and Wang, 2009), few studies have described the histochemical characteristics of the epithelial lining of the digestive tract in Neotropical freshwater species (Leknes, 2010, 2011).

Hemisorubim platyrhynchos belongs to the family Pimelodidae and the order Siluriformes. This is a migratory species without parental care that is widely distributed in the Neotropical region, with reports indicating its presence in the Orinoco, Amazon, Paraguay, Uruguay and Paraná river basins. According to Bressan et al. (2009), *H. platyrhynchos* is a nocturnal carnivorous fish and there has been a reduction in the population size of this species because of habitat destruction as a result of the construction of hydroelectric dams that interrupt the flow of migration required for reproduction. This species is valuable for aquaculture because of the quality and flavor of its meat and the absence of intramuscular bones. Thus, the aim of this study was to describe the anatomical, histological, ultrastructural and histochemical characteristics of the digestive tract of *H. platyrhynchos*, with the goal of increasing available knowledge regarding of the morphofunctional aspects of digestion in carnivorous Neotropical fish.

2. Materials and methods

2.1. Animals

Twenty adult specimens of *H. platyrhynchos*, with a total body length of 35.2 ± 2.3 cm, were obtained from Piraí Pisciculture (Terenos, Mato Grosso do Sul State, Brazil). The animals were anesthetized and euthanized with an overdose of benzocaine, and then dissected with a longitudinal incision along the ventral region. The total length of the digestive tract was measured, and tissue fragments were used for morphological and histochemical studies.

2.2. Gross anatomy

Five adult specimens were used for the analysis and photo documentation of the digestive tract *in situ*. The digestive tract was then removed and dissected for analysis of the internal characteristics of the esophagus, stomach and intestine. The samples were analyzed and documented using a Leica M50 stereomicroscope (Germany) and stored in 10% formalin.

2.3. Histology

Tissue fragments from the esophagus, stomach and intestines were collected from 5 specimens and immediately fixed in Bouin's solution. After fixation, the samples were washed with 70% ethanol, dehydrated in graded ethanol solutions and embedded in historesin. Histological sections (2–3 μm) were stained with hematoxylin–eosin (HE) and 1% toluidine blue (TB), analyzed and photo-documented using an Olympus BX50 microscope (Japan).

2.4. Mucin histochemistry

Tissue fragments from the digestive tracts of 5 specimens were collected, fixed in Bouin's solution and embedded in Paraplast. Then, 5- to 7-μm sections were prepared and processed for the characterization of mucin. To detect neutral mucins, reactions were performed using periodic acid-Schiff (PAS) reagent. To detect acidic mucins, the sections were stained with Alcian blue (AB) at pH 1.0 and 2.5. A sequential staining technique with AB (pH 2.5) and PAS was used to detect the association of acidic and neutral mucins (Cao and Wang, 2009; Suvarna et al., 2012).

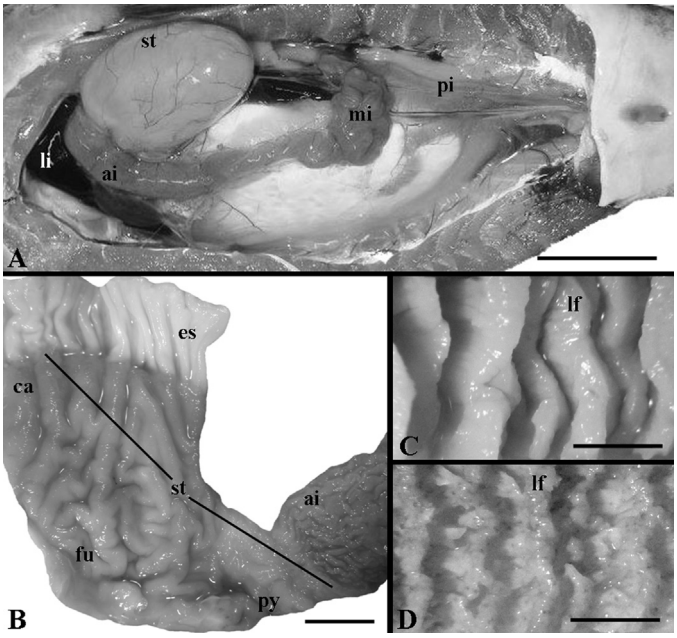

Fig. 1. (A) Ventral view of peritoneal cavity of *Hemisorubim platyrhynchos* showing the organs of the digestive tract: liver (li), stomach (st), anterior intestine (ai), middle intestine (mi) and posterior intestine (pi). (B) Macroscopic image of the luminal surface of the digestive tract: esophagus (es), cardiac (ca), fundic (fu) and pyloric (py) regions of stomach (st) and anterior intestine (ai). (C) Anterior intestine, with thick longitudinal folds (lf). (D) Posterior intestine, showing the thin longitudinal folds (lf). Scale bars: A = 30 mm, B = 8 mm, C = 3 mm, and D = 1 mm.

2.5. Transmission electron microscopy (TEM)

Samples of the digestive tract were removed from 5 specimens and fixed for 24 h at 4 °C in a solution of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4). The samples were then post-fixed for 2 h in 1% osmium tetroxide (pH 7.4), dehydrated in a graded acetone series and embedded in Araldite resin. Resin polymerization was completed in an oven at 60 °C for 48 h. Ultrathin sections (60 and 80 nm) were mounted on copper networks and contrasted with uranyl acetate and lead citrate. The analysis and photographic documentation were performed using a Philips CM100 transmission electron microscope (Netherlands).

2.6. Ethical note

The present study was approved by the Ethical Committee for Research of the Faculty of Sciences at São Paulo State University – UNESP, Bauru, SP, Brazil, under protocol no. 1144/46/01/10.

3. Results

3.1. Gross anatomy

The digestive tract of *H. platyrhynchos* was 17.43 ± 1.27 cm total length and consisted of an oropharyngeal cavity, esophagus, prominent stomach and short intestine with few loops (Fig. 1A). The esophagus was a short tubular organ located dorsally to the liver, and it had a thick wall and pronounced internal longitudinal folds (Fig. 1B). The stomach was a J-shaped saccular organ with a thick wall and consisted of cardiac, fundic and pyloric regions (Fig. 1B).