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# Comparative histology in the liver and spleen of three species of freshwater teleost

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We describe and compare the histology of liver and spleen of *Geophagus brasiliensis* (Perciformes), *Hypostomus francisci* (Siluriformes) and *Hoplias* aff. *malabaricus* (Characiformes), tropical freshwater fishes. In *G. brasiliensis* and *H.* aff. *malabaricus* the hepatocytes were arranged in tubular form whereas in *H. francisci* they cord-like. In all species, hepatocytes presented glycogen, but in *G. brasiliensis* and *H.* aff. *malabaricus* they showed strong stained for hemossiderin in the cytoplasm. In *G. brasiliensis* and *H.* aff. *malabaricus*, melanomacrophage centres (MMCs) were associated to hepatic structures and only in *G. brasiliensis* was observed intrahepatic exocrine pancreas. The spleen, in all species, was characterized by red and white pulp without boundary between the two regions, but only in *H. francisci* was recorded nodular organization in splenic parenchyma. The *G. brasiliensis* and *H.* aff. *malabaricus* presented in the white pulp MMCs linked mainly to ellipsoids. Besides, we observed large MMCs in the spleen in relation to liver of *G. brasiliensis* and *H.* aff. *malabaricus*. In liver, highest values of reticular fibers and collagen were observed in *G. brasiliensis*. In spleen, highest values of reticular fibers and collagen were recorded in *H.* aff. *malabaricus* and *H. francisci*, respectively. Histological differences confirm the hypothesis that the phylogenetic distance is reflected in liver and spleen.

**Keywords:** Extracellular matrix, Hepatocytes, Intrahepatic exocrine pancreas, Melanomacrophage centres, Red and White pulp.

Nós descrevemos e comparamos a histologia do fígado e do baço de *Geophagus brasiliensis* (Perciformes), *Hypostomus francisci* (Siluriformes) e *Hoplias* aff. *malabaricus* (Characiformes), peixes neotropicais de água doce. Em *G. brasiliensis* e *H.* aff. *malabaricus* os hepatócitos organizaram-se na forma tubular enquanto que em *H. francisci* eles apresentaram-se como cordões celulares. Em todas as espécies, os hepatócitos apresentaram glicogênio, mas em *G. brasiliensis* e *H.* aff. *malabaricus*, eles mostraram forte marcação para hemossiderina no citoplasma. Em *G. brasiliensis* e *H.* aff. *malabaricus*, centros melanomacrofágicos (CMMs) foram associados a estruturas hepáticas e somente em *G. brasiliensis* foi observado pâncreas exócrino intrahepático. O baço, em todas as espécies, foi caracterizado pela polpa vermelha e branca sem limites entre as duas regiões, mas somente em *H. francisci* foi registrado uma organização nodular no parênquima esplênico. *G. brasiliensis* e *H.* aff. *malabaricus* apresentaram na polpa branca CMMs associados principalmente a elipsoides. Além disso, nós observamos CMMs grandes no baço em relação ao fígado de *G. brasiliensis* e de *H.* aff. *malabaricus*. No fígado, valores altos de fibras reticulares e colágeno foram observado em *G. brasiliensis*. No baço, valores altos de fibras reticulares e colágeno foram registrados em *H.* aff. *malabaricus* e *H. francisci*, respectivamente. Diferenças histológicas confirmam a hipótese que a distância filogenética está refletida no fígado e no baço.

**Palavras-chave:** Centros melanomacrofágicos, Hepatócitos, Matriz extracelular, Pâncreas exócrino intra-hepático, Polpa branca e vermelha.

## Introduction

Fish liver is described as a multifunctional organ acting in detoxification, production of vitellogenin as well as the deposition and metabolism of carbohydrates and fat (Bruslé, Anadon, 1996). In most teleost fish, the liver is divided into lobes located cranially and ventrally in the body cavity with a reddish-brown color (Bruslé, Anadon, 1996). The spleen has been considered the central component of the immune system and plays an important role in responses against pathogen invasion. It also is a selective filter of the vascular system (Press, Evensen, 1999). The spleen also performs hemocateresis in several teleosts and promotes the maturation of the lymphocytes in both humoral and cellular defenses (Press, Evensen, 1999).

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The head kidney, liver and spleen in some teleosts may present aggregates of macrophages called melanomacrophage centres (MMC). The MMCs are histologically distinguishable within the tissue for presenting macrophages with distinct pigments such as melanin, hemosiderin and lipofuscins (Ribeiro *et al*., 2011; Diaz-Satizabal, Magor, 2015; Gomes *et al*., 2015). The analysis and quantification of granules can be a great indicator of the main metabolic activities by macrophages from MMCs (Ribeiro *et al*., 2011; Diaz-Satizabal, Magor, 2015; Gomes *et al*., 2015). Moreover, presence, number and area of MMCs have been used as biological parameters in toxicology and environmental impact studies (Rabitto *et al*., 2004; Ribeiro *et al*., 2011; Pronina *et al*., 2014; Gomes *et al*., 2015).

Neotropical ichthyofauna, as well as in most of the earth's freshwater ecosystems, is dominated by ostariophysan fishes that contains five orders including Characiformes and Siluriformes. Currently, based in mitochondrial genome analysis these groups are considered as sister taxa within Otophysi. Although distant phylogenetically from the Characiformes and Siluriformes, the Perciformes is also highly diverse (Nakatani *et al*., 2011). The great majority of Neotropical freshwater fishes traced their origins to before the Late Cretaceous separation of Africa and South America (Albert, Reis, 2011). The pearl cichlid, *Geophagus brasiliensis* Quoy & Gaimard, 1824 (Perciformes, Cichlidae), has a diurnal habit with a preference for lentic environments. It is widely distributed in Brazilian rivers and reservoirs where it is an abundant species due to its high trophic plasticity (Abelha, Goulart, 2004). The pearl cichlid is omnivorous and uses its buccal apparatus to feed on nutrients deposited on the bottom of rivers and reservoirs (Abelha *et al*., 2001). The armored catfish, *Hypostomus francisci* (Lütken, 1874) (Siluriformes, Loricariidae) is also widely distributed and uses its mouth to adhere to any surface. It congregates near creek bottoms on rocks, submerged logs, and vegetables and scrapes the periphyton matrix (Casatti, 2002). The trahira, *Hoplias* aff. *malabaricus* (Bloch, 1794) (Characiformes, Erythrinidae), also has a wide distribution in South America. It is carnivorous and is one of the main predators in reservoirs and streams (Oyakawa, Mattox, 2009). These teleost fish have distant phylogenetic origins in evolutionary scale, as well as, possess distinct trophic guild. These characteristics can be reflected on histological differences in several organs. Comparative histological studies of the liver and spleen of these species are scarce.

This study investigates the liver and spleen of three tropical fish to test two hypotheses: 1- We hypothesize that the liver and spleen have distinct characteristics between the three species, and 2- We hypothesize that for each species, there is a predominance of MMC between the different organs that can indicate whether the liver or spleen has a more important role in the immune system.

## Material and Methods

**Sampling.** The *G. brasiliensis* (Fig. 1a; *n* = 29; body weight - BW = 145.4 ± 91.6 g; total length - TL = 18.3 ± 4.4 cm), *H. francisci* (Fig. 1c; *n* = 58; BW = 355.1 ± 249.8 g; TL = 28.7 ± 8.4 cm) and *H.* aff. *malabaricus* (Fig. 1e; *n* = 12; BW = 355.7 ± 239.6 g; TL = 30.5 ± 7.0 cm) were collected quarterly from March 2010 to February 2012 in two sites on the Itapecerica river on the perimeter of Divinópolis city, Brazil (20°13'09"S-44°54'51"W; 20°07'80"S-44°52'83"W). The voucher specimens were deposited at the Department of Zoology MHN-UFMG with access numbers MHN-UFMG 1453, 1456 and 1454 for *G. brasiliensis*, *H. francisci* and *H.* aff. *malabaricus*, respectively. Fish were euthanized by decapitation after anesthesia using tricaine methanesulfonate (MS222 - 300 mgL$^{-1}$) following the principles established by the Brazilian College of Animal Experimentation (COBEA). This work was approved by the ethics committee under protocol Nº 49/2010 CEPEA/UFSJ.

**Histology and histochemistry analysis.** From all animals, samples of liver and spleen were fixed in Bouin's fluid for 12 h, embedded in paraffin, sectioned 4 µm thick and stained with hematoxylin-eosin and Gomori's trichrome. To determine the histochemical contents, Perls' technique was used to identify hemosiderin, lipofuscin and melanin (Leknes, 2015; Gomes *et al*., 2015) and the periodic acid Schiff (PAS) for neutral glycoproteins and glycogen in the liver and spleen. Silver impregnation was used to identify the reticular fibers present in the extracellular matrix both organs (Disbrey, Rack, 1970).

**Morphometry.** Micrographs of liver and spleen were obtained using a trinocular light microscope (Primo Star Zeiss) coupled with a camera. Three animals randomly taken per species were used for quantification of collagen and reticular fibers in liver and spleen, being 10 histological images consecutively taken in a straight line throughout of histological section per animal. The percent area stained by Gomori or silver impregnation was obtained using Axion Vision 4.8 software from Zeiss. The MMC area (µm$^2$) was measured in liver and spleen of *G. brasiliensis* and *H.* aff. *malabaricus* using Axion Vision 4.8 software from Zeiss. The MMC area was calculated using three animals per species for a total of 30 MMCs per organ.

**Statistical analysis.** Values were expressed as the mean ± standard deviation. The means were compared by ANOVA followed by a Tukey post-test that was performed using a GraphPad Instat with confidence intervals of 95%.

## Results

**Liver and spleen morphology.** The liver was located in the cranial region of the abdominal cavity and was situated cranially to stomach and foregut, ventral to swimbladder. The liver of *G. brasiliensis* and *H.* aff. *malabaricus* had three lobes (Figs. 1b, f), while *H. francisci* had several lobes (n=6-10; Fig. 1d). The liver color was reddish-brown in *H.* aff. *malabaricus*, yellow-brown in *G. brasiliensis* and dark yellow in *H. francisci*. The spleen of the three species was also in the abdominal cavity near the stomach and ventral to the swimbladder. The spleen had a dark-brown color with no lobes and regular borders in both species. This organ was fusiform, elongated and triangular in *G. brasiliensis*, *H. francisci* and *H.* aff. *malabaricus*, respectively (Figs. 1b, d, f).

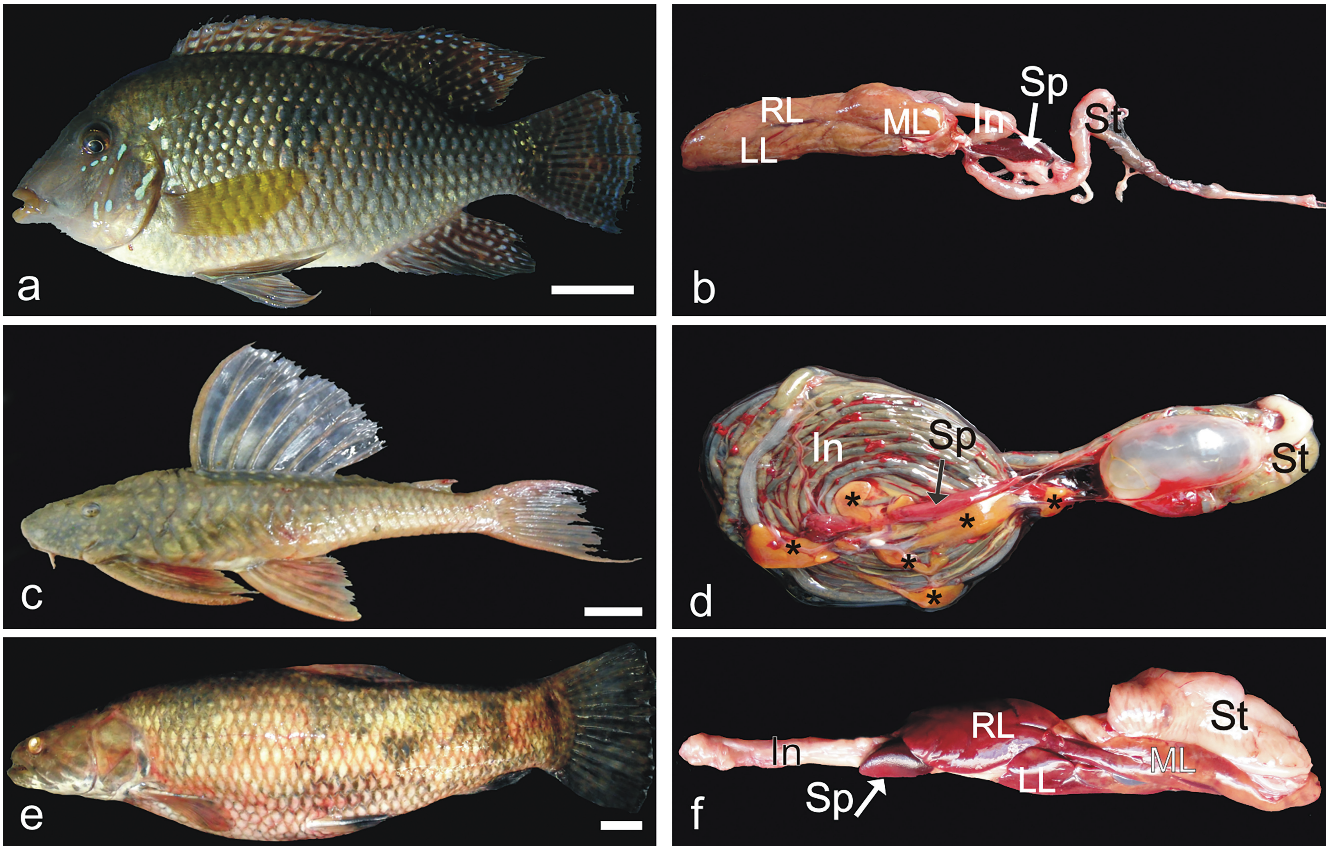

**Fig. 1.** Teleost species with the respective macroscopic characteristics from liver and spleen: (a) *Geophagus brasiliensis*; (c) *Hypostomus francisci* and (e) *Hoplias* aff. *malabaricus*; (b, d and f) ventral view of the liver and spleen (Sp) showing the right (RL), left (LL) and median lobe (ML), stomach (St) and intestine (In). Note fusiform, elongated and triangular forms of the spleen in *G. brasiliensis*, *H. francisci* and *H.* aff. *malabaricus*, respectively (b, d and f). Liver with several lobes in *H. francisci* (asterisks). Bars: (a, c, e) 2 cm; (b, d) 0.5 cm; (f) 1 cm.

For all species, the liver was covered by a thin capsule with simple squamous epithelium and thin connective tissue (Fig. 2a). The hepatic parenchyma was organized in hepatocyte plates surrounded by sinusoids (Fig. 2b). Each plate showed polarized hepatocytes with a sinusoidal and biliary face. According to hepatocyte-sinusoidal arrangement, the hepatic parenchyma was classified into two different forms. In *H. francisci* was observed cord form, when between neighboring sinusoids the hepatocytes were arranged in plates generally with two hepatocytes thick (simple-layered) where hepatic sinusoids were enlarged and straight converging to the centre-lobular vein (Fig. 2e). In *G. brasiliensis* (Fig. 2b) and *H.* aff. *malabaricus* (Fig. 2g) was described tubular form, when between neighboring sinusoids the hepatocytes were organized also in plates generally with three to four cells thick (double-layered) and the sinusoidal capillaries were narrow and branched also connecting to centre-lobular vein.

In *G. brasiliensis*, the hepatocytes were polyhedral with spherical nucleus usually centrally located and a prominent central nucleolus and the eosinophilic cytoplasm (Fig. 2b). Exocrine pancreatic tissue was a pronounced hallmark in liver of *G. brasiliensis* distributed diffusely and separated from the hepatic parenchyma by a thin layer of connective tissue (Figs. 2a, c). The intrahepatic exocrine pancreas showed a peripheral region composed of secretory cells organized in acini and a central region composed by portal vein (Figs. 2a, c). The secretory cells were pyramidal and have a basophilic cytoplasm (Fig. 3a), distinct basal nucleus and many eosinophilic zymogen granules in apical region (Fig. 2c). The hepatic stroma was characterized by thin connective tissue surrounding the sinusoidal capillaries, centre-lobular veins, intrahepatic exocrine pancreas, bile system and MMCs (Figs. 2c-d). The MMCs were located around the centre-lobular veins, intrahepatic exocrine pancreas and bile ducts (Figs. 2b, d). The MMCs were usually nodular and surrounded by a thin connective tissue. The macrophages presented a small peripheral nucleus with different pigments within the cytoplasm (Figs. 2b, d). The centre-lobular veins present simple squamous epithelium and thin connective tissue (Fig. 2b). The bile system consists of bile canaliculi and ducts. The bile canaliculi were observed in the apical border of the neighboring hepatocytes. The bile ducts consist of a simple cubic or columnar epithelium (Fig. 2d). For all species, spleen was surrounded by a capsule that presented a simple cubic epithelium and thin connective tissue, which emerged irregularly trabeculae that extended into parenchyma (Figs. 3a, g-h). In *G. brasiliensis*, the splenic parenchyma had no limits between the white pulp and red pulp (Fig. 3a). The red pulp was predominant and composed by a network of reticular cells nourished by vessel sinusoids. The white pulp was diffuse and characterized by ellipsoids and MMCs that were supported by collagen (Figs. 3b-c). Ellipsoids were structures usually rounded with a capillary center and lined with a simple cubic epithelium (Fig. 3b).

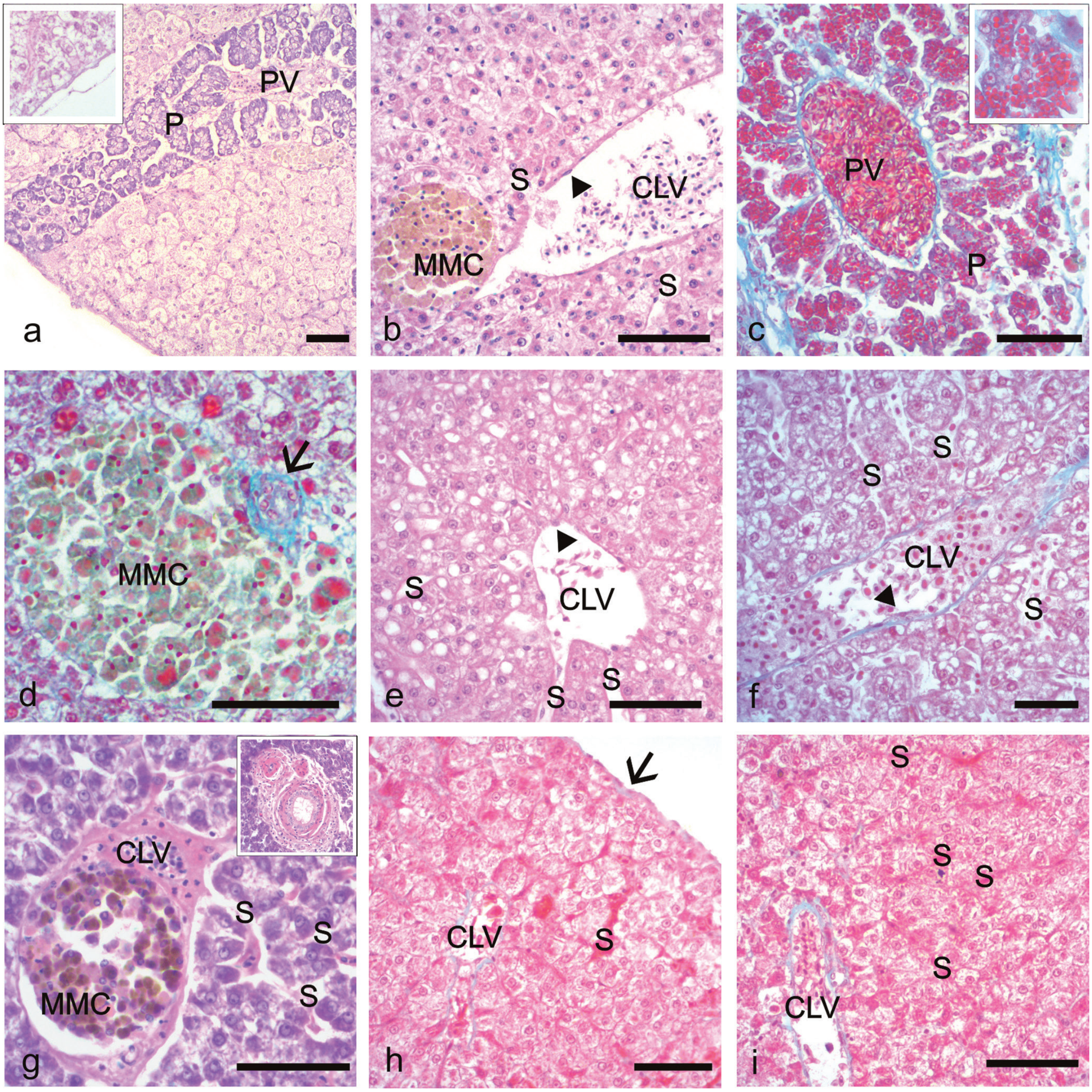

**Fig. 2.** Histological sections of liver from *Geophagus brasiliensis* (a-d), *Hypostomus francisci* (e-f) and *Hoplias* aff. *malabaricus* (g-i) stained with hematoxylin-eosin (a, b, e; g) and Gomori trichrome (c, d, f, h-i). Capsule (black arrow), intrahepatic exocrine pancreatic tissue (P), bile canaliculi (white arrow), melanomacrophage centres (MMC), collagen (green), simple squamous epithelium (arrowhead), centre-lobular and (CLV), portal vein (PV), hepatic sinusoids (S). Insert (a) capsule, (c) secretory cells from intrahepatic exocrine pancreas and (g) large bile duct with a concentric layer of collagen and muscular fibers under the epithelium. Bars: (a-i) 50 µm;

In *H. francisci*, the hepatocytes were polyhedral with fat droplets in the cytoplasm with a cord-like arrangement converging on the centre-lobular vein (Figs. 2e-f). The hepatic sinusoids were enlarged with straight capillaries (Fig. 2e). The bile system was composed by the bile ducts had a simple cubic epithelium, which becomes columnar in large ducts with a concentric layer of collagen and muscular fibers. No MMCs were seen in the hepatic parenchyma. The stroma also composed by thin connective tissue that was associated to centre-lobular veins, sinusoids and bile system (Fig. 2f). The splenic parenchyma from *H. francisci* presented an evident nodular organization. The neighboring nodules were separated by cellular extensions (Figs. 3d-e). Each nodule was recorded presence of red pulp with splenic arteriole and ellipsoids, which were supported by collagen (Figs. 3e-f). This species had no MMC in white pulp. The red pulp also was predominant and histology similar to recorded for *G. brasiliensis*.

In *H.* aff. *malabaricus*, the hepatocytes were polyhedral and organized in tubular form. The sinusoidal capillaries were narrow and irregular and appear throughout the stroma (Figs. 2g-i). The centre-lobular veins, bile system and MMCs were supported by thin connective tissue (Figs. 2g-i). The histological description of the bile system was similar to recorded in the others species (Fig. 2g). The spleen was composed mostly of red pulp and white pulp supported by collagen no evident nodular organization (Figs. 3h-i). The white pulp had MMC and ellipsoids (Figs. 3g, i).

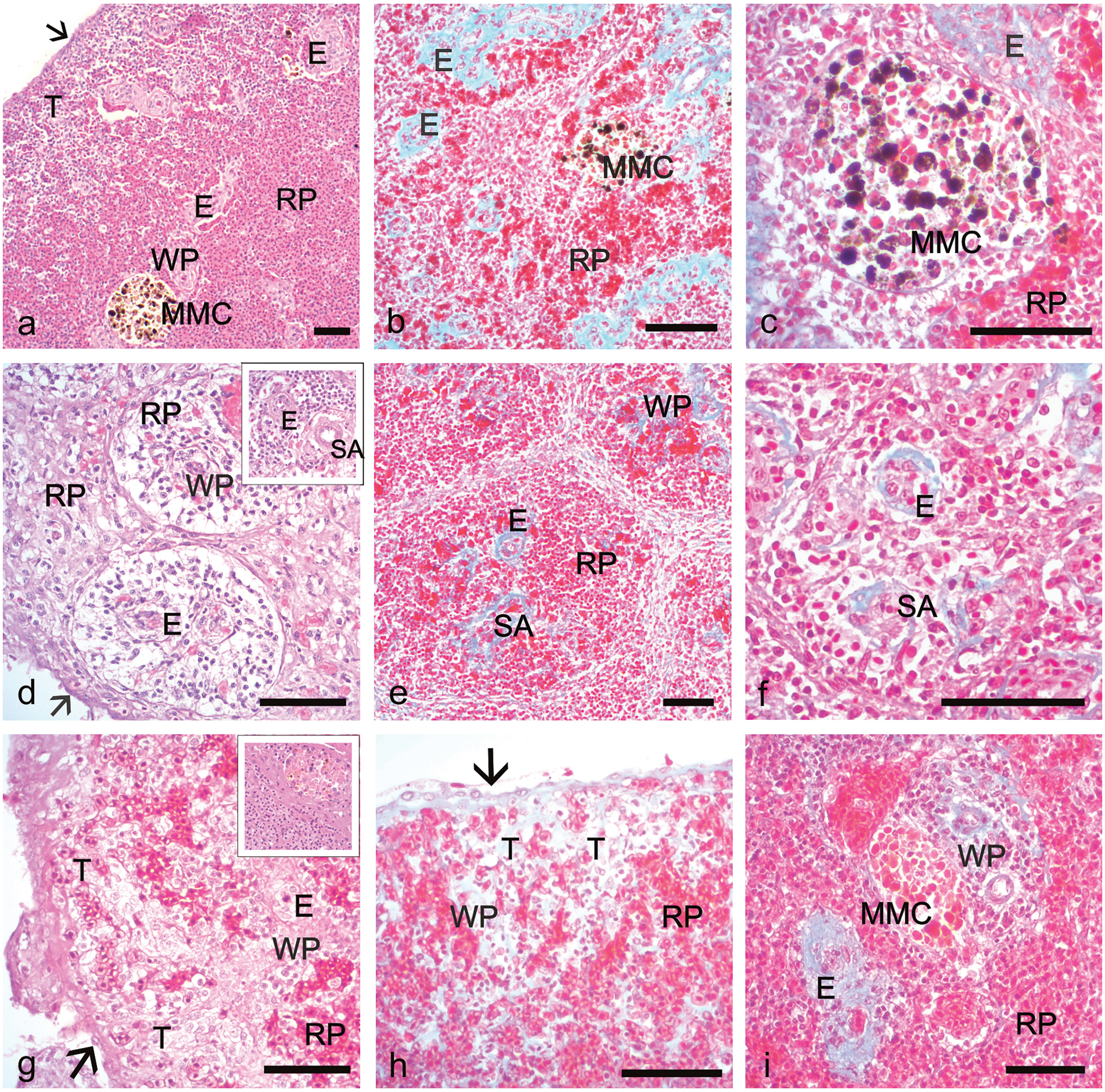

**Fig. 3.** Histological sections of spleen from *Geophagus brasiliensis* (a-c), *Hypostomus francisci* (d-f) and *Hoplias* aff. *malabaricus* (g-i) stained with hematoxylin-eosin (a, d and g) and Gomori trichrome (b-c, e-f, h-i). Capsule (black arrow), trabeculae (T) red pulp (RP) and white pulp (WP), melanomacrophage centres (MMC), ellipsoids (E), splenic arteriole (SA) collagen (green). Insert (d) ellipsoid and splenic arteriole; (g) melanomacrophage centre. Bars: 50 µm;

**Liver and spleen histochemistry.** Liver and spleen histological sections of the three species were submitted to histochemistry for identification of reticular fibers, glycogen, neutral glycoproteins, hemosiderin, melanin and lipofuscins.

In *G. brasiliensis*, the hepatic stroma showed thin networks of reticular fibers disposed in various directions surrounding hepatocytes, intrahepatic exocrine pancreatic tissue (Fig. 4a) and MMCs. The hepatocytes presented thin granules of glycogen and macrophages from MMC showed neutral glycoproteins (Fig. 4b). Secretory cells of the intrahepatic exocrine pancreas were PAS-negative (Fig. 4b). The basement membrane of the blood vessels and bile ducts were positive for neutral glycoproteins (Fig. 4b). The MMCs and hepatocyte cytoplasm were very positive for hemosiderin (Figs. 4c-d) and intrahepatic exocrine pancreatic tissue was negative (Fig. 4d). Moreover, some macrophages from MMCs presented melanin and lipofuscins. In the spleen, reticular fibers mainly supported ellipsoids and MMC (Fig. 5a). Both ellipsoid and MMCs were PAS-positive; the red pulp was negative (Fig. 5b). The hemosiderin, melanin and lipofuscin were easily identified in splenic MMCs (Fig. 5c).

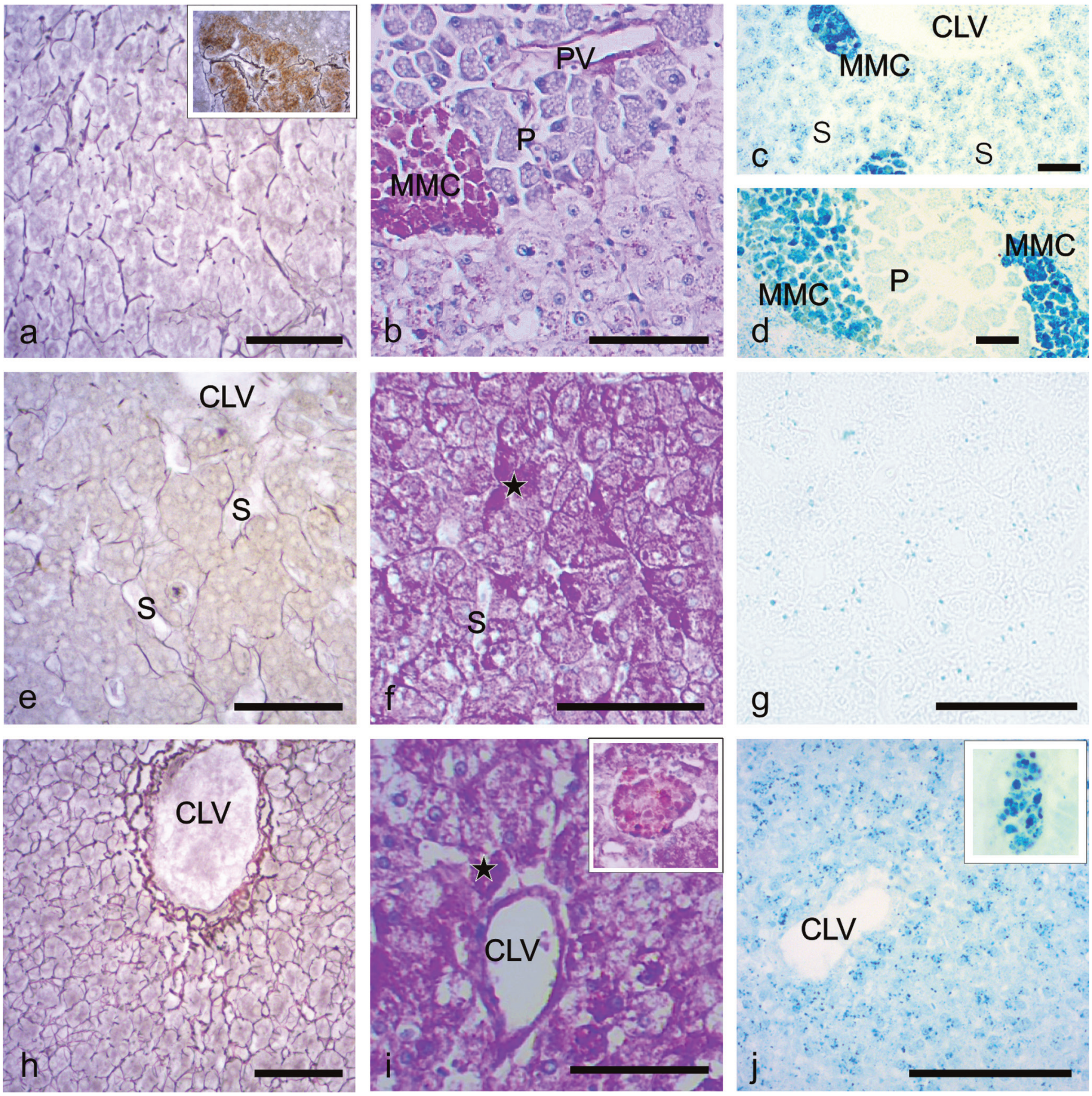

**Fig. 4.** Histological section of liver from *Geophagus brasiliensis* (a-d), *Hypostomus francisci* (e-g) and *Hoplias* aff. *malabaricus* (h-j) submitted to silver impregnation (a, e and h), periodic acid Schiff (PAS) (b, f and i) and Perls (c-d, g and j) histochemistry. Reticular fibers (stained in black), glycogen (stars), intrahepatic exocrine pancreas (P), melanomacrophage centre (MMC), centre-lobular (CLV) and portal (PV) vein, hepatic sinusoids (S) and hemossiderin (blue pigments). Insert (a) Intrahepatic exocrine pancreas and (i-j) MMCs. Bars: 50 µm.

In *H. francisci*, networks of reticular fibers were thin and accompanied the hepatocytes and sinusoidal capillaries (Fig. 4e). Strong and homogeneous stains for glycogen were identified in the hepatocyte cytoplasm (Fig. 4f). On the other hand, staining for hemosiderin was weak or absent in the cytoplasm of the hepatocytes (Fig. 4g). In the spleen, the nodular arrangement of the splenic parenchyma was well evident, where the reticular fibers were observed mainly around of the ellipsoids and cellular cords in red pulp (Fig. 5d). The basal membrane from ellipsoids was PAS-positive, and the red pulp was PAS-negative (Fig. 5e). The MMCs were not observed in this species, however free melanomacrophages scattered throughout the parenchyma were Perls-positive for hemossiderin, melanin and lipofuscin (Fig. 5f).

The *H.* aff. *malabaricus* had a more regular arrangement of thin reticular fibers along with hepatocytes, sinusoidal capillaries and centre-lobular vein (Fig. 4h). The MMCs and bile ducts were also supported by reticular fibers. Hepatocytes showed strong and homogeneous reaction to the PAS assay (Fig. 4i). The MMCs and basal membranes of blood vessels and bile ducts were PAS-positive showing the presence of neutral glycoproteins in these structures (Fig. 4i). The hepatocytes and MMCs were positive for hemosiderin (Fig. 4j) and the MMCs stained also for melanin and lipofuscin. The spleen of this species showed reticular fibers surrounding ellipsoids, cellular cords and MMCs (Fig. 5g). Neutral glycoproteins from the basal membrane were identified in ellipsoids (Fig. 5h). Moreover, macrophages from MMC were PAS-positive (Fig. 5h) and presented also hemossiderin, melanin and lipofuscin (Fig. 5i).

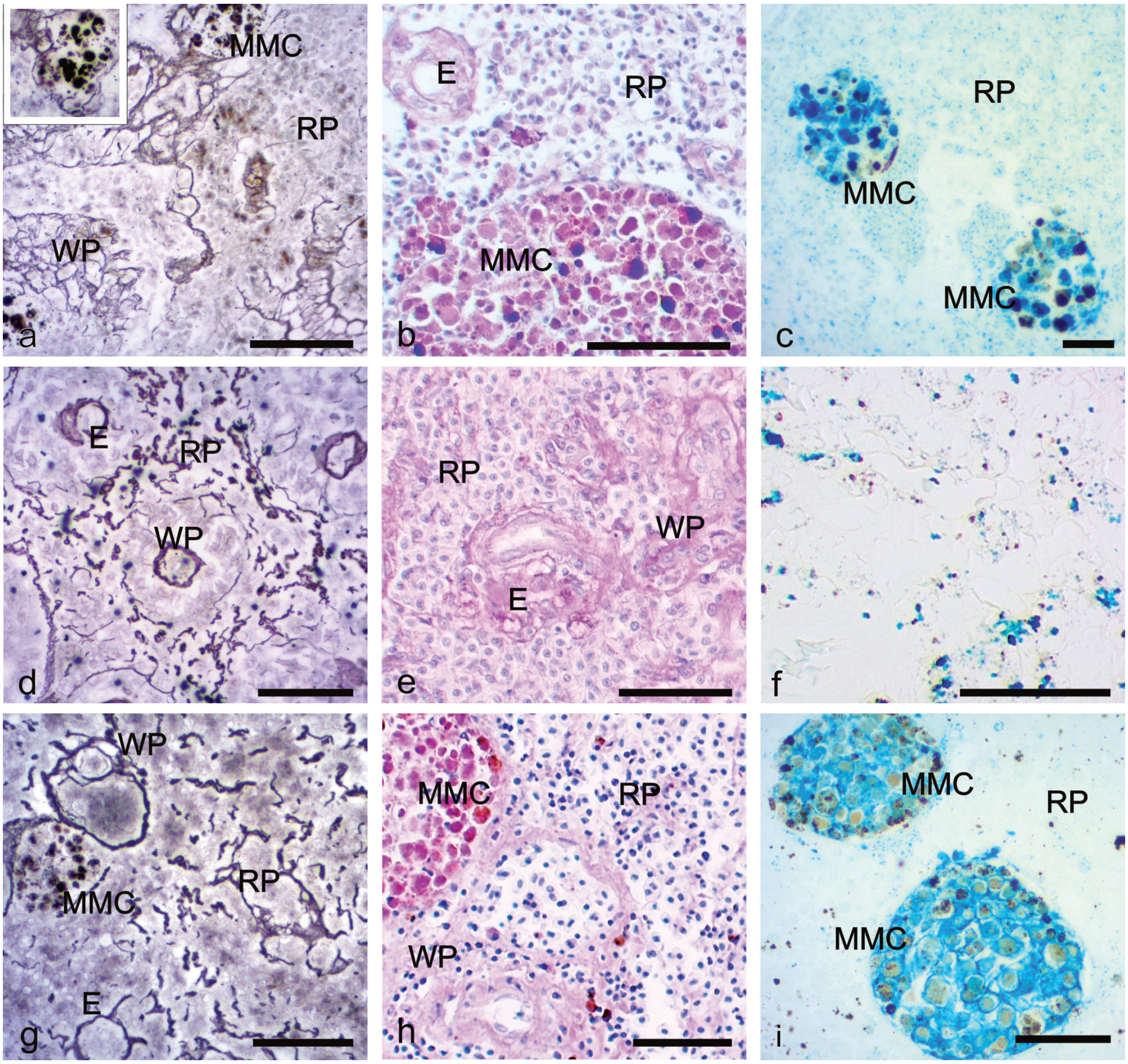

**Fig. 5.** Histological section of spleen from *Geophagus brasiliensis* (a-c), *Hypostomus francisci* (d-f) and *Hoplias* aff. *malabaricus* (g-i) submitted to silver impregnation (a, d and g), periodic acid Schiff (PAS) (b, e and h) and Perls assay (c, f and i), Red pulp (RP) and white pulp (WP), melanomacrophage centre (MMC), ellipsoids (E), thin networks of reticular fibers (stained in black), hemossiderin (blue pigments), melanin (dark pigments) and lipofuscin (yellow-brown pigments). Insert: (a) melanomacrophage centre surrounded by reticular fibers. Bars: 50 µm.

**Morphometry.** The Tab. 1 summarizes the area that was positive for collagen and reticular fibers in the liver and spleen. The liver of *G. brasiliensis* presented more reticular fibers ($p<0.05$) and collagen than other species. The spleen of *H. francisci* and *H.* aff. *malabaricus* exhibited more collagen and reticular fibers ($p<0.05$), respectively.

**Tab. 1.** Stained area for reticular fiber (silver impregnation) and collagen (Gomori trichrome) in liver and spleen of three fish species. In each column, different superscript letters indicate statistical difference with $p<0.05$.

| | Liver | | Spleen | |
|---|---|---|---|---|
| | Reticular fibers | Collagen | Reticular fibers | Collagen |
| *Geophagus brasiliensis* | $24.9 \pm 13.8^{a}$ | $24.5 \pm 15.0^{a}$ | $17.0 \pm 11.5^{a}$ | $10.8 \pm 2.4^{a}$ |
| *Hypostomus francisci* | $10.9 \pm 6.3^{b}$ | $16.0 \pm 12.7^{b}$ | $15.6 \pm 4.0^{a}$ | $40.8 \pm 24.5^{b}$ |
| *Hoplias* aff. *malabaricus* | $10.9 \pm 9.4^{b}$ | $21.2 \pm 10.8^{a}$ | $25.1 \pm 9.8^{b}$ | $15.0 \pm 6.9^{a}$ |

The *G. brasiliensis* and *H.* aff. *malabaricus* presented predominance of MMCs area in size class from 0-2365 µm$^2$ and 0-795 µm$^2$ in the liver, respectively (Figs. 6a-b). In the spleen, *G. brasiliensis* showed a higher frequency of MMCs area in two size classes: 0-1599 and 1600-3199 µm$^2$ (Fig. 7a). The MMC area in *H.* aff. *malabaricus* showed higher frequency for MMCs area from 0-1899 µm$^2$ (Fig. 7b). The MMCs were more predominant in the spleen in relation to liver in *G. brasiliensis* and *H.* aff. *malabaricus.*

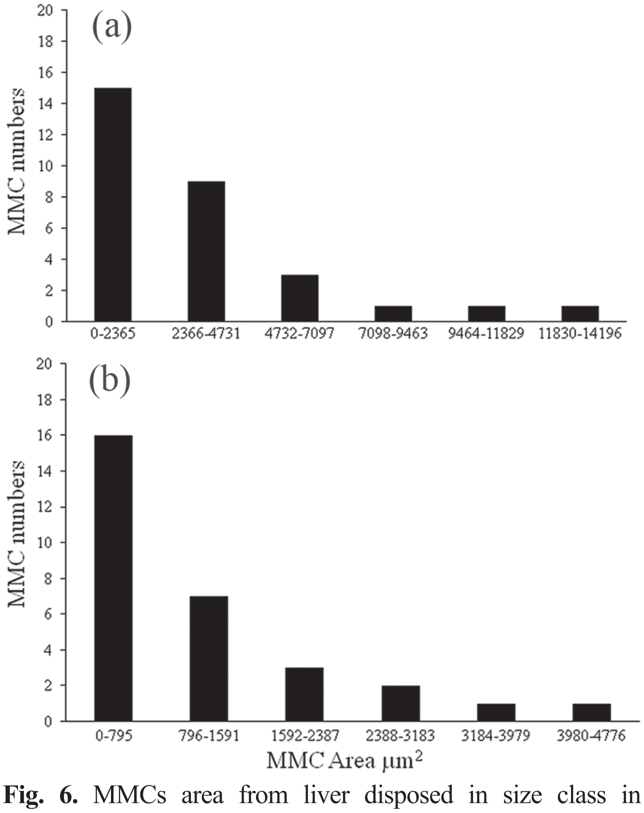

**Fig. 6.** MMCs area from liver disposed in size class in *Geophagus brasiliensis* (a) and in *Hoplias* aff. *malabaricus* (b).

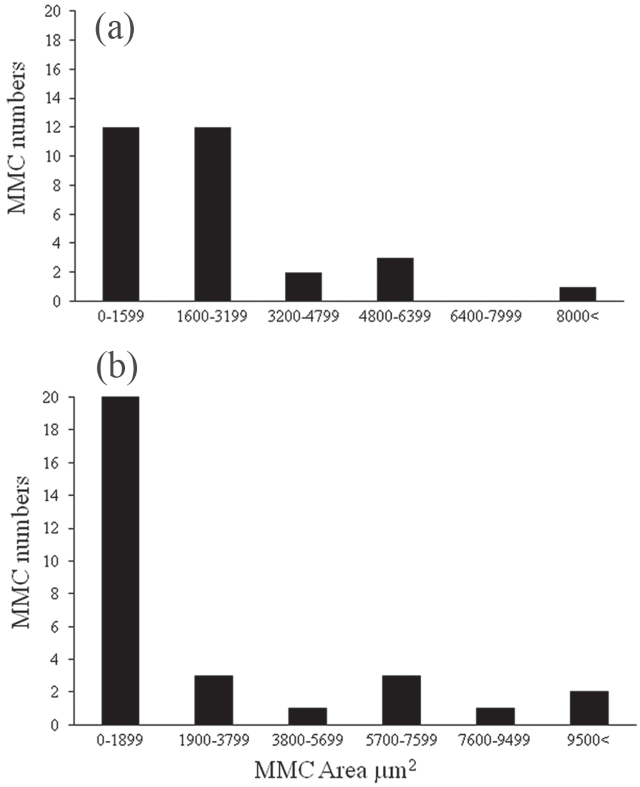

**Fig. 7.** MMCs area from spleen disposed in size class in *Geophagus brasiliensis* (a) in *Hoplias* aff. *malabaricus* (b).

## Discussion

This paper describes for the first time a comparative study of the normal anatomy and histology from liver and spleen in three freshwater teleost species. The anatomy of the liver and spleen was different among the species. The parenchyma and stroma of the liver and spleen showed clear histological differences.

The anatomical location of the liver was similar to other teleost species (Buslé, Anadon, 1996), however the shape and number of hepatic lobes was variable. Teleosts do not have a defined hepatic lobe number and shape (Faccioli *et al.*, 2014). Thus, it adapts to the size and shape of this cavity and the space used by other visceral organs. Moreover, the liver can differ in weight, size and volume according to body weight and length of each animal. The liver of *G. brasiliensis* was yellow-brown, *H.* aff. *malabaricus* was reddish-brown and *H. francisci* was dark-yellow color. We believe that variation in liver color may be related to different dietary habits. In fact, adult individuals of *G. brasiliensis* are omnivores, *H. franscisci* are detritivorous and *H.* aff. *malabaricus* are piscivores (Abelha, Goulart, 2004; Hahn *et al.*, 2004). There are other factors associated with liver color such as health conditions, vascularization and hepatocyte content (Buslé, Anadon, 1996). Spleens of the three species were found in the abdominal cavity near the stomach and ventral to the swimbladder and presented dark-brown color and regular borders similar to other teleosts (Sheibani, 2005). The spleen was fusiform, elongated and triangular in *G. brasiliensis*, *H. francisci* and *H.* aff. *malabaricus*, respectively. On the other hand, the spleen of *Barbus pectoralis* presented reddish-brown color, dorsoventrally flattened, elongated and with irregular surface (Mahabady *et al*., 2012). Fänge, Nilsson (1985) reported that this organ in other classes is associated with the pancreas in the peritoneal cavity.

In the present study, three species had absence of division into hepatic lobules and the lack of portal triads as also recorded in many teleosts (Vicentini *et al*., 2005; Nejedli, Gajger, 2013; Pronina *et al*., 2014; Faccioli *et al*., 2014). The network of blood capillaries showed vessels that were anastomosed and drained by centre-lobular veins. These findings are similar to other teleosts (Akiyoshi, Inoue, 2004; Bombonato *et al.,* 2007). The bile ducts observed in the three species had a simple cubic epithelium, which becomes columnar in large ducts similar to other teleosts (Vicentini *et al*., 2005; Faccioli *et al*., 2014). Only *G. brasiliensis* has an intrahepatic exocrine pancreas that was arranged in clusters of columnar cells, which were organized in acini. Moreover, the acinar cells have a PAS-negative cytoplasm, distinct basal nucleus and many eosinophilic zymogen granules in apical portion from secretory cells. During ontogenesis, the exocrine pancreatic tissue is formed around of the portal vein and penetrates deep and diffusely into the hepatic parenchyma (Vicentini *et al*., 2005). Exocrine pancreatic tissue has been recorded in other teleosts and its proportion depends on the species (Nejedli, Gajger, 2013; Faccioli *et al*., 2014). The exocrine pancreatic tissue is responsible for producing digestive enzymes that act in the intestine and aiding in food digestion (González *et al*., 1993). Moreover, endocrine part of this tissue can also secrete insulin and glucagon (Youson *et al*., 2006).

The hepatocyte-sinusoidal structures of the livers of teleosts were classified in three different types: cord-like, tubular and solid form (Akiyoshi, Inoue, 2004). In *H. francisci* was observed cord-like form and hepatic sinusoids were enlarged and straight converging to the centre-lobular vein. In *G. brasiliensis* and *H.* aff. *malabaricus* was recorded tubular-like form and the sinusoidal capillaries were narrow and branched also connecting also to centre-lobular vein. These architectural differences in the hepatic parenchyma were observed for other teleosts, where such variations were acquired phylogenetically (Akiyoshi, Inoue, 2004).

The hepatocytes of both species were PAS-positive and suggest a reserve of glycogen in these cells. The PAS reaction pattern in the hepatocytes of *G. brasiliensis* presented thin granules of glycogen that were dispersed in the cytoplasm. In *H. francisci* and *H.* aff. *malabaricus*, the staining was homogeneous and mainly peripheral in the cytoplasm. Hepatocytes store glycogen and lipids (Bruslé, Anadon, 1996). Therefore, in relation to the histochemical contents of the hepatocytes, two groups may be described: glycogen-rich or lipid-rich cytoplasm (Akiyoshi *et al*., 2001). The hepatocytes of *H. francisci* showed both types of reserves. Differences are associated with distinct swimming behavior, *i.e*., active fish showed large deposits of glycogen, whereas sedentary fish had large lipid reserves (Akiyoshi *et al*., 2001). The hepatocyte cytoplasm from *G. brasiliensis* and *H.* aff. *malabaricus* presented strong stained for hemosiderin. In *G. brasiliensis*, the intrahepatic exocrine pancreatic tissue was negative for these pigments. Hemosiderin is an iron pigment in the ferric form ($Fe^{+++}$) and results from erythrocyte breakdown. It is often found in fish liver (Wolke, 1992; Velkova-Jordanoska, Kostoski, 2005; Leknes, 2015). Increased hemosiderin in hepatocytes (hemosiderosis) was found in teleosts in a disturbed environment (Pronina *et al*., 2014).

Here, some aspects of the general architecture spleen were similar between the species. They all had a capsule with a simple cubic epithelium and thin connective tissue, which emerged irregularly trabeculae that extended into parenchyma without clear boundaries between the white and red pulp. Interestingly, splenic parenchyma from *H. francisci* presented a nodular arrangement well evident, where the neighboring nodules were separated by cellular extensions. Each nodule was observed white and red pulp. In relation to white pulp, the ellipsoid capillaries were recorded in all species. On the other hand, splenic MMCs were observed only in *G. brasiliensis* and *H.* aff. *malabaricus*. In the teleosts, the red pulp occupies most of the organ (Press, Evensen, 1999). It is organized in irregular compartments separated by cell cords. The white pulp is characterized by lymphocytes randomly distributed in its reticular network associated with ellipsoids. The ellipsoids are terminal branches of splenic arterioles showing a narrow lumen and thick-wall surrounded by a sheath of macrophages, reticular fibers and cells (Press, Evensen, 1999). The ellipsoid capillaries have a filtration function and blood immune complexes (Herraez, Zapata, 1986; Agius, Roberts, 2003). According to Press, Evensen (1999), teleost macrophages transport material from the ellipsoids to the MMC for an inflammatory response.

In ectothermic vertebrates (amphibians, reptiles and fish), melanomacrophage centres are clusters of phagocytic cells that contain pigments as melanin, hemossiderin and lipofuscins (Agius, Roberts, 2003). In teleosts, they are particularly concentrated in the head kidney, liver and spleen from some species (Wolke, 1992; Ribeiro *et al*., 2011; Pronina *et al*., 2014; Diaz-Satizabal, Magor, 2015; Gomes *et al*., 2015). The liver and spleen of *G. brasiliensis* and *H.* aff. *malabaricus* presented MMCs that were associated to hepatic structures and white pulp. However, these same organs in *H. francisci* did not present MMCs. The absence of MMCs in liver and spleen of *H. francisci* probably is compensated by presence of free melanomacrophages on these organs (Vigliano *et al*., 2006). The MMCs are critical elements of the immune system of fish, including phagocytosis and antigen processing (Hartley *et al*., 1996; Agius, Roberts, 2003) as well as destruction, detoxification and recycling of endogenous and exogenous materials (Herraez, Zapata, 1986; Agius, Roberts, 2003; Ribeiro *et al*., 2011). The MMCs of the liver and spleen in *G. brasiliensis* and *H.* aff. *malabaricus* were positive for neutral glycoprotein, hemossiderin, melanin and lipofuscin. The analysis of these pigments indicates the main metabolic activities by macrophages (Leknes, 2015). Melanin can neutralize free radicals (Agius, Roberts, 2003) and other toxic agents derived from the degradation of phagocytized cellular material (Hartley *et al*., 1996; Dubey, Roulin, 2014). This pigment is also important in the production of antibacterial compounds, especially hydrogen peroxide (Wolke, 1992). Lipofuscin and melanin-like pigments are endogenous and accumulate in cells because the cells do not metabolize them (Wolke, 1992). Lipofuscins are results of lipid peroxidation, disturbances of proteostasis by protein oxidation and an impairment of the proteasome that can to promote an accumulation in cytoplasm of non-dividing cells of non-degradable metabolites (Höhn, Grune, 2013). Lipofuscins has been described as most common the pigment present in MMCs from liver and spleen of teleosts (Agius, Roberts, 2003; Ribeiro *et al*., 2011; Diaz-Satizabal, Magor, 2015). The presence of hemosiderin in the MMCs can be associated to phagocytosis of biological iron that came from erythrocytes' hemoglobin (Wolke, 1992). Increase of hemossiderin in splenic MMCs of *Oreochomis niloticus* has been associated to inflammatory process triggered by microorganisms or toxicological stress promoted by cadmium (Manrique *et al*., 2014; Gomes *et al*., 2015).

Increased numbers of MMCs may be influenced by exposure of the fish to contaminants such as heavy metals (Rabitto *et al*., 2004). Moreover, variations in the size, number and histology of the MMCs can occur due to age, nutritional status, disease and environmental conditions (Hartley *et al*., 1996, Ribeiro *et al*., 2011; Pronina *et al*., 2014). In *O. niloticus*, number and area of splenic MMCs were directly related to type of inflammatory agent and that the increases in area and number of MMCs were greater when the stimulation was performed by inoculation of the bacillus Calmette-Guerin (BCG) when compared to subcutaneous implant of glass coverslip or with the implant and BCG in association (Manrique *et al*., 2014). In *O. niloticus*, higher concentration of cadmium (2500 $\mu g.L^{-1}$) showed that percentage areas of MMCs from head kidney and spleen increased after 48 hours of exposure (Gomes *et al*., 2015). Pronina and colleagues (Pronina *et al*., 2014) performed a comparative study of MMCs in liver and spleen of the roach *Rutilus rutilus* from two Lakes: Baikal (safe environmental) and Kotokel (impact environmental). They recorded that the MMC area in the liver of *R. rutilus* from Lake Kotokel was significantly smaller, whereas the number and size of these structures in the spleen was significantly larger than in the roaches from Lake Baikal. In the liver, the MMC area in *G. brasiliensis* and *H.* aff. *malabaricus* showed a predominance of 0-2365 $\mu m^2$ and 0-795 $\mu m^2$ MMCs, respectively. In the spleen of *G. brasiliensis* and *H.* aff. *malabaricus*, we observed a high frequency of 0-3199 $\mu m^2$ and 1899 $\mu m^2$ MMCs, respectively. Thus, the spleen presented a higher frequency of larger MMCs versus the liver in both species. Large MMCs are related to imune response while small MMCs are associated with metabolites such as iron, melanin and lipofuscin (Pronina *et al*., 2014).

The liver and spleen of the three species had a network of reticular fibers that were stained by the silver impregnation methods. In the liver and spleen, this network is associated with hepatocytes-sinusoidal structures and the white pulp, respectively. The arrangement of reticular fibers in the liver is generally accompanied by hepatocyte-sinusoidal structures. In the spleen, MMCs and ellipsoid capillaries were support by reticular fibers. The reticular fiber is formed by type III collagen associated with carbohydrates that has been widely described in the liver and spleen of vertebrates (Ushiki, 2002). Fibroblasts are responsible for the synthesis of reticular fibers, which support cells and blood vessels ultimately forming a framework of stroma in the liver and spleen (Ushiki, 2002). Moreover, these fibers form the basement membrane and are considerably thinner than collagen fibers. Conventional staining methods such as hematoxylin and eosin cannot identify reticular fibers. However, if stained by silver salts they react with the carbohydrate components present in these fibers and stain them black; thicker collagen fibers are brown (Ushiki, 2002). For liver, *G. brasiliensis* had large amount of reticular fibers and collagen. In spleen, the stroma was more developed in *H. francisci* (collagen) and *H.* aff. *malabaricus* (reticular fibers). Increased collagen deposits in the liver could be associated with toxic agents leading to fibrosis and hepatic dysfunction (Babu, 2013).

In summary, the liver and spleen morphology was very different between the species. We observed that the liver of *G. brasiliensis* presented a complex histology showing intrahepatic exocrine pancreas while *H. francisci* had hepatocyte-sinusoidal structure classified in cord-like form without MMCs. Only *H. francisci* had splenic parenchyma with a nodular organization well evident and absence of MMCs. Regarding the liver stroma, *G. brasiliensis* presented many reticular fibers and collagen. In the spleen, *H. francisci* had higher amounts of collagen and *H.* aff. *malabaricus* had more reticular fibers. The histological differences confirmed the hypothesis that the phylogenetic distance is reflected in the liver and splenic organization. These findings may reflect the phylogeny and different eating habits of these species. Besides, we observed large and predominant MMCs in the spleen in relation to liver of *G. brasiliensis* and *H.* aff. *malabaricus*, suggesting that splenic MMCs has a more important role in the immune system while that the centers from liver can be related with metabolism of iron, melanin and lipofuscin. This report expands the descriptive knowledge available about the abdominal organs involved in the fish metabolism and immunity.

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