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ORIGINAL ARTICLE

Histology of the hepatopancreas and anterior intestine in the freshwater prawn *Macrobrachium carcinus* (Crustacea, Decapoda)

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ABSTRACT

The purpose of this study was to describe the structure of the midgut (hepatopancreas and intestine) in the endemic species, *Macrobrachium carcinus*. Thirty specimens were collected, and the midgut was fixed in Bouin's solution for histological and histochemical analyzes by light microscopy. The hepatopancreas consists of two lobes that connect to the end of the stomach by primary ducts, which originate secondary tubules or hepatopancreatic ducts, that branch into hepatopancreatic tubules. The hepatopancreatic duct presents a columnar epithelium composed of R- and F- cells with evident brush borders for absorption and storage. The hepatopancreatic tubule is lined by epithelium with five cell types (E, F, R, B, and M). The distal region presents all cell types, with a predominance of E-cells that correspond to epithelial renewal. The middle region presents F- and B- cells, characteristic of extracellular and intracellular digestion. The proximal region, with B- and R- cells, performs the final digestion, storage, and extrusion of the cells with

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waste material. The intestine is lined by a single cell type with an evident brush border, suggesting luminal absorption. This cellular arrangement along the length of the midgut proposes distinct morpho-functional characteristics of digestion, absorption, and storage in this species.

Keywords

Hepatopancreas, intestine, epithelium, cytoarchitecture, brush border

Introduction

The digestive system of decapod crustaceans comprises an internal tube subdivided into three parts according to their embryological origin: the foregut (esophagus and stomach) and the hindgut originate from the ectodermal layer, and the midgut (hepatopancreas and intestine) originate from the endodermal layer in decapods (McLaughlin, 1983; Felgenhauer, 1992; Icely and Nott, 1992; Ceccaldi, 1998; Sonakowska *et al.*, 2015). Specifically, the midgut is composed of a branched tubular network called the hepatopancreas, and the intestine, which connects the foregut and hepatopancreas to the hindgut (Factor and Naar, 1985; Factor, 1995; Franceschini-Vicentini *et al.*, 2009). The intestine is a tubular organ that originates after the junction between the hepatopancreas and the stomach (Icely and Nott, 1992) and transports the processed contents in the hepatopancreas to the hindgut. Its main function is the regulation and transport of ions and water (Felder and Felgenhauer, 1993; De Jong-Moreau *et al.*, 2000; Sousa and Petriella, 2006).

The hepatopancreas occupies a large part of the cephalothorax and projects into the abdomen in some species. This organ has great relevance for crustaceans, since it is directly involved in the synthesis and secretion of digestive enzymes and, subsequently, in absorption, nutrient assimilation and waste excretion (Barker and Gibson, 1977; Gibson and Barker, 1979; Vogt *et al.*, 1985). In addition, it stores important nutrients, such as lipids, glycogen and other organic and inorganic compounds (Felgenhauer, 1992). These compounds are transported to other organs and used for body growth as well as for the development and maturation of sexual structures (Al-Mohanna and Nott, 1989).

The hepatopancreatic tubule is considered the morpho-functional unit of the hepatopancreas (Johnston *et al.*, 1998; Franceschini-Vicentini *et al.*, 2009; Zhang *et al.*, 2017), and is internally lined by a digestive epithelium composed of four cell types, called the E- (embryonic), F- (fibrillar), B- (blister or vesicular) and R- (reabsorption) cells (Jacobs, 1928; Hirsch and Jacobs, 1930; Vogt, 2019; Štrus *et al.*, 2019). Al-Mohanna *et al.* (1985) described an M-cell as a fifth cell type found in the hepatopancreas of some crustaceans (Icely and Nott, 1992). Each cell type presents distinct characteristics when analyzed by histological and histochemical methods (Longo and Díaz, 2015). In addition, their location along the entire length of the tubule is related to the function performed in digestion and may vary between crustacean species (Al-Mohanna and Nott, 1989; Hu and Leung, 2007; Ribeiro *et al.*, 2014; Štrus *et al.*, 2019).

In general, the morphology of the hepatopancreas reflects the external environmental conditions and presents great plasticity between species (Meyers and Hendricks, 1985; Sousa and Petriella, 2007; Longo and Diaz, 2015). According to Ceccaldi (1998), the various functions of this organ are directly related to their morphological diversity among crustacean species. However, the digestive process and the role of each cell type are not clear for caridean freshwater prawns. Recently, Štrus *et al.* (2019) and Vogt (2019) presented reviews regarding morphology and functional role of epithelial cells in the hepatopancreas with great emphasis on marine species. The morphology of the hepatopancreas provides support for studies on nutritional requirements for the development of sustainable cultivation techniques, as well as knowledge about the biology of the species (Icely and Nott, 1992; Johnston *et al.*, 1998; Franceschini-Vicentini *et al.*, 2009; Díaz *et al.*, 2010; Longo and Díaz, 2015; Ruiz *et al.*, 2019).

Macrobrachium carcinus (Linnaeus, 1758) presents great potential for aquaculture among *Macrobrachium* species and inhabits coastal basins and rivers on the

Atlantic coastline of the America's (Coelho and Ramos-Porto, 1984; Lima *et al.*, 2014). *Macrobrachium carcinus* was on the list of endangered aquatic animals in Brazil due to artisanal fishing (Mantelatto *et al.*, 2016), but currently is mentioned as "Least Concern" (De Grave, 2013). The objective of this study was to describe the histological and histochemical characteristics of the midgut in *M. carcinus*, with emphasis on the cytoarchitecture of the hepatopancreas and intestine, considering the importance of knowledge of the digestive process for the development of sustainable management and the currently deficient data of this species (Valenti, 1993; Coelho-Filho *et al.*, 2018).

Material and Methods

Animals

Specimens of *M. carcinus* were reared in earthen pounds (13 m^3) in Brejinho, Rio Grande do Norte in the Northeast region of Brazil. They were fed with commercial shrimp feed (38% crude protein) twice daily for six months. Animal welfare and handling follow the international protocols according to Diggles (2018) and Daniels *et al.* (2010). Thirty adult specimens (CL between 13.11 cm ± 3.23 and 49.77 g ± 8.51) were collected and euthanized by thermal shock, and subsequently, each animal was dissected to isolate the hepatopancreas and intestine. These organs were fixed in Bouin's solution, for 24 hours. The samples were washed in 70% ethanol to remove the fixative and, then, documented and analyzed with a Leica® M50 stereomicroscope (photographic camera IC80HD, Leica®) for gross anatomy.

Histological studies

Twenty samples previously fixed in Bouin's solution (24 hours) were washed in 70% ethanol, dehydrated in a graduated ethanol series and embedded in Historesin at room temperature (Leica®, Germany). Tissue fragments were sectioned at 3 µm thickness in semi-automatic Leica® RM 2265 microtome. For histological studies, sections were submitted to hematoxylin/eosin (HE) and toluidine blue (TB) 1% staining for a general description of tissues and cellular arrangements.

Histochemical studies

Histochemical studies were performed on ten samples fixed in Bouin's solution, dehydrated in a graduated ethanol series and embedded in Paraplast at 60°C (Oxford, USA). Sections were submitted to periodic acid-Schiff reaction (PAS) for neutral glycoproteins and/or glycogen, and Alcian blue (AB) reaction for acid glycoproteins (carboxylated and sulfated) (AB, pH 2.5) and sulfated acid glycoproteins (AB, pH 1.0), according to Layton and Bancroft (2019). Histological and histochemical studies were analyzed and photographic documentation was performed with a Leica® DM 750 microscope.

Results

General morphology of the hepatopancreas

The hepatopancreas is a compact organ that exhibits orange coloration, and is anatomically divided along the midline of the animal into two large halves, left and right lobes, occupying most of the cephalothorax of *M. carcinus* (Fig. 1A). Each lobe is covered by a capsule of fibrous connective tissue which has associations with small peripheral vessels (extra hepatopancreatic vessels) (Fig. 1B, C) as well as presenting anatomical prominences in the anterior and posterior regions (Fig. 1A). In the hepatopancreas, each primary duct divides into the secondary tubules or hepatopancreatic ducts (Fig. 1D, E) that branch into the hepatopancreatic parenchyma and give rise to numerous blind ending hepatopancreatic tubules.

Histological and histochemical characteristics of the hepatopancreatic ducts

The hepatopancreatic ducts or secondary tubules are lined with columnar epithelium, which is supported by a thin layer of connective tissue that presents isolated muscle cells in circular and longitudinal arrangements (Fig. 1F). The epithelial luminal surface has an irregular shape due to the different heights of R- and F-cells (Fig. 1E–G). In *M. carcinus* an abundance of narrow columnar R-cells is observed with numerous small vacuoles stacked in the cytoplasm, in addition to a few basophilic columnar F-cells (Fig. 1F), along the entire length of the hepatopancreatic duct.

Histochemical analysis reveals a strong reactivity for PAS and moderate for AB (pH 2.5) in the brush border region of epithelial cells, showing the presence of neutral and acid glycoproteins, respectively (Tab. 1;

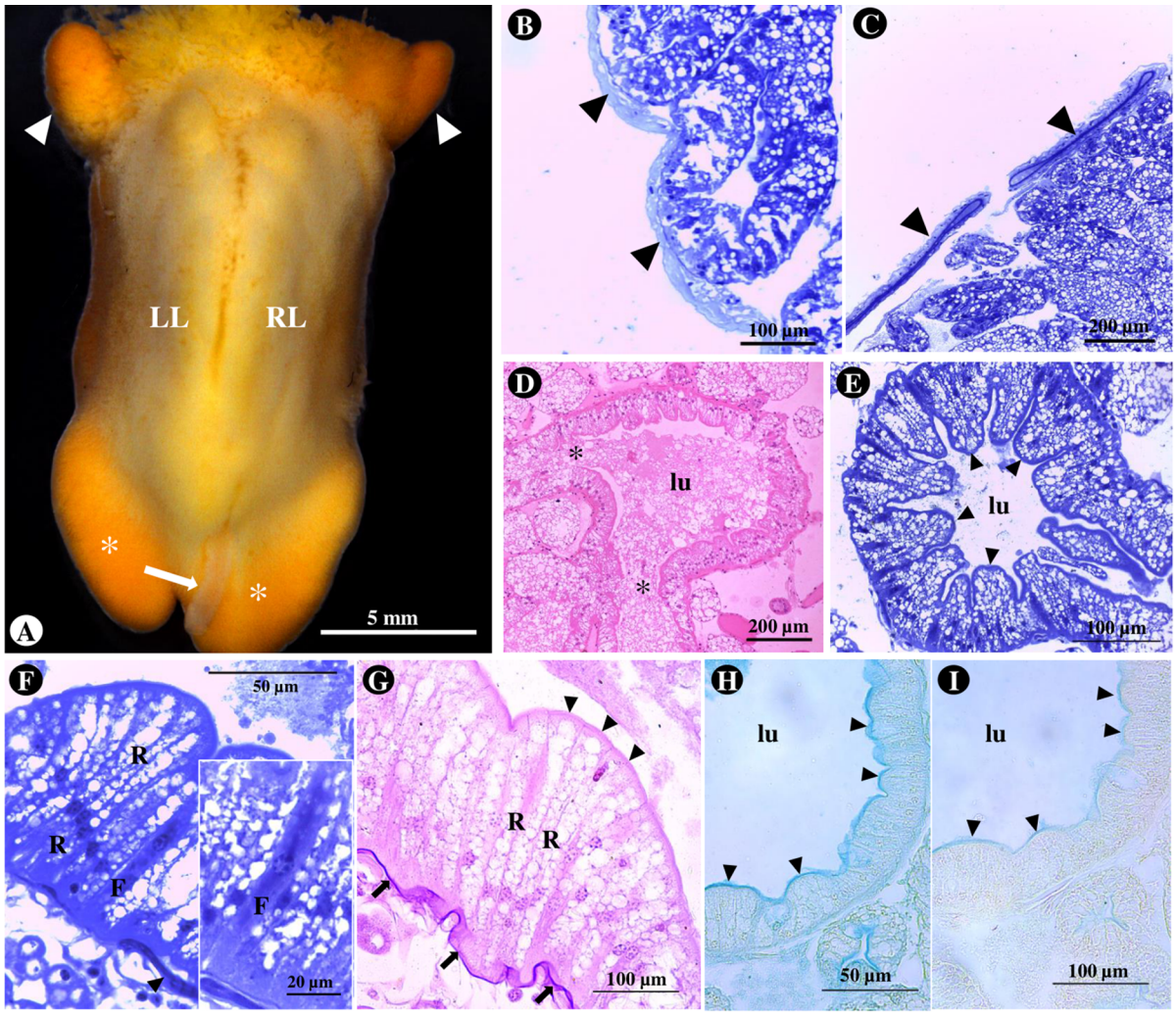

Figure 1. Hepatopancreas of *Macrobrachium carcinus*. (**A**) Dorsal anatomical view of the hepatopancreas showing the right lobe (RL) and the left lobe (LL) with anterior prominences (arrowhead) and posterior ones (asterisks). Note part of intestine arising from lobes (arrow). (**B, C**) Histological section of the hepatopancreas exhibiting in the periphery a connective tissue capsule (in B - arrowhead) and extra-hepatopancreatic vessels (in C - arrowhead). (**D**) Primary duct branching into hepatopancreatic ducts (asterisks). (**E**) Transverse section of a hepatopancreatic duct showing epithelial projections (arrowheads) towards the lumen. (**F**) Detail of ductal epithelium showing elongated R- and F-cells; Inset: detail of basophilic F-cell in the hepatopancreatic duct. (**G–I**) Histochemistry of hepatopancreatic ducts, highlighting the brush border region of epithelial cells (arrowheads) and basement membrane region (in **G** - arrows). Abbreviations: F: F-cells; lu: lumen; R: R-cells. Staining and Reactions: toluidine blue (**B, C, E, F**), HE (**D**), PAS (**G**), AB 2.5 (**H**), AB 1.0 (**I**).

Table 1. Histochemical reactions of the brush border and vacuoles of the midgut organs of *Macrobrachium carcinus*. Staining intensity: (–) negative; (+) weak; (++) moderate; (+++) strong.

Techniques employed	Hepatopancreas			Intestine
	Duct	Tubule		
Brush Border				
PAS	+++	+++		+
AB pH 2.5	++	++		-
AB pH 1.0	+	+		-
Cells	R-cells	F-, B-, and R-cells		Epithelial
Vacuoles				
PAS	-	+++	-	-
AB pH 2.5	-	++	-	-
AB pH 1.0	-	+	-	-
Cells	R-cells	Bv-cells	R-cells	Epithelial

Fig. 1G–I). The reaction of AB (pH 1.0) revealed weak reactivity (Tab. 1; Fig. 1I), showing scarce acid sulfated glycoproteins. A well-defined continuous basement membrane is present between the epithelium and adjacent connective tissue (Fig. 1G).

Histological and histochemical characteristics of the hepatopancreatic tubules

The hepatopancreatic ducts branch into the hepatopancreatic tubules (Fig. 2A), which present three distinct regions, proximal, middle and distal, according to the distance from the hepatopancreatic ducts. Around the tubules, the hemolymph space is observed with some intra-hepatopancreatic vessels (Fig. 2B). The hepatopancreatic tubule is lined by a pseudostratified epithelium composed of five cell types, identified as E-, F-, R-, B-, and M-cells. The tubule lumen displays a star shape because of different heights of epithelial cells, such as B-, R- and F-cells (Figs. 2C, 3A).

The cubic E-cells, with a conspicuous round nucleus in the central region of the cytoplasm (Fig. 2D), occupy the blind end of the hepatopancreatic tubules. F-cells are scattered throughout the length of the tubule, but are more abundant in the proximal and distal zone, close to E-cells (Fig. 2D). F-cells exhibit a columnar shape with basophilic cytoplasm and a central nucleus with evident nucleoli (Fig. 2E).

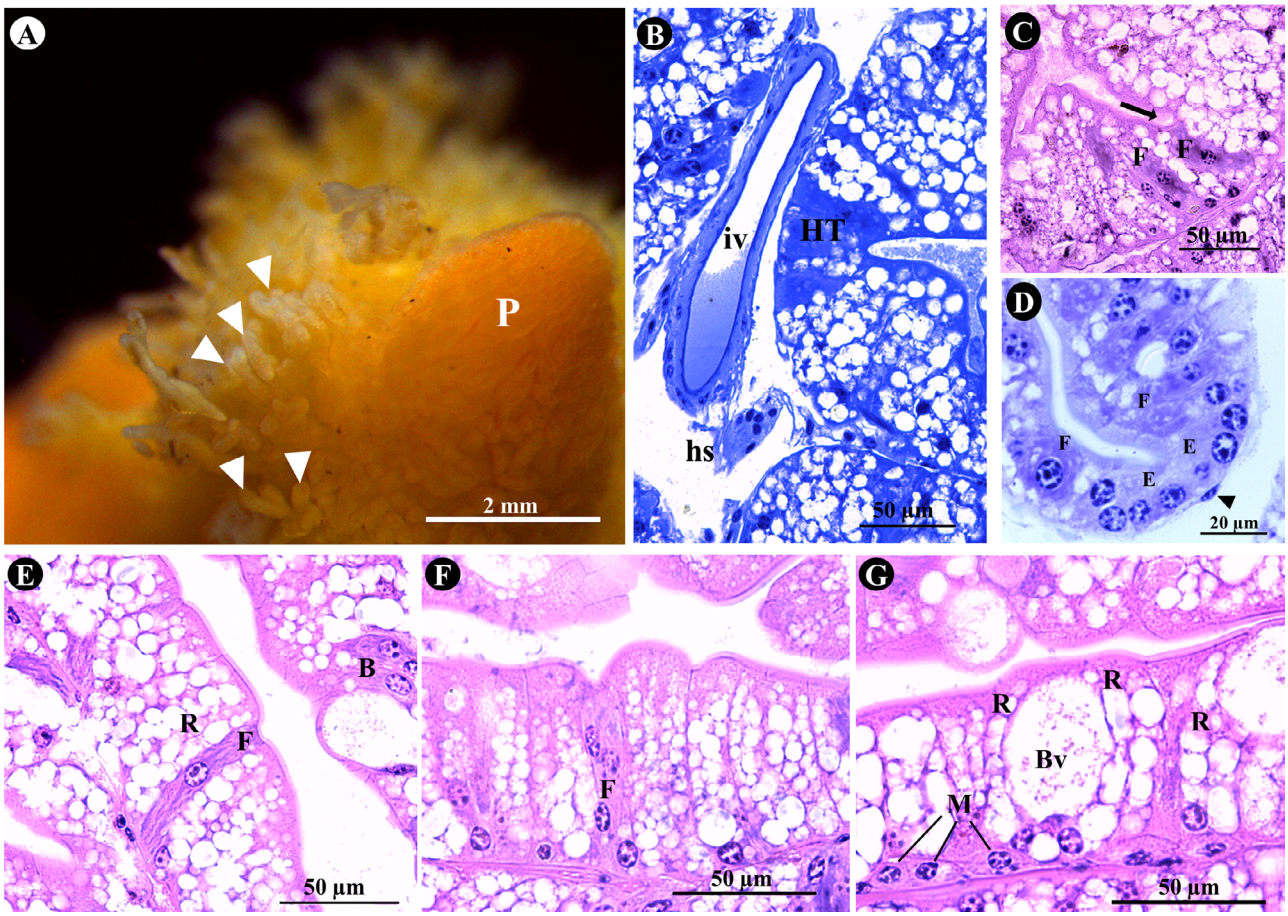

Figure 2. Hepatopancreatic tubules in *Macrobrachium carcinus*. (**A**) Anatomical detail of a prominence showing the terminal hepatopancreatic tubules (arrowheads). (**B**) Detail of an intra-hepatopancreatic vessel (iv) next to a tubule. (**C**) Transverse section of the hepatopancreatic tubule with F-cells in the region of epithelial infolding (arrow). (**D**) Distal region showing E- and F- cells. Note thick connective tissue involving the tubule represented by the fibroblast nucleus (arrowhead). (**E, F**) Middle region showing columnar F-, R-, and B- cells with small vacuoles in the cytoplasm. (**G**) Proximal region showing R-cells with stacked cytoplasmic vacuoles, B-cells with large cytoplasmic vacuoles, and M-cells in the basal region of the epithelium without lumen contact. Abbreviations: B: B-cells; Bv: B-cells with a large vacuole; E: E-cells; F: F-cells; hs: hemolymphatic space; HT: hepatopancreatic tubule; M: M-cells; P: anatomical prominence; R: R-cells. Staining: toluidine blue (**B, D**) and HE (**C, E, F, G**).

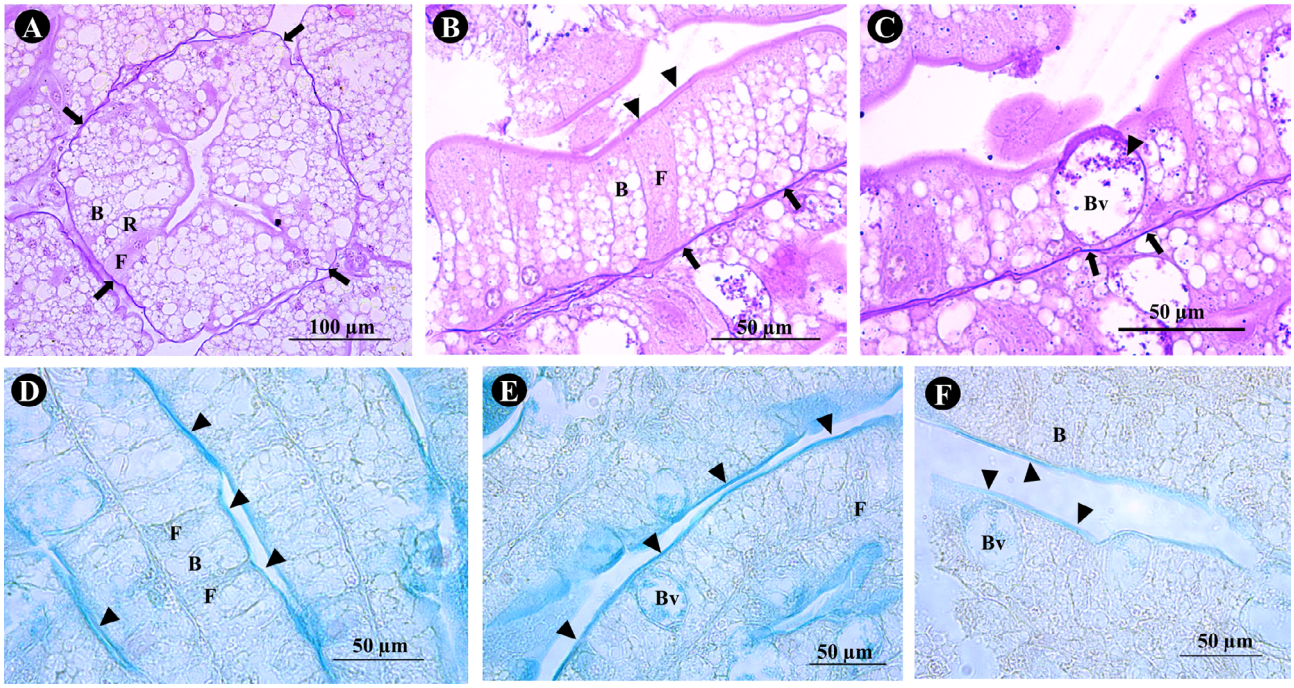

Figure 3. Histochemistry of the hepatopancreatic tubules of *Macrobrachium carcinus*. (**A**) Transverse section showing basement membrane region continues (arrows) around the tubule and R-, F-cells and B-cells with small vacuoles. (**B**) Middle region epithelium showing brush border region (arrowheads) evident on B- and F-cells. (**C**) Detail of B-cell with PAS-positive corpuscles within the large vacuole (arrowhead). (**D, E**) Histochemistry of AB 2.5 showing a moderate reaction on the brush border region (arrowhead). (**F**) Histochemistry of AB 1.0 showing a positive reaction on the brush border region (arrowhead). Abbreviations: B: B-cells; Bv: B-cells with a large vacuole; F: F-cells; R: R-cells. Reactions: PAS (**A, B, C**); AB 2.5 (**D, E**); AB 1.0 (**F**).

B-cells are more evident in the middle and proximal regions and scarce in the distal region of the hepatopancreatic tubules (Fig. 2E, G). In *M. carcinus* it is possible to see B-cells with apical small vacuoles and a basal nucleus (Fig. 2E) and with a large vacuole that occupies the remaining space of the cytoplasm and compresses the nucleus at the periphery (Fig. 2G). They are found throughout the length of the hepatopancreatic tubules, but there is an abundance in the middle region (Fig. 2E, F) and in the proximal region of the tubules (Fig. 2G). In addition, it is possible to see B-cells projecting toward the lumen (Fig. 2G).

R-cells are found dispersed between F- and B-cells in the epithelial lining, throughout the length of the hepatopancreatic tubules. These cells have a columnar shape with numerous vacuoles stacked in the cytoplasm and the nucleus in the basal region (Fig. 2G). The abundance of this cell type increases near the connection with the hepatopancreatic ducts.

M-cells are scarce and have a sparse distribution in the hepatopancreatic tubules, usually found near B- and R-cells. This cell type presents a triangular or rounded shape, with cytoplasm displaying weak basophilia and a central nucleus with evident nucleoli (Fig. 2G). M-cells do not reach the lumen of the tubules and are restricted to the basal region of the epithelium.

The PAS reaction shows a continuous basement membrane associated with myoid cells around the tubules (Fig. 3A). The entire length of the hepatopancreatic epithelium exhibits an evident brush border (Tab. 1; Fig. 3B). It should be noted that B-cells do not present an evident brush border (Fig. 3C), and their large vacuoles contain particles that strongly react with PAS and AB (pH 2.5) (Fig. 3C, E). Analysis of acid glycoproteins present at the brush border exhibit moderate reactivity for acid groups (AB pH 2.5) (Fig. 3D, E) and weak reactivity for specifically acid sulfated groups (AB pH 1.0) (Tab. 1; Fig. 3F).

General morphology, histology, and histochemistry of the intestine

The intestine (Fig. 4A) runs caudally between the hepatopancreatic lobes and connects with the hindgut. The organ is lined by a simple columnar epithelium supported on an irregular PAS-positive basal membrane (Fig. 4D), followed by a thin layer of connective tissue presenting discrete hemolymph spaces (Fig. 4B). The intestinal lining epithelium shows one cell type, which presents two different acidophilic characteristics (Fig. 4C). However, all epithelial cells have a slight brush border (PAS - weak, AB pH 1.0 and pH 2.5 - negative) (Tab. 1), a cytoplasm with small subapical vesicles, and a central nucleus (Fig. 4B–D). Externally, a thick layer of loose connective tissue covers a layer of circular visceral muscle (Fig. 4B). Small vessels are observed in the connective tissue, as well as hemocytes and an irregular system of hemolymph spaces (Fig. 4B, C).

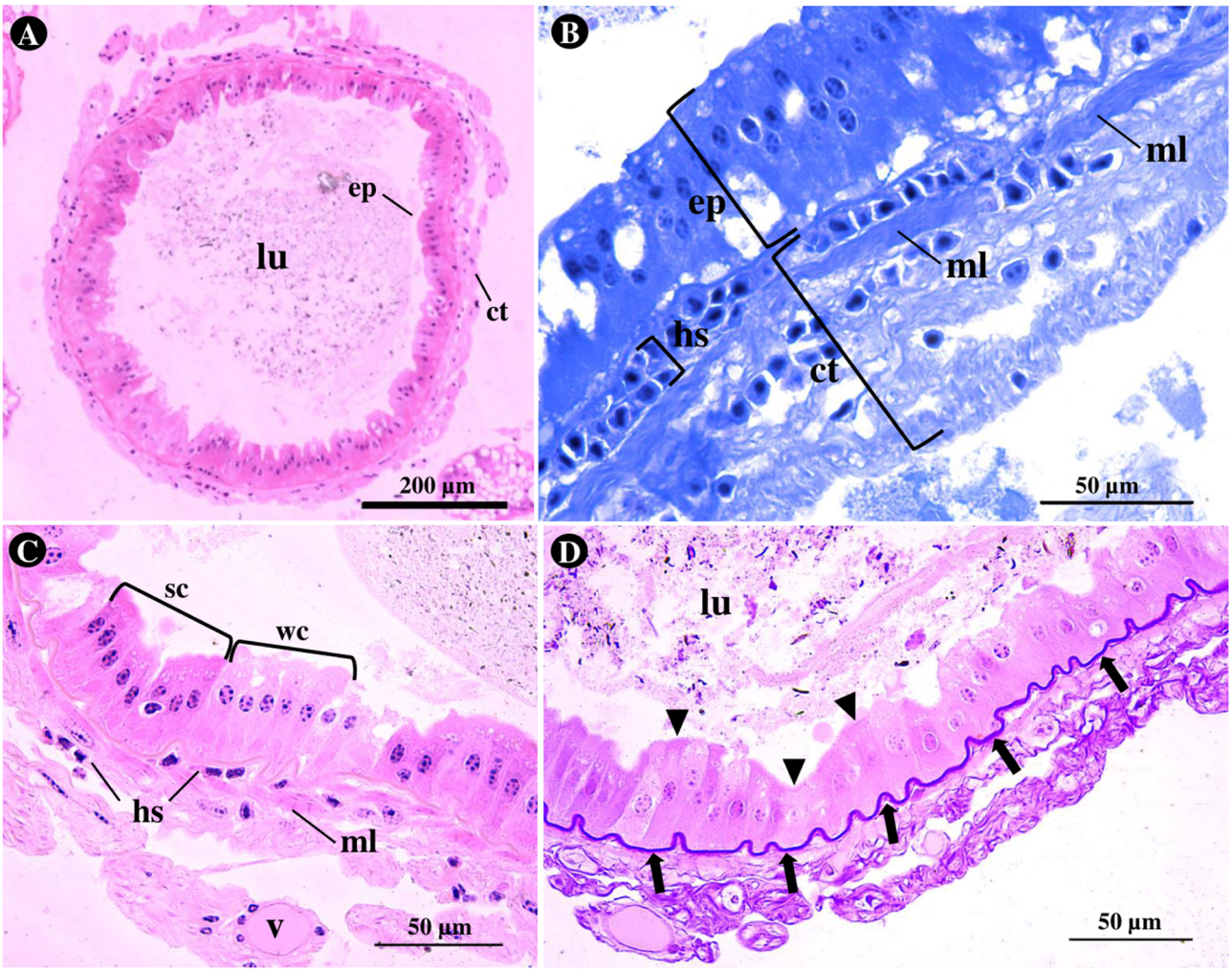

Figure 4. Intestine of *Macrobrachium carcinus*. (**A**) Transverse section showing the epithelium and connective tissue surrounding the intestine. (**B**) Intestinal wall. Note that in the connective layer, a muscular layer, as well as hemolymphatic spaces, are distributed close to the epithelium. (**C**) Histology of intestinal lining epithelium exhibiting one cell type presenting two different acidophilia: the weak acidophilic cells and the strong acidophilic cells. (**D**) PAS histochemistry of the intestinal wall showing the brush border region of epithelial cells (arrowheads) and irregular basement membrane (arrows). Abbreviation: ct: connective tissue; ep: epithelium; hs: hemolymphatic space; lu: lumen; ml: muscle layer; sc: strong acidophilic cells; v: vessel; wc: weak acidophilic cells. Staining and Reactions: HE (**A, C**), toluidine blue (**B**), PAS (**D**).

Discussion

The morphology of the midgut in *M. carcinus* shows great similarities to other Decapoda taxa. However, some authors have reported some differences in anatomy and histology, especially among freshwater prawns (Icely and Nott, 1992; Felgenhauer, 1992; Rőszer, 2014). Different to the hepatopancreatic tubules, the hepatopancreatic ducts or secondary tubules are not clearly described in the literature. The ducts of *M. carcinus* have an irregular luminal shape caused by the height of the epithelial R-cells with a conspicuous brush border. In the digestive tract of vertebrates, both components, luminal irregularity and a brush border, increases the intestinal lining surface area for nutrient absorption (Ross and Pawlina, 2016). In addition, in crustaceans, hepatopancreatic R-cells play an important role in the uptake and metabolism of nutrients, as well as in the storage of reserves (Vogt, 1996; Hu and Leung, 2007). Therefore, hepatopancreatic ducts may be involved in important digestive functions in *M. carcinus*, since they have structures that increase the absorption process and can store essential nutrients in the hepatopancreas. Furthermore, *M. carcinus* ducts present muscle cells around them, which can provide peristaltic movement that contributes to the transport of luminal contents, as observed in the hepatopancreas of *Homarus americanus* H. Milne Edwards, 1837 (Leavitt and Bayer, 1982; Factor, 1995).

In decapod crustaceans, the hepatopancreatic tubules are lined by an epithelium composed of five types of cells, E-, F-, R-, B- and M-cells, which perform multiple functions in the hepatopancreas (Gibson and Barker, 1979; Al-Mohanna and Nott, 1987; Icely and Nott, 1992). The tubules of *M. carcinus* present all five-cell types with some differences in position and frequency in the different regions of the tubules. The location and abundance of each cell type contributes to functional differences of the different tubule regions (Al-Mohanna and Nott, 1989; Hu and Leung, 2007; Ribeiro *et al.*, 2014).

In the distal portion of the hepatopancreatic tubules in *M. carcinus*, E-cells predominate, as in other species (Caceci *et al.*, 1988; Al-Mohanna and Nott, 1989), in addition to sparse F-, R-, and B-cells. E-cells are involved in the process of cell regeneration of the epithelium by cell division and differentiation (Vogt, 1994; Sonakowska *et al.*, 2015). This process in decapods gives rise to R-, F- and B-cells throughout the epithelium (Jacobs, 1928; Caceci *et al.*, 1988; Vogt, 2019). In *M. carcinus*, the abundance of F-cells in the distal region may suggest that they originate directly from the E-cells, as well as the R- and B-cells observed in this region. Therefore, in *M. carcinus* we suggest that the distal region acts in generative and epithelial renewal of the entire hepatopancreatic epithelium, as in *M. amazonicum* (Heller, 1862) (Franceschini-Vicentini *et al.*, 2009; Ribeiro *et al.*, 2014; Ruiz *et al.*, 2019).

The middle region of the hepatopancreatic tubules in *M. carcinus* is mainly composed of F- and B-cells, as also reported for *Neocaridina heteropoda* Liang, 2002 (Sonakowska *et al.*, 2015), and can be considered a region of intense secretion for extracellular digestion and the beginning of intracellular digestion. F-cells are typical secreting cells of the hepatopancreatic epithelium that produce enzymes detected by *in situ* hybridization and immunohistochemistry, such as amylase, chitinase, cellulase, and trypsin; produced by the pancreas and liver in vertebrates (Lehnert and Johnson, 2002; Vogt, 2019). Al-Mohanna and Nott (1989) and Vogt (2019) propose that the B-cells found in the middle region could be related to the activity of luminal absorption for intracellular digestion, whereas those found in the proximal region could be related to the digestion and nutrient assimilation processes. According to Ribeiro *et al.* (2014), vacuolization in B-cells may suggest the functional stage of these cells. Longo and Diaz (2015) and Ruiz *et al.* (2019) suggest that the various small vacuoles showing scarce acid glycoproteins in the B-cell cytoplasm, would indicate the beginning of the digestion process. On the other hand, the presence of a large vacuole in the cytoplasm indicates the end of the digestive process (Franceschini-Vicentini *et al.*, 2009; Ribeiro *et al.*, 2014). In the hepatopancreatic tubules of *M. carcinus*, an abundance of B-cells with apical small vacuoles was observed in the middle and proximal region, suggests that the main digestion processes occur in these regions of the tubules. Furthermore, in the proximal region, most of the B-cells with a large vacuole are found, which would be related to final digestion and the extrusion phase of these cells in

M. carcinus, as suggested for other species (Franceschini-Vicentini *et al.*, 2009; Longo and Diaz, 2015). The final phase or extrusion of B-cells can be classified as holocrine in *M. carcinus*, as it has been described for other crustaceans (Caceci *et al.*, 1988; Vogt, 1994; Sousa *et al.*, 2005), since the complete disconnection of the cell from the basement membrane and its release towards the lumen is observed.

R-cells are the most evident cell type in the hepatopancreatic epithelium of decapods (Icely and Nott, 1992). This cell type in other crustaceans is commonly observed in the tubule proximal region, but in *Penaeus vannamei* Boone, 1931 (Caceci *et al.*, 1988) and *Penaeus semisulcatus* De Haan, 1844 in De Haan, 1833–1850 (Al-Mohanna and Nott, 1989) it is observed throughout the tubule. In *M. carcinus* these cells are distributed throughout the epithelium along the entire length of the tubule and are predominant in the epithelium of the hepatopancreatic ducts. The main functions of these cells involve the uptake and storage of nutrients (Vogt, 1994; 1996; Ribeiro *et al.*, 2014), as well as the important role of nutritional support during the moulting stages (Al-Mohanna and Nott, 1989). Thus, the abundance of these cells in the hepatopancreatic tubules and ducts in *M. carcinus* could contribute to the molting process, as well as supporting our hypothesis of absorption and storage in the epithelium of the hepatopancreatic ducts.

M-cells, a fifth cell type (Al-Mohanna and Nott, 1987), are present in the hepatopancreatic tubules of *M. carcinus*, as reported in *M. amazonicum* (*cf.* Franceschini-Vicentini *et al.*, 2009), *P. semisulcatus* (*cf.* Al-Mohanna and Nott, 1987) *Penaeus monodon* Fabricius, 1798 (Icely and Nott, 1992), *Procambarus blandingii* (Harlan, 1830) (Davis and Burnett, 1964) and *H. americanus* (*cf.* Icely and Nott, 1992). This cell type is frequently in the hepatopancreas of crustaceans and its cell morphology is linked with molting and feeding cycles (Al-Mohanna and Nott, 1987). In *M. carcinus* a sparse distribution of M-cells is observed, as in other decapods (Vogt, 2019). They appear near to B- or R-cells, indicating an important role in synchronization and endocrine regulation in hepatopancreatic epithelium as in other animal life cycles (Vogt, 2019).

In crustaceans, the participation of R- and B-cells in the absorption processes in the hepatopancreas is enhanced by the presence of a brush border over the entire luminal surface of the hepatopancreatic tubules (Ribeiro *et al.*, 2014; Zhang *et al.*, 2017). In *M. carcinus* the brush border in the hepatopancreatic ducts and tubules exhibits acid carboxylated and neutral glycoproteins. Neutral and acid carboxylated mucosubstances, in combination with the activity of alkaline phosphatase, assist in the digestion and emulsification of food and may be related to absorption of easily digested molecules (Monin and Rangneker, 1974; Stroband *et al.*, 1979; Clarke and Witcomb, 1980; Grau *et al.*, 1992; Murray *et al.*, 1996; Petrinec *et al.*, 2005). Acid carboxylate glycoproteins are related to proteolysis and aids in transport across cell membranes (Petrinec *et al.*, 2005; Ross and Pawlina, 2016), which indicates an important role in absorption in the hepatopancreas. In addition, it is important to note the presence of hepatopancreatic ducts and tubules in *M. carcinus* with acid sulfated glycoproteins present on the brush border. These glycoproteins can be important for epithelial resistance and protection against pathogens (Rhodes *et al.*, 1985; Carrassón *et al.*, 2006) and protection from auto-digestion by the secreted enzymes (Domeneghini *et al.*, 2005).

The midgut, in addition to the hepatopancreas, also shows the intestine with particular characteristics. The intestine in most crustaceans is lined by an epithelium composed of a single cell type, which may exhibit different stages in cell morphology (Icely and Nott, 1992; Vogt, 1996; De Jong-Moreau *et al.*, 2000; Martin and Chiu, 2003). The epithelial cells in the intestine of *M. carcinus* present different acidophilia that could represent two main stages in maturation, according to the development of organelles (Icely and Nott, 1992). Future studies that perform transmission electron microscopy are required to support and specify the origin of these differences. These cells are involved in the transport of ions and water, in addition to the secretion of the peritrophic membrane that aids in the movement of fecal pellets through the intestine. The peritrophic membrane is formed from the secretion of apical vacuoles from intestinal cells that have reactivity to PAS histochemistry in crustaceans (Barker and Gibson, 1977; Mykles, 1979; Factor, 1995). In *M. carcinus* the apical vacuoles are present in the intestinal cells, however, the PAS-histochemistry is negative, and the peritrophic membrane in the lumen of the

intestine was not evident. In addition, the intestinal surface of *M. carcinus* has no folds or longitudinal ridges, as described for *H. americanus* (*cf.* Factor, 1995) and *Sicyonia ingentis* (Burkenroad, 1938) (Martin and Chiu, 2003), but exhibits intestinal cells with a well-developed brush border. These characteristics of the intestinal epithelium of *M. carcinus* suggest that this segment of midgut may promote absorption, as proposed for other crustaceans (Ahearn *et al.*, 1985; Ahearn, 1987; Lovett and Felder, 1990), but does not contribute to the development of the peritrophic membrane.

In conclusion, the midgut of *M. carcinus* presents particular cytoarchitecture throughout its length, which indicates possible functional subdivisions for the hepatopancreas and intestine. The proximal, middle and distal regions of the hepatopancreatic tubules perform different digestive processes, according to the distribution of the cell types. The hepatopancreatic ducts present a histological structure that suggests an important role in the process of absorption and storage of compounds in the hepatopancreas, different from that of other crustaceans studied in the literature. In addition, the intestinal segment of the midgut presents characteristics for processes of absorption and/or waste excretion, without participation in the formation of the peritrophic membrane. This specific cytoarchitecture in the midgut of *M. carcinus* is evidence of morpho-functional sites specific to the cell profile that can be key points in future studies of digestive processes and physiology in many crustacean species.

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