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The natural interaction between *Myotis nigricans* (Schinz, 1821) and its trematodes: A histopathological analysis

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ABSTRACT

Bats have a wide diversity of digeneans; however, even with the recent increased interest in studies of parasites on these hosts, there are no data on the microscopic alterations of this host-parasite interaction. The present work characterizes and compares the histological aspects of the liver, gallbladder, and intestine of non-parasitized and parasitized *Myotis nigricans* by digeneans. Ten specimens of *Myotis nigricans* collected in an urban area of Western Amazonia were analyzed for parasites. The digeneans were removed from the hosts and identified. Tissue samples of the liver, gallbladder, and intestines of parasitized and non-parasitized hosts were collected for histological studies. The gallbladder was observed in repletion and presents mucosa formed by simple epithelium that varies from cubic to cylindrical. The hepatic lobes do not have a classic polyhedral-hexagonal aspect. Variations in basophilia, acidophile, and cytoplasmatic granulations were observed in hepatocytes. The parasitism of the intestinal digeneans was restricted to space delimited by the extensions of villi in high association with the intestinal epithelium, not invading the region of the intestinal glands at the base of the villi. Trematodes maintained attached to the villus by the oral sucker and acetabulum, connected by a "pleat" composed of epithelium and lamina propria layers. We observed no signs of inflammatory processes and cellular defense infiltrates in host tissues. Cytochemistry alterations, size variation, and granular deposits in hepatocytes, enterocytes, and goblet cells were observed. Thus, this report is the first study of the natural parasite-host interaction in the liver, gallbladder, and intestine in *M. nigricans* in the neotropical region.

1. Introduction

Myotis nigricans (Schinz, 1821) are widely distributed in the states of Pará, Amazonas, and Amapá in the Brazilian Amazon Region (Bernard et al., 2011). The parasite diversity of these species includes nematodes, trematodes, and cestodes (Santos and Gibson, 2015); and can infect the liver, small and large intestine, gallbladder, and thoracic cavity. The trematode parasites reported for *M. nigricans* in South America corresponds to 6 species: *Anenterotrema liliputianum* (Travassos, 1928), *Parabascus limatulus* (Braun, 1900), *Ochoterenatrema diminutum* (Chanlder, 1938), *Paralecithodendrium conturbatum* (Freitas, 1960), *Urotrema scabridum* Braun, 1900 from intestines and *Metadelphis lenti* (Santos and Gibson, 2015) parasite of the gallbladder (Santos and Gibson, 2015; Fernandes et al., 2019).

Although the diversity of helminth of bats has been studied more in recent years, no records address the pathological aspects of the interaction between parasites and their hosts. There are studies on intestinal histomorphology of bats with different eating habits (Gadelha-Alves et al., 2008), histology of the tongue (Abayomi et al., 2009), and comparison of intestines at various stages of development (Selim and El Nahas, 2015). Still, so far, there are no records of studies comparing the tissue aspects of host bats versus parasitic helminth, and the reports are restricted to other well-known trematode-mammalian interactions, especially species of socioeconomic interest, such as *Schistosoma* and *Fasciola* species demonstrated by Hurtrez-boussès et al. (2001), Amaral et al. (2017) and Oyarzún-Ruiz et al. (2019).

During a survey of the helminth fauna of *M. nigricans* in North of Brazil, we identified *Metadelphis lenti*, *U. scabridum*, *P. aranhai* Lent et al. (1945), *Anenterotrema* sp. and Lecithodendridae trematode infecting this host species. Thus, to understand the host-parasite interaction between those species, we characterized the microscopic morphology of the liver, gallbladder, and intestines of non-parasitized and parasitized bats.

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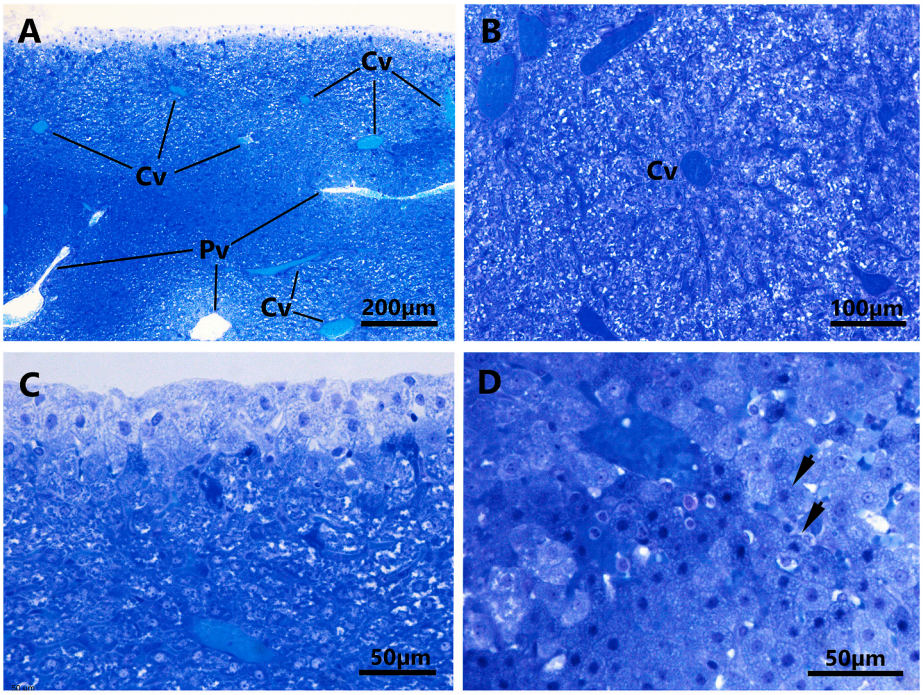

Fig. 1. Histological features of the non-parasitized liver of *Myotis nigricans*. (A) Detail of hepatic lobes, with a view to the centrilobular vein, a segment of the portal triad, and portal vein. (B) Centrilobular vein surrounded by sinusoids capillaries and rows of hepatocytes. (C) Hepatic capsule formed by cubic cells. (D) Hepatocytes with basophilic cytoplasm, few granulations vary in appearance from translucent to basophilic. *Abbreviations:* (Cv), Centrilobular vein, (Pv), Portal vein, (arrowhead), hepatocytes.

2. Material and methods

The specimens of *M. nigricans* were collected in the Federal University of Pará, urban area of Belém, municipality of the state of Pará, Brazil (1°28′23.57″ S 48°26′52.76″ W) in 2017. The hosts were anesthetized and euthanized with lidocaine® injection and later were necropsied and analyzed for parasites. The digeneans were removed from the hosts, processed for light microscopy, and identified. For the morphological analysis, the trematodes were stained with acetic carmine, differentiated in 0.7% hydrochloric acid, neutralized in alkaline ethanol, dehydrated in a graded ethanol series, and cleared using methyl salicylate. Permanent total mounts were made using Damar gum as the embedding medium and studied under an Olympus BX53 microscope (Olympus, Tokyo, Japan) equipped with a digital imaging system and the CellSens Standard 1.9 Software.

We collected two liver samples (1 parasitized and 1 non-parasitized), one gallbladder (non-parasitized), and two samples of the intestine (1 parasitized and 1 non-parasitized). The tissues were fixed in 4% formaldehyde for 24–48 h, dehydrated in an ethanolic series for 1 h in each step (50%–100%), and slowly infiltered in methacrylate resin

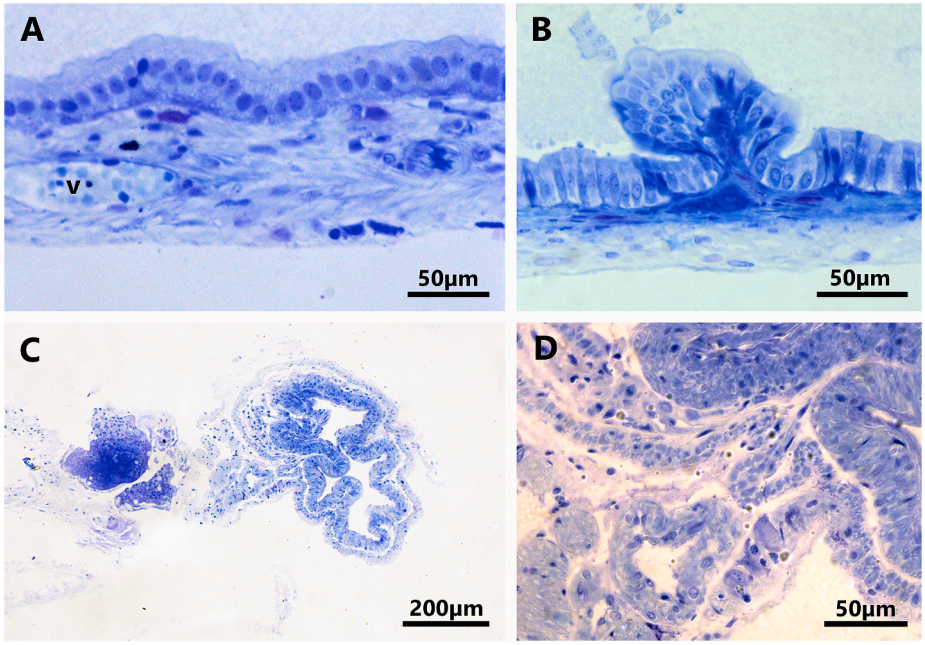

Fig. 2. The non-parasitized gallbladder of *Myotis nigricans*. (A) Detail of the gallbladder mucosa epithelium formed by simple epithelium that varies from cubic to cylindrical. (B) Folds and pleats in gallbladder with mucosa epithelium. (C) General view of gallbladder isthmus. (D) Details of gallbladder isthmus and bile duct segment.

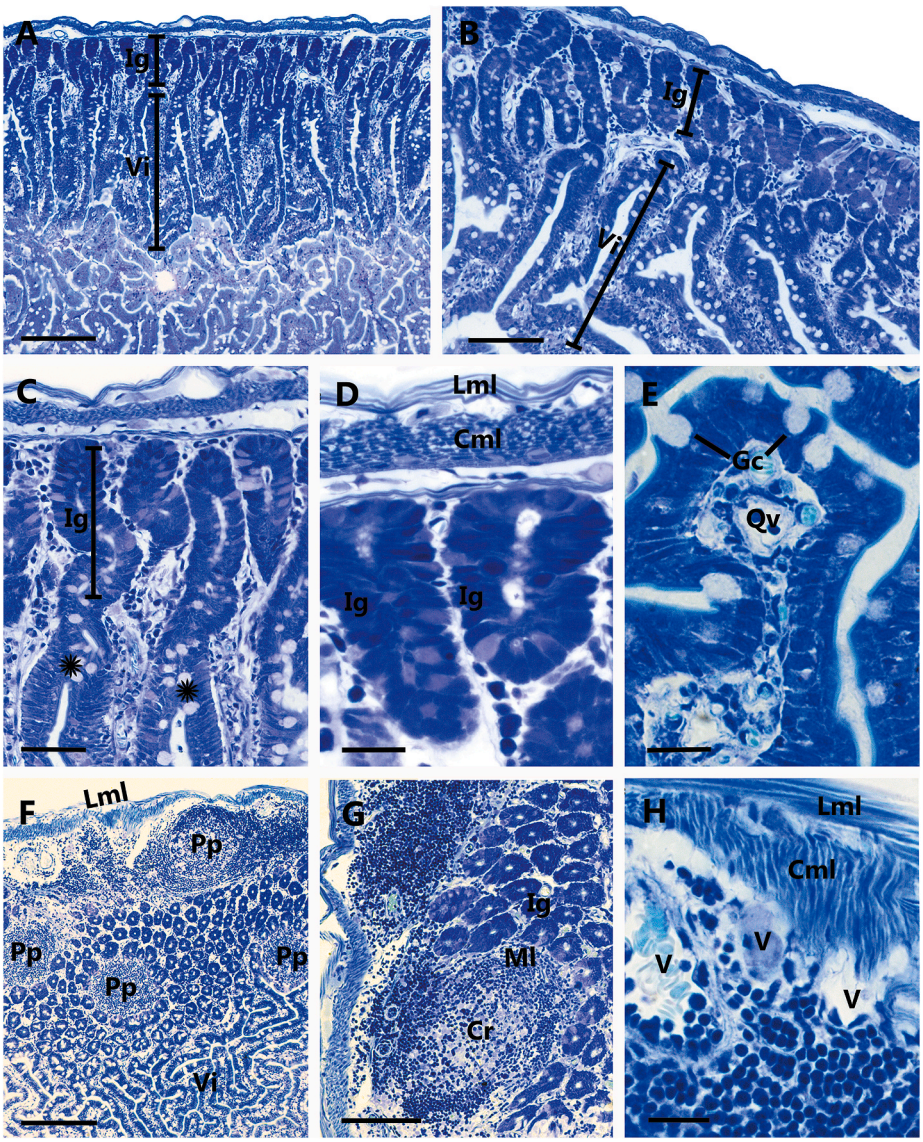

Fig. 3. The non-parasitized intestine of *Myotis nigricans*. (A) and (B) Longitudinal sections of the jejunum. Details of voluminous, long, and sinuous villi and intestinal glands. (C) Intestinal glands and crypts open at the base of the villi, involved by the periglandular capillary plexus. (D) Intestinal glands and submucosa with circular muscular layers and longitudinal muscular layer. (E) Detail of villus epithelium, showing cylindrical simple, goblet cells and quiliferous vessel. (F) and (G) The ileum segment with numerous Peyer patches located between the submucosa and the intestinal glands. (H) A segment of the ilium with the view of vessels and circular and longitudinal muscle layers. Abbreviations and symbols: (Cml) Circular muscular layer; (Cr) cortical region; (Gc) Globet cells; (Ig) intestinal glands; (Lml), longitudinal muscle layer; (Pp) Payer patches; (Qv) quiliferous vessel; (Vi) Villi; (*) opening of intestinal glands.

(Embedding kit Historesin®, Leica, Heidelberg, Baden-Wurttemburg, Germany) in different proportions (5:1, 4:1, 3:1, 2:1, 1:1, pure resin) for 24 h in each step. The inclusion was performed in plastic molds, according to the instructions of the fabricant. Serial sections with 3 μm thickness were done with glass knives on a Leica RM2235 microtome. The serial cuts were arranged neatly on slides and stained with Toluidine Blue 1% and Basic Fuchsin. All sections were analyzed and photographed using a LEICA DM2500 photomicroscope on the Laboratory of Histology and Animal Embryology (LHEA) of the Federal Rural University of Amazonia (UFRA).

3. Results

3.1. Non-parasitized liver, gallbladder, and intestines

The hepatic lobes do not have a classic polyhedral-hexagonal shape, which is difficult to define (Fig. 1A and B). However, the segments of the centrilobular vein (venules) and the hepatic space formed by the bile duct, the portal vein, and hepatic artery branches are easily distinguished (Fig. 1A). The hepatic capsule is composed of cubic cells of reduced basophilia compared to the cells present in the parenchyma (Fig. 1C). The connective tissue is scarce in perilobular areas, and hepatocyte plaques consist of twisted, irregular, and compacted rows of cells bounded by sinusoids. Some hepatocytes are voluminous with basophilic cytoplasm, showing few granulations varying from translucent to basophilic (Fig. 1B–D).

Gallbladder observed in repletion. The vesicle mucosa was formed by single-layer epithelium, with cells varying from cubic to cylindrical (Fig. 2A), scarce or no folds, and pleats (Fig. 2B). Lamina propria and lymphatic plexus are present (Fig. 2A and B). Muscle cells bundle soft and superimposed by the thin adventitious layer containing collagenous fibers (Fig. 2). The region of the neck of the gallbladder has tubular and acinous glands.

Small intestine with characteristics of the jejunum and ileum regions were analyzed. Transverse and longitudinal sections of the jejunum have voluminous, long, and sinuous villi; in some segments, the villi extend and fill the organ's lumen, showing bifurcations or branches (Fig. 3A and B). Intestinal glands occupied one-third of the mucosa's layer, with basophilic cells more marked than the villi (Fig. 3A–D). Crypts of the intestinal glands open at the base of the villi, involved by the periglandular capillary plexus (Fig. 3B and C). Lamina propria of the center of the villi is dense, containing the blood vessels of the villous capillary network and lymphatic capillaries (quiliferous vessel) (Fig. 3E). Submucosa sparse or absent, followed by two thin muscular layers (internal

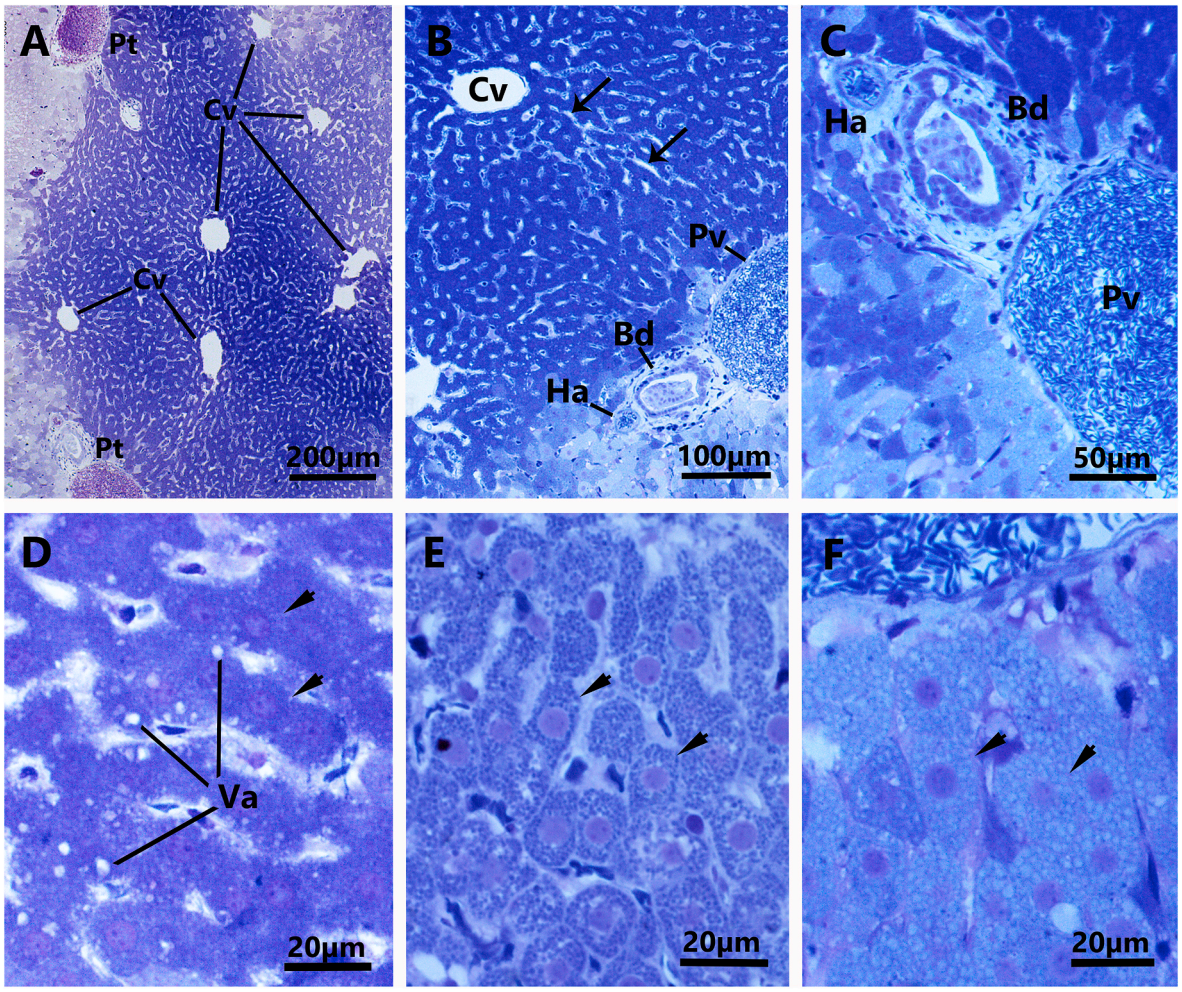

Fig. 4. The liver of *Myotis nigricans* with a parasitized gallbladder. (A) Hepatic parenchyma with significant hepatocyte impairment and visible centrilobular veins and portal triad. (B) Hepatic lobule with the view of the centrilobular vein surrounded by sinusoids capillaries, bile duct, branches of the portal vein, and hepatic artery. (C) Portal triad: bile duct, branches of the portal vein, and hepatic artery. (D), (E) and (F) Hepatocytes with variation in cytoplasmic granular deposits, from clear vacuoles in basophilic cells to dense basophilic granules or chromophobic voluminous granules in cells of low basophilia. Abbreviations and symbols: (arrow), sinusoid capillaries; (arrowhead), granular deposits; (Bd), bile duct; (Cv), centrilobular vein; (Ha), Hepatic artery; (Pv), Portal vein; (Tp) Portal triad; (Va), vacuoles.

circular muscle and the external longitudinal muscle) (Fig. 3B–D). The villus' epithelium is simple, with high cylindrical cells; the microvilli in this region is striated and rich in goblet cells. Intestinal glands are formed by cylindrical and basophilic cells, with Paneth and enteroendocrine cells in the background (Fig. 3B–E).

The ileum region has numerous lymphoid follicles (Peyer patches) of different sizes and is composed of basophilic cells in the submucosa (Fig. 3F–H). The intestinal villi in this region are numerous, long, sinuous with the same structural characteristics as the jejunal area. Intestinal glands occupy half of the mucosa layer and exhibit a basophilic zone more intense than villi (Fig. 3F). The muscle layer is thicker than in the jejune, where the inner circular muscular layer is denser than the external longitudinal muscular layer (Fig. 3F–H). Vessels of the submucosal plexus are located near the lymphoid follicles (Fig. 3H).

3.2. Parasitized liver and intestines

We identified *Metadelphis lenti* parasitizing the gallbladder of the host (Supplementary Fig. S1A). However, the parasites found inside the gallbladder made the organ friable during collection, and their removal for histology was not possible. Thus, we decided to collect samples of liver tissue close to the parasitized gallbladder. There were signs of chronic infection in these analyzed liver samples, with changes in the chemistry of parenchyma, venous congestion process, and increase in the volume of sinusoid capillaries (Fig. 4A, B, and E).

The hepatic parenchyma showed areas with hepatocyte impairment, easily delimited by the toluidine blue metachromasia, with hepatocytes ranging from weak cytoplasmatic basophilia to hypertrophic chromophobic hepatocytes (Fig. 4A–F). The nucleus of hepatocytes varied from basophilic blue to acidophilic (purple color) with chromophobic granules (Fig. 4E and F). The cytoplasm of hepatocytes showed a variation of granular deposits: clear vacuoles in basophilic cells, dense basophilic granules, or chromophobic voluminous granules in cells of low basophilia (Fig. 4E and F). We did not find larvae or juvenile forms in transit, nor lesions suggestive of active migration of parasites or even inflammatory tissue reactions in the hepatic parenchyma.

The trematodes parasites of the intestines were the following: *U. scabridum* (Supplementary Fig. S1B), *P. aranhai* (Supplementary Fig. S1C), *Anenterotrema* sp. (Supplementary Fig. S1D), and Lecithodendridae (Supplementary Fig. S1E). They were between the intestinal villi, and many specimens were in the jejunum and ileum, not invading the intestinal glands (Fig. 5A and B).

The parasites were attached to the villus adjacent to its ventral surface by the oral sucker and ventral sucker. A small area of the villus was projected into the suckers, forming a "pleat" composed of epithelium and lamina propria layers. Thus, the enterocytes and globet cells were directly in contact with the suckers' inner surface (Fig. 5C–E). The ventral and dorsal surfaces of the trematode were in tight adherence

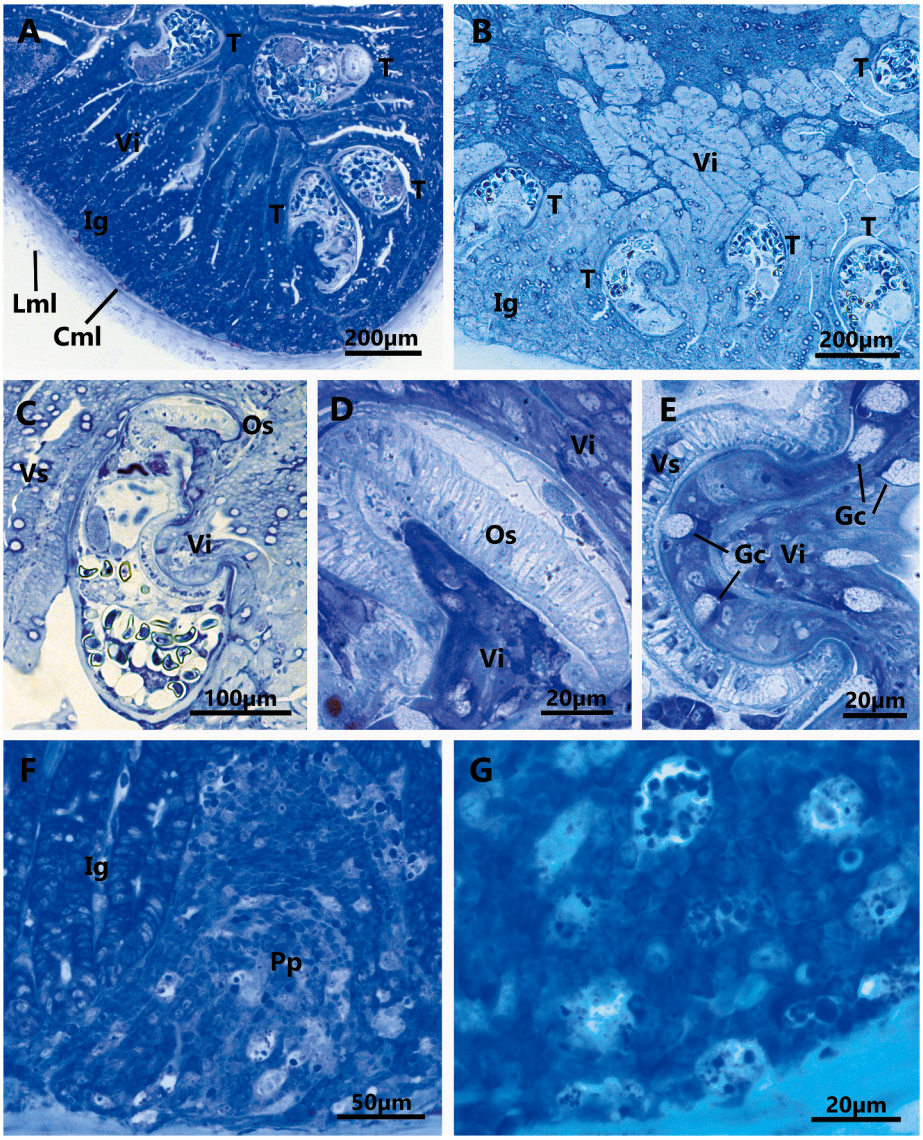

Fig. 5. The parasitized intestine of *Myotis nigricans*. (A) and (B) Trematodes between the intestinal villi do not invade the intestinal glands. (C) Trematode attached to the villus adjacent to its ventral surface, both by the oral sucker and by the ventral sucker. (D) Oral sucker and (E) ventral sucker attached to the epithelium of the intestine, with the view of goblet cells. (F) Peyer patches with visible lymphoid alteration and intestinal glands. (G) Detail of lymphoid cells with nuclear fragmentation and hyperplasia. Abbreviations: (Ig), intestinal glands; (Os), Oral sucker; (Pp), Payer patches; (T), Trematodes; (Vi), Villi; (Vs), ventral sucker.

with the villi surface (Fig. 5A–C). Parasite eggs were also found in the intestinal mucosa.

The villi in the parasitized areas were voluminous, less sinuous, with hypertrophic cells. The epithelium also showed a decrease of goblet cells and reduced basophilia of enterocytes. In the areas of the intestinal glands, the cells were less basophilic; the epithelial cells were hypertrophied, lamina propria reduced, and vessels of the villous plexus were dilated (Fig. 5B and C). The circular and longitudinal muscle layers were thicker in parasitized regions. In some areas, the villi were in the process of degeneration, with compromised tissue and structural integrity. Peyer patches showed lymphoid alteration, and cells presented nuclear fragmentation and hyperplasia (Fig. 5F and G).

4. Discussion

Here we characterize for the first time the microscopic morphology of liver, gallbladder, and intestine of non-parasitized and naturally parasitized *Myotis nigricans*. This host is an insectivorous bat widely distributed in South America and has a large helminth parasite community. There are reports of the following species of digeneans parasites of *M. nigricans*: *Metadelphis lenti* infecting gallbladder from Brazil (Fernandes et al., 2019); and the intestinal parasites *Urotrema scabridum* in Paraguay (Lent et al., 1945) and Argentina (Milano, 2016), and *P. aranhai* for Argentina (Milano, 2016). However, we report for the first time *P. aranhai* infecting *M. nigricans* in the North of Brazil.

The liver does not have classic hepatic lobes of a polyhedral-hexagonal shape. Still, as in most mammals, it is possible to identify the guiding elements of the location of this "physiological unit," such as the centrilobular vein and the portal triad. The lack of polyhedral shape in the hepatic lobes can also be observed in humans, as well in weasels, squirrels, rodents, and bison, according to Prunescu et al. (2002), El-Salkh et al. (2008), Kierszenbaum and Tres (2016) and Al-Aamery et al. (2020). The intestinal tissue aspect of the jejunal and ileum segments are also characteristic of mammals, with a clear distinction between muscle layers, intestinal glands, villi, Peyer's plaques, and other elements.

4.1. Parasite-host interaction in the liver and gallbladder

There are no studies of the pathological effects of natural parasitism and the parasite-host interaction between bats and their helminths. We only found one study characterizing the microscopic liver injury caused by a cestode in *Molossus molossus* (Pallas, 1766) in the state of Amazonas, Brazil (Souza et al., 2019). However, the description of the parasitized organ and its effects on host tissue was not detailed, and the

author's main objective was to identify the parasite using different approaches.

The over infection of *Metadelphis lenti* in the gallbladder of *Myotis nigricans* caused significant hepatic architecture modifications, characteristic of chronic disease influenced by parasitism. We observed congestion and hypertension of the sinusoid capillaries, hypertrophy of the hepatocytes, and granular deposits. However, these results differ from other well-described helminth-liver or helminth-bile ducts interactions. For example, *Schistosoma* spp. and its hosts (from humans to murine) (Andrade, 2009) and *Fasciola* spp. (Gajewska et al., 2005) the parasitism leads to inflammatory, nodular or neoplastic lesions. Therefore, the site of infection of *M. lenti* represents a site to absorb lipid derivatives (cholesterol and organic salts) and glycogen from the liver. They stay away from inflammatory tissue cells of the liver and blood.

We believe that the presence of trematodes in the gallbladder will undoubtedly evolve into changes in bat metabolism. According to Kierszenbaum and Tres (2016), any disturbance in the four essential organs that produce bile (hepatocytes, bile ducts, gallbladder, and intestine) leads to a pathological condition.

A large number of individuals inside the gallbladder (42 trematodes in the same host organ, see Fernandes et al., 2019) can obstruct the flow of bile to the intestine, may induce bile reflux to the liver and systemic circulation, and decreasing the production of bile in hepatocytes, due to the lack of feedback of bile acids in the enterohepatic circulation.

Intriguingly, we did not find any tissues pigments/hemochromatosis, fibrosis, portal hypertension, hypoplasia/atrophy, or hyperplasia of bile canaliculi in infected hosts. The trematodes can cause the backflow of bile to the liver, together with the excretion and secretion products of the parasites, influencing the liver's glycogen metabolism, releasing its products to the bile ducts finally to the site of parasitism (gallbladder).

4.2. Parasite-host interaction in the intestines

Our results showed the natural interaction between the intestinal trematodes *Anenterotrema* sp., *P. aranhai*, *U. scabridum,* and Lecithodendriidae gen. sp. with *M. nigricans*, detailing the process of attachment to the intestinal epithelium. We observed that parasites are directly in contact with intestinal epithelial cells and are attached by the oral and ventral sucker, forming a "pleat" from the villus that occupies the whole internal area of suckers. Thus, the parasite keeps in touch with the villus's physiological microenvironment. Because of this, the digeneans may absorb substances directly from the venous and quiliferous plexus and release excretion/secretion products. These parasites products may change the osmotic balance of the intestinal cells, causing hypertrophy of epithelium and lamina propria, which will reduce the extension of the villi and intestinal glands, leading to changes in pH of these regions/cells, and result in the degeneration of villi.

The thickening of the circular and longitudinal muscle intestinal layers may represent: (i) a host response that increases peristalsis in an attempt to expel parasites, inducing them to move to different loci, or (ii) excretion/secretion products acting increasing intestinal movements and thereby renewing the luminal fluid and nutrient intake on the parasite's surface.

We observed a reduced number of trematode specimens in the mucosa in the ileum area. Considering that the ileum is a naturally recognized region by lymphoid nodules and the production of antibodies to the intestinal surface, it is understandable that trematodes move to areas with less immune stress. Additionally, changes in lymphoid follicles may represent the influence of trematodes and responses to secondary inflammation caused by protozoa or other pathogens. However, these pathogens were not observed during our analysis.

Histopathological studies do not discuss the interaction between bats and their parasitic helminths. Consequently, any alterations caused to the parasitized organs are not known. Interestingly, in our study, the presence of *Metadelphis lenti* and other trematodes in host tissues did not show signs of inflammatory processes and cellular defense infiltrates, suggesting low or no immune reaction from the host to their presence in their infection sites. However, our analysis evidenced consistent cellular alterations, variation of granular deposits in hepatocytes, basophilic alteration of enterocytes, and goblet cell size in the interaction between *M. nigricans* and its trematodes.

This report is the first study of the natural parasite-host interaction in the liver, gallbladder, and intestine in *M. nigricans.* Future histopathological analyses involving helminth-host interaction in bats may aid a better understanding of cellular and tissue alteration caused byhelminths in parasitized organs.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2022.04.012.

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