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Histopathological alterations in gills of Amazonian shrimp *Macrobrachium amazonicum* parasitized by isopod *Probopyrus bithynis* (Bopyridae)

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ABSTRACT: The present study describes, for the first time, histopathological alterations in the gills of *Macrobrachium amazonicum* caused by infestation of *Probopyrus bithynis* (Isopoda: Bopyridae). In every case (100%), the infestation by *P. bithynis* was by a single pair of parasites (male and female) and occurred in the right or left side of the branchial chamber; the gill structures were visibly compressed due to the presence of parasites. The gills of *M. amazonicum* parasitized by *P. bithynis* exhibited a chronic inflammatory response, with the presence of edema, greater quantities of hemocytes, necrosis, epithelial cell hyperplasia, rupture of the pillar cells at the ends of the gill lamellae, desquamation of the cuticle, lamellar fusion and rupture of the lamellar epithelium. Tissue lesions were found in the histological sections of the gills of the parasitized *M. amazonicum*. Structural alterations in the branchial chamber of the hosts caused by the presence of *P. bithynis* can lead to physiological changes that can impair host respiratory performance. Finally, histopathological alterations in the branchial chamber of hosts suggest that *P. bithynis* feed directly on the gill tissues of this shrimp.

KEY WORDS: Gills · Morphology · Isopod · Parasite · Freshwater shrimp · Palaemonidae · Amazon River · Brazil

INTRODUCTION

Macrobrachium amazonicum Heller, 1862 (Decapoda: Palaemonidae) is a freshwater shrimp endemic to South America. It occurs in the basins of the Amazon, Orinoco, Paraguay, Tocantins, Paraná and São Francisco rivers, among others (Oddinetz-Collart 1988, Bialetzki et al. 1997, Sampaio et al. 2007, Kutty & Valenti 2010). It is mainly found in whitewater rivers with a high concentration of nutrients, such as the Amazon River basin. In contrast, it is infrequent in blackwater rivers due to their high levels of acidity and low nutrient content, and in first order rivers (Keppeler &

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Valenti 2006). *M. amazonicum* is a native shrimp species in Brazil, with significant potential for aquaculture production. It is also the freshwater shrimp most commercially exploited by artisanal fishing in the states of Amapá and Pará (Lucena-Frédou et al. 2010). The species exhibits favorable characteristics for aquaculture production, such as rapid growth, ease of reproduction and adaptability to the cultivation system (Maciel & Valenti 2009, Araujo & Valenti 2011). *M. amazonicum* can reach 16 cm in length and weigh 30 g (Coelho et al. 1982, Maciel & Valenti 2009, Araujo & Valenti 2011); however, its growth can be affected by parasitic crustaceans (Beck 1980, Neves et al. 2004), hampering its production in captivity.

Probopyrus bithynis Packard, 1879 (Bopyridae) can be easily seen in the branchial chamber of its hosts. Infestations of *P. bithynis* can affect 11% of the total shrimp population in the Amazon River system (Oddinetz-Collart 1990). This species of parasite has been recorded in *M. amazonicum* (Oddinetz-Collart 1990, Raman et al. 2005), *M. rosenberguii* de Man, 1879 (Raman et al. 2005) and *M. ohione* Smith, 1874 (Truesdale & Mermilliod 1977, Dale & Anderson 1982). The parasites cause problems in their hosts as they permanently lodge in the branchial chamber and impair gaseous exchange by the gills (Raman et al. 2005), but the histopathological alterations caused in the hosts are not known. However, members of the genus *Probopyrus* feed on the lymph of their hosts, perforating the integument with their mandibles, and can cause castration, hinder respiration, reduce metabolic rate, affect concentrations of energy reserves and alter the behavior and mobility of the hosts (Beck 1980, Torres-García & Bortolini-Rosales 2002, Neves et al. 2004, Raman et al. 2005, Hassan et al. 2017, Gopalakrishnan et al. 2017). Thus, these ectoparasites are an important threat to the emerging shrimp industry because they may severely affect the reproductive potential of the host species, both farmed and wild (Gopalakrishnan et al. 2017). However, the pathological alterations caused in the gills of *M. amazonicum* parasitized by *P. bithynis* remain unknown. The aim of the present study was therefore to describe histopathological alterations in the gills of *M. amazonicum* caused by infestation of *P. bithynis*.

MATERIALS AND METHODS

A total of 19 specimens of *Macrobrachium amazonicum* infested by *Probopyrus bithynis* were collected in June 2016 from the lower Amazon River, near the community of Maruim, in the municipal region of Gurupá, in the state of Pará, Brazil (Fig. 1). Catching of the shrimp was authorized by the Brazilian Min-

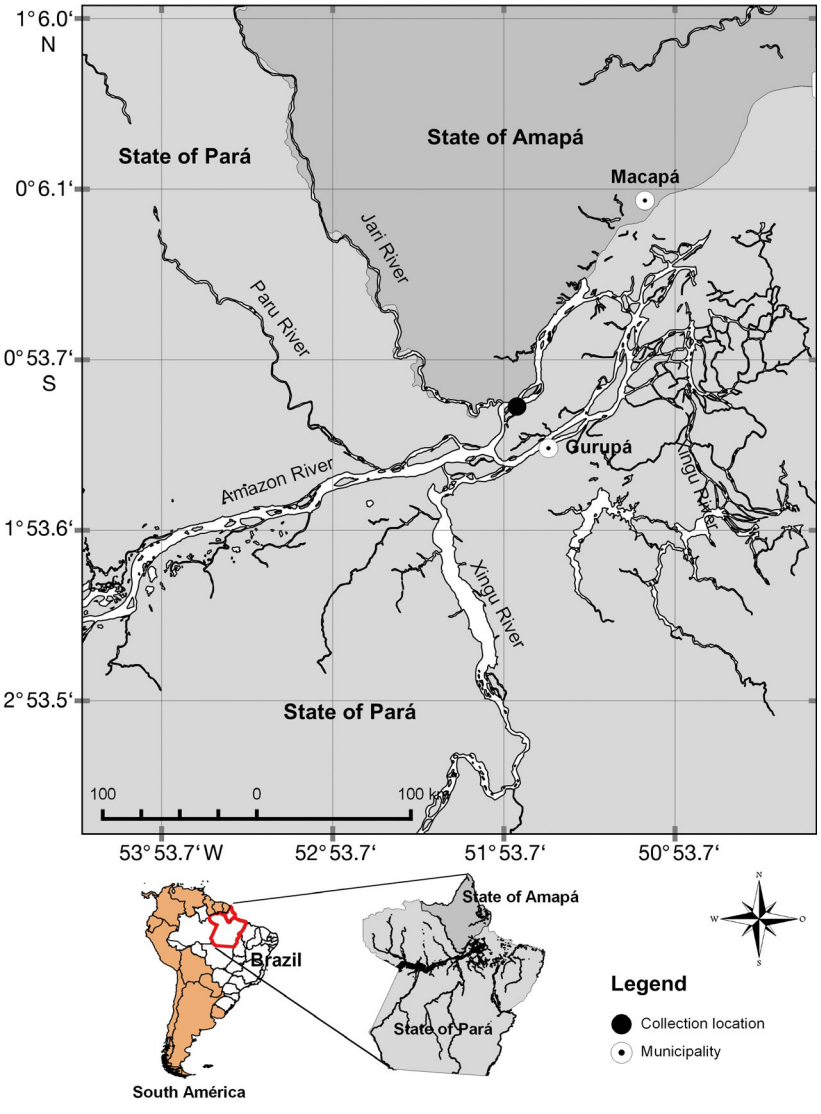

Fig. 1. Geographic location on the Amazon River (Brazil) of the collection site of *Macrobrachium amazonicum* infested with *Probopyrus bithynis*

istry of the Environment (SISBIO: 44268-4) and was performed using trap-like equipment (matapi) that was submerged in the water for approximately 24 h (Araújo et al. 2014). After capture, the parasitized shrimp were euthanized on ice before being placed in 70% ethyl alcohol for 24 h and then preserved in 70% ethyl alcohol/10% glycerol for further analysis.

For removal of the male and female parasites, the branchial chamber was opened with scissors and the exposed parasites were removed with the aid of forceps. Identification of the parasites was performed using the criteria of Lemos de Castro & Loyola-Silva (1985). Six specimens of *M. amazonicum* infested by *P. bithynis* (see Fig. 2) were deposited in the scientific collection of the Zoological Museum of the Universidade Estadual de Campinas (UNICAMP), under registration number ZUEC CRU-2257.

The specimens of *M. amazonicum* (9.1 ± 1.4 cm, 6.6 ± 2.5 g) and *P. bithynis* (0.12 ± 0.07 g) were measured (cm) and weighed (g). Five parasitized shrimps (3 males and 2 females) were used in the histological studies; the control material was the non-parasitized gills from the opposite side. The histological processes were performed by sectioning the third and fourth branchial arches for the paraffin patterns, using 6 branchial filaments in the sagittal and horizontal profiles. The gill filaments were exposed in increasing concentrations of alcohol, initiating the process of dehydration and diaphanization with xylol baths. After this process, the filaments were embedded in histological paraffin (Bell & Lightner 1998). The histological sections were made with a rotating microtome (Leica Biosystems, Leipzig). Four slides were prepared for each specimen of *M. amazonicum*, each of which contained 3 histological sections of 5 µm. The sectioned tissues were stained with hematoxylin and eosin (H&E) and analyzed and photographed using a Leica light microscope coupled to a computer equipped with the IM50 software (Leica). The quantitative evaluation technique in cross-sectional histological sections used to determine the level of depth of the lesion followed Watts et al. (2001), with the depth of injury caused by the attachment of *P. bithynis* calculated according to the sum of the thickness of the serial cuts.

RESULTS

A total of 19 *Macrobrachium amazonicum* specimens exhibiting a dark protuberance in the branchial chamber were parasitized by *Probopyrus bithynis* (Fig. 2). All infestations were by a single pair of parasites (male and female) observed in the right or left side of the branchial chamber; the gill structures were visibly compressed due to the presence of the parasites. The parasites were attached to the second and third branchial arches, with the cephalic region of the parasites directed towards the caudal region of the host body, the dorsal side facing the gills and the ventral part of the parasites in contact with the internal surface of the branchial chamber. The parasites represented around 4% of each host biomass.

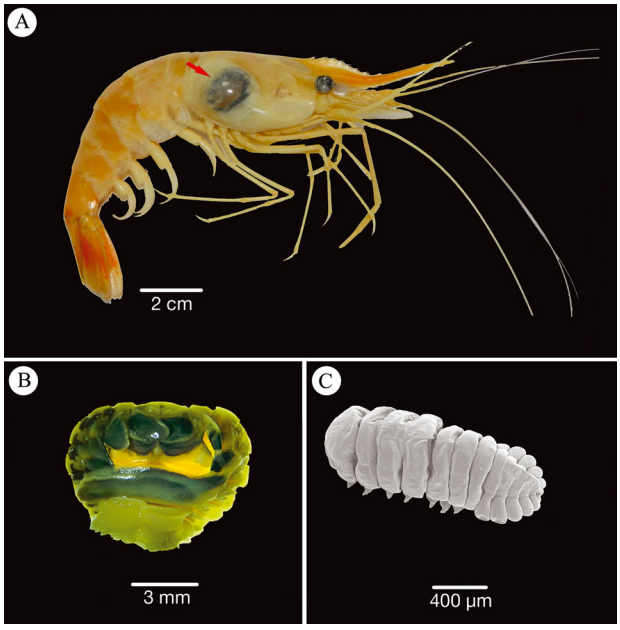

Fig. 2. (A) *Macrobrachium amazonicum* parasitized by *Probopyrus bithynis* (arrow) in the Amazon River (Brazil), and (B) female and (C) male *Probopyrus bithynis* collected from this host

Histological sections of non-parasitized *M. amazonicum* gills revealed that the gill filaments were mainly composed of pillar cells, lamellar epithelium, hemocytes and plasma. The pillar cells had broad apical bases and extremities and the hemocytes had a rounded shape. The epithelium was generally thin, surrounded by a chitinous cuticle (Fig. 3A). Based on the thickness of each histological section (~5 µm), analysis of the serial sections from the parasitized *M. amazonicum* gills in sequence revealed the depth of the lesions to be 46.7 ± 12.2 µm. *M. amazonicum* parasitized by *P. bithynis* exhibited a chronic inflammatory response, with edema, greater quantities of hemocytes and necrosis (Fig. 3B,E). Epithelial cell hyperplasia and pillar cell disruption were also observed (Fig. 3B–E) as well as desquamation of the cuticle, lamellar fusion and rupture of the lamellar epithelium at the extremities of the gill lamellae (Fig. 3D,E).

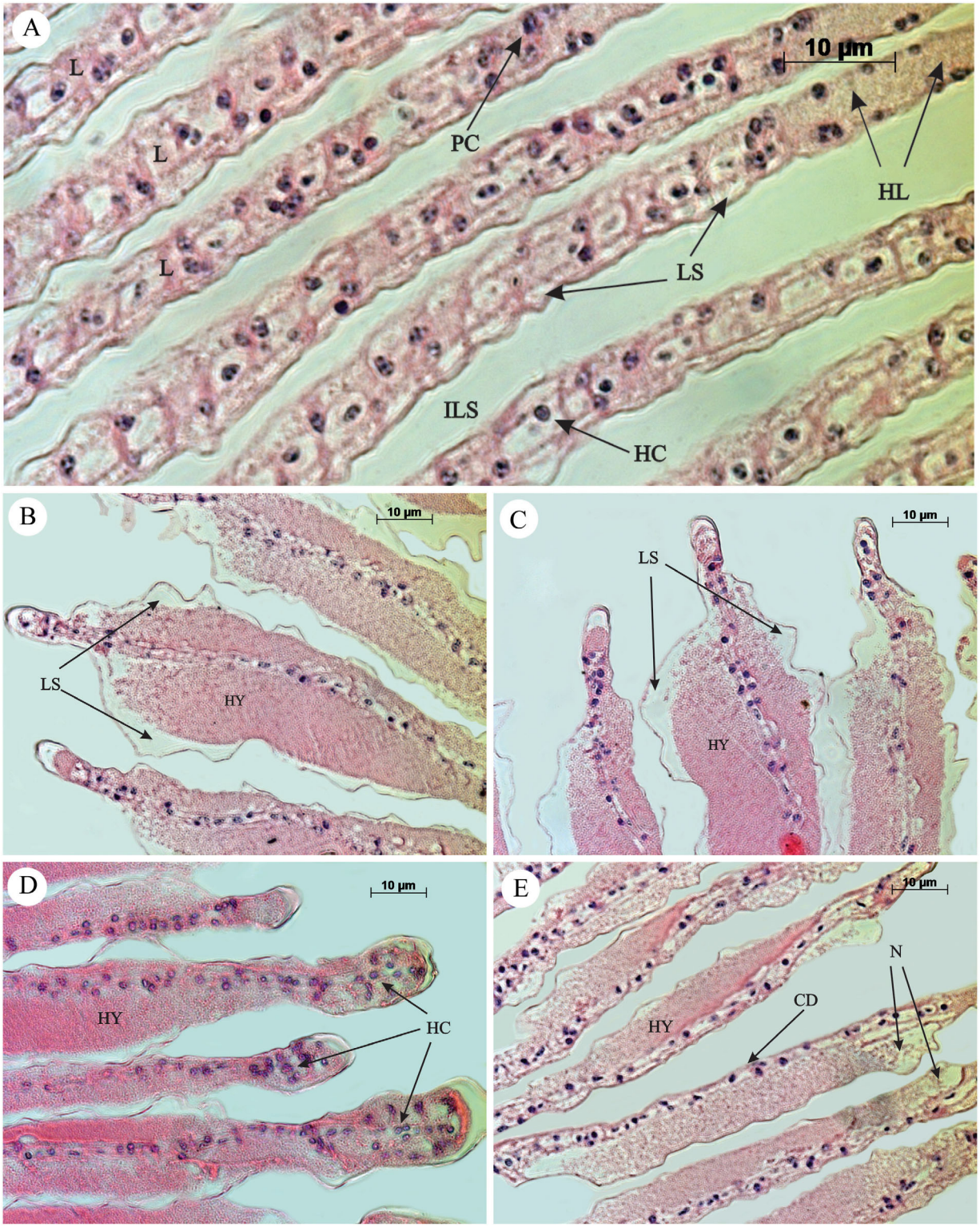

Fig. 3. (A) Longitudinal section of gill filament of *Macrobrachium amazonicum* from the Amazon River (Brazil) non-parasitized by *Probopyrus bithynis* (control). Normal lamellae (L) with uniform interlamellar spaces (ILS), lamellar sinus (LS), pillar cells (PC), hemocytes (HC) and hemolytic gap (HL). (B,C) Lamellar sinus (LS) and hyperplasia (HY) spaces. (D) Hyperplasia (HY) and accumulation of hemocytes (HC) in the distal portion of the lamella. (E) Hyperplasia (HY), desquamation of the cuticle (CD) and necrosis (N) in gill lamellae of hosts infested by *Probopyrus bithynis*. Staining: H&E

DISCUSSION

Bopyrids are common hematophagous ectoparasites in the branchial chambers of *Macrobrachium* spp., which are their definitive hosts (Hassan et al. 2017). In *M. amazonicum*, the presence of *Probopyrus bithynis* was restricted to a single branchial chamber (left or right). This characteristic has also been reported for *M. amazonicum* from the lower Tocantins River, Pará state, infested by *P. bithynis* (Oddinetz-Collart 1990); for *M. lanchesteri* de Man, 1911, parasitized by *Probopyrus* sp. in the Nyatoh River (Malaysia), and for *Palaemonetes hiltoni* Schmitt, 1921, infested by *Probopyrus* cf. *pandalicola* Packard, 1879 from the Pacific coast of Costa Rica (Jiménez & Vargas 1990, Román-Contreras 2004, Hassan et al. 2017). However, Truesdale & Mermilliod (1977) reported that in *M. ohione* parasitized by *P. bithynis*, 2.6% of the hosts had one pair of parasites (female and male) in both the right and left branchial chambers. Thus, finding parasitic infestations in a single branchial chamber was possibly because shrimps with both chambers infested were not caught during sampling, or *M. amazonicum* is not infested in both chambers by *P. bithynis*; however, this issue needs more investigation.

The gills are multifunctional organs that act between the animal and the environment (Evans 2005), and alterations in branchial structure directly affects mechanisms of respiration and osmoregulation (Ardiansyah et al. 2013). A reduction of 20% in oxygen consumption of *Palaemonetes argentinus* Nobili, 1901, due to infestation by *Probopyrus ringueleti* Verdi & Schuldt, 1988, has been reported (Verdi & Schuldt 1988). From a macroscopic perspective, the presence of *P. bithynis* results in a convex-shaped (protuberance) external part of the branchial chamber of *M. amazonicum*, which causes the internal branchial structures to become compressed, distending the branchial filaments and subsequently making respiratory/osmoregulatory functioning difficult, as also described by Schuldt & Rodrigues Capítulo (1985) in *P. argentinus* parasitized by *P. ringueleti*. Similarly, histopathological analysis revealed epithelial cell hyperplasia, pillar cell disruption, accumulation of hemocytes at the extremities of the lamellae and desquamation of the lamellar cuticle on the gills and necrosis. As found in the histological analysis of the control gills in the present study, and also by others researchers (Araujo & Valenti 2011), the gill structures are formed mainly by pillar cells, lamellar epithelium, hemocytes and hemolymph fluid. In the branchial chambers of *Macrobrachium panamense* Rathbun, 1912 infested by *Probopyrus* sp., cellular lysis occurred, caused by the attachment of the parasites. Tissue damage was caused by the appendages of the parasites in the brachial chambers, as well as by direct consumption of hemolymph of the hosts (Torres-García & Bortolini-Rosales 2002). Tissue damage was well evidenced when quantitative evaluation of the lesions was performed, with considerable depth found as a result of parasite pereopod attachment.

Female bopyrids obtain hemolymph of the hosts by puncturing the dorsal branchial chamber cuticle. The thickness of the deformed area of carapace is approximately twice that of the remaining carapace. Such ectoparasites can cause an infiltration of hemocytes, which are packed into layers at the wound site. These layers of cells appear to wall-off and isolate necrotic gill tissue. The connective tissue fibres thicken throughout the packed hemocyte layers, and in several areas necrotic tissue pigment nodules can be found (Bursey 1978). Although the role of hemocytes as a component of the cellular defense mechanism of crustaceans has been established, as has the fact that their greater presence may be a signal of inflammation (Battistella et al. 1996), the accumulation of hemocytes in the distal region of the gill filaments of *M. amazonicum* seems to be more suggestive of circulatory damage resulting from the fixation of the parasite than of inflammatory infiltrate.

The above-mentioned damage resulting from the development of *P. bithynis* in the branchial chamber suggests that the impairment of gill function has the potential to affect the respiratory/osmoregulatory capacity of *M. amazonicum*. These ectoparasites can cause inhibition of ventilation due to their permanent lodging in the branchial chamber and impair gaseous exchange by the gills (Raman et al. 2005). Finally, this first study on histopathological alterations in the branchial chamber of *M. amazonicum* suggests that *P. bithynis* feeds directly of the gill tissues of its hosts.

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