



## *Neoechinorhynchus buttnerae* (Acanthocephala) infection in farmed *Colossoma macropomum*: A pathological approach



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### ABSTRACT

Infection by the acanthocephalan *Neoechinorhynchus buttnerae* is considered the most important obstacle in farmed tambaqui or cachama (*Colossoma macropomum*), the second most cultivated fish species in Brazil. This study describes the occurrence of *N. buttnerae* in farmed this fish based on parasitological and histopathological analysis in highly infected fish. A total of 102 fish were collected from fish farms located in Brazilian states in the northern region. All of the examined fish were heavily infected by *N. buttnerae* and showed no signs of anorexia, but heterogeneous growth and cachexia were frequently found. No cases of mortality were observed in fish farms. Macroscopically, the intestines showed hyperaemia, hardening, thickening of the intestinal wall, yellowish mucosal secretion and a bluetongue aspect. Histological sections showed different degrees of penetration of the acanthocephalan proboscis in the intestinal tissue, surpassing the mucosa, submucosa and muscular layer, provoking dilacerations due to their spines as well as an intense inflammatory reaction composed of macrophages, Langerhans cells and lymphocytes, and granuloma formation located mainly in the submucosa layer. Submucosal oedema, an increased number of goblet cells, metaplasia areas and a reduction of the intestinal villi were also observed. The results present an important register of *N. buttnerae* infection in *C. macropomum* and contribute to the comprehension of pathogeny caused by these worms.

**Statement of relevance:** Characid fish farming has led to significantly increased fish production in Brazil. However, the most important obstacle is the acanthocephalan infection caused by *Neoechinorhynchus buttnerae* in the intestinal tract. The infection does not provoke death but cachexia and growth reduction are frequently observed as well as intense pathological conditions in histological sections.

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### 1. Introduction

*Colossoma macropomum* Cuvier, commonly called tambaqui or cachama, is a member of the Serassalmidae family and the most native farmed Brazilian fish. In 2014, the production data showed an amount of 139,000 tons, representing 56% of the national production of native fish (Instituto Brasileiro de Geografia e Estatística [IBGE], 2014). Cachama farming has spread quickly in Brazil due to easy juvenile production (Gomes et al., 2010), good acceptance of commercial diet and growth

(Dairiki and Silva, 2011), excellent use of natural food (Lopera-Barrero et al., 2011), rusticity (Chagas et al., 2012) and consequently high productivity. Moreover, this fish can support handle in fish farming, variation in the physical and chemical characteristics of water such as low dissolved oxygen (<1 mg·L<sup>-1</sup>), temperature and pH variation, and high levels of ammonia and nitrite (Gomes et al., 2010). In addition, it presents a great acceptability in the market once its meat is appreciated for its flavour, not only in the Amazon region but also in national and international markets (Campos et al., 2015).

Cachama is mainly produced in the Amazon region and transition areas of savanna where the high temperatures favour its fish production. Traditionally, its production is realized in semi-intensive and intensive systems divided into two stages: fingerling (60 days) and

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fattening (240–300 days). Fattening in an intensive system uses artificial aeration and the fish can reach 2.62 kg in weight and a production of 18,530 kg per hectare after 10 months. In this system the fish are generally fed a diet containing 32% crude protein (fish of 160 g to 1.0 kg at 4% of body weight three times a day), 28% crude protein (fish of 1.0 to 2.0 kg at 3% of body weight twice a day) or 28% crude protein again (fish of >2.0 kg at 2% of body weight once a day) (Izel et al., 2013).

Few studies have reported disease problems in cachama farming. Nevertheless, fish farms in the north of Brazil have been facing problems due to acanthocephalan dissemination. Until recently (Noga, 2010), such registers of fish and/or farming damages were considered rare. At the end of the 90s, Malta et al. (2001) described cases of severe infection by the acanthocephalan *Neoechinorhynchus buttnerae* Golvan, 1956, in the Amazon with the first report of economic losses. Recently, parasitism and subsequently acanthocephalosis have spread rapidly among the main cachama farms in the Amazon region (Chagas et al., 2015; Oliveira et al., 2015).

As a result of the acanthocephalan proboscis attachment on the intestinal tissue, the fishes' health and pathological alterations (Belo et al., 2013; Melo et al., 2014) interfere in the nutrient absorption and affect fish growth. In tropical cachama farming this kind of parasitism has been considered the main obstacle responsible for economic losses and high feeding costs.

Due to the fact that *N. buttnerae* is an emergent and important parasite that affects cachama farming, this study evaluated its occurrence in northern Brazil, the most important cachama production area, in order to observe the pathological alterations and parasitic infection in heavily infected fish.

## 2. Materials and methods

A total of 57 fish were examined from fish farms located in northern Brazil, the most important region of cachama producers. A total of 37 fish presenting low zootechnical performance were examined from Rondônia State, Cujubim municipality, northern region (9°28'52.1"S 62°44'11.8"W): juveniles of 30 g, n = 15; subadults of 250 g, n = 15; adults of 2.0 kg, n = 7. A total of 20 fish were examined from the Amazon state, 20 from Manaus (3°05'25.6"S, 59°59'39.3"W): adults of 0.8 kg. In both localities, the fish were reared in earthen ponds with no stocking density control and continuous water flow, and were fed twice a day with a commercial diet with 28% crude protein.

Fish were firstly clinically evaluated followed by euthanasia by cerebral concussion for necropsy and intestinal evaluation. The parasites were processed and identified in accordance with Thatcher (2006) and the parasitological indexes were calculated as proposed by Bush et al. (1997).

Fragments of intestine of infected fish that presented heavy acanthocephalan infection were fixed in 10% buffered formalin solution and processed according to usual histopathological techniques, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin-eosin.

The slides were analysed and photomicrographs were obtained using an Olympus BX60 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) equipped with an image analyser (Image-Pro Plus version 6.1 for Windows – Copyright© 1993–2006 Media Cybernetics, Inc).

## 3. Results

### 3.1. Parasitological analysis and gross pathology

Fish examined from all fish farms showed 100% prevalence of *N. buttnerae* in the anterior and small intestine. Fish from Rondônia State showed a mean intensity of  $262.7 \pm 73.2$ , and those from Amazon state showed a mean intensity of  $476.8 \pm 102.9$ .

During the anamnesis and clinical evaluation, neither anorexia nor mortality was observed despite the high parasite burden. Nevertheless, heterogeneous populations from fish ponds, cachexia and low growth performance were reported by fish farmers and noted after clinical evaluation (Fig. 1a). After necropsy, the fish were found to be highly parasitized by *N. buttnerae* associated with hardening and thickening of the intestinal wall, and subadult and adult animals presented discrete yellowish mucosal secretion of a bluetongue aspect (Fig. 1b) along with high parasitic burden. In contrast, a low mean intensity of infection was observed in juvenile fish.

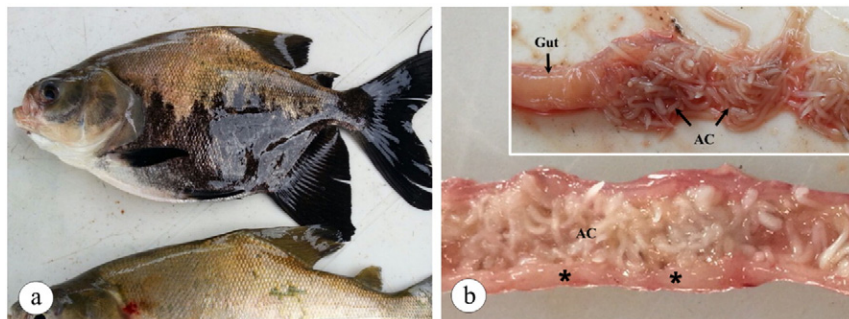
### 3.2. Histopathological analysis

Histological sections showed different degrees of proboscis penetration in the intestinal tissue surpassing the mucosa, submucosa and muscular layer (Fig. 2a–b) with tissue dilacerations due to cephalic spines (Fig. 2c–d). Intense inflammatory reaction composed mainly of macrophages, Langerhans cells and lymphocytes was also reported (Fig. 2e). Granuloma was observed in the submucosa layer of some animals (Fig. 2f). Submucosal oedema, an increased number of goblet cells, metaplasia and a remarkable reduction of the intestinal villi were observed in highly infected fish.

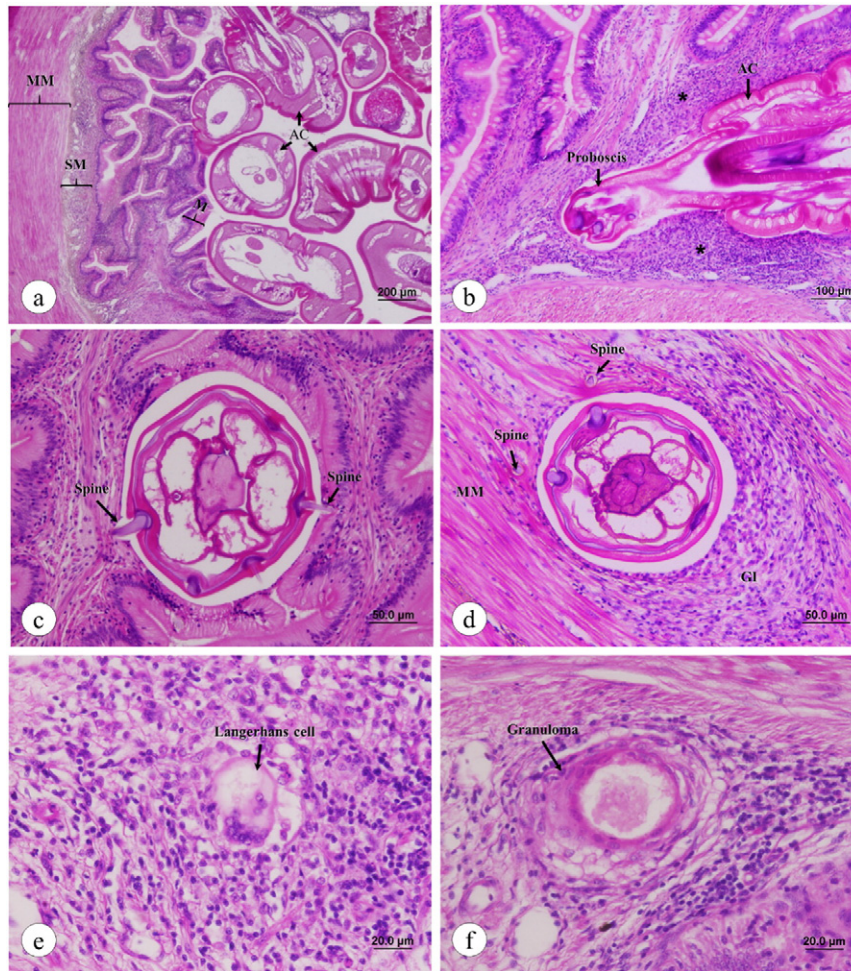
## 4. Discussion

Cachama from fish farms located in different fish regions (Amazonas and Rondônia) showed 100% prevalence of *N. buttnerae*, similarly to that reported by Malta et al. (2001). Although in low prevalence, *N. buttnerae* was also reported parasitizing the hybrid tambatinga (2.8%) (♀ *C. macropomum* × ♂ *Piaractus brachypomus* Cuvier) (Silva et al., 2013) and the hybrid tambacu (4.4%) (♀ *C. macropomum* × ♂ *Piaractus mesopotamicus* Holmberg) from Amapá (Dias et al., 2015).

Studies have demonstrated severe reactions from the host tissue in acanthocephalan infections (Dezfuli et al., 2008; Sanil et al., 2011). Juveniles and adults of cachama under moderate to severe infection by *N. buttnerae* have shown hyperaemia, hardening and thickening of the intestinal tissue, and yellowish mucosal secretion of a bluetongue aspect.



**Fig. 1.** Gross pathology of farmed *Colossoma macropomum* infected by *Neoechinorhynchus buttnerae*. (a) cachexia; (b) longitudinal section of anterior intestine showing the acanthocephalans (AC), hyperaemia and thickening of the intestinal wall (asterisk).



**Fig. 2.** Intestinal histological sections of *Colossoma macropomum* infected by *Neoechinorhynchus buttnerae*. (a) acanthocephalans (AC) in the intestinal lumen, mucosa (M), submucosa (SM) and muscular mucosa (MM); (b) *N. buttnerae* (AC) inserted in the intestine, intense mononuclear inflammatory reaction (asterisks); (c) cephalic spines of proboscis dilacerating the intestinal mucosa; (d) proboscis inserted in the muscular mucosa with dilacerations of muscle fibres, and granulomatous inflammatory reaction in the parasite insertion (GI); (e) presence of Langerhans cells in the mononuclear inflammation of the intestinal mucosa (arrow); (f) granuloma formation in the submucosa and muscular mucosa with epithelioid macrophages and lymphocytic infiltrate.

Such alterations were observed in lower intensity in cachama juveniles, which was associated with low parasitic burden, similarly to that previously reported (Belo et al., 2013). These authors found a positive correlation between the size of fish and number of acanthocephalans and consequently pathological alterations in curimatá (*Prochilodus lineatus* Valenciennes) naturally infected by *N. curemai* Noronha, 1973. The present results confirm the hypothesis that reinfection of fish in contaminated ponds favours the worm life cycle and increases the infection, compromising the host's health.

Weight losses, delayed growth and mortalities in acanthocephalan-parasitized fish were reported by Tonguthai (1997) and Amin et al. (2013). Infected cachama did not show mortality but they were heterogeneous and cachexic. These signs indicate a negative impact of the parasitosis damaging the feeding conversion and compromising the commercialization of fish.

Farmed cachama naturally infected by *N. buttnerae* presented intense inflammatory reaction characterized by monocyte and lymphocyte infiltrate, Langerhans cells and granuloma formation in the intestinal submucosa layer. Inflammatory alterations characterized a chronic inflammation. For better understanding of the physiopathology in characid fish such as pacu (*P. mesopotamicus*), an increase in the number of giant cells and Langerhans cells has explained the evolution of inflammatory reaction 15 days after subcutaneous implantation of glass slides (Belo et al., 2005, 2012). Inflammatory reactions of intestinal submucosa and mononuclear infiltrate in curimatá (*P. lineatus*)

infected by *N. curemai* (Martins et al., 2001) and intestinal haemorrhages in pacu (*P. mesopotamicus*) parasitized by *Metechinorhynchus jucundus* (Ferraz de Lima et al., 1989) were also reported.

A significant increase in the number of monocytes and a decrease in the number of thrombocytes in curimatá (*P. lineatus*) infected by *N. curemai* (Belo et al., 2013) and pacu (*Piaractus mesopotamicus*) with chronic inflammatory reaction (Belo et al., 2014) justify the present findings, which showed intense inflammatory reaction composed mainly of macrophages. In fact, the chronic aspect of intestinal lesions in cachama with participation of macrophages and polycarion formation on the inflamed site is the result of the recruitment of monocytes from circulating blood. On the other hand, thrombocytes present an important role in blood coagulation and the decreased number could be associated with their migration to intestinal tissue in order to close the bleeding caused by the parasites.

Mechanical parasite action in the intestinal lumen induces alterations in the structure and function of the organ (Sanil et al., 2011). In acanthocephalan infection, hyperplasia and hypertrophy of intestinal goblet cells were previously reported (Martins et al., 2001; Dezfuli et al., 2009). Similarly, cachama parasitized by *N. buttnerae* presented submucosal oedema, hyperaemia, an increased number of goblet cells, metaplasia and reduced intestinal villi. Complete desquamation of the intestinal epithelium with severe hyperplasia and hypertrophy of goblet cells, displacement of their sheath and oedema were also found in

curimbatá (*Prochilodus lineatus*) infected by *N. curemai* (Martins et al., 2001). Parasitism due to *Longicollum pagrosomi* Yamaguti, 1935 resulted in destruction of the villi and epithelial covering in red sea bream (*Pagrus major* Temminck & Schlegel) (Kim et al., 2011) and chirruh snowtrout (*Schizothorax esocinus* Heckel) (Irshadullah and Mustafa, 2012).

This study showed different degrees of penetration of the acanthocephalan proboscis in the intestinal epithelium surpassing the submucosa and muscular layer causing dilacerations due to the presence of spines. In contrast to what was observed in the present study, McDonough and Gleason (1981) reported the penetration of the bulb and neck of *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919, in the intestinal epithelium of rainbow darter (*Etheostoma caeruleum* Storer). This resulted in a tunnel and capsule formation surrounding the parasite's neck and proboscis (McDonough and Gleason, 1981). Similarly, Sanil et al. (2011) reported mechanical injuries caused by *Tenuiproboscis* sp. that totally destroyed the intestinal tissue architecture, reaching the visceral cavity of Mangrove red snapper (*Lutjanus argentimaculatus* Forsskal). In these cases, peritonitis and systemic clinical signs were the result of massive infection with intestinal perforation (Bullock, 1963).

The pathological alterations found in this study in fish heavily infected by *N. buttnerae* are of fundamental importance to the understanding of fish health. Nevertheless, the physiopathological study of acanthocephalosis in cachama can enable the establishment of control strategies and the maintenance of fish health.

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