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Original Article

First record of *Neoechinorhynchus buttnerae* and *Piscinoodinium pillulare* infection in *Colossoma macropomum* in the state of Tocantins, Brazil

Primeiro registro de infecção por *Neoechinorhynchus buttnerae* e *Piscinoodinium pillulare* em *Colossoma macropomum* no estado do Tocantins, Brasil

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Abstract

This study aims to report the occurrence of two important parasites in farmed tambaqui *Colossoma macropomum* in the state of Tocantins, the acanthocephalan *Neoechinorhynchus buttnerae* and the dinoflagellate protozoan *Piscinoodinium pillulare*, also suggesting the main treatments to control them. The fish sampled for the study were infected by *N. buttnerae*, and *P. pillulare*, with prevalence from 100% and mean intensity from 51.4 to 354,264, respectively. This was the first report on the occurrence of such parasites in *C. macropomum* in the state of Tocantins. We emphasize the need to adopt good farm management and biosecurity practices to prevent pathogenic agents to enter or leave a property. Reported treatments with synthetic and natural products with positive results are also suggested to treat against those parasites in farmed *C. macropomum*.

Keywords: Acanthocephalan, aquaculture, fish parasites, protozoans.

Resumo

Este estudo teve como objetivo relatar a ocorrência de dois importantes parasitos em tambaqui *Colossoma macropomum* cultivado no estado de Tocantins, o acantocéfalo *Neoechinorhynchus buttnerae* e o protozoário dinoflagelado *Piscinoodinium pillulare* e também destacar os principais tratamentos para controlá-los. Os peixes examinados estavam infectados por *N. buttnerae* e *P. pillulare*, com prevalência de 100% e intensidade média de 51,4 a 354.264,4, respectivamente. Este foi o primeiro relato da ocorrência desses parasitos para *C. macropomum* no estado de Tocantins. Destaca-se a necessidade da adoção de boas práticas de manejo na produção e de manejo sanitário, para evitar a contaminação da propriedade e a transmissão para pisciculturas vizinhas. Em relação ao controle dessas parasitoses são apresentados os tratamentos com resultados positivos para *C. macropomum*, com o uso de produtos sintéticos e naturais.

Palavras-chaves: Acantocéfalo, aquicultura, parasitos de peixe, protozoário.

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Introduction

Colossoma macropomum (tambaqui) is a Serrasalminae fish species native to the Amazon and Orinoco rivers and their tributaries (Gomes et al., 2020; Hilsdorf et al., 2022). It is an economically important fishery in the regions they occur naturally, as well as for aquaculture in Brazil and other Amazonian nations (Hilsdorf et al., 2022), and in Asian countries (Woynárovich & Van Anrooy, 2019). Tambaqui is the most farmed native fish species in Brazil, mainly in the Northem, Northeastern and Western regions, accounting for 262,370 tonnes and 31.2% of the national production in 2021 (Associação Brasileira da Piscicultura – PeixeBR, 2022). Tambaqui is suitable for aquaculture because of its hardiness, tolerance to low dissolved oxygen levels and high ammonia concentration in water, induced artificial spawning up to twice a year per female and easy production of juvenile fish (Valladão et al., 2018; Gomes et al., 2020; Neves et al., 2020; Val & Oliveira, 2021). Additionally, they grow fast and have high market acceptance, which all explains the first position of this native species in the national and international aquaculture (Hilsdorf et al., 2022). The omnivorous feeding habit, acceptance of pelleted feed and intake of the natural food available in the grow-out ponds justify the adoption of the semi-intensive farming system (Valenti et al., 2021; Hilsdorf et al., 2022). Semi-intensive grow-out in earthen ponds is the most practiced farming system for tambaqui, in one or two phases, in mono- or polyculture and, less frequently, in cages (Pedroza-Filho et al., 2016; Valenti et al., 2021). The direct stocking of tambaqui fingerlings of 2 to 5 g at a density of 0.4 to 0.7 fish/m^2 for 12 months is the most adopted practice in the Brazilian farms according to Valenti et al. (2021). However, intensive systems with continuous or supplementary use of aerators are also adopted to increase productivity and shorten the cycle to 10 months (Izel et al., 2013).

Fish health issues may be faced during any phase or farming system of tambaqui. Hilsdorf et al. (2022) have reported parasitic infections, especially in the North region, and heterogenous growth as some of the bottlenecks in tambaqui farming. The main parasites reported for *C. macropomum* are Protozoa, Acanthocephala, Copepoda, Monogenea, Myxozoa and Crustacea species (Ferreira et al., 2018; Maciel et al., 2018; Chagas et al., 2019; Fujimoto et al., 2019; Andrade Porto et al., 2022). Among protozoans, disease outbreaks with high morbidity and mortality in the different culture phases have been associated to *Piscinoodinium pillulare* in the South-eastern and Western regions of Brazil (Martins et al., 2001; Ferreira et al., 2018). *Piscinoodinium pillulare* is a mastigophore protozoal dinoflagellate with no host specificity, infecting host fish and causing integumentary petechiae, necrosis, and inflammation, further to hypertrophy and fusion of the secondary branchial lamellae, resulting in clinical signs consistent to asphyxia (Martins et al., 2015). Fish mortality rates of 40% to 90% have been reported due to *P. pillulare* infection (Martins et al., 2001; Sant'Ana et al., 2012). Another prevalent parasite in tambaqui farming is the acanthocephalan *Neoechinorhynchus buttnerae*, reported in farms in the states of Amazonas, Rondônia, Roraima and Maranhão (Chagas et al., 2015, 2019; Jerônimo et al., 2017; Lourenço et al., 2017; Pereira & Morey, 2018). Their proboscis can pierce the gut wall of the host and cause stiffening and thickening of the mucous and muscular layers, resulting in intense inflammation and mucus production (Jerônimo et al., 2017; Matos et al., 2017). Fish mortalities are not frequent; however, high infection rates can obstruct the gut lumen and jeopardize nutrient absorption and lead to weight loss (Chagas et al., 2015; Silva-Gomes et al., 2017).

In tambaqui farming, disease outbreaks are mostly related to intensification without due management practices (Valladão et al., 2018). Intensive farming without good management practices can lead to unbalanced fish-pathogen-environment relationship and the fish becomes highly susceptible to diseases (Chagas et al., 2019; Costa et al., 2019). It is estimated that Brazilian fish farms may suffer annual direct and indirect economic losses of USD 84 million due to the lack of good farm management practices according to Tavares-Dias & Martins (2017).

In some countries, virtually all chemical products may be legally used to treat such a diversity of diseases, whereas in others, some restrictions may apply (Woynárovich & Van Anrooy, 2019). In Brazil, veterinarian therapeutic products to be used in aquaculture are not widely available (Hilsdorf et al., 2022). Thus, several studies have been conducted testing synthetic products available in the market, as well as the investigation of active ingredients of medicinal plants for therapeutic and prophylactic treatments in tambaqui, to encourage the authorities to approve new products.

With the growth of aquaculture in Brazil and the description and reporting of new diseases and their region of occurrence, studies that expand the epidemiological characterization of diseases in tambaqui farming are crucial. So is the compilation of scientific information on treatment of such diseases. These would contribute to a better understanding of the research done in this area and provide the necessary guidance towards the correct means of treatment and prevention. On this point of view, the aims of the present study were to report the occurrence of

Piscinoodinium pillulare and *Neoechinorhynchus buttnerae* in tambaqui farms in the state of Tocantins (TO), Brazil, and suggest the main treatments available to control such parasitic infection in the host.

Materials and Methods

A technical visit was conducted at a tambaqui farm in Novo Acordo, TO, Brazil, due to possible sanitary issues at the property. The farmer reported fish low weight gain and reduced appetite, fish swimming near the water surface and swelling of the fish bottom lips to help breathing, and changes in the fish skin coloration.

Fish were farmed in one 1,000 m^2 pond stocked at a density of 3 fish/m^2, in a semi-intensive, single phase monoculture system. The fish had been stocked for one year and their measurements have not been taken throughout. In total, the farm had seven earthen ponds (total of 8,500 m^2) stocked with other fish species as well, i.e., *Arapaima gigas* (pirarucu) and *Brycon amazonicus* (matrinxã), in semi-intensive system. The fish farm had been in operation for eight years, mainly for their subsistence and the ponds had never been drained or dried for disinfection. In the tambaqui pond, the water was highly transparent, with excess organic matter in the bottom and the water outlet was through the surface.

Twenty-one tambaqui fish were sampled from the pond using fishing nets and transferred to plastic bags with 1/3 water and 2/3 oxygen and transported to the Fish Health and Disease Laboratory, Embrapa Fisheries and Aquaculture, Palmas, TO. Fish were anesthetised (Roubach et al., 2005) and killed by medullar section to proceed to measurements, necropsy, and sampling of biological material for parasitological analysis according to Eiras et al. (2006). Fish were macroscopically examined for physical alterations and skin lesions. Mucus was collected from head to tail from the fish body surface with a glass slide and the wet mount observed under a light microscope. The branchial arcs were excised, placed in 4% buffered formalin, and vigorously shaken to detach the parasites and fixed for analysis. Fish abdominal cavity was opened, and organs removed and placed on Petri dishes to be analysed under a dissecting microscope. Subsequently, the intestine was separately fixed in 4% buffered formalin for later qualitative and quantitative analysis of the parasites. Fish management was according to the ethics of animal use by the Brazilian Society of Laboratory Animal Science (COBEA).

Sampling, fixing, identification and counting of the parasites were done according to methods described by-Eiras et al. (2006) and Thatcher (2006). For the gills, three 1-mL sub-samples were taken from the fixated material to count the protozoans in a Sedgewick-Rafter chamber, according to Maciel et al. (2018). In the fish mucus, the protozoan *P. pillulare* were counted per score, according to Rach et al. (2000). Acanthocephan parasites were also quantified. The parasitic prevalence index (P), mean intensity (MI), and mean abundance (MA) were calculated according to Bush et al. (1997).

Results and Discussion

Mean body weight ± s.d. and mean standard length ± s.d. of the tambaqui sampled at the farm were 205.9 ± 76.5 g and 22.0 ± 2.4 cm, respectively, which could be considered as initial grow-out phase. However, fish had already been stocked in the pond for 12 months, meaning that the fish weight was much below the expected for the age. In this type of system, it becomes difficult to control pathogenic agents from entering the ponds together with the new batches of fish. The lack of disinfection procedures between farming cycles is a serious management issue that can jeopardize the entire production.

Parasitic prevalence in the sampled fish varied in dependence on the parasites. *Piscinoodinium pillulare* was observed together with *N. buttnerae*. Infection by *P. pillulare* was the highest in the sampled fish (Table 1), with mean intensity values above those reported for tambaqui farmed in cages in Amapá (Santos et al., 2013a) or in

Table 1. Parasitological indices in *Colossoma macropomum* of a fish farm in the municipality of Novo Acordo, State of Tocantins.

Parasite species	Prevalence (%)	Mean intensity	Mean abundance	Site of infection
Piscinoodinium pillulare	100	High score*	-	Tegument
Piscinoodinium pillulare	100	354,264 ± 178,565	354,264 ± 178,565	Gills
Neoechinorhynchus buttnerae	100	51.4 ± 45.7	51.4 ± 45.7	Intestine

*Infection score according to Rach et al. (2000).

earthen ponds in Venezuela (Dezon De Fogel et al., 2004). Parasitic co-infection may be common in some disease outbreaks, but usually only one pathogenic agent is more strongly related to the clinical signs and fish health status. Ferreira et al. (2018) reported co-infection by *P. pillulare* and *Trichodina* spp. in *C. macropomum* farmed in the Federal District, Brazil. Co-infection by the protozoan *P. pillulare* and *Ichthyophthirius multifiliis* was reported by Dezon De Fogel et al. (2004) in tambaqui in Venezuela. In all such cases, *P. pillulare* was the prevailing infection and reported as the main cause of the outbreak, fish morbidity and mortality, which corroborates with the findings of the present study.

The dinoflagellate *P. pillulare* can be found on the fish body surface and gills, as they are naturally present in aquatic systems, without causing any harm when the system is in equilibrium (Martins et al., 2015). Accordingly, Fujimoto et al. (2019) reported low prevalence of *P. pillulare* in *C. macropomum* farmed in earthen ponds. Santos et al. (2013a) reported high infection levels of *P. pillulare* in *C. macropomum* reared in cages, but without fish presenting any clinical signs. These differences may be due to differences of environments. However, in the present study, tambaqui skin colour was greenish-brown and darkened gills, as reported by Arbildo-Ortiz et al. (2020), who additionally reported skin necrosis and petechiae in *C. macropomum*. Furthermore, clinical signs of dysfunctional breathing, such as swimming near the water surface and swollen bottom lips were observed. Such clinical signs had been also previously described in *P. pillulare* infection in other fish species (Martins et al., 2001, 2015; Sant'Ana et al., 2012).

The rapid increase in the number of *P. pillulare* in farming conditions is promoted by low host specificity (Martins et al., 2015), as well as poor management practices, such as high stocking densities, poor water quality and inadequate nutrition (Martins et al., 2001, 2015; Sant'Ana et al., 2012). In this study, fish stocking density was above the recommendation, high transparency in pond water and excess organic matter in the tank bottom, no draining and disinfection of the pond between cycles, which all may have contributed to the outbreak of *P. pillulare* infection. Therefore, risk factors associated to this parasite are directly related to both poor fish growth and water quality (Fujimoto et al., 2019). Unbalance in those variables and their impact on the parasite-host-environment relationship may lead to stress, compromised immune response and fish highly susceptible to such parasitic infection (Santos et al., 2013a). Accordingly, exceeding the pond carrying capacity will result in poor water quality, impaired in fish growth, increased contact, leading to disease outbreak (Baldwin, 2010; Associação Brasileira da Piscicultura – PeixeBR, 2021).

The acanthocephalan *N. buttnerae* were observed in the gut of tambaqui farmed in the state of Tocantins at 100% prevalence, intensity of 19 to 234 parasites per fish, at a total of 1,080 individuals (Table 1). Localized increase in the diameter of the fish intestinal loops was an external sign of the presence of those parasites in the gut. In two of the sampled fish, one and two acanthocephalans were found in the fish stomach, but it was most probably an unusual migration of the parasite. In this study, the mean intensity of *N. buttnerae* was lower than previously reported in tambaqui farmed in the states of Roraima: 188 to 388 (Pereira & Morey, 2018) and Amazonas: 31 to 406 (Malta et al., 2001); 81 to 708 (Matos et al., 2017); 15 to 720 (Silva-Gomes et al., 2017), 107 to 921 (Lourenço et al., 2017); 54 to 931 and 81 to 1.219 (Chagas et al., 2019). Such variation in the parasitic index may be due to different conditions adopted in the farms and stages of the culture cycle when the fish were sampled. Since no draining and disinfection of the pond was practiced in the farm, it is not possible to trace back the origin of the infection, if infected fish were brought into the farm or if they got infected because the pond was already contaminated from previous batches. Incompatible stocking density, i.e., intensification, as well as no adoption of good farm management practices, e.g., draining, disinfection of ponds between cycles, are favorable conditions to the growth of acanthocephalans on intermediate hosts and on newly stocked fish in the following cycle.

Acanthocephalan parasites are heteroxenous, dioecious, and reproduce inside the fish digestive system. Their fertilized eggs are released in the water together with the fish feces, and the eggs are eaten by arthropods, which are eaten by the fish, closing the life cycle that involves an arthropod as intermediate host and a vertebrate as a definitive host (Santos et al., 2013b). The ostracod *Cypridopsis vidua* is the intermediate host for *N. buttnerae*, inside which the parasite grows for 29 days (Lourenço et al., 2017; Chagas et al., 2019). The acanthocephalan *N. buttnerae* causes severe pathogenicity in tambaqui (Jerônimo et al., 2017; Matos et al., 2017). An antiparasitic strategy to control of this endoparasite should the elimination of the intermediate host in environment.

So far, the distribution of *N. buttnerae* in tambaqui had been restricted to the states of Amazonas (Malta et al., 2001; Jerônimo et al., 2017; Lourenço et al., 2017; Chagas et al., 2019; Valladão et al., 2020), Rondônia (Oliveira et al., 2015; Jerônimo et al., 2017), Roraima (Pereira & Morey, 2018) and Maranhão (Chagas et al., 2015), although tambaqui is farmed throughout the North, Northeast, and Central-West regions in Brazil (Associação Brasileira da

Piscicultura – PeixeBR, 2022). In the state of Amapá *N. buttnerae* infection has been reported in the hybrids tambatinga (♀*C. macropomum* x ♂*P. brachypomus*) (Dias et al., 2015) and tambacu (♀*C. macropomum* x ♂*P. mesopotamicus*) (Silva et al., 2013). The present study is the first to report the occurrence of *N. buttnerae* in the state of Tocantins, increasing its distribution area to another state, which must be serve as an alert to the spreading of the disease across the tambaqui farms. Henceforth, sanitary inspection agencies and local farmers should be vigilant to the interstate trading of tambaqui fingerlings, which may be a possible source of disease spread.

Although the main signs in fish reported in this study were related to *P. pillulare* infection, weight loss correlates to *N. buttnerae* infection. Fish growth performance parameters such as weight gain, final biomass, and yield per farmed area in Amazonas had a reduction of more than 200% in fish infected by those acanthocephalans, directly affecting the gross profit of the farms (Silva-Gomes et al., 2017). Therefore, even if the acanthocephalan infection does not cause fish mortality, performance losses must be considered, and prevention and treatment are fundamental.

The official list of authorized drugs to be used in aquaculture is not sufficient to cater to the needs of the different farming systems and fish species farmed in Brazil, thus, to control diseases caused by *P. pillulare* and *N. buttnerae* in tambaqui is challenging. In face of such situation, indiscriminate use of different chemicals, such as benzimidazoles, copper sulphate, potassium permanganate, formalin, among others, has increased in the desperate attempt to control the problem. Nevertheless, the use of such chemicals without knowing the adequate dose or period of administration, may lead to pathogen resistance, and even render the treatments inefficient once they become available. Furthermore, it can lead to immune suppression in the fish, environmental pollution, and food safety issue, as chemical residues may deposit in the fish flesh.

Studies have been conducted with some commercially available synthetic products, as well as with bioactive ingredients from medicinal plants, to stablish protocols to treat and control those parasitic infections by *P. pillulare* and *N. buttnerae* in tambaqui, and results are presented in Tables 2 and 3. The use of chemical treatments to control parasitic infections in farmed fish is a strategy in many countries, but these products are in general toxic to the host fish (Castro et al., 2021), thus essential oils have been used as antiparasitic strategy. *Neoechinorhynchus buttnerae* has detrimental consequences for the health of tambaqui (Jerônimo et al., 2017; Lourenço et al., 2017; Matos et al.,

Table 2. Therapeutic management strategies to control and treatment against infections by *Piscinoodinium pillulare* in *Colossoma macropomum*.

Therapeutic substances	Treatment time	Results	References
120 mL/L of *Terminalia catappa*	Bath for 7 consecutive days	Reduction in parasite load	Claudiano et al. (2009)
40 mg/L of *Mentha piperita*	10 minutes bath for 3 days	Efficacy of 79.9% (mucus)	Ferreira et al. (2019)
40 mg/L of *Mentha piperita*	10 minutes bath for 3 days	Efficacy of 54.6% (gills)	Ferreira et al. (2019)
30 mg/L of *Lippia gracilis*	15 minutes bath for 3 days	Efficacy of 54.6% (gills)	Santos (2021)
50 mg/L of *Aloysia triphylla*	15 minutes bath for 3 days	Efficacy of 78.4% (gills)	Santos (2021)
20 mg/L of *Piper aduncum*	15 minutes bath for 3 days	Efficacy of 83.3% (gills)	Santos (2021)

Table 3. Therapeutic management strategies to control and treatment against infections by *Neoechinorhynchus buttnerae* in *Colossoma macropomum*.

Therapeutic substances	Treatment time	Results	References
30 mg/kg of albendazole*	35 days of feeding	Efficacy of 66.4%	Cordeiro et al. (2022)
1.0 g/kg of trichlorfon	15 days of feeding	Efficacy of 53.4%	Castro et al. (2021)
8.0 g/kg of praziquantel	21 days of feeding	Efficacy of 59.0%	Farias et al. (2021)
200 mg/kg of fembendazol*	30 days of feeding	Efficacy of 15.7%	Sebastião et al. (2022)
300 mg/kg of levamisole*	30 days of feeding	Efficacy of 99.1%	Sebastião et al. (2022)
200 mg/kg of levamisole*	30 days of feeding	Efficacy of 73.4%	Sebastião et al. (2022)
500 mg/kg of levamisole	15 days of feeding	Efficacy of 68.2%	Silva et al. (2021)
3,0 mg/kg of Acantex®	25 days of feeding	Efficacy of 13.46%	Sebastião et al. (2021)
0.54 g/kg of *Mentha piperita*	30 days of feeding	Efficacy of 85.5%	Costa et al. (2020)
1.44 g/kg of *Lippia alba*	30 days of feeding	Efficacy of 67.1%	Costa et al. (2020)
1.44 g/kg de *Zingiber officinale*	30 days of feeding	Efficacy of 11.7%	Costa et al. (2020)
0.86 g/kg of *Lippia grata**	30 days of feeding	Efficacy of 67.1%	Oliveira (2020)
0.76 g/kg of *Lippia origanoides**	30 days of feeding	Efficacy of 67.1%	Oliveira (2020)
1.03 g/kg of *Ocimum gratissimum**	30 days of feeding	Efficacy of 58.7%	Oliveira (2020)

*g/kg live weight/day

2017); however, control and treatment have been limited due to a lack of therapeutic products. Although the use of products extracted from plants can mean alternatives for fish diseases prevention and treatment, their use has been also limited by the efficacy and toxicity to fish. In addition, it is important to note that some results were effectives, however, such studies have not been yet validated in the field, where environmental and farming conditions may affect the results and even oppose those laboratory findings. Furthermore, other therapeutic strategies need to be tested to offer efficient and safe treatment options for these parasitic infections affecting tambaqui. Despite the promising results cited in present study, further studies are needed to assess the pharmacokinetics and pharmacodynamics of these chemotherapeutic and herbal products, in addition to the impact of the costs of the implementing treatments for the cultivation of tambaqui.

Conclusions

This study was the first report of *Piscinoodinium pillulare* and *Neoechinorhynchus buttnerae* parasitic infection for tambaqui in the state of Tocantins, Brazil, emphasising the need to adopt good farm management and sanitary practices. Such practices include adopting recommended stocking densities, redesigning pond water outlet to discharge water from the bottom, monitoring water quality, adopting fish grading for growth and sanitary management, draining and disinfecting ponds between cycles – including liming and use of fertilizer to reduce organic matter load, control parasites and intermediate hosts of the pond bottom, practicing farm biosecurity – adopt a pond to quarantine the fish fingerling or juveniles before going into production, and adopt polyphase system – reducing the time spent by a batch of fish in the same pond. In addition to that, disinfection system for the farm materials and tools should be adopted to avoid the disease spreading between ponds or even between farms in case those materials are shared.

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Ethics declaration

This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and with authorization from the Ethics Committee in the Use of Animals of Embrapa Amapá (Protocol Nº 014- CEUA/CPAFAP).

Conflict of interest

The authors declare they have no conflict of interest.

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