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Perulernaea gamitanae (Copepoda: Lernaeidae) parasitizing tambaqui (*Colossoma macropomum)* (Characidae) and the hybrids tambacu and tambatinga, cultured in northern Brazil

[Perulernaea gamitanae *(Copepoda: Lernaeidae) parasitando tambaqui (*Colossoma macropomum*) (Characidae) e seus híbridos tambacu e tambatinga cultivados no norte do Brasil*]

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ABSTRACT

The infestation rate in *Colossoma macropomum*, hybrid tambacu (*C. macropomum* x *Piaractus mesopotamicus*) and hybrid tambatinga (*C. macropomum* x *Piaractus brachypomum*) with *Perulernaea gamitanae* Thatcher and Paredes, 1985 from two fish farms in Amapá State, Brazil was studied. Lernaeid parasites (n=2887) were collected mainly on the tongue and the mouth cavity and also on cartilage of gill arches and filaments. Inflammation and fibrous nodules were observed on the attachment sites of the parasites. The infestation rate varied according to the fish farm and host. The prevalence of *P. gamitanae* was of 100% in hosts from one fish farm and was lower in the other fish farm. Higher intensity of *P. gamitanae* occurred in hybrids tambacu and tambatinga, but despite the high prevalence its intensity was moderate. This is the first report on epidemiology of *P. gamitanae* in cultured fishes from Brazilian Amazonia, and the occurrence of this crustacean parasite in two new hosts, the hybrids tambacu and tambatinga.

Keywords: freshwater fish, Amazonia, crustacean, infestation, parasite

RESUMO

Estudou-se as taxas de infestação pelo crustáceo Perulernaea gamitanae Thatcher & Paredes, 1985 em tambaqui (Colossoma macropomum) *e seus híbridos tambacu* (C. macropomum x Piaractus mesopotamicus) *e tambatinga* (C. macropomum x Piaractus brachypomum*) de duas pisciculturas no estado do Amapá, Brasil. Os lerneídeos parasitos (n=2.887) foram coletados principalmente na língua e na boca das espécies estudadas. Os crustáceos foram encontrados também nos filamentos e cartilagem dos arcos branquiais. Nos locais parasitados foram observados inflamação e nódulos fibrosos. As taxas de infestações variaram entre espécies e entre pisciculturas. Na piscicultura um a prevalência de* P. gamitanae *foi 100%, e na piscicultura dois, foi menor. A maior intensidade de infestação por* P. gamitanae *ocorreu nos híbridos tambacu e tambatinga. Apesar da elevada prevalência de* P. gamitanae *a intensidade de infestação foi moderada. Este é o primeiro relato sobre níveis epidemiológicos de* P. gamitanae *em peixes de cultivo da Amazônia brasileira, e amplia a ocorrência deste parasito crustáceo para dois novos hospedeiros, os híbridos tambacu e tambatinga.*

Palavras-chave: peixe de água doce, Amazônia, crustáceo, infestação, parasito

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INTRODUCTION

Problems with diseases caused by lernaeid parasites have increased due to epizootics on farmed (Shariff *et al*., 1986; Martins *et al*., 2000; Kim *et al*., 2002; Kir, 2007) and wild fishes (Öktener *et al*., 2008) in cases of heavy infestations, mainly in small fish. In Brazil, *Lernaea cyprinacea* is the most common species of lernaeid, which has spread to several farms (Gabrielli and Orsi, 2000; Martins *et al*., 2000; Martins *et al*., 2002; Mabilia and Souza, 2006) and natural environments (Magalhães, 2006) due to its specificity. Lernaeidae pertaining to the genus *Lamproglena* are emerging parasites with pathogenic potential that have been recently reported to occur in cultured fish in the Southeastern (Alves *et al*., 2000; Martins *et al*., 2002; Lizama *et al*., 2007) and Southern Brazil (Ghiraldelli *et al*., 2006), as well as in wild fish (Azevedo *et al*., 2007). Up to date, *L. cyprinacea* and *Lamproglena* species have not been found in Amazonian fishe, while other Lernaeidae species are restricted to Amazonian hosts (Thatcher, 1991; Thatcher, 1998; Thatcher, 2006).

Perulernaea gamitanae Thatcher and Paredes, 1985 was described in tambaqui *Colossoma macropomum* collected in the Amazon River in Iquitos, Peru, and subsequently, in the same host, in the Brazilian portion of the same river (Thatcher, 1991; Thatcher, 2006). The morphology of nauplius and copepodite stages of *P. gamitanae* was studied from eggs of adult post-metamorphic females obtained from the tambaqui *C. macropomum* (Benetton and Malta, 1999). Recently, Fischer *et al*. (2003) compared levels of parasitism by *P. gamitanae* in tambaqui collected in the Solimões River (State of Amazonas) and Amazon River (State of Para). Nevertheless, reports of infestation rates by *P. gamitanae* in farmed fish are still scarce.

In 2009, 46,454 MT of *tambaqui*, 18,492 MT of hybrid tambacu and 4,204MT of the hybrid tambatinga (*C. macropomum* x *Piaractus brachypomum*) were farm raised in Brazil (MPA, 2010). The intensification of C. *macropomum* and hybrid tambacu and tambatinga culture has contributed to the occurrence and emerging parasites, which may compromise fish health and productivity. The present study investigated infection rates by *P. gamitanae* in *C. macropomum*, hybrids tambacu and tambatinga from two fish farms in the State of Amapá, and also, extended the evaluation of this parasite on other hosts, describing the its abundance in tambacu and tambatinga for the first time.

MATERIAL AND METHODS

Specimens of 14 to 17 month-old tambaqui *Colossoma macropomum* (30.5-48.4cm and 450.0-1932g), 20 month-old hybrid tambacu (33.7-41.6cm and 642.0-1300g) and 12 month-old hybrid tambatinga (36.0-40.3cm and 642.0-1234g) were collected from earthen ponds, for parasitological analysis. Fish were raised intensively in fish farms (fish farm one - $0^{o}02'31.4"S$, $051^{o}07'34.4"W$) located at the Fazendinha district, city of Macapá, State of Amapá, Brazil. Tambaqui and hybrid tambacu were collected from the same pond ($1200m^2$) and hybrid tambatinga from another pond ($4400m^2$). Fish samples were also collected from another farm (fish farm two-$0^{o}01'48.3"S$, $051^{o}07'52.9"W$) from the same area. Specimens of tambaqui *C. macropomum* (30.0-34.0 cm and 800-1000 g) and hybrid tambatinga (32.0-35.2cm and 900-1200g) cultured intensively were collected from earthen ponds, for parasitological analysis. Tambaqui and hybrid tambatinga were collected from the same pond ($2400m^2$) on the second farm.

Fish were measured and necropsied by taking the mouth and gills for the collection and quantification of parasites. All parasites were collected, counted and fixed in 70% alcohol solution. The identification of the parasites was carried out as suggested by Thatcher and Paredes (1985) and Thatcher (2006). Parasitic indexes (prevalence, mean intensity, variation of intensity, mean abundance) were calculated according to Bush *et al*. (1997) in order to evaluate fish infestation rate.

Following the parasitological analysis and collection of the parasites, all ponds were submitted to indefinite bath treatment with $100g/1000m^3$ of Diflubenzuron (Dimilin®) in single application. During fish collection, water physical-chemical variables were analyzed. Temperature and dissolved oxygen (OD) were determined through digital equipment (YSI-Model-55) and pHpH was also determined through digital equipment (pH-meter WTW - D812 model).

RESULTS

Water quality variables in the ponds from farm one varied as follows: pH ranged from 6.8 to 7.8, temperature from 31.0 to 31.4°C and dissolved oxygen 2.9 to 3.1mg/L. In farm two, pond water pH ranged from 6.4 to 6.8, temperature from 29.1 to 31.0°C and dissolved oxygen 0.9 to 2.1mg/L.

In all fish the Lernaeidae parasites were identified as *Perulernaea gamitanae* Thatcher and Paredes (1985) (Figure 1A). Parasites were collected mainly on the tongue and gills of the three fish species (Figure 1C-D), but in the *C. macropomum* parasites were also found on the cartilage of gill arches and filaments, and no hybrid tambatinga had gill infestation. Some specimens of hybrid tambacu also presented the parasites on the palate. In general, the sites of infestation on the gill filaments showed inflammation spots and fibrous nodules (Figure 1C), regardless of the presence or absence of the crustacean.

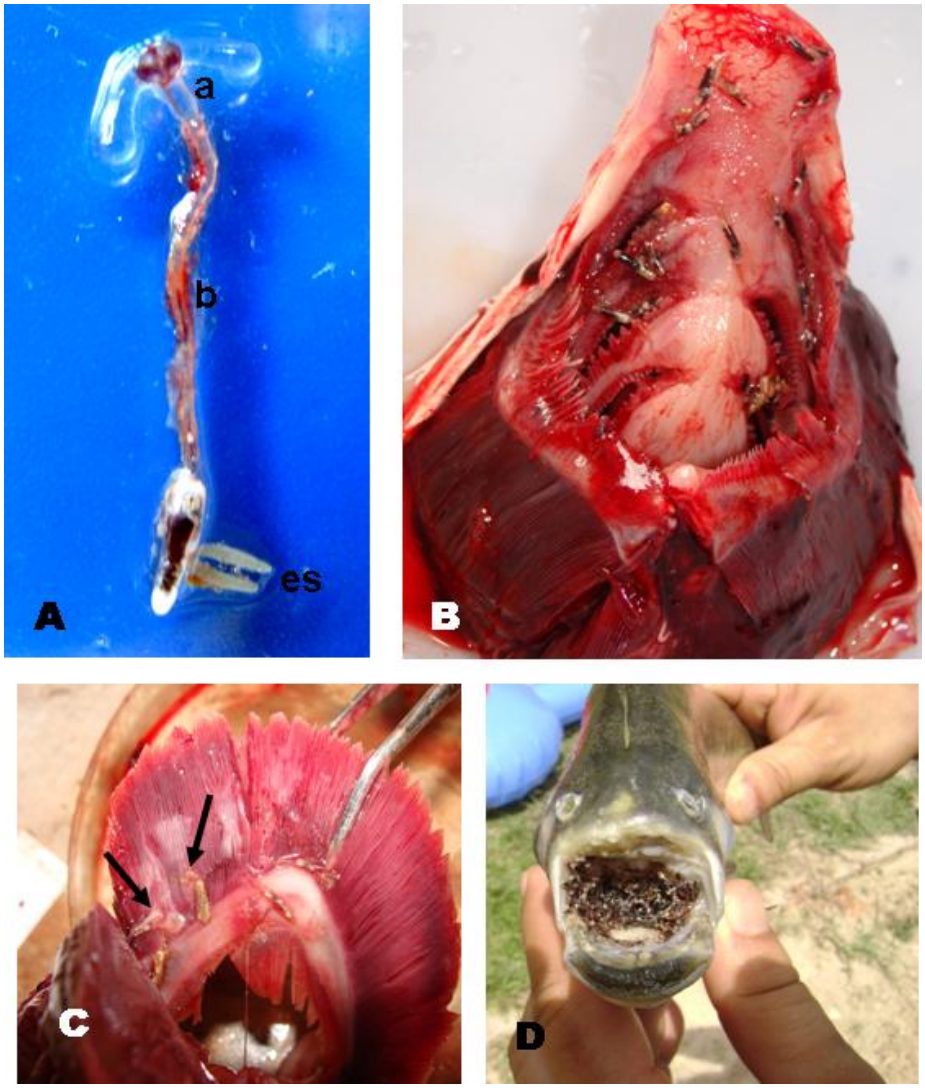

Figure 1. (**A**) *Perulernaea gamitanae* specimen collected from hybrid tambacu. (**B**) Region of the oral cavity of *Colossoma macropomum* with adhered parasites. (**C**) Swollen region (arrow) in gills of hybrid tambacu, with adhered parasites. (**D**) Oral cavity of *C. macropomum* with massive infestation of *P. gamitanae*. a: achor; b: body; es: egg sac.

The parasitism index of *C. macropomum*, and of hybrids tambacu and tambatinga of the collected fish from farm one and two are presented in Table 1 and Table 2, respectively.

These parasites were collected from the mouth cavity and gills of hybrid *tambatinga*, but in *C. macropomum* the infestation occurred only in the mouth cavity (Table 2).

The prevalence of *P. gamitanae* was similar in hybrid tambatinga, but differences occurred in *C. macropomum* and in infestation rates on both fish farms.

The single indefinite bath treatment with 100g/1000m^3 of Diflubenzuron eliminated the parasites in gills and mouth of fish in both fish farms.

Table 1. Parasitological indexes of *Perulernaea gamitanae* in Colossoma *macropomum* and its two hybrids for fish farm one in Macapá (AP), February to April, 2009

Hosts	*C. macropomum*		Hybrid tambatinga		Hybrid tambacu	
Parameters/Locality	Mouth	Gills	Mouth	Gills	Mouth	Gills
EF/PF	39/39	39/38	45/45	0	42/42	42/41
Prevalence (%)	100	97.4	100	0	100	95.2
TNP	354	188	168	0	501	385
Mean intensity	9.1	4.9	3.7	0	11.9	9.6
Range of intensity	1-27	1-15	7-45	0	1-43	2-30
Mean abundance	9.1	4.8	3.7	0	11.9	9.4

PF: parasitized fish; EF: examined fish; TNP: Total number of parasites

Table 2. Parasitological indexes of *Perulernaea gamitanae* in Colossoma *macropomum* and hybrid tambatinga for fish farm two, in Macapá (AP), February to April, 2009.

Hosts	*C. macropomum*		Hybrid tambatinga	
Parameters/Locality	Mouth	Gills	Mouth	Gills
EF/PF	30/6	30/0	50/46	50/30
Prevalence (%)	20.0	0	92.0	60.0
TNP	37	0	1104	150
Mean intensity	6.1	0	24.0	5.0
Range of intensity	1-9	0	12-45	2-10
Mean abundance	1.2	0	22.1	3.0

PF: parasitized fish; EF: examined fish; TNP: Total number of parasites

DISCUSSION

For cultured fish in the Brazilian Amazon the infestation rates by lernaeids are still scant. In *C. macropomum*, hybrid tambatinga and hybrid tambacu collected from fish farm one (Amapá State) the high prevalence (100%) was similar to that reported by Benetton and Malta (1999) for *C. macropomum* (100%) cultured in Manaus. However, in *C. macropomum* and hybrid tambatinga collected from fish farm two, the prevalence was lower and ranged from 20% to 60%. On the other hand, prevalence and infection level by *P. gamitanae* were high compared to the infestation by *P. gamitanae* in *C. macropomum* from Solimões and Amazon Rivers (Fischer *et al*., 2003). This prevalence and infestation was also higher than those reported for *L. cyprinacea*, another Lernaeidae species that commonly parasitize *C. macropomum* (Békési, 1992; Martins and Romero, 1996; Martins *et al*., 2000) and hybrid tambacu (Martins and Romero, 1996; Martins *et al*., 2000; Gabrielli and Orsi, 2000; Schalch and Moraes, 2005) cultured in other regions of Brazil (Martins *et al*., 2000). Studies demonstrated that hybrid tambacu has shown high susceptibility to infestation by *L. cyprinacea* adults when compared to other cultured fish (Tavares-Dias *et al*., 2001).

The parasitized fish in fish farm one were possibly infested by *P. gamitanae* that entered the ponds through the water inlet from the Amazon River that was used to fill the ponds,

infesting 14,000 *C. macropomum*, 10,000 hybrid tambacu and 7,000 hybrid tambatinga.

Shortly after the local transport (Macapá, AP) of the infested fish to farm two, 500 specimens of tambaqui and hybrid tambacu with mean weight of 1500g died, probably due to the low resistance of the fish infested by *P. gamitanae*. The parasite spread in the ponds and also infested the hybrid tambatinga. Hybrid tambatinga had higher rates of parasitism by *P. gamitanae* than *C. macropomum*, but this last species had only the oral cavity parasitized. *P. gamitanae* infestation was controlled with 100g/1000m^3 of Diflubenzuron indefinite bath treatment on both fish farms.

Parasites *P. gamitanae* caused inflammation on the place of attachment, especially in the gills, which could facilitate bacterial and mycotic infections, both relatively common in fish infested by the crustaceans. The species that cause hyperplasia and metaplasia in the gills of the host can reduce respiratory capacity (Thatcher, 1998). Besides, *P. gamitanae* can debilitate fish or cause their death when in confinement (Thatcher, 2006) due to apathy and anorexia. Three consecutive treatments with NaCl (4 to 6%) were applied to the pond to heal the wounds caused by parasites, which made the fish resume feeding, but such treatment did not eradicate the young and adult forms of *P. gamitanae*. Salt (NaCl) has been recommended for the control of several other parasites, as well as to avoid dissemination of parasites and stress in freshwater fish. However, the fish tolerance to salt, which varies depending on the fish species and age (Martins, 2004) must be considered.

During this same period, institutions of technical assistance to fish farmers in the states of Rondônia (Porto Velho and Ji-Paraná), Acre (Rio Branco, Bujari, Porto Acre and Senador Guiomard) and Amazonas (Manaus and Manacapuru) have also recorded infestations by *P. gamitanae* in *tambaqui* from several fish farms.

In Acre and Amazonas States, epizooties of juveniles tambaqui weighing 100 to 200g (Figure 1D) occurred due to the great infestation of *P. gamitanae* in the mouth, impeding fish from eating, causing massive mortality. However, in tambaqui with mean weight of 500g, infestation was mild, with no mortality, while in fish weighing between 2000 and 3000g infestation was mild to low, with no mortality either. In all cases, infestation took place mainly in the mouth and tongue. To control infestations by *P. gamitanae*, fish farmers also have applied 50 to 100g/1000m^3 of Diflubenzuron in ponds, which has eliminated all forms of this parasite. Copepodits of *P. gamitanae* survived up to seven days without a host (Benetton and Malta, 1999), thus the treatment would have to be repeated to eliminate all forms of the parasite. Martins (2004) suggested that any treatment with Diflubenzuron must be repeated three times in order to ensure its efficacy. Diflubenzuron is part of a group of insecticides which cause morphological alterations during the life cycle of the crustacean (Martins, 2004; Mabilia and Souza, 2006; Pelli *et al*., 2008) and was approved by the Environmental Protection Agency (EPA) in 1976 to be employed against plagues in cotton and soy cultures. In 1999, Diflubenzuron began to be used in Brazil to control infestations by crustacean ectoparasites (Martins, 2004). Currently, this product is used to control *L. cyprinacea* in 33.3% of fish farms in Rio Grande of Sul State (Mabilia and Souza, 2006), as well as in other fish farms in Brazil.

In order to be suitable for fish farming, a chemical product must be safe for fish, highly efficient and rapidly degraded, not interfering with the water, be of easy application and low cost (Cruz *et al*., 2009). Studies with juvenile rainbow trout *Oncorhynchus mykiss* and coho salmon *Oncorhynchus kisutch* (McKague and Pridmore, 1978), also *Rhamdia quelen* (Kreutz *et al*., 2008) and *Zungaro zungaro* (Pelli *et al*., 2008), have examined the toxicity of Diflubenzuron and they suggest that its mean lethal concentration for 96h exposure (LC_{50}-96h) is greater than 150mg.L^{-1}. However, the concentration of Diflubenzuron recommended for direct application in ponds is 100 to 200g/1000m^3 of water, which corresponds to 0.1–0.2mg.L^{-1} (Martins, 2004). Hence, this product has low toxicity to fish (Burtle and Morrison, 1987; Moraes and Martins, 2004; Kreutz *et al*., 2008; Pelli *et al*., 2008; Schalch *et al*., 2009). Besides being effective in the treatment against crustacean parasites (Burtle and Morrison, 1987; Routh *et al*., 1993; Moraes and Martins, 2004; Schalch *et al*., 2009), Diflubenzuron is degraded in aerobic conditions

(Routh *et al*., 1993; Pelli *et al*., 2008). This product shows potential of use in fish farms (Pelli *et al*., 2008), but still requires proper registration and regulation for its use as a chemotherapeutic for fish crustacean parasites. In Brazil, only two products are currently evaluated and approved by the Ministry of Agriculture, Livestock and Supply (MAPA) for use in aquaculture. Therefore, as development of fish farming is of great importance for the country, there is an imminent need of decision making regarding the legalization of chemical products employed in the treatment of fish diseases (Cruz *et al*., 2009), such as the Diflubenzuron.

As a result, besides the dispersion of lernaeid *P. gamitanae* among fish farms, the infestation may also take place from wild environment into fish farms, given that many tambaqui breeders are obtained in Amazonian rivers. Thus, parasites may be taken to production ponds in the Northern region, as well as other zones where fish is farmed. Consequently, Lernaeid *P. gamitanae* will probably not be restricted to the Northern region if it finds favorable conditions for development in other Brazilian regions. Thus, it is necessary to use caution when transporting fish, to perform quarantine and diagnosis before introducing fish into ponds, in order to prevent the dissemination of this crustacean in fish farms. *P. gamitanae* could pose a major concern for Amazonian aquaculture, jeopardizing the production of *C. macropomum* and its hybrids (*tambacu* and *tambatinga*), especially the earlier, which is the most cultivated species in the Amazon. Cautionary procedures must be reinforced, since massive infection can undermine the survival of fish juveniles as well as the depreciation of animals ready for commercialization, compromising business and consumption of such fish.

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