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Original Full Paper

Histological lesions by monogeneans in gills of *Piaractus brachypomus* farmed in semi-intensive systems from Peru

Karel Torres-Lozano[1], Milagros Cabrera-Soregui[2], Enrique Garcia-Candela[2], Martín Cuadros-Cuya[2], Andrés Cubas-Rengifo[2], Victor Puicón N[1], Fernando Mesias[2]*

[1]Grupo de Investigación de Parasitología Veterinaria y Zoonosis Parasitaria, Laboratorio de Histopatología animal, Escuela profesional de Medicina Veterinaria, Facultad de Medicina Veterinaria, Universidad Nacional de San Martín, Tarapoto, San Martín, Tarapoto CP 22201, Perú
[2]CITEacuícola Pesquero Ahuashiyacu- Instituto Tecnológico de la Producción, La Banda de Shilcayo, Tarapoto CP 22201, Perú

*Corresponding author: fmesias@itp.gob.pe
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Abstract

Piaractus brachypomus Cuvier, 1818, is Peru's most produced Amazonian fish; however, little is known about histological lesions that monogeneans produce in their gills. When examining 40 juveniles of *P. brachypomus* from two commercial fish farms, *Anacanthorus penilabiatus* (Boeger et al., 1995) and *Mymarothecium viatorum* (Boeger et al., 2002) were found, with a total monogenean prevalence of 100%, with a mean intensity and mean abundance of 225.5 parasites/fish for both species. At the level of the gill tissue, lesions identified were dilation and congestion of the central vein and hyperplasia of the secondary lamella with eosinophilic and lymphocytic infiltration. The histopathological alterations caused by infection of *A. penilabiatus* and *M. viatorum* are reported for the first timein cultured *P. brachypomus* in the Peruvian Amazon. These findings indicate the need to improve good practices and biosafety in producing this fish to prevent or control the impact of these monogeneans.

Keywords: Amazonia, *Anacanthorus*, gills, histopathology, Monogenoidea, *Mymarothecium*.

Introduction

Currently, aquaculture contributes 49.2% of aquatic animals for human consumption worldwide, the animal production activity with the highest growth, at a rate of 3.3% annually (10). Millions of people are dedicated to this sector as their main source of food and income, finding a diversity of fish produced with unique characteristics. In Peru, *Piaractus brachypomus* Cuvier, 1818, is the most produced Amazonian fish (3104 tons), with San Martín as the main production region (14). This species is characterized by its docility, rusticity, size, and high quality of meat, making it attractive to be used in aquaculture and for sale in local markets (7).

In San Martín, *P. brachypomus* is farmed mostly in earthen ponds due to low investment costs. However, this activity can produce stressful conditions in fish, leading some parasites to proliferate rapidly, as the monogeneans, since they do not require an intermediate host and achieve rapid development (13, 20).

Monogeneans of the genera *Mymarothecium* and *Anacanthorus* have been found in high parasitological indexes related to prevalence, mean abundance, and mean intensity in fishes of the genus *Piaractus* (8, 11, 17, 19). On the other hand, at a histological level, few studies have reported lesions caused by these parasites in *Piaractus* fishes. The described lesions include infiltration of inflammatory cells, hyperplasia of secondary lamellae, multifocal necrosis, and partial or total fusion of secondary lamellae, among others have been found (2, 12, 15). Consequently, these microorganisms represent a significant problem in these animals by causing diseases or physical and biological alterations such as petechiae,

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lacerations, lethargy, loss of appetite, and death in more severe cases (24).

Due to the commercial and economic importance of *P. brachypomus* in Peruvian aquaculture, the objective of this research was to characterize histological lesions on the gills of fish parasitized with *Mymarothecium viatorum* (4) and *Anacanthorus penilabiatus* (3) from two commercial fish farms in San Martín region. Furthermore, this study reports, for the first time, evidence of histopathological alterations produced by monogeneans in *P. brachypomus* specimens raised in two semi-intensive centers in the Amazon region of Peru.

Material and Methods

The procedures described below were approved by the "Institutional Bioethics Committee for Research, Biodiversity Management and Animal Welfare" of the Universidad Nacional de San Martín, under Resolution CIEIMBBA N° 12-2021.

Gill samples were taken from forty *P. brachypomus* (21.22 cm ± 3.42 in length and 220.93 g ± 24.6 in weight) in August 2021 (hot season), which were raised in earthen ponds in two aquaculture centers in provinces of San Martin and Moyobamba, from San Martin region, Peru. Fish were collected using fishing nets and carcals and taken to ITP/CITEacuícola Pesquero Ahuashiyacu laboratory in 600-liter polypropylene tanks with constant aeration.

Fish were euthanized by cutting the medulla, opercula were removed, and gill arches were extracted for morphological identification. These organs were placed in Petri dishes with distilled water at 65°C, and continuous lateral movements were made to remove the parasites present. The recovered parasites were then mounted in Hoyer's medium to observe the sclerotized structures with an optical microscope (Leica, Germany) as suggested by Boeger et al. (3, 4). The prevalence, mean abundance, and mean intensity of parasites were calculated according to the methodology recommended by Bush et al. (6).

For histopathological analysis, fish gill arches were stored in 10% buffered formaldehyde and then transferred to 70% alcohol. Then, samples were dehydrated in increasing alcohol solutions (70, 80, 90, and 100%) and clarified with xylol. The materials were embedded in paraffin to make cross sections in a microtome (5 µm) and stained with hematoxylin and eosin (1). Sections were then observed with an optical microscope (Leica, Germany) to identify histopathological lesions and photograph them.

Results

A. penilabiatus and *M. viatorum* were identified in the gills of *P. brachypomus* from both commercial fish farms. In *A. penilabiatus*, the presence of a copulatory organ (Fig. 1) was observed in the shape of a "J" with a wavy shape and slightly curved or coiled in some samples (Fig. 2); on the back of the body, it carried 14 marginal hooks (Fig. 3), which were found divided into two portions, in addition to the absence of both dorsal and ventral bars.

In *M. viatorum*, a composite male copulatory organ was observed, which contains an accessory piece (Fig. 4). This has a straight shape with a slight curvature (Fig. 5). The hooks are divided into two clearly defined parts: 4 dorsal and 10 ventral (Fig. 6). The ventral bar was rod-shaped, with anterior or posterior projections. The dorsal bar or rod was thin "U"-shaped, with anterior projections present or absent and a posterior projection absent.

In all *P. brachypomus* were found monogeneans parasitizing their gills, a total of 9023 monogeneans were identified, recording a global prevalence of 100%, with a mean intensity and a mean abundance of 225.5 parasites/fish, for both indexes (Table 1). According to identified monogenean species, the prevalence was diverse, with a prevalence of 100%, mean intensity, and mean abundance of 81.0 parasites/fish for *A. penilabiatus* and a total of 3241 parasites. For *M. viatorum*, the prevalence was 52.5%, with a mean intensity and mean abundance of 144.5 parasites/fish and a total of 5782 individuals.

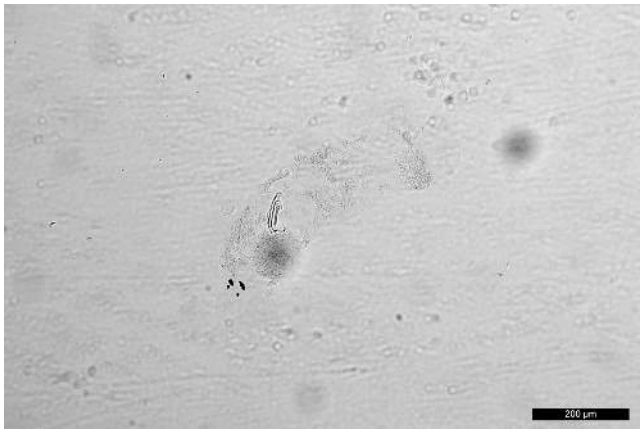

Figure 1. *Anacanthorus penilabiatus*. Full view, copulatory organ visible. Bar =200µm. Magnification: 10x

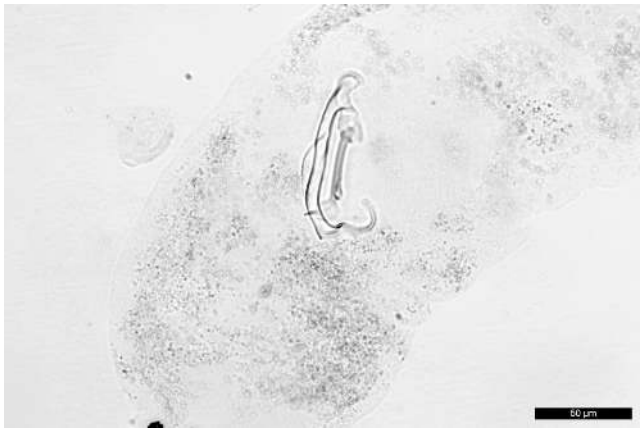

Figure 2. *Anacanthorus penilabiatus*. View of the copulatory organ. Bar =50µm. Magnification: 40x

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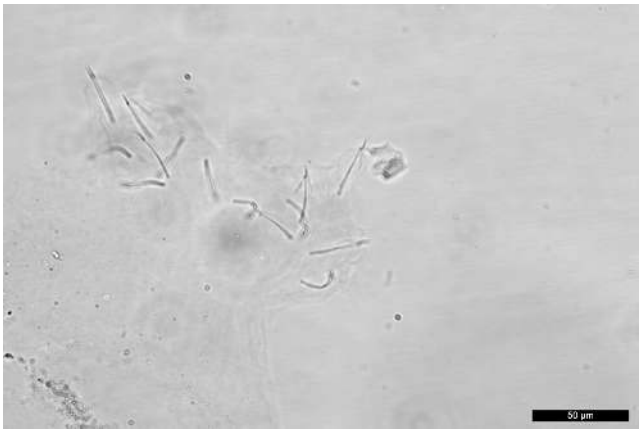

Figure 3. *Anacanthorus penilabiatus.* View of the marginal hooks. Bar =50µm. Magnification: 40x

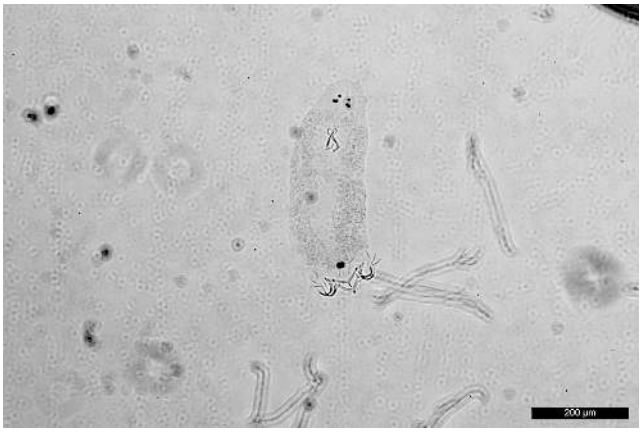

Figure 4. *Mymarothecium viatorum.* Complete view, copulatory organ visible, ventral and dorsal bars Bar =200µm. Magnification: 10x

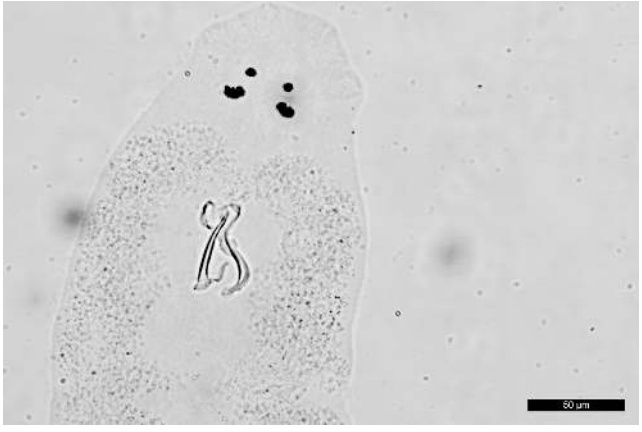

Figure 5. *Mymarothecium viatorum.* View of the copulatory organ. Bar =50µm. Magnification: 40x

Several gills were analyzed for histopathology, which showed several lesions associated with hemodynamic, degenerative, adaptive, growth, and inflammatory disorders (Table 2). Among the most frequent histological alterations were identified dilation (75%) and congestion (41.6%) of the central vein (Fig. 7), eosinophilic and lymphocytic infiltration (41.6%) (Fig. 8), and hyperplasia (41.6%) and fusion (33.3%) of secondary lamellae (Fig. 9), and interlamellar hyperplasia (33.3%). Less frequently, detachment of secondary lamellae epithelium (25%), Monogeneans attached to lamellae (25%) (Fig. 10), telangiectasia and aneurysm (16.6%), lamellae congestion (8.3%), and justalamellar edema (8.3%) and justalamellar detachment (8.3%) were found.

Figure 6. *Mymarothecium viatorum.* View ventral bar (1 pair) and dorsal (2 pairs), marginal hooks. Bar =50µm. Magnification: 40x

Table 1. Prevalence (P), mean intensity (MI) and mean abundance (MA) of infection of *Anacanthorus penilabiatus* and *Mymarothecium viatorum* of *P. brachypomus* gills (n=40) studied in August 2021 in San Martín region.

PARASITES	P (%)	MI	MA	TNP
Monogenean	100	225.5	225.5	9023
Anacanthorus penilabiatus	100	81	81	3241
Mymarothecium viatorum	52.5	144.5	144.5	5782

TNP: Total number of parasites

Discussion

The findings of this study provide critical insights into the parasitological dynamics of *P. brachypomus* cultured in semi-intensive aquaculture systems in the Peruvian Amazon, focusing on infections by *A. penilabiatus* and *M. viatorum.* The observed prevalence of 100% for *A. penilabiatus* and 52.5% for *M. viatorum* highlights the significant parasitic burden in farmed populations of this species, echoing similar reports from other Neotropical regions such as Brazil and Indonesia (2, 17, 18).

In this study, *despite its higher prevalence, A. penilabiatus* exhibited a lower mean intensity and abundance than *M. viatorum*. These results align with previous work by Negreiros and Tavares-Dias (17), who identified similar species in *Piaractus* from Brazil. However, our findings show significantly higher parasite loads in

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Table 2. Classification of histological lesions in gills of *Piaractus brachypomus*, according to the type of lesion and degree of affection

Disorder	Types of lesion	Degree of affection				N	%
		Absent	Slight	Moderate	Severe		
Hemodynamic	Aneurysm	20	4			4/24	16.6
	Dilation of the central vein	6	14	4		18/24	75.0
	Lamella congestion	22		2		2/24	8.3
	Central vein congestion	14	8	2		10/24	41.6
	Justalamelar edema	22	2			2/24	8.3
	Venous sinus dilation	16	6	2		8/24	33.3
Degenerative	Detachment of the justalamellar epithelium	22		2		2/24	8.3
	Detachment of the secondary lamella epithelium	18	4	2		6/24	25.0
	Telangiectasia	20	4			4/24	16.6
Adaptative	Fusion of the lamellae	16	8			8/24	33.3
Growth	Interlamellar hyperplasia	16	8			8/24	33.3
	Secondary lamella hyperplasia	14	8		2	10/24	41.6
Inflammatory	Eosinophilic infiltrate	14	4	6		10/24	41.6
	Lymphocytic infiltrate	14	4	6		10/24	41.6
Other findings	Attached monogenean	18	6			6/24	25.0

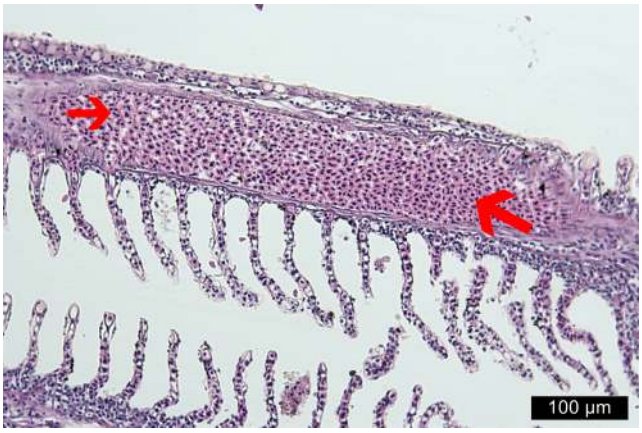

Figure 7. Dilation and congestion of the central vein (arrow). Bar 100µm. Magnification: 20x. H&E stain.

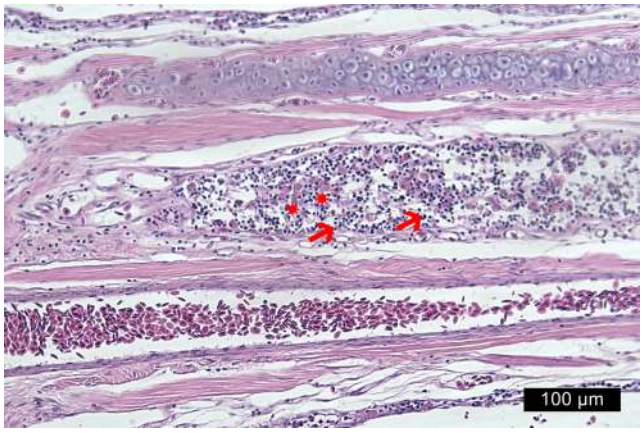

Figure 8. Eosinophilic infiltrate (asterisk), lymphocytic infiltrate (arrow). Bar 100µm. Magnification: 20x.H&E stain.

Peruvian aquaculture systems, likely due to environmental factors such as water quality, stocking density, and pond management practices. Poor water conditions, particularly in earthen ponds, have been shown to exacerbate parasitic infestations by fostering environments conducive to rapid parasite development (11, 13, 20).

Histopathological analysis revealed a range of gill lesions, including hyperplasia of the secondary lamellae, epithelial fusion, and eosinophilic and lymphocytic infiltration. These findings are consistent with earlier reports of histological damage caused by *A. penilabiatus* and *M. viatorum* in cultured *P. brachypomus* and *P. mesopotamicus* (2, 12, 16). The observed hyperplasia and fusion of lamellae can severely impair respiratory efficiency, leading to physiological stress, compromised immune responses, and increased susceptibility to secondary infections (9, 22). Additionally, the congestion and dilation of the central vein in the gills, likely caused by the mechanical action of the parasites' hooks, mirrors pathological effects reported in other species subjected to monogenean infestations (21, 25).

The high parasitic burden observed in this study underscores the urgent need for enhanced management strategies in the Peruvian aquaculture industry. Given the direct life cycle of monogeneans, which facilitates rapid population growth under suboptimal farming conditions, mitigation

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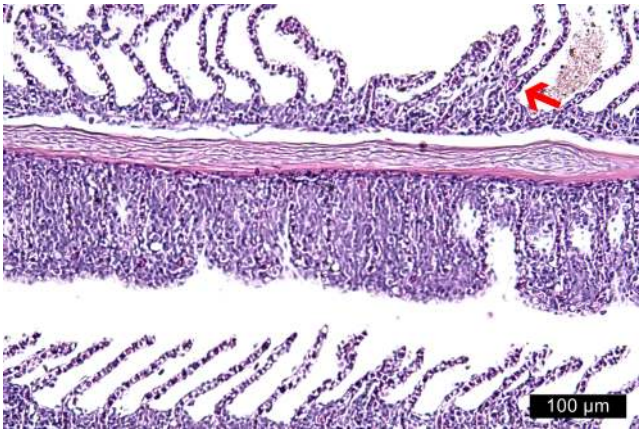

Figure 9. Fusion of the lamellae (arrow). Bar 100µm. Magnification: 20x. H&E stain.

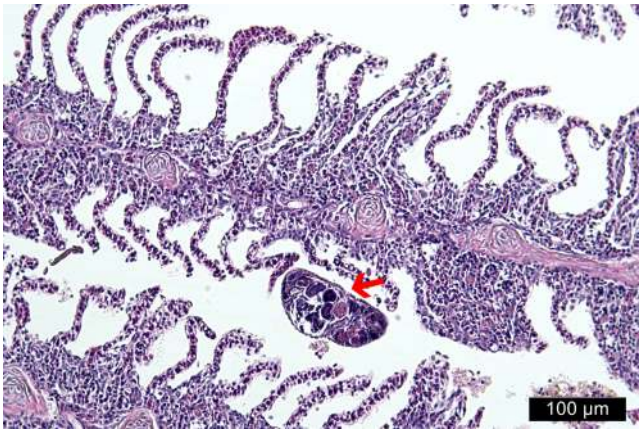

Figure 10. Monogeneum (arrow). Bar 100µm. Magnification: 20x.H&E stain.

measures should focus on improving water quality, reducing stocking densities, and implementing regular parasitic monitoring (15, 23). Furthermore, prophylactic treatments and biosecurity protocols must be rigorously applied to minimize parasite transmission and reduce the overall impact on fish health and farm productivity.

In conclusion, this study provides the first detailed account of histopathological lesions caused by *A. penilabiatus* and *M. viatorum* in farmed *P. brachypomus* in Peru. These parasites' high prevalence and intensity, coupled with the severe tissue damage observed, call for immediate intervention through improved aquaculture practices. Future research should explore the development of targeted antiparasitic treatments and investigate the potential for selective breeding programs to enhance resistance to monogenean infestations in Amazonian fish species.

Conflict of Interest

The authors declare no competing interests.

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Preprint

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