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

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Abstract

Piaractus brachypomus Cuvier, 1818, is the most produced Amazonian fish in Peru; however, little is known about histological lesions that monogeneans produce in their gills. When examining 40 juveniles of *P. brachypomus* from two comercial fish farms, presence of *Anacanthorus penilabiatus* (Boeger, Husak et Martins, 1995) and *Mymarothecium viatorum* (Boeger, Piasecki et Sobecka, 2002) were found, with a total monogenean prevalence of 100%, with a mean intensity and mean abundance of 225.5 parasites/fish for both indices. 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. This is the first report of histopathological alterations caused by infection of *Anacanthorus penilabiatus* and *Mymarothecium viatorum* in cultured groups of *P. brachypomus* in Peruvian Amazon and indicates the need to improve good practices and biosafety in the production of 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, being the animal production activity with the highest growth, at a rate of 3.3% annually (FAO 2022). 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 of this species (PRODUCE, 2023), since it 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 market (Campos-Baca and Kohler, 2005).

In San Martín, production of *Piaractus 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 (Martins and Romero, 1996; Paladini et al. 2017).

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*. (Cohen and Kohn 2005; Franceschini et al. 2013; Oliveira and Tavares-Dias 2016; Negreiros and Tavares-Dias 2019). On the other hand, at a histological level, few studies have reported lesions caused by these parasites in fish of genus *Piaractus*, in which infiltration of inflammatory cells, hyperplasia of secondary lamellae, multifocal necrosis, partial or total fusion of secondary lamellae and among others have been found (Jerônimo et al. 2014; Müller et al. 2016; Anshary et al. 2022). Consequently, these microorganisms represent a great problem in these animals by causing diseases or physical and biological alterations such as petechiae, lacerations, lethargy, loss of appetite and, in more severe cases, death (Tavares-Dias and Martins 2017)

Due to commercial and economic importance of *P. brachypomus* in Peruvian aquaculture, the objective of this research was to characterize histological lesions on gills of fish parasitized with *Mymarothecium viatorum* (Boeger, Piasecki et Sobecka, 2002) and *Anacanthorus penilabiatus* (Boeger, Husak et Martins, 1995) from two comercial fish farms in San Martín region. Furthermore, this study reports, for the first time, evidence on histopathological alterations produced by monogeneans in *P. brachypomus* specimens raised in two semi-intensive centers in Amazon region of Peru.

Methods

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. Fish were collected using fishing nets and carcals, and taken to ITP/CITEacuícola Pesquero Ahuashiyacu laboratory in 600-liter polypropylene tanks with constant aeration. This study was approved by Institutional Committee for Research Ethics, Biodiversity Management and Animal Welfare of Universidad Nacional de San Martín (Resolution No. 394-2021-UNSM/CU-R).

For morphological identification, fish were euthanized by cutting medulla, opercula were removed, and gill arches were extracted. These organs were placed in Petri dishes with distilled water at 65°C, and continuous lateral movements were made to remove the parasites present. These were then mounted in Hoyer's medium to observe with an optical microscope (Leica, Germany) the sclerotized structures as suggested by Boeger et al. (1995, 2002). The prevalence, mean abundance and mean intensity of parasites were calculated according to methodology recommended by Bush et al. (1997).

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 (Adriano et al. 2002). Sections were then observed with an optical microscope (Leica, Germany) to identify histopathological lesions and to photograph them.

Results

Anacanthorus penilabiatus and *Mymarothecium viatorum* were identified in the gills of *Piaractus brachypomus* from both commercial fish farms. In *Anacanthorus penilabiatus*, presence of 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 having absence of both dorsal and ventral bars.

In *Mymarothecium viatorum*, 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). Rod-shaped ventral bar, with anterior or posterior projections. Thin "U"-shaped dorsal bar or rod, anterior projections present or absent, posterior projection absent.

In all *Piaractus 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). The prevalence according to identified monogenean species was diverse, with a prevalence of 100%, mean intensity and mean abundance of 81.0 parasites/fish for *Anacanthorus penilabiatus* and a total of 3241 parasites. For *Mymarothecium viatorum*, prevalence was 52.5%, with a mean intensity and mean abundance of 144.5 parasites/fish and a total of 5782 individuals.

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

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 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 justalamelar detachment (8.3%) were found.

Discussion

Anacanthorus penilabiatus was reported and described for the first time in *Piaractus mesopotamicus* (Holmberg, 1887) (Boeger et al. 1995), it was also reported for the first time for *Colossoma brachypomus* (synonymy of *P. brachypomus*) and *Colossoma macropomum* (Cuvier, 1816), in addition to reporting eggs of *A. penilabiatus* (Pamplona -Basilio et al. 2001) in the aforementioned hosts that belong to the species *P. brachypomus*.

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Table 2. Classification and frequency of histological lesions in gills of *P. brachypomus*, by type of lesion and degree of affection.

DISORDERS	TYPE OF LESION	DEGREE OF AFFECTION				N	%
		None	Slight	Moderate	Severe		
Hemodynamic	Dilation of the central vein	6	14	4		18/24	75.0
	Central vein congestion	14	8	2		10/24	41.6
	Venous sinus dilation	16	6	2		8/24	33.3
	Aneurysm	20	4			4/24	16.6
	Justalamelar edema	22	2			2/24	8.3
	Lamellae congestion	22		2		2/24	8.3
Degenerative	Secondary lamellae epithelium detachment	18	4	2		6/24	25.0
	Telangiectasia	20	4			4/24	16.6
	Justalamellar epithelial detachment	22		2		2/24	8.3
Adaptative	Secondary lamellae fusion	16	8			8/24	33.3
Growth	Interlamellar hyperplasia	16	8			8/24	33.3
	Secondary lamellae 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	Monogeneans attached to lamellae	18	6			6/24	25.0

Mymarothecium viatorum was reported for the first time for *P. brachypomus* in Poland (Boeger et al. 2002). In a study carried out in the state of Ceará in Brazil, it was reported the presence of *Mymarothecium viatorum* for the first time for *P. mesopotamicus* confirming presence of this monogeneous in Neotropical region (Cohen and Kohn, 2005). Studies on parasitism of *P. brachypomus* from Amazon River in the state of Pará, Brazil, collected 27384 metazoan parasites, among which, monogeneans, recording *Mymarothecium viatorum* and other parasites (Oliveira and Tavares-Dias, 2016). Likewise, Cohen and Kohn (2005) describe and highlight the projection of monogenean bars, which is a characteristic that distinguishes it from other genera, this aided in its identification.

The species of *Anacanthorus penilabiatus* and *Mymarothecium viatorum* were reported in a study carried out with fish from research centers in the province of San Martín (Murrieta-Morey et al. 2023). Furthermore, in the state of Acre in Brazil, both species were also reported for *P. brachypomus* (Negreiros and Tavares-Dias, 2019). As is known, monogeneans are parasites with high specificity for their hosts, which is why monogenes of the genus *Anacanthorus* and *Mymarothecium* have been reported in fish of the genus *Piaractus* (Šimková, 2006).

In the present study it is observed that *M. viatorum* presented a higher average intensity and average abundance unlike *Anacanthorus penilabiatus*, however, the latter had a prevalence of 100% in *P. brachypomus* gills, compared to 52.5% of *A. penilabiatus*. These findings are like those reported by Negreiros and Tavares-Dias (2019), where 4 *Piaractus brachypomus* farms from the state of Acre in Brazil were analyzed and *Anacanthorus spathulatus* (Kritsky, Thatcher et Kayton, 1979), *Anacanthorus penilabiatus* and *M. viatorum* were identified, the latter two parasites also were found in the present work. For *A. penilabiatus*; presented a prevalence of 23.8%, average intensity of 13 and average abundance of 3.1, however, in the present work, *A. penilabiatus* showed a prevalence of 100%, average intensity and average abundance of 81 for both. On the other hand, *M. viatorum* presented a prevalence of 60%, mean intensity of 15.8 and mean abundance of 9.5. Unlike the present study, *M. viatorum* had a prevalence of 52.5% with average intensity and average abundance of 144.5 for both indices. The difference in parasite load in fish sampled in both Acre and San Martín could be caused by different factors such as the type of culture, environmental conditions, density, low quality of water with high organic matter, low amount of oxygen, the state farm health, among others (Franceschini et al. 2013; Paladini et al. 2017). Likewise, monogeneans usually proliferate in earthen ponds, where conditions are most suitable, due to their direct life cycle, which does not need an intermediate host (Martins and Romero 1996).

Histological alterations found in this study resemble those described in *P. brachypomus* from Indonesia and in *P. mesopomaticus* from Brazil (Jeronimo et al. 2014; Müller et al. 2016; Anshary et al. 2022). In these countries, tissue lesions were identified in gills infected with *Anacanthorus penilabiatus* and *Mymarothecium viatorum*, also recording findings such as hyperplasia of the interlamellar epithelium, fusion of lamellae, infiltration of inflammatory cells, inflammation of the gill filaments and loss of lamellae.

On the other hand, our results were also similar to those reported in gills of *Colossoma macropomum*, infected by monogeneans (Vargas et al. 2015), where infiltration of

inflammatory cells, hyperplasia and lamellar fusion were identified, being associated with poor water quality. in culture ponds and infection by parasites.

The most common response to gill lesions is epithelial hyperplasia and hypertrophy, which can result in the fusion of lamellae and filaments (Noga 2010). This can be caused by poor water quality, exposure to toxic substances, nutritional deficiencies and parasitic infections (Reimschuessel 2008). Likewise, the insertion of monogenean hooks into gill tissues also causes congestion and dilation of veins, as well as hyperplasia and fusion of lamellae in response to injuries caused by the movement of the parasites (Buchmann and Lindenstrøn 2002).

Conclusions

Based on morphological analysis, the monogeneans found in gills of *P. brachypomus* raised in commercial fish farms were *Mymarothecium viatorum* and *Anacanthorus penilabiatus*.

Based on findings of this research, the need to improve the commercial production of *P. brachypomus* in semi-intensive systems is highlighted, given the identification of numerous histological alterations in the gills of this fish, as well as high prevalence, medium intensity and average abundance of monogenean parasites found.

To mitigate the pathological impact of these monogeneans, it is essential that fish farmers implement prophylactic measures and best aquaculture practices. Furthermore, the need for additional studies to better understand the emergence, impact and pathologies associated with the monogenean parasites *M. viatorum* and *A. penilabiatus* in Amazonian aquaculture in Peru is evident. These efforts are crucial to ensuring the health and sustainability of the aquaculture industry in the region.

Acknowledgements

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Figures

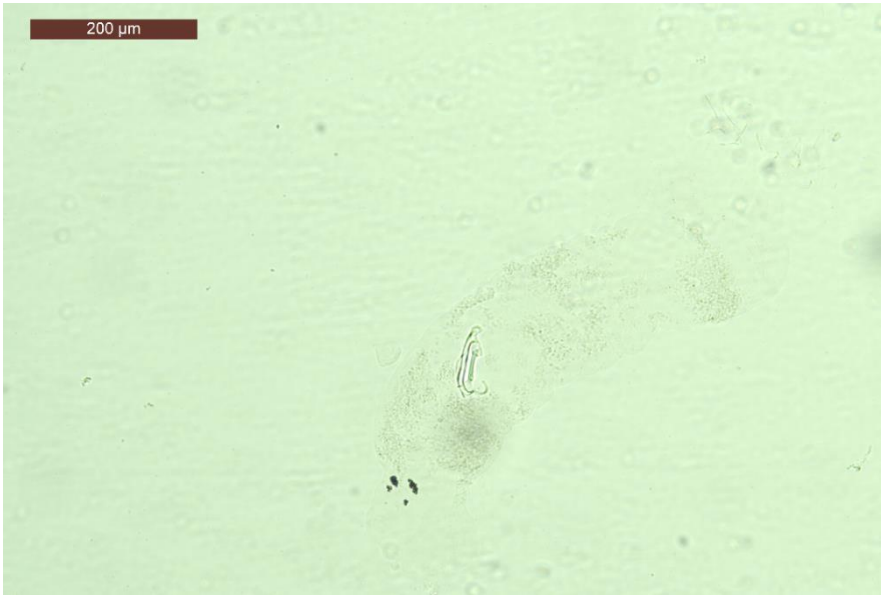

Figure 1. *Anacanthorus penilabiatus.* Copulatory organ, full view. Bar= 200µm. Magnification: 10x.

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

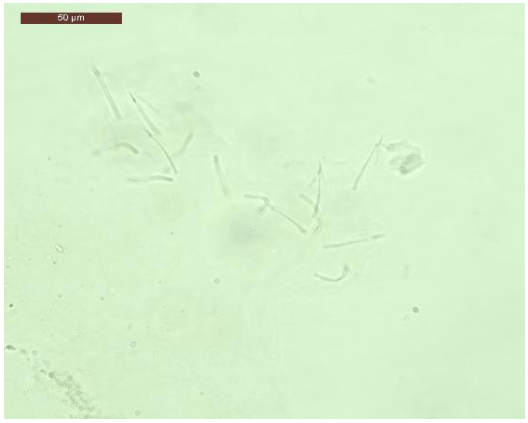

Figure 3. *Anacanthorus penilabiatus.* View of marginal hooks. Bar = 50µm. Magnification: 40x.

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

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

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

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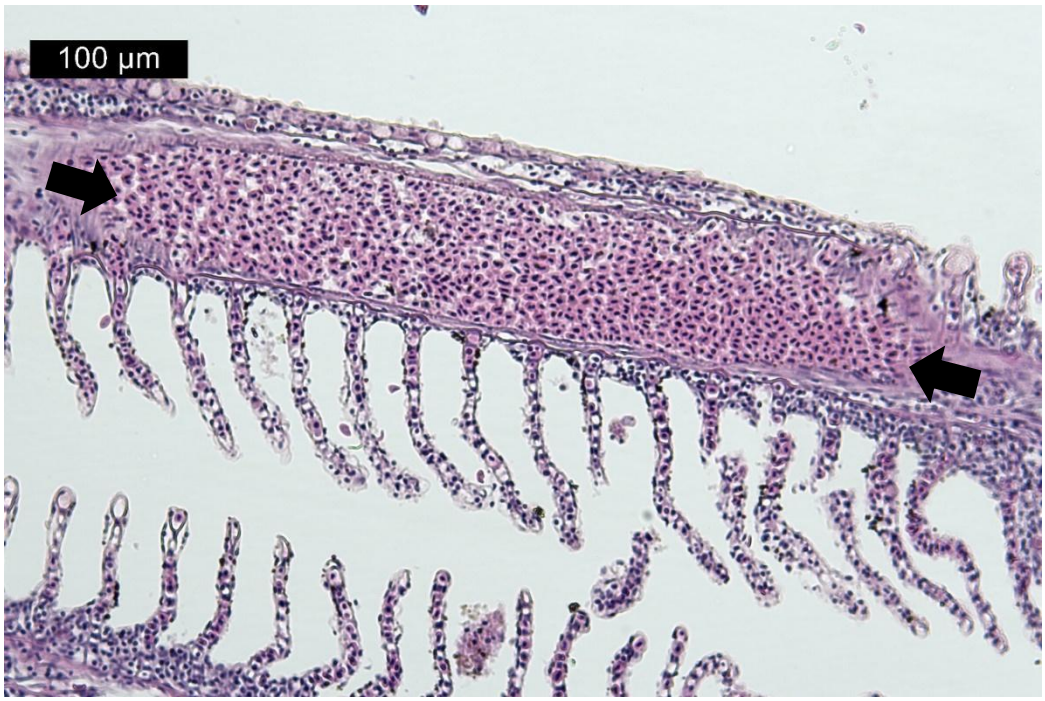

Figure 7. Dilation and congestion (black arrows) of central vein. Bar = 100µm. Magnification: 20x. H&E stain.

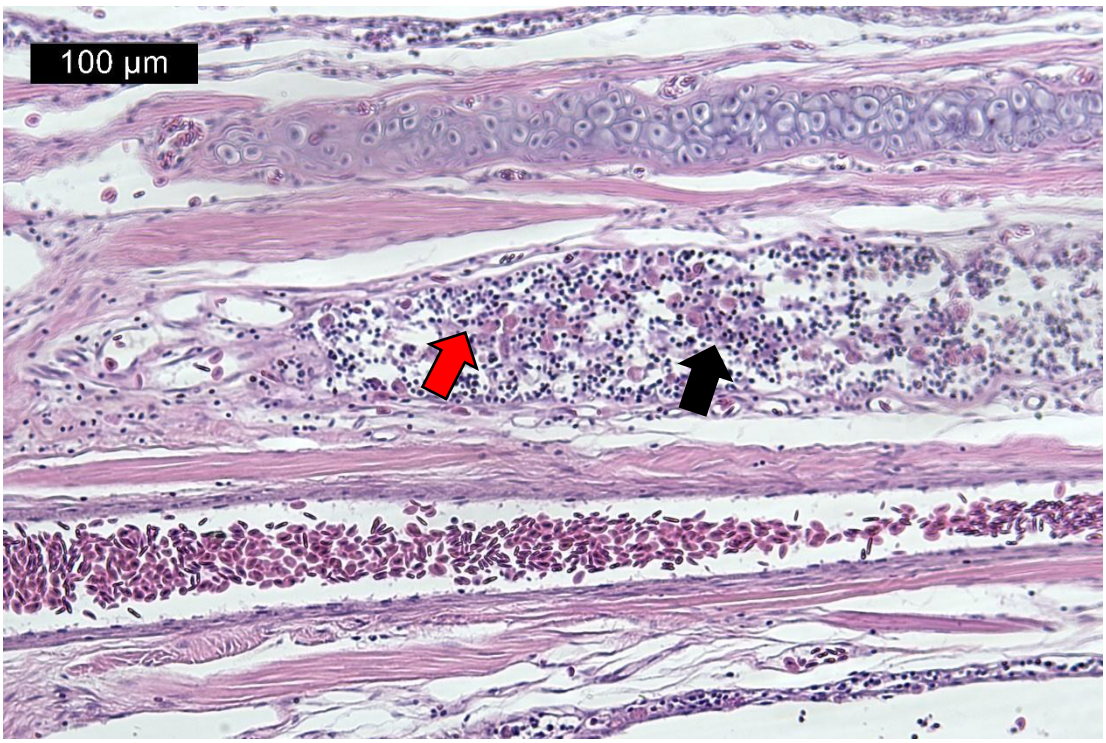

Figure 8. Eosinophilic (red arrow) and lymphocytic (black arrow) infiltrate. Bar = 100µm. Magnification: 20x. H&E stain.

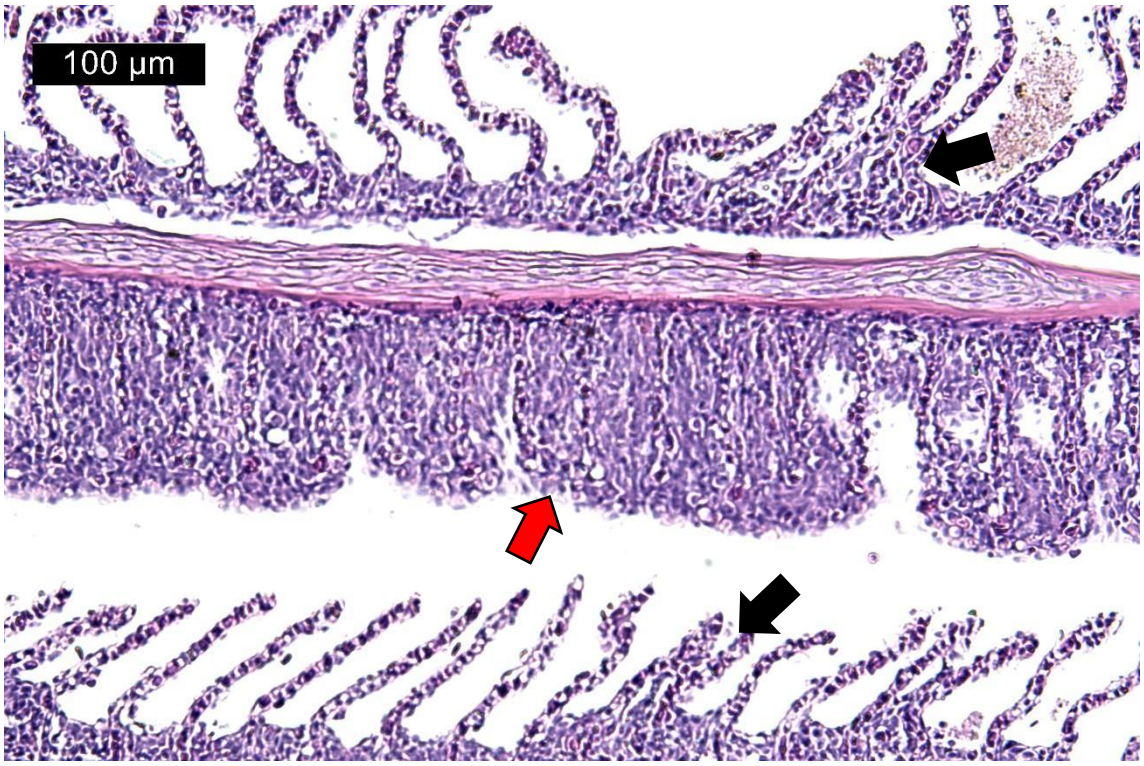

Figure 9. Hyperplasia (red arrow) and fusion (black arrow) of secondary lamellae. Bar = 100µm. Magnification: 20x. H&E stain.

Figure 10. Monogeneans (red arrow) attached to lamellae. Bar = 100µm. Magnification: 20x. H&E stain.