

Original article

NEW RECORD OF MONOGENEAN PARASITE MYMAROTHECIUM VIATORUM (MONOGENEA; DACTYLOGYRIDAE) IN RED-BELLIED PACU (PIARACTUS BRACHYPOMUS) IN INDONESIA

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Summary

Anshary, H., S. Sriwulan & A. Amriana, 2024. New record of monogenean parasite *Myma-rothecium viatorum* (Monogenea; Dactylogyridae) in red-bellied pacu (*Piaractus brachy-pomus*) in Indonesia. *Bulg. J. Vet. Med.*, **27**, No 1, 86–103.

Red-bellied pacu (*Piaractus brachypomus*) is a popular exotic fish cultured globally for ornamental and human consumption purposes. In Indonesia, this fish is mainly cultured in Java, including to supply seed to other regions for the ornamental fish trade or aquaculture. Although this exotic fish in widely cultured, information on parasitic infections is negligible. Red-bellied pacus were purchased from a fish farmer and examined for monogenean parasite infection. Monogenean parasites were identified based on morphological and molecular characteristics. Histopathological examinations were performed on gills from heavily infected fish. DNA was extracted from an individual monogenean; 18S rRNA PCR product was sequenced and a phylogenetic tree constructed. Morphological and molecular traits identified the monogenean as *Mymarothecium viatorum*, the first report of the neotropical *M. viatorum* in this region. The parasite had a prevalence of 79% and a mean intensity of 7.1. There was no significant correlation between the parasite abundance and the condition factor of the fish. *M. viatorum* was more abundant on smaller fish than on larger fish. Gill histopathology showed oedema, severe haemorrhage, and infiltration of inflammatory cells. The occurrence of these parasites could pose a serious threat to the development of red-bellied pacu culture.

Key words: 18S rRNA, ectoparasites, exotic fish, freshwater, monogenean

INTRODUCTION

The rapid growth of the aquaculture industry in the world and global trade of fish commodities have caused the expansion of exotic species aquaculture in many countries (Rodgers *et al.*, 2011). Indonesia is known to have introduced common carp (*Cyprinus carpio*) before 1900, and so far about 42 species of freshwater fish have been introduced and have entered inland waters (Umar & Sulaiman, 2013).

One exotic freshwater fish commonly cultivated in Indonesia, particularly in Java Island, is the red-bellied pacu Piaractus brachypomus. The red-bellied pacu P. brachypomus belongs to the Serrasalmidae family, as do two other commonly cultured species of pacu, P. mesopotamicus and Colossoma macropomum. The juvenile stages of P. brachypomus, P. mesopotamicus, C. macropomum, and the redbelly piranha (Pygocentrus nattereri) resemble each other; however, they have some differences in physical characteristics and food habits (Cagauan, 2007). Pacu is native to freshwater habitats in Bolivia, Brazil, Colombia, Peru, and Venezuela. In their countries of origin, pacus are widely cultivated for consumption purposes and are also very popular in the freshwater aquarium hobby as ornamental fish (Saint-Paul, 2017; CABI, 2021). This species is also commonly cultivated in Latin America, including Bolivia, Argentina, Peru, Brazil, Colombia, Costa Rica, Ecuador, Mexico, Panama, and Venezuela (Campos-Baca & Kohler, 2005).

The red-bellied pacu has been introduced to various countries, such as Canada, China, India, Thailand, Malaysia, Philippines, Papua New Guinea, and Indonesia, initially as an ornamental fish (CABI, 2021). The red-bellied pacu was introduced from Taiwan to Indonesia around 1985 by a private company as an ornamental fish commodity (CABI, 2021). Nowadays, pacus are widely cultivated in Indonesia, including in the major islands of Java, Sumatra, Kalimantan, Sulawesi, and Papua (Anonymous, 2018), and are known to have escaped or been released to the wild (Herder et al., 2012; Ndobe et al., 2019). The two most commonly cultivated species of pacu in Indonesia are the red-bellied pacu, P. brachypomus and the black pacu or tambaqui, C. macropomum.

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Red-bellied pacu production as a food fish has increased significantly in Indonesia, especially in recent years with an increase from 49.105 tons in 2016 to 72.800 tons in 2019, making Indonesia the top producer of pacu fish worldwide, with production volume exceeding that of countries such as China, Columbia, Vietnam, Peru, and Brazil (FAO, 2022). Pacus possess several characteristics considered ideal for aquaculture including an ability to adapt to high-density cultivation conditions, relatively simple hatchery breeding techniques, acceptance of artificial formulated feed, rapid growth, relatively high resistance to diseases, and being market opportunities for human consumption and as ornamental fish (Campos-Baca & Kohler, 2005). This fish can reach large size. with a maximum reported size of 88 cm and weight of 25 kg (Froese & Pauly, 2022). However, stressful conditions in aquaculture can promote the rapid development of some common fish parasites, especially those with rapid development that do not require an intermediate host, such as monogeneans (Klinger & Floyd, 2009; Paladini et al., 2017). In aquaculture facilities, monogenean parasites can cause mortality or reduced weight gain in infected fish, as reported for tambaqui (C. macropomum) infected with a high intensity of monogenean parasites (Mangas et al., 2020).

The introduction of fish to a new environment for aquaculture, whether for human consumption or for ornamental purposes, can also cause the spread of parasites from the country of origin to this new environment, and the consequences can be particularly severe if the parasite moves to new host species (Rodgers *et al.*, 2011). The spread of monogenean parasite infections across borders due to the trade of live fish has been reported; examples include the monogenean parasite Neoheterobothrium hirame infecting Japanese flounder in the waters of Japan (Ogawa, 1999; Anshary et al., 2001), Gyrodactylus salaris on salmon in Norway (Johnsen & Jensen, 1991) and Pseudodactylogyrus spp. on European eels in Europe (Buchmann, 2012). In each of these cases, the introduced parasites caused high mortality of the new host fish species or strain. The problem can become even more serious when infected introduced fish escape to the natural environment; both introduced fish and their parasites can cause ecosystem disturbances and the parasites can have a more severe impact on naïve wild hosts than on the introduced fish which are likely to be infected in the native range of these parasites (Rodgers et al., 2011; Sheath et al., 2015).

The spread of fish disease agents between countries is generally caused by the trade of live fish, and one consequence is that parasitic infections can have a more severe negative impact in their new environment if there is a transfer from the original host to other fish species (Sunarto et al., 2004; Rodgers et al., 2011). Cases of the parasites Lernaea cvprinacea and Myxobulus sp., both of which are thought to have been introduced from Japan, have caused great losses of freshwater fish in Indonesia (Arthur, 2005). Another case is the outbreak of koi herpes virus disease (KHVD) in Indonesia which has caused mass mortality in common carp (Cyprinus carpio) as well as the ornamental koi carp. KHVD entered Indonesia due to the import of koi carp from China through Hong Kong in 2001 and 2002 (Sunarto et al., 2004). The global ornamental fish trade tends to increase every year, involving more than 4000 species of freshwater fish, and is known to have caused the global spread of various

pathogens (Whittington & Chong, 2007). The rapid development of modern aquaculture has resulted in a high interdependence of commodities between countries with the movement of many aquatic animals from one country to another, including non-native species (Peeler *et al.*, 2011). In line with the development and intensification of aquaculture in various parts of the world, the trade in ornamental fish and food fish can be expected to continue to play a major role in the spread of parasites carried by aquatic animals.

Since the pacu was introduced to Indonesia, aquaculture of this fish species has grown rapidly and expanded beyond the main production centres in Java (Jakarta, West Java, East Java, and Central Java) to other regions in Indonesia. This increasing popularity is creating a growing demand for pacu seed for distribution to various regions across Indonesia, including South Sulawesi, both for the ornamental fish trade and for food fish aquaculture. Although Indonesia has become the largest producer of pacu, in particular the red-bellied pacu, information on parasite infections of this exotic fish is rarely reported. The present study aimed to identify the monogenean parasite previously unknown in this region, infecting the exotic red-bellied pacu, using morphological and molecular methods, as well as performing histopathological examinations of fish to analyse disease symptoms.

MATERIALS AND METHODS

Ethics statement

This study was conducted in accordance with the formal approval from the Health Research Ethics Committee of Hasanuddin University for the use of experimental animals (Protocol No. 15211095003).

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Fish samples and parasite examination

A total of 90 red-bellied pacus *Piaractus* brachypomus (Fig. 1) juveniles with a total length range of 3.5 to 6.6 cm $(4.91 \pm 0.6 \text{ SD})$, and weight range of 0.7 to 4.5 g $(1.6 \pm 0.6 \text{ SD})$ were purchased from a fish farm in Makassar which introduced red-bellied pacu from Java Island. The fish were introduced to Sulawesi for the ornamental fish trade (maintained in aquaria) and for food fish (grow-out to market size in concrete tanks). The fish had been air-freighted to Makassar in bags filled with aerated water treated with a few drops of acriflavine and packed in styrofoam boxes.



Fig. 1. Sample of juvenile cultured red-bellied pacu (*Piaractus brachypomus*) introduced to South Sulawesi from a hatchery on Java Island.

In the laboratory, the fish were anesthetised with a few drops of clove oil in 2 L of freshwater, the total length and weight of each fish were measured and then examined for the presence of parasites. The operculum was removed, and each gill was removed from the arch and placed on a petri dish. Parasites on the gills were observed under a stereomicroscope (Olympus SZ51) at $10 \times$ magnification and then a compound microscope (Olympus CX 31) at $400 \times$ magnification for morphological identification of parasites (Heil, 2009).

Monogenean parasites, were isolated, cleaned, placed on microscope slides with a few drops of freshwater, and covered with a coverslip. The parasites were then identified based on observation under a compound microscope (Olympus CX 31) at 400× magnification. To observe the hard parts (hamuli and cirrus) and measure morphological parameters of the monogenean parasites, the parasites were fixed in warm 70% ethanol and cleared in glycerine alcohol (1:1). Morphological identification of monogenean parasites was based on Boeger et al. (2002). Measurements were given as range (mean value) in µm.

Histopathological procedures

Gill arches from infected fish were removed from the fish and placed in a glass jar containing 10% neutral buffered formaldehyde as a fixative for 24 to 48 h. The fixative was then replaced with ethanol 70% and the specimens were stored ready for further histological procedures. The samples were dehydrated with an alcohol series (70-100%), impregnated with xylene-paraffin, and embedded in paraffin wax. Sections of $\approx 5 \ \mu m$ thickness were made, cleared with xylene, stained with haematoxylin and eosin (H & E), and mounted in Canada balsam. The sections were then viewed under a compound microscope, photographed, and histopathological changes were observed.

DNA extraction

Monogenean parasites were first isolated from the gills of infected fish, cleaned from debris, and observed under a stereomicroscope then under a compound microscope to observe the ophisthaptor and hamuli, and the cirrus (male copulatory organ) to determine the identity of the parasite. A single parasite was then preserved in a microcentrifuge tube containing 70% ethanol. For DNA extraction, the ethanol was removed from the tube using a micropipette and the sample was airdried until the ethanol remaining in the tube had completely evaporated. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol to obtain a volume of 100 μ L DNA template.

PCR amplification and sequencing

PCR reaction was performed to amplify the 18S rRNA region of the parasite DNA using two primer pairs: worm A-F: GCGAATGGCTCATTAAATCAGL and Worm B-R: CTTGTTACGACTTTTACT TCC (Littlewood & Olson, 2001); 930F: GCATGGAATAATGGAATAGG (Littlewood et al., 2008) and 1270R: CCG TCAATTCCTTTAAGT (Littlewood & Olson, 2001) at concentrations of 20 pmol. A PCR master mix was made using KOD One PCR Master Mix-Blue (Toyobo) with a final concentration of the KOD enzyme was $1\times$, and 0.3 µM of each primer, with nuclease-free water added to the PCR master mix to achieve the desired total volume of 20 µL for the PCR reaction. All components of the master mix were first mixed in a 1.5 mL microfuge, then pipetted into the PCR tube (0.2 mL) to which 1 µL of DNA template was then added. The PCR profile comprised an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 sec; 52 °C for 30 sec; 72 °C for 2 min), and a final extension at 72 °C for 10 min. PCR products with a clear single band under UV illumination were prepared and sent for sequencing to1st BASE Malaysia through PT Genetika Science Indonesia.

Analysis of the DNA sequence and phylogenetic tree construction

The parasite sample sequence data from PT Genetika Science Indonesia (chromatogram and nucleotide sequences) were analysed. Forward and reverse sequences were aligned and analysed using BioEdit sequence alignment editor version 7.2.5 (Hall, 1999) and CLUSTAL X Multiple Sequence Alignment version 2.1. The alignments were compared manually with the chromatogram when the aligned forward and reverse sequences differed to produce a consensus sequence. The 18S rRNA nucleotide sequence obtained was deposited in GenBank under accession no. OL413221.

The NCBI GenBank BLAST function was run to compare the consensus sequence against homologous DNA sequences deposited as GenBank accessions. A phylogenetic tree was constructed using 18S rRNA nucleotide sequences of parasites available in GenBank containing taxa from the family of Dactylogyridae, and Ancyrocephalidae, which are closely related with the monogenean parasites observed in the present study and Gyrodactylus carassi as an outgroup. The alignment was performed using BioEdit software and a Maximum-likelihood tree was constructed in MEGA X using the Tamura-Nei model (Kumar et al., 2018) with default parameters and 1000 bootstrap replications.

Data analysis

Prevalence and mean intensity of parasite infection were measured following Bush *et al.* (1997). Prevalence was defined as the percentage of fish infected with a certain species of parasite; mean intensity was defined as the average number of a certain species of parasite on the infected fish; and abundance was the average

number of a given species of parasite on the fish examined (infected or not infected). The condition factor was calculated using the formula in Htun-Han (1978):

$$K = (W \times 100)/L^3$$
,

where K= fish condition factor, W= fish weight (g), L= fish total length (mm).

The correlation between parasite abundance and fish length, weight and condition factor were evaluated using a nonparametric statistical test, the two-tailed Spearman's rank correlation coefficient, implemented in the SPSS version 22 statistical package.

RESULTS

Morphology and measurement of Mymarothecium viatorum

Based on morphological examination, the monogenean parasite *Mymarothecium viatorum* was identified on the fish examined. Three specimens were examined and the morphological characters measured (n=3, range and average in μ m) are presented in Table 1.

The distinguishing morphological characteristics of M. viatorum are the ophisthaptor shape and the male copulatory organ shape. Other characteristics include: cephalic lobe well developed; 4 eye spots with posterior pair larger than anterior pair; 14 marginal hooks; two pairs of anchors with ventral anchor slightly larger than dorsal anchor; ventral and dorsal bars with posteromedial projection, which is slightly longer on ventral bar than the medial projection on dorsal bar; and the accessory piece is articulated to the male copulatory organ subterminally by copulatory ligaments (Fig. 3). The monogeneans observed look very similar to the original descriptions of M. viatorum (Boeger et

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al., 2002), particularly the shape of the ophisthaptor and the male copulatory organ.

DNA amplification and sequencing

The combined primer pairs Worm A (F)-Worm B (R) and Worm A-1270R, and 930F-WormB (R) both appeared to produce PCR product bands of the correct respective product sizes (bp), however, only the PCR product from the primer pair 930F-WormB (R) was successfully sequenced. The length of the sequences obtained from the primer pairs was 970 bp. The BLASTn analysis of the consensus sequence obtained from the forward and reverse sequences generated had a 100% similarity with a Mymarothecium viatorum sequence deposited in GenBank (Accession No. KU941838.1; Muller et al., 2016).

Alignment and phylogenetic tree analysis

The maximum likelihood phylogenetic tree constructed resolved the sequence generated from this study into the same clade as *M. viatorum* collected from *P. mesopotamicus* in Brazil by Muller *et al.* (2016) (Fig. 2). The BLASTn and phylogenetic tree analyses confirm the morphological data, identifying the monogenean isolated from cultured *P. brachypomus* in South Sulawesi as *Mymarothecium viatorum*.

Prevalence and mean intensity of M. viatorum

The monogenean parasite found was identified as *M. viatorum* based on the morphological characteristics and molecular sequence of the parasite DNA on 18S rRNA region. The monogenean parasite was found on the gills. Prevalence of *M. viatorum* infection was 78.9% and the mean intensity was 7.1. The larval and adult

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	Piaractus brachypomus	Piaractus brachypomus (Poland)	Piaractus brachypomus (Brazil)	
	(South Sulawesi) Present study	Boeger <i>et al</i> . (2002)	Cohen & Kohn (2005)	
Body length Body width Haptor length	318–491 (384) 72–103 (83) 70–93(85)	382–518 (431) 69–89 (78)	320–550 (389) 80–180 (126)	
Haptor width Pharynx diameter	114–155 (128) 20–21 (20.7)	18–25 (22)	16–25 (20) 3 × 19–26 (22) 3	
Male copulatory organ length	42-43 (42.7)			
Ventral anchor				
total length length to notch superficial root length	30–33 (31.3) 24–26 (25) 14–15 (14.7)	32–40 (37)	32–45 (38)	
deep root length point length base width	4–5 (4.3) 15–19 (16.7) 22–23 (22.7)	19–24 (21)		
Dorsal anchor	()			
total length length to notch superficial root length	28–30 (29) 23–26 (25) 8–9 (8.7)	35–40 (36)	30-40 (34)	
deep root length point length base width	3–4 (3.3) 21–22 (21.3) 22–23 (22.7)			
Ventral bar				
length height width of postero- medial process	42–52 (47) 19–21 (20.3) 7–10 (8.7)	42–71	45–75 (62)	
Dorsal bar				
length height width of postero- medial process	44–47 (45) 16–18 (16.7) 5–8 (6.7)	42–64 (48)	40–62 (51)	
Hook length				
Pair 1	21–25 (22.7)	18–26 (21)	17–25 (21)	
Pair 2	15-20 (16.7)	18-26 (21)	19–24 (17)	
Pair 3 Pair 4	17–22 (19.7) 16–24 (21)	18-26 (21)	20-30 (25)	
Pair 5	16-24 (21) 17-23 (19.3)	18–26 (21) 17–19 (18)	25–31 (28) 12–17 (14)	
Pair 6	17–18 (17.7)	18–26 (21)	19–24 (22)	
Pair 7	18 (18)	18–26 (21)	21–24 (22)	

Table 1. Measurements of the present *Mymarothecium viatorum* and for the same species from different geographic areas (range with an average in parentheses, measurement in μ m).

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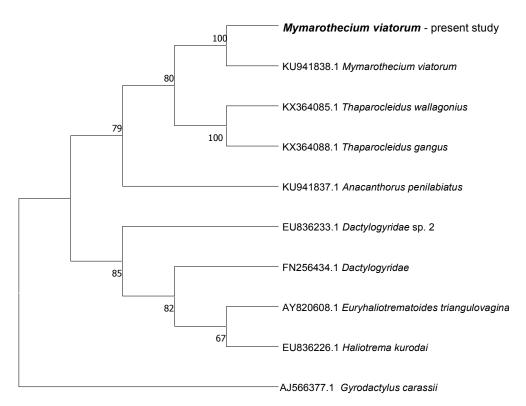


Fig. 2. Phylogenetic tree of Dactylogyridae including *M. viatorum* from the present study based on partial sequences of 18S rRNA. The Maximum Likelihood tree was constructed in MEGA X with *Gyrodactylus carassi* as the outgroup and 1000 bootstrap replications.

stages of the monogenean M. viatorum are presented in Fig. 3. In the larva stage of the monogenean, the ophisthaptor is not yet well developed, and the ventral and dorsal anchors are not fully developed, though the marginal hook and 2 pairs of eyespots have already appeared. As the monogenean parasite develops, the organs become perfectly formed. In juvenile and adult monogeneans, the hamuli and cirrus are well developed and thus can be used as distinguishing morphological characteristics by which this parasite species can be identified.

Gills histopathology

Histopathological damage on the gills of highly infected fish is shown in Fig. 4. Histological observations show that the most significant effect on the gills was oedema on the lamellae and severe haemorrhage on the gill filaments. Infiltration of inflammatory cells (leukocytes) was also observed, indicating that the nonspecific immune system of the red-bellied pacu examined had reacted to the parasitic infection (Fig. 4). During this study, other protozoa parasites such as *Trichodina* sp. were also observed on gills which might also have contributed to the severity on the gill's pathological changes.

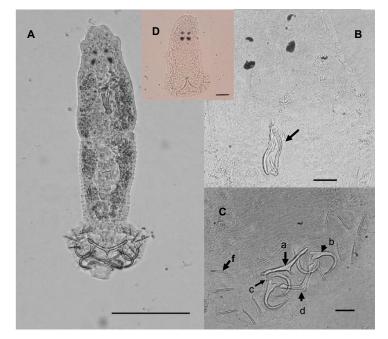


Fig. 3. A. Adult *M. viatorum* found on gills of *P. brachypomus* (whole body, bar=100 μm).
B. Male copulatory organ/Cirrus (bar=20 μm). C. Ophisthaptor of *M. viatorum* (a = ventral bar, b = dorsal anchor, c = ventral anchor, d = dorsal anchor, f = marginal hook, bar = 20 μm).
D. Larval stage of *M. viatorum* on gills of red-bellied pacu *P. brachypomus* (bar=20 μm).

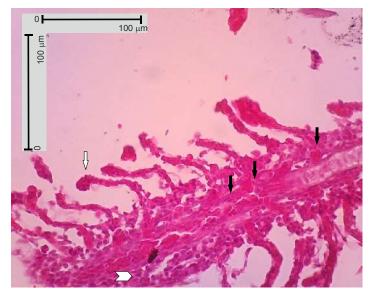


Fig. 4. Histopathological effect on gill tissue of farmed red-bellied pacu *Piaractus brachypomus* with multiple parasite infection: oedema on fish lamella (white arrow); haemorrhage on gill filaments (black arrow); and infiltration of inflammatory cells (leukocytes) (white arrowhead).

Correlation between M. viatorum abundance and fish length, weight and condition factor

The correlations between parasite abundance and fish condition factor and between parasite abundance and fish size (length and weight) were evaluated. There was no significant correlation between parasite abundance and fish condition factor (Spearman's rank correlation coefficient (rs) = 0.189; P=0.076). There was a significant correlation between parasite abundance and the fish size. *M. viatorum* was found more abundant in smaller fish than in bigger fish (Spearman's rank correlation coefficient (rs) = -0.225, P=0.034).

DISCUSSION

Transboundary trade of live fish is a major cause of disease spread, leading several countries to implement strict fish quarantine systems to prevent the spread of pathogens through the trade in live fish and other fishery products (Whittington & Chong, 2007; Rodgers et al., 2011). The OIE or World Organization for Animal Health is a global organisation dealing inter alia with the control of animal-borne pathogens and maintains a list of dangerous pathogens that are likely to be spread through fish (OIE, 2021). Many countries have used this list as a reference in determining the list of prohibited pathogens to be screened for when fish are imported into their respective countries (FAO/ NACA, 2000; Sunarto et al., 2004). As an effort to prevent the spread of disease through the trade-in aquatic animals, certain countries require a fish health certificate stating that fish to be imported are free from infection with certain pathogens (FAO/NACA, 2000). The only monogenean fish parasite on the OIE list is Gyrodactylus salaris. However, there are so many groups of parasites and other pathogens that may be carried by fish that it is not possible to examine all of the pathogens, and therefore the chance of spreading pathogens remains high, and is likely to increase with the growing trade in live fish (Whittington & Chong, 2007). In particular, the development of the aquaculture industry is likely to lead to new diseases emerging in areas where they were previously unknown. Here, we report for the first-time the occurrence of the neotropical monogenean M. viatorum from cultured red-bellied pacu (P. brachypomus) in Indonesia. The phylogenetic tree resolved the monogenean from this study in the same clade as M. viatorum collected from P. mesopotamicus in Brazil. Therefore, the molecular data reinforce the identification of the monogenean infecting P. brachypomus as M. viatorum based on morphological characteristics of the hamuli and cirrus.

The monogenean parasite M. viatorum was first described from P. brachypomus caught in the Odra River in Poland. This fish was originally thought to be a piranha released from an aquarium that had adapted to conditions in the river (Boeger et al., 2002). This parasite is distinguished from other species in the genus Mymarothecium by the presence of a posteromedial projection in both ventral bar and dorsal bar, which is slightly longer on the ventral bar and shorter on the dorsal bar. Superficial root of the dorsal anchor articulates to the ventral bar. In addition, the accessory piece is articulated subterminally to the male copulatory organ by short copulatory ligaments (Boeger et al., 2002). Monogenean parasites have been detected in many places around the world, including nine currently valid species of

Parasite	Host species from the Serrasalmidae family	Country	Reference
M. boegeri	Colossoma macropomum	Brazil	Cohen & Kohn (2005); Tavares-Diaz <i>et al.</i> (2001)
M. dactylotum	Pristobrycon sp. Serrasalmus rhombeus Serrasalmus sp.	Brazil	Kritsky et al.,(1996)
M. galeolum	Pristobrycon eigenmanni Pristobrycon sp. Pygocentrus nattereri Serrasalmus gouldingi	Brazil	Kritsky et al. (1996)
	S. rhombeus	Bolivia	Cordova & Pariselle (2007)
M. ianwhittingtoni	Piaractus mesopotamicus	Brazil	Leao et al. (2017)
M. iiapense	Colossoma macropomum	Peru	Morey et al. (2019)
M. perplanum	S. spilopleura	Brazil	Kritsky et al. (1996)
M. tantaliani	Colossoma macropomum	Peru	Cayulla- Quispe et al. (2021)
M. viatorum	Piaractus brachypomus	Poland	Boeger et al. (2002)
		Brazil	Negreiros & Tavares-Dias (2019); Cohen & Kohn (2005)
		Indonesi a	Present study
	P. mesopotamicus	Brazil	Cohen & Kohn (2005); Muller et al. (2016); Leao et al. (2017)
	hybrid (<i>P. mesopotamicus</i> × <i>P. brachypomus</i>)	Brazil	Franceschini et al. (2013)
	C. macropomum	Brazil	Godoi et al. (2012)
M. whittingtoni	Serrasalmus rhombeus S. spilopleura Serrasalmus sp.	Brazil	Kritsky et al. (1996)

Table 2. Mymarothecium spp. from different fish hosts and regions in the world

the genus *Mymarothecium* (Table 2). The species are *M. dactylotum*, *M. galeolum*, *M. perplanum*, *M. whittingtoni*, *M. viatorum*, *M. boegeri*, *M. ianwhittingtoni*, *M. iiapense*, and M. *tantaliani* (Worms, 2021). These parasites are mainly reported from serraselmid fish in the Neotropical regions, and the only reports from outside the country of origin of the fish host are from Poland in Europe (Boeger *et al.*, 2002) and from Indonesia in Southeast Asia (the present study). *M. viatorum* is the only species of the genus *Mymarothecium* that is known to parasitise non-piranha serrasalmids such as red-bellied pacu *P. brachypomus* and small-scaled pacu *P. mesopotamicus* (Boeger *et al.*, 2002; Cohen & Kohn, 2005; Franceschini *et al.*, 2013; Muller *et al.*, 2016; Leao *et al.*, 2017; Negreiros & Tavares-Dias, 2019).

Cultured fish are susceptible to infection by many parasites that do not require an intermediate host in their life cycle (Wootten, 2012). These types of parasites can easily proliferate in aquaculture environments since cultured fish generally experience some stressors such as poor water quality, poor quality feed, and small tanks with high stocking rate so that their

natural immune defences are weakened (Noga, 2010; Roberts, 2012). Gill-infesting monogeneans can develop rapidly due to their simple life cycle which does not involve an intermediate host (Wootten, 2012). In this study, the prevalence of *M. viatorum* infection was about 79%, which is quite high, and it was the only species of monogenean found on the host. All of the 503 monogenean parasites collected from red-bellied pacu gills had a similar morphology, resembling the M. viatorum specimen described from P. brachypomus in Poland. Though M. viatorum was first reported from P. brachypomus in Poland, it is believed that the parasites spread to Poland as a consequence of the introduction of the exotic fish to the country. The parasite has been reported from the type host P. brachypomus, P. mesopotamicus, and from a hybrid (P. mesopotamicus × Piaractus bra chypomus) in Brazil (Cohen & Kohn, 2005; Franceschini et al., 2013), indicating that the parasite is abundant in neotropical regions. A recent study in Brazil reported three monogenean species from the host P. brachypomus: Anacanthorus spathulatus. M. viatorum, and A. penilabiatus (Negreiros & Tavares-Dias, 2019). Monogenean larvae that are initially attached to the gill lamellae may use marginal hooks to attach to the gills. As the parasite develops, the dorsal and ventral anchors also develop and penetrate deeply into the connective tissue of the gill lamellae causing hyperplasia, gill fusion, and mucus hypersecretion in the gills (Muller et al., 2016). In the long-term, without proper treatment such infections can cause severe damage and even lead to fish mortality. In this study, red-bellied pacu infected with the monogenean M. viatorum and possibly by protozoans on the gills displayed symptoms including

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oedema and haemorrhage on the lamellae and gill filaments. A parasitology study of P. mesopotamicus and a hybrid (P. mesopotamicus x P. brachypomus) in Brazilian farms found various types of parasites. These parasites are M. viatorum, A. penilabiatus, Notozothecium janauachensis (Dactylogyridae, Monogenea); Trichodina spp., I. multifiliis, Chilodonella sp. (Protozoa); Myxobolus spp., Henneguya spp. (Myxozoa); Rondonia rondoni, Contracaecum sp. (Nematoda); and Dolops carvalhoi (Crustacea) (Franceschini et al., 2013). These studies suggest the vulnerability of the red-bellied pacu as a fish host to various parasite infections, including multiple infections.

Correlation between parasitic infection prevalence or intensity and fish size can be due to many factors, including the surface area of the fish body; the relative size of the gills and the volume of water passing through gills during breathing; the type of food eaten; the behaviour of the fish; the level of resistance to and accumulation of parasites (especially larvae); and predation leading to differential mortality of infected fish (Rolbiecki, 2006). In the case of *M*. *viatorum* infection. the abundance of this parasite was negatively correlated with host length. This might be related to the life cycle of the parasite which releases eggs that hatch into infective larvae called oncomiracidia which swim actively to infect new hosts or may be carried by water currents and attach to the gills when fish breathe; it is probably that smaller fish are more frequently in close proximity to the parasitic oncomiracidia, increasing the chances of the parasite larvae encountering its host (Glennon et al., 2006; Whittington & Kearn, 2011). Another possible explanation is that bigger fish may have developed a better immune system so that reinfection tends to be more difficult for the monogenean parasite (Cusack, 1986). A similar pattern with a negative correlation between the intensity of parasite and host length was also reported in Gyrodactylus colemanensis infection on fry of the salmon Salmo gairdneri (Cusack, 1986). The lack of significant correlation between parasite abundance and host fish condition factor, despite the histopathological damage observed on the gills of heavily infected fish, could be due to the small sample size of the present study (n=90). Other studies have also found no significant correlation between parasite abundance and the fish condition factor (Franceschini et al., 2013), and it has been suggested that a non-significant correlation is most likely to occur with a sample size of fewer than 100 fish (Paredes-Trujillo, 2021).

In Indonesia, the pacu has become an alien invasive species. For example, in December 2019, a red-bellied pacu weighing 13 kg was caught in North Sumatra. Adult pacus are omnivorous and eat small fish, crustaceans, molluscs and insects, so that they can be considered a threat to the native fish in the lake (Reenamole & D'cruz, 2015; Anupama et al., 2021). C. macropomum has also been found in Waduk Cirata, West Java (Putri & Tjahyo, 2011), and pacu has also been found in Sulawesian lakes and wetlands (Herder et al., 2012; Ndobe et al., 2019). The pacu has also been reported in the wild in India (Roshni et al., 2014) and the Philippines (Cagauan, 2007), and similar cases may also occur in other areas in Indonesia where the exotic pacu species is currently being cultured. In addition to adverse direct impacts on the environment and native aquatic communities, invasive pacu may also potentially spread parasites to

non-native fish species (Arthur, 2005; Rodgers et al., 2011; Sheath et al., 2015).

Monogenean parasites are known to have high host specificity, and so far, M. viatorum has only been reported from pacu of the family Serraselmidae. Therefore, the parasite M. viatorum detected in farmed pacu in Indonesia may have been introduced to this country through the importation of the red-bellied pacu which began around 1985. Since pacus were first introduced, several species of monogenean have been reported from this fish host including Dactylogyrus sp. (Yulianti et al., 2019), Oncocleidus sp., Tetraonchus sp., and Diplectanum sp. (Kusmawan, 2012). However, infection with monogeneans of the genus Mymarothecium has not been reported, raising the possibility that this parasite may have recently entered Indonesia. Alternatively, the absence of previous records of Mymarothecium sp. from this host could be due to mistaken identity, with parasites erroneously identified as other species. However, it is clear that there is a need for further study on the occurrence of this parasite at other sites, particularly in the areas where pacus are widely cultivated in Indonesia. Furthermore, this finding indicates the risk of the spread of parasites through the ornamental fish trade as well as the culture of food fish, and the need for increased vigilance to prevent and control fish pests and diseases.

CONCLUSIONS

Based on morphological and molecular evidence the monogenean parasite found on the gills of *P. brachypomus* was identified as *Mymarothecium viatorum*. This occurrence of *M. viatorum* is the first report of a neotropical monogenean in Indonesia. The monogenean infection level

was high in terms of both prevalence and mean intensity. These data indicate a need for proper parasite management to reduce the pathological impact of the parasites on this fish as well as to prevent spread. Farmers should apply prophylactic measures as well as good management practices to reduce the high infection rate of parasite infections. There is a need for further study to evaluate the occurrence and impact of the neotropical monogenean parasite *M. viatorum* in Indonesian aquaculture and natural environments including the possibility of host transference.

ACKNOWLEDGEMENTS

We would like to thank the Government of Indonesia through the Ministry of Education and Culture for providing financial support under grant numbers 7/E1/KP.PTNBH/2021 and 752/UN4.22/PT.02.00/2021.

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Paper received 23.12.2021; accepted for publication 26.03.2022

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