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The First Report on the Occurrence of Monogenean Parasite Infestation on Silver Pompano (*Trachinotus Blochii*) Reared in Floating Net Cage Systems in Indonesia

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Abstract

This investigation reports a monogenean outbreak infesting silver pompano (Trachinotus blochii) from floating net cages in Indonesia. The diseased fish showed clinical signs of anorexia, and emaciation and were gasping for air at the water's surface. This study was conducted to determine the first report on the occurrence of monogenean parasite infestation in silver pompano Trachinotus blochii reared in floating net cage systems on Lampung water, Indonesia. The parasites collected from the skin and gill were studied using optical binocular microscopy, and descriptive analysis. The results showed that the monogenean parasite found infesting silver pompano was Neobenedenia girellae and Pyragraphorus hollisae. N.girellae infested the eyes, head, and lateral surface body, while P.hollisae infested the secondary part of the gill lamellae. The monogenean parasites that were found on 5-15 cm silver pompano had a prevalence of 87.5% and an intensity of 15.40, while the fish with a size of 20-30 cm had a 71.43% prevalence and an intensity of 19.16. In the morphological features, the specimen's parasites were identical to those of monogenean groups. Thus, in this study, we confirmed that the parasites belonged to Nebonedenia girellae and Pyragraphorus hollisae. Therefore, this work provides the first detailed description of an ectoparasite that successfully infests pomfret, and molecular approaches such as DNA Barcoding are needed to provide in-depth information for future taxonomic and phylogenetic studies. Furthermore, this finding can serve as an early warning for relevant authorities and farmers that must be managed with aquaculture decision-makers.

Keywords: *Parasite, Monogenean Parasite, Infestation, Floating Net Cage System.*

1. Introduction

The emergence of diseases is one of the main problems in aquaculture. (Naylor et al., 2021). However, the disease is one of the biggest risk factors in the aquaculture industry, sometimes causing significant economic losses (Hanson et al., 2008; Bank et al., 2014; Ibrahim, 2019). The disease is defined as an abnormal process or condition of fish body parts that have characteristics that distinguish them from normal conditions (Kikhney & Moter, 2021). Fish disease is anything that can cause disturbance to fish, either directly or indirectly (Woo & Bruno, 2011); Natnan et al., 2021). This disorder can be caused by other organisms such as parasites (Choe, 2019). Understanding host recognition patterns and mechanisms among parasites has become a dynamic area of research in recent decades (Swann et al., 2015); Mahanta et al., 2018; Scheifler et al., 2022).

Among the many organisms that parasitize teleost fish, the class Monogenea, especially ectoparasites, are often found on the skin, head, tail, and gills of fish (Buchmann & Lindenstrøm, 2002; Hutson et al., 2018). Monogeneans are generally very host-specific compared to other groups of parasites such as copepods (Venmathi Maran et al., 2014; GE, 2019). With the exception of gyrodactylids, gyrodactylids as viviparous and monogeneans are oviparous (Frasca et al., 2018). Monogenean parasites have a direct life cycle i.e., they only parasitize one host during their life cycle (Reed et al., 2009; Šimková et al., 2022). Adult monogenean parasites lay their eggs in the water column which generally attach to various substrates such as floating net cages before hatching into ciliated larvae, called oncomiracidium (El-Naggar et al., 2021).

These free-swimming larvae actively seek suitable hosts and attach to their skin, some of which remain on the skin but most of the adults migrate from the skin to the fish's gills and the head of the host (van Beest & Born-Torrijos, 2020). Fish skin is considered the first physical barrier against external pathogens and stress (Leeches et al.,2007) The effects of ectoparasites on fish skin are specific for each host and parasite (Triki et al., 2016); Scheifler et al., 2019). For example, Rückert et al., (2008) reported that *Benedenia epinepheli* infests the body surface of *Lates calcarifer*. *Neobenedenia girellae* Hargis, 1955, is a monogenean marine ectoparasite that is very important for aquaculture species, which significantly affects its production (Militz et al., 2013).

Researchers focused on monogenean parasites that infest the skin and gills of the Silver Pompano in Hurun Bay, northeast of Lampung, and have a tropical climate with winds blowing from the Indonesian Ocean (Wirasatriya et al., 2021). In addition, the Marine Aquaculture Center of Lampung is one of the role models for the mariculture of silver pompano (*Tracinotus blochii*) in Indonesia. However, until now, information about ectoparasite worm infestations that infest silver pompano is still limited. Therefore, this study aimed to provide scientific information related to ectoparasites infesting silver pompano in the Marine aquaculture centre of Lampung to effectively control monogenean infestations. This is very important for silver pompano fish farmers so they do not experience significant losses due to monogenean parasite infestations.

2. Materials And Methods

A A total of 75 cultured silver pompano (*Tracinotus blochii*) were examined in two different sizes in the waters of Hurun Bay, Lampung (5°31'40.8"LS 105°14'56.1" E) (Figure 1). 40 fish with a length from 5-15 cm and 35 fish 20-30 cm. We examined the same floating net cages in each site during the study period. The cage sizes, and number of Silver pompano and Silver pompano densities were 3 m × 3 m × 3 m, 200 fishes (Figure 2). Parasites were easily observed in the mouth and on the eyes, gills, skin, dorsal fin, anal fin, pectoral fins, and tail of the fish. This parasite attached to its host using its sucker and fed on its host's blood causing the parasite to change color to black and brown. The number of parasites was counted and subsequently, leeches were kept in plastic bottles for further morphological identification. The total length (TL) and body weight (BW) of each silver pompano were determined (Figure 3). Morphological identification was performed by first staining the parasites with a few drops of Semichoen Acetic Carmine solution. After staining, morphological observation was conducted under a binocular microscope and drawn using a Lucida camera. Identification of species was based on the morphological and anatomical guidelines of (Ogawa et al., 1995) and (Bouguerche et al., 2021). Total prevalence and intensity were also calculated in this study using descriptive analysis.

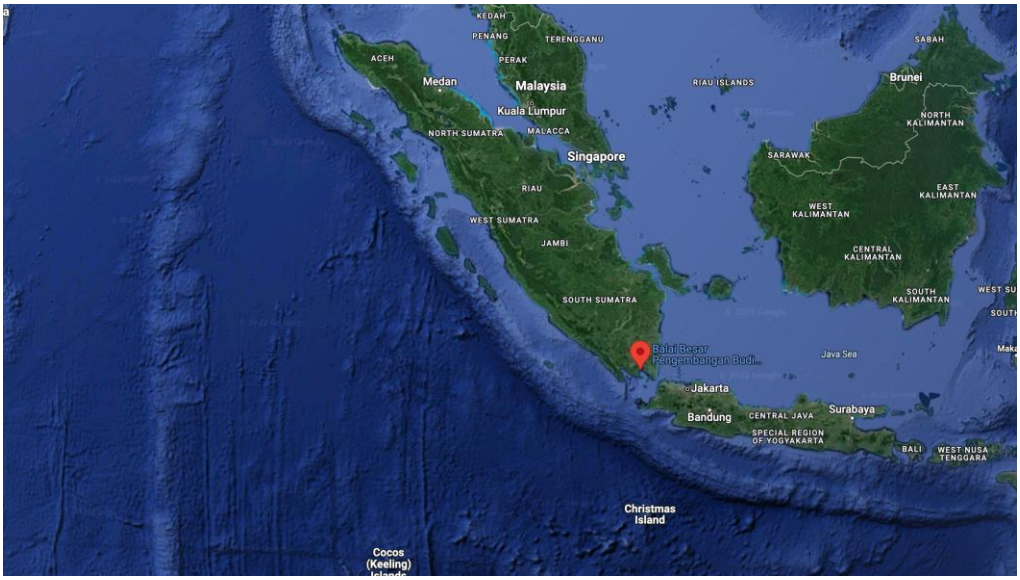

Figure 1. Mariculture center of Lampung, Indonesia.

Figure 2. *Floating Net Cage Systems Lampung Main Center for Marine Aquaculture, Indonesia. Source: personal documents*

Figure 3. *A sampling of Silver Pompano (Trachinotus blochii) for measurement Source: personal documents.*

3. Results and Discussion

A Two species of monogenean parasites were found infesting silver pompano (*Trachinotus blochii*) in Hurun Bay, Lampung, which were *N.girellae* and *P.hollisae*. *N.girellae* infested the eyes, head, and

lateral body surface, whereas *P.hollisae* infested the secondary gill lamellae part (Figures 4 to 8). *Neobenedenia girellae* description of Monogenean infested silver pompano. This species includes the Platyhelminthes phylum, Trematoda class, and monogenean sub-class. These species have a dorsoventral body shape like leaves, a pharynx shaped like a flower, and two pairs of eyespots of different size, the posterior eyespots is larger than the anterior part. The ectoparasite worm has a 3.5-5.3 mm length and 1.87-3.6 mm width. There is an anterior attachment organ with 0.27-0.42 mm length and 0.30-0.43 mm width.

The anterior structure is slightly concave or flat (Kinami et al., 2005). Sub-ventral body of *N. girellae* has a pair of smooth serrated testes and ovaries. Opisthaptor is found on the posterior body part with 0.69-1.2 mm length and 0.59-1.3 mm width. The Opisthaptor on the posterior body part is equipped with hooks, namely anterior hamuli, posterior hamuli, hooklet, and accessory sclerite. The opisthaptor is disk-shaped, with a pair of accessory sclerites, and two pairs of hamuli. The accessory sclerites have branched with blunt tip-sized 0.19-0.27 mm in length. The anterior hamuli have 0.23-0.36 mm length, while the posterior hamuli have 0.06-0.12 mm length.

Pyragraphorus hollisae description includes Trematoda class, Monogenea sub-class, Polyopisthocotylea order, and Pyragraphoridae family. It has an elongated body shape with a tapered tip or often called a torpedo (fusiform), a body length of 1-1.4 mm, and 0.2-0.4 mm in width, and has a buckle sucker, or an oral sucker in the sub-ventral body part. The posterior body has a clamp. It has an anterior clamp with a *fire-tong* type and a posterior *microcotylid* clamp. The clamp anterior has a size of 24x30 – 31x44 µm, while the posterior clamp has a size of 18x27 - 21x33 µm. It has 13 testes and is located in one-third of the posterior part of the body with a diameter of 67-100 µm. Monogenean parasite *N. girellae* was found infesting eyes, head, and lateral body surface, but not in fins on silver pompano (*Trachinotus blochii*) to get the nutrients required by a parasite (Bondad-Reantaso et al., 1995). Genus *Neobenedenia* is an ectoparasite worm that consumes mucus cells (Rubio-Godoy et al., 2011). Teleostei fish have fewer mucus cells on fins compared to the eyes, head, and body surface (Bondad-Reantaso et al., 1995). *N. girellae* is found in almost all marine fish. According to (Kinami et al., 2005), *Neobenedenia* sp. may damage skin and bleed with infestations on the eyes, damaging eye tissue, and leading to blindness.

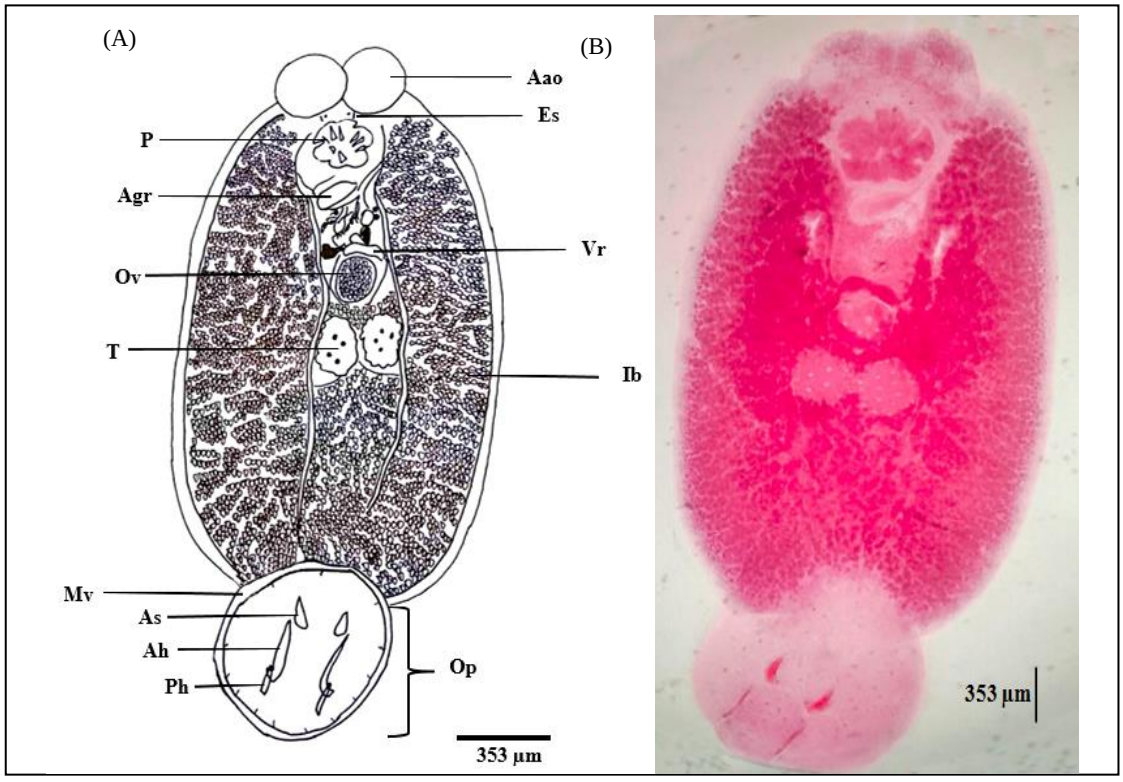

Figure 4. *Neobenedenia girellae* (40x magnification, bar scale 353 µm). (A) Semichoen-Acetic Carmine (B) Lucida camera.

Description : Aao = Anterior attachment organ, Es = Eye spots, P = Pharynx, Agr = Accessory gland reservoir, Vr = Vitelline reservoir, Ov = Ovary, T = Testes, Ib = Intestinal branch, Mv = Marginal valve, As = Accessory sclerite, Op = Opisthaptor, Ah = Anterior hamuli, Ph = Posterior hamuli. Source: personal documenta

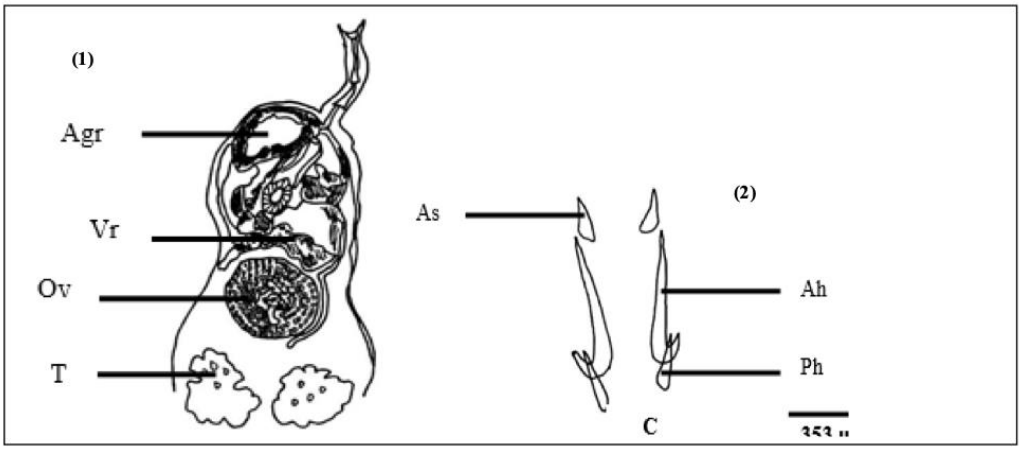

Figure 5. *Organs of Neobenedenia girellae (100x magnification, bar scale 353 µm) 1) Reproductive organs. 2) Hooks Description : Agr = Accessory gland reservoir, Vr = Vitelline reservoir, Ov = Ovary, T = Testes, As = Accessory sclerite, Ah = Anterior hamuli, Ph = Posterior hamuli*
Source: personal documents

Figure 6. The gill lamellae of silver pano (*Trachinotus blochii*) infested *Pyragraphorus hollisae*

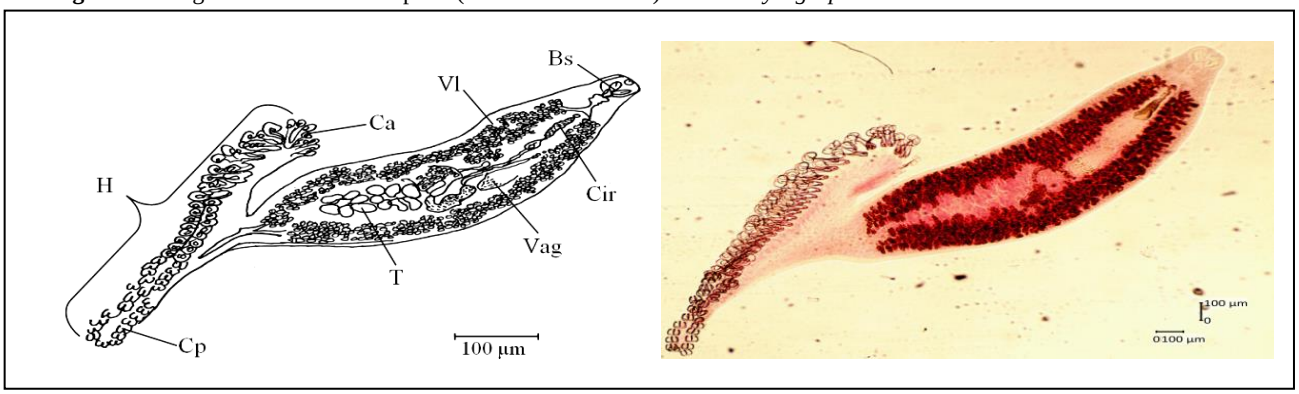

Figure 7. Pyragraphorus hollisae (40x magnification, bar scale 100 µm) with Lucida camera (A) and Semichoen-Acetic Carmine (B). Description : Bs= Buccale Sucker, Cir= Cirrus, Vl= Vitellaria lobulles, Vag= Vagina, T= Testes, H= Haptor, Ca= Anterior clamp, Cp= Posterior clamp.
Source: personal documents

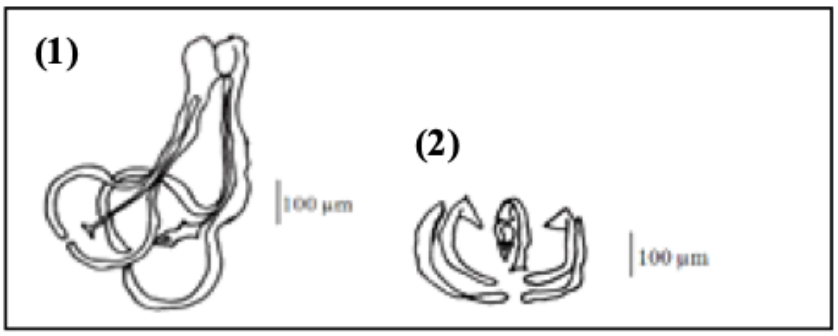

Figure 8. *Clamps* of *Pyragraphorus hollisae* (100x magnification, bar scale 100 µm) drawing with a binocular microscope equipped with a lucida camera. 1) Anterior clamp. 2) Posterior clamp
Source: personal documents

Pyragraphorus hollisae were first reported to infest *Lichia glauca* fish in Mediterranean waters (Euzet, 1970). It infested *Trachinotus rhadopus* in Mexican waters in 1977 and 1999 (Mendoza-Garfias et al., 2017), while in 2021, from carangid fishes in the Mediterranean (Bouguerche et al., 2021). *Pyragraphorus* has the characteristic that having two clamps arranged parallelly, and the body resembles an inverted letter "T" when viewed from the body side (Mendoza-Garfias et al., 2017).

This parasite infested secondary gill lamellae of silver pompano (*Trachinotus blochii*) at Hurun Bay, Lampung (Figure 6). The food sources in the form of tissue components were blood and epithelial cells (Morsy et al., 2011). The infested fishes suffer from anemia (Sitjà-Bobadilla & Alvarez-Pellitero, 2009).

The results revealed that there is a single ectoparasite worm infestation of *Pyragraphorus hollisae*, and mixed infestations with *Neobenedenia girellae* and *Pyragraphorus hollisae*. Silver Pompano (*Trachinotus blochii*) size of 5-15 cm, was infested single ectoparasite *Pyragraphorus hollisae* had 82.5% prevalence and 15,30 intensity, while size 20-30 cm had 51.43% prevalence and 19.61 intensity.

Table 1. Prevalence and intensity of monogenean parasites infest in silver pompano (*Trachinotus blochii*) in floating net cages Hurun Bay Lampung.

Size (cm)	Total of fish samples (Fish)	Species of ectoparasite worms	Total infested fish (Fish)	Total ectoparasite worms (Individual)	Prevalence (%)	Intensity (parasites/fish)
5-15	40	*Neobenedenia girellae*	0	0	0	0
		Pyragraphorus hollisae	33	505	82.5	15.30
		Neobenedenia girellae x Pyragraphorus hollisae	2	34	5	17
		Total	35	539	87.5	15.40*
20-30	35	*Neobenedenia girellae*	0	0	0	0
		Pyragraphorus hollisae	18	353	51.43	19.61
		Neobenedenia girellae x Pyragraphorus hollisae	7	126	20	18
		Total	25	479	71.43	19.16*

*Intensity (parasites/fish) = Total ectoparasites worms/Total infested fish

Silver pompano (*Trachinotus blochii*) with 5-15 cm length infested mixed *Neobenedenia girellae* and *Pyragraphorus hollisae* had 5% prevalence and 17 intensity, while the size of 20-30 cm had 20% prevalence and 18 intensity. The presence of mixed parasite infestations in fish depends on the fish's body's vulnerability against various parasitic infestations (Kotob et al., 2016). Moreover, the infestation of ectoparasite worm itself is affected by age, body size, the host immune system, and water conditions (Tai et al., 2022).

4. Conclusion

Monogenean ectoparasites that were found on silver pompano (*Trachinotus blochii*) in Hurun Bay, Lampung, were *Neobenedenia girellae* and *Pyragraphorus hollisae*. *Neobenedenia girellae* were found infesting the eyes, head, and lateral body surface, whereas *Pyragraphorus hollisae* were found infesting secondary gill lamellae. The total prevalence and intensity of monogenean parasites were 87.5%, and 15.40 found on 5-15 cm, and 71.43% and 19.16 found on 20-30 cm silver pompano. Therefore, we suggest further research to be able to include surveillance and monitoring programs for the development of this ectoparasite by predicting its transmission to susceptible aquatic animals, domestic and wild animals. DNA Barcoding application is also needed so that it can recognize the origin of the sequence of this parasite.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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