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Gyrodactylus (Monogenea: Gyrodactylidae) on marine ornamental fish *Amphiprion percula* from a marine aquaculture facility in Indonesia

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Abstract. *Sufardin, Sriwulan, Anshary H. 2022.* Gyrodactylus *(Monogenea: Gyrodactylidae) on marine ornamental fish* Amphiprion percula *from a marine aquaculture facility in Indonesia. Biodiversitas 23: 1023-1030.* As a popular marine ornamental fish (MOF), clownfishes are widely traded globally due to their beautiful appearance and relative ease of maintenance in the aquarium. There is a large amount of literature on *Amphiprion* biology and ecology, but globally still lacks data on their parasites, especially about monogenean gyrodactylidae. The first investigation of *Gyrodactylus* on a marine ornamental fish was conducted during March-August 2019. The study revealed the occurrence of *Gyrodactylus* infection in clownfish *Amphiprion percula* from Indonesia. A total of 50 fish samples were collected from a fish farm located in Takalar, South Sulawesi, Indonesia. Parasitological examination using the smear method was performed on the skin, fins, and gills. Parasites on each individual fish were counted directly under a stereomicroscope. Morphological measurements of the monogeneans were performed using ImageJ 1.46r software. Parasite infestation data were analyzed statistically using the Kruskal-Wallis test and linear regression. Based on morphology, the parasites infecting *A. percula* were identified as Genus *Gyrodactylus* and resembled described parasites on fish from the black sea. The prevalence of *Gyrodactylus* spp. was 100% on the caudal fin and was classified as a very severe infection with a total of 1908 individual parasites, overall. This study shows the *Gyrodactylus* intensity range is 8-75 parasites/fish and categorized as moderate and severe intensity, which are 6-55 parasite/fish for moderate and 55-100 parasites/fish for severe infection. Parasite infestation was higher on the caudal fin than other organs and was significantly positively correlated with fish size. This first report on the occurrence of *Gyrodactylus* on *Amphiprion percula* from Indonesia adds to the knowledge on gyrodactylid host fishes and infestation patterns.

Keywords: *Amphiprion*, flatworms, *Gyrodactylus*, marine aquaculture, MOF

INTRODUCTION

Clownfishes of the genus *Amphiprion* are popular as marine ornamental fish (MOF) and are widely traded globally (Klann et al. 2021). Indonesia is one of the main MOF trade supply countries (FAO 2009). Wild-caught clownfishes are still highly exported (Akmal et al. 2020), and several species can now be farmed (Olivotto and Geffroy 2017). Their beautiful appearance and relative ease of maintenance in the aquarium make *Amphiprion* attractive to MOF hobbyists and usually used as an attractive model fish (Wood 2001; Wabnitz 2003; Klann et al. 2021). While there is a large amount of literature on *Amphiprion* biology, ecology and husbandry, there appears to be a lack of data on their parasites.

The genus *Gyrodactylus* belongs to the Phylum Platyhelminthes, Family Gyrodactylidae and causes the disease gyrodactyliasis, which is a low-level parasitic worm infection and can occur in both freshwater and seawater fish (Cone et al. 2012). These flatworms or flukes are parasites commonly reported from wild and captive populations of both ornamental and food fishes around the world, including Indonesia (Tu et al. 2015; Putri et al. 2016). Although this parasite has been reported worldwide in many wild and captive fish species (Leis et al. 2021), usually in high prevalence and/or high mean intensity (Putri et al. 2016; Amrullah et al. 2019), to date there are no reports on *Gyrodactylus* infestation in *Amphiprion*.

Gyrodactylus is an important fish pathogen that causes significant economic loss in the aquaculture industry. Moreover, it can infect and may endanger wild fish populations (Amrullah et al. 2019; Ansyari et al. 2020). This fluke parasite can damage the tissue of the host fish due to its ability to penetrate the epidermis cells of the host. Furthermore, *Gyrodactylus* attachment organs and ulcers generated by enzymatic digestion result in the loss of the osmotic integrity of the host epidermis, which seems to be the major cause of host fish mortality (Tu et al. 2015). In addition, epidermal damage caused by this monogenean allows potential secondary infections.

Reed et al. (2012) states that *Gyrodactylus* spp. are amongst the most invasive fish parasites due to their viviparous mode of reproduction and exponential growth rate. They are ubiquitous on teleost fishes and host switching is considered the key mechanism of speciation with over 400 described *Gyrodactylus* species (Konczal et al. 2020). The ability of transmission of these parasites as adults has played a fundamental role in the diversification of *Gyrodactylus*. The continuous transmission ability throughout the lifetime of the parasite increases speciation

through host switching, as this ability allows the parasite to attach itself to less resistant individuals within a new host species or population (Boeger et al. 2014). Therefore, the objective of the present study was to describe the occurrence and analyze the infection rate of *Gyrodactylus* on *Amphiprion percula* as the first record and investigation in the marine ornamental fish from Indonesia.

MATERIALS AND METHODS

Sample collection and preparation

During March to May 2020, the cultured *A. percula* reared in several aquaria at the marine aquaculture facilities, Takalar, Indonesia, were infected by flukes, resulting in mortality of several fish after 1-2 weeks. *A. percula* used was the same age, had been reared about 7 weeks, and originated from cultured results using filtered-seawater. The fish sample was selected based on infected fish tanks, which contained dead fish findings. The study was conducted from June to August 2020. A total of 50 cultured *Amphiprion* fish from Takalar was transported alive by road (1.5 h) to the Parasite and Fish Disease Laboratory, Department of Fisheries Science at Hasanuddin University, Makassar, South Sulawesi, Indonesia for analysis. The fish were transported in aerated plastic containers filled with seawater aeration and kept alive until the parasitological examination was performed. Each specimen was humanely euthanized. The procedures used complied with the ethics protocols for animal research in vigor at Hasanuddin University.

Parasite examination and morphometric analysis

The fish specimens (n: 50, total length 2.45±0.85 cm) were investigated for the presence of monogenean parasites using the smear method as soon as possible after arriving from the sampling site. The fish were killed by being euthanized by submersed on crystal ice. Then, the fish skin, fins, gills, and mucus were examined. Each tissue sample was collected by scraping the relevant organ and transferred to a microscope slide. Saline solution was added with a pipette, the sample was flattened, and a coverslip placed over the sample. The slide was then observed under a compound microscope equipped with image capture (Olympus, Germany) and captured using ×40 and ×100 magnification objectives with oil immersion.

The *Gyrodactylus* parasites present were individually removed from the euthanized fish specimens and then rinsed in distilled water. Specimens were prepared as whole mounts and haptoral hard parts of *Gyrodactylus* were observed under a CX21FS1 Olympus microscope equipped with image capture (40X magnification with oil immersion lens). Measurements were made and analyzed using the calibrated ImageJ 1.46r software based on the captured images. The parasite attachment structure parameters measured followed Gracia-Vazques et al. (2015). These measurements were made on selected specimens and presented as mean with standard deviation and range.

Parasite infestation prevalence and intensity

The number of parasites was calculated directly for each fish by placing each organ on a microscope slide covered with a coverslip and examining the organ under a stereomicroscope. The infestation severity criteria refer to Williams and Bunkley (1996). The prevalence and mean intensity of *Gyrodactylus* infestation were determined by the length and weight classes as well as for the total sample using the formulae.

$$\text{Prevalence } (\%) = \frac{\text{Number of infected fish}}{\text{Number of fish examined}} x\ 100\ \%$$

$$\text{Mean Intensity (Parasites/fish)} = \frac{\text{Number of parasites}}{\text{Number of fish infected}}$$

Prevalence and intensity were compared between length and weight classes using the Kruskal-Wallis test. Linear regression function was used to determine the effect and correlation of *Gyrodactylus* infestation and fish size parameters (length and weight) statistically and implemented in SPSS version 25.

Ethics

This research has been approved by the Health Research Ethics Committee of the Faculty of Public Health, Hasanuddin University, Makassar, Indonesia with the attached number 3649/UN4.14.1/TP.02.02/2021.

RESULTS AND DISCUSSION

Identification of monogenean genus was based partly on morphological characters. *Gyrodactylus* have a fusiform body shape and marginal hook at the posterior end of the body. Observations showed monogenean *Gyrodactylus* with marginal and central hooks (Figure 2) in the opisthaptor for attachment to the surface of fish organs. Monogenean parasites were found in all fish examined (prevalence=100%), mainly on the fins (range of 8-75). In total, 1908 specimens of *Gyrodactylus* were obtained from the fins of *A. percula* (Table 3).

Additional examinations of *Gyrodactylus* specimens showed an embryo, pharynx, and seminal receptacle visible in the ventral view (Figure 2A). Furthermore, this parasite also has cephalic lobes in the anterior of the body (Figure 2B, white arrow). Overall body length (n: 10) was 246.65±64.14 µm and body width (n: 10) was 69.02±12.83 µm (Table 2). The infection of *Gyrodactylus* in the current study adds to the known Gyrodactylid fauna of ornamental fish species, as shown in Table 1.

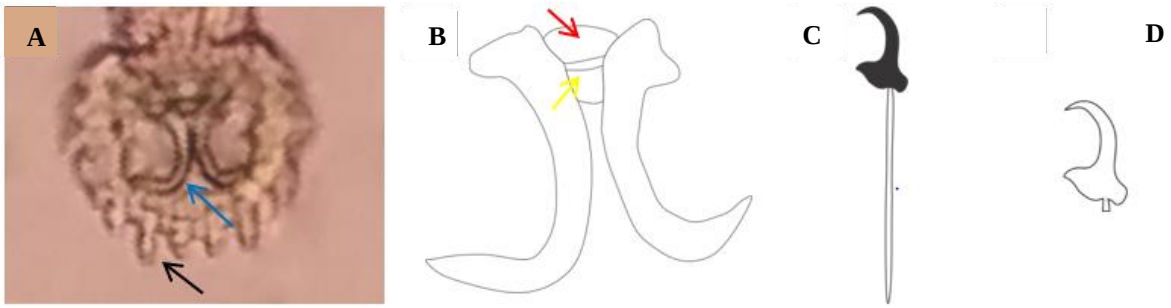

Figure 1. Opisthaptoral hard parts of *Gyrodactylus* on *Amphiprion percula* sp. A) Blue arrow: Opisthaptor with hamuli; black arrow: Marginal Hook. B) Yellow arrow: Drawing of hamuli complex with ventral bar; red arrow: Dorsal bar. C) Marginal hook and D) Marginal hook sickle

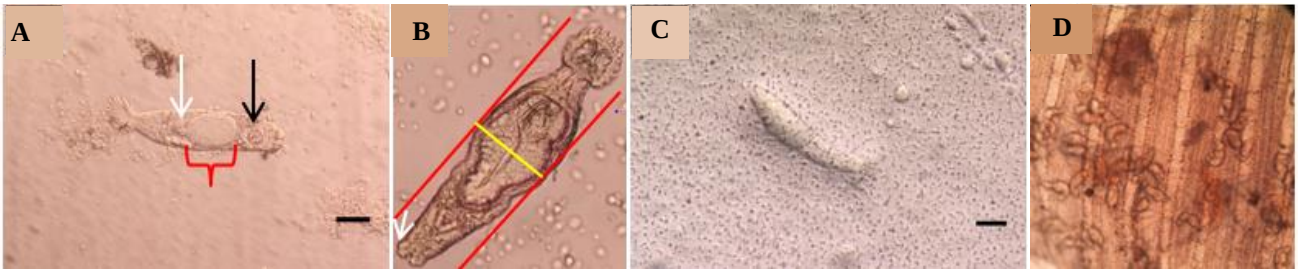

Figure 2. *Gyrodactylus* collected from *Amphiprion percula* from Takalar, Indonesia. A. The whole parasite in ventral view (red arrow: Embryo; black arrow: Pharynx; white arrow: seminal receptacle). B. (Red line: body length; yellow line: body width; white arrow: cephalic lobes). C. Dorsal view of the whole parasite. D. *Gyrodactylus* clustered on the fin of *A. percula*. Magnification: 40X (A, B and C); 10X (D). Scale bars: 50 µm

Table 1. Some records of *Gyrodactylus* infection on ornamental fishes

Species	Host	Location	References
G. kobayashii	*Carassius auratus*	China	Tu et al. (2015)
G. sp.	*Carrasius auratus*	Indonesia	Haryono et al. (2016)
G. sp.	*Carassius auratus*	Pakistan	Iqbal and Imtiaz (2016)
G. sp.	*Carrasius auratus*	Singapore	Trujillo-González et al. (2018)
G. gurleyi	*Carrasius auratus*	Malaysia	Trujillo-González et al. (2018)
G. gurleyi	*Carrasius auratus*	Thailand	Trujillo-González et al. (2018)
G. kobayashii	*Carrasius auratus*	Malaysia	Trujillo-González et al. (2018)
G. sp.	*Carrasius auratus*	Pakistan	Iqbal and Rehaman (2014)
G. mojarrae n. sp.	Cichlid fishes	Mexico	Mendoza-Palmero et al. (2019)
G. pseudobullatarudis	*Xiphophorus hellerii*	Mexico	García-Vásquez et al. (2015)
G. xtachuna	*Poecilia mexicana*	Mexico	García-Vásquez et al. (2015)
G. apazapanensis			
G. actzu			
G. apazapanensis			
G. pungitii			
G. tepari	*Goodea atripinnis*	Mexico	García-Vásquez et al. (2018a)
G. montealbani	*Profundulus oaxacae*	Mexico	García-Vásquez et al. (2018b)
G. zapoteco			
G. neotropicalis	*Astyanax aeneus*	Mexico	Salgado-Maldonaldo et al. (2019)

The records of *Gyrodactylus* from various countries and hosts in Table 1 are dominated by freshwater species, including the reports of *Gyrodactylus* infection in Indonesia. Reports of *Gyrodactylus* infection in marine fish are scarce, especially marine ornamental fish. This study appears to be the first report of marine ornamental fish infection with *Gyrodactylus* in Indonesia, and the first worldwide for *Amphiprion.*

Generally, *Gyrodactylus* has a small body shape, rounded elongated or oval and flat with one end larger (posterior), which is a place attached to the host. The posterior part is the most important organ that is an opisthaptor that has hooks and is equipped with a middle hook (anchor), and has no eyespots (Reed et al. 2012). These features can be seen in the photographs of specimens from this study (Figures 1 and 2). This monogenean

ectoparasite can penetrate the tissue of the host and the epithelium cells, often giving rise to secondary infection as the fish host becomes more readily exposed to pathogens such as fungi, bacteria, and viruses (Landsberg et al. 2013). Another explanation reveals this parasite can penetrate host tissues and epithelial cells and promote the potential for secondary infection which may play an important role in the pathogenicity of *Gyrodactylus* (Tu et al. 2015). The anterior part of *Gyrodactylus* has two lobe-shaped protrusions (Reed et al. 2012). Detection of many parasites, including the monogenean genus *Gyrodactylus*, is based on the identification of major taxonomic features such as the morphometric features of the body (Ye et al. 2017). Morphological measurements of *Gyrodactylus* specimens on *A. percula* are shown in Table 2. The morphometric comparison indicates the parasite in the current study resembles *Gyrodatylus* in fish from the black sea, namely *Gyrodactylus ginestrae* and *Gyrodactylus alviga* (Kvach et al. 2019), based on morphometric features such as the average body size, hamulus, bar, and hook.

In fact, identification of monogenean-gyrodactylid is able molecularly by Polymerase Chain Reaction (PCR) method to confirm the species, firmly. Internal transcribed spacer (ITS) is one of the universal primers that is recommended to be used for the identification and genetic characterization of this worm down to the DNA level. Hansen et al. 2016 state the currently applied standards for the description of species of *Gyrodactylus* involves DNA sequencing of the ribosomal internal transcribed spacer (ITS) region combined with morphometric analyses of the haptoral hard parts of the parasite. The author considered using this approach for further species analysis of this specimen.

Table 3 shows the prevalence and intensity varied between body parts or organs. The investigation only detected *Gyrodactylus* on the fins of *A. percula* with no infection on gills. The caudal fin had the highest prevalence and intensity, while no parasites were found on the pectoral fins. *Gyrodactylus* is an ectoparasite that always lives on the fins of teleost fish or body parts that are directly exposed to the external environment. This parasitic infection causes morbidity and mortality in fish, especially larvae and juveniles in aquaculture systems (Forwood et al. 2016).

The mean intensity and prevalence of *Gyrodactylus* infestation were highest on the caudal fin (Table 3) in both length classes and all weight classes, although the prevalence was also high on dorsal, ventral and anal fins. Fish in the size class 2.5-3.6 cm had similar prevalence (87-90%) on the other fins infested, with higher intensity on ventral fins than on anal fins and the lowest intensity on dorsal fins. In the smaller size class 1.0-2.0 cm, the intensity was similar between dorsal, anal and ventral fins but prevalence varied, ranging from 50% on the ventral fins to 90% on the dorsal fins.

With the exception of the pectoral fins, all fins were infected by *Gyrodactylus.* The prevalence and intensity between fins were not significantly different (P: 0.08 and 0.09 > 0.05) respectively. However, the Kruskal-Wallis test showed significant differences between length classes and the total number of parasites on each organ (P: 0.001 < 0.05). Furthermore, regression analysis of the *Gyrodactylus* infestation against fish size showed a positive correlation (Figure 3 and 4). The correlation was used to determine the relationship between parasite infestation and fish size while regression analysis was used to predict the extent of the effect between variables.

Table 2. Morphological measurements of *Gyrodactylus* on *Amphiprion percula* from Indonesia (this study), and *Gyrodactylus alviga* according to Kvach et al. (2019) (mean± standard deviation followed by the range in parentheses; all measurements in micrometres). *only one specimen examined

Measurement	***Gyrodactylus* on *A. percula* (current study)** ***N*= 10**	***Gyrodactylus ginestrae* Vach et al. 2019** ***N*= 16**	***Gyrodactylus alviga* Vach et al. 2019** ***N*= 15**
TBL	246.65±64.14 (173.51-339.91)	385 (259-483)	400 (363-550)
TBW	69.02±12.83 (56.35-97.42)	69 (51-88)	98 (73-117)
HTL	23.37*	41.8 (39.5-44.0)	65 (63-68)
HA	17.35*	35.1 (31.3-37.9)	30 (30-33)
HPL	11.46*	19.5 (17.9-21.8)	21 (19-22)
HSL	17.76*	28.0 (25.6-30.3)	45 (43-48)
HRL	7.35*	17.0 (15.4-18.8)	21 (19-22)
VBL	3.79*	4.8 (4.1-5.9)	5 (5-6)
VBW	4.32*	19.8 (17.5-21.9)	32 (30-35)
DBL	3.54*	1.3 (1.0-1.6)	4 (4-5)
DBW	1.48*	17.3 (15.08-18.7)	22 (19-24)
MHTL	18.36±6.41 (9.82-28.90)	28.8 (26.6-30.2)	34 (33-34)
MHSL	13.42±6.02 (6.16-23.16)	22.6 (20.7-23.8)	27 (27-28)
MHSiL	4.94±1.41 (2.37-7.23)	5.7 (5.3-6.0)	7
MHToeL	1.84±0.21 (1.63-2.06)	1.6 (1.4-1.8)	-
MHSiDW	3.22±0.40 (2.82-3.62)	2.6 (2.3-2.9)	5
MHA	6.89±0.43 (6.46-7.33)	5.2 (4.8-5.6)	-

Note: TBL: Total body length; TBW: Total body width; HTL: Hamulus total length; HA: Hamulus aperture; HPL: Hamulus point length; HSL: Hamulus shaft length; HRL: Hamulus root length; VBL: Ventral bar length; VBW: Ventral bar width; DBL: Dorsal bar length; DBW: Dorsal bar width; MHTL: Marginal hook total length; MHSL: Marginal hook shaft length; MHSiL: Marginal hook sickle length; MHToeL: Marginal hook toe length; MHSiDW: Marginal hook sickle distal width; MHA: Marginal hook aperture

Table 3. *Gyrodactylus* infestation on *Amphiprion percula* by fish length class and infected organ

Length interval (cm)	Organ	Number of fish examined	∑parasites (Individuals)	Prevalence (%)	Mean Intensity (Parasites/fish) ± SD
2.5-3.6	Caudal fin	30	993	100.00	33.10±12.20
	Dorsal fin	30	101	86.67	3.88±2.10
	Anal fins	30	191	86.67	7.35±5.80
	Ventral fins	30	261	90.00	9.67±5.40
	Pectoral fins	30	0	0	0
	Gills	30	0	0	0
1.0-2.0	Caudal fin	20	197	100.00	9.85±3.13
	Dorsal fin	20	61	90.00	3.39±1.57
	Anal fins	20	66	80.00	4.13±2.41
	Ventral fins	20	38	50.00	3.80±2.45
	Pectoral fins	20	0	0	0
	Gills	20	0	0	0

Table 4. *Gyrodactylus* infestation on *Amphiprion percula* by fish weight class

Weight intervals (g)	Number of fish examined	Number of infected fish	∑parasites (Individuals)	Prevalence (%)	Mean Intensity (Parasites/fish) ± SD
0.18-0.38	23	23	542	100	23.57±0.0556
0.39-0.59	17	17	889	100	52.29±0.0512
0.60-0.80	4	4	167	100	41.75±0.0510
0.81-1.00	6	6	310	100	51.67±0.0557

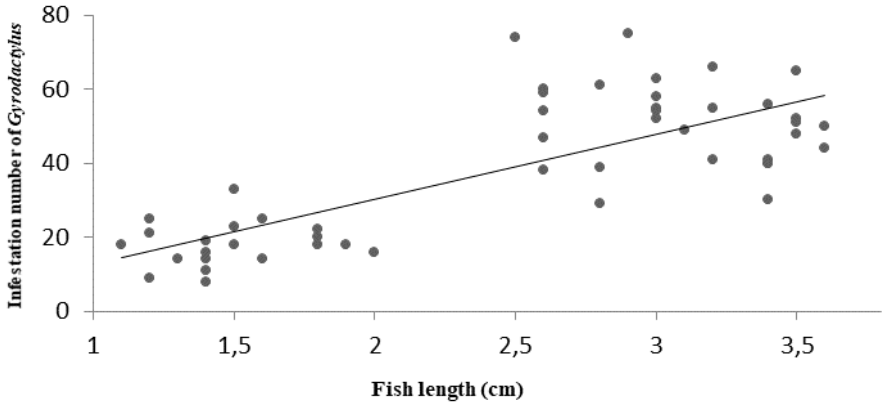

Figure 3. Linear regression model with correlation value of *Gyrodactylus* infestation intensity against *Amphiprion percula* length. Pearson's coefficient: 0.774 (positive correlation) and P value 0.001

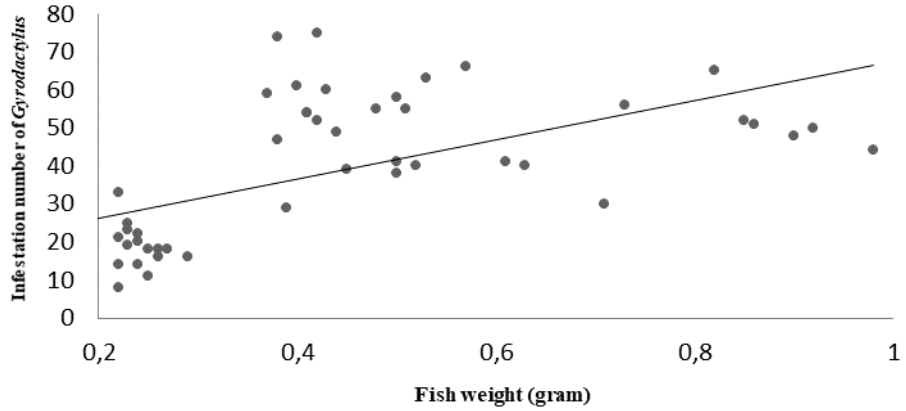

Figure 4. Linear regression model with correlation value of *Gyrodactylus* infestation intensity against *Amphiprion percula* weight. Pearson's coefficient: 0.602 (positive correlation) and P value 0.031

According to Williams and Bunkley (1996), the parasitic infection on *A. percula* was classified as very severe with a prevalence of 100% (Table 3). However, based on intensity, the parasitic infection in *A. percula* can be classified as moderate and severe infection (Table 4). Statistical tests also showed a significant difference in parasitic intensity based on fish size, while the pattern of infestation or distribution of the parasites between organs, also varied between the two length classes. Severe intensity infection causes serious damage to the surface of body tissues and also potential for secondary infection of other pathogens, which causes considerable economic losses in cultured fish (Tu et al. 2015). Some reports even revealed there is no satisfactory way and effective compound to control *Gyrodactylus* infection (de Moraes 2015).

Variations in fish ectoparasite infection rates can be caused by several factors; these include the size and origin of the fish, the biological defenses of fish, and environmental and seasonal changes (Mizuno et al. 2016). The higher total number, prevalence, and intensity of *Gyrodactylus* in larger fish (Tables 3 and 4; Figures 3 and 4) indicate that fish size may play a role in parasitic infection processes. Intuitively, a larger fish offers a larger surface area for parasites to attach and grow. As the same age and life cycle of *Amphiprion* during reared, the current study was proved that variance of host size increases susceptibility against parasitism. According to Ozturk (2005), monogenean parasites such as *Gyrodactylus* generally attack juvenile fish during grow-out, especially seeds of around 1.5-2 months old, and concluded that the age and size of fish affect the occurrence of monogenean parasites, while Haenen et al. (1994) described significant increases in parasitic infection with fish growth, also influenced by the size and age of the fish. A study on *Anabas testudineus* in Aceh, Indonesia (Maulana et al. 2017) also found higher severity of ectoparasite infection in larger fish.

In addition to fish size, other factors may also influence the presence and severity of *Gyrodactylus* infestation. Haenen et al. (2014) state that factors thought to influence the prevalence and intensity of parasites in fish include the size, host, type of food consumed by fish and the movement patterns or capability of parasites. *Gyrodactylus* can move from one fish to another through direct contact between live or dead fish (Cone et al. 2012). The infection of a new host can occur through direct transmission when an infected host makes skin-to-skin contact with another fish and through fin touching (Schelkle 2012). Basic transmission profiles of *Gyrodactylus* include through contact with live hosts and dead hosts, from detached parasites drifting in the water column, and from parasites attached to a solid substrate. Although the plasticity of monogeneans can enable many transmission routes to a new host, the likelihood of transmission from dead hosts can be higher than from living hosts due to the high risk of transmission in running water and the increased opportunities of contacting a new host feeding on the carcass (Cone et al. 2012), and the infection of dead fish may be favorable for gyrodactylid survival (Schelkle 2012). Meanwhile, the distribution and evolutionary patterns of geographically separated *Gyrodactylus* parasites could be explained by parasites living on marine fish which enter brackish and freshwater environments such as estuaries and rivers, where the parasites can then transfer onto freshwater hosts (Garcia-Vasques et al. 2018).

Subsequently, *Gyrodactylus* shows an efficient infection mechanism because it is capable of directional swimming by stretching its body to reach its host. The infective larval stage of the parasite in the same host also causes high infection by penetrating through the host skin (Rohde 2017). The epidermal layer and host body surface can be a barrier to the success of gyrodactylids infection (Grano-Maldonaldo et al. 2018). Schelkle (2012) described parasite transmission as mainly occurring through direct contact between current hosts and potential live or dead new fish hosts; however, they also noted that detached gyrodactylids have a window of opportunity for re-attachment, which lasts for over 20 hours. The parasites may be able to detect water movements associated with an approaching host using mechano-chemical receptors and *Gyrodactylus* can swim to increase the chances of transmission to a new host (Grano-Maldonaldo 2014). The transmission of *Gyrodactylus* does not require an intermediate host, as this genus has a viviparous mode of reproduction, giving birth to full-sized living individuals and thereby allowing rapid population growth on the host. The description by Grano-Maldonaldo et al. (2018a) indicates that *Gyrodactylus* has developed an exceptionally efficient infection mechanism and is capable of directional swimming by flexing the body in order to reach a nearby potential host. Besides, Klinger and Floyd (2013) state *Gyrodactylus* is a viviparous parasite that reproduces by giving birth and embryo development occurs in the reproductive tract (ovary). This reproductive ability allows *Gyrodactylus* to multiply rapidly in an aquatic environment. Furthermore, Grano-Maldonado (2014) described the mode of reproduction of this parasite as viviparous, which is capable of giving birth to full-sized individuals and supports rapid population growth in its host.

This article is the first preliminary report on the occurrence of *Gyrodactylus* on *A. percula* in Indonesia, adding to the knowledge on gyrodactylids and their hosts. In this study, all *A. percula* examined were infected by *Gyrodactylus* of a type resembling *Gyrodactylus* reported in fish from the black sea. Parasite infection intensity differed significantly between fish length and weight classes, as did the organs most severely infested. In order to determine with more certainly the taxonomic identity of the *Gyrodactylus* found in this study, molecular (DNA) analysis is recommended.

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