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STUDY OF MORPHOLOGY, PREVALENCE AND MEAN INTENSITY OF MONOGENEANS INFECTING POND REARED *Pangasianodon hypophthalmus* (STRIPED CATFISH) IN BESUT, TERENGGANU

SARAH SALEHAH AND FAIZAH SHAROM*

School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

Corresponding author: faizah@umt.edu.my

Abstract: This study was conducted to determine the morphology, prevalence and mean intensity of monogeneans on gill filaments of striped catfish *Pangasianodon hypophthalmus* (Pangasiidae). *P. hypophthalmus* are important economic fish in Southeast Asia region and predominantly produced in Asian countries such as Malaysia, Bangladesh, Indonesia, Vietnam, Laos, China and Cambodia through aquaculture. Thirty fish were sampled from an earthen pond located in Kuala Besut, Terengganu, in the period from January to February, 2018. The monogenean parasites were removed from the gill filaments and counted. For the morphological study, the extracted monogeneans were mounted on a slide by using drop of ammonium picrate-glycerin (APG) and then were observed under the Compound Advanced Research Microscope. The drawing was done with the aid of lucida camera attached to compound microscope. The monogenean parasite was identified as *Thaparocleidus* sp based on the characteristics of the morphology and morphometrics of the parasite, which did not differ significantly from the previous descriptions of the same species discovered in other geographic locations. The prevalence and mean intensity levels were 100% and 106.07 parasites per fish, respectively. The monogenean parasites discovered throughout this examination were recorded and the data obtained was summarized.

Keywords: Parasite, *Thaparocleidus* sp, monogenea, *P. hypophthalmus*, freshwater fish, pond culture

Introduction

According to a study by Hudson *et al.,* in (2006), a variety of parasites can be an indication of ecosystem health. The influence of parasites on fish health can be seen through physical, mechanical and reproductive harm they cause. When it comes to fish health evaluation, the role of parasites in their host(s) is frequently overlooked (Iwanowicz, 2011). This is true where there is minimal or information about the species on baseline data of parasite ecology and diversity (Thompson *et al*., 2010).

Among them, one group that is well-known but has not yet been fully explored is the monogenean parasites. During recent years, plenty of attention has been paid to obtaining more details on the ecology of monogeneans. These parasitic ectoparasites are not only known for their captivating life cycle, morphology and their perplexing relationships with metazoan groups, but also for being pathogens to fish, especially in fisheries and aquaculture farms. An awareness in the dynamics of monogenean infection may aid in reducing the loss of fish due to the parasitic diseases (Lim *et al.,* 2016). Whether in the wild or in aquaculture farms, parasitic monogeneans can pose real danger to the fish (Kearn, 2011). So far, monogeneans are the most hostile parasites infecting farmed fish. They are known to infect the gills of their hosts. The infected gills will lead to several problems, for instance difficulty in breathing and problem in respiratory function. The overloading infection may lead to lethargy and result in huge loss of fish (Snieszko & Axelord, 1980).

Especially in the aquaculture industry, these parasitic worms are famous for invading the culture species, such as the striped catfish *Pangasianodon hypophthalmus*. *P. hypophthalmus* are freshwater catfish species that are widely cultured and are one of the productive culture species especially in the Southeast Asia (FAO, 2010). According to Pariselle *et al.*, (2001a; 2004; 2006), one of the monogenean group *Thaparocleidus* spp has been reported to belong to the family of freshwater catfish. Given the importance of maintaining the fish health, especially by minimizing monogenean parasitic infections, it is necessary to

conduct research to determine the level of parasitic infections of *Thaparocleidus* sp which invade the *P. hypophthalmus.*

This study investigates monogenean infection affecting freshwater fish in an earthen pond in Kg Aloi Peroi, Kuala Besut, Terengganu (*Pangasianodon hypophthalmus*), mainly because of their economic importance as food fish to the local people and to the ecosystem itself. On top of that, this study focuses on providing more detailed information about monogenean infection since there is a lack of reports on this parasite affecting pond-cultured *P. hypophthalmus* in Malaysia. The present study will provide more knowledge on monogenean infection on *P. hypophthalmus*. Additionally, the study of the morphology, prevalence and mean intensity of the parasitic monogeneans can provide some new information for future use by the researchers and communities.

Materials and Method

Sample Collection

A total 30 samples of striped catfish (*Pangasianodon hypophthalmus*) were obtained from the earthen pond by fishing. The live catch of *P. hypophthalmus* were kept in aerated aquarium and brought to the laboratory for further investigation. The fish samples were killed by using '*pithing*' method (Faizah, 2012). This method involved cutting the central nervous system below the head of the fish using a sharp scalpel or pointed needle to damage the nerve cord. The samples were measured to find the length and weight by using a ruler and electronic balance scale respectively. The obtained data were then recorded on the sampling data sheet. Then the fish were dissected using dissecting set. By using a sharp pair of scissors, the fish operculum was cut away to expose the gills. The gill arches were removed carefully and placed into a petri dish. The presence of the monogeneans on each gill was counted with the aid of dissecting microscope that was connected to Dino-Eyepiece USB Camera.

Fixing, Staining and Mounting

The extracted monogeneans were fixed in 70% ethanol. The fixed monogeneans were removed and placed on top of microscope slide. With the aids of dropper and dissecting needles, the mucus was removed from the monogeneans and all the excess water was carefully detached from the slide. Few drops of ammonium picrate-glycerin (APG) then were added on to the monogeneans and left soaked for a few second. Excess of APG was absorbed by placing a piece of tissue at the edge of the slide. A coverslip was gently placed on top of the monogeneans to avoid formation of air bubbles. Permanent attachment of the coverslip was done by adding nail polish at each edge of the coverslip. The mounted specimens were observed under compound microscope.

For identification, the morphology of different parts was noted and images were captured by using Advanced Research Compound Microscope (Nikon Eclipse 80i). The measurement of the specimens was obtained using ImageJ software and the drawing was done with the aid of lucida camera. The measurement of the monogeneans and their components are in micrometers (Faizah, 2012).

Calculation of Prevalence and Mean Intensity of Parasites

The formula for the calculation of prevalence and mean intensity of parasites followed Margolis *et al*., (1982) and Bush *et al*., (1997).

$$\text{Prevalence} = \frac{\text{Number of infected host}}{\text{Number of examined host}} \; x \; 100$$

$$\text{Mean Intensity} = \frac{\text{Number of parasites found}}{\text{Number of infected host}}$$

Parasite Morphometry

The mounted monogenean samples were measured and identified based on the variables provided by Pariselle *et al.,* (2006). Morphometric measurements in micrometer (µm) obtained from four mounted monogeneans were recorded. Identification of the parasitic worms follows the appearance and fine structure of sclerotized parts of the monogenean haptor (attachment) (Ergens, 1981; Pariselle *et al.,* 2001a; 2004; 2006).

Results and Discussion

Taxanomic summary

Kingdom	:	Animalia
Phylum	:	Platyhelminthes
Subphylum	:	Monophysthocotylea
Order	:	Dactylogyridea
Family	:	Dactylogyridae
Genus	:	*Thaparocleidus* sp
Host type	:	*P. hypophthalmus*
Site of infections	:	Gill

Locality : Besut (earthen pond culture)

Referring to Pariselle *et al.*, (2001; 2002; 2004), the parasite species characterized belong to Genus *Thaparocleidus* sp., which are included under Family Dactylogyridae. The monogenean species found have morphological resemblance with *Thaparocleidus* sp that infect *P. pangasius* in Bangadesh, India and *P.hypopthalmus* in Chendrawasih, Surabaya, Indonesia. (Pariselle *et al.*, 2001; Anshary & Talunga, 2013).

Table 1: Measurements of *Thapalocleidus* sp

Structures/ Components	Variable	Range		Mean (*µm*)
		Min (*µm*)	Max (*µm*)	
Body	Length, x	521	557.3	545.2
	Width, w	81.2	89.4	84
Copulatory organ	Length, x	64	79	72
Dorsal gripus (DG)	Total length, a	63.2	65.6	64.4
	Shaft length, b	49.2	53.8	52
	Root length, c	21	23.2	22
Dorsal transverse bar (DB)	Length, x	21.5	26	23.5
	Width, w	3.1	3.4	3.4
Cuneus (C)	Length, x	30.7	35.3	33
	C width, w	9	10.4	9.2
Ventral gripus (VG)	Total length, d	20.3	21.6	20.9
	Shaft length, e	15.5	17.3	16.4
	Root length, f	12.6	15	13.8
Ventral transverse bar (VB)	Length, x	34.7	39	36.8
	Width, w	6.2	7	6.7

Thaparocleidus sp (Figure 1) are characterized by the presence of two pairs of eyespots and pharynx at the anterior section (Figure 2A) of the parasitic worms. At the posterior region (Figure 2B), they have two pairs of anchors—dorsal anchor and ventral anchor. At dorsal anchor (Figure 3A), the dorsal gripus is equipped with cuneus and connected by dorsal transverse bar. The ventral anchor (Figure 3B) has a pair of ventral gripus which is connected by ventral transverse bar.

The monogenean body is elongated with blunt anterior and posterior regions measuring length x = 545.2 µm (range 521 – 557.3 µm) and w = 84 µm (range 81.2 – 89.4 µm) wide. The anterior parts of the monogenean have two pairs of eyespots and pharynx. The anterior pair of eyespots is smaller than the posterior pair. The pharynx is spherical in shape.

The copulatory organ of these monogeneans is thin coil-like shape measuring x = 72 µm (range 64 – 79 µm). For the haptor part, there are two pairs of anchor which is the dorsal and ventral anchor. For dorsal anchor region, the measurement for dorsal gripus a, b and c are 64.4 um (range 63.2 – 65.6 µm), 52 µm (range 49.2 – 53.8 µm) and 22 um (range 21 – 23.2 µm), respectively. The pair of dorsal gripus is connected by dorsal transverse bar measuring x = 23.5 µm (range 21.5 – 26 µm) and w = 3.4 µm (range 3.1 – 3.4 µm). At the anterior end of dorsal gripus is cuneus measuring x = 33 µm (range 30.7 – 35.3 µm) and w = 9.2 um (range 9 – 10.4 µm). For ventral anchor region, the measurements for dorsal gripus d, e and f are 20.9 µm (range 20.3 – 21.6 µm), 16.4 µm (range 15.5 – 17.3 µm) and 13.8 µm (range 12.6 – 15 µm), respectively. The ventral transverse bar is wide v-shape like with measurement of x and w – 34.7 µm and 6.2 µm, respectively.

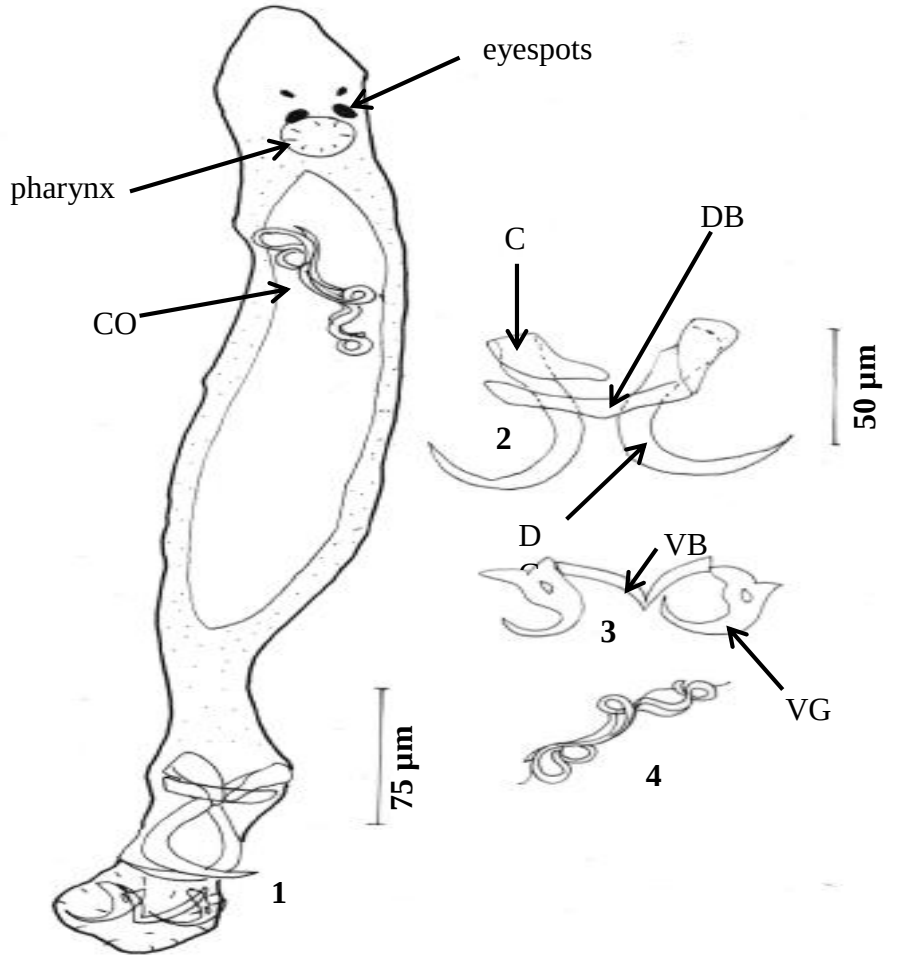

Figure 1: Full drawing of *Thaparocleidus* sp whith bar scale; 1 – full body drawing from anterior to posterior parts, 2 – dorsal anchor, 3 – ventral anchor and 4 – copulatory organ.

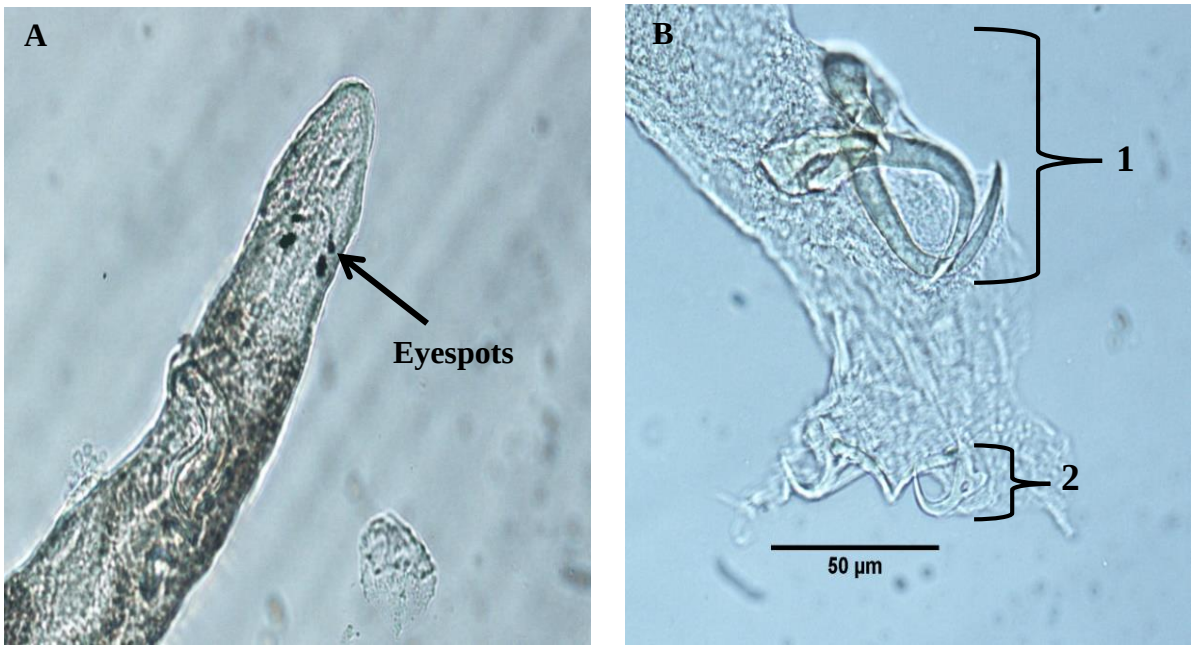

Figure 2: A) Anterior region of *Thaparocleidus* sp. B) Posterior region (Haptor): 1 – Dorsal anchor, 2 – Ventral anchor.

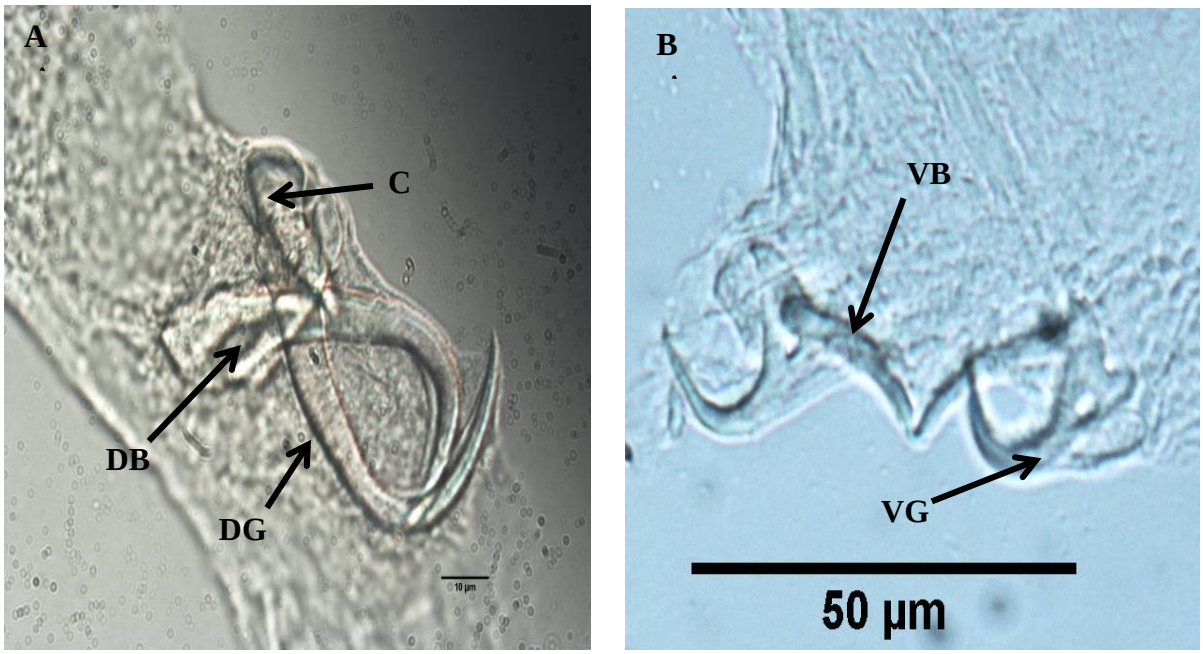

Figure 3: A) Dorsal anchor of the monogenean which consists of cuneus(C), Dorsal gripus (DG) and Dorsal transverse bar (DB). B) The ventral anchor of the monogenean consists of Ventral gripus (VB) and Ventral transverse bar (VB).

Prevalence and Mean Intensity

For the prevalence of monogeneans, this study found that all the examined host *P. hypophthalmus*, were infected with parasitic monogeneans, indicating that all the cultured fish inside the pond were infected with *Thaparocleidus* sp. The prevalence and mean intensity calculation were done based on the data recorded from the samples and are presented in Table 2.

Table 2: The prevalence and mean intensity of *Thaparocleidus* sp in *P. hypophthalmus.*

Parasite	No. of examined host	No. of infected host	No. of parasites found	Prevalence (%)	Mean intensity
Monogenea *Thaparocleidus* sp	30	30	3182	100	106.07

Whether in the wild or in aquaculture industry, fish is also prone to diseases like parasitism. The extent of parasitism in fish varies from slight to harsh based upon the pathogenicity of the causal agent and the intensity of worms affecting the fish. As a result, this is a great threat to the fish industry, causing a decline in fish production, and fish infected by some parasites could be unsuitable for human consumption. Parasites can be divided into two sub-classes—ectoparasite and endoparasite. According to Shariff (1984), diseases caused by the ectoparasite spread quickly and may cause more than 50 percent mortality in short period.

Through research and knowledge gained on taxonomy, morphology and parasitic life cycle, precautions can be taken to prevent diseases from occurring. Based on the results obtained throughout this study, the parasitic monogeneans that infected the striped catfish, *P. hypophthalmus* were identified as *Thaparocleidus* sp. *Thaparocleidus* sp. are parasitic monogeneans that live as ectoparasites on gills and have a direct life cycle. They attach themselves to their host's bodies using anchors that can be found in haptors (Reed *et al.,* 2009). According to Gusev (1985), the identification of monogenean species is mainly based on the morphology of the haptor (attachment organ) and reproductive organ. The morphology of the haptor is considered useful for parasite determination at the genus level, while the reproductive organ is more suitable for identification at the species level, probably because of its higher rate of change (Pouyaud *et al.,* 2006; Wu *et al.,* 2008b).

According to Pariselle *et al.* (2001b), several *Thaparocleidus* species have been reported from Family Pangasiidae in Southeast Asia, namely *P. pangasius, P. kinabatanganensis, P. nieuwenhuisii* and *P. rheophilus.* They also reported that parasitic monogenean *Thaparocleidus sabanensis* was found on the gills of *Pangasius kinabatanganensis* (Roberts & Vidthyanon, 1991) in Kinabatangan River, Sabah, Malaysia. The *Thaparocleidus sabanensis* described has some similarity with the species found recently on the gills of *P. hypophthalmus*. The similarity can be seen on morphological characteristic for both monogenean species. However, the size of *Thaparocleidus* sp found in recent studies was much smaller compared to previous studies. This may be due to the different locations. In addition, a study showed that *Thaparocleidus* sp infecting the *P. hypophthalmus* in Surabaya, Indonesia (Anshary & Kalanga, 2013) also displayed great resemblance with the *Thaparocleidus* sp found recently in terms of the morphological parts for anterior region (granulated eyespots) and posterior region (haptor).

Thaparocleidus are generally gill monogeneans found in siluriform fishes (Cohen & Anna, 2008; Simkova *et al.,* 2013). These parasitic worms are extremely host specific, which means mostly all are restricted only to one host species (Kabata, 1970; Rohde, 1979; Poulin, 1992). Pariselle *et al.,* 2006 stated that around 43 species of *Thaparocleidus* have been reported from Pangasiidae fish. Thus, it can be confirmed that the parasitic monogeneans found on the gills of *P. hypophthalmus* in the earthen pond-culture in Kuala Besut are indeed from the Genus *Thaparocleidus*.

The study area is an earthen pond (21 feet length x 16 feet width with 17000 starting seeds of *P. hypophthalmus*), which commonly receives rainwater and the amount of water is always variable (hinged on the rainfall). Since production, there is no treatment and water exchanging done by the farmer. The *P. hypophthalmus* vary in size and are fed fish pellet and trash fish once a day.

The prevalent rate of parasitic infections of 100% indicates that the entire fish population inside the pond-culture were attacked by the parasitic *Thaparocleidus* sp. The parasite's life cycle is closely related to the high level of parasite prevalence since these parasites have a direct *life cycle* with no intermediary host involved in their life cycle. The eggs hatch into free-swimming larvae and are carried to a new host by water currents and their own ciliated movement (Rohde, 2005). Its prevalence and intensity increase significantly when the parasite life cycle is completed (Ahmed *et al.,* 2007).

Parasites utilize at least two different habitats, one of which is switching between two individual hosts (the ecosystem), and the other being in or on the same host (immediate environment) (Thomas *et al.,* 2002). High density together with poor nutrition of fish also hasten the spread of parasites, in addition to stress factors due to poor water quality, allowing rapidly developing parasites. The area around the pond was also surrounded with overgrown vegetation (Mdegela *et al.,* 2011).

Conclusion

The parasitic monogeneans that invaded the gills of *P. hypophthalmus* in an earthen pond in Kg. Aloi Peroi, Kuala Besut, Terengganu belong to the genus *Thaparocleidus*. The morphology parts of the parasites found in the recent study have several similarities with previous studies of monogeneans on the same fish species (Lim *et al.,* 1961*;* Pariselle *et al.,* 2001a*;* Anshary & Kalanga, 2013). Prevalence and mean intensity of *Thaparocleidus* sp. are relatively high, 100% and 106.07, respectively, indicating low frequency of freshwater catfish maintenance. This can endanger the condition of the fish body. The high density triggers the development of parasites in the cultured system and thus may cause economic loss. This study looks at the morphology; perhaps future work can focus on molecular

studies of the monogeneans and whether the species is the same as the others in previous studies.

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