

Monogenean parasites on cantang grouper (*Epinephelus fuscoguttatus-lanceolatus*) wilture in floating net cage for mariculture center Lombok, West Nusa Tenggara, Indonesia

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Abstract. Cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) is a hybrid grouper species. This hybrid species has several advantages, one of the advantages is it has better resistance to disease. However, it does not guarantee that grouper fish can not be infected by parasite that leads to disease. This study aims to determine the prevalence and intensity of monogenean parasites that infest cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) from floating net-cages farm, at Mariculture Center Lombok, West Nusa Tenggara Indonesia. The study conducted on grouper with size ranging from 15 to 27.5 cm, body weight ranging from 55 to 233 g. The result showed that monogeneans was found in cantang groupers were *Neobenedenia girellae* (3.8-5.4 x 1.5-2.5 mm) that infested the fins, eyes, and fish skin and *Pseudorhabdosynochus seabassi* (0.52-0.68 x 0.12-0.25 mm) only found on gills lamella. The highest prevalence of monogeneans was found in mixed infestation of *P. seabassi* and *N. girellae* 50% in most of categories. The highest intensity of monogeneans was 10.09 individuals/fish in single infestation of *Pseudorhabdosynochus seabassi*.

1. Introduction

Grouper is one of the most important fishery commodities that have a very high export demand in Asia. The number of grouper fish production in Indonesia during 2015 was up to 8,972 tons and had increased to 15,089 tons in the third quarter of 2016 [1]. The total number of exported alive groupers during 2016 reached 4,006,852 tons [2].

Grouper have many species, one of them is Cantang Grouper (*Epinephelus fuscoguttatus-lanceolatus*). Cantang Grouper is a hybrid product of female tiger grouper *E. fuscoguttatus* and male kertang grouper *E. lanceolatus*. In addition, cantang groupers has another advantages, such as better resistance to disease, better tolerance to environmental changes, and can easily live in the narrow space [3].



Cantang groupers which is cultivated using floating net-cages system can not easily avoid ectoparasite infestation. Parasites in fish rarely cause death, but it can lead to several body injuries and open the access of other pathogens such as viruses and bacteria. Monogenean parasites infestation on cultivated activities can cause various losses such as economic, health and ecological losses [4]. Monogenean parasites commonly infest cantang groupers such as *Benedenia epinepheli*, *Diplectanum grouperi*, *Dactylogyrus*, *Neobenedeniagirellae*, *Haliotrema epinepheli*, and *Pseudorhabdosynochus seabasi* [5]. Based on this background, it is necessary to conduct a research on the prevalence and intensity of Monogenean parasites in the cantang groupers which is located at the Mariculture Center Lombok, West Nusa Tenggara.

2. Methodology

Life specimens of *E. fuscoguttatus-lanceolatus* were taken from floating net-cages farm at Mariculture Center Lombok, West Nusa Tenggara. These samples were taken from March to April 2017. The number of samples were taken from 10% of the population (400 fishes). Therefore, samples used in this study were 40 groupers. The fish samples were examined for ectoparasites under dissecting microscope. All the obtained parasites were fixed in 5% of alcohol glycerin, stained with acid carmine, dehydrated in a graded ethanol series, cleaned and finally mounted by Hung's I and Hung's II in accordance to Kuhlmann [6] modified methods. Illustrations were made with the aid of a camera lucida. The Monogeneans infestation prevalence was calculated according to Bush [7] and intensity by Dogiel [8]

3. Results and Discussion

In the present study of the occurrence of monogenean parasite on cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) were monitored. Some of them are *Neobenedeniagirellae* which infested skin, fins, eyes and *Pseudorhabdosynochus seabasi* which attached to the gill's lamella. Occasional haemorrhagic spots were noticed in the head region near to the eyes of the fish. Heavily infested fish showed excessive mucus secretions on the body surface. *Neobenedeniagirellae* and *Pseudorhabdosynochus seabasi*, the two monogeneans species can be seen in figure 1.



Figure 1. (A) Adult worm of *Neobenedeniagirellae*, scale bar = 100 μm (B) *Pseudorhabdosynochus seabasi*, scale bar = 50 μm.

3.1. Description of *Neobenedenia girellae*

Based on 14 adult worm samples that had been examined, the parasite found in the fish was *Neobenedenia girellae* (Monogenean trematode, Monopisthocotylea order, Capsalidae family). *Neobenedenia girellae* had elliptical body and dorsoventral-shaped with total body length 3.8-5.4 mm and width was 1.5-2.5 mm. Anterior attachment organ (sucker) 0.28-0.37 mm long and 0.29-0.34 mm, another sucker on posterior was ophisthaptor 0.84-1.1 mm long and 0.78-1.42 mm wide. Haptor had oval shaped and aseptate with a pair of sclerites accessories, anterior hamuli and posterior hamuli in linier series. Seven pairs of hooklets arranged radially in haptor were not clear. Sclerites accessory hadscoop-shaped and distally-branched with blunt tips 0.15-0.21 mm long. Anterior hamuli 0.20-0.31 mm long, strong, sharp, posterior terminus recurved directed points. Posterior hamuli 0.08-0.11 mm long, straight and fine recurved point.

Two pairs of eyes between Anterior attachment organ and pharynx. Pharynx glandular had six lobes ranging from 0.36-0.42 mm length and 0.41-0.58 mm width. Intesninal crura dendritic medially and laterally, extending posteriorly to the end of proper body, terminating blindly. Two testes was in mid body, lobed, post ovarian, different size 0.28-0.46 mm long. Vas deferens ascending sinistral to ovary and vitelline reservoir. Male copulatory organ situated in posterior to pharynx, consisting of a penis, a large pear shaped muscular sac containing seminal vesicle and an accessory gland reservoir. Penis slightly tapering anteriorly. Ovary 0.18-0.38 mm long, 0.24-0.48 mm wide, medial. Vagina absent. Vitelline reservoir transversely wide anterior to ovary. Vitellaria follicular extending over its body, from behind anterior attachment organ to posterior end of the body.

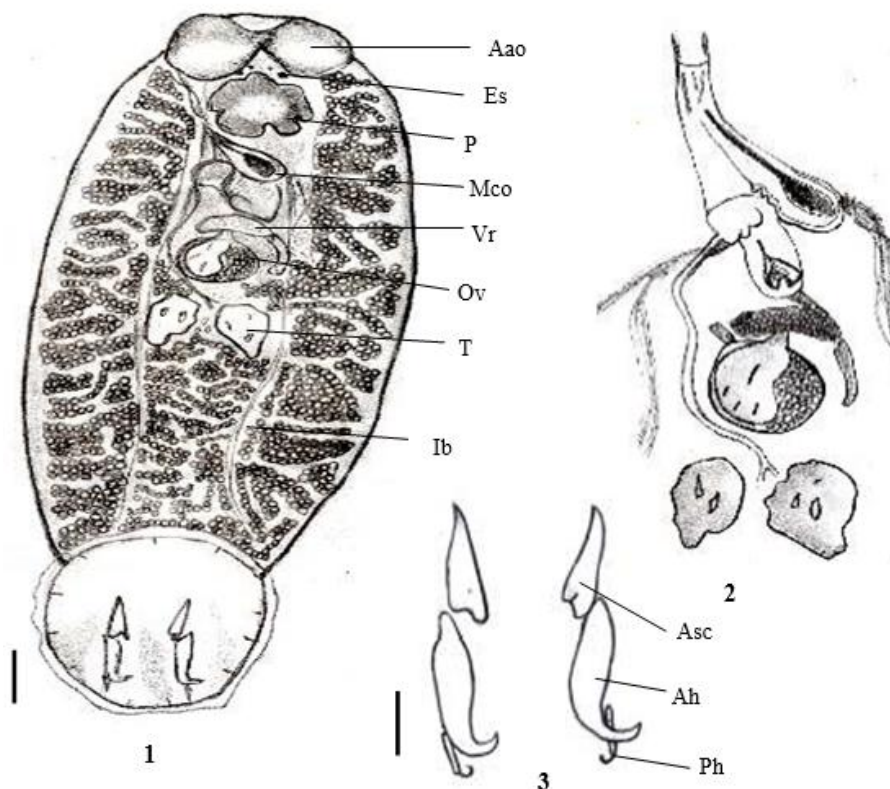


Figure 2. *Neobenedenia girellae*. Images taken with binocular microscope equipped with camera Lucida.

1) *Neobenedenia girellae* ventral view, scale bar 0= 100 μ m 2) Reproductive organs, scale bar = 100 μ m 3) Median sclerites, scale bar = 10 μ m, Description: (Aao) Anterior attachment organ, (Es) Eye spot, (P) Pharynx, (Ov) Ovary, (T) Testis, (Ib) Intestinal branch, (Mv) Marginal valve, (Vr) Vitelline resevoir (Asc) Accessory sclerite, (Ah) Anterior hamuli, (Ph) Posterior hamuli.

3.2 Description of *Pseudorhabdosynochus seabassi*

Pseudorhabdosynochus seabassi was Monogenean Trematode, Dactylogyrydea order, Diplectanidae family. *Pseudorhabdosynochus seabassi* had an ovoid body, slender-shaped, with total length including haptor to ovary 0.52-0.68 mm and width 0.12-0.25 mm. Tegument had directed scales in the posterior half of the body. Two pairs of dissimilar eyes spot were in the anterior of the body. Mouth was in median-subterminal part of the body. This worm had a short esophagus which connects the posterior part of the body with pharynx, a simple intestinal caeca which blindly terminates on the posterior margin of vitelline field. The glandular-shaped pharynx had 0.05-0.071 mm for total length and 0.047-0.107 mm for total width. The subpherical testes was located in the posterior of ovary. Vas deferens was located in the left side of testes, extending along the anterior part of the body and widen in the seminal vesicle part which was located in the central part of the body. Vas deferens also connected the testes to the quadriloculate organ which its terminal section enlarged. The quadriloculate organ consists of four sclerotized chambers. In addition, the fourth chamber has shorter size. The ejaculatory duct had spiral muscular which bands the posterior region of male copulatory bulb organ with male accessory reservoir. The characteristic of each species of *Pseudorhabdosynochus* was different copulatory organs [9, 10].

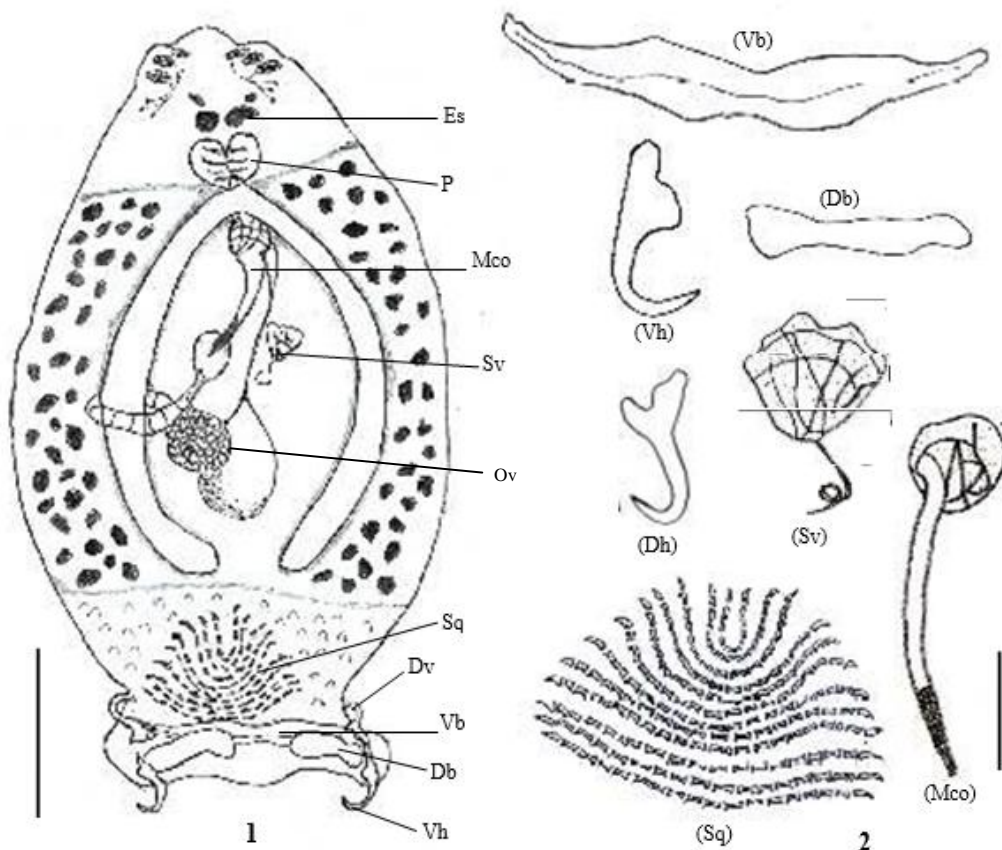


Figure 3. *Pseudorhabdosynochus seabassi*. The images were taken using binocular microscope equipped with camera Lucida.

(1) ventral view, scale bar= 100 μ m, (2) Eye spot (Es), Male copulatory organ (Mco), pharynx (P), Sclerotised vagina (Sv), Squamodisc (Sq), Ventral hamuli (Vh). Bar Scale 100 μ m. Male copulatory organ (Mco), Sclerotised vagina (Sv), Squamodisc (Sq), Ventral hamulus (Vh), Dorsal hamulus (Dh). Bar Scale 20 μ m.

The equatorial ovary covered up the intestinal caecum on some parts. Vagina was divided into two parts. The first part of vagina was located close to ovary, partly sclerotized on the proximal part, and had a lotus flower shape. The second part was sclerotized on the distal part and fibriform-shaped. The vagina was located in sinistral part of the male copulatory organ with a complex sclerotized structure. The sclerotized vagina was 0.104-0.123 mm long and 0.124-0.161 mm wide, comprised sclerotized trumpet, primary canal, primary chamber, secondary canal and secondary chamber. Haptor was located in posterior part of the body, differentiated from the rest of the body, provided with two similar squamodiscs in ventral and dorsal part of the body. This haptor also had two pairs of lateral hamuli, three transverse bars and seven pairs of marginal hooklets but its shape were not clear. The Squamodiscs had a rounded shape and was made of rows of rodlets. Squamodisc had 9-12 concentric rows of rodlet and two inner most rows forming circles. The rodlet sometimes were equipped with visible spurs.

Bars were located between hamuli, strong and concaved on the central part. It also getting thinner in the edge of the body. Bars were straight, with flattened medial extremity and thick cylindrical lateral extremity. Ventral bar was 0.187-0.195 mm long, had flat shaped, massive, with slightly constricted median portion and blunt was extremities. The dorsal hamuli inner was 0.026-0.033 mm long and the outer was 0.04-0.045 mm long. The ventral hamuli inner length was 0.036-0.046 mm and outer length was 0.047-0.057 mm.

3.3 Prevalence, intensity and predilection

The parasite prevalence, intensity, and predilection were recorded. There were three types of Monogenean parasites that infested each individual. *N. girellae* has found on fish body surface, eyes and fins. *P. seabassi* has found only in fish gills lamella. Prevalence and intensity of cantang grouper fish has infested by Monogeneans could be seen in table 1.

Table 1. Prevalence, Intensity and Predilection of Cantang Grouper Fish Infested by Monogeneans.

No	Species of Ectoparasite	Infested fish (ind)	Prevalence (%)	Intensity (ind/fish)	Predilection of parasites
1	<i>Pseudorhabdosynochos Seabassi</i>	11	27.5	10.09	Gills lamela
2	<i>Neobenedenia girellae</i>	4	10	2.75	Eyes, fins and body surface
3	<i>P. seabassi x n. girellae</i>	20	50	9.55	
Total		35	87.5	-	

Grouper fish is a valuable and high-demanded fish in Southeast Asia. *Epinephelus coioides* and *Epinephelus bleekeri* are intensively cultured in sea cages in Vietnam. *E. coioides* is also a common species for grouper aquaculture in China. The hybrid grouper fish, the result of *E. fuscoguttatus* and *E. lanceolatus* crossbreeding, is the main commodity for grouper mariculture in Indonesia. Koesharyani, et al [11] reported that *Cromileptes altivelis* (humpback grouper) were infested by Capsalids monogeneans (*Benedenia epinepheli* and *Neobenedenia girellae*) in Indonesia. Wiyatno, et al [5] also reported *Neobenedenia* sp. mainly infested humpback grouper in sea cages in Situbondo, Indonesia. In Malaysia, *N. girellae* was also found in some species of *E. coioides* (greasy groupers), *E. malabaricus*, and *E. bleekeri* [12]. *N. girellae* was first reported in California [13]. *N. girellae* (Capsalids) had been reported widely across the world, from subtropical to tropical areas [14]. This parasite demonstrated a very low host specificity [15]. The capsalids that infested on the body surface and fins can caused excessive and lost appetite. *N. girellae* was infested almost on head and eyes area. Hard infestation of *N. girellae* can caused opaque eyes and blindness [16].

Diplectanids are monogenean groups that mostly infest the gills of serranid fish and have a wide

world distribution [17]. Justine [18] mentioned that there were 12 monogeneans species on the highfin grouper *Epinephelus maculatus*, including 8 new species of the diplectanid *Pseudorhabdosynochus*. The latter study was recently described from Wu, et al [19] that *P. seabassi* have been found in *Lates calcarifer* that cultured in Guangdong Province, China. The report said that *Pseudorhabdosynochus* which infested grouper fish in Indonesia were rarely found, but according to the research from Ruckert, et al [20] *Pseudorhabdosynochus* sp. that infested *Epinephelus fuscoguttatus* from 25 (cultured grouper) and 30 (wild grouper) parasite species/taxa were identified in Lampung Bay, South Sumatra and the most abundant parasites are the monogeneans *Pseudorhabdosynochus epinepheli* and *Pseudorhabdosynochus lantauensis* for both, cultured and wild grouper. In addition, Wiyatno, et al and Fidyandini, et al [5, 9] stated that the gill worm *Pseudorhabdosynochus* was also found infesting the Humpback grouper (*Cromileptes altivelis*) and Milkfish (*Chanos chanos*) at the Situbondo marine waters. Based on the results, cantang groupers that is cultivated in Mariculture Center Lombok has possibility infested by gill worm *Pseudorhabdosynochus* because the groupers has imported from Situbondo area. *P. seabassi* caused haemorrhagic and abrasive lesions on the body. *Pseudorhabdosynochus* sp. infested all over the gills lamella, a heavy infestation from this parasite on gills lamella caused abrasive lesion and respiratory disorder which could caused death. All of fish samples has been infested by *P. seabassi* were showing clinical symptoms of excessive mucus on the body surface.

Cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) from 40 samples were examined, 35 samples were infested by *N. girellae* and *P. seabassi*. The total prevalence of monogeneans that infested cantang grouper fish was 87.5 %. Total intensity of monogeneans has found in cantang grouper was 8.94 individual/fish from 35 samples infested by monogeneans. The highest number of prevalence appeared in mixed infestation between *N. girellae* and *P. seabassi*, with 50 % prevalence. Therefore, the greatest number of intensity occurred in infestation of *P. seabassi* with 10.09 individual/fish. The mortality which was caused by infestation of monogeneans has rarely occurred in Cantang grouper. The intensity of monogenean parasites that infested grouper fish was classified as upper-middle category and severely did not show any haemorrhagic or abrasive lesions on body surface. The prevalence and intensity of the monogeneans could be associated with poor management of fish maintenance such as dirty sea cage condition and also uncontrolled water quality because it depends only on the season [5]. It also could be associated with the life cycle of Monogeneans that has a direct life cycle. Onchomiracidium has infective phase and swam freely to found the appropriate host. If parasite had found suitable host, it would stick and develop [21]. According to Kennedy [22], the important factor is the presence of host contact with the parasite. The parasite can survive when the host condition is appropriated.

4. Conclusion

The monogeneans which has been found infesting cantang grouper were *Neobenedenia girellae* and *Pseudorhabdosynochus seabassi*. Total Prevalence of infestation ectoparasites was 87.5 %. The prevalence of *Neobenedenia girellae* was 27.5 % with intensity was 2.75 individual/fish. The prevalence of *Pseudorhabdosynochus seabassi* was 10% with intensity was 10.09 individual/fish. The prevalence of mix infestation between *N. girellae* and *P. seabassi* was 50 % and intensity was 9.55 individual/fish. Predilection of monogenean has infested the grouper (*Epinephelus fuscoguttatus-lanceolatus*) that were *N. girellae* on fish body surface, eyes, dorsal fin and pectoral fin. *P. seabassi* only on gills lamella.

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