Survey on ectoparasite occurrence of fish groupers sent to Fish Quarantine Agency for diseases inspection

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Abstract. A parasitology survey on fish groupers was conducted from January to April in 2016 in a Parasitology Laboratory belongs to Fish Quarantine and Inspection Agency, Makassar, to identify parasites from the fish groups. Previous data available at the institute did not distinguish between species of fish examined and the parasites that infect them, most of the fish from different species were only categorized as groupers, while many species of parasites are known to have high host specificity. Fifty-four grouper fish of different species were examined, includes; Cephalopholis sonnerati, C. argus, Plectropomus oligocanthus, P. leopardus, P. areolatus, P. maculatus. The fish species are common groupers that are exported alive to various countries. The fish were sent by exporter companies to the laboratory for disease inspection. The fish species were identified, length and weight were measured, then the whole body surface was visually examined for the presence of large parasites. Operculum was cut, and each gills were removed and placed on a petri dish contained saltwater. Observation of parasites presence was conducted under a stereomicroscope, and identification of parasite to genus level was conducted directly under a compound microscope. Species of parasites found were monogenean; Diplectanum sp., Pseudorhabdosynochus sp., Echinoplectanum leopardi, Echinoplectanum sp., Protozoa; Brooklynella hostilis, Cryptocaryon irritans, Crustacea; Hatschekia sp., Dissonus sp., copepod larvae. Diplectanum sp. was found from P. areolatus and C. sonnerati. The prevalence and mean intensity of the parasite in P. areolatus was 100% and 52.5, respectively, whereas the prevalence and mean intensity of the parasite in C. sonnerati was 50% and 69.6, respectively. Pseudorhabdosynochus sp. was only recorded from Cephalopholis spp. Echinoplectanum leopardi only occurred in P. leopardus. Echinoplectanum sp. was also recorded from P. oligocanthus, P. maculatus and C. sonnerati. Other species of parasites commonly found in the fish examined were B. hostilis, C. irritans, Hatschekia sp. and Dissonus sp. All the parasites are highly pathogenic and may complete their life cycle within a container. Therefore, their occurrence in aquaculture facilities may cause significant damage leading to mortality on fish without proper treatment.

1. Introduction
Groupers are a group of fish that belong to the family of Serranidae, a subfamily of Epinephelinae, which consists of 15 genera and 159 species of marine fish [1]. Groupers are one of the main fishery commodities in Indonesia that have high demand in the local market and foreign market as an export commodity. Groupers are generally exported to foreign countries alive. Groupers have an important role in aquatic ecosystems and are also an important food source for the community in fulfilling their nutritional needs. Some groupers species have also been cultivated in marine areas in Indonesia.
particularly in Bali. Aquaculture industry has developed rapidly in global world in the last decades. As a huge and very populated country, aquaculture industries in Indonesia play an important role in supplying animal protein for local communities and for export, and has been the main sector for economic growth in this region. The rapid development of aquaculture in Indonesia is supported by a huge amount of natural resources across the Indonesia archipelago. A potential area for aquaculture in Indonesia is estimated at about 17,918,254 ha, including 12,123,383 ha for mariculture and 2,964,331 ha for brackish water aquaculture in 2016. However, only 2.36% and 24.15% of the area has been used for mariculture and brackish water aquaculture, respectively [2]. The main fish species for mariculture are groupers, giant seaperch, and milkfish. Grouper production in Indonesia reached 70,294 tons, with a value of IDR 9.7 trillion in 2017 [2].

Along with the increasing population in the world, the aquaculture industry is also developing rapidly with various levels of cultivation technology ranging from simple technology to high technology allowing to stock fish in high density. Such a condition provides a suitable condition for various types of parasites and other pathogens to proliferate and cause disease in fish. Gills are one of the body's vital organs for fish and become a habitat for various types of parasites. Various types of parasitic infections are very likely to be found in finfish aquaculture either in floating cages or in concrete tanks, particularly infection of parasites that have a life cycle with one host or parasites with a life cycle involving several hosts because the cultivation condition is an open system category [3,4].

Fish infection can occur either directly or indirectly through an intermediary host around the floating cage. Infection by parasites with direct life cycles such as ciliate, monogenean, and crustacean can occur in fish cages with high rates of infection in high fish densities in cages [5–7]. Parasitic infections can cause enormous economic losses due to mass mortality in cultured fish [8]. Losses due to parasitic infections are very large [9]. Annual economic losses are estimated to range between 5.8–16.5%, which are equivalent to losses ranging from US $ 62–175 M. In closed systems, for example in concrete tanks, usually only parasites that have a direct life cycle can develop, whereas parasites with a life cycle that involve several intermediate hosts do generally not occur because some intermediate hosts can be eliminated from aquaculture facilities through implementation of good aquaculture practices. However, groupers reared in floating net cages were reported to be infected by 13 species of parasites, 6 species of parasites are known to have only one host in their life cycle (monoxenous), and 7 species have a life cycle of more than one host (heteroxenous) [3].

Although coral reefs are known to have a high diversity and, moreover, Indonesia is known as a center of diversity, researches that have been done related to infection of parasites in groupers that live on coral reefs in this region are negligible. Previous works that have been done on parasites infection of groupers, frequently do not distinguish the species of groupers examined and the particular parasites that infect them. This is very important for parasitology observation to accurately identify the host species and their particular parasites since many species of parasites are recognized to have a high host specificity.

2. Materials and methods

2.1. Fish samples

Materials used in this survey were obtained from exporter companies that frequently send fish samples to Fish Quarantine Inspection Agency (FQIA) for disease inspection. The number and species of fish examined were highly dependent on the availability of samples from exporter companies. The samples are usually brought directly by the company to the laboratory or the authorized officer of the fish quarantine who comes to pick up the sample at the company. Companies usually send samples twice a week at certain days and times of the week. The fish were put in a plastic bag (3-4 fish in each bag) that had been added oxygen and then transported alive to the fish quarantine laboratory by car. After going through the administrative process at the sample receiving office, the fish samples were then taken to the laboratory for parasite examination.
2.2. Parasites examination
In the laboratory, the fish were anesthetized using MS222 or clove oil. The fish was identified, measured length and weight. The operculum was cut off. Each gills filaments were removed from the arch and then placed separately in a petri-dish containing 2% NaCl solution. The gills were observed under a stereomicroscope. The number of parasites on gills were counted under a stereomicroscope. A compound microscope was used to observe a particular organ of parasites examined and to identify the parasites found. Parasites were identified following the identification guide published in some journals [10–14].

2.3. Remark on fish hosts
The fish genus *Plectropomus* spp. are generally large in size, which may reach 1 m in total length. These fish live in shallow waters in both tropical and subtropical regions. The distribution area of the fish is limited to the Indo-Pacific region [1]. *Plectropomus leopardus* inhabits coral reef throughout South East Asia and Great Barrier Reef Australia [15]. *P. leopardus* has been recorded in the Western Pacific region, from Southern Japan to Australia (Queensland and Western Australia) and Eastward to the Caroline Islands and Fiji. *P. leopardus* occupies habitat at depths of 3 to 100 m [1]. This fish lives solitary in the habitats, which are rich in coral reefs on the lagoon and mid-shelf reefs. Juvenile lives demersal in shallow water in coral reef habitats, especially around coral rubble. Adult fish feed on smaller fish, whereas juveniles feed on small fish and invertebrates (crustaceans, squid). *P. oligocanthus* inhabits offshore coral reefs throughout South East Asia, Great Barrier Reef Australia [15]. The fish are also found in the Western Pacific Ocean, Philippines, Indonesia, New Guinea, Northeastern Australia, Belau, Caroline Islands, Marshall Islands, and Solomon Islands. The fish usually prey on crustaceans and small fish that live in rocky and sandy areas. The geographical distribution of *P. areolatus* is Indo-Pacific. The fish is reported in Great Barrier Reef, Western Australia, Chagos, Maldives, Coco-Keeling Islands, Philippines, Indonesia, Taiwan, Ryukyu Islands. The fish is also reported inhabits lagoon and outer reef at a depth of 2 to 20 m [1]. The geographical distribution of *P. maculatus* is the region in Tropical West Pacific, Thailand, Singapore, Philippines, Indonesia, Papua New Guinea, Solomon Islands, and Western Australia to Southern Queensland. The fish is commonly found on the coastal reefs in Queensland and Indonesia. This fish inhabit waters at depths ranging from 5 to 50 meters. *Cephalopholis sonnerati* spreads in the Indo-Pacific from the East Coast of Africa to the Line Islands in the Central Pacific. In the Western Pacific, *C. sonnerati* is found in the regions from Southern Japan to Southern Queensland. This fish is usually caught at depths of 30 to 100 m. *C. argus* has wider distribution area, covering the Red Sea to South Africa and French Polynesia, including Northern Australia, Lord Howe Island, and Japan. This species is common tropical species which might be found in a variety of coral reef habitats from tidal pools to depths of at least 40 m. However, the fish preferred habitat of reef zones at depths of 1 to 10 m [1].

2.4. Data analysis
Data on prevalence and mean intensity of parasite infection are presented in Tables and analyzed descriptively. Prevalence is defined as the number of fish hosts which is infected with 1 or more individuals of a particular species of parasite divided by the number of hosts examined. It is expressed in percentage. Intensity is defined as the number of individuals of a particular species of parasite in a single infected host, whereas mean intensity is the average intensity of a particular species of parasite among the infected host species or a total number of a particular species parasites found in fish examined divided by the number of hosts infected with the parasite species [16].

3. Results and discussion
3.1. Results
During the survey from January to April 2016, six species of groupers were examined; *Plectropomus leopardus* (N= 31), *P. oligocanthus* (N=5), *P. areolatus* (N=4), *P. maculatus* (N= 1), *Cephalopholis*
sonnerati (N= 10), and C. argus (N= 3). A total of 9 type of parasites were found which consist of four monogeneans (Diplectanum sp., Pseudorhabdosynochus sp., Echinoplectanum leopardi, Echinoplectanum sp.), protozoa (Brooklynella sp., Cryptocaryon irritans), crustacean (Hatschekia sp., Dissonus sp.) (Table 1). The six species of fish samples were not always available at the time of fish parasite examination, as presented in Table 1. Only C. sonnerati and P. leopradus were available in the 4 months of examination from January to April, while other fish were not always available during the observation month. In C. sonnerati, Diplectanum sp. and Pseudorhabdosynochus sp. were commonly found. In P. leopradus the most common parasite found was E. leopardi, Hatschekia sp. and Dissonus sp. The other parasite, copepod larvae, were very common and infected all the fish examined with high prevalence and mean intensity.

Pooled data from January to April showed that Diplectanum sp. was found from P. areolatus and C. sonnerati with a prevalence of 100% and 50%, respectively, while mean intensity of infection was 52.5 and 69.6, respectively. Pseudorhabdosynochus sp. was found from C. sonnerati and C. argus with the prevalence of 4% and 32%, and mean intensity of 4 and 32, respectively. Echinoplectanum leopardi was only found from P. leopradi with the prevalence and mean intensity of 83.9% and 16.2, respectively. Echinoplectanum sp was found from P. oligocanthus, P. maculatus and C. sonnerati with the prevalence of infection of 20%, 100%, and 10%, respectively and mean intensity of 10.8, 3, and 2, respectively. Hatschekia sp. was found from P. leopradus, P. oligocanthus, P. areolatus, and C. sonnerati. Dissonus sp was found from P. leopradus, P. oligocanthus, P. maculatus and C. argus. Copepod larvae were found from all the fish examined with high prevalence range from the lowest 33.3% in C. argus to the highest 100% in P. oligocanthus. The highest mean intensity of copepod larva infection was 306 from the gills of C. argus (Table 2). Some fish were infected with the high pathogenic protozoan Brooklynella hostilis and Cryptocaryon irritans with an abundant of the parasite causing Brooklynelliosis and Cryptocaryonosis of the infected fish.

Table 1. Monthly occurrence of parasites on fish species examined

| Fish Species                  | L (cm) | W (g) | Parasite                  | Fish Examined | Fish Infected | Prevalence (%) | MI (range) |
|-------------------------------|--------|-------|---------------------------|---------------|---------------|----------------|------------|
| **JANUARY**                   |        |       |                           |               |               |                |            |
| Tomato Rockcod (Cephalopholis sonnerati) | 25     | 190-350 | Diplectanum sp           | 3             | 2             | 66.7           | 63±8 (55-71) |
|                               |        |       | Larva copepoda           | 2             | 66.7          | 3.5±0.5 (3-4)  |            |
|                               |        |       | Pseudorhabdosynochus sp  | 1             | 33.3          |                | 3.0        |
|                               |        |       | Cryptocaryon irritans    | 1             | 33.3          |                | abundant   |
| Peacock Rockcod (Cephalopholis argus) | 25-27 | 300-350 | Pseudorhabdosynochus sp | 2             | 2             | 100.0          | 46.2       |
|                               |        |       | Brooklynella hostilis    | 1             | 50.0          |                | abundant   |
| Vermicular cod (Plectropomus oligocanthus) | 21-27 | 190-300 | Echinoplectanum sp      | 5             | 5             | 100.0          | 10.8±5.8 (3-18) |
|                               |        |       | Cryptocaryon irritans    | 1             | 20.0          |                | abundant   |
|                               |        |       | Hatschekia sp            | 4             | 80.0          | 4.5±3.2 (1-9)  |            |
|                               |        |       | Larva copepoda           | 5             | 100.0         | 3.8±3.4 (1-10) |            |
|                               |        |       | Dissonus sp              | 1             | 20.0          | 1.0            |            |
| Fish Species                        | Size | Geographical Parameters | Parasites/Protozoans                | Count | Percentage | Mean ± SD (Range) |
|------------------------------------|------|--------------------------|------------------------------------|-------|------------|-----------------|
| **Coral Trout**                    | 25-27| 210-270                  | Echinoplectanum leopardi           | 7     | 85.7%      | 20.3±28.4 (1-83) |
| (Plectropomus leopardus)           |      |                          | Hatschekia sp                      | 6     | 85.7%      | 9±1.8 (1-16)    |
|                                    |      |                          | Larva copepoda                     | 5     | 71.4%      | 9.2±11.8 (1-32) |
|                                    |      |                          | *Brooklynella hostilis*            | 2     | 28.6%      | abundant        |
|                                    |      |                          | *Dissonus* sp                      | 3     | 42.9%      | 1.33±0.5 (1-2)  |
| **Pokadot Cod**                    | 31   | 300                      | Hatschekia sp                      | 1     | 100.0%     | 5±0             |
| (Plectropomus areolatus)           |      |                          | Diplectanum sp                     | 1     | 100.0%     | 103.0           |
|                                    |      |                          | Larva copepoda                     | 1     | 100.0%     | 32.0            |
| **Bar-cheeked Coral Trout**        |       |                          | *Brooklynella hostilis*            | 1     | 100.0%     | abundant        |
| (Plectropomus maculatus)           |       |                          | Echinoplectanum leopardi           | 1     | 100.0%     | 3.0             |
|                                    |       |                          | Larva copepoda                     | 1     | 100.0%     | 3.0             |
|                                    |       |                          | *Dissonus* sp                      | 1     | 100.0%     | 1.0             |
| **FEBRUARY**                       |      |                          | **Tomato Rockcod**                 | 25-31 | 150-590    |                 |
| (Cephalopholis sonnerati)          |      |                          | Larva copepoda                     | 3     | 66.7%      | 4±2 (2-6)       |
|                                    |      |                          | *Pseudorhabdosynochus* sp          | 2     | 66.7%      | 3±1 (2-4)       |
|                                    |      |                          | Hatschekia sp                      | 1     | 33.3%      | 5.0             |
|                                    |      |                          | Echinoplectanum leopardi           | 1     | 33.3%      | 2.0             |
| **Peacock Rockcod**                |       |                          | Larva copepoda                     | 1     | 100.0%     | 306.0           |
| (Cephalopholis argus)              |       |                          | *Dissonus* sp                      | 1     | 100.0%     | 1.0             |
|                                    |       |                          | *Pseudorhabdosynochus* sp          | 1     | 100.0%     | 33.0            |
| **Coral Trout**                    | 25-29| 210-400                  | *Dissonus* sp                      | 12    | 58.3%      | 1.86±1.4 (1-5)  |
| (Plectropomus leopardus)           |      |                          | *Brooklynella hostilis*            | 3     | 25.0%      | abundant        |
|                                    |      |                          | Larva copepoda                     | 10    | 83.3%      | 54.5±77.9 (1-219) |
|                                    |      |                          | Echinoplectanum leopardi           | 10    | 83.3%      | 18±27.6 (1-95)  |
|                                    |      |                          | Hatschekia sp                      | 9     | 75.0%      | 4.3±1.6 (1-7)   |
|                                    |      |                          | *Cryptocaryon irritans*            | 4     | 33.3%      |                 |
| **MARCH**                          |      |                          | **Tomato Rockcod**                 | 23-25 | 250-310    |                 |
| (Cephalopholis sonnerati)          |      |                          | Diplectanum sp                     | 3     | 33.3%      | 74±84.8 (2-193) |
|                                    |      |                          | Larva copepoda                     | 2     | 66.7%      | 38.5±35.5 (5-3-74) |
|                                    |      |                          | *Cryptocaryon irritans*            | 1     | 33.3%      | abundant        |
|                                    |      |                          | *Brooklynella hostilis*            | 1     | 33.3%      | abundant        |
| **Coral Trout**                    | 23-25| 210-310                  | Hatschekia sp                      | 10    | 80.0%      | 3.5±1.9 (2-8)   |
| (Plectropomus leopardus)           |      |                          | *Dissonus* sp                      | 5     | 50.0%      | 2.4±2.3 (1-7)   |
| Species                                      | N | Prevalence | MI   | Notes                |
|----------------------------------------------|---|------------|------|----------------------|
| Larva copepod                                | 8 | 80.0       | 59±115.5 | (3-364)             |
| *Echinoplectanum leopardi*                   | 9 | 90.0       | 6.7±5.0 | (2-18)              |
| *Cryptocaryon irritans*                      | 2 | 20.0       | 15.5±7.5 | (8-23)             |
| *Hatschekia sp*                              | 1 | 50.0       | 3.0    |

| APRIL                                        |   |            |      |                      |
|----------------------------------------------|---|------------|------|----------------------|
| Tomato Rockcod                               | 27| 290       | 1    | 100.0               | 7.0 |
| (*Cephalopholis sonnerati*)                  |   |           |      |                      |
| *Pseudorhabdosynochus sp*                     | 1 | 100.0     | 7.0  |
| Coral Trout                                  | 26| 200       | 2    | 50.0                | 69.0 |
| (*Plectropomus leopardus*)                   |   |           |      |                      |
| *Echinoplectanum leopardi*                   | 2 | 1         | 69.0 |
| Larva copepod                                | 2 | 100.0     | 15.5±7.5 | (8-23)             |
| *Hatschekia sp*                              | 1 | 50.0       | 3.0    |
| Pokadot Cod                                  | 23| 290       | 3    | 100.0               | 35.7±32. |
| (*Plectropomus areolatus*)                   |   |           |      |                      |
| *Diplectanum sp*                             | 3 | 3         | 35.7±32. | 8 (12-82)         |
| Larva copepod                                | 2 | 66.7      | 7±6  | (1-13)             |

Notes: P= prevalence, MI= mean intensity, N= number of fish examined
### Table 2. Prevalence and mean intensity of parasites infection on the fish species examined.

| Parasites            | Species of Fish Examined | Plectropomus leopardus (N=31) | P. oligocanthus (N=5) | P. areolatus (N=4) | P. maculatus (N=1) | Cephalopholis sonnerati (N=10) | C. argus (N= 3) |
|----------------------|--------------------------|-------------------------------|-----------------------|-------------------|--------------------|-----------------------------|----------------|
|                      |                          | P (%) MI (range)              | P (%) MI (range)      | P (%) MI (range)  | P (%) MI (range)   | P (%) MI (range)            | P (%) MI (range) |
| Diplectanum sp       |                          | 0 0                           | 0 0                   | 100               | 0 0                | 0 0                         | 0 0            |
| Pseudorhabdosynochus sp |                        | 0 0                            | 0 0                   | 0 0               | 0 0                | 0 0                         | 0 0            |
| Echinoplectanum leopardi |                     | 83.9 16.2±25.3(1-95)          | 0 0                   | 0 0               | 0 0                | 0 0                         | 0 0            |
| Echinoplectanum sp   |                          | 0 0                           | 0 0                   | 0 0               | 100 3              | 10 2                        | 0 0            |
| Brooklynella hostilis |                        | 6.5 abundant                   | 0 0                   | 0 0               | 100 abundant       | 10 abundant                 | 33.3 abundant  |
| Cryptocaryon irritans |                        | 19.4 abundant                  | 20 abundant           | 0 0               | 0 0                | 20 abundant                 | 0 0            |
| Hatschekia sp        |                          | 77.4 3.7±1.8(1-8)             | 80 25                 | 0 0               | 100 5              | 0 0                         | 0 0            |
| Dissonus sp          |                          | 48.4 1.9±7.1(1-7)             | 20 0                  | 1 0               | 100 1              | 0 0                         | 33.3 1         |
| copepod larvae       |                          | 80.6 364                      | 100 75               | 6.3±5.0(1-13)     | 100 3              | 60 15.2±26.3(2-74)           | 33.3 306       |
3.2. Discussion

A fish parasitology survey on the fish groupers at Fish Quarantine and Inspection Agency was conducted. From the 6 fish species examined, nine types of parasites were identified. The parasites were consisting of four monogeneans: *Diplectanum* sp., *Pseudorhabdosynochus* sp., *Echinoplectanum* leopardi, and *Echinoplectanum* sp.; two protozoa: *Brookynella hostilis*, and *Cryptocaryon irritans*; two crustacean: *Hatchekia* sp. and *Dissonus* sp. Unidentified copepod larvae were also found with high prevalence and mean intensity of all the fish examined, indicating the low host specificity of the parasite larvae. Copepod larvae that infect fish gills cause serious damage to the gills [17].

The monogeneans found were belongs to the family of Diplectanidae characterized by the presence of additional attachment apparatus called “squamodisc” located in the posterior part of the parasite. Generally, the diplectanid parasite is recognized through several characteristics it possesses, among others are adhesive organs in the ventral and dorsal parts of the haptor, which are called squamodisc or lamellodisc; three transverse bars, one located on the ventral part and two on dorsal parts which are connected to two pairs of hooks; one pair of the hook is located on the dorsal and one pair of ventral parts. It also has a germarium or ovary, which is located anteriorly from the testes and loop, which is located on the right side of the intestinal caecum [18,19]. However, this organ was completely lacking at *Pseudorhabdosynochus sinediscus*. Groupers are very commonly found to be infected by diplectanid parasites [18–20].

*Pseudorhabdosynochus* sp., a diplectanid parasite, was found from *C. sonnerati* and *C. argus*. This parasite was not found in *Plectropomus* spp. examined. This is consistent with the report from New Caledonia which never found *Pseudorhabdosynochus* from the species of *Plectropomus* spp. *Pseudorhabdosynochus* are generally strictly host-specific and has a worldwide distribution. *Pseudorhabdosynochus* species is characterized by a quadriloculate shape in the male copulatory organ (MCO), which is a bean shape with four chambers. The vagina is sclerotized, which is a complex structure with several spaces and canals [21–23]. The species *Pseudorhabdosynochus* mainly parasites on groupers, which are abundant in warm waters in the tropics, especially on coral reefs. Several species of *Pseudorhabdosynochus* have been reported to infect fish that are cultivated in floating cages [3,4].

Within the family of Diplectanidae which consist of 50 genera, *Pseudorhabdosynochus* among the biggest member in which about 105 species from these genera have been described [25]. Most of them are reported from serranid fish of the genus of *Epinephelus*. *Pseudorhabdosynochus* has been recorded from various species of marine fish, mostly from *Alphestes*, *Cephalopholis*, *Epinephelus*, *Hyorthodus*, *Mycteroperca*, *Paranthias* and *Variola* [18,26–30]. However, some parasite species are also found from fish that are not of the genus Epinephelie, such as *P. magnisquamodiscum* from *Chaetodon hoeffleri* [31] and *P. serrani* from *Serranus* sp. [32]. *Pseudorhabdosynochus* argus has been reported to infect *C. argus* off New Caledonia and Australia, and *P. minutus* from *C. sonnerati* [33]. *P. argus* has male copulatory organs that are quadriloculate in shape with very long cones, a sclerotized vagina with anterior trumpet, coiled primary canal, and distal parts with two chambers and an accessory part [33]. *P. minutus* is characterized by a small body and sclerotized vagina with two spherical chambers. The prevalence of infection of *P. argus* is reported to reach 100% with an average intensity of 58 [33].

In this study, we reported *Diplectanum* sp. from two species of marine fish *P. areolatus* and *C. sonnerati*. About 73 species of *Diplectanum* has been reported from various fish species in marine and freshwater environment, but mostly the parasites are reported from marine fish [34]. Currently, five species of *Diplectanum* have been reported from freshwater fish, all from *Plagioscon* spp. in Brazil. The parasites are *D. decorum*, *D. gymnopeus*, *D. hilum*, *D. pescadae*, and *D. piscinarius*. *D. nanus* has been reported from *C. sonnerati* in New Caledonia [33]. *D. plecrotopomi* is reported from *P. maculatus* off Western Australia, but the species name has been transferred to the genus *Echinoplectanum*, as *E. plecrotoponi* [10]. *D. nanus* from *C. sonnerati* is characterized by male copulative organs that are very small and funnel-shaped. *Diplectanum* Diesing, 1858, is characterized by the presence of a dorsal and
a ventral squamodisc, three bars and two pairs of hamuli/anchor on the haptor, and a sclerotized tubular copulatory organ [35].

Other diplectanids found in this study were *Echinoplectanum leopardi* and *Echinoplectanum* sp. *E. leopradi* was only found from *P. leopardus* and *Echinoplectanum* sp. was found from *P. oligocanthus*, *P. maculatus* and *C. sonnerati*. So far, seven species of *Echinoplectanum* have been reported in the world; *E. chauvetorum*, *E. echinophallus*, *E. leave*, *E. leopardi*, *E. plectropomi*, *E. pudicum* and *E. raram*. Of the 7 species of *Echinoplectanum* described, six of them are reported from *Plectropomus* spp., suggesting that *Echinoplectanum* infection is associated with *Plectropomus* [10]. The discovery of *E. echinophallus* in *E. marginatus* is suspected due to host switching of the parasites from *Plectropomus* to *Epinephelus* [10]. However, in this study, we found *Echinoplectanum* sp. from *C. sonnerati*. This finding provides new information about the geographical distribution of *Echinoplectanum*, from Indonesian waters, which was previously only reported from Australia, New Caledonia, and Mediterranean Sea.

All monogeneans found have a direct life cycle, starting with eggs, oncomiracidia, juvenile, and becoming adult on gills of fish hosts. In cultivation systems, parasites can develop quickly, especially in suitable environments. In floating cages, parasite eggs will usually attach to the substrate or to the net, and then the newly hatched larvae, oncomiracidia, swim actively to find host quickly, and especially the parasite transmission will be quick on fish cultivated with high stocking density.

Two species of *Brooklynella* have been reported; *Brooklynella hostilis* and *B. sinensis* [36]. *Brooklynella hostilis* was found from 4 fish species examined; *P. leopradus*, *P. maculatus*, *C. sonnerati* and *C. argus*. These fish were all infected with high intensity/abundance of the parasite. This parasite causes a disease called brooklynellois which can cause mass mortality in fish and epizootic, generally infect fish in the tropics. Infected gills have damage to the epithelial tissue. The parasite damages the gills and takes food from the debris using the cytopharyngeal armature organ [37].

*Cryptocaryon* has only one valid species; *C. irritans* [38]. *Cryptocaryon* is a ciliophora parasite that infects a variety of aquaculture fish species and marine ornamental fish in the tropics. Diseases caused by this parasite can spread rapidly in culture system due to its rapid proliferation and fish to fish transmission leading to mortality of infected fish. *C. irritans* is commonly found to infect gills, body surfaces and cause white spots on the body surface, dark body color, lethargy, exophthalmia, increased mucus production, rubbing the body surface on substrate or net. These parasites have often appeared and infected fish in ponds or nets and are considered to be one of the most damaging parasites of marine fish cultivated in temperate and tropical areas [14,39].

About 176 species of *Hatschekia* have reported in the world [40]. In this study, *Hatschekia* sp. was found from *P. leopradus*, *P. oligocanthus*, *P. areolatus*, and *C. sonnerati*. *Hatschekia* sp. have been reported to infect various types of fish in the tropics and subtropics, in at least 8 fish orders; Anguilliformes, Aulopiformes, Beryciformes, Ophidiiformes, Perciformes, Pleuronectiformes, Scorpaeniformes and Tetraodontiformes [41]. Most of the scientific papers available today on *Hatschekia* are related to taxonomy and descriptions of new species from different hosts. This parasite is very often found in groupers with low infection rates.

*Dissonus* spp have been reported from marine fish all over the world. About 12 species have been reported; *Dissonus excavates*, *D. furcatus*, *D. glaber*, *D. heronensis*, *D. hoi*, *D. inaequalis*, *D. kapuri*, *D. manteri*, *D. nudiventris*, *D. ruvetti*, *D. similis*, and *D. spinifer* [42]. *Dissonus* sp was recorded from *P. leopradus*, *P. oligocanthus*, *P. maculatus*, and *C. argus*, with a prevalence range from 20% in *P. oligocanthus* to 48.4% in *P. leopradus* and mean intensity ranges from 1 to 2. *Dissonus* sp. infections can cause fatal damage to the gills. *D. manteri* that infect *P. leopradus* causes hyperplastic on gill filaments as well as the presence of fibroplasia and inflammatory responses [43]. Copepodid and chalimus larvae of *Dissonus* sp., which attach to gills in large amounts, cause injury on the gill filaments, damage the vascular gills, epithelial cell proliferation, and fibrosis [17].

Fish live in the wild generally have various types of parasites, but generally with a low intensity of infection, which will not have or maybe only minimal impact on fish health. However, in cultivation conditions where fish are maintained in confined containers on very high density, it will cause stress to
the cultivated fish, which makes the conditions conducive for the development of pathogens and cause disease. High fish density and fluctuations in environmental conditions and/or stress can support the proliferation of parasites that lead to mortality. In aquaculture, particularly and closed or semi-closed systems, there is a tendency to reduce the overall number of diversity of parasites, and there is a general trend showing a decreased of infection in parasites with complicated/indirect life cycles.

4. Conclusions
Various types of parasites were found to infect some species of groupers from the fish exporter sent to Fish Quarantine and Inspection Agency for disease inspection. The group of parasites was Monogeneans, Crustaceans, and Protozoans which have a direct life cycle and are mostly high pathogenic. Some parasites, particularly from the monogeneans species, showed high host specificity. Direct life cycle and highly pathogenic parasites can be dangerous in aquaculture conditions.

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