Molecular identification and prevalence of ectoparasite worms in barramundi (*Lates calcarifer*) in Lampung Waters

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**Abstract.** Barramundi (*Lates calcarifer*) fish is one of the fishery commodities in Indonesia which has economic value and is the most exported commodity [1]. The problem that often occurs in the process of cultivating barramundi (*Lates calcarifer*) is a parasite from the *Haliotrema* sp. which attacks the gills and can cause a decrease in the level of fish production. One way to determine the parasite infesting barramundi (*Lates calcarifer*) is by conducting a molecular identification. This study aims to determine the parasites by molecular identification and prevalence rate of ectoparasite worms in the gills of barramundi (*Lates calcarifer*) fish in Lampung waters. A sample of 40 fish with a length of 25-35 cm was taken by using a purposive sampling method. The organs observed were fish gills, then molecular identification was carried out by using real-time PCR, and the prevalence calculations. The results of this study indicated that the fish gills were infested by ectoparasitic worms, according to the NCBI GenBank database, called *Haliotrema susanae* with the band at 748 bp, and the prevalence rate of infested fish with gill’s ectoparasites is 80%.

1. Introduction

Barramundi (*Lates calcarifer*) fish is one of the fishery commodities in Indonesia which has economic value and is the most exported commodity [1]. The problem that often occurs in the process of cultivating barramundi (*Lates calcarifer*) is a parasite from *Haliotrema* spp. which attacks the gills, and can cause a decrease in the level of fish production. One way to determine the parasite infesting barramundi (*Lates calcarifer*) is by conducting a molecular identification. In cultivation activities, the use of drugs is only effective against certain types of worm parasites. So that a proper diagnosis is needed, namely in identifying the type of worm that infests based on its molecules. Identification of worms is needed in providing information on parasites in fish that are capable of transmitting diseases to humans, as well as the effect of parasites on fish bodies [2].

Molecular identification of a species has now experienced very rapid development. This is because there is always an improvement from time to time in terms of methods, technology, and tools used in conducting a molecular biological analysis. Therefore the research is far more effective and efficient. One of molecular identification is Polymerase Chain Reaction (PCR) [3]. The purpose of this study was to determine the species of *Haliotrema* infesting barramundi (*Lates calcarifer*) in the waters of Lampung waters.
Lampung, based on morphometric observations by using binocular microscopes and molecular profiles by using the PCR.

2. Material and methods

2.1. Experimental design

During 6-24 Mei 2019, 40 Barramundi were collected in floating net cages the Mari-culture Center, Lampung. The method used in this research is a descriptive exploratory method by taking of fish measuring 25-35 cm with a purposive sampling method.

2.2. Specimen collections

The sample in this study was the parasite *Haliotrema* spp. First, all of the parasites were assumed as *Haliotrema* sp. The specimen was observed by using a binocular microscope to identify the morphological characteristic of the specimen. After the specimen was put in NaCl physiologic, then specimen were placed in 2.5 ml of sterile aquades for PCR test preparation.

2.3. PCR preparation

DNA extraction stage uses the QIAGEN Protease extraction procedure. Gill samples containing worms were crushed by using a pestle until they were evenly mixed, then insert into a 2 ml microtube size and added Proteinase K as much as 20 µl, and ATL buffer solution as much as 180 µl, then incubated at 60°C for 24 hours (until the tissue dissolves). After that, added 200 µl of the AL buffer solution to the sample, and vortex for 15 seconds.

The next step is to add 200 µl 96% ethanol and mixed it by vortex for 15 seconds, and then spin it down. Then it is inserted into the QIAamp Mini spin column and centrifugation at 8,000 rpm for 1 minute. After the centrifugation process is completed, discard 2 ml collection tube containing the filtrate, and replaced it with a new 2 ml collection tube. The next step is to centrifuge at 13,000 rpm for 1 minute. The last step is incubating at room temperature (15-25°C) for 1 minute, then centrifuged at a speed of 8,000 rpm for 1 minute. After that, obtained 50 µl DNA Template (Whole DNA). DNA extraction follows the manufacturer's instructions with several modifications. The primers used in the amplification process are forward primers C1 (5′-ACCCGCTGAATTTAAGCAT-3′), and reverse primer D2 (5′-TGGTCCGTGTTTCAAGAC-3′). [4]

2.4. Research parameters

The research parameters observed were ectoparasite prevalence and intensity. The prevalence and intensity of parasites are calculated using the following formula: [5]

\[
\text{Prevalence} = \frac{\text{Diseased Fish}}{\text{Fish Taken}} \times 100 \%
\]

\[
\text{Intensity} = \frac{\text{Parasite Identified}}{\text{Infested Fish}}
\]

3. Results and discussion

3.1. Analysis of PCR

Based on Figure 1, parasitic DNA bands strung below the 1,000 bp region DNA markers indicate that the presence of molecules less than 1,000 bp in size, as well as the formation of clear, and thick bands in the region of about 700 bp, identified that the concentration of dispersed molecules in the region is
high. In this sample, there was a visible band at 748 bp. According to the NCBI GenBank database, the results of the study is suspected to be a parasite of the *Haliotrema susanae*. Based on morphological identification by using a binocular microscope equipped with Lucida camera and with identification key by Soo (2019) [4], this worms is identified as *Haliotrema susanae* (Figure 2).

**Figure 1.** The results of PCR analysis on the gill of Barramundi (*Lates calcarifer*)

legend: 1kb: marker, 2: gill sample

**Figure 2.** Morphology of *Haliotrema susanae*. Image was taken by a binocular microscope equipped with lucida camera. anterior attachment organ (AO); Eye spot (ES); Oral Sucker (OS); Pharynx (P); Male Copulatory organ (MC); Digestive tract (DT); Prostate Gland (PG); Uterus (U); Ovary (O); Testis; Haptor (H); Scelerites (S); Dorsal hamuli (DH); Ventral hamuli (VH). Scale bar: 30 μm.
3.2. Prevalence and intensity
Parasite Haliotrema susanae is ectoparasitic worm that attached on gill lamellae of Barramundi fish. Haliotrema susanae were often infested on gill lamellae. The prevalence and intensity of this monogenea parasite that was observed infested in Barramundi can be seen in Table 1. Prevalence level and intensity rate of Haliotrema susanae which infested Barramundi (Lates calcarifer) in Mari-culture Center, Lampung, Indonesia respectively were 80% and 28.96 parasites/fish.

Table 1. Prevalence and intensity of Haliotrema susanae which infested barramundi (Lates calcarifer) at Marine Aquaculture Center, Lampung, Indonesia.

| Floating Net Cage | Total Samples (Fish) | Total Infested (Fish) | Total Ectoparasite Worms | Prevalence (%) | Intensity (Parasites/Fish) |
|-------------------|----------------------|-----------------------|--------------------------|----------------|---------------------------|
| Mari-culture Center | 40                   | 32                    | 927                      | 80             | 28.96                     |

The results of the examination of 40 Barramundi measuring 25-35 cm showed 80% prevalence of Haliotrema susanae. According to Soo [4], this prevalence showed that the parasite Haliotrema susanae attack the gills of Barramundi fish. The result of this study indicate that the prevalence value of the parasite Haliotrema susanae on floating net cages is high. The presence of ectoparasite worms in the floating net cages activities is due to poor quality of broodstock management, and uncontrolled water quality because it depends on the season, and the environment of the water. In addition, the high density of parasites on floating net cages can stress the fish, allowing the parasites to develop rapidly where the high stocking density of fish causes competition for space, food and oxygen. Monogeneans that infested fish usually show behavioural changes such as lethargic and floating near the surface of the water. Infested fish get respiratory problems, where the respiratory rate can increase, caused the fish tolerance to low oxygen conditions. Fish with severe respiratory distress will be seen swimming to the surface of the water to breathe. Monogeneans tend to have a direct cycle, which means that no intermediate host is needed to reproduce, therefore the parasites can easily infest fish [6].

The appearance of parasites can be detected by using conventional PCR and Real-Time PCR methods. The result of conventional PCR is the observation of the presence of DNA through an electrophoresis process by using agarose gel [7]. The results showed that the DNA bands were stranded at 748 bp. According to GenBank NCBI data, the 748 bp band is owned by Haliotrema susanae. This also follows the results of research showing that the DNA band of 748 bp is a parasitic species of Haliotrema susanae which has an identity of 81%. This is different from the following Wu et al. [8] research which it showed a DNA band of 821 bp was a parasitic species of Haliotrema epinepheli. Whereas in other studies by Dang et al. [9] Haliotrema platychepali has a band 794 bp.

4. Conclusion
It is concluded that based on molecular identification of parasite Haliotrema susanae in barramundi (Lates calcarifer) in Lampung Waters were stranded at 748 bp and the prevalence level and intensity respectively are 80% and 28.96 (parasites/fish).

5. References
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