Molecular identification and prevalence of endoparasite worms in Silver pompano (*Trachinotus blochii*) in floating net cages of Mari-culture Center, Lampung

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Abstract. Silver pompano (*Trachinotus blochii*) is one type of mari-culture that has high economic value and both national and international markets. The cultivation technique of silver pompano (*T. blochii*) used in floating net cages is inseparable from the common problems in every cultivation activity. One of these obstacles is the problem due to the disruption of fish metabolism by foreign organisms such as parasites. Camallanus is a group of endoparasite worms class Nematoda that are often found in Indonesian waters. Camallanus usually lives in the host in the digestive tract, intestines, and anus. Camallanus is a group of endoparasite worms class Nematoda that are often found in Indonesian waters. Camallanus migrates to intestinal tissue and results in tissue damage[1].

1. Introduction
The Silver pompano (*Trachinotus blochii*) has good marketing prospects in the Asia Pacific region with a high economic value. The Lampung Center for Mari-culture Fisheries has succeeded in developing pilot cultivation or farming demonstration (Demfarm) of silver pompano with satisfactory results. The cultivation technique in floating net cages is inseparable from the common problems in every cultivation activity. One of these obstacles is the problem due to the disruption of fish metabolism by foreign organisms such as parasites. Camallanus is a group of endoparasite worms class Nematoda that are often found in Indonesian waters.
prevention and control in overcoming infection from Camallanus parasites, starting with soaking using acriflavin, an antihelminthic drug such as Levamisole, or with other chemicals. A correct diagnosis is needed, so it is necessary to identify the type of worm that infects based on the molecular analysis. Molecular biology can be used to support classical knowledge (classical taxonomy) about biodiversity, and then molecular biology is used for conservation purposes [2].

Polymerase chain reaction (PCR) is a biomolecular method that has been developed to detect parasite species based on size (bp) in gel electrophoresis. So far, research on endoparasites, especially Camallanus, in Silver pompano (T. blochii) fish is only limited to morphological identification. Meanwhile, the molecular identification of Camallanus parasites has not been widely carried out in Indonesia. The purpose of this study was to determine the species of Camallanus infected Silver pompano in the waters of Lampung, Indonesia, based on morphometric observations by using binocular microscopes, and molecular profiles by using the PCR test.

2. Material and methods

2.1. Experimental design

From 6 May - 11 Oct 2019, 45 Silver pompanos were collected in floating net cages of the Mari-culture Center, Lampung, Indonesia. The method used in this research was a descriptive exploratory method by taking a sample of fish measuring 15-25 cm with a purposive sampling method.

2.2. Specimen collection

The sample in this study was the parasite Camallanus. First, all of the parasites that assumed as Camallanus. The specimen was observed with a binocular microscope and identified the species characteristic. After washing in NaCl, the Camallanus specimen was fixed with sterile aqua dest 2.5ml for PCR preparation.

2.3. PCR preparation

DNA extraction stage used the QIAGEN Protease extraction procedure. Gill samples containing worms were crushed 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 (until the tissue dissolves). After that, we added 200 µl of the ATL buffer solution to the sample and vortex for 15 seconds. The next step is to add 200 µl of 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 centrifuged at 8000 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 centrifugation 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, we obtained 50 µl DNA Template (Whole DNA).

DNA extraction follows the manufacturer's instructions with several modifications. The primers used are Universal primers: ITS-1, 18S, and ITS-2, forward TW81 (5’ –GTGTCCTTAGGTGAACCTGCG-3’), and reverse AB28 (5’ –ATATGCTTAAGTTCAGCGGGT-3’). For control, the master mix is used without DNA. After the electrophoresis process is completed, the gel is removed using gloves, then immerse the gel in a solution of ethidium bromide 0.005% for 10 minutes. The final step is to observe the gel on the UV Transilluminator machine. Positive results of worm parasites when seen DNA band lines (bands) with a size of 700 bp – under 1000bp for worms Camallanus. Negative results if no DNA band lines (bands) are visible.

3. Result and discussion

3.1. Prevalence and intensity

In this study, an examination of the muscular organs of the inner wall of the abdomen, stomach, kidneys, liver, intestines, and anus was carried out. The results of identification of endoparasites found in the anus of Silver pompano in floating net cages the Mari-culture Center, Lampung, Indonesia are
species originating from Phylum: Nematelmintes, Class: Nematoda, Sub Class: Spirudida, Order: Camallanoidea, Family: Camallaninae, Genus: Camallanus, Species: C. carangis [3]. C. carangid is an endoparasite worm that is attached anus of Silver pompano fish. The prevalence and intensity of this parasite that infected Silver pompano fish can be seen in Table 1.

3.2. Analysis of PCR

Based on Figure 1, parasitic DNA bands strung below the 1000 bp region DNA markers indicate that the presence of molecules less than 1000 bp in size. In the BE6 sample, there was a visible band at 972 bp. According to the NCBI GenBank database and morphology, the number of DNA bands from the results of the study is suspected to be a parasite of the species C. carangis.

The result of examined 45 Silver pompano 15-25 cm in size showed an 8.8% prevalence of C. carangis. [4] this prevalence is classified as occasionally. All of C. carangis were found in the anus of Silver pompano.

Recent research related to Camallanus in Indonesia is still limited to identify intensity, prevalence, and morphology. The results of research by Hakim et al. [5] stated that based on the intensity level

![Figure 1](image-url)
Camallanus infects fish of 4 parasites/fish, which means very light. Meanwhile, according to Safitri et al. [6] showed the results of the morphological identification of endoparasites from the target organs, namely the fish intestines that were identified as C. carangis. The results of the study also stated that the prevalence value of Camallanus in silver pompano fish was 4.4%, and included in the infection criteria occasionally, it can be concluded that Camallanus worms are solitary worms, only infecting one type of host and the amount of abundance in nature is also small.

The results showed DNA bands were stranded at 972 bp, that C. carangis has a target size of around 700 bp under 1000 bp, but the PCR test sample shows a band in that figure. The results of the analysis help provide information on possible similarities of the resulting secondary metabolites with other species. A molecular study of the genetic structure of the Camallanus population was carried out by Wu et al. [7], who used the DNA sequence method on ITS rDNA. The Camallanus parasite appears at a fragment size of 743 bp on ITS1 rDNA. The results of these studies also show that there are different phylogenetic relationships between the same species in different geographic areas in China. C. carangis is characterized by the presence of a buccal capsule anteriorly. Each valve on the buccal capsule is provided with nine indentations. The posterior end is tapered, and there is an anal opening on the posterior side. This also supports the data from the molecular to differentiate in terms of morphology with guidance by Rigby et al. [3].

4. Conclusion
Based on molecular identification and prevalence, Camallanus examined from this research was identified as Camallanus carangis. It was stranded at 972 bp., prevalence level is 8.8% included in the category occasionally, and the intensity level is 4 parasites/fish.

5. References
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6. Acknowledgment
Thank you for the help of the technician of Mari-culture Center, Lampung.