

## Identification of Morphology of *Zeylanicobdella Arugamensis* in Tiger Grouper (*Epinephelus Fuscoguttatus*) Using Scanning Electron Microscope Method in Lampung Bay, Indonesia

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Submitted: 1 June 2022 Revised: 21 October 2022 Accepted: 26 October 2022 Publish: 30 October 2022

### Abstract

This research aims to study the morphology of *Zeylanicobdella arugamensis* infesting tiger grouper (*Epinephelus fuscoguttatus*) at Lampung Bay Waters by using scanning electron microscope (SEM). The samples of (*Epinephelus fuscoguttatus*) were obtained from body surface of tiger grouper (*Epinephelus fuscoguttatus*). *Zeylanicobdella arugamensis* infesting tiger grouper has an elongated body silindris with sucker in anterior and posterior. Based on the results of SEM, This parasite has a length of 4 - 4.1 mm and a width of 0.5 mm with anterior suckers and a gap in the middle surrounded by cuticle projections shaped like flower petals and white cilia with a diameter of 0.3 - 0.4 mm and posterior suckers part with a diameter of 0.9 - 1.4 mm where the size is larger than the anterior sucker. On the ventral surface of the body there is a transverse segmented coelom which is separated from the body cavity. Observations using the scanning electron microscope (SEM) method can help determine the body surface structure of the parasite because it has a higher resolution than a light microscope.

**Keywords:** Tiger grouper, *Zeylanicobdella arugamensis*, Scanning Electrone Microscope (SEM), Hirudinea

### INTRODUCTION

The Lampung Sea Cultivation Center is an implementing unit for hatchery technology activities and enlargement of marine fish commodities in Lampung Bay. One of the commodities developed at the Lampung Marine Cultivation Center is tiger grouper (*Epinephelus fuscoguttatus*). Tiger grouper (*E. fuscoguttatus*) is one of the commodities that has advantages such as relatively fast growth, easy adaptation to the cultivation environment so that it is suitable for cultivation on a small and large scale, and has a market share in the domestic and export scope (Heemstra and Randall., 1993).

The main obstacle to aquaculture production is death caused by disease. Economic losses due to disease outbreaks are quite significant and have an impact on the amount of

production, profits and sustainability of the cultivation system (Cao et al., 2007). One of the diseases that attack the tiger grouper (*E. fuscoguttatus*) is *Zeylanicobdella arugamensis*. This disease is often found in growing and developing fish, which are ectoparasites known as sea leeches. Fish infested with this parasite in the acute stage cause clinical symptoms such as weight loss, anemia, and cause secondary infection (Cruz et al., 2004).

Accurate identification is important to be able to determine the right diagnosis for the purposes of monitoring, surveillance, and control. The diagnosis that is often used is conventionally using a light microscope, while to determine the characteristics, morphology of the observed samples, an appropriate method is needed, one of which is the use of the scanning electron microscope

(SEM) method (Gestal et al., 1998). Based on what has been stated and the lack of information about *Zeylanicobdella arugamensis* that attacks tiger grouper, it is necessary to do research on the morphological profile of *Zeylanicobdella arugamensis* in tiger grouper (*Epinephelus fuscoguttatus*) using the scanning electron microscope (SEM) method.

## MATERIAL AND METHOD

### Area Study

This research was conducted at the Lampung Maritime Culture Center, Indonesia. Sampling was carried out in

tiger grouper floating net cages. Parasite staining was carried out at the Microbiology Laboratory of the Faculty of Fisheries and Marine Affairs, Airlangga University. The research location (Figure 1). Parasite drying for scanning electron microscope (SEM) examination was carried out in the integrated laboratory of the Faculty of Medicine, Airlangga University. SEM inspection activities were carried out at the Energy and Environmental Laboratory of the Sepuluh November Institute of Technology Surabaya from April to October 2018.

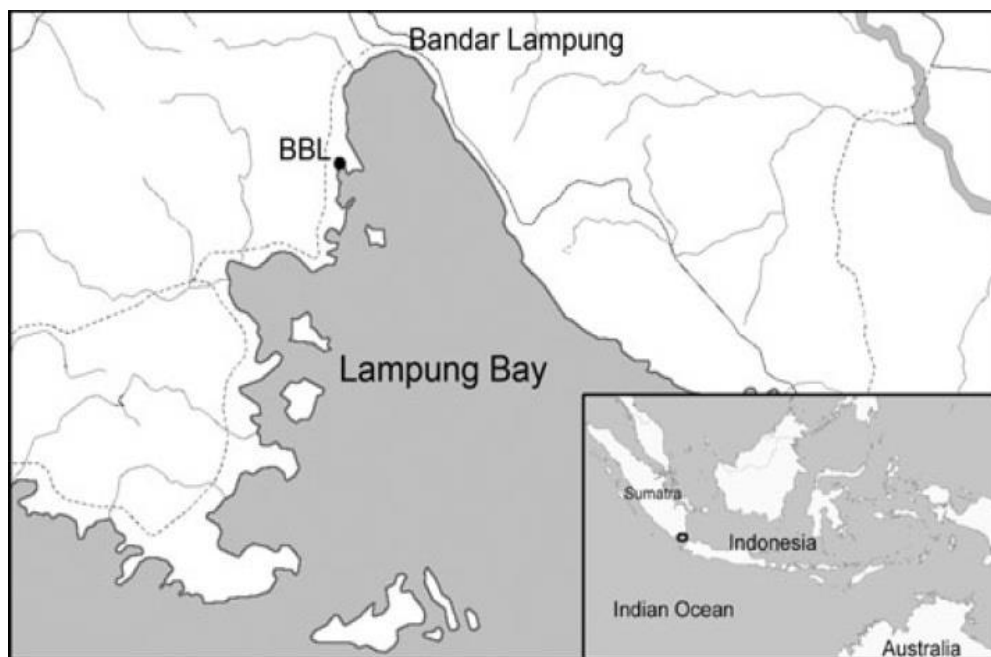


Figure 1. Location of sampling for floating net cages at the Center for Marine Cultivation Development (BBPBL) Lampung, East Java, Indonesia.

### Fish sample

This study used tiger grouper (*Epinephelus fuscoguttatus*) measuring 20-30 cm with a total of 35 individuals at the Lampung Marine Cultivation Center. Sampling of tiger grouper (*Epinephelus fuscoguttatus*) taken according to size from one floating net cage location.

### Sea leeches check

Examination of *Zeylanicobdella arugamensis* on fish samples was carried out using the native method according to Noga (2010). In summary, the tiger grouper was examined on the skin, fins, gills and mouth directly and followed by gentle scraping along the

sides of the body, the fins with a scalpel, while the gills were cut. The results of the scrapings were placed on a glass slide and physiological buffer solution (PBS) was added. The results of the scrapings were observed using a light microscope with at 40 and 100 times magnification. The sea leeches found at this stage were preserved in 5% glycerin alcohol until further use.

#### **Staining and identification procedures**

Staining of the parasite used the Semichoen-Acetic Carmine method referring to Kuhlman (2006) which was modified based on the type of ectoparasite species studied. The identification process was carried out by observing the shape and size of the parasite's body under a light microscope with a magnification times of 40, 100, 400, respectively. The ectoparasite identification keys used in this study were Margolis and Kabata (1984), Kabata (1985), Yamaguti (1963), Chandra (1991), De Silva (1963), and Azmey (2020).

#### **Scanning electron microscope**

The parasites found were fixed using a fixation solution in the form of 2% glutaraldehyde for 2-3 hours at 4°C. The fixed parasites were then washed using phosphate buffer solution pH 7.4 three times with each washing time of 5 minutes at 4°C. The parasites were put into a 1% post-fixation osmic acid solution for 1-2 hours at 4°C and the parasites were washed again as in the third step.

The following is a parasite dehydration with alcohol content of 30%, 50%, 70%, 80%, 90%, and 96%

respectively for 15 - 20 minutes. For 96% alcohol, washing was carried out twice. Parasites that have gone through the dehydration process are put into amyl acetate absolute as a preservative and then in the last stage the drying process is carried out using a critical point drying (CPD) tool. The specimen is attached to a stub and then the specimen is coated with pure gold or carbon using a Vacuum Evaporator. Finally the specimen is ready to be observed and the image is taken using SEM (Hoediasmor, 1985).

#### **Data analysis**

The analysis and presentation of the data in this study used a descriptive method. The data obtained in the form of a morphological profile of *Zeylanicobdella arugamensis* that infested tiger grouper (*Epinephelus fuscoguttatus*) using the scanning electron microscope method, presented descriptively in the form of images.

## **RESULTS**

### ***Z. arugamensis***

This worm parasite belongs to the hirudinea class with the characteristics of a segmented coelom. The class hirudinea is divided into two orders, namely Rhynchobdellae and Arhynchobdellae. The trunk (proboscis) is seen in Semichoen - Acetic Carmine staining and the body length reaches 4 – 10 mm, this is in accordance with the characteristics of order rhynchobdellae. The order arhynchobdellae is characterized by having no proboscis (proboscis) and a body length of 10 - 25 cm. The order Rhynchobdellae is divided into two families, namely

*Piscicolidae* and *Glossiphoniidae*. This parasite has an elongated cylindrical body shape and is divided into two parts, namely anterior and posterior which are often found as parasites on marine and freshwater animals, this is in accordance with the characteristics of *piscicolidae*. The family *glossiphoniidae* has a shape that is not cylindrical and is often found in its young form attached

to the human stomach (in the elderly). This parasite belongs to the genus *Zeylanicobdella* by having five pairs of testes on Semichoen-Acetic Carmine staining. *Zeylanicobdella arugamensis* species have two suckers in the anterior and the dorsal part which are wider than the ventral part, while the posterior sucker has a larger size than the anterior sucker.

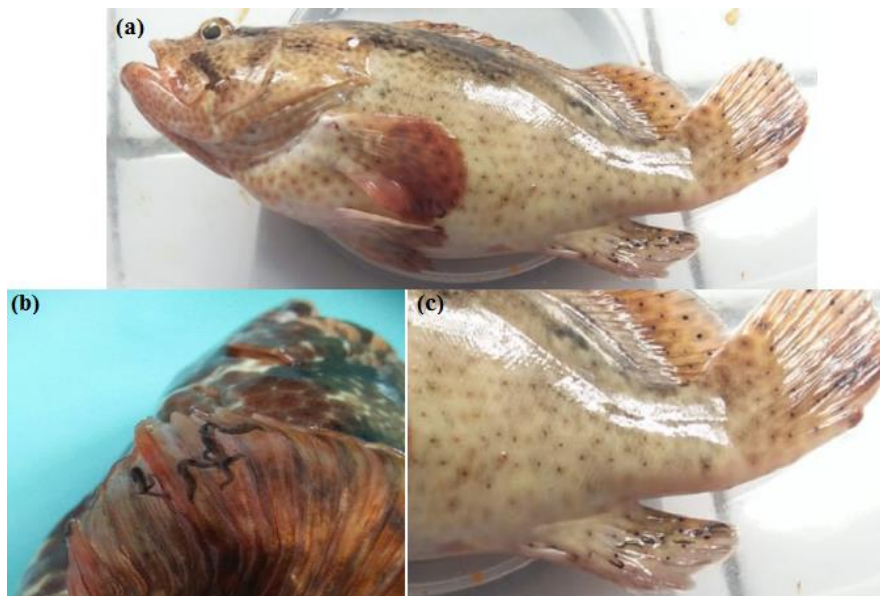


Figure 2. *Zeylanicobdella arugamensis*, in tiger grouper attached to pectoral fins (a), gills (b) and anal fins (c) in fish samples observations

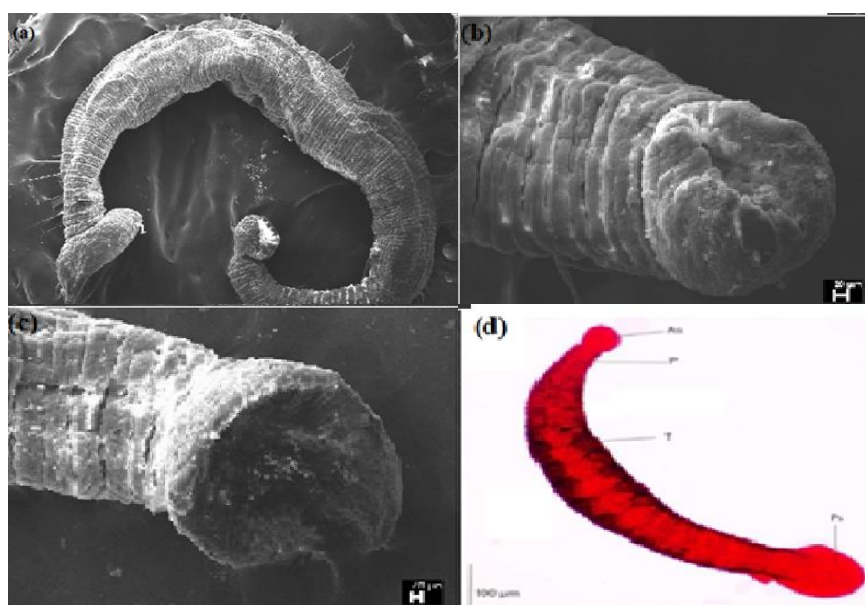


Figure 3. *Zeylanicobdella arugamensis* marine leech, (a) scanning electron microscopy *Zeylanicobdella*



arugamensis. as= anterior sucker, ps= posterior sucker. Scale = 200  $\mu\text{m}$ . (b) scanning electron microscopy of *Zeylanicobdella arugamensis* anterior suction. as= anterior sucker. Scale = 20  $\mu\text{m}$ . (c) *Zeylanicobdella arugamensis* posterior suction electron microscopy. ps = posterior sucker. Scale = 20  $\mu\text{m}$ . (d) semichoen-Acetic Carmine from *Zeylanicobdella arugamensis*. as = anterior sucker, p = proboscis, o = ovary, t = testicle, ps = posterior sucker. Scale = 100  $\mu\text{m}$ .

Measurement of the parasite *Zeylanicobdella arugamensis* in this study using Image J and Image Raster applications. The measurement results *Zeylanicobdella arugamensis* has a cylindrical body shape with a length of 4 - 4.1 mm and a width of 0.5 mm. The body shape of this parasite is shown in Figure 3(a). On the surface of the body of this parasite clearly visible to form a segmentation. In the anterior part, the sucker has an oval shape with a diameter of 0.3 - 0.4 mm and is surrounded by a flower-shaped cuticle and there are small white cilia with a length of 0.01 mm.

The shape of the sucker on the anterior part of the body (anterior sucker) is shown in Figure 3(b). Posteriorly there is a disc-shaped sucker with a diameter of 0.9 - 1.4 mm. Posterior body surface looks segmented and equipped with cilia in the form of small white spots that reach 0.01 mm in length. The shape of the sucker on the back of the body (posterior sucker) can be seen in Figure 3(c).

For Semichoen-Acetic Carmine staining, it has a cylindrical body shape with an anterior suction device equipped with an eyespot and a posterior suction device. On the ventral side there are 5 pairs of testes and proboscis. The results of Semichoen-Acetic Carmine staining (Figure 3d).

### ***Z. arugamensis* with SEM**

Identification of ectoparasite

worms was carried out based on morphological classification. This species belongs to the phylum Annelida, class Hirudinea, order Rhynchobdellida, family Piscicolidae, genus *Zeylanicobdella* and species *Z. arugamensis*. *Z. arugamensis* that infested tiger grouper at the Lampung Bay Marine Cultivation Center has a cylindrical body shape where the body surface has a segmented coelom (Chandra, 1991), stated that the hirudinea class has a segmented coelom (Figure 3a).

This parasite has a body length of 4 - 4.1 mm and a width of 0.5 mm, according to Chandra. (1991) who stated that parasites of the order Rhynchobdellae have a length of 4-20 mm. This worm is divided into two parts, namely anterior and posterior which are equipped with suckers. It was found to infest the tiger grouper, this is in accordance with Chandra, (1991) who stated that the piscicolidae family is often found as a parasite on marine and freshwater animals and has a suction device on the anterior and posterior (Figure 3a).

*Zeylanicobdella arugamensis* has an anterior sucker with an oval shape in the middle, has a slit and is surrounded by a flower-shaped cuticle protrusion (Figure 3b). The posterior sucker has a disc shape, which according to the characteristics of the Piscicolidae family (Figure 3c). The sucker functions as a locomotion and

attaches to the host to suck blood, as a food source for worms. Papillae seen on the body surface of this parasite are small round and have a diameter of up to 0.008 mm, this is in accordance with the statement of Chu et al., (2009) that the papillae of the *Zeylanicobdella arugamensis* parasite function as a mucus-producing agent as the body's adhesive to the substrate attached by the parasite. The visible cilia on the surface of the parasite's body are tiny white spots that serve as sensory organs up to 0.01 mm long.

According to Chandra (1991), that parasites from the hirudinea class are equipped with cilia as body sensors (Figure 3b). The results of Semichoen-Acetic Carmine staining show an anterior suction device, equipped with a pair of eyespots and equipped with stacked rods, this is in accordance Chandra's (1991), that in general, parasites of the order *Rhynchobdellae* have 1 - 4 pairs of eyes on the anterior sucker and trunk. In the ventral part of the parasite's body there are 5 pairs of testes, accordance in Chandra's (1991), that in general parasites of the genus *Zeylanicobdella* have 5 pairs of testes (Figure 3).

## DISCUSSION

Tiger grouper is a fishery commodity that has a high selling price. The production quality of tiger grouper may decrease as it is infested by parasites, one of which is *Zeylanicobdella arugamensis*. The level of parasite transmission can be influenced by several factors, namely the type of fish, the size of the fish, the

age of the fish and the geographical conditions in which the fish are cultivated. The sample of tiger grouper used has a size of 20-30 cm where this size has a large body surface with unfavorable conditions and environment that can help parasite penetration, especially *Zeylanicobdella arugamensis* (Stromnes and Andersen, 2003).

Fish infected by the parasite can cause a decrease in appetite, lesions occur on the body surface so that it can cause death (Ogawa, 2006; Hirayama et al., 2009). *Zeylanicobdella arugamensis* infesting tiger grouper at the Center for Aquaculture Fisheries (BBPBL) Lampung has a cylindrical body shape where the body surface has a segmented coelom, which is a characteristic of the class hirudinea with a segmented coelom. The segments on the body surface of this parasite reach 34 segments, that in general parasites from the Hirudinea class have 34 segments on the body surface. This parasite on its body surface is clearly visible with a segmented structure using a scanning electron microscope because the resolution of this method is higher than that of a light microscope (Sujanto et al., 2015).

This worm has a body length of 4 - 4.1 mm and a width of 0.5 mm, is a characteristic parasite of the order *Rhynchobdellae* with a length of 4-20 mm. This worm is divided into two parts, namely anterior and posterior equipped with suckers which were found to infest the tiger grouper. The piscicolidae family is often found as a parasite on marine and freshwater animals, and has suction devices on the

anterior and posterior (Chandra, 1991). *Zeylanicobdella arugamensis* has an anterior sucker with an oval shape and a slit in the middle surrounded by a cuticle shaped like a flower petal. The posterior sucker is disc-shaped, which is characteristic of the Piscicolidae family.

The sucker functions as a locomotion tool and attaches to the host to suck blood, as a food source for worms. Papillae seen on the body surface of this parasite are small round in shape and have a diameter of up to 0.008 mm, this is in accordance with the statement (Yusron, 1985; Chu et al., 2009) that the papillae of the *Zeylanicobdella arugamensis* parasite function as mucus-producing agents as the body's adhesive to the substrate attached by the parasite. Cilia are visible on the surface of the body of this parasite in the form of small white spots that function as sensory organs with a length of up to 0.01 mm.

## CONCLUSION

*Zeylanicobdella arugamensis* that infested tiger grouper at the Center for Marine Aquaculture (BBPBL) Lampung from the results of examination using a scanning electron microscope (SEM) had a cylindrical body shape with a length of 4 - 4.1 mm and a width of 0.5 mm. On the surface of the body of the parasite *Zeylanicobdella arugamensis* looks segmented with the number of segments filled with 34 equipped with cilia in the form of small white spots that function as sensory organs with a length of up to 0.01 mm and papillae with a diameter of 0.008 mm which produce mucus for

substrate adhesive attached by the parasite. The anterior part of the parasite is oval in shape and there is a gap in the middle with a diameter of 0.3 – 0.4 mm in contact with the cuticle protrusion which is shaped like a flower petal with a diameter of 0.3 – 0.4 mm. The posterior suction device is disc-shaped with a diameter of 0.9 - 1.4 mm, larger than the anterior suction device. Observations using the scanning electron microscope (SEM) method can help determine the body surface structure of the parasite because it has a higher resolution than a light microscope.

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