Morphological profile of L2 Anisakis typica on Indian Mackerel (Rastrelliger kanagurta) from Sedati Fish Auction, Sidoarjo-East Java, Indonesia using Scanning Electron Microscope (SEM)

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Abstract. The aim of this research was to determine Anisakis species that infected Indian mackerel fish in Fish Auction Sedati, Sidoarjo-East Java, Indonesia based on morphometric observation using a binocular microscope and morphological profile using SEM. This study was done on February-March 2019 with 30 Indian mackerel (Rastrelliger kanagurta) were collected from Fish Auction Sedati, Sidoarjo and examined for Anisakis. Anisakis that collected, observed using a binocular microscope and SEM. The result showed there was 10% prevalence of Anisakis. All of Anisakis examined were classified as Anisakis type 1. Based on the body length of Anisakis, all of Anisakis that collected were assumed as Anisakis L2. The conclusion based on morphometric and morphological characteristic showed that Anisakis examined were classified as L2 stage larvae of Anisakis typica.

1. Introduction
Anisakiasis is a zoonotic disease caused by Anisakis infection. The first anisakiasis case in Indonesia was reported by Uga et al. [1] in Sidoarjo. Further research about the identification of Anisakis on consumed fish in Sidoarjo has not been done. Anshary et al. [2] reported that 6 of 18 fish that sold in Makassar were positively infected by Anisakis. Among these six species, one species is widely sold in Sidoarjo, East Java. The fish species is Indian mackerel (Rastrelliger kanagurta). Anshary [3] and Setyobudi et al. [4] showed the presence of Anisakis typica infection in Indian mackerel from the species of Anisakis in Indian mackerel from Sidoarjo is still unknown.

Identification of Anisakis larvae can be done by morphological observation. Morphological observation for identification of Anisakis larvae can be carried out using native observation with binocular microscope and measurement of some body parts. Native observation using a binocular microscope cannot observe the ultrastructure of Anisakis larvae in detail.

Scanning Electron Microscope (SEM) can revealed the ultrastructure using 3D picture. SEM can be used to observe ultrastructure more detail than a binocular microscope. Ultrastructure features such as
boring tooth, mucron, mouth, lips and other organs can be studied more detail Roongruangchai et al. [5]. The aim of this research was to determine Anisakis species that infected Indian mackerel fish in Fish Auction Sedati, Sidoarjo-East Java, Indonesia based on morphometric observation using a binocular microscope and morphological profile using SEM.

2. Material and methods

2.1 Experimental design
During February-March 2019, 30 Indian mackerel fish were collected from Sedati Fish Auction, Sidoarjo, East Java, Indonesia. This research used a survey method with sampling a portion of the population considered to represent the population. Indian mackerel examined was 20-30 cm in size.

2.2 Specimen collection
The parasite that assumed as Anisakis was removed from the wall of the digestive tract (intestine) of Indian mackerel and washed in 0.9% NaCl. First, all of the parasite that assumed as Anisakis specimen was observed with a binocular microscope and identified genus characteristic, following Anderson [6]. After washed in NaCl, Anisakis specimen fixed with glutaraldehyde 2.5% for SEM preparation.

2.3 SEM preparation
Anisakis specimen was fixed in 2.5% glutaraldehyde for 3 hours. After the first fixation was done, specimen washed using phosphate buffer pH 7.4 for 5 minutes 3 times at 4°C. The second fixation was carried out using 1% osmic acid solution for 1-2 hours at 4°C. After the second fixation was done, specimen washed using phosphate buffer pH 7.4 for 5 minutes 3 times at 4°C. The preparation process was continued with dehydration in graded series of alcohol from 30%, 50%, 70%, 80%, 90% and 96% for 15 minutes 2 times at 4°C. Dehydrated specimens were put on Amyl Acetate Absolute. Preparation was continued with drying using Vacuum evaporator and coating with carbon. This SEM preparation was based on Sumarsono [7]. After that, Anisakis specimen was observed with SEM (Carl Zeise (EVO MA 10) British).

3. Results and discussion

3.1 Anisakis observation using binocular microscope
The result of examined 30 Indian mackerel showed 10 % prevalence of Anisakis. The observation using a binocular microscope (Figure 1.) showed boring tooth, nerve ring, oesophagus, ventriculus, intestine and mucron of Anisakis. Some of body part like a boring tooth, rectal gland and mucron were not obvious. The body length of Anisakis that obtained was 5.8 mm on average (4.7-7.7 mm).

![Figure 1. Anisakis observation using binocular microscope (Scale bar : 100 µm); A= Anterior part; B= Body part; C= Posterior part; BT = Boring tooth; NR = Nerve Ring; E = Esophagus; V = Ventriculus; I = Intestine; M= Mucron.](image-url)

Body width of Anisakis obtained was 0.17 mm on average (0.16-0.18 mm). Anisakis obtained from Indian mackerel had thin oesophagus and ventriculus. Ventriculus length was 0.35 mm on average (0.32-
0.37 mm). The length of the oesophagus was 1.05 mm (0.93-1.27 mm). The shape of *Anisakis* ventriculus that found was elongated. The shape of rectal gland of *Anisakis* obtained was not obvious.

### 3.2 SEM observation

SEM observation from the anterior part of *Anisakis* (Figure 2) showed poorly developed triangular lip with an open mouth and a boring tooth. The boring tooth was pointed. Length of the boring tooth was 9.97 µm. There were pairs of dome called papillae and amphid on the ventrolateral lip. Size of the papillae were 8.66 and 9.13 µm. Amphid was shaped like papillae but located in the middle of the ventrolateral lip and smaller than papillae. Size of the amphid were 4.62 and 5.19 µm. Between the ventrolateral lips, there was an excretory pore. The diameter of the excretory pore was 6.46 µm. The cuticle on the anterior part of the body looks like a transversal striation. Distance between each cuticle was 2 µm.

![Figure 2](image2.png)

**Figure 2.** Anterior part of L2 *Anisakis* (Scale bar : 10 µm); BT = boring tooth; M = Mouth; Am = Amphid; P = Papillae; EP = Excretory Pore; Cu = Cuticle.

In the middle part of *Anisakis* body (Figure 3,) there was deirid. Deirid was shaped like papillae but located in *Anisakis* body, near lateral line. Size of the deirid was 4 µm. The cuticle on the body part looks like a longitudinal striation. Distance between each cuticle cannot be measured because it was not clearly visible.

![Figure 3](image3.png)

**Figure 3.** Body part of L2 *Anisakis* (Scale bar : 30 µm); LL = Lateral line; D = Deirid; Cu = Cuticle.
In the posterior part of *Anisakis* body (Figure 4), there were some of the papillae-like structure and a mucron. The size of papillae-like structure was 2-6 µm. The shape of the tail was rounded and short. At the posterior end of *Anisakis* body, there was a mucron. The mucron was looked slightly bent. Length of the mucron was 7.99 µm. The cuticle on the posterior part of *Anisakis* body cannot be seen.

![Figure 4. Posterior part of L2 Anisakis (Scale bar : 20 µm); Mu = Mucron; P = Papillae-like structure.](image)

The result of examined 30 Indian mackerel (20-30 in size) showed 10% prevalence of *Anisakis*. According to William and William [8], this prevalence is classified as often. All of *Anisakis* was found in the wall of the digestive tract (intestine) of Indian mackerel. This was in accordance with Anshary [3] that *Anisakis* is generally found in the wall of the digestive tract, body cavity and liver of fish.

Identification of *Anisakis* using binocular microscope showed that all of *Anisakis* obtained were *Anisakis* type 1. This was based on the presence of mucron at the posterior end of *Anisakis*. The body length of *Anisakis* obtained was about 4-7 mm. Based on the body length, all of *Anisakis* obtained in this research were classified as L2. According to Smith [9], L2 *Anisakis* begin to moult into L3 *Anisakis* when 4 to 6 mm long and start to become L3 *Anisakis* when it reached 8.1 mm. *Anisakis* with a length of about 3-5 mm, has been found in the research of Abdou [10] and Puspitarini *et al.* [11]. Abdou [10] found 3-5 mm *Anisakis* on red sea fish in Egypt, while Puspitarini *et al.* [11] found 4.125 mm *Anisakis* on red snapper in Lampung, Indonesia. The shape of *Anisakis* ventriculus that obtained was elongated. This was similar to *Anisakis typica* on Borges *et al.* [12] research. Length of ventriculus *Anisakis* obtained was 0.35 mm on average. According to Anshary [3] research showed that *Anisakis typica* had 0.3-1.0 mm ventriculus length. Based on the ventriculus shape and length, all of *Anisakis* obtained were assumed as *Anisakis typica*. *Anisakis* obtained have a thin oesophagus. Smith [8] said that L2 *Anisakis* has a thinner oesophagus than L3. A rectal gland of *Anisakis* obtained was not obvious. Smith [8] research also showed that rectal gland of L2 *Anisakis* was difficult to observe.

SEM observation result showed that anterior part showed triangular lip, an mouth, a boring tooth, pairs of papillae, pairs of amphid and cuticle that visible. This characteristic was similar to L3 *Anisakis* characteristic. Smith [9] said L2 *Anisakis* that moult into L3 has some of L3 morphological characteristic. Papillae of *Anisakis* functioned as mechanoreceptors [9]. Amphid of *Anisakis* functioned as chemoreceptors [5]. The body part of *Anisakis* showed a deirid and cuticle. Deirid of *Anisakis* functioned was assumed as sensory receptor [5]. The cuticle on the body part was not too clearly visible. This might be because *Anisakis* was still in the moultng phase and that was not perfectly done. Posterior part of *Anisakis* was showed some of papillae-like structure and a mucron. The position of the mucron was on sideways of the posterior end. It was not in a suitable position. The cuticle on the posterior part was not visible. This also might be because *Anisakis* was still in the moultng phase and that was not perfectly done. Observation using SEM showed some similarities characteristic of *Anisakis* obtained with *Anisakis typica* from Borges *et al.* [12] research. The anterior part of *Anisakis* showed a poorly
developed triangular lip, single papillae on ventrolateral lips and pointed boring tooth. Posterior part of *Anisakis* showed a short and round tail with an mucron. The mucron was slightly bent.

Based on *Anisakis* life cycle, *Anisakis* generally found in fish was on L3 stage. This was in accordance to Anshary [3] that fish act as a second intermediate host that infected by L3 *Anisakis*. However, based on Abdou [10], Puspitarini *et al.* [11] and also this research *Anisakis* also allowed to infected fish in L2 stage. Cheng [13] said L2 Anisakid that live freely in water can also directly infect fish even though fish act as a second intermediate host.

4 Conclusion

Based on morphometric and morphological characteristic by SEM, *Anisakis* examined from this research was identified as *Anisakis typica* and still in L2 stage larvae.

5 References

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