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Anisakis Infection of Belanger's Croaker (*Johnius Belangerii* Cuvier 1830) at The Indian Ocean Coast of Yogyakarta, Indonesia

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Abstract

This research intended to find out the prevalence, intensity, and species of *Anisakis* (Nematoda) larvae infecting Belanger's croaker (*Johnius belangerii*) at Indian Ocean coast of Yogyakarta. Totally, 150 samples of Belanger's croaker fish collected from fishermen were used for this experiment. After measuring the total length and weight, each sample was examined for *Anisakis* larvae infection in the body cavity, internal organ, and muscle. Results showed that Belanger's croaker was susceptible to *Anisakis* infection by moderate prevalence (48.7%) with a mean intensity of 5.7 larvae/individual. All *Anisakis* larvae were found in the abdominal cavity (100%). Morphological identification by Scanning Electron Microscopy showed that the *Anisakis* larvae infecting the Belanger's croaker was *Anisakis* Type I, marked by a boring tooth on the anterior part and a mucron at the caudal end. Molecular identification applying PCR-RFLP of the ITS region, sequencing, and phylogenetic analysis of the mitochondrial DNA cox2 gene confirmed the larvae as *Anisakis typica* var. *indonesiensis*. The presence of *A. typica* in Belanger's croaker is estimated to pose a small risk to human health. *Anisakis typica* is known as not zoonotic *Anisakis* species. Moreover, the infection occurred in a relatively moderate prevalence, low intensity, and larvae were not found at the edible part of fish. From another point of view, these larvae are considered as useful biological indicators for several ecological parameters and further studies.

Keywords: Infection Rates, Prevalence, Zoonosis, Human Health Risk, Food Safety

1. Introduction

The genus *Anisakis* (Nematoda, Family Anisakidae) is strictly parasitic, infecting various marine organisms. These parasites are cosmopolitans and have a wide distribution, although the species are found in limited, partly overlapping, areas (Kuhn *et al*., 2013). *Anisakis* has been studied because of its zoonotic effect on human health, impact to the fisheries industries economy (Pozio, 2013), and was subject of ecological studies, e.g. for fish stock separation (Palm, 2011). *Anisakis* spp. are potentially zoonotic endoparasites; several species are known to cause the anisakiasis, a disease that is transmitted from fish to humans (Sakanari and Mc Kerrow, 1989). The 3rd-stage larvae of *Anisakis* spp. are transmitted to humans consuming raw or uncooked infected fish. Symptoms of *Anisakis* infection in humans include vomiting, nausea, diarrhea, or allergic reactions (Adroher and Benitez, 2020). Ecological studies used *Anisakis* as biological tags of fish migration and movement pattern, stock discrimination, recruitment, reproduction, and for food web structure analyses (MacKenzie, 2002; Podolska *et al*., 2006; Palm, 2011).

Nowadays, various fish species and cephalopods are infected with *Anisakis* larvae. *Anisakis* on fish/cephalopods have been well studied in Europe and America, and e.g. parts of Asia (Kuhn *et al*., 2013). Many pelagic and demersal fish species with high economic value, such as scad (*Decapterus* spp.), frigate tuna (*Auxis thazard*), mackerel (*Scomber* spp.), skipjack tuna (*Katsuwonus pelamis*), hairtail fish (*Trichiurus* spp.), and jack/trevallies (*Caranx* spp.), are potential hosts and have been reported infected with *Anisakis*. Several studies related to local Indonesian *Anisakis* species and infection patterns have been conducted (Hutomo *et al*., 1978; Setyobudi *et al*., 2007; Suadi *et al*., 2007; Palm *et al*., 2008; Setyobudi *et al*., 2011a; Anshary *et al*., 2014; Palm *et al*., 2017, Theisen, 2019), but the number of known local fish hosts is still relatively small when compared with the total number of marine fish species in Indonesian waters. There are 6 genotypes of *Anisakis* found in Indonesian and adjacent waters. Among these nematodes, *Anisakis typica* is the most common, and there are even some local genotypes that may differ from the genotypes that have been published so far. Therefore, in order to avoid identification confusion in the future, the Indonesian *A. typica* genotype was noted as the sub-specific entity namely *A. typica* var. *indonesiensis* (Palm *et al*., 2017). **S**imilar result was shown of *A. typica* T isolated from *Priacanthus tayenus* in Gulf Thailand, and that local

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genotype was considered not a variety but a distinct species status (Eamsobhana *et al*. 2018).

Belanger's croaker is a demersal fish, not yet reported to be infected with *Anisakis* in Indonesia (compare host-parasite checklist for Indonesia in Theisen-2019), even though several studies reported *Anisakis* infection was found on other demersal fish. Interestingly, *Anisakis* infections of Indonesian croakers (Sciaenidae) seem to be rare generally as listed in the local host-parasite checklist for fish parasites (Theisen 2019). Therefore, the detected presence of *Anisakis* spp. on Belanger's croaker makes this fish species a promising biological indicator for subsequent ecological studies (MacKenzie, 2002; Mattiucci *et al*., 2008, Palm 2011; Munster 2015), and can help to improve handling and processing of this consumable fish. The presence of *Anisakis* in marine fish can also be used as an indicator for fish quality. *Anisakis* infection in fish can cause aesthetic deterioration and reduce product value (Aspholm, 1995). Thus, fish with massive infections are regularly avoided by salesmen and consumers, consequently with economical loss, but also preventing human anisakiasis (McClelland, 2002).

Anisakis nematodes can be identified morphologically and molecularly. However, the larvae in fish are morphologically difficult to distinguish. Compared with morphological identification, molecular identification is more efficient and accurate and can overcome its limitations, because reference DNA regions for every valid *Anisakis* species are known and available (Mattiucci and Nascetti, 2008). Molecular techniques that can accurately identify the species of *Anisakis* have been developed rapidly and widely used, such as Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR-RLFP) and direct sequencing (D'Amelio *et al*., 2000; Nadler *et al*., 2005). PCR-RFLP of the Internal Transcribed Spacer (ITS) and 5.8S ribosomal DNA (rDNA) regions can be applied for *Anisakis* species identification (Umehara *et al*., 2006; Anshary *et al*., 2014). However, PCR-RFLP method cannot be used to study in detail the genetic variation of both intra and inter species. Furthermore, ITS region and mitochondrial DNA (mtDNA) cox2 gene analyses have been used to determine the genetic relationship and genetic variation between *Anisakis* species (Farjallah *et al*., 2008; Umehara *et al*., 2010; Eamsobhana *et al*. 2018). Recently, Mattiucci et al. (2017) reviewed and discussed taxonomical aspects related to biodiversity assessment, emphasizing on fish parasitic worm taxa recognized as biological species based on molecular/genetic markers.

2. Material and Methods

2.1. Fish Sampling and Anisakis Larvae Collection

This study sampled 150 Belanger's croaker fish (*Johnius belangerii*) (107 male and 43 female) caught by artisanal fishermen during February–August 2019 from the coast of Yogyakarta, Indonesia (Indian Ocean) (7°53′47″-8°11′56″ SL; 110°17′32″-110°47′32″ EL). The samples were identified based on Carpenter and Niem (2001), then measured for its total length using meter scale (accuracy 0.1 cm) and weight using electronic balance (accuracy 0.1 g), dissected, then observed for *Anisakis* at the abdominal cavity, liver, digestive tract, gonads, and muscle. The collected *Anisakis* were immersed and washed in 0.9% solution of sodium chloride and water, then stored in absolute ethanol for subsequent analysis (Setyobudi *et al*., 2019). The infection parameters, i.e. prevalence (P) and mean intensity (MI), were determined following Bush *et al*. (1997).

2.2. Anisakid Identification

2.2.1. Scanning Electron Microscopy (SEM)

Anisakis larvae were processed and cleaned at 4°C using cacodylate buffer for 6 h, prefixed using 2.5% glutaraldehyde for 12 hours, and then fixed with 2% tannic acid for 6 hours. After fixation process, the samples were washed in cacodylate buffer and gradually dehydrated in 30, 50, 70, 85, 90, and 99.6% ethanol. Specimens were positioned on the stub accordingly, and then covered with Au using Ion Coater SPT-20. The morphological characters of *Anisakis* were observed via a Hitachi SU-3500 SEM.

2.2.2. Molecular Identification

Twenty *Anisakis* larvae individuals were randomly selected for molecular identification. The DNA was extracted using Tissue/Blood DNA minikit extraction (Geneaid) by adhering to the manufacturer's procedure. The genomic DNA was used for further molecular analysis.

2.2.2.1. PCR-RFLP Analysis

The ITS region of rDNA (ITS-1, 5.8 S and ITS-2) of n = 20 worms was amplified using primer A (5′-GTC GAA TTC GTA GGT GAA CCT GCG GAA GGA TCA-3′) and primer B (5′-GCC GGA TCC GAA TCC TGG TTA GTT TCT TTT CCT-3′) (D'Amelio *et al*. 2000). The PCR conditions were: 94°C for 10 min for denaturation; 35 cycles of 94°C for 40 s, 54°C for 40 s, and 72°C for 90 s; and post amplification at 72°C for 7 min. Amplification products were analyzed by RFLP using *Taq*I, *Hha*I, and *Hinf*I DNA endonuclease restriction enzymes (D'Amelio *et al*. 2000). The digestion was carried out using 0.5 µL of endonuclease restriction enzyme, 1 µL of buffer, 3 µL of PCR product and 5.5 µL distilled water. Digestion with the *Hinf*I and *Hha*I enzymes was carried out at 37°C for 90 min, while digestion with the *Taq*I enzyme was carried out at 65°C for 90 min. The PCR-RFLP products were analyzed with 1.5% agarose gel electrophoresis with fluorosafe stain. The observed bands were used to determine the species following the previous research (D'Amelio *et al*., 2000; Anshary *et al*., 2014).

2.2.2.2. mtDNA cox2 Gene Sequencing

Polymerase Chain Reaction of the mitochondrial DNA cox2 gene (n = 4 worms) was conducted using primer 210 (5′-CAC CAA CTC TTA AAA TTA TC-3′) and primer 211 (5′-TTT CTA GTT ATA TAG ATT GRT TYA T-3′ (Nadler and Hudspeth 2000). The amplification was conducted under the reaction conditions: 94°C for 5 min; 35 cycles of 94°C for 40 s, 42°C for 40 s, and 72°C for 75 s; then post amplification at 72°C for 7 min. Then, the product was sequenced through a DNA sequencing service company (1stBase Laboratory in Malaysia through PT Genetika Science Indonesia). The results of nucleotide sequences were verified using BioEdit 7.0.4 software. The nucleotide's alignment was conducted using MEGA X,

followed by the construction of a phylogenetic tree based on mitochondrial DNA cox2 gene nucleotide sequences using the maximum likelihood method and Tamura-Nei model (Kumar *et al.*, 2018).

3. Results

3.1. Anisakis Infection

Belanger's croaker (*Johnius belangerii*, family Sciaenidae) is an economically important fish commonly caught locally. This demersal fish is distributed throughout Indonesian waters, inhabits coastal waters and estuaries, and feeds mainly on penaeid prawn and polychaeta (Simanjutak and Raharjo, 2001). In total, 150 fish samples with body length 14.6–28.2 cm and weight 35.6-339 g were collected from the coast of Yogyakarta, Indonesia. This research showed that Belanger's croaker was susceptible to *Anisakis* larvae infection. *Anisakis* infections of *J. belangerii* at the coast of Yogyakarta were moderate in prevalence (P) and mean intensity (MI) (P= 48.7%, MI=5.7 larvae/fish individual). The increasing prevalence and intensity of *Anisakis* in relation to increasing fish length are shown in Figure 1.

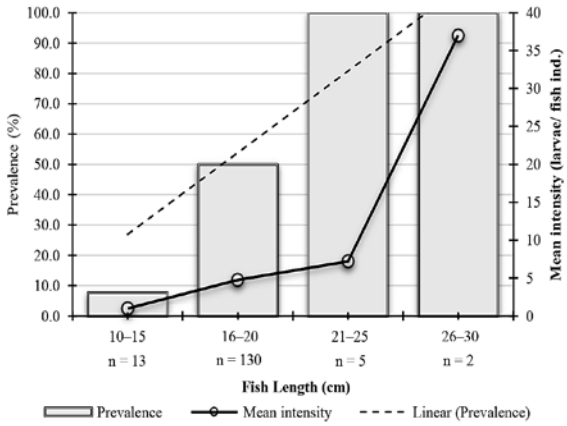

Figure 1. Infection rates of *Anisakis* on Belanger's croaker *Johnius belangerii* caught from the Indian Ocean coast of Yogyakarta

Figure 1 shows that the highest prevalence (100%) was observed in the fish with 21–25 cm length (W=109.7-121.8 g) (n=5), and 26–30 cm (W=282.0-339.0 g) (n=2), whereas the lowest prevalence (7.7%) was found in the fish with 10–15 cm body length (W=32.5-55.0 g) (n=13). The highest mean intensity of *Anisakis* infection was observed in the fish with 26–30 cm length (W=282.0-339.0 g) (37.0 larvae/fish individual), and the lowest was in the fish with 10–15 cm (W=32.5-55.0 g) (2.7 larvae/fish individual). These results indicate a relationship between the infection and body length. Both the prevalence and mean intensity of *Anisakis* infection tend to increase with increase in the body length. All *Anisakis* larvae were found freely or coiled the abdominal cavity and were not found in the other locations. The mean intensity distribution of *Anisakis* infection on Belanger's croaker is shown in Figure 2.

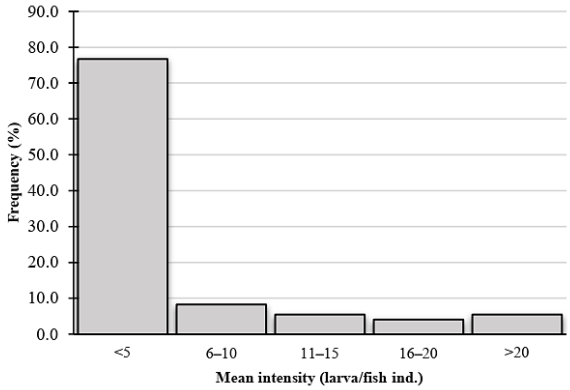

Figure 2. Distribution of intensity of *Anisakis* infection on Belanger's croaker *Johnius belangerii*

As shown in Figure 2, the collected Belanger's croakers were infected by *Anisakis* larvae in low intensity (<5 larvae/fish ind., 76.7 %), and only few were infected in high intensity (>20 larvae/fish ind., 5.5%).

3.2. Anisakis Identification

3.2.1. Scanning Electron Microscopy (SEM)

Morphological identification was carried out by analyzing four *Anisakis* samples via SEM (Plate 1).

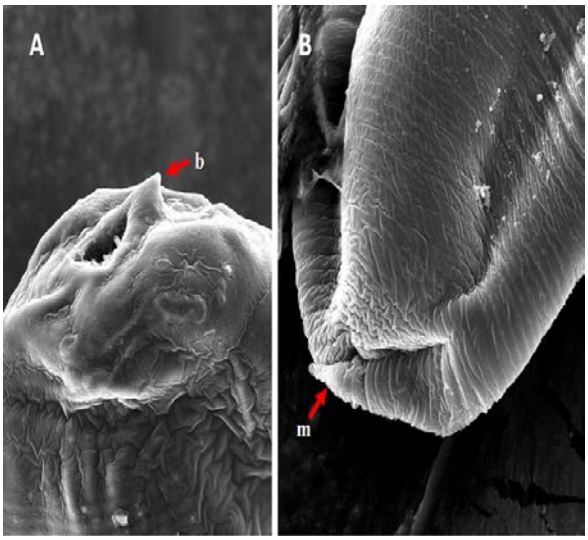

Plate 1. SEM Analysis (A: [b] = boring tooth; B: [m] = mucron) of Anisakis typica from Johnius belangerii

Figure 3 shows that *Anisakis* isolated from Belanger's croakers was Type I, marked by a boring tooth (b) on the anterior part and a mucron (m) on the posterior part.

3.2.2. Molecular Identification

3.2.2.1. PCR-RFLP Analyses

Amplification of the ITS region resulted in a band with a size of ~1 kb (Plate 2). The amplification product of the ITS region was then analyzed by RFLP. The results of digestion restriction enzyme are illustrated in Plate 3.

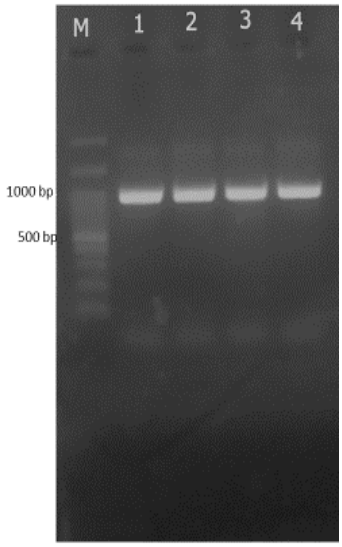

Plate 2. Electrophoresis visualization of the ITS rDNA region of *Anisakis typica* from *Johnius belangerii* (M=marker, 1-4: samples in this study)

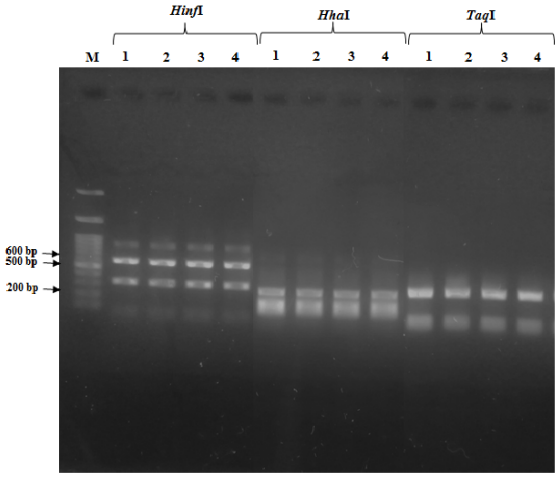

Plate 3. Visualization of digestion restriction enzymes of *Anisakis typica* from *Johnius belangerii* (M = marker, 1-4 = sample in this study)

Plate 3 shows the pattern after digestion of the PCR product using *Taq*I, *Hinf*I, and *Hha*I restriction enzymes. The digestion using *Taq*I formed two bands (400 base pairs (bp), 350 bp), *Hinf*I formed two bands (600 bp, 350 bp), and *Hha*I formed two bands (320 bp, 240 bp). All of the samples produce a similar pattern and corresponded to *Anisakis typica* (D'Amelio *et al*., 2000; Anshary *et al*., 2014).

3.2.2.2. Sequencing mtDNA cox2

PCR of the mtDNA cox2 gene showed band DNA with nucleotide length ± 600 bp. The electrophoresis results are shown in Plate 4.

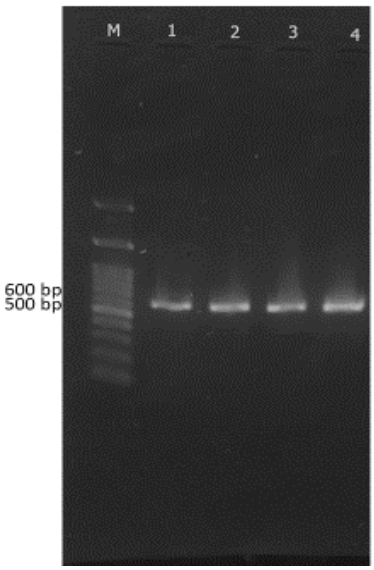

Plate 4. Visualization of *Anisakis typica* from *Johnius belangerii* electrophoresis with the mtDNA cox2 gene (M= marker, 1–4 = samples of this study).

Molecular identification using the mtDNA cox2 target gene confirmed that the *Anisakis* species infecting the Belanger's croaker was *A. typica* .

Anisakis infecting Belanger's croaker is similar *to A. typica* from the Philippines, the Persian Gulf, Thailand, Indonesia, Papua New Guinea, Egypt, Adriatic Sea, Brazil, and Japan. The phylogenetic tree constructed based on mitochondrial DNA cox2 genes sequences explains the genetic relationship of *A. typica* found in Belanger's croaker isolated from the coast of Yogyakarta with references from the NCBI GenBank (Figure 3). The phylogenetic tree shows that the *Anisakis* isolated from Belanger's croaker from Yogyakarta forming into two groups closely related to *A. typica* from the Philippines water, the Gulf of Thailand, the Papua New Guinea, and closely related to *A. typica* from the Persian Gulf.

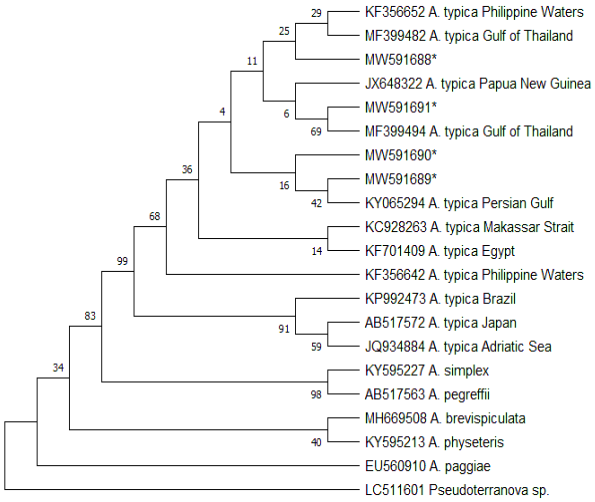

Figure 3. The molecular phylogenetic tree showing the genetic relationship among *Anisakis* species samples based on mtDNA cox2 genes. The phylogenetic tree was constructed using the maximum likelihood method according to the Tamura–Nei model (bootstrap=1000). The GenBank Accession numbers for the sequences from this study are: MW591688-MW591691. *= this study

4. Discussion

4.1. Locality and Host Records

Anisakis is a parasite infecting pelagic and demersal fish. Belanger's croaker *Johnius belangerii* is a paratenic host of *Anisakis* similar to other local demersal fish, such as *Trichiurus* spp., *Selaroides leptolepis*, and *Terapon jarbua* (Palm *et al*., 2017). It has never been reported to be infected in Indonesia (Theisen 2019). Belanger's croaker captured at the coast of Yogyakarta has a moderate prevalence and relatively high infection rate (P = 48.7%; MI = 5.7 larvae/fish individual). Previous studies reported variation in the prevalence, mean intensity and target organ of *Anisakis* larvae infection in marine demersal fish species. Setyobudi *et al*. (2011a) showed the differences of the prevalence of *Anisakis* infecting demersal fish caught at the coast of Yogyakarta; a high prevalence was found in Three-striped tiger fish (*Terapon jarbua*) (66.67%) with a mean intensity of 1.0 larvae/fish ind.

4.2. Host Feeding Ecology and Age/Size

For almost all fish species, host size (in terms of length and weight) is one of the main factors affecting parasitation. Larger fish preying on infected small fish, taking a higher risk of infection and acting as accumulating hosts compared to small host fishes, which are infected only by preying on the first intermediate host, namely Euphausiacea. The prevalence of *Anisakis* infection in Belanger's croaker caught in the waters of the coast of Yogyakarta increased with increasing body size of fish (Figure 1). Most studies on various fish species reported a positive correlation between the host body size with the prevalence and mean intensity of infection (Quiazon *et al*., 2009; Setyobudi *et al*., 2011b; Mladineo *et al*., 2012; Bao *et al*., 2015; Pierce *et al*., 2017; Debendetti *et al.*, 2019; Setyobudi *et al*., 2019). In general, large fish had more time in their life to accumulate *Anisakis* compared to small fish; thus, the former has a higher infection rate than the latter (Abattuoy *et al.*, 2011). In addition, the total amount of different food items consumed and food habits, and also switches in feeding ecology while growing bigger (Munster *et al*., 2015) affect the mean intensity of *Anisakis* infection on fishes. *Anisakis* infection occurs through predation; therefore, adult fish have a higher risk of infection (Mattiucci *et al.*, 2018). For example, cod from Greenland shows the apparent correlation between the size of fish and their food habits with parasitic abundance (Munster *et al*., 2015).

4.3. Microhabitats

The *Anisakis* infection of Belanger's croaker at the coast of Yogyakarta was restricted to the body cavity. In some fish species with high infection rates, *Anisakis* larvae can also be isolated from internal organ, such as the liver, gonads, mesentery, or are attached to the intestinal wall (Mattiucci *et al*., 2018). The variation of infection in different microhabitats could be determined by species and age of fish, parasite species, and the environmental condition of the host after its capture (Lymbery and Cheah, 2007). Similarly, several studies reported that *Anisakis* larvae are mostly found in the abdominal cavity, such as in *Trichiurus lepturus* from Bali (Semarariana *et al*., 2012) and in some fishes in the Makassar Strait (Anshary *et al*., 2014) and Spain (Debendetti *et al*., 2019).

Previous studies indicated that *Anisakis* dominates the host's body cavity, and only a small proportion is found in the muscle (Palm *et al*., 2008, 2017). However, most *Anisakis simplex* (s.s.) larvae, which is the *Anisakis* species with the highest zoonotic potential, causing most anisakiasis cases worldwide, that infect chum salmon (*Onchoryncus keta*) were found in the muscle (98%), and only few (2%) were found in the abdominal cavity and liver (Setyobudi *et al*., 2011b). *Anisakis simplex* and *A. pegreffii* larvae are also known to undergo a post-mortem habitat shift migrating from internal organs towards the muscle tissue of the fish host (Šimat 2015, Cipriani *et al.*, 2016), but this is not known nor assumed for *A. typica*. On the basis of the relative distribution of infection between the abdominal cavity and the muscle of the hosts, some *Anisakis* species migrate to the muscle stimulated by fatty acid content gradients (Smith, 1983), or thermophilic. Larvae of *A. typica* have been found in the muscle tissue of *Auxis rochei*, but only 2.5% (1/40) (Palm *et al.*, 2008).

4.4. Morphology and DNA

Morphological identification of *Anisakis* isolated from Belanger's croaker in the coast of Yogyakarta was led to Type I, which is indicated by a long ventriculus and the existence of a mucron (Berland, 1961). Up to now, the presence of *Anisakis* Type II has not been reported in the Indian Ocean from the south coast of Java (Theisen 2019). Klimpel and Palm (2011) stated that *Anisakis* Type II is not found in Asian waters but has a zoogeography restricted to the Central Atlantic and South Africa.

Currently, molecular methods such as DNA sequencing and PCR-RFLP are widely used to identify *Anisakis* species (D'Amelio *et al*., 2000; Pontes *et al*., 2005). In this study, restrictions of PCR product using three enzymes (*Taq*I, *Hinf*I, and *Hha*I) were successfully used to identify *Anisakis* larvae. PCR-RFLP analysis produces the banding pattern corresponding to *A. typica*. PCR-RFLP can be used to identify species generally by observing the bands formed by digestion of restriction enzymes on the specific site of nucleotide sequence on a certain species. This simple and cheaper method is more appropriate when used for the identify process in a large amount of sample. However, to study the genetic variations of a species, the direct sequencing method was commonly used. For *A. typica*, the digestion of the ITS region using *Hinf*I produces two bands (620 and 350 bp), that using *Hha*I produces four bands (320, 240, 180, and 160 bp), and that using *Taq*I produces two bands (400 and 350 bp).

The *Anisakis* nucleotide sequencing of the mtDNA cox2 gene produced around 590 bp. The nucleotide sequencing showed that the *A. typica* that infects Belanger's croaker at the coast of Yogyakarta has genetic diversity among samples. *Anisakis* isolated from Belanger's croaker from Yogyakarta formed two groups which are closely related to *A. typica* from the Philippines water, the Gulf of Thailand, the Papua New Guinea, and closely related to *A. typica* from the Persian Gulf. Besides, this *A. typica* slightly different from those isolated from Egypt, Brazil, Japan, Adriatic Sea, even other isolates from Makassar Strait and Philippines waters. This finding is in line with Palm *et al.* (2017) regarding the possibility that there are varieties/sister species of *A. typica* (var. *indonesiensis*) isolated locally from Indonesia and adjacent waters. Eamsobhana *et al.* (2018) reported the *Anisakis*

larvae isolated from *P. tayenus* show the similar genetic lineage as the *A. typica* var. *indonesiensis*. Due to the closely related between *A. typica* isolated from the Gulf of Thailand and this finding, supposed that *A. typica* var. *indonesiensis* was distributed from Indian Ocean coast of Yogyakarta in the south to the Gulf of Thailand in the north. Phylogenetic analysis is appropriate in investigating the genetic composition of the *Anisakis* population and its biology (Jabbar *et al*., 2013). *Anisakis typica* has a wide distribution; it has infected several marine fish worldwide circumequatorial, and showed a unique distribution pattern (Klimpel and Palm, 2011). It is distributed from 35–40°N to 36°S in warm and tropical climates. The adult stage of *A. typica* is found in marine dolphins (Mattiucci and Nascetti, 2008; Colon-Llavina *et al*., 2009; Iniguez *et al*., 2009; Klimpel and Palm, 2011). The larval stages are found in many marine fish species and are known to have infected several fishes in Western Indonesia, Japan, Taiwan, Papua New Guinea, the Adriatic Sea in Croatia, China, Brazil, Portugal, Morocco, Mauritania, and several regions in the Mediterranean Sea (Zhu *et al*., 2007; Chen *et al*., 2008; Farjallah *et al*., 2008; Palm *et al*., 2008, 2017; Umehara *et al*., 2010; Borges *et al*., 2012; Smrzlic *et al*., 2012; Koinari *et al*., 2013; Palm et al., 2017; Theisen 2019). Infection by *A. typica* was also reported in marine mammals, i.e. dwarf sperm whales, *Kogia breviceps* from the Brazilian Atlantic coast (Iniguez *et al*., 2011) and *Sotalia fluviatilis* from the Atlantic coast (Mattiucci *et al*., 2002).

4.5. Human Impact, Biohazard and Zoonosis

Anisakiasis, which is mostly caused by *A. simplex* and *A. pegreffii*, has been reported in various Asian and European countries (Baird *et al*., 2014; Aibinu *et al*., 2019). Anisakiasis caused by *A. typica* infection is uncommon. Reports on the presence of *A. typica* in humans suffering from anisakiasis are unknown; thus, its zoonotic effect may be ignored. However, Palm *et al.* (2008) indicated that aside from the body cavity, *A. typica* can also be found in muscles of fish hosts; therefore, these parasites might cause anisakiasis by way of consumption of raw or undercooked fish. Anisakiasis has been widely reported around the world (Lymbery & Cheah, 2007). More than 90% of anisakiasis cases originate in Asia, especially Japan, but several cases have also occurred from western countries (Audicana & Kennedy, 2008; Mattiucci *et al*., 2018). Report on human health risk due to *Anisakis* larvae infection in Indonesia is still rare. However, seropositive against excretory-secretory antigen of third-stage larvae of *Anisakis* spp. have been found from 11% of inhabitants in Sidoarjo, East Java (Uga et al., 1996).

This study indicated that various fish species in Indonesia are susceptible to *Anisakis* infection and new hosts can be recorded locally; however, still only a small number compared to the fish species inhabiting Indonesian waters is investigated. Therefore, further investigation is necessary to understand the geographical distribution, prevalence, and epidemiology of the local *Anisakis*.

5. Conclusion

Belanger's croaker at the coast of Yogyakarta was susceptible to *Anisakis* infection by a moderate prevalence (48.7%) with a mean intensity of 5.7 larvae/fish individual. All *Anisakis* larvae were found in the abdominal cavity (100%). Molecular analysis identifies those larvae as *A. typica (*var. *indonesiensis*).

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