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The Differences of *Anisakis* Larvae Infection on Scads (*Decapterus* spp.) in the Indian Ocean off the Southern Coast of East Java Indonesia

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ABSTRACT

This study investigated the *Anisakis* larvae infection in the scads (*Decapterus* spp.) in the Indian Ocean off the southern coast of East Java. A fish sample of approximately 450 individuals, consisting of three scad species, i.e., the Indian scad (*D. russelli*), the shortfin scad (*D. macrosoma*), and the redtail scad (*D. kurroides*), were obtained from Prigi and Muncar fishing port, East Java. Fish samples were measured individually for length and weight and subsequently dissected and examined in detail to detect the presence of *Anisakis* larvae in the abdominal cavity, intestinal tract, gonad, liver, and muscle. The collected larvae were preserved in 100% ethanol for the further identification process. The study indicates that the *Decapterus* spp. were receptive to the *Anisakis* larvae infection, with varying prevalence and mean intensity across species and locations. The redtail scad (*D. kurroides*) exhibited the highest prevalence and mean intensity; all of the sample were infected with *Anisakis* larvae (*P*= 100%), with a mean intensity of 22.54 larvae/individual.The shortfin scad (*D. macrosoma*), on the other hand, showed the lowest prevalence (*P*= 30.61%) and a mean intensity of MI = 1.29 larvae/individual. Most *Anisakis* larvae (52.30–76.61%) were located within the body cavity, followed by the digestive tract (16.78–18.40%), and only a small number of the larvae were detected in the liver, gonads, and muscles. The morphological identification indicated that the larva infecting the scad was *Anisakis* Type I, and it was confirmed as *Anisakis typica* throughout molecular identification. *Anisakis typica* parasitizes the redtail and shortfin scads in the Indian Ocean off the southern coast of East Java, indicating genetic variation, which is shown by the differences in nucleotide composition. The differences in *Anisakis* larvae infection and its genetic variation might be developed as biological indicators for various ecological investigations of the *Decapterus* spp.

INTRODUCTION

Anisakis, a genus member of the Family Anisakidae, is one of the nematodes that are commonly reported parasitizing of variety marine organisms. *Anisakis* has a complex life cycle and uses various organisms as hosts. The first stage occurs in the egg, while the

immature and adult stages (L4) live freely in the stomachs of cetaceans and marine mammals (definitive hosts) **(Della-Morte *et al.*, 2023)**. *Anisakis* exhibits a complex life process characterized by four moults **(Smith & Wootten, 1978)**. Through predation, fish and squid become infected with anisakid nematodes, but they are regarded as paratenic or transport host since the larvae do not experience any progress or moulting within them. The distribution of *Anisakis* has been investigated more in detail, and has a widespread distribution in the world; however, a particular species is distributed only in a restricted area **(Mattiucci &Nascetti, 2008)**. Morphologically, the nematodes of the genus *Anisakis* are characterized by the presence of a ventriculus and the absence of a ventricular appendix and intestinal caecum **(Quiazon *et al.*, 2020)**. According to the morphological characteristics, *Anisakis* can be grouped as Type I and II, distinguished by the ventriculus length and existence of mucron at the caudal end **(Berland, 1961)**. Today, nine species of *Anisakis* have been identified infecting diverse marine fishes using a molecular approach, i.e. *Anisakis* Type I includes six species: *A.simplex*, *A.pegreffii*, *A. typica*, *A. ziphidarum*, *A. berlandi* & *A.nascettii*, while *Anisakis* Type II includes: *A. brevispiculata*,*A.paggiae*, and *A. physeteris* Baylis, 1923 **(Mattiucci & Nascetti, 2008; Mattiucci *et al.*, 2014; Mattiucci *et al.*, 2018)**. However, up to now, the classification of certain species within the *Anisakis* genus has still been argued and discussed **(Safanova *et al.*, 2021; Takano & Sata, 2022)**.

The presence of *Anisakis* larvae in fishery products is an important problem for consumers, food safety authorities, and the fishing industry sector since it can cause disease and loss of value to the product. Humans can become accidental hosts when they consume raw or undercooked marine fish that are infected with third-stage *Anisakis* larvae. Acute anisakiasis can cause severe abdominal pain, nausea, and vomiting **(Martin-Carillo *et al.*, 2022)**. *Anisakis* not only causes parasitic diseases (anisakiasis) but can also cause allergic reactions **(Morozińska-Gogol, 2019)**. The presence of *Anisakis* larvae leads to pathological effects on infected fish, which can cause an inflammatory response around the larvae, necrosis, and degenerative changes in fish hepatocytes **(Hassan *et al.*, 2013)**. *Anisakis* infection in marine fish is a significant hazard for both human health and the safety of fisheries products. Humans become infected as accidental hosts for *Anisakis* nematodes due to consuming infected raw seafood. This infection can trigger acute gastrointestinal infections, the reactions of allergies, fever, vomiting, diarrhea, and nausea **(Audicana & Kennedy, 2008)**. *Anisakis* can produce a massive infection that enormously harms the host, reducing its visual appeal and resulting in financial losses. Aside from their detrimental effects, *Anisakis* larvae have been utilized as biological indicators in numerous ecological investigations, including those that identify dietary habits, migration patterns, and stock discrimination **(Moser & Hsieh, 1992; Pampillon *et al.*, 2002; Podolska *et al.*, 2006)**.

Anisakis larvae have been isolated in numerous species of marine fish in the Indonesian waters **(Palm *et al.*, 2017; Theisen, 2019; Ayun*et al.*, 2022; Setyobudi *et al.*,**

2023; Syarifah*et al*., 2023); however, their number is limited when compared to the number of fish species that inhabit the area. Understanding the zoogeography and epidemiology of *Anisakis* provides an important information for preventing negative impacts on human health and economic loss in the fisheries industry, as well as developing *Anisakis* as biological indicator.

The scads (*Decapterus* spp.), belonging to the Carangidae, are commercially important pelagic fish with high economic value in Indonesia, including the southern coast of East Java. This fish also makes a significant contribution to capture fisheries production and has been consistently caught for an extended period of time, both by semi-industrial fisheries (large and medium purse seines) and by artisanal fisheries (mini purse seines, seine net). Five species of the scad are primarily found in the Indonesian Sea, such as *D. lajang*, *D. russelli*, *D. macrosoma*, *D. kurroides*, and *D. maruadsi* **(Genisa, 1998)**. The scads are reported to be prone to *Anisakis* infection. Several studies have investigated the existence of *Anisakis* spp. on scad in Indonesia **(Anshary *et al*., 2014; Hafid & Anshary, 2017; Palm *et al*., 2017; Theisen, 2019)**. Additionally, they were investigated in other waters **(Arthur & Lumanlan-Mayo, 1997; Koinari *et al*., 2013; Tiempo*et al*., 2020)**. Currently, research on *Anisakis* larvae infecting the scads (*Decapterus* spp.) in Indonesia is still limited. The aim of this study was to determine the level of infection, specific target organs, and identify the *Anisakis* larvae that infect three species of scads from different locations in the Indian Ocean off the southern coast of East Java.

MATERIALS AND METHODS

1. Sampling and fish samples

A total of four hundred fifty samples of three species of the scad, which are the shortfin scad (*D. macrosoma*), the Indian scad (*D. russelli*), and the redtail scad (*D. kurroides*), were obtained from the southern coast of East Java, Indonesia. The fish samples were obtained from fishermen who captured the fish along Muncar Coast (Muncar fishing port Banyuwangi Regency) and Prigi Coast (Prigi fishing port Trenggalek Regency) (Fig. 1), with a total of 287 and 163, respectively (Table 1). The scads (*Decapterus* spp.) represent a commercially significant fish captured commodity in Prigi fishing port and Muncar fishing port, boasting a high production volume. During the 2017–2021 period, the total production volume of the scads at Prigi port reached around 2,500 tons **(Ginting *et al*., 2022)**. In February 2020, the scads became the fisheries commodity with the highest production volume at Muncar fishing port, reaching more than 235 tons **(Hadi *et al*., 2020)**.

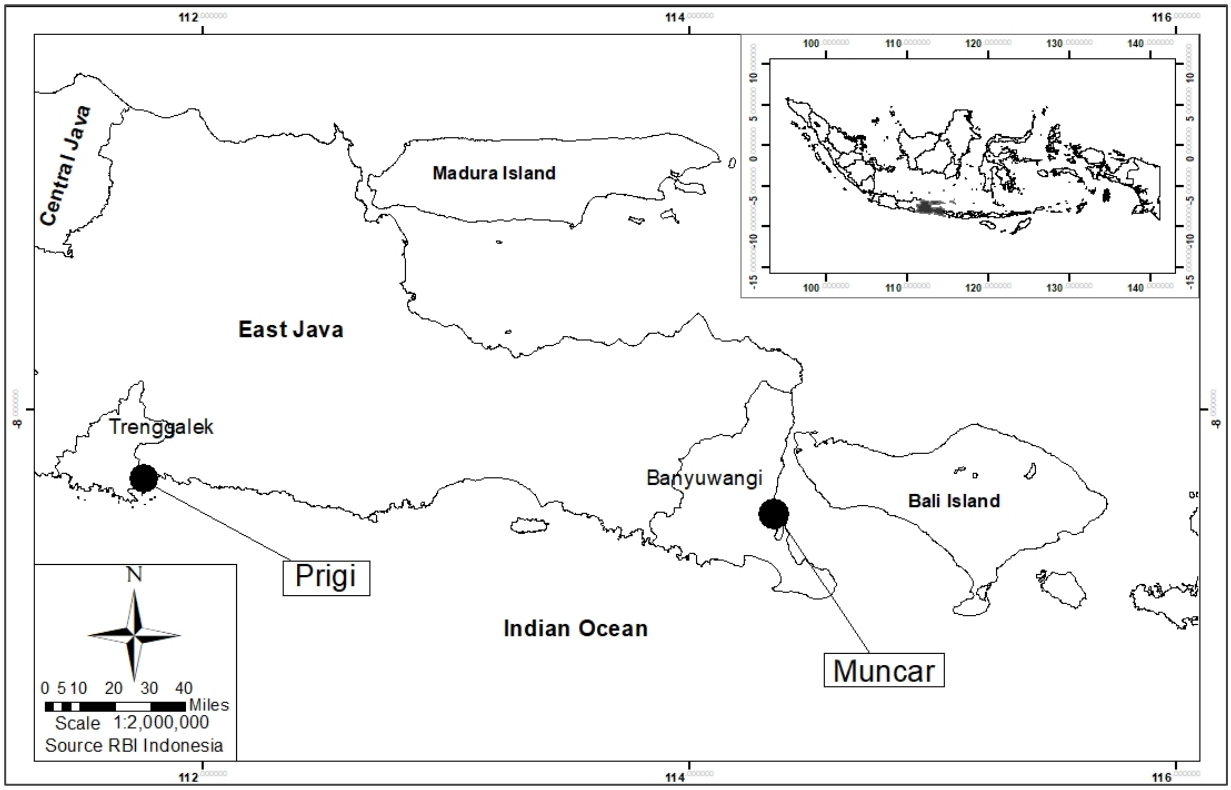

Fig. 1. Sampling location of *Decapterus* spp. in the Indian Ocean off the southern coast of East Java

Table 1. Number and length of the scads collected from the southern coast of East Java

No.	Fish name	Muncar fishing port	Prigi fishing port
		(n, TL)	(n, TL)
1	Indian scad (*D. russelli*)	110 (14.6-24.5 cm)	66 (16.1-26.0 cm)
2	Redtail scad (*D. kurroides)*	41 (18.0-41.5 cm)	65 (19.5-32.6 cm)
3	Shortfin scad (*D. macrosoma)*	136 (16.4-33.8 cm)	32 (19.5-33.3 cm)
	Total	287	163

2. Data collection

2.1. Sample observation and Anisakis collection

The total length of each sample was assessed using a ruler with an accuracy of 0.1cm, while the body weight was determined using a digital balancing with an accuracy of 0.1 g. The observation of *Anisakis* was done through dissecting and examining the internal organs of the fish. The organs that were observed are the body cavities, digestive tract, liver, gonads, and muscle. After cleaning with a 0.9% NaCl solution, the larvae

collected were then preserved in 100% ethanol. Prevalence (P) and mean intensity (MI) were used as parasite population descriptors **(Bush *et al*., 1997)**.

2.2. Morphology identification

A morphological identification was performed on 30 samples of *Anisakis* larvae collected from both Muncar and Prigi fishing port. The clearing solution (glycerin, lactic acid, phenol, and DW, in the ratio of 2: 1: 1: 1) was used for approximately 24 hours. The identification of nematodes was conducted following the manual of **Murata *et al*. (2011)**.

2.3. Molecular identification

Molecular identification of the selected samples was performed using PCR-RFLP analysis of the ribosomal DNA (rDNA) and direct sequencing of mtDNA (mitochondrial DNA) cox2 genes. The DNA of *Anisakis* larvae was obtained by extracting it using the Geneaid DNA Mini Kit (manufactured by Geneaid Company) in accordance with the extraction guidelines. The ITS region was amplified with primer A (forward) (5'-GTC GAA TTC GTA GGT GAA CCT GCG GAA GGA TCA-3'), and primer B (reverse) (5'-GCC GGA TCC GAA TCC TGG TTA GTT TCT TTT CCT-3'). The amplification products were subjected to RFLP analysis utilizing *Hinf*I, *Taq*I, and *Hha*I restriction enzymes **(D'Amelio *et al*., 2000)**. The mixture of 3µL of PCR product, 1µL of buffer, 0.5µL of restriction enzyme, and 5.5µL of distilled water was performed for the digestion process, as follows: 37°C for 90min (*Hha*Iand *Hinf*I enzymes), and 65°C for 90min (*Taq*I enzyme). The forward primers 210(5'-CAC CAA CTC TTA AAA TTA TC-3') and reverse primer 211 (5'-TTT CTA GTT ATA TAG ATT GRT TYA T-3') **(Nadler & Huspeth, 2000)** were used for the amplification process targeting mtDNA cox2 genes.

2.4. Data analysis

The DNA sequencing of the PCR product was conducted via a commercial service company (PT Genetika Science Indonesia). The sequence of mtDNA cox2 region was aligned with the published sequences in GenBank database, and the phylogenetic tree was created using the Mega software **(Kumar *et al*., 2016)**.The sequences of nucleotide were recorded in the GenBank with the Accession Numbers OQ390155-OQ390161.

RESULTS

Decapterus spp. in the Indian Ocean off the southern coast of East Java was susceptible to *Anisakis* larvae infection and showed differences in prevalence and mean intensity between species and regions. The *Anisakis* infection on the scads are shown in Figs.(2, 3).The prevalence and mean intensity of *Anisakis* larvae infection based on host length are shown in Fig.(4).

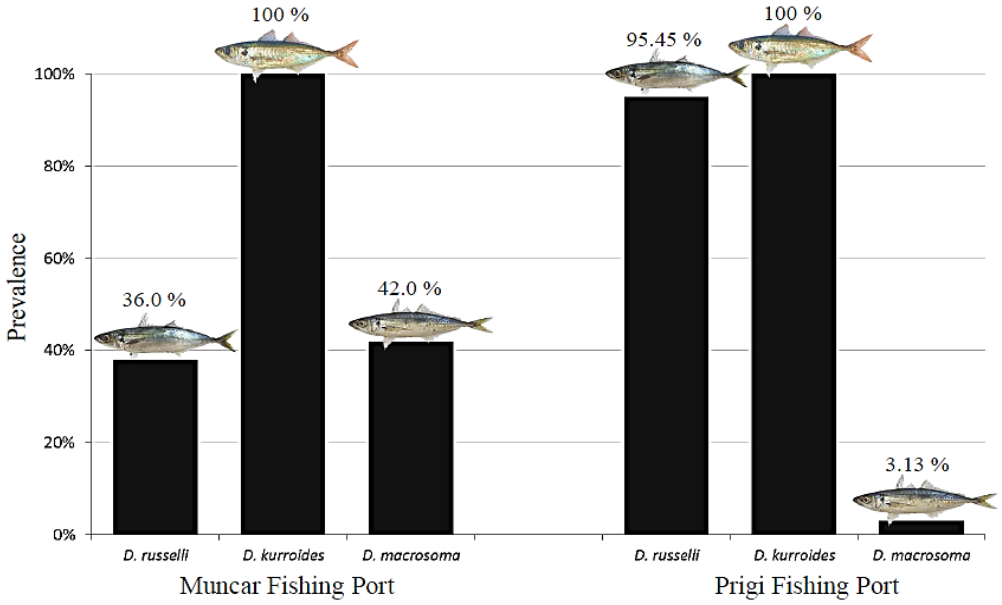

Fig. 2. The prevalence of *Anisakis* on the scad caught at the southern coast of East Java

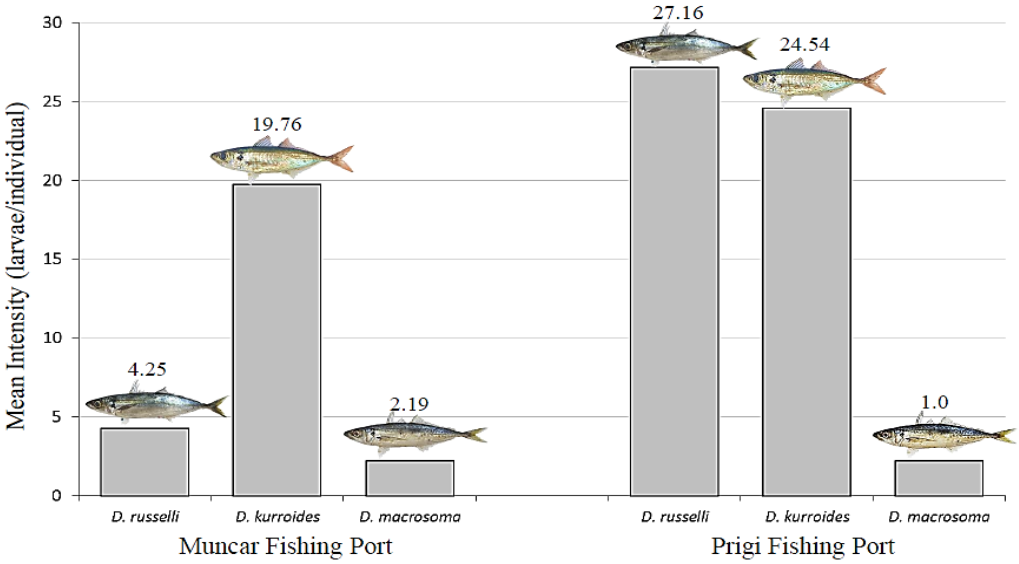

Fig. 3. Mean intensity of *Anisakis* on the scads from the southern coast of East Java

The prevalence and intensity of *Anisakis* larvae infection on scads from Prigi fishing port have a higher values than those from Muncar fishing port, except for the shortfin scad (*D. macrosoma*). The prevalence and intensity of the Indian scad (*D. russelli*) and the redtail scad (*D. kurroides*) vary greatly depending on where they are

found. *Anisakis* found on the redtail scad (*D. kurroides*) both in Muncar and Prigi fishing port has a very high prevalence (100%).

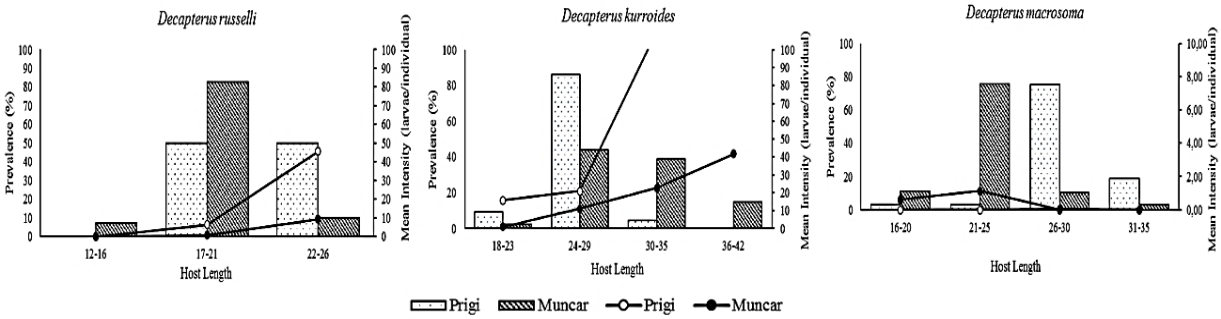

Fig. 4. Relationship between length and *Anisakis* larvae infection on the scads from the southern coast of East Java

The prevalence of *Anisakis* larvae in the scads (*Decapterus* spp.) did not show a positive correlation with the length of the host. The highest prevalence of *Anisakis* (86.15%) occurred in *D. kurroides* (TL 24–29cm), while the lowest prevalence (0%) occurred in *D. russelli* (TL 12–16cm). Meanwhile, the average intensity of *Anisakis* larvae infection in *D. russelli* and *D. kurroides* showed an increase with the increasing host size. The highest average intensity of *Anisakis* (110.33 larvae/ind) occurred in *D. kurroides* with a size of 30- 35cm. Most *D. russelli* and *D. kurroides* were infected with high-intensity *Anisakis* larvae (>20 larvae/ind.), while most *D. macrosoma* were infected with low-intensity *Anisakis* larvae (<5 larvae/ind.). No *Anisakis* larvae with an intensity of more than 16 larvae/ ind. were found to infect *D. macrosoma*. Fig. (5) details the intensity distribution of *Anisakis* larvae on the scads (*Decapterus* spp.) from the southern coast of East Java.

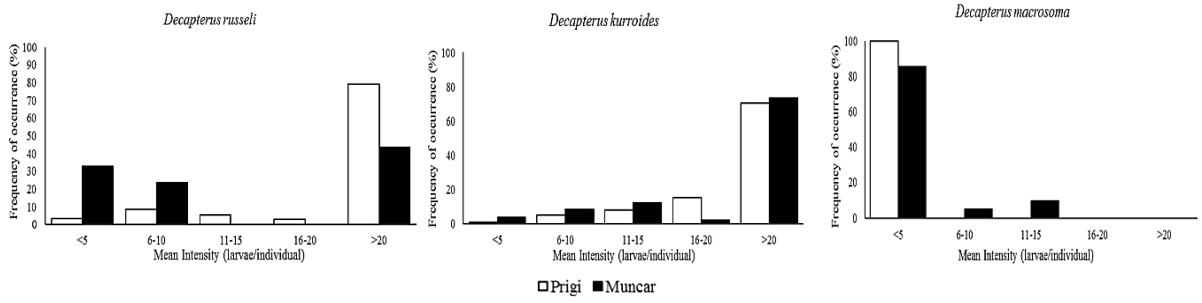

Fig. 5. Intensity of the distribution of *Anisakis* larvae infection on the scads from the southern coast of East Java

Anisakis infection in the scad (*Decapterus* spp.) was found in the body cavity, gonads, liver, digestive tract, and muscle. The distribution of *Anisakis* infection in target organs on scad (*Decapterus* spp.) is displayed in Fig.(6).

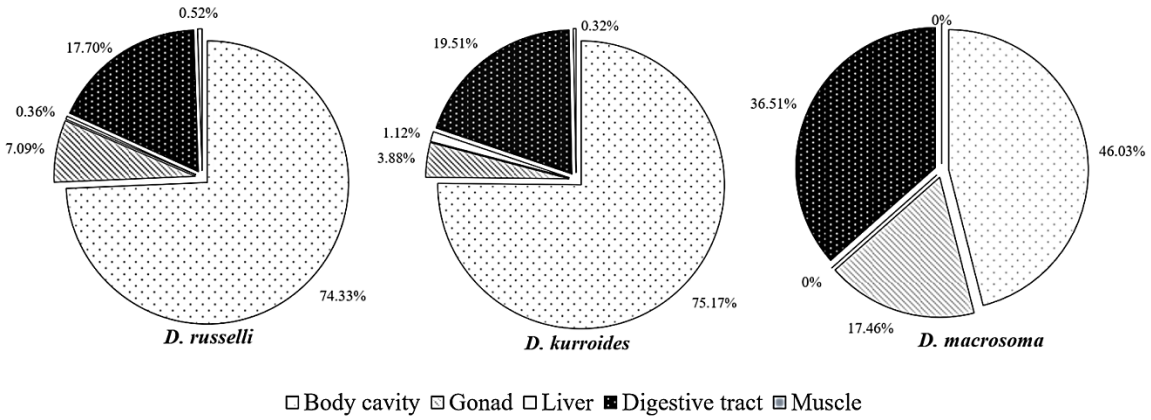

Fig. 6. Distribution of *Anisakis* infection on *Decapterus* spp.

The highest distribution of *Anisakis* infection on the scad was in the body cavity, followed by the digestive tract. Other organs in those scad fish species, i.e., gonads, liver, and muscle, were also infected by *Anisakis*, but only in a small percentage. In addition, in the redtail scad (*D. macrosoma*), there was no *Anisakis* infection in the liver or muscle.

Based on morphological identification, the *Anisakis* parasite found on the scad fish (*Decapterus* spp.) is *Anisakis* Type I, characterized by a long ventriculus and presence of the mucron at the caudal end. Molecular analysis by PCR-RFLP and direct sequencing were implemented to identify the *Anisakis* at the species level. PCR-RFLP analysis on the ITS region produced a different banding pattern, as shown in Table (2) and Figs.(7, 8, and 9).

Table 2. The banding pattern produced by PCR-RFLP analysis on the ITS region using restriction enzymes

Fish species	PCR-RFLP banding pattern			Conclusion
	***Hha*I**	***Hinf*I**	***Taq*I**	
Indian Scad (*D. russelli*)	160-180-240-320 bp	350-620 bp	350-400 bp	*A. typica*
Redtail Scad (*D. kurroides*)	160-180-240-320 bp	350-620 bp	350-400 bp	*A. typica*
Shortfin Scad (*D. macrosoma*)	160-180-240-320 bp	350-620 bp	350-400 bp	*A. typica*
Comparison	550-430 bp	370-300-250 bp	400-320-150 bp	*A. pegreffii*

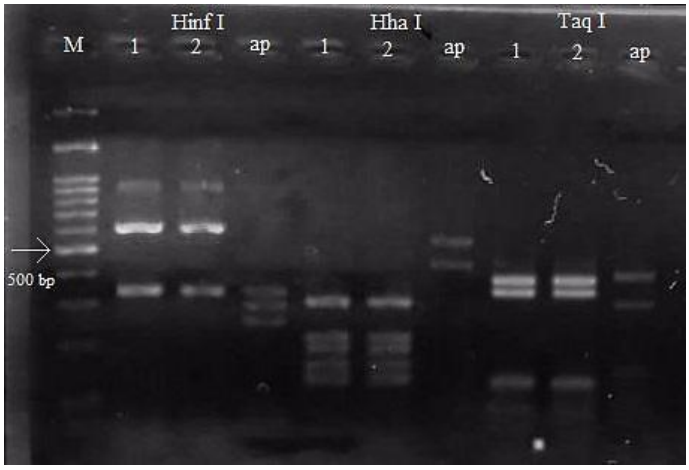

Fig. 7. Retriction fragment length polymorphism pattern obtained by digestion using restriction enzymes on the ITS region of *Anisakis* isolated from the Indian scad (*D. russelli*) (M = marker ; 1, 2 = sample from the Indian scad; ap = *A. pegreffii*)

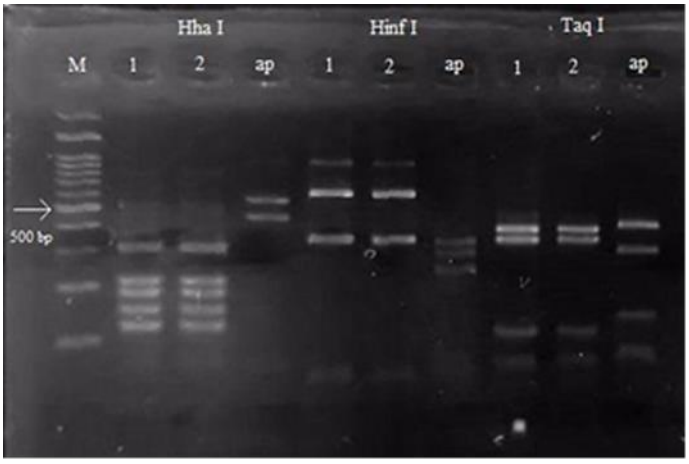

Fig. 8. Retriction fragment length polymorphism pattern obtained by digestion using restriction enzymes on the ITS region of *Anisakis* isolated from the redtail scad (*D. kurroides*) (M = marker; 1, 2 = sample from the redtail scad; ap = *A. pegreffii*)

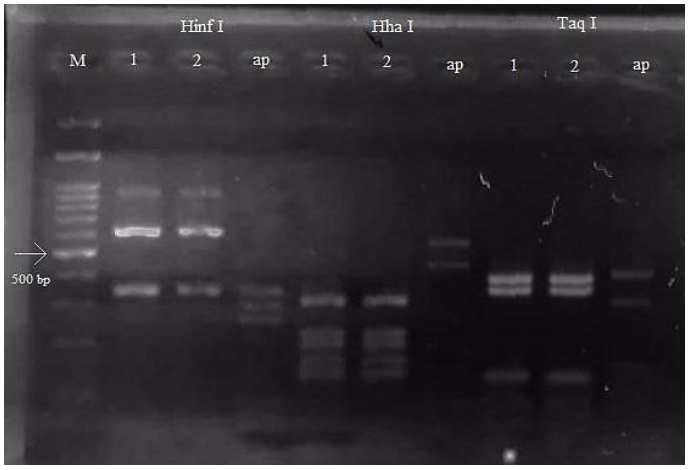

Fig. 9. Retriction fragment length polymorphism pattern obtained by digestion using restriction enzymes on the ITS region of *Anisakis* isolated from the shortfin scad (*D. macrosoma*) (M= marker; 1,2 = sample of the shortfin scad; ap = *A. pegreffii*)

Selected *Anisakis* samples from each species of *Decapterus* spp. were used for molecular identification using the direct sequencing method. The results of *Anisakis* nucleotide sequencing were then carried out by CONTIG using Bioedit software. The results were subjected to BLAST NCBI through the website ncbi.nlm.nih.gov to determine the types of *Anisakis* down to the species level.

Anisakis isolated from the Indian scad (*D. russelli*) from Prigi fishing port and Muncar fishing port resulted in nucleotide sequences of 584 and 559bp. The alignment of the nucleotide sequence of the *Anisakis* sample of the Indian scad (*D. russelli*) from Prigi fishing port and Muncar fishing port shows a 3-bp difference from the total 584bp nucleotide. *Anisakis* isolated from the redtail scad (*D. kurroides*) from Prigi fishing port and Muncar fishing port (1 and 2) resulted in nucleotide sequence lengths of 533, 584, and 569bp. The alignment of the nucleotide sequence of the *Anisakis* sample of the redtail scad (*D. kurroides*) from Prigi fishing port and Muncar fishing port shows a 27-bp difference from the total 569-bp nucleotide. *Anisakis* isolated from the shortfin scad (*D. macrosoma*) from Prigi fishing port and Muncar fishing port resulted in nucleotide sequence lengths of 569 and 571bp. The alignment result of the *Anisakis* sample on the shortfin scad (*D. macrosoma*) shows a 28-bp difference from the total 580-bp nucleotide. Molecular identification using direct sequencing confirms that *Anisakis* isolated from *Decapterus* spp. from the Indian Ocean off the southern coast of East Java is *Anisakis typica*. The phylogenetic analysis shows that all sequences are in the same group as *A. typica* and separated from other anisakid species (Fig.10).

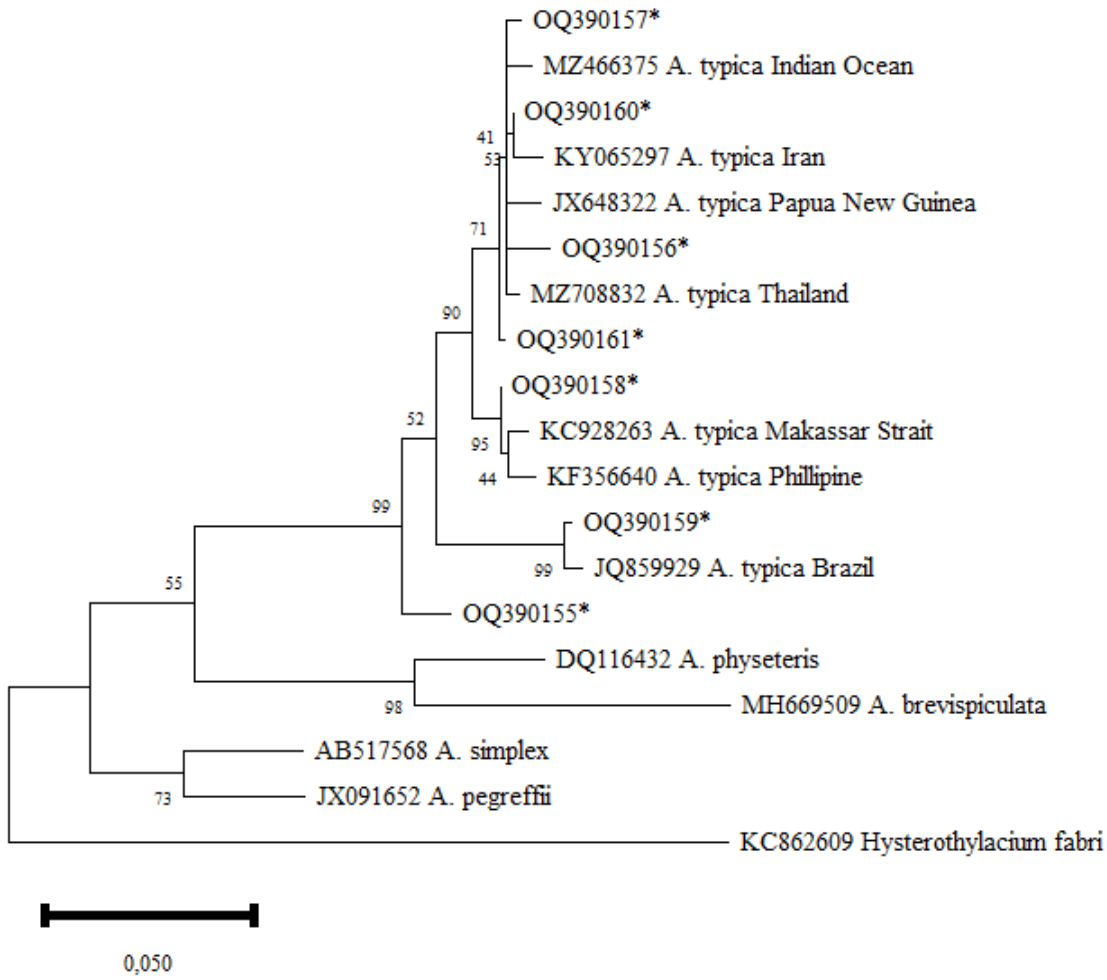

Fig. 10. The phylogram of *A. typica* on *Decapterus* spp. from the Indian Ocean off the southern coast of East Java (constructed using the Maximum Likelihood and Kimura 2-parameter methods)

Table 3. The genetic distance between *Anisakis* species found in this study with other anisakid nematodes based on mtDNA cox2

No.	Accession Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	OQ390155*	-																		
2	OQ390161*	0.037	-																	
3	OQ390158*	0.039	0.017	-																
4	OQ390160*	0.039	0.006	0.019	-															
5	OQ390157*	0.041	0.008	0.021	0.006	-														
6	OQ390156*	0.049	0.015	0.029	0.013	0.015	-													
7	OQ390159*	0.058	0.056	0.053	0.053	0.060	0.068	-												
8	MZ466375 (*A. typica*)	0.035	0.010	0.023	0.008	0.010	0.017	0.058	-											
9	JX648322 (*A. typica*)	0.037	0.009	0.023	0.008	0.009	0.017	0.053	0.011	-										
10	MZ708832 (*A. typica*)	0.037	0.008	0.021	0.006	0.008	0.015	0.060	0.010	0.009	-									
11	KC928263 (*A. typica*)	0.045	0.025	0.008	0.023	0.025	0.033	0.057	0.025	0.027	0.025	-								
12	KF356640 (*A. typica*)	0.045	0.023	0.006	0.021	0.023	0.027	0.055	0.025	0.025	0.023	0.009	-							
13	KY065297 (*A. typica*)	0.047	0.013	0.027	0.008	0.013	0.017	0.062	0.015	0.015	0.013	0.031	0.025	-						
14	JQ859929 (*A. typica*)	0.060	0.058	0.055	0.056	0.058	0.070	0.008	0.060	0.055	0.062	0.060	0.058	0.064	-					
15	AB517568 (*A. simplex*)	0.135	0.144	0.135	0.142	0.146	0.146	0.151	0.142	0.151	0.144	0.142	0.142	0.146	0.146	-				
16	DQ116432 (*A. physeteris*)	0.142	0.158	0.149	0.156	0.158	0.171	0.158	0.156	0.161	0.158	0.156	0.156	0.166	0.151	0.148	-			
17	MH669509 (*A. brevispiculata*)	0.201	0.204	0.199	0.201	0.201	0.204	0.201	0.199	0.206	0.204	0.199	0.207	0.209	0.199	0.180	0.111	-		
18	JX091652 (*A. pegreffii*)	0.119	0.130	0.135	0.132	0.132	0.139	0.141	0.128	0.132	0.130	0.137	0.142	0.137	0.135	0.065	0.139	0.175	-	
19	KC862609 (*H. fabri*)	0.241	0.238	0.235	0.241	0.241	0.241	0.257	0.238	0.240	0.238	0.243	0.244	0.243	0.257	0.221	0.267	0.255	0.226	-

DISCUSSION

The scad (*Decapterus* spp.) in the Indian Ocean off East Java's southern coast were susceptible to *Anisakis* larvae infection. The prevalence and mean intensity of *Anisakis* larvae were higher on the scad fish originated from Prigi fishing port than those sampled from Muncar fishing port, except for the shortfin scad (*D. macrosoma*). Specific to the redtail scad (*D. kurroides*), the fish samples from both fishing ports were infected with *Anisakis* larvae at a high intensity. However, the *Anisakis* larvae infection level in the the shortfin scad (*D. macrosoma*) was lower compared to other species. The differences in prevalence and mean intensity of *Anisakis* larvae might be affected by occurrence of small fish and crustaceans, which serve as food for the scads, and the presence of marine mammals as the final host. According to **Palm *et al*. (2008)**, the high infection rate of *Anisakis* in the north Bali Strait is related to the abundance of dolphins, which are the final hosts of *Anisakis*.

Anisakis infection in *Decapterus* spp. in the Indonesian Sea has been reported, and they exhibit different prevalence and mean intensity. The prevalence of *Anisakis* in the Indian scad (*D. russelli*) was reported in Panggang Island waters with a high value (54.20%) **(Hutomo *et al*., 1978)**, in Bali waters with a moderate value (28.6%) **(Palm *et al*., 2008)** in West Sulawesi with a low value (10%) **(Hafid & Anshary, 2017)**. Moreover, no infection was found in the Makassar Strait **(Anshary *et al*., 2014)**.

This study shows the variations in prevalence and mean intensity of *Anisakis* larvae infection compared to previous studies in different areas of Indonesia. The mean intensity of *Anisakis* in the Indian scad (*D. russelli*) was reported to have low values in the waters of Pulau Panggang (4.43 larvae/ind), Bali waters (1.9 larvae/ind), and West Sulawesi. (1.5 larvae/ind) **(Hutomo *et al*., 1978; Palm *et al*., 2008; Hafid & Anshary,**

2017). While the shortfin scad (*D. macrosoma*) was reported to be infected by *Anisakis* with a moderate prevalence (20%) and low average intensity (1.1 larvae/ind) in Bali waters **(Palm *et al*., 2017)**.The variation in prevalence and mean intensity might be caused by the differences and availability of the intermediate and definitive hosts of *Anisakis* in those particular areas.

This study shows differences in the prevalence and mean intensity of *Anisakis* larvae infection based on host length. The prevalence of *Anisakis* in the Indian scad (*D.russelli*) and the redtail scad (*D. kurroides*) in Prigi fishing port and Muncar fishing port was irregular; however, the mean intensity of *Anisakis* larvae infection had a positive trend in line with the host total length. The shortfin scad (*D. macrosoma*) has an irregular trend in both prevalence and mean intensity. The mean intensity of *Anisakis* infection on the Indian scad (*D. russelli*) and the redtail scad (*D. kurroides*) was positively correlated with the host size; the larger fish are more susceptible to infection than small fish. Larger fish are assumed to have lived longer; therefore, they have greater opportunities to be infected with parasites over their life span **(Al-Zubaidy, 2010)**. Several studies show a positive correlation between the total length of fish and the level of parasite infection in some hosts **(Palm *et al*., 2008; Setyobudi *et al*., 2011b; Ayun *et al*., 2021)**. The number and type of food consumed by the scad fish (*Decapterus* spp.) increase in accordance with their life cycle. *Decapterus russelli* in the Indian waters consumed shrimp, cuttlefish, and other smaller fish (piscivorous) **(Ashwini *et al*., 2016)**. Predation causes *Anisakis* infection, leading to the assumption that large fish have accumulated large amounts of food that could potentially harbor *Anisakis* larvae. Piscivorous will accumulate more *Anisakis* larvae if the prey infects fish or crustaceans through the food chain process without encountering molting **(Klimpel *et al*., 2004)**.

Anisakis larvae in the Indian scad (*D. russelli*), the redtail scad (*D. kurroides*), and the shortfin scad (*D. macrosoma*) were mostly found in the abdominal cavity, with a prevalence of 75.17, 74.33, and 46.03%; only a small number of larvae were discovered in other organs. The presence of *Anisakis* larvae in a particular organ is determined by their microhabitat conditions, which are an environmentthat supports parasites' lives, including the availability of food, oxygen, and other factors **(Smith &Wooten, 1978)**. Factors that influence the microhabitat of parasites are parasite species, fish species, age, environmental conditions, and the duration of parasites inhabiting the host **(Lymbery & Cheah, 2007)**. The majority of *Anisakis* larvae were found in the abdominal cavity, which might be caused by nutrient availability, including fat. The presence of nutrients is an important factor determining the parasite's survival ability **(Smith, 1984)**. Previous research indicates that *Anisakis* tends to occupy the outer wall of the intestine on the scad fish, suggesting a stronger predilection for this location compared to other organs **(Palm *et al*., 2008; Hafid & Anshary, 2017)**. In addition, other studies reported *Anisakis* predominantly found in the viscera or body cavity **(Setyobudi *et al*., 2007; Koinari *et al*., 2013; Anshary *et al*., 2014)**. The presence of *Anisakis* larvae in the abdominal cavity

indicates that the fish act as a paratenic host, and there is no molting from L3 to L4 **(Shih, 2004)**. *Anisakis* has the ability to move and can penetrate any part of the host's body, including the muscle. For example, *Anisakis simplex* (s.s.) was predominantly (98%) found in the muscle of the chum salmon (*Oncorhynchus keta*) **(Setyobudi *et al*., 2011a)**.

Based on morphological identification, the *Anisakis* found in the scads (*Decapterus* spp.) is *Anisakis*type I, which is distinguished by a long ventriculus and the existence of a mucron at the posterior end **(Smith &Wooten, 1978)**. *Anisakis* Type I larvae are characterized by elongated ventricles and mucrons at the tip of the tail, while Type II larvae have shorter ventricles and are without mucrons **(Martin-Carillo *et al*., 2022)**. Several previous studies suggested the presence of *Anisakis* Type I in the Indonesian waters **(Setyobudi *et al*., 2011b; Hafid & Anshary, 2017; Palm *et al*., 2017)**. The presence of *Anisakis* Type II is not yet reported in the Asian waters and is only found in the Mediterranean Sea, South African Continent, Florida Sea, and the Brazilian Sea **(Mattiucci & Nascetti, 2006)**. *Anisakis* larvae identification cannot be easily distinguished morphologically due to their small size and the limitation of essential characteristics as a distinction in taxonomic position; therefore, further identification through molecular methods is necessary. Molecular identification confirms that the *Anisakis* larvae infecting *Decapterus* spp. caught on the southern coast of East Java were *Anisakis typica*.

Sequencing results show differences in nucleotide base sequences between *Anisakis* samples from each *Decapterus* species. The differences in nucleotide sequences of *Anisakis* infecting scads indicate the existence of a hybrid genotype of *Anisakis* sp., or sibling species, which is morphologically the same species but different in genetics, physiology, ecology, reproduction, and behavior. The phenomenon of the hybrid genotype *Anisakis* sp. or *A. typica* sibling species has been reported in the scad fish (*D. ruselli*) in the Makassar Strait Sea **(Anshary *et al*., 2014)**. **Palm *et al*. (2008)** also stated that a 4bp nucleotide difference in the ITS1 region indicates the existence of *A. typica* sibling species.

This study also demonstrated the possibility of *A. typica* sibling species infecting the redtail scad (*D. kurroides*) and the shortfin (*D. macrosoma*) from Prigi and Muncar fishing port. However, until now, no other study has found that there are notable high differences in genotypes (27 bp and 28bp) in a sibling species, as presented by *Anisakis* infecting the redtail scad and the shortfin scad in East Java. The presence of sibling species might be caused by the migration of the intermediate host and the final host of *A. typica*. In addition, **Palm *et al*. (2017)** proposed the specification of *A. typica* under the name *A. typica* var. *indonesiensis* to reduce the possibility of confusion in identifying *Anisakis* larvae that infect fish in the Indonesian Sea due to the high similarity of existing genotypes.

Phylogenetic trees constructed from the mtDNA cox2 genes show cluster formation in *A. typica*. Previous studies revealed different subgroups of clusters

indicating *A. typica* sibling species **(Anshary *et al*., 2014)**. Other studies have shown the genetic differences in the clade *A. typica* in marine fish from Papua New Guinea **(Koinari *et al*., 2013)**. Phylogenetic analysis indicates a strong relationship between *A. typica* found in the scad (*Decapterus* spp.) from the southern coast of East Java and *A. typica* from the Persian Gulf, Iran. This closeness relationship of *A. typica* might be caused by geographic proximity, resulting in gene flow among the seas and the wide range of distribution of *A. typica* around the world, especially in tropical regions, as well as the migration of the final host, namely the dolphins.

The finding of *A. typica* in this study is in line with previous research. *Anisakis typica* was the predominant *Anisakis* species found in the Indonesia Sea, as represented in the Makassar Strait Sea **(Anshary *et al*., 2014)**, the Bali Sea **(Palm *et al*., 2017)**, the coast of southJava **(Setyobudi *et al*., 2019; Ayun *et al*., 2021; Ayun *et al*., 2022; Syarifah *et al*., 2023)**, and the coast of north Java **(Setyobudi *et al*., 2023)**. This species is also commonly reported to be infecting marine fish in tropical regions **(Kong *et al*., 2015)**, such as fish from the Philippines **(Quiazon *et al*., 2011)**, Papua New Guinea **(Koinari *et al*., 2013)**, and the Turkish waters **(Pekmezci *et al*., 2014)**.

The existence of *Anisakis* in Indonesia is related to the presence of its final host, namely marine mammals, such as whales and dolphins. The whale and dolphin migration pattern can spread *Anisakis* worms to other fish around the world. The adult *Anisakis* subsists in the digestive tract of marine mammals and releases the embryonated eggs into the water through the host's feces, and the crustaceans will engulf the eggs. Over than one-third of all known whales and dolphins have been found in Indonesia, which is dispersed throughout coastal to deep waters for migrating and settling **(Salim, 2011)**. The final host of *A. typica* was the dolphins from the Families Delphinidae, Phocoenidae, and Pontoporiidae **(Mattiucci *et al*., 2002)**. The distribution of various dolphin species in Indonesia (*Globicephala macrorhynchus*, *Stenella attenuata*, *Stenella longirostrid*, *Tursiops truncatus*, *Tursiops aduncus*, *Sotalia fluviatilis*, *Stenella coeruleoalba*, and *Steno bredanensis*) has been addressed by **Rudolph *et al*. (1997)**. The close relationship between *A. typica* isolated from the scad in the southern coast of East Java and *A. typica* in the Persian Gulf, Iran, might be caused by the migration and distribution of the final host of *A. typica*, namely dolphins (*Globicephala macrorhynchus*, *Stenella coeruleoalba*, *Stenella attenuata*, and *Tursiops aduncus*). In the Persian Gulf, *Globicephala macrorhynchus* dolphins have been reported **(Hammond *et al*., 2008)**, *Stenella coeruleoalba* **(Taylor *et al*., 2011)**, *Stenella attenuata***(Hammond *et al*., 2012)**, and *Tursiops aduncus* **(Jefferson *et al*., 2015)**, where the four species are also reported to exist in Indonesia and become the final host of *A. typica*.

The existence of *Anisakis* plays a role in the development of natural science about parasites, fishing industry and seafood safety issues, human health concern, and its utilization as a biological indicator for many ecological studies. Humans become accidental hosts and contract the disease by consuming raw or improperly prepared

marine fish or squids that are contaminated with the third-stage larvae **(Ivanovica*et al.*, 2015)**. *Anisakis* spp. can cause pathological effects in infected fish, which can cause an inflammatory response around larvae, necrosis, and degenerative changes in fish hepatocytes **(Hassan *et al.*, 2013)**. Anisakiasis disease has been reported from several countries, particularly those with a habit of consuming raw foods, especially Japan, Korea, and several European countries that cause gastrointestinal disease in humans **(Mineta *et al.*, 2006)**. The *Anisakis* species that infects humans the most is *Anisakis simplex***(Rosales *et al.*, 1999)**. An infection of *A. simplex* can be a potential threat to human health if raw, undercooked, or pickled fish is consumed **(Morozińska-Gogol, 2019)**.Although several fish species in the Indonesian sea have reported having *Anisakis* larvae, there have been no reports of anisakiasis in Indonesia.

Despite their negative impact on humans, parasites have been extensively employed in biological and ecological studies of marine ecosystems as biological indicators. *Anisakis* can serve as a biological indicator of its host's characteristics. These parasites have a crucial role as a biological indicator in the study of population structure, fish stock discrimination, and the organization of rational fishing **(Rybnikova *et al.*, 2018)**. The principle of utilizing parasites as biological markers is that fish can be infected if only they are in or pass through an endemic area of the parasite **(MacKenzie & Abaunza, 2005)**. The fish that are in or pass through a parasite-endemic area are most likely to prey on the infected host that inhabits it. In this case, fish were susceptible to being infected due to prey on contaminated fish. Anisakid nematodes of the genus *Anisakis* are a highly effective biological indicator among the several parasites for determining fish stocks **(MacKenzie, 2002)**.

Anisakis is utilized as a biological indicator in various ecological studies, such as fish stock characterization and discrimination, migration movements, and dietary and eating habits. The advantage of using parasites as biological indicators over artificial markers (tagging and marking) is that they are more appropriate for small fish species, crustaceans, and deep-sea fish species. Another advantage of using parasites as a biological indicator is that they are more economical and do not cause behavior to change, as with artificial markers **(MacKenzie &Abaunza, 1998)**. While the weakness of using parasites as biological indicators compared to artificial markers (tagging and marking) is that it requires completed data on each host's migration and feeding habits.

CONCLUSION

Decapterus spp. in the Indian Ocean off the southern coast of East Java were susceptible to *Anisakis* larvae infection with different prevalences and mean intensities. The redtail scad (*D. kurroides*) infected with *Anisakis* larvae had the highest prevalence and intensity, both originating from the Muncar and Prigi fishing port. The body cavity is the main organ target of *Anisakis* infection on the scads. Molecular identification

confirms that the *Anisakis* larvae infecting the scads from the southern coast of East Java were *Anisakis typica*.

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