Presence of *Anisakis* Larvae on the Demersal Fish Purple-Spotted Bigeye *Priacanthus tayenus* Richardson, 1846 From the North Coast of Java, Indonesia

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Abstract

The purple-spotted bigeye (*Priacanthus tayenus* Richardson, 1846) is a demersal fish species with significant commercial value, high market demand, and abundant production. The objective of this study is to investigate the occurrence and intensity of *Anisakis* larvae infection in purple-spotted bigeye from the northern coast of Java. This research provides information on the distribution of the *Anisakis* infection, which helps fisheries authorities to understand the risks to human health, reduce harm to the fishing industry, and use these parasites as signs of environmental health. In total, 407 samples of purple-spotted bigeye were gathered from the north coast of Java, namely Lamongan (East Java), Rembang (Central Java), and Indramayu (West Java). Fish were collected from the fishermen who landed the fish in the fishing port from July to October 2023. The highest prevalence of *Anisakis* larvae infection was found in purple-spotted bigeye caught from the north coast of Central Java (70.7 %), while the lowest prevalence of *Anisakis* infection was found in samples from West Java (34.8 %). However, the highest mean intensity of *Anisakis* infection was found in samples originating from West Java (16.45 larvae infected host^{-1}), and the lowest in fish from East Java (7.50 larvae infected host^{-1}). *Anisakis* larvae are mostly found in the body cavities of purple-spotted bigeye. Direct sequencing of the ITS-rDNA region confirms the *Anisakis* nematode larvae isolated from the purple-spotted bigeye were *Anisakis typica*.

Keywords: larval prevalence, intensity of infection, nematoda, Priacanthidae

Introduction

Anisakis (Nematode: Anisakidae) are common parasites of marine organisms world-wide (Mattiucci and Nascetti, 2007; Mattiucci and Nascetti, 2008; Dzido et al., 2009; Rokicki et al., 2009). *Anisakis* spp. has four larval stages (L1-L4) and uses marine crustaceans, cephalopods, fishes, and marine mammals to complete their life cycle (Smith and Wootten, 1978; Mattiucci et al., 2018). Anisakid nematodes can infect a broad range of aquatic organisms at various stages of their development, from an egg to an adult (Gregori et al., 2015; Angeles-Hernandez et al. 2020). As an intermediary host, marine crustaceans facilitate the growth and development of larvae to the point where the nematode can infect the final host. Fish and squid are paratenic/transport hosts for *Anisakis* larvae, while the adult stage in the digestive tract of marine mammals, that serve as a definitive host (Bello et al., 2021). Intermediate and paratenic hosts help spread temporal and spatial infections of anisakids, which makes it more likely that the infection will spread to the definitive host. Nine *Anisakis* species have been identified in different marine fish species around the world: these are *A. simplex* (Rudolphi, 1809) Dujardin, 1845; *A. pegreffii* Campana-Rouget and Biocca, 1955; *A. typica* (Diesing, 1860) Baylis, 1920; *A. ziphidarum* Paggi, Nascetti, Webb, Mattiucci, Cianchi & Bullini, 1988; *A. berlandi* Mattiucci, Cipriani, Webb, Paoletti, Marcer, Bellisario, Gibson & Nascetti, 2014; *A. nascettii* Mattiucci, Paoletti & Webb, 2009; *A. brevispiculata* Dollfus, 1966; *A. physeteris* Baylis, 1923; and *A. paggiae* Mattiucci, Nascetti, Dailey, Webb, Barros, Cianchi &

Bullini, 2005 (Mattiucci and Nascetti, 2008; Mattiucci et al., 2014, 2018).

The genus *Anisakis* has wide distribution and shows differences in host preferences and geographical distribution throughout the world. *Anisakis simplex* is widely distributed in Europe and America, although it is also found in several other regions. According to Farjallah et al. (2008), *A. pegreffi* is commonly found in demersal and pelagic fish in the Mediterranean Sea, while *A. typica* is the dominant species in the Asian region with tropical and subtropical climates (Borges et al., 2012; Anshary et al., 2014; Palm et al., 2017; Setyobudi et al., 2019). *Anisakis simplex* is most commonly found in fish that are nektobenthic or demersal in Galician waters, while in the Mediterranean, *A. simplex* hosts are mostly pelagic fish with a few demersal fish hosts (Abollo et al., 2001). Two sibling *Anisakis* species, *A. simplex* (s.s.) and *A. pegreffi*, dominate in distinct regions, which may indicate that their ranges are wider than previously thought. *Anisakis typica* was found to predominate in demersal fish hosts. The distribution of species in the Anisakidae family is enhanced by variations in oceanographic conditions, salinity, water circulation, and temperature brought on by global warming. *Anisakis* infection intensity and prevalence vary depending on the kind of host, time of year, and fishing location (Angeles-Hernandez et al., 2020).

Through the last decade, research has increased on fish and other marine organisms infected with anisakid nematodes. Because of their potential harm to human health, these parasites have emerged as one of the major groups that consumers should be aware of (EFSA-BIOHAZ, 2010). Fish infected with *Anisakis* larvae can transmit the zoonotic disease anisakiasis to humans (Aydin and Pekmezci, 2023). Humans become accidental hosts and contract the disease by consuming raw or improperly prepared marine fish or squid that are contaminated with third-stage larvae (Ivanovic et al., 2015). In the human body, parasites have a short lifespan and eventually perish within a few days or weeks. Parasites cannot survive in the human body for a long time; they will die in a few days or weeks. *Anisakis* larvae can cause gastrointestinal or allergy symptoms after their penetration into the human gastrointestinal tract, whose manifestations vary depending on the larvae species infecting and that impacted organ (Bucci et al., 2013).

The number of cases of anisakiasis has risen significantly globally due to the wide spread of *Anisakis* spp. and the rising popularity of raw fish or undercooked as a tasty food (Liu et al., 2020). Furthermore, anisakiasis has become a new focus of attention in tropical medicine today (Wiwanitkit and Wiwanitkit, 2016). *Anisakis* nematode presence in fish muscle is also unattractive to customers because it can lower the product's value (Aspholm, 1995). Due to their ability to cause human infections and allergies, nematodes belonging to the Genus *Anisakis* pose a significant threat to fisheries products (Gomes et al., 2020). In addition to their negative effects, anisakid nematodes are advantageous because they can be used as a biological indicator for studies on fish migration, feeding habits, and stock characterisation and discrimination (Podolska et al., 2006; Klimpel et al., 2007; Mattiucci and Nascetti, 2007).

The specific and accurate recognition is essential for parasite distribution studies and epidemiology. Identification of *Anisakis* to species level is important to assist in the diagnosis and treatment of infections, determine the source of transmission, and determine measures to control larval infections at all stages of fisheries production (Eamsobhana et al., 2018). Anisakid nematodes can be characterised both morphologically and molecularly. The identification of anisakid nematodes to the genus level is mainly determined by morphological characters, but identification to a lower taxonomic level solely based on morphological characters is difficult due to their small size and the limitation of taxonomically valuable characters, particularly in the larva (Olson et al., 1983; Szostakowska et al., 2002; Mattiucci and Nascetti, 2008). The Anisakidae family has similar and complex morphological characteristics that make morphological identification difficult, especially in the larval stage, therefore molecular identification is required (Pekmezci, 2014; Chaiphongpachara et al., 2022). In order to accurately identify and investigate the systematic evolution, molecular techniques have recently been established of anisakids.

The purple-spotted bigeye (*Priacanthus tayenus* Richardson, 1846; Perciformes) is a demersal fish with significant commercial value, strong market demand, and abundant production (Jabbar, 2017). These fish are distributed widely in the Indo-West Pacific, from the Persian Gulf to the western coast of India; in the East Pacific, from Taiwan southward to the Arafura Sea, and in northern Queensland, Australia. Purple-spotted bigeye inhabits coral or rocky areas, sometimes form aggregations, and smaller fish are mostly found inshore. Fish, crustaceans, and cephalopods are the primary food sources for this species (Ibrahim et al., 2003). Purple-spotted bigeye is one of the predominant demersal fish species in the Java Sea (Indonesian Fisheries Management Area 712). In Indonesia, species in the Priacanthidae had a high production level of around 30,000 tons in 2015 (Nugroho et al., 2017) and have been extensively used as fresh fish for direct consumption, processed into crackers or other fish products, and as a raw material for surimi (Estiyani et al., 2022).

There have been reports of *Anisakis* larvae infecting a variety of marine fish species and cephalopods. Kuhn et al. (2013) reported various marine fish to be susceptible to *Anisakis* infection. Among the fish order, Perciformes and Gadiformes are marine fish that are most frequently infected with *Anisakis* larvae. Nematode larvae were reported to infect *P. areanatus* in Rio de Janeiro, Brazil (Kuraiem et al., 2016). In addition, there have been reports of *Anisakis* larvae infecting *P. tayenus* in the Gulf waters of Thailand (Eamsobhana et al., 2018). A study on the anisakid infection in Indonesian marine fish has been carried out (Setyobudi, Soeparno, Helmiati et al., 2011; Palm et al., 2017). However, in comparison to the number of fish species and marine organisms that inhabit Indonesian waters, the number of studies is still quite limited. Palm et al. (2017) assert that despite the considerable marine biodiversity of Indonesia, data and information about marine fish parasites in Indonesian waters are still inadequate. The present study aims to determine the presence, infection levels, and target organs of *Anisakis* larvae in purple-spotted bigeye (*P. tayenus*), an economically important demersal fish on the northern coast of Java, Indonesia.

Materials and Methods

Ethical approval

No live animals were used in this study. This study used fish sourced from local fishermen and hence no animal ethics permit was required for this research.

Fish sample for nematode collection

A total of 407 purple-spotted bigeye (*P. tayenus*) samples were obtained from the northern coast of Java, namely Lamongan (East Java), Rembang (Central Java), and Indramayu (West Java), from July to October 2023 (Fig. 1). The fish samples that were obtained from the fishermen were taken using a cool box containing ice cubes to the laboratory and stored in the freezer for further observation.

Nematode collection

Fish were measured for total length (accuracy of 0.1 cm) and weight (accuracy of 0.1 g) (Table 1), then dissected for *Anisakis* larval examination. The digestive tract, liver, abdominal cavity, gonads, and muscle were all checked carefully for larvae. Muscle tissue was sliced thinly, then viewed under a cover slip on a slide with a light table to detect larvae. The collected nematode larvae were washed using a physiological solution (0.9 % NaCl), then grouped based on their external appearance, including features such as colour and size. *Anisakis* were kept for molecular analysis in collecting bottles that had been treated with absolute ethanol. The prevalence of larvae (the number of infected fish divided by the number of fish observed, expressed as a percentage), mean intensity of infection (average parasite infection in infected fish) (Bush et al., 1997), target organs of infection, and distribution of anisakids in various fish organs were documented.

DNA extraction

The DNA of selected samples was extracted using FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech. Taiwan) following the company protocols. Larvae were placed in a 1.5 mL Eppendorf tube and crushed until smooth using a micropestle.

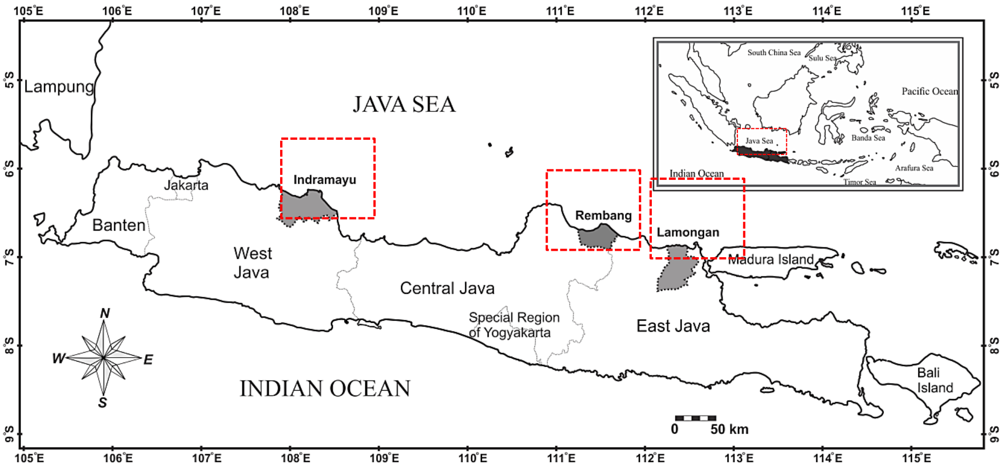

Fig. 1. Sampling locations (red dotted box) for purple-spotted bigeye (*Priacanthus tayenus*) in the northern coast of Java, Indonesia.

Table 1. Locations, the number of samples, fork length, and body weight of *Priacanthus tayenus* collected from the north coast of Java, Indonesia during this study.

Location	Number of samples	Fork length (mm) (mean ± SD)	Body weight (g) (mean ± SD)
East Java	139	227.5 ± 14.9	184.38 ± 31.01
Central Java	133	204.4 ± 18.0	137.13 ± 31.52
West Java	135	191.5 ± 24.8	110.05 ± 44.35

Then, as much as 200 µL of FATG1 buffer and 20 µL of Proteinase K were added and vortexed well. The product was then incubated gradually at 60 °C for 3 h, and then at 70 °C for 10 min. In the next step, as much as 200 µL of absolute ethanol was added and then vortexed. A total of 600 microliters of solution were transferred into a FATG mini column, which had been provided with a collection tube. The tube was centrifuged at a speed of 9,000 ×g for 2 min. After changing the collection tube, 400 µL of W1 buffer was added to the FATG mini column. The next process was adding 750 µL of wash buffer, then centrifuging again at a speed of 9,000 ×g for 2 min. After discarding the liquid in the collection tube, it was centrifuged again at a speed of 9,000 ×g for 6 min. Next, the collection tube was replaced with a sterile microtube. A total of 100 µL of elution buffer was added to the FATG mini column, and the sample was incubated at room temperature for 3 min and centrifuged at 9,000 ×g for 4 min.

Polymerase chain reaction: The ITS region of the rDNA amplification

The ITS (Internal Transcribed Spacer) region of the rDNA was amplified using primers A (5'-GTCGAAGGTGAATTCGTACCTGAAGCGGGATCA-3') and B (5'-GCCGGATCCTCCGAATGGTTATTCTTTTGTTCCT-3') (D'Amelio et al., 2000). Amplification was carried out using a thermal cycler (T100, Bio-Rad), with the amplification conditions as follows: pre-denaturation was carried out at 94 °C for 10 min, denaturation at 94 °C for 40 sec, annealing at 55 °C for 40 sec, extension at 72 °C for 90 sec with 35 cycles, and post-extension at 72 °C for 7 min. The PCR products were electrophoresed to determine the success of the DNA target amplification. The PCR product of the ITS region, was then sent to the 1st Base Laboratory in Singapore via PT. Genetica Science Indonesia for the nucleotide sequencing process. The nucleotide sequences obtained were then aligned using BioEdit software and compared with data available in GenBank to determine the *Anisakis* species. The nucleotide sequences were registered in GenBank, with the Accession Number PP211958-PP211966. The phylogenetic tree was constructed using MEGA11 software with the Kimura 2-Parameter Model method with 1000 bootstraps.

Results

Anisakis nematode larvae found in purple-spotted bigeye (*P. tayenus*) in the northern waters of Java differed in prevalence and average intensity at each of the three sampling locations (Table 2; Fig. 2). In total, 2188 nematodes were found from 407 purple-spotted bigeye (*P. tayenus*). The highest prevalence of *Anisakis* larvae infection was found in samples from Central Java (70.7 %), while the lowest prevalence was found in samples from West Java (34.8 %). However, the mean intensity of *Anisakis* larvae infection was highest in fish from West Java (16.45 larvae infected host^{-1}), while the mean intensity was lowest in samples from East Java (7.50 larvae infected host^{-1}).

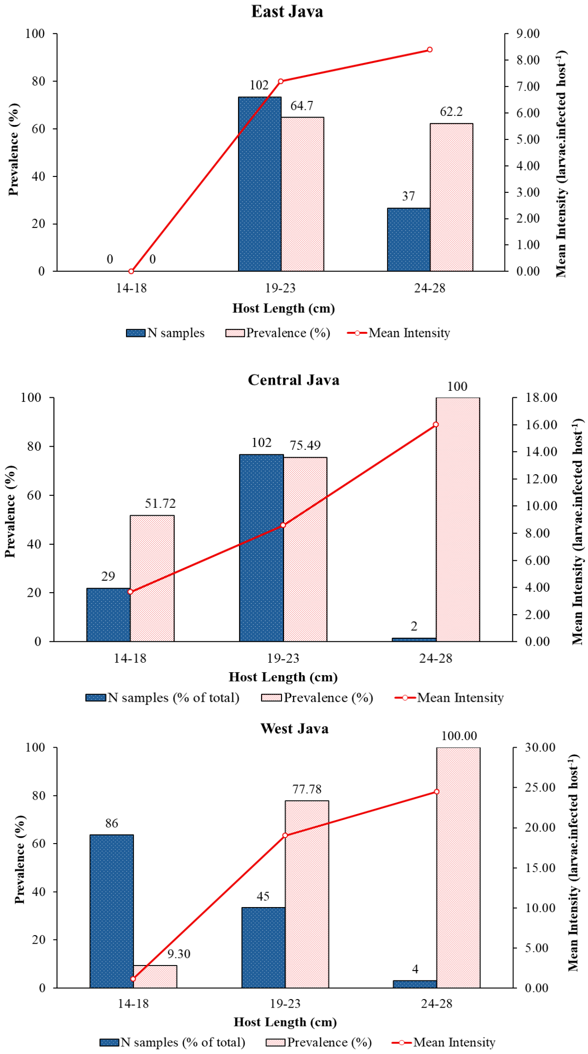

Fig. 2. Number of samples, percentage infection (Prevalence) and mean intensity of *Anisakis* on *Priacanthus tayenus* caught from the north coast of Java, Indonesia. Numbers at the top of the histogram show the number of samples examined and percentage prevalence of infection.

Table 2. Prevalence (%), total number of larvae, mean intensity of infection (± SD) and infection sites of nematode larvae isolated from *Priacanthus tayenus* in the north coast of Java, Indonesia.

Location	P (%)	N	MI	Site of infection (%)				
				Body cavity	Digestive tract	Muscle	Liver	Gonad
East Java	64.02	668	7.50 ± 10.22	69.16	12.57	0.15	12.57	4.79
Central Java	70.68	747	7.94 ± 10.79	79.92	4.28	0.00	13.52	2.28
West Java	34.81	773	16.45 ± 31.87	64.55	16.04	0.00	13.45	5.95

N = total number of *Anisakis* larvae collected; P = prevalence (%); MI = mean intensity (larvae infected host^{-1}).

Anisakis nematode larvae in *P. tayenus* found in the three locations were mostly attached to the body wall or body cavity of the fish (East Java 69 %; Central Java 80 %; West Java 65 %) (Table 2; Fig. 3). *Anisakis* nematode larvae in fish from East Java and West Java were also found in the digestive tract (13 % and 16 %, respectively) and liver (13 % and 13 %, respectively). Meanwhile, *Anisakis* larvae in samples from Central Java were also found in the liver (14 %) and only a few larvae were found in other organs (Fig. 4).

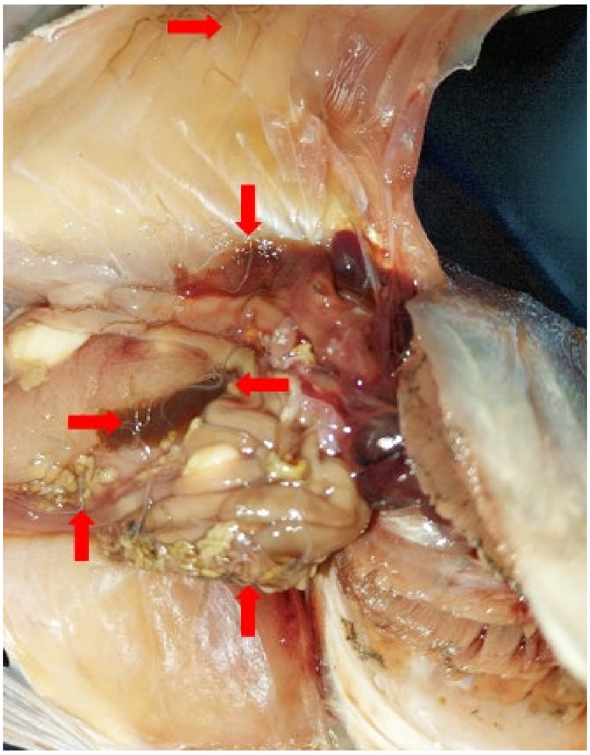

Fig. 3. *Anisakis* larvae infection (red arrows) on *Priacanthus tayenus* caught from the north coast of Java.

The average length of nematode larvae on *P. tayenus* from the three locations was similar, ranging from 10.1 in West Java to 10.6 in East Java (Table 3).

Sequencing and phylogenetic analysis

The amplification of the rDNA (ITS) region using a specific primer generated a ~1 kbp sized product (Fig. 5). The sequence of ITS region of rDNA was aligned with the published sequences in GenBank database, and the phylogenetic tree was created using the MEGA 11 software (Tamura et al., 2021). The molecular identification showed that the *Anisakis* larvae infecting *P. tayenus* from the north coast of Java were *A. typica*. The phylogenetic tree construction is presented in Figure 6.

The phylogenetic tree (Fig. 6) shows the genetic relationship between *A. typica* found on *P. tayenus* in the north coast of Java with other *Anisakis*. In addition, *A. typica* infecting *P. tayenus* from the north coast of Java was in the same cluster as *A. typica* from Malaysia and Thailand. The phylogenetic tree based on the ITS region of rDNA formed two clades. The first clade consists of *Anisakis* species, while the second clade is *Hysterothylacium* (Fig. 6).

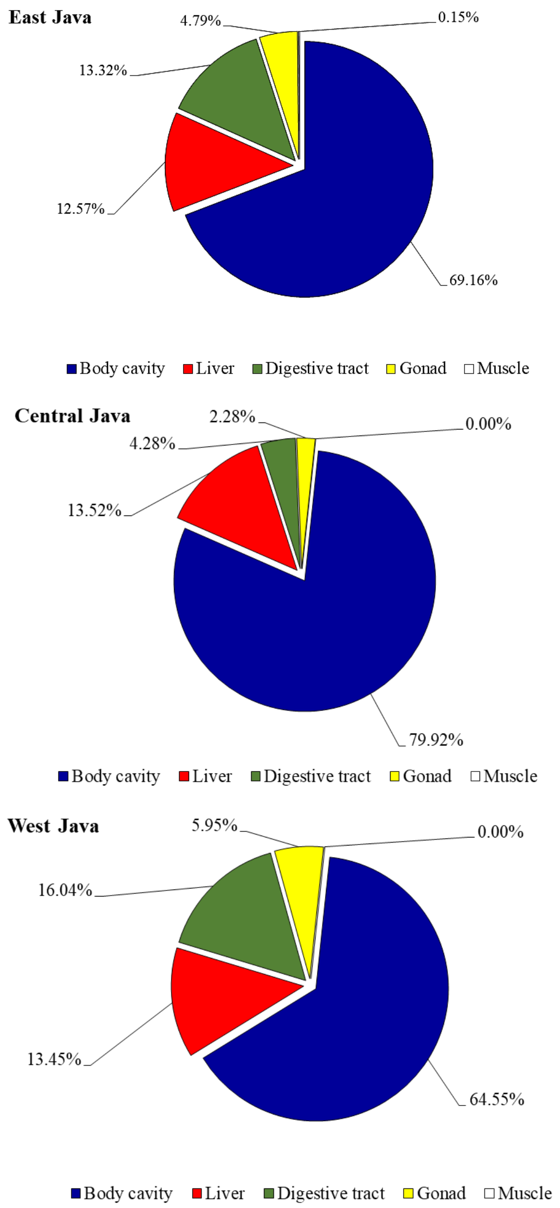

Fig. 4. Percent frequency of *Anisakis* infection on different organs of *Priacanthus tayenus* caught from the north coast of Java.

Table 3. Mean length of nematode larvae (± SD) isolated from *Priacanthus tayenus* in the north coast of Java.

Location	Number of larvae measured	Length of nematode larvae (mm)
East Java	50	10.6 ± 2.1
Central Java	50	10.4 ± 2.5
West Java	50	10.1 ± 2.1

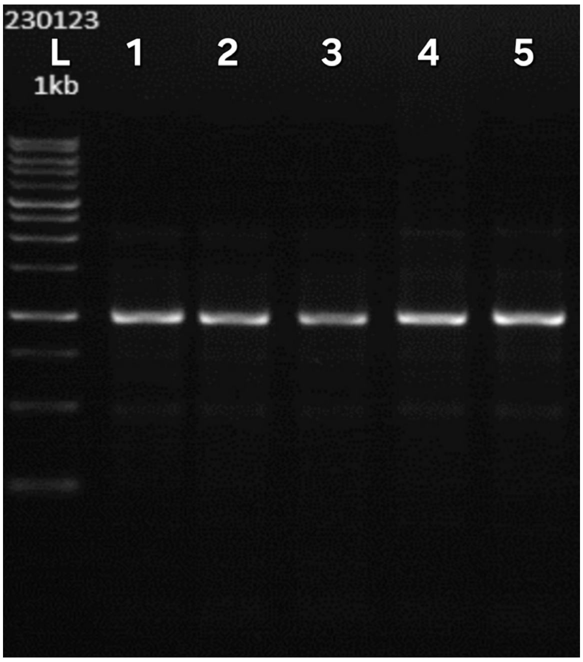

Fig. 5. Amplification results of anisakid nematodes obtained from ITS regions amplified using primer A and primer B (L: DNA ladder; 1: sample from East Java; 2-3: sample from Central Java; 4-5: sample from West Java).

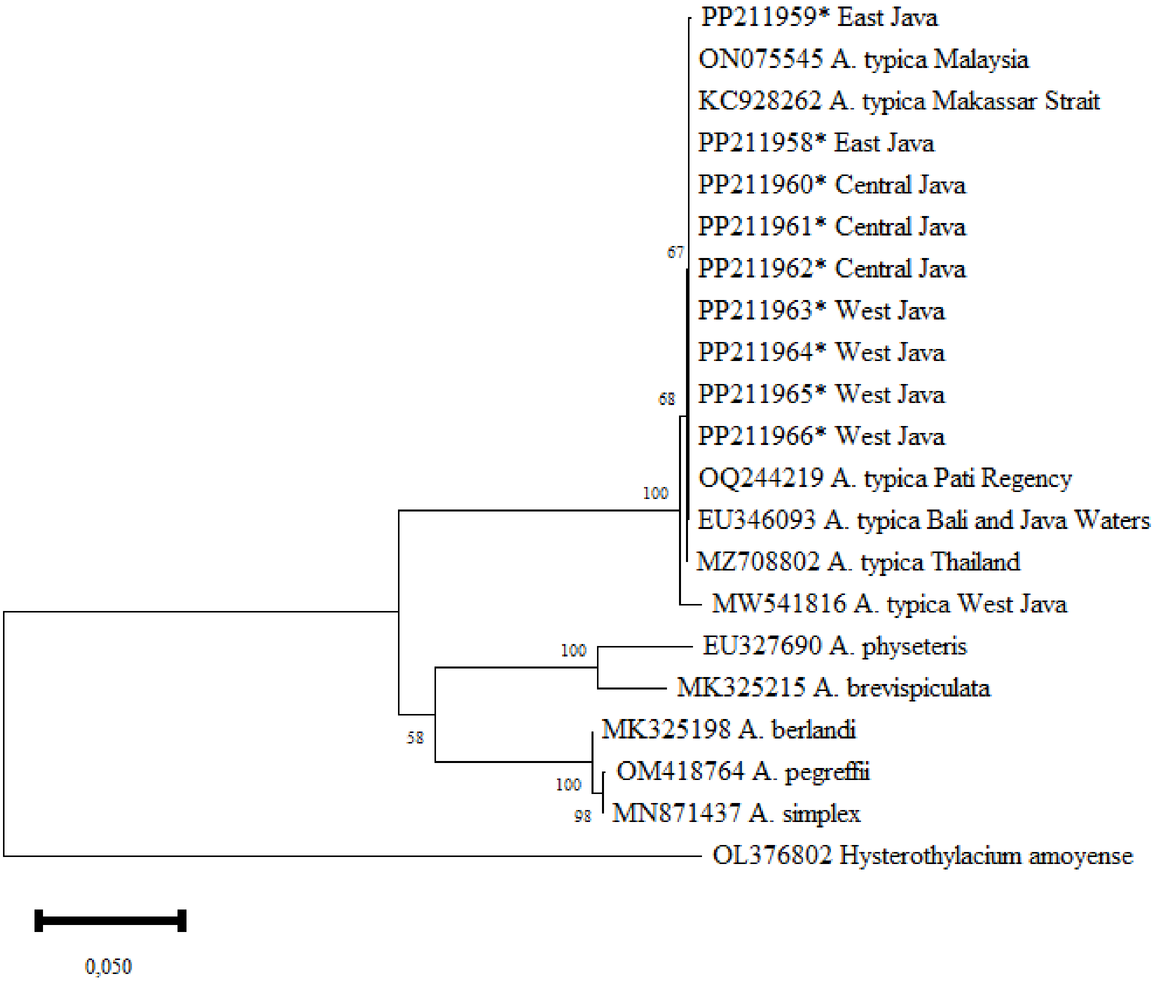

Fig. 6. Maximum likelihood (ML) tree presenting the phylogenetic relationships of *Anisakis typica* isolated from *Priacanthus tayenus* from the north coast of Java, Indonesia (based on ITS region of rDNA). The phylogenetic tree was arranged based on the Kimura 2-parameter model (bootstrap = 1000).

The results of genetic distance analysis between *Anisakis* species found with other nematode species based on the ITS region of rDNA are summarised in Table 4. *Anisakis typica* found in the north coast of Java, includes East Java, Central Java, and West Java are close to *A. typica* found in Pati Regency, Bali, and Makassar Strait. The close genetic relationship is also shown with *A. typica* from Malaysia and Thailand (Table 4). While the farthest genetic distance is between *A. typica* and *H. amoyense* (0.481).

Discussion

Purple-spotted bigeye (*Priacanthus tayenus* Richardson, 1846), caught on the north coast of Java are susceptible to *Anisakis* larvae infection, with differences in the prevalence and mean intensity between East Java, Central Java, and West Java. The purple-spotted bigeye from Central Java and East Java had a high prevalence of *Anisakis* larvae, with 70.7 % and 64.0 %, respectively. While the purple-spotted bigeye from West Java had a lower prevalence (34.81 %), however it had the greatest mean intensity of *Anisakis* larvae (MI = 16.45 larvae infected host^{-1}) compared to fish from Central Java and East Java (Table 2). *Pricanthus tayenus* from the south coast of Java displayed a different *Anisakis* infection rate, with a prevalence of 43.4 % and a mean intensity of 1.3 larvae infected host^{-1} (Palm et al., 2017). *Priacanthus* sp. from the waters of Rio de Janeiro, Brazil, shows the lowest prevalence, which is 20 %, with a mean intensity of 5 larvae infected host^{-1} (Kuraiem et al., 2016). A higher *Anisakis* larvae infection was exhibited on the *Priacanthus* sp. from Karachi, with a prevalence of 58.9 % and a mean intensity of 12.2 larvae infected host^{-1} (Ali and Afsar, 2018).

Reports of *Anisakis* larvae infection on big-eye fish in various waters show varying prevalence and mean intensity. This variation in infection might be caused by differences in the presence and abundance of marine mammals, which are the final hosts, and small marine crustaceans, which serve as intermediate hosts. The abundance of final and intermediate hosts in a certain area might increase the possibility and high level of *Anisakis* infection for fish that inhabit or pass through the area. Dolphins from the families Delphinidae, Phocoenidae, and Pontoriidae have been reported as the final hosts of *Anisakis* (Mattiucci et al., 2002; Kleinertz et al., 2014). Several species of dolphins are known to live in or migrate through Indonesian waters. Rudolph et al. (1997) reported various species of dolphins that live in Indonesia, including *Globicephala macrorhynchus*, *Stenella attenuata*, *Stenella longirostris*, *Tursiops truncatus*, *Tursiops aduncus*, *Sotalia fluviatilis*, *Stenella coeruleoalba*, and *Steno bredanensis*. Various marine organisms, including small crustaceans, inhabit the shallow and productive waters of the Java Sea area. The coastal areas of Java and Indonesian waters are common habitats for crustaceans from the Mysidae family (Sawamoto, 2014).

The prevalence and mean intensity of *Anisakis* larvae in purple-spotted bigeye fish on the north coast of Java tends to increase as host body size increases (Fig. 3). Tolonen and Karlsbakk (2003), stated that increasing the body size and age of the host (fish) causes an increase in the presence of parasites. Since adult fish need more food than young fish, they are more vulnerable to *Anisakis* larvae infection. An increase in the prevalence of *Anisakis* larval infection in larger fish indicates that they are more susceptible to infection than smaller fish (Setyobudi et al., 2023). A positive correlation between the level of infection of *Anisakis* larvae and host body size has also been reported in various previous studies (Konishi and Sakurai, 2002; Cruz et al., 2009; Al-Zubaidy, 2010; Setyobudi, Jeon et al., 2011; Abou-Rahma et al., 2016; Palm et al., 2017; Setyobudi et al., 2019).

Purple-spotted bigeye fish caught on the north coast of Java mostly had *Anisakis* larvae attached to their body cavities, with as many as 65 % prevalence in the body cavities of infected fish from East Java, 80 % from Central Java, and 65 % from West Java. *Anisakis* larvae were also found in other organs (digestive tract, liver, and gonads) but with a relatively low prevalence (Fig. 4). Microhabitat conditions that support parasite life, e.g., availability of food and oxygen, can influence the presence of *Anisakis* larvae in certain organs (Smith and Wooten, 1978). At high infection intensity, *Anisakis* larvae are located in specific parts of the fish host; mostly, in the body cavity (Mattiucci et al., 2018). Nematodes that enter the host's body through the digestive process will then migrate to the body cavity, which is the habitat of the species (Ayun et al., 2021). Piras et al. (2014) reported similar results on ten fish species caught in the western Mediterranean Sea, finding most *Anisakis* larvae in the body cavity and only a few in the fish muscle. The presence of anisakid larvae in the fish's muscle indicates a potential zoonotic risk. The potential for zoonosis in humans might be lower if anisakid larvae are found in visceral organs rather than edible parts of the fish body, such as muscle (Setyobudi et al., 2023).

The body cavity and mesentery are the organs most frequently infected with *A. typica* in Indonesia, followed by the gonads and liver (Palm et al., 2008). The presence of nutrients in the host's body cavity can lead to the dominance of *Anisakis* larvae in that organ. Nutrient availability plays an important role in determining the survival ability of parasites (Smith, 1984). This study did not find *Anisakis* larvae in fish muscles; similar results were reported by Piras et al. (2014), in which *Anisakis* spp. were found in body cavities and only a few in the muscles of some commercial fish caught off the western Mediterranean Sea. *Anisakis* larvae found in the body cavity indicate that the fish acts as a paratenic host, and moulting does not occur from stages L3 to L4 (Shih, 2004). In contrast, Setyobudi, Jeon et al. (2011) found the majority of *A. simplex* (s.s.) larvae infecting chum salmon (*Oncorhynchus keta*) from Korea were predominantly

Table 4. The genetic distance between *Anisakis typica* from this study with other anisakid nematodes based on ITS region of rDNA (%).

No.	AN	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	PP211958*	-																				
2	PP211960*	0.000	-																			
3	PP211961*	0.000	0.000	-																		
4	PP211962*	0.000	0.000	0.000	-																	
5	PP211963*	0.000	0.000	0.000	0.000	-																
6	PP211964*	0.000	0.000	0.000	0.000	0.000	-															
7	PP211965*	0.000	0.000	0.000	0.000	0.000	0.000	-														
8	PP211966*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-													
9	PP211959*	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	-												
10	OQ244219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	-											
11	EU346093	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	-										
12	KC928262	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	-									
13	ON075545	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	-								
14	MZ708802	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	-							
15	MW541816	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.013	0.011	0.011	0.011	0.011	0.011	-						
16	EU327690	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.204	0.206	0.204	0.204	0.204	0.204	0.207	0.211	-					
17	MK325215	0.196	0.196	0.196	0.196	0.196	0.196	0.196	0.196	0.198	0.196	0.196	0.196	0.196	0.197	0.201	0.058	-				
18	MK325198	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.169	0.167	0.167	0.167	0.167	0.168	0.172	0.152	0.133	-			
19	OM418764	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.175	0.173	0.173	0.173	0.173	0.174	0.178	0.152	0.135	0.006	-		
20	MN871437	0.171	0.171	0.171	0.171	0.171	0.171	0.171	0.171	0.173	0.171	0.171	0.171	0.171	0.171	0.176	0.154	0.133	0.004	0.001	-	
21	OL376802	0.481	0.481	0.481	0.481	0.481	0.481	0.481	0.481	0.484	0.481	0.481	0.481	0.481	0.473	0.486	0.461	0.464	0.464	0.465	0.470	-

*This study

found in muscle organs (98 %) and relatively few were found in other organs.

Anisakis body length measurements maybe used to determine the larval stage of the parasite. *Anisakis*, which infects *P. tayenus* from the North Coast of Java, has an average length of 10.1–10.6 mm (Table 3). According to Smith (1983), *Anisakis* larval stage 2 (L-2) begins to moult into larval stage-3 (L3) when the body is 4–6 mm long, and starts to become Anisakis L3 when it reaches 8.1 mm. This shows that the *Anisakis* larvae that infect *P. tayenus* on the north coast of Java are at larval stage 3 (L-3). For comparison, the total body length of *A. typica* (L-3) in mackerel ranges between 14.5 to 20.2 mm, *A. pegrefii* (L-3) ranges between 13.1 and 20.6 mm (Cheypanya et al., 2021), while that of *A. simplex* stage 3 larvae in chum salmon ranges from 19.7 to 28.4 mm (Setyobudi, Jeon et al., 2011).

Identification of *Anisakis* larvae at the species level is important to understand their distribution and molecular epidemiology. This study used direct sequencing targeting the ITS rDNA region for molecular identification of *Anisakis* larvae. The rDNA core genes and ITS genes are commonly used to determine nematode species (Blouin, 2002). Kong et al. (2015) stated that amplification of the ITS rDNA region is an effective method for distinguishing *Anisakis* species that are distant or closely genetically related. Our molecular identification results identify the *Anisakis* larvae infecting *P. tayenus* fish in the waters of the north coast of Java as *A. typica*. Eamsobhana et al. (2018) reported similar results; *A. typica* also infects *P. tayenus* from the Gulf of Thailand. Most of the *Anisakis* larvae, which infect various fish in Indonesian waters, are identified as *A. typica*. Previous studies reported the presence of *A. typica* in marine fish from the southern Makassar Strait (Anshary et al., 2014), Bali's waters (Palm et al., 2017), southern coast of Java (Setyobudi et al., 2019; Ayun et al., 2021; Ayun et al., 2022; Syarifah et al., 2023), and the north coast of Java (Setyobudi et al., 2023).

The ITS rDNA gene reconstruction in this study reveals a close relationship between *A. typica*, isolated from *P. tayenus* on the north coast of Java, and *A. typica* from the waters of Java, Bali, Pati, Malaysia, and the Makassar Strait (Fig. 6). The close geographical location can allow gene flow between these waters, thus influencing the close genetic relationship of *A. typica* in these waters. A close genetic relationship is also shown with *A. typica* from Thailand, which is geographically quite far away. Kuhn et al. (2016) suggest that migration from *Anisakis*' previous host, marine mammals (dolphins), may contribute to the close relationship between *Anisakis* and dolphins. Migration from recent hosts around the world may lead to closely related *A. typica* in distant geographic areas. *Anisakis typica* has a wide distribution and is dominantly distributed in warm and tropical waters (Mattiucci and Nascetti, 2008).

Humans can be infected with *Anisakis* as an incidental host, which is caused by consuming raw or imperfectly cooked infected fish, such as sushi, sashimi, and lomi-lomi. However, this nematode does not reach reproductive maturity in the human body (Sakanari and McKerrow, 1989). Anisakiasis is now a new focus of attention in tropical medicine (Wiwanitkit and Wiwanitkit, 2016). Symptoms of anisakiasis increase when *Anisakis* larvae penetrate the gastric mucosa, causing serious infections in the stomach. In Asia and Europe, particularly Japan, people consume a lot of raw or undercooked seafood like sushi and sashimi, and anisakiasis cases have been widely reported (Madrid et al., 2016). *Anisakis* infection can cause symptoms of nausea, vomiting, diarrhoea, or allergic reactions. Allergic reactions are caused by the presence of chemical compounds found in the flesh of fish infected with *Anisakis* (Alonso-Gómez et al., 2004; Ivanović et al., 2017).

Most cases of anisakiasis in humans are caused by *A. simplex* infection (Arizono et al., 2012), while cases of anisakiasis caused by *A. typica* in humans have never been reported. According to Tunya et al. (2020), cases of *A. typica* infection in humans are rarely reported due to the lack of information available regarding this larval infection. To date, there are no reports regarding cases of anisakiasis and/or anisakidosis in Indonesia, although several fish species in Indonesian waters are susceptible to *Anisakis* infection. In line with consumption patterns and the increasing popularity of seafood served raw or only partially cooked, the risk of anisakiasis may increase. Therefore, a mitigation process requires research on the distribution of *Anisakis* and its pathogenic potency.

Apart from having a negative impact on humans, *Anisakis* infection also causes a decrease in the quality and appearance of fish consumed (Bao et al., 2019). This can impact the economic value of fish by leading to the rejection of fishery products and a decrease in consumer confidence. According to Hassan et al. (2013), *Anisakis* larval infection can cause pathological effects on the host such as an inflammatory response around the larvae, necrosis, and degenerative changes in fish hepatocytes.

Despite the negative impact on human health, the presence of this nematode parasite has been widely used to trace the origin, migration, and distribution of commercial marine fish species (Mattiucci et al., 2015). *Anisakis* can act as a biological indicator in various ecological studies, such as stock characterisation and discrimination, migration behaviour, and fish feeding habits. The use of parasites as biological indicators has advantages over artificial markers because they are more suitable for application to small fish, crustaceans, and deep-sea fish species. In addition, using parasites as biomarkers does not affect fish behaviour, like artificial markers (MacKenzie and Abaunza, 1998). However, the use of parasites as biological markers requires additional information, such as an understanding of migration patterns and the distribution of the final host.

Conclusion

The purple-spotted bigeye, *Priacanthus tayenus* showed susceptibility to *Anisakis* nematode infection, with varying prevalence and mean intensity observed across different locations on the north coast of Java. The highest prevalence of *Anisakis* larvae infection was found in purple-spotted bigeye caught from the north coast of Central Java (70.7 %), while the lowest prevalence of *Anisakis* infection was found in samples originating from West Java (34.8 %). The highest mean intensity of *Anisakis* infection was found in samples originating from West Java (16.45 larvae infected host^{-1}), while the lowest mean intensity of *Anisakis* infection was found in samples originating from East Java (7.50 larvae infected host^{-1}). *Anisakis* larvae were mostly found in the body cavities and rarely found in the muscle of *P. tayenus*. Direct sequencing of the ITS-rDNA region confirms the *Anisakis* nematode larvae isolated from the purple-spotted bigeye were *Anisakis typica* and closely related to *A. typica* from the water of Thailand, Malaysia, Makassar, and Bali.

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