

Anisakid nematodes from the largehead hairtail fish (*Trichiurus lepturus*) from the northern coast of Java, Indonesia

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Manuscript received: 30 November 2022. Revision accepted: 9 March 2023.

Abstract. Setyobudi E, Murwantoko, Utami AMR, Syarifah RF. 2023. Anisakid nematodes from the largehead hairtail fish (*Trichiurus lepturus*) from the northern coast of Java, Indonesia. *Biodiversitas* 24: 1560-1568. Hairtail fish has been frequently reported to be infected by anisakid nematodes. This study aims to determine the presence of anisakid nematodes on the large-head hairtail fish (*Trichiurus lepturus*) from the northern coast of the Java Sea, Indonesia. In total, 306 hairtail fish were collected from the northern coast of Java. Each fish sample was measured its length and weighed, then dissected to examine the presence of anisakid nematode. The anisakid larva examination was conducted in the body cavity, internal organs, digestive tract, and muscles. The anisakid nematodes found were then separated and grouped based on morphological appearance, namely body color and size. Selected samples from each anisakid group and sampling location were extracted and identified molecularly by the direct sequencing method. The findings showed that the larvae of anisakid nematodes infect the *T. lepturus* with different prevalence and average intensity at each location (P: 38.0-100%, MI: 3.33-42.88 larvae/ind.). Mostly anisakid larvae were found in the body cavity and were not found in the muscle. Most of the *T. lepturus* from the northern coasts of Cirebon and Pati were infected with anisakid at low intensity (≤ 5 larvae/individual). The high intensity of anisakid larval infection was shown by the hairtail originating from Rembang and Tuban (>20 larvae/ind.). Molecular identification showed that three anisakids were isolated from hairtails from the northern coast of Java, namely *Anisakis typica*, *Hysterothylacium amoyense*, and *Raphidascaris lophii*. Information on anisakid species and their distribution is important as a reference for fish handling and processing, the assessment of the human health risk, and the development of these nematodes as biological tags.

Keywords: Biological tags, human health risk, infection, prevalence, zoonosis

INTRODUCTION

Large-head hairtail fish (*Trichiurus lepturus*) belonging to the family Trichiuridae is an economically significant fish widely caught in the world (Apriliani et al. 2018; Hashemi et al. 2020; Jeon et al. 2021; Liao et al. 2021). FAO (2022) noted that the global production of *T. lepturus* fish world was as much as 1.14 million tons (1.7% of total finfish production). Hairtail fish are known as important marine fish species in East Asian nations, such as China, Japan, and Korea (Makino et al. 2017; Jeon et al. 2021; Cheng et al. 2022). Hairtail consumption has significantly expanded in these nations at a variety of restaurants, including some that specialize in offering roasted hairtails and other different dishes. Hairtails are an excellent source of nutrients such as proteins, minerals, essential amino acids, and healthful lipids, including unsaturated fatty acids (Shui et al. 2022; Wang et al. 2022). These fish commodities are frequently prepared as fresh products, frozen fillet products, semi-dried products, pickled products, or processed into surimi. The high demand for hairtail as an export commodity has led to an increase in its capture in Indonesia. Hairtail is a common fish target in the southern coast (Fisheries Management Area 573) and northern coast of Java (Fisheries Management Area 712).

Nematodes are a very diverse group of animals, living freely or as parasites on plants and animals (Blaxter and

Koutsovoloulos 2015). Among these groups, anisakid nematodes are common parasites frequently found infecting marine organisms. Anisakid nematodes consisting of two families, i.e., Family Anisakidae with the subfamilies Anisakinae and Contracaecinae and Family Raphidascarididae (Fagerholm 1991). Investigations on the existence of anisakid larvae in marine organisms have revealed that numerous fish species and cephalopods are susceptible to anisakid infection. Anisakid nematodes have a widespread distribution worldwide, found in all oceans, both northern and southern hemispheres, and even in the polar region (Dzido et al. 2009; Severin et al. 2020). In aquatic ecosystems, anisakid nematodes display a complex life cycle that convolutes various hosts, i.e., crustaceans, squid, fish, fish-eating birds, and marine mammals, which act as intermediate, paratenic/transport, and definitive hosts (Mattiucci et al. 2022). The general life cycle of this parasite starts when the nematode larvae are consumed by small marine crustaceans (Mouritsen et al. 2010). Investigations on the presence of anisakid nematodes on marine organisms have developed quite rapidly in recent decades due to human health concerns, commercial fisheries problems, and the development of biological tags. Anisakid nematode is one of the parasite groups that should be aware of its presence in fisheries products (EFSA-BIOHAZ 2010). Besides to the human health concern, the existence of anisakid nematodes has economic importance

due to its impact on the commercial fishing industry (Pozio 2013; Bao et al. 2019; CABI 2022). Apart from the negative side, anisakid nematodes have been extensively employed as biological tags for numerous aspects of fisheries biology, primarily in population studies. Recently, the utilization of parasites for biological indicators for diverse fish and cephalopods has been significantly increasing (Klapper et al. 2016; Nurhidayat et al. 2018).

Several studies exhibit the infection of anisakid nematodes on various marine fish species in Indonesia, although the number of fish hosts recorded is still limited in contrast to the overall of marine fish species in Indonesian waters. Palm et al. (2017) state that more than 100 species of Indonesian marine fish are prone to Anisakis infection. Among the marine fish species, the hairtail is one of those that is frequently reported to be susceptible to anisakid nematode infection. Anisakid infection on the *T. lepturus* has been reported, particularly on the Indian Ocean southern coast of Java (Setyobudi et al. 2011b, 2019; Ayun et al. 2021), but it is still uncommon on the northern coast of Java. This study aims to determine the prevalence, intensity of infection, and species of anisakid nematodes in the Java Sea. The information obtained can be used as a reference in the safe handling and processing of fishery products (food safety) and an assessment of the role of epidemiology in health (human anisakiasis). This information is also very important for using these nematodes as biological tags to study stock differences, migration patterns, food webs, and other ecological topics in the waters of Indonesia.

MATERIALS AND METHODS

Study area

Fish sample collection

In total, 306 hairtail fish samples were collected from the northern coast of Java, Indonesia, i.e., Cirebon (West Java), Pati and Rembang (Central Java), and Tuban (East Java)

(Java) (Figure 1). Sampling location, number, and size of *T. lepturus* are shown in Table 1.

Anisakid nematode collection

Each fish sample was measured for its total length using a ruler (accuracy 0.1 cm) and weighed using electrical balancing (accuracy 1 gr) (Table 1). The anisakid larva examination was conducted in the body cavity, digestive tract, internal organ, and muscle. The anisakid nematodes found were then separated and grouped based on morphological appearance, namely body form, body color and size. The larva was then washed in a physiological solution (0.14% NaCl) to remove the fat attached to the larval body. Anisakid nematodes that have been cleaned are preserved in an absolute ethanol solution for further analysis. The preservation was separated for each larval group.

Anisakid identification

Selected larvae from each group were extracted and identified molecularly by direct sequencing. The DNA genome of anisakid nematodes larvae was extracted using the DNA Mini Kit Tissue Protocol. The ITS rDNA region (ITS1-5.8S-ITS2) was amplified using a primer NC 5 (forward) (NC5 (5'-GTAGGTGAACCTGCGGAAGGATCATT-3') and primer NC2 (reverse) (5'-TTAGTTTCTTTTCCTCCGCT-3') (Zhu et al. 1998; Abattout et al. 2016).

Table 1. Location, number, and size of *Trichiurus lepturus* sampled in this research

Province	Location	Number of samples	L (min-max) (cm)	W (min-max) (g)
West Java	Cirebon	22	50.0-74.0	142-245
Central Java	Rembang	18	65.5-81.8	230-542
	Pati	198	59.5-92.0	121-514
East Java	Tuban	68	53.8-87.0	149-649
		306		

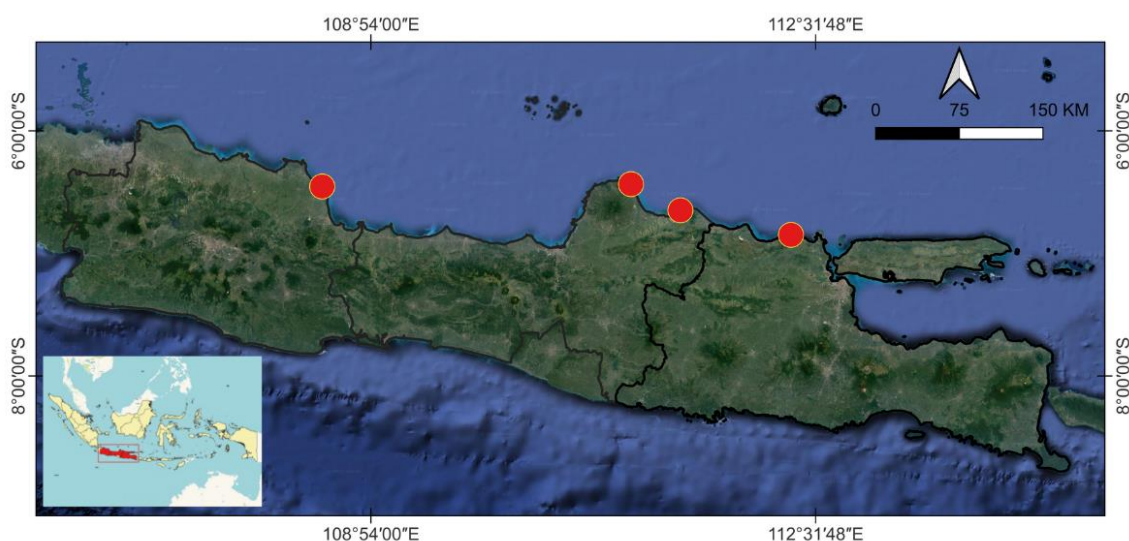


Figure 1. Sampling location of the *Trichiurus lepturus* fish from the northern coast of Java, Indonesia, i.e., Cirebon (West Java), Pati and Rembang (Central Java), and Tuban (East Java)

Amplification was performed under the conditions: pre-denaturation 95°C (10 minutes), and 30 cycles of denaturation 95°C (30 seconds), annealing 55°C (30 seconds), extension 72°C (75 seconds), then the final extension 72°C (7 minutes) (Soewarlan et al. 2014). The amplification results are then electrophoresed to determine the formation and the success of the DNA target amplification. The DNA than was sent to the 1st Base Laboratory in Malaysia via PT. Genetika Science Indonesia for the nucleotide sequencing processes. The amplification results were processed, and phylogenetic trees were built using the BioEdit and Mega X programs (Molecular Evolutionary Genetics Analysis Version X) (Kumar et al. 2018). The NCBI.nlm.go.id website's BLAST analysis was used to identify the anisakid nematode species. The nucleotide sequences obtained were deposited at the NCBI with accession numbers OQ244219, OQ244220, OQ244221, OQ244298, and OQ244299.

Data analysis

Following Bush et al. (1997), the parasite population parameters, namely, prevalence (P) and mean intensity-were calculated (MI). The terms "prevalence" (P) refer to the average number of parasites per host, while "mean intensity" (MI) refers to the proportion of infected hosts among all examined hosts. Additionally, the infection's distribution within each target organ was identified. The

intensity of anisakid nematode infection in each host was categorized into six classes, i.e., ≤ 5 , 6-10, 11-15, 16-20, 21-25, and >25 . The frequency distribution of anisakid nematode infection was presented in a figure.

RESULTS AND DISCUSSION

The results showed that the larvae of anisakid nematodes infect the *T. lepturus* with different prevalence and average intensity at each location (P: 38.0-100%, MI: 3.33-42.88 larvae/ind.). Mostly anisakid larvae are found in body cavities (Figure 2).

Most of the *T. lepturus* from the northern coast of Cirebon were infected at low intensity (≤ 5 larvae/ ind.), i.e., 83%. Similar results were shown by *T. lepturus* from the northern coast of Pati, where most of them were infected with anisakid at low intensity (≤ 5 larvae/ind.), i.e., 70%. The high intensity of anisakid larvae infection was shown by the hairtail originating from Rembang and Tuban. More than 67% of Rembang hairtails and 55% of Tuban hairtails were infected with more than 20 larvae/individuals (Figure 3). As an addition, 10% of the hairtail from East Java was infected with more than 60 larvae/ind.

Tabel 2. Sampling location and infection of anisakid nematods larvae infection on *Trichiurus lepturus*

Province	Location	Prevalence (%)	Mean intensity (larvae/fish)	Description of anisakid larva
West Java	Cirebon	27.3	3.3	Transparent-white, 0.5-1.0 cm
Central Java	Pati	38.4	6.4	Yellowish-white 2.0-3.0 cm
	Rembang	100.0	37.8	Red-brown 1.5-2.0 cm; Yellowish-white 2.0-3.0 cm
East Java	Tuban	98.5	42.9	Red-brown 1.5-2.0 cm; Yellowish-white 2.0-3.0 cm

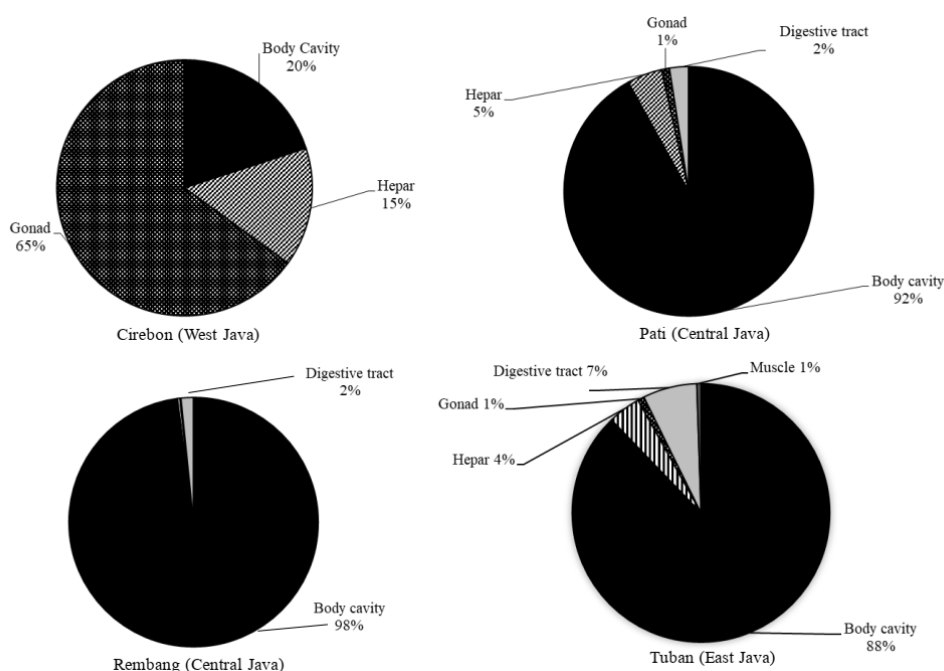


Figure 2. Target organ of anisakid nematode infection on *Trichiurus lepturus* at the northern coast of Java, Indonesia

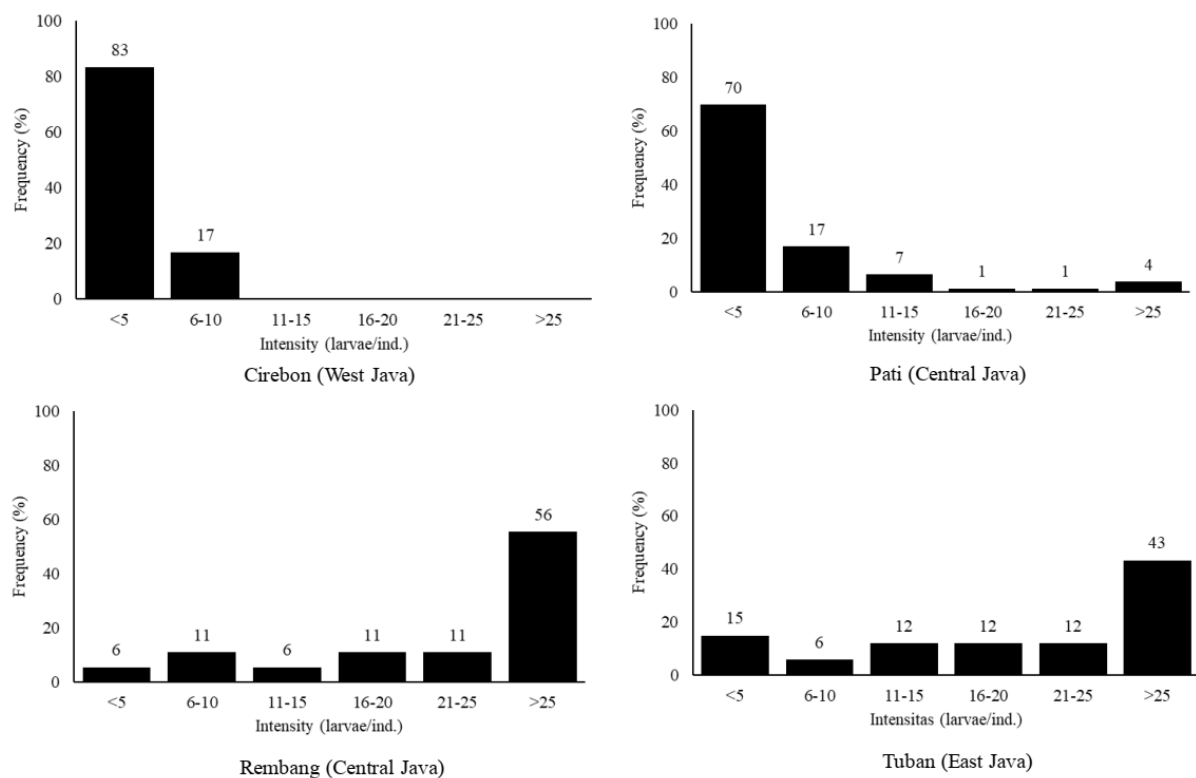


Figure 3. Intensity distribution of anisakid nematode infecting *Trichiurus lepturus* at the northern coast of Java, Indonesia

Table 2. BLAST (Basic Local Alignment Search Tool) results of the ITS rDNA region sequence of anisakid nematodes isolated from *Trichiurus lepturus* at the northern coast of Java, Indonesia

Location	Accession number	Species	Origin	Identity (bp)	Per. ident (%)
Cirebon (West Java)	MH211584 (Zhang et al. 2018)	<i>Raphidascaris lophii</i>	East China Sea	891/892	99.89
	MF539820 (Chen et al. 2018)	<i>Raphidascaris lophii</i>	China	891/892	99.89
	(OQ244299) KX110077 (Li et al. 2017)	<i>Raphidascaris lophii</i>	Yellow Sea	891/892	99.89
	KP419720 (Zhao et al. 2016)	<i>Raphidascaris lophii</i>	China	891/892	99.89
Pati (Central Java)	MN420659 (Tunya et al. 2020)	<i>Anisakis typica</i>	Thailand	848/848	100
	KY524212 (Palm et al. 2017)	<i>Anisakis typica</i>	Indonesia	848/848	100
	(OQ244219) ON188805 (See MS 2022, unpubl. data)	<i>Anisakis typica</i>	South China Sea	848/848	100
	MZ350220 (Syarifah et al. 2021, unpubl. data)	<i>Anisakis typica</i>	South Java, Indonesia	848/848	100
Rembang (Central Java)	MH211527 (Zhang et al. 2018)	<i>Hysterothylacium amoyense</i>	East China Sea	887/887	100
	(OQ244298 and OQ244220) KP252133 (Li et al. 2015)	<i>Hysterothylacium amoyense</i>	East China Sea	887/887	100
	MZ509280 (Bannai and Jori 2021, unpubl. data)	<i>Hysterothylacium amoyense</i>	Iraq	887/887	100
	MW940896 (Bannai et al. 2021, unpubl. data)	<i>Hysterothylacium amoyense</i>	Gulf of Arab	887/887	100
	MN420659 (Tunya et al. 2020)	<i>Anisakis typica</i>	Thailand	907/907	100
	ON188805 (See MS 2022, unpubl. data)	<i>Anisakis typica</i>	South China Sea	907/907	100
	KC928262 (Anshary et al. 2014)	<i>Anisakis typica</i>	Makassar, Indonesia	907/907	100
	HF911524 (Kleinertz et al. 2014)	<i>Anisakis typica</i>	Egypt	907/907	100
Tuban (East Java)	(OQ244221) ON188805 (See MS 2022, unpubl. data)	<i>Anisakis typica</i>	South China Sea	913/913	100
	HF911524 (Kleinertz et al. 2014)	<i>Anisakis typica</i>	Egypt	913/913	100
	KC928262 (Anshary et al. 2014)	<i>Anisakis typica</i>	Makassar, Indonesia	913/913	100
	JX523715 (Zhang et al. 2013)	<i>Anisakis typica</i>	South China Sea	913/913	100

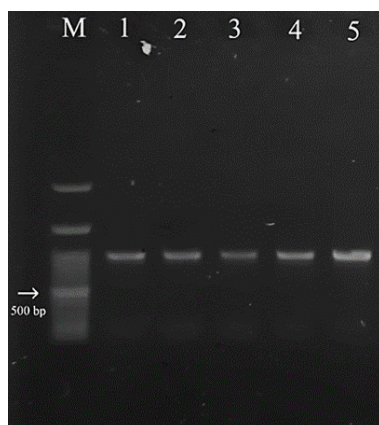


Figure 4. Visualization of the PCR product of the ITS rDNA region of anisakid isolated from *Trichiurus lepturus* off the northern coast of Java. M: Marker; 1: Anisakid isolated from *T. lepturus* from Cirebon (West Java); 2: Anisakid isolated from *T. lepturus* from Pati (Central Java); 3: Anisakid isolated from *T. lepturus* from Rembang (Central Java); 4: Anisakid isolated from *T. lepturus* from Rembang (Central Java); 5: Anisakid isolated from *T. lepturus* from Tuban (East Java)

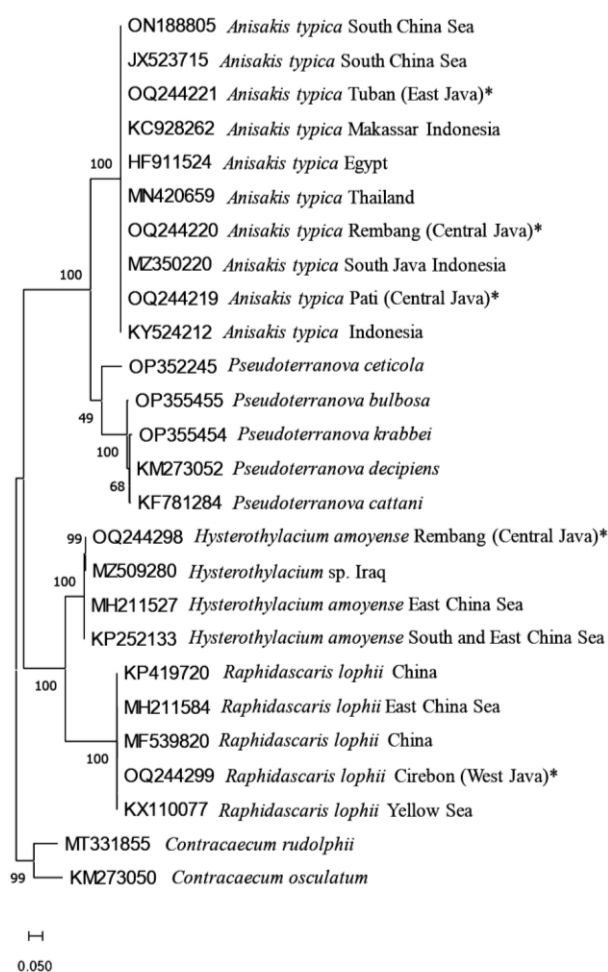


Figure 5. The phylogenetic tree was constructed using the Maximum Likelihood and Kimura 2-parameter methods, showing the phylogenetic relationship of anisakid in this study (indicated by *) and sequences from the GenBank. Evolutionary analysis compiled using Mega X

The samples of anisakid isolated from *T. lepturus* on the northern coast of Java were amplified using the ITS rDNA region. The amplification of the ITS rDNA region produces a fragment of ~1 kbp. Molecular identification by direct sequencing of the ITS rDNA region showed that anisakid nematodes isolated from *T. lepturus* in Cirebon (West Java) were *Raphidascaris lophii*, Pati (Central Java) were *Anisakis typica*, Rembang (Central Java) were *Hysterothylacium amoyense* and *Anisakis typica*, and Tuban (East Java) were *Anisakis typica* (Table 2). The phylogenetic tree formed from the ITS rDNA data set consists of 3 clades. The first clade consists of *A. typica* sequences from various regions and *A. typica* isolated from *T. lepturus* originating from the northern coast of Pati, Rembang (Central Java) and Tuban (East Java). The second clade consists of *H. amoyense* sequences from various regions, including *H. amoyense* from the northern coast of Rembang (Central Java). The third clade consists of *R. lophii* isolated from *T. lepturus* that originated from the northern coast of Cirebon (West Java) and similar species from various regions (Figure 5).

Discussion

The number of studies on anisakid nematodes in Indonesian marine fish is still limited compared to the overall marine fish species in Indonesian waters. Hairtail fish is one of the marine fish species that has been reported to be susceptible to anisakid infection. This research shows the variation in prevalence and mean intensity of anisakid nematode infection from the northern coast of Java (P: 38.0-100%, MI: 3.33-42.88 larvae/ind.) (Table 1). In comparison, anisakids infecting *T. lepturus* in West Java (Eastern Indian Ocean) had a prevalence of 45.5% and a mean intensity of 4.4 larvae/ind (Ayun et al. 2021) and *T. lepturus* in Tanzania (South-West Indian Ocean) had a prevalence of 85% and mean intensity of 5.29 larvae/ind. (Cipriani et al. 2022). The life cycles of anisakid nematodes involve multiple hosts at various trophic levels. The high prevalence of anisakid in the fish hosts may reflect the abundance of small crustaceans and the biomass of other hosts, such as cetaceans. Differences in the prevalence and intensity of anisakid infection can be caused by the geographic distribution and eating habits of a fish species (Hassan et al. 2013). In addition, the prevalence of anisakid larvae increased with host size, suggesting that larger fish are more susceptible to infection than smaller ones. Other studies have shown a relationship between prevalence and host fish length for various host species from different areas (Youssir et al. 2017; Ayun et al. 2021). This might be explained by the fact that larger fish consume a greater number of infected preys.

The majority of *T. lepturus* from the northern coasts of Cirebon and Pati were infected with anisakid at low intensity (5 larvae per individual), 83% and 70%, respectively (Figure 3). The high intensity of anisakid larvae infection was shown by the hairtail originating from Rembang and Tuban. More than 66% of Rembang hairtails and 55% of Tuban hairtails were infected with more than 20 larvae/individuals. In fact, 10% of the hairtail from East Java was infected with more than 60 larvae/ind. The high

intensity of anisakid suggests that fish consume predatory fish that acquire larvae from preceding hosts or crustaceans as the initial intermediate host (Palm et al. 2017).

The majority of anisakid larvae that infected *T. lepturus* on the northern coast of Java are found in body cavities and were not found in the muscle (Figure 2). The absence of anisakid nematodes larvae in the muscle of *T. lepturus* represents a low risk of human health impacts. The zoonotic risk potential was higher if anisakid larvae were found in the edible part compared to the visceral organ. The high rate of anisakid infection in the muscles can increase the risk of human anisakidosis. Several studies have found sites of infection of anisakid nematodes mostly in the body cavity, i.e., *T. lepturus* from the Moroccan Atlantic coast (Youssir et al. 2017) and *T. lepturus* from West Java, Indonesia (Ayun et al. 2021). In contrast, Setyobudi et al. (2011a) showed that the majority of *A. simplex* infections in chum salmon (*Oncorhynchus keta*) were located in the muscle (98%). When anisakid nematodes are consumed by their fish hosts, they pass through the intestinal wall and may persist in the body cavity or migrate to the muscles and internal organs. Larvae can migrate either while the fish is still alive (intravitaly) or after it has died (post-mortem) (Cipriani et al. 2016; Debendetti et al. 2019). Infection rates in different microhabitats could depend on the fish species and age, parasite species, and the environmental condition of the host after it is caught (Lymbery and Cheah 2007; Nadolna-Altyn et al. 2022).

Anisakid larvae have been found infecting a diverse range of marine fishes around the world, and the third-stage larvae of many anisakids are recognized as the causative agents of human anisakidosis. Detection and identification of anisakid larvae in consumed marine fish are critical for determining the prevalence, incidence, and potential for infections in the future. However, according to Chen and Shih (2015), it is nearly impossible to identify anisakid larvae at the species level using morphological characteristics. Anisakid nematodes were identified at the species level using molecular identification. Numerous studies have demonstrated that PCR analysis of the internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA) is an effective method for distinguishing between closely and distantly related anisakid species (Kong et al. 2015; Bannai and Jori 2022). The amplification of the ITS rDNA sequence from anisakid nematodes from *T. lepturus* showed a single band of approximately 1 kb (Figure 4). Molecular identification by direct sequencing of the ITS rDNA region showed that anisakid nematodes isolated from *T. lepturus* in Cirebon (West Java) were *Raphidascaris lophii*, Pati (Central Java) were *Anisakis typica*, Rembang (Central Java) were *Hysterothylacium amoyense* and *Anisakis typica*, and Tuban (East Java) were *Anisakis typica*.

Anisakis typica larvae were the most dominant species among anisakid larvae infecting *T. lepturus* on the northern coast of Java. Similarly, Borges et al. (2012) discovered *A. typica* to be the most common *Anisakis* species found in *T. lepturus* hosts in Brazil. Other studies also found *A. typica* in *T. lepturus* in different areas (Ayun et al. 2021; Cipriani

et al. 2022). In contrast to this finding, Sonko et al. (2020) discovered *A. pegreffii* to be the dominant species in *T. lepturus* in northern Taiwan. *Anisakis typica* is primarily found in tropical and warm waters between 30°S and 35°N (Mattiucci et al. 2022). The final host of *A. typica* has been identified as several species of dolphins from the families Delphinidae, Pontoriidae, and Phocoenidae (Kleinertz et al. 2014; Kuhn et al. 2016). This result is consistent with Palm et al. (2017) regarding the possibility of locally isolated sibling species of *A. typica* var. *indonesiensis*. *Anisakis* infection causes human zoonotic disease (anisakiasis) as an accidental host while consuming undercooked or raw fish. *Anisakis simplex* and *A. pegreffii* are the main agents of human anisakiasis (Lim et al. 2015; Mattiucci et al. 2017; Roca-Geronès et al. 2021).

Raphidascaris lophii is still a poorly studied species, with insufficient detail in the original description to allow for identification or separation from other species in the genus (Xu et al. 2012). This study reported the presence of *R. lophii* in *T. lepturus* on the northern coast of Java. The life cycle of *R. lophii* is still unclear. According to Li et al. (2012), the oceanic life cycle of these species might resemble the life cycle patterns of *H. aduncum*. *Raphidascaris lophii* was reported to be infecting Decapтерus in the Balayan and Tayabas bays of the Philippines. The prevalence of Ascaridosis infection was 27.57% in Tayabas Bay and 15.59% in Balayan Bay (Dela Cruz et al. 2022). Xu et al. (2012) explained that *Raphidascaris* has a whitish color and has medium-sized with a length of 12.5–28.6 mm.

Hysterothylacium larvae are widespread parasites of marine, estuarine, and freshwater fishes. The majority of the species in this genus are commonly non-pathogenic to humans. To date, two cases of human anisakidosis have been reported from Japan and Spain, caused by *H. aduncum* (Yagi et al. 1996; González-Amores et al. 2015). In anisakid nematodes' life cycles, fish served as the final host for *Hysterothylacium*, whereas cetaceans, pinnipeds, fish-eating marine mammals, and birds serve as the final hosts for *Anisakis* spp., *Pseudoterranova* spp., and *Contracaecum* spp., respectively (Bao et al. 2021; Mathison et al. 2022). The genus *Hysterothylacium* comprises 89 recognized species (WoRMS 2021). *Hysterothylacium amoyense* was previously found in *T. lepturus* from Demak (Central Java) (Utami et al. 2021). *Hysterothylacium amoyense* was also found on the *T. haumela* from China and *Platycephalus indicus* from the Gulf of Persian (Kong et al. 2015; Najjari et al. 2016).

The detection of parasitic anisakids in commercially important fish is critical for ensuring seafood safety and quality (Bao et al. 2022). Some anisakid species have a negative impact on human health and can cause gastrointestinal disease. Anisakidosis is caused by parasitic nematode larvae of the Anisakidae family, whose adults live in the digestive tracts of cetaceans and pinnipeds (Angeles-Hernandez et al. 2020). In humans, the larvae cannot develop to the adult stage in this accidental host, but their infection can cause acute symptoms, chronic symptoms, or allergic symptoms (angioedema, asthma). *Anisakis* and *Pseudoterranova* are the most common

genera involved in human infections. The countries with the highest incidence of anisakidosis are Japan, Netherlands, Spain, and Germany (Pravettoni et al. 2012). There is no record of anisakidosis in Indonesia, even though various marine fish species are susceptible to anisakid larvae infection (Setyobudi et al. 2011b; Palm et al. 2017). Apart from the negative impact on human health, the presence of parasites has been used to track the migrations of various commercial marine species (Mattiucci et al. 2015). The basic concept behind using parasites as tags to study host populations is that a host can only get infected by a certain parasite species if it is in its endemic region (MacKenzie 2002). Among anisakid nematodes, the genus *Anisakis* is the most commonly and effectively used biological tag. According to the findings of this study, *T. lepturus* is susceptible to anisakid infection. Although these anisakid nematodes have not been reported to be associated with human health risks in Indonesia, it is necessary to increase awareness of the potential diseases caused by these parasites in the future.

ACKNOWLEDGEMENTS

Thanks to Universitas Gadjah Mada, Yogyakarta, Indonesia, for providing research grant No. 1652/UN1/DITLIT/Dit-Lit/PT.01.03/2022.

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