DEPIK
Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Journal homepage: www.jurnal.usk.ac.id/depik

Investigation of anisakis infection and allergic potential on largehead hairtail (*Trichiurus lepturus*) from south coast of Yogyakarta Indonesia

Ihda Khozainul Busyro[1], Eko Setyobudi[*1,2,4], Indun D. Puspita[1,3,4], Masagus M. P. Putra[*1,3,4]

[1]*Master Program in Fisheries Science, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, 55281 Indonesia*
[2]*Aquatic Resource Management, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, 55281 Indonesia*
[3]*Fish Product Technology, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, 55281 Indonesia*
[4]*Fish Biotechnology Working Group, Agrotropica Learning Center, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, 55281 Indonesia*

ARTICLE INFO

Keywords:
Anisakis
Allergen
Infection
Intensity
Prevalence

DOI: 10.13170/depik.14.3.45035

ABSTRACT

Anisakis spp. are parasites distributed worldwide that often infect marine fish species. This study investigated the prevalence, mean intensity, and Anisakis larvae species, and identified the allergenic potential of *T. lepturus* caught in the southern coasts of Yogyakarta, Indonesia. In total, 50 fish that were caught by the fishermen were measured for length and weight and examined for Anisakis larvae infection. The results showed that larvae were found in the abdominal cavity (87.03%), organs (7.95%), gonads (2.93%), and digestive systems (2.09%). The prevalence of Anisakis infection was high (64%), with a mean intensity of 7.47 larvae/fish. Molecular analysis revealed that the nematode was *Anisakis typica*. Therefore, it poses a potential health risk to humans. Consuming fish infected with Anisakis can cause allergic reactions even after the fish has been cooked. The identification was done through protein profile antigen detection of fish flesh, both infected and non-infected, using SDS-PAGE. In this study, the heating process was applied (60℃, 90℃, and 120℃) for 15 minutes. The result of allergenic potential detection from *T. lepturus* infected by Anisakis showed four protein bands correlated with Anisakis allergens Ani s 1 (24 kDa), Ani s 2 (97 kDa), Ani s 3 (41 kDa), and Ani s 7 (139 kDa) in all the infected fish, both fresh and processed.

Introduction

Anisakis sp. belongs to the Anisakidae family, which has zoonotic properties that cause allergic reactions and are generally found in marine fish. According to Sánchez-Alonso *et al.* (2018), humans can be infected by Anisakis larvae due to consuming raw or improperly processed fish contaminated with Anisakis larvae. In addition, the consumption of infected fish can cause allergic reactions. Anisakis larvae produce the allergens Ani s 1 to Ani s 14, some of which are major allergens.

Anisakis larvae have infected various marine fish species in Indonesia. Indaryanto *et al.* (2024), in their review, showed that the distribution of anisakis larvae in Indonesia covers a wide area from Aceh (Zarry *et al.*, 2017), the south coast of Yogyakarta (Setyobudi *et al.*, 2011), the coast of Bali (Palm *et al.*, 2008), Sabu Beach Nusa Tenggara Timur (Soewarlan *et al.*, 2014), the coast of Sulawesi Selatan (Anshary *et al.*, 2014) and the coast of Kupang City (Dawa *et al.*, 2024). One of the fish species that is sensitive to Anisakis larvae is the largehead hairtail (*Trichiurus lepturus*). The prevalence of *Anisakis* sp. infecting largehead hairtail on the south coast of the Purworejo district is 62,68% (Setyobudi *et al.*, 2007).

Largehead hairtail is highly valued in the market and makes a substantial contribution to the fishing industry. It is a significant fishery product in areas like Cilacap, Central Java (Suadi *et al.*, 2007). Due to its high quality and flavour, largehead hairtail is highly sought after in global markets, particularly in countries like China, Korea, and Japan. The fish is preferred for a variety of culinary applications, including soups and stir-fried dishes, due to its firm

* Corresponding author.
Email address: setyobudi_dja@ugm.ac.id; primaputra@ugm.ac.id

p-ISSN 2089-7790; e-ISSN 2502-6194
Received 21 April 2025; Received in revised form 11 July 2025; Accepted 14 August 2025
Available online 21 September 2025

texture, mild flavour, and adaptability in cooking (Shabrina *et al*., 2022).

Based on previous research on the potential of anisakis allergies, it was found that the cooking and freezing processes of infected fish are insufficient to prevent allergic reactions, as the allergen is thermostable. The release of allergens into fish flesh could still potentially occur during storage, even if anisakis are no longer found in the flesh (Triwijayani, 2019). Even in dead conditions, nematode larvae could still cause an allergic reaction (Moneo *et al*., 2007). Kochanowski *et al.* (2020) have shown in their study that marine fish products, even processed under 121℃ for 60 min, still have the allergen of Anisakis larvae remain active.

Few studies have been conducted in Indonesia on the potential presence of Anisakis allergens in largehead hairtail. This study aims to investigate the presence and resistance of Anisakis allergens during the heat treatment process of largehead hairtail, providing information that will serve as a reference for handling and processing this species.

Materials and Methods

Anisakis collection

In total, 50 largehead hairtail (*T. lepturus*) were collected from the south coast of Yogyakarta (landed in Depok beach) from December 2022 to July 2023. The fish were obtained in fresh condition, brought to the laboratory using a cold box, and then stored in a freezer for further observation. The fish samples were measured for total length using a ruler and for weight using analytical scales. Furthermore, the fish were dissected, and the organs were examined for parasites. The observed parts were the flesh, gastrointestinal tract, liver, abdominal cavity, and gonads. Anisakis were washed using 0.9% NaCl solution and then grouped by external appearance, such as colour and size. Anisakis larvae were stored in a collection tube for further analysis.

Anisakis infection analysis

The data analysis includes the type of anisakis that infect fish, their prevalence, average intensity of infection, target organs, and distribution across different organs. The Bush *et al.* (1997) reference was used to calculate the prevalence and mean intensity of infection.

$$Prevalence = \left(\frac{infected\ fish\ total}{fish\ total}\right) 100\%$$

$$Mean\ Intensity = \left(\frac{total\ of\ parasites}{total\ of\ infected\ fish}\right)$$

Molecular identification

Molecular identification in this study was done using the PCR Direct Sequencing Method. DNA extraction was performed using instructions from the Genomic DNA Mini Kit Tissue Protocol. The target of amplification was the Internal Transcribed Spacer (ITS) region. It amplified using primer A (5'-CCA TCC AAC ATC TCA GCAT GAT GAA A-3') and primer B (5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3') (D'Amelio *et al.* 2000). Amplification process was carried out using Thermalcycler (Biorad, T100TM) with the condition of 94˚C (pre-denaturation) for 5 minutes, following 35 cycles at 94˚C (denaturation) for 40 seconds, 42˚C (annealing) for 40 seconds, 72˚C (extension) for 75 seconds, and 72˚C (final extension) for 7 minutes. The products of amplification were electrophoresed in a 1.5% agarose gel containing 0.75 µL of FloroSafe DNA stain (1st base) using gel electrophoresis (Mupid-exU) for 15 minutes, and the result was photographed on a UV-Transilluminator (Pacific Image Electronic). The PCR products were sequenced in a DNA sequencing service company (Singapore's 1st Base Laboratory through PT. Genetika Science Indonesia).

Allergen heat resistance test

In total 0.25 grams of Anisakis larvae and 0.25 grams of muscle near the site of infection were then taken as samples and heated by boiling using 20 mL of distilled water at 60℃, 90℃, and 120℃ for 15 minutes. The protein allergens were analyzed using SDS-PAGE.

Anisakis allergen identification using SDS-PAGE

Protein isolation

Phosphate Buffer Saline-Tween Phenyl Methyl Sulfoid Fluoride (PBS TPMSF) and quartz sand were added to the prepared sample, which had been crushed in a mortar and processed at 4°C. The sample was then put in a falcon tube and sonicated for 10 minutes. After adding 100% ethanol (1:1) to the microtube containing the supernatant, it was centrifuged for 15 minutes at 4°C and 6,000 x g followed by incubation at -20°C overnight. Centrifuge for 10-15 minutes at 10,000 g and 4°C. Let the pellet air dry and discard the supernatant. Add 0.02 M Tris-HCl pH 6.8 (1:1).

Gel preparation

Gel preparation started with preparing the glass plates. Two layers of gel were then made: a stacking gel for sample collection and a separating gel for protein separation. Upper Gel Buffer (UGB), T-acryl, aquadest, Ammonium Persulfate (APS), and N,

N,N', N'-tetramethyl Ethylene Diamine (TEMED) were mixed to make the stacking gel, and Lower Gel Buffer (LGB), T-acryl, aquadest, APS, and TEMED were mixed to create the separating gel.

Sample injection

A microtube was filled with 10 µL of protein isolate sample, 10 µL of Tris-HCl, and 20 µL of Reducing Sample Buffer (RBS), and then heated for 3 minutes at 100°C. After cooling, the sample was added to each well with a volume of 20 µL. The protein standard was then put into the well on the left. The lower reservoir was linked to the anode, while the higher reservoir was linked to the cathode. The power supply was turned on with an electric current of 30 mA and 130 V. When the blue marker was 0.5 cm above the gel plate's bottom limit, the run was stopped.

Colouring, washing, and molecular weight determination

The gel was submerged in the staining solution for 30-60 minutes. It was then soaked in a destaining solution to remove colour, and a result was obtained. The electrophoresis result was scanned, and the molecular weight was determined. The allergen information from the allergome database was compared with the results.

Data analysis

DNA sequencing data analysis

The DNA sequencing product was analysed using BioEdit software. The nucleotide alignment data obtained were matched with data available in GenBank at NCBI (National Centre for Biotechnology Information) using BLAST (Basic Local Alignment Search Tool) to determine the type of Anisakis analysed. The phylogenetic tree was compiled using Mega 11.0 software by aligning the sample DNA sequence with several Anisakis DNA sequences in GenBank.

Allergen data analysis

The gel electrophoresis product was calibrated for molecular weight by aligning it with the Precision Plus Protein Standard (Bio-Rad). The protein's molecular weight was then calculated using Gel Analyzer 23.1.1 software and compared with the molecular weight of Ani's allergen protein.

Results

A total of 50 largehead hairtail samples, caught at Depok Coasts, Yogyakarta, had lengths ranging from 48.5 to 69 cm and weights ranging from 92.6 to 333.2 grams, and were used in this study. The studies indicated that *T. lepturus* was very susceptible to Anisakis infection. Table 1 depicts the results from the observation of fish infections with Anisakis.

The prevalence of Anisakis in *T. lepturus* at Depok Beach, Yogyakarta, was notably high (64%), with a mean intensity of 7.47 larvae/fish. Figure 1 shows how the prevalence and mean intensity of Anisakis in *T. lepturus* correlated with body length. The maximum prevalence (100%) was observed in the 65.1-70 cm body length category and observed as the highest mean intensity (18.00 larvae/fish) (Figure 2). The minimum prevalence (50%) was recorded in the 45-50 cm and 60.1-65 cm.

The distribution of Anisakis infection in *T. lepturus* body indicated the highest prevalence was observed in the abdominal cavity (87%), followed by organs (8%), gonads (3%), and the digestive system (2%), while no Anisakis infection was detected in the flesh (Table 2).

Anisakis larvae were identified molecularly to obtain accurate species identification. Molecular identification of Anisakis larvae is shown in Figure 3, and the DNA sequencing results are presented in Table 3. Molecular identification of Anisakis larvae through direct sequencing revealed that the larvae collected from *T. lepturus* are identified as *Anisakis typica* with 98-99% similarity in GenBank with *A. typica* species from the USA, Bangladesh, China, Indonesia, Malaysia, Thailand, and Papua New Guinea, with the corresponding accession numbers: MF668910.1, ON065558.1, MT020146.1, MW541817.1, MZ895793.1, MN420659.1, and JX648312.1. Following the identification of *A. typica* through BLAST results from GenBank, a phylogenetic tree was constructed to illustrate the relationships among Anisakis species (Figure 4). The phylogenetic tree analysis indicates that *A. typica* is clustered with other *A. typica* and is distinct from other Anisakis clusters, while *A. simplex* and *A. pegreffii* form exclusive clusters.

Results on Anisakis allergens generate 14 distinct types with varying molecular weights by the SDS-PAGE technique. The electrophoresis results indicated that *A. typica* expressed 11 protein bands, non-infected fish muscle displayed eight protein bands, and infected fish muscle revealed 12 protein bands. Figure 5 shows the results of the molecular weight of Anisakis larvae in several treatments. The molecular weights of Ani's allergens shown in well A are as follows: Ani s 1 (24 kDa), Ani s 2 (97 kDa), Ani s 3 (41 kDa), and Ani s 7 (139 kDa). These results indicate that *A. typica*, which infects *T. lepturus* from the Coast of Depok, Yogyakarta, can produce allergens. Furthermore, the observation of the infected fish muscle sample, which was subjected to heat treatment at 60 ℃ (Ia), 90 ℃ (Ib), and 120 ℃ (Ic) (Figure 5), illustrates that an increase in

temperature results in more fading of the formed band. The code (Ic), subjected to a temperature treatment of 120℃, exhibits a weak and nearly invisible protein band. This suggests that heat treatment may reduce the risk of allergies associated with consuming fish infected with Anisakis. Even though the band was faded, the allergen was still detected. It could cause allergies in people with particular sensitivities who consume Anisakis-infected fish, even after the fish has been cooked.

Discussion

Anisakis is a parasite that commonly infects economically valuable fish, mainly marine species. This observation on *T. lepturus* in the south coast of Depok Beach, Yogyakarta, revealed that among 50 fish examined, 34 were infected with Anisakis larvae. The infection was identified in the cavities, organs, gonads, and gastrointestinal system. The prevalence of Anisakis infection in *T. lepturus* from Depok Beach was notably high, recorded at 64% with a mean intensity of 7.74 larvae/fish. Our findings in the present study were higher compared to the prevalence of Anisakis in *T. lepturus* from the South Coast of Purworejo and Cilacap (62.68% and 53.33%) (Setyobudi *et al*., 2007 and Suadi *et al*., 2007), Pangandaran Coasts (45.5%) (Ayun *et al*., 2021), Pati and Cirebon Coasts (38.4% and 27.3%) (Setyobudi *et al*., 2023), and Bali Coast (31.5%) (Semarariana *et al*., 2012). However, it was much lower compared to the North Coast, Tuban (98.5%) (Setyobudi *et al*., 2023). Based on the observation time, we conclude that the prevalence of Anisakis in *T. lepturus* increased over time and was much higher on the northern Coast of Java.

The mean intensity of Anisakis in *T. lepturus* has been reported from the South Coast of Java, exhibiting a low value at Purworejo (3.3 larvae/fish), Cilacap (4 larvae/fish), and Pangandaran (4.4 larvae/fish) (Ayun *et al*., 2021; Setyobudi *et al*., 2007; Suadi *et al*., 2007). The mean intensity of Anisakis infection in the Waters of North Coast Java was reported as moderate (42.9 larvae/fish) in Tuban. In comparison, Pati (6.4 larvae/fish) and Cirebon (3.3 larvae/fish) exhibited low values (Setyobudi *et al*., 2023). Additionally, the mean intensity of Anisakis infection in Bali's Coasts was also reported as a low value (9.2 larvae/fish) (Semarariana *et al*., 2012).

The size of the fish is a determinant that affects Anisakis larval infection. The prevalence and mean intensity of Anisakis vary according to the length of the fish. Anisakis infection in *T. lepturus* from the Southern Coast of Yogyakarta correlates positively with fish length. In general, larger fish tend to accumulate a greater number of Anisakis larvae, hence showing a higher infection rate than smaller fish. The same phenomena were also reported by Setyobudi *et al*. (2007). Dietary habits and food intake might also increase adult fish infection rates (Mattiucci *et al*., 2018). On the other hand, the prevalence of Anisakis infection in fish is increased by the existence of intermediate and definitive hosts of Anisakis (Muttaqin & Abdulgani, 2013). This fact was proven by Palm *et al*. (2008), who found an elevated frequency of Anisakis infection in the North Bali Strait, which correlates with the large number of dolphins serving as the definitive hosts.

The distribution of Anisakis infection in *T. lepturus* indicated that the abdominal cavity exhibited the highest infection rate (87.03%), while the digestive system showed the lowest (2.09%). Most large larvae are found free-living in the body cavity. However, small numbers of encapsulated larvae are found in the liver, digestive tract, and gonads. The distribution of Anisakis infection could result from the migration of worms within the fish's body during its life or post-mortem. Anisakis larvae, when present in high infection intensity, mainly disseminate throughout different areas of the fish or host's body, predominantly residing in the cavity. Anisakis larvae are commonly found in the liver, gonads, mesentery, or attached to the intestinal mucosa of many fish species (Mattiucci *et al*., 2018). The previous work indicates that Anisakis larvae were primarily found in fish's internal organs and body cavities (Setyobudi *et al*., 2007; Anshary *et al*., 2014).

Anisakis is recognised to cause a zoonotic parasitic disease that has become an emerging problem in developed countries. To date, there have been no reported cases of Indonesian anisakiasis; nevertheless, a shift towards the consumption of raw or undercooked fish, such as sashimi and sushi, could raise the risk of human anisakiasis. Among Anisakis species, *A. simplex* and *A. pegreffii* are the major agents of human anisakiasis (Mattiucci et al. 2011; Umehara et al. 2007), while *A. typica* is still very rarely (not yet) reported as an anisakiasis agent.

Anisakis is a nematode that poses a significant health risk when accidentally ingested by humans. Marine fish infected with Anisakis could lead to anisakiasis and trigger allergic responses. Nowadays, 14 different types of allergens (Ani s 1 to Ani s 14) exhibit varying molecular weights and protein properties (Moneo *et al*., 2007). The gel electrophoresis results revealed specific protein bands with identical molecular weights to the allergens Ani s 1 (24 kDa), Ani s 2 (97 kDa), Ani s 3 (41 kDa), and Ani s 7 (139 kDa).

Anisakis larvae cannot be guaranteed to perish during the cooking process unless they are cooked at the appropriate temperature. Prior research has indicated that cooked fishery products retain live Anisakis larvae due to the heat resistance of Anisakis up to 90℃ (Sánchez-Alonso *et al.*, 2021). The cooking and freezing of fish infected by Anisakis are ineffective in preventing allergic responses due to the thermostable allergen. The possibility for allergen release into the muscle remains during the preservation phase and could happen even after the larvae are no longer found in the fish flesh (Triwijayani, 2019).

The allergic potential caused by eating infected fish might occur when consumed raw and after cooking, because some allergens are thermostable. Every Anisakis allergen found in this study has thermostable properties. This explains why consuming fish infected with Anisakis larvae, even after undergoing processing, still carries the potential of allergies. According to Kochanowski *et al.* (2020), heat treatment detected many Anisakis allergens. These allergens include Ani s 2, Ani s 3, and Ani s 5 and the possible allergens paramyosin, tropomyosin, and SXP/RAL-2 family protein 2 isoform 1. In addition, according to Abattouy *et al.* (2012), two blots included the somatic allergen Ani s 3 (tropomyosin). This allergen is a protein with thermostable characteristics that might cause allergic reactions in certain people who eat cooked contaminated fish. Ani s 1 allergen was also found, a significant thermostable allergen generated by the excretory glands.

In this study, we found a positive correlation between the temperature raised and the fading of the protein band identified. At a temperature of 90℃, the protein band remained clear. However, at 120℃, the protein band shown on SDS-PAGE seemed to diminish in intensity. These findings demonstrate that applying heat treatment can decrease the potential of allergies caused by Anisakis in fish.

Allergic reactions can also occur due to fishery products contaminated with allergens, without any live larvae being found. Anisakis, which infects fish, releases metabolites into the fish muscle tissue in the form of antigens. The antigens will then trigger a reaction from the host's immune system. Solas *et al.* (2008), in their research, found that antigens from the Anisakis allergen were spread in the muscle tissue of infected fish. The antigens spread in fish muscle cause allergic reactions, including urticaria, vomiting, diarrhoea, dyspnea, anaphylaxis, or angioedema in some sensitive individuals. Thus, although larvae are no longer found in fish muscle either naturally or through the handling process, the potential for allergies caused by Anisakis larvae still exists. Likewise, raw seafood such as sushi and processed seafood are not free from the potential for allergies. However, everyone has a different sensitivity to antibody responses due to genetic factors (Kennedy *et al.*, 1991). Therefore, food safety related to Anisakis allergens is crucial to prevent allergic reactions and ensure consumer health.

Conclusion

In this study, the largehead hairtail (*Trichiurus lepturus*) was infected by *Anisakis* sp. with a pretty high prevalence (64%) and mean intensity of 7,47 larvae/fish. The most infected location was the abdominal cavity (87,03%). Furthermore, Anisakis allergens were found in the flesh of the largehead hairtail. Even after the cooking process, the Anisakis allergen was still detected.

References

Abattouy, N., A. Valero, J. Martín-Sánchez, M. C. Peñalver, & J. Lozano. 2012. Sensitization to Anisakis simplex Species in Morocco Sensitization to Anisakis simplex Species in the Population of Northern Morocco. In *J Investig Allergol Clin Immunol* (Vol. 22, Issue 7).

Anshary, H., Sriwulan, M. A. Freeman, K. Ogawa. 2014. Occurrence and molecular identification of *Anisakis* Dujardin, 1845 from marine fish in Southern Makassar Strait, Indonesia. *Korean Journal of Parasitology*, *52*(1), 9–19.

Ayun, N. Q., L. S. Dewi, Murwantoko, E. Setyobudi. 2021. The occurrence of *Anisakis* larvae on hairtail, *Trichiurus lepturus* caught from the Pangandaran Waters, West Java, Indonesia. *Biodiversitas*, *22*(5), 1378–1384. https://doi.org/10.13057/biodiv/d220339

Ayun, N. Q., R. F. Syarifah, Murwantoko, E. Setyobudi. 2022. *Anisakis* infection of belanger's croaker (*Johnius Belangerii* Cuvier 1830) at the Indian Ocean coast of Yogyakarta, Indonesia. Jordan *Journal of Biological Sciences*, 15: 26–36. https://doi.org/10.54319/jjbs/150105

Bush, A. O., K. D. Lafferty, J. M. Lotz, A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *The Journal of Parasitology*, *83*(4), 575–583.

Dawa, U.P.L., B. U. M. T. J. Awang, D.S. Gadi, M.M. Lakapu. 2024. Identification and prevalence of Anisakis sp. parasites in tuna at the Oeba Fish Auction Site (FAS)—Kupang City. JPHPI *27* (10): 955-963. https://doi.org/10.17844/jphpi.v27i10.54739

D'amelio, S., K. D. Mathiopoulos, C. P. Santos, O. N. Pugachev, S. C. Webb, M. Picanco, L. Paggi. 2000. Genetic markers in ribosomal DNA for the identification of members of the genus Anisakis (Nematoda: Ascaridoidea) defined by polymerase chain reaction-based restriction fragment length polymorphism. *International Journal for Parasitology*, *30*, 223–226.

Indaryanto, F.R., M. M. Kamal, N. A. Butet, R. Affandi, R. Tiuria. 2024. Article Review: Identifications and Geographic Distribution of Six Anisakis Species (Nematoda: Anisakidae) in Indonesia. Jurnal Veteriner *25* (1): 143-164. https://doi.org/10.19087/jveteriner.2024.25.1.143

Kennedy, M. W., D. L. Wassom, A. E. Mcintosh, J. C. Thomas. 1991. H-2 (I-A) control of the antibody repertoire to secreted antigens of *Trichinella spiralis* in infection and its relevance to resistance and susceptibility. In *Immunology* (Vol. 73).

Kochanowski, M., M. Różycki, J. Dąbrowska, A. Belcik, J. Karamon, J. Sroka, T. Cencek. 2020. Proteomic and bioinformatic

investigations of heat-treated *Anisakis simplex* third-stage larvae. *Biomolecules*, *10*(7), 1–36. https://doi.org/10.3390/biom10071066

Mattiucci S, L. Paggi, G. Nascetti, C.P. Santos, G. Costa, A.P. Di Beneditto, R. Ramos, M. Argyrou, R. Cianchi, L. Bullini. 2002. Genetic markers in the study of *Anisakis typica* (Diesing, 1860): larval identification and genetic relationships with other species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae). *Systematic Parasitology,* 51 (3): 159-170

Mattiucci S, Paoletti, F Borini, M. Palumbo, R.M. Palmieri, V. Gomes, A. Casati, G. Nascetti. 2011. First molecular identification of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in a paraffin-embedded granuloma taken from a case of human intestinal anisakiasis in Italy. *BMC Infect Dis.,* 11: 82. DOI:10.1186/1471-2334-11-82

Mattiucci, S., P. Cipriani, A. Levsen, M. Paoletti, G. Nascetti. 2018. Molecular epidemiology of *Anisakis* and *anisakiasis*: an ecological and evolutionary road map. *Advances in Parasitology* (Vol. 99, pp. 93–263). Academic Press. https://doi.org/10.1016/bs.apar.2017.12.001

Moneo, I., M. L. Caballero, R. Rodriguez-Perez, A. I. Rodriguez-Mahillo, M. Gonzalez-Muñoz. 2007. Sensitization to the fish parasite *Anisakis simplex*: Clinical and laboratory aspects. *Parasitology Research*, *101*(4), 1051–1055.

Muttaqin, M. Z., N. Abdulgani. 2013. Prevalensi dan derajat infeksi *Anisakis* sp. pada saluran pencernaan ikan Kakap Merah (*Lutjanus malabaricus*) ditempat pelelangan ikan Brondong Lamongan. *Jurnal Sains dan Seni Pomits*, *2(1)*, 30–33.

Palm, H. W., I. M. Damriyasa, Linda, & I. B. M. Oka. 2008. Molecular genotyping of *Anisakis* Dujardin, 1845 (Nematoda: Ascaridoidea: Anisakidae) larvae from marine fish of Balinese and Javanese waters, Indonesia. *Helminthologia*, *45*(1), 3–12. https://doi.org/10.2478/s11687-008-0001-8

Sánchez-Alonso, I., N. Carballeda-Sangiao, M. González-Muñoz, S. C. Arcos, A. Navas, M. Careche. 2021. Thermal patterns of heat treated *Anisakis* L3-infected fishery products allow separation into low, intermediate and high risk groups of potential use in risk management. Food Control, 124. https://doi.org/10.1016/j.foodcont.2020.107837

Sánchez-Alonso, I., N. Carballeda-Sangiao, M. González-Muñoz, A. Navas, S. C. Arcos, A. Mendizábal, M. Tejada, M. Careche. 2018. Pathogenic potential of Anisakis L3 after freezing in domestic freezers. Food Control, 84, 61–69. https://doi.org/10.1016/j.foodcont.2017.07.010

Semarariana, I. W. Y., I. N. A. Suratma, & I. B. M. Oka. 2012. Infeksi larva cacing Anisakis spp. pada ikan Layur (*Trichiurus lepturus*). Indonesia Medicus Veterinus, 1(2), 293–304.

Setyobudi, E., S. Helmiati, D. Soeparno. 2007. Infection of Anisakis sp. in hairtail (Trichiurus sp.) in the Southern Coast of Purworejo Regency. Jurnal Perikanan (Journal of Fisheries Sciences), IX (1), 142-148.

Setyobudi, E., C. H. Jeon, C. H. Lee, K. B. Seong, J. H. Kim. 2011. Occurrence and identification of *Anisakis* spp. (Nematoda: Anisakidae) isolated from chum salmon (*Oncorhynchus keta*) in Korea. Parasitology Research, 108(3), 585–592. http://dx.doi.org/10.1007/s00436-010-2101-x

Setyobudi, E., Murwantoko, A. M. R. Utami, & R. F. Syarifah. 2023. Anisakid nematodes from the largehead hairtail fish (*Trichiurus lepturus*) from the northern coast of Java, Indonesia. Biodiversitas, 24(3), 1560–1568. https://doi.org/10.13057/biodiv/d240328

Setyobudi, E., S. Soeparno, S. Helmiati. 2011. Infection of *Anisakis* sp. larvae in some marine fishes from the southern coast of Kulon Progo, Yogyakarta. Biodiversitas Journal of Biological Diversity, 12(1).

Shabrina, L., Y. H. Sipahutar, W. Sumiyanto, H. Mulyani. 2022. Determination of CCP for frozen layur fish (*Trichiurus laepturus*) processing and compliance with requirements for export document to China during the Covid-19 pandemic in PT. LP Belawan-North Sumatra. The 23rd National Conference on Fisheries and Aquaculture, 23-24 August 2022, Jakarta, Indonesia. DOI: http://dx.doi.org/10.15578/psnp.12099.

Soewarlan, L. C., E. Suprayitno, Hardoko, H. Nursyam. 2014. Identification of anisakid nematode infection on skipjack (*Katsuwonus pelamis* L.) from Savu Sea, East Nusa Tenggara, Indonesia. International Journal of Biosciences (IJB), 5(9), 423–432.

Solas, M. T., M. L. Garci´a, G. Garci´a, A. I. Rodriguez-Mahillo, M. Gonzalez-Munoz, C. D. L. Heras, M. Tejada. 2008. *Anisakis* antigens detected in fish muscle infested with *Anisakis simplex* L3. In *Journal of Food Protection*, 71(6), 1273-1276.

Suadi, S. Helmiati, R. Widaningroem. 2007. Parasit *Anisakis* sp. pada populasi Layur (*Trichiurus* sp.) yang didaratkan di pelabuhan ikan Cilacap. *J.Fish.Sci.*, *IX (2)*, 226–232.

Syarifah, R. F. Murwantoko, E. Setyobudi. 2023. Prevalence and Intensity of Larvae of the Genus *Anisakis* sensu lato (Nematoda, Anisakidae) in Bigeye Scad, *Selar crumenophthalmus* (Bloch 1793), from the Indian Ocean off Java, Indonesia. *Asian Fisheries Science,* 36(4): 192–202. DOI: https://doi.org/10.33997/j.afs.2023.36.4.002.

Syarifah RF, I. Rohmah, L. Ramatia, N. Astuti, Murwantoko, D.W.L., Sari DWK, E. Setyobudi. 2024. The Differences of Anisakis Larvae Infection on Scads (*Decapterus* spp.) in the Indian Ocean off the Southern Coast of East Java Indonesia. *Egyptian Journal of Aquatic Biology and Fisheries*, 28 (4): 1001-1022. DOI: 10.21608/ejabf.2024.370891.

Triwijayani, A. U. 2019. *Thesis.* Identification of *Anisakis sp.* In fresh hairtail, frozen dongte and frozen godengo and detection of allergens produced. Universitas Gadjah Mada.

Umehara, A, Y. Kawakami, T. Matsui, J. Araki, A. Uchida. 2007. Molecular identification of the etiological agent of the human anisakiasis in Japan. *Parasitol Int.* 56: 211–215.

Zarry, M.G., Hambal, M., Zuhrawaty, N.A. 2017. Identifikasi Endoparasit pada Ikan Jebong (*Abalistes stellaris*) di Tempat Pelelangan Ikan (TPI) Lampulo Kota Banda Aceh. Jurnal Ilmiah Mahasiswa Veteriner 1(2): 188-195.

How to cite this paper:
Busyro, I. K., E. Setyobudi, I. D. Puspita, M. M. P. Putra. 2025. Investigation of anisakis infection and allergic potential on largehead hairtail (*Trichiurus lepturus*) from south coast of Yogyakarta, Indonesia. Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan, 14(3): 333–341.

Table 1. Anisakis infection status of *T. lepturus*

Name	N (total)	L (cm)	W (gr)	Infection status
Trichiurus lepturus	50	48,5-71	92,6-333,2	Infected

Table 2. Infection location of *Anisakis* in *T. lepturus*

Location	Total infection(s)	Percentage
Abdominal cavity	208	87,03%
Organs	19	7,95%
Gonads	7	2,93%
Digestive system	5	2,09%
Flesh	0	0,00%

Table 3. *Anisakis* BLAST in *T. lepturus*

Accesion number	Species	Location	Identity (bp)	Percentage (%)
JX648312.1	*Anisakis typica*	Papua New Guinea	593/596	99,50%
MN420659.1	*Anisakis typica*	Thailand	593/597	99,33%
MZ895793.1	*Anisakis typica*	Malaysia	593/597	99,33%
MW541817.1	*Anisakis typica*	Indonesia	592/597	99,33%
MT020146.1	*Anisakis typica*	China	593/597	99,33%
MF668910.1	*Anisakis typica*	USA	580/583	98,66%
ON065558.1	*Anisakis typica*	Bangladesh	586/593	98,82%

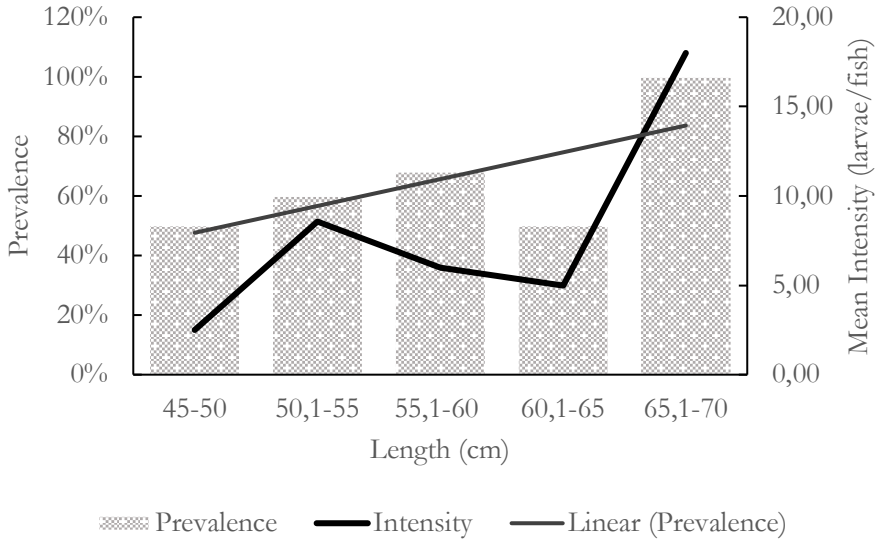

Figure 1. Prevalence and mean intensity of *Anisakis* infection in *T. lepturus*

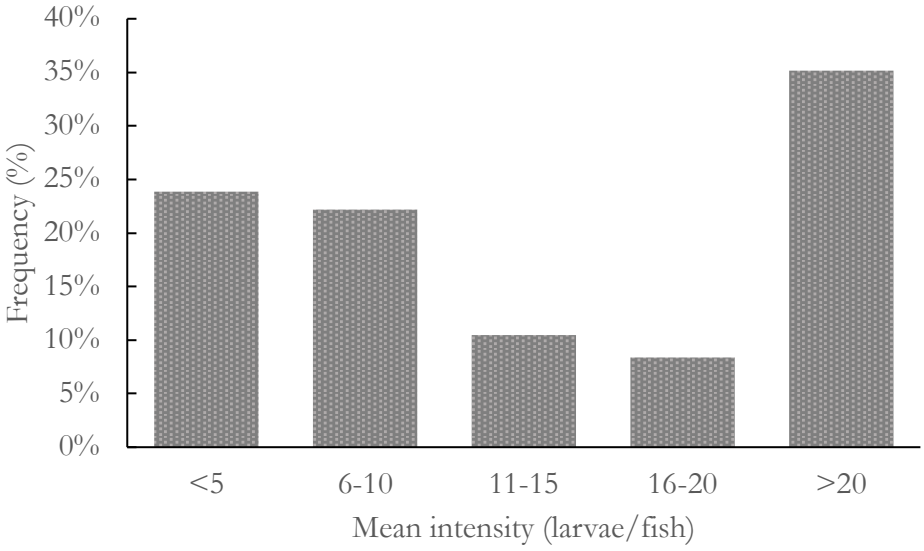

Figure 2. *Anisakis* distribution intensity in *T. lepturus*

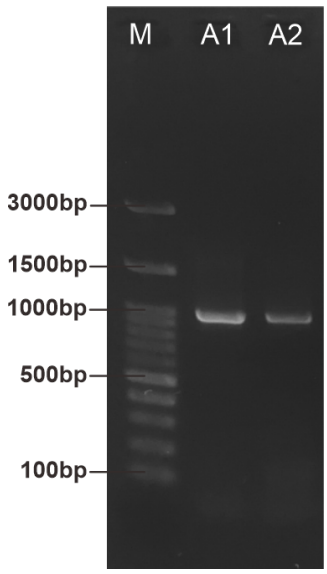

Figure 3. Electrophoresis of gen amplification ITS *Anisakis* larvae

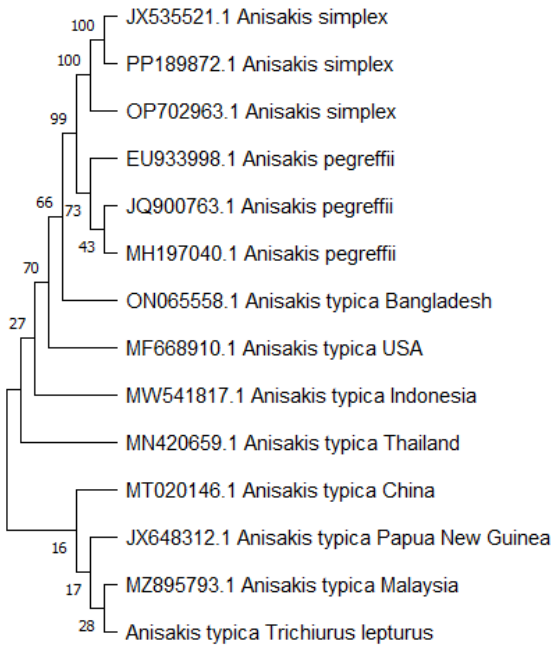

Figure 4. *A. typica* phylogram isolated from *T. lepturus* on the Southern Coast of Yogyakarta

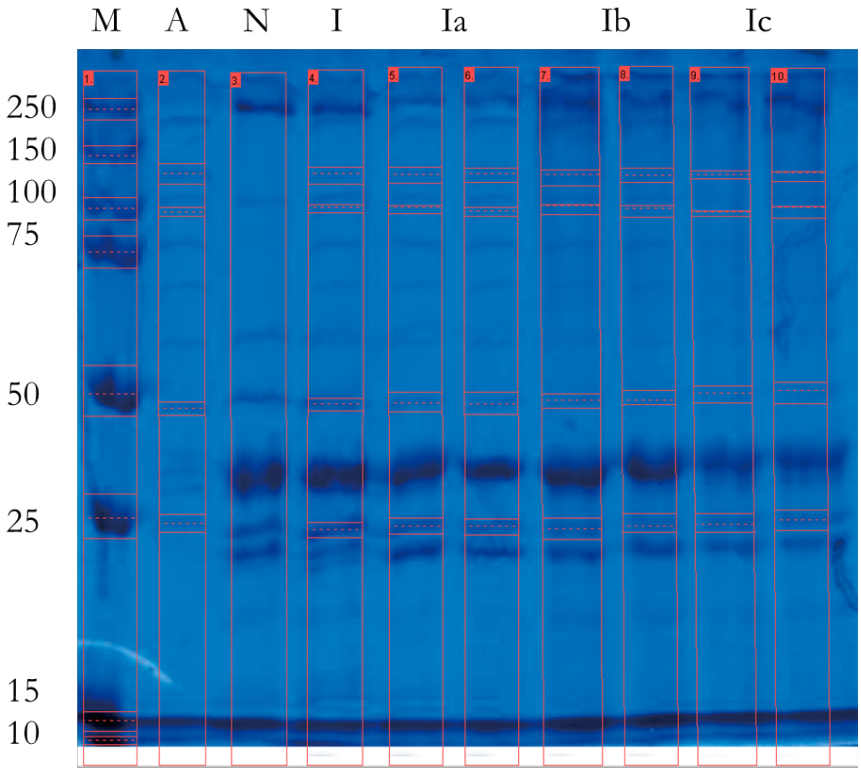

Figure 5. The SDS-PAGE Results on Anisakis Allergens (M: Marker, A: *A. typica*, N: non-infected muscle control, I: Infected muscle control, Ia: Infected muscle treated 60 ℃ (replication 1,2), Ib: Infected muscle treated 90 ℃ (replication 1,2), Ic: infected muscle treated 120 ℃ (replication 1,2))