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Article Review: Identifications and Geographic Distribution of Six Anisakis Species (Nematoda: Anisakidae) in Indonesia

(KAJIAN PUSTAKA: IDENTIFIKASI DAN DISTRIBUSI SECARA GEOGRAFIS ENAM SPESIES ANISAKIS (NEMATODA: ANISAKIDAE) DI INDONESIA)

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Abstract

Anisakis is a zoonotic nematode that causes anisakiasis in Europe and Asia. Food safety has recently become a major concern for consumers; therefore, accurate identification of Anisakis larvae is necessary for the correct diagnosis of Anisakis infection in humans and fish and for improving food safety. From 2001 to 2023, Anisakis examinations were carried out in 33 areas of Indonesian territory. Morphometric Anisakis of Indonesia generally have a body length of 0.10-34.50 mm with a diameter of 0.30-0.42 mm. Based on morphological and genetic identification, identified six species anisakis in Indonesia (*A. simplex, A. physeteris, A. pegreffii, A. berlandi, A. typica* and *Anisakis* sp. HC-2005), or identified six Anisakis genotypes based on genetic identification, namely *A. typica* (s.s), *A. typica var Indonesiansis, A. physeteris, A. pegreffii, A. berlandi* and *Anisakis* sp. HC-2005. *Decapterus* sp., *Thyrsitoides marleyi*, *Euthynnus afifinis*, *Auxis rochei, Gempylus serpens, Katsuwonus pelamis* and *Trichiurus lepturus* have a high prevalence (>90%), indicating that they are often infected with Anisakis. Anisakis spread throughout the waters in Indonesia with various types of fish as hosts and *A. typica* is the predominant species in Indonesia.

Key word: *Anisakis typica*; identification; marine fish parasites; food safety; zoonotic

Abstrak

Anisakis adalah nematoda zoonosis penyebab kejadian anisakiasis di Eropa dan Asia. Keamanan pangan belakangan ini mendapat perhatian utama dari para konsumen maka dari itu identifikasi larva Anisakis secara akurat perlu dilakukan untuk diagnosis infeksi Anisakis

yang benar pada manusia dan ikan di samping untuk meningkatkan keamanan pangan. Sejak tahun 2001 hingga 2023, pemeriksaan Anisakis dilakukan di 33 area territorial Indonesian. Morphometri Anisakis yang ada di Indonesia umumnya memiliki panjang 0,10-34,50 mm, dengan garis tengah/diameter 0,30-0,42 mm. Berdasarkan morfologi dan identifikasi genetik, berhasil diidentifikasi enam spesies anisakis di Indonesia (*A. simplex, A. physeteris, A. pegreffii, A. berlandi, A. typica*, dand *Anisakis* sp. HC-2005), atau teridentifikasi enam genotipe Anisakis berdasarkan pada identifikasi genetik, antara lain *A. typica* (s.s), *A. typica var Indonesiansis, A. physeteris, A. pegreffii, A. berlandi,* dan *Anisakis* sp. HC-2005. *Decapterus* sp., *Thyrsitoides marleyi*, *Euthynnus afifinis*, *Auxis rochei, Gempylus serpens, Katsuwonus pelamis*, dan *Trichiurus lepturus* memiliki prevalensi yang tinggi (>90%), menandakan bahwa mereka kerap menginfeksi bersama-sama Anisakis. Anisakis tersebar dalam perairan di Indonesia dengan berbagai jenis ikan sebagai induk semang dan *A. typica* merupakan spesies yang paling dominan di Indonesia.

Kata-kata kunci: *A. typica;* identifikasi; parasit ikan laut; keamanan pangan; zoonosis

INTRODUCTION

Helminth parasites are a group of parasites found in many organisms, including fish. The helminth parasitic group comprises trematodes (monogeneans and digeneans), cestodes, nematodes and acanthocephalans. Helminth parasites from the digenean and nematode groups are more commonly found inside the body (endoparasites) in Teleostei fish, while the monogenean group is located outside of the fish body (ectoparasites) (Chambers *et al.,* 2001; Cribb *et al.,* 2002). Nematodes are a group of parasites most commonly found in marine fish which are found in internal organs (body cavity, gonads, heart, intestines, liver, mesentery, pylorus caeca, stomach, and swim bladder) and gill cavities; however, they are not found in fish fillets or meat (Koepper *et al.,* 2022).

Some parasites in animals can infect humans and are called zoonotic parasites. The zoonotic helminth parasites, including *Clonorchis* sp*.,* and *Opisthorchis* sp*.,* from the trematode group which can cause liver damage or even liver cancer; *Diphylobothrium* sp*.,* from the cestode group, which can cause abdominal pain and diarrhea; and *Anisakis* sp., *Pseudoterranova* sp. (Jahncke and Schwarz, 2002), and *Histerothylacium* sp. (Koepper *et al.* 2022) from the nematode group which can also cause allergies, nausea, and acute abdominal pain.

After the first human infection reported in 1960 in the Netherlands, many cases of anisakid infections in humans have been reported worldwide because they cause anisakiasis. Most cases (more than 90%) have been reported in Japan and some from the Netherlands, France, and Spain (Santos *et al.* 2022). Anisakiasis cases have also been reported in several other countries, including the Malaysia, United States of America, Mexico, Canada, England, Belgium, Egypt, Korea, Philippines, Chile, Australia, and New Zealand. The main potential risk of Anisakis infection is stomach or intestinal infections that cause acute abdominal pain and can also cause allergic reactions. There are three types of anisakiasis, namely intestinal or gastric anisakiasis (if anisakid larvae infect the human intestine or stomach, it can cause acute abdominal pain for several hours or several days), ectopic anisakiasis (if anisakid larvae penetrate other body tissues such as the lungs, pancreas, eyes, tongue and others), and gastro allergy anisakiasis (Soewarlan 2016). The unspecific symptoms of anisakiasis make misdiagnosis of this disease common. Anisakiasis was frequently diagnosed prior to the presence of other conditions such Crohn's disease, appendicitis, gastric ulcer,

gastric tumor, cholecystitis, and peritonitis. Patients who get gastrointestinal problems must provide a thorough history, including dietary information (Amir *et al*., 2016).

The introduction of foods and cooking techniques from other nations as well as culinary vacations are becoming popular globally. Consuming seafood that is raw or barely cooked may be part of this trend. Transmission Anisakis to humans occurs when raw or undercooked fish are accidentally consumed, such as 1) sushi and sashimi from Japan; 2) tuna sparid carpaccio, marinated salted sardines, or pickled anchovies from the Mediterranean; 3) smoked or fermented herring (maatjes) from the Netherlands; 4) dry salmon (gravlax) from Norway; and 5) raw salmon (lomi-lomi) from Hawaii or ceviche from South America (Mladineo and Poljak, 2014). Therefore, many cases of anisakiasis occur in countries with a culture of consuming raw or undercooked fish. In Indonesia, raw or undercooked fish are also known, namely *Naniura* (raw fish cooked by soaking kaffir lime juice) from Lake Toba, North Sumatra; *Lawa Bale* from Polewali Mandar, West Sulawesi; *Gohu* from Ternate, North Maluku; *Rusip* from Bangka Belitung; and *Pacco* from Luwu, South Sulawesi.

There are no reports of Anisakis in Southeast Asian tropical nations like Brunai, Myanmar, Cambodia, and Vietnam (Wiwanitkit and Wiwanitkit, 2016). There are some reports on parasite study in fish in Thailand (Chaiphongpachara *et al*., 2022) and Philippines (Quiazon *et al*., 2013). Hemsrichart (1993) and Amir *et al*. (2016) both reported on the first anisakiasis case in Thailand and Malaysia, respectively.

The first observation of Anisakis in Indonesia was reported by Yamaguti (1954). Since then, many observations of Anisakis in Indonesian waters have been made. Anisakiasis was first reported by Uga *et al.* (1996), based on a seroepidemiological survey of zoonotic parasite infections among visitors to Sidoarjo City Hospital, East Java who suffered from diarrhea, and 11% positive for *Anisakis* sp., with high antibody titers in the adolescent group. In 2018, the Food and Drug Supervisory Agency (BPOM RI) identified Anisakis in canned mackerel fish products. The species of Anisakis parasites were formerly difficult to distinguish using a light microscope due to their comparable physical traits; as a result, the specifics of Anisakis species remained unknown, at least prior to the development of molecular techniques. However, nowadays, molecular methods serve as instruments to help with accurately identifying Anisakis species. Palm *et al*. (2008) made the first molecular identification of Anisakis in Indonesia, concluding that it is *A. typica*.

This research was aimed to summarize the research on Anisakis identification and distribution conducted in Indonesia. Although it does not thoroughly present the development of Anisakis in Indonesia, and will contribute to future research, increase public awareness, and provide input to government policy on fish food safety.

RESEARCH METHODS

The method used in this literature study is a qualitative method. Literature data was obtained from electronic databases from national and international scientific research journals, textbooks, as well as student research results (thesis, theses and dissertations) on the Internet using the Google search engine (Table 1.). The keyword used in the literature study is anisakis in fish in Indonesia, the scientific articles obtained are then traced more deeply. Collection data was collected by searching, selecting, presenting and analyzing, and developing and processing it into a simple framework and then presented in tabular form, explained descriptively, narratively, concisely, and systematically (Sari *et al.,* 2021).

Table 1. Literature data on the identification and inventory of Anisakis based on electronic databases between 2001 and 2023

No.	Reference	No.	Reference	No.	Reference
1	Lester *et al.* (2001)	20	Utami (2014)	39	Alim (2018)
2	Moore *et al*. (2003)	21	Anshary *et al.* (2014)	40	Hutama *et al*. (2018)
3	Jakob and Palm (2006)	22	Juniardi *et al.* (2014)	41	Puspitarini *et al.* (2018)
4	Suadi *et al.* (2007)	23	Indaryanto *et al.* (2014)	42	Ulkhaq *et al.* (2019)
5	Palm *et al*. (2008)	24	Pradipta *et al.* (2015)	43	Setyobudi *et al*. (2019)
6	Awik *et al.* (2010)	25	Widjanarko (2015)	44	Linda *et al.* (2019)
7	Setyobudi *et al.* (2011)	26	Hibur *et al.* (2016)	45	Fajarullah (2019)
8	Anshary (2011)	27	Hidayati *et al.* (2016)	46	Manurung *et al.* (2020)
9	Saputra (2011)	28	Bahri (2016)	47	Soewarlan *et al*. (2020)
10	Kleinertz *et al.* (2012)	29	Hafid and Anshary (2016)	48	Komariah *et al.* (2020)
11	Tamba *et al.* (2012)	30	Zarry *et al.* (2017)	49	Asmara (2021)
12	Semarariana *et al.* (2012)	31	Yani (2017)	50	Muttaqin *et al*. (2021)
13	Muttaqin and Abdulgani (2013)	32	Utami *et al.* (2017)	51	Ayun *et al.* (2021)
14	Herman *et al.* (2013)	33	Suryani (2017)	52	Labhu *et al.* (2022)
15	Kuhn *et al.* (2013)	34	Liananda *et al.* (2017)	53	Takubak *et al.* (2022)
16	Abdiani (2013)	35	Palm *et al.* (2017)	54	Koepper *et al.* (2022)
17	Koinari *et al*. (2013)	36	Bahari (2018)	55	Rifani *et al.*(2022)
18	Kurniawati (2014)	37	Detha *et al.* (2018)	56	Situngkir (2022)
19	Rahmawati (2014)	38	Paremme *et al.* (2018)	57	Junita *et al.* (2023)

RESULTS AND DISCUSSION

Taxonomy and Morphological Characteristic

Nematodes are also called roundworms because of their elongated cylindrical morphology. Their bodies are elongated, caterpillar-shaped and cylindrical covered with a rigid and non-existent cuticle there are eyelashes (Hassani 2015). The Anisakis family consists of two subfamilies: Anisakinae (Anisakis and Pseudoterranova) and Contracaecinea (Contracaecum and Phocascaris).

The subfamily Contracaecinea is comprised of the genera Contracaecum and Phocascaris. difficult to distinguish, requiring assistance from an electron microscope or genetic identification (Abollo and Pascual, 2002; Arai and Smith, 2016). Its life cycle involves aquatic invertebrates and fish (freshwater fish and marine fish) as intermediate or paratenic hosts as well as marine mammals (seals) and birds as definitive hosts and also involving several land animals (Abollo and Pascual, 2002; Sari *et al.,* 2021). Subfamily Anisakinae, both the Anisakis and Pseudoterranova genera, are known to infect marine fish as well as humans or are zoonotic, especially *Anisakis simplex (ss), A. pegreffii, A. physeteris* and *Pseudoterranova decipiens* (Audicana *et al*., 2003; Chen and Shih, 2015; Mladineo *et al*., 2016).

The structure of the anterior section, the digestive area (esophagus, ventricle, intestinal caecum, and ventricular appendage), and the caudal or posterior (mucron and spicules) are the primary morphological features used to identify Family Anisakidae (see Figure 1.). The identification key of Family Anisakidae species based on morphological character (Zhang *et al*., 2013; Arai and Smith, 2016; Mattiucci *et al.,* 2018; Theisen 2020):

1. Family Anisakidae (Figure 1b.)
   a. ventriculus are short, round, have a ventricular appendage (VA), and the intestinal caecum is long..... subfamily Contracaecinea

b. ventriculus are long and does not have ventriculus appendage (VA) ... 2

2. Subfamily Anisakinae (Figure 1a.)
   a. Intestine caecum are short ..... genus *Pseudoterranova*
   b. Does not have intestine caecum..... 3
3. Genera *Anisakis*
   a. Have mucron at posterior and ventriculus are long..... 4
   b. Does not have mucron at posterior . 5
4. Anisakis type I
   a. ventriculus are long and sigmoid .... *A. simplex* (s.s), *A. pegreffii*, atau *A. berlandi*
   b. ventriculus are long and thin walled ..... *A. ziphidarum*, *A. nascettii*
   c. ventriculus are long ..... *A. typica*
5. Anisakis type II (Figure 1c.)
   a. Does not have mucron, ventriculus are short and stout..... ..... Anisakis type II (*A. paggiae, A. physeteris* atau *A. brevispiculata*)
   b. Does not have mucron, ventriculus are long, posterior or tail sharply attenuated behind the anus..... *Anisakis* sp. CA-2012

Observation of the size and shape of the ventricles and spicules is relatively more difficult to observe and requires a microscope with adequate specifications. *Anisakis pegreffii* ventricular length (VL) is shorter than *A. simplex* (s.s), which has a range of 0.50 to 0.78 mm and 0.90 to 1.50 mm, respectively. The ratio of the lengths of the esophagus and ventriculus in *A. pegreffii* was what caused its shorter ventriculus. This ratio is higher in *A. pegreffii* [(1.00:3.08)–(1.00:4.7)] than in *A. simplex* (s.s.) [(1.0:1.9)–(1:2.8)], with no overlap in ratios seen, especially in the L4 and adult stages (Quiazon *et al*., 2008). *Anisakis pegreffii, A. berlandi* (*A. simplex* C), and *A. simplex* (s.s) all had statistically significantly different VL measurements, with average measurements ranging from 0.76 (0.55-0.90 mm), 1.11 (0.80-1.35 mm), and 1.29 (1.08-1.44 mm, respectively (Mattiucci *et al.,* 2014). *A. nascettii* is very similar morphological characters to *A. ziphidarum* and can be determined after the genetic identification (Mattiucci *et al.,* 2009). Davey (1971) recognizes the validity of only three species, namely *A. simplex, A. typica* and *A. physeteris*. the identification key of Anisakis species:

1. Oesophagus short, may be broader than long. Spicules shorter than 0.4 mm..... *A. physeteris* Baylis, 1923.
   Oesophagus oblong, often sigmoid, always longer than broad. Spicules longer than 0.7 mm ..... (2)
2. Longer spicule rarely more than twice as long as the shorter; ratio about 1 : 1.6 ..... *A. simplex* (Rud., 1809, det. Krabbe, 1878).
   Longer spicule at least 2 times as long as shorter; ratio about 1:3 ..... *A. typica* (Diesing, 1860)

According to Mattiucci *et al*. (2009), a morphological key for identifying and validating Anisakis type II is as follows::

1. Spicules 0.35–0.40 mm..... *A. physeteris*
2. Spicules 0.30–0.34 mm; length of ventriculus 0.56–0.60 mm ..... *A. brevispiculata*
3. Spicules 0.17–0.22 mm; ventriculus 0.35–0.40 mm in length, violin-shaped; three thin denticulate caudal plates, width: wpl1: 0.049– 0.051 mm; wpl2: 0.030–0.040; wpl3: 0.040– 0.045 ..... *A. paggiae*

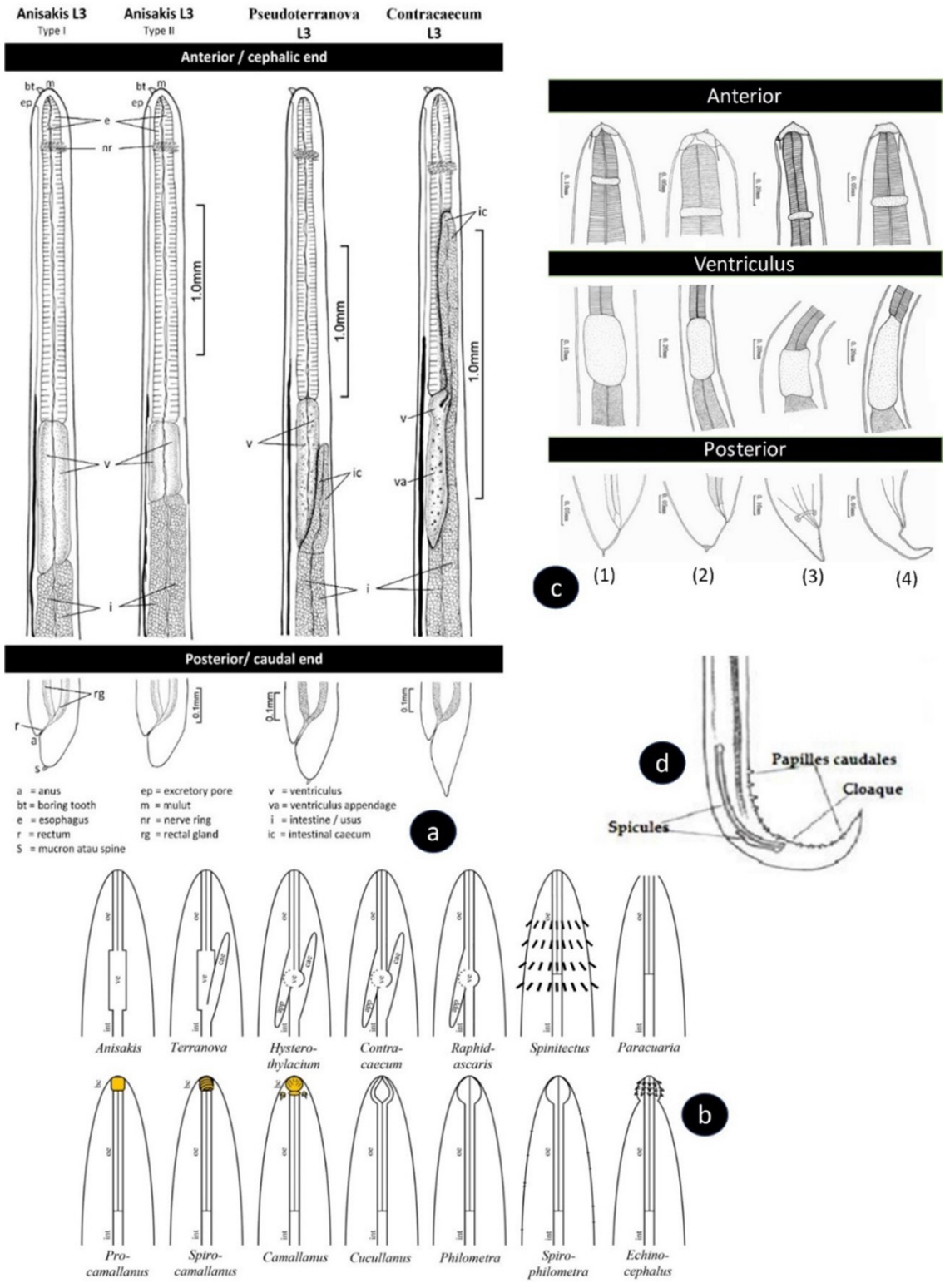

Figure 1. Morphological differences a) Subfamily Anisakinae (modifications from Arai and Smith 2016); b) Nematoda abundant in Indonesia (Theisen 2020); c) Morphological of Anisakis from South China Sea: 1) *A. Typica*, 2) *A. pegreffii*, 3) *A. physeteris*, 4) *Anisakis* sp. CA-2012 (Zhang *et al*. 2013); d) Spicules in Posterior (Hassani 2015)

Table 2. Morphometry *Anisakis* of Indonesia

Anisakis	Fish/host and location	Morphological Character	Measurement: Range(average)	Reference
A. simplex	*Rastrelliger brachysoma,* East Java	Body length	15–27 mm	Herman *et al.* (2013)
		Body diameter	0.40–0.90 mm	
	Euthynnus affinis, Nangro Aceh Darussalam	Body length	10–30 mm	Hidayati *et al.* (2016)
	Trichiurus savala, East Java	Body length	10.0–25.0 mm	Rahmawati (2014)
		Body diameter	0.40–0.90 mm	
	Decapterus macrosoma, East Java	Body length	10.0–19.0 mm	Hutama *et al.* (2018)
		Body diameter	0.40–0.90 mm	
	Priacanthus macracanthus, East Java	Body length	10.0–19.0 mm	Liananda *et al.* (2017)
A. typical	*Auxis rochei,* West Sulawesi	Ventriculus length	0.30–1.00 mm (0.58 mm)	Hafid and Anshary (2016)
		Mucron	0.02–0.03 mm (0.024 mm)	
	Decapterus russellii West Sulawesi	Ventriculus length	0.52–0.65 mm (0.57 mm)	
		Mucron	0.03 mm	
	Rastrelliger brachysoma, Banten	Body length	0.984 mm	Indaryanto *et al.* (2015)
		Body diameter	0.648 mm	
	Katsuwonus pelamis, Kupang	Body length	7.27–14.42 mm	Linda *et al.* (2019)
		Body diameter	0.2–0.48 mm	
		Esophagus length	0.01–0.80 mm	
		Ventriculus length	0.02–0.08 mm	
		Ventriculus diameter	0.01–0.02 mm	
		Mucron	0.001–0.012 mm	
Anisakis sp.	*Auxis thazard*, South Sulawesi	Ventriculus length	0.01–0.098 mm	Saputra (2011)
	Euthynnus affinis, Bangka Belitung	Body length	2.30 mm	Rifani *et al.* (2022)
	Leiognathus dussumieri, South Sulawesi	Body length	11.60 mm	Yamaguti (1954)
		Body diameter	0.24 mm	
		Head	0.105 mm	
		Tail	0.14 mm	
		Esophagus length	0.924 mm	
		Esophagus diameter	0.126 mm	
		Spicules	Left 0.29 mm Right 0.22 mm	
	Auxis rochei, Ende	Body length	8.0–11.0 mm	Labhu *et al.* (2022)
	Epinephelus coioides, East Java	Body length	14.0–22.00 mm	Alim (2018)
		Ventriculus length	0.64–0.67 mm	
		Mucron	0.168 mm	
Anisakis sp. Type II	*Katsuwonus pelamis*, East Java	Body length	10.0–34.5 mm	Suryani (2017)
		Body diameter	0.30–0.42 mm	
Anisakis physeteris	*Lutjanus argentimaculatus,* Lampung	Body length	4.125 mm	Puspitarini *et al.* (2018)
		Body diameter	0.25 mm	
		Esophagus length	0.75 mm	
		Ventriculus length	0.25 mm	

The morphometry of *Anisakis* in Indonesia have been carried out for *A. simplex*, *A. typica*, *A. physeteris, Anisakis* type I and *Anisakis* type II (Table 2). The morphological characteristics of Anisakis vary greatly, this is because they have different host species in their life cycle (Roca-Genores *et al.,* 2018). Fish parasitic nematodes present a taxonomic and systematic problem, the solution cannot be obtained only by its morphological study but also by studying their epidemiology and ecology and biogeography of their hosts. Nevertheless, the morphology remains to be the main means of identifying these parasites and forming a basis their taxonomy (Hassani 2015).

Molecular Identification

Frequently used molecular identification methods for Anisakis species are restriction fragment length polymorphism (PCR-RFLP)-based PCR (Table 3) and sequencing PCR (Tabel 4). Identify Anisakis species with the target ITS region (ITS1-5.8S-ITS2) using the restriction enzymes *HinfI*, *HhaI*, and *TaqI* (D'amalio *et al*., 2000; Pontes *et al.,* 2005; Chen and Shih, 2015) with the following molecular taxonomy keys:

1. The *HinfI* enzyme produces a band on the fragment
   a. 370 – 300 – 250 bp .....
   A. pegreffii [1]
   b. 620 – 250 – 80 bp .....
   ***A. simplex* (s.s)** [1]
   c. 620 – 250 – 80 bp .....
   ***A. simplex* C** [1]
   d. 380 – 290 – 270 bp .....
   A. physeteris [1]
   e. 520 – 340 – 120 bp .....
   A. schupakovi [1]
   f. 370 – 320 – 290 bp .....
   A. ziphidarum [1]
   g. 620 – 350 bp .....
   A. typica [1]
   h. 920 bp .....
   A. paggiae [2]
   i. 620 – 250 – 80 bp .....
   A. brevispiculata [2]
   J. 620 – 370 – 300 – 250 bp .....
   Recombinan genotype A. pegreffii dan *A. simplex* (s.s) [2]
   k. 620 – 320 bp .....
   Anisakis sp. A [3]
   l. 1.000 bp .....
   Anisakis sp. CA-2012 [4]
2. The *HhaI* enzyme produces a band on the fragment
   a. 550 – 430 bp .....
   A. pegreffii [1]
   b. 550 – 430 bp .....
   ***A. simplex* (s.s)** [1]
   c. 550 – 400 – 130 bp .....
   A. simplex C [1]
   d. 550 – 430 bp .....
   A. physeteris [1]
   e. 400 – 300 – 150 bp .....
   A. schupakovi [1]
   f. 550 – 430 bp .....
   A. ziphidarum [1]
   g. 320 – 240 – 180 – 160 bp .....
   A. typica [1]
   h. 550 – 430 bp.....
   A. paggiae [2]
   h. 390 – 330 – 190 bp .....
   A. brevispiculata [2]
   J. **550 – 430 bp** .....
   Recombinan genotype A. pegreffii dan *A. simplex* (s.s) [2]
   k. 550 – 300 – 80 bp .....
   Anisakis sp. A [3]
   l. 430 – 240 – 200 – 120 bp .....
   Anisakis sp. CA-2012 [4]
3. The *TaqI* enzyme produces a band on the fragment
   a. 400 – 320 – 150 bp .....
   A. pegreffii [1]
   b. 430 – 400 – 100 bp .....
   ***A. simplex* (s.s)** [1]
   c. 430 – 400 – 100 bp .....
   ***A. simplex* C** [1]
   d. 300 – 280 – 140 bp .....
   A. physeteris [1]

e. 220 – 190 – 130 – 100 bp ..... *A. schupakovi* [1]
f. 330 – 300 – 140 bp ..... *A. ziphidarum* [1]
g. 400 – 350 bp ..... *A. typica* [1]
h. 430 – 180 – 110 bp ..... *Anisakis* sp. HC-2005 [4]

4. Identification of *A. simplex* (s.s.); *A. simplex* C; *A. brevispiculata* suggested using two enzymes restriction
   a. The *HinfI* enzyme produces bands at 620 – 250 – 80 bp fragments.....b
   b. The *HhaI* enzyme produces a band on the fragment
      - 550 – 430 bp ..... *A. simplex* (s.s) [1,2]
      - 550 – 300 – 130 bp ..... *A. simplex* C[1,2]
      - 390 – 330 – 190 bp ..... *A. brevispiculata*[2]

(Note: 1) D'Amalio *et al*. 2000; 2) Chen and Shih 2015; 3) Pontes *et al*. 2005; 4) Zhang *et al.* 2013; Same band pattern in **bold**)

Geographical Distribution In Indonesia

Nine species of Anisakis have been identified worldwide and are divided into two groups: Anisakis type I and Anisakis type II. Anisakis type I consists of *A. simplex* (s.s), *A. pegreffii, A. berlandi* (*A. simplex* C), *A. typica, A. ziphidarum,* and *A. nascettii*), while Anisakis type II consists of *A. paggiae, A physeteris* and *A. brevispiculata*) (Mattiucci *et al*., 2014). Six anisakis species have been identified in Indonesia based on morphology analysis: *A. simplex, A. physeteris, A. pegreffii, A. berlandi, A. typica,* and sibling species *Anisakis* HC-2005; or six genotypes of anisakis have been identified based on genetic analysis: *A. typica (s.s), A. typica var Indonesiansis, A. physeteris, A. pegreffii, A. berlandi,* and *Anisakis* HC-2005.

Based on the predilection or distribution of Anisakis in the fish body in Table 5, it can be seen that Anisakis is found in many internal organs (liver, gonads, body cavity, swim bladder) and the digestive tract (intestine and stomach). From this data it can be seen that the percentage of *A. simplex* infection in muscles is higher compared to other Anisakis species. So it is suspected that the Anisakis simplex species is more susceptible to infecting humans or zoonosis. To reduce the risk of zoonosis, it is highly recommended to clean and remove the internal organs of the fish before consumption and of course cook it thoroughly.

Table 3. Primers used in PCR-RFLP with targets ITS region

Primer	Reference
NC5 (5' -GTAGGTGAACCTGCGGAAGGATCATT-3') NC2 (5'-TTAGTTTCTTTTCCTCCGCT-3')	Anshary (2011); Anshary *et al.* (2014); Chen dan Shih (2015); Hafid and Anshary (2016)
A (5'-GTCGAATTCGTAGGTGAACCTGCGGAAGGATCA-3 ') B (5'GCCGGATCCGAATCCTGGTTAGTTTCTTTTCCT-3')	D'Amalio (2000); Pontes *et al.* (2005); Zhang *et al*. (2013)

Table 4. Primers used in PCR-sequencing with targets mtDNA COX2 or ITS region

Tager	Primer	Reference
mtDNA COX2	210 (5'- CAC CAA CTC TTA AAA TTA TC-3´) 211 (5'-TTT CTA GTT ATA TAG ATT GRT-TYAT-3´)	Hafid and Anshary (2016); Setyobudi *et al.* (2019); Anshary *et al.* (2014)
	F (5'-TGTAGTTTGCTTTTCGGTGTTCT-3') R (5'-GCCCCACAAATCTCTGAACA-3')	Utami *et al.* (2017)
ITS region	TK1 (5- GGC AAA AGT CGT AAC AAG CT-3) NC2 (5- TTA GTT TCT TTT CCT CCG CT-3)	Kleinertz *et al.* (2012); Palm *et al.* (2017); Kuhn *et al.* (2013)
	NC5 (5'- GTA GGT GAA CCT GCG GAA GGA TCA TT-3') NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3')	Palm *et al.* (2008)

Tabel 5. Predilection of Anisakis

	Digestive Tract	Internal Organs	Muscles	Ektoparasite
A. HC2005	100.00%	0.00%	0.00%	0.00%
A. simplex	50.2%	37.4%	12.4%	0.0%
A. typical	30.81%	68.87%	0.3%	0.00%
A. type 1	47.14%	49.32%	2.26%	1.27%
A. sp	39.47%	57.03%	1.42%	2.07%
Total	44.29%	52.44%	2.40%	0.87%

Diverse fish species and other marine life are abundant in Indonesian waters. With the exception of the Papua region, research on the inventory and identification of Anisakis in Indonesia has been conducted in 16 provinces or 42 areas (Table 6 and Figure 2). However, according to Koinari *et al*. (2013) study of Anisakis species in Papua New Guinea can be used as a representation of Anisakis in Papua.

Anisakis typica is the most common species in Indonesia and is also present in Thailand (Chaiphongpachara *et al*., 2022), Papua New Guinea (Koinari *et al*., 2013), and the Philippines (Quiazon *et al*., 2013). *Anisakis typica*, which belongs to the families Delphinidae, Phocoenidae, and Pontoporidae, is a frequent parasite of numerous dolphin species from warmer temperate and tropical regions (Mattiucci *et al*., 2002). The phylogenetic tree resulting from the identification of Anisakis species in Indonesia demonstrates substantial divergence, and it is evident that *A. typica* is separated into two or three sub-clusters. *A. typica* was suggested to be divided into two genotypic groups by Palm *et al*. (2017): *A. typica* (s.s), which is related to *A. typica* from Brazilian dolphins, and *A. typica* var. Indonesiensis (or *At. Indonesiensis*) which is frequently found in Indonesian. Furthermore, it in line with previous studies that identified *Anisakis* sp. 1, *Anisakis* sp. 2 (Palm *et al.,* 2008) and *A. typica* (Kuhn *et al.,* 2013) as *At. Indonesiensis*. The distribution pattern of *A. typica* can be separated into two groups between South America and Asian (*At. Indonesiensis*) countries. Asian countries such as the Philippines, Indonesia, Papua New Guinea, and Egypt, whereas another group belongs to South America such as Argentina and Brazil. *Rastrelliger* sp. (*R. kanagurta* and *R. branchysoma*) are indicated an important intermediate host of *A. typica* in Southeast Asia, this fish is a pelagic schooling fish widely distributed in the Indian Ocean and Indo-West Pacific region (Chaiphongpachara *et al*., 2022).

The subarctic and temperate waters of the Northern Hemisphere are where *A. simplex* goes through its life cycle. It has been observed in the Pacific and Atlantic oceans, both western and eastern. The Atlantic coast of Spain and Portugal appears to be its southern most point of dispersion in the waters of the North-Eastern Atlantic. Its southern most point of distribution in the North Pacific Ocean appears to be along the North American coast in the eastern section and the Pacific coast of Japan in the western area (Mattiucci *et al*., 2018). Lester *et al*. (2001) and Moore *et al*. (2003) used parasite indicators, including *A. simplex*, to study the movement and stock structure of *Scomberomorus commerson*. The results showed that fish from Kupang, Indonesia could cross to Australia but no adult fish moved from Australia to Kupang. The article by Amir *et al*. (2016) and Jabal *et al*. (2020) provides interesting fact regarding the spread of *A. simplex*. The Anisakiosis case reported by Amir *et al*. (2016) is intriguing since the patient disclosed a history of consuming Empurau fish (*Tor tambroides*) sushi at one of the Japanese restaurants in Kuala Lumpur two days before to a clinical presentation. The fish he consumed was a freshwater fish from Sarawak in Malaysian Borneo, but

anisakiasis is only linked to marine fish. It was determined to be an *A. simplex* using COX2 mtDNA PCR scanning, provided more evidence of *A. simplex* presence in Malaysia. Does this suggest that anadromous fish can spread Anisakis to fish in fresh waters and what is the process of its transmission, considering the fact that of research Jabal *et al*. (2020) found that eels (*Anguilla* spp.) in the Lake Lindu were infected with Anisakis.

First record in Indonesia, *A. pegreffii* and *A. berlandi* (*A. simplex* C) in *Auxis rochei* from Bali (Palm *et al.,* 2017). *Anisakis pegreffii* previously indicated as *A. simplex* A, the dominant species of Anisakis in the Mediterranean Sea, being widespread in all the fish species (Mattiucci *et al.,* 2018). Using genetic analysis and ecological traits it was subsequently shown that *A. simplex* is a complex of three sibling species: *A. simplex* (s.s), *A. pegreffii*, and *A. simplex* C (Mattiucci *et al*., 2005).

Anisaakis physeteris is a zoonotic species and one of the type II Anisakis. *Auxis rochei* from Bali (Palm *et al*., 2017), *Lutjanus argentimaculatus* from Lampung (Puspitarini *et al*., 2018), and *Euthynnus affinis* from Cilacap (Utami *et al*., 2017) are three fish that were found to be *A. physeteris* in Indonesia. Anisakis type II were also detected infecting *Katsuwonus pelemis* from Malang (Suryani 2017), form Gresik (Muttaqin *et al*., 2021), from Kupang (Detha *et al.,* 2018), and from Kendari (Asmara 2021). *Anisakis physeteris* has been reported, at the adult stage, from the sperm whale, *Physeter macrocephalus* from the Tyrrhenian, Central Adriatic, Ionian, and Aegean Sea waters of the Mediterranean Sea, from the Adriactic Croatian coast, along the Iberian Atlantic waters, from a sperm whale stranded along the cottish coasts of the North Sea and from the Caribbean Sea (Mattiucci *et al.,* 2018).

Lepturacanthus savala from Bali, Indonesia (Kuhn *et al.,* 2013), *Epinephelus areolatus* from Central Java, Indonesia (Kleinertz *et al*., 2014), *Sufflamen fraenatum* from Bali (Palm *et al.,* 2017), *Carcharhinus* sp., *Euthynnus yaito* and *Saurida elongate* from Guangdong and Hainan, China (Zhang *et al.,* 2013), *Hoplostethus cadenati* and *Merluccius* from African continent (Kijewska *et al.,* 2009) were all identified to be infected with *Anisakis* sp. HC-2005. Zhang *et al*. (2013) hypothesized that *Anisakis* sp. CA-2012 and *Anisakis* sp. HC-2005 are the same species and belong to the *Anisakis* type III group, which also includes *Anisakis* sp. PB-2010, and *Anisakis* sp. PB-2009. Hermida *et al*. (2013), on the other hand, reported that *Anisakis* sp. HC-2005, *Anisakis* sp. PB-2010, and *Anisakis* sp. PB-2009 are all members of the *Anisakis* type I group. *Anisakis* sp. HC-2005 has the following morphological measurements: length 5.36-12.14 mm, width 0.22-0.38, and ventricle 0.42-0.59 (Zhang *et al*., 2013).

Fish species having a very high prevalence (>90%) of Anisakis infection include *Decapterus* sp., *Thyrsitoides marleyi*, *Euthynnus afifinis, Auxis rochei, Gempylus serpens, Katsuwonus pelamis* and *Trichiurus lepturus*. It is best to avoid eating these fish uncooked or undercooked in order to prevent anisakiosis. While *Alepisaurus ferox, Arius maculatus, Carangoides* sp., *Drepane punctata, Gymnocranius microdon, Lates calcarifer, Leiognathus fasciatus, Pampus argenteus, Platax orbicularis, Priacanthus* sp. *Scarus* sp., *Tylosurus crocodilus, Sphyraena* sp., and *Siganus virgatus* often do not find Anisakis in their bodies

The identification of Anisakis species will continue to be developed based on the iption have been provided because Anisakis is a parasitic organism that lives on marine fish and has a complicated life cycle that involves many different fish species, marine mammals, cephalopods and other marine organisms. As a consequence, it has a variety of morphological characteristics and a wide distribution spectrum. fairly broad in comparison to the host's distribution.

Tabel 6. Geographic distribution of *Anisakis* species in Indonesia from 2001-2023

No	Location	*Anisakis* species	P (%)	Fish/ Parasite Host	Ref
Aceh					
1	Banda Aceh	*Anisakis* sp.	12	*Abalistes stellaris*	30
2	Aceh Besar	*A. simplex*	87	*Euthynnus affinis*	27
3	Aceh Barat	*Anisakis* sp.	100	*Euthynnus affinis*	28
North Sumatera					
4	Sibolga	*A. simplex*	2,86	*Selar crumenophthalmus*	46
5	Belawan	*Anisakis sp.*	6.66	*Scomberomorus commerson*	56
Bangka Belitung					
6	Pulau Bangka	*A.* type I	27.56	*Auxis rochei*	57
		Anisakis sp.	25	*Euthynnus affinis*	55
Lampung					
7	Lampung	*A. physeteris*	10	*Lutjanus argentimaculatus*	41
Banten					
8	Tangerang	*Anisakis* sp.	8.0	*Chanos chanos*	22
		A. typica	15.0 – 90.0	*Alectis indica, Carangoides Chrysophrys, Trichiurus lepturus*	54
9	Serang	*A. typica*	2.2	*Rastrelliger brachysoma*	23
DKI Jakarta					
10	Muara Angke	*A. simplex*	70	*Scomber japonicus,*	24
		A. typica	2.2 – 8.6	*Rastrelliger brachysoma;* *Epinephelus sexfasciatus*	23, 35
West Java					
11	Pelabuhan Ratu	*Anisakis* sp.	10.5 - 100	*Brama dussumieri, Gempylus serpens, Thyrsitoides marleyi, Trichiurus lepturus*	3
		A. typica	6.6 – 80.0	*Rastrelliger brachysoma;* *Johnius borneenis, Mene maculate, Upeneus asymmetricus, Upeneus moluccensis*	23, 54
12	Pangandaran	*A. typica*	45.5	*Trichiurus lepturus*	51
Central Java					
13	Cilacap	*Anisakis* sp.	3.33 – 26.67	*Priacanthus macracanthus, Rastrelliger sp., Selaroides leptolepis, Scomberomorus commerson* *Trichiurus sp.*	20, 4
		A. typica	2.3 – 73.3	*Opisthopterus tardoore, Pomadasys maculatus, Priacanthus tayenus, Scomberoides tol,* *Selaroides leptolepis Terapon jarbua* *Epinephelus areolatus*	35, 10
		A. physeteris	G	*Euthynnus affinis*	32
		A. HC-2005	G	*Epinephelus areolatus*	10
14	Karimunjawa	*A. typica*	8.6	*Epinephelus ongus*	35
Yogyakarta					
15	Kulonprogo	*Anisakis* sp.	33.33 – 66.67	*Caesio* sp., *Lutjanus malabaricus, Parupeneus sp., Terapon jarbua, Trichiurus lepturus*	7
16	Gunung Kidul	*A.* type I	94	*Eletheronema tetradactylum, Decapterus russeli*	25
17	Bantul	*A.* type I	18	*Alepes kleinii*	36
East Java					
18	Malang	*A.* type II	60	*Katsuwonus pelamis*	33
19	Tuban	*Anisakis* sp.	Not counted	*Epinephelus sexfasciatus*	6

Tabel 6 (Lanjutan). Geographic distribution of *Anisakis* species in Indonesia from 2001-2023

No	Location	*Anisakis* species	P (%)	Fish/ Parasite Host	Ref
20	Lamongan	*Anisakis* sp.	9.6 - 80	*Lutjanus malabaricus* *Rastrelliger brachysoma*	13, 14
		A. simplex	10 – 74.99	*Euthynnus affinis* *Trichiurus savala* *Decapterus macrosoma* *Priacanthus macracanthus*	18 19 40 34
21	Banyuwangi	*Anisakis* sp.	6.25 – 66.67	*Caranx* sp., *Euthynnus* sp., *Euthynnus* sp.	42
		A. typica	15.8	*Rastrelliger* sp.	43
22	Gersik	*A.* type II	60 – 80.0	*Epinephelus sexfasciatus, Lutjanus malabaricus*	50
23	Prigi	*A. typica*	18.7	*Rastrelliger* sp.	43
24	Pacitan	*A.* type I	Not counted	Alim (2018)	39
25	Sidoarjo	*A. typica*	G	*Euthynnus affinis*	45
Bali					
26	Kedonganan, Badung	*Anisakis* sp.	31.25 – 83.8	*Selar crumenophthalmus, Trichiurus lepturus,* *Decapterus* sp.	11, 12, 48
		A. typica	2.9 – 81.1	*Auxis rochei* *Auxis rochei, Auxis thazard, Brama cf. orcini, Decapterus macrosoma, D. tabl, Epinephelus areolatus, E. longispinis, Lutjanus argentimaculatus, L. vitta, Nemipterus furcosus, Megalaspis cordyla, Selar crumenophthalmus, Trichiurus lepturus* *Epinephelus areolatus* *Auxis rochei, Auxis thazard, Caesio cuning, Lepturacanthus savala, Trichiurus lepturus*	5 35 10 15
		A. physeteris	G	*Auxis rochei*	35
		A. pegreffii	G	*Auxis rochei*	35
		A. berlandi	G	*Auxis rochei*	35
		A.. HC-2005	G	*Lepturacanthus savala, Sufflamen fraenatum*	15,35
27	Anturan	*A. typica*	2.9 – 74.3	*Auxis rochei*	5
East Nusa Tenggara					
28	Kupang	*Anisakis sp.*	76.67	*Auxis thazard, Katsuwonus pelamis* *Epinephelus sp, Lutjanus sanguineus*	26 38, 53
		A. type I	22 - 26	*Epinephelus* sp.; *Auxis rochei*	37,47
		A. type II	22	*Epinephelus* sp	37
		A. simplex	Not counted	*Scomberomorus commerson*	1, 2
		A. typica	G	*Katsuwonus pelamis*	44
29	Ende	*A.* type I	26.31	*Auxis rochei*	52
West Sulawesi					
30	Mamuju	*A. typica*	10.0 – 43.3	*Auxis rochei, Decapterus russeli*	29
		Anisakis sp.	13.33 - 30	*Katsuwonus pelamis, Thunnus albacore*	31
South Sulawesi					
31	Makasar	*A.* type I	70.0	*Auxis thazard*	9
		A. typica	3.12 – 92.3	*Auxis thazard* *Katsuwonus pelamis, Euthynnus affinis, Cephalopholis cyanostigma, Caranx sp., Auxis thazard*	8 21

Tabel 6 (Lanjutan). Geographic distribution of *Anisakis* species in Indonesia from 2001-2023

No	Location	*Anisakis* species	P (%)	Fish/ Parasite Host	Ref
32	Talakar	*A. typica*	5.0	*Rastreliger kanagurta*	21
Southeast Sulawesi					
33	Kendari	*A.* type I	20	*Katsuwonus pelamis*	49
		A. type II	10	*Katsuwonus pelamis*	49
East Kalimantan					
34	Tarakan	*Anisakid*	60.0	*Euthynnus* sp.	16
Papua New Guinea					
35	**PNG**	*A. typica*	G	*Decapterus macarellus, Gerres oblongus, Pinjalo lewisi, Pinjalo pinjalo, Selar crumenophthalmus, Scomberomorus maculatus, Thunnus albacares*	17

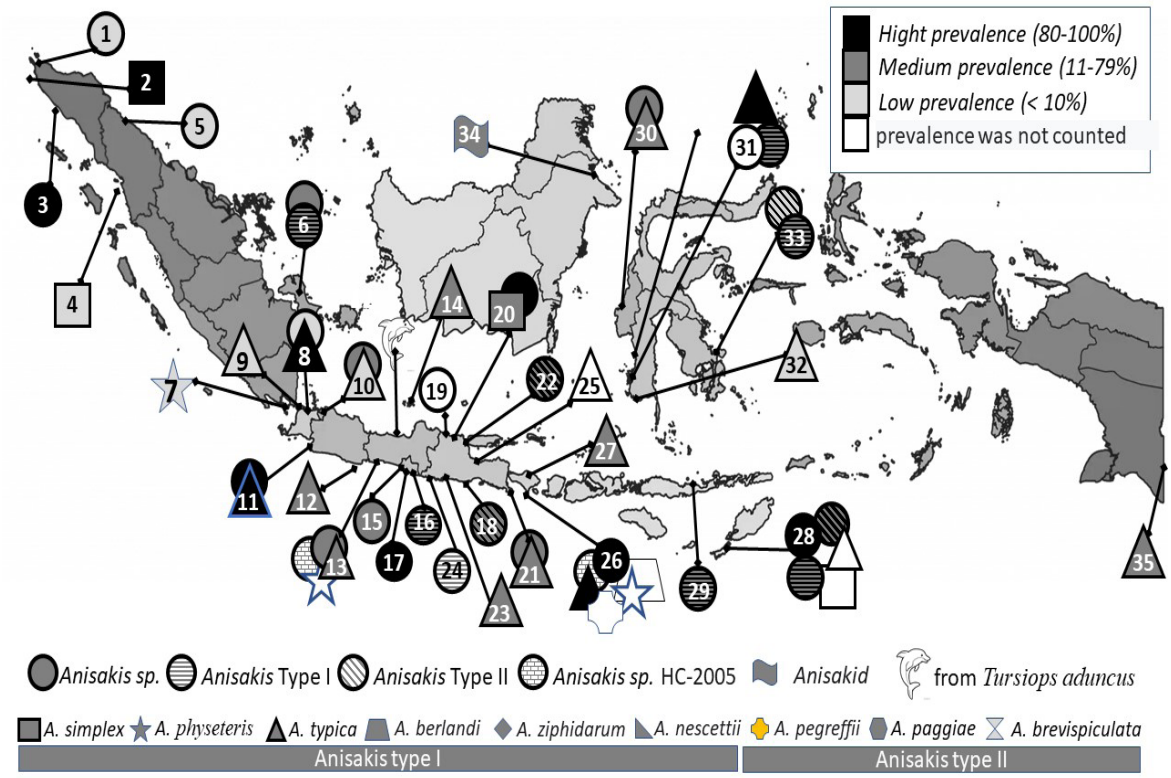

Figure 2. Distribution map of *Anisakis* species in Indonesia

CONCLUSION

Morphometric Anisakis in Indonesia generally have a length of 0.10-34.50 mm with a diameter of 0.30-0.42 mm. Based on morphological and genetic identification, identified six species anisakis in Indonesia (*A. simplex, A. physeteris, A. pegreffii, A. berlandi, A. typica* and *Anisakis* sp. HC-2005), or identified six Anisakis genotypes based on genetic identification, namely *A. typica* (s.s), *A. typica var Indonesiansis, A. physeteris, A. pegreffii, A. berlandi* and *Anisakis* sp. HC-2005. *Decapterus* sp., *Thyrsitoides marleyi*, *Euthynnus* afiffinis, *Auxis rochei, Gempylus serpens, Katsuwonus pelamis*, and *Trichiurus lepturus* have a high prevalence (>90%) indicating that they are often infected with Anisakis.

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