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

RIZKA FAUZIANA SYARIFAH, MURWANTOKO MURWANTOKO, EKO SETYOBUDI*
Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia

E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2023.36.4.002

*E-mail: setyobudi_dja@ugm.ac.id | Received: 17/10/2023 Accepted: 22/12/2023

Abstract

This research investigates the prevalence and mean intensity of *Anisakis* larvae on the bigeye scad, *Selar crumenophthalmus* (Bloch, 1793), along the Indian Ocean off Java. In total, 498 fish samples were collected from four locations: the Indian Ocean off East Java, Yogyakarta Special Territory, Central Java, and West Java. Each fish sample was measured for length and weight, then examined for *Anisakis* larvae in the abdominal cavity, liver, gonads, digestive tract, and muscle. Morphological characterisation was done using scanning electron microscope (SEM). The ITS rDNA region was amplified using PCR, then used to perform RFLP for identification and sequenced to form a phylogenetic tree. The results demonstrated that the bigeye scad was vulnerable to infection by *Anisakis* nematodes with varying prevalence and mean intensity at each location. The bigeye scad from the Indian Ocean off East Java had the highest prevalence and mean intensity (P = 75.44 %; MI = 10.38 larvae per-host), while from West Java had the lowest prevalence (P = 1.45 %; MI = 3.33 larvae per-host). The prevalence of *Anisakis* in the bigeye scad tends to decrease as one moves to the west from the sampling locations. Most larvae were found in the abdominal cavity (70 %–100 %), whereas a relatively low prevalence was found in other organs. The direct sequencing in the ITS rDNA region showed that *Anisakis* larvae found in the bigeye scad were *A. typica* (Diesing, 1860), which was indicated as *A. typica* var. *indonesiensis* (*A. typica* sp. B). *Anisakis* infection can be utilised as a biological indicator for movement patterns and fish stock differentiation.

Keywords: Anisakid, intensity, Indian Ocean, prevalence, pelagic

Introduction

Anisakis (Family Anisakidae) occurs worldwide and infects various marine organisms as hosts at almost all levels of the food web (Mattiucci et al., 2018). The member of the genus *Anisakis* has four moults during its life cycle. Small marine crustaceans serve as intermediate hosts, squid and fish as paratenic or transport hosts, and marine mammals as final or definitive hosts where adult *Anisakis* lay their eggs (Smith and Wootten, 1978). Nine species of *Anisakis*, classified as Type I and Type II, have been reported to infect various marine fishes. *Anisakis* Type I includes six species: *A. simplex* (Rudolphi, 1809) Dujardin, 1845, *A. typica* (Diesing, 1860) Baylis, 1920, *A. pegreffii* Campana-Rouget and Biocca, 1955, *A. ziphidarum* Paggi, Nascetti, Webb, Mattiucci, Cianchi & Bullini, 1988, *A. berlandi* Mattiucci, Cipriani, Webb, Paoletti, Marcer, Bellisario, Gibson & Nascetti, 2014, and *A. nascettii* Mattiucci, Paoletti & Webb, 2009, whereas *Anisakis* Type II includes three species: *A. brevispiculata* Dollfus, 1966, *A. physeteris* Baylis, 1923, and *A. paggiae* Mattiucci, Nascetti, Dailey, Webb, Barros, Cianchi & Bullini, 2005 (Mattiucci and Nascetti, 2008; Mattiucci et al., 2014, 2018). However, until now, the taxonomic position of several members of the *Anisakis* genera has been much debated and reviewed using various approaches (Safonova et al., 2021; Takano and Sata, 2022).

Anisakis is well known worldwide, with different distribution areas for each species (Nadler et al., 2006;

Farjallah et al., 2008; Murata et al., 2011; Quiazon et al., 2011; Mattiucci et al., 2014, 2018). Among the genus *Anisakis*, *A. typica* is the most commonly reported *Anisakis* species that infects fish in Indonesian waters (Palm et al., 2008, 2017; Anshary et al., 2014; Setyobudi et al., 2019; Ayun et al., 2022). Palm et al. (2017) discovered genotype differences in *A. typica* at four positions in the ITS-1 region in various fish species in Indonesia. The genotype was previously reported from *A. typica* infecting fish in Balinese waters (Palm et al., 2008). These genotypic differences were classified as *A. typica* var. *indonesiensis* to avoid further mis-identification (Palm et al., 2017).

Anisakis infection in marine species impacts product safety and human health. *Anisakis* infection results in human zoonotic disease (anisakiasis) when consuming undercooked or raw fish (Mineta et al., 2006). Anisakiasis has become a significant public health concern due to the severe health consequences it causes, including acute gastrointestinal infections, the reaction of allergies, vomiting, fever, diarrhoea, and nausea (Audicana and Kennedy, 2008). *Anisakis simplex* and *A. pegreffii* are the most commonly reported to infect humans (Rosales et al., 1999; Fumarola et al., 2009). There have been no reports of anisakiasis in Indonesia, even though several marine fish species are susceptible to *Anisakis* infection (Palm et al., 2017; Setyobudi et al., 2019).

Anisakis induces pathogenic effects in the fish host, i.e., an inflammatory reaction around the larvae, necrosis, and degenerative hepatocyte alterations (Hassan et al., 2013). The massive *Anisakis* infection seriously harms the host, lowering its aesthetic value and creating economic losses. On the other hand, the presence of *Anisakis* can be used as a biological indicator for fish migration patterns, stock differentiation, food habits, and other ecological studies (Podolska et al., 2006; Mattiucci and Nascetti, 2007).

Anisakid larvae can be identified by morphological and molecular approaches. Morphological identification was challenging because of the lack of taxonomically significant characters, particularly in the larval stage, so it was necessary to complete molecular identification. Anisakid nematode species were successfully identified using molecular identification techniques such as restriction fragment length polymorphism (RFLP) of PCR products, direct sequencing of the ITS rDNA region (D'Amelio et al., 2000; Nadler et al., 2006), and the mtDNA cox2 gene (Valentini et al., 2006; Mattiucci et al., 2014).

Although *Anisakis* have been reported in various marine fish species (Anshary et al., 2014; Palm et al., 2017; Setyobudi et al., 2019), the study of *Anisakis* infection in fish is still limited and not comparable to the number of fish species inhabiting Indonesian waters. Information on Anisakis zoogeographic and its distribution on marine fish species could be used to prevent human anisakiasis, avoid economic losses in fishing activities, and develop parasites as a biological indicator. The bigeye scad, *Selar crumenophthalmus* (Bloch, 1973), is an economically valuable and commonly consumed pelagic fish in Indonesia. *Anisakis* infection of bigeye scad has been observed worldwide (Zhu et al., 2007; Koinari et al., 2013; Kuhn et al., 2013), including in Balinese waters (Kuhn et al., 2013; Palm et al., 2017). This research attempts to determine the prevalence, mean intensity, and target organs of *Anisakis* larvae infection in the bigeye scad originating along the Indian Ocean off Java.

Materials and Methods

Sampling location and nematode collection

The bigeye scad was collected from fisherman's catches in four areas along the Indian Ocean off Java, i.e., the Indian Ocean off East Java, Yogyakarta Special Territory, Central Java, and West Java (Fig. 1). The fish were transported to the Water Resources Management Laboratory and Fisheries Products Quality and Safety Laboratory, Department of Fisheries Universitas Gadjah Mada, using a coolbox for larval examinations.

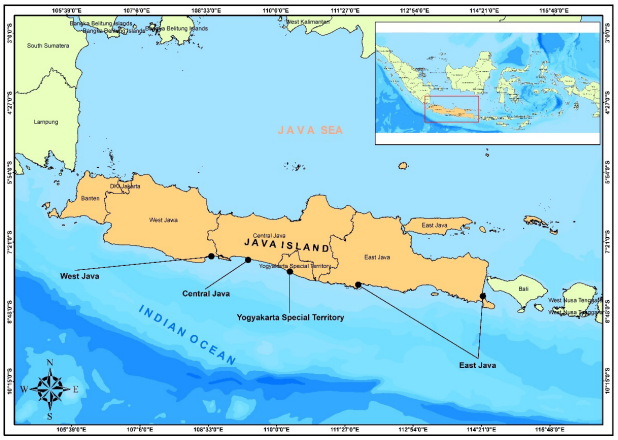

Fig. 1. Sampling location of bigeye scad, *Selar crumenophthalmus*, captured from the Indian Ocean off Java to determine the prevalence and intensity of *Anisakis* infection.

Each fish sample was measured for length and weight, then dissected for *Anisakis* larval examination. The presence of larvae was examined in the abdominal cavity, liver, gonads, digestive tract, and muscle. The larvae were extracted from the muscles by filleting, then squeezed between two glass slides. The existence of larvae was checked using light. The larvae were collected, washed with a solution of 0.9 % NaCl, and then stored in 100 % ethanol. *Anisakis* larvae were separated from other anisakids using identification guidelines from Anderson (2000).

Scanning electron microscopy (SEM)

Anisakis larvae were processed at 4 °C, cleaned for ± 2

hours in a cacodylate buffer, prefixed for ± 12 hours in 2.5 % glutaraldehyde solution, and then fixed for 6 hours in 2 % tannic acid solution. Samples were washed using a cacodylate buffer and then dehydrated using alcohol (50 %, 70 %, 85 %, 90 % and 100 %). Samples were attached to the conductive carbon adhesive tapes, and then coated with Au using an ion coater (JEOL JEC-3000FC, Japan). The morphological characters of *Anisakis* were observed, including anterior and posterior parameters, using a SEM (JSM-6510LA, JEOL, Japan).

PCR-RFLP

The extraction of DNA followed the Geneaid Genomic DNA Mini Kit Tissue Protocol. PCR primers A (forward) (5'-GTC GAA TTC GTA GGT GAA CCT GCG GAA GGA TCA-3') and B (5'-GCC GGA TCC GAA TCC TGG TTA GTT TCT TTT CCT-3') were used to amplify the ITS region (ITS1-5.8S-ITS2) of rDNA (D'Amelio et al., 2000). The denaturation process was conducted at 94 °C for 10 min, followed by 35 cycles at 94 °C for 40 s, 54 °C for 40 s, 72 °C for 90 s, and post-amplification at 72 °C for 7 min. The PCR-RFLP was conducted using *TaqI*, *HinfI*, and *HhaI* endonuclease restriction enzymes (D'Amelio et al., 2000). The reaction was carried out with 3 µL of PCR product, 0.5 µL of restriction enzyme, 1 µL of buffer, and 10 µL of distilled water. The digestion using *TaqI* enzyme was conducted at 65 °C for 90 min, while the digestion using *HinfI* and *HhaI* enzymes were conducted at 37 °C for 90 min. Electrophoresis on 1.5 % agarose gel was used to analyse the final products.

DNA sequencing

In total, 320 µL PCR products were sent to the 1st Base Laboratory in Malaysia via PT Genetika Science Indonesia for sequencing processes. The programs BioEdit and Molecular Evolutionary Genetics Analysis version X (Mega X) were used to process the amplification results and construct the phylogenetic trees. The *Anisakis* species was determined using a BLAST analysis on the https://blast.ncbi.nlm.nih.gov/ website.

Data analysis

The parasite population parameters were calculated following Bush et al. (1997), i.e., prevalence (P) and mean intensity (MI). Prevalence (P) refers to the ratio of the number of infected hosts to the total hosts examined, and the mean intensity (MI) refers to the average number of parasites per host. The distribution of infection in each target organ was also determined.

Results

A total of 498 samples of bigeye scad were collected from four sampling locations, i.e., the Indian Ocean off East Java, Yogyakarta Special Territory, Central Java, and West Java. *Anisakis* larvae were susceptible to infecting the bigeye scad, although with varying prevalence and mean intensity for each location. The bigeye scad from the Indian Ocean off East Java had the highest prevalence and mean intensity (P = 75.44 %; MI = 10.38 larvae per-host), while that from West Java had the lowest prevalence (P = 1.45 %; MI = 3.33 larvae per-host) (Table 1).

Most larvae were found in the abdominal cavity (70 %–100 %), whereas a relatively low prevalence was found in other organs at a relatively low prevalence (Fig. 2). Most bigeye scads were infected with fewer than five larvae per host (Table 2). The prevalence and mean

Table 1. The prevalence and mean intensity of *Anisakis* on the bigeye scad, *Selar crumenophthalmus*, from the Indian Ocean off Java.

Location	Number of fish	Total length (cm)	Weight (g)	Numbers of *Anisakis*	Prevalence (%)	Mean intensity (larvae per-host)
East Java	114	15.5-28.4	38.0-302.5	893	75.44	10.38
Yogyakarta Special Territory	120	17.1-27	55.7-255.2	67	34.17	2.31
Central Java	57	19.6-26.8	89.1-235.7	22	17.54	2.20
West Java	207	17.1-26.5	65.6-239.3	10	1.45	3.33
Total	498			992		

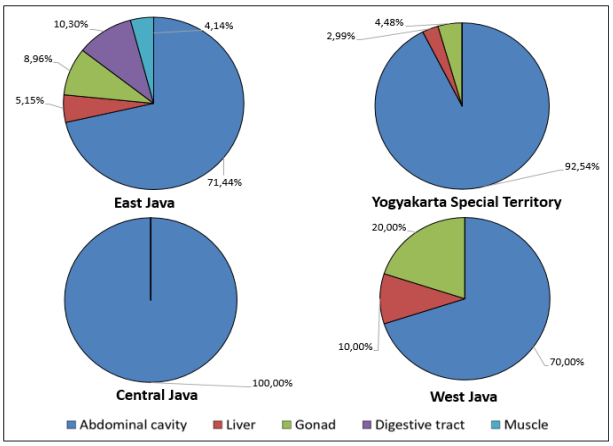

Fig. 2. The distribution of *Anisakis* infection on the bigeye scad, *Selar crumenopthalmus*, from the Indian Ocean off Java.

Table 2. The frequency of *Anisakis* infection on the bigeye scad, *Selar crumenopthalmus*, from the Indian Ocean off Java.

Location	Frequency of infection (%)						
	≤5	6–10	11–15	16–20	21–25	26–30	>30
East Java	52.33	19.77	8.14	6.98	2.33	2.33	8.14
Yogyakarta Special Territory	89.66	10.34	0.00	0.00	0.00	0.00	0.00
Central Java	90.00	0.00	10.00	0.00	0.00	0.00	0.00
West Java	100.00	0.00	0.00	0.00	0.00	0.00	0.00

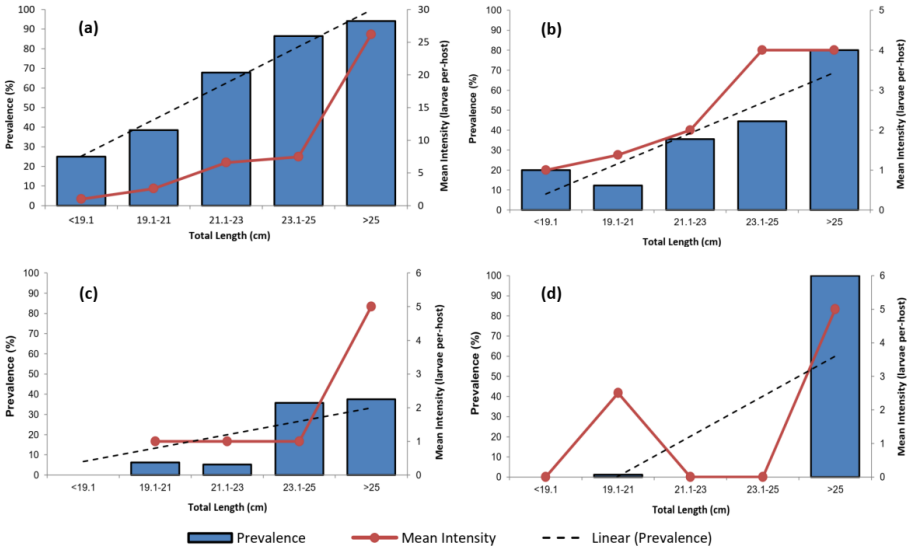

Fig. 3. Relationship between length and *Anisakis* larvae infection on the bigeye scad, *Selar crumenopthalmus*, from the Indian Ocean off Java. (a) East Java, (b) Yogyakarta Special Territory, (c) Central Java, (d) West Java.

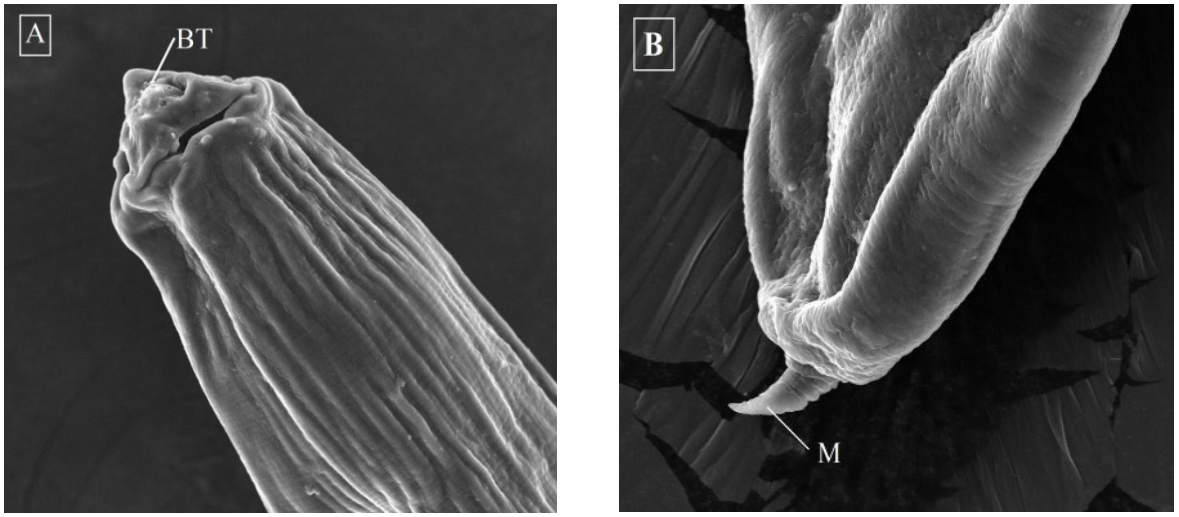

Fig. 4. Morphological characteristics of *Anisakis* on the bigeye scad, *Selar crumenopthalmus*, from the Indian Ocean off Java using 500x and 1,000x magnification SEM. Details of the structure: (A) cephalic region, (BT) boring tooth, (B) caudal end, (M) mucron.

intensity of *Anisakis* are proportional to the length of the host (Fig. 3). The highest number of *Anisakis* larvae infections in a single fish was 73, found in bigeye scad collected from the Indian Ocean off East Java.

Four selected samples were characterised morphologically using scanning electron microscopy (SEM). *Anisakis* larvae isolated from bigeye scad were characterised by the occurrence of mucron on the posterior end and a boring tooth on the cephalic region (Fig. 4).

Amplification of the ITS rDNA region (ITS1, 5,8 s, and ITS2) results in products with a base size of ~1 kb. Digestion using *HhaI* restriction enzyme produced bands at 320 bp, 240 bp, 180 bp, and 160 bp, using *HinfI* produced 620 bp and 350 bp bands, and using *TaqI* produced 400 bp and 350 bp bands (Fig. 5; Table 3). The

a. *TaqI* b. *HinfI* c. *HhaI*

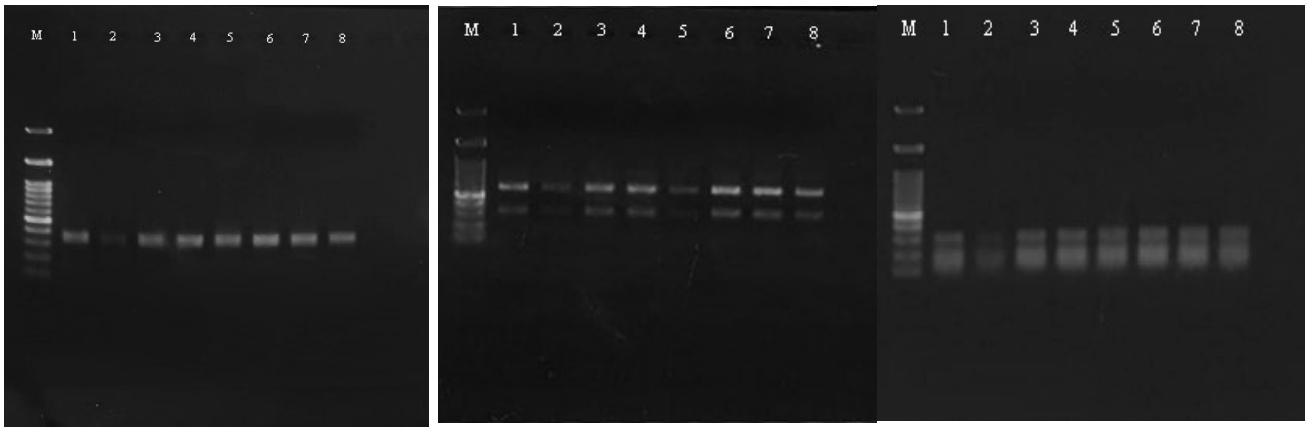

Fig. 5. Visualisation of restriction enzyme digestion of *Anisakis* larvae on the bigeye scad, *Selar crumenopthalmus*, from the Indian Ocean off Java. (a) using *TaqI*, (b) using *HinfI*, (c) using *HhaI*, (M) Marker, (1–2) *Anisakis* on the bigeye scad from the Indian Ocean off East Java, (3–4) *Anisakis* on the bigeye scad from the Indian Ocean off Yogyakarta Special Territory, (5–6) *Anisakis* on the bigeye scad from the Indian Ocean off Central Java, (7–8) *Anisakis* on the bigeye scad from the Indian Ocean off West Java.

Table 3. Banding pattern of the PCR-RFLP using the restriction endonuclease enzyme of *Anisakis* larvae on the bigeye scad, *Selar crumenopthalmus*, from the Indian Ocean off Java.

Location	Digestion enzymes			Species
	HinfI	*HhaI*	*TaqI*	
East Java	350-620 bp	160-180-240-320 bp	350-400 bp	*A. typica*
Yogyakarta Special Territory	350-620 bp	160-180-240-320 bp	350-400 bp	*A. typica*
Central Java	350-620 bp	160-180-240-320 bp	350-400 bp	*A. typica*
West Java	350-620 bp	160-180-240-320 bp	350-400 bp	*A. typica*

PCR-RFLP method produced banding patterns corresponding to *A. typica* (D'Amelio et al., 2000).

The direct sequencing in the ITS rDNA region showed that the *Anisakis* larvae found in the bigeye scad were *A. typica*. The Genbank accession numbers for the ITS rDNA region of *Anisakis* sequences in the bigeye scad are MZ350216, MZ350217, MZ350218, MZ350219, MZ350220, MZ350221, MZ350222, and MZ350223 (Table 4).

The phylogenetic tree was constructed from the ITS rDNA sequences (Fig. 6). The phylogenetic tree showed that *A. typica* in the bigeye scad from the southern coast of Java formed one clade with *A. typica* var. *indonesiensis* (Palm et al., 2017), that is currently suggested to be referred as *A. typica* sp. B (Cipriani et al., 2022). Furthermore, *A. typica* from this study was closely related to that of *A. typica* from the Persian Gulf, Iran (Genbank accession no. KY081898).

Discussion

A total of 992 *Anisakis* larvae were collected from 498 bigeye scad samples from the Indian Ocean off Java. The bigeye scad was vulnerable to *Anisakis* nematode infection, with varying prevalence and mean intensity at each location. The bigeye scad from the Indian Ocean off East Java had the highest prevalence and mean intensity (P = 75.44 %; MI = 10.38 larvae per-host), while that from West Java had the lowest prevalence (P = 1.45 %; MI = 3.33 larvae per-host). The bigeye scad from the Indian Ocean off Yogyakarta Special Territory and Central Java had different prevalence and mean intensity of *Anisakis* larvae infections (P = 34.17 %; MI = 2.31 larvae per-host and P = 17.54 %; MI = 2.20 larvae per-host). The prevalence of *Anisakis* in the bigeye scad tends to decrease as one moves to the west from the sampling locations. A similar trend was also shown in the mean intensity of infection, except for the bigeye scad originating from West Java. In comparison, the level of infection in bigeye scad from Balinese waters was higher (P = 81.1 %; MI = 3 larvae per-host)(Palm et al., 2017). In various places, including Guangdong (China) (Zhu et al., 2007), Papua New Guinea (Koinari et al., 2013), Indies (Dundas et al., 2019), Hawaii and Moorea (Kuhn et al., 2013), there have been reports of *Anisakis*-infected bigeye scad.

The availability of food and distribution of the *Anisakis*'s final host could influence the infection levels of *Anisakis* at each location, thus affecting the accumulation of *Anisakis* infection. According to Mattiucci et al. (2004), the occurence of *Anisakis* in a particular region was associated with the movement of the paratenic and final hosts. The existence and life

Table 4. *Anisakis* BLAST results in the ITS rDNA region of the bigeye scad, *Selar crumenophthalmus*, from the Indian Ocean off Java.

Location	Accession number	Species	Region	Identity (bp)	Percentage (%)
East Java (MZ350220 and MZ350221)	HF911524	*A. typica*	Egypt	921/935	99
	KC928262	*A. typica*	Makassar (Indonesia)	920/934	99
	KF673776	*A. typica*	China	917/926	99
Yogyakarta Special Territory (MZ350222 and MZ350223)	MT020146	*A. typica*	China	914/921	99
	AB432909	*A. typica*	Japan	919/933	99
	MN420659	*A. typica*	Thailand	906/911	99
Central Java (MZ350218 and MZ350219)	KC928261	*A. typica*	Makassar (Indonesia)	938/945	99
	JN005760	*A. typica*	Portuguese	938/945	99
	AB432908	*A. typica*	Taiwan and Japan	937/944	99
West Java (MZ350216 and MZ350217)	JX648312	*A. typica*	Papua New Guinea	902/907	99
	MT271945	*A. typica*	Thailand	892/894	99
	MN420659	*A. typica*	Thailand	892/894	99

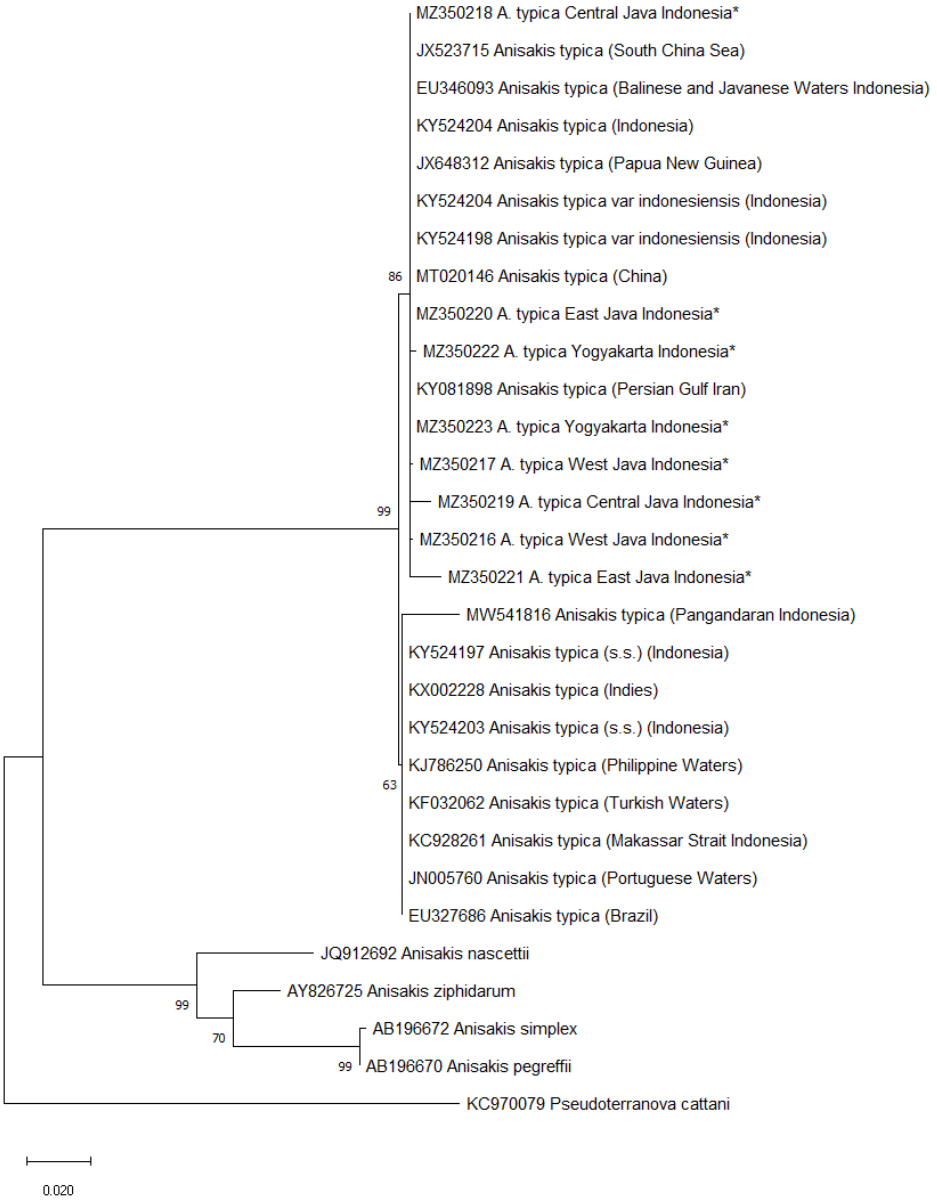

Fig. 6. Maximum likelihood (ML) tree presenting the phylogenetic relationships of *A. typica* reported in this study (indicated by *) and *Anisakis* species sequences from the GenBank, inferred from the ITS rDNA data set. *Pseudoterranova cattani* (George-Nascimento & Urrutia, 2000), was used as an outgroup. The numbers on the branches represent the percentage of bootstrap resampling. The phylogenetic tree was arranged based on the Kimura 2-parameter model (bootstrap = 1000).

cycle of Anisakidae depend on the salinity of the water and the presence of small crustaceans such as shrimp (Bilska-Zając et al., 2015). Another study by Cipriani et al. (2016) revealed a substantial difference in the prevalence of *A. pegreffii* infection among European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), in different Mediterranean Sea fishing regions. The Central and South Adriatic Seas had significant infection rates, whereas the South Sicilian, Ionian, and Alboran Seas were free of infection. Parasite species density is primarily determined by geographical location. Cipriani et al. (2016) found that the parasite's life was affected by both the abiotic (how water moves) and biotic (living) properties of water.

Most larvae were found in the abdominal cavity (70 %–100 %), whereas a relatively low prevalence was found in other organs at a relatively low prevalence. According to Palm et al. (2008), the body cavity and mesentery are the most common sites of infection for *A. typica* in Indonesia, followed by the gonads and liver. Other reports have shown the presence of *Anisakis* larvae in the fish body cavities (Palm et al., 2017; Setyobudi et al., 2019; Tunya et al., 2020; Ayun et al., 2021). According to Smith (1984), the microhabitat of *Anisakis* is associated with the presence of fat. The fish's bodily cavity contains various nutrients, one of which is fat, which is necessary for the survival of *Anisakis*. In contrast, Setyobudi et al. (2011) observed that the majority of *A. simplex* infections in chum salmon, *Oncorhynchus keta* (Walbaum, 1792), originating from Korea, were identified in the muscle (98 %) and only a few larvae were found in other organs. The low infection level in the muscle indicates a reduced risk of anisakiasis. According to Cipriani et al. (2016), the common cause of human infection is *Anisakis* larvae in the host's flesh. *Anisakis* larvae can penetrate the intestinal wall, attach to the organ's surface, and migrate into the muscle. Larvae can migrate intravitally (while the fish is still alive) or post-mortem (after it has died) (Quiazon et al., 2011; Cipriani et al., 2016).

Most bigeye scads were infected with fewer than five larvae per host. The low intensity of *Anisakis* infection suggests that fish consume crustaceans directly as the first intermediate host, whereas larger predatory fish acquire larvae from the preceding host (Palm et al., 2017). *Anisakis* has a relatively long-life span, and there is no difference between the rainy and dry seasons in *Anisakis* infection (Palm et al., 2017). In tropical waters, the slight seasonal variations of *Anisakis* infection might be caused by a lack of climate variation (Strømnes and Andersen, 2000).

There was a correlation between the increase in body length of bigeye scad and the prevalence of *Anisakis* infection along the Indian Ocean off Java. Longer fish have a longer life span, which increases the probability of *Anisakis* infection (Al-Zubaidy, 2010). In addition, the amount of food consumed increases in line with their body length, resulting in an accumulation of *Anisakis* infection. Other studies have discovered a correlation between *Anisakis* infection and increased fish body length (Al-Zubaidy, 2010; Palm et al., 2017; Setyobudi et al., 2019).

Anisakis larvae were characterised by round bodies that were transparent, pointy at the ends, and which were 2–5 cm long. A micrograph using SEM showed the presence of mucron at the posterior end and the boring tooth in the cephalic region of *Anisakis* larvae. Boring tooth perforate the small intestine walls, allowing larvae to remain attached to the mucosa when the intestines contract to digest food. Molecular identification was used to identify *Anisakis* larvae at the species level. The PCR-RFLP and direct sequencing of the ITS rDNA region showed that the *Anisakis* larvae found in the bigeye scad were *A. typica*. According to Blouin (2002), core rDNA genes and ITS genes are commonly used to distinguish between nematode species. The ITS region can develop faster than the ribosomal coding region because it does not code for the product. Therefore, the level of variation in the area could be used to detect genetic variation among species (Umehara et al., 2006).

The ITS rDNA *Anisakis* sequences were amplified and compared to the sequences of *A. typica* (s.s) (Genbank accession no. KY524197 and KY524203) and *A. typica* var. *indonesiensis* (Genbank accession no. KY524198 and KY524204) (Palm et al., 2017) from GenBank to determine the *Anisakis* subspecies. *Anisakis typica* in this study and *A. typica* (s.s.) differ by 5–12 nucleotide bases, while *A. typica* var. *indonesiensis* differs by 1–8 nucleotide bases. This research showed the sequences of *A. typica* have a high degree of similarity with *A. typica* var. *indonesiensis,* as reported by Palm et al. (2017). The last report, Cipriani et al. (2022), suggested that A. *typica* var. *indonesiensis* be referred to as *A. typica* sp. B. The taxonomic position of several members of the Anisakis genus has been reviewed. Based on the genetic data analysis, Safonova et al. (2021) suggests restoring the genus *Peritrachelius typicus* for *Anisakis typica*. However, Takano and Sata (2022) expressed doubts about recognizing the position of *P. typicus* (Diesing, 1861) Jaegerskiold, 1894 replaced *A. typica*. The World Register of Marine Species (2023) notes *Anisakis typica* (Diesing, 1860) Baylis, 1920 as the name of a recognized and accepted species.

In this study, the phylogenetic tree of *A. typica* demonstrates a close relationship with *A. typica* from the Persian Gulf, Iran (Genbank accession no. KY081898). The closeness of relationships between individuals could be generated through genetic transfer between nearby areas. Migration from its hosts worldwide could induce the strong affinity of *A. typica* populations in distant geographical areas. *Anisakis typica* is a widespread species of fish found in warm and tropical waters, between 30 °S and 35 °N (Mattiucci et al., 2002). Several species of dolphins from the families Delphinidae, Phocoenidae, and

Pontoriidae have been reported as the final hosts of *A. typica* (Mattiucci et al., 2002; Kleinertz et al., 2014). Several marine mammals have been identified as final hosts of *A. typica* namely short-snouted spinner dolphin, *Stenella clymene* (Gray, 1850), melon-headed whale, *Peponocephala electra* (Gray, 1846), and sperm whale, *Kogia breviceps* (de Blainville, 1838) (Iñiguez et al., 2011), spinner dolphin, *Stenella longirostris* (Gray, 1828) (Nadler et al., 2006), pantropical spotted dolphin, *Stenella attenuata* (Gray, 1846), rough-toothed dolphin, *Steno bredanensis* (G. Cuvier in Lesson, 1828), and bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) (Valentini et al., 2006), striped dolphin, *Stenella coeruleoalba* (Meyen, 1833) and fraser's dolphin, *Lagenodelphis hosei* Fraser, 1956 (Bilska-Zając et al., 2015), tucuxi, *Sotalia fluviatilis* (Gervais & Deville in Gervais, 1853) (Mattiucci et al., 2002), guiana dolphin, *Sotalia guianensis* (Van Beneden, 1864) (Iñiguez et al., 2009), short-finned pilot whale, *Globicephala macrorhynchus* Gray, 1846 (Mattiucci et al., 2005), from various areas in the world.

Several species of dolphins and whales have been reported in Indonesia (Rudolph et al., 1997). Among those species, sereval were reported as final hosts of *Anisakis*, i.e., the striped dolphin (*S. coeruleoalba*), spinner dolphin (*S. longirostris*), pantropical spotted dolphin (*S. attenuata*), fraser's dolphin (*L. hosei*), rough-toothed dolphin (*S. bredanensis*), bottlenose dolphin (*T. truncatus*), short-finned pilot whale (*G. macrorhynchus*), melon-headed whale (*P. electra*), and sperm whales (*K. breviceps*). The presence of *Anisakis* in Indonesia was influenced by the migration of its definitive host, marine mammals (dolphins and whales). References reported the migration of cetaceans from the Indian Ocean to the Pacific Ocean via the Lesser Sunda Islands, around 900 km long (Klinowska, 1991; Faizah et al., 2006). Furthermore, Braulik et al. (2010) and Owfi et al. (2016) reported the striped dolphin (*S. coeruleoalba*), spinner dolphin (*S. longirostris*), pantropical spotted dolphin (*S. attenuata*), rough-toothed dolphin (*S. bredanensis*), and melon-headed whale (*P. electra*) in Iran. These marine mammals were also found in Indonesia and reported as the final hosts of *Anisakis*.

Parasites are utilised as biological markers to offer information on the movements and populations of their hosts (Mackenzie, 2002). The variation in the distribution of the host allows the host to remain uninfected throughout the distribution area. The parasite then acts as a marker, marking the fish's movement (Speare, 1995). Among parasitic organisms that identify fish populations, the larval Anisakid nematode is one of the most effective biological markers (Mackenzie, 2002). Numerous studies have been conducted on applying *Anisakis* as a biological indicator (Podolska et al., 2006; Mattiucci and Nascetti, 2007). As a biological indicator, *Anisakis* was utilised in numerous investigations, including the characterisation and differentiation of stocks, migration movements, feeding patterns, and eating habits. Kijewska et al. (2009) utilised mtDNA sequences to determine the subpopulation of Atlantic and Pacific *A. simplex* s.s. fish samples. In a different study, Cross et al. (2007) utilised the mtDNA gene to determine the geographic distribution of the *Anisakis* nematode. The cox1 gene from *A. simplex* s. s. in *Clupea herengus* (Linnaeus, 1758), caught off the northwest coast of Scotland, was analysed to determine intrapopulation variation. Highly haplotype differences and low nucleotide diversity were seen, which shows that mtDNA has undergone many changes and is a good way for parasites to spread.

Anisakis typica in this study formed a one clade with *A. typica* var. *indonesiensis*, according to the phylogenetic tree based on the ITS rDNA region. Suggests that *A. typica* in the bigeye scad from the Indian Ocean off Java originated within the same population as *A. typica* var. *indonesiensis*. According to Kijewska et al. (2009), the parasites and their host's life cycles influence their life structure, genetic variety, and gene flow between populations. The migration of *A. typica* along the Indian Ocean off Java could be driven by the movement of larvae carried by ocean currents. Another possibility is the distribution of *A. typica*'s hosts, including paratenic, intermediate, and final hosts. The homogenisation effect of gene flow, amplified by the host's high dispersal capacity, causes low nucleotide diversity between populations separated by thousands of kilometres (Mattiucci and Nascetti, 2008). It is hypothesised that an increase in the number of *Anisakis* samples tested from the Indian Ocean off Java would support the notion that the *Anisakis* samples originate from the same population. This hypothesis requires additional data, such as food habits, the presence of *Anisakis* in different hosts, the presence of hosts, and migration from each host.

Conclusion

The bigeye scad, *Selar crumenophthalmus*, was vulnerable to infection by *Anisakis* nematodes, with varying prevalence and mean intensity at each location. The bigeye scad from the Indian Ocean off East Java had the highest prevalence and mean intensity (P = 75.44 %; MI = 10.38 larvae per-host), while that from West Java had the lowest prevalence (P = 1.45 %; MI = 3.33 larvae per-host). The prevalence of *Anisakis* in the bigeye scad tends to decrease as one moves to the west from the sampling locations. Most larvae were found in the abdominal cavity (70 % – 100 %), whereas a relatively low prevalence was found in other organ. The direct sequencing in the ITS rDNA region showed that the *Anisakis* larvae found in the bigeye scad were *A. typica*, which was indicated as *A. typica* var. *indonesiensis*. *Anisakis* infection can be utilised as a biological indicator for fish movement patterns and stock differentiation.

Acknowledgements

This research was funded by Universitas Gadjah Mada,

Number: 2039/UN1/DITLIT/DIT-LIT/LT/2020. This research is part of the thesis of the first author.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Rizka Fauziana Syarifah: Data collection, data analysis, and writing. Murwantoko Murwantoko: Writing, supervision. Eko Setyobudi: Experimental design, writing, supervision.

References

Al-Zubaidy, A.B. 2010. Third-stage larvae of *Anisakis simplex* (Rudolphi, 1809) in the Red Sea fishes, Yemen Coast. Journal of King Abdulaziz University 21:95–112. https://doi.org/10.4197/Mar.21-1.5

Anderson, R.C. 2000. Nematode parasites of vertebrates: Their development and transmission, 2nd ed. CABI Publishing, Wallingford, UK. pp. 1-650.

Anshary, H., Sriwulan, Freeman, M.A., Ogawa, K. 2014. Occurrence and molecular identification of *Anisakis* Dujardin, 1845 from marine fish in southern Makassar strait, Indonesia. Korean Journal of Parasitology 52:9–19. https://doi.org/10.3347/kjp.2014.52.1.9

Audicana, M.T., Kennedy, M.W. 2008. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. Clinical Microbiology Reviews 21:360–379. https://doi.org/10.1128/CMR.00012-07

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

Ayun, N.Q., Syarifah, R.F., Murwantoko, Setyobudi, E. 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

Bilska-Zając, E., Różycki, M., Chmurzyńska, E., Karamon, J., Sroka, J., Kochanowski, M., Kusyk, P., Cencek, T. 2015. Parasites of Anisakidae family—Geographical distribution and threat to human health. Journal of Agricultural Science and Technology A 5:146–152. https://doi.org/10.17265/2161-6256/2015.02a.010

Blouin, M.S. 2002. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. International Journal for Parasitology 32:527–531. https://doi.org/10.1016/S0020-7519(01)00357-5

Braulik, G.T., Ranjbar, S., Owfi, F., Aminrad, T., Dakhteh, M., Kamrani, E., Mohsenizadeh, F. 2010. Marine mammal records from Iran. Journal of Cetacean Research and Management 11:49–63

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. The Journal of Parasitology 83:575–583. https://doi.org/10.2307/3284227

Cipriani, P., Acerra, V., Bellisario, B., Sbaraglia, G.L., Cheleschi, R., Nascetti, G., Mattiucci, S. 2016. Larval migration of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in European anchovy, *Engraulis encrasicolus*: Implications to seafood safety. Food Control 59:148–157. https://doi.org/10.1016/j.foodcont.2015.04.043

Cipriani, P., Giulietti, V., Shayo, S.D., Storesun, J.E., Bao, M., Palomba, M., Mattiucci, S., Levsen, A. 2022. Anisakid nematodes in *Trichiurus lepturus* and *Saurida undosquamis* (Teleostea) from the South-West Indian Ocean: Genetic evidence for the existence of sister species within *Anisakis typica* (s.l.), and food-safety considerations. Food and Waterborne Parasitology 28:1-18. https://doi.org/10.1016/j.fawpar.2022.e00177

Cross, M.A., Collins, C., Campbell, N., Watts, P.C., Chubb, J.C., Cunningham, C.O., Hatfield, E.M.C., MacKenzie, K. 2007. Levels of intra-host and temporal sequence variation in a large CO1 sub-units from *Anisakis simplex* sensu stricto (Rudolphi 1809) (Nematoda: Anisakisdae): Implications for fisheries management. Marine Biology 151:695–702. https://doi.org/10.1007/s00227-006-0509-8

D'Amelio, S., Mathiopoulos, K.D., Santos, C.P., Pugachev, O.N., Webb, S.C., Picanço, M., Paggi, L. 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. https://doi.org/10.1016/S0020-7519(99)00178-2

Dundas, N.S., Fitzpatrick, D.M., McKibben, J.S., Amadi, V.A., Pinckney, R.D. 2019. Identification of helminth parasites from *Selar crumenophthalmus* in Grenada, West Indies. Journal of Food Protection 82:1244–1248. https://doi.org/10.4315/0362-028X.JFP-18-470

Faizah, R., Dharmadi, Purnomo, F.S. 2006. Distibusi dan kepadatan lumba-lumba *Stenella longirosttis* di Laut Sawu, Nusa Tenggara Timur. Jurnal Penelitian Perikanan Indonesia 13:175–181. https://doi.org/DOI:10.15578/JPPI.12.3.2006.175-181 (in Indonesian).

Farjallah, S., Slimane, B. ben, Busi, M., Paggi, L., Amor, N., Blel, H., Said, K., D'Amelio, S. 2008. Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of Mediterranean Sea. Parasitology Research 102:371–379. https://doi.org/10.1007/s00436-007-0771-9

Fumarola, L., Monno, R., Ierardi, E., Rizzo, G., Giannelli, G., Lalle, M., Pozio, E. 2009. *Anisakis pegreffi* etiological agent of gastric infections in two Italian women. Foodborne Pathogens and Disease 6:1157–1159. https://doi.org/10.1089/fpd.2009.0325

Hassan, M.A., Mohamed, A.E.-M.H., Osman, H.A.M. 2013. Some studies on Anisakidae larvae in some marine fish species. Researcher 5:172–180.

Iñiguez, A.M., Carvalho, V.L., Motta, M.R.A., Pinheiro, D.C.S.N., Vicente, A.C.P. 2011. Genetic analysis of *Anisakis typica* (Nematoda: Anisakidae) from cetaceans of the northeast coast of Brazil: New data on its definitive hosts. Veterinary Parasitology 178:293–299. https://doi.org/10.1016/j.vetpar.2011.01.001

Iñiguez, A.M., Santos, C.P., Vicente, A.C.P. 2009. Genetic characterization of *Anisakis typica* and *Anisakis physeteris* from marine mammals and fish from the Atlantic Ocean off Brazil. Veterinary Parasitology 165:350–356. https://doi.org/10.1016/j.vetpar.2009.07.012

Kijewska, A., Dzido, J., Rokicki, J. 2009. Mitochondrial DNA of *Anisakis simplex* s.s. as a potential tool for differentiating populations. Journal of Parasitology 95:1364–1370. https://doi.org/10.1645/GE-1950.1

Kleinertz, S., Hermosilla, C., Ziltener, A., Kreicker, S., Hirzmann, J., Abdel-Ghaffar, F., Taubert, A. 2014. Gastrointestinal parasites of free-living Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in the Northern Red Sea, Egypt. Parasitology Research 113:1405–1415. https://doi.org/10.1007/s00436-014-3781-4

Klinowska, M. 1991. Dolphins, porpoises and whales of the world: the IUCN Red Data Book. IUCN, Gland, Switzerland and Cambridge, UK. 423 pp.

Koinari, M., Karl, S., Elliot, A., Ryan, U., Lymbery, A.J. 2013. Identification of *Anisakis* species (Nematoda: Anisakidae) in marine fish hosts from Papua New Guinea. Veterinary Parasitology 193:126–133. https://doi.org/10.1016/j.vetpar.2012.12.008

Kuhn, T., Hailer, F., Palm, H.W., Klimpel, S. 2013. Global assessment of molecularly identified *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae) in their teleost intermediate hosts. Folia Parasitologica 60:123–134.

https://doi.org/10.14411/fp.2013.013

Mackenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology 124:153–163. https://doi.org/10.1017/S0031182002001518

Mattiucci, S., Abaunza, P., Ramadori, L., Nascetti, G. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. Journal of Fish Biology 65:495–510. https://doi.org/10.1111/j.0022-1112.2004.00465.x

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., Nascetti, G. 2018. Molecular epidemiology of *Anisakis* and Anisakiasis: An ecological and evolutionary road map. Advances in Parasitology 99:93–263. https://doi.org/10.1016/bs.apar.2017.12.001

Mattiucci, S., Cipriani, P., Webb, S.C., Paoletti, M., Marcer, F., Bellisario, B., Gibson, D.I., Nascetti, G. 2014. Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). Journal of Parasitology 100:199–214. https://doi.org/10.1645/12-120.1

Mattiucci, S., Nascetti, G. 2007. Genetic diversity and infection levels of anisakid nematodes parasitic in fish and marine mammals from Boreal and Austral hemispheres. Veterinary Parasitology 148:43–57. https://doi.org/10.1016/j.vetpar.2007.05.009

Mattiucci, S., Nascetti, G. 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Advances in Parasitology 66:47–148. https://doi.org/10.1016/S0065-308X(08)00202-9

Mattiucci, S., Nascetti, G., Dailey, M., Webb, S.C., Barros, N.B., Cianchi, R., Bullini, L. 2005. Evidence for a new species of *Anisakis* Dujardin, 1845: morphological description and genetic relationships between congeners (Nematoda: Anisakidae). Systematic Parasitology 61:157–171. https://doi.org/10.1007/s11230-005-3158-2

Mattiucci, S., Paggi, L., Nascetti, G., Santos, C.P., Costa, G., di Beneditto, A.P., Ramos, R., Argyrou, M., Cianchi, R., Bullini, L. 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 51:159–170. https://doi.org/10.1023/a:1014554900808

Mineta, S., Shimanuki, K., Sugiura, A., Tsuchiya, Y., Kaneko, M., Sugiyama, Y., Akimaru, K., Tajiri, T. 2006. Chronic anisakiasis of the ascending colon associated with carcinoma. Journal of Nippon Medical School 73:169–174. https://doi.org/10.1272/jnms.73.169

Murata, R., Suzuki, J., Sadamasu, K., Kai, A. 2011. Morphological and molecular characterization of *Anisakis* larvae (Nematoda: Anisakidae) in *Beryx splendens* from Japanese waters. Parasitology International 60:193–198. https://doi.org/10.1016/j.parint.2011.02.008

Nadler, S., D'Amelio, S., Dailey, M., Paggi, L., Siu, S., Sakanari, J. 2006. Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from northern Pacific marine mammals. The Journal of Parasitology 91:1413–1429. https://doi.org/10.1645/GE-522R.1

Owfi, F., Braulik, G.T., Rabbaniha, M. 2016. Species diversity and distribution pattern of marine mammal s of the Persian Gulf and Gulf of Oman - Iranian Waters. Iranian Journal of Fisheries Science 15:927–944

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

Palm, H., Theisen, S., Damriyasa, I., Kusmintarsih, E., Oka, I., Setyowati, E., Suratma, N., Wibowo, S., Kleinertz, S. 2017. *Anisakis* (Nematoda: Ascaridoidea) from Indonesia. Diseases of Aquatic Organisms 123:141–157. https://doi.org/10.3354/dao03091

Podolska, M., Horbowy, J., Wyszynski, M. 2006. Discrimination of Baltic herring populations with respect to *Anisakis simplex* larvae infection. Journal of Fish Biology 68:1241–1256. https://doi.org/10.1111/j.0022-1112.2006.01004.x

Quiazon, K.M.A., Yoshinaga, T., Ogawa, K. 2011. Distribution of *Anisakis* species larvae from fishes of the Japanese waters. Parasitology International 60:223–226. https://doi.org/10.1016/j.parint.2011.03.002

Rosales, M.J., Mascaró, C., Fernandez, C., Luque, F., Sanchez Moreno, M., Parras, L., Cosano, A., Ramón Muñoz, J. 1999. Acute intestinal anisakiasis in Spain: a fourth-stage *Anisakis simplex* larva. Memórias do Instituto Oswaldo Cruz 94:823–826. https://doi.org/10.1590/S0074-02761999000600020

Rudolph, P., Smeenk, C., Leatherwood, S. 1997. Preliminary checklist of Cetacea in the Indonesian Archipelago and adjacent waters. Zoologische Verhandelingen 312:1–48

Safonova, A., Voronova, A., Vainutis, K.S. 2021. First report on molecular identification of *Anisakis simplex* in *Oncorhynchus nerka* from the fish market, with taxonomical issues within Anisakidae. Journal of Nematology 53:1-10. https://doi.org/10.21307/jofnem-2021-023

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

Setyobudi, E., Rohmah, I., Syarifah, R.F., Ramatia, L., Murwantoko, Sari, D.W.K. 2019. Presence of *Anisakis* nematode larvae in Indian mackerel (*Rastrelliger* spp.) along the Indian Ocean southern coast of East Java, Indonesia. Biodiversitas Journal of Biological Diversity 20:313–319. https://doi.org/10.13057/biodiv/d200136

Smith, J.W. 1984. The abundance of *Anisakis simplex* L3 in the body-cavity and flesh of marine teleosts. International Journal for Parasitology 14:491–495. https://doi.org/10.1016/0020-7519(84)90030-4

Smith, J.W., Wootten, R. 1978. *Anisakis* and anisakiasis. Advances in Parasitology 16:93–163. https://doi.org/10.1016/S0065-308X(08)60573-4

Speare, P. 1995. Parasites as biological tags for sailfish *Istiophorus platypterus* from east coast Australian waters. Marine Ecology Progress Series 118:43–50

Strømnes, E., Andersen, K. 2000. "Spring rise" of whaleworm (*Anisakis simplex*; nematoda, ascaridoidea) third-stage larvae in some fish species from Norwegian waters. Parasitology Research 86:619–624. https://doi.org/10.1007/PL00008541

Takano, T., Sata, N. 2022. Multigene phylogenetic analysis reveals non-monophyly of *Anisakis* s.l. and *Pseudoterranova* (Nematoda: Anisakidae). Parasitology International 91:102631. https://doi.org/10.1016/j.parint.2022.102631

Tunya, R., Wongsawad, C., Wongsawad, P., Chai, J.Y. 2020. Morphological and molecular characteristics of *Anisakis typica* larvae in two species of threadfin bream, *Nemipterus hexodon* and *N. japonicus*, from the Gulf of Thailand. Korean Journal of Parasitology 58:15–25. https://doi.org/10.3347/kjp.2020.58.1.15

Umehara, A., Kawakami, Y., Matsui, T., Araki, J., Uchida, A. 2006. Molecular identification of *Anisakis simplex* sensu stricto and *Anisakis pegreffii* (Nematoda: Anisakidae) from fish and cetacean in Japanese waters. Parasitology International 55:267–271. https://doi.org/10.1016/j.parint.2006.07.001

Valentini, A., Mattiucci, S., Bondanelli, P., Webb, S.C., Mignucci-Giannone, A.A., Colom-Llavina, M.M., Nascetti, G. 2006. Genetic relationships among *Anisakis* species (Nematoda: Anisakidae)

inferred from mitochondrial cox2 sequences, and comparison with allozyme data. Journal of Parasitology 92:156–166. https://doi.org/10.1645/GE-3504.1

World Register of Marine Species (WoRMS). 2023. *Anisakis typica* (Diesing, 1860) Baylis, 1920. At https://www.marinespecies.org/aphia.php?p=taxdetails&id=122891. Accessed 17 April 2023.

Zhu, X.Q., Podolska, M., Liu, J.S., Yu, H.Q., Chen, H.H., Lin, Z.X., Luo, C.B., Song, H.Q., Lin, R.Q. 2007. Identification of anisakid nematodes with zoonotic potential from Europe and China by single-strand conformation polymorphism analysis of nuclear ribosomal DNA. Parasitology Research 101:1703–1707. https://doi.org/10.1007/s00436-007-0699-0