Journal of Sustainability Science and Management
Volume 17 Number 7, July 2022: 104-120
eISSN: 2672-7226

Anisakis typica (DIESING, 1860), DOMINANT ANISAKID NEMATODE PRESENT IN SHORTFIN SCAD, *Decapterus macrosoma* (BLEEKER, 1851) FROM TERENGGANU WATERS, MALAYSIA

MING SHE SEE[1], FAIZAH SHAROM HARISON[2], HAZLINA AHAMAD ZAKERI[1,3] AND NOR OMAIMA HARUN[1,3]*

[1]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
[2]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
[3]BIOSES Research Interest Group, Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

**Corresponding author: omaima@umt.edu.my*
Submitted final draft: 4 April 2022 *Accepted: 21 April 2022* http://doi.org/10.46754/jssm.2022.07.008

Abstract: Anisakid (*Anisakis* spp.) nematode is a fish-borne parasite that can cause zoonotic disease, Anisakiasis and IgE hypersensitivity in humans. Thus, the ecology and epidemiology of *Anisakis* nematode are important to control this zoonotic infection. Although *Anisakis* larvae have been reported in Malaysia, it has not been studied through morphological characteristics and molecular analyses. The objective of this study was to identify the *Anisakis* species and its infection status in *Decapterus macrosoma* from Terengganu waters, Malaysia. All *Anisakis* larvae found were morphologically similar to each other and identified as *Anisakis typica*. High infection level (P = 100%, M.I. = 23.31±17.26) of *A. typica* was found in *D. macrosoma*. There was a bioaccumulation of *A. typica* in *D. macrosoma*, showing significant correlation of body weight ($p = 0.05$) and total body length ($p = 0.02$) with the amount of *A. typica* present in *D. macrosoma.* Interestingly, the gonad was the specific site of infection of *A. typica* in *D. macrosoma*, where the intensity of *A. typica* was significantly ($p < 0.003$) higher than other visceral organs. Therefore, the migration of *A. typica* depended on the fatty tissue of the organ.

Keywords: *Anisakis typica*, *Decapterus macrosoma*, bioaccumulation, fatty tissue, Terengganu.

Introduction

Anisakiasis is a fish-borne zoonotic disease caused from consuming raw or undercooked *Anisakis* larvae (L3) of infected fish or seafood (Kim *et al.*, 2013; Lim *et al.*, 2015; Tsukui *et al.*, 2016; Mattiucci *et al.*, 2017; València, 2017). Common symptoms of anisakiasis include nausea, vomiting and epigastric pain (Mattiucci, 2015). In addition, there are 14 types of allergenic proteins found in anisakid nematodes which can cause IgE hypersensitivity to certain patients (Kobayashi *et al.*, 2007; Mattiucci *et al.*, 2018). It has been reported in Spain that one in every three fish are found to be infected with anisakid nematodes (València, 2017). In Malaysia, the first case of anisakiasis was reported in 2016, when a 64-year-old Chinese man was diagnosed having abdominal discomfort after consuming sushi and empurau fish from Sarawak which was infected with *Anisakis simplex* larva (Amir *et al.*, 2016).

Anisakis simplex and *Anisakis pegreffii* commonly cause anisakiasis in Spain, Morocco, Japan, Indonesia and Taiwan (Chou *et al*., 2011; Palm *et al*., 2017; Shawket *et al*., 2017; Cavallero, 2018; Cipriani *et al*., 2018; Yamamoto, 2019; Roca-Geronès *et al*., 2020). However, species such as *A. simplex* and *A. pegreffii* are rarely found in warm waters located along the equatorial (Mattiucci *et al.*, 2018). Instead, *A. typica*, a heterogeneous species of *A. simplex* and *A. pegreffii* is found in temperate and tropical waters (Mattiucci *et al.*, 2002; Cavallero *et al.*, 2012; Chen & Shih, 2015). Reports stated wide distribution of *A. typica* in the Indian Ocean (Mattiucci *et al.*, 2002), Atlantic Ocean (Hermida *et al.*, 2012), Central Pacific (Kuhn *et al.*, 2013), Red Sea (Kleinertz *et al.*, 2014)

and Taiwan Sea (Chen & Shih, 2015) since its definitive host, the oceanic dolphin, is present in the area (Mattiucci & Nascetti, 2008; Iñiguez *et al*., 2011). Malaysia's neighbouring countries like Indonesia, Thailand and the Philippines have *A. typica* as the dominant species, followed by *A. pegreffii* (Eamsobhana *et al*., 2018; Tunya *et al*., 2020; Ayun *et al*., 2021; Hien *et al*., 2021). Australia and New Caledonia have the most diverse *Anisakis* such as *A. simplex*, *A. pegreffii*, *A. typica*, *Anisakis berlandi*, *Anisakis brevispiculata* and *Anisakis paggiae* (Shamsi *et al*., 2018; Shamsi, 2021). Meanwhile, Taiwan and Japan have reported four species of *Anisakis* nematodes: The dominant species is *A. simplex*, followed by *A. pegreffii*, *A. typica* and *A. berlandi* (Gomes *et al*., 2020; Sonko *et al*., 2020). The larvae of *A. simplex*, *A. pegreffii* and *A. typica* are morphologically classified as *Anisakis* Type I larvae (Berland, 1961). *A. typica* larvae have a cylindrical mucron which can be differentiated from *A. simplex* larvae that have a cone-shape mucron (Tunya *et al*., 2020).

Anisakid nematodes have an indirect life cycle, which are classified as heteroxenous parasites. Marine mammals such as delphinoids serve as definitive hosts of anisakid nematodes (Mattiucci & Nascetti, 2008). Adult anisakid nematodes are found in the stomach of the marine mammal: Cetaceans such as *Orcinus orca* and *Pontoporia blainvillei* and sea lion such as *Zalophus californianus* (Hrabar *et al*., 2021; Irigoitia *et al*., 2021). The female anisakid nematodes release their eggs into the host faeces, which are then excreted from host body into open water. After hatching, the first-stage larvae (L1) become the second-stage larvae (L2) in free living form (Mattiucci *et al.,* 2018). The L2 stage larvae (L2) are eaten by the first intermediate host (crustacean: *Crangon crangon*), becoming the third-stage larvae (L3) in hemocoel (Pawlak *et al*., 2019). Following the food web, infected crustaceans are eaten by the cephalopods and fish and the third-stage (L3) larvae bore into the digestive tract to migrate into the visceral organ of the intermediate host. L3 larvae only develop into mature males and females in marine mammals, where the sexual life cycle will take place (Mattiucci *et al.,* 2018). Starting from the 1950s, studies on *Anisakis* have not only been found in the marine environmental host. Other than marine animal hosts, animals such as amphibians, birds, reptiles and mammals including humans have been infected by the *Anisakis* nematodes (Measures, 2014). Shamsi *et al*. (2017) also observed that the pathogenicity of L3-stage *Anisakis* sp. depended on the species of *Anisakis* larvae that infected the unusual hosts (other than teleost fish). For example, *A. typica* (L3) were not encapsulated in the digestive system of Blue-lipped sea krait, *Carcharhinus brevipinna* and Spinner shark, *Laticauda laticauda* while *A. berlandi* (L3) and *A. pegreffii* (L3) were encapsulated in Grey petrel, *Procellaria cinereal* and Little penguin, *Eudyptula minor* (Shamsi *et al*., 2017).

Decapterus is a pelagic coastal fish found throughout the year and is widely present in global waters, including western Indian Ocean and western Pacific Ocean (Saikliang, 1997) and East China Sea, Indo-Pacific and Taiwan coastal region until southern Japan (Shiraishi *et al.*, 2010). Three species of *Decapterus: Decapterus macrosoma*, *Decapterus macarellus* and *Decapterus russelli* have been identified in Southeast Asia including Indonesia and Malaysia, where they are widely consumed as local delicacies (Arnaud *et al.*, 1999; Borsa, 2003; Rohit & Shanblogue, 2005). In Malaysia, *D. macrosoma* or Shortfin Scad is also known as *selayang*, *basung* or sardine. It is commonly used as the main ingredient for fish crackers and canned fish products in Malaysia (Kang *et al.*, 2018; Nartasha & Sarbon, 2019). *Decapterus macrosoma* has a body length ranging from 11.5 cm to 31.5 cm (Afdhila *et al.*, 2019; Rada *et al.*, 2019) and the male *D. macrosoma* is more predominant than the female (Asni *et al.*, 2019; Ahmadi, 2020). However, cases where this fish was infected with *A. typica* have been reported in Indonesia, causing food-safety problems in the fisheries products (Palm *et al.*, 2008; Palm *et al.*, 2017). In addition, in Malaysia, the anisakid nematodes were found in eight brands of canned fish products in 2018 (ProMED, 2018; Ruxyn, 2018). Coincidentally, in Indonesia and Peru,

Anisakis nematodes were detected in canned fish products (News Desk, 2017; Marwati, 2018). These problems cause health concerns for consumers and affect the sales of canned fish products, with more studies on *Anisakis* infection having been done in ASEAN countries in recent years. Therefore, the aim of this study was to identify the *Anisakis* species, its prevalence (P), mean intensity (M.I.) and mean abundance (M.A.) in *D. macrosoma* from Terengganu waters, Malaysia.

Materials and Methods

Parasite Collection

A total of 30 Shortfin Scad, *Decapterus macrosoma* were purchased from the fish market of Kuala Terengganu Fisheries Department, Terengganu, Malaysia between February to April 2021. The fish samples were transported in a cool box filled with ice to the Histology Lab, Faculty of Science and Marine Environment, Universiti Malaysia Terengganu (UMT). The body weight and total body length of fish samples were recorded upon reaching the lab. The fish samples were dissected under a light dissecting microscope (Olympus SZX7). The *Anisakis* third stage larvae (L3) were identified and removed from the surface of the fish organs, such as gut, liver, pyloric cecum, intestine and gonad and washed with (0.95% NaCl) saline water. The amount of *Anisakis* nematodes found from each organ was record individually. *Anisakis* larvae were identified at the genus level using the diagnostic morphological keys from Berland (1961). The identification of *Anisakis* nematodes (L3) was based on the morphological characteristic of the lips surrounding the anterior end, the presence of boring tooth, the shape of ventriculus, the shape of the tail and the mucron at the posterior end. The ultrasonic cleanser machine (Sonic-P27) was used to remove the outer coat of the larvae to get a finer result for the genetic and morphological study. A total of 676 *Anisakis* larvae were isolated and stored in 70% absolute ethanol for further analysis.

Molecular Analysis

20 *Anisakis* larvae were randomly selected from each host and underwent DNA extraction using GF-1 Viral Nucleic Acid Extraction Kit (Vivantis) according to the manufacturer's instructions. The extracted DNA was stored at -20°C until further analysis. Internal transcribed spacer (ITS) from the genomic DNA was isolated and amplified by using the primer NC5 (5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3') and NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') (Zhu *et al.*, 2000). The PCR was performed in 50 µl of reaction mix, containing approximately 0.025 μl^{-1} Taq DNA polymerase, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.1 at 20°C), 0.01% Triton™ X-100, 1 µM of each primer and 2.5 µM of sample. The amplification conditions were as follows (Zhu *et al.*, 2000): 94°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds and final extension at 72°C for 5 minutes.

After that, the PCR products were electrophoresed with 1,000 bp DNA ladder in 1% agarose gel with cybersafe under 90 V for 60 minutes. The targeted ITS genomic DNA sequences produced DNA fragments with 972 bp length. The UV transilluminator (OmegaLumG, Aplegen) was used to visualize the positive bands. Six PCR products with positive bands were sent for DNA sequencing. Bioedit Alignment Sequence Editor Ver. 7.0.5.3 was used to edit and assemble the forward and reverse sequence of ITS1-5.8S-ITS of rDNA. The ITS genomic DNA sequenced from the specimens were compared to the ITS genomic DNA sequence retrieved from GenBank for species identification. Retrieved ITS genomic DNA sequences are listed in Table 1. CLUSTAL X Version 2.1 Multiple Sequence Alignments was used to align obtained sequences with previous characterized sequences of *Anisakis* spp. registered in GenBank (https://www.ncbi.nlm.nih.gov/genbank). Maximum likelihood (ML) and neighbour-joining (NJ) phylogenetic trees with 1,000-replicates bootstrap were generated by using *MEGA* version 5.

Table 1: List of the sequences used in the phylogenetic analysis

Accession Number	*Anisakis* Species	Host	Region	Size	References
MZ895523	*Anisakis typica*	*Decapterus macrosoma*	Malaysia	934	Present study
MZ895793	*Anisakis typica*	*Decapterus macrosoma*	Malaysia	943	Present study
ON075544	*Anisakis typica*	*Decapterus macrosoma*	Malaysia	923	Present study
ON075545	*Anisakis typica*	*Decapterus macrosoma*	Malaysia	941	Present study
ON075546	*Anisakis typica*	*Decapterus macrosoma*	Malaysia	939	Present study
ON075547	*Anisakis typica*	*Decapterus macrosoma*	Malaysia	940	Present study
JX648312	*Anisakis typica*	*Decapterus macarellus*	Papua New Guinea	907	Koinari *et al*., 2013
MN420659	*Anisakis typica*	*Nemipterus japonicus*	Thailand	910	Tunya *et al*., 2020
MT020146	*Anisakis typica*	*Nemipterus japonicus*	China	921	Guo *et al*., 2020
JX523715	*Anisakis typica*	*Platycephalus indicus*	China	921	Zhang *et al*., 2013
KC928262	*Anisakis typica*	*Katsuwonus pelamis*	Indonesia	952	Anshary *et al*., 2014
AB479120	*Anisakis typica*	*Steno bredanensis*	Japan	933	Umehara *et al*., 2010
JN005760	*Anisakis typica*	*Pagellus bogaraveo*	Portugal	954	Hermida *et al*., 2012
EU718476	*Anisakis typica*	*Merluccius polli*	Africa	907	Kijewska *et al*., 2009
JQ934866	*Anisakis typica*	*Thunnus thynnus*	Croatia	888	Vardic Smrzlic *et al*., 2012
EU624345	*Anisakis paggiae*	*Theragra chalcogramma*	Japan	921	Quiazon *et al*., 2009
KM658332	*Anisakis brevispiculata*	*Scomber australasicus*	Taiwan	854	Chan and Shih, 2015
MH211473	*Anisakis pegreffii*	*Lophius litulon*	China	908	Zhang *et al*., 2018
MT448531	*Anisakis simplex*	*Gadus morhua*	Greenland	953	Severin *et al*., 2020
JQ912692	*Anisakis nascettii*	Marine fish	Rome	884	Mattiucci *et al*., 2014
JQ912691	*Anisakis ziphidarum*	Marine fish	Rome	888	Mattiucci *et al*., 2015
MH211517	*Hysterothylacium aduncum*	*Lophius litulon*	China	998	Zhang *et al*., 2018

Morphological Study

Scanning Electron Microscope (JEOL JSM6360 LA) (SEM) was used for more distinct morphological structure study of *Anisakis* nematode. The *Anisakis* nematodes were taken out from 70% absolute ethanol (HmbG) and underwent fixing and dehydration by using the procedure provided from INOS, UMT (Abdullah, M.N., 2021) and Kashi *et al*. (2014) with slight modifications: The nematode samples were fixed in 2.5% glutaraldehyde (Merck) in 0.1 M sodium cacodylate buffer (EMS), pH 7.2 for 2 hours. Then, the samples were taken out from the solution and washed with 0.1 M sodium cacodylate buffer (EMS) for 1 hour by changing the solution every 15 minutes. Next, the nematode samples were placed into 1% osmium tetroxide (Sigma-Aldrich) in 0.1 M sodium cacodylate buffer (EMS), pH 7.2 for 2 hours as the post-fixation process. After that, the nematode samples were washed with 0.1 M sodium cacodylate buffer (EMS) for 15 minutes by changing the solution every 5 minutes. The nematode underwent dehydration with different ethanol concentration (Sigma-Aldrich) from 35%, 50%, 60%, 70%, 80%, 90%, 95% until 100% for 10 minutes per solution. After dehydration, the nematode samples were placed in HMDS solution (AcroSeal®) for 3 minutes and air dried for 5 minutes. The prepared samples were mounted on stub by using a tissue double-sided tape (APOLLO). Next, the samples were coated with aurum using auto fine coater (Quorum). The distinct morphology structure of the sample was viewed and captured under scanning electron microscope (JEOL JSM6360 LA). Significant characterising organs such as lips bulge, external papillary structure, amphids and boring tooth, viewed from the SEM were labelled on the captured picture (Weerasooriya *et al*., 1986; Molina-Fernández *et al*., 2018).

Statistical Analysis

For the determination of the prevalence, mean intensity and mean abundance of the *Anisakis* larvae in *Decapterus macrosoma*, these formula from Bush (1997) were used:

$$Prevalence\ (P) = \frac{Number\ of\ individual\ infected\ host}{Total\ host\ sample} \times 100\%$$

$$Mean\ intensity\ (M.I.) = \frac{Number\ of\ individual\ parasite}{Number\ of\ individual\ infected\ host}$$

$$Mean\ abundance\ (M.A.) = \frac{Number\ of\ individual\ parasite}{Total\ host\ sample}$$

The data were presented as mean ± standard deviation. Statistical analysis were done using SPSS version 25 statistical software. Correlation study was also done between the size of fish and prevalence and mean intensity of *Anisakis* by using Pearson's correlation method. Non-normally distributed data underwent log transformation. Kruskal-Wallis test was used to understand the differences among visceral organs infected and paired *t* test was used to determine the significant preference in the distribution of anisakid nematode on visceral organs. The significance level was set at $p \leq 0.05$.

Results and Discussion

Molecular Identification of L3 Anisakis Larvae

20 PCR products of *Anisakis* larvae showed positive band in gel electrophoresis. The electrophoresis of six selected PCR products for the DNA sequencing is shown in Figure 1.

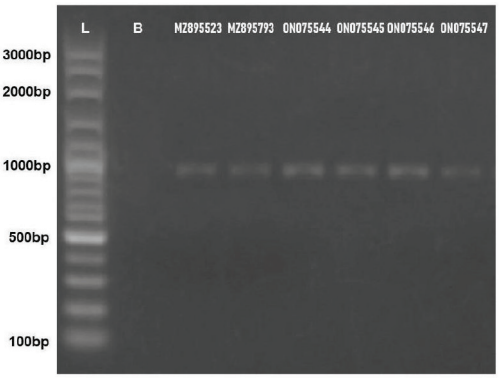

Figure 1: Gel electrophoresis of PCR product from ITS region of L3 *Anisakis typica* from shortfin scad, *Decapterus macrosoma*. L, 1,000 bp DNA ladder (Vivantis); B, blank

The amplification of entire ITS regions of *Anisakis* larvae from *Decapterus macrosoma* generated approximately 943 bp nucleotides. The ITS1-5.8S-ITS2 of rDNA gene sequences of the present *Anisakis* larvae were deposited into Genbank with accession numbers MZ895523, MZ895793, ON075544, ON075545, ON075546 and ON075547. The ITS region of the specimen was assigned to the species, *Anisakis typica* due to the high blast score (100% similarity) with *A. typica* accession numbers MN420659, MT020146, KC928262 and AB479120 when comparing the nucleotide sequences of rDNA in GenBank (Figure 2 and Figure 3).

From the maximum likelihood and neighbour-joining phylogenetic trees constructed from this study, *Anisakis typica* was a distinct lineage among *Anisakis* species (Chen & Shih, 2015). *Anisakis typica* was genetically related to *Anisakis ziphidarum* and *Anisakis nascettii a*nd heterogeneous with *Anisakis pegreffii* and *Anisakis simplex* (Anshary *et al.*, 2014). Moreover, *Anisakis* sp.1 was reported to be present in *Nemipterus japonicus* in Malaysian waters and it might be the sibling species of *A. typica* from the Central Pacific water based on the *mt*DNA COX2 region (Mattiucci & Nascetti, 2008; Mattiucci *et al.*, 2018). Our study was the first report of the presence of *A. typica* in *D. macrosoma* from Terengganu, Malaysia that had high similarity with other characterised *A. typica* in the GenBank (accession numbers MN420659, MT020146, KC928262 and AB479120). The polygenetic tree and genetic distance showed that *A. typica* from *D. macrosoma* was closely related to the *A. typica* found in the nearest countries located in the South China Sea (0.000) and Australian waters (0.000-0.0029) (Table 2).

Different *Anisakis* species are present in different geographical areas (Mattiucci *et al.*, 2007; Mattiucci *et al.*, 2018; Rahmati *et al.*, 2020). Thus, studying the geographic distribution of *Anisakis* spp. is important to ascertain the hot spot of Anisakiasis and

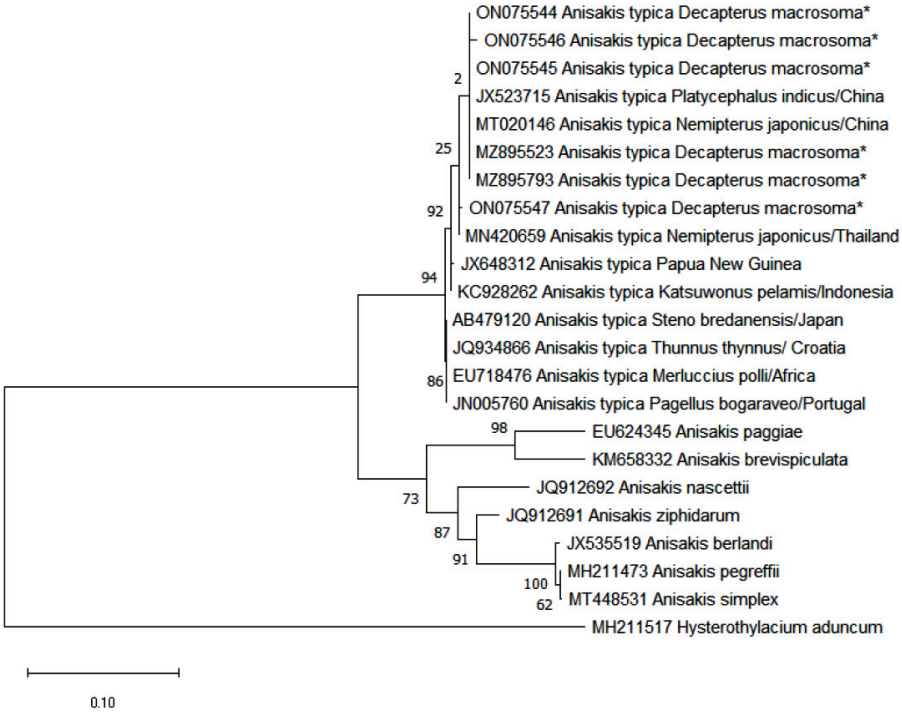

Figure 2: Maximum likelihood phylogenetic tree for *Anisakis* spp. based on ITS region of rDNA under the K2P (Kimura-2-parameter). Accession numbers are listed in the text and *Hysterothylacium aduncum* was used as out group. Number of nodes is 1,000 bootstrap replication. *state for *Anisakis* nematode in present study

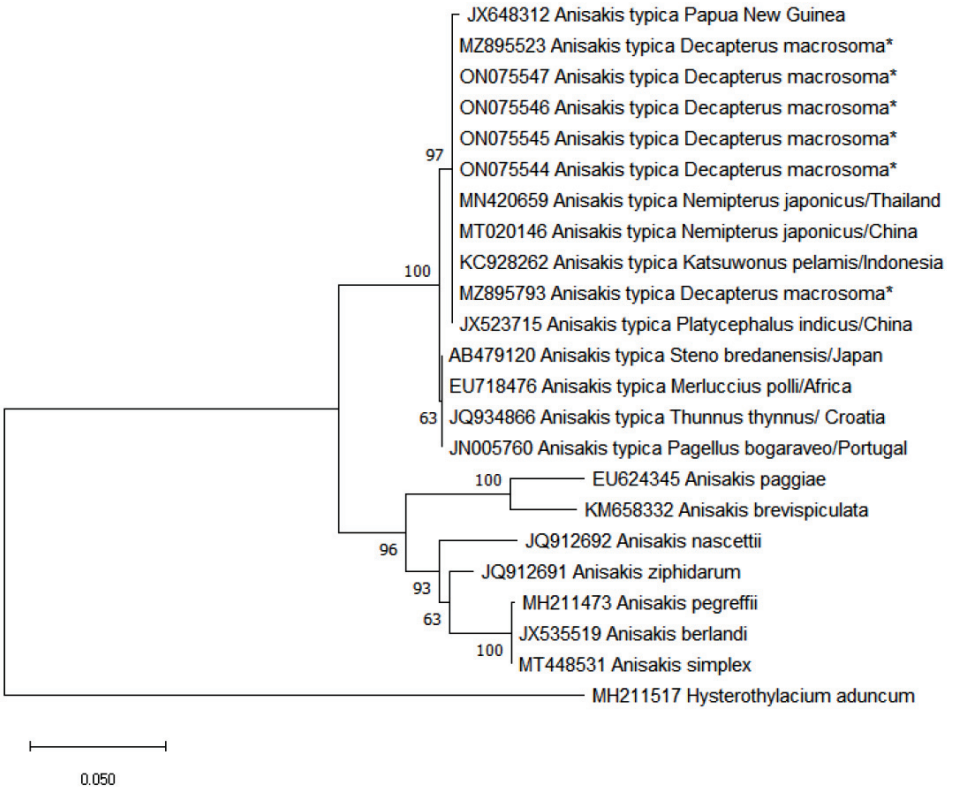

Figure 3: Neighbour-joining phylogenetic tree of *Anisakis* spp. based on ITS region of *r*DNA under the K2P (Kimura-2-parameter). Accession numbers are listed in the text and *Hysterothylacium aduncum* was used as out group. Number of nodes is 1,000 bootstrap replication. *state for *Anisakis* nematode in present study

Table 2: Genetic distance of *Anisakis* typica based on ITS1-5.8S-ITS2 of rDNA

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MZ895523_Anisakis typica*		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
MZ895793_Anisakis typica*	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
ON075544_Anisakis typica*	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
ON075545_Anisakis typica*	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
ON075546_Anisakis typica*	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
ON075547_Anisakis typica*	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
JX523715_Anisakis typica/China	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
MT020146_Anisakis typica/China	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
MN420659_Anisakis typica/Thailand	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0020	0.0029	0.0029	0.0029	0.0029
KC928262_Anisakis typica/Indonesia	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0020	0.0029	0.0029	0.0029	0.0029
JX648312_Anisakis typica/Papua New Guinea	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029		0.0036	0.0036	0.0036	0.0036
AB479120_Anisakis typica/Japan	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0087		0.0000	0.0000	0.0000
JQ934866_Anisakis typica/Croatia	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0087	0.0000		0.0000	0.0000
EU718476_Anisakis typica/Africa	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0087	0.0000	0.0000		0.0000
JN005760_Anisakis typica/Portugal	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0058	0.0087	0.0000	0.0000	0.0000	

control the infection (Rahmati *et al*., 2020). The dominant *Anisakis* species in *Merluccius merluccius* and *Xiphias gladius* from Northeast Atlantic is *A. simplex* (Mattiucci & D'Amelio, 2014). In the Mediterranean Sea, *A. pegreffii* (79%) was reported as the dominant species, followed by *A. typica* (9.21%), *A. physeteris* (6.38%) and *A. simplex* (4.6%) (Farjallah *et al*., 2008). The nearest countries to Malaysia such as Indonesia, Thailand and Vietnam have *A. typica* population in their water area (Anshary *et al*., 2014; Eamsobhana *et al*., 2018, Tunya *et al*.,

2020; Hein *et al*., 2021). Moreover, Anshary *et al*. (2014) found a high prevalence (92.3%) of *A. typica* in *Katsuwonus pelamis*. In the present study, there was only a single *Anisakis* species (i.e., *A. typica*) found in the *D. macrosoma* populations from Terengganu waters. This phenomenon has also been observed in the Indian Ocean located from 30 °S to 35 °N in temperate and tropical waters possibly due to the presence of Oceanic dolphins, which are the definitive host for *A. typica* (Mattiucci *et al.*, 2002; Mattiucci & Nascetti, 2008; Iñiguez *et al.*, 2011; Cavallero *et al.*, 2012). Nonetheless, Irrawaddy dolphins (*Orcaella brevirostris*) present on the east coast of the Peninsular Malaysia, may become the reservoir of *A. typica* in Terengganu waters (Mahmud *et al*., 2018; Jackson-Ricketts *et al*., 2020; Ismail *et al*., 2021).

Morphological Study

30 *Anisakis* samples were randomly selected from each fish samples and classified into *Anisakis* Type I larvae (Berland, 1961) which was characterized by long ventriculus, oblique connection between ventruculus and intestine and presence of mucron at the posterior end. It was mentioned in the previous study that there was difficulty to differentiate between *Anisakis* species because their morphology was highly similar (Koinari *et al.*, 2013). However, microphotographs are able to describe the L3 larvae of *Anisakis pegreffii* and *Anisakis typica* based on morphological and molecular studies (Chen & Shih, 2015). Based on Tunya *et al.* (2020), the posterior mucron of L3 larvae of *A. typica* was cylindrical while *Anisakis simplex* was cone-shape with wider and shorter mucron than *A. typica*. Under the Scanning Electron Micrograph (SEM) (JEOL JSM6360 LA), the anterior end of *A. typica* had three lips (labial protuberances) with papillae. The dorsal lip had two cephalic papillae and each ventrolateral lip had one cephalic papilla at the site. Besides that, the amphid was also located on both of the ventrolateral lips. The mouth was an open and triradiate form of a triangular aperture. The boring tooth was located between two ventrolateral lips (Figure 4 (a)). At the ventral part, there was an excretory pore below the boring tooth. The excretory pore was pointing forward with the mouth in same area. Then, there was an irregularly distributed striation from the anterior until the posterior end of *A. typica*. The posterior end of *A. typica* was a conical-like structure with a mucron end, having a C-shaped anus pointing toward the posterior end (Figure 4 (b)). Consequently, the *Anisakis* specimens in this study were defined as *A. typica* based on the morphology described in the previous studies (Koinari *et al.*, 2013; Gregori *et al.*, 2015; Chen & Shih, 2015; Tunya *et al.*, 2020).

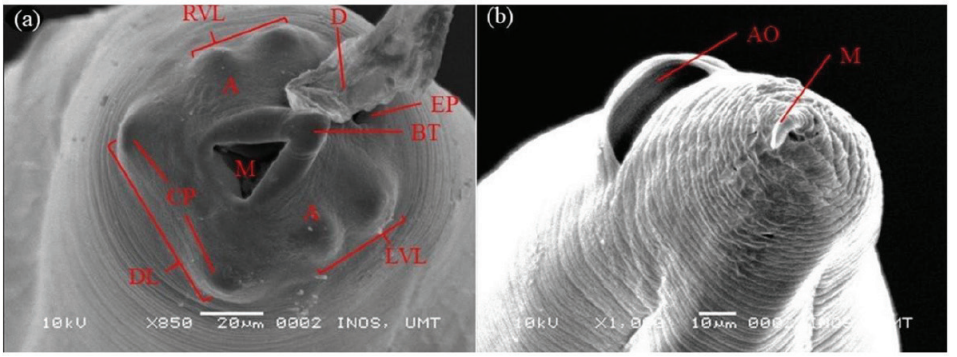

Figure 4: Scanning electron micrographs of mouth part (a) and tail part (b) of L3 *Anisakis typica* from shortfin scad, *Decapterus macrosom*a. (a) Using 850X magnification with 10 kV accelerating voltage. A, amphid; BT, boring tooth; CP, cephalic papillae; D, debris; DL, dorsal lip; EP, excretory pore; LVL, left ventrolateral lip; M, mouth; RVL, right ventrolateral lip. (b) Using 1,000 X magnification with 10 kV accelerating voltage. AO, anus opening; M, mucron. Pictures were taken using SEM (JEOL JSM6360 LA)

Anisakis Larvae Infection in Decapterus macrosoma

The mean body weight and mean total body length of fish samples (n = 30) were 132.51±12.96 g and 23.16±0.93 cm, respectively. The prevalence, mean intensity, abundance of *Anisakis* larvae found in *Decapterus macrosoma* are shown in Table 3.

Clade 1 *Anisakis* species such as *Anisakis simplex, Anisakis pegreffii* and *Anisakis berlandi* are commonly found in pelagic, benthopelagic and benthic demersal fish (Matttiucci *et al.*, 2018) while *Anisakis typica* is mainly present in pelagic and reef-associated teleost fish species (Kuhn *et al.*, 2013). Consequently, there was a high infection level of *A. typica* in *D. macrosoma* in our study (P = 100%, M.I. = 23.31±17.26) (Table 3). Besides that, the reservoir of *A. typica* (Delphinidae) was found in epipelagic zone (Gregori *et al.*, 2015). The upwelling and downwelling conditions in mesozooplankton communities (Farjallah *et al.*, 2008; Kuhn *et al.*, 2013) were also the factors that caused high prevalence of *A. typica* in the pelagic fish such as *D. macrosoma* (Table 3) and *Scomber japonicus* (85.7%) from Taiwan waters (Hien *et al.*, 2021).

In recent studies, the bioaccumulation of *Anisakis* larvae occurred in *Alosa alosa*, *Engaraulis encrasicolus* and *Gadus morhua* (Bao *et al.*, 2015; Münster *et al.*, 2015; Cipriani *et al.*, 2018). The larger size of *D. macrosoma* had higher infection level of *A. typica*. The body weight (r = 0.36, p = 0.05) and total length (r = 0.42, p = 0.02) of *D. macrosoma* also showed a positive linear correlation with the number of *Anisakis* larvae collected from the infected fish (Figure 5). This could be caused by the transmission of *Anisakis* nematodes into another host (predator) during the prey-predator system (Mattiucci *et al.*, 2018). Moreover, a larger

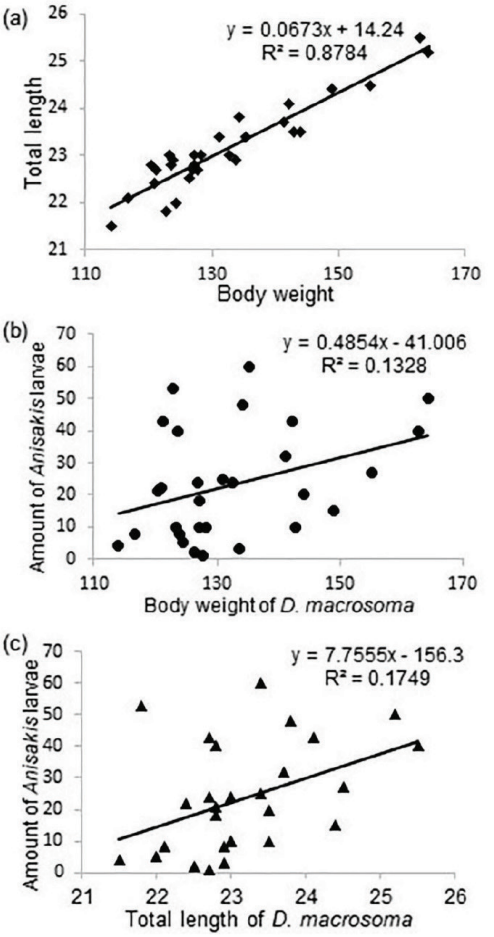

Figure 5: Correlation of size of *Decapterus macrosoma* and number of *Anisakis*. (a) is body weight and total length of shortfin scad, *D. macrosoma*, (b) is body weight and number of *Anisakis* larvae in shortfin scad, *D. macrosoma*, (c) is total length and number of *Anisakis* larvae in shortfin scad, *D. macrosoma*

Table 3: Prevalence, mean intensity, mean abundance and range of *Anisakis* larvae in shortfin scad, *Decapterus macrosoma*

Fish Species	Shortfin Scad (*Decapterus macrosoma*)
Prevalence of *Anisakis* larvae	100%
Mean intensity of *Anisakis* larvae	23.31±17.26
Mean abundance of *Anisakis* larvae	24.6
Range	1-60

or older fish that consumes a large number of small fish (intermediate host) may cause the bioaccumulation of *Anisakis* larvae, increasing the infected amount of *A. typica* larvae when size of *D. macrosoma* increases.

During infection, *Anisakis* larvae such as *A. simplex* and *A. pegreffii* migrate to the muscular part of the host (Strømnes, 2014) while *A. typica* is rarely found in fish flesh (Palm, 2017). A previous study has shown that the digestive method and incubation method could yield more accurate and higher nematode counts in the fish host. However, visual examination was used in the present study because the migration of *A. typica* into the muscle was blocked by the enzyme activity (unfavourable temperature and pH value), reducing the movement of *A. typica* during the incubation in specific infected organ (Šimat *et al*., 2015). In our study, *A. typica* accumulated in the visceral organs of *D. macrosoma*. The highest distribution of *Anisakis* nematodes was in the gonad of *D. macrosoma* (49%), followed by the gut (22%), intestine (13%), pyloric caeca (12%) and lastly, the liver (4%) (Figure 6). The infected number of *Anisakis* nematodes showed significant differences among the visceral organs (Kruskal-Wallis: $X^2 = 34.47$, $p = 5.98\times10^{-7}$). The gonad of *D. macrosoma* was surrounded by fatty tissue (Figure 7) and *Anisakis* nematode infection on the gonad showed significant difference ($p < 0.003$) among other visceral organs such as gut, pyloric caeca, liver and intestine. Coincidentally, a report has stated that the growth and migration behaviour of *Anisakis* spp. were dependent on lipid concentration in the organ (Šimat *et al.*, 2015). There is a co-relationship between the migration of *Anisakis* spp. and fat tissue (Münster *et al.*, 2015; Šimat *et al.*, 2015), corroborating our observation of a high percentage of *A. typica* found on organ surrounded by fatty tissues.

Conclusion

In conclusion, shortfin scad, *Decapterus macrosoma* from Terengganu waters was exclusively infected with *A. typica* L3 larvae. All the extracted ITS1-5.8S-ITS2 of rDNA gene sequences from the present study were identified as *Anisakis typica*. The microphotograph of *A. typica* showed a triangular open mouth, boring tooth, excretory pore, one dorsal lip with two papillae, two ventrolateral lips with one papilla and one amphid at the anterior end and that the posterior end was a conical-like structure with a mucron end, with a C-shaped anus.

All of the fish samples (n = 30) with 132.51±12.96 g body weight and 23.16±0.93 cm body length was infected with this zoonotic nematode (100% prevalence) with an average of 23.31±17.26 individual nematodes present in one infected fish. The intensity of *A. typica*

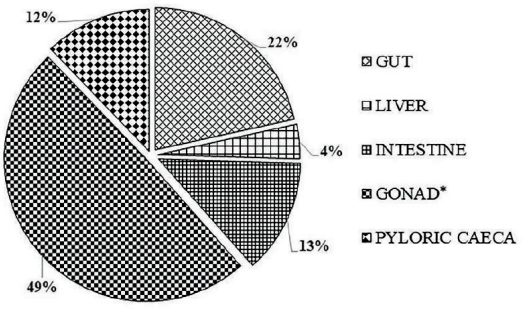

Figure 6: Percentage of amount of *Anisakis* larvae found on different organs in shortfin scad, *Decapterus macrosoma.* *represent significant difference

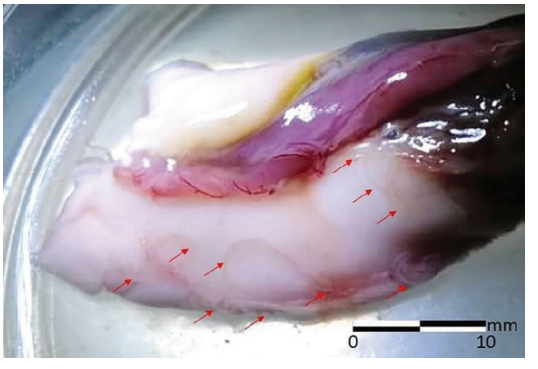

Figure 7: *Anisakis* larvae accumulated in fatty tissue on gonad of shortfin scad, *Decapterus macrosoma*. Arrow represent *Anisakis* larvae

in *D. macrosoma* was significantly correlated to the body weight ($r = 0.36$, $p = 0.05$) and total length ($r = 0.42$, $p = 0.02$) of host, showing that the infection of *A. typica* could happen in prey-predator relationship and cause bioaccumulation in larger host. Besides that, *A. typica* was found in the fatty tissues of the gonad of the infected fish, reflecting the *Anisakis* migration behaviour.

Further studies such as the migration behaviour of *A. typica* within the host environment condition can be conducted to understand the affecting factors that inhibit *A. typica* migration to the muscle tissue of fish host and prevent migration of other *Anisakis* species. The investigation on allergen profile of *A. typica* is also recommended to understand the excretory substance of *A. typica* that causes the immune sensitivity in humans.

Acknowledgements

This study was funded by the Research Intensified Grant Scheme (RIGS) 2019 managed by the Centre for Research and Innovation Management, Universiti Malaysia Terengganu under vot no. 55192/4.

References

Afdhila, R., Muhammadar, A. A., & Chaliluddin, M. A. (2019) Length-weight relationship and condition factors of layang fish (*Decapterus macrosoma*) that landed at Lampulo Ocean Fishing Port, Banda Aceh. *IOP Conference Series: Earth and Environmental Science*, *348*, 012079. DOI: 10.1088/1755-1315/348/1/012079

Ahmadi. (2020) Length-weight relationship, body condition and fishing gear selativity of shortfin sacd (*Decapterus macrosoma*) landed in Banjarmasin Fishing Port, Indonesia. *Fisheries & Aquatic Life*, *28*, 113-124. DOI: 10.2478/aopf-2020-0014

Amir, A., Ngui, R., Ismail, W. H. W., Wong, K. T., Ong, J. S. K., Lim, Y. A. L., Lau, Y. L., & Mahmud, R. (2016). Anisakiasis causing Acute Dysentery in Malaysia. *The American Journal of Tropical Medicine and Hygiene*, *95*(2), 410-412. DOI: 10.4269/ajtmh.16-0007

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. *The Korean Journal of Parasitology*, *52*, 9-19. DOI: 10.3347/kjp.2014.52.1.9

Arnaud, S., Bonhomme, F., & Borsa, P. (1999). Mitochondrial DNA analysis of the genetic relationships among populations of scad mackerel (*Decapterus macarellus, D. macrosoma and D. russelli*) in South-East Asia. *Marine Biology, 135*, 699-707. DOI: 10.1007/S002270050671

Asni, A., Hamsiah, Rustam & Ilmiah. (2019). Biological aspects of Shortfin Scad (*Decapterus macrosoma*) in Makassar Waters, South Sulawesi, Indonesia. *IOP Conference Series: Earth and Environmental Science*, *253*, 012008. DOI: 10.1088/1755-1315/253/1/012008

Ayun, N. Q., Dewi, L. S., Antoko, M., & Setyobudi, E. (2021). The occurrence of *Anisakis* larvae on hairtail, *Trichiurus lepturus* caught from the Pangandaran water, Weat Java, Indonesia. *Biosiversitas Journal of Biological Diversity*, *22*, 1378-1384. DOI: 10.13057/biodiv/d220339

Bao, M., Roura, A., Mota, M., Nachón, D. J., Antunes, C., Cobo, F., MacKenzie, K., & Pascual, S. (2015). Macroparasites of allis shad (*Alosa alosa*) and twaite shad (*Alosa fallax*) of the Western Iberian Peninsula Rivers: Ecological, phylogenetic and zoonotic insights. *Parasitology Research*, *114*, 3721-3739. DOI: 10.1007/s00436-015-4601-1

Berland, B. (1961). Nematodes from some Norwegian marine fishes. *Sarsia*, *2*, 1-50. DOI: 10.1080/00364827.1961.10410245

Borsa, P. (2003). Genetic structure of round scad mackerel *Decapterus macrosoma*

(Carangidae) in the Indo-Malay archi pelago. *Marine Biology*, *142*, 575-581. DOI: 10.1007/s00227-002-0974-7

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shoastak, A. W. (1997). Parasitology meets ecology on its own terms. *Journal of Parasitology, 83*(4), 575-583. DOI: 10.2307/3284227

Cavallero, S. M. (2018). Anisakiasis in Italy: Analysis of hospital discharge records in the years 2005-2015. *PLOS ONE*, *13*(12), e0208772. DOI: 10.1371/journal.pone.0208772

Cavallero, S., Ligas, A., Bruschi, F., & D'Amelio, S. (2012). Molecular identification of *Anisakis* spp. from fish collected in the Tyrrhenian Sea (NW Mediterranean). *Veterinary Parasitology*, *187*, 563-566. DOI: 10.1016/j.vetpar.2012.01.033

Chen, H. Y., & Shih, H. H. (2015). Occurrence and prevalence of fish-borne *Anisakis* larvae in the spotted mackerel *Scomber australasicus* from Taiwanese waters. *Acta Tropica*, *145*, 61-67. DOI: 10.1016/j.actatropica.2015.02.011

Chou, Y. Y., Wang, C. S., Chen, H. G., Chen, H. Y., Chen, S. N., & Shih, H. H. (2011). Parasitism between *Anisakis simplex* (Nematoda: Anisakidae) third-stage larvae and the spotted mackerel *Scomber australasicus* with regard to the application of stock identification. *Veterinary Parasitology*, *177*, 324-331. DOI: 10.1016/j.vetpar.2010.12.003

Cipriani, P., Sbaraglia, G., Palomba, L., Giulietti, L., Bellisario, B., Buselic, I., Mladineo, I., Cheleschi, R., Nascetti, G., & Mattiucci, S. (2018). *Anisakis pegreffii* (Nematoda: Anisakidae) in European anchovy *Engraulis encrasicolus* from the Mediterranean Sea: Considerations in relation to fishing ground as a driver for parasite distribution. *Fisheries Research*, *202*, 59-68. DOI: 10.1016/j.fishres.2017.03.020

Eamsobhana, P., Yong, H. S., Song, S. L., Tungtrongchitr, A., & Roongruangchai, K. (2018). Genetic differentiation of *Anisakis* species (Nematoda: Anisakidae) in marine fish *Priacanthus tayenus* from Gulf of Thailand. *Tropical Biomedicine*, *35*, 669-677.

Farjallah, S., Slimane, B. D., 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. DOI: 10.1007/s00436-007-0771-9

Gomes, T. L., Quiazon, K. M. A., Kotale, M., Itoh, N., & Yoshinaga, T. (2020). *Anisakis* spp. in fishery products from Japanese water: Updated insights on host prevalence and human infection risk factors. *Parasitology International*, *78*, 102137. DOI: 10.1016/j.parint.2020.102137

Gregori, M., Roura, Á., Abollo, E., González, Á. F., & Pascual, S. (2015). *Anisakis simplex* complex (Nematoda: Anisakidae) in zooplankton communities from temperate NE Atlantic waters. *Journal of Natural History*, *49*, 755-773. DOI: 10.1080/00222933.2014.979260

Guo, N., Chen, H. X., Zhang, L. P., Zhang, J. Y., Yang, L. Y., & Li, L. (2020). Infection and molecular identification of ascaridoid nematodes from the important marine food fish Japanese threadfin bream *Nemipterus japonicus* (Bloch) (Perciformes: Nemipteridae) in China. *Infection, Genetic and Evolution*, *85*, 104562. DOI: 10.1016/j.meegid.2020.104562

Hermida, M., Mota, R.. Pacheco, C. C., Santos, C. L., Cruz, C., Saraiva, A., & Tamafnini, P. (2012). Infection levels and diversity of Anisakid nematodes in blackspot seabream, *Pagellus bogaraveo*, from Portuguese waters. *Parasitology Research*, *110*, 1919-1928. DOI: 10.1007/s00436-011-2718-4

Hien, H. V., Dung, B. T., Ngo, H. D., & Doanh, P. N. (2021). First morphological and molecular identification of third-stage larvae of *Anisakis typica* (Nematoda: Anisakidae)

from marine fishes in Vietnamese water. *Journal of Nematology*, *52*, 1-9. DOI: 10.21307/jofnem-2021-010

Hrabar, J., Smodlaka, H., Rasouli-Dogaheh, S., Petric, M., Trumbić, Ž., Palmer, L., Sakamaki, K., Pavelin, T., & Mladineo, I. (2021). Phylogeny and pathology of Anisakids parasitizing stranded California Sea Lions (*Zalophus californianus*) in Southern California. *Frontiers in Marine Science*, *8*, 636626. DOI: 10.3389/fmars.2021.636626

Irigoitia, M. M., Palomba, M., Braicovich, P. E., Lanfranchi, A. L., Denuncio, P. E., Gana, J. C., Mattiucci, S., & Timi, J. T. (2021). Genetic identification of *Anisakis* spp. (Nematoda: Anisakidae) from cetaceans of the Southwestern Atlantic Ocean: Ecological and zoogeographical implications. *Helminthology*, *120*, 1699-1711. DOI: 10.1007/s00436-021-07088-w

Ismail, F. D., Azizul, I., Jaaman, S. A., Muda, A. M., Momin, M. V., Abdullah, B., Zhang, X., & Muhamad, H. M. (2021). Seasonal occurrence, distribution and group size of Irrawaddy Dolphins (*Orcaella brevirostris*) in the Bay of Brunei, Brunei Darussalam. *Sains Malaysiana*, *50*, 3159-3169. DOI: 10.17576/jsm-2021-5011-01

Iñiguez, A. M., Carvalho, V. L., Motta, M. R. A., Phinheiro, 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. DOI: 10.1016/j.vetpar.2011.01.001

Jackson-Ricketts, J., Junchompoo, C., Hines, E. M., Hazen, E. L., Ponnampalam, L. S., Ilangakoon, A., & Monanunsap, S. (2020). Habitat modeling of Irrawaddy dolphins (*Orcaella brevirostris*) in the Eastern Gulf of Thailand. *Ecology and Evolution*, *10*, 2778-2792. DOI: 10.1002/ece3.6023

Kang, P. Y., Ishak, N. H., & Sarbon, N. M. (2018). Optimization of enzymatic hydrolysis of shortfin scad (*Decapterus macrosoma*) myofibrillar protein with antioxidant effect using alcalase. *International Food Research Journal*, *25*(5), 1808-1817. http://www.ifrj.upm.edu.my/25%20(05)%202018/(6).pdf

Kashi, A. M., Tabermanesh, K., Chaichian, S., Joghataei, M. T., Moradi, F., Tavangar, S. M., Najafabadi, A. S. M., Lotfibakhshaiesh, N., Beyranvand, S. P., Anvari-Yazdi, A. F., & Abed, S. M. (2014). How to prepare biological sample and live tissues for scanning electron microscopy (SEM). *Galen Medical Journal*, *3*, 63-80. DOI: 10.31661/gmj.v3i2.267

Kijewska, A., Dzido, J., Shukhgalter, O., & Rokicki, J. (2009). Anisakid parasites of fishes caught on the African shelf. *Journal of Parasitology*, *95*, 639-645. DOI: 10.1645/GE-1796.1

Kim, S. H., Park, C. W., Kim, S. K., Won, S., Park, W. K., Kim, H. R., Nam, K. W., & Lee, G. S. (2013). A case of *Anisakiasis* invading the stomach and the colon at the same time after eating anchovies. *Clinical Endoscopy*, *46*(3), 293-296. DOI: 10.5946/ce.2013.46.3.293

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. DOI: 10.1007/s00436-014-3781-4

Kobayashi, Y., Shimakura, K., Ishizaki, S., Nagashima, Y., & Shiomi, K. (2007). Purification and *c*DNA cloning of a new heat-stable allergen from *Anisakis simplex*. *Molecular and Biochemical Parasitology*, *155*, 138-145. DOI: 10.1016/j.molbiopara.2007.06.012

Koinari, M., Karl, S., Elliot, A., Ryana, U., & Lymbercyc, A. J. (2013). Identification of *Anisakis* species (Nematoda: Anisakidae) in marine fish hosts from Papua New Guinea. *Veterinary Parasitology*, *193*, 126-133.

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. DOI: 10.14411/fp.2013.013

Lim, H., Jung, B. K., Cho, J., Yooyan, T., Shin, E. H., & Chai, J. Y. (2015). Molecular diagnosis of cause of *Anisakiasis* in humans, South Korea. *Emerging Infectious Diseases*, *21*(2), 342-344. DOI: 10.3201/eid2102.140798

Mahmud, A. I., Jaaman, S. A., Muda, A. M., Muhamad, H. M., Zhang, X., & Scapini, F. (2018). Factor influencing the behaviour of Irrawaddy dolphins *Orcaella brevirostris* (Owen in Gray, 1866) in Brunei Bay, Malaysia. *Journal of Ethology*, *36*, 169-180. DOI: 10.1007/s10164-018-0549-9

Marwati. (2018). Universitas Gadjah Mada. *Anisakis* in Fish Natural Phenomenon. https://www.ugm.ac.id/en/news/15980-anisakis-in-fish-is-natural-phenomenon

Mattiucci, S. (2015). Anisakis. In L. H. Xiao, U. Ryan, & Y. Y. Feng (Eds.), *Biology of Foodborne Parasites* (1st ed., pp. 255-274). CRC Press.

Mattiucci, S., Abaunza, P., Damiano, S., Garcia, A., Santos, M., & Nascetti, G. (2007). Distribution of *Anisakis* larvae, identified by genetic markers and their use for stock characterization of demersal and pelagic fish from European waters: An update. *Journal of Helminthology*, *81*, 117-127. DOI: 10.1017/S0022149X07754718

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., & Nescetti, G. (2018). Molecular epidemilology of *Anisakis* and *Anisakiasis*: An ecological and evolutionary road map. *Advances in Parasitology*, *99*, 96-263. DOI: 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. DOI: 10.1645/12-120.1

Mattiucci, S., & D'Amelio, S. (2014). Anisakiasis. In F. Bruschi (Ed.), *Helminth Infections and their Impact on Global Public Health* (1st ed., pp. 325-365). Springer.

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. DOI: 10.1016/S0065-308X(08)00202-9

Mattiucci, S., Paggi, L., Nascetti, G., Portes Santos, C., Costa, G., DiBeneditto, 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. DOI: 10.1023/A:1014554900808

Mattiucci, S., Paoletti, M., Colantoni, A., Carbone, A., Gaeta, R., Proietti, A., Frattaroli, S., Fazii, P., Bruschi, F., & Nescetti, G. (2017). Invasive anisakiasis by the parasite *Anisakis pegreffii* (Nematoda: Anisakidae): Diagnosis by real-time PCR hydrolysis probe system and immunoblotting assay. *BMC Infectious Disease*, *17*, 530. DOI: 10.1186/s12879-017-2633-0

McGladdery, S. E. (1986). *Anisakis simplex* (Nematoda, Anisakidae) infection of the musculature and body cavity of Atlantic herring (*Clupea harengus*). *Canadian Journal of Fisheries and Aquatic Sciences*, *43*, 1312-1317. DOI: 10.1139/f86-164

Measures, L. N. (2014). Anisakiosis and pseudoterranovosis: Reston, Va., U.S. Geological Survey Circular 1393, 43 p., 2 appendixes. http://dx.doi.org/10.3133/cir1393.

Molina-Fernández, D., Adroher, F. J., & Benítez, R. (2018). A scanning electron microscopy study of *Anisakis physeteris* molecularly identified: From third stage larvae from fish to fourth stage larvae obtained in vitro. *Parasitology Research*, *117*, 2095-2103. DOI: 10.1007/s00436-018-5896-5

Münster, J., Klimpel, S., Fock, H. O., MacKenzie, K., & Kuhn, T. (2015). Parasites as biological tags to track an ontogenetic shift in the feeding behaviour of Gadus morhua off West and East Greenland. *Parasitology Research*, *114*(7), 2723-2733. DOI: 10.1007/s00436-015-4479-y

News Desk. (2017). *Outbreak News Today*. Peru: Chinese canned fish pulled from market due to *Anisakis* worms. http://outbreaknewstoday.com/peru-chinese-canned-fish-pulled-market-due-anisakis-worms-99850/

Nurtasha, A. B., & Sarbon, N. M. (2019). Microstructure, extractability and physicochemical properties of shortfin scad (*Decapterus macrosoma*) bone collagen as influenced by acetic acid concentration. *International Food Research Journal, 26*(2), 451-458. http://www.ifrj.upm.edu.my/26%20(02)%202019/(10).pdf

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

Palm, H. W., Theisen, S., Damriyasa, I. M., Kusmintarsih, E. S., Oka, I. B. M., Setyowati, E. A., Suratma, N. A., Wibowo, N. A., & Klenertz, S. (2017). *Anisakis* (Nematoda: Ascaridoidea) from Indonesia. *Diseases of Aquatic Organisms*, *123*(2), 141-157. DOI: 10.3354/dao03091

Pawlak, J. Nadolna-Ałtyn, K., Szostakowska, B., Pachur, M., Bańkowska, A., & Podolska, B. (2019). First evidence of the presence of *Anisakis simplex* in *Crangon crangon* and *Contracaecum osculatum* in *Gammarus* sp. by in situ examination of the stomach contents of cod (*Gadus morhua*) from the Southern Baltic Sea. *Parasitology*, *146*, 1699. DOI: 10.1017/S0031182019001124

Pontes, T., D'Amelio, S., Costa, G., & Paggi, L. (2005). Molecular characterization of larval anisakid nematodes from marine fishes of Madeira by a PCR-based approach, with evidence for a new species. *Journal of Parasitology*, *91*, 1430-1434. DOI: 10.1645/GE-565R1.1

ProMED. (2018) PRO/MBDS. Anisakiasis - Malaysia: Ex China, canned sardines (20180629.5881848). *International Society for Infectious Diseases*. http://www.promedmail.org/direct.php?id=20180629.5881848

Quiazon, K. M., Yoshinaga, T., Santos, M. D., & Ogawa, K. (2009). Identification of larval *Anisakis* spp. (Nematoda: Anisakidae) in *Alaska pollock* (*Theragra chalcogramma*) in Northern Japan using morphological and molecular markers. *Journal of Parasitology*, *95*, 1227-1232. DOI: 10.1645/GE-1751.1

Rada, B. G., Ramos, E. B., Riva, C. J., & Royo, N. R. (2019). Preliminary study on spawning period and length at maturity of short- fin scad, *Decapterus macrosoma*, (Bleeker, 1851, Perciformes: Carangidae) from the Coastal Waters of San Fernando, Romblon. *The Philippine Journal of Fisheries*, *26*, 35-43. DOI: 10.31398/TPJF/26.1.2018-0014

Rahmati, A. E., Kiani B., Afshari, A., Moghaddas, E., Williams, M., & Shamsi, S. (2020). World-wide prevalence of *Anisakis* larvae in fish and its relationship to human allergic anisakiasis: A systematic review. *Fish Parasitology*, *119*, 3585-3594. DOI: 10.1007/s00436-020-06892-0

Roca-Geronès, X., Segovia, M., Godínez-González, C., Fisa, R., & Montoliu, L. (2020). *Anisakis* and *Hysterothylacium* species in Mediterranean and North-East Atlantic fishes commonly consumed in Spain: Epidemiological, molecular and morphometric discriminant analysis. *International Journal*

of Food Microbiology, 325, 108642. DOI: 10.1016/j.ijfoodmicro.2020.108642

Rohit, P., & Shanblogue, S. L. (2005). Age and growth of *Decapterus russelli* and *D. macrosoma* along Karnataka coast, India. *Journal of the Marine Biological Association of India*, *47*, 180-184. http://eprints.cmfri.org.in/id/eprint/2058

Ruxyn, T. (2018, April 20). *MOH Confirms 2 Brands of Canned Sardines from China are Contaiminated with Parasites*. SAYS. https://says.com/my/news/moh-confirms-recalls-two-brands-of-canned-sardines-from-china-found-to-contain-dead-worms

Saikliang, P. (1997). Fishery resources and the state of exploitation of some economic fish species in the South China Sea area case study: Malaysia and Thai Waters. In SEAFDEC/TD (Ed.), *Fishery Resources and State of Stocks Exploitation in the Waters of the Gulf of Thailand, East Coast of Peninsular Malaysia and Andaman Sea* (pp. 59-90). Samut Prakarn, Thailand: Training Department, Southeast Asian Fisheries Development Center.

Severin, N. L., Yurchenko, M., Sorensen, J. S., Zuo, S., Karami, A. M., Kania, P. W., & Buchmann, K. (2020). Anisakid nematode larvae in the liver of Atlantic cod *Gadus morhua* L. from West Greenland. *Parasitology Research*, *119*, 3233-3241. DOI: 10.1007/s00436-020-06807-z

Shamsi, S. (2021). The occurrence of *Anisakis* spp. In Australian waters: Past, present and future trends. *Fish Parasitology*, *120*, 3007-3033. DOI: 10.1007/s00436-021-07243-3

Shamsi, S., Chen, Y. C., Poupa, A., Ghadam, M., & Justine, J. L. (2018). Occurrence of anisakid parasites in marine fishes and whale off New Caledonia. *Parasitology Research*, *117*, 3195-3204. DOI: 10.1007/s00436-018-6018-0

Shamsi, S., & Suthar, J. (2016). A revised method of examining fish for infection with zoonotic nematode larvae. *International Journal of Food Microbiology*, *227*, 13-16. DOI: 10.1016/j.ijfoodmicro.2016.03.023

Shawket, N., Aasri, A. E., Elmadhi, Y., M'Bareck, I., Kharrim, K. E., & Belghyti, D. (2017). *Anisakis simplex* (Nematode: Anisakidae) from horse mackerel (*Trachurus trachurus*) in Atlantic coast of Morocco. *Asian Pacific Journal of Tropical Disease*, *7*, 463-466. DOI: 10.12980/APJTD.7.2017D6-439

Shiraishi, T., Tanaka, H., Ohshimo, S., Ishida, H., & Morinaga, N. (2010). Age, growth and reproduction of two species of scad, *Decapterus macrosoma* and *D. macarellus* in the Waters off Southern Kyushu. *Japan Agricultural Research Quarterly*, *44*, 197-206. DOI: 10.6090/jarq.44.197

Shokoofeh, S., Briand, M. J., & Justine, J. L. (2017). Occurrence of *Anisakis* (Nematode: Anisakidae) larvae in unusual host in Shouthern hemisphere. *Parasitology International*, *66*, 837-840. DOI: 10.1016/j.parint.2017.08.002

Sonko, P., Chen, S. C. C., Chou, C. M., Huang, Y. C., Hsu, S. L., Barčák, D., Oros, M., & Fan, C. K. (2020). Multidisciplinary approch in study of the zoonotic *Anisakis* larval infection in the blue mackerel (*Scomber australasicus*) and the largehead hairtail (*Trichirus lepturus*) in Northern Taiwan. *Journal of Microbiology, Immunology and Infection*, *53*, 1021-1029. DOI: 10.1016/j.jmii.2019.04.012

Strømnes, E. (2014). An *in vitro* study of lipid preference in whaleworm (*Anisakis simplex*, Nematoda, Ascaridoidea, Anisakidae) third-stage larvae. *Parasitology Research*, *113*, 1113-1118. DOI: 10.1007/s00436-013-3748-x

Šimat, V., Miletić, J., Bogdanović, T., Poljak, V., & Mladineo, I. (2015). Role of biogenic amines in the post-mortem migration of *Anisakis pegreffii* (Nematoda: Anisakidae Dujardin, 1845) larvae into fish fillets. *International Journal of Food Microbiology*, *214*, 179-186. DOI: 10.1016/j.ijfoodmicro.2015.08.008

Tsukui, M., Morimoto, N., Kurata, H., & Sunada, F. (2016). Asymptomatic anisakiasis of the colon incidentally diagnosed and treated during colonoscopy by retroflexion in the ascending colon. *Journal of Rural Medicine*, *11*(2), 73-75. DOI: 10.2185/jrm.2914

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. *The Korean Journal of Parasitology*, *58*, 15-25. DOI: 10.3347/kjp.2020.58.1.15

Umehara, A., Kawakami, Y., Ooi, H. K., Uchida, A., Ohmae, H., & Sugiyama, H. (2010). Molecular identification of *Anisakis* type I larvae isolated from hairtail fish off the coasts of Taiwan and Japan. *International Journal of Food Microbiology*, *143*, 161-165. DOI: 10.1016/j.ijfoodmicro.2010.08.011

València, E. D. (2017, August 16). *Uno de cada tres pescados consumido en España está infectado de Anisakis*. Levante-EMV. https://www.levante-emv.com/vida-y-estilo/salud/2017/08/16/tres-pescados-consumido-espana-infectado-13748331.html

Vardic Smrzlic, I., Valic, D., Kapetanovic, D., Kurtovic, B., & Teskeredzic, E. (2012). Molecular characterisation of Anisakidae larvae from fish in Adriatic Sea. *Parasitology Research*, *111*, 2385-2391. DOI: 10.1007/s00436-012-3094-4

Weerasooriya, M. V., Fujino, T., Ishii, Y., & Kagei, N. (1986). The value of external morphology in the identification of larval anisakidnematodes: A scanning electron microscope study. *Z Parasitenkd*, *72*, 765-778. DOI: 10.1007/BF00925097

Yamamoto, K. T. (2019). Comparison of risk factors between human intestinal and gastric Anisakiasis. *Parasitology International*, *75*, 102024. DOI: 10.1016/j.parint.2019.102024

Zhang, K., Xu, Z., Chen, H. X., Guo, N., & Li, L. (2018). Anisakid and raphidascaridid nematodes (Ascaridoidea) infection in the important marine food-fish *Lophius litulon* (Jordan) (Lophiiformes: Lophiidae). *International Journal of Food Microbiology*, *284*, 105-111. DOI: 10.1016/j.ijfoodmicro.2018.08.002

Zhang, L., Du, X., An, R., Li, L., & Gasser, R. B. (2013). Identification and genetic characterization of *Anisakis* larvae from marine fishes in the South China Sea using an electrophoretic-guided approach. *Electrophoresis*, *34*, 888-894. DOI: 10.1002/elps.201200493

Zhu, X., D'Amelio, S., Paggi, L., & Gasser, R. B. (2000). Assessing sequence variation in the internal transcribed spacers of ribosomal DNA within and among members of the *Contracaecum osculatum* complex (Nematoda: Ascaridoidea: Anisakidae). *Parasitology Research*, *86*, 677-683. DOI: 10.1007/PL00008551