Ascaridoid nematodes infecting commercially important marine fish and squid species from Bangladesh waters in the Bay of Bengal

Miguel Bao [a,*], Paolo Cipriani [a,b], Lucilla Giulietti [a], Mohammad Ashraful Alam [c], Marialetizia Palomba [b,d], Simonetta Mattiucci [b], Arne Levsen [a]

[a] Section of Contaminants and Biohazards, Institute of Marine Research (IMR), PO Box 1870, Nordnes, N-5817 Bergen, Norway
[b] Department of Public Health and Infectious Diseases, Section of Parasitology, "Sapienza-University of Rome", Rome, Italy
[c] Bangladesh Fisheries Research Institute, Riverine Station, Chandp ur-3602, Bangladesh
[d] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121 Naples, Italy

ARTICLE INFO

ABSTRACT

Parasitic ascaridoid nematodes occur in a wide range of marine organisms across the globe. Some species of the anisakid family (Ascaridoidea: Anisakidae) can cause gastrointestinal disease in humans (i. e. anisakidosis). Despite their importance as potentially hazardous parasites, the occurrence and infection characteristics of ascaridoids are still poorly known from many host species and geographical areas. This study investigated the diversity and infection levels of ascaridoid parasites in various commercial fish and squid host species off Bangladesh. Fish and squid specimens were visually inspected for nematodes using the UV-press method. Nematodes were assigned to genus level based on morphology and identified by sequence analyses of the entire ITS region and partial 28S rDNA and mtDNA *cox2* genes. Third-stage larvae (L3) of *Anisakis typica* occurred at low prevalence (*P* = 10% and 8%, respectively) in the viscera of *Selar crumenophthalmus* and *Trichiurus lepturus*, while *Hysterothylacium amoyense* occurred in the viscera of *Sardinella fimbriata* (*P* = 1%) and the viscera and muscle of *Harpadon nehereus* (*P* = 32%) and *T. lepturus* (*P* = 76%). *Lappetascaris* sp. Type A L3 occurred in the mantle of the squid *Uroteuthis duvaucelii* (*P* = 11%). *Anisakis* and *Lappetascaris* species, and *H. amoyense* were firstly identified in the Bay of Bengal. The potentially zoonotic *A. typica* was only found in fish viscera. *Hysterothylacium amoyense* and *Lappetascaris* sp., both generally regarded as non-zoonotic, occurred at low prevalence in the muscle or mantle of fish or squid, respectively. Since consumption of raw or lightly processed seafood seems to be rare in Bangladesh, the risk of acquiring anisakidosis from consuming fishery products from off Bangladesh appears to be low. Due to its reddish appearance, the visual presence of *H. amoyense* larvae in fish flesh may represent a food quality issue.

1. Introduction

Anisakiasis is an underestimated emerging fish-borne zoonotic disease of global concern (Bao et al., 2019). This gastrointestinal disease is caused by fish parasitic nematodes of the genus *Anisakis* (Nematoda: Ascaridoidea: Anisakidae) in the third larval stage (L3) (Audicana and Kennedy, 2008). The term anisakidosis is also used when the disease is caused by any member of the family Anisakidae

---

(e. g. the genera *Anisakis*, *Pseudoterranova* and *Contracaecum*) (Audicana and Kennedy, 2008). The aetiological agent may be accidentally transmitted to humans through consumption of parasitized raw, marinated or undercooked fishery products which contain a viable parasite (Audicana and Kennedy, 2008; Bao et al., 2019).

The disease can be classified into different clinical forms depending on the location of the larva and symptoms. The parasite may try to invade the gastrointestinal tract provoking acute/chronic gastric, intestinal or extra-gastrointestinal anisakiasis with mild to severe symptoms (e.g. abdominal pain, vomiting, intestinal obstruction, granuloma formation, etc.) (Nieuwenhuizen, 2016). Transient form of disease occur where the parasite larvae do not invade the human digestive tract, and after hours to weeks abandons the body, usually alive (Shamsi and Andrew, 2011; Smith, 1999). Asymptomatic or symptomless cases can also occur and are usually not diagnosed (Carrascosa et al., 2015; Takasaki et al., 2020). Gastro-allergic anisakiasis may occur when allergic symptoms (ranging from urticaria, angioedema to life-threatening anaphylaxis) predominate (Daschner et al., 2000). In addition, allergy to *Anisakis* may occur when the larval allergens that may be present in a fishery product (with no necessity of live parasite) trigger an allergic response in sensitized individuals (Adroher-Auroux and Benítez-Rodríguez, 2020; Audicana and Kennedy, 2008; Bao et al., 2019; EFSA-BIOHAZ, 2010). Occupational allergy has been also described in fishmongers (Añíbarro and Seoane, 1998) and fish-processing workers (Nieuwenhuizen et al., 2006).

It is estimated that the total number of worldwide anisakidosis (almost all anisakiasis) cases up to December 2017 may be over 76,000, mainly from developed countries such as Japan, South Korea, Spain and Italy (Bao et al., 2019). It appears that there is an increasing trend in annual hospitalizations in Spain (Herrador et al., 2019), South Korea (Kim et al., 2019) and Japan (Murata et al., 2021; Watahiki et al., 2020), which was not observed in Italy (Cavallero et al., 2018). A retrospective survey carried out over the period 2010 to 2014 in France showed a decrease in clinical cases (Yera et al., 2018). In addition, recent studies suggest that the estimated annual cases could be between 8000 and 21,000 in Spain (Bao et al., 2017; Herrador et al., 2019) and over 7000 in Japan (Murata et al., 2021).

To the best of our knowledge, the disease has been reported in littoral countries of the Bay of Bengal such as Malaysia (Amir et al., 2016) and Thailand (Hemsrichart, 1993) but not yet in Bangladesh, India, Indonesia, Myanmar or Sri Lanka (Bao et al., 2019; FAO/WHO, 2014; Wiwanitkit and Wiwanitkit, 2016). Recently, Wiwanitkit and Wiwanitkit (2016) reviewed the situation of anisakiasis in Southeast Asia and concluded that the disease is possible and should become a new focused interest in tropical coastal medicine.

The Bay of Bengal is one of the world's 64 Large Marine Ecosystems (BOBLME, 2013). Over 400 million people of its littoral
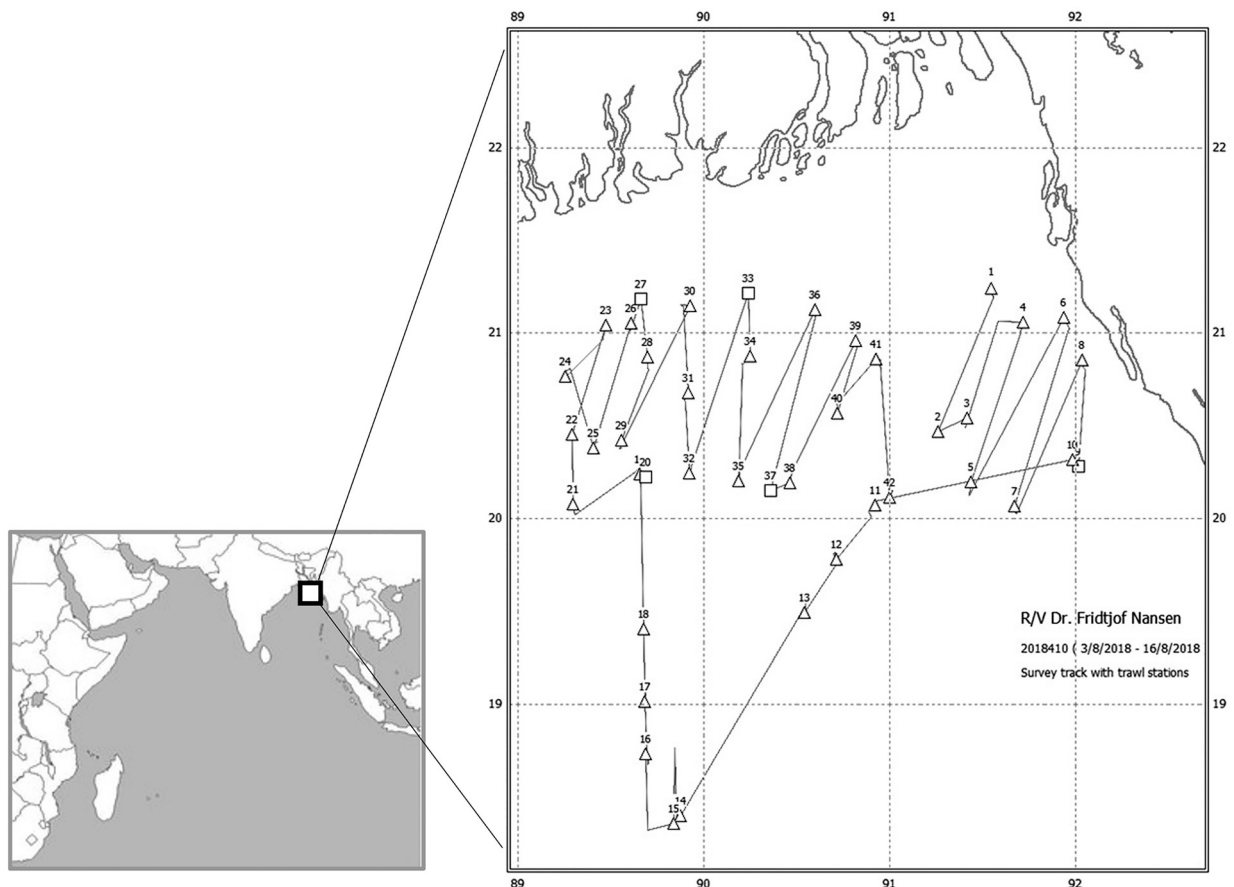

Fig. 1. Course track and trawl stations made by the research vessel R/V "Dr. Fridtjof Nansen" in the sampling area.

countries harvest the coastal and marine fishery resources to support their livelihood (BOBLME, 2013). The Bay of Bengal has a distinct tropical marine ecosystem with profuse drainage from upstream rivers into the northern part and abundant swamp and mangroves that further supports diverse marine fish assemblages (about 511 marine fish species) including coral reefs, tropical dolphins and sharks (Murshed-e-Jahan et al., 2014). Bangladesh has one of the biggest delta areas, contributing to very rich fresh and brackish water fisheries and aquaculture industries (3,084,399 metric tons/year), in addition to marine fisheries (599,846 metric tons/year) (Shamsuzzaman et al., 2017). More than 17 million people depend on the fishery sector for their livelihood, and fish supplements about 60% of the people's daily animal protein intake (Shamsuzzaman et al., 2017).

The study of fish parasites in Bangladesh has a relatively short history, dating back to a few scattered records contained in the works of Thomas Southwell and colleagues at the first quarter of the 20th Century (Arthur and Ahmed, 2002). Arthur and Ahmed (2002) provided the first parasite-host list, highlighting that some or all ascaridoid records may involve misidentification. Later, Chandra (2006) recorded 290 species of fish parasites in Bangladesh; however, most of these were from freshwater. Anisakids and raphidascaridids such as *Hysterothylacium* spp. (Nematoda: Ascaridoidea: Raphidascarididae) are very common parasites of marine fishes and squids of all oceans and may occur in freshwater too (Klimpel and Rückert, 2005; Li et al., 2016a; Mattiucci et al., 2018; Shamsi et al., 2013). However, many of the commercially important Bangladeshi marine fish species have not been examined for ascaridoid parasites yet.

The aim of the present study was to investigate various marine fish and squid species caught within the exclusive economic zone of Bangladesh, for the presence and spatial distribution of potentially zoonotic ascaridoid parasites.

2. Material and methods

2.1. Fish and squid sampling

A total of 14 teleost fish ($N = 464$) and 2 squid ($N = 31$) species were caught in August 2018 during a research cruise onboard the research vessel R/V "Dr. Fridtjof Nansen" (NORAD-FAO project GCP/INT/730/NOR; survey number 2018410) using pelagic and bottom trawl on the continental shelf off Bangladesh, and extending offshore to deeper waters for mesopelagic trawling transects (Fig. 1; Table 1). Fishes and squids from each catch were identified to species level by local taxonomists. Total length (mantle length for squids), total weight and sex were recorded for each specimen (Table 1).

Table 1
Fish and squid biometrics and trawl stations from where they were caught.

Fish and squidˆ species (Family)	Common name	N	Station	TL	TW	Sex (f, m, u)
Bregmaceros mcclellandi (Bregmacerotidae)	Unicorn cod	100	28	5*	0.91**	NS
Cubiceps pauciradiatus (Nomeidae)	Bigeye cigarfish	60	13	11.1 ± 0.7 (10–12.5)	13.2 ± 2.4 (9–19)	32/24/4
Dussumieria elopsoides (Dussumieriidae)	Slender rainbow sardine	30	23	20.8 ± 0.4 (20.0–21.5)	79.1 ± 4.2 (71–89)	29,1,0
Eupleurogrammus muticus (Trichiuridae)	Smallhead hairtail	3	31	62.2 ± 1.3 (61.0–63.5)	100.5 ± 3.5 (98–103)	2/1/0
Harpadon nehereus (Synodontidae)	Bombay-duck	50	27, 33	25.9 ± 2.8 (12.5–31.5)	113.7 ± 35.5 (62–288)	3/0/47
Megalaspis cordyla (Carangidae)	Torpedo scad	15	31	25.3 ± 2.2 (22.5–29.0)	140.9 ± 32.6 (103–190)	7/5/3
Mene maculata (Menidae)	Moonfish	30	23	25.1 ± 1.4 (19.0–25.0)	186.8 ± 23.7 (112–212)	5/25/0
Sardinella fimbriata (Clupeidae)	Fringescale sardinella	100	6	16.4 ± 0.5 (15.5–17.5)	39.9 ± 3.6 (31.0–51.0)	59/41/0
Selar crumenophthalmus (Carangidae)	Bigeye scad	30	24	24.3 ± 1.0 (22.5–26.5)	196.5 ± 32.3 (134–273)	19/11/0
Trichiurus lepturus (Trichiuridae)	Largehead hairtail	38	4, 9, 31	57.8 ± 10.4 (42.5–82.0)	133.1 ± 115.0 (29–497)	26/9/3
*Ommastrephes bartramii*ˆ (Ommastrephidae)	Neon flying squid	22	17	8.1 ± 1.1 (6.5–10.5)	17.0 ± 6.8 (9–32)	NS
*Uroteuthis duvaucelii*ˆ (Loliginidae)	Indian squid	9	10	8.2 ± 0.9 (7.0–9.5)	24.8 ± 5.8 (17–32)	NS
Chirocentrus dorab (Chirocentridae)	Dorab wolf-herring	2	36	37.8 ± 1.8 (36.5–39.0)	154.5 ± 33.2 (131–178)	0/1/1
Scomberomorus guttatus (Scombridae)	Indo-Pacific king mackerel	4	30, 36	39.9 ± 10.9 (32.0–56.0)	426.8 ± 358.0 (192–960)	1/0/3
Scomberomorus commerson (Scombridae)	Narrow-barred Spanish mackerel	1	30	55	890	0/0/1
Scomberoides tol (Carangidae)	Needlescaled queenfish	1	36	34.5	226	1/0/0

N = number of fishes sampled; Station = trawl station from where fish/squid were caught; TL = total length (mantle length for squids) in cm (mean ± SD = standard deviation (minimum and maximum range values)); TW = total weight in g (mean ± SD = standard deviation (minimum and maximum range values)); f = female, m = male, u = unknown; NS = not sexed. * Due to small body size, only 1 fish per sample was measured for TL. ** average TW per fish (TW of sample = 91 g/100 fish).

2.2. Parasite inspection

Fish and squid specimens were dissected, and the body cavity and internal organs initially inspected for ascaridoid nematodes by the naked eye under good light conditions. Thereafter, the internal organs and fillets were placed in individual transparent plastic bags and inspected using the UV-press method (Karl and Leinemann, 1993; Levsen and Lunestad, 2010). The method uses the fluorescence of dead by freezing anisakid nematodes (Pippy, 1970). The pressed samples were stored frozen at −20 °C for at least 24 h before thawing and examining each bag under 366 nm UV-light in a dark room (Karl and Leinemann, 1993; Levsen and Lunestad, 2010). Ascaridoid parasites may be tentatively assigned to genus level (e.g. *Anisakis*, *Hysterothylacium*), depending on differences in shape and size/thickness, shade as well as intensity and brightness of fluorescence (Bao et al., 2021) (see also "morphological identification" subsection below).

The nematodes recorded were stored frozen at −20 °C for further morphological and molecular study.

2.3. Morphological identification

A subsample of 114 out of 270 ascaridoid nematodes, randomly selected depending on their macroscopic appearance (see Results section), from the viscera and flesh or mantle of infected fish or squid hosts, were subjected to morphological identification using light microscopy. The ascaridoids were assigned to genus and their developmental stage determined according to morphological characters such as: presence/absence of lips or boring tooth depending on developmental stage (fourth larval stage (L4)/adult or third larval stage (L3)); presence/absence and appearance of ventricle, intestinal caecum, ventricular appendix and terminal mucron, as well as the position of the excretory pore relative to boring tooth (if present) and nerve ring (Berland, 1989; Li et al., 2016b; Li et al., 2012; Li et al.,

Table 2

Morphological identity and developmental stage of the parasites found per host species, and sequence similarity scores obtained by BLAST (Basic Local Alignment Search Tool) for the present ascaridoid ITS, *cox*2 and 28S gene sequences, with the conclusion for parasite species identity.

Host species	Morphology	Gene	BLAST (N, bp)	Acc. nr.	Parasite species
E. muticus	1 L3 *Anisakis* sp. larval type I	NA			
	1 L3 *H. amoyense/H. zhoushanense*	NA			
H. nehereus	16 L3 *H. amoyense/H. zhoushanense*	ITS (n = 13)	100% *H. amoyense* (381 to 804 bp)	KP252131	*H. amoyense*
		cox2 (n = 4)	97.01–97.97% *H. amoyense* (356 to 583 bp)	MF120253	
		28S (n = 3)	100% *H. amoyense* (724 to 725 bp)	MF094276	
S. fimbriata	1 L3 *H. amoyense/H. zhoushanense*	ITS (n = 1)	100% *H. amoyense* (292 bp)	KP252131	*H. amoyense*
		cox2 (n = 1)	97.35% *H. amoyense* (432 bp)	MF120253	
		28S (n = 1)	100% *H. amoyense* (724 bp)	MF094276	
S. crumenophthalmus	3 L3 *Anisakis* sp. larval type I	ITS (n = 1)	100% *A. typica* (897 bp)	JQ912690	*A. typica*
		cox2 (n = 2)	97.74–99.30% *A. typica* (574 bp)	JQ859931 and JQ859923	
		28S (n = 2)	100% *A. typica* (731 and 751 bp)	KX098562	
T. lepturus	4 L3 *Anisakis* sp. larval type I	ITS (n = 2)	100% *A. typica* (720 to 814 bp)	JQ912690	*A. typica*
		cox2 (n = 2)	98.74–99.48% *A. typica* (578 bp)	KC928267	
	87 L3 + 2 L4 *H. amoyense/H. zhoushanense*	ITS (n = 4)	99.87–100% *H. amoyense* (743 to 809 bp)	KP252131	*H. amoyense*
		cox2 (n = 6)	97.01–97.60% *H. amoyense* (462 to 587 bp)	MF120253	
		28S (n = 5)	100% *H. amoyense* (711 to 725 bp)	MF094276	
U. duvaucelii	1 L3 *Lappetascaris* sp. type A	ITS (n = 1)	100% *Hysterothylacium* sp. or *Lappetascaris* sp. (874 bp)	MT365537 or MW750364	*Lappetascaris* sp.
		cox2 (n = 1)	98.78% *Lappetascaris* sp. (575 bp)	MW775335	
		28S (n = 1)	Matches <96.5%		

Morphology: number of ascaridoids identified to species or larval type and developmental stage (i.e. L3 = third larval stage, L4 = fourth larval stage); Gene: ribosomal (ITS or 28S) or mitochondrial (*cox2*) DNA sequences; BLAST: blast results (percent identity (%) and species identity), bp: number of base pairs; Acc. Nr.: Accession number of the highest match from published deposited samples; Parasite species = conclusion for species identity. NA: not available.

2008; Nagasawa and Moravec, 2002).

2.4. Molecular identification

A subsample of 30 ascaridoid nematodes, randomly selected among those initially identified to genus level, were specifically identified by sequence analyses of the mitochondrial cytochrome *c* oxidase II (mtDNA *cox*2) gene, entire internal transcribed spacers (ITS rDNA) region and/or 28S rDNA gene (see Table 2 and Table A.1).

Genomic DNA was extracted using the DNeasy® Blood & Tissue Kit (QIAGEN® GmbH, Hilden, Germany) according to the manufacturer's instructions.

The mtDNA *cox*2 gene of 16 ascaridoids was amplified using the primers 211F (5′-TTTTCTAGTTATATAGATTGRTTTYAT-3′) and 210R (5′-CACCAACTCTTAAAATTATC-3′) (Nadler and Hudspeth, 2000), following procedures of Mattiucci et al. (2014), modified by Bao et al. (2021).

The entire ITS rDNA region (ITS1, 5.8S rDNA gene and ITS2) of 22 ascaridoids was amplified using the primers NC5 (5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and NC2 (5′-TTAGTTTCTTTTCCTCCGCT-3′) following Zhu et al. (2000) with modifications, i. e. the annealing temperature was set up at 54 °C instead of 55 °C.

In addition, the partial 28S rDNA gene (28S) of 12 ascaridoids was amplified using the primers 28SF (5′-AGCGGAGGAAAAGAAACTAA-3′) and 28SR (5′-ATCCGTGTTTCAAGACGGG-3′) (Nadler and Hudspeth, 1998), following procedures of Li et al. (2018).

Purification and sequencing of PCR products of correct size were carried out by Eurofins (Cologne, Germany) using the primers 210R, NC5 and 28SF for the amplification of the *cox*2, ITS and 28S genes, respectively.

The obtained sequences were searched for similarity by BLAST (Basic Local Alignment Search Tool), using Blastn query with the nucleotide collection (nr/nt) database and the megablast program selection, through web servers of the National Center for Biotechnology Information (USA) (Altschul et al., 1990).

2.5. Parasite infection analyses

Parasite infection descriptors such as prevalence, mean abundance and mean intensity were calculated as described in Bush et al. (1997). Any correlations between parasite abundance and fish size (total length and weight) were calculated for the most parasitized host species using Spearman rank test. The significance level was set at 0.05 and all calculations and tests were run in Statistica v 13.4.0.14.

3. Results

3.1. Necropsy and gross parasite identification

A total of 269 parasitic nematodes were detected in the fish examined. At first, $n = 261$ nematodes were grossly classified as ascaridoid sp. A and 8 as ascaridoid sp. B (see infection level section below for further details). Ascaridoid sp. A had a reddish appearance. These larvae were encapsulated or moving freely around the viscera (Fig. A.1.), as well as the fish flesh (Fig. A.2). The parasites were not fluorescent under UV-light. Ascaridoid sp. B had a whitish appearance. The larvae were found in or around the viscera and showed brightly bluish fluorescence under UV-light. In addition, one single larva, initially classified as ascaridoid sp. C, was found in the mantle of the squid *U. duvaucelii*, and appeared bluish upon UV light exposure.

3.2. Parasite species identification

A total of 105 ascaridoid sp. A, 8 ascaridoid sp. B and 1 ascaridoid sp. C specimens were morphologically assigned to *Hysterothylacium amoyense* or *Hysterothylacium zhoushanense*, *Anisakis* type I and *Lappetascaris* sp. type A (Fig. A.3) larvae, respectively (Table 2). Out of 30 specimens molecularly identified, according to the obtained mtDNA *cox2,* rDNA ITS and/or 28S gene sequences, 25 *H. amoyense/H. zhoushanense*, 4 *Anisakis* sp. larval type I and 1 *Lappetascaris* sp. morphotypes were assigned to the species *H. amoyense*, *A. typica* and *Lappetascaris* sp., respectively (Table 2). Sequences of the former were deposited in GenBank with the accession numbers (ITS: ON065558–60, ON098267–85), (*cox2*: ON109753–67, ON109536) and (28S: ON098258–66, ON117607–09) (further details at Table A.1).

The ITS sequences of the *H. amoyense* specimens in the present study were identical except for 1 larva from *Trichiurus lepturus* that had 1 nucleotide different from the others at alignment position 377 (G instead of A). They matched 99.87% to 100% with the *H. amoyense* sequence deposited in GenBank (KP252131) (Table 2). The 28S sequences were identical among them and to *H. amoyense* GenBank sequence MF094276 (Table 2). The *cox2* sequences matched at 97.01%–97.97% with the *H. amoyense* sequence deposited in GenBank (MF120253) (Table 2). Further details at the discussion section.

3.3. Parasite infection levels

The majority of ascaridoids, i. e. 261 *H. amoyense* and 8 *A. typica*, were found in just four fish species, i.e. *Harpadon nehereus*, *Sardinella fimbriata*, *Selar crumenophthalmus* and *T. lepturus* (Table 3). A single *Lappetascaris* sp. type A larva occurred in the mantle of the squid *Uroteuthis duvaucelii*. The most infected fish species was *T. lepturus* showing 76% overall ascaridoid prevalence and 6.0 (7.8)

mean abundance (standard deviation). *Hysterothylacium amoyense* was found in the viscera and sometimes in the muscle of *H. nehereus* and *T. lepturus*, whilst *A. typica* only occurred in the viscera of *S. crumenophthalmus* and *T. lepturus* (Table 3). *Anisakis* type I (*N* = 1, likely *A. typica*) and *H. amoyense* or *H. zhoushanense* (N = 1, likely *H. amoyense*) larvae were found in the viscera of *Eupleurogrammus muticus*. No ascaridoids were found in *Bregmaceros mcclellandi*, *Chirocentrus dorab*, *Cubiceps pauciradiatus*, *Dussumieria elopsoides*, *Megalaspis cordyla*, *Mene maculata*, *Ommastrephes bartramii*, *Scomberomorus commerson*, *Scomberomorus guttatus* and *Scomberoides tol* (Table 3).

Mean abundance of ascaridoid nematodes in *T. lepturus* (N = 38) was weakly but significantly positively related to fish host length (Spearman $R = 0.48$, $p = 0.002$) and body weight (Spearman $R = 0.45$, $p = 0.005$).

4. Discussion

The parasitic nematode fauna of marine fish species from Bangladesh waters is poorly known, and some earlier records were possibly misidentified (Arthur and Ahmed, 2002; Chandra, 2006). To the best of our knowledge, herein we present the first report of molecularly identified ascaridoid nematodes of the genera *Anisakis* and *Lappetascaris* and of *H. amoyense* found in fishes and squids from off Bangladesh (and Bay of Bengal).

Anisakis spp. have heteroxenous life cycles in the marine environment, in which marine mammals (mainly cetaceans) act as definitive hosts, small crustaceans as intermediate hosts, and fish and squids as second intermediate or paratenic hosts (Mattiucci et al., 2018). The anisakid species *A. typica* was reported from the viscera of *S. crumenophthalmus* and *T. lepturus* with low prevalence of infection; 10% and 8%, respectively. As far as we know, this is the first molecular report of an *Anisakis* species in the Bay of Bengal. *E. muticus* appears to be new fish paratenic or intermediate host record of *Anisakis* sp. (possibly *A. typica*).

Anisakis typica has been reported in fishes (e. g. *S. crumenophthalmus* and *T. lepturus*) from Indonesian (Kuhn et al., 2013; Palm et al., 2017; Palm et al., 2008), South and East China Sea (Guo et al., 2020; Kong et al., 2015), Papua New Guinea (Koinari et al., 2013) and northern Taiwan waters (Sonko et al., 2020). It has been also reported in fishes from Japanese and northern Australian waters (Jabbar et al., 2012; Takano et al., 2021). A new taxon closely related to *A. typica*, and temporarily indicated as *Anisakis* sp. 1, was reported as L3 from the fish *Nemipterus* sp. from western Malaysian waters (Mattiucci et al., 2018). It appears that this provisional taxon (also

Table 3
Infection levels of the ascaridoid parasites found in fish and squid species collected off Bangladesh in the Bay of Bengal.

Host	Parasite	Muscle			Viscera			Total		
		P	mA	mI	P	mA	mI	P	mA	MI
B. mcclellandi (*N* = 100)	Not infected	0%	–	–	0%	–	–	0%	–	–
C. pauciradiatus (*N* = 60)	Not infected	0%	–	–	0%	–	–	0%	–	–
D. elopsoides (*N* = 30)	Not infected	0%	–	–	0%	–	–	0%	–	–
E. muticus (*N* = 3)	Ascaridoid spp. (N = 2)	0%	–	–	67%	0.7 (0.6)	1.0 (0.0) {1–1}	67%	0.7 (0.6)	1.0 (0.0) {1–1}
	Anisakis sp. (N = 1)	0%	–	–	33%	0.3 (0.6)	1	33%	0.3 (0.6)	1
	H. amoyense or *H. zhoushanense* (N = 1)	0%	–	–	33%	0.3 (0.6)	1	33%	0.3 (0.6)	1
H. nehereus (*N* = 50)	*H. amoyense* (N = 31)	12%	0.1 (0)	1.0 (0) {1–1}	30%	0.5 (0.9)	1.7 (1.1) {1–4}	32%	0.6 (1.1)	1.9 (1.1) {1–4}
M. cordyla (*N* = 15)	Not infected	0%	–	–	0%	–	–	0%	–	–
M. maculata (*N* = 30)	Not infected	0%	–	–	0%	–	–	0%	–	–
S. fimbriata (*N* = 100)	*H. amoyense* (N = 1)	0%	–	–	1%	0.0 (0.1)	1	1%	0.0 (0.1)	1
S. crumenophthalmus (N = 30)	*A. typica* (N = 3)	0%	–	–	10%	0.1 (0.3)	1.0 (0.0) {1–1}	10%	0.1 (0.3)	1.0 (0.0) {1–1}
T. lepturus (*N* = 38)	*A. typica* (N = 4)	0%	–	–	8%	0.1 (0.4)	1.3 (0.6) {1–2}	8%	0.1 (0.4)	1.3 (0.6) {1–2}
	H. amoyense (N = 228)	5%	0.1 (0.2)	1.0 (0.0) {1–1}	76%	5.9 (7.7)	7.8 (7.9) {1–33}	76%	6.0 (7.8)	7.9 (8.0) {1–34}
	Ascaridoid spp. (N = 232)	5%	0.1 (0.2)	1.0 (0.0) {1–1}	76%	6.1 (7.8)	7.9 (8.1) {1–34}	76%	6.1 (8.0)	8.0 (8.2) {1–35}
O. bartramii (*N* = 22)	Not infected	0%	–	–	0%	–	–	0%	–	–
U. duvaucelii (*N* = 9)	*Lappetascaris* sp. (N = 1)	11%	0.1 (0.3)	1	0%	–	–	11%	0.1 (0.3)	1
C. dorab (*N* = 2) *	Not infected	0%	–	–	0%	–	–	0%	–	–
S. guttatus (*N* = 4) *	Not infected	0%	–	–	0%	–	–	0%	–	–
S. commerson (*N* = 1) *	Not infected	0%	–	–	0%	–	–	0%	–	–
S. tol (*N* = 1) *	Not infected	0%	–	–	0%	–	–	0%	–	–

Host = host species sampled, number (N) of individuals sampled in parenthesis; Parasite = Ascaridoid species identified, number (N) of parasites found in parenthesis; Muscle = infection data for the muscle; Viscera = infection data for the viscera; Total = infection data in viscera and muscle; P = prevalence of infection as percentage; mA = mean abundance, standard deviation in parenthesis; mI = mean intensity, standard deviation in parenthesis and range (minimum – maximum number of parasites in the sample) with curly brackets. * Just the belly flaps (i. e. anterior-ventral portion of the fillets) were examined in these fishes.

reported as *A. typica* var. *indonesiensis, A. typica* sp. T or *A. typica*) is frequent in fishes (e.g. *M. cordyla, S. crumenophthalmus, S. tol* and *T. lepturus*) from Indonesian (Anshary et al., 2014; Kuhn et al., 2013; Mattiucci et al., 2018; Palm et al., 2017; Palm et al., 2008), Thailand (Eamsobhana et al., 2018; Tunya et al., 2020), Vietnamese (Van Hien et al., 2021) and South China Sea (Guo et al., 2020) waters but has not been found in the present study. Interestingly, one L3 from *S. crumenophthalmus* (acc. Num. ON109756) showed a highest match of 97.7% with an *A. typica* sequence deposited in GenBank (acc. Num. JQ859931), lower than the other three specimens here identified, matching ≥98.3% with the same specimen, and being therefore identified as *A. typica*. Once aligned with these specimens, this sequence showed several differences, suggesting that it could likely represent an undescribed *A. typica* genotype.

Palm et al. (2017) reported *A. typica/ A. typica* var. *indonesiensis* and *A. typica* prevalence of 81,1% and 2,9% for *S. crumenophthalmus* and *T. lepturus* from Indonesia, respectively. *Anisakis* spp. prevalence raised up to 30% in the viscera of *T. lepturus* from the Gulf of Thailand (Purivirojkul, 2009). In terms of definitive host, it appears that *A. typica* is a common parasite of various dolphin and kogiid species in warmer and tropical waters (Kuhn et al., 2016; Mattiucci et al., 2018). It has been reported from the pygmy sperm whale (*Kogia breviceps*) and dwarf sperm whale (*Kogia sima*) from Philippines' waters (Quiazon, 2016; Quiazon et al., 2013). In addition, *A. typica* (possibly *Anisakis* sp. 1) has been reported from the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) in the Northern Red Sea (Kleinertz et al., 2014).

Anisakis spp. are very common parasites of marine fishes from all oceanic waters (Mattiucci et al., 2018). The low infection level of *Anisakis* sp. found in the present study might be explained by an absence/scarcity of suitable cetacean definitive hosts around sampling areas of Bangladeshi waters. Other unknown biological/physical factors (e. g. presence of suitable crustacean intermediate hosts, influence of river exports, etc.) might be affecting parasite recruitment and transmission dynamics in the area as well. Interestingly, a complete disappearance of *Anisakis* spp. larvae in recent years in fishes from New South Wales (Australia) waters was recently reported despite a common and increasing population of marine mammals in the region (reviewed by Shamsi (2021)). Shamsi (2021) hypothesized that the remarkable finding may be explained by environmental changes or a dramatic decline in the population of crustacean intermediate hosts. Further parasitological studies in the same and other important fish species (e.g. *Tenualosa ilisha*) caught in different seasons, areas (e. g. outer shelf and deep sea) and commercial sizes are recommended to better understand *Anisakis* epidemiology of Bangladesh fishes.

Species of *Hysterothylacium* are very common in marine fishes worldwide (Klimpel and Rückert, 2005; Li et al., 2016a; Shamsi et al., 2013). They are parasites of cold-blooded organisms using mainly fishes as both intermediate/paratenic and/or definitive hosts, and small crustaceans as first intermediate hosts (Balbuena et al., 1998; Bao et al., 2021; Køie, 1993; Levsen et al., 2016). The raphidascaridid *H. amoyense* was found in the viscera of *H. nehereus*, *S. fimbriata* and *T. lepturus*. The parasite was also found in the muscle of *H. nehereus* and *T. lepturus*. To the best of our knowledge, this is the first molecular report of an *Hysterothylacium* species from off Bangladesh waters. The moray eel *Muraenesox cinereus* from Chinese waters was reported as definitive host of *H. amoyense* (originally described as *Contracaecum amoyensis* Hsü, 1933 from the *M. cinereus* type host) (Li et al., 2018; Li et al., 2008). The conger eel *Conger myriaster* was also reported as definitive host (Li et al., 2018). No adult nematodes were found here but two fourth-stage larvae (L4) were present in the viscera of two *T. lepturus*. Third-stage larvae (L3) of *H. amoyense* and *H. zhoushanense* are very similar and cannot be distinguished only based on morphological characters (Li et al., 2016b), therefore, analysis of the rDNA ITS and 28S, as well as mtDNA *cox2* sequences, identified the larvae as *H. amoyense*.

The ITS sequences of the specimens in the present study were identical except for 1 larva from *T. lepturus* that had 1 nucleotide different from the others at alignment position 377 (G instead of A). They were identical to sequences of larval *H. amoyense* from fishes (i.e. *Lophius litulon, C. myriaster, Halieutaea stellata, Nemipterus japonicus, Platycephalous indicus*) of China or Iran from published works (Chen et al., 2018; Guo et al., 2020; Li et al., 2016b; Najjari et al., 2016; Zhang et al., 2018), and deposited in GenBank (accession numbers MH211518 - MH211527; MF539810, MF539811, MF539813; KT749421, KT749422; KP252130 - KP252133; MT020111 - MT020134). Larvae have been also suggested from hagfish *Eptatretus burgeri* from Taiwan (Luo et al., 2016) as well as *T. lepturus* and *Scomber japonicus* from China (Kong et al., 2015) by PCR-RFLP analyses of the ITS region but no sequence has been provided. There is currently no ITS sequence available from an adult *H. amoyense* in the GenBank, and as explained above, L3 of *H. amoyense* cannot be morphologically distinguished from *H. zhoushanense*. Thus, Shamsi et al. (2016) proposed to refer to the larval type of Li et al. (2016b) and those described by authors from fishes (i.e. *Otolithes ruber, Psettodes erumei, Saurida tumbil* and *S. commerson* from Irani waters, and with identical ITS1 & ITS2 sequences (as to those in the present study), as *Hysterothylacium* larval type XV. However, there is currently a 28S sequence (accession number MF094276) from an adult *H. amoyense* identified from its definitive host *M. cinereus* in Chinese waters (Li et al., 2018). The 28S sequences of our larval specimens are identical to the former, therefore confirming *H. amoyense* identity. In addition, the *cox2* sequence of the same parasite isolate is also available in GenBank (accession number MF120253) (Li et al., 2018). Blast analyses of the present *cox2* sequences showed 97.01–97.80% similarity to the former sequence. Thus, this is the first molecular report of *H. amoyense* in *H. nehereus*, *S. fimbriata* and *T. lepturus* fish hosts, and extends the geographical distribution of the parasite to Bangladesh waters. By syllogism, those larval individuals presented above from published studies (Chen et al., 2018; Guo et al., 2020; Li et al., 2016b; Najjari et al., 2016; Zhang et al., 2018) with identical ITS sequence as those presented in here can be considered as *H. amoyense*.

The raphidascaridid genus *Lappetascaris* was erected by Rasheed (1965) to include the type species *L. lutjani*, recovered from the intestine of Pakistani fishes *Lutjanus* sp. and *T. ilisha* (Rasheed, 1965). The parasite was later reported in fishes from India (De, 1990) and more recently Brazil (Vicente et al., 2002). Unfortunately, no sequence of *L. lustjani*, or any other *Lappetascaris* species (i. e. *Lappetascaris suraiyae* and *L. chandipurensis*, see http://www.marinespecies.org/aphia.php?p=taxdetails&id=990484), are available in GenBank for comparison with the *Lappetascaris* larva sequenced here. Third-stage larvae of *Lappetascaris* sp. was firstly described from the cyprinid fish *Paraulaubuca* cf. *typus* from the Mekong river in Laos (Moravec and Scholz, 1991). Later, Nagasawa and Moravec (1995) described the morphology of L3 of *Lappetascaris* from the mantle of squid *Todarodes pacificus* from the Sea of Japan, also

reported by Takahara and Sakurai (2010) and Gomes et al. (2020) in same host and area, and concluded that the two larval types belong to different congeneric species. Nagasawa and Moravec (2002) included the former larvae into a *Lappetascaris* sp. Type A larvae further described from the muscle of the squids *Thysanotheuthis rhombus*, *O. bartramii*, *Onychoteuthis borealijaponica* and *Gonatopsis borealis* from Central and Western North Pacific Ocean. *Lappetascaris* sp. Type B was additionally described as morphologically similar but significantly smaller and was found encapsulated in the stomach of *O. bartramii* (Nagasawa and Moravec, 2002). Recently, *Lappetascaris* sp. Type A larvae was reported from the muscle and other tissues of the squids *Histioteuthis reversa* and *Histioteuthis bonnellii* from Mediterranean Sea (Culurgioni et al., 2010; Palomba et al., 2021). The ITS sequence of *Lappetascaris* sp. Type A larva obtained here is identical to those of *H. reversa* and *H. bonnellii* (accession numbers MW697754 and MW697755; MW750359 to MW750364) and to those from octopus *Eledone cirrhosa* and *Eledone moschata* from Mediterranean Sea morphologically identified as *Lappetascaris* sp. or *Hysterothylacium* sp., and deposited in GenBank as *Hysterothylacium* sp. (accession numbers MT365529 to MT365537) (Guardone et al., 2020; Palomba et al., 2021). According to phylogenetic studies of the ITS region and *cox2* gene locus, *Lappetascaris* sp. appears to be closely related to other ascaridoids of the genus *Hysterothylacium* (i.e. *H. brucei*, *H. tetrapteri*, *H. kajikiae* and *H. corrugatum*) which have predator billfishes of the family Xiphiidae and Istiophoridae as definitive hosts (Guardone et al., 2020; Palomba et al., 2021). Thus, *Lappetascaris* sp. Type A is tentatively identified for the first time in *U. duvaucelii* and extends the geographical distribution of the parasite to Bangladeshi waters. Further morphological and molecular studies on adult *Lappetascaris* spp. from type fish hosts (e. g. *T. ilisha*) as well as adult raphidascaridids from billfishes are recommended to clarify its identity and taxonomic status.

Parasitic ascaridoids in commercial fish are important from food safety and quality perspectives (Bao et al., 2021; Bao et al., 2019; Bao et al., 2018). Anisakiasis is an emerging fish-borne zoonosis of worldwide concern caused by species of the genus *Anisakis* (Bao et al., 2017). Heating (e. g. to >60 °C at the core of the product for at least 1 min) and freezing (to not more than −20 °C at the core of the product for not less than 24 h) of fish fillets are the recommended methods to devitalize the parasite and prevent anisakiasis (EFSA-BIOHAZ, 2010). Out of the 9 fully described *Anisakis* species, *A. simplex* (*s. s.*) and *A. pegreffii* have been molecularly confirmed as causative agents so far (Kołodziejczyk et al., 2020; Mattiucci et al., 2013). Recently, *A. typica* was identified as cause of anisakiasis in Japan (The 86th Annual Meeting of the Japanese Society of Parasitology (2017) cited in Suzuki et al. (2021)). The potentially zoonotic *A. typica* was found only in the viscera of *T. lepturus* and *S. crumenophthalmus* (and possibly *E. muticus*) in low numbers (i. e. prevalence ≤10% for the two former fish species). However, a few specimens of *A. typica* (*s. l.*) were reported in the muscle of *Auxis rochei rochei* and *S. crumenophthalmus* from Indonesia (Palm et al., 2017; Palm et al., 2008). Anshary et al. (2014) observed an *Anisakis* (likely *A. typica/Anisakis* sp. 1) migrating into the fish musculature.

To date, anisakiasis has not been reported in Bangladesh, but it was reported in other littoral countries of the Bay of Bengal such as Malaysia and Thailand (Amir et al., 2016; Hemsrichart, 1993). Uga et al. (1996) reported seroprevalence to *Anisakis* spp. of 11% (*n* = 26 out of 244 individuals attending the hospital for routine examinations or presenting diarrhoea) tested by ELISA in Indonesia, even though anisakiasis has neither been reported there yet. As far as we know, there is currently no tradition of consumption of raw, marinated, cold-smoked or generally lightly cooked fish in Bangladesh. In addition, the parasite was present with low prevalence in fishes (i. e. *S. crumenophthalmus*, *T. lepturus* and probably *E. muticus*) but was not found in the muscle. Thus, the risk of anisakiasis appears to be low but cannot be completely discarded (see also further comments below).

The genus *Hysterothylacium* is generally considered non-zoonotic, however, this aspect is yet to be thoroughly elucidated and therefore remains somehow controversial (Bao et al., 2021; Shamsi et al., 2018). *Hysterothylacium amoyense* was observed to probably cause pathological effects (i.e. tumour-like lesions and lymphocytic infiltration in stomach) in mice model (Najjari et al., 2016). The parasite was found in the muscle of *N. nehereus* and *T. lepturus* with a prevalence of 12% and 5%, respectively. However, it is generally accepted that the pathogenic potential of anisakids (e. g. *Anisakis*) surpasses those of raphidascaridids (e. g. *Hysterothylacium* and *Lappetascaris*), specially comparing hundreds of well reported clinical anisakidosis cases to few and dubious raphidascaridosis. Thus, the risk of fish-borne zoonosis caused by ascaridoids from consumption of the Bangladeshi fish/squid species studied here appears to be low. However, prevention methods (i. e. heating or freezing) should prevail if fishery products are meant to be consumed as raw or lightly cooked. The reddish colour of *H. amoyense* larvae, making them easily visible in fillets, may represent a food quality issue (see Figs. A.1 and A.2). Prompt evisceration and removal of visible parasites from flesh are recommended.

5. Conclusions

Nematodes of the genera *Anisakis* (i. e. *A. typica*), *Lappetascaris* (i. e. *Lappetascaris* sp. Type A) and *Hysterothylacium* (i. e. *H. amoyense*) were firstly identified in waters of the Bay of Bengal (i. e. Bangladesh). The raphidascaridid nematode *H. amoyense* was the most abundant parasite species and is generally regarded as non-zoonotic. The potentially zoonotic *A. typica* was only present in fish viscera in low abundances, suggesting that its life cycle is hampered by unknown ecological and/or physical factors. Moreover, there is currently no tradition of consumption of raw or lightly processed fishery products in Bangladesh. Thus, the risk of fish-borne zoonosis caused by ascaridoids appears to be low. Prevention methods (heating or freezing) to kill any possible worm in the fish before consumption should prevail.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix

Fig. A.1. *Hysterothylacium amoyense* third stage larvae in situ in the viscera of *Trichiurus lepturus*.

Fig. A.2. *Hysterothylacium amoyense* third stage larva in situ in the muscle of *Harpadon nehereus*.
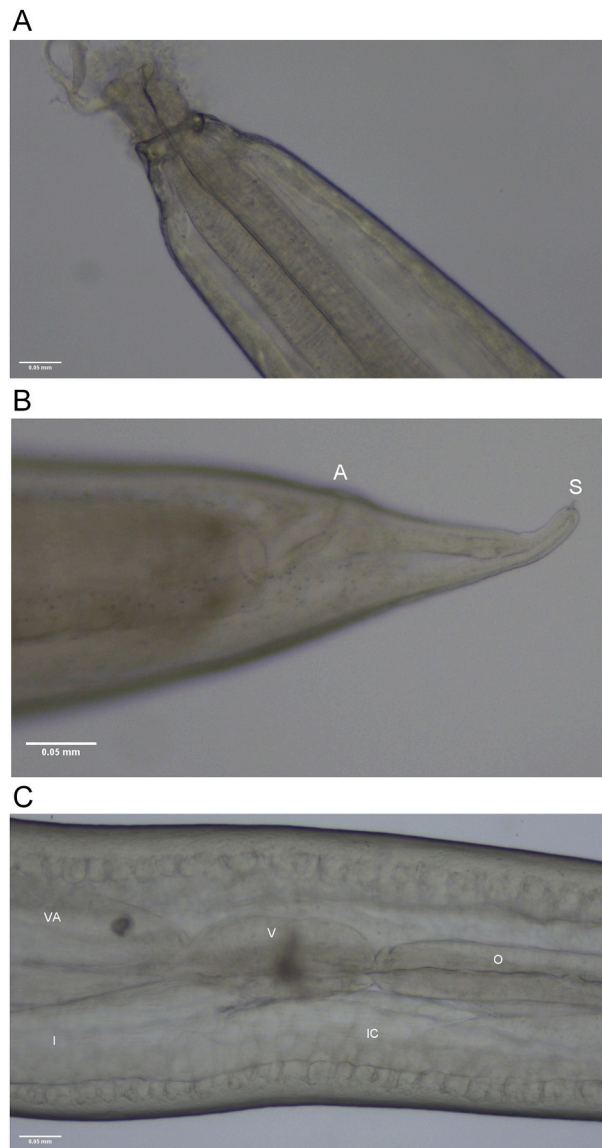
Fig. A.3. *Lappetascaris* sp. type A third larval stage from *U. duvaucelii*. A) cephalic extremity with ventral part protruding more anteriorly than dorsal one. B) ventricle (V) part connected to the end of the muscular oesophagus (O), ventricular appendix (VA) and intestine (I), and intestinal caecum (IC) extending anteriorly alongside the ventricle and oesophagus. C) caudal extremity showing anus (A) and terminal spike (S).

Table A.1

GenBank accession numbers (i. e. ITS number, *cox2* number, 28S number) of ascaridoid parasite isolates from fish and squid host species of Bangladesh.

Ascaridoid species	Isolate	Host species	ITS number	*cox2* number	28S number
H. amoyense	HARP12_H	*Harpadon nehereus*	NA	ON109760	NA
	HNMUS23H	*H. nehereus*	ON098267	ON109762	ON098262
	HNVIS1H	*H. nehereus*	ON098269	ON109767	ON098265
	HNMUS43H	*H. nehereus*	ON098268	ON109763	ON098263
	HARPOON15ST27vis1	*H. nehereus*	ON098270	NA	NA
	HARPOON34ST33vis1	*H. nehereus*	ON098271	NA	NA
	BOMBAY DUCK18ST27–2	*H. nehereus*	ON098272	NA	NA
	BOMBAY DUCK18ST27–1	*H. nehereus*	ON098273	NA	NA
	HARPOON35ST33mus1	*H. nehereus*	ON098274	NA	NA
	HARPODON22_ST27_MUS1	*H. nehereus*	ON098275	NA	NA
	HARPODON4_ST29_VIS1	*H. nehereus*	ON098276	NA	NA
	HARPODON34_ST33_VIS2	*H. nehereus*	ON098277	NA	NA

Table A.1 (*continued*)

Ascaridoid species	Isolate	Host species	ITS number	*cox*2 number	28S number
	HARPODON34_ST33_VIS3	*H. nehereus*	ON098278	NA	NA
	HARPODON4_ST29_VIS3	*H. nehereus*	ON098279	NA	NA
	SFVIS24H	*S. fimbriata*	ON098280	ON109761	ON098261
	Hystero1 T.Lepturus 28	*Trichiurus lepturus*	NA	ON109757	ON098258
	Hystero2 T.Lepturus 28	*T. lepturus*	NA	ON109758	ON098259
	Hystero1 T.Lepturus 17	*T. lepturus*	NA	ON109759	ON098260
	TLVIS20H	*T. lepturus*	ON098281	ON109764	ON098264
	TLVIS18H	*T. lepturus*	NA	ON109765	NA
	TLVIS14H	*T. lepturus*	NA	ON109766	NA
	TL17VISH	*T. lepturus*	ON098282	NA	ON098266
	TLEPT34ST9mus1	*T. lepturus*	ON098283	NA	NA
	TLEPT29ST9mus1	*T. lepturus*	ON098284	NA	NA
A. typica	SCVIS15A	*S. crumenophthalmus*	NA	ON109756	ON117607
	SCVIS20N	*S. crumenophthalmus*	ON065558	ON109753	ON117608
	TLEPT29ST9vis30	*T. lepturus*	ON065559	ON109754	NA
	TLEPT31ST9vis1	*T. lepturus*	ON065560	ON109755	NA
Lappetascaris sp.	UDMAN6A2	*U. duvaucelii*	ON098285	ON109536	ON117609

References

Adroher-Auroux, F.J., Benítez-Rodríguez, R., 2020. Anisakiasis and *Anisakis*: an underdiagnosed emerging disease and its main etiological agents. Res. Vet. Sci. https://doi.org/10.1016/j.rvsc.2020.08.003.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

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. Am. J. Trop. Med. Hyg. 95, 410–412. https://doi.org/10.4269/ajtmh.16-0007.

Añíbarro, B., Seoane, F.J., 1998. Occupational conjunctivitis caused by sensitization to *Anisakis simplex*. J. Allergy Clin. Immunol. 102, 331–332. https://doi.org/10.1016/S0091-6749(98)70108-3.

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 J. Parasitol. 52, 9–19. https://doi.org/10.3347/kjp.2014.52.1.9.

Arthur, J.R., Ahmed, A.T.A., 2002. Checklist of the Parasites of Fishes of Bangladesh, FAO Fisheries Technical Paper 369/1. Rome.

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

Balbuena, J.A., Karlsbakk, E., Saksvik, M., Kvenseth, A.M., Nylund, A., 1998. New data on the early development of *Hysterothylacium aduncum* (Nematoda, Anisakidae). J. Parasitol. 84, 615. https://doi.org/10.2307/3284732.

Bao, M., Pierce, G.J., Pascual, S., González-Muñoz, M., Mattiucci, S., Mladineo, I., Cipriani, P., Bušelić, I., Strachan, N.J.C., 2017. Assessing the risk of an emerging zoonosis of worldwide concern: anisakiasis. Sci. Rep. 7, 43699. https://doi.org/10.1038/srep43699.

Bao, M., Pierce, G.J., Strachan, N.J.C., Martínez, C., Fernández, R., Theodossiou, I., 2018. Consumers' attitudes and willingness to pay for *Anisakis*-free fish in Spain. Fish. Res. 202, 149–160. https://doi.org/10.1016/j.fishres.2017.06.018.

Bao, M., Pierce, G.J., Strachan, N.J.C., Pascual, S., González-Muñoz, M., Levsen, A., 2019. Human health, legislative and socioeconomic issues caused by the fish-borne zoonotic parasite *Anisakis*: challenges in risk assessment. Trends Food Sci. Technol. 86, 298–310. https://doi.org/10.1016/J.TIFS.2019.02.013.

Bao, M., Cipriani, P., Giulietti, L., Drivenes, N., Levsen, A., 2021. Quality issues related to the presence of the fish parasitic nematode *Hysterothylacium aduncum* in export shipments of fresh Northeast Arctic cod (*Gadus morhua*). Food Control 121, 107724. https://doi.org/10.1016/j.foodcont.2020.107724.

Berland, B., 1989. Identification of fish larval nematodes from fish. In: Möller, H. (Ed.), Nematode Problems in North Atlantic Fish. Report from a Workshop in Kiel, 3-4 April 1989, pp. 16–22.

BOBLME, 2013. Marine Protected Areas in Bangladesh - a Framework for Establishment and Management BOBLME-2013-Ecology-04.

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

Carrascosa, M.F., Mones, J.C., Salcines-Caviedes, J.R., Román, J.G., 2015. A man with unsuspected marine eosinophilic gastritis. Lancet Infect. Dis. 15, 248. https://doi.org/10.1016/S1473-3099(14)70892-8.

Cavallero, S., Martini, A., Migliara, G., De Vito, C., Iavicoli, S., D'Amelio, S., 2018. Anisakiasis in Italy: analysis of hospital discharge records in the years 2005-2015. PLoS One 13, e0208772. https://doi.org/10.1371/journal.pone.0208772.

Chandra, K., 2006. Fish parasitological studies in Bangladesh: a review. J. Agric. Rural Dev. 4, 9–18. https://doi.org/10.3329/jard.v4i1.762.

Chen, H.-X., Zhang, L.-P., Gibson, D.I., Lü, L., Xu, Z., Li, H.-T., Ju, H.-D., Li, L., 2018. Detection of ascaridoid nematode parasites in the important marine food-fish *Conger myriaster* (Brevoort) (Anguilliformes: Congridae) from the Zhoushan fishery. China. Parasit. Vectors 11, 274. https://doi.org/10.1186/s13071-018-2850-4.

Culurgioni, J., Cuccu, D., Mereu, M., Figus, V., 2010. Larval anisakid nematodes of *Histioteuthis reversa* (Verril, 1880) and *H. bonnellii* (Férussac, 1835) (Cephalopoda: Teuthoidea) from Sardinian Channel (western Mediterranean). Bull. Eur. Assoc. Fish Pathol. 30, 220–228.

Daschner, Á., Alonso-Gómez, A., Cabañas, R., Suárez de Parga, J.M., López-Serrano, M.C., 2000. Gastroallergic anisakiasis: borderline between food allergy and parasitic disease-clinical and allergologic evaluation of 20 patients with confirmed acute parasitism by *Anisakis simplex*. J. Allergy Clin. Immunol. 105, 176–181. https://doi.org/10.1016/S0091-6749(00)90194-5.

De, N.C., 1990. Some new data on the morphology of the nematode *Lappetascaris lutjani* Rasheed, 1965 (Nematoda: Anisakidae). Folia Parasit. 37 (4), 295–299.

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. Trop. Biomed. 35, 669–677.

EFSA-BIOHAZ, 2010. Scientific opinion on risk assessment of parasites in fishery products. EFSA J. 8, 1543. https://doi.org/10.2903/j.efsa.2010.1543.

FAO/WHO, 2014. Multicriteria-Based Ranking for Risk Management of Food-Borne Parasites, Microbiological Risk Assessment Series. Food and Agriculture Organization of the United Nations/World Health Organization.

Gomes, T.L., Quiazon, K.M.A., Itoh, N., Yoshinaga, T., 2020. *Anisakis* spp. in fishery products from Japanese waters: updated insights on host prevalence and human infection risk factors. Parasitol. Int. 78, 102137 https://doi.org/10.1016/j.parint.2020.102137.

Guardone, L., Bilska-Zając, E., Giusti, A., Malandra, R., Cencek, T., Armani, A., 2020. Larval ascaridoid nematodes in horned and musky octopus (*Eledone cirrhosa* and *E. moschata*) and longfin inshore squid (*Doryteuthis pealeii*): safety and quality implications for cephalopod products sold as fresh on the Italian market. Int. J. Food Microbiol. 333, 108812 https://doi.org/10.1016/j.ijfoodmicro.2020.108812.

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. Infect. Genet. Evol. 85, 104562 https://doi.org/10.1016/j.meegid.2020.104562.

Hemsrichart, V., 1993. Intestinal anisakiasis: first reported case in Thailand. J. Med. Assoc. Thail. 76, 117–121.

Herrador, Z., Daschner, Á., Perteguer, M.J., Benito, A., 2019. Epidemiological scenario of anisakidosis in Spain based on associated hospitalizations: the tip of the iceberg. Clin. Infect. Dis. 69, 69–76. https://doi.org/10.1093/cid/ciy853.

Jabbar, A., Asnoussi, A., Norbury, L.J., Eisenbarth, A., Shamsi, S., Gasser, R.B., Lopata, A.L., Beveridge, I., 2012. Larval anisakid nematodes in teleost fishes from Lizard Island, northern great barrier reef, Australia. Mar. Freshw. Res. 63, 1283. https://doi.org/10.1071/MF12211.

Karl, H., Leinemann, M., 1993. A fast and quantitative detection method for nematodes in fish fillets and fishery products. Arch. Leb. 44, 105–128.

Kim, J.Y., Yi, M., Yong, T.-S., 2019. Parasitic infections and medical expenses according to health insurance review assessment claims data in South Korea, 2011–2018. PLoS One 14, e0225508. https://doi.org/10.1371/journal.pone.0225508.

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. Parasitol. Res. 113, 1405–1415. https://doi.org/10.1007/s00436-014-3781-4.

Klimpel, S., Rückert, S., 2005. Life cycle strategy of *Hysterothylacium aduncum* to become the most abundant anisakid fish nematode in the North Sea. Parasitol. Res. 97, 141–149. https://doi.org/10.1007/s00436-005-1407-6.

Køie, M., 1993. Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Can. J. Zool. 71, 1289–1296. https://doi.org/10.1139/z93-178.

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. Vet. Parasitol. 193, 126–133. https://doi.org/10.1016/j.vetpar.2012.12.008.

Kołodziejczyk, L., Szostakowska, B., Sobecka, E., Szczucki, K., Stankiewicz, K., 2020. First case of human anisakiasis in Poland. Parasitol. Int. 76, 102073 https://doi.org/10.1016/j.parint.2020.102073.

Kong, Q., Fan, L., Zhang, J., Akao, N., Dong, K., Lou, D., Ding, J., Tong, Q., Zheng, B., Chen, R., Ohta, N., Lu, S., 2015. Molecular identification of *Anisakis* and *Hysterothylacium* larvae in marine fishes from the East China Sea and the Pacific coast of Central Japan. Int. J. Food Microbiol. 199, 1–7. https://doi.org/10.1016/j.ijfoodmicro.2015.01.007.

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 Parasitol. 60, 123–134. https://doi.org/10.14411/fp.2013.013.

Kuhn, T., Cunze, S., Kochmann, J., Klimpel, S., 2016. Environmental variables and definitive host distribution: a habitat suitability modelling for endohelminth parasites in the marine realm. Sci. Rep. 6, 30246. https://doi.org/10.1038/srep30246.

Levsen, A., Lunestad, B.T., 2010. *Anisakis simplex* third stage larvae in Norwegian spring spawning herring (*Clupea harengus* L.), with emphasis on larval distribution in the flesh. Vet. Parasitol. 171, 247–253. https://doi.org/10.1016/j.vetpar.2010.03.039.

Levsen, A., Paoletti, M., Cipriani, P., Nascetti, G., Mattiucci, S., 2016. Species composition and infection dynamics of ascaridoid nematodes in Barents Sea capelin (*Mallotus villosus*) reflecting trophic position of fish host. Parasitol. Res. 115, 4281–4291. https://doi.org/10.1007/s00436-016-5209-9.

Li, L., Xu, Z., Zhang, L., 2008. Redescription of three species of *Hysterothylacium* (Nematoda: Anisakidae) from marine fishes from the Yellow Sea, China, with the synonymy of *Hysterothylacium muraenesoxin* (Luo, 1999). Zootaxa 1878, 55. https://doi.org/10.11646/zootaxa.1878.1.2.

Li, L., Liu, Y.-Y., Zhang, L.-P., 2012. Morphological and genetic characterization of *Hysterothylacium zhoushanensis* sp. nov. (Ascaridida: Anisakidae) from the flatfish *Pseudorhombus oligodon* (Bleeker) (Pleuronectiformes: Paralichthyidae) in the East China Sea. Parasitol. Res. 111, 2393–2401. https://doi.org/10.1007/s00436-012-3095-3.

Li, L., Gibson, D., Zhang, L.-P., 2016a. An annotated catalogue of the ascaridoid nematode parasites of Chinese vertebrates. Syst. Parasitol. 93, 1–35. https://doi.org/10.1007/s11230-015-9617-5.

Li, L., Zhao, W.-T., Guo, Y.-N., Zhang, L.-P., 2016b. Nematode parasites infecting the starry batfish *Halieutaea stellata* (Vahl) (Lophiiformes: Ogcocephalidae) from the east and South China Sea. J. Fish Dis. 39, 515–529. https://doi.org/10.1111/jfd.12374.

Li, L., Lü, L., Nadler, S.A., Gibson, D.I., Zhang, L.-P., Chen, H.-X., Zhao, W.-T., Guo, Y.-N., 2018. Molecular phylogeny and dating reveal a terrestrial origin in the early carboniferous for ascaridoid nematodes. Syst. Biol. 67, 888–900. https://doi.org/10.1093/sysbio/syy018.

Luo, H.-Y., Chen, H.-Y., Chen, H.-G., Shih, H.-H., 2016. Scavenging hagfish as a transport host of Anisakid nematodes. Vet. Parasitol. 218, 15–21. https://doi.org/10.1016/j.vetpar.2016.01.005.

Mattiucci, S., Fazii, P., De Rosa, A., Paoletti, M., Megna, A., Glielmo, A., De Angelis, M., Costa, A., Meucci, C., Calvaruso, V., Sorrentini, I., Palma, G., Bruschi, F., Nascetti, G., 2013. Anisakiasis and gastroallergic reactions associated with *Anisakis pegreffii* infection, Italy. Emerg. Infect. Dis. 19, 496–499. https://doi.org/10.3201/eid1903.121017.

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). J. Parasitol. 100, 199–214. https://doi.org/10.1645/12-120.1.

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., Nascetti, G., 2018. Molecular epidemiology of *Anisakis* and anisakiasis: an ecological and evolutionary road map. Adv. Parasitol. 93–263. https://doi.org/10.1016/bs.apar.2017.12.001.

Moravec, F., Scholz, T., 1991. Observations on some nematodes parasitic in fresh-water fishes in Laos. Folia Parasitol. 38, 163–178.

Murata, R., Suzuki, J., Kodo, Y., Kobayashi, K., Sadamasu, K., Takano, T., Iwaki, T., Waki, T., Ogawa, K., 2021. Probable association between *Anisakis* infection in the muscle of skipjack tuna (*Katsuwonus pelamis*) and human anisakiasis in Tokyo, Japan. Int. J. Food Microbiol. 337, 108930 https://doi.org/10.1016/j.ijfoodmicro.2020.108930.

Murshed-e-Jahan, K., Belton, B., Viswanathan, K.K., 2014. Communication strategies for managing coastal fisheries conflicts in Bangladesh. Ocean Coast. Manag. 92, 65–73. https://doi.org/10.1016/j.ocecoaman.2014.01.003.

Nadler, S.A., Hudspeth, D.S.S., 1998. Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata: Secernentea): implications for morphological evolution and classification. Mol. Phylogenet. Evol. 10, 221–236. https://doi.org/10.1006/mpev.1998.0514.

Nadler, S.A., Hudspeth, D.S.S., 2000. Phylogeny of the Ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology hypotheses of structural and sequence evolution. J. Parasitol. 86, 380–393. https://doi.org/10.1645/0022-3395(2000)086[0380:POTANA]2.0.CO;2.

Nagasawa, K., Moravec, F., 1995. Larval anisakid nematodes of Japanese common squid (*Todarodes pacificus*) from the sea of Japan. J. Parasitol. 81, 69. https://doi.org/10.2307/3284008.

Nagasawa, K., Moravec, F., 2002. Larval anisakid nematodes from four species of squid (Cephalopoda: Teuthoidea) from the central and western North Pacific Ocean. J. Nat. Hist. 36, 883–891. https://doi.org/10.1080/00222930110051752.

Najjari, M., Sadjjadi, S.M., Derakhshanfar, A., Ebrahimipour, M., 2016. *Hysterothylacium amoyense* in *Platycephalous indicus*: a Persian Gulf fish and its experimental infection of mouse model. Comp. Clin. Pathol. 25, 1143–1149. https://doi.org/10.1007/s00580-016-2318-x.

Nieuwenhuizen, N., 2016. Anisakis - immunology of a food-borne parasitosis. Parasite Immunol. 38, 548–557. https://doi.org/10.1111/pim.12349.

Nieuwenhuizen, N., Lopata, A.L., Jeebhay, M.F., Herbert, D.R., Robins, T.G., Brombacher, F., 2006. Exposure to the fish parasite *Anisakis* causes allergic airway hyperreactivity and dermatitis. J. Allergy Clin. Immunol. 117, 1098–1105. https://doi.org/10.1016/j.jaci.2005.12.1357.

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. 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. Dis. Aquat. Org. 123, 141–157. https://doi.org/10.3354/dao03091.

Palomba, M., Mattiucci, S., Crocetta, F., Osca, D., Santoro, M., 2021. Insights into the role of deep-sea squids of the genus *Histioteuthis* (Histioteuthidae) in the life cycle of ascaridoid parasites in the Central Mediterranean Sea waters. Sci. Rep. 11, 7135. https://doi.org/10.1038/s41598-021-86248-5.

Pippy, J.H.C., 1970. Use of ultraviolet light to find parasitic nematodes *in situ*. J. Fish. Res. Board Can. 27, 963–965. https://doi.org/10.1139/f70-107.

Purivirojkul, W., 2009. An investigation of larval ascaridoid nematodes in some marine fish from the Gulf of Thailand. Agric. Nat. Resour. 43, 85–92.

Quiazon, K.M.A., 2016. *Anisakis* Dujardin, 1845 infection (Nematoda: Anisakidae) in pygmy sperm whale *Kogia breviceps* Blainville, 1838 from West Pacific region off the coast of Philippine archipelago. Parasitol. Res. 115, 3663–3668. https://doi.org/10.1007/s00436-016-5169-0.

Quiazon, K.M.A., Santos, M.D., Yoshinaga, T., 2013. *Anisakis* species (Nematoda: Anisakidae) of dwarf sperm whale *Kogia sima* (Owen, 1866) stranded off the Pacific coast of southern Philippine archipelago. Vet. Parasitol. 197, 221–230. https://doi.org/10.1016/J.VETPAR.2013.05.019.

Rasheed, S., 1965. On a remarkable new nematode, *Lappetascaris* gen. et sp. nov. (Anisakidae: Ascaridoidea) from marine fishes of Karachi and an account of *Thynnascaris inquies* (Linton, 1901) n.comb. and *Goezia intermedia* n.sp. J. Helminthol. XXXIX, 313–342.

Shamsi, S., 2021. The occurrence of *Anisakis* spp. in Australian waters: past, present, and future trends. Parasitol. Res. 120, 3007–3033. https://doi.org/10.1007/s00436-021-07243-3.

Shamsi, S., Andrew, B.R., 2011. First report of human anisakidosis in Australia. Med. J. Aust. 194, 199–200. https://doi.org/10.1016/j.mcp.2010.09.003.

Shamsi, S., Gasser, R., Beveridge, I., 2013. Description and genetic characterisation of *Hysterothylacium* (Nematoda: Raphidascarididae) larvae parasitic in Australian marine fishes. Parasitol. Int. 62, 320–328. https://doi.org/10.1016/J.PARINT.2012.10.001.

Shamsi, S., Ghadam, M., Suthar, J., Ebrahimzadeh Mousavi, H., Soltani, M., Mirzargar, S., 2016. Occurrence of ascaridoid nematodes in selected edible fish from the Persian Gulf and description of *Hysterothylacium* larval type XV and *Hysterothylacium persicum* n. sp. (Nematoda: Raphidascarididae). Int. J. Food Microbiol. 236, 65–73. https://doi.org/10.1016/j.ijfoodmicro.2016.07.006.

Shamsi, S., Steller, E., Chen, Y., 2018. New and known zoonotic nematode larvae within selected fish species from Queensland waters in Australia. Int. J. Food Microbiol. 272, 73–82. https://doi.org/10.1016/J.IJFOODMICRO.2018.03.007.

Shamsuzzaman, M.M., Islam, M.M., Tania, N.J., Al-Mamun, M.A., Barman, P.P., Xu, X., 2017. Fisheries resources of Bangladesh: present status and future direction. Aquac. Fish. 2, 145–156. https://doi.org/10.1016/j.aaf.2017.03.006.

Smith, J.W., 1999. Ascaridoid nematodes and pathology of the alimentary tract and its associated organs in vertebrates, including man: a literature review. Helminthol. Abstr. 49–96.

Sonko, P., Chih-Cheng Chen, S., Chou, C.M., Huang, Y.C., Hsu, S.L., Barčák, D., Oros, M., Fan, C.K., 2020. Multidisciplinary approach in study of the zoonotic *Anisakis* larval infection in the blue mackerel (*Scomber australasicus*) and the largehead hairtail (*Trichiurus lepturus*) in northern Taiwan. J. Microbiol. Immunol. Infect. 53, 1021–1029. https://doi.org/10.1016/j.jmii.2019.04.012.

Suzuki, J., Murata, R., Kodo, Y., 2021. Current status of Anisakiasis and *Anisakis* larvae in Tokyo, Japan. Food Saf. 9, 89–100. https://doi.org/10.14252/foodsafetyfscj.d-21-00004.

Takahara, H., Sakurai, Y., 2010. Infection of the Japanese common squid, *Todarodes pacificus* (Cephalopoda: Ommastrephidae) by larval anisakid nematodes. Fish. Res. 106, 156–159. https://doi.org/10.1016/j.fishres.2010.05.009.

Takano, T., Iwaki, T., Waki, T., Murata, R., Suzuki, J., Kodo, Y., Kobayashi, K., Ogawa, K., 2021. Species composition and infection levels of *Anisakis* (Nematoda: Anisakidae) in the skipjack tuna *Katsuwonus pelamis* (Linnaeus) in the Northwest Pacific. Parasitol. Res. 120, 1605–1615. https://doi.org/10.1007/s00436-021-07144-5.

Takasaki, T., Yamada, T., Kinoshita, J., Motomura, Y., 2020. Asymptomatic colonic anisakiasis: is it so rare? Case Rep. Gastroenterol. 14, 593–597. https://doi.org/10.1159/000508822.

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 J. Parasitol. 58, 15–25. https://doi.org/10.3347/kjp.2020.58.1.15.

Uga, S., Ono, K., Kataoka, N., Hasan, H., 1996. Seroepidemiology of five major zoonotic parasite infections in inhabitants of Sidoarjo, East Java, Indonesia. Southeast Asian. J. Trop. Med. Public Health 27, 556–561.

Van Hien, H., 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. J. Nematol. 53 https://doi.org/10.21307/JOFNEM-2021-010.

Vicente, J.J., Mincarone, M.M., Pinto, R.M., 2002. First report of *Lappetascaris lutjani* Rasheed, 1965 (Nematoda, Ascaridoidea, Anisakidae) parasitizing *Trachipterus arawatae* (Pisces, Lampridiformes) on the Atlantic Coast of Brazil. Mem. Inst. Oswaldo Cruz 97, 93–94. https://doi.org/10.1590/S0074-02762002000100015.

Watahiki, M., Uchida, K., Kanatani, J., Kato, T., Kimata, K., Isobe, J., Oishi, K., Tozaki, K., Sekiguchi, K., Horita, Y., Morishima, Y., Sugiyama, H., 2020. Molecular identification of parasites isolated from the stomach of patients with *Anisakis* food poisoning in Toyama prefecture, Japan, in 2018. Jpn. J. Infect. Dis. 74, 85–86. https://doi.org/10.7883/yoken.JJID.2020.289.

Wiwanitkit, S., Wiwanitkit, V., 2016. Anisakiasis in Southeast Asia: a story of new tropical disease? Asian Pac. J. Trop. Med. 6, 382–383. https://doi.org/10.1016/j.apjtb.2015.11.011.

Yera, H., Fréalle, É., Dutoit, E., Dupouy-Camet, J., 2018. A national retrospective survey of anisakidosis in France (2010-2014): decreasing incidence, female predominance, and emerging allergic potential. Parasite 25, 1–6. https://doi.org/10.1051/parasite/2018016.

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). Int. J. Food Microbiol. 284, 105–111. https://doi.org/10.1016/j.ijfoodmicro.2018.08.002.

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). Parasitol. Res. 86, 677–683. https://doi.org/10.1007/PL00008551.