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A contribution on first report of morphogenetic characterization of *Anisakis typica* parasitizing Indian sand whiting, *Sillago sihama* from Central west coast of India

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Summary

The search for hitherto undiscovered larvae of *Anisakis* sp. from marine habitat in the Indian sub-continent yielded *A. typica* (Dujardin) larvae hitherto unconfirmed. The present study is the maiden attempt to report 3rd stage larvae of *A. typica* from the reef-associated *Sillago sihama* in Arabian Sea off the coast of Goa, which has been identified recently as reef-populated area within the maritime boundary of India. The morphometry of 3rd stage larvae has been presented with a record of molecular characterization. In the context of current study, the natural prevalence of *A. typica* larvae in marine piscine hosts of Arabian Sea in India was 6.84 % and of co-occurring *Rotundocollarette capoori* (Yadav, Kapoor and Malhotra) in the same fish was 13.65 %. The roundworms were confirmed to be *A. typica* by application of the molecular and genetic characterization based on ITS1, ITS2 and 18S rDNA sequence analysis. The infestation of reef-associated fishes in this study by anisakid worms study provided an opportunity to explore mechanism of ecological associations of coral reefs with parasitization in future.

Keywords: *Anisakis typica*; Genetic characterization; Molecular; *Rotundocollarette capoori;* Reef-associated fish; Goa

Introduction

The molecular evidence of larvae of *Anisakis typica* being zoonotic agents is missing in the International literature (Leuckart, 1876; Van Thiel, 1960; Van Thiel *et al*., 1962; World Health Organization, 2004). The worms of genus *Anisakis* (Anisakidae, Ascaridoidea) have been reported to cause anisakiasis, an inflammation of the human gastrointestinal tract, in many countries (Smith & Wootten, 1978; Nieuwenhuizen *et al*., 2013; Baird *et al.*, 2014, Shamsi & Barton, 2023; Rahamati *et al*., 2020). The world-wide occurrence of *A. typica* and its larvae have, however, been concluded by various authors (Della-Morte *et al.,* 2023; Bao *et al.,* 2015, 2022; Shamsi, 2021; Arizono *et al*., 2012; Borges *et al.,* 2012). But there are no reports on *A. typica* or larvae of *Anisakis* from fish in Indian waters, though certain reports on infections by other anisakid worms from India, *viz. Hysterothylacium* in different marine fishes appeared in nineties (Rajyalaxmi, 1992; Rajyalaxmi *et al*., 1991, 1992). The infestations by the agents of anisakiasis from the fish along Indian coast of Bay of Bengal have been scarcely explored. In the study area, the parasite life cycle starts with adult nematodes occurring in the stomach of marine mammals such as cetaceans (Nieuwenhuizen & Lopata, 2013; Pampiglione *et al*., 2002). Non-embryonated eggs pass with faecal material into the waters of Arabian Sea.)

These nematodes by the latter authors were characterized morphometrically by a dorsal tooth atop cephalic complex. Their

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differentiation from the reported roundworms from adjacent regions of Indonesia (Suryani *et al*., 2021), Thailand (Chaiphongpachara *et al*., 2022), Java, Japan (Suzuki *et al*., 2021) and Australia (Shamsi, 2021; Yann, 2006) have been substantiated by morphogenetic characterization based on the analysis of sequences of ITS1, ITS2 and 18S rDNA in this investigation. Stray reports have appeared during previous years to account for the seasonal prevalence of *Anisakis* spp. From different areas of the country, but without any specific diagnostic characteristics of microphotographs or SEM pictures on record to confirm their 'generic' as well as 'species' status.

Several earlier workers who attempted to review occurrence of *Anisakis* sp. in Indian subcontinent did not deal with morphotaxonomic aspects of the worms collected. The genetic compatibility of larva of *A. simplex* with its host *Carassius gibelio* was worked out by Ahmed *et al*. (2022) but no reference to the morphotaxonomy of *A. typica* was discussed. It was confirmed in the study by Ahmed *et al*. (2022) that larva of *Anisakis simplex* has not been parasitic in *C. gibelio. A. simplex* is a well known parasite of carp fish, although it has yet not been reported in *C. gibelio*. Ahmed *et al*. (2022).

The authors have admittedly emphasized themselves that *A. simplex* is a well known parasite of carp fish, although it is still not reported in *C. gibelio* in India.

The report of one larval nematode species of genus *Anisakis* sp. L_3 larvae in *C. batrachus* was included in Tripura Report by Koiri and Roy (2016). But no diagnostic features were outlined to confirm its species status. Such half-baked reports on Pre-Monsoon, Monsoon and Post-Monsoon data on a "so-called" species which is not established by appropriate publication of morphological data worth the name of a Figure, must not be taken up as *A. simplex* larvae from *C.batrachus*. Virtually no name of any nematode discovered was mentioned in Guchhait *et al*., (2018). Ruchi and Patricia (2023) included unpublished record of a paper that was purportedly published in *International Journal of Zoological Investigations*. These were named as 3rd stage larva "Type of *A. simplex*" without a description or diagnostic characteristics outlined. In addition, as many as eleven species of anisakids, particularly *Hysterothylacium* were reported by Rajyalakshmi and co-workers, but none recorded anisakiasis with *A. typica* or its larvae: Rajyalakshmi (2005), Lakshmi (1993), Lakshmi *et al*., (1993a,b).

Materials and Methods

Study area

The expeditions to collection sites for coastal fish were conducted at Jetty at Porvorim, Panjim, North Goa at 15.505082°N, 73.834789°E for latitude and longitude, respectively, 19Kms away from the recently discovered coral reef-associated *'Grande'* Island by Indian Scientists on March 5, 2024. Site map of recently discovered Grande Island is given as Figure1.

Collection of A. typica *from the examined fish*

The study comprised examination of 49 *Sillago sihama* and 42 *Johnius dussumieri*. The freshly caught fish in the coastal region were brought to the laboratory. The fish were euthanized by hitting in the region of the spinal cord, with a heavy, sharp object in the fastest manner to avoid unnecessary torment. All fish appeared to be healthy by the criteria that they were able to swim and breathe

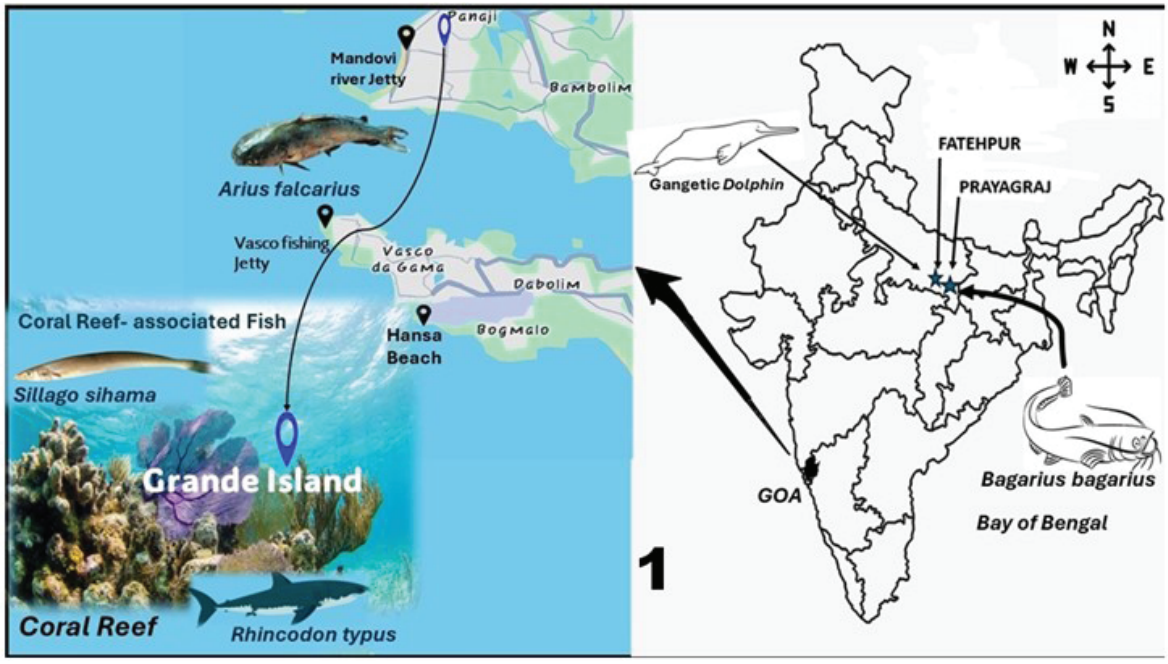

Fig. 1. Site map of recently discovered Grande Island.

Table 1. Morphometric measurements of 3rd stage larvae of *Anisakis typica* collected from the Northern whiting, *Sillago sihama* in the present study.

Characters		Measurements (mm)	
		Male	Female
Body	L:	3.89-5.366 (4.98±0.28)	8.49-9.82 (7.57±2.79)
Body	W:	0.036-0.043(0.039±0.01)	0.18-0.37(0.27±0.009)
Buccal tooth	L:	0.0054-0.0072(0.0063±0.004)	0.009-0.012(0.010±0.003)
Head	L:	0.0043-0.0068 (0.0055±0.001)	0.010-0.014(0.012±0.006)
	W:	0.012-0.014(0.013±0.004)	0.019-0.022(0.020±0.004)
Buccal cavity	Deep:	0.016-0.019(0.018±0.002)	0.018-0.020(0.19±0.004)
	W:	0.014-0.018(0.016±0.009)	0.016-0.019(0.017±0.003)
Oesophagus i. Anterior		0.25-0.27(0.26±0.06) 0.036-0.043(0.039±0.002)	0.27-0.32(0.29±0.08) 0.041-0.052(0.046±0.02)
ii.Posterior		0.18-0.19(0.18±0.007) x0.054-0.064 (0.059±0.006)	0.21-0.25(0.23±0.1) x 0.060-0.064 (0.062±0.01)
Intestine	W:	-	0.28-0.609 (0.57±0.08) x 0.065-0.099 (0.087±0.03)
Intestinal caecum	L:	0.21-0.23(0.22±0.08)	0.28-0.31(0.29±0.08)
	W:	0.0072-0.010(0.009±0.001)	0.009-0.012(0.011±0.004)
Ventriculus	L:	0.018-0.021(0.019±0.006)	0.022-0.023(0.023±0.007)
	W:	0.036-0.050(0.043±0.002)	0.041-0.047(0.043±0.002)
Ventricular appendix	L:	0.10-0.12(0.11±0.007)	0.135-0.151(0.15±0.02)
	W:	0.036-0.054(0.045±0.001)	0.051-0.066(0.058±0.007)
Distance of anus from tail tip		0.033-0.039(0.034±0.004)	0.039-0.041(0.040±0.01)
Mucron	L:	0.0032-0.0036(0.0034)	0.005-0.006(0.0058±0.001)
	W:	0.0018-0.0028(0.0023)	0.003-0.005(0.004±0.001)
Ratio of Caeca : Ventricular Appendix		1:1.92	1:1.84
Weight/Length ratio of ventriculus		1:2.2	1:2.12
Length ratio of ventriculus : ceca		1:0.086	1:0.080

normally, without gasping for air, eating and interacting with other co-habitants. The body parts, pyloric ceca, liver, alimentary canal, gall bladder and gills were examined for parasites, particularly nematodes. The small intestine of *S. sihama* yielded 3rd stage larvae (N= 367), which were diagnosed to belong to genus *Anisakis*, and 8 worms of another agent of anisakiasis, larval *R. capoori*. The nematodes were killed in lukewarm water; washed thoroughly in 0.85 % normal saline, and fixed in Berland's solution. A small piece of the mid-body of each of the three individual nematodes were removed with a scalpel, and preserved in 100 % ethanol for molecular analysis. The morphometric analysis conducted on roundworms of *S. sihama* revealed 6.84 % worms of *A. typica* and 13.65 % of co-occurring *R. capoori* in the same fish during 2021 – 2024.

The nematodes were freshly washed with warm water; kept in normal saline during the period of their extraction from the body of their hosts, and processed immediately following the method of Malhotra (1986). Micrographs were taken with the help of BIOVIS Image Analyzer, and camera lucida illustrations were prepared. Specimens were observed in varied magnifications under Nikon Trinocular Research Microscope.

18 worms to be used for Scanning Electron Micrographic analysis were fixed in Glutaraldehyde (2.5 % in 0.1M phosphate buffer), and processed after rehydration (Malhotra *et al*., 2012). SEM analysis was performed on Jeol JSM 6510LV at the University Sophisticated Instrument Facility (USIF), Aligarh Muslim University, Aligarh, India.

Molecular analyses

The total genomic DNA was isolated from the nematode samples collected from *S. sihama* by using Qiagen DNeasy Blood and Tissue Kit (Qiagen,USA), following manufacturer's instructions. The

Table 2. Comparative chart of measurements of *Anisakis* 3rd stage larvae infesting different hosts, inclusive of those given in earlier reports.

Authors	*Anisakis* spp.	Host	Body		No. of Examined Hosts	Oesophagus Length	Ventriculus Length	Mucron Length
			Length	Width				
Hurst (1984)	*Anisakis simplex* 3rd stage larvae	Fish (*Thyrsites atun*)	20.26±3.04	-	-	1.99±0.21	0.69±0.09	0.023± 0.004
Larizza and Vovlas (1995)	*Anisakis simplex*	*Merluccius merluccius*	21.60±3.47	0.41±0.05	-	2.65±0.29	0.70±0.08	0.11±0.01
Quiazon, Yoshinaga, Ogawa, and Yukami (2008)	*Anisakis pegreffii*	*Delphinus delphis*	11.10-26.78	0.38-0.60	-	1.04-2.11	0.50-0.78	0.02-0.03
Quiazon, Yoshinaga, Ogawa, and Yukami (2008)	*Anisakis simplex*	*Delphinus delphis*	12.75-29.94	0.45-0.75	-	1.18-2.58	0.90-1.50	0.02-0.03
Setyobudi, Jeon, Lee, Seong, and Kim (2011)	*Anisakis simplex*	*Oncorhynchus keta*	23.62±1.87	0.56±0.04	-	2.06±0.25	1.14±0.13	0.021±0.004
Pardo-Gandarillas, Lohrmann, Valdivia and Ibáñez (2009)	*Anisakis physeteris*	*Etmopterus spinax*	27-33	0.63-0.74	-	1.82-2.89	0.53-0.65	0.17-0.32
Murata, Suzuki,Sadamasu and Kai (2011)	*Anisakis paggiae*	*Beryx splendens*	18.22±2.28	0.54±0.07	-	1.59±0.19	-	-
Setyobudi, Jeon, Lee, Seong, and Kim(2011)	*Anisakis simplex*	*M. merluccius lessepsianus*	20.4±1.2	0.55±0.02	-	2.12±0.20	0.82±0.1	0.025±0.02
Abou-Rahma *et al.* (2016)	*Anisakis simplex*	*Merluccius merluccius lessepsianus*	14.1-25.6	0.48-(20.4±1.2) 0.62 (0.55±0.02	60	1.18–2.68 (2.12±0.2)	0.71–0.92 (0.82±0.1)	0.019–0.032 (0.025 ± 0.02)
Roca-Geronesa, Segoviab, Godinez- Gonzaleza, Fisaa and Montoliua (2020)	*Anisakis pegreffii*	*Trachurus trachurus (Horse mackerrel)*	16.9±0.52	0.45±0.06	-	1.70-1.86	0.19-0.31	0.05-0.12
Roca-Geronesa, Segoviab, Godinez- Gonzaleza, Fisaa and Montoliua (2020)	*Anisakis simplex*	*Trachurus trachurus (Horse mackerrel)*	17.7±0.21	0.45±0.001	-	1.39-2.45	0.71-1.15	0.05-0.13
Hien, HV, Dung, BT, Ngo, HD & Doanh, PN (2021)	*Anisakis simplex*	*Merluccius merluccius*	18.60±1.3	0.29±0.00	-	18.60+1.1x 0.64±0.04	1.54±0.03x 0.64±0.04	0.025±0.005
Mostafa (2023)	*Anisakis simplex*	*Delphinus delphis*	18.0±2.1	0.45±0.02	-	1.35±0.02	1.35±0.02	0.018±0.002
Author's study (2024)	*Anisakis typica* 3rd stage Larvae	*Sillago sihama*	7.57±1.79	0.25±0.06	49	0.44±0.05	0.018-0.023 (0.023±0.007)	0.033-0.041 (0.040±0.01)

Table 3. Frequency of consumption of various non-vegetarian food items reported by households in Kerala.

Attributes	Percentage	Fresh Prawn	Dried Fish	Dried prawn
Never	100%	62	-	196
Others	90-100%		182	
On special occasions	30-80%	209	104	77
More than once a week	10-30%	91	121	141
Almost Everyday	0-10%	49	-	-

sequences were searched for homology on the DNA databases by using BLAST (Basic Local Alignment Search Tool) (Altschul *et al*., 1990) on the NCBI GenBank. The sequences were aligned with the sequences of reference organisms derived from databases (www.ncbi.nlm.nih.gov/). The DNA sequences were aligned for phylogenetic analysis using the CLUSTAL W computer program (Thompson *et al*., 1997), and DNA sequences were edited in DNASTAR (Frontiers in Bioscience; DNAStar, Inc, USA). The evolutionary distances were computed by Kimura's two parameter method (Kimura, 1980). The phylogenetic tree was constructed by the neighbor-joining method (Saitou & Nei, 1987) using MEGA version 4.0 (Tamura *et al*., 2011). The tree was evaluated using the bootstrap test (Felsenstein, 1985) based on 1,000 replications. The nucleotide sequences determined in this study were deposited in the National Center for Biotechnology Information (NCBI) under the accession numbers KF636791-18S, KF633459-ITS1 and KF633462-ITS2. PCR was used to amplify the 18S rDNA regions using primer sets Nem18SF, 5' CGCGAATRGCTCATTACAACAGC-3' (forward); and Nem18SR, 50-GGGCGGTATCTGATCGCC-3' (reverse) (Floyd *et al*., 2005). The primers utilized in the study were designed as according to Malhotra *et al*. (2012). The rDNA regions comprising ITS1 and ITS2 sequences were amplified with primers, 18SF (5'TTGATTAGGTCCCTGCCCTTT3') and 26SR (5'TTTCACTCGCCGTTACTAAGG3') for ITS1 gene, and SS2,(5'TTGCAGACACATTGAGCACT3') and NC2,(5'TTAGTTTCTTTTCCGCT3') for ITS2 gene. Each PCR reaction was performed under the following conditions: after initial denaturation at 94°C for 5 min., 35 cycles of 94°C for 30 s (denaturation), 50°C for 30 sec (annealing), 72°C for 30 s (extension), followed by a final extension at 72°C for 8 min. The PCR products were run on a 1 % agarose gel and visualized by Ethidium bromide staining. Purification and sequencing was done by Macrogen, Italy. The information about identified /verified names of submitted sequences has been given to GenBank. Adult nematodes were identified to species based on the available keys and descriptions (Hodda, 2022; Liang *et al*., 2016; De Ley and Blaxter, 2002; Coomans, 2002; Bruce and Cannon, 1990).

Ethical Approval and/or Informed Consent

No procedures performed in studies involved human participants and the ethical standards of the institutional ethical committee and with the 1964 Helsinki declaration and its later amendments, were followed.

Results and Discussion

The specimens of 3rd stage larvae of *Anisakis* used in the present investigation were compared morphometrically (Table 1) to reveal that the size of mucron in the specimens of *A. typica* of authors and co-workers was smallest *A. simplex* reported by Larizza and Vovlas (1995) (Table 2); than *A. physeteris* recorded by Pardo-Gandarillas *et al*. (2009); than *A. pegreffii* recorded by Roca-Geronesa *et al*. (2020); and then *A. simplex*, as reported by Roca-Geronesa *et al*. (2020). However, the larger length of mucron was encountered in the specimens of *A. typica* than *A. simplex* measured by Hurst (1984); than *A. physeteris* recorded by Pardo-Gandarillas *et al*. (2009); than *A. pegreffii* reported by Roca-Geronesa *et al*. (2020); and then *A. simplex* as measured by Roca-Geronesa *et al*. (2020). On the other hand, the measurements of the body of worms, as well as size of oesophagus, and ventriculus of the specimens of *A. typica* of authors were smaller than those reported by Hurst (1984), Larizza and Vovlas (1995), Quizon *et al*. (2008), Setyobudi *et al*. (2011), Abou-Rahma *et al*. (2016), Roca-Geronesa

Table 4. Per capita monthly consumption of fish v/s other meat in Malappuram, Kerala.

S. No.	Items consumed	Per capita consumption (kg/month)
1.	Fish	2.6
2.	Chicken	0.8
3.	Beef	0.6
4.	Mutton	0.4
5.	Pork	0.0

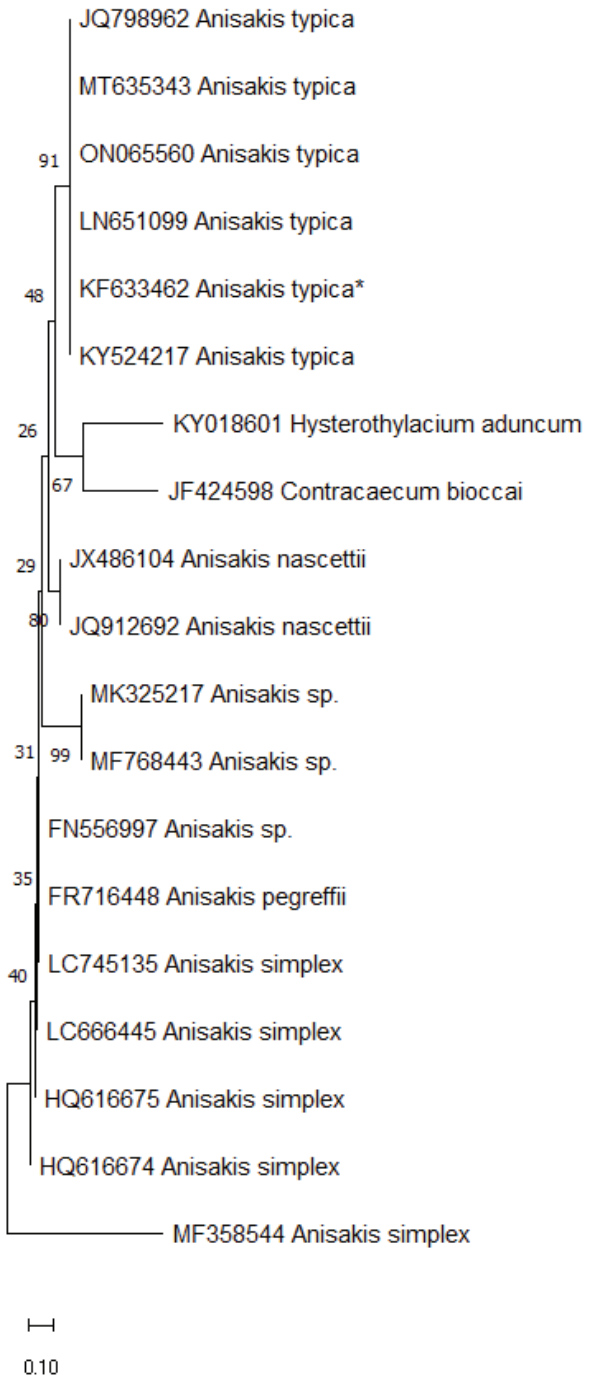

Fig. 2. Neighbour-joining tree based on nucleotide ITS2 sequences and reference species. Nucleotide ITS2 sequence data are as described in the text with GenBank accession numbers. Bootstrap values based on 1000 replicates were used. Scale bar represents an interval of the Kimura two-parameter (K2P) model. *Current investigation

et al. (2020); Hien *et al*. (2021), and Mostafa (2023) for *A. simplex*, while the body size, as well as size of oesophagus and ventriculus of *A. pegreffii* by Quizon *et al*. (2008), and by Roca-Geronesa *et al*. (2020) was larger than the specimens of *A. typica* of authors. Simultaneously, the size of oesophagus and ventriculus were smaller in *A. physeteris* than *A. typica* of the authors and co-workers given in Tables 1 and 2.

In Asian context, Chaiphongpachara *et al*. (2022) recorded outbreak of *A. typica* from the Gulf of Thailand; Suryani *et al*. (2021); and Palm *et al*. (2008) too recorded its outbreak from an Indian Mackerel, *i.e., R. kanagurta* from Indonesia, in Asian sub-continent. Summarily, a total of 9 species of *Anisakis* are known to have existed world-over: *A. simplex s.s., A. pegreffii*, *A. simplex* C., *A. typica, A. ziphidarum, A. nascettii, A. brevispiculata, A. paggiae* and *A. physeteris* (Jeon and Kim, 2015; Setyobudi *et al*., 2011; Mattiucci *et al*., 2008) in oceans around India.

The report of Suzuki *et al.* (2021) concluded that though several cases of human anisakiasis are being recorded in Japan each year, their probable association with two commonly occurring species of *Anisakis viz*., *A. pegreffii* and *A. simplex* (*s.s.*) were apparent. However, no genetic confirmation of occurrence of *A. typica* has been available.

None of the fish in India has been reported harbouring *A. typica,* to date, which has immense zoonotic significance attached to its prevalence world over (Koie *et al*., 1995; Matos *et al*., 2001; Mello *et al*., 2011; Reis *et al*., 2003; Arizono *et al*., 2012; Mattiucci *et al*., 2013; Suzuki *et al.,* 2021). Although the surveys continued in Arabian Sea by the authors and co-workers for long, and members of Anisakidae that have the potential of transformation along evolutionary lines were recently discovered with essential constituents

Table 5. Most purchased fish species in Malappuram, Kerala.

S. No.	Fish Species	Purchased by (%)
1.	Sardine	91
2.	Mackerel	60
3.	Sole fish	20
4.	Cod fish	14
5.	Squid	8.5
6.	Prawns	8
7.	Tuna	6.5
8.	Pomfret	5.5
9.	*Tilapia*	4.5
10.	Seer fish	4
11.	Shark	3.5
12.	Malabar trevally	3
13.	Threadfin bream	2.5
14.	Clams	1.5
15.	Pearl spot	1
16.	Ribbon fish	1

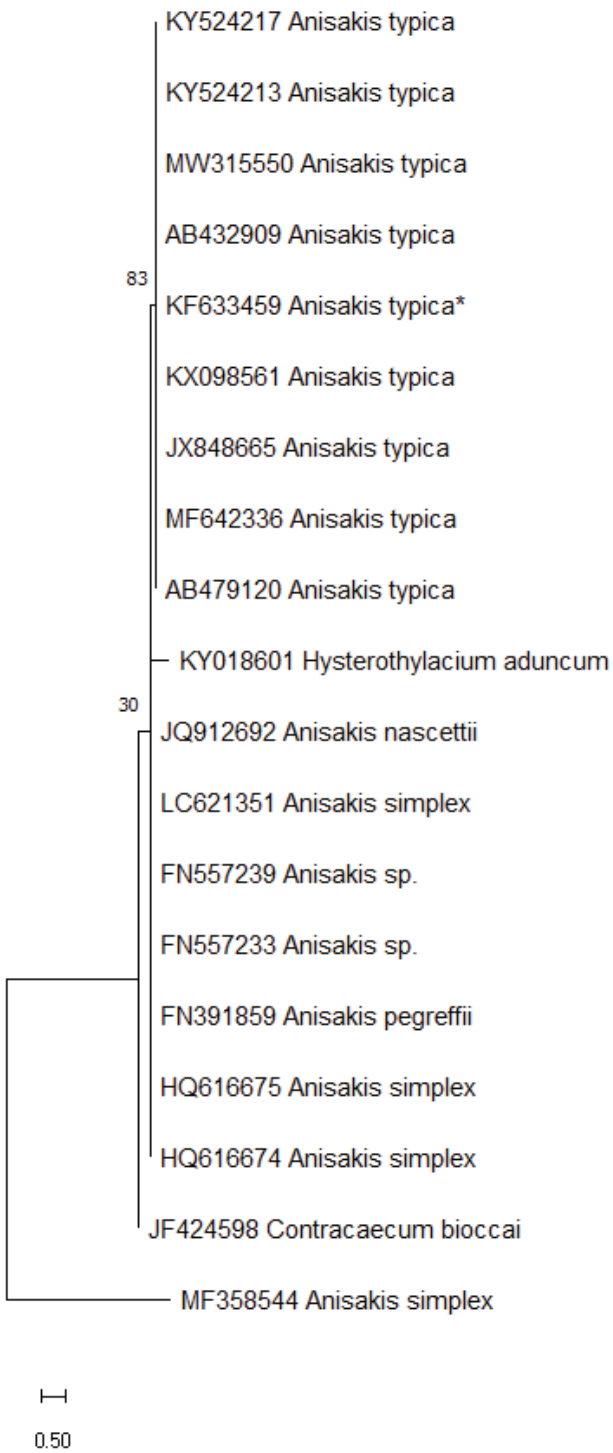

Fig. 3. Neighbour-joining tree based on nucleotide ITS1 sequences and reference species. Nucleotide ITS1 sequence data are as described in the text with GenBank accession numbers. Bootstrap values based on 1000 replicates were used. Scale bar represents an interval of the Kimura two-parameter (K2P) model. *Current investigation

of ventriculus, excretory pore, genital pore along with oral aperture being atop cephalic complex in *Rotundocollarette capoori* (Yadav *et al*., 2022), in addition to buccal tooth, and the raphidascaridoid worms, *Rostellascaris spinicaudatum* in marine fish as well as in another fish (Jaiswal *et al*., 2024) along Central west coast of India. The Indian sand whiting, *Sillago sihama* (Family: Percomorphoidea) is a significant component of coastal and estuarine fisheries of India. Parasitic fauna of this fish in Indian Ocean has been scarcely studied (Malhotra *et al*., 2012), while fish are the known target of commercialization of fresh, frozen, canned, smoked, salted or dried (Reddy, 1991; Vishal, 2024). The mainstays of the Japanese Sushi shop and eating at cheap Kaitenzushi (Conveyor belt sushi restaurants) have ensured effective encroachment into Indian feedings habits. This certainly could extend as risk to human consumption in India. Indian cuisine similar to Japanese cuisine 'Sushi', is traditionally altered for preparation with seafood (Reddy, 1991; Padiyar *et al*., 2024; Vishal, 2024), particularly Indian sand whiting or other fish or crab meat. It became dry, if overcooked, and is preferred as a substitute of pork, as explained by Wakeelah (Reddy, 1991). The dietary shift in Indian sand whiting was recorded by Reddy (1991). The fishmeal production becomes a mechanism for transmission of larval and developmental stages of nematodes to fish. Indian mackerel (*Rastrelliger* spp.) from the waters around coastal areas of other countries in Bay of Bengal, like Bangladesh, have been the common subjects of study to investigate the infesting ascaridoid worms of genus *Anisakis* (Nematoda: Anisakidae), but the Indian coastal waters were not surveyed. Instead, the reports on worms of Anisakidae infesting Indian Mackerel appeared from Indian Ocean, Southern coast of East Java and Bangladesh (Bao *et al*., 2022; Suryani *et al*., 2021). Being a coastal state and leading fish producer of the country, both fresh and dried fish are important items of Kerala diet (Tables 3, 4, 5)(Sajeev *et al*., 2021). Fish consumption per capita of 2.26Kg in rural and 2.21Kg in urban areas has been concluded in Kerala State (NSSO, 2012).

Molecular characterization

The read length of all 18S sequences was 972 bp, of all ITS1 sequences was 380 bp, and of all ITS2 sequences was 456 bp.

The larger clade of *A. typica* incorporating specimen under current investigation (KF633462) was entirely segregated in the tree based on ITS2 sequences (Table 6). The monophyletic association with the 100 % support of Boot strap value comprised KF633462, KY524217 (*p* distance, 0.00), LN651099 (*p* distance, 0.00), ON065560 (*p* distance, 0.00), JQ798962 (*p* distance, 0.00), and MT635343 (*p* distance, 0.00)(Fig. 2).

Neighbour joining tree distinctly delineated the larger clade that comprised as many as six specimens of *A. typica*, though the second clade contained 3 sub-clades, the first one comprising twin *A. nascettii* (JQ912692 and JX486104); with twin *Anisakis* spp. (MK325217 and MF768443) with bootstrap values depicting 99 % alignment, and lastly, *A. pegreffii* (FR716448); with LC745135

Table 6. Representative groups of ITS2 gene sequences selected for the study.

GenBank Accession No.	Gene	Taxon	Reference
JQ798962	ITS2	*Anisakis typica*	Borges *et al*. (2012)
MT635343	ITS2	*Anisakis typica*	Borges *et al*. (2021)
ON065560	ITS2	*Anisakis typica*	Bao et al. (2022)
LN651099	ITS2	*Anisakis typica*	Shamsi *et al*. (2015)
KF633462*	ITS2	*Anisakis typica*	Present study
KY524217	ITS2	*Anisakis typica*	Palm *et al.* (2017)
JQ912692	ITS2	*Anisakis nascettii*	Mattiucci *et al.* (2014)
FN556997	ITS2	*Anisakis* sp.	Shamsi *et al.* (2012)
FR716448	ITS2	*Anisakis pegreffii*	Shamsi (Unpublished)
LC745135	ITS2	*Anisakis simplex*	Ikebuchi (Unpublished)
LC666445	ITS2	*Anisakis* sp.	Hirata *et al.* (Unpublished)
MK325217	ITS2	*Anisakis* sp.	Shamsi *et al.* (2019)
MF768443	ITS2	*Anisakis* sp.	Quiazon *et al.* (Unpublished)
MF358544	ITS2	*Anisakis simplex*	Cipriani *et al.* (2017)
HQ616674	ITS2	*Anisakis simplex*	Meloni *et al.* (2011)
JF424598	ITS2	*Contracaecum bioccai*	D'Amelio *et al.* (Unpublished)
KY018601	ITS2	*Hysterothylacium aduncum*	Pawlak *et al.* (2018)
KY524217	ITS2	*Anisakis typica*	Palm *et al*. (2017)

*Current investigation

and LC666445 of *A. simplex* along with *Anisakis* sp. (FN556997) showing 100 % bootstrap value clustered together to form non-*A. typica* sub-clade. *Hysterothylacium aduncum* (KY018601) formed an outgroup. The monophyletic association with the 100 % support of Boot strap value comprised KF633462, KY524217 (*p* distance, 0.00), LN651099 (*p* distance, 0.00), ON065560 (*p* distance, 0.00), JQ798962 (*p* distance, 0.00), and MT635343 (*p* distance, 0.00) based on Internal Transcribed Spacer gene. Three smaller sub-clades that comprised 2, 4 and 2 specimens, respectively, confirmed divergence of nucleotides within genus *Anisakis,* with the genetic distance being *p* = 0.00 for the specimens, namely, *A. nascettii* (JQ912692); *A. pegreffii* (FR716448); *A. simplex* (LC745135); *A. simplex* (LC666445), and *p* distance, 0.313 (JX486104); 0.1774 (FN556997); *A. simplex* (MK325217), *p* distance, 0.4206; and (MF768443) *p* distance, 0.1086.

Neighbour joining tree clearly indicated two distinct clusters among specimens from different populations based on ITS1 gene (Fig. 3; Table 7). *A. typica* specimens from this study were grouped

Table 7. Representative groups of ITS1 gene sequences selected for the study.

GenBank Accession No.	Gene	Taxon	Reference
KX098561	ITS1	*Anisakis typica*	Andres *et al.* (2016)
JX848665	ITS1	*Anisakis typica*	Jabbar *et al.* (2017)
MF642336	ITS1	*Anisakis typica*	Shamsi *et al.* (2017)
AB479120	ITS1	*Anisakis typica*	Umehara *et al*. (Unpublished)
AB432909	ITS1	*Anisakis typica*	Umehara *et al*. (Unpublished)
MW315550	ITS1	*Anisakis typica*	Suthar and Shamsi (2021)
KF633459	ITS1	*Anisakis typica*	Present study*
JQ912692	ITS1	*Anisakis nascettii*	Mattiucci *et al*. (2014)
LC621351	ITS1	*Anisakis simplex*	Sato *et al.* (Unpublished)
FN557239	ITS1	*Anisakis* sp.	Shamsi (Unpublished)
FN557233	ITS1	*Anisakis* sp.	Shamsi (Unpublished)
FN391859	ITS1	*Anisakis pegreffii*	Shamsi *et al.,* (2012)
MF358544	ITS1	*Anisakis simplex*	Cipriani *et al*., (2017)
JF424598	ITS1	*Contracaecum bioccai*	D'Amelio *et al.,* (Unpublished)
HQ616674	ITS1	*Anisakis simplex*	Meloni *et al.,*(2011)
HQ616675	ITS1	*Anisakis simplex*	Meloni *et al*.,(2011)
KY018601	ITS1	*Hysterothylacium aduncum*	Pawlak *et al*.(2018)
KY524217	ITS1	*Anisakis typica*	Palm *et al.*(2017)
KY524213	ITS 1	*Anisakis typica*	Palm *et al.* (2017)

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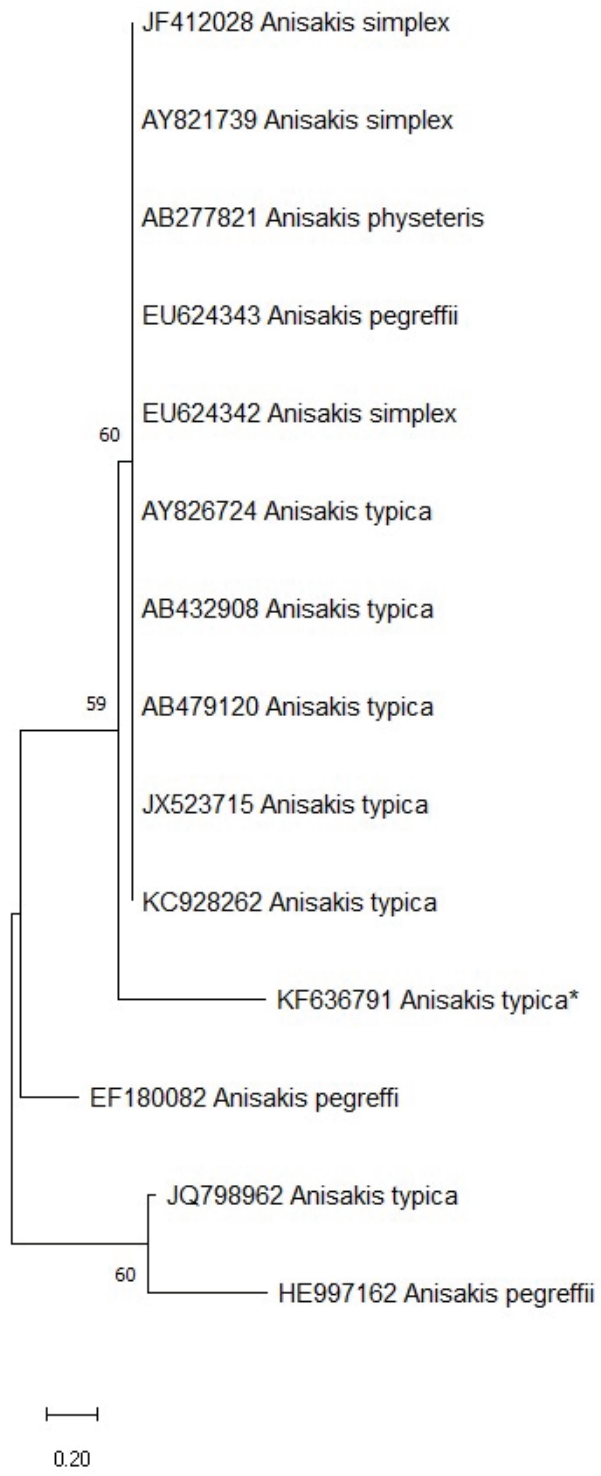

Fig. 4. Neighbour-joining tree based on nucleotide 18S rDNA sequences and reference species. Nucleotide 18S rDNA sequence data are as described in the text with GenBank accession numbers. Bootstrap values based on 1000 replicates were used. Scale two-parameter (K2P) model.
*Current investigation

at the top of the clade, with the specimens from the present study (KF633459; *p* distance 0.00) exhibiting monophyletic association with AB432909; *p* distance 0.004) and MW315550; *p* distance 0.00). Bootstrap supports were found to be 98 % for the remaining 4 specimens (AB479120; MF642336; JX848665 (*p* distance 0.0001); KX098561 (*p* distance 0.0039)) nucleotides within the same clade. The divergence of populations of *A. typica* was apparent under the remaining 5 distinct clusters from Japan (LC621351; K_2p distance, 0.035), Australia (FN557239, *p* distance, 0.008; FN557233, *p* distance, 0.008; FN391859, *p* distance 0.013) and one human being from Italy (JQ912692, *p* distance, 0.119), based on ITS1 gene. *Hysterothylacium aduncum* (KY018601) was taken as outgroup.

The monophyletic association of specimens of *A. typica* aligned closely to form major clade, based on 18S rDNA gene comprising six specimens conspecific (*p* distance= 0.0-0.026) to the worms of *A. typica* (KF636791, *p* distance= 3.438) under study (Fig. 4; Table 8). As many as six *A. typica* specimens were part of the larger clade that comprised total nine conspecific (*p* distance=0.001-0.006) along with three other specimens (*A. simplex*; *A. pegreffii* and *A. physeteris*) based on 18S rDNA gene. The twin *A. pegreffii* (HE997162) and *A. typica* (KF636791) conformed to the outgroup (*p* Distance=2.09).

Conclusions

The maiden investigation has been presented herein to record *Anisakis typica* 3rd stage larvae within the maritime zones of Indian sub-continent. Although *A. typica* was reportedly widely distributed in warmer temperate and tropical seas under influence of diverse migratory habits of their definitive fish hosts, yet these were not recorded in Indian seas earlier. The requirements of food safety would warrant strict monitoring so that the best practices could be galvanized to generate an effective safety environment in public health. The coral reef-linked associations of the marine fish coral trout *Paracamallanus areolatus* inhabiting Red Sea in Egypt highlighting these flourishing associations in foreign seas with *A. typica* brought to knowledge the coral reef-associated promotion of *A. typica* and cohabitant populations of agents of anisakiasis in Indian subcontinent in the present study. Thus the significance of intense activity of the etiological agent of the gastrointestinal zoonotic disease, anisakiasis (*viz*, *A. typica* and *R. capoori*) in the reef-associated Goan coastal areas, were noticeable. No other infections co-occurred in these reef-associated coastal fish fauna.

Author Contributions

Conceptualization, S.K.M., Collection & Investigation, N.J. & A.Y., Writing—Original Draft Preparation, S.K.M.; Writing—Review & Editing, N.J. & A.Y.; Visualization, A.Y. All authors read and approved the final version of the manuscript.

Table 8. Representative groups of 18S rDNA gene sequences selected for the study.

GenBank Accession No.	Gene	Taxon	Reference
JF412028	18S rDNA	*Anisakis simplex*	Lalle *et al*.(Unpublished)
AY821739	18S rDNA	*Anisakis simplex*	Nadler *et al.,* (2004)
AB277821	18S rDNA	*Anisakis physeteris*	Umehara *et al.,*(2008)
EU624343	18S rDNA	*Anisakis pegreffii*	Quiazon *et al.,*(2008)
EU624342	18S rDNA	*Anisakis simplex*	Quiazon *et al.,*(2008)
AY826724	18S rDNA	*Anisakis typica*	D'Amelio *et al.,*(Unpublished)
AB432908	18S rDNA	*Anisakis typica*	Umehara *et al*., (2008)
AB479120	18S rDNA	*Anisakis typica*	Umehara *et al.,* (2010)
JX523715	18S rDNA	*Anisakis typica*	Zhang *et al.,*(2013)
KC928262	18S rDNA	*Anisakis typica*	Anshary *et al.,* (2014)
KF636791*	18S rDNA	*Anisakis typica*	Jaiswal *et al.,* (2013)
EF180082	18S rDNA	*Anisakis pegreffii*	Nadler *et al.,* (2007)
JQ798962	18S rDNA	*Anisakis typica*	Borges *et al.,* (2012)
HE997162	18S rDNA	*Anisakis pegreffii*	De Luca *et al*., (Unpublished)

*Current investigation

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Conflict of Interest

Authors have no potential conflict of interest pertaining to this submission to Helminthologia whether financial or non-financial, professional, or Proceedings from a Conference.

References

Ahmed, W., Gupta, S., Mukherjee, I., Babu, V., Singh, R. (2022): Comparative studies of codon usage profile of *Anisakis simplex* (Nematoda) and *Carassius gibelio* (Prussian carp). *J Environ Biol*, 43(1): 123 – 132. DOI: 10.22438/eb/43/1/MRN-1845

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. DOI: 10.1016/S0022-2836(05)80360-2

Arizono, N., Yamada, M., Tegoshi, T., Yoshikawa, M. (2012): *Anisakis simplex sensu stricto* and *Anisakis pegreffii*: biological characteristics and pathogenetic potential in human anisakiasis. *Foodborne Pathog Dis,* 9(6): 517 – 521. DOI: 10.1089/fpd.2011.1076

Baird, F.J., Gasser, R.B., Jabbar, A., Lopata, A.L. (2014): Foodborne anisakiasis and allergy. *Mol Cell Probes,* 28(4): 167 – 174. DOI: 10.1016/j.mcp.2014.02.003

Bao, M., Cipriani, P., Giulietti, L., Alam, M.A., Palomba, M., Mattiucci, S., Levsen, A. (2015): *Anisakis* infection in allis shad, *Alosa alosa* (Linnaeus, 1758), and twaite shad, *Alosa fallax* (Lacépède, 1803), from Western Iberian Peninsula Rivers: zoonotic and ecological implications. *Parasitol Res,* 114: 2143 – 2154. DOI: 10.1007/s00436-015-4403-5

Bao, M., Cipriani, P., Giulietti, L., Alam, M.A., Palomba, M., Mattiucci, S., Levsen, A. (2022): Ascaridoid nematodes infecting commercially important marine fish and squid species from Bangladesh waters in the Bay of Bengal. *Food Waterborne Parasitol*, 27: e99517. DOI: 10.1016/j.fawpar.2022.e00157

Borges, J.N., Cunha, L.F., Santos, H.L., Monteiro-Neto, C., Santos, C.P. (2012): Morphological and Molecular Diagnosis of Anisakid Nematode Larvae from Cutlassfish (*Trichiurus lepturus*) off the Coast of Rio de Janeiro, Brazil. *PLoS One,* 7(7): e40447. DOI: 10.1371/journal.pone.0040447

Bruce, N.L., Cannon, L.R.G. (1990): Ascaridoid nematodes from sharks from Australia and the Solomon Islands, Southwestern Pacific Ocean. *Invertebr Syst,* 4: 763 – 783. DOI: 10.1071/IT9900763

Chaiphongpachara, T., Adisakwattana, P., Suwandittakul, N. (2022): Intraspecific Genetic Variation of *Anisakis typica* in Indian Mackerel caught from the Gulf of Thailand, Samut Songkhram Province. *ScientificWorldJournal,* 2022: 2122619. DOI: 10.1155/2022/2122619

Coomans, A. (2002): Present status and future of nematode systematics. *Nematology*, 4: 573 – 582

Della-Morte, D., Ambrosi, C., Chiereghin, F.M., Pastore, I.D., Pacifici, F., Scaramella, M., Gentile, L., Mulas, F., Quintavall, E.G. (2023): Methods for inactivation of seafood *Anisakis* larvae and prevention of human anisakiasis: a mini-review. *Eur Rev Med Pharm Sci,* 27: 5246 – 5256. DOI: 10.26355/eurrev_202306_32643

De Ley, P., Blaxter, M. (2002): Systematic position and phylogeny. In: Lee, B.L. (Ed), *The Biology of Nematodes.* Taylor & Francis, New York: pp. 1 – 30

Felsenstein, J. (1985): Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* 39: 783 – 791. DOI: 10.2307/2408678

Floyd, R.M., Rogers, A.D.J., Lambshead, P.J.D., Smith, C.R. (2005): Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Mol Ecol Notes,* 5: 611 – 612. DOI: 10.1111/j.1471-8286.2005.01009.x

Guchhait, A., Sanyal, K.B., Mukherjee, D., Dash, G. (2018): Preva-

lence of Helminth Infestation in *Clarias batrachus* (Linnaeus, 1758) with Season and Size Variation in West Bengal, India. *Int J Ecol Environ Sci*, 44 (4): 395 – 401

Hodda, M. (2022): Phylum Nematoda: a classification, catalogue and index of valid genera, with a census of valid species. *Zootaxa*, 5114 (1): 1 – 289. DOI: 10.11646/zootaxa.5114.1.1

Jeon, C.H., Kim, J.H. (2015): Pathogenic potential of two sibling species, *Anisakis simplex* (s.s.) and *Anisakis pegreffii* (Nematoda: Anisakidae): *In vitro* and *in vivo* studies. *Biomed Res Int*, 2015: 983656. DOI: 10.1155/2015/983656

Kalaiselvi, N. (2024): *Dr N Kalaiselvi, DG CSIR, Visits CSIR-NIScPR. CSIR India CSIR – Natl. Instt. Oceanogr*. Unveiled the Coral Reef Monitoring and Surveillance Robot, C-Bot, developed by the Natl. Instt. Oceanogr., 1p.

Kimura, M.A. (1980): A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol,* 16(2): 111 – 120. DOI: 10.1007/BF01731581

Køie, M., Berland, B., Burt, M.D.B. (1995): Development to third-stage larvae occurs in the eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). *Can J Fish Aquat Sci*, 52: 134 – 139. DOI: 10.1139/f95-519

Lakshmi, R.I. (1993): *Hysterothylacium kiranii* n. sp. (Nematoda: Anisakidae) from the body cavity of *Channa* (*Ophiocephalus*) *punctatus*. *Indian J Parasit,* 16: 83

Lakshmi, R.I., Rao, H.K., Shyamasundari, K. (1993a): *Hysterothylacium carutti* n. sp. (Nematoda: Anisakidae) from the marine fish *Johnius carutta* Bloch of Bay of Bengal, Visakhapatnam. *Uttar Pradesh J Zool*, 13: 1 – 10

Lakshmi, R.I., Rao, H.K., Shyamasundari, K. (1993b): *Hysterothylacium longicaecum* n. sp. (Nematoda: Anisakidae) from the intestine and stomach of freshwater fishes of Visakhapatnam. *J Parasitol Appl Anim Biol*, 2: 1 – 5

Leuckart, R. (1876): *Die menschlichen Parasiten und die von ihren herriihrend Krankheiten* [*Human parasites and the diseases they cause*]. Leipzig, 2 Part 3: 513 – 882 (In German)

Liang, K.L., O'Connor, C., Veiga, J.P., McCarthy, T.V., Keeshan, K. (2016): TRIB2 regulates normal and stress-induced thymocyte proliferation. *Cell Disc*. 2: 15050. DOI: 10.1038/celldisc.2015.50

Malhotra, A., Jaiswal, N., Malakar, A.K., Verma, M.S., Singh, H.R., Lakra, W.S., Malhotra, Sandeep K., Shamsi, S. (2012): The morphology and genetic characterization of *Iheringascaris goai* n. sp. (Nematoda: Raphidascarididae) from the intestine of the silver whiting and spotted catfish off the central west coast of India. *J Helminthol,* 86(3): 353 – 362. DOI: 10.1017/S0022149X11000472

Matos, E.R., Corral, L., Matos, P., Casa, G., Azevedo, C. (2001): Incidência de parasitas do Phylum Myxozoa (Sub-reino Protozoa) em peixes da região amazônica, com especial destaque para o gênero *Henneguya* [Icthyoparasitism of the phylum Myxozoa on fishes of the Amazon basin]. *Rev Cienc Agrar,* 36: 83 – 99. DOI: 10.1590/S1413-95962002000500010 (In Portuguese)

Mattiucci, S., Fazii, P., Rosa, A.D., Paoletti, M., Megna, A.S., Glielmo, A., Angelis, M.D., 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(3): 496 – 499. DOI: 10.3201/eid1903.121017

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. *Adv Parasitol*, 66: 47 – 148. DOI: 10.1016/S0065-308X(08)00202-9

Melo, M., De, F., De, C. (2011): *Raphidascaris* (*Sprentascaris*) *lanfrediae* sp. nov. (Nematoda : Anisakidae) from the fish *Satanoperca jurupari* (Osteichthyes: Cichlidae). *Mem Inst Oswaldo Cruz*, 106(5): 553 – 556. DOI: 10.1590/s0074-02762011000500006

Nieuwenhuizen, N.E., Lopata, A.L. (2013): *Anisakis* - a food-borne parasite that triggers allergic host defences. *Int J Parasit,* 43: 1047 – 1057. DOI: 10.1016/j.i.para.2013.08.01

NSSO (2012): *Household consumption of various goods and services in India* (*Rep No*: 541). Government of India. Retrieved from https://mospi.gov.in/sites/default/files/publication_reports/nss_report_541.pdf.

Padiyar, P.A., Dubey, S.K., Bayan, B., Mohan, C.V., Belton, B., Jena, J., Susheela, M., Murthy, L.N., Karthikeyan, M., Murthy, C.K. (2024): *Fish consumption in India: Patterns and trends*. New Delhi, India: WorldFish. Retrieved from https://digitalarchive.worldfishcenter.org/items/0a406686-8aae-4054-bc5e-d81216343ed0.

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(1): 3 – 12. DOI: 10.2478/s11687-008-0001-8

Pampiglione, S., Rivasi, F., Criscuolo, M., Brindisi, A.S.L., Beneditis, A.C., Villan, M., Gentile, A., Russo, S., Testini, M. (2002): Human anisakiasis in Italy: a report of eleven new cases. *Path Res Prac*,198: 429 – 434. DOI: 10.1078/0344-0338-00277

Quiazon, K.M., Yoshinaga, T., Ogawa, K., Yukami, R. (2008): Morphological differences between larvae and in vitro-cultured adults of *Anisakis simplex* (*sensu stricto*) and *Anisakis pegreffii* (Nematoda: Anisakidae). *Parasitol Int*, 57: 483 – 489. DOI: 10.1016/j.parint.2008.06.003

Rahamati, A.R., 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. *Parasitol Res,* 119(11): 3585 – 3594. DOI: 10.1007/s.00436-020-06892-0

Rajyalakshmi, I. (2005): A new nematode *Hysterothylacium aetobatum* from ray fish, *Aetobatus narinari* (Euphrasen). *Parasitol Latinoamer,* 60: 170 – 173. DOI: 10.4067/S0717-77122005000200012

Reddy, C.R. (1991): *Some Biological Aspect of Sillago sihama from Karwar Waters*. Ph. D. Thesis: the Karnataka University.

Reis, E.C., Roth, E.K., Syphan, J.L., Tarbell, S.E., Holubkov, R. (2003): Effective pain reduction for multiple immunization in-

jections in young infants. *Arch Pediatr Adolesc Med*, 157(11): 1115 – 1120. DOI: 10. 1001/archpedi.157.11.1115

Roca-Geronèsa, X., Segoviab, M., Godínez-Gonzáleza, C., Fisaa, R., Montoliua, I. (2020): *Anisakis* and *Hysterothylacium* species in Mediterranean and North-East Atlantic fishes commonly consumed in Spain: Epidemiological, molecular and morphometric discriminant analysis. *Int J Food Microb*, 325: 108 – 642. DOI: 10.1016/j.ijfoodmicro.2020.108642

Ruchi, S., Patricia, F. (2023): Identification of *Anisakis* Spp. Infestation and its Effect in Greater Lizardfish (*Saurida tumbil*). *Int J Zool Invest*, 9(2): 79 – 85. DOI: 10.33745/ijzi.2023.v09i02.008.

Saitou, N., Nei, M. (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, 4(4): 406 – 425. DOI: 10.1093/oxfordjournals.molbev.a040454

Sajeev, M.V., Ramesh, T.J., Chetan, N., Gopika, R. (2021): Factors Influencing Fish Purchase and Consumption Behaviour of Koraga and Soliga Tribes, Karnataka, India*. Indian J Ext Edu*, 59(4): 86 – 89. DOI: 10.48165/IJEE.2023.59418

Setyobudi, E., Jeon, C.H., Lee, C.H., Seong, K.B., Kim, J.H. (2011): Occurrence and identification of *Anisakis* spp. (Nematoda : Anisakidae) isolated from *Oncorhynchus keta* in Korea. *Parasitol Res,* 10: 585 – 592. DOI: 10.1007/s00436-010-2101-x

Shamsi, S. (2021): The occurrence of *Anisakis* spp. in Australian waters: past, present, and future trends. *Parasitol Res,* 120(9): 3007 – 3033. DOI: 10.1007/s00436t-021-07243-3

Shamsi, S., Barton, D.P. (2023): A critical review of anisakidosis cases occurring globally. *Parasitol Res*, 122(8): 1 – 13. DOI: 10.1007/S00436-023-07881-9

Smith, J.W., Wootten, R. (1978): *Anisakis* and anisakiasis. *Adv Parasitol,* 16: 93 – 163. DOI: 10.1016/s0065-308x(08)60573-4

Suryani, N., Subekti, S., Koesdarto, S., Amiin, M.K. (2021): Morphological profile of L2 *Anisakis typica* on Indian Mackerel (*Rastrelliger kanagurta*) from Sedati Fish Auction, Sidoarjo-East Java, Indonesia using Scanning Electron Microscope (SEM). *IOP Conf. Ser.: Earth Environ. Sci,* 679(1): 012059. DOI: 10.1008/1755-1315/679/1/012059

Suzuki, J., Murata, R., Kodo, Y. (2021): Current status of Anisakiasis and *Anisakis* larvae in Tokyo, Japan. *Food Saf* (Tokyo). 9(4): 89 – 100. DOI: 10.14252/foodsafetyfscj.D-21-00004

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S.(2011): MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*, 28(10): 2731 – 2739. DOI: 10.1093/molbev/msr121

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgens, D.G. (1997): The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl Acids Res,* 25(24): 4876 – 4882. DOI: 10.1093/nar/25.24.4876

Van Thiel, P.H., Kuipers, F.C., Roskam, R.T. (1960): A nematode parasitic to her ring, causing acute abdominal syndromes in man. *Trop Geog Med*, 2: 97 – 113

Van Thiel, P.H. (1962): Anisakiasis. *Parasitology,* 52: 16 – 17

Vishal, A. (2024): Ramen in Ludhiana, sushi in Kothrud. *Forbes India*, Retrieved from https://www.forbesindia.com/article/lifes/ramen-in-ludhiana-sushi-in-kothrud/91515/1.

World Health Organization (2004): *Report of Joint WHO/FAO workshop on food-borne trematode infections in Asia, Ha Noi, Vietnam, 26 – 28 November, 2002*. WHO, WPRO: 1 – 58

Yadav, A., Kapoor, N., Malhotra, Sandeep, K. (2022): Faunal change over 3 decades to reveal *Rotundocollarette capoori* n. gen., n.sp., a unique anisakid from *Johnius dussumieri* from the Central west coast of India at Goa. *J Parasit Dis,* 46: 1110 – 1126. DOI: 10.1007/s12639-022-01533-y

Yann, C.F. (2006): *Molecular and biological characterization of anisakids in Western Australia.* B.Sc. Honours Thesis, Murdoch, Australia: Murdoch University.