

# First morphological and molecular identification of third-stage larvae of *Anisakis typica* (Nematoda: Anisakidae) from marine fishes in Vietnamese water

Hoang Van Hien<sup>1</sup>, Bui Thi Dung<sup>1</sup>,  
Ha Duy Ngo<sup>1</sup> and Pham Ngoc  
Doanh<sup>1,2,\*</sup>

<sup>1</sup>Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

<sup>2</sup>Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

\*E-mail: pndoanh@yahoo.com

This paper was edited by Zafar Ahmad Handoo.

Received for publication November 24, 2020.

## Abstract

Anisakid nematodes are parasites of cetaceans, their larval stages live in marine fishes. The third-stage larvae of some *Anisakis* species are also the etiological agents of human anisakiasis caused by consumption of raw or undercooked infected fish. Thus, identification of *Anisakis* larvae at the species level is crucial for their ecology and epidemiology. In Vietnam, although *Anisakis* larvae have been reported, they have not been identified to the species level. The aim of this study was, therefore, to identify third-stage larvae of *Anisakis* collected from marine fishes in Vietnamese water, based on morphological characteristics and molecular analysis. All *Anisakis* larvae found in this study were morphologically similar to each other and identical to *Anisakis typica*. In addition, molecular analysis based on ITS1-5.8S-ITS2 sequences confirmed them as *A. typica*. Vietnamese *A. typica* population was genetically close to those from Asian countries and Australia. The third-stage larvae of *A. typica* were collected from eight fish species from three localities in the South of Vietnam. Among them, seven were recorded as new intermediate hosts of *A. typica*. This is the first identification of *A. typica* larvae in Vietnamese water with records of new fish hosts.

## Keywords

Anisakid larvae, Intermediate fish host, Molecular analyzes, Morphology, Vietnam.

Nematodes of the genus *Anisakis* (Nematoda: Anisakidae) are parasites of marine organisms. The life cycle of these nematodes requires marine mammals, mainly cetaceans, as the definitive hosts, and crustaceans, fish, and cephalopods as intermediate/paratenic hosts (Klimpel and Palm, 2011). Humans are accidental hosts due to ingestion of raw or undercooked fish containing the third infective-stage larvae (L3). Human anisakiasis patients suffer from abdominal pain, nausea, vomiting, and/or diarrhea (Dorny et al., 2009). In addition, allergic reactions may occur due to exposure to the nematode antigens (Aibinu et al., 2019; Audicana et al., 2002). Given the influence on human health, *Anisakis* nematodes are

of interest. Anisakid larvae can be morphologically identified at the genus level by typical characteristics of anterior and posterior regions, and are classified into two types, type I and II, based on the length of the ventriculus and presence/absence of the tail spine (mucron): *Anisakis* type I larva has a longer ventriculus and a mucron, while type II larva has a shorter ventriculus and no mucron (Berland, 1961). Type I consists of *A. simplex*, *A. pegreffii*, *A. typica*, *A. ziphidarum*, and *A. nascettii*, while type II consists of *A. paggiae*, *A. physeteris*, and *A. brevispiculata* (Mattiucci and Nascetti, 2008). Previously, it was not easy to identify anisakid larvae at the species level, because there is a lack of distinct morphological characteristics

required for species identification (Farjallah et al., 2008). Recent studies indicated morphological differences between *Anisakis* species (Chen and Shih, 2015; Sonko et al., 2019; Tunya et al., 2020). In addition, molecular tools allow the accurate identification of anisakid larvae by using sequences of the internal transcribed spacer (ITS) region of ribosomal DNA (D'amelio et al., 2010; Mattiucci and Nascetti, 2008).

In Vietnam, data on *Anisakis* nematodes are scarce. There have been a few reports on *Anisakis* larvae without morphological description and identification to species level (Arthur and Te, 2006; Ngo et al., 2009). During our recent comprehensive survey for parasites of marine fishes in Vietnamese water, we collected *Anisakis* larvae from eight fish species. The aim of the present study was to identify these *Anisakis* specimens from Vietnamese water by morphological and molecular approaches.

## Materials and methods

### Fish examination and larval collection

Marine fish were bought in 10 fish ports located in 10 provinces along the seashore of Vietnam where fishing vessels docked (Fig. 1). All fish specimens were placed on ice and transferred to the laboratory under good aeration. Fish were dissected, their body cavities and internal organs were examined under a stereomicroscope. Third-stage larvae were isolated from the body cavity and visceral organs. The larvae were washed in phosphate-buffered saline. For morphological identification, larvae were preserved in 4% formalin. Representative specimens were preserved in 70% ethanol for DNA isolation.

### Morphological study

*Anisakis* larvae were soaked in a solution of glycerin-phenol-lactic acid-distilled water (2:1:1:1) for about 48 hr until the body parts were transparent. Then, the larvae were observed and measured under a light microscope (ECLIPSE H600 L Nikon). For scanning electron microscopy, *Anisakis* larvae were prepared according to Madden and Tromba (1976) and Morsy et al. (2017). Larvae were identified according to the reported references (Berland, 1961; Chen and Shih, 2015; Mattiucci et al., 2009; Sonko et al., 2019; Tunya et al., 2020).

### Molecular and phylogenetic analysis

DNA of three representative larvae from three localities was extracted using QIAamp DNA stool

Minikit (Qiagen, Hilden, Germany). Two primers NC5-GTAGGTGAACCTGCGGAAGGATCATT (forward) and NC2-TTAGTTTCTTTTCCTCCGCT (reverse) were used in a polymerase chain reaction (PCR) to amplify the rDNA region of the first to the second internal transcribed spacer (ITS1-5.8S-ITS2) (Zhu et al., 2000). PCR products were electrophoresed in a 1.0% agarose gel and visualized by ethidium bromide staining. Positive PCR products were sent to Macrogen Company (Korea) for sequencing. The nucleotide sequences obtained in this study were deposited in GenBank under accession numbers LC592876-LC592878.

BLAST searches were performed at NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to find sequence similarities. Sequences of *Anisakis* species available in GenBank were downloaded for analysis. The analysis involved 42 nucleotide sequences, including a sequence (KM491173) of *Contraeaecum osculatum* as an out-group. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model in MEGA software v.7.0. (Kumar et al., 2016). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter=0.8044)). All positions containing gaps and missing data were eliminated. There were a total of 656 positions in the final dataset.

## Results and discussion

A total of 3,775 fish of 138 species from 10 study sites were examined. *Anisakis* larvae were found from eight fish species from three localities, Khanh Hoa, Vung Tau, and Bac Lieu, in the South of Vietnam. Prevalences of infection ranged from 10 to 50% with intensity varied from 1 to 19 larvae/fish (Table 1), non-infected fish species were presented in the supplemental material.

All *Anisakis* larvae were morphologically similar to each other. The body of the larvae was cylindrical in shape, attenuated at both ends, and measured 17.2 to 20.3 (18.6 ± 1.1) mm long and 0.26 to 0.34 (0.29 ± 0.03) mm width ( $n=30$  larvae). The lips were inconspicuous, with a prominent boring tooth at the anterior extremity. The esophagus had an anterior muscular part and measured 1.52 to 1.58 (1.54 ± 0.02) mm long and a glandular ventriculus measured 0.58 to 0.82 (0.64 ± 0.04) mm long. Long intestinal caeca with clear demarcation were present. The body of

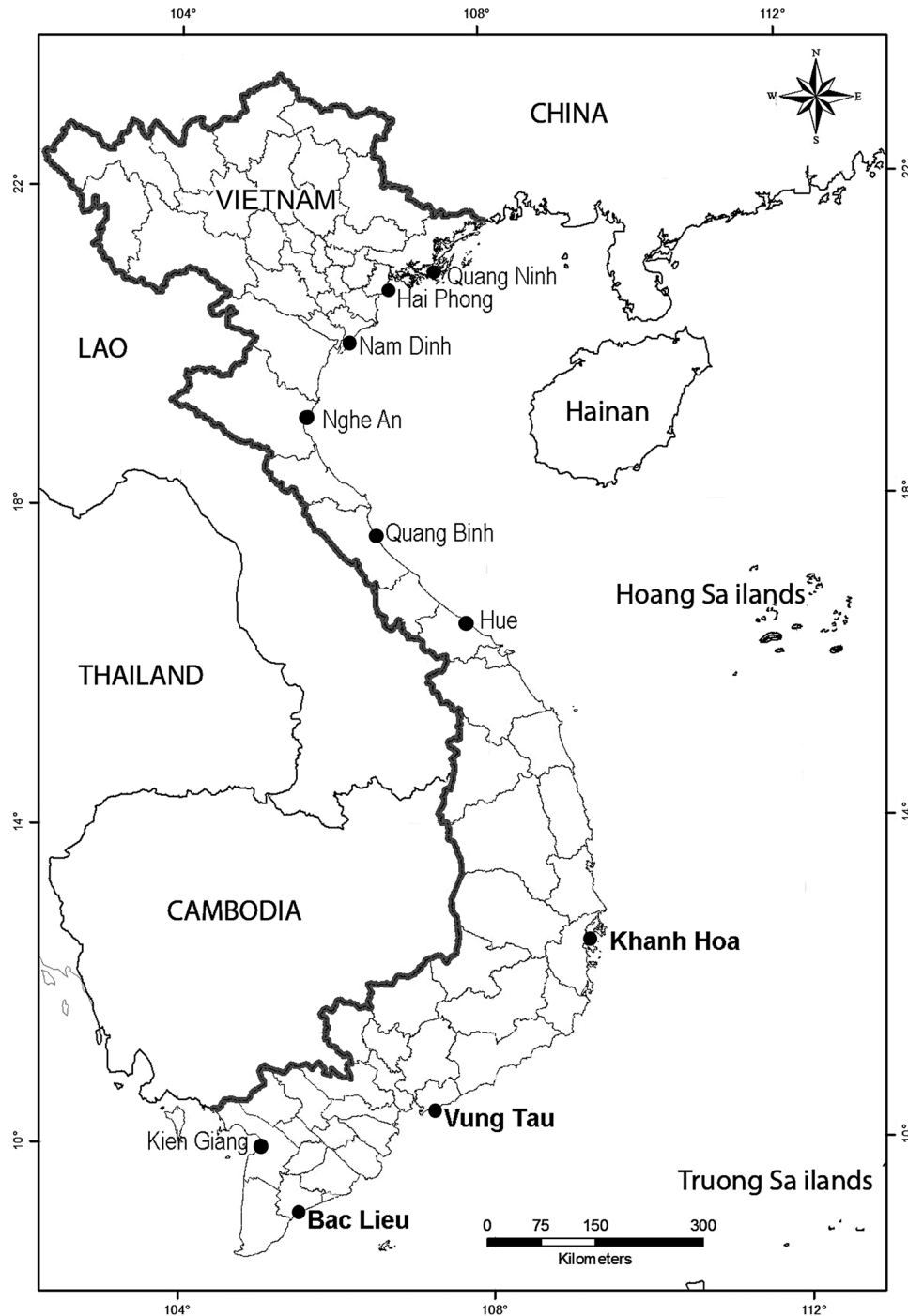


Figure 1: Study sites along the seashore of Vietnam. Three localities where fishes were infected with *Anisakis* larvae are print in bold.

larvae ended at a short cylindrical mucron measuring 0.021 to 0.030 ( $0.025 \pm 0.005$ ) mm long (Figs. 2, 3). These characteristics of the third-stage larvae were identical to *Anisakis* larvae type I (Berland, 1961). It has previously been noted that it is difficult to distinguish between *Anisakis* species belonging to

type I because they look quite similar to each other (Farjallah et al., 2008). However, recent studies based on morphological and molecular approaches provided descriptions and microphotographs showing differences between L3 larvae of *A. pegreffii* and *A. typica* (Chen and Shih, 2015; Sonko et al., 2019).

Table 1. Prevalence of *Anisakis* larvae infection in marine fishes in Vietnamese water.

Locality	No. of fish examined	No. of fish species	Infected fish species	No. of infected/ examined fish (%)	Density
Quang Ninh	615	62	0		
Hai Phong	478	41	0		
Nam Dinh	122	23	0		
Nghe An	303	50	0		
Quang Binh	520	75	0		
Hue	211	28	0		
Khanh Hoa	766	82	<i>Dcapterus macarellus</i>	10/20 (50.0)	1-19
			<i>Trichiurus lepturus</i>	6/20 (30.0)	1-5
			<i>Sargocentron rubrum</i>	1/10 (10.0)	1
			<i>Lutjanus johnii</i>	1/10 (10.0)	1
			<i>Megalaspis cordyla</i>	2/12 (16.7)	1; 3
			<i>Priacanthus hamrur</i>	1/8 (12.5)	2
			<i>Pristipomoides filamentosus</i>	3/10 (30.0)	1; 1; 1
Vung Tau	40	6	<i>Megalaspis cordyla</i>	1/5 (20.0)	6
Bac Lieu	390	58	<i>Carangoides malabaricus</i>	1/10 (10.0)	1
Kien Giang	330	63	0		
Total	3775	138	8		1-19

In addition, Tunya et al. (2020) suggested that the protruded mucron of L3 larvae can be used to identify anisakid larvae at the species level: the protruded mucron of *A. simplex* was cone-shape, while that of *A. typica* was cylindrical-shape, which is narrower and longer than that of *A. simplex*. According to these, L3 larvae of *Anisakis* specimens found in this study were identified as *A. typica*.

Because the *Anisakis* larvae collected in the present study were all morphologically similar to each other, three larvae representative for three locations were used for molecular analyses. Three ITS1-5.8S-ITS2 sequences obtained from three L3 larvae were 771 bp and completely identical (100%) with each other. In agreement with morphological identification, the BLAST searches revealed that the ITS1-5.8S-ITS2 sequences of *Anisakis* larvae from Vietnam showed the highest similarity (100%) with that of *A. typica* available in GenBank. The analysis of genetic distances demonstrated that inter-specific genetic distances between *A. typica* and other *Anisakis* species were: *A. paggiae* 17.2%; *A. ziphidarum* 17.3%; *A. pegreffii* 18.0%; *A. simplex* 18.0%; *A. physeteris*

18.4%; and *A. brevispiculata* 18.7%. In the phylogenetic tree (Fig. 4), *A. typica* made a distinct clade that was far distant from other *Anisakis* species. Vietnamese *A. typica* were genetically close to those from China, Thailand, Indonesia, Papua New Guinea, and Australia, to make a common group that was separated from another group of America, Brazil, Turkey, and Portugal. Our analysis is in agreement with a previous report that the separation of *A. typica* populations related to geographical origins (Tunya et al., 2020).

Molecular studies of *Anisakis* from various parts of the world Oceans confirmed the validity of nine *Anisakis* species (Klimpel and Palm, 2011). Most of them distribute in the Atlantic and the Mediterranean Sea with several records of some species in the South American, African, and Australian sea, and SW Pacific Ocean. A unique distribution pattern has been known for *A. typica* which has been reported in warmer temperate and tropical waters (Mattiucci and Nascetti, 2006). In Asian countries, *A. typica* larvae have been reported in Japan, Korea, China, Taiwan, Indonesia, and Thailand (Lee et al., 2016; Palm et al., 2008, 2017; Sonko et al., 2019; Tunya et al., 2020; Umehara et al., 2010;

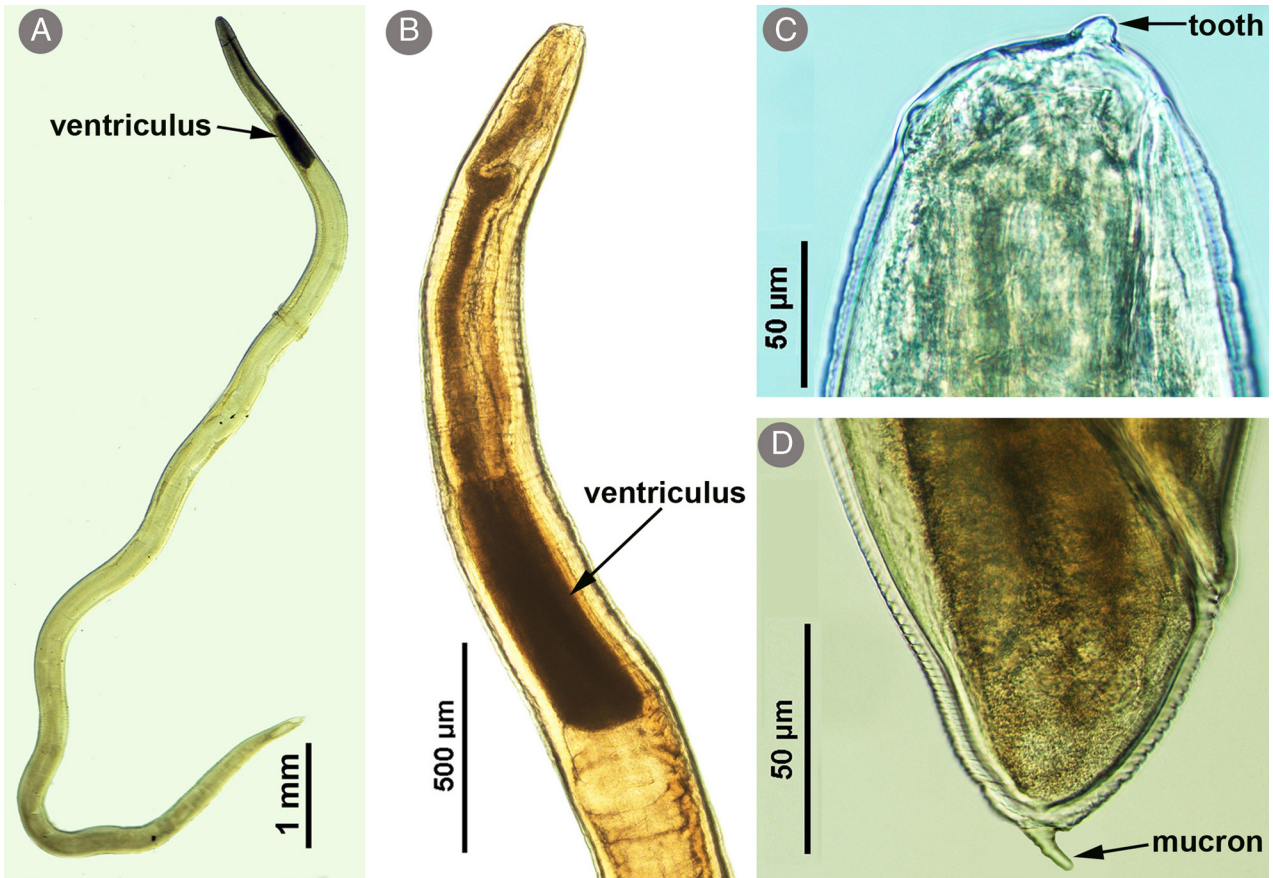


Figure 2: Light micrographs of *Anisakis typica* larva. A. Whole larva; B. Anterior part of the body showing a long ventriculus; C. Anterior part of the body showing a boring tooth; D. Posterior end of the body showing a mucron.

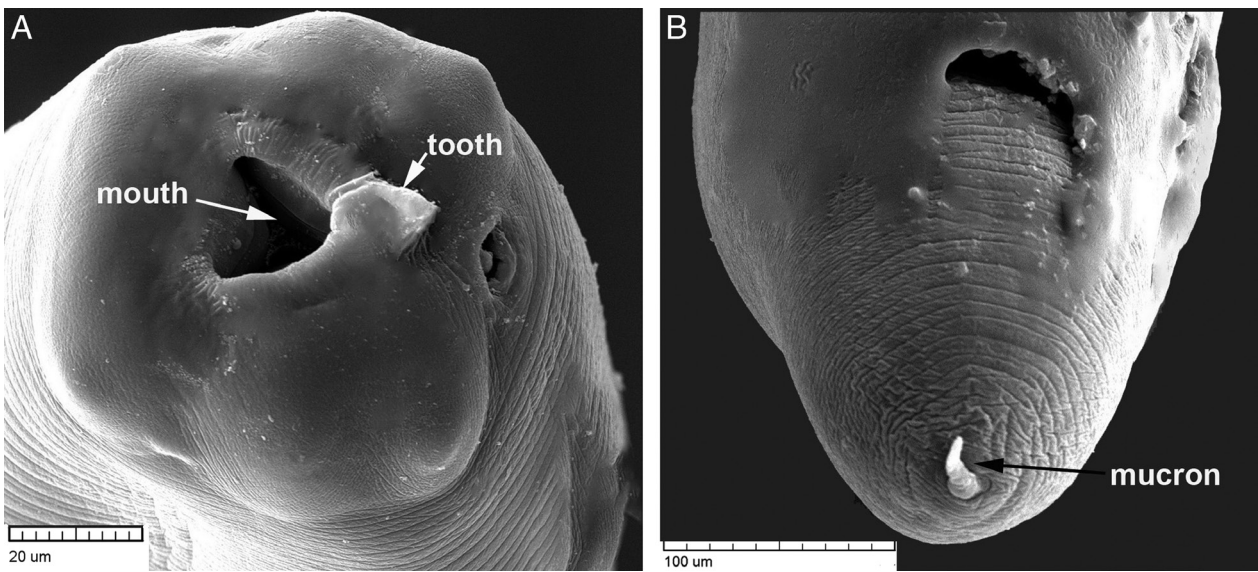


Figure 3: Scanning electron micrographs of *Anisakis typica* larva. A. Anterior end showing a mouth and a boring tooth; B. Posterior end showing a mucron.

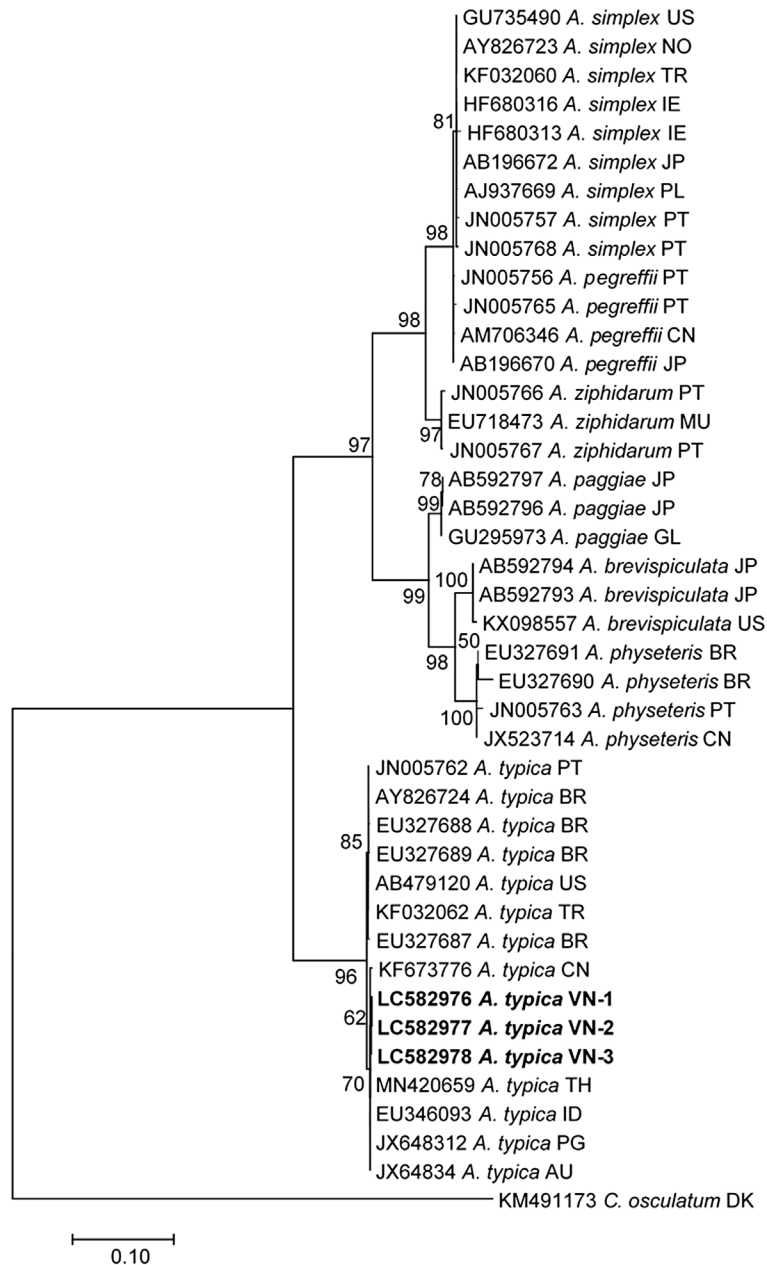


Figure 4: Phylogenetic tree reconstructed from ITS1-5.8S-ITS2 sequences of *Anisakis typica* from Vietnam and other *Anisakis* species. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap values are shown above the nodes. The nucleotide sequences obtained in this study are printed in bold, and others from the GenBank database are shown with Accession No., species name, and two letter country code of their geographical origin (AU: Australia, BR: Brazil, CN: China, DK: Denmark, GL: Greenland, IN: Indonesia, IE: Ireland, JP: Japan, MU: Mauritius, NO: Norway, PG: Papua New Guinea, PL: Poland, PT: Portugal, TH: Thailand, TR: Turkey, US: United States of America, VN: Vietnam).

Zhu et al., 2007). In the present study, we firstly identified *A. typica* larvae in Vietnamese water. Although a limited number of larval samples were molecularly analyzed, we speculated, based on their

morphological similarity, that the *Anisakis* larvae found in this study are all *A. typica*. It is highly possible that *A. typica* is the most dominant species or the only *Anisakis* species in the South of Vietnamese water

Table 2. Intermediate fish hosts of *Anisakis typica* in the World and in Vietnam.

No.	Host species	Localities	References
1	<i>Sotalia guianensis</i>		
2	<i>Auxis thazard</i>		
3	<i>Thunnus thynnus</i>	Brazil Coast	
4	<i>Pseudoperca numida</i>		
5	<i>Trachurus picturatus</i>		
6	<i>Scomber japonicus</i>	Portugal	Mattiucci et al. (2002), Marques et al. (2006), Pantoja et al. (2015)
7	<i>Platichthys flesus</i>		
8	<i>Scomberomorus commerson</i>		
9	<i>Euthynnus affinis</i>		
10	<i>Sarda orientalis</i>	Somalia	
11	<i>Coryphaena hippurus</i>		
12	<i>Stenella attenuata</i>		
13	<i>Globicephala macrorhynchus</i>	Florida	Mattiucci et al. (2005)
14	<i>Scomber scombrus</i>		
15	<i>Merluccius merluccius</i>	North Africa	Farjallah et al. (2008)
16	<i>Phycis phycis</i>		
	<i>Scomber japonicus</i>	Turkey	Pekmezci et al. (2014)
17	<i>Micromesistius poutassou</i>		
	<i>Trichiurus</i> spp.	Japan	Umehara et al. (2010)
	<i>Scomber japonicus</i>		Suzuki et al. (2010)
18	<i>Trichiurus lepturus</i>	Korea	Lee et al. (2009)
19	<i>Todarodes pacificus</i>		
20	<i>Astroconger myriaster</i>		Cho et al. (2015)
21	<i>Decapterus macarellus</i>		
22	<i>Gerres oblongus</i>		
23	<i>Pinjalo lewisi</i>		
24	<i>Pinjalo pinjalo</i>	Papua New Guinea	Koinari et al. (2013)
25	<i>Selar crumenophthalmus</i>		
26	<i>Scomberomorus maculatus</i>		
27	<i>Thunnus albacares</i>		
28	<i>Auxis rochei rochei</i>	Indonexia	Palm et al. (2008)
29	<i>Decapterus russelli</i>		
30	<i>Nemipterus hexodon</i>	Thailand	Tunya et al. (2020)
31	<i>Nemipterus japonicus</i>		
32	<i>Scomber australasicus</i>	Taiwan	Umehara et al. (2010), Sonko et al. (2019)
	<i>Trichiurus lepturus</i>		
1	<i>Carangoides malabaricus</i>		
2	<i>Dcapterus macarellus</i>		

3	<i>Sargocentron rubrum</i>		
4	<i>Lutjanus johnii</i>	Vietnam	In this study
5	<i>Megalaspis cordyla</i>		
6	<i>Priacanthus hamrur</i>		
7	<i>Pristipomoides filamentosus</i>		
	<i>Trichiurus lepturus</i>		

similar to reports in Thailand water where *A. typica* was the only species found in the Gulf of Thailand (Eamsobhana et al., 2018; Tunya et al., 2020).

Regarding intermediate fish hosts, the third-stage larvae of *A. typica* are found in various fish species. They differ from place to place depending on geographical locations. Outside of Vietnamese water, 32 fish species have been reported as intermediate hosts of *A. typica* (Table 2). In this study in Vietnam, *A. typica* larvae were found from eight fish species, *Carangoides malabaricus*, *Dcapterus macarellus*, *Sargocentron rubrum*, *Lutjanus johnii*, *Megalaspis cordyla*, *Priacanthus hamrur*, *Pristipomoides filamentosus*, and *Trichiurus lepturus*. Among these, only *T. lepturus* has been previously reported as an intermediate host of *A. typica* in Korean and Taiwanese waters, the other seven species are reported as new hosts.

## Conclusion

The present study firstly identified *A. typica* larvae, based on morphological characteristics and molecular analysis, from eight marine fish species in the South of Vietnamese water and recorded seven fish species as new intermediate hosts of this *Anisakis* nematode. Genetically, the ITS1-5.8S-ITS2 sequences of Vietnamese *A. typica* were close to those from Asian countries and Australia, to make a common group separated from another group from America and Europe.

## Acknowledgments

This study was supported by National Program under code number VAST.DA47.DA.12/16–19. Conflict of interest: none.

## References

Aibinu, I. E., Smooker, P. M., Lopata and A. L. 2019. *Anisakis* nematodes in fish and shellfish from infection to allergies. International Journal for Parasitology: Parasites Wildlife 9:384–93.

Arthur, J. R. and Te, B. Q. 2006. Checklist of the parasites of fishes of Viet Nam FAO, Rome.

Audicana, M. T., Ansotegui, I. J., de Corres, L. F. and Kennedy, M. W. 2002. *Anisakis simplex*: dangerous – dead and alive?. Trends in Parasitology 18:20–5.

Berland, B. 1961. Nematodes from some Norwegian marine fishes. Sarsia 2:1–50.

Chen, H. Y. and Shih, H. H. 2015. Occurrence and prevalence of fish-borne *Anisakis* larvae in the spotted mackerel *Scomber australasicus* from Taiwanese waters. Acta Tropica 145:61–7.

Cho, J., Lim, H., Jung, B. K., Shin, E. H. and Chai, J. Y. 2015. *Anisakis pegreffii* Larvae in Sea Eels (*Astroconger myriaster*) from the South Sea, Republic of Korea. Korean Journal of Parasitology 53:349–53.

D’amelio, S., Busi, M., Ingrosso, S. and Paggi, L. 2010. “Anisakiasis”, In Liu, D. Y. (Ed.), Molecular detection of foodborne pathogens Taylor & Francis CRC Press, pp. 757–68.

Dorny, P., Praet, N., Deckers, N. and Gabriel, S. 2009. Emerging food-borne parasites. Veterinary Parasitology 163:196–206.

Eamsobhana, P., Yong, H. S., Song, S. L., Tungtrongchitr, A. and Roongruangchai, K. 2018. Genetic differentiation of *Anisakis* species (Nematoda: Anisakidae) in marine fish *Priacanthus tayenus* from Gulf of Thailand. Tropical Biomedicine 35:669–77.

Farjallah, S., Slimane, B. D., Busi, M., Paggi, L., Amor, N., Blel, H., Said, K. and D’Amelio, S. 2008. Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of Mediterranean Sea. Parasitology Research 102:371–9.

Klimpel, S. and Palm, H. W. 2011. “Anisakid nematode (Ascaridoidea) life cycles and distribution: Increasing zoonotic potential in the time of climate change?”, In Mehlhorn, H. (Ed.), Progress in parasitology. Parasitology research monographs, Vol. 2 Springer, Berlin, pp. 201–22.

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

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. Molecular Biology and Evolution 33:1870–4.

Lee, M. H., Cheon, D. S. and Choi, C. 2009. Molecular genotyping of *Anisakis* species from Korean



- sea fish by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *Food Control* 20:623–6.
- Lee, W. J., Seo, D. J., Oh, H., Jeon, S. B., Jung, D. and Choi, C. 2016. Simultaneous detection and prevalence of allergens in *Anisakis* species isolated from marine fishes. *Journal of Food Protection* 79:789–94.
- Madden, P. A. and Tromba, F. G. 1976. Scanning electron microscopy of the lip denticles of *Ascaris suum* adults of known ages. *Journal of Parasitology* 62:265–71.
- Marques, J. F., Cabral, H. N., Busi, M. and D'Amelio, S. 2006. Molecular identification of *Anisakis* species from Pleuronectiformes off the Portuguese coast. *Journal of Helminthology* 80:47–51.
- Mattiucci, S. and Nascetti, G. 2006. Molecular systematics, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: an update. *Parasite* 13:99–113.
- Mattiucci, S. and 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. *Advance in Parasitology* 66:47–148.
- Mattiucci, S., Paoletti, M. and Webb, S. C. 2009. *Anisakis nascettii* n. sp. (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. *Systematic Parasitology* 74:199–217.
- Mattiucci, S., Nascetti, G., Dailey, M., Webb, S. C., Barros, N. B., Cianchi, R. and Bullini, L. 2005. Evidence for a new species of *Anisakis* Dujardin, 1845: morphological description and genetic relationships between congeners (Nematoda: Anisakidae). *Systematic Parasitology* 61:157–71.
- Mattiucci, S., Paggi, L., Nascetti, G., Santos, C. P., Costa, G., Di Benedetto, A. P., Ramos, R., Argyrou, M., Cianchi, R. and Bullini, L. 2002. Genetic markers in the study of *Anisakis typica* (Diesing, 1860): larval identification and genetic relationships with other species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae). *Systematic Parasitology* 51:159–70.
- Morsy, K., Badr, A. M., Abdel-Ghaffar, F., Deeb, S. E. and Ebead, S. 2017. Pathogenic potential of fresh, frozen, and thermally treated *Anisakis* spp. type II (L3) (Nematoda: Anisakidae) after oral inoculation into Wistar rats: a histopathological study. *Journal of Nematology* 49:427–36.
- Ngo, H. D., Ha, N. V., Tu, N. D. and Thanh, N. V. 2009. Preliminary study on the parasitic helminth fauna on marine fishes in the coastal waters of Hai Phong province. *Vietnam Journal of Biology* 31:1–8.
- Palm, H. W., Damriyasa, I. M., Linda and 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.
- Palm, H. W., Theisen, S., Damriyasa, I. M., Kusmintarsih, E. S., Oka, I. B., Setyowati, E. A., Suratma, N. A., Wibowo, S. and Kleinertz, S. 2017. *Anisakis* (Nematoda: Ascaridoidea) from Indonesia. *Diseases of Aquatic Organisms* 123:141–57.
- Pantoja, C. S., Borges, J. N., Santos, C. P. and Luque, J. L. 2015. Molecular and morphological characterization of Anisakid nematode larvae from the sandperches *Pseudoperca numida* and *Pinguipes brasiliensis* (Perciformes: Pinguipedidae) off Brazil. *Journal of Parasitology* 101:492–99.
- Pekmezci, G. Z., Onuk, E. E., Bolukbas, C. S., Yardimci, B., Gurler, A. T., Acici, M. and Umur, S. 2014. Molecular identification of *Anisakis* species (Nematoda: Anisakidae) from marine fishes collected in Turkish waters. *Veterinary Parasitology* 201:82–94.
- Sonko, P., Chen, S. C., Chou, C. M., Huang, Y. C., Hsu, S. L., Barčák, D., Oros, M. and Fan, C. K. 2019. 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. *Journal of Microbiology, Immunology and Infection* 53:1021–9.
- Suzuki, J., Murata, R., Hosaka, M. and Araki, J. 2010. Risk factors for human *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakid nematodes. *International Journal of Food Microbiology* 137:88–93.
- Tunya, R., Wongsawad, C., Wongsawad, P. and Chai, J. Y. 2020. Morphological and molecular characteristics of *Anisakis typica* larvae in two species of threadfin bream, *Nemipterus hexodon* and *N. japonicus*, from the Gulf of Thailand. *Korean Journal of Parasitology* 58:15–25.
- Umehara, A., Kawakami, Y., Ooi, H. K., Uchida, A., Ohmae, H. and Sugiyama, H. 2010. Molecular identification of *Anisakis* type I larvae isolated from hairtail fish off the coasts of Taiwan and Japan. *International Journal of Food Microbiology* 143:161–5.
- Zhu, X., D'Amelio, S., Paggi, L. and Gasser, R. B. 2000. Assessing sequence variation in the internal transcribed spacers of ribosomal DNA within and among members of the *Contraecum osculatum* complex (Nematoda: Ascaridoidea: Anisakidae). *Parasitology Research* 86:677–83.
- Zhu, X. Q., Podolska, M., Liu, J. S., Yu, H. Q., Sonko, H. H., Lin, Z. X., Luo, C. B., Song, H. Q. and Lin, R. Q. 2007. Identification of anisakid nematodes with zoonotic potential from Europe and China by single-strand conformation polymorphism analysis of nuclear ribosomal DNA. *Parasitology Research* 101:1703–7.